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Permalink

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Journal

American Journal of Botany, 103(3)

ISSN

0002-9122

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Publication Date

2016-03-01

DOI

10.3732/ajb.1600074

Peer reviewed

INTRODUCTION TO THE SPECIAL ISSUE

Pollen—Tiny and ephemeral but not forgotten: New ideas on their ecology and evolution¹

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Ecologists and evolutionary biologists have been interested in the functional biology of pollen since the discovery in the 1800s that pollen grains encompass tiny plants (male gametophytes) that develop and produce sperm cells. After the discovery of double fertilization in flowering plants, botanists in the early 1900s were quick to explore the effects of temperature and maternal nutrients on pollen performance, while evolutionary biologists began studying the nature of haploid selection and pollen competition. A series of technical and theoretic developments have subsequently, but usually separately, expanded our knowledge of the nature of pollen performance and how it evolves. Today, there is a tremendous diversity of interests that touch on pollen performance, ranging from the ecological setting on the stigma, structural and physiological aspects of pollen germination and tube growth, the form of pollen competition and its role in sexual selection in plants, virus transmission, mating system evolution, and inbreeding depression. Given the explosion of technical knowledge of pollen cell biology, computer modeling, and new methods to deal with diversity in a phylogenetic context, we are now more than ever poised for a new era of research that includes complex functional traits that limit or enhance the evolution of these deceptively simple organisms.

KEY WORDS functional trait; gametophytic competition; haploid selection; male gametophyte; natural selection; performance trait; pollen competition; pollen germination; pollen tube growth; sexual selection

If one had developed a life history theory for land plants 420 million years ago, it would have been a theory based on gametophyte viabilities and fertilities and a reproductive process in which genes were transmitted from one gametophyte generation to the next through a cryptic and short-lived diploid sporophyte. As it turns out, today more than 90% of land plants are sporophyte-dominant flowering plants (The Plant List, 2013), in which highly reduced male and female gametophytes live and reproduce almost entirely within sporophytic tissues such as the anther, carpel, and ovule. Yet, despite their small size and morphological simplicity, angiosperm gametophytes are exposed to a remarkable diversity of forms of natural and/or sexual selection during their fleeting lifespans.

In flowering plants, “pollen performance” refers to the traits of the male gametophyte that influence its ability to achieve its highly specialized functions: reaching a stigma; germinating; developing a pollen tube that acquires sufficient resources to be able to reach the

base of the style and to enter the ovary; and locating and entering the ovule to deliver sperm to a receptive egg and central cell. Although pollen structure and biology have a long history of investigation, the evolutionary importance of performance was not fully appreciated until the 1920s and 1930s, when experimental studies by C. Correns, D. A. Jones, J. T. Buchholz, A. F. Blakeslee, and others indicated that pollen competition can strongly affect gene or chromosome frequencies (reviewed by Jones, 1928). Most of these studies inferred prezygotic processes but did not directly identify the specific male gametophytic traits that determine performance. Performance differences among competing pollen genotypes were inferred from segregation distortion among the seeds produced following mixed pollinations.

It was not until the 1960s and 1970s, with classic studies by Brewbaker (1967), Hoekstra and Bruinsma (1975), Baker and Baker (1979), Mulcahy (1974, 1979), and Mulcahy and Mulcahy (1975), that researchers became interested in identifying specific male gametophytic traits involved in the ecological and evolutionary lability of performance. It should be noted, however, that as early as the late 1800s, Darwin (as always!) had witnessed the differential performance of outcross and self-pollen tubes (Darwin, 1876), and E. Strasburger and students had discovered that angiosperms with tricellular pollen were largely restricted to herbaceous and/or

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doi:10.3732/ajb.1600074

weedy groups, whereas those with bicellular pollen were broadly distributed across angiosperms (Strasburger, 1884).

Several dramatic intellectual developments in the 1970s served to eclipse these function- and diversity-based studies, catalyzing the next generation of research on pollen performance. Chief among these were the theoretical advances by David Lloyd and his colleagues (Lloyd, 1980; Lloyd et al., 1980), who explored the influences of serial maternal investment and mating systems on the evolution pollen performance, and the applications by a variety of plant evolutionary biologists of sexual selection, kin selection, and parent–offspring conflict theories to plants (Charnov, 1979; Willson, 1979; Nakamura, 1980; Westoby and Rice, 1982; Queller, 1983, 1984; Stephenson and Bertin, 1983; Willson and Burley, 1983; Mazer, 1987; Snow and Spira, 1991). These developments motivated a large number of empirical studies through the mid-1990s (reviewed by Hormaza and Herrero, 1992; Walsh and Charlesworth, 1992; Stephenson et al., 1992; Snow, 1994; Willson, 1994; Skogsmyr and Lankinen, 2002; Bernasconi et al., 2004). Most botanists now accept that both natural and sexual selection can affect postpollination processes in angiosperms (Moore and Pannell, 2011; Lankinen and Green, 2015).

Much of the present-day research on male gametophyte ecology and evolution has its roots in one or both of these intertwined lineages of empirical discovery of functional diversity and theoretical exploration. This special issue on the ecology and evolution of pollen performance was conceived following a symposium on the same topic at the Botany 2014 meetings in Boise, Idaho. Our goal was to bring into focus recent trait- and function-based research on male gametophyte performance. The research community of today may be less cohesive than that between the late 1970s and 1990s, but a surprising number of researchers in disparate fields continue to address the ecological and evolutionary significance of variation in pollen performance. At the same time, a vibrant and interconnected community of researchers is making rapid progress in understanding the biophysics and the cell and molecular biology of pollen and pollen tube growth (Cheung and Wu, 2008; Winship et al., 2011; Firon et al., 2012; Rounds and Bezanilla, 2013; Sanati Nezhad and Geitmann, 2013; Vogler et al., 2013; Cai et al., 2015; Higashiyama and Takeuchi, 2015; Russell and Jones, 2015; Hafidh et al., 2016). As we approach a detailed understanding of the functional cell biology of male gametophytes, it seems timely for the ecological and evolutionary community to incorporate mechanistic, trait-based thinking into our research, which to date has been largely based on outcomes of performance rather than on performance traits per se. We hope that this special issue will catalyze new collaborations that unite cell, organismal, and population biologists in a shared effort to reveal the genetic, environmental, and developmental causes and consequences of all of the levels of variation displayed by pollen.

We should point out that there were several aspects of gametophyte performance—and its evolution—that we chose not to include among the contributions assembled here. First, we decided not to solicit new examples of the many interesting cases of self- or heteroincompatibility and cytoplasmic male sterility, as these aspects of performance relate to one or a few genes of major effect, of which the evolutionary dynamics have been well explored. Instead, we chose to focus on research that examines factors that affect the performance of conspecific pollen between the time of pollen dispersal and fertilization in a variety of ecological settings (Fig. 1).

Thus, we also avoided the great number of studies on pollen morphology that are typically motivated by questions concerning phylogenetic relationships, morphological evolution, or biogeography rather than pollen function.

Since the 1990s, there has been a great deal of progress in our understanding of the intrinsic and extrinsic factors contributing to male gametophytic success (or failure) in nature. As you will see, the 18 studies in this issue reflect a broad range of disciplines, methods, and conceptual approaches to the question of how pollen tube performance and functional traits vary and evolve within and across taxa (Fig. 1). The studies also encompass a broad range of taxonomic diversity (Fig. 2), and some studies were explicitly comparative. Understanding of the phylogenetic pattern of diversification of pollen and pollen tube structures, sizes, life spans, and growth rates has generated new hypotheses and predictions. Some of the major questions motivating the research included in this issue, along with brief synopses of the papers that address them, are discussed next.

WHAT ARE THE CAUSES OF VARIATION IN POLLEN QUALITY OR THE INTENSITY OF POLLEN COMPETITION?

The degree to which pollen competition can shape the evolution of pollen performance is most obviously determined by how many male gametophytes typically compete for access to each egg. But, as many of the papers in this issue show, the intensity of pollen competition is shaped by both pollen quantity and quality, as well as by a number of other ecological variables and maternal and paternal effects (Fig. 1). In fact, there are many conditions in which pollen competition is expected to be weak, and therefore any study of pollen performance should start with an understanding of the context of pollination and its effect on male gametophyte demography within the pistil.

One might suppose chronic pollen limitation to be more prevalent in plants growing at range margins or in extreme environments where pollinators might be unreliable from year to year or pollen quality might be reduced. Instead, two studies on pollen limitation indicate that the effects of range limits or marginal environments are not so simple. First, Wagner et al. (2016 in this issue) provide an exceptionally detailed study of three widely distributed species that occupy high elevations in the European Alps. They used both natural and supplemental pollinations to compare performance from the time of pollen arrival to pollen germination to pollen tube growth and seed set. In all three species, there was little pollen limitation at the level of stigmatic pollen loads and germinated pollen numbers. High pollen tube to ovule and seed to ovule ratios were also observed, except in *Cerastium uniflorum*, where the pollen tube to ovule ratio was reduced below one and resulted in a very low seed to ovule ratio. Since supplemental pollinations only slightly increased the seed to ovule ratio in *C. uniflorum* and had no effect in the other two species, Wagner et al. suggests that low seed set in these species was due to overproduction of ovules as a strategy to deal with stochastic resource limitation in alpine areas (Burd, 2008; Rosenheim et al., 2014). Interestingly, resource limitation may have also affected the ability of styles to support sufficient pollen tube growth.

As much as the intrinsic attributes of individual flowers, plants or taxa may influence the quantity or quality of the pollen received (e.g., Arceo-Gómez et al., 2016 and Mazer et al., 2016 in this issue), so might the context in which plants find themselves. This principle is well illustrated by studies designed to identify the factors that

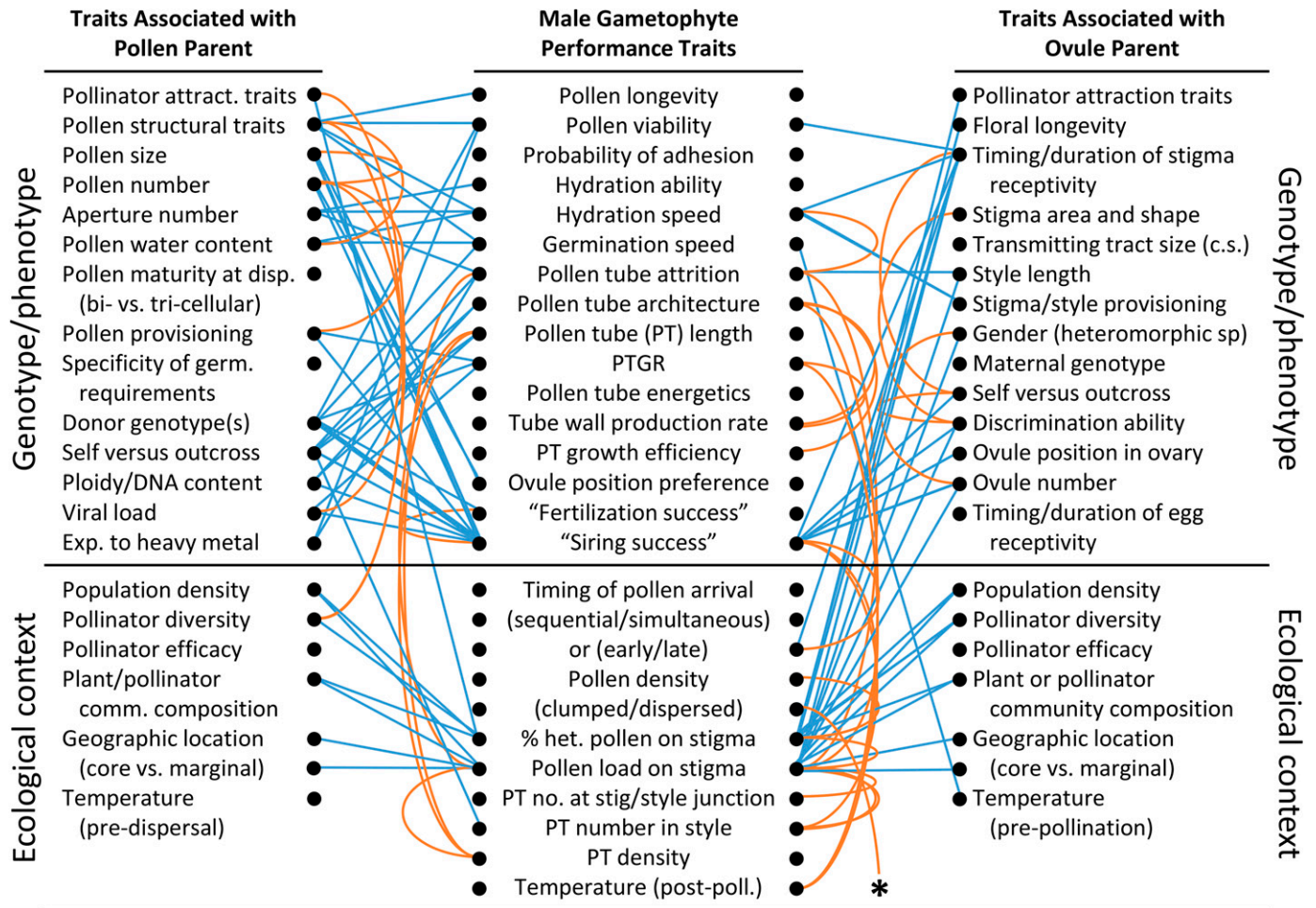


FIGURE 1 Pollen performance traits and proximal sources of variation in performance traits. Associations between traits that were documented or inferred from research in this special issue are connected by lines (orange, within generation; blue, between generations). “Donor genotype(s)” and “Maternal genotype” broadly includes paternal or maternal effects other than self/outcross effects, shown separately. Association between pollen performance and offspring performance is asterisked. For information about direction, magnitude, or causality, please see the articles.

determine the range limits of wild plant species. Indeed, many such studies have identified differences between plants in central vs. marginal portions of their geographic range with respect to commonly measured fitness components (e.g., seed germination, survivorship, or fecundity). Castilla et al. (2016 in this issue) address whether marginal populations of the gynodioecious *Daphne laureola* (Thymelaeaceae) differ from core populations with respect to less well-studied traits: the number of pollen grains per stigma and the number of pollen tubes in the styles of females vs. hermaphrodites. In addition, they estimate pollen quality received by plants in marginal vs. core populations by examining the relationship between the number of pollen grains deposited and the number of pollen tubes penetrating the style. Castilla et al. found that, while both sexes (particularly females) in marginal populations received more pollen per stigma, the quality of this pollen was lower than in core populations. Determining whether this difference in pollen quality is genetically based or the result of geographic variation in the timing of pollen deposition and/or in the environmental conditions when pollination occurs (both of which may affect pollen germination or pollen tube growth) requires additional investigation.

Another factor shaping the quantity and quality of the pollen population reaching the stigma of a flower is the specificity of the pollen-pollinator relationship. When plant species share pollinators, the potential for the transfer of heterospecific pollen can be very high. Given that the receipt of heterospecific pollen (HP) can reduce seed production, identifying the sources of variation in the number and diversity of HP receipt can indicate the qualities of species and populations that may make them most vulnerable to its negative effects. Arceo-Gómez et al. (2016) investigated whether the floral and ecological attributes of 19 plant species, including flower biomass, floral symmetry, pollinator functional diversity, plant community (dry scrubland, serpentine outcrops, and dolomite outcrops), and plant population densities affected the number or diversity of HP observed on stigmas. In addition to detecting high intraspecific variation in the quantity and diversity of HP received in their multicontinent survey, they found that plant species differed greatly in HP receipt based on their degree of ecological generalization: the higher the diversity of floral visitors, the greater the quantity and diversity of HP received. Larger-flowered species also received more HP, while, surprisingly, floral longevity and conspecific population density

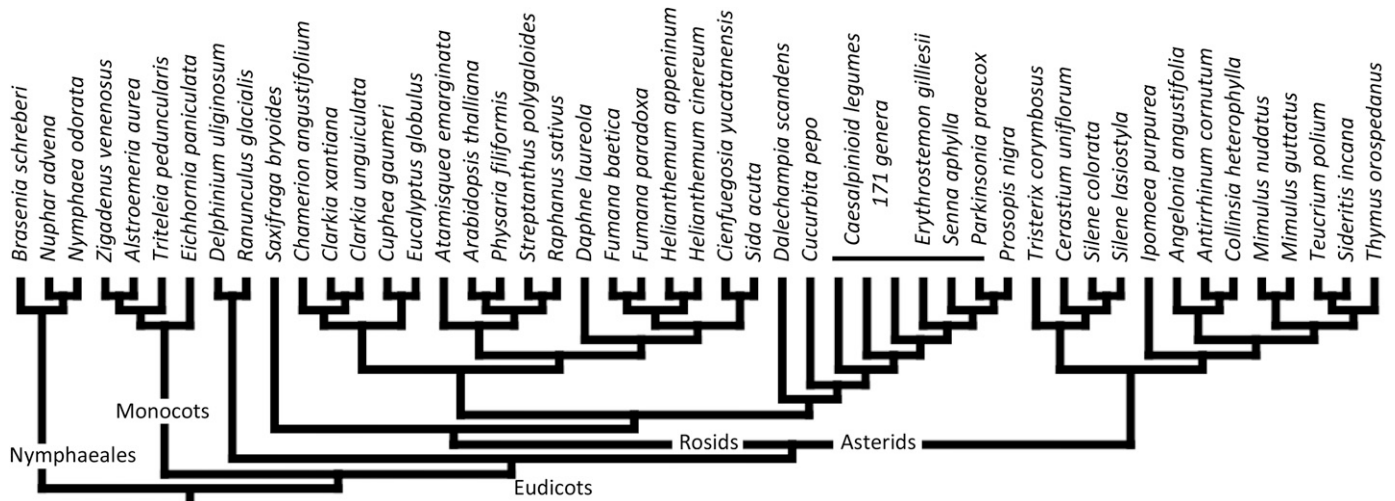


FIGURE 2 Taxa investigated in this issue span the phylogenetic breadth of flowering plants. The study of Banks et al. in this issue on the densely sampled caesalpinioid grade is shown with only a few branches. Relationships are based on those of Zanne et al. (2013).

had no detectable effect on either HP diversity or quantity. Arceo-Gómez et al. further analyzed in each species the shape of the relationship between the number of heterospecific (HP) vs. conspecific pollen (CP) received; the observations that these variables are not independent of each other and that the shape of the relationship differs among taxa suggest that natural selection may operate on floral or life history traits that affect the HP:CP ratio (favoring low phenotypic values of this parameter): an intriguing area for future research.

Style length is a trait that can increase the intensity of competition by magnifying small differences in pollen tube growth rate. However, longer styles can also intensify competition by allowing more room (and time) for attrition (failure to reach the ovary before senescence of the style) to occur. Alternatively, longer styles may increase pollen capture and the size of the stigmatic pollen load, which may itself affect the intensity of gametophytic competition. Mazer et al. (2016) attempted to distinguish between the direct and indirect effects of style length on pollen capture and pollen attrition rates among naturally pollinated, senescing styles from populations of two insect-pollinated species of *Clarkia*. Observing pollen germination rates and pollen tube growth, they found that style length was positively correlated with pollen receipt, but also that attrition rates within the style were never affected by style length independent of the size of the pollen load. There was strong evidence for the occurrence of an often hypothesized “gate” at the stigma/style junction—stigmas with >100 pollen grains had a significantly higher mean attrition rate for pollen tube entry into the style relative to stigmas that had <100 pollen grains. The authors concluded that the higher attrition rates associated with higher pollen numbers (in longer styles) must be caused by phenomena such as greater stigma clogging or stronger negative pollen–pollen interactions at early stages of pollen tube growth. In the end, despite the increased attrition observed in longer styles, the number of pollen tubes reaching the style base increased with style length. This pattern should result in higher fertilization rates among relatively long styles, since mean pollen tube number at the style base never exceeded mean ovule number in either species.

WHAT SPECIFIC POLLEN TRAITS AFFECT MALE GAMETOPHYTE PERFORMANCE?

Every study of performance provides some information on male gametophyte characters, but up until recently, the fitness traits most frequently reported had little to do with mechanistic aspects of performance. For example, “fertilization success” (often measured at seed set or later) tells us little about causal aspects of gametophyte biology. Even when pollen germination or tube growth is the focus of study, researchers often report only (1) relative performance, without phenotypic values of the performance-related trait, or (2) population-level estimates of pollen germination percentage or mean or maximum pollen tube growth rates (as estimates of paternal sporophyte vigor). Some recent studies have shifted the focus onto functional traits of individual gametophytes by measuring structural and physiological aspects of pollen grains and pollen tubes in both noncompetitive and competitive settings.

Prior to the molecular genetics era, pollen morphology contributed greatly to our understanding of the evolutionary relationships and history of land plants. Yet, the functional significance of that diversity was not often explored outside of a few model species. Pollen walls must accommodate great contraction and expansion of the male gametophyte during pollen desiccation in the anther, presentation and dispersal, and upon rehydration on the stigma (Heslop-Harrison, 1979). Banks and Rudall (2016, in this issue) explore the diversity of pollen structure and function among all 171 genera of the paraphyletic caesalpinioid legumes (the species-rich papilionoid and mimosoid legumes are currently nested within the caesalpinioid clade, but were not studied). In this survey, 102 of 120 distinctive pollen morphologies were present in the two early-divergent caesalpinioid tribes, Cercideae and Detarieae, compared with only 19 morphologies in the remaining two tribes (despite similar species numbers). Much of the diversity was related to features that accommodate size changes (harmomegathy), such as apertures and wall innovations, and there were some repeated associations between striate/verrucate pollen ornamentation and vertebrate pollination. Finally, a number of Detarieae have independently

evolved a thick, pectic oncus (“Zwischenkörper”), which, as shown by Vieira and Feijó (2016 in this issue), can have consequences for the speed and control of the pollen hydration and germination. Because the vast majority of studies of pollen structure to date have been conducted to sort out taxonomic relationships among plants rather than to identify the functions of alternative structural elements, there is much room to study trade-offs between features that promote efficient dispersal and reception vs. those that enable high pollen longevity, viability, and effective germination timing.

We are grateful to Vieira and Feijó for providing an in-depth experimental study of the pollen hydration process, a critical and rarely studied aspect of pollen adhesion and eventual germination. In many species, pollen competition is most intense at the germination stage. Pollen grains of many, if not most, angiosperms are dehydrated and dormant at pollination, and the dehydrated cells cannot take up water by active mechanisms such as membrane-based osmoregulation. In fact, the mechanisms that control water uptake during pollen hydration are largely unknown. Vieira and Feijó propose that pectins in the aperture area of pollen grains control water flux during hydration and early pollen germination by phase-transition to a hydrogel (a hydrogel has intermediate properties between a solid and a liquid, and can hold water). In a first test of this hypothesis, they used experimental manipulations of hydration conditions known to affect hydrogel behavior coupled with immunolocalization of pectins in pollen grain walls of *Eucalyptus globulus*. They show that as the strongly dehydrated pollen of *Eucalyptus* begins to take up water, there is localized swelling of the pectins in the aperture area accompanied by changes in pectin structure. These and other results are consistent with the idea that apertural pectins have hydrogel-like properties that enable controlled water transfer into the vegetative cell. Vieira and Feijó suggest that this mechanism allows sufficient time for correct self-organization of molecules and cellular components necessary to resume metabolism. The fairly straightforward imaging protocol that they developed could help experimentalists to quantify the velocity of water uptake in pollen, which could be useful both for mechanistic studies of the genetic basis of hydration and to evolutionary ecologists interested in comparative performance at the earliest stages of competition.

Many investigators have documented the effects of ecologically important and variable extrinsic factors such as temperature and humidity on pollen tube germination or growth. One environmental factor that intrigued us, due to its potential to serve as a selective agent influencing the evolution of pollen morphology, is the sugar concentration of the moist surface on which pollen must germinate. Prieu et al. (2016 in this issue) recognized that the rapid absorption of water by pollen grains, which must rehydrate upon landing on the stigmatic surface, puts them at risk for rupturing if this process results in a dangerously rapid change in volume. This vulnerability could, in turn, result in strong selection favoring structural traits, such as aperture number, that influence the ability of a pollen grain to avoid rapid changes in volume. Prieu et al. used the pollen of triaperturate, wild type *Arabidopsis thaliana* and that of mutants with either zero, four, or 4–8 apertures per pollen grain to examine the effect of various concentrations of mannitol on the ability of these gametophytes to remain intact. At all levels of mannitol exposure, the larger the number of apertures, the greater the probability of pollen rupture; and, among all pollen grain types, the higher the mannitol concentration, the lower the probability of

rupture. Together, these results indicate that aperture number may commonly influence pollen performance beyond its effect on the avoidance of desiccation (as other investigators have detected). Moreover, the evident role of mannitol concentration on the likelihood of pollen rupture indicates that selection may also operate on stigmatic variation, favoring maternal sporophyte genotypes that provide a biochemical environment that promotes rapid rehydration while minimizing the likelihood of pollen rupture.

Pollen size has often been shown to be associated with performance traits such as pollen tube length and growth rate. McCallum and Chang (2016 in this issue) report the exceptionally clear results of a greenhouse study designed to detect the functional significance of genetically based variation in pollen size in *Ipomoea purpurea*. Following an artificial selection experiment that succeeded in creating divergent lines with respect to pollen grain size, they conducted mixed pollinations containing both large and small pollen grains followed by the analysis of microsatellite markers among the resulting seeds to determine paternity. They found that the size of individual pollen grains had a strong and positive effect on siring success, although the success of large- relative to small-grained pollen donors differed among pollen recipients. This context-specific effect of pollen size on performance indicates that other factors, such as pollen–pistil interactions, pollen genotype \times environment interactions, or other genetically based differences among pollen donors that influence pollen performance must also play a role in determining the outcome of these competitive interactions and in maintaining the high level of genetic variation in pollen grain size that made this elegant experiment possible in the first place. Marshall and Evans (2016 in this issue) also found a pollen size effect on pollen performance (see next section).

While many plant evolutionary biologists have recognized that the fertilization success of microgametophytes depends in part on rapid pollen tube growth rates, little attention has been directed toward the potential importance of efficient growth, where efficiency is measured as the ability of this architecturally simple organism to achieve a major task (penetrating the style to reach the ovary before its competitors) while using as few resources as possible. Any consideration of the efficiency of postpollination microgametophyte performance soon turns to the question of how to identify the traits that contribute to such efficiency and how to measure efficiency per se. Williams et al. (2016 in this issue) tackled this question by first characterizing and then interpreting intra- and interspecific variation in pollen tube architecture and pollen tube growth rate (PTGR) with the insight that the evolution of increasing competitive ability among microgametophytes might result not only in higher PTGR, but also in correlated changes in other features of the male gametophyte that depend on pollen tube wall construction, such as pollen tube circumference (C) and wall thickness (W).

To characterize covariation in PTGR, C , and W , Williams et al. developed a novel index of pollen tube growth efficiency by examining the scaling of the rate of wall construction relative to PTGR in three species of water lilies (*Nymphaea odorata*, *Nuphar advena*, and *Brasenia schreberi*) with similar pollen tube wall composition. If the rate of wall construction in a growing pollen tube is estimated as the product of C , W , and mean PTGR, then the ratio of the wall construction rate (a “cost”) to growth rate (PTGR, a “benefit”) represents the efficiency with which pollen tubes grow; the lower this ratio, the greater the efficiency. By comparing related taxa with respect to all of these traits, Williams et al. guide us to see how individual gametophytes and species differ with respect to the efficiency

of pollen tube growth—and the architectural means by which they achieve it—and to determine whether trade-offs between different components of pollen performance are expressed. Within species, they found that pollen tube growth efficiency was surprisingly independent of variation in PTGR—faster-growing pollen tubes were no less efficient than slow-growing ones. Among the focal taxa, however, not only did efficiency and pollen tube growth rates differ significantly, but the taxa also differed with respect to the combination of architectural features contributing to their efficiency.

ARE INTERACTIONS AMONG MALE GAMETOPHYTES IMPORTANT?

Just as the nature of interactions among conspecific diploid individuals (e.g., density-independent mortality and positive and negative density-dependent interactions [facilitation and competition, respectively]) can be identified by the shape of the survivorship curve of a population, so might the interactions among male gametophytes. Accordingly, the population dynamics of male gametophytes following the pollination of a single flower might reveal evidence of the intensity and timing of negative or positive interactions among pollen tubes. Harder et al. (2016 in this issue) took this approach to examine—within and among nine species in six angiosperm families—variation in the relations between the numbers of pollen grains received by (or, in one species, just penetrating) stigmas and the numbers of pollen tubes reaching the base (or, in one species, two-thirds the length) of the style: in other words, a dose-response relation. The data for this investigation came from a variety of studies designed to address other questions, but the similarity among them with respect to the parameters measured allowed species to be compared with respect to their male gametophyte population dynamics as well as with respect to the degree of intraspecific variation in such dynamics among flowers within individuals, between individuals, and (for some species) among populations.

Harder et al. begin by developing a suite of imaginative and thoughtful nonlinear models relating the mean and standard deviation of pollen-tube number to pollen receipt. By determining which of these models (peaked vs. asymptotic) provided the best fit for each species, they detected qualitative differences among species—and in some cases among conspecific individuals or populations—with respect to the shape of these relations. They found that in most species, pollen tubes competed sequentially, whereby their growth was arrested if earlier-arriving pollen tubes either prevented access to stylar space or depleted stylar resources. Some species showed evidence of positive density dependence, in which, at low densities, pollen tubes appeared to facilitate each other's growth. Others displayed density-independent mortality before exhibiting competition among male gametophytes. In short, even within this small slice of angiosperm diversity, Harder et al. were able to distinguish among conspecific, haploid gametophytes a range of interactions that are surely as diverse as those displayed by the populations of diploid individuals in which these gametophytes grow. The degree to which each of these distinct types of population dynamics represents the outcome of natural selection on microgametophytic traits or, alternatively, may influence their future evolution, remains an intriguing question.

Direct pollen–pollen interactions have been the most difficult aspect of competition to study, and a subject of intense interest. This is because the genotypic identity of individual pollen tubes has

been impossible to ascertain *in situ* within maternal tissues of stigma, style and ovary. Swanson et al. (2016 in this issue) have finally accomplished this long-awaited goal by following mixed pollinations of *Arabidopsis thaliana* over the entire series of reproductive stages from pollen viability to pollination, germination, pollen tube growth, fertilization and seed development. They were able to use two GUS-transformed reporter lines to identify pollen, pollen tubes and embryos from each line *in situ*. First, they found nonrandom mating; when pollinating Columbia flowers with equal proportions of Landsberg (Ler) and Columbia (Col) pollen, they observed a >13-fold seed-siring advantage for Col pollen. That advantage was attributed to Col's higher pollen viability and germination, faster germination, and faster pollen tube growth, which were observed directly in a separately conducted set of mixed pollinations. There were no further differences between the two pollen sources in postzygotic stages, from fertilization to seed set. Though other studies have been able to localize nonrandom mating in mixed crosses to the prezygotic stage, this is the first to have definitively identified the causal prezygotic traits, in this case pollen viability, germination and tube growth rate. Furthermore, Swanson et al. identified the components of interference *in situ*: in mixed pollinations, Ler pollen tubes were shorter than the Ler single-donor control at all times, whereas Col pollen was the same in the mixed and the single-donor control up until 9 h after pollination, by which time most ovules are usually fertilized. Although the reciprocal mixed crosses have yet to be conducted, this study provides one of the most complete documentations of the power of pollen competition to influence allele frequencies among offspring. Since QTLs for nonrandom mating have also been found in this system (Carlson et al., 2011), the results here may help in the search for genes that contribute to competition-mediated nonrandom mating, such as those that have been found in corn (Valdivia et al., 2007).

The mechanisms for direct pollen–pollen interactions have been studied occasionally, and both positive (e.g., Brewbaker and Kwack, 1963) and negative, or allelopathic (Murphy, 2000) interactions have been reported. One underappreciated issue that is currently receiving much attention is whether heterospecific pollen can affect conspecific pollen performance on a stigma. Wipf et al. (2016 in this issue) formulated and tested a novel hypothesis that metal hyperaccumulators might have elevated heavy metal concentrations in their pollen and therefore be toxic to nonaccumulator pollen when interacting on a stigma. Pollen grains from the nickel hyperaccumulator, *Streptanthus polygaloides*, were found to have higher levels of nickel than that of a nonaccumulator, *Mimulus guttatus*. In experimental mixed load pollinations on *M. guttatus* stigmas, pollen germination of conspecific nonaccumulator pollen was slightly enhanced, whereas the proportion of conspecific pollen tubes and seed number were decreased. However, changes in the concentration of Ni in *Streptanthus* pollen did not intensify the effect. Despite the nonsignificant result, this is the first application of the concept of “elemental allelopathy” to pollination, and the solid experimental protocols and discussion of hypotheses point to a new and productive line of future research.

DOES HAPLOID SELECTION REDUCE INBREEDING DEPRESSION (OF THE SPOROPHYTE)?

The expression of deleterious alleles occurs during pollen tube growth and can promote purifying selection when stigmatic pollen

loads are large because dysfunctional or slow pollen tubes are assumed to carry a higher genetic load than pollen that performs well; hence, fertilization will generally be achieved by pollen tubes with relatively fewer deleterious alleles. This effect should be detectable in single-donor crosses, such that the quality of resulting sporophyte embryos will be higher and less variable in high vs. low competition settings. Yet the handful of studies that have been designed to test this hypothesis have yielded mixed results. Pélabon et al. (2016 in this issue) note that one reason might be that researchers usually use large vs. small pollen loads to manipulate the intensity of pollen competition. This confounds the test because of sample size effects: larger loads are more likely to contain gametophytes of high quality. To remove the pollen number effect from the intensity of competition effect, Pélabon et al. used clumped vs. dispersed pollen loads of equal size to achieve high vs. low competition, respectively. This is possible because of the long stigmatic surfaces of *Dalechampia scandens*. Surprisingly, they found no effect of competition intensity on the mean or variance of several early embryo and seedling performance traits and concluded that the role of pollen competition on sporophyte evolution may be overestimated. That conclusion was reflected also in a recent review that appeared after submission of their paper. Baskin and Baskin (2015) found that 16 of 30 studies reported no effect of pollen competition intensity on seed germination.

One outcome of the many studies of inbreeding depression that have been conducted to compare the quality of selfed vs. outcrossed seeds in self-compatible species is that it is widely accepted that deleterious, nonlethal mutations may negatively affect plant performance during all diploid stages of the life cycle. By contrast, the negative effects of such alleles on the gametophytic phase of the plant life cycle have not been well documented. To measure the effects of mutational load expressed both among sporophytes and among the pollen grains they produce, Husband (2016 in this issue) took advantage of the heteroploid species *Chamerion angustifolium* (Onagraceae) to create selfed and outcrossed seeds from both diploid and tetraploid (autopolyploid) lineages; he then raised these seeds to maturity and tested the pollen that they produced both in vitro and by pollinating plants of the same ploidy level. The comparison of gametophyte performance in these diploid vs. tetraploid lineages permitted the decoupling of parental effects on pollen performance due to the mutational load expressed by the sporophyte from the effects on pollen performance due to the gametophyte's genotype.

With these distinct pollen sources at hand (pollen from diploid vs. tetraploid plants that were themselves the product of self vs. outcrossed pollinations), Husband could test several predictions regarding the effects of mutational load on pollen performance, from pollen tube growth through siring success. For example, if pollen performance is under sporophytic control (i.e., determined by the genotype of the pollen-producing parent), then selfing is expected to have a stronger negative effect in diploids than in tetraploids due to the potential for deleterious recessive alleles to be masked in sporophytes of the latter. By contrast, if pollen performance is under gametophytic genetic control, then the negative effects of self-fertilization should be greater in the tetraploid than in the diploid lines because the latter cannot mask mutations and would be expected to have purged them. Husband also tested the prediction that, in general (and again due to masking of deleterious recessives), the gametophytes derived from the outbred tetraploid lines will perform better (e.g., have faster-growing pollen tubes) than

those derived from the outbred diploid lines. The detection of mutational load in this study provides evidence that autopolyploids may enjoy a siring advantage over their diploid progenitors due to the former's ability to mask deleterious mutations, thereby facilitating their establishment when they first appear. One complication of the study that could point to future research is that we know very little about the direct effects of ploidy on cell dimensions and tube tip growth of male gametophytes.

HOW DO INTERACTIONS WITH FEMALE TISSUES SHAPE POLLEN PERFORMANCE?

A difficult aspect of determining causality of pollen performance evolution is that performance is affected by changes in paternal provisioning of pollen, male gametophyte gene expression and energetics ("vigor"), and maternal traits that determine the timing, duration, and distance over which pollen tube growth takes place. Furthermore, physiological discrimination among pollen by stigmas, styles and ovules can also evolve. Thus, "pollen–style interactions" are often invoked to explain why performance traits such as germination speed and PTGR are so often found to be variable, when they are expected to be relatively invariant because they are so strongly related to fertilization success.

The various genetic actors involved in pollen–style interactions often have conflicting interests. In an ambitious attempt to examine sexual conflict in the context of pollen competitive interactions and mating system, Lankinen et al. (2016 in this issue) examine potential sexual conflict over stigmatic receptivity in *Collinsia heterophylla*. In this mixed mating species, pollen release begins as flowers open followed by stigma receptivity a few days later, but these phases overlap, such that self-pollination regularly occurs with or without outcross pollination. In competition, outcross pollen outcompetes self-pollen. Earlier work had shown that some pollen donors can fertilize ovules on plants with early receptivity, but this paternal benefit also resulted in reduced seed set for the maternal plant. Sexual conflict theory predicts that a maternal (pistil) trait, such as delayed receptivity, should evolve to minimize the negative effects of early receptivity. In a carefully thought out experimental crossing design, mixed self/outcross and outcross/outcross pollinations were done at four stages of flower anthesis, followed by independent assessment of pollen germination and timing of stigma receptivity in all flowers (i.e., germination could occur on an apparently nonreceptive stigma and vice versa). Thus, the average timing of stigma receptivity could be calculated from both the pollen-parent and the ovule-parent points of view. For early-stage pollinations, plants with late receptivity had higher seed set, consistent with selection against "selfish" pollen (i.e., early germinators), and there was no evidence that pollen induced early receptivity. Pollen that favored delayed receptivity (i.e., that germinated late) had higher siring success, especially when in competition with self-pollen at early stages. Interestingly, higher siring success was not a result of faster PTGRs. Given their findings, the authors suggest that researchers consider both mating system and sexual conflict effects in future studies of plant reproductive evolution.

In what one reviewer described as a "heroic" effort, Marshall and Evans (2016 in this issue) designed a large-scale selection experiment to test whether there was paternally based genetic variation in performance of *Raphanus sativus* pollen. In *R. sativus*, pollen parents differ in both PTGR and in their tendency to fertilize ovules in

the stylar or basal portion of the ovary. Marshall and Evans selected on the latter character and found a response to selection, indicating heritable genetic variation for pollen performance, in two of four replicate selection experiments. The variation was correlated with pollen size, such that relatively large pollen grains tended to sire seed in the basal region of the ovary (see also McCallum and Chang [2016 in this issue] for genetically based pollen size effects). Despite finding variation in seed siring ability among pollen donor families in all replicates, the results of selection were not replicated in all experiments, possibly because environmental effects differed between experiments. This serves as a reminder of how difficult it is to design and control for the many environmental and developmental variables that affect pollen performance.

Relative pollen quality can be, apparently, in the eye of the beholder. Cruzan and Barrett (2016 in this issue) examined covariation among populations (sampled from Brazil and Jamaica) between mating system and the degree of postpollination discrimination among pollen types in the self-compatible, tristylous *Eichhornia paniculata*. Following controlled pollinations with mixtures of pollen from different anther types (short, mid, and long), Cruzan and Barrett examined variation among populations in outcrossing rate, in stigma-load capacity (the number of pollen grains adhering to a well-pollinated stigma), and in the ability of styles to discriminate between legitimate vs. illegitimate pollen. They found that stylar discrimination among pollen grains based on their legitimacy differed greatly among populations and covaried with outcrossing rate—a novel pattern with many evolutionary implications. The effects of such discrimination were consequential, as the combination of stigma-load capacity and stylar discrimination explained more than 80% of the variance among populations in outcrossing rate. Interestingly, highly selfing populations were less discriminating than those with high outcrossing rates, which may represent an adaptation due to the relatively low level of genetic variation among pollen genotypes generally encountered by individual stigmas of the former and/or to the high risk of inbreeding depression to which chronically outcrossing populations are vulnerable. In sum, the ability of *E. paniculata* styles to discriminate among alternative pollen sources appears to be highly genetically variable, with differences among populations arguably the result of natural selection, rendering this trait an intriguing candidate for future studies of selection in both controlled and natural environments (see Lankinen et al. [2016] in this issue for additional evidence of genetic variation among maternal genotypes in their discriminatory ability).

Most studies of the factors influencing individual fitness in natural populations do not provide estimates of components of male fitness, and this gender-bias is shared by studies of disease ecology as well. The effects of disease resistance on pollen production and pollen performance, as well as on fruit and seed production, are rarely simultaneously investigated. Taking advantage of a well-studied interaction between zucchini yellow mosaic virus (ZYMV) and squash (*Cucurbita pepo* subsp. *texana*), Harth et al. (2016 in this issue) examined the effects of ZYMV infection on the quantity and fertilization ability of pollen produced by infected (susceptible) and noninfected (resistant) genotypes, and they used their observations to draw inferences regarding the potential for the spread of resistance alleles in this subspecies. ZYMV infection reduced staminate flower production, pollen production per flower, and fruit set compared with that of noninfected plants. In addition, ZYMV infection resulted in the production of pollen that was less likely to achieve fertilization than pollen from noninfected (resistant) plants

following mixed pollinations in which the two pollen types competed within a style. Consequently, differences in staminate flower production, pollen per flower, and pollen performance between infected and noninfected plants can hasten the spread of resistance alleles. This finding has important implications for the escape of disease resistant transgenes when there is pollen flow from cultivated crops to wild relatives.

CONCLUSIONS

There is ongoing debate concerning the consequences of the ecology and evolution of male gametophytes for the sporophytes in which they grow, and vice versa. The articles in this special issue reveal that there is much room left for understanding how (and which) phenotypic traits are affected in both generations (Fig. 1). There is substantial overlap in gene expression between generations, as first noted over 30 years ago. On the other hand, gametophyte performance phenotypes can also evolve without necessarily having any effect on sporophyte traits or fitness. Pollen has a highly divergent pattern of gene expression relative to sporophytic tissues, in part because there are also a substantial number of unique pollen-expressed genes (Russell and Jones, 2015; Rutley and Twell, 2015). As we enter the genomics and bioinformatics era, it has been inspiring that biologists from such diverse fields are continuing to discover new aspects of gametophyte biology and function, and we hope that the discoveries presented here will serve to stimulate and encourage the current and next generation of plant reproductive biologists.

ACKNOWLEDGEMENTS

We are grateful first to the symposium speakers at Botany 2014 for their participation and enthusiasm: Tia-Lynn Ashman, Anja Geitmann, Lawrence Harder, Åsa Lankinen, Susan Mazer, Rob Swanson, and Joe Williams, and also to Spencer Barrett, Shu-Mei Chang, Chris Ivey, Jeff Karron, and others for wonderful discussions afterward. An outstanding group of reviewers provided immeasurable help and insight. Finally, we thank Pam Diggle and Amy McPherson for their perceptive comments during both the organization and the editing of this issue. The work of both authors on pollen performance has been supported by the National Science Foundation (IOS 1052291 to J.H.W. and IOS-0718227 to S.J.M.)

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