SPECIAL ISSUE ARTICLE

Botany
American Journal of

Pollen as the link between floral phenotype and fitness

Øystein H. Opedal[1] 🟢 | Rocío Pérez-Barrales[2] | Vinícius L. G. Brito[3] |
Nathan Muchhala[4] | Miquel Capó[5] | Agnes Dellinger[6]

[1]Department of Biology, Lund University, Sölvegatan 37, Lund 22362, Sweden

[2]Botany Department, University of Granada, Granada 18071, Spain

[3]Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia 38405-315, Brazil

[4]Department of Biology, University of Missouri at Saint Louis, Saint Louis, MO, USA

[5]Departamento de Sistemas y Recursos Naturales, Universidad Politécnica de Madrid, Madrid, Spain

[6]Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria

Correspondence
Øystein H. Opedal, Department of Biology, Lund University, Sölvegatan 37, Lund 22362, Sweden.
Email: oystein.opedal@biol.lu.se

Abstract
Pollen plays a key role in plant reproductive biology. Despite the long history of research on pollen and pollination, recent advances in pollen-tracking methods and statistical approaches to linking plant phenotype, pollination performance, and reproductive fitness yield a steady flow of exciting new insights. In this introduction to the Special Issue "Pollen as the Link Between Phenotype and Fitness," we start by describing a general conceptual model linking functional classes of floral phenotypic traits to pollination-related performance metrics and reproductive fitness. We use this model as a framework for synthesizing the relevant literature, highlighting the studies included in the Special Issue, and identifying gaps in our understanding and opportunities for further development of the field. The papers that follow in this Special Issue provide new insights into the relationships between pollen production, presentation, flower morphology, and pollination performance (e.g., pollen deposition onto stigmas), the role of pollinators in pollen transfer, and the consequences of heterospecific pollen deposition. Several of the studies demonstrate exciting experimental and analytical approaches that should pave the way for continued work addressing the intriguing role of pollen in linking plant phenotypes to reproductive fitness.

KEYWORDS
fitness function, flower–pollinator fit, heterospecific pollen transfer, path analysis, plant–pollinator interactions, pollen performance, pollen presentation, pollen production, pollen transfer, pollinator-mediated selection

Pollen plays a key role in plant reproductive biology and is studied in a range of fields including cell and molecular biology (Bedinger, 1992), biochemistry (Stanley and Linskens, 1974), ecology (Ollerton, 2021), systematic biology (Cardinal-McTeague and Gillespie, 2016), and evolutionary botany (Williams and Mazer, 2016). As male gametophytes, pollen share some aspects of its biology with that of animal sperm, while other aspects are fundamentally distinct. Chief among these is the involvement of pollinators in pollen transfer among flowers in most flowering plants (Ollerton, 2021). Pollination by animals has myriad consequences for plant ecology and evolution, and as carriers of genes and mediators of fitness gains through pollen export and receipt, pollen sits at the very center of the pollination process.

Natural selection arises when phenotypic traits are linked to individual performance and, in turn, fitness (Opedal, 2021). "Performance" is used here in the sense of measurable quantities linking a particular kind of phenotypic trait to fitness (Arnold, 1983), and "fitness" in the context of pollination usually refers to reproductive fitness as measured by seed production. The relevant performance component(s) can be defined with reference to the hypothesized function of the traits under study. For example, the performance of individual pollen grains (pollen performance) can be measured as the success in reaching a stigma, germinating, developing a pollen tube that reaches an ovary, and fertilizing an ovule (Williams and Mazer, 2016). In the context of animal pollination, however, pollination-related performance components (pollination performance) are best understood

---

from the perspective of the parental plant and include metrics such as pollen pickup from pollinators (a component of female pollination performance), placement onto pollinators and subsequent deposition onto conspecific stigmas (components of male pollination performance). While these perspectives are obviously related, the pollination perspective leads naturally to questions about floral traits functionally involved in the pollination process (pollination functional traits), while the pollen perspective leads to questions about pollen traits determining the fate of the individual gametophyte (pollen functional traits), such as pollen size and pollen-tube growth rates. This distinction is partly, but not completely, aligned with the conventional distinction between pre-pollination and post-pollination processes (Minnaar et al., 2019; Christopher et al., 2020).

As reflected in the title, this special issue focuses on the role of pollen in linking floral phenotypes to fitness. This idea is summarized graphically in the general model represented in Figure 1. While this model is on the one hand conceptual, we will see that it also can function as an empirical roadmap and as a representation of an analytical framework allowing joint analyses of many of the processes discussed in the papers that follow in this special issue. Our discussion will center more on pollination performance and pollination functional traits than on pollen performance and pollen functional traits. This choice partly reflects our own research areas and expertise and also the fact that the pollen performance perspective was well covered in a previous special issue of the *American Journal of Botany* (see Fig. 1 of Williams and Mazer, 2016). In their introductory

paper, Williams and Mazer (2016) also provide a brief historical account of research on pollen biology. Our focus is similar to that of Minnaar et al. (2019), and their discussion of "pathways to paternity" falls naturally into the pollen-export pathway in our model (Figure 1).

In the following, we use our graphical model (Figure 1) as a framework for outlining some of the key topics related to the role of pollen in linking floral phenotypes to fitness, including brief summaries of the papers that address them in the current special issue. We end by pointing to gaps in our current understanding and propose ways forward for addressing these gaps.

FUNCTION AND PERFORMANCE IN POLLINATION, OR HOW POLLEN LINKS FLORAL PHENOTYPE TO FITNESS

Our conceptual model (Figure 1) can be seen as a graphical representation of a path-analytical fitness function linking phenotypic traits through hypothesized causal relationships to components of performance and fitness. This kind of model borrows conceptual ideas from Wrightian path analysis (Wright, 1921; Shipley, 2016) and can be formalized as a series of statistical models yielding estimates of trait–performance, performance–fitness, and trait–performance–fitness relationships. Trait–performance relationships are those between a performance component and a trait (e.g., between stigma pollen load and flower size), and performance–fitness
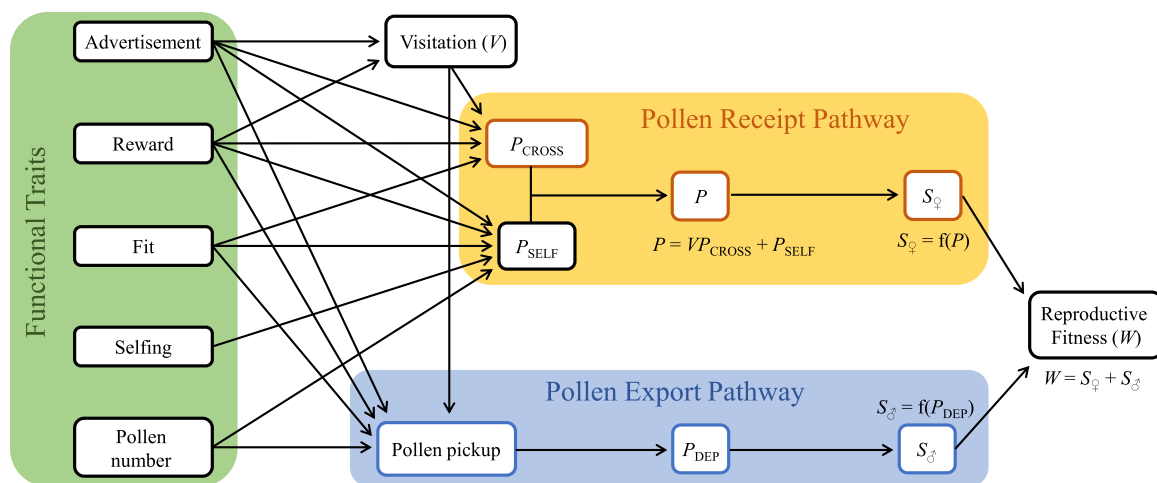

FIGURE 1 Graphical model linking classes of pollination functional traits (left) to reproductive fitness on the right ($W = S_♀ + S_♂$). Tracing the arrows backward, the number of seeds produced as a maternal parent ($S_♀$) is a function of the number of pollen grains (P) deposited onto stigmas through cross- (P_{CROSS}) and self-pollination (P_{SELF}). Because cross-pollen receipt requires pollinator visitation, the expected cross-pollen load can be written as a product of the probability of visitation (V) and pollen deposition conditional on visitation (P_C), and the total pollen load (P) as $P = VP_C + P_S$. Floral traits can indirectly increase the number of pollen grains (P) by increasing visitation of pollinators responding to advertisement or reward traits, or directly by improving mechanical fit and thus the efficiency of pollen transfer. The rate of self-pollination is also determined by floral traits affecting within-flower pollen transfer. The number of seeds sired through pollen export ($S_♂$) depends on the amount of pollen deposited by pollinators onto conspecific stigmas (P_{DEP}), which in turn depends on variation in pollen placement onto pollinators (pollen pickup) as a function of pollinator attraction, flower–pollinator fit, and pollen production.

relationships are those between a fitness component and a performance component (e.g., how variation in stigma pollen load translates into seed production) (Arnold, 1983; Opedal, 2021). This and related analytical approaches are gaining increasing popularity in evolutionary ecology (Shaw et al., 2008; Lefcheck, 2016; Henshaw et al., 2020) and provide several specific advantages in the study of pollination (Stanton et al., 1991; Conner, 1996; Aigner, 2005; Bolstad et al., 2010; Opedal, 2021). Most importantly, causal-inference methods such as path analysis require prior knowledge about the (hypothesized) causal relations between variables, which is often available in pollination ecology. Rodríguez-Otero et al. (2023) use the type of path–analytical fitness function illustrated by our model to link floral traits to several pollination performance components (pollinator visitation, pollen deposition, pollinarium removal), and one reproductive fitness component (seed set) in a set of food-deceptive orchids.

It is important to keep in mind that the model represented in Figure 1 is not fixed but can be easily tailored to the biology of the study system and to the data available in a specific study. For example, in this special issue, García-Muñoz et al. (2023) developed structural equation models that link floral morphology, reproductive investment (pollen and ovule production), and fitness (seed production) in the selfer *Erysimum incanum* s.l. maintained in a pollinator-free greenhouse environment. Structural equation modeling is closely related to the fitness–function approach described above and allowed the authors to assess support for hypothesized causal relationships among variables. Because the analysis concerned plants kept in a greenhouse, the fitness function could be simplified by omitting cross-pollination. Similarly, for self-incompatible species, it would often be natural to omit self-pollen deposition from the fitness function (although sexual interference can act also in self-incompatible species; Webb and Lloyd, 1986). Finally, for simplicity, our model treats selfed and outcrossed maternal seed production jointly (Figure 1, pollen-receipt pathway), which corresponds to female fitness as typically measured in field studies. A simple extension would be to split this fitness component into selfed and outcrossed components (e.g., Briscoe-Runquist et al., 2017).

Trait–performance and performance–fitness relationships can be studied independently (e.g., in field vs. greenhouse/lab studies), and subsequently combined into a complete fitness function (Arnold, 1983). Indeed, any study exploring one or more links between phenotype, performance, and fitness can be seen as estimating one component relationship of a fitness function. In this sense, Figure 1 can be seen as an empirical roadmap where the important role of pollen in all links between components helps to clarify the title and focus of this special issue. In the following sections, we give several examples of how additional trait classes (e.g., pollen morphology, floral shape) and performance components (e.g., heterospecific pollen deposition) can be integrated into this general framework.

Pollen production and presentation

Building the kind of fitness function represented in Figure 1 requires careful consideration of the expected causal relations among variables. From the male perspective, the first step in successful cross-pollination is pollen placement by an anther onto a pollinator ("pollen pickup"). The rate at which this occurs depends on the local abundance of (potential) pollinators, the foraging preferences of these pollinators, their mechanical fit with the visited flowers, and the amount (and perhaps properties) of pollen presented (Minnaar et al., 2019).

Pollen production varies greatly among animal-pollinated plants, and a long history of theoretical and empirical work has explored the causes and consequences of this variation. Cruden (1977) compiled estimates of the ratio of the number of pollen grains to ovules (pollen:ovule ratio) to assess and support the expectation that the production of pollen relative to ovules increases along a gradient from cleistogamous to highly outcrossing species to the extent that pollen:ovule ratios can be treated as a reliable proxy of variation in mating system. Recent meta-analyses, including in this issue, have revealed large-scale patterns and correlates of pollen:ovule ratios. For example, Cunha and Aizen (2023) tested and found support for the expectation that per-flower pollen production will increase with flower display size to compensate for increased pollen discounting when pollinators visit multiple flowers on the same plant (Harder and Barrett, 1995). Also in this issue, Harder and Johnson (2023) argue that some of the conclusions of previous meta-analyses on this topic may be premature because the analysis of ratios can obscure effects present for one or both underlying variables (here pollen and ovule production). Instead, Harder and Johnson (2023) propose that pollen and ovule numbers be analyzed jointly in a bivariate model, pointing out that when the analysis is performed on a logarithmic scale, the pollen:ovule ratio can be directly inferred from the parameters of such models (given that log Pollen number – log Ovule number = log [Pollen number/Ovule number]). Whether and how this analytical approach will change the qualitative results of previous analyses remains to be seen, but this potential issue illustrates the importance in biology of careful consideration of measurements and their meaning (Houle et al., 2011). Harder and Johnson (2023) also tested and found support for the hypothesis that pollen number per flower decreases in taxa with more efficient pollen transfer as measured by the proportion of pollen reaching stigmas, supporting a direct link from pollen production via pollen transfer to fitness, i.e., a trait–performance–fitness relationship (Figure 1).

In terms of within-population trait–performance relationships for pollen number (Figure 1), we expect variation in pollen number per flower to affect pollen placement onto pollinators and self-pollen deposition onto stigmas. Estimates of these relationships in natural populations are rare, likely reflecting the general focus on selection through

female function (Minnaar et al., 2019; Christopher et al., 2020). Stanton et al. (1991), in an early application of path analysis in pollination ecology, found that greater pollen production increased visits by small native bees, which in turn led to greater siring success. In one of the few studies quantifying phenotypic selection on pollen number through the male function, Morgan and Conner (2001) detected positive selection. If we assume for now that pollen placement onto pollinators and subsequent deposition onto stigmas are correlated, this result indirectly supports a positive relationship between pollen production and placement. This assumption may not be justified, however. For example, in this issue, Pearson et al. (2023) report positive relationships between individual pollen production by *Echinacea* plants and placement onto a range of pollinators, but this variation was not associated with subsequent variation in siring success. This lack of a performance–fitness relationship can perhaps be explained by differences in pollinator behavior and pollen-transfer processes, as discussed below (and see Stanton et al., 1991).

In their analysis of the selfing *Erysimum incanum*, García-Muñoz et al. (2023) considered patterns of covariation among pollen production, ovule production, floral morphology, and seed production. Although pollen number had a limited direct effect on seed production in a greenhouse, this study did show that pollen number can covary with flower morphology and with ovule production, thus affecting reproductive fitness.

Beyond pollen number, the dynamics of pollen placement onto pollinators will also depend on the way in which pollen is presented by flowers. For example, aligning the daily period of pollen presentation to the activity peak of pollinators may both optimize pollen removal and, to some extent, reduce reproductive interactions with co-flowering species (Stone et al., 1998). In this issue, Štenc et al. (2023) detected alignment between peaks in pollen presentation and pollinator visitation for three co-flowering species. Less is known about within-population variation in daily pollen presentation schedules (Eisen et al., 2017), which would be an interesting trait to include in pollination fitness functions (Figure 1). Similarly, plant species differ in the size of the pollen "packages" released per pollinator visit. The optimum amount of pollen released per visit is expected to depend on several factors (Harder and Thomson, 1989). When pollen is the primary reward, plants face the challenge of avoiding excessive pollen loss due to grooming while ensuring attractiveness to pollinators. In this issue, Heiling et al. (2023) modeled pollen packaging strategies under the influence of these constraints, confirming the expectation that optimum package size depends on the relative preference of pollinators for larger packages, but also additional factors such as total pollen production in the population and possible relationships with per-plant visitation rates. The role of pollen as a reward in some species could be easily incorporated into fitness functions (Figure 1) by including links from pollen production (or daily presentation) to pollinator visitation.

Pollen transfer

Once placed onto a pollinator, pollen can be lost through a series of filters or barriers (Minnaar et al., 2019). For example, in the classic study of *Erythronium grandiflorum* by Thomson and Thomson (1989), less than 1% of the pollen removed from anthers reached a conspecific stigma. Even in unusually precise systems such as pollinarium-bearing orchids, pollen is often lost during transfer (Capó et al., 2023; Harder and Johnson, 2023). These results suggest that the link from floral phenotype (affecting pollen placement) to male reproductive fitness via deposition onto conspecific stigmas may be highly variable and hard to detect in analyses linking floral traits to pollen placement (male performance) and number of seeds sired (male reproductive fitness, see Pearson et al., 2023). This variability may be one reason why relatively few studies have detected phenotypic selection on floral traits through the male function (Ashman and Morgan, 2004; but see e.g., Briscoe Runquist et al., 2017).

Variation in pollen transfer can arise through many processes, including interactions on the body of the pollinator (Minnaar et al., 2019). In this special issue, Moir and Anderson (2023) provide empirical evidence that pollen of sequentially visited flowers can be deposited in layers onto the pollinator's body, and that pollen deposition may decline from early- to later-visited flowers. Using the recently developed fluorescent quantum-dots technique (Minnaar and Anderson, 2019) to label pollen of individual flowers, Moir and Anderson (2023) analyzed the layers of pollen on the bodies of fly pollinators for labeled pollen and found more labeled pollen in the top layers. When a fly had visited two labeled flowers, it carried more pollen from the first flower than from the second flower, suggesting that existing pollen grains from earlier visits inhibit additional pollen placement. These results provide the first empirical evidence for pollen layering as a mechanism mediating male–male competition during pollen transfer. How pollen layering affects subsequent deposition onto stigmas remains an open question.

Another important source of variation in pollen-transfer efficiency is the identity of pollinators. These differences are clear from a small but growing body of studies assessing landscape-scale effects of pollinator mobility on population differentiation (Dellinger et al., 2022; Feigs et al., 2022; Gamba and Muchhala, 2023). In this issue, Lewis et al. (2023) used population genetics to test whether differences in the mobility of hawkmoth and bee pollinators explain population differentiation in two species of *Oenothera*. As expected, they found increased population differentiation with less-mobile pollinators (bees in their case), but little effects on population differentiation through (ballistic short-distance) seed dispersal (see also Gamba and Muchhala, 2020). Further multispecies studies assessing the relative importance of pollen versus seed-mediated gene dispersal are needed to better understand the impact of

pollinator declines and changes in pollinator assemblages on the persistence and evolution of plant populations.

Besides landscape-scale effects of pollinator mobility, differences in pollinator foraging patterns and behavior may also affect small-scale pollen-transfer efficiency and siring success within populations (Minnaar et al., 2019; Kern et al., 2023). In two studies in this issue, aspects of pollination efficiency were compared across different bee species. Pearson et al. (2023) combined field observations, single-visit pollination experiments and paternity analyses to show that bee species differentially influence siring success and that these differences are not always captured by measures of visitation rate or pollen removal. Similarly, Weinman et al. (2023) studied the composition of the pollen loads that bees carry on their bodies and in their scopae and found that pollen of many plant species were packed in bee scopae (and thus not available for transfer to stigmas) without occurring on bee bodies. These two studies accentuate the value of combining molecular approaches with refined pollination experiments that go beyond the mere documentation of visitation and of studying bee foraging strategies to decipher subtle pre- and post-pollination phenotype–performance–fitness relationships (Christopher et al., 2020).

Pollinator attraction

From the perspective of the maternal plant, the first step in successful cross-pollination is to attract at least one pollinator individual. Trait–performance relationships for pollinator attraction traits arise when pollinators make foraging decisions based on direct assessment of reward availability (Bolstad et al., 2010) or on floral or plant-level advertisements acting as "honest signals" (e.g., plant height, Lortie and Aarssen, 1999; flower size, Armbruster et al., 2005). In this issue, trait-performance relationships for attraction traits are demonstrated in two studies of terrestrial orchids. Capó et al. (2023) quantified the relationships between plant height, floral display size, pollinarium removal, and fruit set of five species. Although plant size also reflects overall vigor, which leads to variation in reproductive success independently of pollinators (e.g., Trunschke et al., 2017), the positive relationships between pollinarium removal and these "vigor" traits also support a role of pollinator-mediated selection in their evolution. Rodríguez-Otero et al. (2023) took the analysis of trait–performance–fitness relationships one step further by developing a complete fitness function and using it to estimate selection gradients. These authors also detected overall positive trait–performance-relationships and selection on advertisement traits. Interestingly, the trait–performance relationships were comparable between female (pollen deposition) and male pollination performance (pollinarium removal), suggesting at least some connection between the pollen-receipt and pollen-export pathways to fitness (Figure 1).

Flower–pollinator fit

While the initial step for the maternal plant involves attraction of the pollinator, receiving pollen then depends on the presence of pollen on the pollinator's body and on whether parts of the pollinator's body carrying pollen make contact with a receptive stigma. To the extent that floral traits determine the probability of contact and position of contact with pollinator bodies, these flower–pollinator fit traits will then link floral phenotype to pollination performance (Opedal, 2021). Rodríguez-Otero et al. (2023) included spur length and flower size as a fit trait in their orchid fitness function and showed that trait–performance relationships and selection on fit traits tend to fluctuate dramatically in both strength and direction, presumably in response to variation in flower–pollinator fit across populations and species visited by different pollinators (Opedal, 2021). While most previous studies have focused on individual fit traits, Dellinger et al. (2023) took on the challenge of linking complex floral shape variation as detected by morphometric techniques to pollen removal from anthers and deposition onto stigmas. They used a dimension-reduction technique, the two-block partial least squares method (Rohlf and Corti, 2000), to combine a large set of measurements (here landmarks) into a single axis related to variation in pollination performance. This approach revealed a detectable trait–performance relationship for pollen removal from anthers, but not for pollen deposition onto stigmas. Dellinger et al. (2023) also provide a general roadmap for further work along this line.

Pollen placement on pollinators is perhaps best understood through the lens of pollination accuracy (Armbruster et al., 2009). For example, Stewart et al. (2022) compared the precision of pollen placement (one component of pollination accuracy) across a diverse set of plant species in Thailand and found that pollen placement on pollinators was most precise in phenotypically more specialized flowers, as indicated (among other traits) by bilateral flower symmetry and fused corollas. In this issue, Pérez-Barrales and Armbruster (2023) compared patterns of pollination accuracy across sites where *Linum* species occur either alone or with congeners to assess, and to some extent support, divergence in fit traits among co-flowering species.

When a plant species is visited by more than one pollinator species (or functional group), trait–performance relationships are likely to differ among them. In this issue, Furtado et al. (2023) compared the pollination efficiency of bee and hummingbird pollinators of *Palicourea rigida*, leveraging the pollen-size dimorphism of their distylous flowers to assess pollen placement on pollinators and single-visit deposition onto stigmas. They found that the two pollinator functional groups differed in per-visit pollen deposition, especially for the short-styled morph. Also in this issue, Pearson et al. (2023) and Weinman et al. (2023) document differences in pollination efficiency among bee pollinator taxa. These kinds of differences could be incorporated into fitness functions

by splitting the pollen-deposition performance component into pollinator-specific contributions (cf. Schemske and Horvitz, 1988).

Heterospecific pollen transfer

Whenever coflowering plants share pollinators, pollen loads deposited onto stigmas may contain pollen of several species. Heterospecific pollen deposition is generally thought to have negative effects on seed production through mechanisms such as stigma clogging, interference in the style, or conspecific pollen loss (Ashman and Arceo-Gómez, 2013; Moreira-Hernández and Muchhala, 2019). In practice, however, if pollinators also tend to deposit more conspecific pollen when they deposit more heterospecific pollen, increased heterospecific pollen deposition may actually correspond to greater seed set (e.g., Lopes et al., 2021), especially if the recipient species has evolved a degree of tolerance to heterospecific pollen. For example, in this issue, Hao et al. (2023) studied patterns and consequences of heterospecific pollen deposition onto stigmas in three *Silene* species and found that stigmas receiving more heterospecific pollen also tended to receive more conspecific pollen and that hand-pollination with mixtures of conspecific and heterospecific pollen did not usually reduce seed production compared to those with only conspecific pollen. Such tolerance likely evolves in response to natural exposure to heterospecific pollen. For example, Moreira-Hernández et al. (2023) studied pollen transfer within and between sympatric *Burmeistera* and compared the effect of experimental pollen mixtures among several species that differ in the amount of heterospecific pollen they typically receive in the wild. As predicted, a species that receives more heterospecific pollination in the wild appears to have evolved greater tolerance to it. How tolerance to heterospecific pollen evolves is not entirely clear, but likely relates to changes in pollen–pistil interactions and incompatibility systems (Broz and Bedinger, 2021).

Analytically, heterospecific pollen deposition can readily be included into pollination fitness functions as a performance component with logical links to pollinator visitation and advertisement, reward, and flower-pollinator fit traits (Figure 1). As we have just seen, the performance–fitness relationship (how heterospecific pollen deposition affects seed set and the relationship between conspecific pollen deposition and seed set) has been well studied (e.g., Hao et al., 2023; Moreira-Hernández et al., 2023; Pérez-Barrales and Armbruster, 2023 [all in this issue]) and could easily be included by modelling seed set as a function of conspecific pollen deposition, heterospecific pollen deposition, and, presumably, their interaction.

Trait–performance relationships for heterospecific pollen deposition have been less well studied, although analyses of population means suggest that such relationships may occur at least occasionally (Peuker et al., 2020; Lopes et al., 2021). The positive relationships often observed between conspecific and heterospecific pollen loads (e.g., Tur et al., 2016) also suggest that the trait–performance relationships might be similar in shape. Similar trait–performance relationships would most likely occur if conspecific and heterospecific pollen grains are placed, on average, on the same part of the pollinator (Muchhala and Thomson, 2012). This assumption may be justified in systems with scattered pollen placement (e.g., in buzz-pollination), while in systems with more precise pollen placement, there is increasing evidence for reproductive character displacement acting to reduce heterospecific pollen transfer (Armbruster et al., 1994; Grossenbacher and Whittall, 2011; Eisen and Geber, 2018). Pérez-Barrales and Armbruster (2023) studied heterospecific pollination in the light of local assemblage structure and potential evolution of reproductive character displacement in co-flowering *Linum* species. Their focal species, *Linum suffruticosum*, occurs in sites without other *Linum* species and in sites with co-flowering and pollinator-sharing congeners. In this system, heterospecific pollen deposition negatively affected conspecific pollen tube formation, but different *Linum* species were only partially differentiated in their pollinator assemblages and in floral traits affecting pollen placement on shared pollinators at sympatric sites.

Performance–fitness relationships: from pollen deposition to seed set

While male reproductive success requires pollen export to conspecific stigmas, such pollen export does not necessarily guarantee that ovules will be fertilized. The relationship between conspecific pollen deposition and seed production (i.e., the performance–fitness relationship of our general fitness function; Figure 1) is usually asymptotic, with the asymptote reflecting the number of ovules and/or the upper limit of resources available for seed maturation (Ashman et al., 2004). In terms of building a fitness function, the pollen-to-seeds curve can be estimated either in the field or in a controlled greenhouse environment, though the exact shape may differ due to the influences of environmental factors in the field (Hildesheim et al., 2019). One source of variation in pollen-to-seed curves is the nature of pollen competition. A large body of work has explored the consequences and evolutionary importance of pollen competition, and we refer readers to Williams and Mazer (2016) and references therein.

Pollen functional traits

Beyond pollen number, many pollen functional traits could affect the probability of placement onto pollinator bodies, deposition onto conspecific stigmas, and competitive ability. Traits such as pollen size, pollen nutrient content, and pollen-tube growth rate would be straightforward to integrate into a fitness function (Figure 1). Pollen

traits can also serve to attract pollinators when pollen functions as a floral reward. In this issue, Nebauer et al. (2023) studied the role of pollen sterols in the foraging decisions, nutrition, and fitness of bumble bees. Surprisingly, they found that while bumble bees could detect pure sterols, they did not discriminate between different sterols or between pollen with different sterol concentrations. Analytically, including pollen nutrient composition into a fitness function would likely benefit from dimension-reduction approaches such as the two-block partial least square approach used by Dellinger et al. (2023) or the reduced-rank regression approach recently developed for analyzing trait–performance relationships and selection on floral scent chemistry (Opedal et al., 2022).

Along with moving gametes between individuals, pollen can also move a host of different pathogens. In this issue, Fetters and Ashman (2023) reviewed viral plant infections associated with pollen, summarizing the characteristics of the pollen virome and its transmission, with particular attention to plant, pollen, pollinator, and landscape traits likely to be associated with variation in virus transmission. While the effect of fungal anther pathogens on pollination has been studied (Jennersten, 1988; Antonovics and Alexander, 1992), too little is known about the effect of pollen viruses on plant traits and fitness to be able to make clear predictions about virus effects on pollination. Infections could potentially affect plants in ways that would alter pollen transfer, creating opportunities for selection on floral traits (Giles et al., 2006). In principle, such effects could be integrated into a pollination fitness function (Figure 1).

TECHNICAL ADVANCES IN POLLEN IDENTIFICATION AND QUANTIFICATION

Studies of trait–performance–fitness relationships of the kind discussed above often require quantification and identification of pollen on stigmas or on pollinators. Recently, methods for automated pollen identification have advanced considerably, including machine-learning algorithms for analyzing pollen photos (Olsson et al., 2021) and pipelines for meta-barcoding of pollen samples from bees (Bell et al., 2016) and the environment (Polling et al., 2022). A paper in *Applications in Plant Sciences* forms part of the current special issue and presents a new pollen quantification technique. Bailey et al. (2023) evaluated the use of high-energy violet light for pollen grain classification. The authors sampled pollen pellets from 16 *Apis mellifera* hives and compared identification methods based on high-energy violet light and visible light to assess the relationship between pollen composition and color. The protocol was able to differentiate 35 pollen pellet colors and aided in distinguishing their composition even within the same color class. This method is low-cost, easy to standardize, and highly reproducible because pollen colors can be identified by their numerical composition (RGB or hexadecimal).

CONCLUSIONS AND PERSPECTIVES

The papers that follow in this special issue attest to the key role of pollen in linking floral phenotypic variation to pollination performance and reproductive fitness. We hope that our discussion of the diverse insights and results from these studies in the light of a general path-analytical conceptual framework helps to illustrate the connections between topics that are often studied separately.

We highlight the value of a path-analytical framework for conceptualizing studies of plant–pollinator interactions and floral evolution. In studies focusing on one or more individual trait–performance, trait–fitness, or performance–fitness relationship, developing a hypothetical fitness function (causal diagram) can aid interpretations by clarifying the expected links to other components of performance and fitness. Because the approach is modular, individual trait–performance and performance–fitness relationships can be studied in isolation and later linked together. For example, several studies included in this special issue estimate trait–performance relationships by relating floral phenotype to pollen placement or deposition in the field (e.g., Capó et al., 2023; Dellinger et al., 2023; Pérez-Barrales and Armbruster, 2023; Rodríguez-Otero et al., 2023). The corresponding performance–fitness relationship can be studied through experimental manipulation of pollen loads in the field or greenhouse to infer pollen-to-seed curves or the influence of heterospecific pollen deposition on the relationship between conspecific pollen deposition and seed set (e.g., Hao et al., 2023; Moreira-Hernández et al., 2023; Pérez-Barrales and Armbruster, 2023). While these kinds of results are increasingly available, it is still rare to see them combined into a complete fitness function of the kind illustrated in Figure 1, and we hope that this special issue will inspire further work along this line.

Two key points that arise when designing studies of trait–performance–fitness relationships are to consider (1) the (hypothesized) function of each kind of phenotypic trait, and (2) the intermediate performance components that link these trait classes to reproductive fitness. If the pollination component is made only implicit by going straight from phenotype to fitness, as is often the case in studies of pollinator-mediated selection (Opedal, 2021), we risk missing important mechanisms such as those associated with pollen loss and male–male interactions during pollen transfer (Minnaar et al., 2019). On this note, it is common to see statements about pollination performance components being poor surrogates or proxies for fitness. However, pollination performance components are not necessarily studied because they are "proxies" of reproductive fitness, but because they in themselves are informative about pollination function, which is central to understanding the evolution of flowers and plant reproduction. In fact, weak performance–fitness relationships equally means that male reproductive fitness may be a poor proxy of pollination performance, highlighting the importance of studying both components.

Perspectives

One general insight from the studies included in this special issue and the general literature is that different pollinator taxa may mediate different trait–performance–fitness relationships (e.g., Aigner, 2005; Sahli and Conner, 2011; Furtado et al., 2023; Nakazawa and Kishi, 2023; Pearson et al., 2023). One way to see this is that each pollinator taxon mediates a unique pollination fitness function that can, in principle, be estimated. Estimating such refined, taxon-specific fitness functions will require creative, multi-method study designs that draw on both classical pollination–ecological observations, experiments, and molecular techniques (Castilla et al., 2017). As a step in this direction, it seems important to at least consider pollinator-specific responses to floral advertisements and patterns of pollen deposition (Schemske and Horvitz, 1988; Stanton et al., 1991).

The development of new pollen-labeling techniques such as quantum dots has opened new opportunities for quantifying the fates of pollen grains after pollen placement onto a pollinator (Minnaar and Anderson, 2019), but their empirical implementation has been relatively slow. We are convinced that such pollen-labeling methods will facilitate studies of pollen transfer (Kern et al., 2023) and the system-specific performance–fitness relationships associated with pollen loss during transfer (Minnaar and Anderson, 2021). Detailed pollen tracking methods may also allow us to link pollination performance more closely to pollen performance and hence gain a more holistic understanding of when and why specific pollen grains are more competitively successful than others. Combining such approaches with paternity assessments and population genetics holds further promise for realistically estimating trait–performance–fitness relationships under the influence of both stochastic and deterministic components of pollen transfer.

Finally, we conclude by reporting on an incident that occurred during the process of putting together this special issue, which is of relevance for the broader scientific community. Two years ago, one of us was approached by a for-profit journal with the invitation to guest edit a special issue and invited the rest of us to contribute. In our proposal, we explicitly stated our goal of reaching broadly across the pollination biology community, including young researchers and researchers from the global south. After sending out invitations to colleagues, we learned of the exorbitant processing charges and open-access publication fees that each author would need to cover; an amount that would serve to exclude the very groups we were trying to reach. After discussions among ourselves, some of the authors contributing to this special issue and other colleagues, we decided to retract the special issue from the original journal and instead we approached the editorial board of the *American Journal of Botany* (*AJB*) with our special issue proposal. A year and a half later, we are extremely happy to have taken this step. We believe that drawing broad attention to publication bias in for-profit journals, and the value of professional societies and the power they have in supporting a more diverse and inclusive scientific community is as important as assessing the role of pollen in plant reproduction. We thank the editors of *AJB* for the opportunity to publish this special issue and their support and trust throughout the process.

ORCID

Øystein H. Opedal http://orcid.org/0000-0002-7841-6933

REFERENCES

Aigner, P. A. 2005. Variation in pollination performance gradients in a *Dudleya* species complex: Can generalization promote floral divergence? *Functional Ecology* 19: 681–689.

Antonovics, J., and H. M. Alexander. 1992. Epidemiology of anther-smut infection of *Silene alba* (=*S. latifolia*) caused by *Ustilago violacea*: patterns of spore deposition in experimental populations. *Proceedings of the Royal Society, B,Biological Sciences* 250: 157–163.

Armbruster, W. S., M. E. Edwards, and E. M. Debevec. 1994. Floral character displacement generates assemblage structure of western Australian triggerplants (*Stylidium*). *Ecology* 75: 315–329.

Armbruster, W. S., L. Antonsen, and C. Pélabon. 2005. Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. *Ecology* 86: 3323–3333.

Armbruster, W. S., T. F. Hansen, C. Pélabon, R. Perez-Barrales, and J. Maad. 2009. The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of Botany* 103: 1529–1545.

Arnold, S. J. 1983. Morphology, performance and fitness. *American Zoologist* 23: 347–361.

Ashman, T. L., and M. T. Morgan. 2004. Explaining phenotypic selection on plant attractive characters: Male function, gender balance or ecological context? *Proceedings of the Royal Society, B, Biological Sciences* 271: 553–559.

Ashman, T. L., and G. Arceo-Gómez. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany* 100: 1061–1070.

Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.

Bailey, C. P., C. A. Sonter, J. L. Jones, S. Pandey, S. Haberle, K. Santos, M. L. Absy, and R. Rader. 2023. Does sorting by color using visible and high-energy violet light improve classification of taxa in honey bee pollen pellets? *Applications in Plant Sciences* 11: e11514.

Bedinger, P. 1992. The remarkable biology of pollen. *Plant Cell* 4: 879–887.

Bell, K. L., N. de Vere, A. Keller, R. T. Richardson, A. Gous, K. S. Burgess, and B. J. Brosi. 2016. Pollen DNA barcoding: current applications and future prospects. *Genome* 59: 629–640.

Bolstad, G. H., W. S. Armbruster, C. Pélabon, R. Pérez-Barrales, and T. F. Hansen. 2010. Direct selection at the blossom level on floral

reward by pollinators in a natural population of *Dalechampia schottii*: full-disclosure honesty? *New Phytologist* 188: 370–384.

Briscoe Runquist, R. D., M. A. Geber, M. Pickett-Leonard, D. A. Moeller, C. G. Eckert, and A. A. Winn. 2017. Mating system evolution under strong pollen limitation: evidence of disruptive selection through male and female fitness in *Clarkia xantiana*. *American Naturalist* 189: 549–563.

Broz, A. K., and P. A. Bedinger. 2021. Pollen–pistil interactions as reproductive barriers. *Annual Review of Plant Biology* 72: 615–639.

Capó, M., J. Borràs, S. Perelló-Suau, J. Rita, and J. Cursach. 2023. Phenotype–fitness relationships and pollen-transfer efficiency of five orchid species with different pollination strategies. *American Journal of Botany* 110: e16198.

Cardinal-McTeague, W. M., and L. J. Gillespie. 2016. Molecular phylogeny and pollen evolution of Euphorbiaceae tribe Plukenetieae. *Systematic Botany* 41: 329–347.

Castilla, A. R., N. S. Pope, M. O'Connell, M. F. Rodriguez, L. Trevino, A. Santos, and S. Jha. 2017. Adding landscape genetics and individual traits to the ecosystem function paradigm reveals the importance of species functional breadth. *Proceedings of the National Academy of Sciences, USA* 114: 12761–12766.

Christopher, D. A., R. J. Mitchell, and J. D. Karron. 2020. Pollination intensity and paternity in flowering plants. *Annals of Botany* 125: 1–9.

Conner, J. K. 1996. Understanding natural selection: an approach integrating selection gradients, multiplicative fitness components, and path analysis. *Ethology Ecology & Evolution* 8: 387–397.

Cruden, R. W. 1977. Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.

Cunha, N. L. d., and M. A. Aizen. 2023. Pollen production per flower increases with floral display size across animal-pollinated flowering plants. *American Journal of Botany* 110: e16180.

Dellinger, A. S., D. Hanusch, M. Oswald, D. Fernández-Fernández, and J. Schönenberger. 2023. Using geometric morphometrics to determine the "fittest" floral shape: a case study in large-flowered, buzz-pollinated *Meriania hernandoi*. *American Journal of Botany* 110: e16183.

Dellinger, A. S., O. Paun, J. Baar, E. M. Temsch, D. Fernández-Fernández, and J. Schönenberger. 2022. Population structure in Neotropical plants: integrating pollination biology, topography and climatic niches. *Molecular Ecology* 31: 2264–2280.

Eisen, K., A. L. Case, and C. M. Caruso. 2017. Variation in pollen-dispensing schedules of *Lobelia siphilitica*. *International Journal of Plant Sciences* 178: 79–84.

Eisen, K. E., and M. A. Geber. 2018. Ecological sorting and character displacement contribute to the structure of communities of *Clarkia* species. *Journal of Evolutionary Biology* 31: 1440–1458.

Feigs, J. T., S. I. J. Holzhauer, S. Huang, J. Brunet, M. Diekmann, P.-O. Hedwall, K. Kramp, and T. Naaf. 2022. Pollinator movement activity influences genetic diversity and differentiation of spatially isolated populations of clonal forest herbs. *Frontiers in Ecology and Evolution* 10: 908258.

Fetters, A. M., and T. L. Ashman. 2023. The pollen virome: a review of pollen-associated viruses and consequences for plants and their interactions with pollinators. *American Journal of Botany* 110: e16144.

Furtado, M. T., R. Matias, R. Pérez-Barrales, and H. Consolaro. 2023. Complementary roles of hummingbirds and bees: pollen pickup, pollen deposition, and fruit production in the distylous *Palicourea rigida*. *American Journal of Botany* 110: e16194.

Gamba, D., and N. Muchhala. 2020. Global patterns of population genetic differentiation in seed plants. *Molecular Ecology* 29: 3413–3428.

Gamba, D., and N. Muchhala. 2023. Pollinator type strongly impacts gene flow within and among plant populations for six Neotropical species. *Ecology* 104: e3845.

García-Muñoz, A., C. Ferrón, C. Vaca-Benito, J. Loureiro, S. Castro, A. J. Muñoz-Pajares, and M. Abdelaziz. 2023. Ploidy effects on the relationship between floral phenotype, reproductive investment and fitness exhibited by an autogamous species complex. *American Journal of Botany* 110: e16197.

Giles, B. E., T. M. Pettersson, U. Carlsson-Graner, and P. K. Ingvarsson. 2006. Natural selection on floral traits of female *Silene dioica* by a sexually transmitted disease. *New Phytologist* 169: 729–739.

Grossenbacher, D. L., and J. B. Whittall. 2011. Increased floral divergence in sympatric monkeyflowers. *Evolution* 65: 2712–2718.

Hao, K., Q. Fang, and S.-Q. Huang. 2023. Do *Silene* species with exposed stigmas tolerate interference by heterospecific pollen? *American Journal of Botany* 110: e16147.

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

Harder, L. D., and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512–515.

Harder, L. D., and S. D. Johnson. 2023. Beyond pollen:ovule ratios: evolutionary consequences of pollinator dependence and pollination efficiency for pollen and ovule production in angiosperms. *American Journal of Botany* 110: e16177.

Heiling, J. M., R. E. Irwin, and W. F. Morris. 2023. Conflicting constraints on male mating success shape reward size in pollen-rewarding plants. *American Journal of Botany* 110: e16158.

Henshaw, J. M., M. B. Morrissey, and A. G. Jones. 2020. Quantifying the causal pathways contributing to natural selection. *Evolution* 74: 2560–2574.

Hildesheim, L. S., Ø. H. Opedal, W. S. Armbruster, and C. Pelabon. 2019. Quantitative and qualitative consequences of reduced pollen loads in a mixed-mating plant. *Ecology and Evolution* 9: 14253–14260.

Houle, D., C. Pélabon, G. P. Wagner, and T. F. Hansen. 2011. Measurement and meaning in biology. *Quarterly Review of Biology* 86: 3–34.

Jennersten, O. 1988. Insect dispersal of fungal disease: effects of *Ustilago* infection on pollinator attraction in *Viscaria vulgaris*. *Oikos* 51: 163–170.

Kern, B. R., Carley L. N., and Moeller D. A. 2023. Direct tracking of pollen with quantum dots reveals surprising uniformity in dispersal distance across eleven populations of an annual plant. *American Journal of Botany* https://doi.org/10.1002/ajb2.16201 [in press].

Lefcheck, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7: 573–579.

Lewis, E. M., J. B. Fant, M. J. Moore, and K. A. Skogen. 2023. Hawkmoth and bee pollinators impact pollen dispersal at the landscape but not local scales in two species of *Oenothera*. *American Journal of Botany* 110: e16156.

Lopes, S. A., P. J. Bergamo, S. Najara Pinho Queiroz, J. Ollerton, T. Santos, and A. R. Rech. 2021. Heterospecific pollen deposition is positively associated with reproductive success in a diverse hummingbird-pollinated plant community. *Oikos* 2022; e08714.

Lortie, C. J., and L. W. Aarssen. 1999. The advantage of being tall: higher flowers receive more pollen in *Verbascum thapsus* L. (Scrophulariaceae). *Ecoscience* 6: 68–71.

Minnaar, C., and B. Anderson. 2019. Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods in Ecology and Evolution* 10: 604–614.

Minnaar, C., and B. Anderson. 2021. A combination of pollen mosaics on pollinators and floral handedness facilitates the increase of outcross pollen movement. *Current Biology* 31: 3180–3184.e3.

Minnaar, C., B. Anderson, M. L. de Jager, and J. D. Karron. 2019. Plant–pollinator interactions along the pathway to paternity. *Annals of Botany* 123: 225–245.

Moir, M., and B. Anderson. 2023. Pollen layering and male–male competition: Quantum dots demonstrate that pollen grains compete for space on pollinators. *American Journal of Botany* 110: e16184.

Moreira-Hernández, J., H. Ghai, N. Terzich, R. Zambrano-Cevallos, N. H. Oleas, and N. Muchhala. 2023. Limited reproductive interference despite high rates of heterospecific pollen transfer among co-occurring bat-pollinated *Burmeistera*. *American Journal of Botany* 110: e16199.

Moreira-Hernández, J. I., and N. Muchhala. 2019. Importance of pollinator-mediated interspecific pollen transfer for angiosperm evolution. *Annual Review of Ecology, Evolution, and Systematics* 50: 191–217.

Morgan, M. T., and J. K. Conner. 2001. Using genetic markers to directly estimate male selection gradients. *Evolution* 55: 272–281.

Muchhala, N., and J. D. Thomson. 2012. Interspecific competition in pollination systems: costs to male fitness via pollen misplacement. *Functional Ecology* 26: 476–482.

Nakazawa, T., and S. Kishi. 2023. Pollinator sex matters in competition and coexistence of co-flowering plants. *Scientific Reports* 13: 4497.

Nebauer, C. A., M. C. Schleifer, F. A. Ruedenauer, S. D. Leonhardt, and J. Spaethe. 2023. Perception, regulation, and fitness effects of pollen phytosterols in the bumble bee, *Bombus terrestris*. *American Journal of Botany* 110: e16165.

Ollerton, J. 2021. Pollinators and pollination: nature and society. Pelagic Publishing, Exeter, UK.

Olsson, O., M. Karlsson, A. S. Persson, H. G. Smith, V. Varadarajan, J. Yourstone, and M. Stjernman. 2021. Efficient, automated and robust pollen analysis using deep learning. *Methods in Ecology and Evolution* 12: 850–862.

Opedal, Ø. H. 2021. A functional view reveals substantial predictability of pollinator-mediated selection. *Journal of Pollination Ecology* 30: 273–288.

Opedal, Ø. H., K. Gross, E. Chapurlat, A. Parachnowitsch, N. Joffard, N. Sletvold, O. Ovaskainen, and M. Friberg. 2022. Measuring, comparing and interpreting phenotypic selection on floral scent. *Journal of Evolutionary Biology* 35: 1432–1441.

Pearson, A. E., Z. Zelman, L. A. Hill, M. A. Stevens, E. X. Jackson, M. M. N. Incarnato, R. M. Johnson, et al. 2023. Pollinators differ in their contribution to the male fitness of a self-incompatible composite. *American Journal of Botany* 110: e16190.

Pérez-Barrales, R., and W. S. Armbruster. 2023. Incomplete partitioning of pollinators by *Linum suffruticosum* and its coflowering congeners. *American Journal of Botany* 110: e16181.

Peuker, M. A., H. Burger, S. Krausch, U. Neumuller, M. Ayasse, and J. Kuppler. 2020. Floral traits are associated with the quality but not quantity of heterospecific stigmatic pollen loads. *BMC Ecology* 20: 54.

Polling, M., S. Melati, L. A. de Weger, A. G. C. L. Speksnijder, M. J. F. Koenders, H. de Boer, and B. Gravendeel. 2022. DNA metabarcoding using nrITS2 provides highly qualitative and quantitative results for airborne pollen monitoring. *Science of the Total Environment* 806: 150468.

Rodríguez-Otero, C., M. Hedrén, M. Friberg, and Ø. H. Opedal. 2023. Analysis of trait–performance–fitness relationships reveals pollinator-mediated selection on orchid pollination traits. *American Journal of Botany* 110: e16128.

Rohlf, F. J., and M. Corti. 2000. Use of two-block partial least-squares to study covariation in shape. *Systematic Biology* 49: 740–753.

Sahli, H. F., and J. K. Conner. 2011. Testing for conflicting and nonadditive selection: floral adaptation to multiple pollinators through male and female fitness. *Evolution* 65: 1457–1473.

Schemske, D. W., and C. C. Horvitz. 1988. Plant–animal interactions and fruit production in a neotropical herb: a path analysis. *Ecology* 69: 1128–1137.

Shaw, R. G., C. J. Geyer, S. Wagenius, H. H. Hangelbroek, and J. R. Etterson. 2008. Unifying life-history analyses for inference of fitness and population growth. *American Naturalist* 172: E35–47.

Shipley, B. 2016. Cause and correlation in biology. a user's guide to path analysis, structural equations and causal inference with R. Cambridge University Press, Cambridge, UK.

Stanley, R. G., and L. F. Linskens. 1974. Pollen. Biology biochemistry management. Springer Verlag, Berlin, Germany.

Stanton, M., H. J. Young, N. C. Ellstrand, and J. M. Clegg. 1991. Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. *Evolution* 45: 268–280.

Štenc, J., L. Janošík, E. Matoušková, J. Hadrava, M. Mikát, and Z. Janovský. 2023. Pollinator visitation closely tracks diurnal patterns in pollen release. *American Journal of Botany* 110: e16179.

Stewart, A. B., C. Diller, M. R. Dudash, and C. B. Fenster. 2022. Pollination-precision hypothesis: support from native honey bees and nectar bats. *New Phytologist* 235: 1629–1640.

Stone, G. N., P. Willmer, and J. A. Rowe. 1998. Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology* 79: 2808–2827.

Thomson, J. D., and B. A. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution* 43: 657–661.

Trunschke, J., N. Sletvold, and J. Ågren. 2017. Interaction intensity and pollinator-mediated selection. *New Phytologist* 214: 1381–1389.

Tur, C., A. Saez, A. Traveset, and M. A. Aizen. 2016. Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecology Letters* 19: 576–586.

Webb, C. J., and D. G. Lloyd. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *New Zealand Journal of Botany* 24: 163–178.

Weinman, L. R., T. Ress, J. Gardner, and R. Winfree. 2023. Individual bee foragers are less efficient transporters of pollen for the plants from which they collect the most pollen into their scopae. *American Journal of Botany* 110: e16178.

Williams, J. H., and S. J. Mazer. 2016. Pollen—Tiny and ephemeral but not forgotten: New ideas on their ecology and evolution. *American Journal of Botany* 103: 365–374.

Wright, S. 1921. Correlation and causation. *Journal of Agricultural Research* 20: 557–585.