Université de Lille

Thèse pour l'obtention du grade de Docteur de l'Université de Lille

Discipline: Biologie Évolutive et Ecologie

U.F.R de Biologie

Pollinators: demanding partners.

Investigating the interplay between plant-pollinator interactions and plant trait evolution

Présentée et soutenue publiquement le 10 décembre 2015 par

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Résumé

La grande majorité des plantes à fleurs se reproduisent grâce à leurs pollinisateurs. L'évolution chez les plantes est pourtant souvent étudiée sans prendre en compte ces interactions, et cette thèse vise à mieux comprendre leur impact sur l'évolution des plantes. Dans un premier temps, je présenterai les effets connus des pollinisateurs sur le taux d'autofécondation des plantes et sur son évolution. Dans un second chapitre, j'ai modélisé l'évolution du taux d'autofécondation des plantes lorsque celui-ci affecte la démographie des plantes et des pollinisateurs, et l'investissement des plantes dans la pollinisation. Cette étude montre que l'évolution vers l'autofécondation peut mener à l'extinction des plantes. Dans un troisième temps, je m'intéresserai à l'évolution des caractéristiques florales pour des espèces qui dépendent obligatoirement du transfert de pollen entre individus : les espèces dioïques. Cette étude montre que l'attractivité des plantes peut évoluer différemment chez les individus mâles et femelles, surtout dans les grandes populations qui subissent peu de limitation en pollen. Ce résultat suggère que le dimorphisme sexuel ne menacerait pas le maintien des populations dioïques. Enfin, même si elles prennent en compte les pollinisateurs de manière sommaire, les précédentes études ont montré leur effet sur la démographie et l'évolution chez les plantes. Je présenterai le développement en cours d'une méthode de quantification des mécanismes sous-jacents au comportement du pollinisateur, en particulier en ce qui concerne les traits floraux. Elle permettrait une meilleure compréhension de la manière de modéliser les interactions plantes-pollinisateurs.

Mots clés

Interactions plantes-pollinisateurs, traits floraux, taux d'autofécondation, système de reproduction

Abstract

The mode of pollination is often neglected regarding the evolution of plant traits, although the reproduction of most flowering plants is based on their interactions with pollinators. This thesis aims at a better understanding of the interplay between animal-pollination and the evolution of plant traits. First, I will present a detailed review on the interplay between plant mating system and pollinator behavior, which highlights the impact of pollinators on the immediate ecological selfing rate and on its evolution. Second, I modeled the evolution of plant selfing rate when it affects both the demography of plants and pollinators and the investment of plants in pollination. This study provides new theoretical evidence that evolution towards selfing can lead to an evolutionary suicide in some conditions. Third, I will present a modeling analysis of the impact of animal-pollination for species that compulsorily rely on outcross pollination: entomophilous dioecious species. This study revealed that under pollinator-mediated selection, attractiveness of males and females should evolve in large populations that do not suffer from pollen limitation. This result suggests that dimorphism may not be a threat to dioecious populations. Finally, although the previous models integrated pollinators in a basic way, they highlighted strong interplays between pollinators, plant demography, and the evolution of plant traits. I will present a work in progress aiming at developing a methodology to quantify the mechanisms underlying pollinator foraging behavior, especially regarding plant traits. This would allow for a better understanding of how to model plant-pollinators interactions.

Keywords

Plant-pollinator interactions, floral traits, selfing rates, reproductive system

Remerciements

Mathilde, Sylvain, grâce à vous ces 3 années de thèse ont été un vrai plaisir. La soutenance étant passée, je peux dire que je n'hésiterai pas une minute si c'était à recommencer. Je peux même dire que c'est grâce à vous si je n'ai pas implosé pendant le rush de dernière minute, quand il était si tentant de tout laisser tomber et de partir vivre dans une grotte (ou au moins dans un appart sans internet). Vous êtes vraiment ... vraiment.

Mathilde, merci de m'avoir donné un exemple à suivre. Merci de ta patience et d'avoir été présente à chaque fois que j'avais besoin d'un conseil, d'un avis ou juste de me mettre les idées au clair. Merci d'avoir toujours été là en renfort à chaque fois que je me sentais perdue, et que je ne pensais pas être capable d'y arriver.

Sylvain, merci de m'avoir fait découvrir le monde de la recherche quand j'étais en L2. C'est grâce à toi qu'aujourd'hui je ne suis pas en dépression dans une classe de collège, et je ne sais pas si je pourrais te remercier assez un jour ! Merci d'avoir toujours pris le temps de passer voir si "tout allait bien", et surtout merci de ne pas avoir (trop) désespéré de ne jamais avoir de réponse positive à cette question !

Merci à tous les deux de m'avoir permis de vivre cette expérience extraordinaire, vous êtes vraiment des encadrants géniaux. Vous avez toujours su être présents et me (re)donner confiance quand j'en avais le plus besoin. Merci de m'avoir donné autant d'ultimatums (jamais respectés), d'avoir partagé autant de discussions autour d'un café, et tout simplement de m'avoir consacré autant de temps.

Si ma thèse s'est aussi bien passée, c'est aussi grâce au laboratoire GEPV/EEP, à sa bonne humeur et à ses discussions sans prise de tête. Au GEPV, j'ai fait mes premiers pas dans la recherche dans les meilleures conditions possibles, et la transition avec le monde réel risque d'être difficile.

Merci à toute la promo de M2. Grâce à vous le GEPV restera toujours associé aux fous rires, aux Chœurs de l'Armée Rouge en 221b et à notre affiche à nous (La recherche par l'armée des Masters, membres de la main d'œuvre bon marché !). Je serai toujours fière d'avoir un jour été ministre de la culture et de la pensée unique de notre dictature éclairée et bienveillante. Léa, ton dessin trône toujours sur mon bureau. Quand j'ai envie de tout lâcher, je relis la légende "puisses-tu survivre et réussir dans ce monde de brutes", et j'essaie de ne pas te décevoir. Adeline, je pense à toi à chaque fois que je reçois des corrections. Le stylo rouge ne s'améliore pas avec le temps, et j'attends toujours de m'y habituer... Merci surtout à Alex, Lieselot, Pierre, Léa et Adeline, c'est grâce à vous que j'ai tant aimé la recherche. Vous verrez, on s'en sortira !

Merci Diala de m'avoir montré la voie. Tu as été vénérée par toute notre promo (à juste titre !), et tu nous as prouvé que tout était possible. Merci pour ta bonne humeur, tes coups de gueule et tes coups de main. Merci d'avoir toujours pris le temps de discuter, et pour toutes les pauses café partagées. Merci également de m'avoir mise en garde contre le "thesis-blues". Je ne t'avais pas crue à l'époque, mais après le dépôt ça m'a bien aidée de savoir que je n'étais pas la seule ! Sans toi, le labo paraît bien calme. Heureusement que tu viens régulièrement nous amener ton grain de folie (des paillettes!).

Merci à tous ceux qui m'ont permis de me sentir chez moi dans ce labo. Déjà, merci à tous les permanents de ne jamais nous prendre de haut nous autre pauvres petits étudiants, et de toujours favoriser la discussion et l'échange. Et puis merci à tous ceux qui contribuent à la bonne ambiance du labo. Je me souviendrai longtemps des discussions aberrantes de la salle café (merci Joël !). Merci à tous ceux qui m'ont permis de glander dans les canapés les jours de flemme, et à tous ceux qui m'ont aidée à me motiver.

Merci également à tous ceux qui ont permis à ces travaux de thèse de voir le jour, que ce soit au travers de collaborations, de simples discussions, de conseils ou en m'ayant juste simplifié la vie ! Anne-Cath, merci de m'avoir fait découvrir cestmafournée, l'organisation des Journal Club est devenue plus intéressante en ayant des recettes à tester. Grâce à toi (ou à cause de toi?), je penserai au GEPV à chaque gâteau préparé ! Sandrine, un énorme merci pour ton soutien pendant toute ma thèse mais surtout pendant la période de rédaction. Ça a été un vrai soulagement de pouvoir compter sur toi pour m'aider à organiser le grand jour, et pour pouvoir me défouler quand ça coinçait. Et bien sûr, merci à l'Université de Lille et à la région Nord-Pas-de-Calais pour leur soutien financier, sans lequel rien de tout ça ne serait arrivé.

Pour finir, bien sûr, un immense merci à Matthieu. Mathilde et Sylvain ont tous les deux trouvé que j'avais été particulièrement calme pendant ma thèse. Toi tu as eu droit à l'envers du décor, c'est toi qui as subi mes pétages de plombs, qui a ramassé les morceaux quand rien n'allait, (et surtout) qui m'a aidée à me motiver quand je voulais juste abandonner. Merci de me supporter depuis si longtemps !

Contents

Chapter 1

Introduction

1.1 Reproductive systems diversity

Biologists have long been fascinated by the amazing diversity of mating patterns and sexual systems in flowering plants. How natural selection can maintain such diversity within flowering plants has been a central question in evolutionary biology for decades but is only partly understood. This diversity is depicted through three major aspects of plant reproductive systems: (i) the distribution of male and female functions between individuals (unisexual vs. cosexual individuals), (ii) the selfing rate (the fraction of selfed embryos produced by an individual plant) and (iii) the mode of pollination (wind-pollinated vs. animal-pollinated).

Unlike in the animal kingdom, most flowering plant species are hermaphroditic, *i.e.* all individuals possess both male and female functions (over approximately 70% Chapman and Reiss 1999). However, the remaining species show various alternative sexual systems, composed of different combinations of hermaphroditic, female and male plants. Dioecy, *i.e.* the coexistence of male and female unisexuate individuals within the same species, occurs in only about 6% of all species (Renner and Ricklefs 1995). Polymorphic populations can also consist of hermaphroditic and male individuals (androdioecy) or of hermaphroditic and female individuals (gynodioecy). The complexity of plant sexual systems is further increased because plant individuals of some plant species can produce a combination of unisexual and cosexual flowers, or can separate male and female function through time. It has been suggested that dioecy could be favored because a specialization of plant individuals in one sex function could allow a better fitness than splitting resources between male and female functions (Maynard Smith 1978). Moreover, the separation of male and female functions prevents self-fertilization and its associated disadvantages (see below). On the other hand, hermaphroditism allows for reproductive assurance, since in case of a reduction or loss of conspecific pollen or pollen vector,

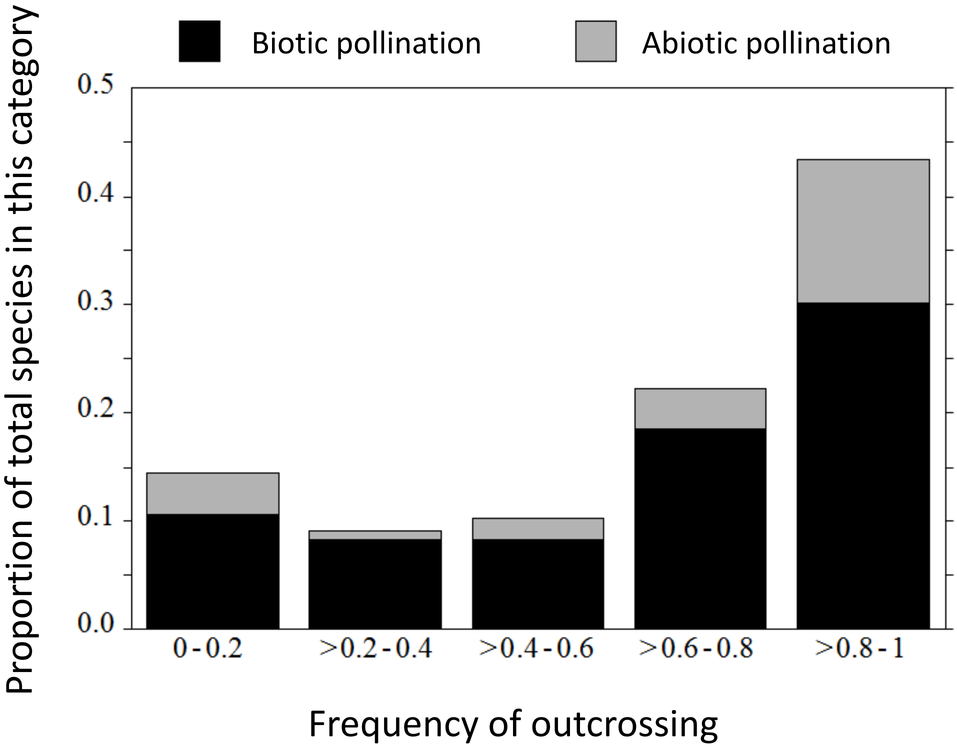


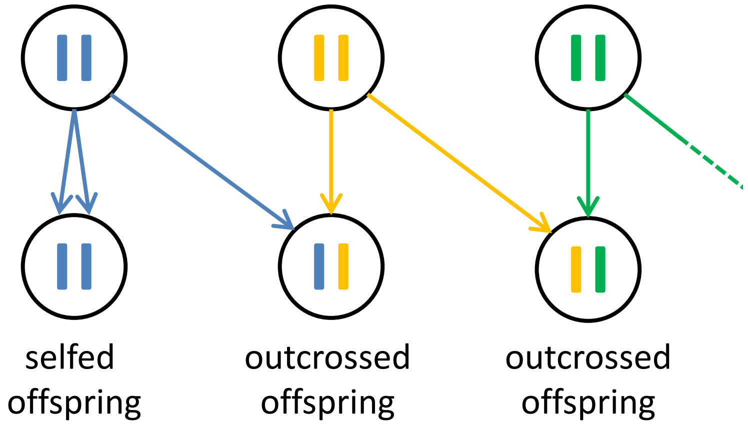
Figure 1.1: Distribution of outcrossing rates in 345 angiosperm species (from Goodwillie et al. 2005). Biotic (animal-pollination) and abiotic (wind-pollination) are depicted in black and grey respectively.

hermaphroditic individuals can produce seeds by themselves (through selfing, see below), whereas the reproductive success of unisexuate individuals would be strongly decreased. Moreover, in hermaphroditic populations, each other individual is a potential mate, which could contribute to reproductive assurance.

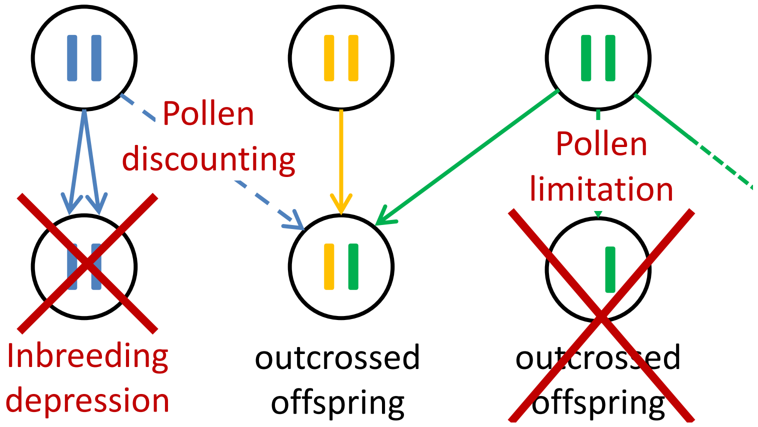
Benefits and disadvantages of hermaphroditism are deeply linked with the possibility of hermaphrodites to self-fertilize some of their ovules. Despite the potential ability of all hermaphroditic individuals to self-fertilize their ovules, flowering plants exhibit a great diversity in mating systems, ranging from obligate outcrossing (no output is produced through self-pollination), through mixed mating (simultaneous selfing and outcrossing) to complete selfing (Fig. 1.1). Some species have complete control over their selfing rate: compulsorily outcrossing species (*e.g.* hermaphroditic self-incompatible species), and completely selfing species (*e.g.* cleistogamous species that produce closed flowers only, Culley and Klooster 2007). For all other species, self-pollen deposition is likely to occur (but sometimes at unpredictable rates) both within-flowers or between flowers. Selfing should be selected for for two reasons. First selfing provides "reproductive assurance", which should be of primary importance for immobile individuals: selfing allows for offspring production even when mates are rare or pollen vector lacking (pollen limitation, Cheptou 2004; Porcher and Lande 2005). Second, selfers benefit from the "automatic transmission advantage": a 50% transmission advantage of their genome, since they are both the maternal and paternal parents of the seed they produce (Fisher 1941). On the other hand, inbreeding depression (the relative decrease in fitness of selfed *v.s.* outcrossed progeny) is assumed to prevent the evolution of selfing because of a reduction of fitness in selfed offspring compared to outcrossed ones (Charlesworth 2006). Moreover, pollen discounting, a negative relationship between selfing rate and pollen export, reduces the automatic advantage of selfing: selfers have a lower outcross siring success than outcrossers. Therefore, pollen discounting hinders the evolution of high selfing rates (Goodwillie et al. 2005). Yet, few of those models predict the stable maintenance of mixed-mating systems (Goodwillie et al. 2005). Recent studies highlighted the potential impact of pollination ecology on the evolution of plant selfing rate (Devaux et al. 2014; Jordan and Otto 2012), and future studies will be needed to understand the interplay between the evolution of selfing rates and the other components of plant reproductive systems.

The mode of pollination, although directly affecting pollination events, has received less attention than the evolution of other plant reproductive characteristics. The reproduction of a vast majority of flowering plant species is based on their interactions with pollinators (*i.e.* entomophily, $\approx 90\%$, Ollerton et al. 2011). As reported by Barrett (2010), little theoretical and empirical attention has been paid to the advantages of animal-pollination *v.s.* wind-pollination. Wind-pollination is often described as a random and wasteful process, leading to a huge loss of male gametes during pollen dispersal. However, a succinct comparison of the pollen loss in wind-pollinated and animal-pollinated species does not support with this explanation (Friedman and Barrett 2009): pollen transfer efficiency may not be considerably lower in wind-pollinated species than in animal-pollinated plants. Moreover pollen limitation (reduction in plant reproductive success due to inadequate quantity or quality in pollen receipt) in wind-pollinated plants

Box. 1: the genetic background of selfing evolution



Selfers benefit from an “automatic advantage” of transmission: they transmit two copies of their genomes to the selfed seeds, and one copy to the seeds they sire through outcrossing (Fisher 1941).



This advantage of selfing is reduced because of inbreeding depression: deleterious recessive alleles are more likely to be expressed in homozygous form in selfed offspring, which could decrease the fitness of selfed compared to outcrossed offspring (Charlesworth 2006).The advantage of selfing can be further reduced because of pollen discounting: *i.e.* if increasing selfing rate decreases their outcross siring success.

may not be as common as it is in animal-pollinated species (Friedman and Barrett 2009). If animal-pollination truly does not allow improved pollen transfer compared to wind-pollination, the wide occurrence of animal-pollination would be a conundrum. Indeed, animal-pollination often relies on plant attractive traits (floral color, shape, scent, rewards. . .) that could be extremely costly (see below), and because the energy allocated to pollinator attraction is not needed for wind pollination, and could be re-allocated to female or male function (Friedman and Barrett 2009). Moreover, pollinators could be unpredictable partners, and in case of pollinator shortage, plants suffer high pollen limitation, reducing their fitness and leading to an increase of plant selfing rate (*e.g.* strong increase of selfing rate in plants left without pollinators, Bodbyl Roels and Kelly 2011).

Although many studies are dedicated to the understanding of how and why these three components of reproductive systems (sex functions of individuals, selfing rate and mode of pollination) have evolved along the evolutionary history of flowering plants, these three categories of reproductive strategy are often studied separately. In the following, I will argue, reviewing both empirical and theoretical studies, that there is a strong interplay between animal-pollination and the other two components of plant reproduction, at two distinct time scales. First, I will show that pollinator behavior influences plant demography, and that their behavior is determined by plant traits. Second, I will present some evidence that pollinators influence the evolution of plant reproductive and mating traits. More specifically, pollinator-mediated selective pressures on plants will be investigated for two strikingly different situations: either in hermaphroditic species, in which selfing rates could evolve as an answer to pollinator-mediated selective pressures, or in dioecious species, in which plants compulsorily rely on pollen transfer between sexes. In dioecious species, selection on floral traits may not act similarly on male and female individuals, leading to sexually dimorphic plant populations. The evolution of dimorphism could in return affect pollinator behavior, and threaten pollen transfer and plant demography.

1.2 Pollinators influence plant demography

Plant-pollinator interactions are defined as mutualistic interactions: the visits of pollinators allow pollen dispersal among plant individuals, and in return pollinators use resources made available by the plants for their own survival and reproduction. Thus, pollinators affect plant demography through two important ways: (i) they determine the plant reproductive success and (ii) the benefit of the interactions with pollinators usually comes with a substantial cost that may decrease the amount of energy that could be devoted to reproduction.

Pollinators and plant reproductive success

Pollination in hermaphroditic plant species

Pollinator movements within and among plants determine the dynamics of the pollination process, by directly affecting the amount of pollen transferred among plants of the same species (favoring the reproduction with other individuals: outcrossing), and the amount of pollen transferred within flowers (intra-floral selfing) or among flowers on the same plant (geitonogamous selfing, Fig. 1.2). Pollinator behavior is strongly affected by plant traits. Indeed, plant traits influence both the number of pollinator visits that a plant receives but also the number of flowers visited on a plant and the time spent within a given flower. A review of the impact of plant traits on pollinator behavior and on the subsequent selfing rates is given in Chapter 2.

The number of visits that an individual plant receives is likely to increase its outcross pollen reception and export. Indeed, pollinators are likely to deposit substantial outcross pollen on the first flower probed on a plant (Karron et al. 2009). One should note, however, that outcross pollination depends on the presence of conspecific pollen on pollinators. Thus, pollinator behavior such as grooming may decrease outcross pollination (Holmquist et al. 2012). Similarly, when foraging within a plant community comprising of several co-flowering plant species, pollinator constancy (*i.e.* its propensity to visit flowers of a single floral type within a foraging bout) is also likely to affect outcross pollen receipt (Ashman and Arceo-Gómez 2013). Since outcross pollination depends on the plant visitation rate, it is primarily affected by pollinator abundance (Eckert et al. 2010; Thomann et al. 2013). Plant population density is also expected to affect the pollen transfer, since pollinators can spread their visits among a higher number of plants and/or switch more frequently between plant individuals when plant density is high (optimal foraging theory, Essenberg 2012). Moreover, plant floral traits, such as flower size or flower number could enhance

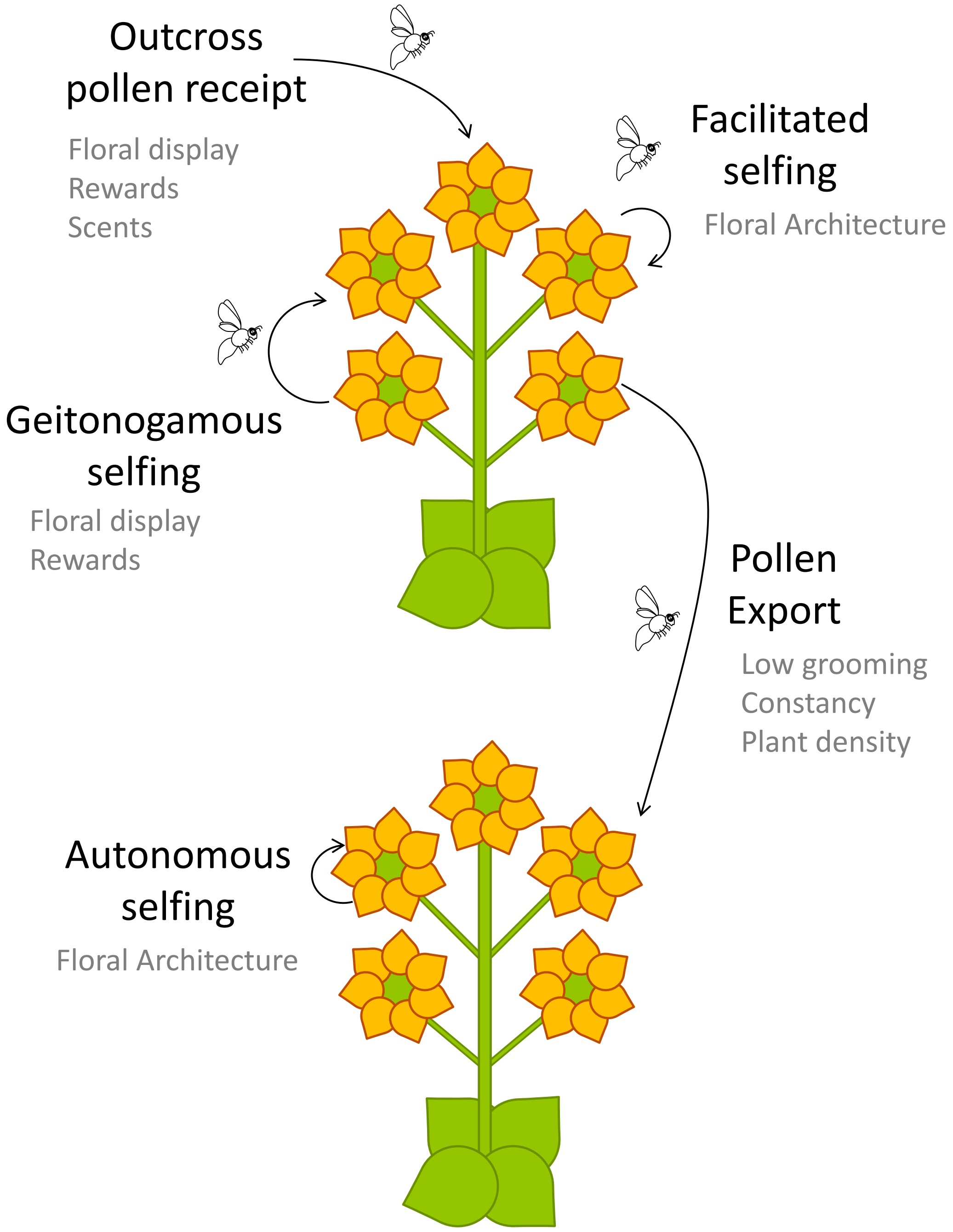


Figure 1.2: Impact of pollinator visits on plant selfing and outcrossing rates. Outcrossing corresponds to pollination occurring between two individuals of the same population (pollen receipt from another individual and pollen export). Autonomous selfing corresponds to autogamous (within-flower) self-pollination occurring without pollinator visits; it is divided into three modes depending on the timing of outcross- *v.s.* self-pollination: prior, competing (simultaneous), and delayed autonomous selfing. Facilitated selfing corresponds to autogamous (within-flower) self-pollination induced by pollinator visits. Geitonogamous selfing corresponds to self-pollination among flowers induced by pollinators probing several open flowers on the same plant. Plant traits favoring each of those components are depicted in grey. For more details, see Chapter 2.

pollinator visitation rates (but see the impact of flower numbers on selfing rate below Martin 2004; Mitchell et al. 2004).

On the other hand, plant self-pollination increases with the number of flowers a pollinator probes successively on a plant (Karron et al. 2009; Rademaker et al. 1999). Pollinators tend to probe more flowers on plants with larger floral displays (Mitchell et al. 2004), hence one can observe a positive correlation between plant floral display and plant selfing rate (Brunet and Sweet 2006; Karron et al. 2012). Nectar production and replenishment dynamics also affect geitonogamous selfing rate, with for example, a reduced geitonogamy in plant individuals that maintain nectarless flowers within an otherwise nectar-producing inflorescence (Bailey et al. 2007; Ferdy and Smithson 2002). Indeed, pollinator experiencing rewardless flowers may leave a plant early, thereby reducing geitonogamy. Besides, as stated above, plant density could affect the probability that pollinators leave a plant, with pollinators probing more flowers on each individual when the distance between plants is higher (Essenberg 2012).

Pollination in dioecious plant species

Because a pollination event in an entomophilous dioecious species is only realized when pollinators visit a male and then a female plant, the proportion of pollinator visits leading to potential seed production is lower than in hermaphroditic species (Vamosi et al. 2006). Thus, pollinator behavior among sexes may strongly affect pollen transfer. Moreover, the evolution of separate sexes is commonly associated with the evolution of sexual dimorphism (Barrett and Hough 2013), especially with regards to floral traits (Delph et al. 1996). Males tend to invest more than females in numerous floral traits, such as flower size, flower number or scent quantity (Ashman 2009; Delph 1996; Delph et al. 2002). Because pollinator behavior is greatly influenced by floral traits, pollinators may disproportionately visit plant individuals that display more flowers or larger ones (Martin 2004; Mitchell et al. 2004). Hence, as shown by Vamosi and Otto (2002), a sexual dimorphism in floral traits could induce unbalanced visitation rates to males and females, thus reducing pollen transfer from male to female individuals, and even threatening small plant populations (Vamosi and Otto 2002). However, this study considered a fixed population size, and hypothesized that a visit to only one female plant by a pollinator carrying

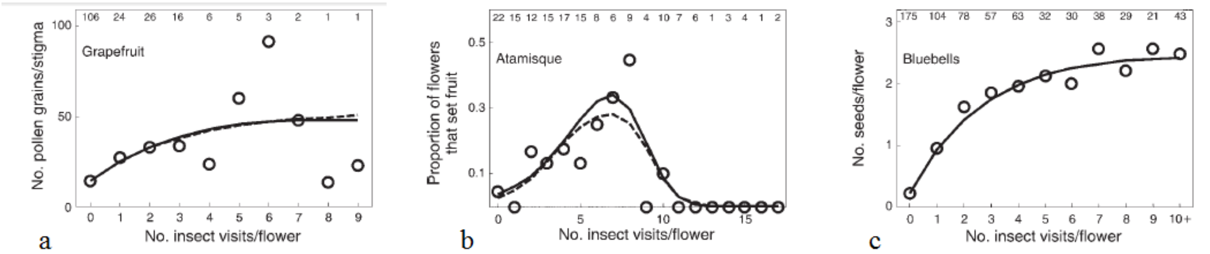


Figure 1.3: The impact of visitation rates on plant reproductive success in grapefruit (*Citrus paradise*), atamisque (*Capparis atamisquea*) and bluebells (*Mertensia paniculata*). The reproductive success measurement depends on the species biology: pollen load for grapefruit, fraction of flowers that set fruit for atamisque (each fruit contains a single seed) and number of seeds per flower for bluebells (up to 4 seeds per fruit). Circles represent the data and curves represent the net benefits models that better fit the data Morris et al. (from 2010).

some pollen allows population maintenance. In order to validate the hypothesis that animal-pollination threatens dimorphic plant populations, one should further investigate the interplay between dimorphism and plant population dynamic.

Pollinators, costly partners

The reproductive fitness of a plant depends on the benefit it obtains through pollination, but also on the energy invested into its interactions with pollinators. Bronstein (2001) highlighted the ubiquity of the cost of mutualistic interactions. Yet, only few models include a cost of mutualism, although it could affect the demographical dynamics of mutualists (Holland and DeAngelis 2010). One reason for the omission of costs of mutualism in theoretical studies is that linking the cost to plant and pollinator densities is not an easy task, even in the restricted case of pollination mutualism. Morris et al. (2010) investigated the way one might include the cost of pollination in models, and showed that a large number of relationships can emerge, depending on one's assumptions on the nature of the cost. Moreover, empirical studies show extremely contrasted patterns between species (Fig. 1.3). Below, I present some potential costs of pollination and the expected impact of plant and pollinator densities on these costs.

Currently, the most detailed data on the costs of pollination comes from highly specialized pollinating seed-consumer mutualisms. Indeed, measuring the cost of the interaction is relatively straightforward in such systems, since both the benefit and the cost are measured in the same currency: seeds (Bronstein 2001). In such mutualism, it has been shown that the interactions with pollinators can cause a high energy loss, with up to 60% of seeds lost to pollinator offspring (reviewed in Bronstein 2001). In this case, the higher the visitation rate, the higher the number of eggs laid by pollinators in the inflorescences (Klank et al. 2010; Pellmyr 1989).

In most pollination mutualism, the costs of interaction are more subtle than in seed-consumer mutualisms, but pollinators still use resources produced by the plant. Many pollinator species forage for pollen or nectar, and are attracted by flowers that offer a large amount of reward (Carlson and Harms 2006; Hernandez-Conrique et al. 2007; Keasar et al. 2008). The consumption of pollen by pollinators decreases the benefit of each visit, because it hinders pollen transfer between individuals. This may not represent a negligible cost. Indeed, only a small amount of the total pollen production reaches other plant stigmas (Friedman and Barrett 2009). Similarly, the consumption of nectar may be especially costly for the plant species (Southwick 1984; Pyke 1991; but see Harder and Barrett 1992), and it has been shown that nectar removal could decrease seed production (Pyke 1991). Pollinators could select for a high investment in reward production, as in *Petunia axillaris* where pollinators spend more time into flowers that produce a large amount of the nectar, leading to a higher seed production in lineages that produced more nectar (Brandenburg et al. 2012). The cost of resource production is likely to increase with the plant visitation rate. Indeed, if plants replenish its resource content between each visits (*e.g.* nectar production can be enhanced by its consumption by pollinators, Castellanos et al. 2002; Ordano and Ornelas 2004), the higher the visitation rate, the higher the amount of resources used by pollinators.

Besides, even in the (rare) cases in which entomophilous plants do not offer reward to pollinators, animal-pollination often relies on the production of attractive floral structures by plants. The production of floral structures and their maintenance rely on a substantial energy investment (Andersson 2006; Mazer et al. 2010). Since pollinators may disproportionately visit plant individuals that display more or larger flowers (Martin 2004; Mitchell et al. 2004), animal-pollination could select for a high investment into attractive floral structures. The cost of attractive structure production may be independent of plant or pollinator densities, as it might be the case in species that cannot modify their flowering length or their floral display size with the number of visits they receive (Van Doorn 1997).

However, many species have the ability to increase their floral display or the length of their flowering period when their visitation rate is low (Harder and Johnson 2005; Van Doorn 1997). In such case, we can expect the cost of pollination to increase at low pollinator density.

Given the diverse nature of the costs and the diversity of relationships between these costs and visitation rate, it may be impossible to satisfactorily include all the potential costs of mutualism into general demographic models of mutualism. Most models simply ignore the cost of interaction, or consider it decreases the net benefit of each visit, and thus do not specifically integrate it. As shown by Morris et al. (2010), this strategy is correct, but only if both the benefit and the cost of the interaction are saturating functions of plant visitation rate, and assuming the cost is always lower than the benefit, regardless the plant visitation rate. As soon as one wishes to account for other kinds of relationships, explicit consideration of benefits and costs must be considered, and one should model plant-pollinator interactions just as a consumer-resource interaction (Holland and DeAngelis 2010).

1.3 Pollinators influence the evolution of plant traits

As shown above, plant traits influence pollinator behavior, which in turn affects plant mating success. Consequently, animal-pollination is likely to affect plant trait evolution. In the following, I will briefly present the state of the art on the interplay between animal-pollination and the evolution of plant traits in two contrasting situations: in a first case I will investigate how hermaphroditic plants could avoid pollinator pressure through selfing, and in a second case, I will investigate how plant traits evolve under pollinator-mediated selection in a system where plant compulsorily rely on outcross pollen transfer: dioecious species.

The evolution of plant mating system

Animal-pollination strongly affects plant instantaneous selfing rate, but it is also likely to affect its evolution. Indeed, species pollinated by animals are almost twice as likely to exhibit a mixed mating system as species pollinated by wind (Goodwillie et al. 2005, Fig. 1.1), implying that the mode of pollination could interfere with the evolution of selfing. This pattern can be explained either because some mechanism prevents the evolution of mixed-mating system in abiotically pollinated species, or because the evolutionary forces that lead to mixed-mating are more pronounced in biotically pollinated species. Several ecological factors that strongly affect the evolution of selfing may depend on pollinator abundance and behavior.

First, pollen limitation (the reduction in plant reproductive success due to inadequate quantity or quality in pollen receipt) favors the evolution of higher selfing rates (Cheptou 2004; Porcher and Lande 2005). Pollinator abundance strongly affects pollen limitation (Ashman et al. 2004; Eckert et al. 2010; Thomann et al. 2013), and Bodbyl Roels and Kelly (2011) showed that selfing could rapidly evolve with pollinator loss. Thus, pollinators could affect selfing evolution because of their unpredictability: variations in pollen limitation could select for the maintenance of mixed-mating systems (Morgan and Wilson 2005).

Second, pollen discounting (the reduction in outcrossed male siring success associated with an increase in selfing rate) can be related to pollinator behavior. Indeed, a pollinating insect brings mostly outcross pollen to the first flower probed on the plant, but then it loses self-pollen on the next flowers it visits on the same plant, and this pollen is lost for export (Karron and Mitchell 2012). Since pollinators tend generally to visit many flowers on each plant, this behavior could increase pollen discounting and select against higher selfing rates (Goodwillie et al. 2005). Devaux et al. (2014), for example, showed that floral display evolution could be strongly affected by pollinator behavior and abundance, because of their impact on plant geitonogamous selfing rate.

Third, recent studies suggested that trade-offs between selfing and other components of plant fitness (*e.g.* viability) could play an important role in mating system evolution, especially in the stable maintenance of mixed-mating systems (Jordan and Otto 2012). One key component of plant-pollinator interactions is the cost of their interactions for the plants. Increased selfing rate decreases the reliance of plants on pollinators, and could decrease the selective pressures for the maintenance of such costs. Yet, because few theoretical studies explicitly take into account plant-pollinator interactions, the impact of this cost of pollination on the evolution of plant selfing rate remains to be investigated.

The evolution of floral traits in dioecious plant species

Pollinators have been shown to select for plant traits such as phenology, number of open flowers or corolla size (Harder and Johnson 2009). However, selection on floral traits may not affect similarly male and female fitness components if, as in the animal kingdom, secondary sexual trait evolution is driven by sexual selection: males would tend to be limited by mate availability, *i.e.* by the number of reproductive events, whereas females would be limited by the resources needed to produce their offspring (Bateman 1948).

The wide occurrence of sexual dimorphism in dioecious species suggests that sexual selection also applies to flowering plants (Delph 1996). An empirical study of selection acting on floral traits in dioecious *Silene latifolia* suggested that selection could indeed differ between male and female individuals (Delph and Herlihy 2012). Yet, several features of plant reproduction may affect the accurate application of sexual selection to plants (Burd 1994). First, pollination of entomophilous dioecious plant species depends on a "third partner": the pollinator. The modifications of pollinator behavior possibly resulting from such differences between male and female flowers (which could exhibit different attractivity from the pollinator point of view) could strongly affect plant fitness, and thus selection on plant traits. Second, many flowering plants suffer from pollen limitation (Ashman et al. 2004). Because of pollen limitation, females could be as limited by mate access as males and sexual selection may not suit well to plant species (Burd 1994).

Vamosi and Otto (2002) highlighted this limitation on the application of sexual selection on plants. Indeed, their model of the evolution of plant traits predicts that the selective gradient on female floral traits depend on pollen limitation. Especially, when female fitness is limited by the number of reproductive events, selective pressures on male and female individuals are similar. Yet, Vamosi and Otto (2002) assumed plant density to be fixed, whereas the evolution of floral traits is likely to affect plant demography. The interplay between demography and evolution of floral traits mediated by pollinators thus remains to be investigated in order to properly test if the evolution of dimorphism demographically threatens dioecious populations.

1.4 Modeling plant-pollinator interactions

As presented above, only a few theoretical studies explicitly consider plant-pollinator interactions. However, empirical studies highlighted the important role played by pollinators on plant fitness components. Moreover, previous theoretical studies highlighted the impact of ecological factors on plant demography and evolution. Pollinators may influence such factors (*e.g.* pollen limitation), and it seems crucial to better understand how pollinator behavior and abundance may influence plant demography and the evolution of plant traits.

Merging demography and evolution in the case of plant-pollinator interactions is crucial. Indeed, demography is likely to affect the evolution of traits and vice-versa. Especially, some traits are likely to induce density-dependent fitness. For example, the advantage for an individual to invest much energy in pollinator attraction is likely to depend on the relative densities of plants and pollinators. Moreover, the evolution can affect demography. In particular, the evolution of reward production could lead to "evolutionary suicide" (the demographic extinction of the population) or to "evolutionary murder" (the demographic extinction of the partner population) in the case of mutualism, because "cheater" (individuals that do not provide reward, or provide less reward to their partners) may invade the mutualistic populations (Ferrière et al. 2002).

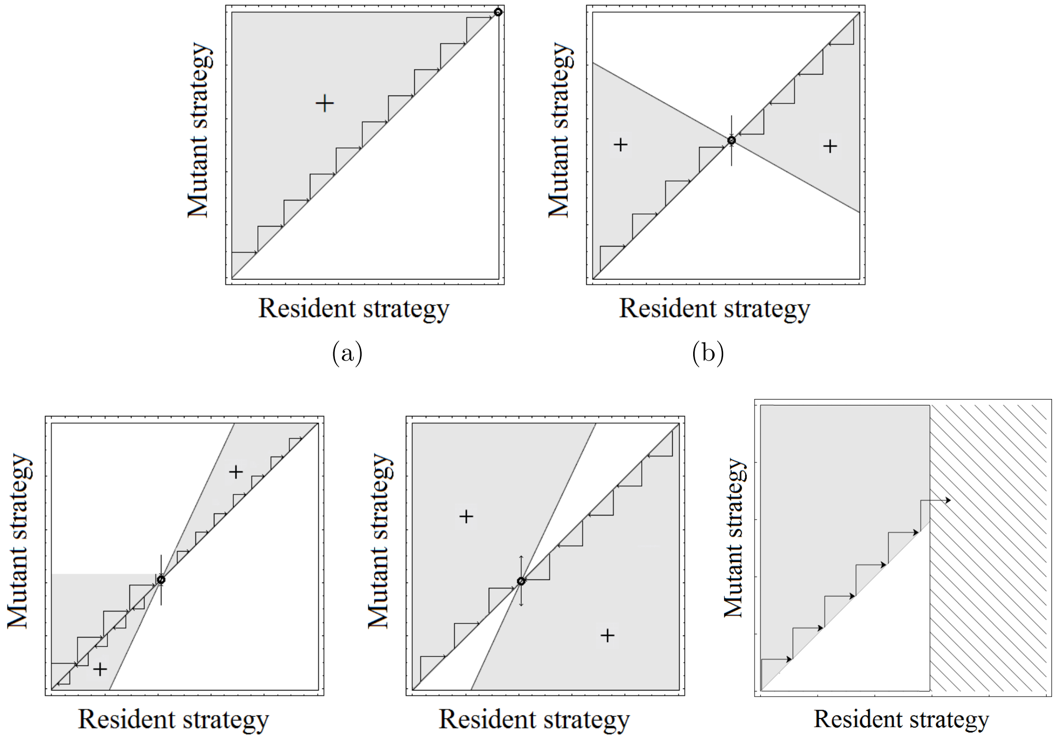
Adaptive Dynamic framework allows the study of the interplay between demography and evolution (Brännström et al. 2013; Geritz et al. 1998). As we will use this framework in some of the theoretical studies presented in this document, insights on its general assumptions and on the results reading are given in Box. 2. However, this technique makes some really stringent assumptions. Especially, it assumes a complete time-scale separation between the introduction of a mutant and the ecological dynamics, *i.e.* it assumes rare mutation events. Yet, evolution can strongly modify plant traits in few generations (*e.g.* Bodbyl Roels and Kelly 2011). Because one might want to ensure that the results of such models are not affected by the framework's assumptions, several modeling approaches should be used in a complementary manner (*e.g.* use stochastic simulations in addition to the deterministic approach).

The study of the evolution of plant trait using Adaptive Dynamic framework relies

Box. 2: Adaptive Dynamic framework

The technique consists in analyzing whether a population that initially displays a fixed trait value (the resident strategy) can be invaded by a mutant with a different strategy. The evolutionary outcome of a trait is determined by following a series of mutational steps: the introduction of a mutant always occurs once the resident population has reached its demographic equilibrium, and only one mutant appears at a time in the population (Brännström et al. 2013; Geritz et al. 1998).

Those evolutionary trajectories are classically represented with pairwise invisibility plots (PIP). A short description of the possible evolutionary outcome and their PIP representation is given below (Brännström et al. 2013; Otto and Day 2007). The resident strategy is given on the x-axis, and the mutant strategy on the y-axis. Regions of invasion are depicted in grey and regions of non-invasion in white. Striped zones indicate strategies for which population cannot demographically reach equilibrium. Examples of evolutionary trajectory (a series of successful invasions of mutants) are represented by the arrows.



(a) (b)

(c) (d) (e)

Figure 1.4: PIPs examples

Fig. 1.4a depicts a simple case of directional selection: a mutant with a higher trait values is always selected for. On the opposite, the evolutionary trajectory of Fig. 1.4b represents a case in which an intermediate trait value is selected for. Successive mutations converge to this strategy (which is thus convergent stable: CS), and once the population has reached this strategy, no mutant can invade the population (the strategy is evolutionary stable: ESS).

Fig. 1.4c represents a case of divergent selection: according to the current value of the population trait, evolution will either select for lower trait values or to higher trait values. No intermediate state is stable. Fig. 1.4d represents an evolutionary branching point: evolution leads to an intermediate trait value, and in a population with this strategy, other mutants can invade the population. This can lead to polymorphic populations. Fig. 1.4e represents an evolutionary suicide: evolution leads to the demographic extinction of the population.

on the accurate creation of a demographic model of plant-pollinator interactions. Then mutant's fitness is given by its growth rate in this population at demographical equilibrium (Malthusian fitness). Developing demographic model of plant-pollinator interactions implies to solve several problems.

First, one must decide how to explicitly describe plant-pollinator interactions. Only a few models explicitly consider pollination mutualism, and they often show several limits. Indeed, models of plant-pollinators interactions often neglect pollen dynamic, and assume the pollinator to always carry pollen. For example, Fishman and Hadany (2010) investigated the impact of pollinator behavior on the plant visitation rate, when taking into account the ability of pollinators to deposit a mark on the flower they visit. This mark then prevents further visits by other pollinators that detect the mark while landing on the flower and do not pollinate it. Although considering the impact of fine pollinator behavior, this model assumes pollinators to constantly carry pollen, which allows plant fertilization as soon as the pollinator enters the flower. Similarly, DeAngelis and Holland (2006) simulated detailed pollinator behavior to understand how visitation rates might depend on plant and pollinator densities. Yet, they assumed a pollinator to carry pollen, and allow for the fertilization of all ovules at one time (subsequent visits do not improve seed set).

Second, beyond the choice one has to make about the impact of visits on the benefit and cost of mutualism, another crucial difficulty arises: how should the inter-specific interactions be modeled? What visitation rates should be expected? How do plant traits affect those visitation rates?

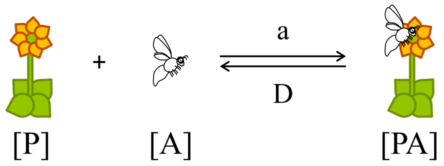
I would like to stop a moment here to discuss the way one decides to model inter-specific interactions. The following discussion somehow relates to the evolution of my personal attitude regarding the integration of inter-specific interactions. In the Chapters included in this thesis that will be presented below, I used several approaches, depending both on the particular requirements of each model, but also on my personal perception of this field of research.

Most models depict inter-specific mutualistic interactions using functional responses that were at first created for predator-prey interactions (*e.g.* Holling 1959) before being adapted for mutualistic interactions (*e.g.* Holland and DeAngelis 2010). Although predator-prey interactions were studied for decades, there is still a huge debate on the relationship one might use to accurately describe the predation rate (*e.g.* Arditi and Ginzburg 2012 *v.s.* Abrams 2015). At first, Holling (1959) proposed a simple relationship of predator-prey interactions. The biological mechanisms underlying the Holling functional response are well known. Predators search for a prey, and once they successfully attacked a prey, they spend some time to manipulate it. This approach considers predators to act independently from each other. This kind of functional response is by far the most commonly used in demographical models. However, some evidence point out that the predation rate might depend on predator density, and several other functional responses were proposed, either predator dependent (the consumption of the prey depends on the predator density or on the prey and the predator densities) or ratio dependent (the consumption of the prey depends on the ratio of predator and prey resources). There is no clear consensus on which functional response might be used (reviewed in Arditi and Ginzburg 2012, but see Abrams 2015).

When applying those functional responses to plant-pollinator interactions, one should note that another difficulty emerges. Crucially, for a visit to allow ovule fertilization, the pollinator must carry pollen: hence it must have visited another plant from the same species (in the case of dioecious populations, it must even visit sequentially a male and then a female). In order to resolve this issue, one can either neglect the need of pollinator to carry pollen, thus considering a pollinator always brings some pollen to a plant, or adapt a functional response to integrate the need of several interactions in order to pollinate a plant (Chapter 3). In both cases, the functional responses are chosen because they provide the wanted mathematical properties, but they do not rely on the mechanisms of pollination. Adapting the existing functional responses is the easiest way to model the interactions, but they may not always represent well the system of interaction. Moreover, this method can be used only when one can determine the expected relationships between plant and pollinator densities and visitation rates.

A second approach can be to consider simple mechanisms of interactions within a population. With this approach, we consider each individual to be in one category of individuals: searching another individual to interact with, interacting with an individual, resting... In this case, one needs to determine the categories of individuals to be integrated

Box. 3: Derivation of a simple functional response



Let the total density of plants and pollinator be P and A respectively. Individuals are either free (densities $[P]$ and $[A]$) or interacting (density $[PA]$). Free pollinators ($[A]$) are attracted by unoccupied plants ($[P]$) at rate a, and leave plants after an interaction at rate D.

From those interactions, we can deduce the following dynamics:

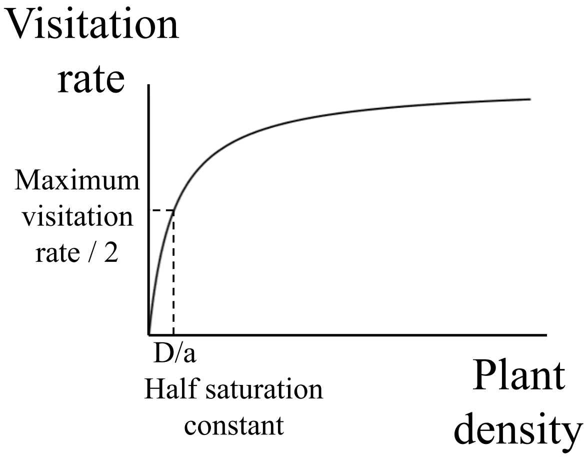
$$\begin{cases} \dfrac{d\,[P]}{dt} = \dfrac{d\,[A]}{dt} = -\,a\ \ [P]\,[A] + D\ \ [PA]\,, \\ \dfrac{d\,[PA]}{dt} = a\ \ [P]\,[A] - D\ \ [PA]\,. \end{cases}$$

Assuming the interactions to occur almost instantaneously at the time-scale of plant and pollinator density variations (quasi steady state approximation), the above equations equal 0, implying

$$[PA] = \tfrac{a}{D}\,[P]\,[A]\,.$$

We assume free individual plants are much more numerous than plants in interaction (*i.e.* no competition for plants between pollinators): $P \approx [P]$. Total pollinator density is given by $A = [A] + [PA]$. Plant visitation rate is given by the rate of creation of plant-pollinator complexes: $a\,[PA]$. Using the above equations, one can deduce that:

$$a\,[PA] = a\frac{A\ P}{\frac{D}{a} + P}.$$



Thus, using these simple rules of interaction, one can mechanistically obtain a type II Holling functional response (Holling 1959).

into the model, and then to determine the rate of interaction at the population level by considering a mean population behavior (all individuals act the same way). This method is more complicated to set up, but it allows a better understanding of the assumptions the model does on each partner (*e.g.* Box. 3). This method has been used to get mechanistic derivation of the usual functional responses (*e.g.* Geritz and Gyllenberg 2012). Moreover, it also allows the deduction of visitation rates in populations for which it is difficult to get intuitive insights on the relationships between densities and visitation rates. For example Fishman and Hadany (2010) used this method to predict the visitation rates of plant individuals when pollinators mark the flowers they are visiting, and avoid the marked flowers. Similarly, we used this method to predict visitation rates in dioecious populations, where the use of functional responses is not straightforward, because three partners are interacting (Chapter 4).

A last approach could be to explicitly model individual pollinator foraging choices within a plant population (*e.g.* Essenberg 2012; Pyke et al. 1977). Those models consist in determining the probabilistic rules of pollinator behavior, and determining their impact on pollinator movements within and between plants. Those models focus on pollinator energy income (Optimal Foraging), and predict which pollinator movement is the most likely to provide the more energy. However, those models have limited predictive abilities, because most empirical data violate several of their assumptions: plant populations are large, without boundaries, whereas the populations used in controlled areas are small and delimited. Moreover, these models do not consider the whole trajectory of a pollinator but only its transitions within and among plants, and the pollinator is assumed to visit many plant individuals within a foraging bout. Yet, at least in small populations, this assumption is likely to be false. For example, Mitchell et al. (2004) showed that in a population of 36 plant individuals, most pollinator only visited one flower.

Integrating fine pollinator behavior seems to be a promising research avenue for two reasons. First, data on pollinator foraging are more and more precise, and one can hope one day acquiring GPS data of those insects, as was done for analyzing the movement patterns of ants (Mersch et al. 2013). Second, our computational power incredibly increased, allowing for analysis that were once too much time-consuming. Put together, one could determine the fine behavior of pollinators that affect plant visitation rates, by comparing real pollinator movements and simulated ones (see Chapter 5 for a currently under progress work in this sense). This comparison relies on the Approximate Bayesian Computation (ABC) framework (Beaumont 2010; van der Vaart et al. 2015).

1.5 Presentation of the thesis content

During my thesis, I studied the interplay between animal-pollination and plant trait evolution, especially traits involved in the mating system, using a theoretical approach. These modeling approaches draw their inspiration from many empirical studies, and data of pollinator behavior.

In Chapter 2, I will present a detailed review on the interplay between plant mating system and pollinator behavior, which I briefly mentioned in this introduction. First, we show that pollinators determine the immediate ecological selfing rate. Indeed, the selfing rate depends on the dynamics of the pollination process: the amounts of pollen transferred among plants of the same species (outcrossing), among flowers on the same plant (geitonogamous selfing), and within flowers on the same plant (intrafloral selfing). Those transfers highly depend on plant floral display (the number and size of flowers of an individual plant), and on flower characteristics. Second, we reviewed the theoretical studies that analyzed the impact of pollinator behavior on the evolution of plant selfing rate. Finally, we investigated the interplay between pollinator behavior and plant mating systems at the community level. This work was conducted in collaboration with Emmanuelle Porcher from the Natural History Museum (Paris) and Céline Devaux from the Institute of Evolutionary Sciences (ISEM, Montpellier) and has been published in the Journal of Evolutionary Biology (Special Issue: Sex uncovered: the evolutionary biology of reproductive systems).

In Chapter 3, I will present a study that investigated the impact of pollinators on the evolution of plant prior selfing rate. Previous theoretical studies did not integrate one key consequences of the evolution of selfing in entomophilous plant species: selfing species typically show a “selfing syndrome”. This refers to the fact that selfing species often evolve floral modifications, such as a smaller floral display, a lower nectar production or a reduced herkogamy (Goodwillie et al. 2010; Sicard and Lenhard 2011). Thus, the cost of pollination is likely to decrease with plant selfing rate. In Chapter 3, I will present the outcome of the evolution of autonomous prior selfing (selfing that occurs before outcrossing, not mediated by pollinators) when it affects both the demography of plants and pollinators and the investment of plants in pollination. Including the selfing syndrome in the model brings original results, including a new explanation for the stable maintenance of mixed mating systems and the theoretical evidence that evolution towards selfing can lead to evolutionary suicide under some conditions. This study thus highlighted important interactions between animal-pollination and selfing evolution, even when assuming basic pollinator behavior. This study was published in Evolution (Lepers et al. 2014).

I will present in Chapter 4 an analysis of the impact of animal-pollination for species that compulsorily rely on outcross pollination: entomophilous dioecious species. This will allow determining the interplay between animal-pollination and the selective pressures suffered through male and female fitness. We investigated how the attractiveness of males and females should evolve under pollinator-mediated selection. Higher attractiveness allows a higher visitation rate of an individual plant, but because of the energetic cost of attractiveness, it reduces the investment of that plant into reproduction. This model also reveals that pollen transfer and plant density are higher in populations with females being more or equally attractive than males. This model highlights on the one hand that the evolution of male strategy only depends on male-male competition, favoring a high investment into traits linked to attractiveness. On the other hand, female evolution depends on the level of pollen limitation: females evolve high attractiveness if pollen is limiting, otherwise females allocate more resources in ovule production. The lower population densities are expected in populations with low dimorphism, which suffer from high pollen limitation. Thus, dimorphism may not threaten dioecious species. On the opposite, sexual dimorphism may reflect an efficient pollination in the population. This chapter is the object of a manuscript in preparation.

Finally, in Chapter 5, I will present an ongoing project that aims at defining and quantifying the mechanisms underlying pollinator foraging behavior, and especially the impact of the number of flowers displayed by plants on pollen transfer. This project consists in the creation of spatially explicit simulations of pollinator foraging freely in a space that includes plants with different numbers of flowers. Those simulations will be compared with real visitation bouts, using a Bayesian framework (Beaumont 2010), and will allow determining the required assumptions one must make on pollinator behavior in order to accurately predict visitation patterns within a plant species. This project in progress is conducted in collaboration with V. Bansaye (CMAP, École Polytechnique, France) and G. Berthelot (INSEP-IRMES, France).

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Chapter 2

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

JOURNAL OF Evolutionary Biology

eseb European Society for Evolutionary Biology

doi: 10.1111/jeb.12380

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

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Keywords:

floral traits;
inbreeding depression;
mutualistic networks;
plant-pollinator interactions;
plasticity;
pollinator foraging behaviour;
selfing rates.

Abstract

Most flowering plants rely on pollinators for their reproduction. Plant-pollinator interactions, although mutualistic, involve an inherent conflict of interest between both partners and may constrain plant mating systems at multiple levels: the immediate ecological plant selfing rates, their distribution in and contribution to pollination networks, and their evolution. Here, we review experimental evidence that pollinator behaviour influences plant selfing rates in pairs of interacting species, and that plants can modify pollinator behaviour through plastic and evolutionary changes in floral traits. We also examine how theoretical studies include pollinators, implicitly or explicitly, to investigate the role of their foraging behaviour in plant mating system evolution. In doing so, we call for more evolutionary models combining ecological and genetic factors, and additional experimental data, particularly to describe pollinator foraging behaviour. Finally, we show that recent developments in ecological network theory help clarify the impact of community-level interactions on plant selfing rates and their evolution and suggest new research avenues to expand the study of mating systems of animal-pollinated plant species to the level of the plant-pollinator networks.

Introduction

Flowering plants are stimulating models for studying the evolutionary biology of reproductive systems, owing to their wide diversity of mating systems (Barrett, 2003; Charlesworth, 2006). Typical topics of interest comprise the evolution of selfing rates (Goodwillie *et al.*, 2005), including self-incompatibility (Goldberg *et al.*, 2010), the evolution of separate sexes (Spigler & Ashman, 2012) and sex chromosomes (Charlesworth, 2013) and the maintenance of sexual reproduction (Silvertown, 2008). The reproduction of the vast majority of Angiosperm species is unique in its reliance on animals as pollen vectors (~90%, Ollerton *et al.*, 2011), yet the study of plant mating system evolution has long remained uncoupled from pollination ecology, focusing historically on genetic drivers. As mentioned by Charlesworth (2006), the failure to include ecological mechanisms into the evolution of plant mating may be due to their diversity: '*Models of mating system evolution have emphasised genetic effects, even though* (…) *ecological circumstances, such as pollinator abundance or plant density, must often be important. Their complexity and variety, however, creates difficulties in developing any general theories*'.

The gap between the study of plant mating systems and pollination ecology has shrunk over the past decades, with many studies focusing mostly on animal-pollinated plants (reviewed e.g. in Harder & Barrett, 1996, 2006; Goodwillie *et al.*, 2005; Mitchell *et al.*, 2009; Eckert *et al.*, 2010; Karron *et al.*, 2012; Thomann *et al.*, 2013) but also on wind-pollinated plants (Friedman & Barrett, 2009). This rich literature has revealed general patterns and processes out of the complexity mentioned by Charlesworth (2006). One such pattern is the increase in selfing rates under pollen limitation and its

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long-term evolutionary consequences (Wright *et al.*, 2013). This intuitive expectation can now be tested as pollinator abundance decline and reduced pollination service become a worldwide reality (Potts *et al.*, 2010; Gonzalez-Varo *et al.*, 2013). Several papers discuss pollinator shortage as a cause of outcross pollen limitation (see Glossary) and increased immediate ecological selfing rates, which may determine future evolutionary changes in plant mating systems (Eckert *et al.*, 2010; Thomann *et al.*, 2013). The latter prediction is supported by rapid evolution of the ability to self-fertilize observed in *Mimulus guttatus* in the absence of pollinators (Bobdyl Roels & Kelly, 2011).

Pollinator foraging behaviour also emerges as an important factor influencing plant selfing rates and their evolution. The predicted impact of foraging behaviour on plant mating systems is however less clear than that of pollinator abundance because selection on pollinator vs. plant traits do not always act in the same direction. In plants, selection favours floral traits that influence pollen transfer to promote reception of conspecific and compatible pollen, increase the quantity and quality of seeds produced (including the ratio of selfed to outcrossed seeds) and the amount of pollen exported to conspecifics. In pollinators, selection favours traits that maximize the rate of energy gain through foraging behaviour (optimal foraging theory, Pyke, 1984), which may lead to nonoptimal pollen transfer for plants. Hence, although plants and pollinators are involved in true mutualistic interactions with reciprocal benefits, conflicts of interest are widespread (Bronstein, 2001; Dufay & Anstett, 2003; De Jong & Klinkhamer, 2005, p. 229). Constraints imposed by pollinators on plants, as well as constraints imposed by plants on pollinators, may induce plastic and evolutionary responses, such that the two partners are engaged in a permanent Red Queen-like coevolutionary race. The coevolution of plant and pollinator traits has been a major topic of research since Darwin (1876), but constraints imposed by pollinator foraging behaviour on the ecology and evolution of plant mating systems are seldom integrated into experimental and theoretical studies. For example, most models of the evolution of plant selfing rates assume that these rates can evolve freely between zero and one. Another limit of studies connecting mating systems and pollination ecology is that they are often restricted to pairs of plant-pollinator species. Recent developments in ecological network theory have revealed that the community context, multispecies interactions, and the position of a species in a plant-pollinator network can be key to understand the influence of pollinators on plant mating systems (Vanbergen *et al.*, 2014).

Here, we review how the interplay between pollinator behaviour and plant floral traits influences the immediate, ecological plant selfing rates (hereafter 'ecological selfing rates'), the evolution of selfing rates, and their distribution in plant-pollinator networks (summarized in Fig. 1). We are interested in self-compatible plants, mostly with perfect flowers, that are pollinated by insects or birds (Buchmann & Nabhan, 1996), particularly bees and hummingbirds for which we have more data. In the first section, we show that the ecological selfing rates of about one half of flowering plant species is controlled partly by pollinator abundance and behaviour, which should therefore be included in theoretical investigation of plant mating system evolution. We argue, as do Karron *et al.* (2012), that the reciprocal effects of pollinator behaviour on floral traits are much better documented than their consequences for plant self-fertilization. Because we focus on pollinator behaviour, we intentionally exclude pollinator abundance, pollen limitation and their influence on the selection of floral traits, which are presented extensively elsewhere (Elzinga *et al.*, 2007; Eckert *et al.*, 2010; Schiestl & Johnson, 2013; Thomann *et al.*, 2013). One should keep in mind that these two components strongly determine ecological selfing rates and their evolution.

The second part of this review examines how theoretical studies of the evolution of plant selfing rates include pollinator behaviour and model the plant traits that can alleviate the constraints it might impose. We show that mechanistic models combining pollinator behaviour and the genetic consequences of selfing improve on pure ecological or pure genetic models by providing quantitative predictions of evolutionarily and ecologically stable plant mating strategies. We propose a method to account for pollination ecology in models of the evolution of selfing rates and provide experimental research avenues to help clarify the role of plant-pollinator interactions in the distribution of plant selfing rates. The last section shows that community-level processes can influence the ecology and evolution of plant mating systems, and outline how to use current knowledge on competing vs. facilitative interactions among plant species and pollinator species for a better understanding of the distribution and evolution of plant selfing rates.

The interplay between pollinator behaviour and floral traits determines ecological plant selfing rates

The selfing rate or self-fertilization rate s is the fraction of selfed embryos produced by an individual plant. This fraction depends primarily on the rate of self-pollination, the relative amounts of self vs. outcross pollen transferred by pollinators or through nonpollinator means (e.g. stigma dragging). The self-pollination rate can be further modified by pre and post-fertilization selection (see Glossary), which is beyond the scope of this review. Only two categories of plant species have complete control over their selfing rate, although pollinators may still influence their seed set: (i) obli-

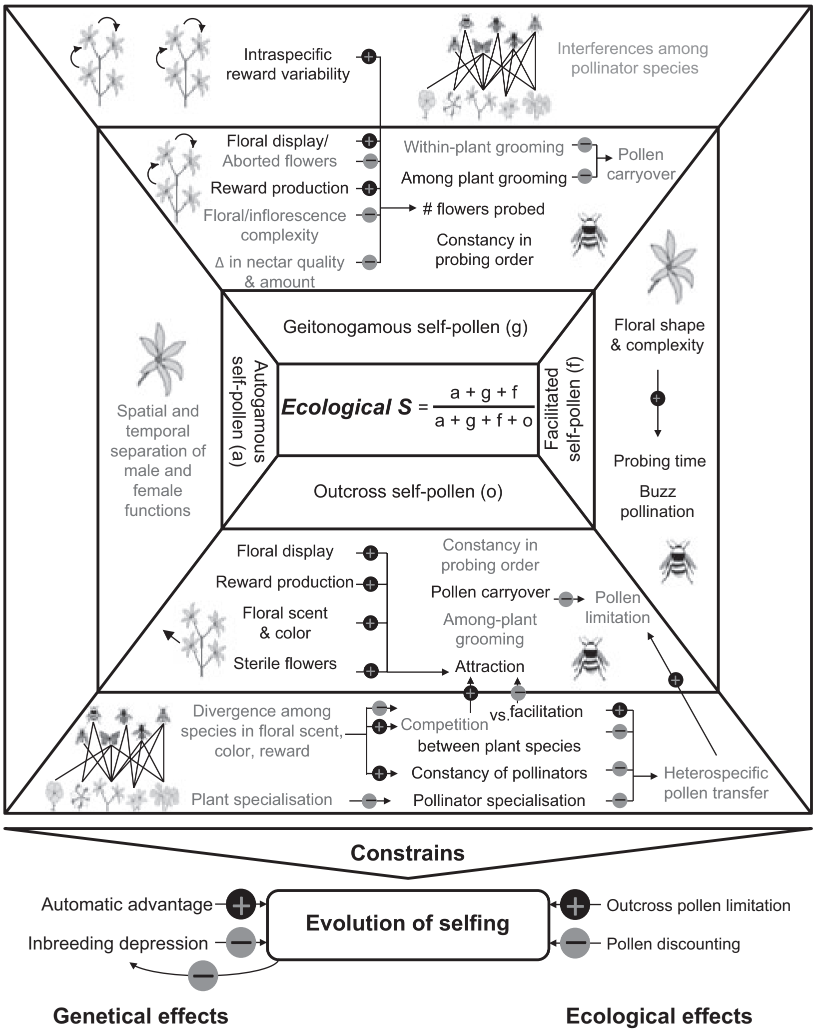

Fig. 1 Summary of the constraints imposed by pollinator behaviour on the ecology and evolution of plant selfing rates. The upper panel describes the plant and pollinator traits that influence the deposition of each of the three origins of self-pollen, as well as outcross pollen (see Glossary). Traits with a positive (respectively negative) impact on amounts of pollen deposited are in black (respectively grey). The inner square recalls the definition of the rate of self-fertilization. The intermediate square groups plant and pollinator traits operating at the flower or individual plant levels. The outer square groups plant and pollinator characteristics operating at the population or network levels.

gately outcrossing species ($s = 0$), such as self-incompatible or dioecious species, representing ca. 50% of species (Igic & Kohn, 2006) and (ii) completely selfing species ($s = 1$) relying on prior autonomous selfing (see Glossary), such as species producing closed flowers only, which are however extremely rare (Goodwillie *et al.*, 2005; Culley & Klooster, 2007).

In the remaining half of animal-pollinated plant species, selfing rates are invariably influenced by pollinators, via the quantities of both outcross and self-pollen transferred among and within flowers (Figs 1 and 2). Outcross pollen deposition on plant stigmas is fully constrained by pollinators. In contrast, self-pollen deposition on stigmas depends only partially, but sometimes strongly, on pollinators as it includes: (i) facilitated self-pollination, the transfer of self-pollen within flowers caused by pollinator visits, (ii) geitonogamous self-pollination caused by pollinator visits among flowers on a plant, and (iii) autonomous, that is, without the help of pollinators, prior, competing and delayed self-pollination within flowers (Fig. 2 and definitions of the glossary). Note that the amount of self-pollen deposited ‘autonomously’ by a plant on stigmas may still be influenced by previous outcross pollen limitation, as is sometimes the case with delayed autonomous selfing (e.g. Ruan *et al.*, 2010). Besides, even if the *amount* of autonomous self-pollen is under the plant control, the *rate* of self-pollination depends on pollinators via the amounts of self and outcross pollen they deposit on stigmas.

In the following, we examine how pollinator behavioural traits interact with plant traits to constrain patterns of outcross and self-pollen deposition and thereby the ecological selfing rates of plants. We review how pollinators can respond plastically to variation in floral traits to optimize their energy gains, and which

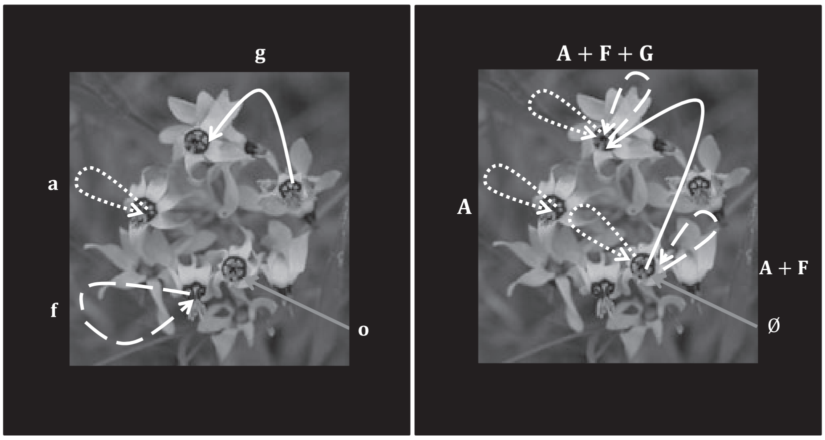

Fig. 2 Clarification (left panel) and one estimation method (right panel, see also Box 1) of selfing components for self-compatible animal-pollinated plants with perfect flowers (following Lloyd, 1992).

floral traits can vary plastically or genetically to modify pollinator behaviour and control plant selfing rates. We consider one self-compatible plant species specialized (see Glossary) on one pollinator species or functional group (Fenster *et al.*, 2004), as is frequently done in studies of the relationship between pollinators and plant mating systems. Pollinator choice among multiple plant species (preference and constancy, see Glossary), competition among plant species for shared pollinators, and their impacts on plant selfing rates are discussed only in the last section.

Pollinator traits influencing outcross and self-pollen transfer

Self-pollination rates are influenced by (i) the number of pollinator visits to individual plants and flowers, (ii) the efficiency of pollen transfer from stamens to pollinators and from pollinators to stigmas, within and among flowers and (iii) the composition of pollen loads on pollinators, particularly the fraction of self vs. outcross pollen. All these components ultimately depend on pollinator abundance, morphological and phenological matching of plants and pollinators and pollinator foraging behaviour among and within plants. We focus on pollinator behaviour (Fig. 1) and do not address pollinator abundance, phenology or morphology (see e.g. Waser, 1978; Harder & Barrett, 1996 for details on pollen placement on pollinators; or O'Neil, 1997; Elzinga *et al.*, 2007; Bartomeus *et al.*, 2011 for phenology).

Pollinator grooming behaviour influences pollen transfer efficiency within and among plants; its intensity and timing greatly determine patterns of pollen deposition of outcross and self-pollen throughout a foraging bout (see Glossary). Grooming generally causes pollen to be deposited on fewer flowers (e.g. Harder *et al.*, 2000; Castellanos *et al.*, 2003), hence reduces pollen carryover (see Glossary), with contrasting impacts of within- vs. between-plant grooming. Between-plant grooming reduces pollen dispersal among plants (Holmquist *et al.*, 2012) and is likely to increase outcross pollen limitation. Within-plant, between-flower grooming reduces pollen carryover within plants and should increase geitonogamous selfing rates (Matsuki *et al.*, 2008). The scant data available suggest that most grooming occurs between plants (see e.g. Harder, 1990; Mitchell *et al.*, 2004; Johnson *et al.*, 2005), but the pattern may vary among pollinator species and depend on pollen availability, as shown by a higher probability of grooming between flowers with increasing pollen availability (Harder, 1990). More intensive and more frequent grooming is observed for pollen-collecting visitors (e.g. bees) than for nectar-collecting visitors (e.g. birds or moths; Thomson, 1986; Castellanos *et al.*, 2003). As a result, nectar-feeding species tend to transfer pollen more efficiently than do pollen-feeding species (Conner *et al.*, 1995; Sahli & Conner, 2007; but see King *et al.*, 2013). More generally, the extent of pollen carryover is negatively related to geitonogamous selfing rates (Geber, 1985; Robertson, 1992; Morris *et al.*, 1994): pollinators that deposit most of their outcross pollen on the first few flowers transfer mostly self-pollen on the subsequent flowers of the plant. Grooming can partially cause the negative relationship between pollen carryover and geitonogamy (Rademaker *et al.*, 1997; Matsuki *et al.*, 2008), but pollen carryover is also strongly governed by interactions between pollinator morphology and floral design (Harder & Barrett, 1996).

The number of flowers a pollinator probes successively on a plant increases geitonogamous self-pollination (Rademaker *et al.*, 1999; Karron *et al.*, 2009). Pollinators tend to probe more flowers on plants with larger floral displays (see Glossary), but rarely probe more than a dozen flowers per plant, so that the fraction of flowers probed per plant is often a decreasing function of display size (reviewed in Snow *et al.*, 1996; Ohashi & Yahara, 2001; Harder *et al.*, 2004). Multiple reasons can explain why pollinators leave plants before visiting all open flowers on a plant (reviewed in Snow *et al.*, 1996); the most general one involves the maximization of energy gains (optimal foraging). For pollinators, which have limited short-term memory, visiting only a fraction of available flowers reduces the risk of visiting a given flower twice (Ohashi & Yahara, 1999, 2001, 2002). Similarly, pollinators leave a plant earlier when they encounter empty, rewardless flowers (e.g. Bailey *et al.*, 2007 and references therein). Self-pollination also depends on the order in which flowers of a plant are visited by pollinators. As the first few flowers visited by pollinators contribute the most to outcross pollen transfer, outcross pollination is expected to be larger when independent pollinators visit flowers randomly on a plant than when all pollinators visit flowers in the same order (Devaux *et al.*, unpublished manuscript, for a model).

All of the above characteristics of pollinators affect the transfer of outcross and self-pollen among flowers, but not within-flower facilitated selfing (see Glossary), which remains little studied. Facilitated selfing has been demonstrated unambiguously in a small number of species (e.g. Anderson *et al.*, 2003; Duncan *et al.*, 2004; Johnson *et al.*, 2005; Owen *et al.*, 2007; Vaughton *et al.*, 2008), but its broader contribution to total selfing rates in natural populations is currently unknown (see Box 1 for a method). It is unclear whether facilitated selfing is primarily controlled by flower characteristics, such as spatial (herkogamy) or temporal (dichogamy) separation of anthers and stigmas (Lloyd & Webb, 1986; Webb & Lloyd, 1986; Brunet & Eckert, 1998) or if it can be influenced by pollinator behaviour. Buzz pollination by native bees causes high rates of facilitated self-pollination in the partially self-incompatible *Dianella revoluta* (Duncan *et al.*, 2004). Furthermore, reduced time spent at individual flowers by hawkmoths has been associated with decreased seed production in self-compatible *Petunia* lines (Brandenburg *et al.*, 2012), but we do not know whether any of the effect can be attributed to facilitated selfing. From the available experimental studies, we can nevertheless argue that increased numbers of flowers probed per plant or increased probing time per flower should contribute to increased facilitated selfing at the plant level.

Box 1: A method for estimating facilitated selfing

Estimation of facilitated selfing is challenging because it requires devices to mark pollen or follow pollinator visits, while controlling for resource allocation, outcross pollen limitation and pollinator attraction. However, facilitated selfing could contribute significantly to observed selfing rates and their evolution. Indirect evidence of facilitated selfing is available, but only for plant species with specific floral types (Fig. 2): Johnson *et al.* (2005) make use of the absence of autonomous selfing in *Disa cooperi*, Vaughton *et al.* (2008) of the absence of geitonogamous selfing in *Bulbine vagans* and Owen *et al.* (2007) of the absence of both in *Bulbine bulbosa*; on the other hand, Anderson *et al.* (2003) use *Roridula* species for which facilitated selfing is performed by insects (hemipterans) that do not contribute to the other selfing components, performed by bees.

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

Estimating the three components of selfing or self-pollination (Fig. 2) requires a detailed description of single-pollinator visits to individual plants. The method requires N replicate caged plants with F individually marked open perfect flowers. Identical numbers of flowers control for resource allocation if selfing (not self-pollination) rates are to be estimated. Each plant should be visited by a single pollinator carrying no pollen, which precludes outcross pollination and the visitation order of the pollinator should be recorded (some flowers can be visited several times, and some flowers may remain unvisited). The components of selfing and self-pollination can then be estimated by analysing the seed set of the $N \times F$ flowers or by counting pollen grains deposited within each flower stigmas, respectively.

The simplest estimation method requires discarding all flowers visited more than once and counting pollen grains, not seeds, per flower to eliminate the delayed autonomous selfing component. Pollen loads provide information about (i) autonomous selfing for unvisited flowers (ii) autonomous and facilitated selfing jointly for flowers visited once as the first flower of the sequence, thus by a pollinator carrying no self-pollen, and (iii) all three components of selfing for flowers visited once as the second flower of the sequence, by a pollinator carrying self-pollen from one previously visited flower (Fig. 2, right panel). More sophisticated methods could use seed sets from all F flowers but would need to control for delayed autonomous selfing (possibly via the number of visits per focal flower or per plant, depending on the underlying mechanisms) and for the quantity of geitonogamous self-pollen deposited on flowers as a function of their visitation rank. For practical reasons, F needs to be small enough to keep track of the entire pollinator visitation sequence and large enough such that pollinators do not visit all open flowers, but generate variation in the number of flowers visited per plant; some flowers may have to be removed and some pollinators may have to be excluded from the experimental cage to avoid too long visitation sequences.

Floral traits influencing self-pollination rates via pollinator behaviour

Floral traits affecting self-pollination can be divided into three broad categories: (i) traits related to autonomous selfing (Lloyd & Schoen, 1992), (ii) traits modifying mechanically pollinator-mediated outcross and self-pollen transfer, without altering pollinator behaviour (e.g. floral design, pollen size and stickiness, and within and among flowers temporal separation of male and female functions; Webb & Lloyd, 1986; Lloyd & Schoen, 1992) and (iii) traits modifying pollinator behaviour directly. We focus on the latter floral traits, which typically influence two types of foraging behaviours: patterns of flower visitation among plants (attraction, preference and constancy, see Glossary), which are mostly discussed in the last section and patterns of flower visitation within plants (number and sequence of flowers visited, as well as probing time).

Floral display (see Glossary) may be the most studied plant trait influencing pollinator behaviour. Plants with larger floral displays attract more pollinators, thereby receiving more outcross and geitonogamous pollen (Snow *et al.*, 1996; Rademaker *et al.*, 1999; Karron *et al.*, 2004; Williams, 2007). Floral display can be modified plastically, for example via flower wilting when pollinators are abundant (Harder & Johnson, 2005), or flower abortion inducing pollinators to leave the plant (Ito & Kikuzawa, 2003); both mechanisms reduce the risk of geitonogamous selfing. Floral display can also be modified via evolutionary changes in the total flower production, in the distribution of open flowers among days (individual phenology) or in the longevity of flowers (Elzinga *et al.*, 2007; Devaux & Lande, 2010). How these changes in floral display modify selfing rates depends on the rates and patterns of flower visitation by pollinators, but the general trend is again an increase in plant selfing rates with larger floral displays.

Many other floral traits are also cues for pollinator attraction and influence visitation rates and outcross pollen receipt. These include flower size, reward production, floral shape, colour, and scent (Cozzolino & Scopece, 2008) or more species-specific characteristics, such as colourful leaves (Keasar *et al.*, 2009), sterile anthers (staminodes, Sandvik & Totland, 2003) or sterile flowers (e.g. *Centaurea cyanus*, Garcia-Jacas *et al.*, 2001). Nectar production and its replenishment dynamics have received particular attention, because they are critically related to geitonogamy. For example, bumblebees experiencing unrewarding (nectarless) plants probe more flowers on subsequent rewarding plants, such that the geitonogamous selfing rates of rewarding plants increase with the frequency of unrewarding plants in the population (Ferdy & Smithson, 2002). Conversely, maintaining nectarless flowers within an otherwise nectar-producing inflorescence may encourage pollinators to leave a plant early, thereby reducing geitonogamy (Hirabayashi *et al.*, 2006; Bailey *et al.*, 2007 and references therein; Whitehead *et al.*, 2012). Pollinator behaviour within a plant, hence geitonogamy, can also be modified by floral complexity (more flowers visited in plants with simpler flowers, Ohashi, 2002) or inflorescence architecture (e.g. lower selfing in racemes vs. umbels, Harder *et al.*, 2004; Jordan & Harder, 2006; or in more tightly twisted inflorescences, Iwata *et al.*, 2012).

Again, floral traits influencing facilitated self-pollination are poorly known, apart from structural features preventing self-pollination, such as anther caps (Peter & Johnson, 2006) and spatial separation of sexes. In contrast, the production of complex floral design or shape can induce longer probing time (Ohashi, 2002), which can translate into higher seed set (Brandenburg *et al.*, 2012), potentially because of higher facilitated selfing.

There is ample and long-standing evidence that plant selfing rates are constrained by pollinator morphology and foraging behaviour (Harder & Barrett, 1996), which may favour plant traits that can alleviate constraints imposed by pollinators. Evolution of plant traits under pollinator-mediated selection has been extensively studied (Elzinga *et al.*, 2007 for plant phenology; Schiestl & Johnson, 2013 for floral signals), but with surprisingly little emphasis on plant mating systems, except in verbal models (Eckert *et al.*, 2010; Thomann *et al.*, 2013). In the next section, we review the few theoretical studies that include pollinator behaviour in models of the evolution of plant mating systems. We argue that combining pollination ecology and the genetics of inbreeding depression is necessary to accurately describe and understand plant mating system evolution. We conclude by pointing out the experimental data needed to extend theoretical work on the evolution of plant mating system under pollinator constraints.

The role of pollinators in the evolution of plant selfing rates

We clarify how the interplay between plants and pollinators determines plant fitness components, besides selfing rates and review how models of the evolution of selfing rates implicitly or explicitly integrate plant-pollinator interactions.

Constraints imposed by pollinators on plant fitness components and the evolution of plant selfing rates

The fitness of a plant is determined by the number of outcrossed and selfed seeds it produces and the number of pollen grains it successfully exports to other plants (male outcrossed siring success), weighted by the relative offspring fitnesses. Hence, the fitness of animal-pollinated plants depends critically on pollinators, also via resource allocation to attraction traits, and on inbreeding depression (see Glossary, Lloyd, 1979; Lande & Schemske, 1985; Charlesworth & Willis, 2009).

Two major phenomena involving pollinators and influencing plant fitness components are often included in studies of the evolution of plant selfing rates without the explicit modelling of pollinators (reviewed in Goodwillie *et al.*, 2005): pollen limitation and pollen discounting (see Glossary). Pollen limitation is a key component favouring the evolution of higher selfing rates (Cheptou, 2004; Porcher & Lande, 2005b) and depends greatly on pollinator abundance (Ashman *et al.*, 2004; Eckert *et al.*, 2010; Thomann *et al.*, 2013). Several authors have proposed that stronger outcross pollen limitation can mimic declines in pollinator density and hamper the evolution of complete outcrossing (Sakai & Ishii, 1999; Masuda *et al.*, 2001; Morgan & Wilson, 2005). Pollen discounting, a negative relationship between selfing rate and pollen export, can be caused by pollinators transferring large amounts of self-pollen among multiple flowers of plants, which are therefore lost for outcrossing (Karron & Mitchell, 2012), and hinders the evolution of high selfing rates (Goodwillie *et al.*, 2005).

More generally, correlations or functional relationships among fitness components are important drivers of the evolution of plant mating systems. Several models show that the maintenance of mixed mating can result from relationships between male fertility, female self fertility and female outcross fertility (including pollen discounting, Johnston *et al.*, 2009), between viability and selfing rate (Jordan & Otto, 2012), or between selfing and a cost of interaction with pollinators (Lepers *et al.*, unpublished manuscript). Correlations among plant fitness components are partly governed by pollinators, particularly by their foraging behaviour within plants in response to floral traits (e.g. floral display, Best & Bierzychudek, 1982). Yet, models that use these correlations without integrating pollinators explicitly are useful to address the effects of pollinators on selfing rates and have helped understand the qualitative role of pollination in the evolution of plant mating. Assuming a positive correlation between the number of selfed and outcrossed ovules is relevant for many animal-pollinated species in which more pollinator visits increase both geitonogamous self-pollination and the number of outcrossed seeds (Johnston *et al.*, 2009 and references therein). Similarly, a negative correlation between reward production and selfing rate (as in Lepers *et al.*, unpublished manuscript), or between the production of costly open (vs. closed) flowers and selfing rate (as in Masuda *et al.*, 2001; data in Oakley *et al.*, 2007) can be used to understand the evolution of selfing syndromes (see Glossary; reviewed by Sicard & Lenhard, 2011).

Further progress towards more reliable, quantitative predictions of equilibrium mating systems requires mechanistic models of the constraints that pollinator behaviour imposes on plant selfing rates, which are still few. Morgan *et al.* (2005) used optimal foraging theory to model evolution of selfing by assuming a decreasing rate of geitonogamous selfing with increasing plant density, which was justified because pollinators are more likely to switch between plants when flight distances are smaller (Cresswell, 1997; Mustajarvi *et al.*, 2001). Another approach has included the demography of plant and pollinator populations, highlighting the possibility of demographic extinction of pollinator and plant populations during the transition to higher selfing rates (Lepers *et al.*, unpublished manuscript), due to reduced production of rewards for pollinators. The most comprehensive mechanistic models tackle the evolution of floral traits influencing pollinator behaviour, and therefore plant selfing rates. For example, models that jointly describe the evolution of daily floral display and pollinator foraging behaviour show that pollinators can generate stable intermediate geitonogamous selfing rates (De Jong *et al.*, 1992; Masuda *et al.*, 2001; Devaux *et al.*, unpublished manuscript). Similarly, models of the evolution of nectar content have analysed how the production of rewardless flowers can decrease the geitonogamous selfing rate of individual plants (Bailey *et al.*, 2007) and conversely how pollinator learning can increase the geitonogamous selfing rates of rewarding plants that co-occur with nonrewarding plants (Ferdy & Smithson, 2002).

Models combining pollinator foraging and the evolution of floral traits are promising tools to study the ecological drivers of plant mating system evolution, but they can still be improved. The number of flowers probed, hence the geitonogamous selfing rate of self-compatible hermaphrodite species, critically depends on pollinator foraging behaviour, but the latter is simplified in existing models: pollinators are assumed to visit all flowers on a plant (De Jong *et al.*, 1992), the number of pollinator visits per plant is assumed proportional to floral display (Masuda *et al.*, 2001, 2004), the probability to leave a plant is assumed unrelated to floral display (Devaux *et al.*, unpublished manuscript), and pollinators are assumed to leave a plant immediately after visiting a rewardless flower (Bailey *et al.*, 2007); these assumptions are at odds with empirical observations (Robertson, 1992; Duan *et al.*, 2005; Ishii & Harder, 2006). Such assumptions are unavoidable, and highlight the difficulty of including realistic but sufficiently general models of pollination ecology in models of the evolution of plant selfing rates (but see Ferdy & Smithson, 2002 for a model incorporating pollinator learning).

The interplay between pollinators and the dynamics of inbreeding depression determines the evolution of plant selfing rates

Inbreeding depression (see Glossary and Box 2), the relative fitness of selfed vs. outcrossed offspring, is a central evolutionary force that has received much attention in the population genetics approach to studying plant mating system evolution (reviewed in

Charlesworth & Willis, 2009). The joint evolution of inbreeding depression and plant mating systems, that is, the purging of deleterious mutations with increasing selfing rates, has been extensively studied both experimentally (Husband & Schemske, 1996) and theoretically, but has rarely been included in ecological models of plant mating system evolution. Allowing for an evolving rather than a constant inbreeding depression in models of pollination ecology can nevertheless greatly alter the outcomes of models of the evolution of selfing. First, conditions favouring the maintenance of outcrossing are much more restricted in the presence of evolving rather than constant inbreeding depression (Porcher & Lande, 2005a,b). Second, purging creates a strong positive feedback on the evolution of selfing: an increase in the population selfing rate decreases inbreeding depression (Fig. 3), thereby strongly favouring the further evolution of increased selfing. This can destabilize equilibria that appear evolutionarily stable in models where inbreeding depression does not depend on the selfing rate. Porcher *et al.* (2009) demonstrated that incorporating a genetic model for inbreeding depression and the possibility of purging destabilizes intermediate selfing rates that would otherwise be maintained by temporal variation in inbreeding depression (Cheptou & Schoen, 2002).

The joint evolutionary dynamics of inbreeding depression and plant mating system are often overlooked in ecologically oriented models because their analysis requires complex genetic models over and above the complexity of ecological processes. A method based on an approximation for the purging of inbreeding depression (e.g. Lande *et al.*, 1994) provides a powerful way around this complexity (Box 2), on the condition that genomic mutation rates to deleterious alleles causing inbreeding depression remains moderate (Box 2, Porcher & Lande, 2013). This approximation has been used in some ecological models to study the joint role of ecological and genetic constraints in plant-mating system evolution (Johnston, 1998; Devaux *et al.*, unpublished manuscript; Lepers *et al.*, unpublished manuscript). These models show that ecological and genetic mechanisms interact strongly to determine evolutionary outcomes. For example, Devaux *et al.* (unpublished manuscript) identified two types of equilibrium selfing rates when modelling the evolution of floral display and geitonogamous selfing under pollina-

Box 2: An approximation for evolutionary equilibria of plant selfing rates: how to include the purging of inbreeding depression into ecological models of the evolution of selfing

The evolutionary dynamics of inbreeding depression greatly influence plant mating system evolution and should not be overlooked. The joint evolution of inbreeding depression and selfing rates can be modelled using detailed genetic models of inbreeding depression, which has rarely been carried out in ecological theoretical studies (but see Porcher & Lande, 2005a,b; Porcher *et al.*, 2009) because it requires complex models and long computation time. Alternatively, an approximation assuming that plant selfing rates evolve by small mutational steps allows modelling the dynamics of purging without a full genetic model for inbreeding depression (Lande *et al.*, 1994; Johnston, 1998). This approximation amounts to letting the level of inbreeding depression vary with the selfing rate and finding joint equilibria of the mating system and inbreeding depression, instead of assuming constant inbreeding depression (see Porcher & Lande, 2013 for more details). A numerical or analytical relationship between inbreeding depression and population selfing rate can be obtained from any genetic model (e.g. Kondrashov, 1985; Charlesworth *et al.*, 1990). Analytical relationships are derived from polynomial regressions (Johnston, 1998; Lepers *et al.*, unpublished manuscript) with relatively simple models of inbreeding depression (e.g. based on a single locus, Charlesworth *et al.*, 1990).

A change in the selfing rate modifies inbreeding depression, but also other components of plant fitness, via the automatic advantage of selfing (Fisher, 1941), reproductive assurance in pollen-limited environment, or pollen discounting. The approximation examines the indirect selection gradient on small changes in the selfing rate to find joint equilibria of the mating system and inbreeding depression, which occur at the intersection of the inbreeding depression function and a constraint function (Fig. 3). This constraint function summarizes all other drivers of the evolution of the selfing rate, particularly ecological mechanisms, some of which are governed by pollinators and their behaviour (Porcher & Lande, 2005b; Devaux *et al.*, unpublished manuscript; Lepers *et al.*, unpublished manuscript). Singular strategies (selfing rates) correspond to fitness maxima or minima, which can be distinguished from the sign of the second partial derivative with respect to selfing rate at this strategy. Graphically, the singular strategy is evolutionarily stable (i.e. a maximum) if the constraint function is smaller (respectively larger) than inbreeding depression when the equilibrium selfing rate is increased (respectively decreased; Fig. 3).

The approximation ignores genotypic association among the loci controlling the selfing rates and those controlling inbreeding depression and is therefore accurate only for moderate, but biologically realistic, genomic mutation rates to deleterious alleles causing inbreeding depression ($U < 0.2$, Porcher & Lande, 2013). For larger mutation rates, differential purging occurs between genotypes with different selfing rates, a phenomenon that is not accounted for in the approximation, which thus becomes inaccurate. Large U may be found in perennial plants (see Porcher & Lande, 2013) and may better account for the observed similar levels of inbreeding depression in completely outcrossing and mixed-mating populations (Winn *et al.*, 2011).

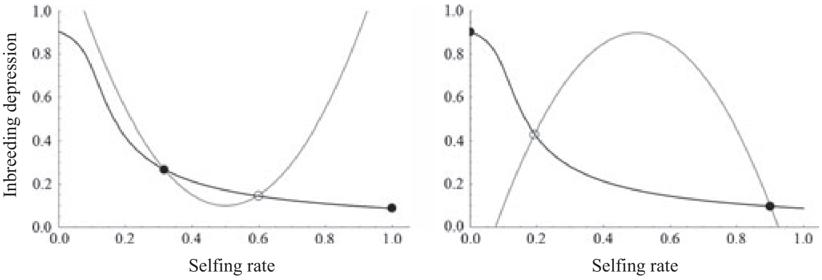

Fig. 3 Evolutionarily stable (closed circles) and unstable (open circles) selfing rates found at the intersection of the inbreeding depression curve (black line) and an ecological constraint function on plant fitness components (grey line).

tor constraints: (i) ecological equilibria constrained by pollinator behaviour only, which exist regardless of inbreeding depression, and (ii) evolutionarily stable equilibria maintained by a trade-off between pollinator attraction and avoidance of geitonogamous selfing and governed by evolving inbreeding depression. This model also suggests that a baseline rate of unavoidable geitonogamous selfing constrained by pollinators can trigger purging of inbreeding depression and create conditions favouring evolution of increased autonomous selfing. In view of this interaction between ecological and genetic mechanisms, we encourage modellers interested in the ecological drivers of plant mating system evolution to account for evolving inbreeding depression, which can be easily performed using approximation methods (Box 2).

Experimental limits to theoretical models

No model has yet analysed the joint evolution of plant selfing rates and pollinator characteristics: the morphologies and foraging behaviours of pollinators are generally assumed to be constant (but plastic) functions of floral signals. Univariate pollinator-mediated selection on multiple floral traits and their genetic architecture are well documented (Karron *et al.*, 1997; Kingsolver *et al.*, 2001; Geber & Griffen, 2003; Ashman & Majetic, 2006; Galliot *et al.*, 2006). Several papers have shown that floral traits could adapt rapidly (Ashman *et al.*, 2004; Thomann *et al.*, 2013), and a recent review has suggested that plants could adapt more rapidly to pollinator-mediated selection than pollinators do to floral traits, which can explain why we frequently observe pollination syndromes and floral convergence in plants (Schiestl & Johnson, 2013 and references therein). Thus, the rarity of coevolutionary models could reflect the lack of data on traits and behaviour of pollinators and their adaptive potential, although a few models of coevolution of plant and pollinator traits do exist (Kiester *et al.*, 1984; Zhang *et al.*, 2013), but do not address the evolution of plant mating.

Modelling the joint evolution of plant mating and pollinator traits would require the following experimental steps: (i) for plants, estimation of facilitated self-pollination and elucidation of pollinator characteristics and behaviour involved (see Box 1 for a method); (ii) for plants again, estimation of the multivariate selection induced by pollinators on several floral traits simultaneously, as both direct and indirect (correlated) selection is responsible for the observed phenotypic distribution of floral traits; (iii) for pollinators, accurate description of the genetic architecture (number of and correlation among genes) and the selection gradients on morphological and behavioural traits imposed by plants or their competitors; and (iv) estimation of the adaptive potential of both floral and pollinator traits.

The reciprocal contribution of plant-pollinator networks to plant selfing rates

The interactions between plants and pollinators determine immediate ecological self-pollination rates, as well as the evolution of plant selfing rates. Most studies, both experimental and theoretical, address this topic by focusing on pairs of interacting species, whereas plants and their pollinators are part of complex interaction networks (Bascompte *et al.*, 2003; Strauss & Irwin, 2004; Pocock *et al.*, 2012), which should influence plant mating systems and their evolution. The combination of estimates from independent populations scattered across the globe shows a U-shaped distribution of plant selfing rates, with a strong bias towards highly outcrossing species and numerous species with intermediate selfing rates (Goodwillie *et al.*, 2005; Igic & Kohn, 2006). Whether this distribution is representative of local plant communities is debatable, nevertheless it is likely that selfing rates exhibit interspecific variation within communities. In the following, we argue that (i) in a network, the distribution of selfing rates of plant species that are not completely outcrossing or completely selfing can be explained partly by the multispecies interactions operating at an ecological time scale (Fig. 1), and (ii) conversely, on longer time scales, the evolution of plant mating systems, particularly the transition to higher selfing, can constrain the architecture of plant-pollinator networks.

The influence of the architecture of mutualistic networks on plant ecological selfing rates and their evolution

Here, we review the scarce available data and make predictions about the expected impact of multispecies interactions on plant mating. We also point out the data that are needed to test these predictions and better understand community-level effects on plant selfing rates.

Plant-pollinator networks are shaped by pollinator optimal foraging and morphological or phenological matching between partners (e.g. Junker *et al.*, 2013). Within a given network, plants differ in the identity of their pollinators, which is a primary mechanism leading to differences in selfing rates in a plant community: different pollinator species have different foraging behaviour and pollen transfer efficiencies and therefore contribute to variation in selfing rates (Morinaga *et al.*, 2003; Brunet & Sweet, 2006; Matsuki *et al.*, 2008; but see Eckert, 2002). Mutualistic interaction networks are also characterized by their nestedness (see Glossary), which implies asymmetric relationships between plant and pollinator species: specialist plant (respectively pollinator) species interact (more often) with generalist pollinator (respectively plant) species (Bascompte *et al.*, 2003; Thébault & Fontaine, 2010). This architecture determines the level of interferences among specialist plant species because of shared (generalist) pollinators, and among specialist pollinator species because of shared (generalist) plant species. The combination of all interference components determines the number of pollinator visits per plant and the quantity of heterospecific, outcross and self-pollen deposited per pollinator visit, hence immediate ecological selfing rates (Wilcock & Neiland, 2002; Vanbergen *et al.*, 2014).

In pollinator-sharing plant species, the rate of heterospecific vs. conspecific outcross pollen transfer is governed by the floral constancy of pollinators (Ashman & Arceo-Gomez, 2013 and see Glossary). Within-bout floral constancy of individual pollinators is likely to maintain high outcrossing rates by increasing the relative amount of conspecific vs. heterospecific pollen deposited on stigmas. We are unaware of any experimental study of the relationship between floral constancy and selfing rates, but floral constancy is influenced by the structure of both plant and pollinator communities, which should contribute to variation in the selfing rate of a given plant species among communities. Similarities in floral traits among plant species of a community tend to lower floral constancy: De Jager *et al.* (2011) observed higher probabilities of pollinators switching between co-occurring *Oxalis* species of similar colour. Conversely, Gegear & Laverty (2001, 2005) predicted and verified experimentally that the diversity of plant species in a community increases floral constancy, particularly when plant species differ in multiple floral traits. Interference among pollinators in a community can also alter floral constancy, and thus the amount of outcross pollination. For example, experimental removal of an abundant pollinator caused weaker interspecific competition for food resources among the remaining pollinator species, which decreased their floral constancy, thus plant seed set, in natural populations of *Delphinium barbeyi* (Brosi & Briggs, 2013); the consequences on selfing rates were, however, not examined.

In addition to heterospecific pollen transfer, interference among (specialist) plant species sharing pollinators also impacts pollinator visitation rates, with two contrasting patterns: (i) decreased visitation rates (competition), caused by a combination of higher density of competitor plants and higher pollinator preference for competitor plants (Rathcke, 1983; Vamosi *et al.*, 2006; Flanagan *et al.*, 2011), and (ii) increased visitation rates (facilitation), resulting from more effective attraction cues, maintenance of larger populations of pollinators (Sargent & Ackerly, 2008; Liao *et al.*, 2011), or availability of complementary resources for pollinators (Ghazoul, 2006). Competitive and facilitative interactions influence selfing rates because they control the amount of outcross pollen deposited on stigmas (Vamosi *et al.*, 2006; Alonso *et al.*, 2010). Which of these predominates among plant species likely depends on phylogenetic distances among species (facilitation is more likely among closely related species, Moeller, 2004; Schuett & Vamosi, 2010; Sargent *et al.*, 2011) and the overlapping of population flowering phenologies within (Fründ *et al.*, 2011) and among days (Motten, 1986; Devaux & Lande, 2009; Runquist, 2013).

Predicting how heterospecific pollen transfer and competition for pollinator visitation jointly impact plant mating is straightforward: both mechanisms cause conspecific outcross pollen limitation, which should be associated with increased selfing. Only a couple of studies have demonstrated increased (ecological) selfing rates due to competition for pollinators: in *Mimulus ringens* (Bell *et al.*, 2005) and *Laguncularia racemosa* (Landry, 2013). At broader time scales, highly selfing populations of *Arenaria uniflora* are thought to have evolved to avoid competition with *A. glabra* (Fishman & Wyatt, 1999). In contrast, the effect of pollinator sharing on selfing rates in plant species with facilitative interactions is less intuitive, because heterospecific pollen transfer and increased pollinator visitation rates should compensate one another. In *Clarkia* communities characterized by facilitative interactions, increased autonomous selfing is selected for under low plant species diversity (Moeller & Geber, 2005), which suggests weak outcross pollen limitation and limited impacts of heterospecific pollen transfer in highly diverse plant communities. The negative effects of heterospecific pollen transfer can be avoided by increased floral constancy of pollinators (Gegear & Laverty, 2005), separate pollen placement on pollinator bodies (Waser, 1978; Huang & Shi, 2013 and references therein) or higher tolerance to heterospecific pollen transfer (Ashman & Arceo-Gomez, 2013).

Finally, we may predict lower selfing rates on average for generalist than for specialist plant species for two reasons. First, generalist plant species should receive more independent visits by pollinators, which should deposit larger amounts of outcross pollen. In a nested network, a significant fraction of this outcross pollen may, however, be heterospecific, because generalist plant species are visited by specialist as well as generalist pollinators. Fang & Huang (2013) for example observed higher rates of heterospecific pollen transfer in generalist vs. specialist plants. Heterospecific pollen transfers may explain why the general negative relationship between pollinator diversity and outcross pollen limitation remains weak (Davila *et al.*, 2012). Second, interference among pollinators can alter pollinator behaviour within plants, thereby influencing selfing rates via geitonogamous pollen transfer. Optimal foraging selects for pollinators that leave plants before interacting with competitors and predators. Hence, generalist plant species could exhibit lower geitonogamous selfing rates because interference among multiple pollinator species is likely to reduce the number of flowers each pollinator probes on a plant. One experimental study provides indirect support for this phenomenon: Greenleaf & Kremen (2006) showed that in sunflower (*Helianthus annuus*) fields containing male-fertile and male-sterile individuals, honey bees that interacted with wild bees on male-sterile plants flowers were more likely to switch to a male-fertile plant than when they interacted with a conspecific, thereby enhancing pollen transfer among individuals. Further investigation is needed to confirm or rule out the prediction of larger outcrossing rates in generalist vs. specialist plant species, for which at present there is little direct experimental evidence.

There is thus mounting evidence that the architecture of plant-pollinator networks can influence plant selfing rates and their evolution (Fig. 1). Experimental data are, however, needed to explore the relationship between selfing rates and network architecture, particularly plant specialization to pollinators (see Glossary), and broaden our understanding of the underlying mechanisms. Particularly, quantified plant-pollinator networks are crucial for an accurate assessment of plant specialization (Ings *et al.*, 2009). Note, however, that most networks are based on visitation data, which may not be representative of actual pollen transfer networks (Alarcón, 2010; King *et al.*, 2013). We also need reliable marker-based estimates of selfing rates for plant species of the same network (David *et al.*, 2007), to document the community-level distribution of plant selfing rates, now only available in different ecological contexts (Goodwillie *et al.*, 2005). As a first step, selfing rates could be regressed on specialization (number of visiting pollinator species) across all plant species of a given plant-pollinator network at a given time. Alternatively, analyses could focus on a single or a few plant species and make use of the documented spatial or temporal variation in mutualistic networks. For example, Vanbergen *et al.* (2014) estimated the selfing rate of *Cirsium palustre* and characterized plant-pollinator networks across a gradient of grazing intensity. They observed higher selfing rates, associated with less densely connected networks, in ungrazed vs. intensively grazed habitats. However, for a given grazing intensity, selfing rates were positively related to network connectance. No general conclusion can be drawn from this single study with conflicting patterns, but the work of Vanbergen *et al.* (2014) does confirm that the architecture of plant-pollinator networks impacts plant selfing rates.

Finally, studies of pollinator floral constancy are still rare: existing data deal with the specialization of pollinator species only, whereas floral constancy is defined at the individual level. Floral constancy and specialization can overlap (a pollinator species specialized on a single plant species can only be constant), but remain distinct features of pollinator behaviour (a generalist pollinator can be or not constant within a foraging bout, see Glossary). Hence, we believe that pollinator specialization is informative to study the dynamics of plant-pollinator networks, but not to understand realized pollination patterns at the network level. Estimates of floral constancy should ideally quantify the probability of pollinator switching, and go beyond binomial classifications (in/fidelity, as in Brosi & Briggs, 2013).

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

Evolutionary changes in selfing rates, particularly autonomous selfing, may in the short-term involve minor modifications of floral traits (e.g. herkogamy, Webb & Lloyd, 1986), and have little consequence for pollinator visitation rates and patterns. In the long term, however, they can induce more conspicuous changes and dramatically reduce pollinator visits, for example, via a decrease in floral size, nectar and scent production, or pollen/ovule ratio (Sicard & Lenhard, 2011), which could eventually alter the architecture of plant-pollinator networks. Few studies so far have examined how plant mating systems shape plant-pollinator networks, with the exception of Ollerton *et al.* (2006) and Davila *et al.* (2012), who found no difference in plant specialization between self-compatible and self-incompatible species (a qualitative approach). Yet, comparing self-compatible and self-incompatible species may not be appropriate to detect a relationship between plant specialization and selfing rate, because self-compatible species exhibit a wide range of selfing rates, from complete outcrossing to complete selfing (Goodwillie *et al.*, 2005).

We present here a preliminary analysis that corroborates our prediction that predominantly selfing species should be visited by fewer pollinator species than predominantly outcrossing species. For this analysis, we brought together data on plant mating system from the Biolflor database (Klotz *et al.*, 2002) with three

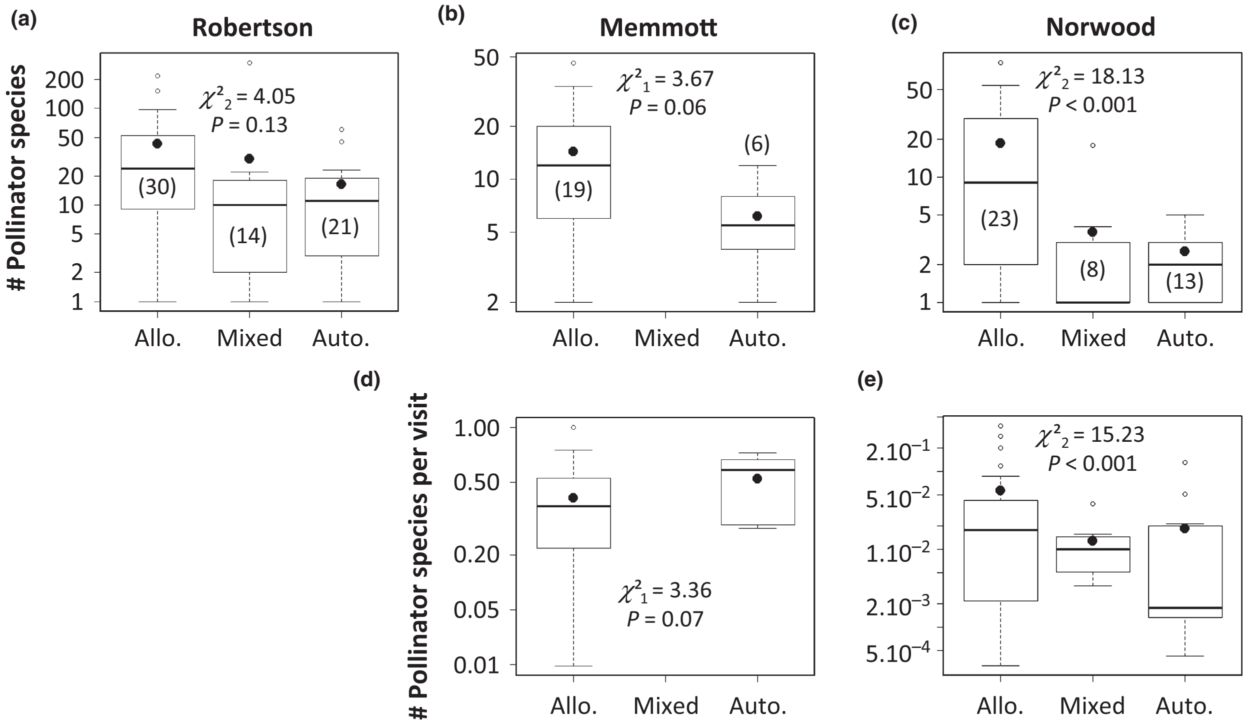

Fig. 4 Relationship between mating system and plant specialization in three plant-pollinator networks. Box plots (with whiskers representing 1.5× interquartile) and mean (closed circles) of the number of pollinator species per plant (a–c) and number of pollinator species per visit (d, e). Mating systems were obtained from the Biolflor database and divided into three classes to obtain balanced and sufficient sample sizes: allogamous ('Allo.', comprising 'allogamous' and 'facultative allogamous' species of the database), mixed mating ('Mixed'), and autogamous ('Auto.', comprising both 'facultative autogamous' and 'autogamous' species of the database). *P*-values for the 'mating system' effects were obtained by analysing the variation in pollinator richness (either per plant or per visit), assuming it follows a quasipoisson distribution, as a function of the mating system alone (a–c) or the mating system, the number of visits and their interaction (d,e).

published networks providing qualitative or quantitative plant-pollinator interactions: a forest understory (Robertson, 1929), a meadow (Memmott, 1999) and the Norwood farm (Pocock *et al.*, 2012) networks. The same tendency of fewer pollinator species on predominantly selfing plant species is observed across the three networks (Fig. 4), although differences among the mating system classes are not all significant. A higher diversity of pollinator species on outcrossing species can result from two nonexclusive phenomena: a lower visitation rate to highly selfing species, which is mechanically associated with fewer pollinator species via a sampling effect, and a higher 'true' specialization of selfing plant species.

This example emphasizes the need for quantified interaction networks, to separate the contributions to plant specialization due to overall pollinator (or plant) abundance vs. diversity of visiting pollinator species. Several authors (e.g. Blüthgen *et al.*, 2007) have stressed the influence of plant or pollinator abundance on the measurement of specialization, but specialization is still frequently measured as a number of species, so that highly selfing plant species receiving few visits may be mistaken for extremely specialist species. In the two quantified networks available here, the number of pollinator species was always strongly and significantly correlated with the total number of visits on a plant (not shown). There was also a tendency, although not significant, for fewer visits to highly selfing plant species vs. highly outcrossing plant species. When controlling for the effect of the number of visits a residual effect of mating system on the number of pollinator species remained, but only in the Norwood network for which predominantly outcrossing plant species were visited by a higher diversity of pollinators.

This analysis has several shortcomings (crude classification of plant mating systems, single trait approach ignoring correlates of mating systems that may also influence plant-pollinator interactions, species considered as independent samples, etc.), and does not provide a causal relationship between plant selfing rates and the plant-pollination networks. However, it offers new research directions, both theoretical and experimental, to understand the contribution of plant mating systems to the architecture of plant-pollinator networks.

Concluding remarks

The study of plant mating systems in the context of plant-pollinator networks is still in its infancy, but there is already conclusive evidence that ecological plant selfing rates and their evolution are shaped by the conflict of interest between plants and their pollinators, and by interactions between pollinator behaviour and plant mating systems at the network level. The relative contribution of such ecological constraints vs. genetic drivers to the evolution of plant mating systems, as well as the role of temporal variation in plant-pollinator networks, remains largely unknown and should be explored both empirically and theoretically. Regardless of what drives the evolution of plant selfing rates, it is associated with changes in floral traits, as is the case in the selfing syndrome: the consequences of plant mating system evolution on the architecture of plant-pollinator networks is another topic that warrants further investigation.

Acknowledgments

We thank Colin Fontaine and Russell Lande for insightful discussions and suggestions, François Ory for drawings used in Fig. 1, as well as two anonymous reviewers who helped improve an earlier version of the manuscript. This work was supported by a Region Nord-Pas-de-Calais grant and the ANR "Trans" grant #ANR-11-BSV7-013-03 to C.L, by the French CNRS program PICS grant #5273 to E.P. and by a grant from the Balzan Foundation.

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Glossary

Floral constancy (or fidelity) refers to the propensity for an individual pollinator to visit flowers of a single floral type (hence one or a few plant species) within a foraging bout. It can be measured by the number of transitions among floral types visited within a bout (Waser, 1986; Chittka *et al.*, 1999). Constancy is widespread across pollinator species and is explained, at least partially, by the limited short-term memory of pollinators for visual and olfactory cues and by their limited motor learning for handling flowers (Chittka *et al.*, 1999).

Floral display is the total number of flowers that are open simultaneously on a plant on a given day. Note that floral display sometimes also includes floral size (e.g. Goodwillie *et al.*, 2010), despite an existing trade-off between number and size of flowers (Sargent *et al.*, 2007).

A **foraging bout** is the time a pollinator spends visiting flowers during a single sequence, between the moment it leaves its nest and the moment it returns to it.

Inbreeding depression is the relative decrease in fitness of selfed vs. outcrossed progeny, caused mostly by a combination of highly deleterious, nearly recessive alleles and mildly deleterious, nearly additive alleles. Recessive deleterious mutations are more likely to be eliminated by natural selection (purging) in inbred homozygotes, which creates a negative relationship between inbreeding depression and the population selfing rate (Lande & Schemske, 1985; see Box 2).

Nestedness characterizes networks with many specialist species and few extremely generalist species, as well as asymmetric specialization (specialist species tend to interact with generalist species).

Pollen carryover is the extent to which pollen collected on a flower is transported and deposited on stigmas of other flowers (of the same plant or different plants) during a foraging bout; it depends on both the pollen uptake rate and pollen deposition rate (De Jong *et al.*, 1993).

Pollen discounting is the reduction in outcrossed male siring success associated with an increase in selfing rate, due to decreased amounts of exported pollen (Nagylaki, 1976).

Pollen limitation is the reduction in plant reproductive success (fruit or seed set) due to inadequate quantity or quality in pollen receipt; it is usually tested for through supplementary pollination (see Knight *et al.*, 2005 for a review).

Pollinator preference is the propensity for an individual pollinator to visit a plant species disproportionately to the availability of resources this species provides (Cock, 1978); it is usually measured as the number of visits per plant relative to other plant species.

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

Selfing rate is the rate of self-fertilization, the fraction of selfed vs. outcrossed embryos produced by an individual plant. It is defined at fertilization (primary selfing rate) but usually measured at a later stage (in seeds, seedlings or even adults, secondary selfing rate). Primary and secondary selfing rates can differ from the self-pollination rate due to (i) self-incompatibility, which can be partial or cryptic (e.g. via differences in growth rates of selfed vs. outcrossed pollen tubes) and (ii) post-fertilization selection processes, for example selective flower abscission, fruit abortion or inbreeding depression.

Selfing and outcrossing components of self-compatible animal-pollinated plants with perfect flowers (following Lloyd, 1992). **Autonomous selfing** (a, dotted white lines; left panel of Fig. 2) corresponds to autogamous (within-flower) self-pollination occurring without pollinator visits; it is divided into three modes depending on the timing of outcross- vs. self-pollination: prior, competing (simultaneous), and delayed autonomous selfing. **Facilitated selfing** (f, dashed white lines; left panel of Fig. 2) corresponds to autogamous (within-flower) self-pollination induced by pollinator visits. **Geitonogamous selfing** (g, solid white lines; left panel of Fig. 2) corresponds to self-pollination among flowers induced by pollinators probing several open flowers on the same plant. **Outcross pollination** (o, solid grey lines) refers to pollen deposited on a flower that originates from other plants in the population.

Selfing syndrome is a characteristic set of morphological and functional plant traits that enhance pollen transfer efficiency within flowers and/or decrease pollinator visitation. Selfing syndrome usually includes small flowers, thus reduced anther-stigma distance (herkogamy), reduced petal size, and reduced corolla width (following Sicard & Lenhard, 2011).

Specialization has many definitions but is generally inversely related to the total number of species an individual, a population or a species interacts with. This number can be weighted by the frequency of interactions with each partner species.

Received 14 October 2013; revised 31 January 2014; accepted 20 March 2014

Chapter 3

How does pollination mutualism affect the evolution of prior self-fertilization? A model

ORIGINAL ARTICLE

doi:10.1111/evo.12533

How does pollination mutualism affect the evolution of prior self-fertilization? A model

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Received May 31, 2013
Accepted September 28, 2014

The mode of pollination is often neglected regarding the evolution of selfing. Yet the distribution of mating systems seems to depend on the mode of pollination, and pollinators are likely to interfere with selfing evolution, since they can cause strong selective pressures on floral traits. Most selfing species reduce their investment in reproduction, and display smaller flowers, with less nectar and scents (referred to as selfing syndrome). We model the evolution of prior selfing when it affects both the demography of plants and pollinators and the investment of plants in pollination. Including the selfing syndrome in the model allows to predict several outcomes: plants can evolve either toward complete outcrossing, complete selfing, or to a stable mixed-mating system, even when inbreeding depression is high. We predict that the evolution to high prior selfing could lead to evolutionary suicides, highlighting the importance of merging demography and evolution in models. The consequence of the selfing syndrome on plant–pollinator interactions could be a widespread mechanism driving the evolution of selfing in animal-pollinated taxa.

KEY WORDS: Adaptive Dynamics, demography, inbreeding depression, mixed-mating, pleiotropy, selfing syndrome.

Flowering plants exhibit a great diversity in mating systems, ranging from obligate outcrossing to complete selfing through mixed-mating species (simultaneous selfing and outcrossing). The recurrent transitions from outcrossing to selfing, despite negative effects of selfing on long-term diversification (Igic and Busch 2013; Wright et al. 2013), have motivated numerous empirical and theoretical studies (Goodwillie et al. 2005; Busch and Delph 2012). Seminal studies have investigated how the genetic implications of self-pollination should impact the evolution of selfing. On the one hand, selfers benefit from a 50% transmission advantage of their genome compared to outcrossers, known as the "automatic transmission advantage" (Fisher 1941): selfers transmit two copies of their genes through their own seeds and one copy through pollen export, whereas outcrossers transmit only one copy through their own seeds, and one copy through pollen export. On the other hand, inbreeding depression is assumed to prevent the evolution of selfing because of a reduction of fitness in selfed offspring compared to outcrossed ones (Charlesworth 2006). More recently, theoretical studies have started to consider the impact of several ecological mechanisms of pollination on selfing evolution, such as pollen limitation (Holsinger 1991; Cheptou 2004; Morgan and Wilson 2005), pleiotropic effects of selfing on viability or fertility components (e.g., pollen discounting: Lloyd 1992; Harder and Wilson 1998; Johnston et al. 2009; Jordan and Otto 2012; or simultaneous increase in the number of selfed and outcrossed ovules: Johnston et al. 2009) and indirect effect of floral display on geitonogamous self-pollination (Devaux et al. 2014a). Because they predict that mixed-mating systems can be evolutionarily stable under some conditions, such models allow a better understanding of the observed variation in selfing rates among plant species.

One major feature of plant pollination ecology is the mode of pollination, (i.e., whether the plant is pollinated by animals or abiotically) and this trait apparently shapes the distribution of mating systems, with biotically pollinated species being about twice more likely to exhibit a mixed-mating system than abiotically pollinated ones (Goodwillie et al. 2005). Two nonexclusive explanations could account for this pattern: either some mechanism prevents the evolution of mixed-mating system in abiotically pollinated species, or the evolutionary forces that lead to mixed-mating are more pronounced in biotically pollinated species. Both these explanations imply that the mode of pollination could interfere with the evolution of selfing. Accordingly, at least two characteristics of animal-pollinated species potentially impact the cost/benefit balance of selfing versus outcrossing and should consequently influence the evolution of self-pollination.

First, those species depend on pollinator availability for their reproduction, and low pollinator abundance or predictability should influence the evolution of self-pollination, with for example a rapid evolution of autonomous selfing caused by pollinator exclusion (Bodbyl Roels and Kelly 2011). Second, pollination mediated by animals is known to select for a high investment in attractive floral structures, since pollinators are often attracted by large floral display (Sato and Yahara 1999; Martin 2004; Goodwillie et al. 2010) and by flowers that offer a large amount of reward (Carlson and Harms 2006; Hernandez-Conrique et al. 2007; Keasar et al. 2008). However, such floral traits are likely to be costly for the plants (Ashman and Schoen 1997). For example, nectar production seems to require much energy (Southwick 1984; Pyke 1991), and since its production can be stimulated by its consumption, each pollinator visit should cause a cost to the plant (Castellanos et al. 2002). This cost of pollination is likely to decrease with selfing rate, because selfing species typically show a "selfing syndrome," that is floral modifications, such as a smaller floral display, a lower nectar production or a reduced herkogamy (see Sicard and Lenhard 2011, for a review). Floral modifications associated to selfing syndrome may evolve rapidly with self-pollination, as suggested by Bodbyl Roels and Kelly (2011) who showed an experimental evolution of floral traits affecting herkogamy in a few generations. The observed variation of floral morphology among populations correlated with the ability to self-fertilize (flower size, Elle and Carney 2003) is also consistent with a rapid evolution of such traits. Those modifications could be selected for because they facilitate self-pollen deposition (e.g., reduced herkogamy, Bodbyl Roels and Kelly 2011 or higher reproductive assurance with smaller flowers, Elle and Carney 2003), and/or because they allow a decrease of the cost of pollination. For instance, the cost of a flower that remains closed and produces seeds only through selfing can be twice as low as the cost of an opened flower, since it produces no reward for pollinators and displays reduced attractive structures (Oakley et al. 2007).

Although animal-pollination is likely to interfere with the evolution of selfing, theoretical studies that model mutualistic interactions of pollination usually disregard the ability of plant to self-pollinate (e.g., Fishman and Hadany 2010; Holland and DeAngelis 2010), or are concerned about other features of the interaction (e.g., the response to climate change on plant-pollinator phenologies, Gilman et al. 2012). On the other hand, many models of selfing evolution neglect the mode of pollination and even when theoretical studies consider biotic pollination, they do not explicitly model the population dynamics of pollinators and they do not consider the cost of pollination (De Jong et al. 1992; Morgan et al. 2005).

Coupling the mating system and the pollination process raises several questions. What are the consequences of selfing for the demography of plant and pollinator populations? Is selfing evolution affected by the mutualistic pollination interactions? Can mutualistic pollination interactions explain the over-representation of mixed-mating systems in animal-pollinated taxa compared to abiotically pollinated ones? In the current study, we focus on autonomous selfing (i.e., not mediated by pollinators) that occurs before outcrossing. This is called prior selfing, in contrast with competing or delayed selfing, which occur during and after pollinator visitation, respectively. Prior selfing is indeed the most likely form of selfing to select for a decrease in the cost of pollination, since it reduces the plant reliance on pollinators, whereas competing and delayed selfing are likely to maintain the floral traits that promote outcrossing (Kalisz and Vogler 2003). In a first step, we investigated the impact of prior selfing on the stability and demography of plant-pollinator populations. We propose an unidirectional consumer-resources model (Holland and DeAngelis 2010), that explicitly includes the ability of plants to self-fertilize a fraction of their ovules. We assume selfing to affect both the reproductive output and the cost of the interaction. In a second step, we investigated the impact of plant–pollinator interaction on prior selfing evolution, by performing Adaptive Dynamics analyzes (Geritz et al. 1998; Brannström et al. 2013). In particular, we determined the effect of the reproductive assurance and of the cost of pollination on prior selfing evolution in animal-pollinated plant species.

The Dynamics of Plant and Pollinator Populations

DEMOGRAPHIC MODEL DESCRIPTION

The model explicitly describes a two-species pollination mutualism, in which plants provide some resources (e.g., nectar or pollen) consumed by pollinators, and pollinators provide a nontrophic service to the plants (pollen transfer from one plant to another). We considered this mutualistic interaction as a unidirectional consumer–resources interaction (Holland and DeAngelis 2010), and modeled the benefit for plants as the achievement of their outcrossing. We assumed the plants to devote a part of their ovules to prior selfing (i.e., selfing occurs before outcrossing, within a flower, with no contribution of pollinators). As detailed below, the realized selfing rate is defined as the amount of selfed seeds (which can be lower than the amount of selfed ovules because of inbreeding depression) over the total number of seeds produced (the outcrossed part being possibly lowered through pollen limitation). Thus, for a fixed *amount* of selfed ovules, the *realized selfing rate* can vary, depending on both the inbreeding depression and the achievement of outcrossing through pollinators. We later use the term "prior selfing fraction" as Morgan et al. (2005) to describe the rate of ovules that is self-fertilized, regardless of their fate (i.e., developing into seed vs. dying because of inbreeding depression).

Let P and A denote plant and animal pollinator densities. The population dynamics of plants and pollinators are given by the following system of ordinary differential equations (details of parameters signification and default values are given in Table 1):

$$\begin{cases} \dfrac{dP}{dt} = r\,P\,(s\,(1-\delta) + (1-s)\,R[P,A]) - m\,P\left(1+\dfrac{P}{k}\right) & \\ \qquad -c\,P\,F[s]C[P,A]\,, & \text{(1a)} \\ \dfrac{dA}{dt} = \rho A - \mu A\left(1+\dfrac{A}{K}\right) + \beta\,AF[s]\,B[P]\,. & \text{(1b)} \end{cases}$$

The first term of equation (1a), $rP(s(1-\delta)+(1-s)\,R[P,A])$, represents the reproduction rate of the plant population. Each plant produces ovules at a rate r, and a fixed part s of these is devoted to prior selfing. Inbreeding depression decreases the reproduction rate with selfed offspring dying with probability δ. The remaining fraction $(1-s)$ of ovules is fertilized through outcrossing and thus relies on pollinator visitation. We assumed the pollinator species to be the unique vector for outcrossing pollination, with the number of outcrossed seeds saturating with pollen reception (Morris et al. 2010). We assumed pollen transfer to increase with pollinator density (higher visitation rate), but also with plant density, to depict the need of successive visits of two plant individuals in order to effectively transfer pollen. Hence, we modeled the fertilization of ovules through outcrossing with a Holling type II functional response that depends on the rate of effective plant–pollinator interactions: $R[P,A] = \frac{PA}{h_1+PA}$. Thus, outcrossing reproductive output can be pollen limited, because of low plant or pollinator densities. We assumed no pollen discounting, that is selfing does not affect the outcross siring success. Importantly, because only prior selfing is considered in our model, there is no plastic response by plants to alleviate pollen limitation. We further analyzed the effect of pollen limitation by comparing our model to a modified version where all outcrossed ovules are fertilized, that is where $R[P,A]=1$. The first term of equation (1b), ρA, is the intrinsic reproduction of pollinators. The value of ρ indicates the degree of generalism of pollinators, with $\rho = 0$ depicting specialist pollinators (i.e., the plant species is required for their reproduction), and $\rho \gg 0$ representing generalist pollinators. The second terms of equations (1a) and (1b), $mP(1+\frac{P}{k})$ and $\mu A(1+\frac{A}{K})$ represent the density-dependent self-limitation of plant and pollinator populations, respectively, with m and μ the mortality rates at low densities.

The third terms of equations (1a) and (1b), $c\,P\,F[s]\,C[P,A]$ and $\beta\,A\,F[s]\,B[P]$, represent respectively the cost of pollination for the plant and the benefit for the pollinator. We assumed the benefit of pollination for pollinators to increase with each of their visits on a plant, by using $B[P] = \frac{P}{h_3+P}$, to depict the increasing number of visits one pollinator can realize as a function of plant density. We made the general assumption that the interaction with pollinators causes a cost to the plants, using three different relationships between the pollination cost per plant, and the densities of plants and pollinators: (i) The per capita cost increases with pollinator density and decreases with plant density, using $C[P,A] = \frac{A}{h_2+P}$ (later referred as "increasing cost," after its relationship with pollinator density). Such scenario is expected in plant species that pay a cost when receiving a visit (regardless of the effect on pollination of the visit). For example, the total cost for some nectar-producing species can increase with the number of visits they receive, because nectar production is stimulated by consumption (Castellanos et al. 2002; Ornelas and Lara 2009; Morris et al. 2010). Similarly, for plant species involved in a nursery pollination mutualism, the higher the visitation rate, the higher the number of eggs laid by pollinators in the inflorescences (Pellmyr 1989; Klank et al. 2010). The cost is assumed to decrease with plant density, since pollinators can spread their visits among a higher number of plants and/or switch more frequently among plant individuals when plant density is high (optimal foraging theory, Essenberg 2012), thus depleting nectar from fewer flowers, or laying less eggs on a given plant; (ii) The per capita cost of pollination is independent of plant and pollinator densities, that is $C[P,A] = 1$, later referred as "fixed cost." This kind of relationship seems to fit the cost of attractiveness for some plant species. Floral display production (Andersson 2006) and maintenance (Grison-Pigé et al. 2001) has been shown to be costly, and this cost may be fixed for species that cannot modify their flowering length or their floral display size with the number of visits they receive (Van Doorn 1997); (iii) The per capita cost decreases with pollinator and plant densities, as depicted by $C[P,A] = (1-(A/(h+A)))(1/(h_2+P))$ (later named "decreasing cost"). Such cost can describe the ability of many plant species to increase their floral display or the length of their flowering period when their visitation rate is low (Van Doorn 1997; Harder and Johnson 2005). For such species, the cost of pollination will decrease with increasing pollinator density, since high pollinator density will ensure high visitation rate, thus shortening the flowering length or decreasing the floral display. The cost of pollination is assumed to decrease with increasing plant density, because most pollinator species are more attracted by large patches, thus high plant

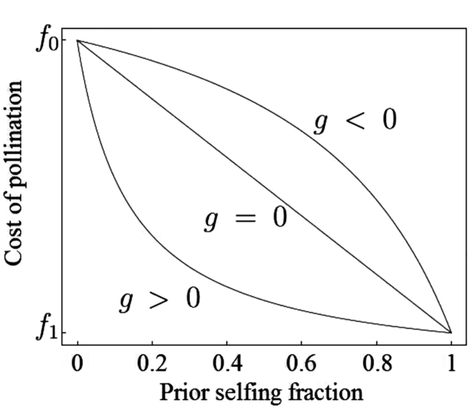

Figure 1. Selfing syndrome shape modeled as concave ($g = -0.7$), linear ($g = 0$), and convex ($g = 7$).

density is likely to enhance pollinator attraction, allowing each plant to decrease its investment in attractiveness (Dauber et al. 2010; Nattero et al. 2011).

Regardless of how the pollination cost varies with densities, we assumed this cost (and the benefit for the pollinators) to be a decreasing function of prior selfing fraction, as expected under a selfing syndrome. We assumed the relationship between the cost of pollination and selfing to follow the function

$$F[s] = \left(s f_1 + \frac{(1-s)\, f_0}{1 + g\, s} \right). \tag{2}$$

In the absence of any empirical data, we chose a general function that allows the notification of the two endpoints: the cost of pollination when plants are complete outcrossers (f_0) and complete selfers (f_1). The selfing syndrome ($F[s]$) can take three possible shapes (Fig. 1): concave ($g < 0$), linear ($g = 0$) or convex ($g > 0$). When assuming the shape to be concave ($g < 0$), the decrease of the cost of pollination is initially weak when the prior selfing fraction is low, and the cost decreases sharply when the prior selfing fraction is high. On the opposite, when assuming the shape to be convex ($g > 0$), the cost of pollination decreases sharply when prior selfing fraction is initially low, and weakly when the prior selfing fraction is high. A linear shape ($g = 0$) depicts a similar decrease in the cost of pollination regardless of the prior selfing fraction.

DEMOGRAPHIC MODEL ANALYSIS

No analytical explicit solutions of the system of equation (1) were tractable and we thus numerically investigated the model. We tested every combination of possible values of prior selfing s and inbreeding depression δ by step of 0.1. Selfing syndrome shape g was modeled as concave ($-0.9 \leq g < 0$, by step of 0.05), linear ($g = 0$) and convex ($0 < g \leq 9$, by step of 0.5). For each set of parameters, we analyzed the stability properties of equilibria and conducted a phase-plane analysis (Otto and Day 2007). Densities of plants and pollinators at equilibrium are noted $\widehat{P}$ and $\widehat{A}$, respectively, and equilibria are noted $\{\widehat{P}, \widehat{A}\}$.

Since plants are assumed to suffer a cost that depends on plant and pollinator densities (except in case of "fixed cost"), the interaction between plants and pollinators can be mutually beneficial (plant and pollinator are truly mutualistic) or beneficial for the pollinators and detrimental for plants if the cost of pollination exceeds the pollination benefit, pollinators thus being parasites. One should note that when assuming no pollen limitation ($R[P, A] = 1$), plant–pollinator interactions are always parasitic, since plants supply reward to the pollinator although receiving no benefit. Such situation is unlikely to be found in nature, and is used here only to highlight the impact of pollen limitation (although it could be adapted to nectar robber insect species, that deplete the nectar from flowers without pollinating it, Richardson 2004). We considered the interaction as mutualistic when a stable equilibrium with both plants and pollinators exists ($\{\widehat{P}, \widehat{A}\}$, $\widehat{P} > 0$, $\widehat{A} > 0$) with higher densities compared with one in the absence of the other species ($\{\widehat{P}, 0\}$ and $\{0, \widehat{A}\}$), regardless of the nature of the interaction during transient phases. The greater the difference of density with/without the other species, the higher the strength of interaction (Holland and DeAngelis 2009).

RESULTS OF THE DEMOGRAPHIC MODEL ANALYSIS

All investigated situations have common features, regardless of the selfing syndrome shape and of the relationship between the pollination cost and plant/pollinator densities. First, for both high inbreeding depression (δ) and high prior selfing (s), plant reproduction is greatly reduced, so that no equilibrium with plants could be found (not shown). In other cases, the model predicts an equilibrium of plant-pollinator coexistence ($\{\widehat{P}, \widehat{A}\}$, $\widehat{P} > 0$, $\widehat{A} > 0$). The demographic properties of this equilibrium mainly depend on the nature of the pollination cost ($C[P, A]$). When assuming a "fixed cost," the equilibrium of plant-pollinator coexistence is globally stable (i.e., any initial densities of plant and pollinator lead to this equilibrium, Fig. 2J–L). On the opposite, when assuming "increasing cost" or "decreasing cost," the fate of the plant population strongly depends on initial plant and pollinator densities when prior selfing fraction is low (Fig. 2A, B, G, H). In both scenarios, for high initial plant density, the cost of pollination per plant is low, so that stable coexistence of plants and pollinators is reached ($\{\widehat{P}, \widehat{A}\}$, $\widehat{P} > 0$, $\widehat{A} > 0$), whereas for low initial plant density, each individual plant suffers a high cost of pollination, resulting in the extinction of plant population ($\{0, \widehat{A}\}$). This threshold depends to a lesser extent on the pollinator density. The relationship between the threshold and the pollinator density can be observed when analyzing the effect of the degree of generalism of pollinators on densities at equilibrium (Fig. 3). A higher

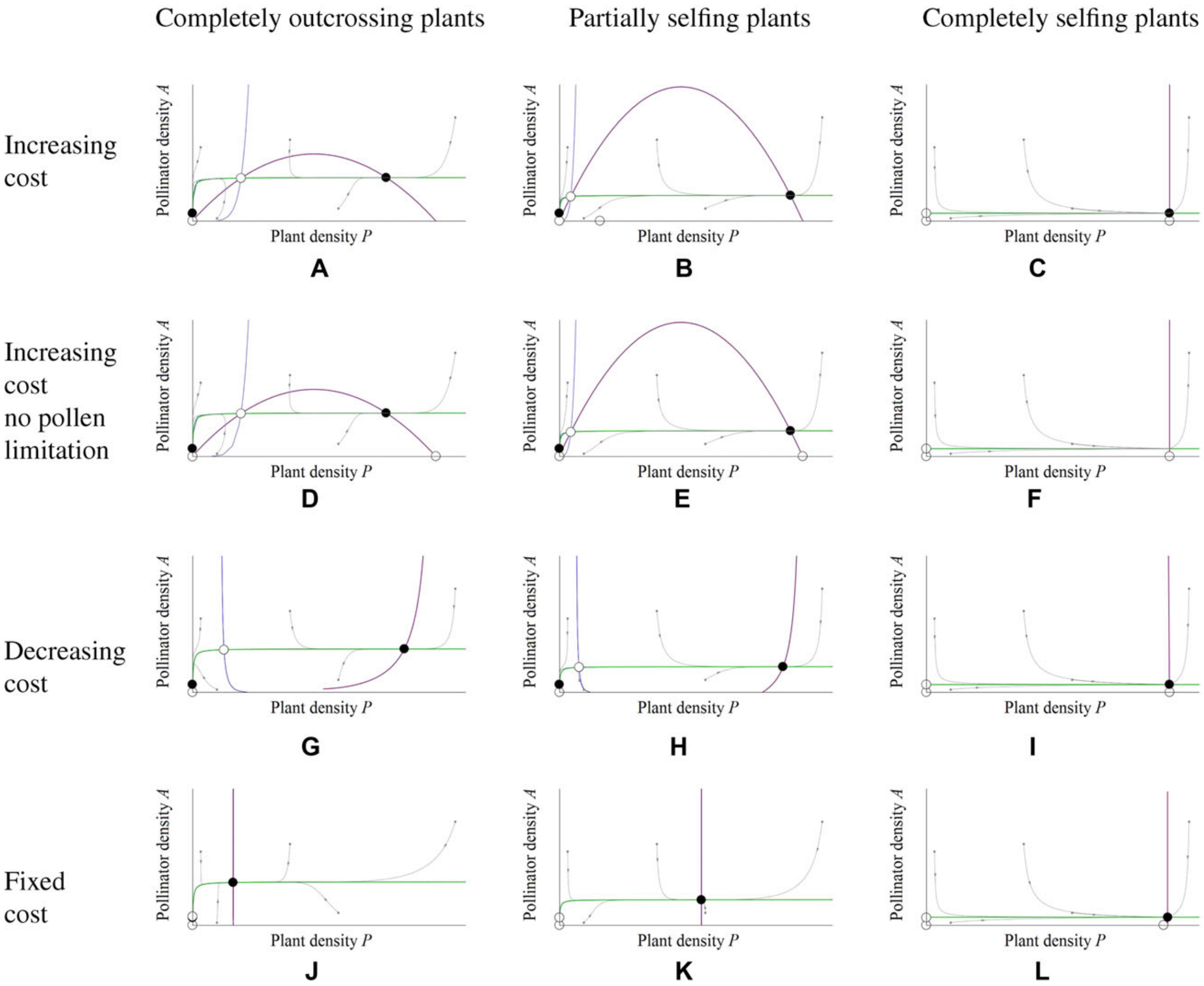

Figure 2. Phase-plane diagrams for the population dynamics of pollination mutualism between a plant species (density P) and a generalist pollinator species ($\rho \gg 0$, density A). The cost of pollination is modeled as: (A–F) increasing with pollinator density and decreasing with plant density ("increasing cost" model); (G–I) decreasing with pollinator and plant densities ("decreasing cost" model); (J–L) Independent of pollinator and plant densities ("fixed cost" model). Figures (D–F) show results with no pollen limitation. Plant species is modeled as a completely outcrossing species in the first column ($s = 0$), a partially outcrossing species in the second column ($s = 0.5$) and a completely selfing species in the third column ($s = 1$). Zero-growth isoclines for P and A are denoted with purple and green lines, respectively. Filled and open black circles represent stable equilibria (stable nodes) and unstable equilibria (saddle points and unstable nodes), respectively. Blue lines represent the separatrix that subdivides the phase-plane space into different basins of attraction when coexistence stable equilibrium is not globally stable. Transient dynamics of some population trajectories (initial conditions represented by filled gray circle) are represented by gray arrows. Parameters have their default values (Table 1), with $\delta = 0.2$, $g = 0$, $\rho = 0.2$. $c = 0.1$ for "increasing cost," $c = 50$ for "decreasing cost," and $c = 0.1$ for "fixed cost." Scales of axes are fixed among diagrams.

degree of generalism implies a higher intrinsic growth rate of the pollinator, hence, for a given plant density (i.e., a given benefit of pollination for pollinator), the higher the pollinator degree of generalism, the higher its density (Fig. 3B, D). When assuming "increasing cost," the threshold below which the plant population goes extinct increases with pollinator degree of generalism, that is with increasing pollinator density and cost of pollination (Fig. 3A), leading to a higher stability of the plant population with specialist pollinators. On the opposite, when assuming "decreasing cost," the threshold decreases with pollinator degree of generalism, because an increase in pollinator density decreases the cost of pollination (Fig. 3C).

Second, the prior selfing fraction strongly affects the demographical properties and the strength of the mutualism, regardless of the relationship between the cost and plant-pollinator densities. When assuming outcrossing to rely on pollinators (i.e., potential

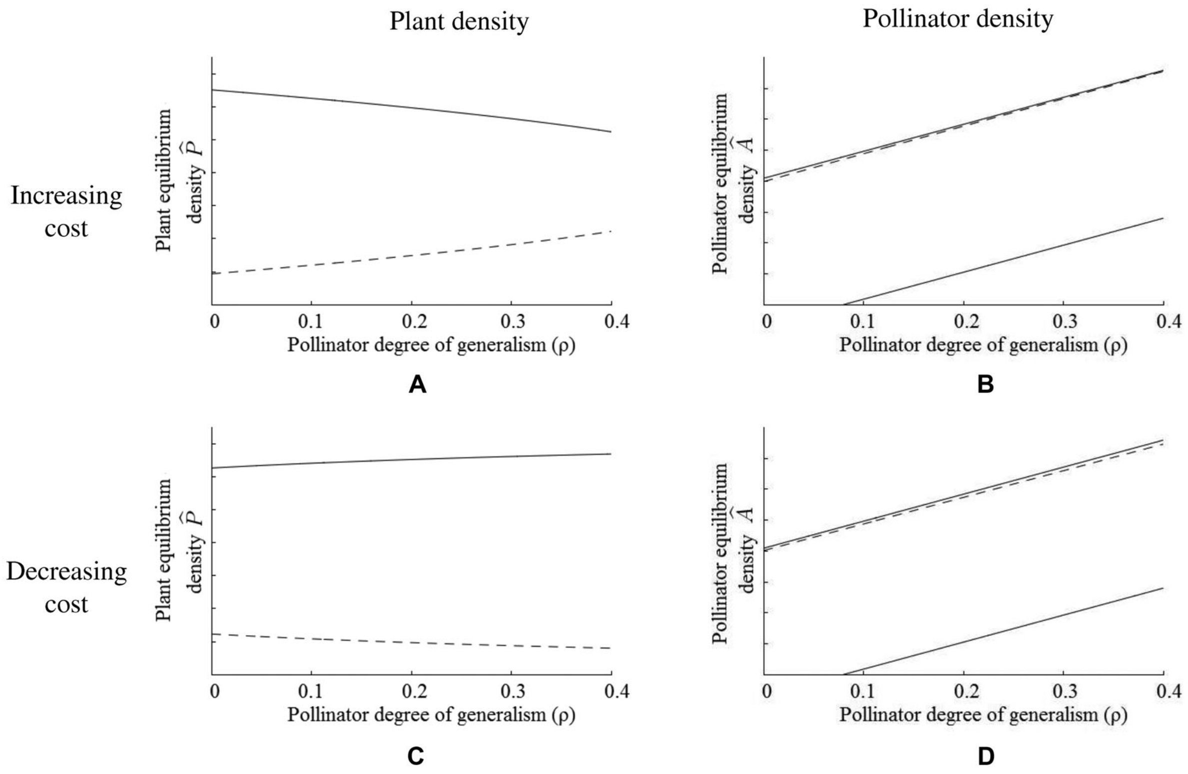

Figure 3. **Density (A, C) of plants and (B, D) pollinators at equilibrium depending on the pollinator degree of generalism (ρ). The cost of pollination is modeled as: (A, B) "increasing cost;" (C, D) "decreasing cost." Each graph includes two plain lines representing the stable equilibria densities and one dashed line representing the densities at the unstable equilibrium. In graphs (A, C) one of the stable equilibria is merged with the x-axis, that is $\widehat{P} = 0$, the plant population is extinct. In those graphs, the unstable equilibrium can be used as an indicator of the plant density threshold that subdivides the phase-plane into two basins of attraction (the separatrix passes through this equilibrium). If plant density is higher than this density, the plant population persists (the upper stable equilibrium) whereas if the plant density is lower than this threshold, the plant population goes extinct (the stable equilibrium merged with the x-axis). In graphs (B, D) the pollinator densities at the upper stable equilibrium and on the unstable equilibrium are close, as shown also in Figure 2.**

pollen limitation), selfing acts as a reproductive assurance in absence of pollinators. Thus, without pollinators ($\widehat{A}$ fixed to 0), populations persist only when plants produce a large number of selfed seeds (i.e., high selfing and low inbreeding depression, see the occurrence of an unstable equilibrium $\{\widehat{P}, 0\}$, $\widehat{P} > 0$ on the x-axis only for nonnull prior selfing, Fig. 2). Such an equilibrium is unstable since the introduction of pollinators in this plant population leads to stable coexistence of plants and pollinators. This advantage of selfing does not exist if one considers that no pollen limitation occurs, so that plant population is stable in absence of pollinators regardless of its prior selfing fraction (Fig. 2D–F). The stability of plant and pollinator population at equilibrium also depends on prior selfing fraction. The higher this fraction, the lower the benefit for pollinators (plant decreases their investment in the interaction with pollinators, because of the selfing syndrome $F[s]$). Thus, the difference in pollinator density with and without plants, that is the strength of the mutualism, decreases (Fig. 2 column 1 vs. 2). From the plant perspective, increasing prior selfing has two opposite effects. On the one hand, it decreases their benefit, since a lower part of their reproduction comes from outcrossing. On the other hand, it also decreases the cost of pollination, so the decrease of mutualism strength for plants depends on the shape and magnitude of the cost decrease. To summarize, an increased prior selfing fraction implies a weaker mutualism, but also leads to more stable plant population. Indeed, an increase in prior selfing fraction allows a decrease in the cost of pollination, which decreases the density threshold under which plant population goes extinct when assuming "increasing cost" or "decreasing cost" (Fig. 2, decrease of the threshold between columns 1, 2, and 3).

Evolution of Prior Selfing

DESCRIPTION OF THE MODEL OF SELFING EVOLUTION

In the previous section, the amount of selfing was considered as a fixed parameter of the model. In a second step, we built a phenotypic model allowing for the evolution of the prior selfing fraction. We considered the appearance of a mutant with prior selfing fraction s', whose density is denoted M, in an initially monomorphic population with prior selfing fraction s (referred later as the resident population). We analyzed this model using the Adaptive Dynamics framework (Geritz et al. 1998; Brännström et al. 2013). This approach assumes a time-scale separation between the appearance of a mutant and the ecological dynamics, that is the appearance of a mutant always occurs once the resident population has reached its demographical equilibrium. Moreover, it considers mutations to be of small effect (i.e., s' is close to s), and the initial behavior of the mutant density is assumed representative of its capacity to invade the resident population. Hence, for the analysis, we considered the mutant density (M) negligible compared to the resident density (P), that is mating only occurs through selfing or with resident partners, and competition occurs only with resident individuals. The initial growth rate of the mutant is described by the following equation (detailed computation is given in Appendix A):

$$\begin{aligned}\frac{dM}{dt} &= r\,M\left(s'(1-\delta) + \frac{(1-s+1-s')}{2}R\big[\widehat{P},\widehat{A}\big]\right)\\ &\quad - m\,M\left(1+\frac{\widehat{P}}{k}\right) - c\,M\,F[s']C\left[\widehat{P},\widehat{A}\right]. \qquad (3)\end{aligned}$$

Parameters and functions have the same definitions and default values than in equation (1) (Table 1). One should note that all selfed offspring are considered as mutants, whereas only half of the outcrossed output has a mutant phenotype, to account for the automatic advantage of selfing (Fisher 1941; Cheptou 2004). As previously, we analyzed the impact of pollen limitation on the evolution of selfing by using a model with no pollen limitation ($R[\widehat{P},\widehat{A}] = 1$). Pollinators are modeled as generalist ($\rho \gg 0$) or specialist ($\rho = 0$) species. The relationship between the cost of pollination and the plant and pollinator densities is modeled as an "increasing cost," a "decreasing cost" or as a "fixed cost," using the same three functions than in the demographic model for $C\big[\widehat{P},\widehat{A}\big]$. For each cost function, the impact of the selfing syndrome shape ($F[s]$) was analyzed by using concave ($-0.9 \leq g < 0$, by step of 0.05), linear ($g = 0$), and convex ($0 < g \leq 9$, by step of 0.5) functions.

We also performed individual-based simulations in order to evaluate the results of the Adaptive Dynamics, allowing strong effect mutations, and the coexistence of several mutants in a same population at the same time. The algorithm is given in Appendix B.

INBREEDING DEPRESSION

Two separate cases were considered regarding inbreeding depression. In the first case, we considered inbreeding depression as a fixed parameter, thus assuming its magnitude to be independent of selfing. For short evolutionary times, the hypothesis that no purging occurs may be correct, as suggested by Jordan and Otto (2012) and is assumed in many models of selfing evolution (see Goodwillie et al. 2005, for a review), including Adaptive Dynamics models (Cheptou 2004; Morgan et al. 2005). Indeed, between closely related species, inbreeding depression does not seem to strongly correlate with selfing rate (Winn et al. 2011). However, selfing species show in average lower inbreeding depression than outcrossing and mixed-mating ones, which is consistent with purging (Winn et al. 2011), and theory predicts a fast decrease of inbreeding depression with the evolution of selfing rate (Charlesworth et al. 1990). Moreover, it has been shown theoretically that this decrease has a strong effect on selfing rate evolution (Porcher and Lande 2005a). In a second case, we thus considered inbreeding depression to vary with selfing. For the sake of simplicity, our model does not integrate an explicit genetic model, but rather polynomial regressions based on the results of Charlesworth et al. (1990). These functions describe the variation of inbreeding depression with selfing rate when inbreeding is caused by deleterious mutations (the values of dominance coefficient, mutation rate, selection coefficient and related function are given in Appendix C). As we assumed a time-scale separation between ecological and evolutionary dynamics, we assumed the time between each mutation to be long enough for purging to occur. Thus, each time a mutant invades the resident population, a new value of inbreeding depression was assigned to the population. We assumed the invasion to be fast enough to consider the inbreeding depression fixed during the invasion phase.

ANALYSIS OF EVOLUTIONARILY STABLE PRIOR SELFING FRACTION

We determined evolutionarily stable prior selfing fraction by using techniques of Adaptive Dynamics (Geritz et al. 1998; Brännström et al. 2013). We first determined the gradient of invasibility of a rare mutant (prior selfing s') in a resident population with prior selfing fraction s, which is depicted by the per capita growth rate of a mutant. The gradient vanishes when the amount of prior selfing is a maximum or a minimum of fitness. We considered such a strategy as a possible evolutionary outcome when it was convergent stable (CSS: successive mutations lead to that strategy). When it was possible, we also examined whether this strategy was evolutionarily stable (ESS: no mutant can invade the population once this strategy is reached) or a branching point. We do not present the results of the individual-based simulation model, as they are similar to those found by the deterministic one, thus confirming that the hypotheses of the Adaptive Dynamics framework are robust.

RESULTS OF THE MODEL OF SELFING EVOLUTION

The evolutionary model led to four possible outcomes: complete selfing, complete outcrossing, mixed-mating and evolutionary

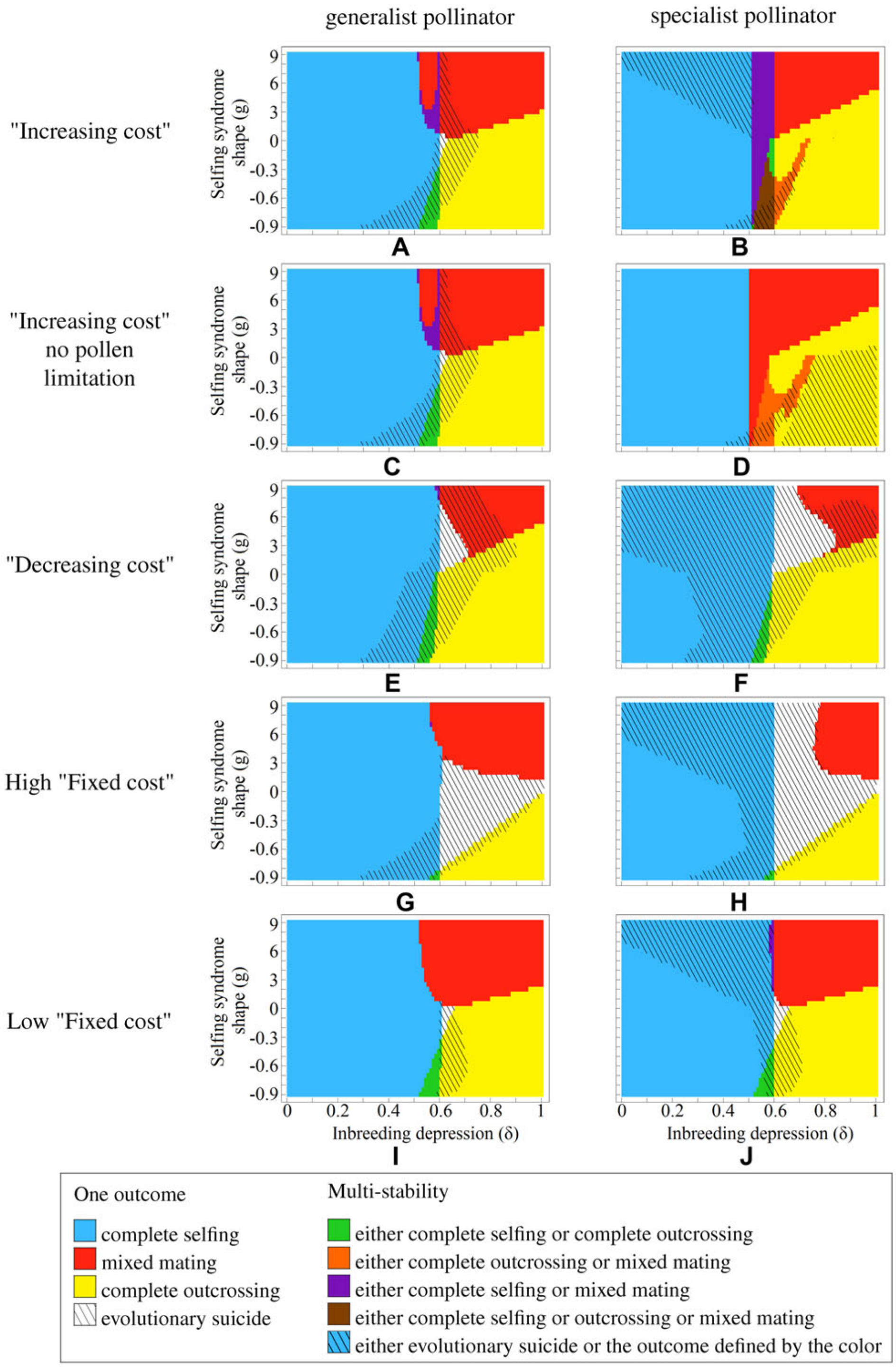

Figure 4. **Evolutionary outcomes of selfing evolution depending on inbreeding depression (δ) and selfing syndrome shape (*g*). The function of cost is: (A–D) "increasing cost;" (E–F) "decreasing cost;" (G–J) "fixed cost." Figures (C–D) show results with no pollen limitation. Pollinator is assumed to be generalist (left column) or specialist (right column). Parameter values yielding to complete outcrossing ($s < 0.0001$) are depicted in yellow, complete selfing ($s > 0.9994$) in blue, mixed mating ($0.0001 > s > 0.9994$) in red, bistability with complete outcrossing and complete selfing in green, bistability with complete outcrossing and mixed mating in orange, bistability with mixed mating and complete selfing in purple, and tristability with complete outcrossing, mixed mating, and complete selfing in brown. Striped zones represent parameters values that can lead to evolutionary suicide depending on initial prior selfing fraction. White striped zones depict zones where the only outcome is evolutionary suicide. Evolutionary outcomes are considered as such when CSS. Most of the outcomes are also ESS, except in the tristability zone (brown) and in the bistability zone with complete outcrossing and mixed mating (orange), where some intermediate prior selfing fraction are branching points.**

Table 1. Summary of parameters and variables used in equation (1).

Symbols	Default value(s)	Signification
P; A	/	Density of plants and pollinators, respectively.
r; ρ	0.2; 0.2 or 0	Intrinsic growth rate of plants and pollinators.
s; s'	[0, 1]	Plants resident and mutant prior selfing fraction.
δ	[0, 1]	Plants inbreeding depression.
$R[P, A]$	/	Function for the outcrossing reproduction for plants.
m; μ	0.08; 0.08	Intrinsic death rate of plants and pollinators.
k; K	1000; 35	Density-dependence parameter of plants and pollinators.
c	0.03, 0.1 or 50	Cost of pollination for plants.
$C[P, A]$	/	Function for the cost of pollination for plants.
β	0.55	Benefit of pollination for pollinator.
$B[P, A]$	/	Function for the benefit of pollination for pollinators.
h_1; h_2; h; h_3	1; 3; 200; 5	Half-saturation constants for plants outcrossing, for cost of pollination for plants and for benefit of pollination for pollinators.
$F[s]$	/	Function for the selfing syndrome.
f_1	0	Cost of pollination of completely selfing plants species.
f_0	1	Cost of pollination of completely outcrossing plants species.
g	[−0.9, 9]	Selfing syndrome shape.

suicide (Figs. 4 and 5). The evolutionary outcome sometimes depends on the initial value of prior selfing fraction, especially for intermediate inbreeding depression (bi- or tristability, Fig. 4). Those outcomes are nearly always CSS and ESS, although few branching points were found in specific cases (not detailed). The outcome mainly depends on the selfing syndrome shape g and on inbreeding depression δ. Our results show that for inbreeding depression lower or equal to 0.5, mutants with higher prior selfing fraction have both an increased reproduction and a lower cost of pollination, so they are always selected for. Hence, with inbreeding depression lower than 0.5, the evolutionary end point is complete selfing, regardless of the cost of pollination (Fig. 4, left part of each graph), except in cases of bistability, in which evolution may lead to an evolutionary suicide (striped zones on graphs of Fig. 4, see below). When inbreeding depression is higher than 0.5, mutants with higher prior selfing fraction have a lower reproduction rate. In those situations, the selfing syndrome shape (g) determines the outcome (see below).

Impact of the selfing syndrome shape

The evolutionary outcome when inbreeding depression is strong ($\delta > 0.5$) depends on how the cost of pollination decreases with increasing selfing ($F[s]$). Mutants with higher prior selfing fraction are selected for if the decrease of their cost of pollination exceeds the negative effects of inbreeding depression. In the opposite case, lower prior selfing fraction is selected for.

When the shape of selfing syndrome is convex ($g > 0$, Fig. 1), mutants with higher prior selfing fraction are favored when resident prior selfing fraction is low, because a small increase in the prior selfing fraction allows a strong decrease in the cost. On the contrary, when the resident prior selfing fraction is high, an increase of the prior selfing fraction provides a weaker decrease in the cost, and mutants with higher prior selfing fraction are selected against because of inbreeding depression. This explains why evolution leads to a stable mixed mating strategy (Fig. 4, red zones).

When the shape of selfing syndrome is concave ($g < 0$, Fig. 1), in a resident population with low prior selfing fraction, mutants with higher prior selfing fraction are selected against because their cost of pollination is only weakly decreased, ultimately leading to complete outcrossing (Fig. 4, right and bottom zones of each graph). In contrast, when the resident prior selfing fraction is high, mutants with higher prior selfing fraction benefit from a great decrease in their cost of pollination, which can counterbalance (depending on inbreeding depression) the decrease in reproduction, and are thus selected for. If inbreeding depression is too high, a population with high prior selfing fraction is demographically nonviable, and either increasing selfing is never favored and the only evolutionary outcome is complete outcrossing (Fig. 4, yellow zones) or the evolution to higher prior selfing fraction can lead to evolutionary suicide (striped zones, Fig. 4, see below). With intermediate inbreeding depression, this selection for higher prior selfing fraction can lead either to complete

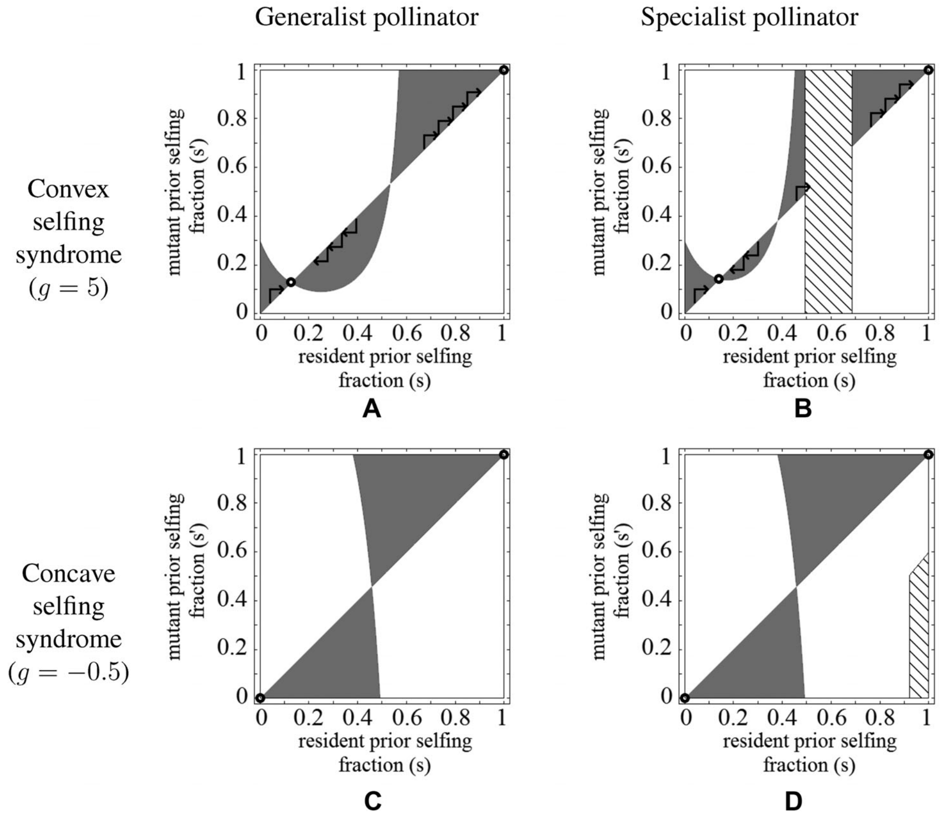

Figure 5. Pairwise invasibility plot with dynamic inbreeding depression. Results are shown for the case with pollen limitation and assuming a "fixed cost." Pairwise Invasibility Plot (PIP) are presented for (A, C) generalist pollinators or (B, D) specialist pollinator. The selfing syndrome shape is assumed to be (A–B) convex ($g = 5$) or (C–D) concave ($g = -0.5$). Regions of invasion (gray), and noninvasion (white) of a mutant are plotted against the prior selfing fraction of the mutant (s') and the prior selfing fraction of the resident (s). Striped zones indicate prior selfing fraction where demographical equilibrium with positive plant density ($\widehat{P} > 0$) does not exist. We denoted the evolutionary end points (ESS and CSS) by open circles, except when the outcome is evolutionary suicide. Examples of evolutionary trajectory (a series of successful invasions of mutants) is represented by the arrows on the panels A and B. We used default parameters values (Table 1), with $c = 0.03$. The purging of inbreeding depression is calculated for alleles with dominance coefficient equal to 0.1, genomic mutation rate equal to 1 and selection coefficient equal to 0.9, from Charlesworth et al. (1990).

selfing (Fig. 4, green and brown zones), or to a high intermediate prior selfing fraction (Fig. 4, orange and brown zones, see below).

Selfing evolution, plant-pollinator demography, and extinction

We found two distinct processes leading to evolutionary suicides. In the first case, an evolutionary suicide of the plant population follows the extinction of the specialist pollinator species. Because specialist pollinators depend on reward production for their survival, the evolution of increasing prior selfing fraction (i.e., decrease of reward production) leads to their extinction (evolutionary murder of the pollinator by the plant). The steeper the decrease of plant cost of pollination with selfing (increasing convexity $g \gg 0$), the more likely evolutionary suicide. Indeed, if the cost decreases rapidly with increasing selfing, then pollinator population can go extinct for low plant prior selfing fraction. Since outcrossing occurs only through pollinators, if the pollinator extinction occurs when the amount of selfed seeds is not high enough, this leads to the extinction of the plant population as well (Fig. 4, right panel). The extent of evolutionary suicides caused by evolutionary murders can be evaluated by comparing Figure 4B and 4D. When assuming no pollen limitation (Fig. 4D), the plant population persists in the absence of pollinators, so that plants do not suffer from the evolutionary murder. One should note that assuming no pollen limitation can hamper the evolution to complete selfing in case pollinators go extinct. When assuming outcrossing to depend on pollinators, complete selfing is always selected for if pollinators go extinct. Such selective pressure does not exist when plant pollination is not limited by pollinator density (compare Fig. 4B and 4D at intermediate inbreeding depression values).

In the second case, an evolutionary suicide is caused by the decrease in plant density with increasing prior selfing fraction. Indeed, a mutant with a higher prior selfing fraction is selected for in the resident population because of the decrease in its cost of pollination. However, once the mutant has invaded the population, the overall reproductive output of the population decreases because the selfed output suffers from inbreeding depression (Appendix D), which can lead to the extinction of the plant population. Such vortex of extinction is enhanced by the magnitude of the cost of pollination. The higher the cost of pollination, the higher the selective force to decrease this cost, thus the more likely the prior selfing fraction will evolve to higher values despite the decrease in its reproductive output (compare Fig. 4G, H and I, J).

Impact of the pollinator degree of generalism

The inclusion of demography in this model highlights the role of pollinator specialization on the evolution of plant mating systems. In case of an "increasing cost," because the density of pollinators is lowered by pollinator specialization (Fig. 3B–D), so is the cost imposed at the population level, regardless of the selfing syndrome shape. Thus, the benefit of a mutant with higher prior selfing fraction is lower (the decrease of its cost has a lower impact) compared to similar scenarios involving a generalist pollinator. Hence, the evolution of outcrossing may be favored in case of a specialist pollinator (larger yellow zones on Fig. 4B compared to Fig. 4A). Moreover, the decrease of the pollination cost can hamper the evolution toward higher prior selfing fraction, thus favoring mixed mating (larger red zone, or emergence of orange and brown zones on Fig. 4B–D compared to Fig. 4A–C), and prevent the evolution of the plant population toward evolutionary suicide (smaller striped zones on Fig. 4B compared to Fig. 4A). On the opposite, when assuming a "decreasing cost" of pollination with pollinator density, the decrease of pollinator density with increasing specialization (Fig. 3D) leads to a higher per plant cost of pollination. In that case, mutants with higher prior selfing fraction are strongly selected for, enhancing a vortex of extinction (larger white striped zones on Fig. 4B compared to Fig. 4A).

Impact of purging

Charlesworth et al. (1990) predicted that inbreeding depression should be low ($\delta < 0.5$) regardless of the value of prior selfing fraction, for many combinations of dominance coefficients, mutation rates and selection coefficients (Appendix C). In those cases, as predicted with fixed inbreeding depression, the model predicts the evolution toward complete selfing for all shapes of selfing syndromes and all initial conditions.

However, for some combinations of highly recessive deleterious mutations (dominance coefficient 0.02 or 0.1) and high genomic mutation rates (0.2 or 1), inbreeding depression is predicted to be strong for mostly outcrossing plants and weaker for mostly selfing species (Appendix C). With such a purging scenario, the evolutionary outcome depends on the resident prior selfing fraction. In the case of low resident prior selfing fraction, strong inbreeding depression generally prevents the evolution toward higher prior selfing fraction, promoting complete outcrossing (Fig. 5C and 5D). On the opposite, with high resident prior selfing fraction, inbreeding depression is low, thus leading to the evolution toward complete selfing (Fig. 5). As previously, the higher the decrease of the pollination cost with a small amount of selfing, the greater the selection to higher prior selfing fraction. Thus, similarly to situations with fixed inbreeding depression, stable mixed-mating strategies are selected for when the selfing syndrome is convex (Fig. 5A and 5B). Similarly, the selection of higher prior selfing fraction can lead to evolutionary suicide, if specialist pollinators get extinct while inbreeding depression is still strong (Fig. 5B).

Discussion

Our model combines the plant ability to self-fertilize a fraction of its ovules (so called the prior selfing fraction) and the mutualistic interaction between the plant and its pollinators. This model is likely to have a broad biological significance, since approximately 180,000 flowering plant species are biotically pollinated (Bodbyl Roels and Kelly 2011). Among these species, a wide diversity of mating systems has been described, from complete outcrossing to complete selfing, through mixed-mating strategies (Goodwillie et al. 2005). In this study, we decided to focus on prior selfing. As explained below, although this remains to be tested, some of our model predictions are unlikely to apply to other forms of self-pollination. However, as stated by Goodwillie et al. (2005) "although strict prior selfing is probably rare, a continuum from prior to competing selfing undoubtedly exists, and autonomous self-pollination may often occur before outcrossing." Our predictions should thus at least partly apply to a broad range of plant species. Moreover, this study shows that modeling selfing evolution while explicitly considering the dynamics of plant and pollinator populations can bring original results.

The acknowledged benefit of pollination for plants is the achievement of their reproduction through outcrossing. Our model explicitly integrates the pollinator demography, and describes the fertilization of ovules through pollen transfer between two mating partners by the pollinators. Thus, unlike in other models, pollen limitation is not a fixed parameter but emerges because of low plant or pollinator densities. Interestingly, our predictions are robust to the modification of the function of reproduction, and are not qualitatively modified when it is assumed that there is no pollen limitation, especially when pollinators are generalist (see Fig. 4C and D). This suggests that in our model, plant demography and selfing evolution are more affected by the cost imposed by pollinators rather than by pollen limitation. One should note, however, that pollen limitation is likely to select for higher rate of delayed selfing (Morgan et al. 2005). This suggests that the weak impact of pollen limitation on selfing evolution predicted by the current model may not apply to all forms of self-pollination.

We assumed the interaction to be costly for the plants. The existence of a cost of the interaction is likely to fit to mutualisms in general (Bronstein 2001), and pollination mutualisms in particular. First, the pollinators visits can be costly, as described in nursery pollination systems or for some nectar rewarding species. In the case of nursery pollination systems, in which pollinators lay eggs within flowers (Bronstein 2001; Holland et al. 2002; Dufay and Anstett 2003), the higher pollinator density, the more seeds consumed by pollinator's larvae, thus the higher the cost of pollination (Pellmyr 1989; Klank et al. 2010). Similarly, nectar production can be enhanced by its consumption by pollinators (Castellanos et al. 2002; Ordano and Ornelas 2004). This production can be costly (Southwick 1984; Pyke 1991), so plant growth rate possibly varies with the number of visits received, the first visits favoring seed production, but subsequent visits decreasing the number of seeds produced (Morris et al. 2010). For this reason, some species may allocate few resources to reward production (e.g., low cost of nectar production, Harder and Barrett 1992), but the interaction with pollinators is still likely to cause a cost, because it requires the production of attractive structures for pollinators (costly flower production and maintenance Andersson 2006; Mazer et al. 2010). The cost of pollinator attraction may require a fixed amount of energy (fixed floral display regardless of the visitation rates), whereas a great number of plant species can plastically modify their floral display size and the length of their flowering period in response to a low pollinator visitation rate, leading to situations where the pollination cost increases with decreasing pollinator density (Van Doorn 1997; Harder and Johnson 2005). In order to account for this diversity of situations, we considered three different relationships between the pollination cost and plant/pollinator densities. Interestingly, the main results of our model remain qualitatively similar regardless of the type of pollination cost (the demographic stability and the evolutionary outcomes of selfing were only marginally affected by how the cost varied with densities).

One cornerstone assumption of our model is the occurrence of a selfing syndrome (i.e., a decrease in the cost of pollination with increasing selfing rate). Such assumption is particularly adapted to the study of prior selfing, since floral modifications seem to depend on the timing of self-fertilization, with early selfing being correlated with the stronger decrease in floral investment (Goodwillie and Ness 2005). Prior selfing is indeed likely to be associated with shorter flower maintenance time and lower investment in attractive cues, by reducing the plant reliance on pollinators (Kalisz and Vogler 2003). This underlines the need of future studies that will investigate how other forms of selfing, with a different relationship with pollination cost, should evolve in animal-pollinated plants. Moreover, in the current study, we considered a simple decrease of pollination cost with increasing selfing rate, and we neglected the possible side effects of the investment in pollination on selfing. For example, the production of a larger floral display or a larger amount of nectar (i.e., an investment in pollination, and thus an increased pollination cost), have been shown to modify the pollinator behavior, by increasing the number of flowers probed on one plant (e.g., Harder and Barrett 1995; Ohashi and Yahara 2002), which often lead to a higher selfing rate by increasing geitonogamy (Karron et al. 2009). Our model thus neglects the fact that higher investment in pollination of outcrossing species may have strong feed-backs on selfing rates. The complex interplay between selfing through geitonogamy and investment in pollination is only starting to be understood (Devaux et al. 2014a,b) and its impact on selfing evolution is still an open question.

PURGING AND SELFING EVOLUTION

Models that include the purging of inbreeding depression often predict the instability of mixed mating, and the selection of completely selfing species or completely outcrossing species, depending on the resident selfing rate (reviewed in Goodwillie et al. 2005, but see Porcher and Lande 2005a; Johnston et al. 2009). The inclusion of the purging of inbreeding depression in our model makes the conditions of the evolution to stable mixed mating more stringent, but a strong decrease of costs with selfing still allows the evolution to intermediate prior selfing fraction. One must note, however, that the values of inbreeding depression found by mutation-selection balance (Charlesworth et al. 1990) used in our model with purging do not seem congruent with the empirical distribution of inbreeding depression with selfing rate (Winn et al. 2011). Indeed, even if completely selfing species seem to present lower inbreeding depression than outcrossing species, the magnitude of purging seems to widely vary among species that share the same mating system (Winn et al. 2011). In particular, among species with mixed mating system, many exhibit a strong inbreeding depression, which is consistent with our model predictions. However, the maintenance of mixed mating in species that show an inbreeding depression lower than 0.5 remains to be explained.

CONSEQUENCES OF MERGING PLANT SELFING AND MUTUALISTIC INTERACTIONS

Considering explicitly the dynamics of plant–pollinator interactions within a model of selfing evolution allows us to draw several general conclusions. First, our demographical model predicts that selfing weakens the strength of the mutualism. Indeed, completely outcrossing plants entirely rely on the pollinator for their reproduction, and can therefore be assimilated to obligate mutualists, whereas mixed mating and selfing plants can be depicted as facultative mutualists, since they can persist in the absence of the pollinator, even if a part of their ovules remains unfertilized. Hence, in environments with frequent shifts in pollinator availability, our model predicts the extinction of completely outcrossing species, and the persistence of partially or completely selfing species, suggesting that those shifts could be a strong selective force that maintains intermediate selfing rates, even if those are not evolutionary optimal strategies in stable environments.

Second, we found that evolution of selfing can lead to either the extinction of both mutualistic partners or the extinction of the plant species only. In the first case, our model predicts the extinction of specialist pollinators when the associated plant species evolves toward a high prior selfing fraction because of the decrease of reward production. In case self-pollination only does not produce enough seeds, this can be followed by the extinction of the plant population. It seems rather uncommon that a pollinator species depends on one plant species on its entire range (but see e.g., Pellmyr 2003), but it may apply well at the population scale (e.g., more than a quarter of pollinators are found to be locally specialist, Bosch et al. 2009). This pattern is similar to the predictions made about the evolution of cheating in models of mutualism evolution, where selection for decreased costs can lead to the extinction of obligate partners (Ferrière et al. 2002). This may provide a new hypothesis to explain the observed lower diversification rate of selfing species (Igic and Busch 2013; Wright et al. 2013), but probably only for species in which selfing is mainly performed through prior selfing. Indeed, delayed (and in a minor propensity, competing) selfing can provide a plastic response to pollinator scarcity and thus avoid plant extinction when pollinators are extinct. In the second case, the evolution toward higher selfing rates leads to the extinction of the plant (but not the pollinator) population, because inbreeding depression reduces seed production too sharply. Delayed selfing cannot prevent this type of evolutionary suicide, since it is not due to pollen limitation.

Third, our results highlight a novel factor that could facilitate the loss of self-incompatibility. The loss of self-incompatibility is one of the most frequent transitions in the evolutionary history of plant mating systems (Igic et al. 2008), which is generally thought to be due to low inbreeding depression or pollen limitation (e.g., Porcher and Lande 2005b). In our model, self-incompatible plant would correspond to a zero resident prior selfing fraction. Our results show that a convex relationship between the cost of pollination and the prior selfing fraction leads to an easier evolution to a positive selfing rate. In other words, if a small amount of selfing allows a strong decrease in the cost of pollination, this should facilitate the loss of self-incompatibility.

Fourth, most models of the evolution of selfing predict the evolution to complete selfing or complete outcrossing (Busch and Delph 2012; Cheptou 2012). These predictions remain even when plant demography is taken into account (Cheptou 2004) but the inclusion of pollinators is often neglected (Goodwillie et al. 2005). However, Morgan et al. (2005) showed that under the assumption that pollinators only affect the number of ovules fertilized, evolution leads to complete selfing or complete outcrossing, and suggested that mixed-mating could be the result of maladaptive geitonogamy, as proposed by Barrett et al. (2003). There are three main categories of models that predict the stable occurrence of mixed-mating systems: (i) models that include very specific hypotheses about inbreeding depression (such as overdominance, Charlesworth and Charlesworth 1990, or temporal variations of inbreeding depression, Cheptou and Mathias 2001), (ii) models that include pleiotropic effects of selfing, such as pollen discounting (Holsinger 1991; Harder and Wilson 1998; Porcher and Lande 2005a; Johnston et al. 2009; Jordan and Otto 2012), correlation between the number of outcrossed ovules and selfed ovules (Johnston et al. 2009), or pleiotropic effects of selfing on viability (Jordan and Otto 2012), and (iii) models that include pollen limitation that varies with time (e.g., Morgan and Wilson 2005). Such mechanisms are likely to occur in both biotically and abiotically pollinated species, and thus cannot account for the difference of mating systems between the modes of pollination. The explicit consideration of animal-pollination and associated cost raises a new explanation to the stable occurrence of mixed mating (with the realized selfing rate reaching a wide diversity of intermediate values), without assuming pollen discounting or making any strong hypothesis on inbreeding depression. This does not rule out the impact of other mechanisms on selfing evolution but could explain why biotically pollinated species display twice as much mixed mating systems as abiotically pollinated ones. Indeed, the selfing syndrome is widely described in selfing species (Sicard and Lenhard 2011), and can be considered as a form of pleiotropy, but mediated by the pollinators. In the only experimental study on selfing syndrome, Bodbyl Roels and Kelly (2011) found that the evolution to higher selfing rates is correlated with reduced herkogamy, but not with smaller flowers. A rapid evolution of floral morphology should nevertheless be found in species that display a correlation between selfing rate and flower size among populations, or even within population (e.g., Lyons and Antonovics 1991; Dart et al. 2012). We predict that the decrease of the cost of pollination associated with higher prior selfing could be a cornerstone in the evolution of selfing in animal-pollinated taxa. More specifically, we predict the occurrence of stable mixed-mating systems when a small amount of selfing allows a strong decrease in the cost of pollination (convex selfing syndrome), and this even if inbreeding depression is high. However, although selfing syndrome has been described in many species, its shape is poorly known. Empirical studies that quantify such cost of pollination and how it quantitatively varies along a gradient of selfing rates in mixed-mating species are thus critically needed to go further on the understanding of mating system evolution.

ACKNOWLEDGMENTS

We especially thank Pierre Collet for his help in the analysis of the dynamical system. We thank two anonymous reviewers and the Associate Editor Mark Johnston for their insightful suggestions. This work was supported by the French Agence Nationale de la Recherche ANR-11-BSV7-013-03 and by a grant from the Region Nord-Pas de Calais to CL.

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Associate Editor: Mark Johnston

Appendix A: Underlying Model for Mutant Initial Growth Rate

We considered a mutant (density M) with prior selfing fraction s', in a plant population (density P) with prior selfing fraction s. We transformed the system of equation (1), to consider the impact of mutants on plants and pollinators growth rate, and to include the mutant dynamics. This model is phenotypic, and crossings between resident and mutant individuals produce half mutants and half residents. Similar theoretical framework has already been used to model selfing rate evolution (e.g., Cheptou 2004). This assumption does not narrow the results of this study, since it was shown that in absence of particular genotypic architecture, phenotypic models give the same conclusion than phenotypic ones (Lloyd 1977). We present here the equations with the assumptions of pollen limitation ($R[P, A] = \frac{PA}{h_1+PA}$) and "Increasing cost" ($C[P, A] = A/(h_2 + P)$. Similar computations can be conducted with the other hypotheses.

Equations (A1), (A2), and (A3) respectively represent the resident plants, the pollinators and the mutants population dynamics:

$$\begin{aligned}\frac{dP}{dt} &= rPs(1-\delta) + rP(1-s)\left(\frac{PA}{h_1+(P+M)A} + \frac{1}{2}\frac{MA}{h_1+(P+M)A}\right)\\ &\quad + rM(1-s')\left(\frac{1}{2}\frac{PA}{h_1+(P+M)A}\right) - mP\left(1+\frac{P+M}{k}\right),\\ &\quad - c\ P\ F[s]\frac{A}{h_2+(P+M)}\end{aligned} \tag{A1}$$

$$\begin{aligned}\frac{dA}{dt} &= \rho A - \mu A\left(1+\frac{A}{K}\right) + \beta\ F[s]\ A\frac{P}{h_3+(P+M)}\\ &\quad + \beta F[s']\ A\frac{M}{h_3+(P+M)},\end{aligned} \tag{A2}$$

$$\begin{aligned}\frac{dM}{dt} &= rMs'(1-\delta) + rP(1-s)\left(\frac{1}{2}\frac{MA}{h_1+(P+M)A}\right)\\ &\quad + rM(1-s')\left(\frac{MA}{h_1+(P+M)A} + \frac{1}{2}\frac{PA}{h_1+(P+M)A}\right)\\ &\quad - mM\left(1+\frac{P+M}{k}\right) - c\ M\ F[s']\frac{A}{h_2+(P+M)}.\end{aligned} \tag{A3}$$

The first three terms of equations (A1) and (A3) represent the reproduction rate of residents and mutants, respectively. The first terms represent the selfed offspring. The second terms represent the outcrossed ovules of residents (in factor of $(1-s)$) that gives resident and mutant offspring, respectively. The third terms represent the outcrossed ovules of mutants (in factor of $(1-s')$) that gives resident and mutant offspring, respectively. One should note that mutant and resident have the same probability of successfully export pollen, that is we assumed no pollen discounting. The fifth and sixth terms of equations (A1) and (A2) represent the density-dependent death rate and cost of pollination, respectively. Equation (A2) is similar to equation (1b), but considers both the benefit made with residents (third term) and with mutants (fourth term).

We analyzed this model using the Adaptive Dynamics framework (Cheptou and Dieckmann 2002; Brännström et al. 2013). This approach considers the initial growth rate of mutants, so that mutant density (M) is negligible compared to the resident population density (P). As we neglected M, resident plant and pollinator densities (Eqs. A1 and A2) became independent of mutant density, reflecting the time-scale separation between the apparition of a mutant and the ecological dynamic. Their equilibrium densities can therefore be determined with system of equation (1). Thus, equation (A3) can be simplified as following to describe the initial growth rate of the mutant:

$$\begin{aligned}\frac{dM}{dt} &= rM\left(s'(1-\delta) + \frac{(1-s+1-s')}{2}\frac{\widehat{P}\widehat{A}}{h_1+\widehat{P}\widehat{A}}\right)\\ &\quad - mM\left(1+\frac{\widehat{P}}{k}\right) - c\ M\ F[s']\frac{\widehat{A}}{h_2+\widehat{P}}.\end{aligned} \tag{A4}$$

Appendix B: Algorithm of the Stochastic Model

We performed individual-based simulations with the following algorithm. Each individual is characterized by a phenotype s_i that determines its prior selfing fraction. We assumed that the population is known at time t.

1. The rate of each event (birth or death of a plant or a pollinator) is determined:
    - For each plant morph P_i with a given prior selfing fraction s_i, the rate of birth, noted P_{i+} is:
      $P_{i+} = rP_is_i(1-\delta) + rP_i(1-s)\frac{P_iA}{h_1+P_t}$
      $+ \sum_{j=0,\ j\neq i}^{n} \frac{1}{2}rP_i((1-s_i)+(1-s_j))\frac{P_jA}{h_1+P_t}$
    - For each plant morph, the rate of death, noted P_{i-}, is:
      $P_{i-} = mP_i(1+\frac{P_t}{k}) + cF\,[s_i]\,A\frac{P_i}{h_2+P_t}$
    - The rate of birth of a pollinator, noted A_+, is:
      $A_+ = \rho A + \sum_{j=0}^{n}\beta F\left[s_j\right]A\frac{P_j}{h_3+P_t}$
    - The rate of death of a pollinator, noted A_-, is:
      $A_- = \mu A(1+\frac{A}{K})$
2. We define the total event rate at the population level by: $Ev = \sum_{i=0}^{n}P_{i+} + \sum_{i=0}^{n}P_{i-} + A_+ + A_-$, and the probability of each event is: $\frac{P_{i+}}{Ev}$, $\frac{P_{i-}}{Ev}$, $\frac{A_+}{Ev}$, $\frac{A_-}{Ev}$.
3. The next event time is $t' = t + \tau$ where τ is an independent random variable that is exponentially distributed with parameter Ev.
4. A value θ is randomly drawn from an uniform distribution bounded by 0 and Ev.
    (a) If $0 < \theta \leq \sum_{i=0}^{n}P_{i+}$, then a plant birth happens:
        - we determine the prior selfing fraction of the parent: if $0 < \theta \leq P_{1+}$ the prior selfing fraction is s_1, if $P_{1+} < \theta \leq P_{1+} + P_{2+}$, it is s_2 etc.
        - each new individual has a fixed probability to be a mutant, and its prior selfing fraction is then randomly drawn from a normal distribution with mean the prior selfing fraction of its parent and with standard deviation s_d. The new value must be comprised between 0 and

1, so if the random value is out of these bounds, a new sampling is done.

(b) If $\sum_{i=0}^{n} P_{i+} < \theta \leq \sum_{i=0}^{n} P_{i+} +; \sum_{i=0}^{n} P_{i-}$ then a plant dies. The phenotype of this plant is determined by the same way than for plant birth.

(c) If $\sum_{i=0}^{n} P_{i+} + \sum_{i=0}^{n} P_{i-} < \theta \leq \sum_{i=0}^{n} P_{i+} + \sum_{i=0}^{n} P_{i-} + A_{+}$, a pollinator is born.

(d) If $\sum_{i=0}^{n} P_{i+} + \sum_{i=0}^{n} P_{i-} + A_{+} < \theta \leq \sum_{i=0}^{n} P_{i+} + \sum_{i=0}^{n} P_{i-} + A_{+} + A_{-}$, a pollinator dies.

We used this model with the probability of mutation of each new individual being 10^{-3}. We modeled low effect mutations ($s_d = 10^{-2}$) or strong effect mutations ($s_d = 0.1$). We used the same default values than for determistic models, and we verified that populations were large enough to avoid extinctions linked to demographic stochasticity (no extinction of the populations when the prior selfing fraction is constant for 100 replicates). We determined the number of plants of each morph and the number of pollinators for the last 1000 iterations, and calculate the averaged prior selfing fraction. We chose the number of iteration long (around a billion) so that populations reach equilibrium (low variations in the last 1000 iterations). Ten replicates were done for each set of parameters. The result of the individual-based model were similar to the deterministic results.

Appendix C: Dynamical Inbreeding Depression

Appendix D: Plant and Pollinator Densities at Evolutionarily Stable Prior Selfing Fraction

Plant and pollinator densities at evolutionarily stable prior selfing fraction depend on the outcome reached, on inbreeding depression and on the selfing syndrome shape. On the other side, the evolutionarily stable prior selfing fraction also depend on those parameters.

With low inbreeding depression, complete selfing is reached. Pollinator population does not depend anymore on the plant population (no rewards offered), and its density thus only depends on its degree of generalism. Plant population density depends on the magnitude of inbreeding depression (quasi continuum at $s = 1$). With high inbreeding depression, the evolutionary outcome depends on the selfing syndrome shape. If the selfing syndrome is concave ($g < 0$), complete outcrossing is reached, and the plant and pollinator densities do not depend on inbreeding depression nor on selfing syndrome shape (g), and are represented by one point (not apparent). If the selfing syndrome is convex ($g > 0$), mixed mating can be reached. The higher the inbreeding depression, the lower the evolutionarily stable prior selfing fraction (Fig. D1), and the greater the convexity, the higher the evolutionarily stable prior selfing fraction (not shown). In that case, plant and pollinator density decreases with increasing prior selfing fraction (because of inbreeding depression for plants, and of for pollinators because of the decrease in their benefit). When the evolutionarily stable prior selfing fraction is intermediate, the realized selfing rate is reduced because of inbreeding depression, and can be close to zero for the highest values of inbreeding depression (Fig. D1)

Table C2. Polynomial regressions of degree 4 calculated from the observed values of inbreeding depression found by Charlesworth et al. (1990).

Dominance coefficient	Mutation rate	Selection coefficient	Polynomial regression	Inbreeding depression
0.02	0.2	0.2	$\delta = -0.2905s^4 - 0.7936s^3 + 3.0383s^2 - 2.8052s + 0.9379$	High inbreeding depression ($\delta > 0.5$) at low prior selfing fraction
0.1	1.0	0.2	$\delta = -0.2172s^4 + 1.4298s^3 - 1.7587s^2 + 0.0133s + 0.8607$	
0.1	1.0	0.9	$\delta = -1.9238s^4 + 4.5573s^3 - 3.1732s^2 - 0.0173s + 0.8618$	
0.1	0.2	0.2	$\delta = -0.7191s^4 + 1.6146s^3 - 0.8317s^2 - 0.4999s + 0.6126$	
0.1	0.2	0.9	$\delta = 0.3955s^4 - 1.201s^3 + 1.4365s^2 - 0.8899s + 0.3297$	Low inbreeding depression ($\delta < 0.5$) regardless the prior selfing fraction
0.1	0.1	0.2	$\delta = 0.2193s^4 - 0.742s^3 + 1.0238s^2 - 0.7531s + 0.3275$	
0.1	0.1	0.9	$\delta = 0.5846s^4 - 1.4201s^3 + 1.2754s^2 - 0.5822s + 0.1796$	
0.2	0.5	0.05	$\delta = 0.0019s^4 + 0.0028s^3 + 0.0802s^2 - 0.2543s + 0.3076$	
0.2	0.5	0.2	$\delta = 0.0041s^4 - 0.0203s^3 + 0.1393s^2 - 0.3004s + 0.3117$	
0.2	0.5	0.9	$\delta = 0.0624s^4 - 0.2505s^3 + 0.4377s^2 - 0.4447s + 0.3143$	
0.2	0.2	0.2	$\delta = -0.0044s^4 - 0.046s^3 + 0.1416s^2 - 0.1745s + 0.1388$	
0.35	0.2	0.2	$\delta = -0.0446s^4 + 0.1039s^3 - 0.0712s^2 - 0.0018s + 0.0417$	

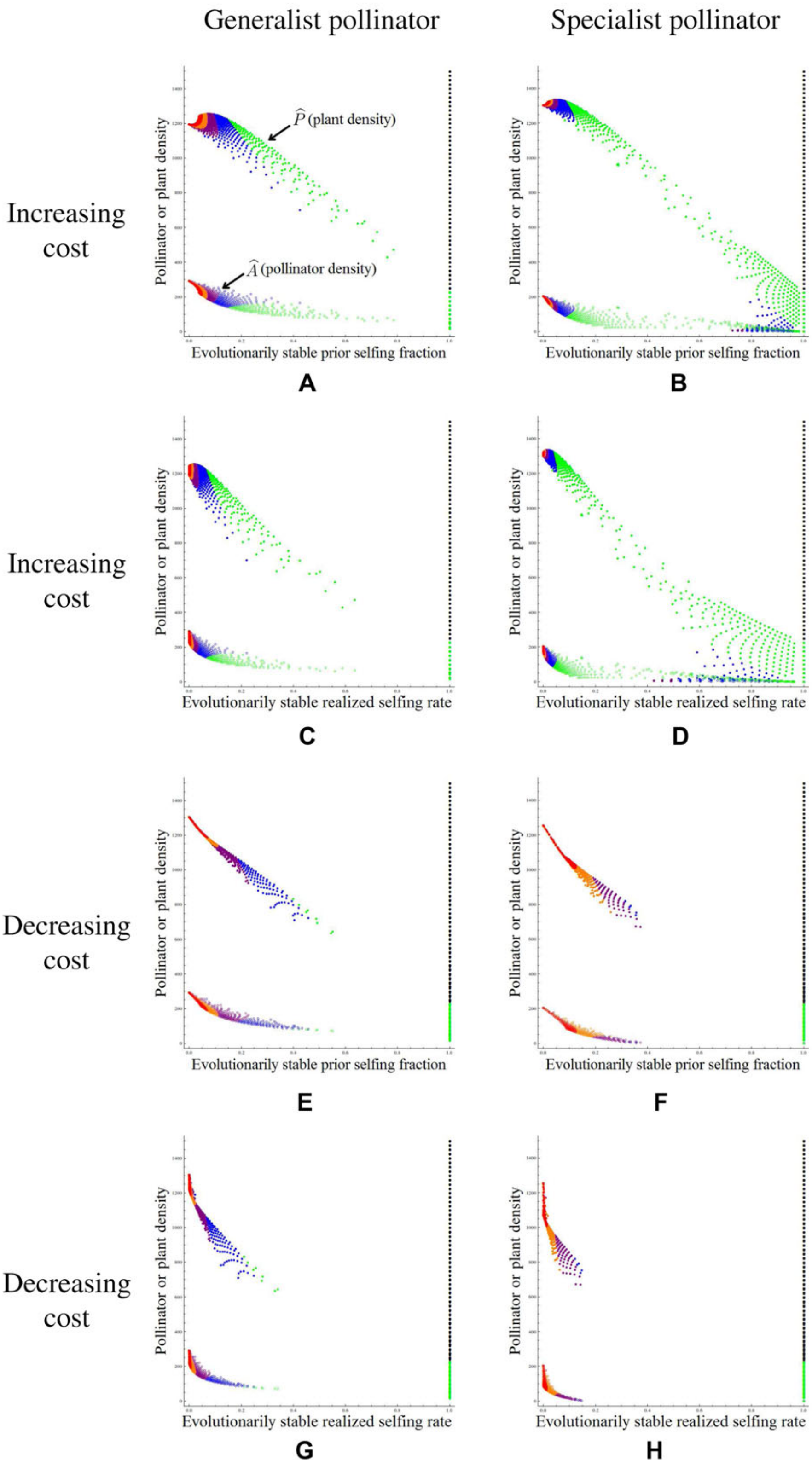


Figure D1. Plant (filled symbols) and pollinator (empty symbols) densities at evolutionarily stable prior selfing fraction. The densities are plotted as a function of (A, B, E, F) the selfed ovules fraction (the amount of ovules dedicated to prior selfing) or as a function of (C, D, G, H) the realized selfing rate (the fraction of seeds produced through selfing). The cost function is modeled as (A–D) "Increasing cost" (E–H) "Decreasing cost." The colors depict the inbreeding depression magnitude (δ), with $0 < \delta \leq 0.5$ in black; $0.5 < \delta \leq 0.6$ in green; $0.6 < \delta \leq 0.7$ in blue; $0.7 < \delta \leq 0.8$ in purple; $0.8 < \delta \leq 0.9$ in orange and $0.9 < \delta \leq 1$ in red.

Chapter 4

Is dioecy an evolutionary suicide for plants?

Lepers C., Van De Paer C., Abu Awad D., Massol F., Billiard S. and Dufay M.

Abstract

Commonly, dioecious angiosperms species display a sexual dimorphism, especially in floral traits. Floral traits strongly affect pollinator behavior, and a dimorphism in floral traits could induce unbalanced visits to male and female individuals and decrease the efficiency of pollen transfer. We modeled the evolution of floral attractiveness both in males and females, accounting for the impact of dimorphism on plant and pollinator demography. We show that the evolution of attractiveness in males is driven only by intra-sexual competition, whereas in the case of females, the evolution depends on the level of pollen limitation. We show that depending on the level of pollen limitation either males or females are expected to be the most attractive sex. Because pollen limitation strongly depends on the visitation rate needed by females to fertilize their ovules, the extent of dimorphism is likely to depend on female reproductive characteristics. Merging demography and evolution highlights the fact that strong sexual dimorphism evolves in large populations that suffer from low pollen limitation, suggesting that dimorphism may not necessarily represents some threat to the survival of the plant population.

Keywords

Dioecy, dimorphism, plant-pollinator interactions, floral traits, evolution

4.1 Introduction

Unlike in the animal kingdom, dioecy, *i.e.* the coexistence of male and female unisexuate individuals within the same species, is rather rare in flowering plants, occurring in only about 6% of all species, although it has evolved in 38% of Angiosperm families (Renner and Ricklefs 1995). The rarity of dioecy in flowering plants has attracted the attention of evolutionary biologists and its causes are still debated. Because species richness is generally lower in dioecious taxa when compared to their hermaphroditic or monoecious sister taxa, Heilbuth (2000) suggested that dioecious plant species suffered from higher extinction rate or lower speciation rate (Heilbuth 2000). On the reverse, Käfer et al. (2014) found that the observed diversity could be better explained by a low transition rate to dioecy and frequent reversions to hermaphroditism. Regarding the first hypothesis, we can find in the literature several factors that could explain an increased extinction rate, implying dioecy could be an evolutionary suicide in plant species. First, the existence of separate sexes prevents the reproductive assurance that benefits self-compatible hermaphroditic species. Second, only females produce seeds, which may result in a disadvantage at the population level, either because fewer offspring are produced or because a large production of seeds is carried by a limited number of individuals, thus increasing offspring competition (Charlesworth 2009; Heilbuth et al. 2001). Third, because a pollination event in an entomophilous dioecious species is only realized when pollinators visit a male and then a female plant, the proportion of pollinators visits leading to potential seed production is lower than in hermaphroditic species (Vamosi et al. 2006). Moreover, pollination efficiency can be reduced even further in case of sexual dimorphism. Indeed, the evolution of separate sexes is commonly associated with the evolution of sexual dimorphism (Barrett and Hough 2013), especially in regards to floral traits (Delph et al. 1996). Males tend to invest more than females in numerous floral traits, such as flower size, flower number or scent quantity (Ashman 2009; Delph 1996; Delph et al. 2002). Pollinator behavior is greatly influenced by floral traits, and pollinators may disproportionately visit plant individuals that display more flowers or larger ones (Martin 2004; Mitchell et al. 2004). Hence, a dimorphism in floral traits between males and females could induce unbalanced visitation rates to males and females, thus reducing pollen transfer from male to female individuals (Vamosi and Otto 2002).

Despite its potential effects on the evolutionary success of dioecy, little theoretical attention has been dedicated to the evolution of sexual dimorphism in plants and its impact on pollination. Dimorphism is likely to affect pollen transfer, which is likely to affect access to sexual partners for both male and female individuals. The latter is a central point of sexual selection, while also impacting seed production, and thus plant demography. The evolution of plant attractiveness is therefore likely to strongly affect plant demography, and conversely. Vamosi and Otto (2002) modeled the evolution of plant attractiveness in a dioecious species, assuming fixed plant and pollinator densities, and concluded extreme sexual dimorphism could evolve. Their model predicts that dimorphism could increase the probability of extinction of small dioecious populations, in the event of a shortage of pollinators. Yet, Vamosi and Otto (2002) neglected the interplay between demography and evolution, and provided no insight on how dimorphism affects demography at equilibrium and vice versa.

In this study, we investigate how sexual dimorphism evolves when plant-pollinator interactions are explicitly taken into account, as well as the impact of sexual dimorphism on plant demography. Close attention is paid on analyzing how different categories of sexual dimorphism (males being more attractive than females and conversely) affect pollinator visitation patterns, and their impact on plant demography at equilibrium. Moreover, we determine the conditions for the evolution of sexual dimorphism by independently considering the evolution of males and females. We also explore whether evolution can lead to sexual dimorphism that would destabilize plant populations. By merging demography and evolution, we demonstrate that, although evolution can lead to extreme values of dimorphism in plant attractiveness, this dimorphism may not be a major cause of demographic fragility of dioecious population in a stable environment.

4.2 Modeling dioecious populations: demography and evolution of attractiveness

Demographic model

This model explicitly describes plant-pollinator interactions, with males and females co-occurring simultaneously in the plant population. We assumed plant-pollinator interactions to be mutualistic, with pollinators providing pollen transfer between plant individuals and plants providing some resources (*e.g.* nectar) that is consumed by pollinators. The overall dynamics of male plants (density M), female plants (density F) and pollinators (density P) is given by the following system of ordinary differential equations (details of parameters signification and default values are given in Table 4.1):

$$\left\{\begin{aligned} \frac{dM}{dt} &= \frac{1}{2}\ r\ F\frac{V_{fm}}{h+V_{fm}} - d_m\ M\left(1+\frac{M+F}{K}\right), &\text{(4.1a)}\\ \frac{dF}{dt} &= \frac{1}{2}\ r\ F\frac{V_{fm}}{h+V_{fm}} - d_f\ F\left(1+\frac{M+F}{K}\right), &\text{(4.1b)}\\ \frac{dP}{dt} &= \rho\ P\ \left(1-\frac{P}{\kappa}\right) + \beta_m\ M\ V_m + \beta_f\ F\ V_f. &\text{(4.1c)} \end{aligned}\right.$$

The first term of equations (4.1a) and (4.1b), $r\ F\frac{V_{fm}}{h+V_{fm}}$, depicts seed production by females. Each female produces ovules at a rate r. Fertilization of the ovules is a saturating function of pollen receipt, *i.e.* of the visits of pollinators transferring pollen from males (V_{fm}, see below). Every visit of a pollinator carrying pollen allows the fertilization of some ovules until all ovules are fertilized. The visitation rate needed to fertilize half of a female's ovules (h) determines their reproductive efficiency. For low visitation rates, pollen limitation may occur, reducing seed production. The lower the visitation rate needed to fertilize its ovules, the lower the pollen limitation for a given visitation rate. There is therefore a direct relationship between pollen limitation and female reproductive efficiency (h). We assume the plant sex ratio at birth to be unbiased: half of the fertilized ovules produce male individuals and the remaining half produces female individuals. The last term of equations (4.1a) and (4.1b) represents the density-dependent self-limitation of

Symbols	Default value(s)	Signification
M; F	/	Density of males and females, respectively
P	/	Density of pollinators
a_m; a_f	10	Plants attractiveness for pollinators
r	0.05	Ovules production rate of females
γ	0.0025	Males efficiency to transfer pollen to pollinators
h	0.01, 0.1 or 0.5	Half-saturation constants for plant ovules fertilization
D_m; D_f	1	Rate at which pollinators leave a plant
λ	10	Rate of pollen loss
u_m; u_f	0	Probability that pollinator loses pollen when visiting a plant
d_m; d_f	0.1	Intrinsic death rate of male and female plants
k; K	1000; 300	Density-dependence parameter of plants and pollinators
ρ	0.2	Intrinsic growth rate of pollinators
β_m; β_f	0	Benefit for pollinators of a visit on a male or a female plant
I	$[0, 1]$	investment into attractiveness (1 - investment into reproduction)
c_a	20	conversion parameter between investment and effective attractiveness
c	0.05	conversion parameter between investment and effective reproductive characteristic (r or γ)
s	0.3, 0 or 3	shape parameter between investment and effective reproductive characteristic (r or γ)

Table 4.1: Summary of parameters and variables used in equations (4.1)

males and females, respectively, with d_m and d_f the mortality rate without competition.

The dynamics of pollinators depends on their intrinsic growth rate, $\rho \ P \ \left(1 - \frac{P}{\kappa}\right)$, which is assumed to be density-dependent. The benefit acquired by pollinators, given by the terms β_m and β_f for visits to male and female respectively, is assumed to increase linearly with each visit. The benefit can differ between sexes, depending on the reward available on male and female plants. This benefit increases with plant visitation rates (V_m and V_f), regardless of their impact on plant reproduction (*i.e.* regardless of the pollen load of pollinators).

Visitation rates

The population-level visitation rates are derived from individual-level interactions between plants and pollinators. Features of those interactions are summarized in Fig. 4.1. Both plant and pollinator densities are assumed as constant at the time scale of pollinator visits, *i.e.* we considered a time-scale separation between the time needed for a pollinator

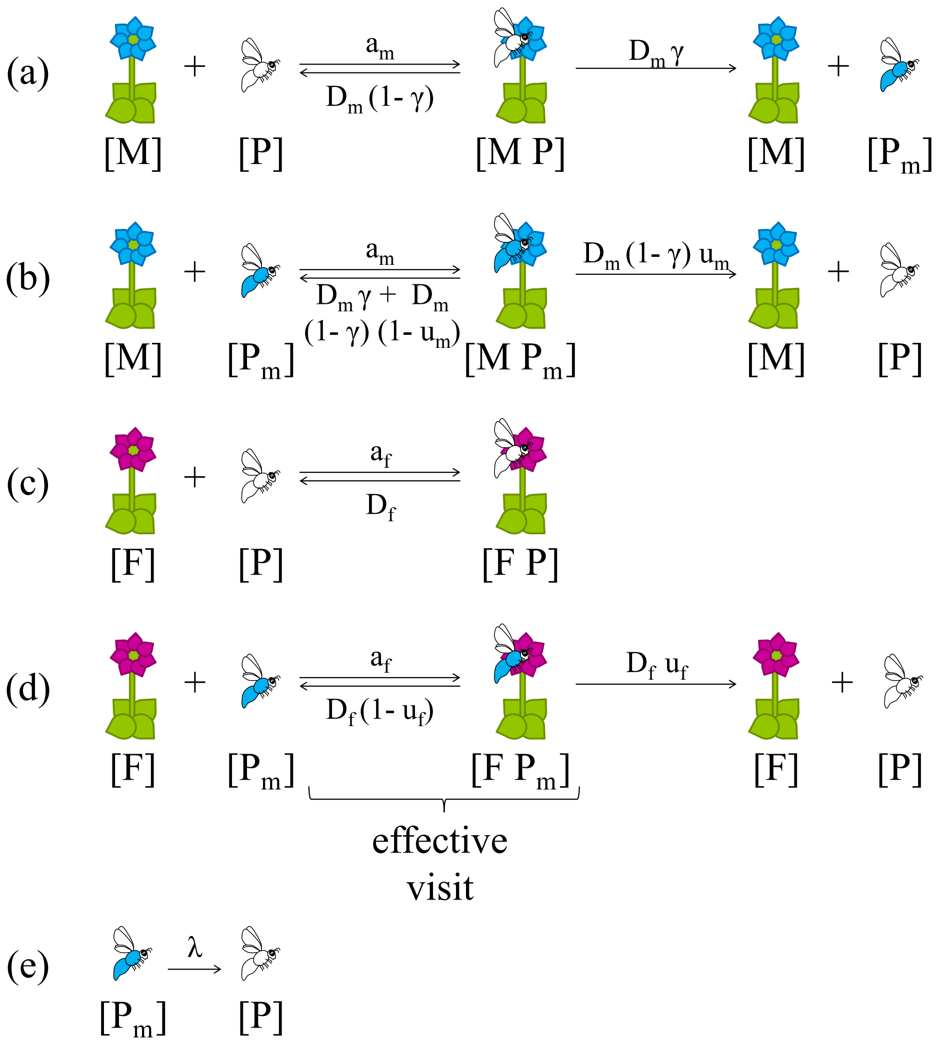


Figure 4.1: A "kinetic" scheme of plant-pollinator interactions for a dioecious plant species. The rates of interactions are given above and under the arrows. The subscripts i depict either male or female characteristics (to be replaced by m and f according to the plant sex).
"Free" pollinators (density $[P]$ and $[P_m]$) are attracted by unoccupied plant individuals (males, density $[M]$ or females, density $[F]$) according to plant attractiveness denoted a_i (equations a-d). Pollinators leave a plant they are interacting with at a rate D_i (equations a-d). After a visit on a male plant, pollinator carry pollen with probability γ (density $[P_m]$, equations a,b). We assume effective visit to occur when a pollinator carrying pollen visits a female (equation d). Pollinators may lose the pollen they carry while visiting a plant, with probability u_i (equations b,d). A pollinator that carries pollen may also lose it without any interaction with a plant (*e.g.* by grooming), at rate λ (equation e).

to visit a plant individual and the time-scale at which plant and pollinator densities vary. Detailed computation of the visitation rates is given in appendix 4.A. The visitation rate of an individual plant i (to be replaced by m or f according to plant sex) by pollinators (carrying or not pollen) is given by:

$$V_i = \left(\frac{a_i}{1 + \frac{a_m}{D_m} M + \frac{a_f}{D_f} F} \right) \; P, \tag{4.2}$$

The per capita visitation rate of a female individual by pollinators carrying pollen (*i.e.* the effective visitation rate, Fig. 1d) is given by:

$$V_{fm} = \left(\frac{a_f}{1 + \frac{a_m}{D_m} M + \frac{a_f}{D_f} F} \right) \; \left(\frac{a_m \; \gamma \; M}{a_m \; \gamma \; M + \lambda + a_m (1-\gamma) \; u_m \; M + a_f \; u_f \; F} \right) \; P. \tag{4.3}$$

Visitation rates have the following biological interpretations. For a visit to occur, a pollinator has to be attracted by one plant individual (depending on its attractiveness, a_i), compared to all the possible pollinator actions: remaining free or being attracted by another unoccupied plants $(1 + \frac{a_m}{D_m} M + \frac{a_f}{D_f} F)$. For a visit to be effective, the pollinator visiting a female plant must carry pollen (Fig. 1d). The proportion of pollinators carrying pollen is given by the rate at which male individuals attract and transfer pollen to pollinators $(a_m \; \gamma \; M)$ over all the possible states of pollinators: carrying pollen $(a_m \; \gamma \; M)$ or having lost the pollen without visiting a plant (λ) or while visiting a plant (male $a_m \; (1-\gamma) \; u_m \; M$ or female $a_f \; u_f \; F$).

Model analysis

We analyzed the impact of a sexual dimorphism in attractiveness on visitation rates and on plant demography. To do so, we studied the impact of female attractiveness while fixing male strategy, and vice versa. We then analyzed how visitation rates and demography are affected by attractiveness dimorphism between sexes, in both directions. No analytical explicit solutions of the system of equations (4.1) were tractable, we thus investigated the demographic model numerically. The set of explored parameter values is given in Table 4.1. For each set of parameters, we analyzed the stability properties of equilibria and conducted a phase-plane analysis (Otto and Day 2007). Below, densities of male and female plants and pollinators at equilibrium are noted $\widehat{M}$, $\widehat{F}$ and $\widehat{P}$ respectively, and equilibria are noted $\left\{\widehat{M}, \widehat{F}, \widehat{P}\right\}$.

Parameters values: resource limitation

Two scenarios are investigated. In the first scenario, we assumed plants to have unlimited resources, hence there is no correlation among traits, so that an increase in plant attractiveness does not affect its reproductive characteristics. In the second scenario, we assume plants to have limited resources that could be invested either to attractiveness or reproduction, thus creating a trade-off between these traits. Plants therefore invest a part I of their resources into attractiveness:

$$a = c_a\ I, \tag{4.4}$$

with c_a being the conversion parameter between investment and effective attractiveness to the pollinator. The remaining part of their resources $(1 - I)$ is invested in their reproduction, with ovule production (r) in females or efficiency to transfer pollen to pollinators (γ) in males being:

$$c\,\frac{1 - I}{1 - I + s\ I}, \tag{4.5}$$

with c the conversion parameter between investment and effective reproductive characteristics, which may differ between males and females. The investment into reproduction can have a linear $(s = 1)$, concave $(s < 1)$ or convex $(s > 1)$ impact on the reproductive characteristic (Fig. 4.2).

Model of attractiveness evolution

In the previous section, plant traits are considered as fixed parameters of the model. Here, we present a phenotypic model allowing for the evolution of plant attractiveness.

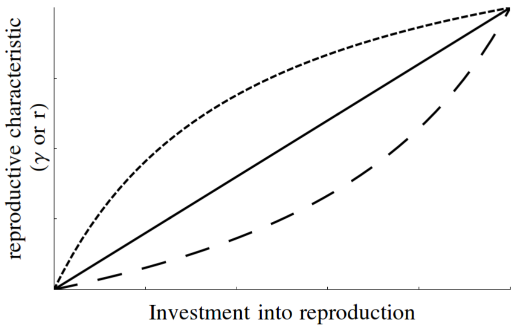


Figure 4.2: Relationship between the decrease in the investment into reproduction $(1-I)$ and reproductive strategy (γ or r), modeled as convex ($s=3$, dashed lines), linear ($s=1$, plain lines) and concave ($s=0.3$, dotted lines).

We consider the introduction of a mutation, affecting only males (mutant density M_x) or only females (mutant density F_x), *i.e.* we assumed no correlated evolution between the sexes. The mutant's traits are represented with the subscript i_x, with i denoting the sex of the mutant (for example the attractiveness of a male mutant is denoted by a_{m_x}).

We analyze this model using the Adaptive Dynamics framework (Brännström et al. 2013; Geritz et al. 1998). This approach assumes a time-scale separation between the introduction of a mutant and the ecological dynamics, *i.e.* the introduction of a mutant always occurs once the resident population has reached its demographic equilibrium, and only one mutant appears at a time in the population. Moreover, it considers mutations to be of small effect (for example a_{i_x} is close to a_i), and the initial fitness of the mutant is assumed representative of its capacity to invade the resident population. Hence, for the analysis, mutant density is assumed to be negligible compared to the resident density, *i.e.* mating only occurs with resident partners, and competition occurs only with resident individuals. The per capita fitness of a rare male mutant, defined as its ability to transmit its genes to offspring, is described by the following equation:

$$W_{M_x} = \left(\frac{1}{2}\; r\; \widehat{F}\; \frac{\widehat{V_{fm_x}}}{h + \widehat{V_{fm}}} - d_{m_x}\; M_x\; \left(1 + \frac{\widehat{M} + \widehat{F}}{K} \right) \right) / M_x, \tag{4.6}$$

and similarly, the per capita fitness of a rare female mutant is described by:

$$W_{F_x} = \left(\frac{1}{2}\; r_x\; F_x\; \frac{\widehat{V_{f_x m}}}{h_x + \widehat{V_{f_x m}}} - d_{f_x}\; F_x\; \left(1 + \frac{\widehat{M} + \widehat{F}}{K} \right) \right) / F_x, \tag{4.7}$$

with:

$$\widehat{V_{f_xm}} = \left(\frac{a_{f_x}}{1+\frac{a_m\widehat{M}}{D_m}+\frac{a_f\widehat{F}}{D_f}}\right)\left(\frac{a_m\ \gamma\ \widehat{M}}{a_m\ \gamma\ \widehat{M}+\lambda+a_m(1-\gamma)\ u_m\ \widehat{M}+a_f\ u_f\ \widehat{F}}\right)\ \widehat{P},$$

$$\widehat{V_{fm_x}} = \left(\frac{a_f}{1+\frac{a_m\widehat{M}}{D_m}+\frac{a_f\widehat{F}}{D_f}}\right)\left(\frac{a_{m_x}\ \gamma_x\ M_x}{a_m\ \gamma\ \widehat{M}+\lambda_x+a_m(1-\gamma)\ u_m\ \widehat{M}+a_f\ u_f\ \widehat{F}}\right)\ \widehat{P},$$

$$\widehat{V_{fm}} = \left(\frac{a_f}{1+\frac{a_m\widehat{M}}{D_m}+\frac{a_f\widehat{F}}{D_f}}\right)\left(\frac{a_m\ \gamma\ \widehat{M}}{a_m\ \gamma\ \widehat{M}+\lambda+a_m(1-\gamma)\ u_m\ \widehat{M}+a_f\ u_f\ \widehat{F}}\right)\ \widehat{P}.$$

Parameters and functions have the same definitions and default values as in equation (4.1) (Table 4.1). The first term of equation (4.6) denotes the siring success of a male mutant (*i.e.* the seed produced by female resident individuals with pollen from a mutant individual). The first term of equation (4.7) denotes the seed production by a female mutant (with pollen from resident males). One should note that in this model each parent contributes equally to the genome of the offspring. The second term of both equations represents the per capita mortality rate of mutants. Visitation rates have the same biological interpretation as in the previous section: the first terms depict the probability that the pollinator chooses the individual over other individuals, and the second term corresponds to the probability that the pollinator carries pollen from a given category of males.

Model analysis

As previously, we investigate two scenarios concerning resource limitation. In a first case, we assumed plants to have unlimited resources (no correlation between traits). In this case, we assumed the mutation to affect only attractiveness (mutant's strategy a_{i_x}). In a second case, we assumed plants to have limited resources that could be used either to attractiveness or reproduction, thus creating a trade-off between those traits. In this case, we assumed the mutation to affect the resource allocation strategy (mutant's strategy I_x), thus modifying both attractiveness (mutant's attractiveness $a_{i_x} = c_a I_x$) and reproductive characteristics (mutant's ovule production (r_x) or efficiency to transfer pollen to pollinators (γ_x) being $c\ \dfrac{1-I_x}{1-I_x+s\ I_x}$). The investment in reproduction can have a linear ($s = 1$), concave ($s < 1$) or convex ($s > 1$) impact on the ability plant reproduction (Fig. 4.2).

All other characteristics of the mutants are similar to those of the residents. We first determine the gradient of invasibility of a rare mutant in a resident population (attractiveness a_i or investment strategy I), which is depicted by the sign of the per capita mutant's fitness. The gradient vanishes at strategies that are a maximum or a minimum of fitness. Such a strategy is considered as a possible evolutionary outcome when it was convergent stable (CS: successive mutations lead to that strategy) and evolutionarily stable (ESS: no mutant can invade the population once this strategy is reached).

4.3 Results

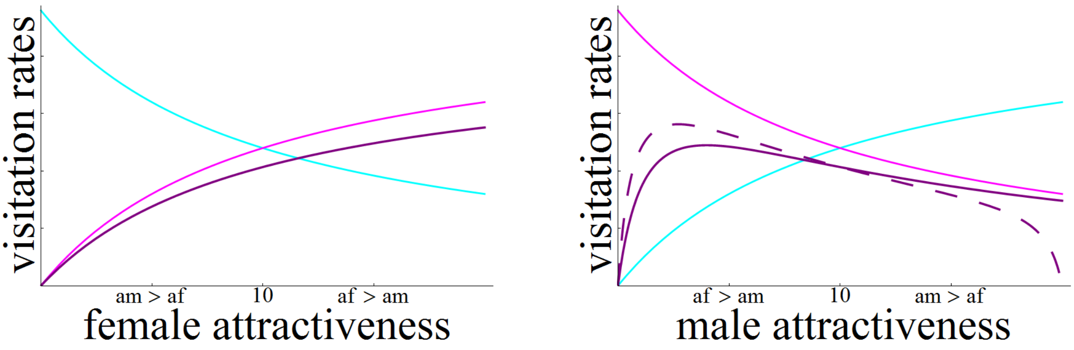
What is the impact of sexual dimorphism on plant demography?

Plant demography depends on two features: the effective visitation rate to female individuals, *i.e.* visits by pollinators carrying pollen, and the relationship between visitation rates and seed production. In the following, we analyze the impact of dimorphism on those features, modifying female attractiveness and keeping male strategy fixed, and conversely.

Effective visitation rate

Regardless of the scenario of resource limitation (*i.e.* whether attractiveness can increase independently of all other model parameters or whether there is a trade-off between attractiveness and reproductive traits), for a given population size (fixed plant and pollinator densities), the effective visitation rate (V_{fm}) is enhanced in populations in which females are more or as equally attractive as males (Fig. 4.3). Indeed, the effective visitation rate is higher if, after visiting a male, a pollinator carrying pollen then brings pollen to several females. The higher the male efficiency to transfer pollen (γ) and the lower the rate of pollen loss (on a plant u_i or while flying λ), the higher the likelihood that a pollinator will carry pollen after visiting a male, and the more the effective visitation rate is increased by high female attractiveness (data not shown).

Resource limitation does not influence the effective visitation rate when we consider variations in female attractiveness, since although it affects ovule production, it does not interfere with pollen transfer in populations with fixed density (Fig. 4.3a). When analyzing the variation in male attractiveness, in a scenario of resource limitation, males that



(a) (b)

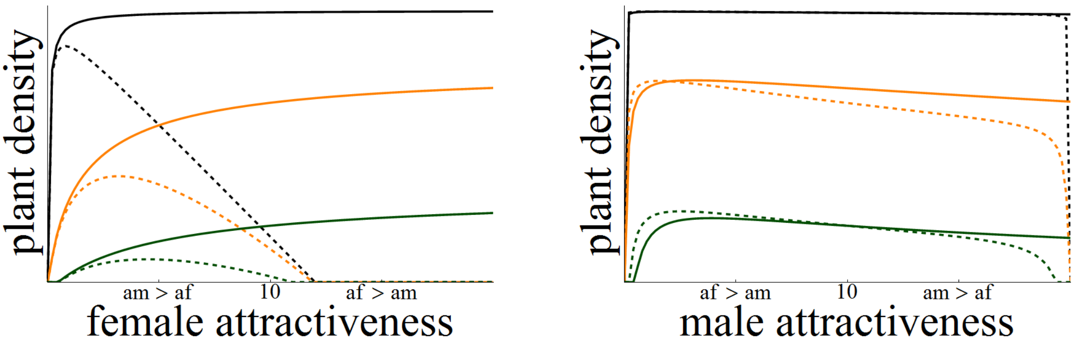
Figure 4.3: Visitation rates (to males in blue, to females in pink and effective visits in purple) as a function of (a) female attractiveness or (b) male attractiveness. Plain lines depict the scenario of unlimited resources, and dashed lines the scenario of resource limitation (trade-off between attractiveness and investment in reproduction). Plant and pollinator densities are fixed ($M = 250$, $F = 250$, $A = 300$). (a) Male attractiveness (a_m) and male efficiency to transfer pollen (γ) are fixed, with $a_m = 10$ and $\gamma = 0.0025$. (b) Female attractiveness (a_f) and female ovule production (r) are fixed, with $a_f = 10$ and $r = 0.5$.

invest few resources into attractiveness will increase their investment into reproduction. Thus a weak investment of males into attractiveness is beneficial for two reasons: (i) pollinators will preferentially visit females and (ii) pollinators are more likely to carry pollen because males invest a lot into reproduction (Fig. 4.3b, compare plain and dashed purple curves).

Densities at equilibrium

The impact of the effective visitation rates on plant demography depends both on the female reproductive efficiency and on the production rate of ovules. The higher the effective visitation rate needed to fertilize female's ovules (high values of h), the lower the reproductive efficiency. Regardless of the scenario of resource limitation, the higher the female reproductive efficiency, the higher the plant density at equilibrium. This is because for similar effective visitation rates and ovule production, higher female reproductive efficiency leads to higher seed production.

When assuming no resource limitation, plant density at equilibrium follows the same trend as the effective visitation rate (there is no variation in ovule production), and the highest densities are obtained for populations in which females are more or as equally attractive as males (Fig. 4.4). On the other hand, when assuming resource limitation,



(a) (b)

Figure 4.4: Plant density as a function of (a) female attractiveness or (b) male attractiveness, for low ($h = 0.5$, green), intermediate ($h = 0.05$, orange) and high ($h = 0.001$, black) reproductive efficiency. The higher effective visitation rate needed to fertilize all ovules of a female (high values of h), the lower the reproductive efficiency. Plain lines depict the scenario of unlimited resources, and dashed lines the scenario of resource limitation (trade-off between attractiveness and investment in reproduction). (a) Male attractiveness (a_m) and male efficiency to transfer pollen (γ) are fixed, with $a_m = 10$ and $\gamma = 0.0025$. (b) Female attractiveness (a_f) and female ovule production (r) are fixed, with $a_f = 10$ and $r = 0.5$.

the highest plant densities are obtained when females invest little in attractiveness thus investing most of their resources into ovule production (Fig. 4.4a). If females invest most of their resources into attractiveness, though it does increase the effective visitation rates (see above) and most of their ovules are likely to be fertilized, the lower investment into ovule production translates into a low seed production, thus a low plant density.

Similarly, the highest plant densities are also observed when males invest most of their resources into reproduction, and have a low attractiveness (4.4b). Indeed, a low male attractiveness compared to female attractiveness enhances female visitation rates, and a high investment into reproduction increases the likelihood that a pollinator will carry pollen when visiting a female. Thus, plant density at equilibrium is expected to be higher when both sexes invest more in reproduction than in attractiveness.

What sexual dimorphism can we expect in a dioecious population?

First, from equation (4.6), a mutation that affects males will invade only if:

$$W_{M_x} > W_M \quad \Rightarrow \quad \frac{a_{m_x}}{a_m}\,\frac{\gamma_x}{\gamma} > 1 \quad \underset{resource\ limitation}{\Rightarrow} \quad \frac{I_x}{I}\,\frac{\frac{1-I_x}{1-I_x+s\ I_x}}{\frac{1-I}{1-I+s\ I}} > 1. \tag{4.9}$$

One should note that the evolution of male attractiveness depends neither on population characteristics nor on female strategy. The most favorable male is the one that attracts pollinators and transfers its pollen to pollinators more than the others. With unlimited resources, γ_x and γ are equal, so evolution would infinitely lead to higher attractiveness. Under the scenario of resource limitation, the evolutionary outcome depends on the relationship between the investment of a male into reproduction and its efficiency to transfer pollen to pollinators (γ, Fig. 4.2). In case of a concave relationship, males will invest more than half of their resources into attractiveness at the ESS, because increasing the investment into attractiveness has low negative impact on the transfer of pollen (Fig. 4.5, dotted line). In case of a linear relationship, males will invest exactly half of their resources into attractiveness at the ESS (Fig. 4.5, plain line). Finally, in case of a convex relationship, males will invest less than half of their resources into attractiveness at the ESS (Fig. 4.5, dashed line).

Regarding the females, from equation (4.7), a mutation will invade only if:

$$\begin{aligned} W_{F_x} > W_F \quad &\Rightarrow \quad \frac{r_x}{r}\left(1-\delta+\delta\,\frac{a_{f_x}}{a_f}\right) > 1 \\ &\underset{resource\ limitation}{\Rightarrow} \quad \frac{\frac{1-I_x}{1-I_x+s\ I_x}}{\frac{1-I}{1-I+s\ I}}\left(1-\delta+\delta\frac{I_x}{I}\right) > 1, \end{aligned} \tag{4.10a}$$

with $\delta = (1 - \frac{\widehat{V_{f_x m}}}{h+\widehat{V_{f_x m}}})$, the pollen limitation of a female mutant (defined as 1 minus the seed set).

Without resource limitation, evolution would infinitely lead to greater attractiveness of female plants. Thus, when neither males nor females are limited by resources, we expect no dimorphism, with both males and females being extremely attractive for pollinators. On the contrary, in the scenario of resource limitation, the higher the pollen limitation, the more females invest into attractiveness (Fig. 4.5). Therefore, if pollen limitation is negligible ($\delta \to 0$), the invasibility of a mutant does not depend on its attractiveness, and female mutants are selected for as soon as they produce more ovules than resident ones ($\frac{r_x}{r} > 1$). In this case, we expect a large dimorphism at the ESS, with females investing no resources into attractiveness (Fig. 4.5), and males investing around half of their resources into attractiveness.

With extreme pollen limitation ($\delta \to 1$), male and female invasibility gradients are similar. In this case, the female ESS is similar to that of a male: the investment of females into attractiveness will be high (around half of their resources) and the evolutionary outcome will depend on the relationship between the investment of a female into reproduction and its ovule production rate (r , Fig. 4.2). Thus, with high pollen limitation, if the relationship is the same for males and females (*i.e.* same value of s), we expect no dimorphism at the ESS (Fig. 4.5, compare same line types for males and females). Some dimorphism may, however, evolve if the relationship between investment into reproduction and reproductive output differs between males and females (Fig. 4.5, compare different line types for males and females). For example, a linear relationship in females and a concave relationship in males would lead to a population where at the ESS males are more attractive than females. On the contrary, a linear relationship in females and a convex relationship in males would lead to a population where females are more attractive than males at the ESS.

How does the co-variation of plant attractiveness and pollen limitation affect the evolution of female attractiveness?

As previously shown, dimorphism at the ESS greatly depends on the occurrence and the strength of pollen limitation. However, pollen limitation is not a fixed parameter of our model but varies along with the attractiveness strategies of males and females. In the following, we will focus on three evolutionary trajectories of female attractiveness that lead to different levels of dimorphism (depicted by the three dots in Fig. 4.5 and Fig. 4.6). As the evolution of male strategy depends neither on demographic properties nor on female strategy, it is assumed to be at the ESS.

In all scenarios, the more females invest into attractiveness, the higher their effective visitation rate (see above). On the other hand, the more females invest into reproduction, the higher their ovule production. If pollen is not overly limiting, higher ovule production

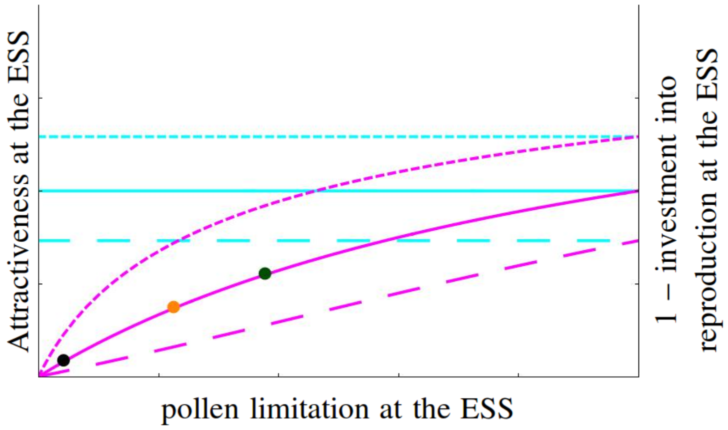


Figure 4.5: Evolutionary outcome as a function of pollen limitation at the ESS (δ). Female strategy at ESS is depicted by pink curves and male strategy by blue lines. The relationship between investment into reproduction and reproductive strategy (γ or r) is assumed to be convex ($s = 3$, dashed lines), linear ($s = 1$, plain lines) or concave ($s = 0.3$, dotted lines). Three possible outcomes for the evolution of female attractiveness (assuming a linear relationship between investment into reproduction and male or female reproductive characteristics, $s = 1$ and assuming male strategy to be at the ESS) depending on the female reproductive efficiency considered are depicted by the dots ($h = 0.001$ in black, $h = 0.05$ in orange and $h = 0.5$ in green).

will increase seed set, and therefore allow for mutants with a lower attractiveness will be selected for in a population in which females are initially highly attractive. Indeed, as presented above, pollen limitation is likely to be low in such a population, for two reasons: (i) high female attractiveness promotes high effective visitation rates, and (ii) the low density in such populations decreases the competition among plants for pollinators. Thus, the decrease of their attractiveness, which leads to an increase in their investment into ovule production, allows a higher seed production. On the opposite, in a population in which females initially invest only a little into attractiveness, pollen limitation is likely to be high. In such a population, higher investment into attractiveness is selected for because it increases the female visitation rate, which allows a better fertilization of its ovules, whereas higher investment into reproduction does not allow higher seed production because of pollen limitation. As shown in Fig. 4.6, the outcome of the evolution of attractiveness depends on female characteristics, and especially on their reproductive efficiency. Indeed, pollen limitation strongly depends on the visitation rate required to fertilize its ovules (h). The lower the visitation rate needed to fertilize its ovules, the lower the pollen limitation. Thus, in a population in which females only require a low visitation rate to fertilize ovules, the investment into attractiveness is unnecessary and selection favors a great dimorphism (black trajectory, Fig. 4.6). In populations with lower female reproductive efficiency, higher attractiveness is selected for, in order to promote higher female visitation rates (orange and green trajectories, Fig. 4.6).

The evolutionary outcome also depends on male characteristics (not shown). The lower the male attractiveness, the more pollinators will visit females rather than males. Thus, the lower the male attractiveness, the higher the pollen transfer and the lower the pollen limitation. Similarly, the higher the male efficiency to transfer pollen to pollinators (γ) and the lower the rate of pollen loss (λ or u), the lower the pollen limitation. Thus, males that invest more into reproduction and less into attractiveness reduce pollen limitation and enhance the selection for low female attractiveness, and high female ovule production.

Thus, population with no dimorphism or with females being more attractive than males are predicted only in populations suffering from extremely high pollen limitation or in which male and female investment into reproduction have different impacts on their reproductive characteristics (Fig. 4.5). Because such an extreme pollen limitation is not demographically sustainable, populations displaying a dimorphism with females being more attractive than males is predicted only if decreasing the investment in reproduction leads to a small negative effect on ovule production in females (concave relationship), but a strong negative impact on the efficiency of pollen transfer by the males (convex relationship, data not shown).

4.4 Discussion

Dioecious plant species often display sexual dimorphism, especially on floral traits (Barrett and Hough 2013; Delph et al. 1996). In most dioecious plant species, males seem to invest more in floral traits than females, especially considering flower number and flower size, but also with regards to the quantity of reward and floral scents (Ashman 2009; Delph et al. 1996; 2002). Although in fewer species, females can also display larger flowers than males, especially in tropical areas (Delph et al. 1996). In animal-pollinated species, these floral traits are involved in pollinator attraction (Martin 2004; Mitchell et al. 2004). Because attractiveness and rewards are expected to increase the number and duration

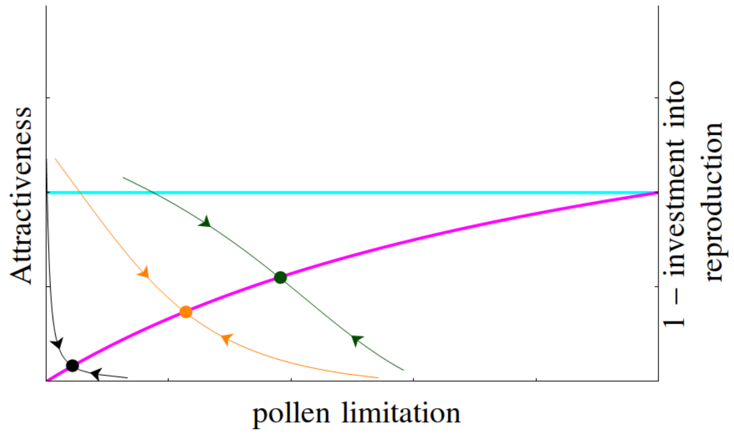


Figure 4.6: Three evolutionary trajectories (thin lines) corresponding to $h = 0.001$ (black), $h = 0.05$ (orange) and $h = 0.5$ (green). Female strategy at ESS is depicted in pink and male strategy in blue. The trajectories are calculated assuming male strategy to be at the ESS ($a_m = 10$, $\gamma = 0.0025$) and assuming a linear relationship between investment into reproduction and male or female reproductive characteristics, $s = 1$.

of pollinator visits, the sex with the strongest investment in such traits is the one under stronger selective pressure to increase access to mates.

Sexual dimorphism is expected to arise from selection that acts either on one sex only or unequally in both sexes. Bateman's principle suggests sexual dimorphism could arise if the relationship between mating success (access to mating partners) and reproductive success (number and quality of offspring) differs between male and female individuals: males would tend to be limited by mate availability, *i.e.* by the number of reproductive events, whereas females would be limited by the resources needed to produce their offspring (Bateman 1948). Although it has mainly been used to describe sexual selection and subsequent evolution of sexual dimorphism in animal species, some authors now considered that this principle also applies to flowering plants (Barrett and Hough 2013). Indeed, it seems acceptable to assume that female reproductive success is more limited by resources invested in seed production than by pollen deposition, whereas male reproductive success would be more limited by access to mates. Therefore, sexual selection is expected to act on floral traits involved in pollinator attraction, which could explain why male plants seem to be more attractive to pollinators than females in many species.

Yet, several features of plant reproduction are strikingly different from animal reproduction, and could affect the correct application of Bateman's principle to plants. First, male plants do not actively seek female partners and female plants do not directly choose males. Indeed, unlike in animals, the way sexual selection operates on male and female traits depends on a "third partner", the pollinators, which must visit both male and female plants for pollination to be achieved. Because most insects show floral constancy (Chittka et al. 1999), meaning that they tend to perform sequences of successive visits on homogeneous categories of flowers, pollination could be hampered if only or mostly one sex receives pollinators visits because flowers of male and female plants are not sufficiently similar. Second, pollen limitation seems to be common in plant species, suggesting that females could be as limited by mate access as males (Ashman et al. 2004; Burd 1994). This therefore implies that because of pollen limitation, the assumptions needed for the application of Bateman's principle is quite not suited for plant species (Burd 1994).

Our results suggest that the arguments both supporting and refuting the applicability of Bateman's principle to plants are justified, depending on the degree of pollen limitation. When pollen limitation is low, a strong sexual dimorphism is expected, with males investing more resources into attractiveness than females. In this case, male evolution is driven by male-male competition for mates, whereas females are selected for their ovule production (see also Vamosi and Otto 2002). However, as soon as there is pollen limitation, female evolution is also driven by mating success, and in case of extreme pollen limitation, selection acts similarly on males and females. This leads to selection for an increase in female attractiveness in order to reduce pollen limitation. Pollen limitation can therefore hamper the evolution of sexual dimorphism, a result which seems consistent with the few studies conducted on floral traits selection. Selection pressures on floral traits seems to differ between male and female plants in the dioecious species *Silene latifolia*, with a pattern consistent with observed sexual dimorphism (Delph and Herlihy 2012). There is also evidence that the strength of selection on attractive traits can increase with greater pollen limitation (Ashman and Morgan 2004).

One should note that, for the sake of simplicity, we assumed independent evolution in males and females. Indeed, correlation among male and female traits is not complete (Steven et al. 2007), and at least in *Silene latifolia*, the variance in floral traits expressing sexual dimorphism was explained by sex-specific QTL loci (Delph et al. 2010). This suggests that sexual dimorphism can arise in dioecious plant species for at least some floral traits. Correlation of traits among sexes would impede the evolution of sexual dimorphism, and accentuate sexual conflict. However this is beyond the scope of this study, and would need deeper analysis.

The evolutionary outcome strongly depends on the impact of investment into reproduction on actual reproductive characteristic (the values of s, Fig. 4.5 and Fig. 4.6) for both males and females. However, no data is available on the relationship between investment in reproduction and reproductive characteristics. A concave relationship depicts the fact that decreasing investment into reproduction may at first have low impact on reproduction. This kind of relationship should be expected for example for males in species that produce more gametes than can be exported. A linear relationship is expected if a decrease in the investment into reproduction leads to a decrease of gamete production, translating into a decrease of reproductive success. This may be the case for females that produce costly gametes. Empirical studies that quantify relationships between the investment into reproduction and male and female reproductive success are thus critically needed for a better understanding of the evolution of dimorphism.

Our model predicts that males should be more attractive than females in most dioecious plant populations, a result which agrees with the observed trend in natural populations. However, our model predicts that populations favoring the evolution of high female attractiveness, *i.e.* low dimorphism or dimorphism with females being more attractive than males, are usually demographically non viable. This suggests that sexual selection on attractiveness may not allow for the evolution of a dimorphism where females are more attractive than males. Yet, such dimorphism, although rarer, is observed in nature, suggesting that it could be due to other selective pressures. For example, Delph et al. (1996) suggested that functional constraints could lead to large female flowers, as perianth size could be correlated to the number of ovules.

To our knowledge, no previous study explicitly integrated pollinator behavior and pollinator dynamics on the evolution of sexual dimorphism. In the model proposed by Vamosi and Otto (2002), the analysis of the conditions for the evolution of sexual dimorphism in plants was carried out assuming a fixed pollen limitation, and the impact of dimorphism on small plant populations, with limited events of pollination. In their study, pollinators were integrated as a fixed parameter, which prevented to take into account (i) explicit visitation patterns of pollinators on male and female plants and (ii) feed-backs between plant-pollinator dynamics and the evolution of floral traits. Vamosi and Otto (2002) have suggested that extreme sexual dimorphism could lead plant populations to extinction and they argued that this could explain the rarity of dioecious species in flowering plants.

By merging demography and evolution in our model, we draw different conclusions. Indeed, in our model pollen limitation varies along with plant and pollinator densities and with plant characteristics. By explicitly considering plant demography, we find that populations evolving to high dimorphism with males being more attractive than females are the largest ones, and suffer from low pollen limitation. Thus, sexual dimorphism with males being more attractive than females does not seem to threaten dioecious populations. One should note that although low plant attractiveness or low population density decreases pollinator visitation rates in our model, we assumed it had a low impact on pollinator density. For a given attractiveness, a plant individual in a small population receives more visits than in a large population, because this individual is in competition with fewer individuals. This may be inconsistent with some empirical studies that showed that small population may receive fewer pollinator visits than large populations (*e.g.* Klinkhamer and De Jong 1990). However, this does not modify the generality of our results, since populations with a large dimorphism are the largest ones, and would thus receive more visits, leading to a decrease of pollen limitation, and favoring higher dimorphism. On the contrary, in populations with a low dimorphism, which are usually small, a decrease in pollinator density would increase pollen limitation, selecting against a higher dimorphism.

Moreover, our model shows that the higher the female reproductive efficiency (*i.e.* low visitation rate is needed to fertilize their ovules), the larger the expected dimorphism at ESS, with females investing only few resources into attractiveness. In case of sudden pollinator shortage, dimorphism should hamper pollen transfer (reduced effective visitation rate with high dimorphism, see also Vamosi and Otto 2002). However, we predict that extreme dimorphism should go along with high female efficiency, implying that in spite of low pollen transfer, there should still be some seed production. This female characteristic could therefore counteract the deleterious impact of dimorphism on pollinator visitation rates, and highlights the fact that dimorphism may not represent an evolutionary suicide, even in stochastic environments.

Acknowledgements

This work was supported by a grant from the Region Nord-Pas de Calais to C.L.

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4.A Appendix: Visitation rates derivation

Let the total density of male and female plants be M and F respectively, and the density of pollinators be P. As discussed in the main text and scheme in Figure 4.1, plant and pollinator individuals can be divided among:

- Free pollinators without pollen (density $[P]$) *v.s.* free pollinators carrying pollen (density $[P_m]$).
- Unoccupied male (density $[M]$) and female (density $[P]$) plants.
- Plant and pollinators in interaction. The density of plant individuals visited by pollinators without pollen will be denoted as $[MP]$ and $[FP]$. The density of individuals visited by a pollinator carrying pollen will be denoted as $[MP_m]$ and $[FP_m]$.

We assume the interactions among plants and pollinators to increase linearly with their densities (according to plant attractiveness). This implies that the environment is homogeneous, so that a pollinator randomly meets males and females individuals randomly, and interacts with them according to their attractiveness. The interactions scheme in Figure 4.1 yields the following system of ODE:

$$\left\{\begin{aligned}
\frac{d[M]}{dt} &= -\,a_m\ [M]\,[P] - a_m\ [M]\,[P_m] + D_m\ [MP] + D_m\ [MP_m]\,, && (4.11a)\\
\frac{d[F]}{dt} &= -\,a_f\ [F]\,[P] - a_f\ [F]\,[P_m] + D_f\ [FP] + D_f\ [FP_m]\,, && (4.11b)\\
\frac{d[P]}{dt} &= -\,a_m\ [M]\,[P] - a_f\ [F]\,[P] + D_m\,(1-\gamma)\ [MP] + D_f\ [FP] && \\
&\quad + D_m\,(1-\gamma)\,u_m\ [MP_m] + D_f\,u_f\ [FP_m] + \lambda\ [P_m]\,, && (4.11c)\\
\frac{d[P_m]}{dt} &= -\,a_m\ [M]\,[P_m] - a_f\ [F]\,[P_m] + D_m\,\gamma\ [MP] + D_m\,\gamma\ [MP_m] && \\
&\quad + D_m\,(1-\gamma)(1-u_m)\ [MP_m] + D_f\,(1-u_f)\,[FP_m] - \lambda\ [P_m]\,, && (4.11d)\\
\frac{d[MP]}{dt} &= a_m\ [M]\,[P] - D_m\ [MP]\,, && (4.11e)\\
\frac{d[MP_m]}{dt} &= a_m\ [M]\,[P_m] - D_m\ [MP_m]\,, && (4.11f)\\
\frac{d[FP]}{dt} &= a_f\ [F]\,[P] - D_f\ [FP]\,, && (4.11g)\\
\frac{d[FP_m]}{dt} &= a_f\ [F]\,[P_m] - D_f\ [FP_m]\,, && (4.11h)
\end{aligned}\right.$$

First, we assumed the interactions to occur almost instantaneously at the time-scale of plant and pollinator density variations. This separation of time-scale allows us to consider the fast processes to be at equilibrium at the time scale of demographic variations (quasi steady state approximation). Thus, all the above ODE equal 0, implying

$$\begin{aligned}[MP] &= \frac{a_m}{D_m}[M][P] \ , \quad [MP_m] = \frac{a_m}{D_m}[M][P_m] \ , \\ [FP] &= \frac{a_f}{D_f}[F][P] \text{ and } \quad [FP_m] = \frac{a_f}{D_f}[F][P_m] \, . \end{aligned} \tag{4.12a}$$

Substituting equation (4.12) into (4.11d) yields

$$\frac{d[P_m]}{dt} = 0 \Rightarrow [P_m] = \frac{a_m \ \gamma \ [M]}{a_m\gamma[M] + \lambda + a_m(1-\gamma)u_m[M] + a_f u_f [F]} \left([P] + [P_m]\right). \tag{4.13}$$

Second, assuming that the density of occupied plant individuals at any time is negligible relative to the density of unoccupied ones, the total densities of male and female plants (M and F) and the total density of pollinators (P) can be written as a function of the variables involved in individual interactions:

$$M = [M] + [MP] + [MP_m] \approx [M] \, , \tag{4.14a}$$

$$F = [F] + [FP] + [FP_m] \approx [F] \, , \tag{4.14b}$$

$$P = [P] + [P_m] + [MP] + [MP_m] + [FP] + [FP_m] \, . \tag{4.14c}$$

Substituting equations (4.12), (4.14a) and (4.14b) into equation (4.14c) yields

$$[P] + [P_m] = \frac{P}{\left(1 + \frac{a_m}{D_m} \ [M] + \frac{a_f}{D_f} \ [F]\right)} \approx \frac{P}{\left(1 + \frac{a_m}{D_m} \ M + \frac{a_f}{D_f} \ F\right)}.$$

The visitation rate of one male individual (V_m) by all pollinators is given by the rate of creation of new complex of interaction $[MP]$ and $[MP_m]$:

$$\begin{aligned} V_m = \frac{a_m \ [M] \ ([P] + [P_m])}{[M]} &= \frac{a_m}{\left(1 + \frac{a_m}{D_m} \ [M] + \frac{a_f}{D_f} \ [F]\right)} \ P \\ &\approx \frac{a_m}{\left(1 + \frac{a_m}{D_m} \ M + \frac{a_f}{D_f} \ F\right)} \ P. \end{aligned}$$

Similarly, the visitation rate of one female individual (V_f) by all pollinators is given by the rate of creation of new complex of interaction $[FP]$ and $[FP_m]$:

$$V_f = \frac{a_f \ [F]\left([P]+[P_m]\right)}{[F]} = \frac{a_f}{\left(1+\frac{a_m}{D_m}\ [M]+\frac{a_f}{D_f}\ [F]\right)}\ P \approx \frac{a_f}{\left(1+\frac{a_m}{D_m}\ M+\frac{a_f}{D_f}\ F\right)}\ P.$$

The visitation rate of one female individual by pollinators carrying pollen (V_{fm}) is given by the rate of creation of new complex of interaction $[FP_m]$:

$$V_{fm} = \frac{a_f\,[F]\,[P_m]}{[F]} = a_f\ \left([P]+[P_m]\right)\ \frac{a_m\ \gamma\ [M]}{a_m\ \gamma\ [M]+\lambda+a_m\ (1-\gamma)\ u_m\ [M]+a_f\ u_f\ [F]},$$

$$V_{fm} \approx \left(\frac{a_f}{\left(1+\frac{a_m}{D_m}\ M+\frac{a_f}{D_f}\ F\right)}\right)\ \left(\frac{a_m\ \gamma\ M}{a_m\ \gamma\ M+\lambda+a_m\ (1-\gamma)\ u_m\ M+a_f\ u_f\ F}\right)\ P.$$

One should notice those visitation rates increase linearly with pollinator density, meaning there is no competition among pollinators for the plants. For high pollinator densities, one should note the assumption that the density of occupied plant individuals at any given time is negligible relative to the density of unoccupied ones does not hold any more, and visitation rate should be modified accordingly.

4.B Appendix: Fitness of a rare mutant

Mutation with an effect in males

A rare mutation that affects only males (mutants are denoted M_x) emerges in the resident population. The new interactions involving the mutant, or pollinators carrying pollen from the mutant are:

$$\begin{aligned}
[M_x]+[P] &\xleftrightarrow[D_{m_x}(1-\gamma_x)]{a_{m_x}} [M_xP] && \xrightarrow{D_{m_x}\gamma_x} [M_x]+[P_x]\\
[M_x]+[P_m] &\xleftrightarrow[D_{m_x}(1-\gamma_x)(1-u_{m_x})]{a_{m_x}} [M_xP_m] && \xrightarrow{D_{m_x}\gamma_x} [M_x]+[P_x]\\
& && \xrightarrow{D_{m_x}(1-\gamma_x)u_{m_x}} [M_x]+[P]\\
[M_x]+[P_x] &\xleftrightarrow[D_{m_x}(\gamma_x+(1-\gamma_x)(1-u_{m_x}))]{a_x} [M_xP_x] && \xrightarrow{D_{m_x}(1-\gamma_x)u_{m_x}} [M_x]+[P]\\
[F]+[P_x] &\xleftrightarrow[D_f(1-u_f)]{a_f} [FP_x] && \xrightarrow{D_fu_f} [F]+[P]\\
[P_x] &\xrightarrow{\lambda_x} [P],
\end{aligned}$$

From those interactions, we deduce the new equations:

$$\begin{aligned}
\frac{d\,[P_x]}{dt} &= -a_m\,[M]\,[P_x]-a_f\,[F]\,[P_x]-a_{m_x}\,[M_x]\,[P_x]\\
&\quad+D_m(1-\gamma)(1-u_m)\,[MP_x]+D_f(1-u_f)\,[FP_x]\\
&\quad+D_{m_x}(1-\gamma_x)(1-u_{m_x})\,[M_xP_x]-\lambda_x\,[P_x]\\
&\quad+D_{m_x}\gamma_x\,[MP]+D_{m_x}\gamma_x\,[MP_m]+D_{m_x}\gamma_x\,[MP_x]\\
\frac{d\,[MP_x]}{dt} &= a_m\,[M]\,[P_x]-D_m\,[MP_x]\\
\frac{d\,[FP_x]}{dt} &= a_f\,[F]\,[P_x]-D_f\,[FP_x]\\
\frac{d\,[M_xP]}{dt} &= a_{m_x}\,[M_x]\,[P]-D_{m_x}\,[M_xP]\\
\frac{d\,[M_xP_m]}{dt} &= a_{m_x}\,[M_x]\,[P_m]-D_{m_x}\,[M_xP_m]\\
\frac{d\,[M_xP_x]}{dt} &= a_{m_x}\,[M_x]\,[P_x]-D_{m_x}\,[M_xP_x]\,.
\end{aligned}$$

As previously, we assumed the interactions to occur almost instantaneously at the time-scale of plant and pollinator density variations. This separation of time-scale allows us to consider the fast processes to be at equilibrium at the time scale of demographic variations (quasi steady state approximation). Thus, all the above ODE equal 0:

$$[MP_x]=\frac{a_m}{D_m}\,[M]\,[P_x]\ \ ,\ \ [FP_x]=\frac{a_f}{D_f}\,[F]\,[P_x]\ \ ,\ \ [M_xP]=\frac{a_{m_x}}{D_{m_x}}\,[M_x]\,[P]\,,$$

$$[M_xP_m]=\frac{a_{m_x}}{D_{m_x}}\,[M_x]\,[P_m]\,\text{and}\quad [M_xP_x]=\frac{a_{m_x}}{D_{m_x}}\,[M_x]\,[P_x]\,,$$

leading to:

$$[P_x] = \frac{a_{m_x}\ \gamma_x\ [M_x]}{a_m\gamma[M]+a_{m_x}\gamma_x[M_x]+\lambda_x+a_m(1-\gamma)u_m[M]+a_{m_x}(1-\gamma_x)u_{m_x}[M_x]+a_f u_f[F]}\ ([P]+[P_m]+[P_x]).$$

As previously, we assumed the density of occupied plant individuals at any time is negligible relative to the density of unoccupied ones: $M_x \approx [M_x]$. The total density of pollinators (P) can be written as:

$$\begin{aligned} P &= [P]+[P_m]+[P_x]+[MP]+[MP_m]+[MP_x] \\ &\quad +[M_xP]+[M_xP_m]+[M_xP_x]+[FP]+[FP_m]+[FP_x] \qquad (4.4a) \\ \Rightarrow [P]+[P_m]+[P_x] &= \frac{P}{\left(1+\frac{a_m}{D_m}\ [M]+\frac{a_{m_x}}{D_{m_x}}\ [M_x]+\frac{a_f}{D_f}\ [F]\right)} \\ \Rightarrow [P]+[P_m]+[P_x] &\approx \frac{P}{\left(1+\frac{a_m}{D_m}\ M+\frac{a_{m_x}}{D_{m_x}}\ M_x+\frac{a_f}{D_f}\ F\right)}. \end{aligned}$$

The visitation rate of one female individual by pollinators carrying pollen from the mutant (V_{fm_x}), *i.e.* the siring success of a male mutant, is given by the rate of creation of new complex of interaction $[FP_x]$. Assuming the density of mutants to be negligible compared to the density of residents, V_{fm_x} is given by:

$$\begin{aligned} V_{fm_x} &= \frac{a_f\,[F]\,[P_x]}{[F]}, \\ &= a_f\ \frac{a_{m_x}\ \gamma_x\ [M_x]}{a_m\gamma[M]+a_{m_x}\gamma_x[M_x]+\lambda_x+a_m(1-\gamma)u_m[M]+a_{m_x}(1-\gamma_x)u_{m_x}[M_x]+a_f u_f[F]}\ ([P]+[P_m]+[P_x]), \\ V_{fm_x} &\approx \left(\frac{a_f}{1+\frac{a_m}{D_m}\ M+\frac{a_f}{D_f}\ F}\right)\ \left(\frac{a_{m_x}\ \gamma_x\ M_x}{a_m\ \gamma\ M+\lambda_x+a_m\ (1-\gamma)\ u_m\ M+a_f\ u_f\ F}\right)\ P. \end{aligned}$$

Mutation with an effect in females

A rare mutation that affects only females (mutants are denoted F_x) emerges in the resident population. The new interactions involving the mutants are:

$$[F_x]+[P] \xleftrightarrow[D_{f_x}]{a_{f_x}} [F_xP]$$

$$[F_x]+[P_m] \xleftrightarrow[D_{f_x}(1-u_{f_x})]{a_{f_x}} [F_xP_m] \qquad \xrightarrow{D_{f_x}u_{f_x}} [F_x]+[P]$$

From those interactions, we deduce the new equations:

$$\begin{aligned}
\frac{d\left[F_xP\right]}{dt} &= a_{f_x}\left[F_x\right]\left[P\right] - D_{f_x}\left[F_xP\right] \\
\frac{d\left[F_xP_m\right]}{dt} &= a_{f_x}\left[F_x\right]\left[P_m\right] - D_{f_x}\left[F_xP_m\right].
\end{aligned}$$

As previously, we assumed the interactions to occur almost instantaneously at the time-scale of plant and pollinator density variations. This separation of time-scale allows us to consider the fast processes to be at equilibrium at the time scale of demographic variations (quasi steady state approximation). Thus, all the above ODE equal 0:

$$\left[F_xP\right] = \frac{a_{f_x}}{D_{f_x}}\left[F_x\right]\left[P\right] \text{ and } \left[F_xP_m\right] = \frac{a_{f_x}}{D_{f_x}}\left[F_x\right]\left[P_m\right].$$

As previously, we assumed the density of occupied plant individuals at any time is negligible relative to the density of unoccupied ones: $F_x \approx [F_x]$. The total density of pollinators (P) can be written as:

$$\begin{aligned}
P &= [P] + [P_m] + [MP] + [MP_m] + [FP] + [FP_m] + [F_xP] + [F_xP_m] \\
\Rightarrow [P] + [P_m] &= \frac{P}{\left(1 + \frac{a_m}{D_m}\ [M] + \frac{a_f}{D_f}\ [F] + \frac{a_{f_x}}{D_{f_x}}\ [F_x]\right)} \\
\Rightarrow [P] + [P_m] &\approx \frac{P}{\left(1 + \frac{a_m}{D_m}\ M + \frac{a_f}{D_f}\ F + \frac{a_{f_x}}{D_{f_x}}\ F_x\right)}.
\end{aligned}$$

The visitation rate of one female mutant by pollinators carrying pollen (V_{f_xm}), *i.e.* the reproductive success of a female mutant, is given by the rate of creation of new complex of interaction $[F_xP_m]$. Assuming the density of mutants to be negligible compared to the density of residents, V_{f_xm} is given by:

$$\begin{aligned}
V_{f_xm} &= \frac{a_{f_x}\ \left[F_x\right]\left[P_m\right]}{\left[F_x\right]}, \\
&= a_{f_x}\ \frac{a_m\ \gamma\ [M]}{a_m\gamma[M]+\lambda+a_m(1-\gamma)u_m[M]+a_f u_f[F]+a_{f_x}u_{f_x}[F_x]}\ ([P]+[P_m]), \\
V_{f_xm} &\approx \left(\frac{a_{f_x}}{1 + \frac{a_m}{D_m}\ M + \frac{a_f}{D_f}\ F}\right)\ \left(\frac{a_m\ \gamma\ M}{a_m\ \gamma\ M + \lambda + a_m\ (1-\gamma)\ u_m\ M + a_f\ u_f\ F}\right)\ P.
\end{aligned}$$

Chapter 5

How does floral display modify pollinator behavior?

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Abstract

Pollinator foraging behavior strongly depends on plant traits, but the underlying mechanisms of this behavior are poorly known. In this study, we built individual-based models that include different basic pollinator behaviors, in particular regarding their attraction to floral display. We simulated pollinator foraging trajectories with these different models, and compared them to empirical data of bumblebees foraging in experimental arrays of *Mimulus ringens* (Mitchell et al. 2004). Models comparisons and calibration will be conducted under the Approximate Bayesian Computational (ABC) framework. This study will allow us determining the required assumptions one must make on pollinator behavior in order to accurately predict visitation patterns within a plant species.

Keywords

Plant-pollinator interactions, Bayesian framework, foraging, floral traits

5.1 Introduction

A major challenge in ecological modeling is to make reliable predictions about what will happen to real populations in real landscapes (van der Vaart et al. 2015b). However, there is often a wide separation between empirical studies that statistically describe precise individual behavior, and models that often consider simplified behaviors. Individual Based Models (IBMs) could be the key to shrink this gap.

Individuals do not act independently of each other, and the actions of individuals both influence and are influenced by the actions of other individuals. In particular, one central problem in ecology is to understand the impact of interspecific interactions on the dynamics of the populations. One of the strengths of IBMs is that population dynamics emerge from explicit interactions among individuals. Such models are mechanistic, *i.e.* they capture the underlying biological processes, and therefore they are expected to provide correct predictions even in novel environmental conditions (van der Vaart et al. 2015b).

The interplay between plant-pollinator interactions and plant reproductive success has received much attention from the empirical perspective. These studies highlighted the impact of plant traits on pollinator behavior. For example, pollinators may disproportionately visit individuals that display more flowers or larger ones (Martin 2004; Mitchell et al. 2004). Besides, pollinators tend to probe more flowers on plants with larger floral displays (Harder et al. 2004; Ohashi and Yahara 2001; Snow et al. 1996). These pollinator behaviors have been shown to strongly affect plant reproductive success and mating system. For example, the number of flowers a pollinator probes successively on a plant increases between-flowers self-pollination (Karron et al. 2009; Rademaker et al. 1999). It has also been suggested that differences of floral traits in dioecious plant species could hamper pollen transfer among sexes (Vamosi and Otto 2002).

Models that include plant-pollinator interactions have emphasized their importance both for plant demography (*e.g.* Fishman and Hadany 2010; Holland and DeAngelis 2010) and for the evolution of plant traits (*e.g.* Bailey et al. 2007; Devaux et al. 2014; Lepers et al. 2014). However, such deterministic models often consider a simplistic pollinator behavior, which may not reflect biological reality. On the opposite, the expansion of the optimal foraging theory allowed a better understanding of the mechanism underlying pollinator behavior (Pyke et al. 1977). Those models focus on pollinator energy income, and predict which pollinator movement is the most likely to provide the more energy. However, those models have limited predictive abilities, because most empirical data violate several of their assumptions: plant populations are large, without boundaries, whereas the populations used in empirical studies are often small and delimited. Moreover, the pollinator is assumed to visit many as within a foraging bout, and these models consider only pollinator choice among plants instead of considering the pollinator trajectory both on plants and on any other points of the space. Yet, at least in small populations, this assumption is likely to be false. For example, Mitchell et al. (2004) showed that in a population of 64 plant individuals, most pollinators only visited one flower within the entire population.

In this study, we built several individual based models that include different basic pollinator behaviors. A single pollinator will move in a continuous space that includes plants, and we will record the pollinator trajectory. The aim of this study is two-fold: (i) provide a better understanding of the mechanisms underlying pollinator behavior, especially concerning plant traits, and (ii) predict the impact of plant traits on plant fitness depending on pollinator behavior. For example, in the long run, this model could be used to predict the influence of sexual dimorphism in dioecious species when the pollinator behavior is finely taken into account. This would especially allow for comparison between models that assume a simplistic pollinator behavior, and models that include extensive pollination mechanisms. Similarly, this model could provide insights on the impact of plant community on plant mating system, *e.g.* determine what mechanisms underlie pollinator constancy (the propensity for an individual pollinator to visit flowers of a single floral type).

In a first step, we aim at predicting pollinator visitation bouts within a plant population that comprises individuals that display different flower numbers. We especially investigate what mechanisms affect pollinator behavior in regards with this plant trait (plant attractiveness, probability to stay longer on a plant). For this purpose, we simulated pollinator trajectories with several models of pollinator behavior, and compared those trajectories with data collected in experimental arrays of *Mimulus ringens* (Mitchell et al. 2004). Those data comprise extensive collection of pollinators (mainly Bumblebees) movement among plants in the arrays. This project is currently under progress, and I will present here only preliminary results, as well as the intended improvements.

5.2 Pollinator movements modeling

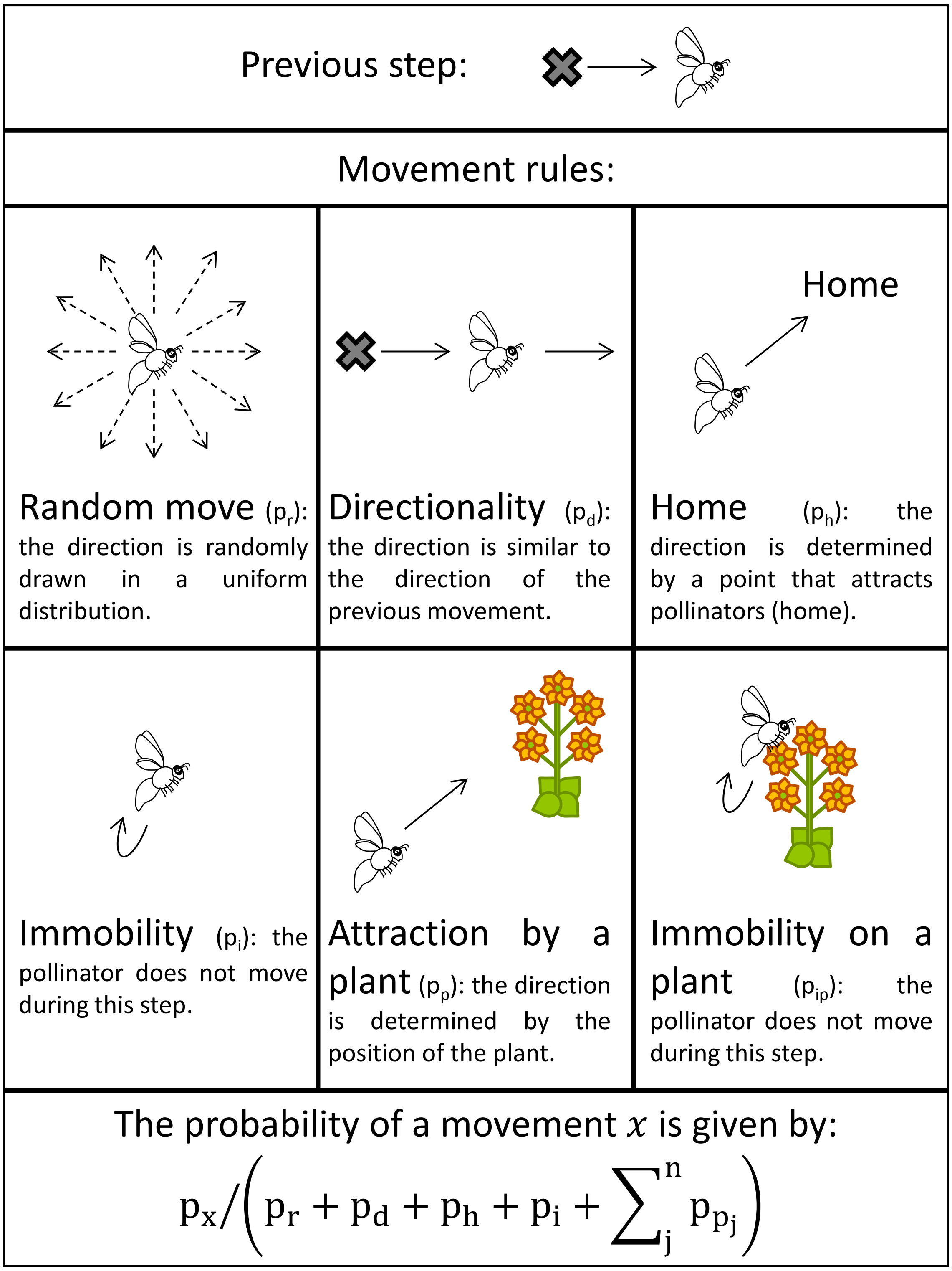
We modified the model by Berthelot et al. (in prep) and adapted it to plant-pollinators interactions. We performed individual-based simulations that track the motion of a unique pollinator that moves in a continuous space that includes plant individuals. We considered a discrete spatial motion: at each step, the pollinator can stop either on a plant or on any other point in the space (*i.e.* we track the whole pollinator trajectory, rather than its visitation bout). We assumed a plant to be visited if the pollinator stops within an area of a range r surrounding the plant position.

Each simulation is performed over t steps. At each step, the direction of the pollinator is randomly drawn in a multinomial distribution depending on the weights of different modalities: random movement (weight p_r), directionality (weight p_d), attraction by a special direction (weight p_h), immobility (weight p_i) and attraction by a plant (weight p_p). When a pollinator previously stopped on a plant, the immobility weight depends on the plant characteristic (weight p_{ip}). The detail of each of those rules is given in Fig. 5.1. The distance of the movement is drown randomly from a log-normal law (mean m and standard deviation sd).

Pollinator behavior regarding plants

This model is general and allows for any number (n) and position of plants within the space. The weight of attraction and the weight of immobility can be set independently for each individual plant. We may especially expect those weights to depend on plant traits, such as floral display or reward production. Indeed, a pollinator is likely to be more attracted by plants with larger floral displays, and to stay more on plants that offer rewards.

Regarding the movement of the pollinator to a plant, we considered four cases (Fig. 5.2). First, a pollinator that is attracted by a plant can either go in the direction of



Previous step:

Movement rules:

Random move (p_r): the direction is randomly drawn in a uniform distribution.

Directionality (p_d): the direction is similar to the direction of the previous movement.

Home (p_h): the direction is determined by a point that attracts pollinators (home).

Immobility (p_i): the pollinator does not move during this step.

Attraction by a plant (p_p): the direction is determined by the position of the plant.

Immobility on a plant (p_{ip}): the pollinator does not move during this step.

The probability of a movement x is given by:

$$p_x / \left(p_r + p_d + p_h + p_i + \sum_j^n p_{p_j} \right)$$

Figure 5.1: Rules of pollinator movements.

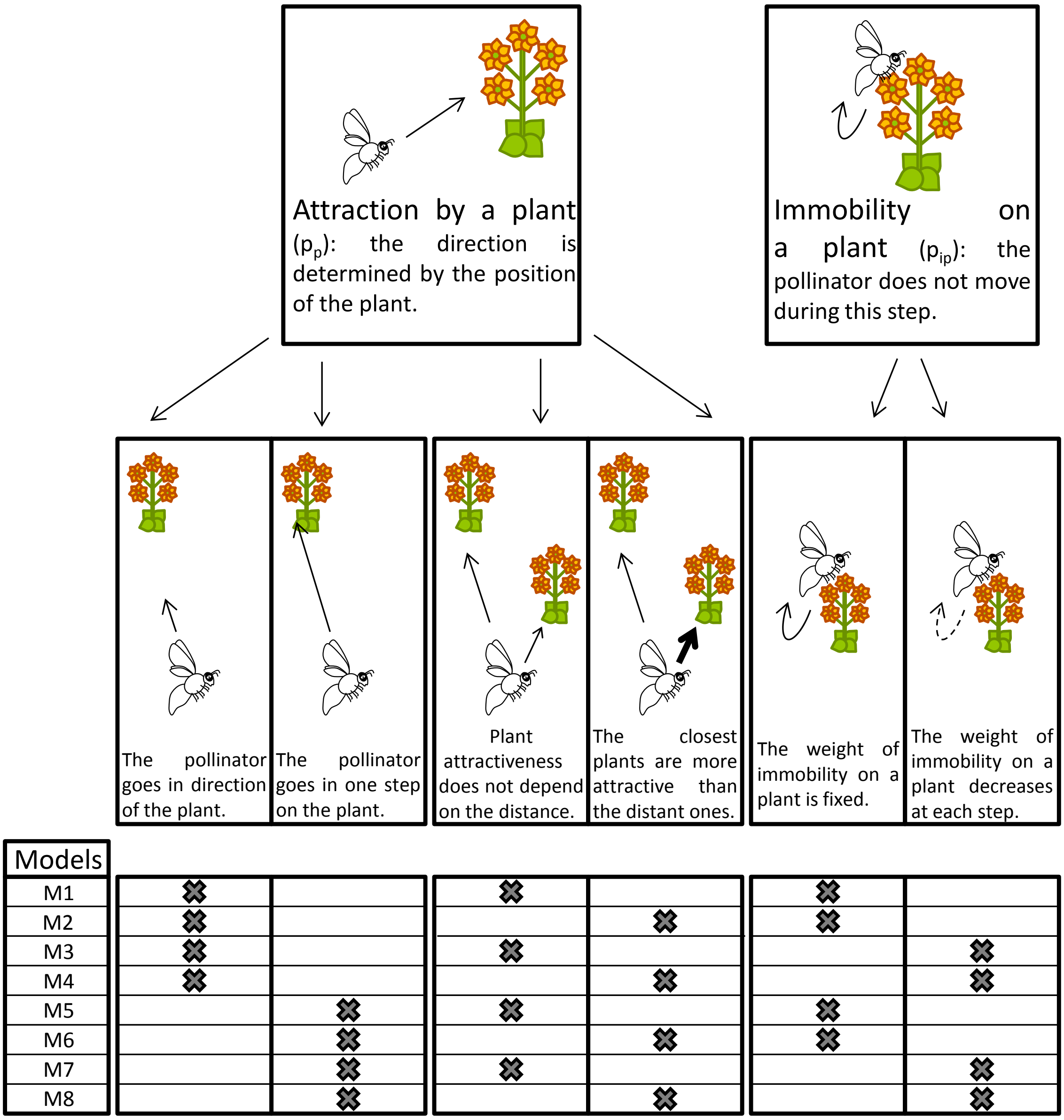
the plant (but not directly on the plant, models M1 to M4), or it can move directly on this plant (models M5 to M8). Second, pollinator attraction to a plant depends (models M2, M4, M6 and M8) *v.s.* does not depend (models M1, M3, M5 and M7) on the distance between the plant and the pollinator. When plant attractiveness depends on pollinator position, we assumed the plant(s) that are the closest to the pollinator to have an increased attractiveness compared to the plants that are further away (nearest neighbors attractiveness hypothesis).

Second, we assumed the weight of immobility on a plant either to be fixed (models M1, M2, M5, M6) or to decrease with the time already spent on the plant (models M3, M4, M7 and M8). Indeed, the time spent on an individual plant is likely to be proportional to the number of flowers visited on this individual. The more a pollinator stays on a plant, the lower the number of unvisited flowers on this individual. If the weight of immobility on a plant depends on the number of unvisited flowers available on this plant, it is likely to decrease with the time spent on an individual.

Data used to evaluate pollinator behavior

We used the published visitation bouts used by Mitchell et al. (2004). In their study, Mitchell et al. (2004) conducted pollination surveys, following individual foraging bouts of pollinators (bumblebees) on arrays of 36 plants (*Mimulus ringens* individuals). Plants floral display was manipulated each day, and plant individuals displayed either two, four, eighth or sixteen flowers (Fig. 5.3). The exact sequence of floral probes and plant visits was recorded, recording the identity of each visited plant, its position, and the number of flowers probed on that plant. This data set comprises 206 complete individual foraging bouts, that is foraging bouts for which the pollinator arrival and departure from the experimental array was recorded.

We used those data to evaluate which model predicts visitation bouts that are the closest to the observed visitation bouts. To this aim, we integrated 64 plants in our model. In order to determine if the weight of attractiveness and/or the weight of immobility on a plant depend on its floral display, we investigated several scenarios (Fig. 5.4). First, the weight of attractiveness of a plant (p_p) is either null (scenario 0 p_p), the same for every plant (scenario 1 p_p) or increased with plant floral display (scenario 4 p_p). Second, the



Models	The pollinator goes in direction of the plant.	The pollinator goes in one step on the plant.	Plant attractiveness does not depend on the distance.	The closest plants are more attractive than the distant ones.	The weight of immobility on a plant is fixed.	The weight of immobility on a plant decreases at each step.
M1	✖		✖		✖	
M2	✖			✖	✖	
M3	✖		✖			✖
M4	✖			✖		✖
M5		✖	✖		✖	
M6		✖		✖	✖	
M7		✖	✖			✖
M8		✖		✖		✖

Figure 5.2: Assumptions of the 8 models of pollinator behavior.

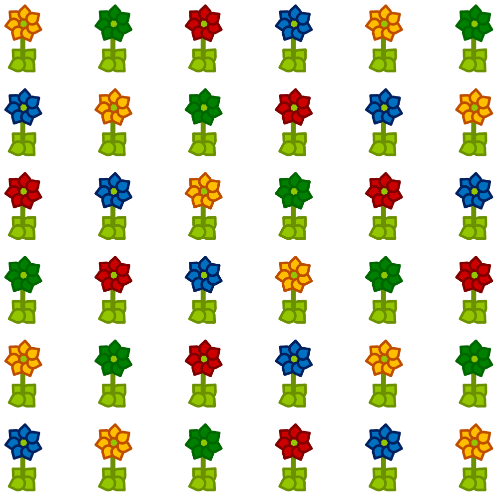


Figure 5.3: Plant floral display follows a regular arrangement (Latin square design). Colors depict plants with either two, four, eighth or sixteen flowers. Flower number depended on the day and garden of the pollination record (see Mitchell et al. 2004, for more details).

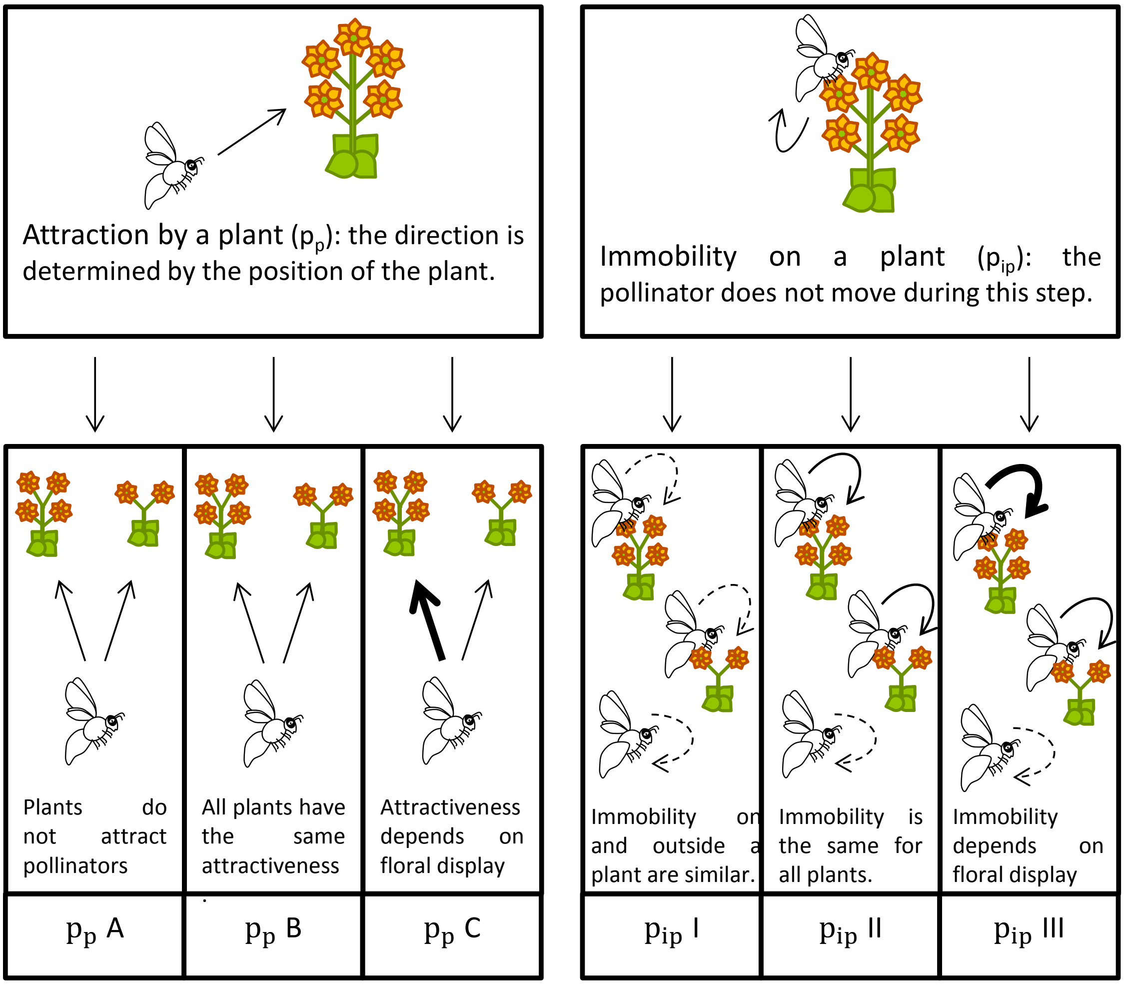
weight of immobility of a pollinator on a plant (p_{ip}) is either the same than outside a plant (p_i, scenario $0p_{ip}$), the same for all plants (scenario $1p_{ip}$) or increased with the plant floral display (scenario $4p_{ip}$).

For each model and parameter set, we simulated 206 visitation bouts, and compared it to the 206 real visitation bouts. For the moment, we only run simulations under model M5 and M8, each with three combination of scenarios regarding floral display: scenarios 0 p_p and 0 p_{ip} (107 412 runs with model M5 and 78 980 with model M8); scenarios 1 p_p and 1 p_{ip} (102 684 runs with model M5 and 77 234 with model M8); and scenarios 4 p_p and 4 p_{ip} (108 826 runs with model M5 and 72 198 with model M8).

Analysis of pollinator motion

We used an Approximate Bayesian Computation (ABC) framework to test for the different models. One key advantage of ABC, compared with other Bayesian methods, is that it is not necessary to analytically express how the likelihood of the data depends on the model parameters (van der Vaart et al. 2015a). ABC analysis allows (i) to determine which model gives the best fit to the data and (ii) to obtain the posterior distribution of the parameters using simulation under a defined model (Beaumont 2010). Fig. 5.5 details the procedure we used.

With this method, simulations are run using independent random parameter values that are sampled from their prior distributions. The prior distributions of each parameter of the model (*i.e.* the range of values in which parameters are likely to lie) can take on



Attraction by a plant (p_p): the direction is determined by the position of the plant.

Immobility on a plant (p_{ip}): the pollinator does not move during this step.

Plants do not attract pollinators

All plants have the same attractiveness

Attractiveness depends on floral display

Immobility on and outside a plant are similar.

Immobility is the same for all plants.

Immobility depends on floral display

Figure 5.4: Scenarios of pollinator behavior regarding the impact of plant floral display on plant attractiveness (scenarios 0 p_p, 1 p_p and 4 p_p) and immobility on a plant (scenarios $0p_{ip}$, 1 p_{ip} and $4p_{ip}$).

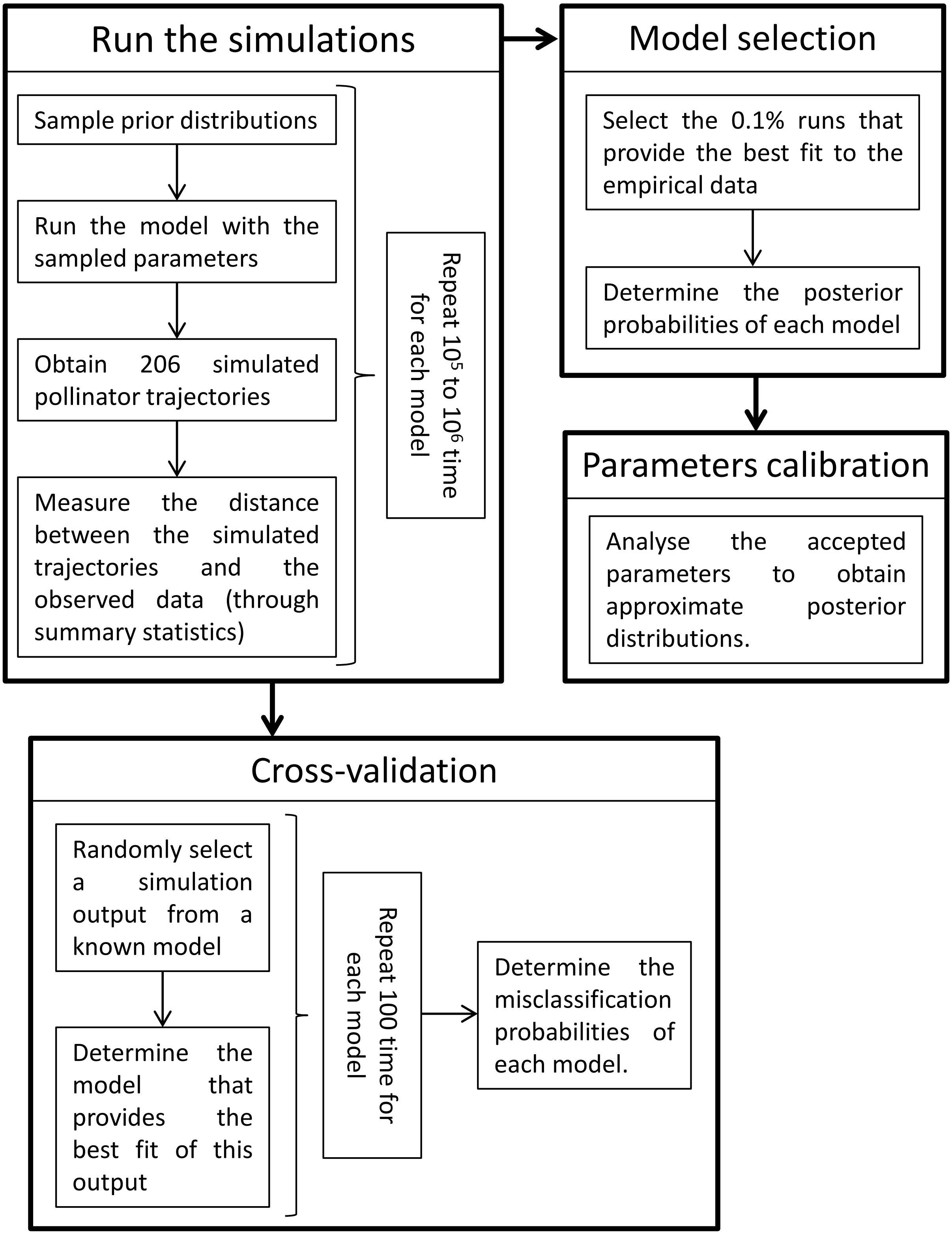


Figure 5.5: The procedure for the ABC analysis.

any shape that accurately reflects what is actually known (van der Vaart et al. 2015b). We do not have insights on the values that are expected for the weight of each movement, thus we choose uniform distributions ranging from 0 to 1.

Then, only the 0.1% simulations that provide the best fit to the empirical data are accepted. In order to determine which simulations are the closest from the empirical data, we used several summary statistics (see below). We first determine the model that provides the best fit of the empirical data. For this, we estimated the ratios of simulations accepted for each model, which give their probability given the data (Bayes factor, van der Vaart et al. 2015b). We also considered two other methods to estimate posterior probabilities of the models: using a multinomial logistic regression or using neural networks. Model selection using Bayes Factors automatically accounts for differences in model complexity (Beaumont 2010). This is because if the same number of simulations is done for all models, more parameters mean that a model's parameter space will be sampled less exhaustively (van der Vaart et al. 2015a).

To check the accuracy of ABC's estimates, we performed a cross-validation. The aim of this method is to check whether our method correctly determines which model and parameters were used to simulate a trajectory. This method uses randomly selected model outputs as “pseudo-data” and then uses the remaining runs to do ABC. Because the model and parameter values that produced the “pseudo-data” are known, this makes it possible to check whether ABC is accurately estimating them. This provides the mean misclassification probabilities of each model: the probability that this simulation is better explained by a model that is different from the model that gave that output. This analysis gives insights on whether the models produce different movement patterns and whether the summary statistics can gather these differences.

Summary statistics

Since available data on pollinator behavior rely on visitation patterns on plants, we focus here on statistics that could be evaluated on the available data set. Thus, we sub-sampled the pollinator trajectories, in order to keep only the foraging bouts of each pollinator. We assumed that several flowers had been visited on a given plant if the pollinator stops for several steps within the range of this plant.

For each simulation and real pollinator foraging bout, we computed the total number of plants that are visited and the number of flowers visited on each plant. We also calculated how many time each plant has been visited during one pollinator visitation bout (number of revisits). Those statistics were measured for all plants, but also on groups of plant depending on their position within the array (exterior, middle or central) and depending on plant traits (floral display). Moreover, we recorded the distance travelled within each pair of plants visited consecutively and the angle performed by the pollinator within each group of three plants visited consecutively. We also created transition matrix between positions (transition rate between exterior position to central position etc), and between plant traits (transition rate between trait 1 to trait 2, etc.).

Along the simulated trajectories, a pollinator can visit a plant, then stay outside the range of this plant and come back to this plant without having visited another individual plant meanwhile. Thus, we evaluated the above statistics using two different rules. We either considered the two visits to be independent (*i.e.* two visits on a plant), or we considered the two visits to be linked, in which case we considered it as one visit of the individual plant, but with two flowers that were visited.

Each statistic was evaluated on the real and on the simulated visitation bouts. We then calculated the distance between all real data (the 206 visitation bouts) and the simulated bouts (the 206 trajectories simulated with a parameter set). For this, we measured the norm L2 of each distribution: we created intervals, and summed up the squared deviation of simulated data from real data for each interval. The same method was used for the transition matrices. The lower the norm, the more simulated visitation bouts are close to the empirical data.

5.3 Results

Preliminary analyzes were conducted, using only two models: models M5 and M8 (Fig. 5.2). For each of these models, we considered only three combined scenarios regarding the impact of the floral display of plants: scenarios 0 p_p and 0 p_{ip} (no impact of plants on pollinator behavior); scenarios 1 p_p and 1 p_{ip} (same weights for all plants); and scenarios 4 p_p and 4 p_{ip} (four weights depending on plant floral display).

Method	model M5 0 p_p $0p_{ip}$	model M5 1 p_p $1p_{ip}$	model M5 4 p_p $4p_{ip}$	model M8 0 p_p $0p_{ip}$	model M8 1 p_p $1p_{ip}$	model M8 4 p_p $4p_{ip}$
rejection	0.0719	0.2582	0.0001	0.0875	0.5817	0.0006
neural network	0.2366	0.0153	0.0003	0.0859	0.6610	0.0009
multinomial logistic	0.0066	0.0000	0.0040	0.9850	0.0002	0.0043

Table 5.1: Posterior probabilities of each model

The choice of the model needs deeper analysis. Indeed, depending on the method used (rejection, multinomial logistic regression or neural network), different conclusions can be drawn (Table 5.1). When considering the rejection method, 58% of the accepted simulations are from model M8, with scenarios 1 p_p and $1p_{ip}$, and 26% of the accepted simulations are from model M5, with scenarios 1 p_p and $1p_{ip}$. Thus, this analysis provides no strong insight on the model that gives the best fit of empirical data. However, it suggests that assuming all plants to have the same weight of attractiveness and the same weight of immobility provides the best fit to empirical data.

Posterior probabilities obtained using neural network method predict that there is 66% chance that the model M8, with scenario 1 p_p and 1 p_{ip} provides the best fit to the real data. However there is also 24% chance that model M5, with scenarios 0 p_p and 0 p_{ip} gives the best fit to the real data. Thus this method provides no insight on the assumptions that provide the best fit to empirical data, neither on the model nor on the assumptions on plant attractiveness or on the weight of immobility.

Posterior probabilities obtained using multinomial logistic regression predict that there is 98% chance that the model that provides the best fit of real data is the model M8, with scenario 0 p_p and $0p_{ip}$. This prediction completely disagrees with the results obtained with the other methods.

Thus, deeper analysis is needed, especially to determine whether the trends presented above are the same regardless the summary statistic we consider, or if the probability of each model depends on the statistics considered.

Moreover, when considering the cross-validation, we can see there are many misclassifications between models. Especially, simulations from model M8 are often mixed up with

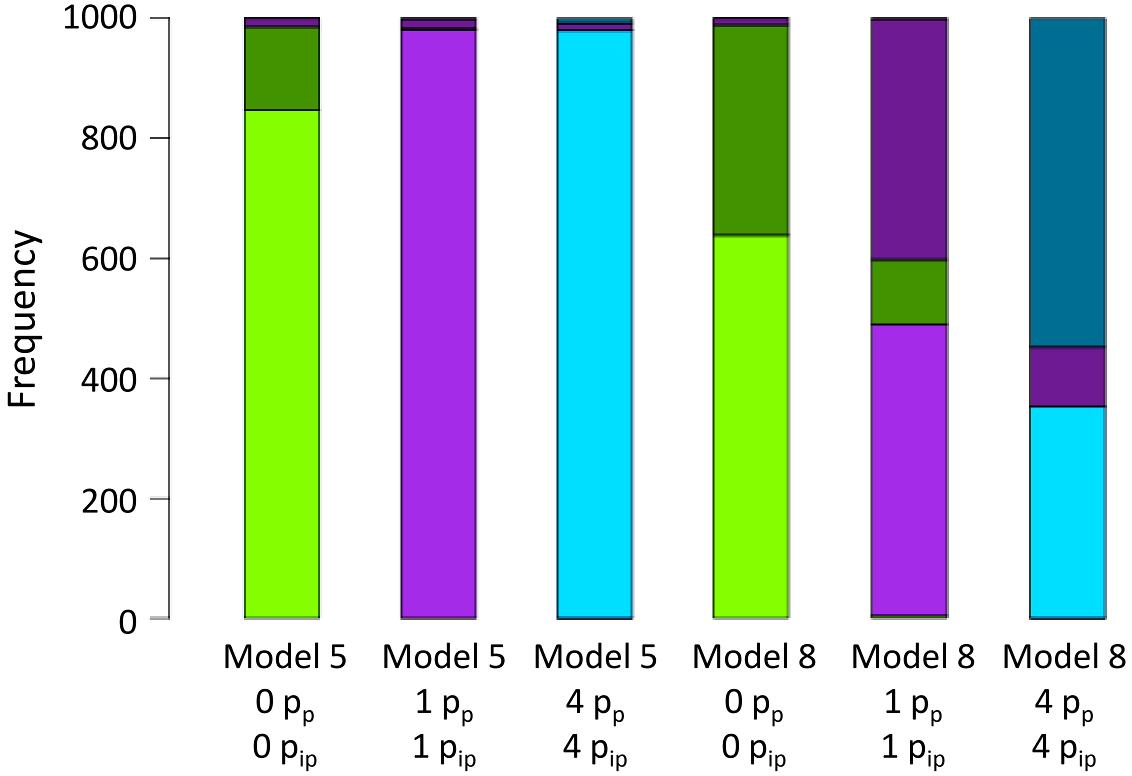


Figure 5.6: Misclassification proportions for the two models (model M5: pale colors; model M8 deeper colors) and the three scenarios regarding the impact of floral display (0 p_p and 0 p_{ip} in green, 1 p_p and 1 p_{ip} in purple, and 4 p_p and 4 p_{ip} in blue). If all models were classified correctly 100% of the times, all six bars would have a single color.

simulations from model M5 (see Fig. 5.6). However, it seems the classification according to the assumptions made on the impact of floral display is quite good: the simulations are correctly classified between 0 p_p and $0p_{ip}$, 1 p_p and $1p_{ip}$, and 4 p_p and $4p_{ip}$, depending on plant attractiveness (see Fig. 5.6). Thus, it seems the assumptions made on the impact of floral display have a strong impact on visitation patterns, whereas the assumptions made on the impact of distance on plant attractiveness and the assumptions made on the impact of the time spent on a plant do not create different patterns.

5.4 Perspectives

Given those preliminary results, several points need improvement. First, one limitation of our study is that we have not sampled our priors sufficiently, and more simulations need to be done. Indeed, our model comprises 6 parameters of movement that do not depend on plants (mean and standard deviation of the log-normal law, in addition to the weights of each movement: random movement p_r, directionality p_d, attraction by a special direction p_h, and immobility p_i). Additionally, 2 to 10 parameters depend on plant traits (weight of plant attractiveness p_p, weight of immobility on a plant p_{ip}, the decrease of the plant attractiveness with distance and the decrease of the weight of immobility with time). With so many parameters to calibrate, extensively sample the parameter space may be really difficult. For example, van der Vaart et al. (2015a) calibrated a 14 parameters individual based model, and they run one million simulations per model in order to get enough confidence in their model. Other methods exist to sample more efficiently the prior distributions: MCMC-ABC (Markov Chain Monte Carlo) and SMC-ABC (Sequential Monte Carlo). Those methods use the output of each simulation to gradually improve the sampling: values of the parameters are drawn above or below the current parameter value, and if the simulation provides a better fit to the empirical data, subsequent samplings are drawn with this new value of the parameter (van der Vaart et al. 2015a). However, both MCMC and SMC require simulations to be run sequentially, and it may be difficult or impossible to parallellise them on large computing clusters. Moreover, there is probably a strong dependency between our parameters, and those methods may not work well in this case (van der Vaart et al. 2015a).

One other possibility to restrain the number of simulations would be to use the same parameter sets for all models, and modify only the parameters that differ between the models (*e.g.* draw parameters for the model without weight of plant attractiveness, and use the same parameters for the model with one weight of plant attractiveness, by only sampling a value for this parameter). This method would allow us to determine if adding a parameter in the model could lead to a better fit of the data. However, the pairing that this method would create among simulations will probably make the statistical analysis difficult.

Second, our results are likely to depend on the assumptions made on the prior distributions of the parameters (Beaumont 2010). Indeed, using wrong prior distributions would lead to numerous simulations with inappropriate parameters values. For the ongoing analysis, some parameters prior distributions will be narrowed, and some parameters values will be fixed (*e.g.* the log-normal distribution parameters, and the weight of random move). Indeed, for now, one problem for the calibration of the model may be that there is no defined scale for the weights of the different movements (*i.e.* if all weights are multiplied by the same number, the models will be similar).

Third, to check the accuracy of our ABC's estimates, we performed only cross-validation. This method uses randomly selected model outputs as "pseudo-data", and determines the misclassification probabilities of this simulation (*i.e.* the probability our method predict this simulation is better explained by a model that is different from the model that gave that output). Another way to check the accuracy of our estimates would be to use the method of coverage. This method does not select randomly the "pseudo-data", but sample output only in the "best" runs, *i.e.* the runs selected as the closest to the real data (van der Vaart et al. 2015a). This method could perhaps be more adapted, since the cross-validation currently done could be biased because of simulations that are extremely distant from the observed data.

The accuracy of our model and of the estimated parameter set will also be tested with another independent data set. Indeed, other visitation bouts involving the same species (64 *Mimulus ringens* plant individuals, pollinated by multiple Bumblebee species) could be analyzed (Karron et al. 2006). In this data set, all plants display the same number of flowers. If our model gathers the important mechanism of pollinator behavior, our model should be able to predict accurately the foraging bouts of pollinators within those arrays.

Finally, it is difficult for now to determine if the mitigated results are linked to insufficient sampling effort, or if the summary statistics we currently use do not provide enough information for an accurate differentiation of the visitation patterns created by the different models, or simply because the different models do not create different visitation patterns of the plants. Besides, the differences between models are perhaps difficult to detect because most of the visitation bouts that have been recorded in the experimental arrays are extremely short (one to two flowers visited Mitchell et al. 2004). Thus, this data set may be insufficient for the estimation of that much parameters.

5.5 Acknowledgements

This work was supported by a grant from the Region Nord-Pas de Calais to C.L.

Numerical results presented in this paper were computed on the biomed virtual organization of the European Grid Infrastructure (http://www.egi.eu) via DIRAC portal (http://diracgrid.org). We thank the European Grid Infrastructure and supporting National Grid Initiatives (listed here: http://lsgc.org/en/Biomed:home#Supporting_National_Grid_Initiatives) for providing the technical support, computing and storage facilities.

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Chapter 6

Conclusion

Most plants use pollinators as pollen vectors ($\approx$ 90% Ollerton et al. 2011) and flowering plants show an amazing diversity of flower size, color, shapes or scents, traits used as signals that allow plant-pollinator interactions. Since pollinator behavior directly affects pollen transfer, a strong interplay between pollinators and the evolution of plant traits is expected, for instance traits that affect plant selfing rate.

6.1 Pollinators, drivers of evolution

It is more and more striking that pollinators must be accounted for when studying the evolution of traits in flowering plant. For example, seminal studies of the evolution of plant selfing rate focused on the genetic factors influencing its evolution. They showed that if inbreeding depression is higher than 0.5, complete outcrossing should be favored, and otherwise, complete selfing should evolve (reviewed in Goodwillie et al. 2005). However, when analyzing plant selfing rates, studies revealed that many species display mixed-mating systems (Goodwillie et al. 2005). This observed deviation from the predicted selfing rates might be explained by ecological factors that were neglected in the previous studies.

On one hand, many species self-fertilize a part of their ovules despite high inbreeding depression (Winn et al. 2011). In Chapter 2, we showed that such intermediate selfing rates could be explained because plants do not have complete “choice” over the pollen they receive. Some species may display intermediate selfing rates because of variations in pollen receipt (translating into variations in pollen limitation) due to unpredictable pollinator abundance (Morgan and Wilson 2005). Moreover, plants do not have control over the number of flowers probed by pollinators: selfing could be due to “maladaptive” geitonogamy. Devaux et al. (2014) highlighted the fact that intermediate selfing rate could result of unavoidable geitonogamy, because large floral displays may be required to attract enough pollinators. Our study presented in Chapter 3 provides another mechanism that favors mixed-mating systems in animal-pollinated species: because pollinator attraction is costly, some plant species may evolve intermediate selfing rates to reduce the cost of the interaction with pollinators.

On the other hand, many species show mixed-mating systems although their inbreeding depression seems low. So far, I think no convincing explanation was provided to explain the maintenance of outcrossing in populations that have low inbreeding depression. Some studies suggested such intermediate selfing rates could be selected for because of correlations among fitness components (Johnston et al. 2009; Jordan and Otto 2012). For example, it could be due to a positive correlation between outcross and self pollen reception. Devaux et al. (2014) showed that plants that favor high selfing rate should evolve to open all their flowers on the short period of time to enhance geitonogamous selfing. However, because pollinators always bring some outcross pollen, these species do not achieve complete selfing. It would be interesting to determine to which extent this phenomenon could be found in nature, however this explanation is not fully convincing for at least two reasons. First, plant species can modify their autonomous selfing rate rapidly (Bodbyl Roels and Kelly 2011), for example by reducing plant herkogamy (the distance between male and female organs). Second, plants would invest high energy into flower production in order to favor geitonogamous self-pollination. This means that, for a given plant population with low inbreeding depression, a plant that increases its geitonogamous selfing rate would still invest resources into flower and reward production, whereas a plant that increases its selfing rate through autonomous selfing would be able to invest less resources into pollinator attraction, and to reallocate this energy into seed production. Thus, in such population, autonomous selfing should be favored compared to geitonogamous selfing, and the mechanisms that prevent the evolution of autonomous selfing remain unclear. This phenomenon would need further investigations, and provides promising research avenues on plant mating systems evolution.

The inclusion of pollinator-mediated selective pressure in models of the evolution of plant selfing rate provided new mechanisms to explain the observed diversity of plant selfing rates. It highlights the importance of a better understanding of the impact of pollinators on plant fitness components and enhances the importance to understand how to account for the effect of pollinators on evolutionary trajectories.

6.2 Merging demography and evolution

The understanding of the impact of pollinators on traits evolution necessitates the inclusion of plant demography. Indeed, pollinator-mediated selective pressures are likely to depend on plant demography (*e.g.* mating success is likely to vary with the density of plants). Conversely, plant demography is likely to depend on the evolution of plant traits, which may modify its reproductive success. The studies presented in Chapters 3 and 4 highlight the importance of the joint consideration of demography and evolution.

First, in chapter 3, I showed that evolution strongly affects population dynamics and stability. More importantly, the evolutionary trajectories are strongly affected by demography, and this study points out that the evolution toward higher selfing rate can lead plant populations to the extinction. Indeed, selection may favor plants that reduce their costs of pollination through increased selfing, despite the negative effect of selfing, leading to evolutionary suicide.

Second, the results from chapter 4 offer a striking example of the importance of merging demography and evolution. Indeed, the model of Vamosi and Otto (2002) and the model presented in Chapter 4 use different modeling approaches. However, the two models predict the same selective pressures on plant traits: males are driven by male-male competition only, whereas females tend to invest in ovule production more than in attractiveness if pollen limitation is low, or for a high investment into attractiveness similarly to males otherwise. Although our results on the selective pressures are similar, these two studies draw quite different conclusions regarding the impact of the evolution on population dynamics. By separately studying evolution and demographic implications, Vamosi and Otto (2002) proposed that dimorphism could threaten plant populations. This may be true for small plant populations, in which extreme dimorphism implies that no female are visited by pollinators carrying pollen. Yet, our study predicts that such strong dimorphism should evolve only if pollen limitation is low. That is, dimorphism evolves if pollen is not limiting: in populations with high density and with efficient females that do not need much visits to fertilize all their ovules. Thus, our conclusion is quite at the opposite from the study of Vamosi and Otto (2002), and we argue that sexual dimorphism does not necessarily tend to threaten plant populations. The conclusions given by the two models apply in different situations: because we used a deterministic framework, our conclusions apply well in stable populations, whereas the stochastic approach used by Vamosi and Otto (2002) may be more appropriate in small disturbed populations.

These studies emphasized the importance of the interplay between demography and evolution. Because plant-pollinator interactions may strongly affect plant demography, it seems of primarily importance to be able to accurately describe such interactions. However, the inclusion of pollinators in models is often basic, and one major challenge will be to find ways to accurately describe plant-pollinators interactions.

6.3 Accurate modeling of plant-pollinators interactions

As already expressed in the introduction, integrating plant-pollinator interactions in models is not straightforward, and each model makes different assumptions on pollinator behavior. Integrating in a simplistic way the impact of pollinators on plant demography and on the evolution of plant traits is appealing. Indeed, simple deterministic models can easily provide analytically tractable results. Moreover, such simple models can provide clear patterns that do not depend (too much) of each parameter of the model. However, if one wants to trust the results provided by those models, he must trust the assumptions made by the model. One of the most important tasks of a theoretician may be to ground the assumptions of his models on empirical work, or to provide some evidence that these assumptions may be biologically relevant in some conditions. Especially, when describing the impact of plant-pollinators interactions on the plant on the fitness of a mutant in the population, it is crucial to fully understand how the pollinator will answer to the modification of the plant trait. Otherwise, one may predict biased evolutionary trajectories that may not reflect the actual selective pressure.

So far, it is difficult to get insights on the mechanisms that cause variations of pollinator movements for at least two reasons. First, understanding these mechanisms rely on the creation of individual-based models that require huge computational power. Indeed, accurate calibration of such models may require millions of simulations for each models (van der Vaart et al. 2015). With the expansion of virtual organizations such as the European Grid Infrastructure, this difficulty shrinks, and one could now achieve enough simulations to draw clear patterns from those individual-based models and to get enough statistical significance.

Second, tracking foraging pollinators is extremely time-consuming and the tracking of individuals is for now limited to the record of pollinator stops on plant individuals. For example, Mitchell et al. (2004) followed individual pollinator foraging bouts within a population of plants, and recorded the position of the insect each time it visited a flower. This study, required many hours of observation, and yet only 206 foraging pollinators have been observed. One can hope that in a few years, automatically tracking methods will spread to pollinators, and that experimental data of pollinator behavior will allow the complete recording of pollinator trajectories, which would allow for more extensive analysis. So far, automatically tracking pollinator behavior seems out of the reach, but it may soon be possible. Indeed, Mersch et al. (2013) showed that it was possible to automatically record the movement of ants that have previously been tagged. This allowed for the obtention of a huge data set of interactions between ants within a colony. This data set provided some insights on the social organization of the colony, and further analyses could perhaps allow predictions of ant movements within the colony. As the GPS records are becoming more common, the study of movement patterns will need the development of new methods of analysis. Currently, GPS tracking on large wild individual provides large data sets of animal trajectories, and will soon allow for a better understanding of their large-scale movement patterns (Berthelot et al. in prep). The development of these new methods to pollinators may provide exceptional datasets, that could give new insights on the mechanisms of pollination.

In the end, we may soon be able to analyze extremely complex models of plant-pollinator interactions. Simple (deterministic) models and of complex individual-based models complement each other. Indeed, complex models are needed to get faith in the assumption one makes when creating simpler models. In return, simpler models will more easily provide general and clear results.

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