Constraints imposed by pollinator behaviour on the ecology and evolution of plant mating systems

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2   **CONSTRAINTS IMPOSED BY POLLINATOR BEHAVIOUR ON THE ECOLOGY AND EVOLUTION OF**

3   **PLANT MATING SYSTEMS**

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1 **ABSTRACT**

2 Most flowering plants rely on pollinators for their reproduction. Plant-pollinator interactions,

3 although mutualistic, can create a conflict of interest between both partners and constrain

4 plant mating systems at multiple levels, including the immediate ecological plant selfing rates,

5 their distribution in and contribution to pollination networks, and their evolution. Here, we

6 first review experimental evidence that pollinator behaviour influences ecological plant

7 selfing rates in pairs of interacting species, and that reciprocally plants can modify pollinator

8 behaviour through plastic and evolutionary changes in floral traits. We also examine how

9 theoretical studies include pollinators, implicitly or explicitly, to investigate the role of their

10 foraging behaviour in plant mating system evolution. In doing so, we call for more

11 evolutionary models combining ecological and genetic factors, and additional experimental

12 data, particularly to describe pollinator foraging behaviour. We finally show that recent

13 developments in ecological network theory clarify the impact of community-level interactions

14 on plant selfing rates and their evolution, and allow for new research avenues to expand the

15 study of mating systems of animal-pollinated plant species to the level of the plant-pollinator

16 networks.

17

1   **INTRODUCTION**

2   Flowering plants are stimulating models for studying the evolutionary biology of reproductive

3   systems, owing to their wide diversity of mating systems (Barrett, 2003; Charlesworth, 2006).

4   Typical topics of interest comprise the evolution of selfing rates (Goodwillie *et al.*, 2005),

5   including self-incompatibility (Goldberg *et al.*, 2010), the evolution of separate sexes (Spigler

6   & Ashman, 2012) and sex chromosomes (Charlesworth, 2013), and the maintenance of sexual

7   reproduction (Silvertown, 2008). The reproduction of the vast majority of Angiosperm

8   species is unique in its reliance on animals as pollen vectors (~90%, Ollerton *et al.*, 2011), yet

9   the study of plant mating system evolution has long remained uncoupled from pollination

10   ecology, focusing primarily on genetic drivers. As mentioned by Charlesworth (2006), the

11   failure to include ecological mechanisms into the evolution of plant mating may be due to

12   their diversity: "*Models of mating system evolution have emphasised genetic effects, even*

13   *though (…) ecological circumstances, such as pollinator abundance or plant density, must*

14   *often be important. Their complexity and variety, however, creates difficulties in developing*

15   *any general theories*".

16   The gap between the study of plant mating systems and pollination ecology has shrunk

17   over the past decades, with many studies focusing mostly on animal-pollinated plants

18   (reviewed e.g. in Harder & Barrett, 1996, 2006; Goodwillie *et al.*, 2005; Mitchell *et al.*, 2009;

19   Eckert *et al.*, 2010; Karron *et al.*, 2012; Thomann *et al.*, 2013) but also on wind-pollinated

20   plants (Friedman & Barrett, 2009). This rich literature has revealed general patterns and

21   processes out of the complexity mentioned by Charlesworth (2006). One such pattern is the

22   increase in selfing rates under pollen limitation and its long-term evolutionary consequences

23   (Wright *et al.*, 2013). This intuitive expectation can now be challenged as pollinator

24   abundance decline and reduced pollination service become a worldwide reality (Potts *et al.*,

25   2010; Gonzalez-Varo *et al.*, 2013). Several papers discuss pollinator shortage as a cause of

1 outcross pollen limitation (see glossary) and increased immediate ecological selfing rates,

2 which may determine future evolutionary changes in plant mating systems (Eckert *et al.*,

3 2010; Thomann *et al.*, 2013). The latter prediction is supported by rapid evolution of the

4 ability to self-fertilize observed in *Mimulus guttatus* in the absence of pollinators (Bobdyl

5 Roels & Kelly, 2011).

6   Pollinator foraging behaviour also emerges as an important factor influencing plant

7 selfing rates and their evolution. The predicted impact of foraging behaviour on plant mating

8 systems is however less clear than that of pollinator abundance because selection on

9 pollinator vs. plant traits do not always act in the same direction. In plants, selection favours

10 floral traits that influence pollen transfer to promote reception of conspecific and compatible

11 pollen, increase the quantity and quality of seeds produced (including the ratio of selfed to

12 outcrossed seeds) and the amount of pollen exported to conspecifics. In pollinators, selection

13 favours traits that maximize the rate of energy gain through foraging behaviour (optimal

14 foraging theory, Pyke, 1984), which may lead to non-optimal pollen transfer for plants.

15 Hence, although plants and pollinators are involved in true mutualistic interactions with

16 reciprocal benefits, conflicts of interest are widespread (Bronstein, 2001; Dufay & Anstett,

17 2003; De Jong & Klinkhamer, 2005, p. 229). Constraints imposed by pollinators on plants, as

18 well as constraints imposed by plants on pollinators, may induce plastic and evolutionary

19 responses, such that the two partners are engaged in a permanent Red Queen-like co-

20 evolutionary race. The coevolution of plant and pollinator traits has been a major topic of

21 research since Darwin, but constraints imposed by pollinator foraging behaviour on the

22 ecology and evolution of plant mating systems are seldom integrated into experimental and

23 theoretical studies. For example, most models of the evolution of plant selfing rates assume

24 that these rates can evolve freely between zero and one. Another limit of studies connecting

25 mating systems and pollination ecology is that they are often restricted to pairs of plant-

1   pollinator species. Recent developments in ecological network theory have revealed that the

2   community context, multi-species interactions, and the position of a species in a plant-

3   pollinator network can be key to understand the influence of pollinators on plant mating

4   systems (Vanbergen *et al.*, 2014).

5   Here, we review how the interplay between pollinator behaviour and plant floral traits

6   influences the immediate, ecological plant selfing rates (hereafter "ecological selfing rates"),

7   the evolution of selfing rates, and their distribution in plant-pollinator networks (summarized

8   in Fig. 1). We are interested in self-compatible plants, mostly with perfect flowers, that are

9   pollinated by insects or birds (Buchmann & Nabhan, 1996), particularly bees and

10   hummingbirds for which we have more data. In the first section, we show that the ecological

11   selfing rates of about one half of flowering plant species is controlled partly by pollinator

12   abundance and behaviour, which should therefore be included in theoretical investigation of

13   plant mating system evolution. We argue, as do Karron *et al.* (2012), that the reciprocal

14   effects of pollinator behaviour on floral traits are much better documented than their

15   consequences for plant self-fertilization. Because we focus on pollinator behaviour, we

16   intentionally exclude pollinator abundance, pollen limitation and their influence on the

17   selection of floral traits, which were presented extensively elsewhere (Elzinga *et al.*, 2007;

18   Eckert *et al.*, 2010; Schiestl & Johnson, 2013; Thomann *et al.*, 2013). One should keep in

19   mind that these two components strongly determine ecological selfing rates and their

20   evolution.

21   The second part of this review examines how theoretical studies of the evolution of

22   plant selfing rates include pollinator behaviour and model the plant traits that can alleviate the

23   constraints it might impose. We show that mechanistic models combining pollinator

24   behaviour and the genetic consequences of selfing improve on pure ecological or pure genetic

25   models by providing quantitative predictions of evolutionarily and ecologically stable plant

1   mating strategies. We propose a method to account for pollination ecology in models of the

2   evolution of selfing rates, and provide experimental research avenues to help clarify the role

3   of plant-pollinator interactions in the distribution of plant selfing rates. The last section shows

4   that community-level processes can influence the ecology and evolution of plant mating

5   systems, and outline how to use current knowledge on competing vs. facilitative interactions

6   among plant species and pollinator species for a better understanding of the distribution and

7   evolution of plant selfing rates.

8

9   **THE INTERPLAY BETWEEN POLLINATOR BEHAVIOUR AND FLORAL TRAITS DETERMINES**

10 **ECOLOGICAL PLANT SELFING RATES**

11 The selfing rate or self-fertilization rate s is the fraction of selfed embryos produced by an

12 individual plant. This fraction depends primarily on the rate of self-pollination, the relative

13 amounts of self vs. outcross pollen transferred by pollinators or through non-pollinator means

14 (e.g. stigma dragging). The self-pollination rate can be further modified by pre and post-

15 fertilization selection (see glossary), which is beyond the scope of this review. Only two

16 categories of plant species have complete control over their selfing rate, although pollinators

17 may still influence their seed set: (1) obligately outcrossing species ($s = 0$), such as self-

18 incompatible or dioecious species, representing ca. 50% of species (Igic & Kohn, 2006) and

19 (2) completely selfing species ($s = 1$) relying on prior autonomous selfing (see glossary), such

20 as species producing closed flowers only, which are however extremely rare (Goodwillie *et*

21 *al.*, 2005; Culley & Klooster, 2007).

22   In the remaining half of animal-pollinated plant species, selfing rates are invariably

23 influenced by pollinators, via the quantities of both outcross and self-pollen transferred

24 among and within flowers (Figs. 1 and 2). Outcross pollen deposition on plant stigmas is

25 always fully constrained by pollinators. In contrast, self-pollen deposition on stigmas depends

1   only partially, but sometimes strongly, on pollinators as it includes: (1) facilitated self-

2   pollination, the transfer of self-pollen within flowers caused by pollinator visits, (2)

3   geitonogamous self-pollination caused by pollinator visits among flowers on a plant, and (3)

4   autonomous, i.e. without the help of pollinators, prior, competing and delayed self-pollination

5   within flowers (Fig. 2 and definitions of the glossary). Note that the amount of self-pollen

6   deposited "autonomously" by a plant on stigmas may still be influenced by previous outcross

7   pollen limitation, as is sometimes the case with delayed autonomous selfing (e.g. Ruan *et al.*,

8   2010). Besides, even if the *amount* of autonomous self-pollen is under the plant control, the

9   *rate* of self-pollination depends on pollinators via the amounts of self and outcross pollen they

10   deposit on stigmas.

11   In the following, we examine how pollinator behavioural traits interact with plant traits

12   to constrain patterns of outcross and self-pollen deposition and thereby the ecological selfing

13   rates of plants. We review how pollinators can respond plastically to variation in floral traits

14   to optimize their energy gains, and which floral traits can vary plastically or genetically to

15   modify pollinator behaviour and control plant selfing rates. We consider one self-compatible

16   plant species specialized (see glossary) on one pollinator species or functional group (Fenster

17   *et al.*, 2004), as is frequently done in studies of the relationship between pollinators and plant

18   mating systems. Pollinator choice among multiple plant species (preference and constancy,

19   see glossary), competition among plant species for shared pollinators, and their impacts on

20   plant selfing rates are discussed only in the last section.

21   *Pollinator traits influencing outcross and self-pollen transfer*

22   Self-pollination rates are influenced by (1) the number of pollinator visits to individual plants

23   and flowers, (2) the efficiency of pollen transfer from stamens to pollinators and from

24   pollinators to stigmas, within and among flowers, and (3) the composition of pollen loads on

25   pollinators, particularly the fraction of self vs. outcross pollen. All these components

1   ultimately depend on pollinator abundance, morphological and phenological matching of

2   plants and pollinators, and pollinator foraging behaviour among and within plants. We focus

3   on pollinator behaviour (Fig. 1) and do not address pollinator abundance, phenology or

4   morphology (see e.g. Waser, 1978; Harder & Barrett, 1996 for details on pollen placement on

5   pollinators; or O'Neil, 1997; Elzinga *et al.*, 2007; Bartomeus *et al.*, 2011 for phenology).

6   Pollinator grooming behaviour influences pollen transfer efficiency within and among

7   plants; its intensity and timing greatly determine patterns of pollen deposition of outcross and

8   self-pollen throughout a foraging bout (see glossary). Grooming generally causes pollen to be

9   deposited on fewer flowers (e.g. Harder *et al.*, 2000; Castellanos *et al.*, 2003), hence reduces

10   pollen carryover, with contrasting impacts of within- vs. between-plant grooming. Between-

11   plant grooming reduces pollen dispersal among plants (Holmquist *et al.*, 2012) and is likely to

12   increase outcross pollen limitation. Within-plant, between-flower grooming reduces pollen

13   carryover within plants and should increase geitonogamous selfing rates (Matsuki *et al.*,

14   2008). The scant available data suggest that most grooming occurs between plants (see e.g.

15   Harder, 1990; Mitchell *et al.*, 2004; Johnson *et al.*, 2005), but the pattern may vary among

16   pollinator species and depend on pollen availability, as shown by a higher probability of

17   grooming between flowers with increasing pollen availability (Harder, 1990). More intensive

18   and more frequent grooming is observed for pollen-collecting visitors (e.g. bees) than for

19   nectar-collecting visitors (e.g. birds or moths; (Thomson, 1986; Castellanos *et al.*, 2003). As a

20   result, nectar-feeding species tend to transfer pollen more efficiently than do pollen-feeding

21   species (Conner *et al.*, 1995; Sahli & Conner, 2007; but see King *et al.*, 2013). More

22   generally, the extent of pollen carryover is negatively related to geitonogamous selfing rates

23   (Geber, 1985; Robertson, 1992a; Morris *et al.*, 1994): pollinators that deposit most of their

24   outcross pollen on the first few flowers transfer mostly self-pollen on the subsequent flowers

25   of the plant. Grooming can partially cause the negative relationship between pollen carryover

1   and geitonogamy (Rademaker *et al.*, 1997; Matsuki *et al.*, 2008), but pollen carryover is also

2   strongly governed by interactions between pollinator morphology and floral design (Harder &

3   Barrett, 1996).

4   The number of flowers a pollinator probes successively on a plant increases

5   geitonogamous self-pollination (Rademaker *et al.*, 1999; Karron *et al.*, 2009). Pollinators tend

6   to probe more flowers on plants with larger floral displays (see glossary), but rarely probe

7   more than a dozen flowers per plant, so that the fraction of flowers probed per plant is often a

8   decreasing function of display size (reviewed in Snow *et al.*, 1996; Ohashi & Yahara, 2001;

9   Harder *et al.*, 2004). Multiple reasons can explain why pollinators leave plants before visiting

10   all open flowers on a plant (reviewed in Snow *et al.*, 1996); the most general one involves the

11   maximization of energy gains (optimal foraging). For pollinators, which have limited short-

12   term memory, visiting only a fraction of available flowers reduces the risk of visiting a given

13   flower twice (Ohashi & Yahara, 1999, 2001, 2002). Similarly, pollinators leave a plant earlier

14   when they encounter empty, rewardless flowers (e.g. Bailey *et al.*, 2007 and references

15   therein). Self-pollination also depends on the order in which flowers of a plant are visited by

16   pollinators. As the first few flowers visited by pollinators contribute the most to outcross

17   pollen transfer, outcross pollination is expected to be larger when independent pollinators

18   visit flowers randomly on a plant than when all pollinators visit flowers in the same order

19   (Devaux *et al.*, unpublished manuscript, for a model).

20   All above characteristics of pollinators affect the transfer of outcross and self-pollen

21   among flowers, but not within-flower facilitated selfing (see glossary), which remains little

22   studied. Facilitated selfing has been demonstrated unambiguously in a small number of

23   species(e.g. Anderson *et al.*, 2003; Duncan *et al.*, 2004; Johnson *et al.*, 2005; Owen *et al.*,

24   2007; Vaughton *et al.*, 2008), but its broader contribution to total selfing rates in natural

25   populations is currently unknown (see Box 1 for a method). It is unclear whether facilitated

1   selfing is primarily controlled by flower characteristics, such as spatial (herkogamy) or

2   temporal (dichogamy) separation of anthers and stigmas (Lloyd & Webb, 1986; Webb &

3   Lloyd, 1986; Brunet & Eckert, 1998) or if it can be influenced by pollinator behaviour. Buzz-

4   pollination by native bees causes high rates of facilitated self-pollination in the partially self-

5   incompatible *Dianella revoluta* (Duncan *et al.*, 2004). Furthermore, reduced time spent at

6   individual flowers by hawkmoths has been associated with decreased seed production in self-

7   compatible *Petunia* lines (Brandenburg *et al.*, 2012), but we do not know whether any of the

8   effect can be attributed to facilitated selfing. From available experimental studies, we can

9   nevertheless argue that increased numbers of flowers probed per plant or increased probing

10   time per flower should contribute to increased facilitated selfing at the plant level.

11   ***Floral traits influencing self-pollination rates via pollinator behaviour***

12   Floral traits affecting self-pollination can be divided into three broad categories: (1) traits

13   related to autonomous selfing (Lloyd & Schoen, 1992), (2) traits modifying mechanically

14   pollinator-mediated outcross and self-pollen transfer, without altering pollinator behaviour

15   (e.g. floral design, pollen size and stickiness, and within and among flowers temporal

16   separation of male and female functions; Webb & Lloyd, 1986; Lloyd & Schoen, 1992) and

17   (3) traits modifying pollinator behaviour directly. We focus on the latter floral traits, which

18   typically influence two types of foraging behaviours: (3.1) patterns of flower visitation among

19   plants (attraction, preference and constancy, see glossary), which are mostly discussed in the

20   last section and (3.2) patterns of flower visitation within plants (number and sequence of

21   flowers visited, as well as probing time).

22   Floral display (see glossary) may be the most studied plant trait influencing pollinator

23   behaviour. Plants with larger floral displays attract more pollinators, thereby receiving more

24   outcross and geitonogamous pollen (Snow *et al.*, 1996; Rademaker *et al.*, 1999; Karron *et al.*,

25   2004; Williams, 2007). Floral display can be modified plastically, for example via flower

1   wilting when pollinators are abundant (Harder & Johnson, 2005), or flower abortion inducing

2   pollinators to leave the plant (Ito & Kikuzawa, 2003); both mechanisms reduce the risk of

3   geitonogamous selfing. Floral display can also be modified via evolutionary changes in the

4   total flower production, in the distribution of open flowers among days (individual

5   phenology) or in the longevity of flowers (Elzinga *et al.*, 2007; Devaux & Lande, 2010). How

6   these changes in floral display modify selfing rates depends on the rates and patterns of

7   flower visitation by pollinators but the general trend is again an increase in plant selfing rates

8   with larger floral displays.

9   Many other floral traits are also cues for pollinator attraction and influence visitation

10   rates and outcross pollen receipt. These include flower size, reward production, floral shape,

11   colour, and scent (Cozzolino & Scopece, 2008), or more anecdotal characteristics, such as

12   colourful leaves (Keasar *et al.*, 2009), sterile anthers (staminodes, Sandvik & Totland, 2003)

13   or sterile flowers (e.g. *Centaurea cyanus*, Garcia-Jacas *et al.*, 2001). Nectar production and its

14   replenishment dynamics have received particular attention, because they are critically related

15   to geitonogamy. For example, bumblebees experiencing unrewarding (nectarless) plants

16   probe more flowers on subsequent rewarding plants, such that the geitonogamous selfing rates

17   of rewarding plants increased with the frequency of unrewarding plants in the population

18   (Ferdy & Smithson, 2002). Conversely, maintaining nectarless flowers within an otherwise

19   nectar-producing inflorescence may encourage pollinators to leave a plant early, thereby

20   reducing geitonogamy (Hirabayashi *et al.*, 2006; Bailey *et al.*, 2007 and references therein;

21   Whitehead *et al.*, 2012). Pollinator behaviour within a plant, hence geitonogamy, can also be

22   modified by floral complexity (more flowers visited in plants with simpler flowers, Ohashi,

23   2002) or inflorescence architecture (e.g. lower selfing in racemes vs. umbels, Harder *et al.*,

24   2004; Jordan & Harder, 2006; or in more tightly twisted inflorescences, Iwata *et al.*, 2012).

1   Again, floral traits influencing facilitated self-pollination are poorly known, apart from

2   structural features preventing self-pollination, such as anther caps (Peter & Johnson, 2006)

3   and spatial separation of sexes. In contrast, the production of complex floral design or shape

4   can induce higher probing time (Ohashi, 2002), which can translate into higher seed set

5   (Brandenburg *et al.*, 2012), potentially because of higher facilitated selfing.

6   There is ample and long-standing evidence that plant selfing rates are constrained by

7   pollinator morphology and foraging behaviour (Harder & Barrett, 1996), which may favour

8   plant traits that can alleviate constraints imposed by pollinators. Evolution of plant traits

9   under pollinator-mediated selection has been extensively studied (Elzinga *et al.*, 2007 for

10   plant phenology; Schiestl & Johnson, 2013 for floral signals), but with surprisingly little

11   emphasis on plant mating systems, except in verbal models (Eckert *et al.*, 2010; Thomann *et*

12   *al.*, 2013). In the next section, we review the few theoretical studies that include pollinator

13   behaviour in models of the evolution of plant mating systems. We argue that combining

14   pollination ecology and the genetics of inbreeding depression is necessary to describe

15   accurately and to understand plant mating system evolution. We conclude by pointing out

16   experimental data needed to extend theoretical work on the evolution of plant mating system

17   under pollinator constraints.

18

19   **THE ROLE OF POLLINATORS IN THE EVOLUTION OF PLANT SELFING RATES**

20   We clarify how the interplay between plants and pollinators determines plant fitness

21   components, besides selfing rates, and review how models of the evolution of selfing rates

22   implicitly or explicitly integrate plant-pollinator interactions.

23   *Constraints imposed by pollinators on plant fitness components and the evolution of plant*

24   *selfing rates*

1   The fitness of a plant is determined by the number of outcrossed and selfed seeds it produces

2   and the number of pollen grains it successfully exports to other plants (male outcrossed siring

3   success), weighted by the relative offspring fitnesses. Hence the fitness of animal-pollinated

4   plants depends critically on pollinators, also via resource allocation to attraction traits, and on

5   inbreeding depression (see glossary, Lloyd, 1979; Lande & Schemske, 1985; Charlesworth &

6   Willis, 2009)

7   Two major phenomena involving pollinators and influencing plant fitness components

8   are often included in studies of the evolution of plant selfing rates without explicit modelling

9   of pollinators (reviewed in Goodwillie *et al.*, 2005): pollen limitation and pollen discounting

10   (see glossary). Pollen limitation is a key component favouring the evolution of higher selfing

11   rates (Cheptou, 2004; Porcher & Lande, 2005b) and depends greatly on pollinator abundance

12   (Ashman *et al.*, 2004; Eckert *et al.*, 2010; Thomann *et al.*, 2013). Several authors have

13   proposed that stronger outcross pollen limitation can mimic declines in pollinator density and

14   hamper the evolution of complete outcrossing (Sakai & Ishii, 1999; Masuda *et al.*, 2001;

15   Morgan & Wilson, 2005). Pollen discounting, a negative relationship between selfing rate and

16   pollen export, can be caused by pollinators transferring large amounts of self-pollen among

17   multiple flowers of plants, which are therefore lost for outcrossing (Karron & Mitchell, 2012),

18   and hinders the evolution of high selfing rates (Goodwillie *et al.*, 2005).

19   More generally, correlations or functional relationships among fitness components are

20   important drivers of the evolution of plant mating systems. Several models show that the

21   maintenance of mixed mating can result from relationships between male fertility, female self

22   fertility and female outcross fertility (including pollen discounting, Johnston *et al.*, 2009),

23   between viability and selfing rate (Jordan & Otto, 2012), or between selfing and a cost of

24   interaction with pollinators (Lepers *et al.*, unpublished manuscript). Correlations among plant

25   fitness components are partly governed by pollinators (see below), particularly by their

1   foraging behaviour within plants in response to floral traits (e.g. floral display, Best &

2   Bierzychudek, 1982). Yet, models that use these correlations without integrating pollinators

3   explicitly are useful to address the effects of pollinators on selfing rates and have helped

4   understand the qualitative role of pollination in the evolution of plant mating. Assuming a

5   positive correlation between the number of selfed and outcrossed ovules is relevant for many

6   animal-pollinated species in which more pollinator visits increase both geitonogamous self-

7   pollination and the number of outcrossed seeds (Johnston *et al.*, 2009 and references therein).

8   Similarly, a negative correlation between reward production and selfing rate (as in Lepers *et*

9   *al.*, unpublished manuscript), or between the production of costly open (vs. closed) flowers

10   and selfing rate (as in Masuda *et al.*, 2001; data in Oakley *et al.*, 2007) can be used to

11   understand the evolution of selfing syndromes (see glossary; reviewed by Sicard & Lenhard,

12   2011).

13   Further progress towards more reliable, quantitative predictions of equilibrium mating

14   systems requires mechanistic models of the constraints that pollinator behaviour imposes on

15   plant selfing rates, which are still few. Morgan *et al*. (2005) used optimal foraging theory to

16   model evolution of selfing by assuming a decreasing rate of geitonogamous selfing with

17   increasing plant density, which was justified because pollinators are more likely to switch

18   between plants when flight distances are smaller (Cresswell, 1997; Mustajarvi *et al.*, 2001).

19   Another approach has included the demography of plant and pollinator populations,

20   highlighting the possibility of demographic extinction of pollinator and plant populations

21   during the transition to higher selfing rates (Lepers *et al.*, unpublished manuscript), due to

22   reduced production of rewards for pollinators. The most comprehensive mechanistic models

23   tackle the evolution of floral traits influencing pollinator behaviour, and therefore plant

24   selfing rates. For example, models that jointly describe the evolution of daily floral display

25   and pollinator foraging behaviour show that pollinators can generate stable intermediate

1   geitonogamous selfing rates (de Jong *et al.*, 1992; Masuda *et al.*, 2001; Devaux *et al.*,

2   unpublished manuscript). Similarly, models of the evolution of nectar content have analysed

3   how the production of rewardless flowers can decrease the geitonogamous selfing rate of

4   individual plants (Bailey *et al.*, 2007) and conversely how pollinator learning can increase the

5   geitonogamous selfing rates of rewarding plants that co-occur with non-rewarding plants

6   (Ferdy & Smithson, 2002).

7   Models combining pollinator foraging and the evolution of floral traits are promising

8   tools to study the ecological drivers of plant mating system evolution, but they can still be

9   improved. The number of flowers probed, hence the geitonogamous selfing rate of self-

10   compatible hermaphrodite species, critically depends on pollinator foraging behaviour, but the

11   latter is simplified in existing models: pollinators are assumed to visit all flowers on a plant

12   (de Jong *et al.*, 1992), the number of pollinator visits per plant is assumed proportional to

13   floral display (Masuda *et al.*, 2001, 2004), the probability to leave a plant is assumed

14   unrelated to floral display (Devaux *et al.*, unpublished manuscript), and pollinators are

15   assumed to leave a plant immediately after visiting a rewardless flower (Bailey *et al.*, 2007);

16   these assumptions are at odds with empirical observations (Robertson, 1992b; Duan *et al.*,

17   2005; Ishii & Harder, 2006). Such assumptions are unavoidable, and highlight the difficulty

18   of including realistic but sufficiently general models of pollination ecology in models of the

19   evolution of plant selfing rates (but see Ferdy & Smithson, 2002 for a model incorporating

20   pollinator learning).

21   ***The interplay between pollinators and the dynamics of purging inbreeding depression***

22   ***determines the evolution of plant selfing rates***

23   Inbreeding depression (see glossary and Box 2), the relative fitness of selfed vs. outcrossed

24   offspring, is a central evolutionary force that has received much attention in the population

25   genetics approach to studying plant mating system evolution (reviewed in Charlesworth &

1   Willis, 2009). The joint evolution of inbreeding depression and plant mating systems, i.e. the

2   purging of deleterious mutations with increasing selfing rates, has been extensively studied

3   both experimentally (Husband & Schemske, 1996) and theoretically, but has rarely been

4   included in ecological models of plant mating system evolution. Allowing for an evolving

5   rather than constant inbreeding depression in models of pollination ecology can nevertheless

6   greatly alter the outcomes of models of the evolution of selfing. First, conditions favouring

7   the maintenance of outcrossing are much more restricted in the presence of evolving rather

8   than constant inbreeding depression (Porcher & Lande, 2005a; b). Second, purging creates a

9   strong positive feedback on the evolution of selfing: an increase in the population selfing rate

10   decreases inbreeding depression (Fig. 3), thereby strongly favouring the further evolution of

11   increased selfing. This can destabilize equilibria that appear evolutionarily stable in models

12   where inbreeding depression does not depend on the selfing rate. Porcher *et al.* (2009)

13   demonstrated that incorporating a genetic model for inbreeding depression and the possibility

14   of purging destabilizes intermediate selfing rates that would otherwise be maintained by

15   temporal variation in inbreeding depression (Cheptou & Schoen, 2002).

16   The joint evolutionary dynamics of inbreeding depression and plant mating system are

17   often overlooked in ecologically-oriented models because their analysis requires complex

18   genetic models over and above the complexity of ecological processes. A method based on an

19   approximation for the purging of inbreeding depression (e.g. Lande *et al.*, 1994) provides a

20   powerful way around this complexity (Box 2), on the condition that genomic mutation rates to

21   deleterious alleles causing inbreeding depression remains moderate (Box 2, Porcher & Lande,

22   2013). This approximation has been used in some ecological models to study the joint role of

23   ecological and genetic constraints in plant mating system evolution (Johnston, 1998; Devaux

24   *et al.*, unpublished manuscript; Lepers *et al.*, unpublished manuscript). These models show

25   that ecological and genetic mechanisms interact strongly to determine evolutionary outcomes.

1   For example, Devaux *et al.* (unpublished manuscript) identified two types of equilibrium

2   selfing rates when modelling the evolution of floral display and geitonogamous selfing under

3   pollinator constraints: (1) ecological equilibria constrained by pollinator behaviour only,

4   which exist regardless of inbreeding depression, and (2) evolutionarily stable equilibria

5   maintained by a trade-off between pollinator attraction and avoidance of geitonogamous

6   selfing, and governed by evolving inbreeding depression. This model also suggests that a

7   baseline rate of unavoidable geitonogamous selfing constrained by pollinators can trigger

8   purging of inbreeding depression and create conditions favouring evolution of increased

9   autonomous selfing. In view of this interaction between ecological and genetic mechanisms,

10   we encourage modellers interested in the ecological drivers of plant mating system evolution

11   to account for evolving inbreeding depression, which can be easily done using approximation

12   methods (Box 2).

13   ***Experimental limits to theoretical models***

14   No model has yet analysed the joint evolution of plant selfing rates and pollinator

15   characteristics: the morphologies and foraging behaviours of pollinators are generally

16   assumed to be constant (but plastic) functions of floral signals. Univariate pollinator-mediated

17   selection on multiple floral traits and their genetic architecture are well documented

18   (Kingsolver *et al.*, 2001; Geber & Griffen, 2003; Ashman & Majetic, 2006; Karron *et al.*,

19   1997; Galliot *et al.*, 2006). Several papers have shown that floral traits could adapt rapidly

20   (Ashman *et al.*, 2004; Thomann *et al.*, 2013), and a recent review has suggested that plants

21   could adapt more rapidly to pollinator-mediated selection than pollinators do to floral traits,

22   which can explain why we frequently observe pollination syndromes and floral convergence

23   in plants (Schiestl & Johnson, 2013 and references therein). Thus, the rarity of coevolutionary

24   models could reflect the lack of data on traits and behaviour of pollinators, and their adaptive

1   potential, although a few models of coevolution of plant and pollinator traits do exist (Kiester

2   *et al.*, 1984; Zhang *et al.*, 2013), but do not address the evolution of plant mating.

3   Modelling the joint evolution of plant mating and pollinator traits would require the

4   following experimental steps: (1) for plants, estimation of facilitated self-pollination and

5   elucidation of pollinator characteristics and behaviour involved (see Box 1 for a method); (2)

6   for plants again, estimation of the multivariate selection induced by pollinators on several

7   floral traits simultaneously, as both direct and indirect (correlated) selection is responsible for

8   the observed phenotypic distribution of floral traits; (3) for pollinators, accurate description of

9   the genetic architecture (number of and correlation among genes) and the selection gradients

10   on morphological and behavioural traits imposed by plants or their competitors; and (4)

11   estimation of the adaptive potential of both floral and pollinator traits.

12

13   **THE RECIPROCAL CONTRIBUTION OF PLANT-POLLINATOR NETWORKS TO PLANT SELFING**

14   **RATES**

15   The interactions between plants and pollinators determine immediate ecological self-

16   pollination rates, as well as the evolution of plant selfing rates. Most studies, both

17   experimental and theoretical, address this topic by focusing on pairs of interacting species,

18   whereas plants and their pollinators are part of complex interaction networks (Bascompte *et*

19   *al.*, 2003; Strauss & Irwin, 2004; Pocock *et al.*, 2012), which should influence plant mating

20   systems and their evolution. The combination of estimates from independent populations

21   scattered across the globe indicates a U-shaped distribution of plant selfing rates, with a

22   strong bias towards highly outcrossing species and numerous species with intermediate

23   selfing rates (Goodwillie *et al.*, 2005; Igic & Kohn, 2006). Whether this distribution is

24   representative of local plant communities is debatable, nevertheless it is likely that selfing

25   rates exhibit interspecific variation within communities. In the following we argue that (1) in

1　a network, the distribution of selfing rates of plant species that are not completely outcrossing

2　or completely selfing can be explained partly by the multispecies interactions operating at an

3　ecological time scale (Fig. 1), and (2) conversely, on longer time scales, the evolution of plant

4　mating systems, particularly the transition to higher selfing, can constrain the architecture of

5　plant-pollinator networks.

6　***The influence of the architecture of mutualistic networks on plant ecological selfing rates***

7　***and their evolution***

8　Here, we review the scarce available data and make predictions for the expected impact of

9　multispecies interactions on plant mating. We also point out the data needed to test these

10　predictions and better understand community-level effects on plant selfing rates.

11　　　Plant-pollinator networks are shaped by pollinator optimal foraging and morphological

12　or phenological matching between partners (e.g. Junker *et al.*, 2013). Within a given network,

13　plants differ in the identity of their pollinators, which is a primary mechanism leading to

14　differences in selfing rates in a plant community: different pollinator species have different

15　foraging behaviour and pollen transfer efficiencies and therefore contribute to variation in

16　selfing rates (Morinaga *et al.*, 2003; Brunet & Sweet, 2006; Matsuki *et al.*, 2008; but see

17　Eckert, 2002). Mutualistic interaction networks are also characterized by their nestedness (see

18　glossary), which implies asymmetric relationships between plant and pollinator species:

19　specialist plant (respectively pollinator) species interact (more often) with generalist

20　pollinator (respectively plant) species (Bascompte *et al.*, 2003; Thébault & Fontaine, 2010).

21　This architecture determines the level of interferences among specialist plant species because

22　of shared (generalist) pollinators, and among specialist pollinator species because of shared

23　(generalist) plant species. The combination of all interference components determines the

24　number of pollinator visits per plant and the quantity of heterospecific, outcross and self-

1   pollen deposited per pollinator visit, hence immediate ecological selfing rates (Wilcock &

2   Neiland, 2002; Vanbergen *et al.*, 2014).

3   In pollinator-sharing plant species, the rate of heterospecific vs. conspecific outcross

4   pollen transfer is governed by the floral constancy of pollinators (Ashman & Arceo-Gomez,

5   2013 and see glossary). Within-bout floral constancy of individual pollinators is likely to

6   maintain high outcrossing rates by increasing the relative amount of conspecific vs.

7   heterospecific pollen deposited on stigmas. We are unaware of any experimental study of this

8   relationship between floral constancy and selfing rates, but floral constancy is influenced by

9   the structure of both plant and pollinator communities, which should contribute to variation in

10   the selfing rate of a given plant species among communities. Similarities in floral traits among

11   plant species of a community tend to lower floral constancy: de Jager *et al.* (2011) observed

12   higher probabilities of pollinators switching between co-occurring *Oxalis* species of similar

13   colour. Conversely, Gegear & Laverty (2001, 2005) predicted and verified experimentally

14   that the diversity of plant species in a community increases floral constancy, particularly

15   when plant species differ in multiple floral traits. Interference among pollinators in a

16   community can also alter floral constancy, and thus the amount of outcross pollination. For

17   example, experimental removal of an abundant pollinator caused weaker interspecific

18   competition for food resources among the remaining pollinator species, which decreased their

19   floral constancy, thus plant seed set, in natural populations of *Delphinium barbeyi* (Brosi &

20   Briggs, 2013); the consequences on selfing rates were however not examined.

21   In addition to heterospecific pollen transfer, interference among (specialist) plant

22   species sharing pollinators also impacts pollinator visitation rates, with two contrasting

23   patterns: (1) decreased visitation rates (competition), caused by a combination of higher

24   density of competitor plants and higher pollinator preference for competitor plants (Rathcke,

25   1983; Vamosi *et al.*, 2006; Flanagan *et al.*, 2011), and (2) increased visitation rates

1   (facilitation), resulting from more effective attraction cues, maintenance of larger populations

2   of pollinators (Sargent & Ackerly, 2008; Liao *et al.*, 2011), or availability of complementary

3   resources for pollinators (Ghazoul, 2006). Competitive and facilitative interactions influence

4   selfing rates because they control the amount of outcross pollen deposited on stigmas

5   (Vamosi *et al.*, 2006; Alonso *et al.*, 2010). Which of these predominates among plant species

6   likely depends on phylogenetic distances among species (facilitation is more likely among

7   closely related species, Moeller, 2004; Schuett & Vamosi, 2010; Sargent *et al.*, 2011) and the

8   overlapping of population flowering phenologies within (Fründ *et al.*, 2011) and among days

9   (Motten, 1986; Devaux & Lande, 2009; Runquist, 2013).

10   Predicting how heterospecific pollen transfer and competition for pollinator visitation

11   jointly impact plant mating is straightforward: both mechanisms cause conspecific outcross

12   pollen limitation, which should be associated with increased selfing. Only a couple of studies

13   have demonstrated increased (ecological) selfing rates due to competition for pollinators: in

14   *Mimulus ringens* (Bell *et al.*, 2005) and *Laguncularia racemosa* (Landry, 2013). At broader

15   time scales, highly selfing populations of *Arenaria uniflora* are thought to have evolved to

16   avoid competition with *A. glabra* (Fishman & Wyatt, 1999). In contrast, the effect of

17   pollinator sharing on selfing rates in plant species with facilitative interactions is less

18   intuitive, because heterospecific pollen transfer and increased pollinator visitation rates

19   should compensate one another. In *Clarkia* communities characterized by facilitative

20   interactions, increased autonomous selfing is selected for under low plant species diversity

21   (Moeller & Geber, 2005), which suggests weak outcross pollen limitation and limited impacts

22   of heterospecific pollen transfer in highly diverse plant communities. The negative effects of

23   heterospecific pollen transfer can be avoided by increased floral constancy of pollinators

24   (Gegear & Laverty, 2005), separate pollen placement on pollinator bodies (Waser, 1978;

1   Huang & Shi, 2013 and references therein) or higher tolerance to heterospecific pollen

2   transfer (Ashman & Arceo-Gomez, 2013).

3   Finally, we may predict lower selfing rates on average for generalist than for specialist

4   plant species for two reasons. First, generalist plant species should receive more independent

5   visits by pollinators, which should deposit larger amounts of outcross pollen. In a nested

6   network, a significant fraction of this outcross pollen may however be heterospecific, because

7   generalist plant species are visited by specialist as well as generalist pollinators. Fang &

8   Huang (2013) for example observed higher rates of heterospecific pollen transfer in generalist

9   vs. specialist plants. Heterospecific pollen transfers may explain why the general negative

10   relationship between pollinator diversity and outcross pollen limitation remains weak (Davila

11   *et al.*, 2012). Second, interference among pollinators can alter pollinator behaviour within

12   plants, thereby influencing selfing rates via geitonogamous pollen transfer. Optimal foraging

13   selects for pollinators that leave plants before interacting with competitors and predators.

14   Hence, generalist plant species could exhibit lower geitonogamous selfing rates because

15   interference among multiple pollinator species is likely to reduce the number of flowers each

16   pollinator probes on a plant. One experimental study provides indirect support for this

17   phenomenon: Greenleaf & Kremen (2006) showed that in sunflower (*Helianthus annuus*)

18   fields containing male-fertile and male-sterile individuals, honey bees that interacted with

19   wild bees on male-sterile plants flowers were more likely to switch to a male-fertile plant than

20   when they interacted with a conspecific, thereby enhancing pollen transfer among individuals.

21   Further investigation is needed to confirm or rule out the prediction of larger outcrossing rates

22   in generalist vs. specialist plant species, for which at present there is little direct experimental

23   evidence.

24   There is thus mounting evidence that the architecture of plant-pollinator networks can

25   contribute to plant selfing rates and their evolution (Fig. 1). Experimental data are however

1   needed to explore the relationship between selfing rates and network architecture, particularly

2   plant specialization to pollinators (see glossary), and broaden our understanding of the

3   underlying mechanisms. Particularly, quantified plant-pollinator networks are crucial for an

4   accurate assessment of plant specialization (Ings *et al.*, 2009). Note however that most

5   networks are based on visitation data, which may not be representative of actual pollen

6   transfer networks (Alarcón, 2010; King *et al.*, 2013). We also need reliable estimates of

7   selfing rates, based on genetic markers for plant species of the same network (David *et al.*,

8   2007), to document the community-level distribution of plant selfing rates, now only available

9   in different ecological contexts (Goodwillie *et al.*, 2005). As a first step, selfing rates could be

10   regressed on specialization (number of visiting pollinator species) across all plant species of a

11   given plant-pollinator network at a given time. Alternatively, analyses could focus on a single

12   or a few plant species and make use of the documented spatial or temporal variation in

13   mutualistic networks. For example, Vanbergen *et al.* (2014) estimated the selfing rate of

14   *Cirsium palustre* and characterized plant-pollinator networks across a gradient of grazing

15   intensity. They observed higher selfing rates, associated with less densely connected

16   networks, in ungrazed vs. intensively grazed habitats. However, for a given grazing intensity,

17   selfing rates were positively related to network connectance. No general conclusion can be

18   drawn from this single study with conflicting patterns, but the work of Vanbergen *et al.*

19   (2014) does confirm that the architecture of plant-pollinator networks impacts plant selfing

20   rates.

21   Finally, studies of pollinator floral constancy are still rare: existing data deal with the

22   specialization of pollinator species only, whereas floral constancy is defined at the individual

23   level. Floral constancy and specialization can overlap (a pollinator species specialized on a

24   single plant species can only be constant), but remain distinct features of pollinator behaviour

25   (a generalist pollinator can be or not constant within a foraging bout, see glossary). Hence, we

1   believe that pollinator specialization is informative to study the dynamics of plant-pollinator

2   networks, but not to understand realized pollination patterns at the network level. Estimates of

3   floral constancy should ideally quantify the probability of pollinator switching, and go beyond

4   binomial classifications (in/fidelity, as in Brosi & Briggs, 2013).

5   *The influence of plant mating systems on plant-pollinator network architecture*

6   Evolutionary changes in selfing rates, particularly autonomous selfing, may in the short-term

7   involve minor modifications of floral traits (e.g. herkogamy, Webb & Lloyd, 1986), and have

8   little consequence for pollinator visitation rates and patterns. In the long term however, they

9   can induce more conspicuous changes and dramatically reduce pollinator visits, e.g. via a

10 decrease in floral size, nectar and scent production, or pollen/ovule ratio (Sicard & Lenhard,

11 2011), which could eventually alter the architecture of plant-pollinator networks. Few studies

12 so far have examined how plant mating systems shape plant-pollinator networks, with the

13 exception of Ollerton *et al.* (2006) and Davila *et al.* (2012), who found no difference in plant

14 specialization between self-compatible and self-incompatible species (a qualitative approach).

15 Yet, comparing self-compatible and self-incompatible species may not be appropriate to

16 detect a relationship between plant specialization and selfing rate, because self-compatible

17 species exhibit a wide range of selfing rates, from complete outcrossing to complete selfing

18 (Goodwillie *et al.*, 2005).

19   We present here a preliminary analysis that corroborates our prediction that

20 predominantly selfing species should be visited by fewer pollinator species than

21 predominantly outcrossing species. For this analysis, we brought together data on plant

22 mating system from the Biolflor database (Klotz *et al.*, 2002) with three published networks

23 providing qualitative or quantitative plant-pollinator interactions: a forest understory

24 (Robertson, 1929), a meadow (Memmott, 1999) and the Norwood farm (Pocock *et al.*, 2012)

25 networks. The same tendency of fewer pollinator species on predominantly selfing plant

1   species is observed across the three networks (Fig. 4), although differences among the mating

2   system classes are not all significant. A higher diversity of pollinator species on outcrossing

3   species can result from two non-exclusive phenomena: a lower visitation rate to highly selfing

4   species, which is mechanically associated with fewer pollinator species via a sampling effect,

5   and a higher "true" specialization of selfing plant species.

6   This example emphasizes the need for quantified interaction networks, to separate the

7   contributions to plant specialization due to overall pollinator (or plant) abundance vs.

8   diversity of visiting pollinator species. Several authors (e.g. Blüthgen *et al.*, 2007) have

9   stressed the influence of plant or pollinator abundance on the measurement of specialization,

10   but specialization is still frequently measured as a number of species, so that highly-selfing

11   plant species receiving few visits may be mistaken for extremely specialist species. In the two

12   quantified networks available here the number of pollinator species was always strongly and

13   significantly correlated with the total number of visits on a plant (not shown). There was also

14   a tendency, although not significant, for fewer visits to highly-selfing plant species vs. highly-

15   outcrossing plant species. When controlling for the effect of the number of visits a residual

16   effect of mating system on the number of pollinator species remained, but only in the

17   Norwood network for which predominantly outcrossing plant species were visited by a higher

18   diversity of pollinators.

19   This analysis has several shortcomings (crude classification of plant mating systems,

20   single trait approach ignoring correlates of mating systems that may also influence plant-

21   pollinator interactions, species considered as independent samples, etc.), and does not provide

22   a causal relationship between plant selfing rates and the plant-pollination networks. However,

23   it offers new research directions, both theoretical and experimental, to understand the

24   contribution of plant mating systems to the architecture of plant-pollinator networks.

25

1 CONCLUDING REMARKS

2 The study of plant mating systems in plant-pollinator networks is still in its infancy, but there

3 is already conclusive evidence that ecological plant selfing rates and their evolution are

4 shaped by conflicts of interest between plants and their pollinators, and by interactions

5 between pollinator behaviour and plant mating systems at the network level. The relative

6 contribution of such ecological constraints vs. genetic drivers to the evolution of plant mating

7 systems, as well as the role of temporal variation in plant-pollinator networks, remains largely

8 unknown and should be explored both empirically and theoretically. Regardless of what

9 drives the evolution of plant selfing rates, it is associated with changes in floral traits, as is the

10 case in the selfing syndrome: the consequences of plant mating system evolution on the

11 architecture of plant-pollinator networks is another topic that warrants further investigation.

12

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19

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20

1   **GLOSSARY**

2

3   **Floral constancy** (or fidelity) refers to the propensity for an individual pollinator to visit

4   flowers of a single floral type (hence one or a few plant species) within a foraging bout. It can

5   be measured by the number of transitions among floral types visited within a bout (Waser,

6   1986; Chittka *et al.*, 1999). Constancy is widespread across pollinator species and is

7   explained, at least partially, by the limited short-term memory of pollinators for visual and

8   olfactory cues and by their limited motor learning for handling flowers (Chittka *et al.*, 1999).

9   **Floral display** is the total number of flowers that are open simultaneously on a plant on a

10   given day. Note that floral display sometimes also includes floral size (e.g. Goodwillie *et al.*,

11   2010), despite an existing trade-off between number and size of flowers (Sargent *et al.*, 2007).

12   A **foraging bout** is the time a pollinator spends visiting flowers during a single sequence,

13   between the moment it leaves its nest and the moment it returns to it.

14   **Inbreeding depression** is the relative decrease in fitness of selfed vs. outcrossed progeny,

15   caused mostly by a combination of highly deleterious, nearly recessive alleles and mildly

16   deleterious, nearly additive alleles. Recessive deleterious mutations are more likely to be

17   eliminated by natural selection (purging) in inbred homozygotes, which creates a negative

18   relationship between inbreeding depression and the population selfing rate (Lande &

19   Schemske, 1985; see Box 2).

20   **Nestedness** characterizes networks with many specialist species and few extremely generalist

21   species, as well as asymmetric specialization (specialist species tend to interact with

22   generalist species).

23   **Pollen carryover** is the extent to which pollen collected on a flower is transported and

24   deposited on stigmas of other flowers (of the same plant or different plants) during a foraging

1   bout; it depends on both the pollen uptake rate and pollen deposition rate (de Jong *et al.*,

2   1993).

3   **Pollen discounting** is the reduction in outcrossed male siring success associated with an

4   increase in selfing rate, due to decreased amounts of exported pollen (Nagylaki, 1976).

5   **Pollen limitation** is the reduction in plant reproductive success (fruit or seed set) due to

6   inadequate quantity or quality in pollen receipt; it is usually tested for through supplementary

7   pollination (see Knight *et al.*, 2005 for a review).

8   **Pollinator preference** is the propensity for an individual pollinator to visit a plant species

9   disproportionately to the availability of resources this species provides (Cock, 1978); it is

10   usually measured as number of visits per plant relative to other plant species.

11   **Self-pollination rate** is the fraction of self-pollen vs. conspecific outcross pollen deposited on

12   plant stigmas.

13   **Selfing rate** is the rate of self-fertilization, the fraction of selfed vs. outcrossed embryos

14   produced by an individual plant. It is defined at fertilization (primary selfing rate) but usually

15   measured at a later stage (in seeds, seedlings or even adults, secondary selfing rate). Primary

16   and secondary selfing rates can differ from the self-pollination rate due to (1) self-

17   incompatibility, which can be partial or cryptic (e.g. via differences in growth rates of selfed

18   vs. outcrossed pollen tubes) and (2) post-fertilization selection processes, for example

19   selective flower abscission, fruit abortion or inbreeding depression.

20   **Selfing and outcrossing components** of self-compatible animal-pollinated plants with

21   perfect flowers (following Lloyd, 1992). **Autonomous selfing** (a, dotted white lines; left

22   panel of Fig. 2) corresponds to autogamous (within-flower) self-pollination occurring without

23   pollinator visits; it is divided into three modes depending on the timing of outcross- vs. self-

24   pollination: prior, competing (simultaneous), and delayed autonomous selfing. **Facilitated**

25   **selfing** (f, dashed white lines; left panel of Fig. 2) corresponds to autogamous (within-flower)

1   self-pollination induced by pollinator visits. **Geitonogamous selfing** (g, solid white lines; left

2   panel of Fig. 2) corresponds to self-pollination among flowers induced by pollinators probing

3   several open flowers on the same plant. **Outcross pollination** (o, solid black lines) refers to

4   pollen deposited on a flower that originates from other plants in the population. Estimates

5   obtained from the method proposed in Box 1 are given with upper-case letters next to three

6   flowers (right panel of Fig. 2).

7   **Selfing syndrome** is a characteristic set of morphological and functional plant traits that

8   enhance pollen transfer efficiency within flowers and/or decrease pollinator visitation. Selfing

9   syndrome usually includes small flowers, thus reduced anther-stigma distance (herkogamy),

10   reduced petal size, and reduced corolla width (following Sicard & Lenhard, 2011).

11   **Specialization** has many definitions but is generally inversely related to the total number of

12   species an individual, a population or a species interacts with. This number can be weighted

13   by the frequency of interactions with each partner species.

1   **BOX 1:** A METHOD FOR ESTIMATING FACILITATED SELFING

2   Estimation of facilitated selfing is challenging because it requires devices to mark pollen or

3   follow pollinator visits, while controlling for resource allocation, outcross pollen limitation

4   and pollinator attraction. However, facilitated selfing could contribute significantly to

5   observed selfing rates and their evolution. Indirect evidence of facilitated selfing is available,

6   but only for plant species with specific floral types (Fig. 2 of the glossary): Johnson *et al*.

7   (2005) make use of the absence of autonomous selfing in *Disa cooperi*, Vaughton *et al*.

8   (2008) of the absence of geitonogamous selfing in *Bulbine vagans* and Owen *et al*. (2007) of

9   the absence of both in *Bulbine bulbosa*; on the other hand, Anderson *et al*. (2003) use

10   *Roridula* species for which facilitated selfing is performed by insects (hemipterans) that do

11   not contribute to the other selfing components, performed by bees.

12   *A simple, but adjustable, experiment to estimate all components of selfing*

13   Estimating the three components of selfing or self-pollination (Fig. 2 in the glossary) requires

14   a detailed description of single-pollinator visits to individual plants. The method requires *N*

15   replicate caged plants with *F* individually-marked open perfect flowers. Identical numbers of

16   flowers control for resource allocation if selfing (not self-pollination) rates are to be

17   estimated. Each plant should be visited by a single pollinator carrying no pollen, which

18   precludes outcross pollination and the visitation order of the pollinator should be recorded

19   (some flowers can be visited several times, and some flowers may remain unvisited). The

20   components of selfing and self-pollination can then be estimated by analysing the seed set of

21   the $N \times F$ flowers or by counting pollen grains deposited within each flower stigmas,

22   respectively.

23   The simplest estimation method requires discarding all flowers visited more than once

24   and counting pollen grains, not seeds, per flower to eliminate the delayed autonomous selfing

25   component. Pollen loads provide information about (1) autonomous selfing for unvisited

1   flowers (2) autonomous and facilitated selfing jointly for flowers visited once as the first

2   flower of the sequence, thus by a pollinator carrying no self-pollen, and (3) all three

3   components of selfing for flowers visited once as the second flower of the sequence, by a

4   pollinator carrying self-pollen from one previously visited flower (Fig. 2 of the glossary, right

5   panel). More sophisticated methods could use seed sets from all F flowers but would need to

6   control for delayed autonomous selfing (possibly via the number of visits per focal flower or

7   per plant, depending on the underlying mechanisms) and for the quantity of geitonogamous

8   self-pollen deposited on flowers as a function of their visitation rank. For practical reasons F

9   needs to be small enough to keep track of the entire pollinator visitation sequence and large

10   enough such that pollinators do not visit all open flowers, but generate variation in the number

11   of flowers visited per plant; some flowers may have to be removed and some pollinators may

12   have to be excluded from the experimental cage to avoid too long visitation sequences.

1   **BOX 2:** AN APPROXIMATION FOR EVOLUTIONARY EQUILIBRIA OF PLANT SELFING RATES:

2   **how to include the purging of inbreeding depression into ecological models of the**

3   **evolution of selfing**

4   The evolutionary dynamics of inbreeding depression greatly influence plant mating system

5   evolution and should not be overlooked. The joint evolution of inbreeding depression and

6   selfing rates can be modeled using detailed genetic models of inbreeding depression, which

7   has rarely been done in ecological theoretical studies (but see Porcher & Lande, 2005a; b;

8   Porcher *et al.*, 2009) because it requires complex models and long computation time.

9   Alternatively, an approximation assuming that plant selfing rates evolve by small mutational

10   steps allows modeling the dynamics of purging without a full genetic model for inbreeding

11   depression (Lande *et al.*, 1994; Johnston, 1998). This approximation amounts to letting the

12   level of inbreeding depression vary with the selfing rate, and finding joint equilibria of the

13   mating system and inbreeding depression, instead of assuming constant inbreeding depression

14   (see Porcher & Lande, 2013 for more details). A numerical or analytical relationship between

15   inbreeding depression and population selfing rate can be obtained from any genetic model

16   (e.g. Kondrashov, 1985; Charlesworth *et al.*, 1990). Analytical relationships are derived from

17   polynomial regressions (Johnston, 1998; Lepers *et al.*, unpublished manuscript) with

18   relatively simple models of inbreeding depression (e.g. based on a single locus, Charlesworth

19   *et al.*, 1990).

20   A change in the selfing rate modifies inbreeding depression, but also other components

21   of plant fitness, via the automatic advantage of selfing (Fisher, 1941), reproductive assurance

22   in pollen-limited environment, or pollen discounting. The approximation examines the

23   indirect selection gradient on small changes in the selfing rate to find joint equilibria of the

24   mating system and inbreeding, which occur at the intersection of the inbreeding depression

25   function and a constraint function (Fig. 3). This constraint function summarizes all other

1   drivers of the evolution of the selfing rate, particularly ecological mechanisms, some of which

2   are governed by pollinators and their behaviour (Porcher & Lande, 2005b; Devaux *et al.*,

3   unpublished manuscript; Lepers *et al.*, unpublished manuscript). Singular strategies (selfing

4   rates) correspond to fitness maxima or minima, which can be distinguished from the sign of

5   the second partial derivative with respect to selfing rate at this strategy. Graphically, the

6   singular strategy is evolutionarily stable (i.e. a maximum) if the constraint function is smaller

7   (respectively larger) than inbreeding depression when the equilibrium selfing rate is increased

8   (respectively decreased; Fig. 3).

9   The approximation ignores genotypic association among the loci controlling the selfing

10   rates and those controlling inbreeding depression, and is therefore accurate only for moderate,

11   but biologically realistic, genomic rates to deleterious mutations causing inbreeding

12   depression ($U < 0.2$, Porcher & Lande, 2013). For larger mutation rates, differential purging

13   occurs between genotypes with different selfing rates, a phenomenon that is not accounted for

14   in the approximation, which thus becomes inaccurate. Large U may be found in perennial

15   plants (see Porcher & Lande, 2013) and may better account for the observed similar levels of

16   inbreeding depression in completely outcrossing and mixed-mating populations (Winn *et al.*,

17   2011).

1   **FIGURE LEGENDS**

2   Figure 1. Summary of the constraints imposed by pollinator behaviour on the ecology and

3   evolution of plant selfing rates. The upper panel describes the plant and pollinator traits that

4   influence the deposition of each of the three origins of self pollen, as well as outcross pollen

5   (see glossary). Traits with a positive (respectively negative) impact on amounts of pollen

6   deposited are in black (respectively grey). The inner square recalls the definition of the rate of

7   self-fertilization. The intermediate square groups plant and pollinator traits operating at the

8   flower or individual plant levels. The outer square groups plant and pollinator characteristics

9   operating at the population or network levels.

10

11   Figure 2. Clarification (left panel) and one estimation method (right panel, see also Box 1) of

12   selfing components for self-compatible animal-pollinated plants with perfect flowers

13   (following Lloyd, 1992).

14

15   Figure 3. Evolutionarily stable (closed circles) and unstable (open circles) selfing rates found

16   at the intersection of the inbreeding depression curve (black line) and an ecological constraint

17   function on plant fitness components (gray line).

18

19   Figure 4. Relationship between mating system and plant specialization in three plant-

20   pollinator networks.

21   Box plots (with whiskers representing 1.5× interquartile) and mean (closed circles) of the

22   number of pollinator species per plant (A-C) and number of pollinator species per visit (D-E).

23   Mating systems were obtained from the Biolflor database and divided into three classes to

24   obtain balanced and sufficient sample sizes: allogamous ("Allo.", comprising 'allogamous'

25   and 'facultative allogamous' species of the database), mixed mating ("Mixed"), and

1 autogamous ("Auto.", comprising both 'facultative autogamous' and 'autogamous' species of

2 the database). *P*-values for the 'mating system' effects were obtained by analysing the

3 variation in pollinator richness (either per plant or per visit), assuming it follows a

4 quasipoisson distribution, as a function of the mating system alone (A-C) or the mating

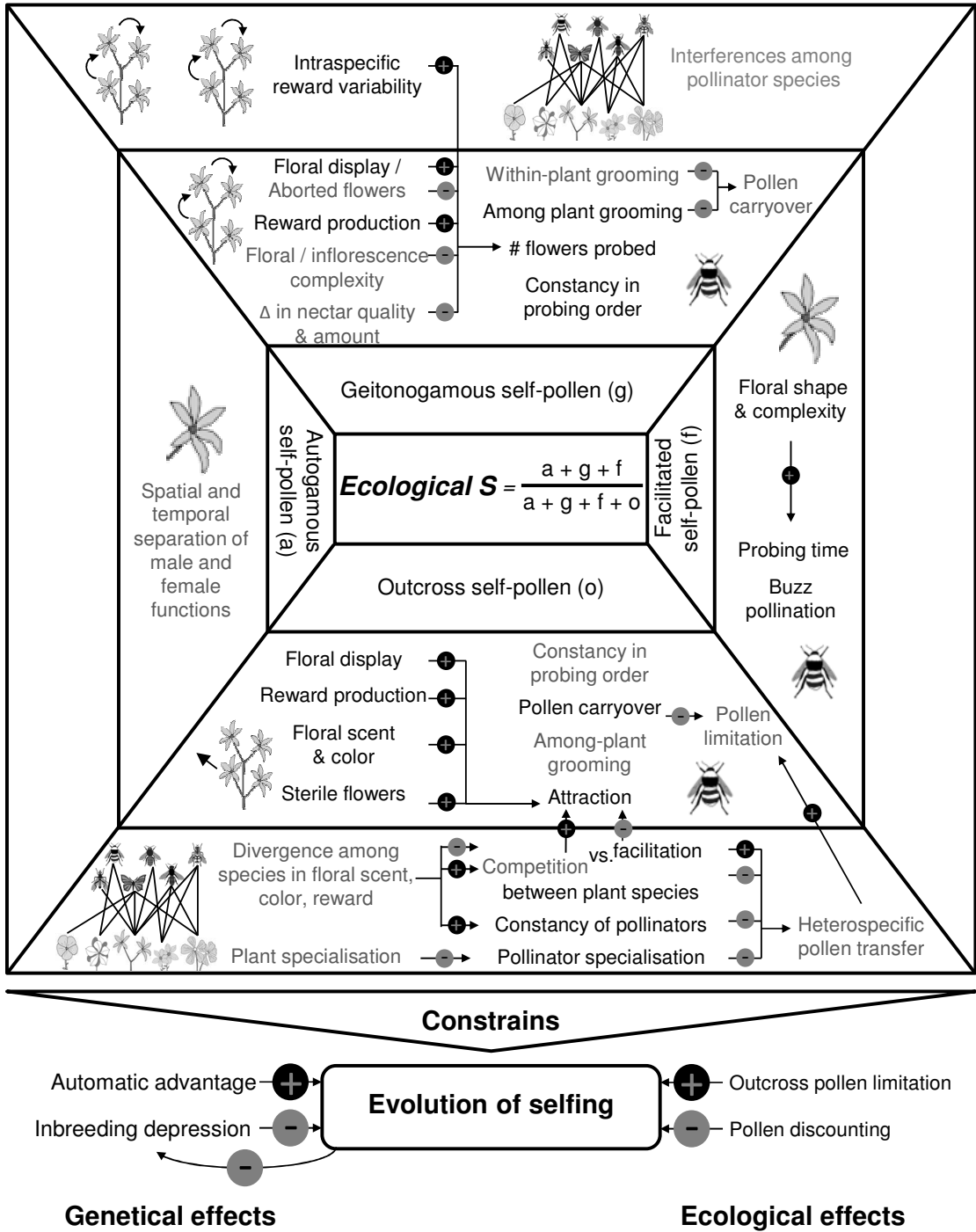5 system, the number of visits and their interaction (D-E).

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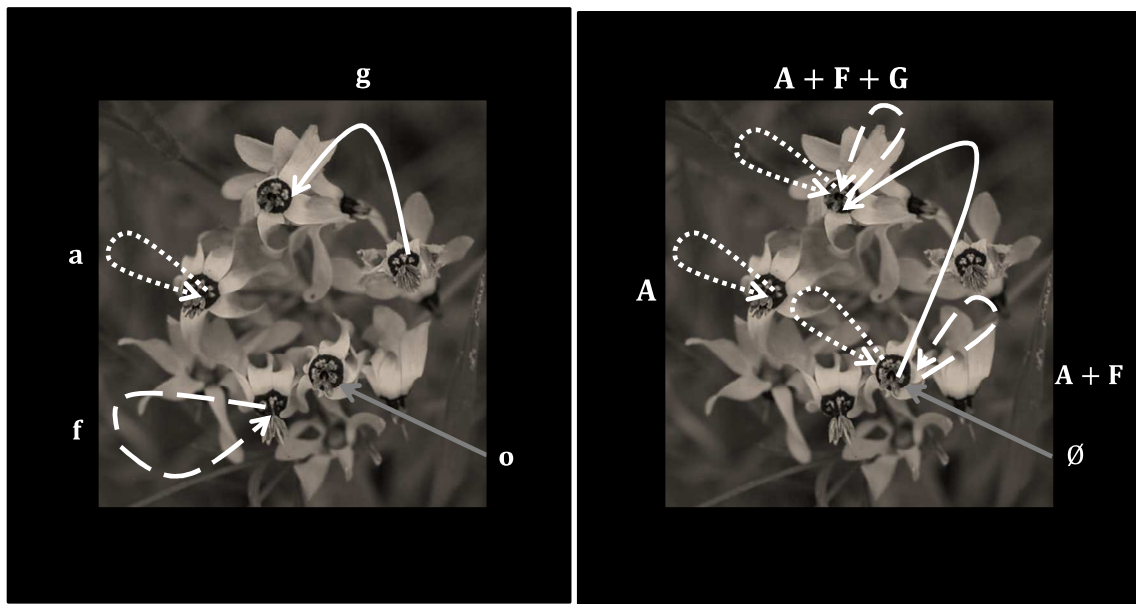
3   **Figure 1**

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5 **Figure 2 (to be included in the glossary)**

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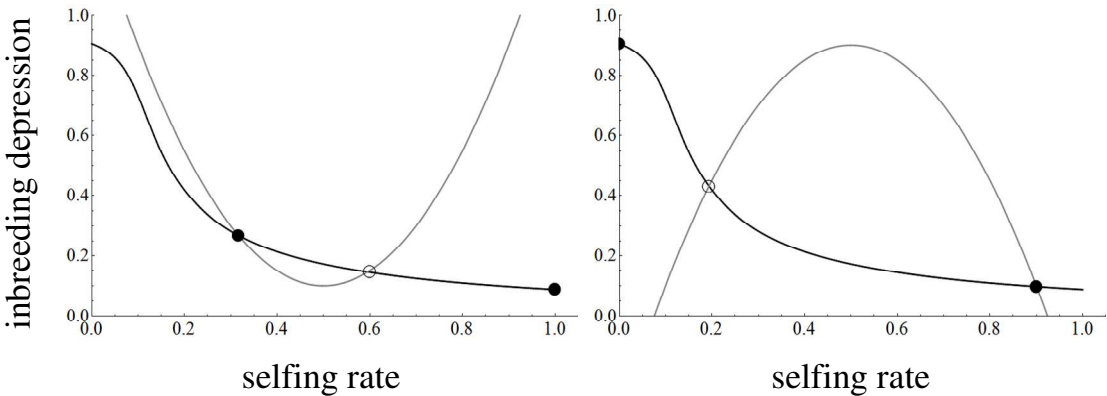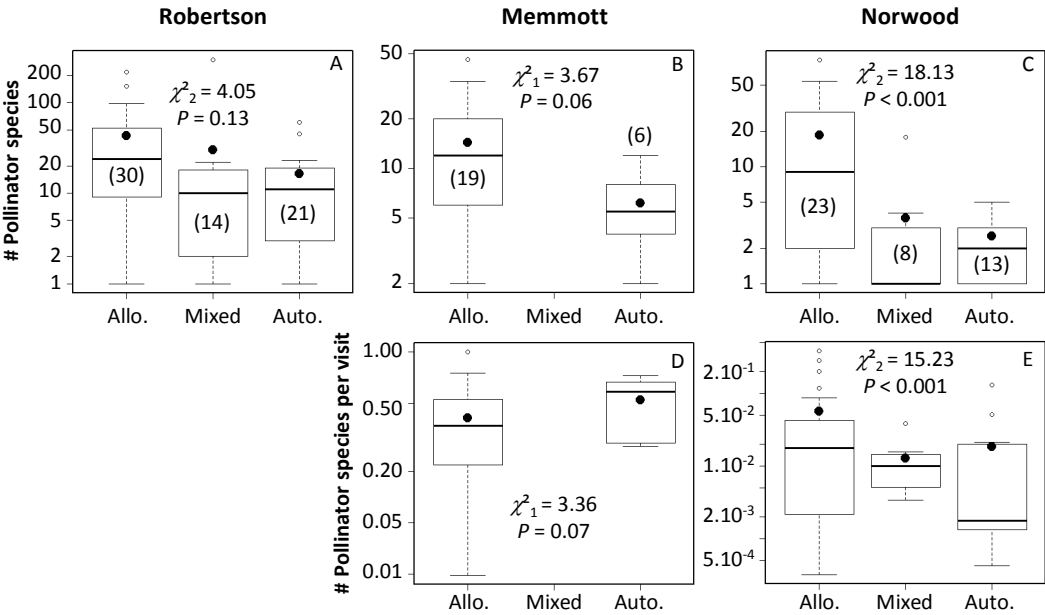7   **Figure 3 (to be included in Box 2)**

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3   **Figure 4**

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