

# Darwin’s ‘mystery of mysteries’: the role of sexual selection in plant speciation

Mohammadjavad Haghghatnia<sup>1,2</sup>, Antonin Machac<sup>3</sup>, Roswitha Schmickl<sup>1,2</sup> and Clément Lafon Placette<sup>1,\*</sup> 

<sup>1</sup>*Department of Botany, Faculty of Science, Charles University, Benatska 2, Prague CZ-128 01, Czech Republic*

<sup>2</sup>*Institute of Botany, The Czech Academy of Sciences, Žitánek 1, Průhonice 252 43, Czech Republic*

<sup>3</sup>*Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, Prague 14220, Czech Republic*

## ABSTRACT

Sexual selection is considered one of the key processes that contribute to the emergence of new species. While the connection between sexual selection and speciation has been supported by comparative studies, the mechanisms that mediate this connection remain unresolved, especially in plants. Similarly, it is not clear how speciation processes within plant populations translate into large-scale speciation dynamics. Here, we review the mechanisms through which sexual selection, pollination, and mate choice unfold and interact, and how they may ultimately produce reproductive isolation in plants. We also overview reproductive strategies that might influence sexual selection in plants and illustrate how functional traits might connect speciation at the population level (population differentiation, evolution of reproductive barriers; i.e. microevolution) with evolution above the species level (macroevolution). We also identify outstanding questions in the field, and suitable data and tools for their resolution. Altogether, this effort motivates further research focused on plants, which might potentially broaden our general understanding of speciation by sexual selection, a major concept in evolutionary biology.

*Key words:* sexual selection, plant speciation, reproductive isolation, reproductive strategies, macroevolution.

## CONTENTS

I. Introduction	2
II. Sexual selection mechanisms and reproductive strategies in plants	2
(1) Premating sexual selection (pre-pollination stage)	3
(2) Postmating sexual selection (post-pollination stage)	4
(3) Reproductive strategies influencing sexual selection	5
(a) Pollination mode	5
(b) Sexual polymorphism versus hermaphroditism	5
(c) Selfing versus outcrossing	6
III. Sexual selection and reproductive barriers	7
(1) Pollinator shift in response to pre-pollination sexual selection	7
(2) Pollen rejection and post-pollination sexual selection	8
(3) Ecological speciation and speciation by sexual selection	10
IV. Sexual selection and plant speciation (macroevolutionary scale)	11
(1) Macroevolutionary research	11
(2) Bridging the micro- and macroevolution of speciation by sexual selection in plants	12
V. Conclusions	14
VI. Acknowledgements	14
VII. References	14

\* Author for correspondence (Tel.: +420 774 300 495; E-mail: [lafonplc@natur.cuni.cz](mailto:lafonplc@natur.cuni.cz)).

## I. INTRODUCTION

Why some evolutionary lineages are dramatically more species rich than others remains an enigma in biology. Sexual selection is often considered as one of the factors that promote speciation and diversification (Panhuis *et al.*, 2001; Kraaijeveld, Kraaijeveld-Smit & Maan, 2011; Maan & Seehausen, 2011), yet current theory and empirical results often suggest a surprisingly tenuous connection. For example, sexually dimorphic lineages tend to be more speciose (Futuyma & Kirkpatrick, 2017), and some theory exists on how sexual selection might lead to speciation (Fisher, 1930; Turelli, Barton & Coyne, 2001). But it has also been reported that sexual selection might be insufficient to govern the emergence of new species and sometimes may even hinder the speciation process (Servedio & Bürger, 2014; Servedio & Boughman, 2017; Irwin, 2020; Mendelson & Safran, 2021). Importantly, sexual selection can be hard to distinguish from other processes, mediated by environment and by geographic isolation, such as ecological or mutation-order speciation (Panhuis *et al.*, 2001; Kraaijeveld, Kraaijeveld-Smit & Maan, 2011; Maan & Seehausen, 2011). Thus, how to reconcile these mixed conclusions regarding the role of sexual selection on speciation remains an outstanding challenge, especially in plants. To address this, we need to study the processes that unfold across the continuum of evolutionary scales, from population divergence to the emergence of reproductive isolation and macroevolutionary diversification.

Sexual selection, defined as non-random variance in mating success (Kokko & Jennions, 2008), explains the evolution of sexual traits through their positive impact on reproductive success through a competitive advantage during mating, despite their often negative impact on survival (Darwin, 1871). It includes two dominant processes: the first is intrasexual (usually male–male) competition for mating; and the second is the choice of mates by the other sex, usually females (Darwin, 1871; Willson, 1979; Panhuis *et al.*, 2001; Rosenthal & Ryan, 2022). While sexual selection has been extensively studied in animals, its mechanisms and consequences remain largely understudied in plants.

Historically, the existence of sexual selection in plants has been controversial (Moore & Pannell, 2011), given that plant species are mostly hermaphrodites. Interestingly, sexual selection mechanisms have been demonstrated in hermaphroditic animal species (Beekman *et al.*, 2016) and, correspondingly, sexual selection is now generally recognised to operate in plants (Moore & Pannell, 2011). Yet, the consequences of sexual selection on plant evolution and plant speciation remain largely understudied.

In plants, whether sexual selection can lead to sexual trait divergence and the establishment of reproductive barriers has yet to be documented through empirical evidence. Divergent mate preferences in animals can act as a reproductive barrier (Boughman, 2001), and a similar process may take place in plants (Baek *et al.*, 2015; Li *et al.*, 2018). Nevertheless, the specificities of sexual selection in plants (the sexual mechanisms themselves, the diversity of

life-history traits, and the mating systems involved) may also contribute to the establishment of reproductive barriers that might be specific to plants.

Finally, an outstanding question in evolutionary biology is whether, and how, microevolutionary processes, such as sexual selection-driven reproductive barriers, translate into large-scale processes such as speciation and diversification (macroevolution; Jablonski, 2000). Darwin (1871) proposed that groups with more elaborate secondary sexual characters should be species rich, implying that the divergence of traits under sexual selection can lead to lineage diversification. While this hypothesis has been extensively tested in animals (Boul *et al.*, 2006; Mendelson, Imhoff & Venditti, 2007; Cally *et al.*, 2021), the role of sexual selection in large-scale speciation in plants remains surprisingly unaddressed (see Table 1).

In this review, we address the following questions: (i) what plant traits are under sexual selection, and how do reproductive strategies affect sexual selection; (ii) can sexual selection produce reproductive barriers and thus facilitate population divergence; and (iii) can this divergence lead to large-scale speciation? Some of these questions have been investigated in plants, but in isolation, and have yet to be evaluated together with a view to bridging the gaps between them. Our study details the connections between these research questions and aims to integrate previous work to formulate a more comprehensive perspective on plant speciation by sexual selection, ranging from micro- to macroscales.

## II. SEXUAL SELECTION MECHANISMS AND REPRODUCTIVE STRATEGIES IN PLANTS

*[A]though plants ‘neither sing nor dance’, they do exhibit female choice and male competition as intensely as animals do.* (Ganeshaiah & Shaanker, 2001, p. 423).

Darwin (1871) proposed that sexual selection involves differences in reproductive success caused by competition for mating. By focusing on competition for mating, this concept therefore excludes processes related to natural selection acting on sexual traits. Darwin further suggested that sexual selection may involve either intrasexual (male–male) competition for mating, and/or intersexual selection (female mate choice). Female choice has been proposed to be costly and yet advantageous, as this cost may be compensated by an improved general fitness of the progeny (‘good genes’ hypothesis; Byers & Waits, 2006) or by an improved reproductive success of male progeny due to the heritability of male competition abilities, hence indirectly increasing female fitness (‘sexy son’ hypothesis; Weatherhead & Robertson, 1979). A link between male–male competition and female choice was further proposed under the concept of Fisherian runaway selection, which assumes that male traits evolve under recurrent directional selection by females; in other words, representing a case of coevolution between male traits and female preference (Fisher, 1930).

Table 1. Summary of current knowledge regarding the role of sexual selection in plant speciation. PTGR, pollen tube growth rate; SC, self-compatible; SI, self-incompatible.

Process	State of knowledge	Main evidence
Sexual selection mechanisms	Moderate amount of evidence	Floral and pollen traits evolve in response to male–male competition for pollinator attraction (Cocucci <i>et al.</i> , 2014; Paterno <i>et al.</i> , 2020) PTGR and pollen germination are associated with siring success (Snow & Spira, 1991a,b, 1996; Skogsmyr & Lankinen, 1999) Stressing the maternal plant increases random mating (Marshall & Diggle, 2001; Shaner & Marshall, 2003)
Reproductive strategies influencing sexual selection	Some evidence	Higher pollen performance in outcrossing <i>versus</i> selfing <i>Clarkia</i> species (Mazer <i>et al.</i> , 2018) Relaxation of sexual selection in selfing species (Gutiérrez-Valencia <i>et al.</i> , 2022)
Reproductive barriers promoted by sexual selection	Some evidence	Bilateral mating incompatibilities (Arnold <i>et al.</i> , 1993; Hodges & Arnold, 1995) Unilateral SI × SC incompatibility (Covey <i>et al.</i> , 2010; Baek <i>et al.</i> , 2015) Negative correlation between style length and interspecific seed set in <i>Silene latifolia</i> (Nista <i>et al.</i> , 2015)
Sexual selection and local adaptation	No data	<b>Suggestion to address the knowledge gap:</b> testing for pleiotropic effects of sexual selection at (i) the trait level (e.g. artificial selection on pollen performance and measure its impact on adaptive traits such as drought response), and (ii) the gene/genome level (to search for possible links between genomic targets of local adaptation and genomic targets of sexual selection)
Sexual selection and plant diversification	No data or indirect evidence	Mating systems (SI <i>versus</i> SC) influence diversification rates (Goldberg <i>et al.</i> , 2010) <b>Suggestion to address the knowledge gap:</b> sexual selection directly measured at the population level could be correlated with speciation rates estimated from present-day phylogenies

The concept of sexual selection has been expanded to postmating processes, notably: (i) cryptic female choice (Eberhard, 1996), a process in which the fertilisation of certain sperm is favoured to the detriment of other sperm; and (ii) postmating male–male competition (competition between sperm cells for fertilisation of the egg cell; Eberhard, 2009). These expansions of the concept of sexual selection are particularly pertinent in the case of plants, which, for obvious reasons, do not show courtship behaviours, male fights for access to mating or visible female choice (Willson, 1979).

Sexual selection in plants (mostly angiosperms) acts at the pre- and postmating stages similarly to in the animal kingdom. In plants, the deposition of pollen on the stigma (i.e. pollination) is considered analogous to mating in animals. Sexual selection can therefore act before mating on traits that promote pollen transfer to conspecific stigmas, which corresponds to male–male competition (Willson, 1979; Delph & Ashman, 2006). Sexual selection in plants also takes place after pollination, whereby the traits mediating pollen performance as well as the mechanisms of female selection may impact the reproductive success of both males and females (Queller, 1984; Willson, 1994; Moore & Pannell, 2011; Tonnabel *et al.*, 2021). Interestingly, plants show a much wider diversity of reproductive strategies than animals, likely associated with dramatic differences in the intensity of sexual selection (Friedman & Barrett, 2011; Collet *et al.*, 2014). This makes plants an important taxon for the study of general principles that govern sexual selection and speciation, potentially providing key insights for evolutionary theory. Below, we review the currently known mechanisms

of sexual selection in plants before and after mating and evaluate how reproductive strategies can affect sexual selection.

### (1) Premating sexual selection (pre-pollination stage)

Using flowers to attract pollinators is a major part of pre-pollination sexual selection. Angiosperm flowers show an enormous diversity (e.g. petal size, biomass, petal and stamen number, colour, and scent) (Armbruster, 2014; Davis *et al.*, 2014; Vasconcelos *et al.*, 2019; Nikolov, 2019). Darwin (1877) noted that the perianth (petals and/or sepals) size was often larger in functionally male or hermaphrodite flowers than in female flowers in taxa with unisexual flowers.

This observation is in line with an influential theory, ‘Bateman’s principle’, which postulates that sexual selection acts on male mating success more than on the success of females (Willson, 1994; Collet *et al.*, 2014; Paterno *et al.*, 2020). It hypothesises that the reproductive success of females is limited by resources, and the frequency of mating therefore has minimal impact on their reproductive fitness, whereas male reproductive success is limited by mating opportunities and thus should increase with the number of mating events (Bateman, 1948; Collet *et al.*, 2014). When applied to plants, mating success is a crucial bottleneck for males: it was estimated for one plant community that less than 3% of pollen produced reaches a conspecific stigma (Gong & Huang, 2014). Thus, from the male perspective, the more pollinators that visit the plant, the higher the chances that pollen from a particular male will access a

female, and the higher their reproductive success. By contrast, from the female perspective, as only a fraction of all pollen is required to fertilise all seeds, a low pollinator visitation rate may be sufficient (Elle & Meagher, 2000; Collet *et al.*, 2014). This imbalance will mean that floral traits influencing both pollen import and export by pollinators will be selected to a great extent by the male function (the ‘fleurs-du-male’ hypothesis; Queller, 1997). As a consequence, male fecundity should be strongly associated with floral traits, such as conspicuous flowers (Stanton *et al.*, 1991; Paterno *et al.*, 2020). Consistently, recent research showed that larger hermaphrodite angiosperm flowers show increased investment in male organs and pollinator attraction (larger petals), whereas smaller flowers tend to be female (Paterno *et al.*, 2020). In other words, there is male–male competition over pollinator visitation.

Sexual selection might also shape traits that facilitate pollen removal and transfer. Studies have shown the possibility of intrasexual selection through pollen–pollen competition during the movement of pollen to and from the pollinator’s body (pollen transfer) (Cocucci *et al.*, 2014; Duffy & Johnson, 2014; Lynn *et al.*, 2020). For example, Cocucci *et al.* (2014) showed that pollinaria (aggregated pollen) in the Apocynaceae subfamily Asclepiadoideae physically compete for access to attachment sites on the body of pollinators.

Therefore, increasing mating opportunities appears to be under strong selection mostly for male parts of the plant (Willson, 1979; Willson & Burley, 1983). Divergent premating sexual selection may lead to the divergence of floral traits between related lineages, and if so, this could result in the establishment of reproductive barriers and, eventually, to plant speciation. This hypothesis will be further explored in Section III.

## (2) Postmating sexual selection (post-pollination stage)

When pollen is deposited on the stigma, there arises the possibility of competition between males (pollen) to reach the ovules (Mulcahy, 1979; Arnold, 1994; Willson, 1994; Moore & Pannell, 2011) and of female choice, i.e. the selection of pollen by females (Mazer *et al.*, 2018). At this stage, therefore, traits related to both male performance and female choice may be under sexual selection.

Competition between males may occur when the number of pollen grains deposited on a stigma exceeds the number of ovules (Delph, Weinig & Sullivan, 1998; Winsor, Peretz & Stephenson, 2000), which is the case for the majority of species (Gong & Huang, 2014; İltaş, Le Vève, Slovak & Lafon Placette, *in preparation*). In male–male competition for access to ovules, pollen performance traits including pollen viability, pollen size, pollen germination rate, chemical interference, and pollen tube growth rate (PTGR) are likely to be under selection (Snow & Spira, 1991a; Spira *et al.*, 1992; Johnston, 1993; Delph *et al.*, 1998; Snow, Spira & Liu, 2000; Lankinen & Skogsmyr, 2002; Delph & Ashman, 2006; McCallum & Chang, 2016). Of these, pollen germination rate and PTGR are the most studied

performance traits that might mediate pollen–pollen competition in the style (Mazer *et al.*, 2010; McCallum & Chang, 2016). In *Hibiscus moscheutos* (Malvaceae) and *Viola tricolor* (Violaceae), experiments showed that pollen with higher germination rates and PTGRs can reach unfertilised ovules faster and achieve greater fertilisation success than those with lower rates (Snow & Spira, 1991a,b, 1996; Skogsmyr & Lankinen, 1999). Thus, sexual selection could act on the PTGR as a competitive trait.

After the deposition of pollen on the stigma, female choice might affect the success of pollen donors. Although the relative siring success of different pollen donors was not affected by the maternal plant in one study (Marshall, Shaner & Oliva, 2007), stressing the maternal plants led to more random mating (Marshall & Diggle, 2001; Shaner & Marshall, 2003), suggesting that healthy females can affect seed paternity. The traits involved in pollen selection by females may include style length, stigmatic area, stigma receptivity, and chemical signalling in the stigma and style (Mulcahy, 1979; Armbruster *et al.*, 1995; Lankinen & Strandh, 2016; Mazer *et al.*, 2016; Tonnabel *et al.*, 2021), of which style length and chemical signalling are the best investigated traits.

Pistil traits may enhance pollen competition as a female choice mechanism (Lankinen & Green, 2015). For example, there is evidence that a longer style provides females with a mechanism to discriminate among pollen with different performance levels; it might lead to a longer interaction between pollen tubes and pistil that allows females to control pollen tube growth in the style *via* molecular interactions (Mulcahy & Mulcahy, 1975; Lankinen & Skogsmyr, 2002; Ramesha *et al.*, 2011). Hence, sexual selection might favour females with longer styles (Ö. İltaş, A. Le Vève, M. Slovak, C. Lafon Placette, *in preparation*). However, while a meta-analysis confirmed that style length was significantly correlated with rate of non-random mating, the degree of non-random mating was highest in species with shorter styles (Ruane, 2009), arguing against the proposal that a longer style may increase female selectivity. In a mathematical modelling study, Travers & Shea (2001) used measurements of *in vivo* pollen performance (PTGR and pollen germination rate) and style length. They predicted that faster pollen germination would increase siring success when style length was shorter, and faster PTGR would increase siring success when styles were longer (Travers & Shea, 2001). Thus, the higher degree of non-random mating in species with shorter styles found by Ruane (2009) might be explained by a greater role of pollen germination rate rather than PTGR in male–male competition.

Female control of pollen germination and tube growth likely involves complex chemical signalling in the stigma and style, allowing the female sporophyte to recognise compatible pollen, support the growing pollen tube in the style, and help the pollen tube to target the ovary (reviewed in Tonnabel *et al.*, 2021). Germination of pollen at the stigmatic surface is controlled *via* a complex signalling interaction between specific pollen proteins/molecules and proteins at the stigmatic surface (reviewed in Wheeler, Franklin-Tong & Franklin, 2001;



Zheng *et al.*, 2018; Johnson, Harper & Palanivelu, 2019), leading to female provisioning of the pollen grain with resources, especially water for the hydration process and germination of the pollen on the stigma. After this stage, the continued chemical interactions between pistil and pollen tubes control pollen tube growth into the style transmitting tract (Zheng *et al.*, 2018). When pollen tubes reach the style, guiding signals and nutrients from the pistil extracellular matrix guide the pollen tube towards the ovary. In the final step, signals produced by the ovules guide pollen tubes towards the ovule and lead to fertilisation (reviewed by Dresselhaus & Franklin-Tong, 2013; Johnson *et al.*, 2019).

At each of these steps (pollen germination, pollen tube growth, and pollen tube guidance) female choice has the potential to act at a molecular level (Tonnabel *et al.*, 2021). For example, there is evidence for mate recognition mechanisms such as the self-incompatibility (SI) system, in which the female can accept/reject compatible pollen at the germination (sporophytic SI; e.g. Brassicaceae) and tube growth stage (gametophytic SI; e.g. Solanaceae), and this can affect the siring success of pollen donors carrying alleles recognised as self by the female plant (Richman & Kohn, 2000; Doucet, Lee & Goring, 2016). Nevertheless, while this may appear to be an example of female choice and fits the definition for sexual selection of non-random variance in mating success (Kokko & Jennions, 2008; Tonnabel *et al.*, 2021), this may not be the case. Darwin (1871) proposed that sexual selection acts on differences in reproductive success caused by competition for mating, and in the SI system, female choice is only based on ‘self’ *versus* ‘non-self’ criteria (even though ‘self’ may be other individuals with the same S-allele) in an inbreeding-avoidance strategy. This does not involve any competition for mating, even though rare S-alleles are beneficial to males as they increase the probability to be accepted by females against more frequent S-alleles (Durand *et al.*, 2020), resembling male–male competition. The consequences are however different, with self-incompatibility leading to balancing selection on S-alleles, rather than directional selection expected in a sexual selection scenario. Also, self recognition may impact gene flow and lead to species barriers in nature (Pickup *et al.*, 2019; Harkness & Brandvain, 2021), which we will consider in Section III. In general, evidence for sexual selection acting on female chemical control of pollen germination/tube growth/tube guidance, i.e. female choice of male competitive traits (‘sexy-son’ hypothesis) or male traits directly improving female fitness (‘good genes’ hypothesis), remains sparse.

Therefore, in principle, sexual selection could operate after pollination through both male–male competition and female choice. Males with higher pollen performance traits may be at an advantage against other males (Mazer *et al.*, 2010; Baskin & Baskin, 2015), while females, *via* pistil traits and chemical signalling, could exhibit choosiness among different pollen (Lankinen & Strandh, 2016; Mazer *et al.*, 2016; Madjidian *et al.*, 2020). We will further explore below whether such mechanisms can establish postmating reproductive barriers, and potentially lead to speciation.

### (3) Reproductive strategies influencing sexual selection

Compared to animals, and probably due to their sessile nature, plants (particularly angiosperms), have evolved myriad unique reproductive strategies that are likely to influence how sexual selection works, as well as its intensity. In this section we evaluate the impact of various life-history traits on the potential for sexual selection.

#### (a) Pollination mode

Plants are immobile and thus rely on biotic (pollinators) and abiotic (wind and water) vectors for transferring their (male) pollen to the (female) ovary (Friedman & Barrett, 2009), and these different vectors might change the intensity of sexual selection. In particular, the use of wind *versus* animal pollination is likely to affect pre-pollination male–male competition, as the differences between these two strategies lie mostly in the premating phase.

In animal-pollinated plant species, conspicuous flowers and inflorescences (e.g. larger petals; Paterno *et al.*, 2020) or floral scents used to signal the availability of nutritional rewards function to attract pollinators and thus contribute to male reproductive success (Arnold, 1994; Willson, 1994; Delph & Ashman, 2006; Cocucci *et al.*, 2014; Paterno *et al.*, 2020). Thus, for animal-pollinated species, a large set of traits, ranging from floral display, reward, or pollen structures enabling attachment to pollinators, are likely to improve male mating success under male–male competition for pollinators. This is likely to facilitate the evolution of premating sexual traits *via* sexual selection. By contrast, in wind-pollinated plants pollination takes place randomly, and male reproductive success is linearly correlated with male reproductive investment (Charnov, 1979; Aljiboury & Friedman, 2022). As a result, higher production of pollen or traits affecting pollen dissemination (e.g. aerodynamic properties) are the only traits that can increase the probability of fertilising ovules in competition with other males (Cruden, 1977; Eppley & Pannell, 2007; Friedman & Barrett, 2009; Tonnabel, David & Pannell, 2019), as most of the pollen produced will be wasted (Darwin, 1876; Ackerman, 2000). Therefore, in wind-pollinated species, sexual selection has very few opportunities to act at the premating stage, and likely does so mostly on pollen production and dissemination.

After the pollen reaches the stigma (postmating), sexual selection is likely unaffected by pollination mode. In a meta-analysis of 51 studies on 23 different species from 17 different families (mostly Brassicaceae, Poaceae, and Cucurbitaceae), no significant difference was found in the non-random siring rate between wind- and animal-pollinated species (Ruane, 2009), suggesting that the pollination mode does not affect the intensity of postmating sexual selection.

#### (b) Sexual polymorphism versus hermaphroditism

Most angiosperm species are hermaphrodites, with both female (ovules) and male (pollen) parts on the same flower

(Barrett, 2002; Charlesworth, 2006). However, some species have evolved from hermaphroditism towards gynodioecy and androdioecy, i.e. the co-occurrence of hermaphrodite and female or male individuals, respectively (Darwin, 1877; Pannell, 2002), or even towards the complete separation of the sexes (male and female individuals only; dioecy) (Charlesworth, 1999). Dioecious species are found in only 6% of angiosperms (Renner, 2014), although it has evolved from hermaphroditism multiple times independently (Käfer, Marais & Pannell, 2017).

In hermaphroditic species, the evolution of sex-specific traits is likely a trade-off between male and female interests due to resource and morphological constraints, similarly to trade-offs proposed in hermaphroditic species of animals (Schärer & Janicke, 2009). Thus, the partial or complete separation of the sexes is likely to favour the evolution of fitness optima, such as higher pollen production and pollen transfer for males (Wilson & Harder, 2003) or higher seed production for females (Freeman, Klickoff & Harper, 1976). Sexual selection is expected to lead to fitness optima, with more competitive males (more conspicuous flowers, higher pollen performance) (Kwok & Dorken, 2022) and more selective females (longer styles, stricter chemical female choice) compared to hermaphrodites. This means that the effects of sexual selection may be clearer in dioecious species compared to hermaphrodites. Modelling studies have shown that males are more likely to evolve showy flowers than females in dioecious animal-pollinated plants, especially with pollinator abundance (Vamosi & Otto, 2002). However, a morphological analysis on tropical forest trees found that the flowers of almost all animal-pollinated dioecious species were relatively small, unspecialised, and of inconspicuous colour in comparison to hermaphroditic plants (Bawa & Opler, 1975; Bawa, 1994). While this apparently contradicts the suggestion that intrasexual selection will act more on male flower traits in dioecious compared to hermaphroditic species, this finding might be explained by the fact that the small flowers of dioecious species were associated with visitation by a wide range of insect pollinators while the hermaphroditic species were visited by larger specialists (Bawa & Opler, 1975; Bawa, 1994). Attracting a wide range of pollinators might improve pollen transfer and thus male reproductive success more than relying on a few specialised pollinators.

In androdioecious and gynodioecious species, as unisexual individuals co-occur with hermaphrodites, the sex ratio may be biased towards one or other sex (Pannell, 2002), and this may influence the intensity of intrasexual competition for mating. Interestingly, a reciprocal effect was found in dioecious species with an XY sex-determination system: more intense pollen competition led to a sex ratio more biased towards female progeny. This was explained by pollen grains with an X chromosome outcompeting pollen grains bearing a Y chromosome during mating (Delph, 2019). Just as for fully dioecious species, gyno/androdioecy may allow the specialisation of individuals into solely male or female function, respectively, and under sexual selection we can expect

exacerbation of traits for male–male competition or female choice. For example, in the genus *Silene*, some female flowers of gynodioecious species have larger stigmatic surfaces and longer stigma papillae than in hermaphroditic flowers (Bock, 1976; Dulberger & Horovitz, 1984).

### (c) *Selfing versus outcrossing*

While more than 90% of plant species are hermaphrodites (Bawa, 1980), almost 65% of all species are outcrossing, 11% selfing, and 24% show mixed mating (Igic & Kohn, 2006).

The well-known evolutionary transition from outcrossing to selfing in flowering plants (reviewed in Igic, Lande & Kohn, 2008) can take place when the advantage of reproductive assurance [e.g. reproducing under pollen limitation (Darwin, 1876; Eckert, Samis & Dart, 2006) and selfing transmission (Fisher, 1941)] outweighs the cost of inbreeding depression (i.e. reduced offspring fitness compared with outbreeding; Charlesworth, 2006). This transition frequently involves breakdown of the SI system, and hence the evolution of SC (Takayama & Isogai, 2005). The so-called ‘selfing syndrome’ of floral traits that evolve after the transition to selfing is distinguished by small and inconspicuous flowers, reduced scent, reduced pollen production and a lower pollen:ovule (P/O) ratio (reviewed in Sicard & Lenhard, 2011; Willi, 2013; Tsuchimatsu *et al.*, 2020).

The transition from outcrossing to selfing is likely accompanied by relaxation of sexual selection (Cutter, 2019; Gutiérrez-Valencia *et al.*, 2022). At the pre-mating stage, the high pollen transfer efficiency ensured by selfing strongly reduces male–male competition for pollen transfer (Harvey & May, 1989; Willis, 1999). Therefore, a selective pressure on males for attractive traits such as flower size or scent will be relaxed, leading to the reduction of these traits as part of the ‘selfing syndrome’. At the post-mating stage, as both pollen and the female sporophyte are from the same individual and thus carry the same pool of alleles, variation in male competitiveness/female choice is virtually null, likely making sexual selection inefficient. In addition, the mating success or failure of a specific pollen grain is less likely to impact fitness in a scenario where male gametophytes are virtually identical to each other (due to homozygosity and lack of efficient recombination) and to the female gametophytes. One prediction is that male competitive traits (pollen germination, PTGR, etc.) and female choice traits (style length, chemical selection of pollen grains) would be under weaker selection in selfers compared to outcrossers. Also note that natural selection may actually favour changes that accompany the transition to selfing as a resource allocation trade-off. For example, in the selfer *Arabidopsis thaliana* it was shown that the reduction in pollen number is caused by an allele which has been under positive selection (Tsuchimatsu *et al.*, 2020). Additionally, it is likely that any form of asexual reproduction, which occurs in about 80% of angiosperms (Klimeš *et al.*, 1997), will have a similar effect as selfing of reducing the strength of sexual selection.

A study that examined the divergence in pollen performance in two *Clarkia* sister species, predominantly outcrossing *C. unguiculata* and self-pollinating *C. exilis*, revealed that *C. unguiculata* had a higher PTGR than *C. exilis* (Mazer *et al.*, 2018). Similarly, in another model system, *Mimulus guttatus* (outcrosser) had a higher PTGR than *M. nasutus* (selfer) (Diaz & Macnair, 1999). It was also shown that *M. guttatus* pollen did not outcompete *M. guttatus* pollen when *M. guttatus* was used as the maternal plant, suggesting a role of female choice. Indeed, style length comparisons between selfer and outcrosser species revealed that outcrossers had longer styles (Diaz & Macnair, 1999; Runions & Geber, 2000), consistent with the idea that sexual selection is weaker in selfing species in comparison to outcrossers. Interspecific crosses between several SI and SC *Arabidopsis* species revealed that pollen of SI species could germinate on an SC stigma, while in the reciprocal crosses pollen of SC species did not germinate when the maternal plant was an SI species (Li *et al.*, 2018). This suggests that females of SI species are choosier than SC females in the signalling crosstalk between pollen and stigma. A similar case of SI/SC unilateral pollen rejection was reported in the genus *Solanum*, involving the inhibition of pollen tube growth and pollen tube targeting to the ovules rather than of pollen germination (Baek *et al.*, 2015; Lafon-Placette *et al.*, 2016). Importantly, many phenotypic and genetic changes occur after the transition to selfing due to factors other than sexual selection, and disentangling these factors remains a challenge for future research.

### III. SEXUAL SELECTION AND REPRODUCTIVE BARRIERS

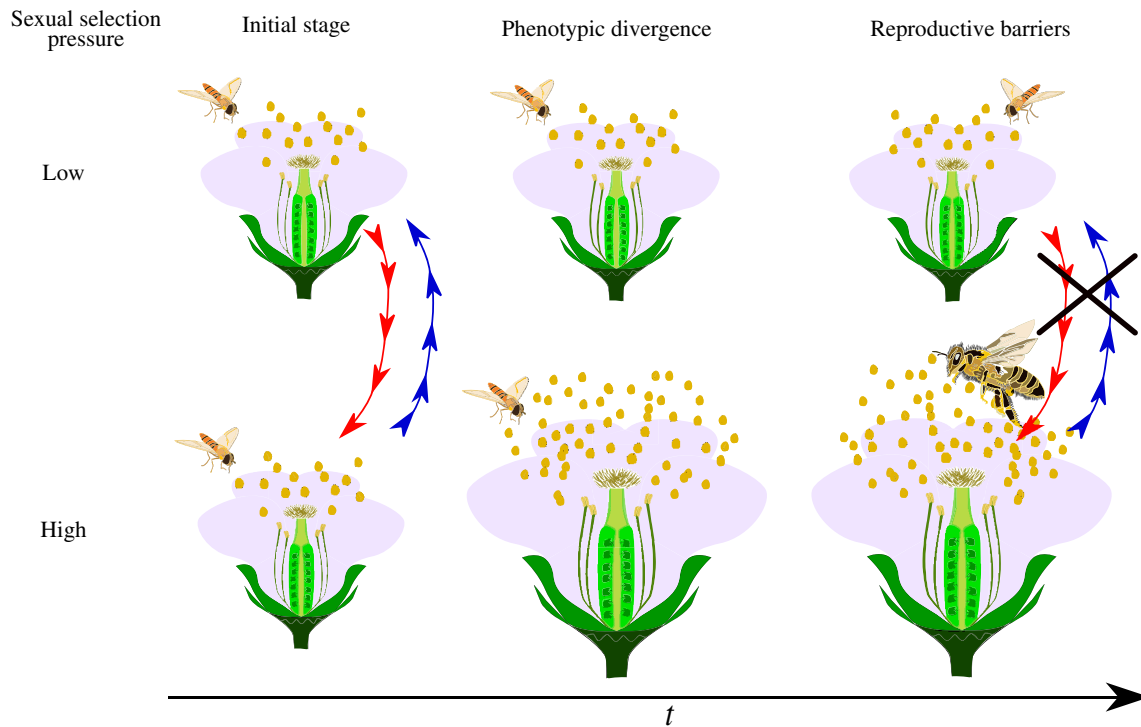
Speciation is the process by which genetically and phenotypically distinct lineages emerge *via* the evolution of reproductive isolation (Dobzhansky, 1935; Mayer, 1942; Turelli *et al.*, 2001; Mallet, 2010; Seehausen *et al.*, 2014). Isolating barriers are classified into prezygotic (e.g. geographical isolation, gametic isolation, or isolation by different pollinators) and postzygotic (hybrid inviability or sterility) (Dobzhansky, 1970; Coyne, 1992; Coyne & Orr, 1998; Orr & Turelli, 2001). A prezygotic barrier means that zygotes are never created, whereas a postzygotic barrier means that individuals of two species may produce zygotes, but these hybrid offspring are inviable or infertile (Dobzhansky, 1970; Coyne, 1992; Coyne & Orr, 1998; Orr & Turelli, 2001). Prezygotic barriers in plants can occur before mating, such as pollinator shifts [e.g. genus *Mimulus* (Schemske & Bradshaw, 1999); *Satyrium longicauda* (Castañeda-Zárate, Johnson & van der Niet, 2021)], or after mating by pollen rejection through blocking pollen germination on the stigma (Li *et al.*, 2018), delaying or stopping pollen tube growth in the style (Baek *et al.*, 2015), or misguiding pollen away from the ovary (Lafon-Placette *et al.*, 2016). It has been suggested that sexual selection could play a role in plant speciation

(Panhuis *et al.*, 2001; Ritchie, 2007) by driving the evolution of these reproductive barriers (Lafon-Placette *et al.*, 2016). In this following section, we evaluate whether sexual selection is a plausible driver of these reproductive barriers in plants at the pre- and postmating stage.

#### (1) Pollinator shift in response to pre-pollination sexual selection

To mate, plants are reliant on vectors (pollinators) for transferring pollen to the stigma. Most angiosperms (87.5%) use animals as vectors (Friedman & Barrett, 2009; Ollerton, Winfree & Tarrant, 2011), and pollinator-mediated selection on floral traits is argued as a mechanism underlying plant adaptation and speciation. It is thought that flowers evolved in angiosperms to attract pollinators (Stebbins, 1970; Kiestler, Lande & Schemske, 1984; Crane, Friis & Pedersen, 1995; Thien *et al.*, 2009). A shift in pollinators *via* floral trait divergence could act as a strong hybridisation barrier; if two lineages no longer share the same pollinator, gene flow between them will be limited or absent. For example, a change in floral reward chemistry from nectar to oil is thought to have led to a pollinator shift in the long-spurred African orchid, *S. longicauda* from moth to oil-collecting bee pollination (Castañeda-Zárate *et al.*, 2021). Similarly, in monkeyflowers, *Mimulus lewisii* is predominantly pollinated by bees, while another, *M. cardinalis*, is hummingbird pollinated (Ramsey, Bradshaw Jr & Schemske, 2003). These differences in pollinator preferences have been explained by floral trait divergence between the two species, which differ in petal colour and nectar volume (Schemske & Bradshaw, 1999; Ramsey *et al.*, 2003).

According to Bateman's principle, male reproductive success will be limited by mating opportunities and therefore the evolution of floral traits is largely driven by selection on males (see Section II.1; Bateman, 1948; Willson, 1994; Collet *et al.*, 2014; Paterno *et al.*, 2020). In other words, increasing pollinator attraction (higher visitation rate) will benefit males (pollen transfer) more than females (Stanton *et al.*, 1991; Queller, 1997; Paterno *et al.*, 2020). It is important to note, however, that in some taxa or under certain ecological circumstances, plants can experience pollen limitation (Knight *et al.*, 2005). In this case, pollinator visitation may also be a limiting factor for female reproductive success, leading to female–female competition for pollinators. In particular, pollinator shift has been proposed to occur in response to decreased abundance of a given pollinator (Thomson & Wilson, 2008) since a shift to a new pollinator should increase the chances of pollination. Thus, pollinator shifts may take place in response to high male–male (and female–female) competition for pollinator attraction (Fig. 1), with low resources (i.e. pollinator visitation) leading to higher intrasexual competition, and hence selecting for flower traits such as more conspicuous display and higher rewards (Ratnieks & Balfour, 2021). We argue that this will lead to floral trait divergence and ultimately to the establishment of reproductive barriers between individuals showing the ancestral and



**Fig. 1.** Pollinator shifts in response to male–male competition (pre-pollination sexual selection). Higher male–male competition for pollinators, for example resulting from local pollinator scarcity, may select for a larger floral display and higher production of pollen grains (bottom row). This may lead to floral divergence (phenotypic divergence) and, ultimately, to the establishment of reproductive barriers between individuals with the ancestral and derived pollinator syndromes.  $t$  is evolutionary time; red and blue arrows indicate gene flow between the two lineages; black cross indicates a barrier to gene flow.

derived pollinator syndromes. This process may act in interaction with other speciation mechanisms, such as reinforcement, i.e. positive selection for a barrier to prevent costly hybridisation. Interestingly, changes in pollinator attraction between *Phlox* species have been shown to act as a reinforcement mechanism (Hopkins & Rausher, 2012; Hopkins *et al.*, 2014). In a contact zone between *Phlox cuspidata* and *P. drummondii*, a change in flower morphology in *P. drummondii* promotes assortative mating (Hopkins & Rausher, 2012). In this contact zone, male–male competition for pollinators could occur both intra- and interspecifically, and as a result, sexual selection may be more intense. In this context, an allele affecting flower colour that promotes assortative mating would potentially increase mating success (pollinator visitation). Thus, we speculate that pollinator shifts may occur as a result of an interaction between sexual selection and reinforcement mechanisms.

## (2) Pollen rejection and post-pollination sexual selection

Pollen from a given lineage may be rejected by the pistil from another lineage, leading to a postmating reproductive barrier. Pollen rejection can take place at different stages: (i) at the germination stage when pollen lands on the stigma (stigmatic pollen rejection); (ii) as pollen tubes grow through the

style (stylar pollen rejection); and (iii) during targeting of pollen tubes to the ovary (ovarian pollen rejection) (Baek *et al.*, 2015; Lafon-Placette *et al.*, 2016; Li *et al.*, 2018). Below, we consider these stages of pollen rejection as reproductive barriers and discuss the possible role of sexual selection among other evolutionary scenarios for establishing these barriers.

While interspecific pollen rejection at the stigma, style or ovary stage may appear to be a form of female choice and sexual selection, this may not always be the case. The process of genetic drift, i.e. random changes in the number of allelic variants in a population (Lynch *et al.*, 2016), may be an alternative explanation for the establishment of pollen rejection. Sexual reproduction involves the location of suitable mates, and the chemical communication between pollen and the female organs (stigma, style, ovules) fulfils this role. If two lineages evolve separately, this chemical communication is likely to evolve separately simply by genetic drift. If sufficient evolutionary time elapses, pollen–female communication will likely diverge enough between lineages that pollen receptors from one lineage will not match female ligands from the other, eventually leading to pollen germination/tube growth/tube guidance incompatibilities in hybridisation events, and hence a reproductive barrier. For example, Swanson *et al.* (2016) compared pollen performance in two *A. thaliana* accessions: Columbia (Col), and Landsberg erecta



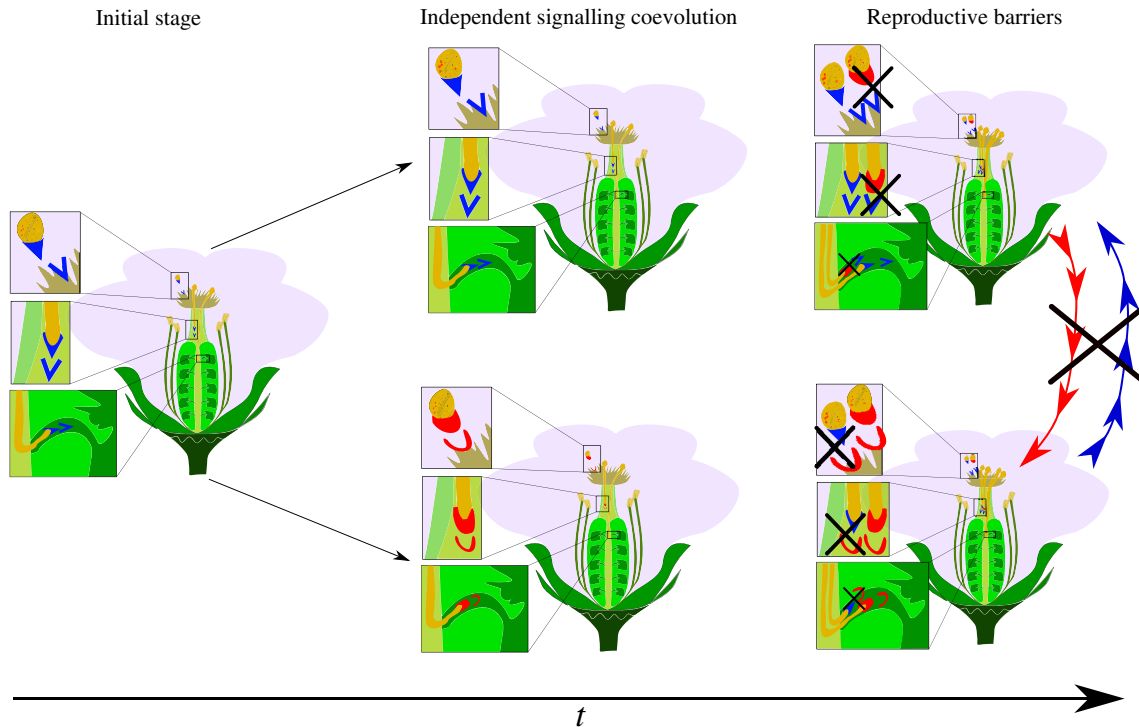
(Ler). In cross-pollination using pollen of Col and Ler with Col pistils, Ler pollen on a Col pistil showed reduced germination and tube growth, leading to significantly higher siring success for Col pollen (98%) than Ler pollen (11%) in a mixed pollination experiment (Swanson *et al.*, 2016). *A. thaliana* is a selfer, and hence is expected to be under relaxed sexual selection (Cutter, 2019). Thus, while this case initially seems to provide an example of female choice, sexual selection is unlikely to be the underlying evolutionary driver and the best scenario is neutral divergence of pollen–pistil communication between Col and Ler by genetic drift, leading to a reproductive barrier.

However, pollen rejection could also be a result of independent runaway sexual selection (Fisherian model; see Section II.1) in two lineages (Ritchie, 2007). For example, in plants, style length as a female choice trait and a faster PTGR as a male trait could coevolve to reach an optimum (Ö. İtaş, A. Le Vève, M. Slovak, C. Lafon Placette, in preparation). Some studies have found positive correlations between pistil length and PTGR (Williams & Rouse, 1990; Herrero & Hormaza, 1996), whereas studies in *Hibiscus* and *Ipomopsis* failed to find a correlation (Snow & Spira, 1996; Alarcón & Campbell, 2000). If female preference (style length, chemical signalling) and male traits (pollen germination, tube growth, chemical reception) coevolve separately in two lineages they might reach two different optima, and as a result, the two lineages will exhibit bilateral mating incompatibilities (Fig. 2). For example, crossing experiments between two predominantly outcrossing iris species, *Iris fulva* and *I. hexagona*, showed a low number of hybrids, while further analysis using mixed pollination (1:1 pollen mixture of both species) to test for interspecific pollen competition resulted in no hybrid progeny, suggesting that the low number of hybrids was due to selective fertilisation by intraspecific pollen (Arnold, Hamrick & Bennett, 1993). Further research revealed that a reduced PTGR of heterospecific pollen may explain failed hybridisation between these two irises (Hodges & Arnold, 1995). The same results were found for two sympatric *Hibiscus* species, *H. moscheutos* and *H. laevis* (Klips, 1999). However, artificial crosses in *Helianthus* gave contrasting results in which a lower number of hybrids could not be explained by PTGR differences (no intraspecific and interspecific difference in PTGRs Rieseberg, Desrochers & Youn, 1995). Disentangling the roles of divergent runaway selection versus neutral divergence by genetic drift as a cause for pollen rejection remains difficult to do, as both have the same outcome, i.e. bilateral pollen rejection. Genomics may help resolve this issue, by identifying the genes responsible for bilateral pollen rejection and assessing selective processes acting on them. Indeed, reproductive genes may evolve faster than other genes simply because they are expressed in a sex-specific manner and therefore selection on them is relaxed: when carried by the other sex, these alleles will be masked to selection (Dapper & Wade, 2016). However, this may be only partly true in plants, since the haploid state in the gametophytic phase is subject to stronger purifying selection than the diploid state, as shown for

pollen-specific genes in *Capsella grandiflora* (Arunkumar *et al.*, 2013). In this study, pollen-specific genes also showed higher rates of positive selection than sporophytic genes. Thus, if genes responsible for bilateral pollen rejection would also show signs of positive selection, one could safely assume that this hybridisation barrier arose as a result of divergent runaway selection rather than drift. This hypothesis remains however to be tested.

Differential sexual selection intensity (i.e. divergence in sexual selection) might be another explanation for the establishment of barriers through pollen rejection in plants. For example, the species *Silene latifolia* shows a wide natural variation in style length, and *S. latifolia* style length was negatively correlated with seed set when pollinated with *S. declinis* (Nista, Brothers & Delph, 2015). This could suggest that populations of *S. latifolia* have experienced different intensities of sexual selection that have led to divergence in style length that translates into different strengths of hybridisation barrier with a related species. Interestingly, the decreased seed set was found between sympatric populations of the two species, suggesting that sexual selection may play an important role in preventing hybridisation in sympatry.

In general, we predict that if two lineages experience different intensities of sexual selection, pollen competitive traits and female choosiness will diverge quantitatively, and as a consequence, these two lineages will face unilateral incompatibilities (Fig. 3). An extreme case of such divergence is relaxed sexual selection inherent to the transition to selfing, as male–male competition will be much reduced in a selfer as compared to an outcrosser (Cutter, 2019). An investigation in wild tomatoes identified a unilateral prezygotic barrier that occurs between the pollen of a self-compatible (SC) species (*Solanum lycopersicum*) and the female of a self-incompatible (SI) related species (*S. pennellii*), whereas the SI pollen was able to fertilise an SC female (Covey *et al.*, 2010; Baek *et al.*, 2015). These results suggest that SI females are choosier than SC females, and that SI pollen is more performant than SC pollen; this has been coined the ‘weak inbreeder/strong outbreeder’ hypothesis (Brandvain & Haig, 2005). As a result, the SI population with a higher intensity of sexual selection is more likely to reject the SC pollen and therefore hybridisation is prevented. Note that it is not clear whether divergent sexual selection alone is involved in this hybridisation barrier, or whether this type of reproductive isolation could simply be a by-product of the SI system. Indeed, some studies suggest that molecular components of the SI system are directly involved in the SI × SC unilateral incompatibility, notably in *Solanum* (Markova *et al.*, 2016; Pease *et al.*, 2016). Work on several *Arabidopsis* species showed that the locus responsible for the SI × SC unilateral incompatibility was not linked to the S-locus (Li *et al.*, 2018), suggesting that this barrier is not a by-product of the SI system. Finally, a study surveying cases of unilateral gene flow in the wild found that all cases of SI × SC species pairs with available information showed unilateral gene flow (Pickup *et al.*, 2019). This suggests that SI × SC unilateral



**Fig. 2.** Pollen rejection in response to independent coevolution of female preference and male traits (post-pollination sexual selection). The separate coevolution of female choice (ligands) and male trait (receptors) in two lineages might establish bilateral incompatibilities, as the ligand and receptor of each lineage will no longer match. Blue triangle/V-shape indicate the receptors and ligands for the ancestral allele; red semicircle/U-shape indicate the receptors and ligands for the derived allele;  $t$  is evolutionary time; red and blue arrows indicate gene flow between the two lineages; black cross indicates a pollen rejection/barrier to gene flow.

incompatibility, and thus potentially sexual selection, could play an important role in species boundaries in nature.

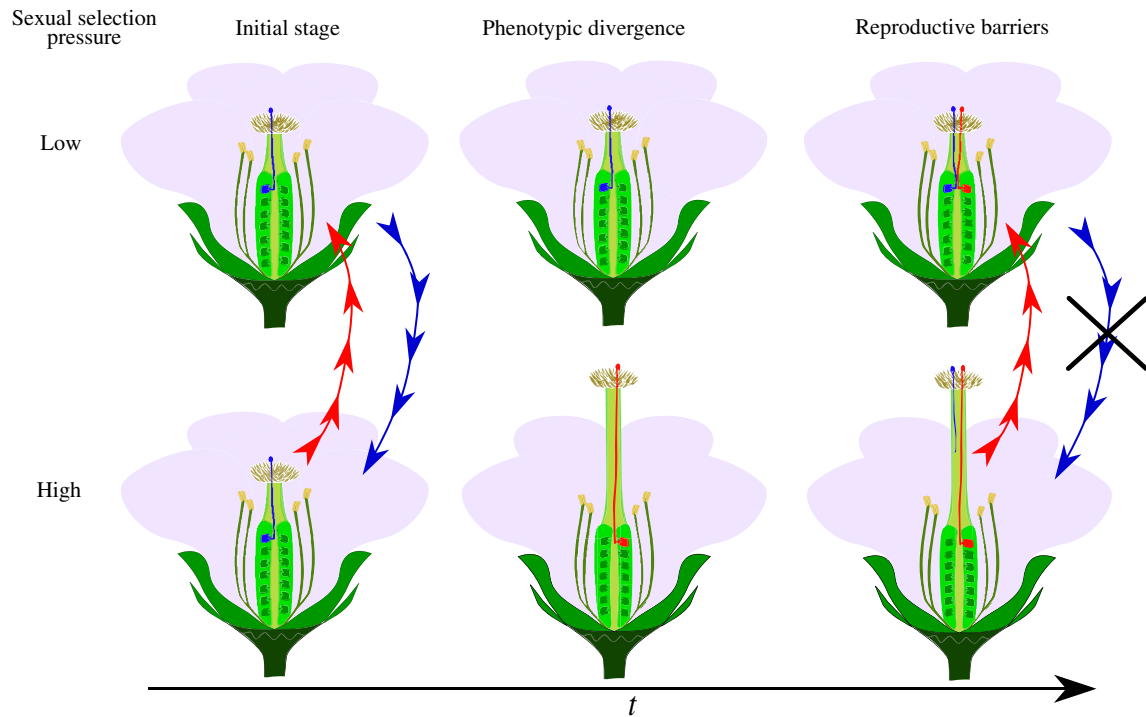
Taken together, plants can show prezygotic barriers before mating through the evolution of traits for different pollinators (pollinator shifts) (Schemske & Bradshaw, 1999; Ramsey *et al.*, 2003; Castañeda-Zárate *et al.*, 2021) and after mating due to pollen rejection (Sarker, Elleman & Dickinson, 1988; Dickinson, 1995; Hulskamp, Schneitz & Pruitt, 1995; Zheng *et al.*, 2018; Tonnabel *et al.*, 2021). Before mating, intense male–male competition for pollinator visitation and pollen transfer may drive rapid divergence of floral traits and potentially lead to pollinator shifts, providing a strong reproductive barrier between lineages. After mating, many evolutionary scenarios can explain pollen rejection as a reproductive barrier. Sexual selection (independent runaway selection or divergent intensity of sexual selection) may be a plausible scenario, but one should first rule out the neutral divergence of molecular mechanisms as an evolutionary cause for interspecific pollen rejection. To achieve this, we propose that (i) the traits involved should be investigated for a role within species in male–male competition/female choice as defined by sexual selection theory; and (ii) pollen rejection should be explained within a sexual selection theoretical framework, i.e. with evidence for independent runaway selection or divergent sexual selection intensity.

To disentangle neutral divergence *versus* independent runaway selection as an explanation for pollen rejection, we predict that pollen rejection established by runaway-like mechanisms should arise faster in clades with more intense sexual selection.

### (3) Ecological speciation and speciation by sexual selection

Animal studies have suggested that sexual selection alone may be insufficient to drive the emergence of new species, and that it is likely to act in concert with local adaptation and ecological speciation (Servedio & Bürger, 2014; Servedio & Boughman, 2017; Irwin, 2020; Mendelson & Safran, 2021). Sometimes, sexual selection may be antagonistic to the latter processes. Below, we evaluate possible interactions between sexual selection, natural selection and speciation in plants.

A clear case of an interaction between sexual selection and natural selection is when traits/genes under sexual selection are also involved in local adaptation. For example, pollen production and performance are affected by different environmental variables such as drought, nutrient availability, or herbivory (Delph, Johannsson & Stephenson, 1997; Liu *et al.*, 2023). Adaptations to such environmental variables



**Fig. 3.** Pollen rejection in response to divergent sexual selection intensity. Two lineages experience different intensities of sexual selection, and as a response pollen competitive traits (pollen tube growth rate, PTGR) and female choosiness (style length) diverge quantitatively. As a result, these two lineages show unilateral incompatibilities, with the pollen of one lineage being unsuccessful in the style of the other lineage, but the reciprocal is not true. Red circle, pollen for the ancestral trait; blue circle, pollen for the novel trait;  $t$  is evolutionary time; red and blue arrows indicate gene flow between the two lineages; black cross indicates a pollen rejection/barrier to gene flow.

may include pollen traits that are actually deleterious to pollen performance (e.g. accumulation of chemical compounds; Descamps, Quinet & Jacquemart, 2021), or adaptation may impact genes with pleiotropic effects, affecting all cells, including pollen. Natural selection would thus favour lower pollen performance while sexual selection would favour higher pollen performance, leading to antagonistic effects between the two. This could explain why, in natural populations, genetic variation is found in pollen performance: directional selection such as sexual selection is expected to decrease variation for this trait, but if natural selection acts antagonistically, this would maintain polymorphism (Delph *et al.*, 1997). Conceivably, sexual selection may slow down ecological divergence between populations by retarding local adaptation, and this could prevent ecological speciation.

Alternatively, sexual and natural selection may act synergistically on plant speciation. This could happen when male traits are a proxy for the fitness of the progeny, also known as ‘good genes’ selection (Byers & Waits, 2006). It is likely that pollen tube growth, a cell growth process with high metabolic demands (Selinski & Scheibe, 2014), is correlated with other aspects of the life cycle involving plant growth. In fact, genes expressed in pollen are also involved in other stages of plant life (Beaudry *et al.*, 2020). If there is female selection for pollen performance, then it is likely that it will also

select for progeny fitness, fitting the ‘good genes’ scenario. In this case, sexual and natural selection may act in the same direction, and this is likely to accelerate the ecological divergence between two populations, and ultimately, ecological speciation.

While these considerations are key to understanding the role of sexual selection in plant speciation, they are admittedly speculative. Further empirical research is needed to unravel how sexual selection might interact with local adaptation and how this interplay could affect speciation, including whether these processes typically act in synergy or antagonism and under what circumstances.

#### IV. SEXUAL SELECTION AND PLANT SPECIATION (MACROEVOLUTIONARY SCALE)

##### (1) Macroevolutionary research

Sexual selection is known to influence evolution above the species level (macroevolution) (Futuyma & Kirkpatrick, 2017). While the mechanisms behind the effects of sexual selection on large-scale speciation and extinction remain subject to research, a significant body of empirical results for plants support correlations between a variety of sexual traits

(sexual dimorphism, reproductive system, flower colouration) and diversification rates inferred from molecular phylogenies (Hodges & Arnold, 1995; Dodd, Silvertown & Chase, 1999; Kay *et al.*, 2006; Goldberg *et al.*, 2010).

Diversification refers to the net difference between speciation and extinction and can be estimated from the branching patterns of present-day phylogenies (Nee, May & Harvey, 1994; Ricklefs, 2007; Title & Rabosky, 2019). Consequently, differences in diversification, as defined here, might be due to differences in speciation, extinction, or some combination of these. Early studies correlated sexual traits with diversification rates and sometimes with differences in species richness across sister clades within a phylogeny (Heilbuth, 2000; Paradis, 2012; Jetz *et al.*, 2012; Rabosky & Matute, 2013; Maddison & FitzJohn, 2015; Rabosky & Goldberg, 2015; Futuyma & Kirkpatrick, 2017). While these correlations seem tentatively to suggest a link between sexual selection and diversification, later work revealed that such correlations might have other causes. These correlations could result from an asymmetry in diversification rates across trait values (due to sexual selection) but also from an asymmetry of transitions between trait values (due to trait evolution) (Maddison, 2006; Käfer *et al.*, 2017). To tease apart these effects, a range of methods have been developed that model the two asymmetries simultaneously (FitzJohn, 2010; Beaulieu & O'Meara, 2016). While these methods have their limitations (e.g. Type I errors when the assumption of time-constant diversification rates is violated) (Machac, 2014; Rabosky & Goldberg, 2015), empirical research based on this wide range of methods [sister-clade comparisons, binary state speciation and extinction (SSE), quantitative SSE, hidden SSE] has identified a compelling battery of traits that suggest signatures of sexual selection at the macroevolutionary level.

Flower symmetry, floral structure, nectar production, and the presence of an inflorescence represent several well-known examples of sexual traits that seem to be statistically associated with faster diversification. Other traits that might also be interpreted as relating to sexual selection include animal pollination, bilateral floral symmetry, and the presence of nectar spurs, all of which have also been hypothesised to promote speciation rates because of their likely effects on mating specificity in plants. Empirical research confirmed that self-incompatibility (Goldberg *et al.*, 2010), floral nectar spurs (Hodges & Arnold, 1995), and bilateral floral symmetry (Kay *et al.*, 2006) promote diversification, at least in some plant taxa. Moreover, Dodd *et al.* (1999) used sister-clade comparisons to show that animal pollination is associated with faster diversification than abiotic modes of pollination (e.g. wind pollination). These findings correspond with much empirical research implicating a role of plant–pollinator interactions in plant speciation (Stebbins, 1975; Grant, 1981; Coyne & Orr, 2004; Kay *et al.*, 2006).

Identifying traits that fit the theoretical and mechanistic definition of sexual selection is not trivial, as not all reproduction-related traits will be under sexual selection.

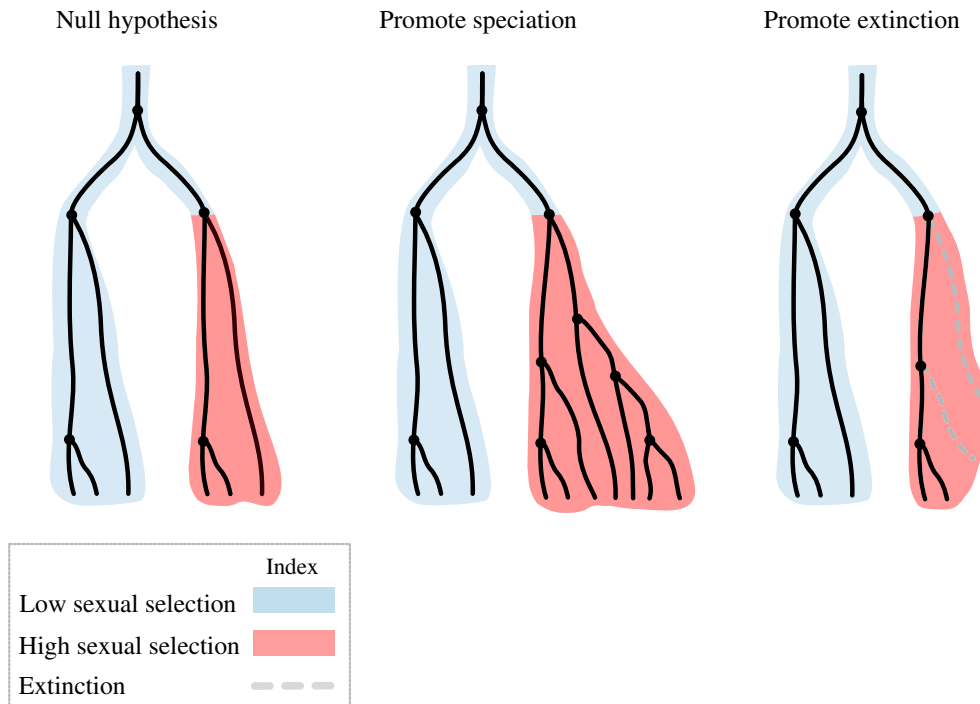
Moreover, different traits could have mutually contradictory effects. For example, even though selfing is often thought to promote diversification (Igic *et al.*, 2008), Goldberg *et al.* (2010) reported that selfing might simultaneously increase both speciation and extinction rates, at least in the family Solanaceae. Selfing might therefore produce a short-term advantage through increased speciation, which might be balanced by the long-term extinction risk, making outcrossing advantageous in the long term. Importantly, transitions to selfing from outcrossing are related to many other life-history and population genetic changes, such as assured reproduction, increased capacity to colonise new regions, and population genetic structure, all of which may contribute to speciation independently of sexual selection. This example illustrates the complexity of attempting to isolate the effects of any particular trait and to determine whether it operates through sexual selection on plant speciation. This is a shortcoming of the correlative research that typifies macroevolutionary studies, and this field could benefit from the more mechanistic results available from population genetics, involving selection strength, genetic architecture of traits, etc.

Identifying the mechanisms behind widely known statistical correlations (e.g. between sexual dimorphism and diversification) has been notoriously difficult, partly because previous studies rarely investigated the mechanisms across scales that bridge the gap between microevolutionary processes and macroevolutionary patterns.

## (2) Bridging the micro- and macroevolution of speciation by sexual selection in plants

Evolution has been extensively studied below (microevolution) as well as above the species level (macroevolution) and while much research exists within each of these two levels, the chasm between them has been hard to close (Reznick & Ricklefs, 2009). One way to bridge the gap between the microevolutionary processes and macroevolutionary patterns might be to focus on sexual traits or reproductive strategies that are linked to sexual selection below the species level but also correlate with diversification patterns above the species level. These traits (e.g. the P/O ratio, style length) might serve as a common currency to translate sexual selection and its cascading effects across the levels of evolutionary investigation. Possible disconnects between these levels would indicate that sexual selection within species does not translate into macroevolutionary patterns, presumably because its effects are less important than other selective processes (e.g. temperature-driven speciation rates, extinction rates mediated by geographic range size; Fig. 4) (Brown *et al.*, 2004; Jablonski, 2008). By considering traits under sexual selection, we might achieve an integrative perspective that encompasses population-level processes (strength of sexual selection) which translate into divergence between populations, the emergence of reproductive barriers, speciation and ultimately, the evolution and diversification of new clades.





**Fig. 4.** Influence of sexual selection on evolution above the species level in plants. The null hypothesis is that branching patterns in clades with low or high sexual selection intensity are homogeneous. Alternatively, higher sexual selection in one clade may lead to higher branching, promoting speciation, or it might lead to lower branching, promoting extinction.

Another way to bridge micro- and macroevolutionary levels might be to consider the mechanisms of sexual selection, and to postulate likely links. For example, premating male–male competition may lead to floral divergence (Cocucci *et al.*, 2014; Lynn *et al.*, 2020; Paterno *et al.*, 2020), causing pollinator shifts (Ramsey *et al.*, 2003; Castañeda-Zárate *et al.*, 2021) that, in turn, result in faster speciation rates. Runaway selection may lead to faster divergence of pollen–pistil communication mechanisms that result in the emergence of pollen–pistil incompatibilities (Arnold *et al.*, 1993; Carney, Hodges & Arnold, 1996; Klips, 1999) and speciation. Finally, sexually driven divergence in pollen performance and female choosiness (Díaz & Macnair, 1999; Runions & Geber, 2000; Mazer *et al.*, 2018; Madjidian *et al.*, 2020) may produce unilateral pollen–pistil incompatibilities (Covey *et al.*, 2010; Baek *et al.*, 2015) which result in speciation. For example, the change in pollination mode from biotic to abiotic is correlated with a decreasing speciation rate (Dodd *et al.*, 1999). We may mechanistically connect the levels of evolutionary investigation by stating that premating barriers arise faster as a consequence of sexual selection having more opportunities to act (floral attraction traits) in animal-pollinated species compared to wind-pollinated ones, and this may lead to faster speciation rates due to frequent pollinator shifts. Also, as a similar intensity of postmating sexual selection was found in wind- and animal-pollinated species (Ruane, 2009), and as speciation rates do vary between

these species (Dodd *et al.*, 1999), we may predict from this example that postmating barriers driven by sexual selection such as pollen rejection do not play a major role in plant speciation.

While each of these mechanisms have been supported by empirical work, no studies to our knowledge have attempted to demonstrate a chain of these processes within the same study system. Yet, suitable systems for such work exist (e.g. well-studied plant families with abundant phenotypic and phylogenetic data such as Brassicaceae or Solanaceae), that would permit the examination of sexual selection from micro- to macroscales.

Despite the promise of the trait-based approach, we caution that some traits might approximate sexual selection better than others. While reproductive strategies might provide the first approximation of the effects of sexual selection, future studies might benefit from more direct proxies, such as style length and stigmatic surface area as proxies for female choice. The P/O ratio might serve as a direct proxy for the intensity of premating male–male competition. The ratio between pollen deposited on the stigma and ovule number might be a useful proxy for the intensity of postmating male–male competition. Measuring these more direct proxies and relating them to life-history traits and diversification across a whole clade (e.g. Brassicaceae, Solanaceae) is feasible and may produce compelling results. Importantly, since these more direct proxies have specific effects, we will be able to make accurate predictions as to how they lead to the

formation of reproductive barriers, and hence bridge the micro- and macroevolutionary scales.

## V. CONCLUSIONS

(1) While a role of sexual selection in plants is now relatively well accepted, its role in plant speciation remains largely unexplored (see Table 1). Compared to animals, plants show a wider range of reproductive strategies, making them a unique system to study sexual selection mechanisms and their impacts on speciation.

(2) Many authors have associated pollen rejection, a common reproductive barrier in plants, with female choice/sexual selection. However, the most parsimonious explanation for pollen rejection is neutral divergence in the mechanisms for mate compatibility. For a hybridisation barrier to fit into a theoretical frame of sexual selection, one requires evidence for a role of the mechanism in *bona fide* sexual selection within a lineage together with evidence for divergent sexual selection between reproductively isolated lineages.

(3) Whether reproductive barriers driven by sexual selection translate into large-scale speciation remains unknown in both plants and animals, despite the use of relatively direct proxy traits for sexual selection mechanisms in numerous macroevolutionary studies in the latter. In plants, we first need to identify traits that act as direct proxies for sexual selection mechanisms. Reproductive strategies (pollination mode, sexual polymorphism, mating system) may prove too indirect and introduce too many confounding factors to be used to investigate the role of sexual selection in plant speciation.

(4) Only by using direct proxy traits with a clear role in sexual selection mechanisms, will we be able to bridge micro- and macroevolution in order to understand if and by which mechanisms sexual selection plays a role in plant speciation. This will require evidence for a role in sexual selection below the species level and a role in diversification patterns above the species level, together with the ability to link these processes mechanistically at all evolutionary levels of investigation.

(5) Such work may reveal that sexual selection plays an important role in plant speciation, or may instead show that classical drivers of speciation such as geographic or ecological isolation are dominant.

## VI. ACKNOWLEDGEMENTS

We thank members of Lab Alliance for carefully reading and improving the manuscript. M. H. and C. L. P. were supported by Charles University Research Centre program no. PRIMUS/19/SCI/02. M. H. and R. S. were supported by the long-term research development project no. RVO 67985939 of the Czech Academy of Sciences. A. M. was supported by the Standard Grant no. 23-05977S from the Czech Science Foundation.

## VII. REFERENCES

- ACKERMAN, J. D. (2000). Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. In *Pollen and Pollination* (eds A. DAFNI, M. HESSE and E. PACINI), pp. 167–185. Springer Vienna, Vienna.
- ALARCÓN, R. & CAMPBELL, D. R. (2000). Absence of conspecific pollen advantage in the dynamics of an *Ipomopsis* (Polemoniaceae) hybrid zone. *American Journal of Botany* **87**, 819–824.
- ALJIBOURY, A. A. & FRIEDMAN, J. (2022). Mating and fitness consequences of variation in male allocation in a wind-pollinated plant. *Evolution* **76**, 1762–1775.
- ARMBRUSTER, W. S. (2014). Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AOB Plants* **6**, plu003.
- ARMBRUSTER, W. S., MARTIN, P., KIDD, J., STAFFORD, R. & ROGERS, D. G. (1995). Reproductive significance of indirect pollen-tube growth in *Dalechampia* (Euphorbiaceae). *American Journal of Botany* **82**, 51–56.
- ARNOLD, M. L., HAMRICK, J. L. & BENNETT, B. D. (1993). Interspecific pollen competition and reproductive isolation in *Iris*. *Journal of Heredity* **84**, 13–16.
- ARNOLD, S. J. (1994). Bateman's principles and the measurement of sexual selection in plants and animals. *The American Naturalist* **144**, 126–149.
- ARUNKUMAR, R., JOSEPHS, E. B., WILLIAMSON, R. J. & WRIGHT, S. I. (2013). Pollen-specific, but not sperm-specific, genes show stronger purifying selection and higher rates of positive selection than sporophytic genes in *Capsella grandiflora*. *Molecular Biology and Evolution* **30**, 2475–2486.
- BAEK, Y. S., COVEY, P. A., PETERSEN, J. J., CHETELAT, R. T., MCCLURE, B. & BEDINGER, P. A. (2015). Testing the SI × SC rule: pollen–pistil interactions in interspecific crosses between members of the tomato clade (*Solanum* section *Lycopersicon*, Solanaceae). *American Journal of Botany* **102**, 302–311.
- BARRETT, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**, 274–284.
- BASKIN, J. M. & BASKIN, C. C. (2015). Pollen (microgametophyte) competition: an assessment of its significance in the evolution of flowering plant diversity, with particular reference to seed germination. *Seed Science Research* **25**, 1–11.
- BATEMAN, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368.
- BAWA, K. S. (1980). Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* **11**, 15–39.
- BAWA, K. S. (1994). Pollinators of tropical dioecious angiosperms: a reassessment? No, not yet. *American Journal of Botany* **81**, 456–460.
- BAWA, K. S. & OPLER, P. A. (1975). Dioecism in tropical forest trees. *Evolution* **29**, 167–179.
- BEAUDRY, F. E. G., RIFKIN, J. L., BARRETT, S. C. H. & WRIGHT, S. I. (2020). Evolutionary genomics of plant gametophytic selection. *Plant Communications* **1**, 100115.
- BEAULIEU, J. M. & O'MEARA, B. C. (2016). Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic Biology* **65**, 583–601.
- BEEKMAN, M., NIEUWENHUIS, B., ORTIZ-BARRIENTOS, D. & EVANS, J. P. (2016). Sexual selection in hermaphrodites, sperm and broadcast spawners, plants and fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**, 20150541.
- BOCK, C. (1976). Polymorphisme floral et sterilité male chez le *Silene acaulis* (L.) Jacq. *Comptes Rendues de l'Académie des Sciences (Paris)* **282**, 1865–1868.
- BOUGHMAN, J. W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**, 944–948.
- BOUL, K. E., FUNK, W. C., DARST, C. R., CANNATELLA, D. C. & RYAN, M. J. (2006). Sexual selection drives speciation in an Amazonian frog. *Proceedings, Biological Sciences* **274**, 399–406.
- BRANDVAIN, Y. & HAIG, D. (2005). Divergent mating systems and parental conflict as a barrier to hybridization in flowering plants. *The American Naturalist* **166**, 330–338.
- BROWN, J. H., GILLOOLY, J. F., ALLEN, A. P., SAVAGE, V. M. & WEST, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
- BYERS, J. A. & WAITS, L. (2006). Good genes sexual selection in nature. *Proceedings of the National Academy of Sciences* **103**, 16343–16345.
- CALLY, J. G., STUART-FOX, D., HOLMAN, L., DALE, J. & MEDINA, I. (2021). Male-biased sexual selection, but not sexual dichromatism, predicts speciation in birds. *Evolution* **75**, 931–944.
- CARNEY, S. E., HODGES, S. A. & ARNOLD, M. L. (1996). Effects of differential pollen-tube growth on hybridization in the Louisiana irises. *Evolution* **50**, 1871–1878.
- CASTAÑEDA-ZARATE, M., JOHNSON, S. D. & VAN DER NIET, T. (2021). Food reward chemistry explains a novel pollinator shift and vestigialization of long floral spurs in an orchid. *Current Biology* **31**, 238–246.e7.
- CHARLESWORTH, D. (1999). Theories of the evolution of Dioecy. In *Gender and Sexual Dimorphism in Flowering Plants* (eds M. A. GEBER, T. E. DAWSON and L. F. DELPH), pp. 33–60. Springer Berlin, Heidelberg.
- CHARLESWORTH, D. (2006). Evolution of plant breeding systems. *Current Biology* **16**, 726–735.
- CHARNOV, E. L. (1979). Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences* **76**, 2480–2484.

- COCUCCI, A. A., MARINO, S., BARANZELLI, M., WIEMER, A. P. & SÉRSIC, A. (2014). The buck in the milkweed: evidence of male–male interference among pollinaria on pollinators. *New Phytologist* **203**, 280–286.
- COLLET, J. M., DEAN, R. F., WORLEY, K., RICHARDSON, D. S. & PIZZARI, T. (2014). The measure and significance of Bateman's principles. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20132973.
- COVEY, P. A., KONDO, K., WELCH, L., FRANK, E., SIANTA, S., KUMAR, A., NUÑEZ, R., LOPEZ-CASADO, G., VAN DER KNAAP, E., ROSE, J. K. C., MCCLURE, B. A. & BEDINGER, P. A. (2010). Multiple features that distinguish unilateral incongruity and self-incompatibility in the tomato clade. *The Plant Journal* **64**, 367–378.
- COYNE, J. A. (1992). Genetics and speciation. *Nature* **355**, 511–515.
- COYNE, J. A. & ORR, H. (1998). The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **353**, 287–305.
- COYNE, J. A. & ORR, H. A. (2004). *Speciation*. Sinauer Associates, Sunderland.
- CRANE, P. R., FRIIS, E. M. & PEDERSEN, K. R. (1995). The origin and early diversification of angiosperms. *Nature* **374**, 27–33.
- CRUDEN, R. W. (1977). Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31**, 32–46.
- CUTTER, A. D. (2019). Reproductive transitions in plants and animals: selfing syndrome, sexual selection and speciation. *New Phytologist* **224**, 1080–1094.
- DAPPER, A. L. & WADE, M. J. (2016). The evolution of sperm competition genes: the effect of mating system on levels of genetic variation within and between species. *Evolution* **70**, 502–511.
- DARWIN, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. John Murray, London.
- DARWIN, C. (1876). *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. John Murray, London.
- DARWIN, C. (1877). *The Different Forms of Flowers on Plants of the Same Species*. John Murray, London.
- DAVIS, C. C., SCHAEFER, H., XI, Z., BAUM, D. A., DONOGHUE, M. J. & HARMON, L. J. (2014). Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 5914–5919.
- DELPH, L. F. (2019). Pollen competition is the mechanism underlying a variety of evolutionary phenomena in dioecious plants. *New Phytologist* **224**, 1075–1079.
- DELPH, L. F. & ASHMAN, T.-L. (2006). Trait selection in flowering plants: how does sexual selection contribute? *Integrative and Comparative Biology* **46**, 463–472.
- DELPH, L. F., JOHANSSON, M. H. & STEPHENSON, A. G. (1997). How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology* **78**, 1632–1639.
- DELPH, L. F., WEINIG, C. & SULLIVAN, K. (1998). Why fast-growing pollen tubes give rise to vigorous progeny: the test of a new mechanism. *Proceedings of the Royal Society of London Series B: Biological Sciences* **265**, 935–939.
- DESCAMPS, C., QUINET, M. & JACQUEMART, A.-L. (2021). Climate change–induced stress reduce quantity and alter composition of nectar and pollen from a bee-pollinated species (*Borago officinalis*, Boraginaceae). *Frontiers in Plant Science* **12**, 755843.
- DIAZ, A. & MACNAIR, M. R. (1999). Pollen tube competition as a mechanism of prezygotic reproductive isolation between *Mimulus nasutus* and its presumed progenitor *M. guttatus*. *The New Phytologist* **144**, 471–478.
- DICKINSON, H. (1995). Dry stigmas, water and self-incompatibility in *Brassica*. *Sexual Plant Reproduction* **8**, 1–10.
- DOBZHANSKY, T. (1935). A critique of the species concept in biology. *Philosophy of Science* **2**, 344–355.
- DOBZHANSKY, T. (1970). *Genetics of the Evolutionary Process*. Columbia University Press, New York City.
- DODD, M. E., SILVERTOWN, J. & CHASE, M. W. (1999). Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* **53**, 732–744.
- DOUCET, J., LEE, H. K. & GORING, D. R. (2016). Pollen acceptance or rejection: a tale of two pathways. *Trends in Plant Science* **21**, 1058–1067.
- DRESSELHAUS, T. & FRANKLIN-TONG, N. (2013). Male–female crosstalk during pollen germination, tube growth and guidance, and double fertilization. *Molecular Plant* **6**, 1018–1036.
- DUFFY, K. J. & JOHNSON, S. D. (2014). Male interference with pollination efficiency in a hermaphroditic orchid. *Journal of Evolutionary Biology* **27**, 1751–1756.
- DULBERGER, R. & HOROVITZ, A. (1984). Gender polymorphism in flowers of *Silene vulgaris* (Moench) Garcke (Caryophyllaceae). *Botanical Journal of the Linnean Society* **89**, 101–117.
- DURAND, E., CHANTREAU, M., LE VEVE, A., STETSENKO, R., DUBIN, M., GENETE, M., LLAURENS, V., POUX, C., ROUX, C., BILLIARD, S., VEKEMANS, X. & CASTRIC, V. (2020). Evolution of self-incompatibility in the Brassicaceae: lessons from a textbook example of natural selection. *Evolutionary Applications* **13**, 1279–1297.
- EBERHARD, W. (1996). *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton.
- EBERHARD, W. G. (2009). Postcopulatory sexual selection: Darwin's omission and its consequences. *Proceedings of the National Academy of Sciences* **106**, 10025–10032.
- ECKERT, C. G., SAMIS, K. E. & DART, S. (2006). Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In *Ecology and Evolution of Flowers* (eds L. D. HARDER and S. C. H. BARRETT), pp. 183–203. Oxford University Press, Oxford.
- ELLE, E. & MEAGHER, T. R. (2000). Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae). II. Paternity and functional gender. *The American Naturalist* **156**, 622–636.
- EPPLEY, S. M. & PANNELL, J. R. (2007). Density-dependent self-fertilization and male versus hermaphrodite siring success in an androdioecious plant. *Evolution* **61**, 2349–2359.
- FISHER, R. A. (1930). *The Genetical Theory of Natural Selection*. The Clarendon Press, Oxford.
- FISHER, R. A. (1941). Average excess and average effect of a gene substitution. *Annals of Eugenics* **11**, 53–63.
- FITZJOHN, R. G. (2010). Quantitative traits and diversification. *Systematic Biology* **59**, 619–633.
- FREEMAN, D. C., KLIKOFF, L. G. & HARPER, K. T. (1976). Differential resource utilization by the sexes of dioecious plants. *Science* **193**, 597–599.
- FRIEDMAN, J. & BARRETT, S. C. (2009). Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* **103**, 1515–1527.
- FRIEDMAN, J. & BARRETT, S. C. H. (2011). The evolution of ovule number and flower size in wind-pollinated plants. *The American Naturalist* **177**, 246–257.
- FUTUYMA, D. & KIRKPATRICK, M. (2017). *Evolution*. Sinauer Associates, Sunderland.
- GANESHIAH, K. N. & SHAANKER, R. (2001). Sexual selection in plants: the process, components and significance. *Proceedings of the Indian National Science Academy-Part B: Biological Sciences* **67**, 423–432.
- GOLDBERG, E. E., KOHN, J. R., LANDE, R., ROBERTSON, K. A., SMITH, S. A. & IGIC, B. (2010). Species selection maintains self-incompatibility. *Science* **330**, 493–495.
- GONG, Y.-B. & HUANG, S.-Q. (2014). Interspecific variation in pollen–ovule ratio is negatively correlated with pollen transfer efficiency in a natural community. *Plant Biology* **16**, 843–847.
- GRANT, V. (1981). *Plant Speciation*. Columbia University Press, New York.
- GUTIÉRREZ-VALENCIA, J., FRACASSETTI, M., HORVATH, R., LAENEN, B., DÉSAMORE, A., DROUZAS, A. D., FRIBERG, M., KOLÁŘ, F. & SLOTT, T. (2022). Genomic signatures of sexual selection on pollen-expressed genes in *Arabidopsis thaliana*. *Molecular Biology and Evolution* **39**, msab349.
- HARKNESS, A. & BRANDVAIN, Y. (2021). Non-self recognition-based self-incompatibility can alternatively promote or prevent introgression. *New Phytologist* **231**, 1630–1643.
- HARVEY, P. H. & MAY, R. M. (1989). Out for the sperm count. *Nature* **337**, 508–509.
- HEILBUTH, J. C. (2000). Lower species richness in dioecious clades. *The American Naturalist* **156**, 221–241.
- HERRERO, M. & HORMAZA, J. I. (1996). Pistil strategies controlling pollen tube growth. *Sexual Plant Reproduction* **9**, 343–347.
- HODGES, S. A. & ARNOLD, M. L. (1995). Spurring plant diversification: are floral nectar spurs a key innovation? *Proceedings of the Royal Society of London Series B: Biological Sciences* **262**, 343–348.
- HOPKINS, R., GUERRERO, R. F., RAUSHER, M. D. & KIRKPATRICK, M. (2014). Strong reinforcing selection in a Texas wildflower. *Current Biology* **24**, 1995–1999.
- HOPKINS, R. & RAUSHER, M. D. (2012). Pollinator-mediated selection on flower color allele drives reinforcement. *Science* **335**, 1090–1092.
- HULSKAMP, M., SCHNEITZ, K. & PRUITT, R. E. (1995). Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*. *The Plant Cell* **7**, 57–64.
- IGIC, B. & KOHN, J. R. (2006). The distribution of plant mating systems: study bias against obligately outcrossing species. *Evolution* **60**, 1098–1103.
- IGIC, B., LANDE, R. & KOHN, J. R. (2008). Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* **169**, 93–104.
- ILTAŞ, Ö., LE VÈVE, A., SLOVAK, M. & LAFON PLACETTE, C. (2023). Runaway selection and the coevolution between pollen tube growth and style length: a molecular love story. in preparation.
- IRWIN, D. E. (2020). Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. *The American Naturalist* **195**, E150–E167.
- JABLONSKI, D. (2000). Micro- and macroevolution: scale and hierarchy in evolutionary biology and paleobiology. *Paleobiology* **26**, 15–52.
- JABLONSKI, D. (2008). Biotic interactions and macroevolution: extensions and mismatches across scales and levels. *Evolution* **62**, 715–739.
- JETZ, W., THOMAS, G. H., JOY, J. B., HARTMANN, K. & MOOERS, A. O. (2012). The global diversity of birds in space and time. *Nature* **491**, 444–448.

- JOHNSON, M. A., HARPER, J. F. & PALANIVELU, R. (2019). A fruitful journey: pollen tube navigation from germination to fertilization. *Annual Review of Plant Biology* **70**, 809–837.
- JOHNSTON, M. O. (1993). Tests of two hypotheses concerning pollen competition in a self-compatible, long-styled species (*Lobelia cardinalis*: Lobeliaceae). *American Journal of Botany* **80**, 1400–1406.
- KÄFER, J., MARAIS, G. A. B. & PANNELL, J. R. (2017). On the rarity of dioecy in flowering plants. *Molecular Ecology* **26**, 1225–1241.
- KAY, K. M., VOELCKEL, C., YANG, J. Y., HUFFORD, K. M., KASKA, D. D. & HODGES, S. A. (2006). Floral characters and species diversification. In *Ecology and Evolution of Flowers* (eds L. D. HARDER and S. C. H. BARRETT), pp. 311–325. Oxford University Press, Oxford.
- KIESTER, A. R., LANDE, R. & SCHEMSKE, D. W. (1984). Models of coevolution and speciation in plants and their pollinators. *The American Naturalist* **124**, 220–243.
- KLIMES, L., KLIMESOVÁ, J., HENDRIKS, R. & VAN GROENENDAEL, J. (1997). Clonal plant architecture: a comparative analysis of form and function. *The Ecology and Evolution of Clonal Plants* **2917**, 1–29.
- KLIPS, R. A. (1999). Pollen competition as a reproductive isolating mechanism between two sympatric *Hibiscus* species (Malvaceae). *American Journal of Botany* **86**, 269–272.
- KNIGHT, T. M., STREETS, J. A., VAMOSI, J. C., MAZER, S. J., BURD, M., CAMPBELL, D. R., DUDASH, M. R., JOHNSTON, M. O., MITCHELL, R. J. & ASHMAN, T.-L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36**, 467–497.
- KOKKO, H. & JENNIONS, M. D. (2008). Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* **21**, 919–948.
- KRAAIJEVELD, K., KRAAIJEVELD-SMIT, F. J. L. & MAAN, M. E. (2011). Sexual selection and speciation: the comparative evidence revisited. *Biological Reviews* **86**, 367–377.
- KWOK, A. & DORKEN, M. E. (2022). Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proceedings of the Royal Society B: Biological Sciences* **289**, 20220919.
- LAFFON-PLACETTE, C., VALLEJO-MARÍN, M., PARISOD, C., ABBOTT, R. J. & KÖHLER, C. (2016). Current plant speciation research: unravelling the processes and mechanisms behind the evolution of reproductive isolation barriers. *New Phytologist* **209**, 29–33.
- LANKINEN, Å. & GREEN, K. (2015). Using theories of sexual selection and sexual conflict to improve our understanding of plant ecology and evolution. *AOB Plants* **7**, plv008.
- LANKINEN, Å. & SKOGSMYR, I. (2002). Pollen competitive ability: the effect of proportion in two-donor crosses. *Evolutionary Ecology Research* **4**, 687–700.
- LANKINEN, Å. & STRANDH, M. (2016). Differential selection on pollen and pistil traits in relation to pollen competition in the context of a sexual conflict over timing of stigma receptivity. *AOB Plants* **8**, plw061.
- LI, L., LIU, B., DENG, X., ZHAO, H., LI, H., XING, S., FETZER, D. D., LI, M., NASRALLAH, M. E., NASRALLAH, J. B. & LIU, P. (2018). Evolution of interspecific unilateral incompatibility in the relatives of *Arabidopsis thaliana*. *Molecular Ecology* **27**, 2742–2753.
- LIU, X., XIAO, Y., ZI, J., YAN, J., LI, C., DU, C., WAN, J., WU, H., ZHENG, B., WANG, S. & LIANG, Q. (2023). Differential effects of low and high temperature stress on pollen germination and tube length of mango (*Mangifera indica* L.) genotypes. *Scientific Reports* **13**, 611.
- LYNCH, M., ACKERMAN, M. S., GOUT, J.-F., LONG, H., SUNG, W., THOMAS, W. K. & FOSTER, P. L. (2016). Genetic drift, selection and the evolution of the mutation rate. *Nature Reviews Genetics* **17**, 704–714.
- LYNN, A., PIOTTER, E., HARRISON, E. & GALEN, C. (2020). Sexual and natural selection on pollen morphology in *Taraxacum*. *American Journal of Botany* **107**, 364–374.
- MAAN, M. E. & SEEHAUSEN, O. (2011). Ecology, sexual selection and speciation. *Ecology Letters* **14**, 591–602.
- MACHAC, A. (2014). Detecting trait-dependent diversification under diversification slowdowns. *Evolutionary Biology* **41**, 201–211.
- MADDISON, W. P. (2006). Confounding asymmetries in evolutionary diversification and character change. *Evolution* **60**, 1743–1746.
- MADDISON, W. P. & FITZJOHN, R. G. (2015). The unsolved challenge to phylogenetic correlation tests for categorical characters. *Systematic Biology* **64**, 127–136.
- MADJIDIAN, J. A., SMITH, H. G., ANDERSSON, S. & LANKINEN, Å. (2020). Direct and indirect selection on mate choice during pollen competition: effects of male and female sexual traits on offspring performance following two-donor crosses. *Journal of Evolutionary Biology* **33**, 1452–1467.
- MALLET, J. (2010). Group selection and the development of the biological species concept. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 1853–1863.
- MARKOVA, D. N., PETERSEN, J. J., QIN, X., SHORT, D. R., VALLE, M. J., TOVAR-MÉNDEZ, A., MCCLURE, B. A. & CHETELAT, R. T. (2016). Mutations in two pollen self-incompatibility factors in geographically marginal populations of *Solanum habrochaites* impact mating system transitions and reproductive isolation. *American Journal of Botany* **103**, 1847–1861.
- MARSHALL, D. L. & DIGGLE, P. K. (2001). Mechanisms of differential pollen donor performance in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany* **88**, 242–257.
- MARSHALL, D. L., SHANER, M. G. & OLIVA, J.-P. (2007). Effects of pollen load size on seed paternity in wild radish: the roles of pollen competition and mate choice. *Evolution* **61**, 1925–1937.
- MAYER, E. (1942). *Systematics and the Origin of Species from the Viewpoint of a Zoologist*. Columbia University Press, New York.
- MAZER, S. J., HENDRICKSON, B. T., CHELLEW, J. P., KIM, L. J., LIU, J. W., SHU, J. & SHARMA, M. V. (2018). Divergence in pollen performance between *Clarkia* sister species with contrasting mating systems supports predictions of sexual selection. *Evolution; International Journal of Organic Evolution* **72**, 453–472.
- MAZER, S. J., HOVE, A. A., MILLER, B. S. & BARBET-MASSIN, M. (2010). The joint evolution of mating system and pollen performance: predictions regarding male gametophytic evolution in selfers vs. outcrossers. *Perspectives in Plant Ecology, Evolution and Systematics* **12**, 31–41.
- MAZER, S. J., MOGHADDASI, A., BELLO, A. K. & HOVE, A. A. (2016). Winning in style: longer styles receive more pollen, but style length does not affect pollen attrition in wild *Clarkia* populations. *American Journal of Botany* **103**, 408–422.
- MCCALLUM, B. & CHANG, S.-M. (2016). Pollen competition in style: effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. *American Journal of Botany* **103**, 460–470.
- MENDELSON, T. C., IMHOFF, V. E. & VENDITTI, J. J. (2007). The accumulation of reproductive barriers during speciation: postmating barriers in two behaviorally isolated species of darters (Percidae: *Etheostoma*). *Evolution* **61**, 2596–2606.
- MENDELSON, T. C. & SAFRAN, R. J. (2021). Speciation by sexual selection: 20 years of progress. *Trends in Ecology & Evolution* **36**, 1153–1163.
- MOORE, J. C. & PANNELL, J. R. (2011). Sexual selection in plants. *Current Biology* **21**, 176–182.
- MULCAHY, D. L. (1979). The rise of the angiosperms: a genealogical factor. *Science* **206**, 20–23.
- MULCAHY, D. L. & MULCAHY, G. B. (1975). The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. *Theoretical and Applied Genetics* **46**, 277–280.
- NEE, S., MAY, R. M. & HARVEY, P. H. (1994). The reconstructed evolutionary process. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **344**, 305–311.
- NIKOLOV, L. A. (2019). Brassicaceae flowers: diversity amid uniformity. *Journal of Experimental Botany* **70**, 2623–2635.
- NISTA, P., BROTHERS, A. N. & DELPH, L. F. (2015). Differences in style length confer prezygotic isolation between two dioecious species of *Silene* in sympatry. *Ecology and Evolution* **5**, 2703–2711.
- OLLERTON, J., WINFREE, R. & TARRANT, S. (2011). How many flowering plants are pollinated by animals? *Oikos* **120**, 321–326.
- ORR, H. A. & TURELLI, M. (2001). The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. *Evolution* **55**, 1085–1094.
- PANHUIS, T. M., BUTLIN, R., ZUK, M. & TREGENZA, T. (2001). Sexual selection and speciation. *Trends in Ecology & Evolution* **16**, 364–371.
- PANNELL, J. R. (2002). The evolution and maintenance of androecy. *Annual Review of Ecology and Systematics* **33**, 397–425.
- PARADIS, E. (2012). Shift in diversification in sister-clade comparisons: a more powerful test. *Evolution* **66**, 288–295.
- PATERNO, G. B., SILVEIRA, C. L., KOLLMANN, J., WESTOBY, M. & FONSECA, C. R. (2020). The maleness of larger angiosperm flowers. *Proceedings of the National Academy of Sciences* **117**, 10921–10926.
- PEASE, J. B., GUERRERO, R. F., SHERMAN, N. A., HAHN, M. W. & MOYLE, L. C. (2016). Molecular mechanisms of postmating prezygotic reproductive isolation uncovered by transcriptome analysis. *Molecular Ecology* **25**, 2592–2608.
- PICKUP, M., BRANDVAIN, Y., FRAÏSSE, C., YAKIMOWSKI, S., BARTON, N. H., DIXIT, T., LEXER, C., CEREGHETTI, E. & FIELD, D. L. (2019). Mating system variation in hybrid zones: facilitation, barriers and asymmetries to gene flow. *New Phytologist* **224**, 1035–1047.
- QUELLER, D. (1997). Pollen removal, paternity, and the male function of flowers. *The American Naturalist* **149**, 585–594.
- QUELLER, D. C. (1984). Pollen-ovule ratios and hermaphrodite sexual allocation strategies. *Evolution* **38**, 1148–1151.
- RABOSKY, D. L. & GOLDBERG, E. E. (2015). Model inadequacy and mistaken inferences of trait-dependent speciation. *Systematic Biology* **64**, 340–355.
- RABOSKY, D. L. & MATUTE, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. *Proceedings of the National Academy of Sciences* **110**, 15354–15359.
- RAMESHA, B. T., YETISH, M. D., RAVIKANTH, G., GANESHIAH, K. N., GHAZOUL, J. & SHAANKER, R. U. (2011). Stylish lengths: mate choice in flowers. *Journal of Biosciences* **36**, 229–234.
- RAMSEY, J., BRADSHAW, H. D. JR. & SCHEMSKE, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**, 1520–1534.



- RATNIEKS, F. L. W. & BALFOUR, N. J. (2021). Plants and pollinators: will natural selection cause an imbalance between nectar supply and demand? *Ecology Letters* **24**, 1741–1749.
- RENNER, S. S. (2014). The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of Botany* **101**, 1588–1596.
- REZNICK, D. N. & RICKLEFS, R. E. (2009). Darwin's bridge between microevolution and macroevolution. *Nature* **457**, 837–842.
- RICHMAN, A. D. & KOHN, J. R. (2000). Evolutionary genetics of self-incompatibility in the Solanaceae. *Plant Molecular Evolution* **42**, 169–179.
- RICKLEFS, R. E. (2007). Estimating diversification rates from phylogenetic information. *Trends in Ecology & Evolution* **22**, 601–610.
- RIESEBERG, L. H., DESROCHERS, A. M. & YOUN, S. J. (1995). Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *American Journal of Botany* **82**, 515–519.
- RITCHIE, M. G. (2007). Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics* **38**, 79–102.
- ROSENTHAL, G. G. & RYAN, M. J. (2022). Sexual selection and the ascent of women: mate choice research since Darwin. *Science* **375**, eabi6308.
- RUANE, L. G. (2009). Post-pollination processes and non-random mating among compatible mates. *Evolutionary Ecology Research* **11**, 1031–1051.
- RUNIONS, C. J. & GEBER, M. A. (2000). Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. Size and development of floral organs. *American Journal of Botany* **87**, 1439–1451.
- SARKER, R. H., ELLEMAN, C. J. & DICKINSON, H. G. (1988). Control of pollen hydration in *Brassica* requires continued protein synthesis, and glycosylation in necessary for intraspecific incompatibility. *Proceedings of the National Academy of Sciences* **85**, 4340–4344.
- SCHÄRER, L. & JANICKE, T. (2009). Sex allocation and sexual conflict in simultaneously hermaphroditic animals. *Biology Letters* **5**, 705–708.
- SCHEMSKE, D. W. & BRADSHAW, H. D. (1999). Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences* **96**, 11910–11915.
- SEEHAUSEN, O., BUTLIN, R. K., KELLER, I., WAGNER, C. E., BOUGHMAN, J. W., HOHENLOHE, P. A., PEICHEL, C. L., SAETRE, G.-P., BANK, C., BRÄNNSTRÖM, Å., BRELSFORD, A., CLARKSON, C. S., EROUKHMANOFF, F., FEDER, J. L., FISCHER, M. C., *ET AL.* (2014). Genomics and the origin of species. *Nature Reviews Genetics* **15**, 176–192.
- SELINSKI, J. & SCHEIBE, R. (2014). Pollen tube growth: where does the energy come from? *Plant Signaling & Behavior* **9**, e977200.
- SERVEDIO, M. R. & BOUGHMAN, J. W. (2017). The role of sexual selection in local adaptation and speciation. *Annual Review of Ecology, Evolution, and Systematics* **48**, 85–109.
- SERVEDIO, M. R. & BÜRGER, R. (2014). The counterintuitive role of sexual selection in species maintenance and speciation. *Proceedings of the National Academy of Sciences* **111**, 8113–8118.
- SHANER, M. G. & MARSHALL, D. L. (2003). Under how wide a set of conditions will nonrandom mating occur in *Raphanus sativus* (Brassicaceae)? *American Journal of Botany* **90**, 1604–1611.
- SIGARD, A. & LENHARD, M. (2011). The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. *Annals of Botany* **107**, 1433–1443.
- SKOGSMYR, I. & LANKINEN, Å. (1999). Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. *Evolutionary Ecology Research* **1**, 971–985.
- SNOW, A. A. & SPIRA, T. P. (1991a). Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **78**, 1419–1426.
- SNOW, A. A. & SPIRA, T. P. (1991b). Pollen vigour and the potential for sexual selection in plants. *Nature* **352**, 796–797.
- SNOW, A. A. & SPIRA, T. P. (1996). Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution* **50**, 1866–1870.
- SNOW, A. A., SPIRA, T. P. & LIU, H. (2000). Effects of sequential pollination on the success of “fast” and “slow” pollen donors in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **87**, 1656–1659.
- SPIRA, T. P., SNOW, A. A., WHIGHAM, D. F. & LEAK, J. (1992). Flower visitation, pollen deposition, and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **79**, 428–433.
- STANTON, M., YOUNG, H. J., ELLSTRAND, N. C. & CLEGG, J. M. (1991). Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. *Evolution* **45**, 268–280.
- STEBBINS, G. L. (1970). Adaptive radiation of reproductive characteristics in angiosperms. I: pollination mechanisms. *Annual Review of Ecology and Systematics* **1**, 307–326.
- STEBBINS, G. L. (1975). *Flowering Plants: Evolution above the Species Level*. Harvard University Press, Cambridge.
- SWANSON, R. J., HAMMOND, A. T., CARLSON, A. L., GONG, H. & DONOVAN, T. K. (2016). Pollen performance traits reveal prezygotic nonrandom mating and interference competition in *Arabidopsis thaliana*. *American Journal of Botany* **103**, 498–513.
- TAKAYAMA, S. & ISOGAI, A. (2005). Self-incompatibility in plants. *Annual Review of Plant Biology* **56**, 467–489.
- THIEN, L. B., BERNHARDT, P., DEVAL, M. S., CHEN, Z., LUO, Y., FAN, J.-H., YUAN, L.-C. & WILLIAMS, J. H. (2009). Pollination biology of basal angiosperms (ANITA grade). *American Journal of Botany* **96**, 166–182.
- THOMSON, J. D. & WILSON, P. (2008). Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Sciences* **169**, 23–38.
- TITLE, P. O. & RABOSKY, D. L. (2019). Tip rates, phylogenies and diversification: what are we estimating, and how good are the estimates? *Methods in Ecology and Evolution* **10**, 821–834.
- TONNABEL, J., DAVID, P., JANICKE, T., LEHNER, A., MOLLET, J.-C., PANNELL, J. R. & DUFAY, M. (2021). The scope for postmating sexual selection in plants. *Trends in Ecology & Evolution* **36**, 556–567.
- TONNABEL, J., DAVID, P. & PANNELL, J. R. (2019). Do metrics of sexual selection conform to Bateman's principles in a wind-pollinated plant? *Proceedings of the Royal Society B: Biological Sciences* **286**, 20190532.
- TRAVERS, S. E. & SHEA, K. (2001). Individual variation, gametophytic competition and style length: does size affect paternity? *Evolutionary Ecology Research* **3**, 729–745.
- TSUCHIMATSU, T., KAKUI, H., YAMAZAKI, M., MARONA, C., TSUTSUI, H., HEDHLY, A., MENG, D., SATO, Y., STÄDLER, T., GROSSNIKLAUS, U., KANAOKA, M. M., LENHARD, M., NORDBORG, M. & SHIMIZU, K. K. (2020). Adaptive reduction of male gamete number in the selfing plant *Arabidopsis thaliana*. *Nature Communications* **11**, 2885.
- TURELLI, M., BARTON, N. H. & COYNE, J. A. (2001). Theory and speciation. *Trends in Ecology & Evolution* **16**, 330–343.
- VAMOSI, J. C. & OTTO, S. P. (2002). When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. *Proceedings of the Royal Society of London Series B: Biological Sciences* **269**, 1187–1194.
- VASCONCELOS, T. N. C., CHARTIER, M., PRENNER, G., MARTINS, A. C., SCHÖNENBERGER, J., WINGLER, A. & LUCAS, E. (2019). Floral uniformity through evolutionary time in a species-rich tree lineage. *New Phytologist* **221**, 1597–1608.
- WEATHERHEAD, P. J. & ROBERTSON, R. J. (1979). Offspring quality and the polygyny threshold: 'the Sexy Son Hypothesis'. *The American Naturalist* **113**, 201–208.
- WHEELER, M. J., FRANKLIN-TONG, V. E. & FRANKLIN, F. C. H. (2001). The molecular and genetic basis of pollen–pistil interactions. *New Phytologist* **151**, 565–584.
- WILLI, Y. (2013). Mutational meltdown in selfing *Arabidopsis lyrata*. *Evolution* **67**, 806–815.
- WILLIAMS, E. G. & ROUSE, J. L. (1990). Relationships of pollen size, pistil length and pollen tube growth rates in *Rhododendron* and their influence on hybridization. *Sexual Plant Reproduction* **3**, 7–17.
- WILLIS, J. H. (1999). The contribution of male-sterility mutations to inbreeding depression in *Mimulus guttatus*. *Heredity* **83**, 337–346.
- WILLSON, M. F. (1979). Sexual selection in plants. *The American Naturalist* **113**, 777–790.
- WILLSON, M. F. (1994). Sexual selection in plants: perspective and overview. *The American Naturalist* **144**, S13–S39.
- WILLSON, M. F. & BURLEY, N. (1983). *Mate Choice in Plants: Tactics, Mechanisms, and Consequences*. Princeton University Press, Princeton.
- WILSON, W. G. & HARDER, L. D. (2003). Reproductive uncertainty and the relative competitiveness of simultaneous hermaphroditism versus dioecy. *The American Naturalist* **162**, 220–241.
- WINSOR, J. A., PERETZ, S. & STEPHENSON, A. G. (2000). Pollen competition in a natural population of *Cucurbita foetidissima* (Cucurbitaceae). *American Journal of Botany* **87**, 527–532.
- ZHENG, Y.-Y., LIN, X.-J., LIANG, H.-M., WANG, F.-F. & CHEN, L.-Y. (2018). The long journey of pollen tube in the pistil. *International Journal of Molecular Sciences* **19**, 3529.

(Received 14 December 2022; revised 2 June 2023; accepted 7 June 2023)