

INVITED PAPER

For the Special Issue: Ecology and Evolution of Pollen Performance

Diverse ecological relations of male gametophyte populations in stylar environments¹

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PREMISE OF STUDY: Pollen on a stigma represents a local population of male gametophytes vying for access to female gametophytes in the associated ovary. As in most populations, density-independent and density-dependent survival depend on intrinsic characteristics of male gametophytes and environmental (pistil) conditions. These characteristics and conditions could differ among flowers, plants, populations, and species, creating diverse male-gametophyte population dynamics, which can influence seed siring and production.

METHODS: For nine species, we characterized the relations of both the mean and standard deviation of pollen-tube number at the style base to pollen receipt with nonlinear regression. Models represented asymptotic or peaked relations, providing information about the incidence and magnitude of facilitation and competition, the spatial and temporal characteristics of competition, and the intensity and relative timing of density-independent mortality.

KEY RESULTS: We infer that pollen tubes of most species competed sequentially, their tips ceasing growth if earlier tubes had depleted stylar space/resources; although two species experienced simultaneous competition. Tube success of three species revealed positive density dependence (facilitation) at low density. For at least four species, density-independent mortality preceded competition. Tube success varied mostly within plants, rather than among plants or conspecific populations. Pollen quality influenced tube success for two of three species; affecting density-independent survival in one and density-dependent performance in the other.

CONCLUSIONS: The diverse relations of pollen-tube success to pollen receipt evident among just nine species indicate significant contributions of the processes governing pollen germination and tube growth to the reproductive diversity of angiosperms.

KEY WORDS competition; facilitation; male gametophyte; pollen quality; pollen tube; progamic; population ecology

The evolution of flowers and closed carpels represent seminal transitions that characterize angiosperms (Mulcahy, 1979; Williams, 2008). Subsequent evolutionary exploration of alternative solutions to promote reproductive success generated the remarkable diversity of flowers and inflorescences evident among contemporary species. Many morphological aspects of this diversity represent contrasting adaptations that facilitate pollen dispersal by dissimilar

pollinators (e.g., Thomson and Wilson, 2008; Schiestl and Johnson, 2013; Rosas-Guerrero et al., 2014; Van der Niet et al., 2014). However, pollen dispersal is only the first step in angiosperm reproduction. The subsequent steps from pollen receipt to ovule fertilization (progamic stage) and from zygote to released seed also exhibit considerable variety (e.g., Moles et al., 2005; Friedman et al., 2008; Williams, 2008; Gibbs, 2014). Nevertheless, much of the latter diversity probably remains undiscovered, as it primarily involves physiological, rather than morphological, adaptation (Swanson et al., 2004). Not coincidentally, diversity during all three reproductive stages is associated with interactions between parties with conflicting interests, including pollen and pollen vectors (Harder et al., 2001), pollen tubes with each other and the pistils they occupy (Mulcahy, 1979; Williams, 2008; Gibbs, 2014), and embryos with each other, their endosperms and their maternal sporophytes (Friedman et al., 2008).

Components of the progamic phase of angiosperm reproduction exhibit considerable variation among species associated with

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differences in pollen and pistil characteristics and pollen–pistil interaction (Heslop-Harrison and Shivanna, 1977; Erbar, 2003; Edlund et al., 2004; Hiscock and Allen, 2008; Williams, 2008; Cruden, 2009; Williams et al., 2014, 2016 [in this issue]); however, the associated consequences for the mean and variation in pollen-tube success and ovule fertilization have received limited attention. Recent surveys by Alonso et al. (2012, 2013) demonstrated contrasting relations of the numbers of pollen tubes at the bases of styles to pollen receipt by the associated stigmas among species and between sex phenotypes within species, but the causes of this variation are unclear. More recently, some of us (Harder, in press) showed theoretically that the nature of pollen-tube competition and its timing relative to density-independent tube failure influences relations of both the mean and variation in tube success to pollen receipt. We also developed a statistical method for discriminating among classes of male-gametophyte mortality, which we illustrated for two species with contrasting dose-response relations. This approach can reveal insight into the nature of the interactions of male gametophytes with each other and the pistils they inhabit.

In this study, we compared the relation of pollen-tube success to pollen receipt for nine angiosperm species to illustrate the diversity of outcomes generated as male gametophytes navigate to ovaries. We specifically applied previously developed methods (Harder, in press) to examine variation in the population ecology of male gametophytes within and among species. In this analysis, we examined four aspects of male-gametophyte performance: how many of the pollen grains deposited on a stigma produce pollen tubes that reach the base of the style, and so could fertilize ovules; variation in this relation among flowers within plants; among-plant variation; and heterogeneity among conspecific sporophyte populations. We considered the pollen deposited on a stigma as a local population of male gametophytes and evaluated the consequences of density-independent mortality and density-dependent interactions (facilitation and competition) for subsequent pollen-tube success. Before outlining the statistical approach used to distinguish these influences and describing the results, we will summarize diagnostic consequences of these processes to identify specific predictions and facilitate subsequent interpretation.

Overview of the population ecology of male gametophytes—In general, the fate of a male gametophyte can depend on intrinsic and extrinsic influences that act regardless of the presence of other gametophytes (density-independent effects) and other influences associated with the abundance of other gametophytes (density-dependent effects). Density-independent (DI) factors include pollen viability (Dafni and Firmage, 2000; Hedhly et al., 2005), overall genotype (Losdat et al., 2014; Swanson et al., 2016 [in this issue]), self-incompatibility genotype (Hiscock and Allen, 2008; Gibbs, 2014), and phenotypic effects established during pollen development (Stephenson et al., 2003), all of which represent aspects of pollen quality. By definition, DI survival does not vary with density, so the average proportion of successful pollen tubes is constant (invariant), regardless of pollen receipt (Fig. 1B, horizontal portion of black line). This constant represents the slope (first derivative) of the relation of mean tube success to pollen receipt in the absence of density-dependent effects (Fig. 1A, ascending portion of black line). In contrast, density-dependent effects depend, in the first instance, on the quantity of other male gametophytes in the same pistil. Positive density dependence, or facilitation, occurs when the presence of other gametophytes enhances average gametophyte

success, as has been observed during both pollen germination and tube growth (e.g., Cruzan, 1986, and references therein; Niesenbaum and Schueller, 1997). It is evident from an accelerating relation of tube number and a positive relation of proportional tube success to pollen receipt (ascending portion of dashed curve, Fig. 1A and B, respectively). Negative density dependence, or competition, arises when a gametophyte's performance is hampered by the presence of other gametophytes (e.g., Cruzan, 1986; Niesenbaum and Schueller, 1997; Skogsmyr and Lankinen, 1999; Bochenek and Eriksen, 2011). Competition could occur sequentially, if a tube grows into a stylar region in which resources have already been depleted by a previous tube, or simultaneously, if multiple tube tips attempt to access the same space/resources concurrently. Such inhibition causes a decelerating relation of tube number and a negative relation of proportional tube success to pollen receipt (solid gray curves and ultimate portion of dashed curve in Fig. 1A and B, respectively).

The nature of competition, including its timing relative to DI mortality, determines the details of the mean dose-response relation and the variation in tube success among pistils (Fig. 2). A pollen tube's continued growth requires adequate space and resources adjacent to its tip (Herrero and Dickinson, 1979; Rounds et al., 2011; Sanati Nezhad et al., 2013; Losada and Herrero, 2014), so competition occurs when tubes are sufficiently close to limit space/resources. Accordingly, both the spatial proximity of tubes within a stylar cross section and the timing of the arrival of new tube tips will determine how many tubes continue toward the ovary. We illustrate these influences by considering the four combinations of repulsed vs. random spatial distributions and sequential vs. simultaneous competition, recognizing that both dichotomies likely represent extremes of continua. These effects can be further modified by whether DI mortality has already reduced the number of tubes attempting to access a stylar cross section, diminishing the incidence and intensity of competition, or whether it occurs after competition.

The eight combinations of the spatial (repulsed or random) and temporal (sequential or simultaneous) details of competition and the timing of DI mortality (before or after competition) create distinctive relations of the mean and standard deviation of tube number at the bases of styles to pollen receipt among pistils (Fig. 2, Table 1; Harder, in press). If growing tube tips tend to avoid other tubes (repulsed tubes), increasing pollen receipt causes a discontinuous transition for the mean and variation in tube success when tube density equals the stylar carrying capacity (Fig. 2A, B, dashed lines). Below this capacity, each tube can access sufficient space/resources by avoiding other tubes, and competition does not occur (Fig. 2A, B, gray area). Consequently, only DI mortality influences tube success, and the mean and standard deviation in tube success increase linearly. In contrast, once tube density exceeds the carrying capacity, tips of repulsed tubes cannot avoid each other, and competition precludes success of some or all tubes, depending on whether tube tips compete sequentially or simultaneously. With sequential competition of repulsed tubes, only those that enter the style while space/resources remain succeed, creating an asymptotic dose-response relation (Fig. 2A, black solid and dashed lines). In contrast, with simultaneous competition, if tube number exceeds the stylar capacity, no tube has access to sufficient resources, and all tubes fail (Fig. 2A, gray solid and dashed lines). Random, rather than repulsed, attempts to access space/resources within a stylar cross section modify these general relations because as few as two

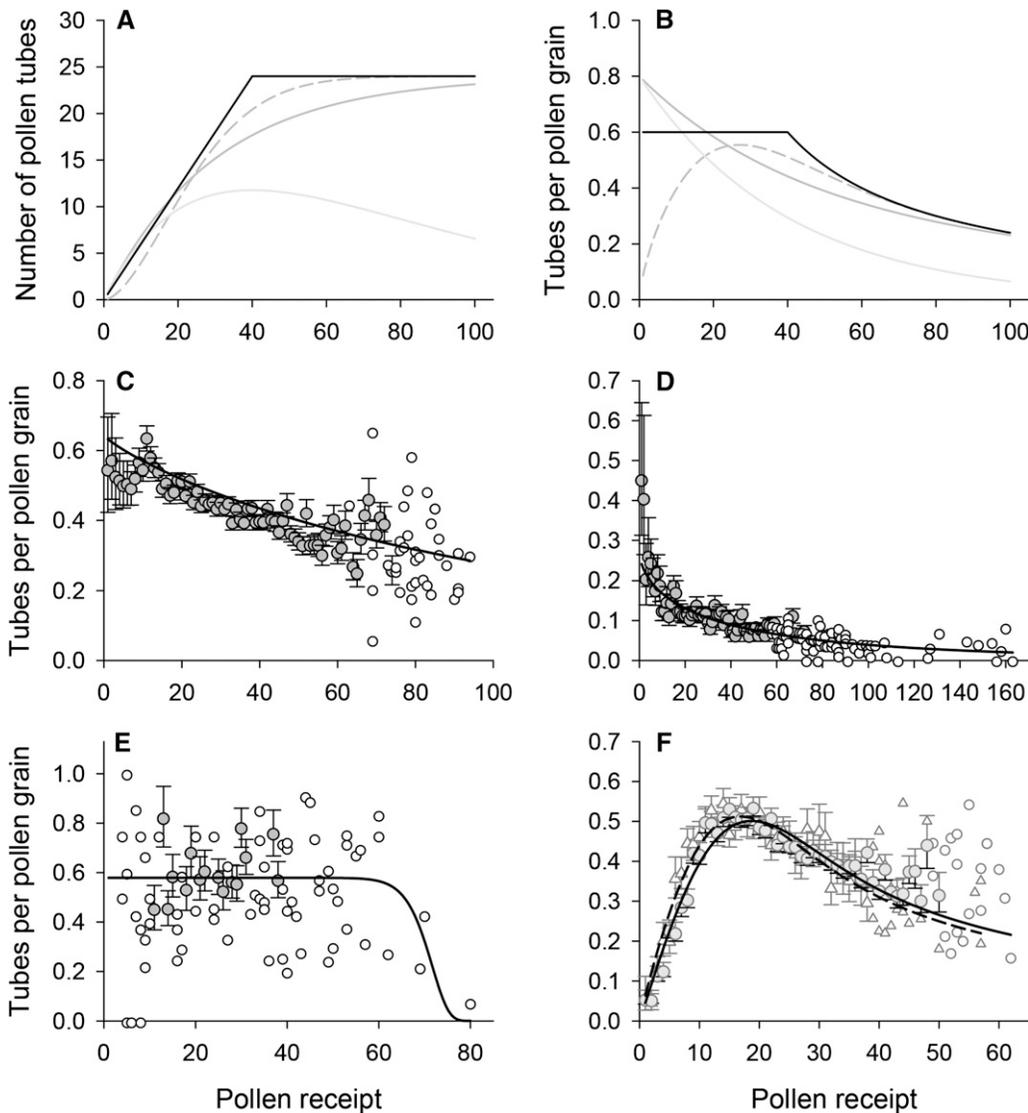


FIGURE 1 Evidence of density-independent and density-dependent relations of pollen-tube success to pollen receipt. Panel (A) illustrates theoretical dose-response relations and (B) presents the corresponding proportional tube success. In these examples, the black line illustrates a density-independent increase to an asymptote that imposes density-dependent success; the gray lines illustrate different examples of joint density independence and universal density dependence, including strict negative density dependence (competition: solid gray lines) and initial positive density dependence (facilitation) turning to competition with increasing pollen receipt (dashed line). The remaining panels illustrate empirical examples of proportional tube success, including (C) *Atamisquea emarginata*, (D) *Physaria filiformis*, (E) *Senna aphylla*, and (F) *Prosopis nigra*. In (C–F), symbols with error bars represent the mean (\pm SE) proportional tube success for pollen counts represented by ≥ 5 pistils, symbols without error bars depict results from individual pistils for pollen counts with fewer samples, and the lines represent the trends based on the best-fitting regressions for the corresponding dose-response relations. For the solid curves in (B) and in (C–E), the tube success for receipt of a single pollen grain indicates average density-independent survival (\hat{v}), whereas for the dashed curve in (B) and the curves in (F) it is represented by the maximal proportion of successful tubes. Panel (F) contrasts the relations for three sites (CON, COS, VIP) with homogeneous results (circles and solid curve) with that from a divergent fourth site (IMH: triangles and dashed curve).

tubes can compete in a style. Consequently, with a random distribution of tube attempts, mean tube success increases in a consistently decelerating, rather than linear, manner with increasing pollen receipt and reaches a lower maximum than for repulsed tubes. Nevertheless, random sequential and simultaneous competition still

generate asymptotic and peaked mean dose-response relations, respectively (Fig. 2C).

Density-independent mortality variously modifies these effects of competition on the mean and variation in overall tube success, depending on whether it occurs before (early DI) or after (late DI) competition (compare solid and dashed curves in Fig. 2). Early DI mortality reduces the number of tubes that can eventually compete within individual styles and introduces variation in the number of competing tubes among pistils that all received p pollen grains. The first effect lessens competition within styles below that expected for receipt of p grains, but it does not alter the maximum number of tubes that can enter the ovary, t_{\max} . The second effect moderates the effects of competition on the mean and variation in tube success among styles. For example, consider a set of pistils that each receive $p > t_{\max}$ pollen grains, which germinate sequentially, producing tubes that avoid each other, if space/resources allow. Owing to variation in tube number caused by early DI mortality, some styles may contain $t < t_{\max}$ tubes, which do not compete, and others will have $t > t_{\max}$ tubes, of which only $t = t_{\max}$ survive competition. Consequently, average tube success over the set of styles will be less than t_{\max} , even though $p > t_{\max}$. This effect will tend to smooth what would otherwise be a discontinuous transition in mean tube success at $p = t_{\max}$ (compare black solid and dashed lines of the same shading in Fig. 2A), with corresponding consequences for variation in tube success among styles (Fig. 2B). In contrast to early DI, late DI mortality does not affect competition, but instead claims tubes that have already survived competition

(Fig. 2A, C, dashed lines). This loss universally reduces overall tube success, with the consequence that maximal success falls below a pistil's capacity to support tubes, regardless of pollen receipt (e.g., compare asymptotes of black solid and dashed lines in Fig. 2A, C).

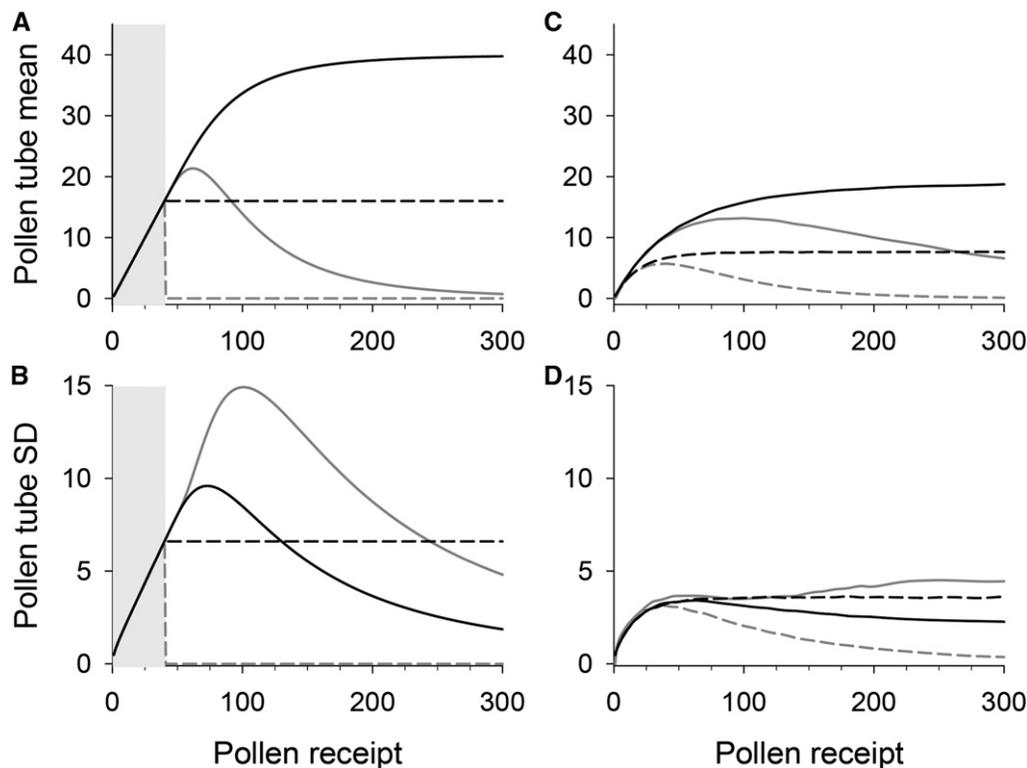


FIGURE 2 Theoretical relations of the mean (A and C) and standard deviation (SD: B and D) of the number of pollen tubes at the base of a style to pollen receipt by the associated stigma for different types of competition and timings of density-independent mortality. (A) and (B) depict outcomes for repulsed pollen tubes, which do not compete when tube number does not exceed the styler capacity to support tubes (gray area: note the linear increases in A, which are indicative of density-independent mortality). (C) and (D) illustrate outcomes for tubes that attempt to access space/resources randomly in styler cross sections. The SDs represent the effects of beta-binomial variation in density-independent mortality (B and D) and of random distribution of attempts by tubes to access space/resources (D only). Black lines represent sequential competition, whereas gray lines depict simultaneous competition. Solid lines represent cases in which density-independent mortality occurs before the possibility of competition; dashed lines depict cases with density-independent mortality after competition. Redrawn from Harder (in press).

The preceding overview illustrates that the relation of among-style variation in tube success to pollen receipt depends on the nature of competition and the timing of DI mortality (Fig. 2B, D). In general, tube success can vary among pistils because of stochasticity associated with DI mortality, random competition, differences in the composition of male-gametophyte populations, and

the diversity of these relations, we consider data for nine species that were collected for other purposes. In addition to revealing interspecific diversity in the population ecology of male gametophytes, these data allow assessment of intraspecific variation in tube success. Overall tube success can differ among a recipient plant's flowers if they receive pollen loads of variable quality that are deposited in heterogeneous sequences. Pollen performance could also differ among recipient plants in association with their genotypes, nutrient status, etc. Because most of the study species are represented by replicate samples for individual plants, we could discriminate the contributions of such among- vs. within-plant variation in tube success. In addition, some species were represented by samples from multiple sites or pollination treatments, which allowed us to assess the effects of general environmental conditions and pollen quality on the population ecology of male gametophytes.

TABLE 1. Expected qualitative relations of mean proportional tube success and the mean and standard deviation of absolute pollen-tube success to pollen receipt for different spatial (repulsed vs. random) and temporal (sequential vs. simultaneous) characteristics of tube competition and different timings of density-independent (DI) tube mortality relative to the occurrence of competition (before vs. after).

Timing of tube interaction	Distribution of attempted resource access	Low density proportional tube success	Mean	Standard deviation	
				DI before	DI after
Sequential	Repulsed	Constant	A	I	A
Sequential	Random	Declining	A	A (I)	A
Simultaneous	Repulsed	Constant	I	I	I
Simultaneous	Random	Declining	I	I (A)	I

Notes: A, asymptotic relation; I, an intermediate peak within the range of pollen receipt. Relations indicated in parenthesis arise with extensive among-flower variation in tube success. Based on theoretical results of Harder (in press).

the styler conditions for tube growth. We represent this variation by beta-binomial distributions: the binomial component describes variation caused by DI mortality, whereas the beta component describes variation in the binomial mean owing to other causes (Harder, in press). If DI mortality acts after competition, the standard deviation in tube success varies largely in parallel with the mean (Fig. 2B, D, dashed lines). In contrast, with DI mortality before competition, the associated binomial variation decreases as an increasing proportion of styles support the maximal number of tubes. Nevertheless, if tubes attempt to access space/resources randomly, beta variation remains relatively extensive, despite receipt of abundant pollen, because of the added variation associated with this spatial process (Fig. 2B, D, solid lines). This effect would be accentuated if pistils receive pollen loads of heterogeneous quality or differ extensively in the styler conditions that enable pollen-tube growth.

Whether the variety of possible relations of the mean and variation in tube success to pollen receipt described above is actually evident among angiosperms remains to be determined. As a preliminary assessment of

MATERIALS AND METHODS

Study species and data characteristics—To explore the diversity of ecological relations for male gametophytes, we used data collected for other purposes for nine species, as described in the source publications identified in Table 2. These species include *Alstroemeria aurea* Graham (Alstroemeriaceae), *Atamisquea emarginata* Miers ex Hook. & Arn. (Capparidaceae), *Chamerion angustifolium* (L.) Holub (Onagraceae), *Erythrostemon* (*Caesalpinia*) *gilliesii* (Hook.) Klotzsch (Fabaceae, Caesalpinioideae), *Parkinsonia praecox* (Ruiz & Pav.) Hawkins (= *Cercidium australe*: Fabaceae, Caesalpinioideae), *Physaria filiformis* (Rollins) O’Kane & Al-Shehbaz (Brassicaceae), *Prosopis nigra* Hieron. (Fabaceae, Mimosoideae), *Senna* (*Cassia*) *aphylla* H.S. Irwin & Barneby (Fabaceae, Caesalpinioideae) and *Tristerix corymbosus* (L.) Kuijt (Loranthaceae). The basic data available for most species included the numbers of pollen grains on the stigmas of individual flowers and of pollen tubes at the bases of the associated styles or in the ovary (*Atamisquea*). The exception involved *Tristerix*, for which the number of pollen tubes that penetrated the stigma represents the effective pollen load, and pollen tubes were counted about two-thirds of the way (2 cm) along the style. Except for *Erythrostemon* and *Senna*, multiple flowers were collected per plant, and for all of these species, except *Physaria*, plant identity was recorded. This structured replication allowed us to discriminate variation in tube success among flowers within plants, associated largely with pollen-load characteristics (see Herrera, 2002, 2004), from variation among plants, associated largely with recipient characteristics (see Cruzan, 1990; Kerwin and Smith-Huerta, 2000). For *Atamisquea*, *Parkinsonia*, *Physaria*, and *Prosopis*, samples were collected from four sites each, allowing assessment of among-site variation in tube success. Such variation could reflect variation in the local growing conditions of pollen-producing plants (see Stephenson et al., 2003), population differences in characteristics of maternal plants (e.g., Murdy and Brown Carter, 1987) and/or ambient conditions during pollen-tube germination and growth (see Hedhly et al., 2005).

The data for *Alstroemeria*, *Chamerion*, and *Tristerix* arose from experiments designed to assess effects of pollen quantity and quality on tube success. The *Alstroemeria* experiment involved hand pollination of flowers on intact recipient plants with pollen from donor plants that were either intact or had been defoliated about 2 weeks prior (Aizen and Raffaele, 1998: adjacent ramets within genets provided resources to defoliated plants). Pollen from both donor treatments was used to pollinate different flowers on individual recipient plants, so treatment differences represent the effect of donor quality on tube performance, after among-recipient variation has been isolated. The *Chamerion* (Richards et al., 2009) and *Tristerix* experiments (Aizen, 2003) both assessed natural pollen limitation of tube performance by comparing open-pollinated flowers and those that additionally received supplemental cross-pollen. They also assessed effects of pollen quality, although with different approaches. The *Tristerix* experiment included bagged flowers, which could only self-pollinate autonomously, on the same plants exposed to the other pollination treatments. The *Chamerion* experiment included hand-pollinated flowers that received solely self- or cross-pollen, in addition to other treatments. Note that hand pollination could increase the incidence of simultaneous competition if more pollen was deposited at one time than is typical of natural pollination.

Statistical methods—We used nonlinear regression to analyze the joint relations of the mean and standard deviation of pollen-tube

number to pollen receipt, $\mu(p)$ and $\sigma(p)$, respectively (proc nlmixed, SAS/STAT 13.2: SAS Institute, 2014). We assumed that tube number (t) varied among pistils that received p pollen grains according to a beta-binomial distribution,

$$\Pr(t|p, \bar{s}, \phi) = \frac{\Gamma(p+1)\Gamma(a+b)\Gamma(t+a)\Gamma(p-t+b)}{\Gamma(t+1)\Gamma(p-t+1)\Gamma(a)\Gamma(b)\Gamma(p+a+b)}, \quad (1)$$

where \bar{s} is the average probability of tube success, $\Gamma()$ is the incomplete gamma function, $a = \bar{s}/\phi$, $b = (1 - \bar{s})/\phi$, and $\phi > 0$ characterizes variation in s (Richards, 2008). This distribution has a mean of $\mu(p) = p\bar{s}$ and variance of

$$\sigma^2(p) = \mu(p) \left(1 - \frac{\mu(p)}{p} \right) \left(1 + \frac{[p-1]\phi}{1+\phi} \right). \quad (2)$$

Our regression analysis is unusual in that we explicitly modeled variation in the standard deviation (σ), as well as the mean. Standard regression analysis assumes the specific association between the variance (σ^2) and the mean (μ) that characterizes the assumed sampling distribution (e.g., normal distribution, σ^2 independent of μ ; Poisson distribution, $\sigma^2 = \mu$: see Stroup, 2013). In contrast, the fact that the variance of the beta-binomial distribution depends on ϕ , in addition to the mean (see Eq. 2), allows modeling of the standard deviation as an independent function of pollen receipt, p . Specifically, rearrangement of Eq. 2 to isolate ϕ yields

$$\phi(p) = \frac{\sigma^2(p) - \mu(p) \left(1 - \frac{\mu(p)}{p} \right)}{\mu(p) \left(p - \mu(p) \right) - \sigma^2(p)}. \quad (3)$$

Therefore, characterization of the relations of the mean and standard deviation in tube success to pollen receipt, $\mu(p)$ and $\sigma(p)$ respectively, allows representation of a p -specific ϕ (via Eq. 3), which in turn defines the specific beta-binomial distribution associated with receipt of p pollen grains (via Eq. 1).

For each species, we analyzed six models that considered combinations of one of two relations for the mean, asymptotic (A) or peaked (P), and one of three relations for the standard deviation, asymptotic (A), peaked (P), or a fixed function of the mean (B). Each model is identified by two letters representing the mean and standard deviation functions (e.g., model AP includes an asymptotic mean function and a peaked standard deviation function). The asymptotic function used for both the mean and standard deviation models was

$$f_A = \alpha \left(1 - \exp \left[-\beta \left(\frac{p}{\alpha} \right)^\gamma \right] \right), \quad (4a)$$

where α is the asymptote. For $\gamma \leq 1$, this function describes a constantly decelerating relation, whereas if $\gamma > 1$ the relation is sigmoidal, which would indicate facilitation following limited pollen receipt. The peaked function that we used was

$$f_P = \beta p \exp \left(-\left[\frac{p}{\alpha} \right]^\gamma \right), \quad (4b)$$

which reaches a maximum of $t_{\max} = \alpha \beta \gamma^{-1/\gamma} e^{-1/\gamma}$ at $p^* = \alpha \exp \left(-\frac{\ln(\gamma)}{\gamma} \right)$.

Thus, this function describes an (intermediate) peak within the observed range of pollen receipt only if the range includes p^* (i.e.,

$p^* < \text{maximum } p$); otherwise, it describes a decelerating (essentially asymptotic) relation. Thus, we distinguish a peaked regression model (P: Eq. 4b) from an observed relation with an intermediate peak (I). We refer to the parameters of Eq. 4 as α_μ , β_μ , and γ_μ for functions describing mean tube success and as α_σ , β_σ , and γ_σ for functions describing the standard deviation of success. Note that, as with standard regression analysis, the fitting of systematic functions of the independent variable allows estimation of variation in summary statistics of the dependent variable (μ for standard regression, μ and σ in our analyses) without replication for any value of the independent variable. However, replication is needed to calculate observed p -specific standard deviations for graphical illustration.

For most species, multiple flowers were sampled for individual plants, allowing separate estimation of within- and among-plant variation in tube success. Such cases used mixed-effects models to assess variation in plant mean tube success, so that for plant i

$$\mu_i(p, z_i) = \frac{p\mu(p)e^{z_i}}{p - \mu(p) + \mu(p)e^{z_i}},$$

where z_i was a normally distributed deviate (mean = 0, within-plant variance = σ_i^2). Correspondingly, $\bar{s}_i = \mu_i(p)/p$, which was incorporated in plant-specific $a_i = \bar{s}_i/\phi(p)$ and $b_i = (1 - \bar{s}_i)/\phi(p)$ parameters of the beta-binomial distribution (note the use of a common $\phi(p)$ for all plants). Given an estimate of $\hat{\sigma}_i^2$, the among-plant variance of $\mu_i(p)$ was calculated by numerical integration as

$$\hat{\sigma}_A^2(p) = \int f_N(z | 0, \sigma_i^2) \mu_i^2(p, z) dz - \hat{\mu}_G^2(p),$$

where $f_N(z | 0, \sigma_i^2)$ is the probability density of the normal distribution and

$$\hat{\mu}_G(p) = \int f_N(z | 0, \sigma_i^2) \mu_i(p, z) dz$$

is the estimated grand mean pollen-tube number (with among-plant variation, fits of Eq. 4a or 4b for the mean are slightly biased). The model likelihood is given by

$$L(\theta) = \prod_i \int_{z_i} f_N(z_i | 0, \sigma_i^2) \prod_j \Pr[t_{ij} | p_{ij}, \bar{s}_i(p_{ij}), \phi(p_{ij})] dz_i,$$

where p_{ij} and t_{ij} are the numbers of pollen grains and pollen tubes associated with the j th flower sampled on the i th plant, and θ defines the set of model parameters.

We used Akaike's information criterion (AIC) to identify which model(s) best describe variation in pollen-tube number. Models with AIC values within six units of the smallest AIC (best-fitting model) were retained for consideration (Richards, 2005).

For the species represented by samples from multiple sites or pollination treatments, we assessed whether the overall regression models differed among sites or treatments with likelihood-ratio (G) tests. These tests compared the maximum log-likelihood for the best-fitting model for all classes combined (LL_{combined}) to the sum of the maximum log-likelihoods for the individual fits of the same model for all k classes (LL_j), $G = 2 \left(\sum_j LL_j - LL_{\text{combined}} \right)$. This test

statistic was compared with the χ^2 distribution with $\rho(k - 1)$ degrees of freedom, where ρ is the number of parameters estimated during individual analyses (7 with among-plant variation).

Given estimates of the parameters of Eq. 4a or 4b for the mean dose-response relation, we could estimate the mean probability of density-independent survival, \bar{v} . In the absence of facilitation, this probability is reasonably represented by the mean number of tubes that reach the ovary following deposition of a single pollen grain (i.e., $p = 1$), which is $\hat{v} = \alpha(1 - \exp[-\beta\alpha^{-\gamma}])$ for Eq. 4a and $\hat{v} = \beta\gamma\exp(-\alpha^{-\gamma})$ for Eq. 4b. With facilitation (i.e., Eq. 4a with $\gamma > 1$), this value will underestimate DI survival, because tube success when $p = 1$ is also inhibited by the absence of facilitating male gametophytes. In this case, a reasonable estimate is provided by the first derivative of the mean dose-response relation evaluated at the pollen receipt for which the effects of negative and positive density dependence counter-balance each other (i.e., at the inflection point of Eq. 4a), specifically

$$\hat{v} = (\gamma - 1) \exp \left[\frac{1 - \gamma - \ln(\gamma - 1) + \ln(\beta) + \ln(\gamma)}{\gamma} \right].$$

We estimated the 95% confidence interval of \bar{v} using the delta method (Cox, 1998).

RESULTS

Regression analysis for the nine species typically identified multiple models that satisfied the selection criterion (Table 2, Fig. 3: see Appendix S1 for illustrations for all species and Appendix S2 for parameter estimates in the online Supplemental Data). Such model uncertainty occurred primarily when few flowers received abundant pollen, providing either no evidence or limited support for asymptotic or peaked relations for the mean and/or standard deviation. In the absence of such evidence, all models depict a positive, decelerating relation, so their fits differ little (e.g., Fig. 3G, H), despite their contrasting mathematical forms. When support is limited, the peaked function can predict maximum tube number near the maximum pollen receipt and so represents an effectively asymptotic relation (e.g., Fig. 3A, D). However, in other cases of limited support, the fits of the asymptotic and peaked models differed markedly (e.g., Fig. 3B, E, F). Based on these results, we inferred an equivocal positive decelerating relation or a recognizable asymptote or intermediate peak for the mean and standard deviation of pollen receipt for each species (Table 2). These inferences were then compared with the theoretical expectations (Fig. 2) to identify likely features of the population ecology of the male gametophytes for all nine species (Table 3).

Interspecific differences—The nine surveyed species provide evidence that the population ecology of male gametophytes differs extensively among species (Table 3, Figs. 1C–F, 3–5; Appendix S1); although they do not represent all of the possibilities summarized in Table 1. The mean dose-response relations of only *Senna* (Fig. 3E) and *Tristerix* (Fig. 5A) declined following receipt of abundant pollen, which is indicative of simultaneous competition. All of the remaining seven species exhibited decelerating relations. Five of

TABLE 2. AIC fits of six regression models and inferred relations of mean tube number (first letter of model name: A, asymptotic; P, peaked) and the standard deviation in tube number (second letter of model name: A, asymptotic; B, fixed function of mean; P, peaked) to pollen receipt.

Species	Model						Inferred relation		Source
	AB	AA	AP	PB	PA	PP	Mean	SD	
<i>Alstroemeria aurea</i>	55.16	0	0.22	58.73	7.63	6.82	A	A or I	Aizen and Raffaele, 1998
<i>Atamisquea emarginata</i>	55.77	5.25	1.69	56.95	0	8.35	d	d	Aizen and Feinsinger, 1994
<i>Chamerion angustifolium</i>	226.34	8.08	0	227.47	9.11	2.35	A	I	Richards et al., 2009
<i>Erythrostemon gilliesii</i>	0.1	2.76	0.63	0	2.69	0.66	d	A (I)	Aizen and Feinsinger, 1994
<i>Parkinsonia praecox</i>	218.87	1.21	0	492.5	255.47	256.15	A	A or I	Aizen and Feinsinger, 1994
<i>Physaria filiformis</i>	9.74	2.68	1.02	9.35	0	1.11	A	A or I	Joseph, 2012
<i>Prosopis nigra</i>	453.32	0	71.28	1363.16	593.42	599.81	A	A	Aizen and Feinsinger, 1994
<i>Senna aphylla</i>	4.62	10.16	10.46	0	10.59	10.95	I (A)	I (A)	Aizen and Feinsinger, 1994
<i>Tristerix corymbosus</i>	127.51	7.42	12.25	129.74	0	2.54	I	A	Aizen, 2003

Notes: Values for each model indicate the difference of its AIC from that of the best-fitting model: those in bold are <6 and so warrant interpretation. The inferred relations represent interpretations based on the model fits: A, asymptotic; I, intermediate peak; d, decelerating (whether A or I is ambiguous). A peaked function could be interpreted as depicting an asymptotic relation if its estimated peak lies at the maximum pollen receipt or beyond. All analyses involve homogeneous data from which anomalous sites or pollination treatments were excluded (see Table 4). Parameter estimates for all models can be found in Appendix S2.

these species (*Alstroemeria* [Fig. 5D], *Parkinsonia* [Fig. 4A], *Chamerion*, *Physaria* [Fig. 3A], *Prosopis* [Fig. 3C]) exhibited distinct asymptotic relations that characterize sequential competition. For the remaining two species (*Atamisquea emarginata* [Fig. 3G], *Erythrostemon gilliesii*), the mean dose-response relation is not maximized within the range of observed pollen receipt, providing no information about whether pollen tubes compete sequentially or simultaneously.

For all species, except *Senna*, the average proportion of pollen grains that produced successful tubes varied continuously with increasing pollen receipt (e.g., Figs. 1C, 1D, 1F, 5C, 5F; Appendix S1), which is indicative of tubes attempting to access space/resources regardless of the presence of other tubes (random distribution). For five of these eight species (*Atamisquea* [Fig. 1C], *Chamerion*, *Erythrostemon*, *Physaria* [Fig. 1D], *Tristerix* [Fig. 5C]), proportional success declined continuously with increasing pollen receipt, indicating competition even when pistils contained few pollen tubes. In contrast, for *Alstroemeria* (Fig. 5F), *Parkinsonia*, and *Prosopis* (Fig. 1F), proportional success varied positively with receipt of up to 20–30 pollen grains, suggesting that facilitation occurs relatively commonly. *Senna* was unique in that average proportional success did not vary over most of the observed range of pollen receipt (Fig. 1E), a pattern expected if pollen tubes avoid each other (repulsed distribution: Fig. 1B solid line; also see Fig. 2A).

The timing of DI mortality relative to competition is more difficult to infer. Because eight species seem to engage in random attempts to use space/resources, contrasting relations (i.e., one asymptotic, the other with an intermediate peak) of the mean and standard deviation of tube number to pollen receipt indicate DI mortality before competition (Table 1). Such combinations are evident for some of the retained regression models for *Alstroemeria*, *Parkinsonia* (Fig. 4A, B), *Chamerion*, *Physaria* (Fig. 3A, B), and *Tristerix* (Fig. 5A, B). In contrast, similar relations of the mean and standard deviation are possible if DI mortality precedes or follows random competition, or with simultaneous competition among repulsed tubes (Table 1). Therefore, the timing of density-independent mortality is ambiguous for the remaining species.

Estimates of the average proportion of male gametophytes that survived density-independent mortality (\bar{v}) ranged from 0.240 (*Physaria*) to 0.818 (*Erythrostemon*), with a median of 0.605

(Table 3). For many species, DI mortality caused as much or more gametophyte attrition as competition (see Figs. 1C–F, 5F). For example, consider proportional survival of *Atamisquea* male gametophytes (Fig. 1C). Following receipt of one pollen grain, a pollen tube survived in an estimated 63% of pistils, indicating 37% DI mortality. In contrast, in pistils subject to maximal pollen receipt, 28% of pollen grains were represented by pollen tubes at the bases of styles. The reduction in tube survival between these two extremes indicates that competition maximally claimed 35% of gametophytes. Thus, over most of the range of pollen receipt, competition had a lesser effect on gametophyte failure than DI processes.

Intraspecific variation—Pollen-tube success varied extensively among pistils, with coefficients of overall variation (estimated standard deviation/estimated mean) exceeding 0.4 for all species. The ϕ parameter of the beta-binomial distribution, which represents among-pistil variation in the probability of tube survival, exceeded 0 for all species (e.g., Fig. 6). Therefore, the probability of individual tube survival varied significantly among pistils. In addition, except for *Erythrostemon* and *Senna*, ϕ varied with pollen receipt, as indicated by poor fits of the B standard-deviation models (Table 2). In all variable cases, ϕ (Eq. 3) peaked abruptly and then declined with increasing pollen receipt (Fig. 6). For some species, ϕ declined to almost 0 with receipt of abundant pollen, which is symptomatic of a binomial distribution among pistils (e.g., *Prosopis*, Fig. 6A). In other species, ϕ remained elevated regardless of pollen receipt (e.g., *Tristerix*, Fig. 6B), indicating beta-binomial distributions. For most species represented by replicate samples per plant, tube success varied predominately among flowers within plants, rather than among plants (e.g., compare solid [total SD] and dashed curves [within-plant SD] in Fig. 3D, F, H, and black and gray curves in Fig. 4B). *Alstroemeria* (Fig. 5E) and *Chamerion* are exceptions, as tube success varied more among plants over most of the range of pollen receipt.

Consistent with limited among-plant variation, patterns of tube success varied little among sites for the four species sampled at multiple locations (Table 4). In all cases, at least three of the four sites exhibited homogenous regression relations. The heterogeneous cases involve three species sampled by Aizen and Feinsinger (1994) at four sites, CON, COS, IMH, and VIP, which present different patterns of among-site variation. In the simplest case, the

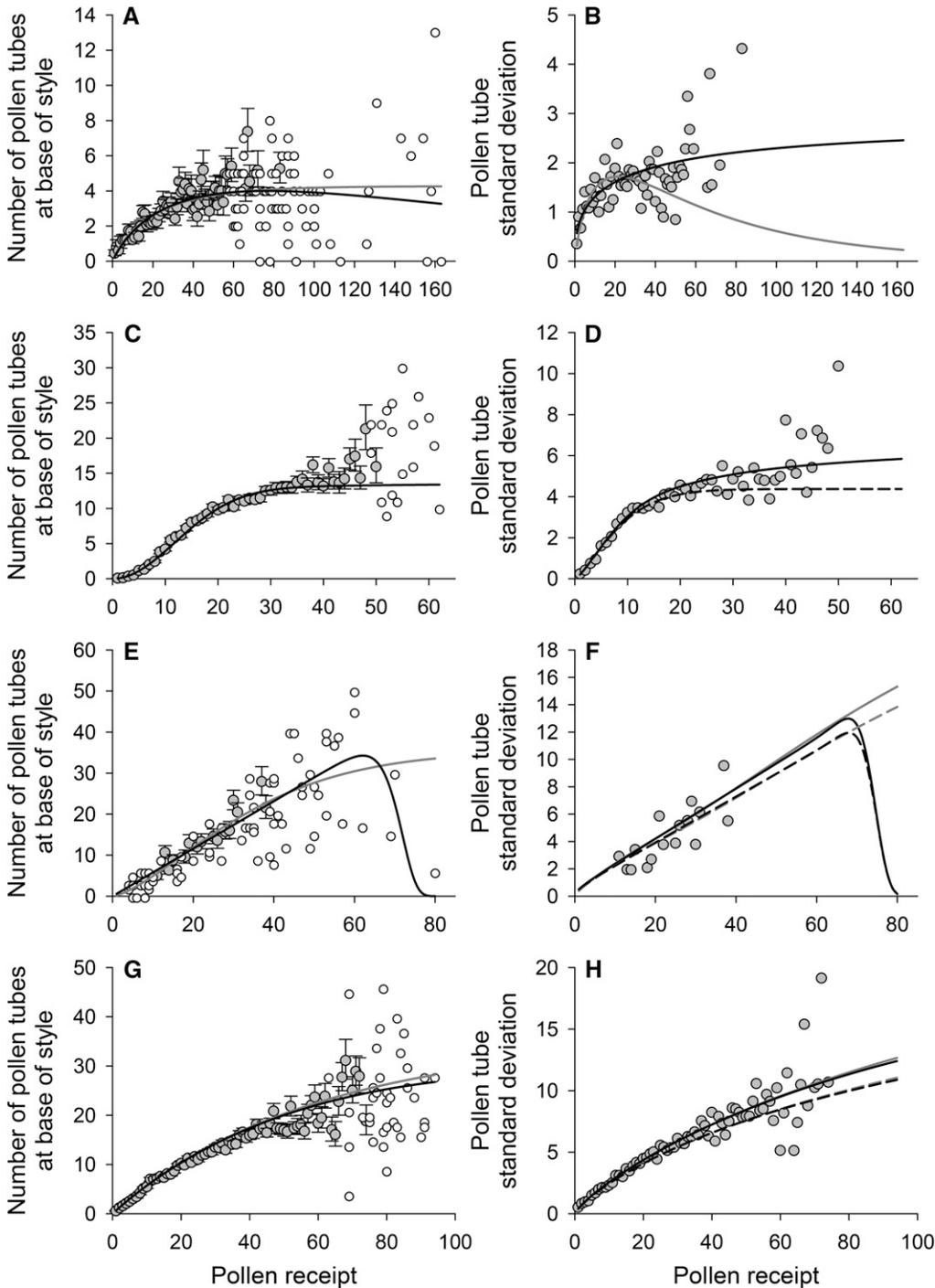


FIGURE 3 Examples of relations of the number of number of pollen tubes at the base of the style (A, C, E, G) and the standard deviation (SD) of tube success among pistils (B, D, F, H) to the number of pollen grains received by associated stigmas. The species illustrated include *Physaria filiformis* (A, B), *Prosopis nigra* (C, D), *Senna aphylla* (E, F), and *Atamisquea marginata* (G, H) (see Table 2 for data sources). Gray symbols represent summary statistics (means \pm SE) for A, C, E, G; SD for B, D, F, H) for values of pollen receipt represented by ≥ 5 pistils; open symbols represent individual pistils. Black lines illustrate regression fits for the best-fitting model; gray lines represent fits for models within 6 AIC units of the best-fitting model (see Table 2). In panels B, D, F, H, solid lines illustrate the fit of the SD of overall variation among pistils; dashed lines depict the estimated within-plant SD. For information concerning the sampling distribution of pollen receipt, see Appendix S1.

asymptotic capacity of *Parkinsonia* plants at CON to support pollen tubes ($\alpha_{\mu} = 14.7$ tubes, lower and upper 95% confidence limits, LCL = 14.45, UCL = 14.93, based on model AI) exceeded that of the three other sites by 37% ($\alpha_{\mu} = 10.7$ tubes, LCL = 10.17, UCL = 11.25; Fig. 4A), which had equivalent relations (Table 4: based on model AI). At the other extreme, for *Prosopis*, the difference in tube success at site IMH from that at the three other sites (Table 4) could not be attributed to a single regression parameter of model AA. A regression that allowed separate estimates for the mean function for IMH fit decidedly better than a model with common estimates for all sites (AIC difference, $\Delta_{AIC} = 9.03$). Compared with the other sites, IMH exhibited higher proportional tube success at low pollen receipt, but lower success at high pollen receipt (Fig. 1F). Based on the second derivatives of the mean dose-response relations ($d^2\hat{t}/dp^2 \pm SE$) following receipt of $p = 1$ (maximal positive density dependence) or 20 pollen grains (maximal negative density dependence), this difference resulted because male gametophytes at IMH experienced significantly stronger facilitation than those at other sites (for $p = 1$: IMH, 0.111 ± 0.007 ; other sites, 0.089 ± 0.005 ; $t_{112} = 3.35$, $P < 0.005$), but the intensity of competition did not differ (for $p = 20$: IMH, -0.044 ± 0.004 ; other sites, -0.043 ± 0.003 ; $t_{112} = 0.24$, $P > 0.8$). In contrast to the site effects on mean tube success, variation among *Prosopis* pistils or plants did not differ appreciably among the four sites, over and above that expected from the direct association of the standard deviation with the mean (comparison of models with separate estimates for the mean only, or for all parameters, $\Delta_{AIC} = 2.06$).

Pollen quality effects—Three data sets include information that allows assessment of pollen-quality effects on tube performance (Table 4).

TABLE 3. Characteristics of the data sets analyzed by nonlinear regression, including the number of flowers sampled, whether facilitation occurred and, if so, its magnitude (1 = no facilitation), inferences about the temporal and spatial distribution of competition and the timing of density-independent (DI) mortality, and the estimated average DI survival probability.

Species	No. flowers	Facilitation γ_{μ} (LCL, UCL)	Competition		DI timing	DI survival $\hat{\nu}$ (LCL, UCL)
			Timing	Distribution		
<i>Alstroemeria aurea</i>	297	1.253 (1.066, 1.441)	Sequential	Random	Before	0.436 (0.358, 0.514)
<i>Atamisquea emarginata</i>	2933	No	Ambiguous	Random	Ambiguous	0.636 (0.581, 0.691)
<i>Chamerion angustifolium</i>	296	No	Sequential	Random	Before	0.356 (0.085, 0.626)
<i>Erythrostemon gilliesii</i>	102	No	Ambiguous	Random	Ambiguous	0.818 (0.669, 0.968)
<i>Parkinsonia praecox</i>	2956	1.999 (1.874, 2.124)	Sequential	Random	Before	0.605 (0.566, 0.644)
<i>Physaria filiformis</i>	840	No	Sequential	Random	Before	0.240 (0.168, 0.313)
<i>Prosopis nigra</i>	3510	2.033 (1.920, 2.145)	Sequential	Random	Ambiguous	0.679 (0.640, 0.718)
<i>Senna aphylla</i>	174	No	Simultaneous	Repulsed	Ambiguous	0.581 (0.531, 0.630)
<i>Tristerix corymbosus</i>	1373	No	Simultaneous	Random	Before	0.757 (0.717, 0.797)

Note: All analyses involve homogeneous data from which anomalous sites or pollination treatments were excluded (see Table 4).

For *Chamerion*, open pollination, supplemental pollination, hand cross-pollination and hand self-pollination resulted in equivalent tube relations (Table 4), indicating no differences in pollen quality based on relatedness of pollen donor and recipient. In contrast, analyses for *Tristerix* and *Alstroemeria* both detected significant effects of pollen quality (Table 4). In both cases, pollen quality affected the mean-dose response relation, rather than variation among flowers, although these effects manifest in different ways (Fig. 5).

Male gametophytes in pistils of *Tristerix* flowers that had been excluded from pollinator access, and so could only self-pollinate autonomously, generally performed poorer than those in flowers exposed to open or supplemental cross-pollination (Fig. 5A–C), which did not differ significantly (Table 4). An analysis that assessed separate estimates for all parameters (except σ_t^2) of model IA identified that only α_{μ} differed between bagged and exposed flowers ($P > 0.1$ for all other parameters). Based on the final analysis with common estimates for all parameters except α_{μ} , the maximum number of successful tubes was 27% lower for bagged flowers ($t_{\max} = 22.6$ tubes, LCL = 18.6, UCL = 26.6) than for those for which cross-pollination was possible ($t_{\max} = 31.1$ tubes, LCL = 28.3, UCL = 33.9; Fig. 5A). In contrast to this negative effect of inbreeding on competition, estimated density-independent survival did not differ between pollination treatments (open and supplemental pollination

combined, $\hat{\nu} = 0.757$, LCL = 0.717, UCL = 0.797; bagged, $\hat{\nu} = 0.805$, LCL = 0.663, UCL = 0.946).

The *Alstroemeria* experiment, which assessed the consequences of defoliation of the pollen-donating plant for the progamic success of its pollen, revealed density-independent, but not density-dependent, effects (Table 4). An analysis that provided separate estimates for all parameters (except σ_t^2) of model AA identified that only β_{μ} differed between crosses with pollen from intact vs. defoliated plants ($P > 0.1$ for all other parameters). Based on the final analysis with common estimates for all parameters except β_{μ} , gametophytes from defoliated donors had 26% lower density-independent survival ($\hat{\nu} = 0.377$, LCL = 0.301, UCL = 0.453) than those from intact donors ($\hat{\nu} = 0.511$, LCL = 0.413, UCL = 0.609). This effect is evident in the more gradual approach to the common mean asymptotic tube number with increasing pollen receipt for gametophytes from defoliated plants (Fig. 5D).

DISCUSSION

Interspecific progamic diversity—The nine species considered in this study illustrate extensive diversity in the population ecology of male gametophytes. As summarized in Table 3, the relations of the mean and standard deviation of tube number to pollen receipt for at least one species are consistent with the expectations of competition between repulsed tubes (*Senna*) or between tubes that attempt to access resources randomly in stylar cross sections (all other species); simultaneous competition (*Senna*, *Tristerix*) or sequential competition (all other species); facilitation at low gametophyte densities (*Alstroemeria*, *Parkinsonia*, *Prosopis*); and density-independent mortality acting before competition (*Alstroemeria*, *Chamerion*, *Parkinsonia*, *Physaria*, *Tristerix*). Unequivocal evidence is not apparent for density-independent mortality acting after competition, although this possibility cannot be excluded by the available results. Of the seven species for which both aspects of competition could be inferred, five provided evidence of sequential, random competition.

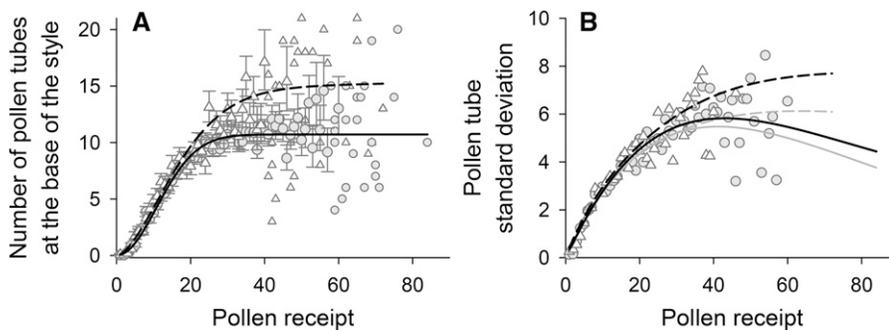


FIGURE 4 Among-site differences in the relations of the mean (A) and standard deviation (SD: B) of tube success for *Parkinsonia praecox* (data from Aizen and Feinsinger, 1994). Gray circles and solid curves illustrate relations for three sites with homogeneous relations (COS, IMH, VIP); open triangles and dashed lines depict relations for a deviant site (CON). The black curves in (B) depict the estimated overall SD and gray curves represent the estimated SD of variation among flowers within plants. For information concerning the sampling distribution of pollen receipt, see online Appendix S1.

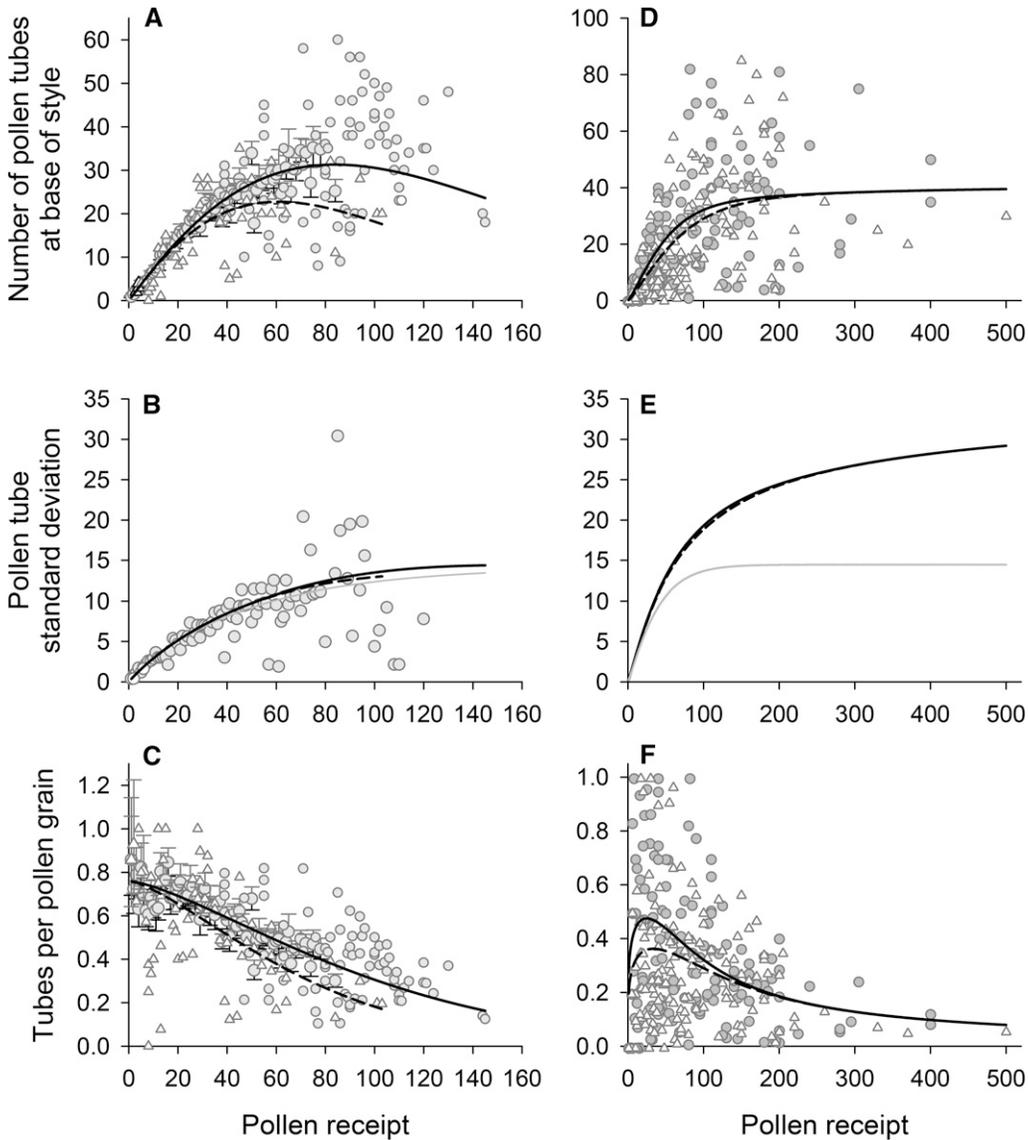


FIGURE 5 Effects of pollen quality on pollen-tube success for *Tristerix corymbosus* (A–C) and *Alstroemeria aurea* (D–F) (see Table 2 for data sources). In (A–C) gray circles and solid lines represent results for open- and supplementally pollinated flowers; white triangles and dashed lines depict results for bagged (autonomously self-pollinated) flowers. In (D–F) gray circles and solid lines represent results for pollen from intact donors; white triangles and dashed lines depict results for pollen from defoliated donors. In A, C, D, and F, curves illustrate the fitted mean relation. In B and E, the black curves depict the estimated overall standard deviation; gray curves represent the estimated standard deviation of variation among flowers within plants. For information concerning the sampling distribution of pollen receipt, see online Appendix S1.

Furthermore, these five species included all three examples of facilitation. Thus, despite considerable variety overall, some patterns of gametophyte ecology occur more frequently than others within this limited collection of species.

Sequential competition between pollen tubes is perhaps the least surprising result. Although stigmatic mechanisms that could synchronize pollen germination have been described, they are known from just a few species (Murphy and Brown Carter, 1987; Ganeshiah and Uma Shaanker, 1988; Douglas and Cruden, 1994; Lankinen and Madjidian, 2011). Even if such mechanisms exist, variation in pollen-tube growth rates (e.g., Herrero and Dickinson, 1980; Cruzan, 1986, 1990; Skogsmyr and Lankinen, 1999) should

quickly erode synchrony, so that tube tips should typically access a stylar cross section sequentially. Thus, simultaneous competition, like that suggested by the results for *Senna* and *Tristerix*, is expected primarily when competition occurs most intensively within, or close to, the stigma, before differential growth separates the tips of growing pollen tubes.

Possibly more surprising is the implication that pollen tubes typically attempt to access space/resources within stylar cross sections regardless of the presence of other tubes, rather than avoiding each other. Thus, growing tube tips may not detect and/or respond to either other tubes or resource gradients. Minimally, this result indicates that continued growth of an individual pollen tube depends on the local conditions adjacent to its tip, rather than throughout the entire stylar cross section, as would occur for repulsed tubes. Indeed, any heterogeneity in the space or resources in a stylar cross section could generate a continuously decelerating mean dose-response relation. Such heterogeneity could arise from the growth patterns of tubes, as implied in our characterization of random tube growth, or variation in stylar conditions. For example, styles with transmitting tissue containing embedded cells that constrain possible paths for pollen tubes (see Erbar, 2003) probably provide varied conditions for tube growth. Whatever the cause, the decelerating dose-response relations for most species indicate that competition often contributes to tube attrition even when only a few tubes occupy a style.

From an empirical perspective, the prevalence of decelerating dose-response relations indicates that the statistical approach of fitting segmented linear relations of Alonso et al. (2012, 2013) will typically characterize the associations of tube success to pollen receipt inadequately.

In addition to negative density-dependent interactions, a third of the studied species exhibited positive density dependence in response to limited pollen receipt (e.g., Figs. 1F, 5F). In such cases, poor pollination is coupled with low probability of tube success, intensifying pollen limitation of seed production compared with that expected from pollen receipt alone, imposing an Allee effect (Taylor and Hastings, 2005). Because an appreciable fraction of

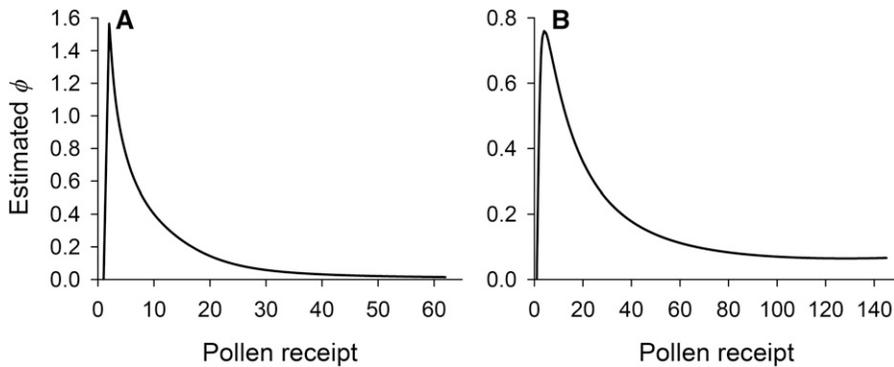


FIGURE 6 Variation in the estimated ϕ parameter of the beta-binomial distribution to pollen receipt for (A) *Prosopis nigra* and (B) *Tristerix corymbosus*.

stigmas receive pollen loads within the range susceptible to this effect (see figures for *Alstroemeria*, *Parkinsonia*, and *Prosopis* in Appendix S1). Allee effects on seed production should be common for species subject to facilitation of male-gametophyte performance. Intriguingly, all cases of facilitation revealed by the proportional success of pollen tubes (e.g., peaked relations of observed means in Figs. 1F, 5F) unequivocally involved asymptotic, rather than peaked, dose-response relations (Table 2). Whether this association occurs generally and results from counteracting effects of positive and negative density dependence awaits further study.

Male gametophytes of all species also experienced considerable DI mortality, with average survival ranging from 0.240 to 0.818. DI mortality could act during pollen germination (e.g., Dafni and Firmage, 2000) or tube growth (e.g., Hiscock and Allen, 2008; Gibbs, 2014; Losdat et al., 2014). Whether species are self-incompatible (SI) or self-compatible (SC) did not obviously affect the severity of DI mortality, as illustrated by their ranking from lowest to highest DI survival: SI, SC, SC, SC, SI, SI, SI, SC, SC. This heterogeneity likely reflects the fact that self-incompatibility systems impose DI mortality only on received pollen that is genetically similar to the pistil. Thus, the incidence of DI mortality related to self-incompatibility depends, in the first instance, on the proportion of self-pollen received by stigmas, as determined by floral and inflorescence traits (e.g., Herlihy and Eckert, 2007; Williams, 2007) and pollinator characteristics (e.g., Brunet and Sweet, 2006; Howard and Barrows, 2014). Interestingly in this context, the incidence of

DI mortality in our survey varied among the species in association with pollinator type: small bee, *Physaria* ($\bar{v} = 0.240$); large bee, *Chamerion* (0.356), *Alstroemeria* (0.436), *Senna* (0.581), *Parkinsonia* (0.605); mixed Hymenoptera, *Atamisquea* (0.636), *Prosopis* (0.679); hummingbird, *Tristerix*, (0.757); hawk moth *Erythrostemon* (0.818). The possibility that pollination quality strongly influences the incidence of DI mortality is also consistent with the evidence that DI mortality occurred primarily before competition.

Quantitative and qualitative effects—Male gametophyte success typically depends on both pollen quantity and quality. Not sur-

prisingly, tube success generally varied positively with pollen receipt for all species. For *Senna*, this relation was linear over most of the range of pollen receipt (Fig. 3E), with the slope determined solely by DI survival. For all other species, the slope varied continuously with pollen receipt (e.g., Fig. 1C, 1D, 1F, 5C, 5F), illustrating pervasive density dependence, in addition to density independence. The positive effect of pollen receipt on the number of successful tubes should generally promote female success. Obvious exceptions are *Senna* (Fig. 3E) and *Tristerix* (Fig. 5A), for which receipt of abundant pollen resulted in fewer successful tubes than more moderate receipt, although few stigmas received sufficient pollen to precipitate this detrimental effect. In contrast, from the male (sporophyte or gametophyte) perspective, contributing to a large stigmatic pollen load generally reduced the probability of individual male gametophytes being represented among the successful pollen tubes, except in the cases of facilitation (Fig. 1C–F). This negative density dependence would promote dispersal of few pollen grains to each of many stigmas to increase the chance of participating in a small pollen load and experiencing less competition. Such bet-hedging is feasible given the extensive variation in pollen receipt exhibited by all species (see Appendix S1) and would favor traits that enable substantial carryover of pollen on pollinators' bodies.

Harder et al. (in press) argued (in contrast to Alonso et al., 2012) that the effects of average pollen quality on tube success could not be specifically identified from individual dose-response relations, because such effects can manifest in diverse ways. Instead, qualitative

TABLE 4. Tests of different regression relations among populations or pollination treatments for seven species.

Species	Comparison	Flowers	Plants	Classes	G	df	P
Site effects							
<i>Atamisquea emarginata</i>	All sites	3923	113	4	41.34	21	0.005
	CON excluded	2933	84	3	22.03	14	0.078
<i>Parkinsonia praecox</i>	All sites	3887	116	4	50.11	21	<0.001
	CON excluded	2956	86	3	11.64	14	0.635
<i>Physaria filiformis</i>	All sites	840		4	22.50	6	0.211
<i>Prosopis nigra</i>	All sites	4711	113	4	42.35	21	0.004
	IMH excluded	3510	83	3	15.84	14	0.323
Pollen quality effects							
<i>Alstroemeria aurea</i>	Donor state	297	43	2	10.10 ^a	1	<0.001
<i>Chamerion angustifolium</i>	All treatments	296	114	4	10.67	21	0.969
<i>Tristerix corymbosus</i>	All treatments	1373	24	3	25.34	14	0.031
	Bagged excluded	1276	24	2	0.02	7	0.999

Notes: Provided information identifies the numbers of plants and flowers sampled, the number of classes of flowers (sites or pollination treatments), and results of likelihood-ratio (G) tests comparing the fits of separate regression models for different classes vs. a common model. See Table 3 for the data sources.

^a Based on a comparison of models with either common or separate estimates of β_{μ} .

effects on tube performance can be identified directly by comparing dose-response relations between groups of stigmas expected to have received pollen of contrasting quality, as in the *Chamerion*, *Tristerix*, and *Alstroemeria* experiments (Fig. 5). For *Chamerion angustifolium*, self- and cross-gametophytes performed equivalently, even though selfed sporophytes of this species, including developing seeds, experience severe inbreeding depression (Husband and Schemske, 1997). In contrast, self-gametophytes of *Tristerix* performed poorer, on average, than gametophytes in pistils subject to some cross pollination through effects on the capacity of pistils to support successful pollen tubes (Fig. 5A–C). This result suggests that the simultaneous competition inferred for *Tristerix* arises when failed tubes (self or nonself) block progress of surviving tubes. Self-tubes seem particularly susceptible to such competition, increasing the chance of obstruction. Finally, the effect of defoliation of *Alstroemeria* pollen donors (Fig. 5C–F) illustrates intergenerational effects on male gametophyte quality (also see Stephenson et al., 2003). Defoliation reduced average pollen size and tube growth rates, but did not affect pollen germination (Aizen and Raffaele, 1998). These effects altered DI success, but did not modify either facilitation or competition among gametophytes from the same treatment. Consequently, donor quality determined the intrinsic capacity of gametophytes and/or their individual interactions with the pistils that they occupied, rather than affecting their interactions with each other. These three experiments provide just a glimpse of influences of pollen quality on male-gametophyte performance; however, they reveal that gametophyte quality is multifaceted.

Intraspecific variation—Tube success varied extensively among pistils for all species. Typically, most variation occurred among flowers within individual plants, rather than among plants. Extensive within-plant variation could exist for two general, nonexclusive reasons: a plant's different flowers could provide varied environments for male-gametophyte populations (e.g., Travers, 1999), or the populations themselves could be heterogeneous in terms of the number and representation of different pollen donors and the quality of their male gametophytes (see Stephenson et al., 2003). Given the generally limited variation in tube success among recipient plants, despite their genetic differences and contrasting growth conditions, stilar conditions may often not vary substantially within individual plants. Instead, extensive within-plant variation seems more consistent with a plant's flowers receiving heterogeneous male gametophyte populations. Particularly relevant is the fact that each stigmatic pollen load represents a sample from the total population of dispersed pollen. Basic sampling theory states that a large sample tends to be more representative of the total population than a small sample. Consequently, averages vary less among large samples than among small samples, as illustrated by the inverse relation of the standard error to sample size. This feature is likely responsible for the declining relations of $\hat{\phi}$ to pollen receipt exhibited by most species (e.g., Fig. 6), a pattern inconsistent with heterogeneous growth conditions among pistils, unless they correlate with pollen receipt. Thus, variation in the composition of stigmatic pollen loads is likely the primary cause of within-plant variation in male gametophyte success. Such heterogeneity would cause variation in tube success among stigmas to be over-dispersed relative to binomial expectations.

The relation of average tube success to pollen receipt varied relatively little among sporophyte populations. This result is consistent

with Herrera's (2002) survey of *Helleborus foetidus* in three widely separated regions in Spain, which attributed only 18.5% of the total variance of pollen-tube number per pistil to variation among 29 populations. Our analyses for four species found homogeneous relations of performance to pollen receipt for most populations (Table 4). The detected differences ranged from an unusually high asymptotic tube number for a *Parkinsonia* population to general differences in average tube performance associated with greater facilitation for a single *Prosopis* population. Thus, the relations of pollen-tube success to pollen receipt appear to be relatively species specific, despite considerable variability among flowers within individual plants in populations.

CONCLUSIONS

Population ecology emerges from the fates of individuals, as determined by their intrinsic capacity and environmental conditions. Our analysis used patterns exhibited by male-gametophyte populations occupying individual pistils to infer aspects of individual performance. Such inference is necessarily indirect, and so subject to misinterpretation. Nevertheless, our interpretations highlight several unknown aspects of male-gametophyte success that warrant consideration. Key among these are the mechanisms responsible for density-dependent tube failure. To date, cellular studies of pollen germination and tube growth have focused on individual tubes and their interactions with stilar tissue (for recent reviews see Malhó, 2006; Krichevsky et al., 2007; Moscatelli and Idilli, 2009; Rounds et al., 2011; Dresselhaus and Franklin-Tong, 2013), with limited consideration of physical and chemical interactions between pollen tubes. Our interpretations indicate that biochemical and cellular studies of pollen-tube growth would benefit from consideration of heterogeneous conditions along styles and within stilar cross sections, including the availability of space and resources for tube expansion and the nature of interaction of growing tube tips with other tubes. The magnitude of among-flower variation in tube success that we observed also signals significant influences of the composition of stigmatic pollen loads on ovule fertilization and seed siring and production, over and above effects of the number of grains received (also see Aizen and Harder, 2007). Furthermore, the variety of population ecology that we have inferred for just nine species suggests corresponding variety of biochemical and cellular processes during pollen germination and tube growth, which cannot be understood fully by focusing analysis on any single species (see Williams et al., 2016 [in this issue]). This diversity also signals considerable evolutionary vagility in traits of pistils and male gametophytes that govern gametophyte performance. For example, much of the observed variety is evident among *Erythrostemon*, *Parkinsonia*, *Prosopis*, and *Senna*, all of which are members of the Fabaceae. Together, these insights underscore that the individual performance of male gametophytes and their resulting population ecology represent significant, if largely hidden, components of the reproductive diversity of angiosperms.

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