Reply

Modest Pollen Limitation of Lifetime Seed Production Is in Good Agreement with Modest Uncertainty in Whole-Plant Pollen Receipt

(A Reply to Burd)

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abstract: We recently introduced a model that predicts the degree to which a plant's lifetime seed production may be constrained by unpredictable shortfalls of pollen receipt ("pollen limitation"). Burd's comment in this issue criticized our analysis, first by arguing that the empirical literature documents much higher levels of pollen limitation than our model predicts and then suggesting that the apparent discrepancy stemmed from our (1) underestimating the costs of securing a fertilized ovule and (2) assuming too little unpredictability in whole-plant pollen receipt. We reply as follows. First, the empirical literature must be consulted carefully. Burd relies on pollen supplementation experiments performed on parts of plants or on whole plants but during only one reproductive season for polycarpic perennials; in both cases, resource reallocation often leads to gross overestimates of pollen limitation. We comprehensively review pollen limitation estimates that are free of these estimation problems and find strong agreement with our model predictions. Second, although cost estimates for different components of seed production are imprecise, errors are likely to be small relative to the >1,000-fold differences observed across plant species, the primary focus of our article. Finally, contrary to Burd's argument, pollen receipt by entire plants is much more predictable than that by individual flowers because the flower-to-flower variation "averages out" when summed across many flowers. Our model uses parameter values that are in broad agreement with the empirical record of modest plant-to-plant variation in pollen receipt and thus predicts the generally modest pollen limitation that is observed in nature.

Keywords: pollen limitation, plant fitness, pollen receipt, pollen supplementation.

Pollen limitation occurs when lifetime seed production by plants is constrained by shortfalls of pollen receipt. If pollen limitation reduces plant fitness, it may shape the evolution of floral traits and plant mating systems and, potentially, have implications for plant conservation (Knight et al. 2005). Despite important progress (Burd 1994; Ashman et al. 2004; Knight et al. 2006; Aizen and Harder 2007), key questions remain regarding proximate and ultimate causes of pollen limitation, the prevalence of pollen limitation in nature, and the magnitude of fitness losses involved.

We recently extended a model developed by Haig and Westoby (1988) and Burd (2008) to explore how the prepollination and postpollination costs of seed production shape the expected importance of pollen limitation for plants faced with unpredictable variation in pollen availability (Rosenheim et al. 2014). Our model predicts, first, that a nonzero risk of pollen limitation is universal. Simply put, plants that never experience a risk of pollen shortfall must be investing too heavily in pollen attraction and would benefit from allocating less there and more to seed maturation. Second, our model predicts that the expected magnitude of pollen limitation depends critically on the relative magnitudes of the prepollination versus postpollination costs of seed production. When prepollination costs are much smaller than postpollination costs, as appears to commonly be the case, plants evolve to overinvest strongly in pollen attraction, essentially purchasing "insurance" against unpredictably low pollen availability. This insurance pushes down the incidence of pollen limitation (a smaller proportion of individuals in the population experiences a shortage of pollen), and the expected magnitude of fitness losses associated with insufficient pollen receipt is, consequently, generally expected to be quite small (less than ~15%). However, for the roughly one-quarter of plant species that incur high prepollination costs (i.e., flowers that are expensive relative to the smaller costs of maturing seeds and fruits), our model predicts instead that plants will underinvest in pollen attraction, resulting in a

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higher proportion of pollen-limited individuals within the population and larger fitness losses due to insufficient pollination.

Burd (2016) has criticized our analysis, focusing not on the differences between plant species in predicted levels of pollen limitation (our emphasis) but rather on the overall level of pollen limitation predicted across all plants. First, Burd argues that our model predicts fitness losses due to pollen limitation that are too small to be consistent with the extensive empirical literature on pollen limitation. Burd then argues that this apparent discrepancy can be explained by (1) errors in estimating the relative magnitudes of prepollination versus postpollination costs of seed production for plants whose pool of resources expands during a reproductive season (income breeders) and (2) our use of an estimate for whole-plant unpredictability in pollen receipt that is too small. Here we respond to these criticisms. We address the first criticism by highlighting prior warnings that the empirical record of pollen supplementation experiments can be very misleading as a guide to the fitness consequences of pollen limitation because most of these studies were not designed in a way that reveals effects of pollen receipt shortfalls on a plant's lifetime seed production (Knight et al. 2006). Studies quantifying pollen limitation's impact on lifetime seed production reveal instead generally modest effects that are in good agreement with our model's predictions. We address the second criticism by directing the reader to appendix E of our original article (Rosenheim et al. 2014), where we give a detailed treatment of a variety of challenges— of which capital breeding is just one—associated with estimating cost components of seed production. Finally, we extend the mathematical argument and simulation model introduced by Burd (2016) to address the question that is central to Burd's argument: how much unpredictability do real plants experience in total pollen receipt? Whereas Burd argues that unpredictability in whole-plant pollen receipt stemming from stochastic receipt of pollen by individual flowers should be higher than that observed across the individual flowers themselves, we demonstrate instead that unpredictability in whole-plant pollen receipt should be lower, decreasing as flower number per plant increases. This occurs because the flower-to-flower variation is "averaged out" when pollen receipt is summed across many flowers.

Empirical Estimates of Fitness Costs of Pollen Limitation

The empirical literature on pollen limitation is vast (Burd 1994; Larson and Barrett 2000; Ashman et al. 2004; Knight et al. 2005), with pollen supplementation experiments often resulting in large proportional increases in reproduction (e.g., mean 67%–75% increase in fruit set [Larson

and Barrett 2000; Knight et al. 2005] or mean 42% increase in seed production [Ashman et al. 2004]). Burd (2016) begins his critique by highlighting exactly this broad literature. Nevertheless, warnings regarding the danger of interpreting augmented seed production in response to pollen supplementation as a direct indicator of fitness effects of pollen limitation have been voiced repeatedly (Janzen et al. 1980; Stephenson 1981; Zimmerman and Pyke 1988; Knight et al. 2006; Wesselingh 2007). The problem is this: when pollen is added to some, but not all, of the flowers produced over a plant's reproductive lifetime, any increase in seed production on the supplemented flowers could come at the expense of decreased seed production on nonsupplemented flowers, as a result of resource reallocation (Obeso 2002). The result is an overestimation of pollen limitation.

Knight et al. (2006) highlighted two kinds of pitfalls for pollen supplementation experiments. The first pitfall occurs when pollen is supplemented to just some of the flowers produced by a plant during a given breeding season. In this case, enhanced allocation of resources to the supplemented flowers can come at the expense of nonsupplemented flowers, creating overestimates of pollen limitation. The magnitude of the error can be large; Knight et al. (2006; see their fig. 3) showed for monocarpic herbaceous plants that mean pollen limitation estimates were inflated ~6.0-fold when pollen was supplemented to only some flowers rather than all flowers produced. The second pitfall occurs when pollen is supplemented to all flowers but during just a single breeding season of a polycarpic perennial plant. In this case, enhanced allocation of resources to flowers during the supplemented year can come at the expense of future plant reproduction, again creating overestimates of pollen limitation. Knight et al. (2006) estimated the magnitude of the error at >9.0-fold (see their fig. 3).

Burd's (2016) test of our model's predictions uses data exclusively from whole-plant pollen supplementation experiments (see his table 1 and fig. 1). Thus, the first pitfall is avoided. However, because four of the five species used by Burd in his test are polycarpic perennials, his estimates do not avoid the second pitfall. The same problem applies to the other studies cited by Burd as reinforcing his view of high levels of pollen limitation in nature; although these studies used whole-plant pollen supplementation, 13 of the 17 species studied were polycarpic perennials. Thus, it is misleading to compare our model's predictions with the results of these studies, which are likely to have produced severely inflated estimates of pollen limitation.

To test our model predictions, it is therefore necessary to use only those studies that supplement pollen to all flowers produced over a plant's full reproductive life. The most appropriate test of our model's prediction linking pollen limitation to prepollination versus postpollination costs of reproduction requires us to build a data set linking cost es-

timates with fitness consequences of pollen limitation; this work is currently under way. In the meantime, however, we can use existing published data to ask whether the mean loss of plant fitness predicted by our model for a sample of 53 animal-pollinated angiosperms is broadly consistent with the mean effect size documented by pollen supplementation studies covering a plant's full lifetime complement of flowers. Our model with base parameter values for variation in whole-plant pollen receipt (normal distribution; coefficient of variation [CV]: 0.33) predicts a mean loss of plant fitness due to pollen shortfalls that is small (mean: 11.9%; range: 1.1%–44.8% across the 53 taxa). In contrast, parameterizing our model with a much higher amount of variability (exponential distribution; CV: 1), as advocated by Burd (2016), yields a mean predicted fitness loss of 48.9% (range: 2.3%–224.1%). What does the empirical record say? Knight et al. (2006) reviewed studies published from 1981 to 2003 in which pollen was supplemented to all flowers of monocarpic species, revealing a mean effect size of pollen limitation on fitness of

7% (95% confidence interval: 2%–12%; $N = 25$ records). We have extended their literature survey through April 2015, using just one mean estimate per plant species to avoid undue influence from more intensively studied species. Our survey reveals strong variation across species and a mean effect size on plant fitness of 25.2% (SD: 43.1%; $N = 21$ records; table 1).

Finally, because researchers studying pollen limitation may gravitate to study systems that show strong pollen limitation effects, it is possible that the published literature may be biased toward higher pollen limitation estimates. In this regard, two community-wide surveys of pollen limitation reported by Lázaro et al. (2015) are significant, as the species included are unlikely to be biased with respect to pollen limitation. Nearly all of the plants studied by Lázaro et al. (2015) were perennials, and thus, as noted above, we can expect the pollen limitation estimates from single-year supplementation experiments to be higher than the true lifetime fitness effects. Nevertheless, Lázaro et al.

Table 1: Summary of experimental studies estimating the influence of pollen limitation on plant fitness (total lifetime reproduction through female function)

Plant taxon	ω[a]	Fitness metric	Reference(s)
Gentianella campestris var. *seucica*	−.309	Seeds/plant	Lázaro et al. 2015
Clarkia parviflora	−.236	Seeds/plant	Runquist and Moeller 2013
Linum catharticum	−.059	Seeds/plant	Lázaro et al. 2015
Nemophila menziesii	.000	Seeds/plant	McCall 2008
Lasthenia fremontii	.055	Seeds/plant	Sargent et al. 2011
Limnanthes douglasii	.064	Seeds/plant	Runquist 2011; J. A. Rosenheim, unpublished data
Linanthus bicolor	.083	Seeds/plant	Goodwillie 2001
Datura stramonium	.088	Seeds/plant	L. S. Adler, unpublished data
Gilia achilleifolia	.129	Fruit set	Schoen 1982
Sabatia angularis	.144	Seeds/plant	Dudash 1993
Clarkia xantiana subsp. *parviflora*	.149	Seeds/plant	Geber and Eckart 2005; Runquist and Moeller 2013
Linanthus jepsonii	.179	Seeds/plant	Goodwillie 2001
Blackstonia perfoliata	.180	Seeds/fruit	Brys et al. 2013
Clarkia xantiana subsp. *xantiana*	.209	Seeds/plant	Geber and Eckart 2005
Centaurea solstitialis	.243	Seeds/plant	Swope 2014
Gentiana nivalis	.283	Seeds/plant	Lázaro et al. 2015
Linanthus parviflorus	.318	Seeds/plant	Goodwillie 1999, 2001
Mimulus guttatus	.372	Seeds/flower	Fishman and Willis 2008
Chaetanthera renifolia	.800	Fruit set	Torres-Diaz et al. 2011
Ipomopsis aggregata	.913	Seeds/plant	Campbell 1991; Campbell and Halama 1993; Juenger and Bergelson 1997; Burkle and Irwin 2009
Castilleja indivisa	1.689	Seeds/plant	Adler 2000
Mean ± SD	.252 ± .341		

Note: Only studies that supplemented pollen to the full lifetime complement of a plant's flowers and that measured total lifetime reproduction through female function (seeds per plant, seeds per flower, or fruit set) were included. Fitness increase was calculated as (fitness metric with supplemented pollen – fitness metric with open pollination)/(fitness metric with open pollination).

[a] Proportional fitness increase with pollen supplementation.

(2015) found that supplemental pollination increased seed set by only 7% ± 4% in an alpine community (Finse site, $N = 24$ plant species; range: −18% to 79%) and 7% ± 5% in a lowland community (Ryghsetra site, $N = 24$ plant species; range: −24% to 84%). Thus, taken together the evidence from nature points to highly variable but generally modest fitness effects from pollen limitation. This empirical record is much better aligned with the predictions from our base parameter set, which incorporates modest uncertainty in pollen receipt at the whole-plant level, than with parameter values reflecting much higher levels of uncertainty.

Estimating Prepollination versus Postpollination Costs of Seed Production

We agree fully with Burd (2016) that actually estimating the magnitude of prepollination versus postpollination costs of seed production is a difficult task. In our original article (Rosenheim et al. 2014), we addressed the particular concern that Burd raised (capital vs. income breeding), along with a series of potentially coequal challenges (in particular, we direct the interested reader to appendix E for a detailed look at estimation problems). Nevertheless, because cost values varied >1,000-fold across species, even relatively rough estimates should, we suggest, provide useful information regarding the relative importance of pollen limitation.

For plants that exhibit substantial vegetative growth between a relatively early, synchronized flowering period and a later, temporally displaced period of seed and fruit maturation, costs incurred early, during flowering, may need to be weighted more heavily than later-incurred costs (e.g., Seger and Eckhart 1996). This does not, however, mean that the resource pools used for flowering and seed maturation are independent; rather, they can be connected through processes of resource storage and remobilization, both within and across growing seasons for perennial plants. Thus, the underlying logic of the allocation problem may change quantitatively but not qualitatively. Researchers have not yet explored the importance of sequential allocations to flowering, growth, and seed maturation for pollen limitation; such an exploration should also consider plasticity of allocations in response to realized pollen receipt. We view these as important frontiers in our understanding of pollen limitation.

Finally, Burd (2016) also raises the possible influence of overproduction of ovules. We agree that this is an important life-history response to uncertain pollen receipt (Burd 1995; Schreiber et al. 2015). As we show elsewhere, however, overproduction of ovules actually lessens the impact of pollen limitation, even when there is uncertainty in late-season resource income available for seed maturation (Rosenheim et al. 2015). Thus, ovule overproduction does not appear to explain why plants exhibit elevated pollen limitation in nature.

Variance in Pollen Receipt

Unpredictability in pollen receipt is the core problem that leads to a risk of pollen limitation. How much unpredictability exists? Our model makes predictions for whole-plant loss of seed production (pollen limitation) based on shortfalls of whole-plant pollen receipt. Burd argues that whole-plant pollen receipt uncertainty is high, much higher than we assumed in our model; we used in our base parameter set a whole-plant pollen receipt that was normally distributed with a CV (defined as the ratio of the SD to the mean) of 0.33. The CV is a dimensionless metric that is useful for this discussion, as it captures the proportional uncertainty of pollen receipt.

An extensive literature demonstrates conclusively that individual flowers face strong uncertainty in pollen receipt in nature (Burd 1995; Alonso et al. 2013). For example, Alonso et al. (2013) report data for 21 species in 10 plant families, showing that the mean CV for pollen receipt by individual flowers is 0.91 ± 0.61 (SD; range: 0.54–2.48). Burd argues that whole-plant uncertainty in pollen receipt will exceed this substantial uncertainty in pollen receipt experienced by single flowers. This is, we believe, the crux of our disagreement with Burd. We now show that the opposite is the case, namely, that whole-plant pollen receipt (the parameter used by our model) is more predictable than pollen receipt by individual flowers and that by deploying larger numbers of flowers, plants lessen their uncertainty in pollen receipt.

As Burd notes, if the number of ovule fertilizations per flower, X_i, is independently and identically distributed in any manner with mean μ_x and variance σ_x^2, then the total number of fertilizations received by a plant with N flowers ($X_1 + X_2 + \cdots + X_N$) will be distributed with mean $\mu_P = N\mu_x$ and variance $= N\sigma_x^2$. As emphasized by Burd, both of these quantities increase linearly with N. From this, Burd concludes that uncertainty in whole-plant pollen receipt will exceed uncertainty in pollen receipt by individual flowers. However, variance in the total number of pollen grains received is not a good metric for the problem posed by uncertain pollination because it does not capture the proportional importance of the variation in pollen receipt (Herrera 2009). For example, adding or subtracting 10 pollen grains could be quite important to a plant that, on average, receives a total of 12 pollen grains but would be less important for a plant that, on average, receives 1,000 pollen grains. A metric that better describes the proportional importance of variation is the CV in the plant's total pollen receipt, CV_P:

$$CV_P = \frac{\sqrt{N\sigma_x^2}}{N\mu_x} = \frac{\sigma_x}{\sqrt{N} \cdot \mu_x}.$$

Unlike the variance in total pollen receipt, the CV for total pollen receipt decreases as the inverse of the square root

of flower number per plant. Thus, just by increasing the number of flowers, plants decrease the uncertainty of total pollen receipt. Whole-plant pollen receipt "averages out" the uncertainty of pollen receipt by summing across individual flowers, yielding an increasingly predictable total pollen harvest; this is exactly as expected under the law of large numbers. Thus, Burd's argument is turned on its head.

We extended Burd's simulation model to show how the mean, variance, and CV for whole-plant pollen receipt change with flower number per plant. Following Burd, each flower on a plant draws its pollen receipt from a geometric distribution with mean $\mu_x = 3$ and $\sigma_x^2 = 12$. As the number of flowers per plant increases, (1) the mean total pollen receipt increases linearly with N, (2) the variance in total pollen receipt increases linearly with N, but (3) the CV decreases as

$$\frac{1}{\sqrt{N}}$$

(fig. 1). Thus, whereas single flowers expect a CV for pollen receipt of 1.15, a plant with 25 flowers expects a CV for total pollen receipt of 0.23, and a plant (e.g., a shrub or tree) with 1,000 flowers expects a CV for total pollen receipt of just 0.04.

The results of our simulation are exactly as expected under the central limit theorem (Durrett 2010), which applies not only to the sample means of pollen receipt per flower, as noted by Burd, but also to the total, whole-plant pollen receipt, as both involve sums of independent random variables. The central limit theorem implies that the distribution of whole-plant pollen receipt will become normal as N increases, as our simulation shows (fig. 1). Although flower number per plant is highly variable across different taxa, it is unusual for plants to produce <20 flowers over their reproductive lives, and many species, including most shrubs and trees, produce hundreds to many thousands of flowers. As a result, the strong within-plant, between-flower component of variance in pollen receipt often makes only modest contributions to total uncertainty in pollen receipt at the whole-plant level.

Although Burd's argument focuses on the contribution of stochastic pollen receipt by individual flowers to unpredictability in whole-plant pollen receipt, there may also be between-plant differences in expected pollen availability due to factors like differences in the local density of conspecifics (pollen donors), local availability of pollen vectors, and differences between plants in how intensively they invest in harvesting pollen (e.g., different investments in floral displays or pollinator rewards). These factors result in each plant having a different underlying distribution of pollen availability for its flowers, with its own particular mean and variance. Within-plant, between-flower variation in pollen receipt and true between-plant variation in pollen availability are predicted to favor different life-history traits related

to ameliorating the costs of pollen limitation (Schreiber et al. 2015).

The empirical literature demonstrates, however, that the dominant contributor to variance in pollen receipt across flowers sampled from many plants in one or more plant populations emerges consistently from the within-plant, between-flower variance component (see table 9.1 in Herrera 2009). This is true both when pollen grains per stigma is measured (mean contribution from within-plant, between-flower variance is 87.5% of the total [range: 78.9–92.0; $n = 3$ studies]) and when pollen tubes per style is measured (mean contribution from within-plant, between-flower variance is 81.2% of the total [range: 67.7–90.7; $n = 11$ studies]). Variance contributed by between-plant effects is generally small.

Finally, what does the empirical record tell us about the CV for total pollen receipt across plants? Is it lower than the CV for pollen receipt across individual flowers, as our simulations suggest it should be? We know of only three published data sets (note that data on seed set, as cited by Burd [2016], will include sources of variation beyond simple pollen receipt). Levin (1990) recorded pollen tubes per style for 10 flowers on each of 50 plants of *Phlox drummondii*, recording a CV of 0.52 for total pollen tubes across plants. Honig et al. (1992) sampled 25 flowers on each of 13 *Staberoha banksii* plants and found a CV of 0.48 for total pollen tubes per plant. Finally, Herrera (2002) sampled pollen tubes per style for 6–12 flowers on each of 10 different *Helleborus foetidus* plants across 22 populations; the mean CV for plants within a population was 0.22 in 2000 and 0.41 in 2001. Although few in number, these studies point to substantially smaller levels of between-plant variation in pollen receipt than has been observed among individual flowers (cf. Burd 1995; Alonso et al. 2013). Furthermore, the true CV among plants is likely smaller than estimated by these studies because the full number of flowers produced by these plants was much larger than the number of flowers sampled per plant by the researchers.

For all these reasons, we feel that our model's baseline value for total between-plant CV of pollen receipt (0.33) was reasonable. Our sensitivity analyses (appendix F in Rosenheim et al. 2014; CV varied from 0.16 to 1.0) and Burd's (2016) analysis highlight the importance of uncertainty in pollen receipt in driving predicted levels of pollen limitation and underscore the need for additional work in this area. Different plant species likely face different levels of unpredictability in whole-plant pollen receipt, contributing to the variation in the observed importance of pollen limitation.

Conclusion

In conclusion, we reject both Burd's primary contention (that our model predicts levels of pollen limitation that
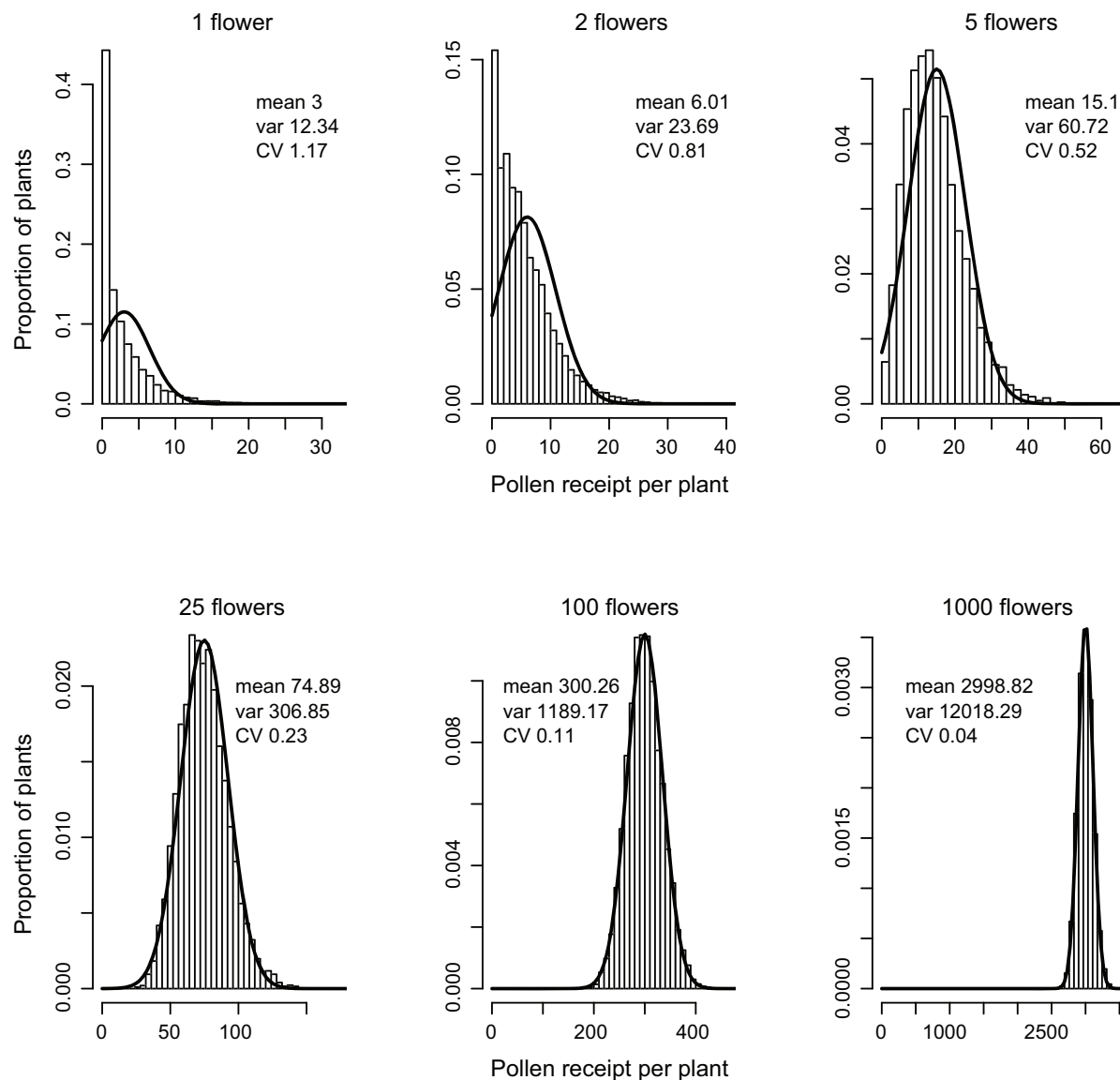
Figure 1: Effect of flower number per plant on the mean, variance, and coefficient of variation (CV) of total pollen receipt across whole plants. Plotted histograms are the simulated consequences of sampling from a between-flower geometric distribution of pollen receipt with a mean of 3 and a variance of 12. Successive panels show the resulting distributions of between-plant total pollen receipt for plants with 1, 2, 5, 25, 100, or 1,000 flowers per plant. Also shown (solid curve) is the normal distribution with the same mean and variance as the associated simulated distribution. As expected under the central limit theorem, the simulated distributions converge to normal distributions as flower numbers per plant increase.

are lower than what is observed empirically) and Burd's primary explanation for that contention (that we used levels of uncertainty in pollen receipt that were too low). Simply by producing multiple flowers, plants reduce substantially the uncertainty they face in their total receipt of pollen. This occurs as a result of the stabilizing effect of averaging across many uncertain "trials" of pollen receipt by individual flowers, as expected under the law of large numbers. Thus, both theoretical considerations and the empirical record suggest that unpredictability in whole-plant pollen receipt is substantially less than unpredictability in pollen receipt by individual flowers. As a result, severe pollen shortfalls are, for most species, predicted to be rare. This prediction is mirrored in the empirical record, which documents mostly modest impacts of pollen limitation on lifetime seed production by plants.

Acknowledgments

Literature Cited

Adler, L. S. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. American Naturalist 156:92–99.

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology 88:271–281.

Alonso, C., C. M. Navarro-Fernández, G. Arceo-Gómez, G. A. Meindl, V. Parra-Tabla, and T.-L. Ashman. 2013. Among-species differences in pollen quality and quantity limitation: implications for endemics in biodiverse hotspots. Annals of Botany 112:1461–1469.

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

Brys, R., B. Geens, T. Beeckman, and H. Jacquemyn. 2013. Differences in dichogamy and herkogamy contribute to higher selfing in contrasting environments in the annual Blackstonia perfoliata (Gentianaceae). Annals of Botany 111:651–661.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60:83–139.

———. 1995. Ovule packaging in stochastic pollination and fertilization environments. Evolution 49:100–109.

———. 2008. The Haig-Westoby model revisited. American Naturalist 171:400–404.

———. 2016. Pollen limitation is common—should it be? (a comment on Rosenheim et al., "Parental optimism versus parental pessimism in plants: how common should we expect pollen limitation to be?"). American Naturalist 187:000–000.

Burkle, L. A., and R. E. Irwin. 2009. The effects of nutrient addition on floral characters and pollination in two subalpine plants, Ipomopsis aggregata and Linum lewisii. Plant Ecology 203:83–98.

Campbell, D. R. 1991. Effects of floral traits on sequential components of fitness in Ipomopsis aggregata. American Naturalist 137:713–737.

Campbell, D. R., and K. J. Halama. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74:1043–1051.

Dudash, M. R. 1993. Variation in pollen limitation among individuals of Sabatia angularis (Gentianaceae). Ecology 74:959–962.

Durrett, R. 2010. Probability: theory and examples. 4th ed. Cambridge University Press, Cambridge.

Fishman, L., and J. H. Willis. 2008. Pollen limitation and natural selection on floral characters in the yellow monkeyflower, Mimulus guttatus. New Phytologist 177:802–810.

Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in Clarkia xantiana. II. Fitness variation across a subspecies border. Evolution 59:521–531.

Goodwillie, C. 1999. Wind pollination and reproductive assurance in Linanthus parviflorus (Polemoniaceae), a self-incompatible annual. American Journal of Botany 86:948–954.

———. 2001. Pollen limitation and the evolution of self-compatibility in Linanthus (Polemoniaceae). International Journal of Plant Sciences 162:1283–1292.

Haig, D., and M. Westoby. 1988. On limits to seed production. American Naturalist 131:757–759.

Herrera, C. M. 2002. Censusing natural microgametophyte populations: variable spatial mosaics and extreme fine-graininess in winter-flowering Helleborus foetidus (Ranunculaceae). American Journal of Botany 89:1570–1578.

———. 2009. Multiplicity in unity: plant subindividual variation and interactions with animals. University of Chicago Press, Chicago.

Honig, M. A., H. P. Linder, and W. J. Bond. 1992. Efficacy of wind pollination: pollen load size and natural microgametophyte populations in wind-pollinated Staberoha banksii (Restionaceae). American Journal of Botany 79:443–448.

Janzen, D. H., P. DeVries, D. E. Gladstone, M. L. Higgins, and T. M. Lewinsohn. 1980. Self- and cross-pollination of Encyclia cordigera (Orchidaceae) in Santa Rosa National Park, Costa Rica. Biotropica 12:72–74.

Juenger, T., and J. Bergelson. 1997. Pollen and resource limitation of compensation to herbivory in scarlet gilia, Ipomopsis aggregata. Ecology 78:1684–1695.

Knight, T. M., J. A. Steets, and T.-L. Ashman. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. American Journal of Botany 93:271–277.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T.-L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology and Systematics 36:467–497.

Larson, B. M. H., and S. C. H. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503–520.

Lázaro, A., R. Lundgren, and Ø. Totland. 2015. Pollen limitation, species' floral traits and pollinator visitation: different relationships in contrasting communities. Oikos 124:174–186.

Levin, D. A. 1990. Sizes of natural microgametophyte populations in pistils of Phlox drummondii. American Journal of Botany 77:356–363.

McCall, A. C. 2008. Florivory affects pollinator visitation and female fitness in Nemophila menziesii. Oecologia 155:729–737.

Obeso, J. R. 2002. The costs of reproduction in plants. New Phytologist 155:321–348.

Rosenheim, J. A., S. J. Schreiber, and N. M. Williams. 2015. Does an "oversupply" of ovules cause pollen limitation? New Phytologist. doi:10.1111/nph.13750.

Rosenheim, J. A., N. M. Williams, and S. J. Schreiber. 2014. Parental optimism versus parental pessimism in plants: how common should we expect pollen limitation to be? American Naturalist 184:75–90.

Runquist, R. D. B. 2011. Pollinator-mediated competition and sources of pollen limitation in Limnanthes douglasii rosea and Limnanthes alba. PhD diss. University of California, Davis.

Runquist, R. D. B., and D. A. Moeller. 2013. Resource reallocation does not influence estimates of pollen limitation or reproductive assurance in Clarkia xantiana subsp. parviflora (Onagraceae). American Journal of Botany 100:1916–1921.

Sargent, R. D., S. W. Kembel, N. C. Emery, E. J. Forrestel, and D. D. Ackerly. 2011. Effect of local community phylogenetic structure

on pollen limitation in an obligately insect-pollinated plant. American Journal of Botany 98:283–289.

Schoen, D. J. 1982. Male reproductive effort and breeding system in an hermaphroditic plant. Oecologia 53:255–257.

Schreiber, S. J., J. A. Rosenheim, L. D. Harder, and N. W. Williams. 2015. Evolutionary and ecological consequences of multiscale variation in pollen receipt on plant reproduction. American Naturalist 185:E14–E29.

Seger, J., and V. M. Eckhart. 1996. Evolution of sexual systems and sex allocation in plants when growth and reproduction overlap. Proceedings of the Royal Society B: Biological Sciences 263:833–841.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12:253–279.

Swope, S. M. 2014. Biocontrol attack increases pollen limitation under some circumstances in the invasive plant *Centaurea solstitialis*. Oecologia 174:205–215.

Torres-Diaz, C., S. Gomez-Gonzalez, G. C. Stotz, P. Torres-Morales, B. Paredes, M. Perez-Millaqueo, and E. Gianoli. 2011. Extremely long-lived stigmas allow extended cross-pollination opportunities in a high Andean plant. PLoS One 6:e19497.

Wesselingh, R. A. 2007. Pollen limitation meets resource allocation: towards a comprehensive methodology. New Phytologist 174:26–37.

Zimmerman, M., and G. H. Pyke. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. American Naturalist 131:723–738.
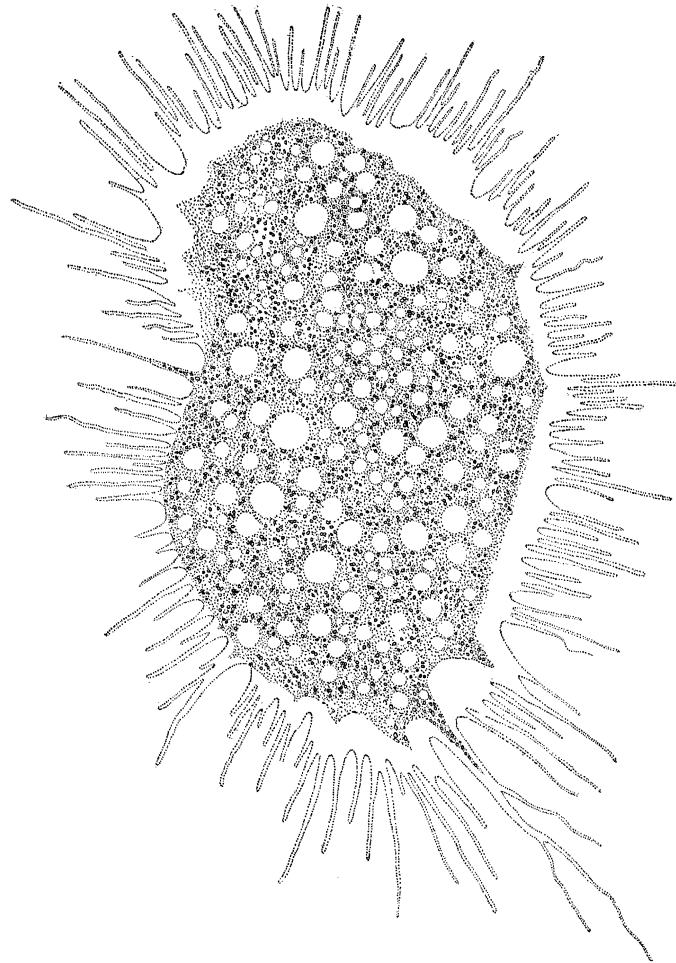
"In December, 1904, I discovered in some water taken by Mr. William G. Lapham from an oozy bank near Afton, Virginia, a large Vampyrella-like specimen [illustrated], which except for the absence of nuclei and the variable size of the vacuoles answered in detail to *Leptophrys elegans*. In size the creature would cover a circular surface whose diameter was 80 micra. It was very active, constantly changing its form laterally, though dorso-ventrally it maintained a film- or sheet-like structure which was about 5 or 10 micra thick." From "Notes on the Genus *Leptophrys*" by William A. Kepner (*The American Naturalist*, 1906, 40:335–342).