# Does an 'oversupply' of ovules cause pollen limitation?

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## Summary

- Lifetime seed production can be constrained by shortfalls of pollen receipt ('pollen limitation'). The ovule oversupply hypothesis states that, in response to unpredictable pollen availability, plants evolve to produce more ovules than they expect to be fertilized, and that this results in pollen limitation of seed production.
- Here, we present a cartoon model and a model of optimal plant reproductive allocations under stochastic pollen receipt to evaluate the hypothesis that an oversupply of ovules leads to increased pollen limitation.
- We show that an oversupply of ovules has two opposing influences on pollen limitation of whole-plant seed production. First, ovule oversupply increases the likelihood that pollen receipt limits the number of ovules that can be fertilized ('prezygotic pollen limitation'). Second, ovule oversupply increases the proportion of pollen grains received that are used to fertilize ovules ('pollen use efficiency'). As a result of these opposing influences, ovule oversupply has only a modest effect on the degree to which lifetime seed production is constrained by pollen receipt, producing a small decrease in the incidence of pollen limitation.
- Ovule oversupply is not the cause of the pollen limitation problem, but rather is part of the evolutionary solution to that problem.

## Introduction

Plants are said to be pollen limited when their lifetime production of seeds is constrained by shortfalls of pollen receipt. An extensive literature suggests that pollen limitation is widespread, with potentially important repercussions for conservation of native plant populations (Larson & Barrett, 2000; Ashman *et al.*, 2004; Knight *et al.*, 2005; Anderson *et al.*, 2011) and production of food for a growing world population (Klein *et al.*, 2007).

There remains, however, considerable uncertainty regarding the ultimate causes of pollen limitation. Leading nonmutually exclusive hypotheses include: pollen limitation occurs because of negative anthropogenic effects on populations of plants (pollen donors) or pollinators (pollen vectors)(Burkle *et al.*, 2013; Hadley *et al.*, 2014); pollen limitation reflects natural fluctuations in factors constraining seed production, as expected under an optimal plant life history in an environment with uncertain pollen receipt (Ashman *et al.*, 2004; Burd, 2008; Rosenheim *et al.*, 2014; Schreiber *et al.*, 2015); and pollen limitation occurs specifically as a result of plants evolving to produce an 'oversupply' of ovules in response to uncertain pollen receipt (Burd, 1995; Ashman *et al.*, 2004; Knight *et al.*, 2005). Each of these hypotheses continues to be discussed as explanations for pollen limitation (Fulkerson *et al.*, 2012; Vamosi *et al.*, 2013; Lázaro *et al.*, 2015), and without any resolution of their relative importance it is unclear how, or if, one should best seek to remedy observed pollination shortfalls.

Uncertainty in pollen receipt is expected to have a strong, direct effect on pollen limitation (arrow 1 in Fig. 1): pollen limitation is caused by unpredictable variation in the availability of pollen, and models consistently demonstrate that as the magnitude of the unpredictability increases, pollen limitation becomes more severe (Burd, 2008; Rosenheim *et al.*, 2014; Schreiber *et al.*, 2015). Under the ovule oversupply hypothesis, however, there is a second, indirect pathway through which uncertain pollen receipt is proposed to shape pollen limitation (arrows 2 and 3 in Fig. 1). Growing empirical and theoretical evidence supports the hypothesis that uncertain pollen receipt favors plants that produce a greater number of ovules than they generally expect to be able to mature into seeds (arrow 2 in Fig. 1; Burd, 1995; Ashman *et al.*, 2004; Burd *et al.*, 2009; Friedman & Barrett, 2011; Schreiber *et al.*, 2015). This oversupply of ovules is advantageous in plant populations that experience considerable within-plant variation in pollen receipt across flowers, because it allows plants to capitalize on reproductive opportunities afforded by the rare flowers that receive unusually abundant pollen. However, flowers with more numerous ovules obviously require greater pollen receipt to achieve full ovule fertilization, raising the possibility that ovule oversupply is a central driver of pollen limitation. The goal of our study was to evaluate the suggestion that ovule oversupply causes pollen limitation (arrow 3 in Fig. 1).

We first present a simple cartoon model of plant reproduction to show why, perhaps counter to our intuition, it is difficult to anticipate whether or not an oversupply of ovules should be
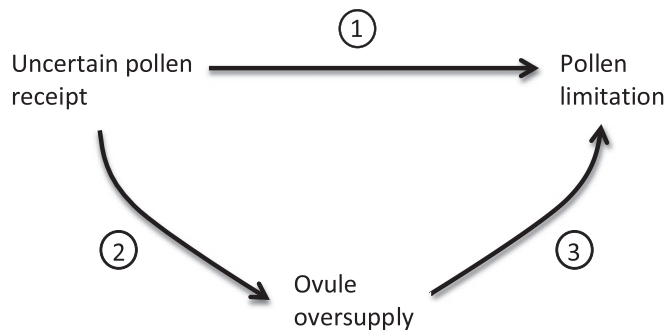
**Fig. 1** Uncertainty in pollen receipt has been hypothesized to lead to pollen limitation through two pathways. Theoretical support for the direct pathway (arrow 1) is well established, as pollen limitation is not expected without unpredictability in pollen receipt. It is also well established that ovule oversupply is favored evolutionarily in response to uncertain pollen receipt (arrow 2). The goal of this study was to evaluate the importance of ovule oversupply as a cause of pollen limitation (arrow 3).

expected to increase pollen limitation. The cartoon model introduces the potential importance of 'pollen-use efficiency,' the fraction of pollen received by a plant that is used to fertilize ovules. We then extend the optimality model of plant reproduction introduced by Schreiber *et al.* (2015), which incorporates separate plant allocations to producing ovules, attracting pollen, and maturing seeds, to evaluate quantitatively the relationship between ovule oversupply and pollen limitation in an environment with stochastic pollen receipt. To distinguish the role of ovule oversupply (arrow 3 in Fig. 1) from the direct pathway of influence of uncertain pollen receipt on pollen limitation (arrow 1 in Fig. 1), we alter ovule supply without altering the magnitude of uncertainty in pollen receipt. We do this in two ways. First, we conduct an *in silico* 'ovule supplementation' experiment, as a direct analog to the pollen supplementation experiments that are the mainstay of pollen limitation studies. This *in silico* experiment produces the clearest inferences regarding the effects of increasing ovule number, because it changes ovule number while holding all other reproductive traits (i.e. allocations to producing ovules, attracting pollen, and maturing seeds) unchanged. Second, we alter the cost of producing each ovule, and calculate the resulting optimal number of ovules to be produced; as expected, the optimal number of ovules produced increases when ovules are cheaper. This produces somewhat less direct inferences regarding the effects of ovule number, because optimal allocations to pollen attraction and seed maturation also change, albeit subtly, as ovule costs change. These changing allocations to pollen attraction and seed maturation can have their own influences on pollen limitation. But the manipulation of ovule cost is useful nonetheless as a mirror of what might be observed in a comparative study of plant taxa exhibiting differences in ovule cost and number.

## Model description

### Cartoon model

We use a simple cartoon model to show that, for a plant experiencing substantial between-flower variation in pollen receipt,

increasing the number of ovules per flower does not necessarily lead to an increase in pollen limitation. The cartoon model is a heuristic tool only. We emphasize that our final conclusions regarding the influence of ovule oversupply on pollen limitation are derived entirely from the optimality model, which embraces a range of pollen receipt possibilities rather than just contrasting two simple scenarios, as we do with the cartoon model. Nevertheless, we hope that the cartoon model will provide an intuitive understanding of why the optimality model produces the results that it does.

Plants require three essential, nonsubstitutable components to produce a mature seed: an ovule; pollen to fertilize the ovule, resulting in a zygote; and the resources needed to provision the zygote, resulting in a mature seed along with any protective or dispersal structures. Fig. 2 depicts a plant with four flowers (A–D); the upper and lower panels differ only in whether each flower has four ovules (Fig. 2a) or eight ovules (Fig. 2b). Pollen receipt varies across the four flowers, creating potential mismatches between the availability of ovules and pollen. Although our cartoon implies that a single pollen grain suffices to fertilize an ovule, in general multiple grains are often needed (Alonso *et al.*, 2011); thus, an individual 'unit' of pollen shown in Fig. 2 can be thought of as a 'packet' of pollen grains sufficient to fertilize a single ovule. Finally, our plant also has stored reserves sufficient to fully provision ten seeds.

Whereas ovules and pollen cannot be reallocated between flowers after pollen receipt is completed (that is, any mismatches that emerge after pollen receipt are permanent), in a fully physiologically integrated plant, or plant module, resources for seed maturation can be allocated to any flower, tracking the availability of zygotes (Zimmerman & Pyke, 1988).

For a shortfall in pollen receipt to limit total seed production by a plant, two conditions must be satisfied. First, zygote production by at least some individual flowers on the plant must be pollen limited, rather than ovule limited; that is, individual flowers must not receive sufficient pollen to fertilize all their ovules. The number of zygotes produced on three of the four flowers in Fig. 2(a) (flowers A, B and C) is limited by the amount of pollen received. Second, the entire plant must be 'zygote limited', rather than being limited by resources available for seed maturation; that is, the plant must fail to produce enough zygotes to utilize fully its resources available for seed maturation. The plant depicted in Fig. 2(a) is zygote limited, because only seven zygotes will be formed (one on flower A, two on flower B, and four on flower D); these seven zygotes will be provisioned to make seeds, leaving resources sufficient for three additional seeds 'wasted' (equivalently, one could say that resources for maturing seeds were used with an efficiency of only 70%). Thus, because the plant depicted in Fig. 2(a) has pollen-limited flowers, and is zygote-limited, total seed production is also pollen-limited: if supplemental pollen were added to each flower, 16 zygotes would be formed, 10 of which would be matured to seeds, an increase of three seeds compared with the natural level of pollen receipt. We define the magnitude of pollen limitation as the proportional increase in seed production achieved by supplementing pollen to all flowers: [(seeds produced with supplemental pollen) − (seeds
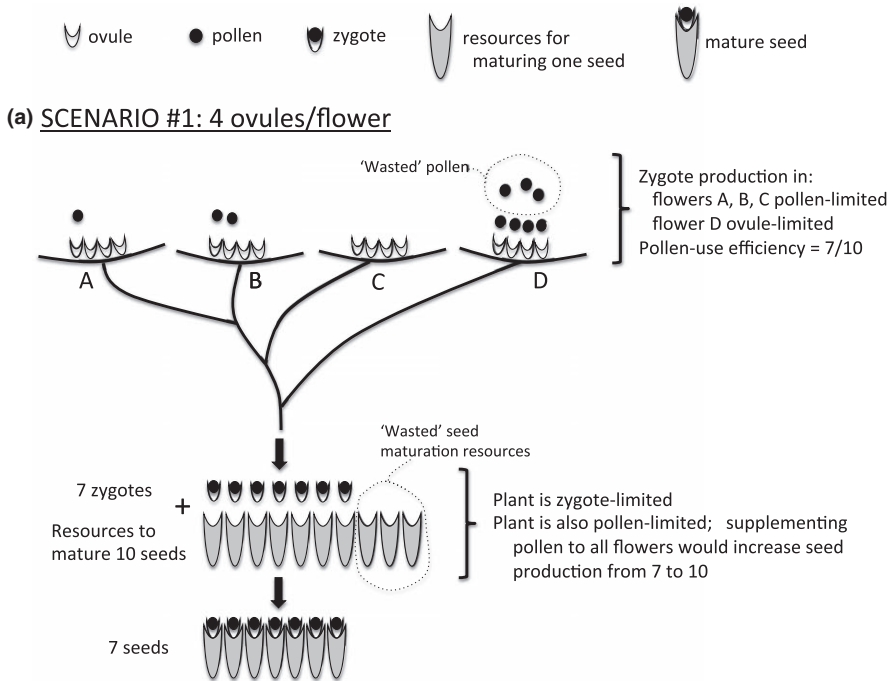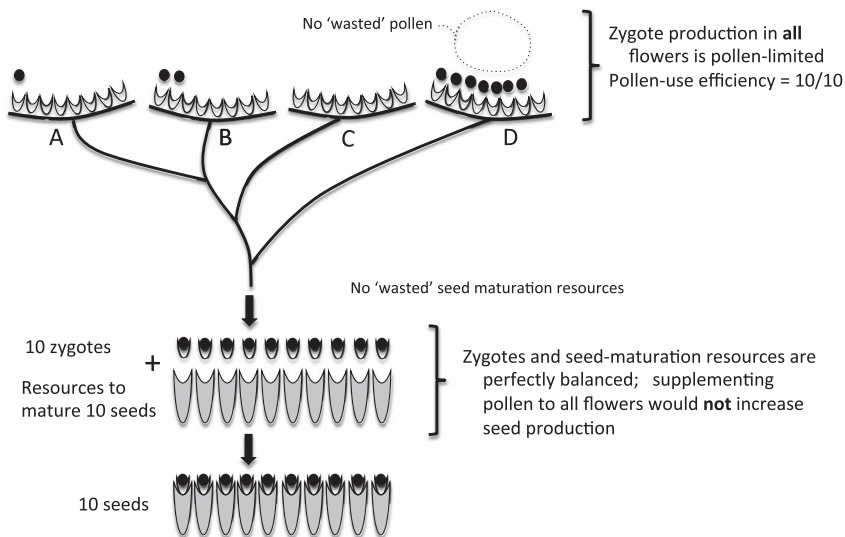
**Fig. 2** Cartoon model showing that increasing the number of ovules per flower does not necessarily increase the degree to which shortfalls in pollen receipt constrain whole-plant seed production. Shown is a plant with four flowers, each of which has either (a) four or (b) eight ovules. Pollen receipt varies across flowers. Increasing the number of ovules per flower increases the fraction of flowers whose zygote production is limited by pollen receipt (3/4 flowers in (a) versus 4/4 flowers in (b)), but also increases the efficiency of pollen use (7/10 in (a) versus 10/10 in (b)). In this hypothetical example, increased pollen use efficiency more than offsets the greater pollen limitation of zygote production, such that doubling the number of ovules per flower eliminates pollen limitation of whole-plant seed production.

produced under natural pollination)]/(seeds produced under natural pollination); in this case pollen limitation $= (10 - 7)/7 = 0.43$. Our fitness metric is slightly different from that introduced by Larson & Barrett (2000), but for the modest pollen limitation effects that our optimality model predicts (see Results), the two measures are very similar. Note that pollen limitation implies that resources available for seed maturation were used inefficiently: pollen limitation $= 1/$(seed resource use efficiency) $- 1$.

It is, however, events occurring at flower D (Fig. 2a) that hold the key to our evaluation of the ovule oversupply hypothesis.

Flower D is not pollen limited, but rather is ovule limited: because pollen receipt exceeds the total number of ovules, three packets of pollen are 'wasted', failing to produce zygotes. As a result, the total plant-wide efficiency of pollen use, which we define as (the number of pollen packets that fertilize ovules)/(the total number of pollen packets received), is equal to 7/10 or 70%. When pollen limits reproductive success, any process that enhances pollen-use efficiency clearly has the potential to reduce the impact of pollen limitation.

It should now be clear why it is difficult to anticipate whether or not increasing the supply of ovules should be expected to

increase pollen limitation: an increasing supply of ovules seems likely to have opposing effects on the degree to which the two conditions necessary for pollen limitation of total seed production by the plant, pollen limitation of flowers and zygote limitation of plants, will be satisfied. First, the most immediate effect of ovule oversupply is to increase the importance of pollen limitation for individual flowers. As ovule number per flower increases, more pollen is needed to achieve full fertilization, and there are clearly greater opportunities for ovules to remain unfertilized. Thus, if in our cartoon model we assume that ovule cost is negligibly small compared with the cost of pollen attraction or seed maturation, such that we can increase ovule number without changing pollen receipt or seed maturation, then doubling the number of ovules per flower to eight results in all of the flowers on the plant being pollen limited (Fig. 2b). However, a simultaneous effect of increasing the number of ovules per flower is to decrease the importance of zygote limitation by increasing the likelihood that every packet of pollen received by a plant finds an ovule to fertilize. Thus, with eight ovules per flower, our plant does not waste any of the 10 pollen packets received (pollen-use efficiency = 100%), as even flower D, which receives seven packets of pollen, has more than enough receptive ovules. Thus, with eight ovules per flower, the plant forms 10 zygotes, sufficient to utilize fully its resources available for seed maturation, and produces 10 seeds. In this hypothetical example, having a stronger oversupply of ovules eliminates pollen limitation entirely: adding supplemental pollen would not generate any increase in seed production.

In sum, we have the possibility of a counterintuitive result: although having more ovules per flower often results in more pollen limitation at the level of the individual flower, it does not appear universally to result in more pollen limitation at the level of the whole plant. More generally, if ovule oversupply has opposing effects on the two conditions that jointly underlie pollen limitation of total seed production by a plant (pollen receipt limits the number of zygotes produced by individual flowers, and zygote production limits total seed production by the plant), then we need a formal quantitative assessment to evaluate the net effect of ovule oversupply on the expression of pollen limitation.

## Optimality model

We now present a model that allows us to explore quantitatively how an oversupply of ovules influences the degree to which pollen limitation constrains seed production for a semelparous plant. We manipulated ovule oversupply in two ways. First, we simulated the effects of taking plants expressing optimal reproductive allocations under stochastic pollen receipt and performing with them an *in silico* 'ovule supplementation experiment'. In direct analogy to the pollen supplementation experiments that ecologists routinely perform to quantify pollen limitation in nature, we simulated the consequences of giving additional ovules to every flower on a plant, at no cost to the plant. This approach obviously is not realistic, but it has the advantage of manipulating ovule number while holding everything else constant (i.e. there were no concurrent changes in resource allocations to producing

ovules, attracting pollen, or maturing seeds); thus, it provides the strongest inferences regarding the causal effects of ovule oversupply. Second, we varied the cost of producing an ovule as a biologically relevant means of creating different levels of ovule oversupply (smaller ovules, which are cheaper to produce, are associated with a greater number of ovules per flower in interspecific comparisons; Greenway & Harder, 2007). As will be shown later, our model predicts that cheaper ovules are associated with a higher optimal number of ovules per flower, thereby creating the desired 'treatments' for our modeling experiment. Across a range of ovule costs, we determined the optimal allocation to ovule production, pollen attraction, and seed maturation for plants experiencing unpredictable pollen receipt, and then simulated the stochastic process of pollen receipt to explore consequences of the optimal reproductive allocations for the expression of pollen limitation.

Here, we briefly describe our model of optimal reproductive allocations; for a full description, see Schreiber *et al.* (2015). Plants begin with a total of $R$ units of resource available for female reproductive function ($R = 100$ in the baseline parameter set), of which fractions $a_o$, $a_p$, and $a_s$ are allocated to producing ovules, attracting pollen, and maturing seeds, respectively, across $k$ flowers ($a_o + a_p + a_s = 1$). Each ovule costs $c_o$, attracting enough pollen to fertilize one ovule costs $c_p$, and provisioning one zygote to produce a mature seed costs $c_s$. Thus, the average flower receives enough pollen to fertilize $a_p R/c_p k$ ovules. We introduce stochasticity in pollen receipt at two levels: between-plant; and within-plant, between-flower. Between-plant stochasticity is introduced with a gamma-distributed random variable $Y$, with mean 1 and coefficient of variation $CV_{plant}$, such that mean pollen receipt per flower for a random plant is:

$$\overline{P} = \frac{a_P R Y}{c_P k}$$

Within-plant stochasticity is introduced with $k$ independent gamma-distributed random variables, $Z_1^Y, \ldots, Z_k^Y$, each with mean $Y$ and coefficient of variation $CV_{flower}$, such that the $i$th flower on a plant receives sufficient pollen to fertilize ovules.

$$P_i = \frac{a_P R Z_i^Y}{c_P k}$$

If neither ovules nor pollen can be redistributed between flowers, but resources for seed maturation can be, then a plant's total seed production (our measure of plant fitness, excluding fitness through male function) is:

$$S = \min\left\{\frac{a_S R}{c_S}, \sum_{i=1}^{k} \min\left\{\frac{a_O R}{c_O k}, \frac{a_P R Z_i^Y}{c_P k}\right\}\right\}$$

This expression includes two 'min' terms. The right-most of these terms says that the number of zygotes produced on flower $i$ equals the minimum of the number of ovules produced there and the number of pollen packets received there. The left-most 'min'

term says that the total number of seeds produced is the minimum of the total number of seeds that can be fully provisioned, given the resources available after allocations to ovules and pollen attraction are completed, and the total number of zygotes produced, summing across all $k$ flowers. Monte Carlo methods and the optim function in R (R Core Team, 2014) were used to find the allocations to ovules (and, thus, the number of ovules produced per flower), pollen attraction, and seed maturation that maximized the expected value of $S$, assuming that plants make a single, irreversible allocation decision at the outset of reproduction.

We used numerical methods to examine the effects of adding supplemental ovules to each flower, or changing the cost of ovule production, $c_o$, on the following: the percentage of all plants that were zygote limited (i.e. that produced fewer zygotes than they could fully provision to mature seeds); the percentage of all flowers that were pollen limited (i.e. that did not receive sufficient pollen to fertilize all ovules); ovule-use efficiency (i.e. the percentage of ovules that were fertilized); pollen-use efficiency (i.e. the percentage of pollen packets received that fertilized ovules); zygote-use efficiency (i.e. the percentage of zygotes that were matured to seeds); and pollen limitation of seed production by the plant (i.e. the percentage increase in total seed production if all ovules on all flowers were fertilized).

Finally, because some plants may flower early in the growing season and then continue to harvest resources that are used for seed provisioning later in the season (i.e. 'income breeding' as opposed to 'capital breeding'; Charlesworth & Morgan, 1991), we explored a model that included stochastic postpollination seed maturation resource income, with mean expected resource income, $Inc$, varying from 0 (baseline) up to 100 units, which doubled the expected total amount of resources available for reproduction. We modeled $Inc$ as a gamma-distributed random variable with coefficient of variation = 0.5.

We parameterized our model using values grounded in empirical observations for flowering plants. We chose baseline parameter values to match trait values typically observed in nature, and used sensitivity analyses to explore the variation commonly observed about those values. Greenway & Harder (2007) showed that median seed size is 93-fold greater than the ovules from which they developed, and Rosenheim *et al.* (2014) found that the median flower cost (prepollination costs of seed production) for animal-pollinated angiosperms is *c.* 14% of the total cost of seed production, with the remaining 86% of the cost associated with maturing the seed (postpollination costs of seed production). Both studies found considerable variation in the relative costs of ovules, flowers, and seeds. Thus, our baseline cost parameters were $c_o = 0.01$, $c_p = 0.1$, and $c_s = 1$, and our sensitivity analyses varied both $c_o$ and $c_s$ up and down ten-fold ($c_o = 0.001{-}0.1$; $c_s = 0.1{-}10.0$) relative to flower costs. Substantial variation in pollen receipt across different flowers within a plant population has been observed in many studies, with observed median $CV_{flower}$ near 1.0, and values ranging from *c.* 0.4 to 2.5 (Burd *et al.*, 2009; Alonso *et al.*, 2013). Variation in pollen receipt observed at the level of whole plants has been relatively little explored; the few published studies suggest a median for $CV_{plant}$

near 0.4 and only relatively modest between-system variation (observed values range from 0.22 to 0.52; Rosenheim *et al.*, 2016). Thus, our baseline parameter values for unpredictability in pollen receipt were $CV_{flower} = 1.0$ and $CV_{plant} = 0.4$, with sensitivity analyses spanning the full range of observed values ($CV_{flower}$: 0.4–2.5; $CV_{plant}$: 0.2–0.6). Finally, for the base parameter set we set flower number, $k = 25$, and allowed $k$ to vary over a broad range of values (5–125).

Under our baseline parameter values, the optimal strategy for plants was to produce *c.* 10 ovules in each of their 25 flowers (see Results). In our ovule supplementation experiment, we supplemented each flower with 1–40 additional ovules (25–1000 ovules added per plant), allowing us to explore a broad range of ovule oversupply conditions.

## Results

Under our baseline parameter values, and in the absence of stochasticity in pollen receipt, each plant was capable of producing a total of 90 seeds (each seed costs 1.11 units of resource: 0.01 for the ovule, 0.1 for pollen attraction, and 1.0 for seed maturation; thus 90 seeds cost 99.9 units of resource, nearly fully utilizing the 100.0 units of resource that each plant started with). To produce 90 seeds, each of the 25 flowers would need to host three or four ovules. Under stochastic pollen receipt, the optimal number of ovules produced per flower was instead *c.* 10 (Fig. 3d); thus, our model successfully created the expected ovule oversupply condition (arrow 2 in Fig. 1).

However, we find no support for the hypothesis that increasing ovule numbers causes increased pollen limitation. Instead, our model suggests that increasing the oversupply of ovules through *in silico* ovule supplementation causes a consistent decrease in pollen limitation at the level of the whole plant (Fig. 3). Pollen limitation decreased with ovule supplementation for the baseline parameter values (Fig. 3j) and across all 16 alternate parameter value sets explored in our sensitivity analyses (Supporting Information Figs S1–S3). The effect size was consistently small; for example, under the baseline parameter values, pollen limitation dropped from 0.042 to 0.037, a drop of just (0.042 − 0.037)/0.042 = 11.8%. Across the 17 scenarios explored, ovule supplementation decreased pollen limitation by just 13.9% on average (range: 2.3–32.2%). This small effect size reflected a nearly even balancing of two opposing effects of ovule oversupply. First, a stronger oversupply of ovules caused the percentage of flowers that were pollen limited to increase (Fig. 3f). Thus, as anticipated, zygote production became more heavily pollen limited as the number of ovules per flower increased. This should act to increase the importance of pollen limitation for total seed production. Second, however, a stronger oversupply of ovules also increased pollen-use efficiency (Fig. 3h), leading to a decrease in the incidence of zygote-limited plants (Fig. 3e). This should act to decrease the importance of pollen limitation for total seed production. These two effects largely offset one another, resulting in a modest net influence of ovule oversupply on the degree to which pollen limitation constrains seed production. And, as anticipated by our cartoon model, the net influence of
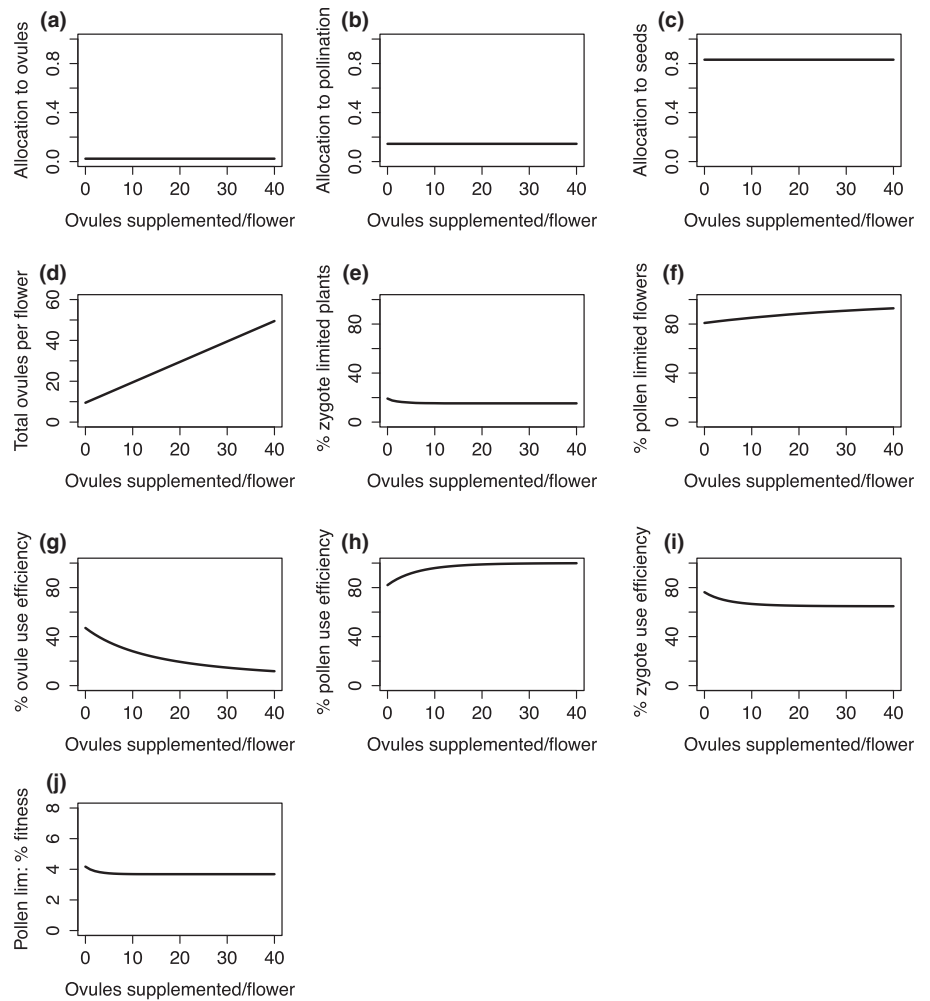
**Fig. 3** *In silico* ovule supplementation experiment: baseline parameter values. Shown are the optimal total plant-wide proportions of reproductive resources allocated to (a) ovules, (b) pollen attraction, and (c) seed maturation, (d) the total number of ovules per flower, (e) the percentage of plants whose total seed production is zygote limited (i.e. zygote number < number of seeds that can be produced, given the amount of resources available for seed maturation), (f) the percentage of flowers that are pollen limited (i.e. pollen receipt < ovule number), (g) ovule-use efficiency (percentage of ovules that are fertilized), (h) pollen-use efficiency (percentage of pollen packets that are used to fertilize ovules), (i) zygote-use efficiency (percentage of zygotes that are provisioned to mature seeds), and (j) pollen limitation of whole-plant seed production (per cent fitness gain that would be achieved if all flowers received supplemental pollen sufficient to fertilize all ovules). Results are shown for the baseline parameter set ($c_o = 0.01$, $c_p = 0.1$, $c_s = 1$, $CV_{plant} = 0.4$, $CV_{flower} = 1.0$, and $k = 25$).

strengthening the oversupply of ovules is actually a decrease in the importance of pollen limitation.

As expected, increasing ovule oversupply reduced the proportion of ovules that were fertilized (Fig. 3g; ovule-use efficiency) and reduced the proportion of zygotes that were successfully matured into seeds (Fig. 3i; zygote-use efficiency). Thus, ovule oversupply did have the expected effects on ovule and zygote dynamics, even if these effects propagated only weakly to shape actual seed production.

These results were largely confirmed in our second *in silico* experiment, in which we manipulated optimal ovule number per flower by changing the cost of producing an ovule ($c_o = 0.001$–0.1; Figs S4–S7). Decreasing the cost of an ovule resulted in a strong increase in ovule number (Fig. S4d), but simultaneously resulted in slight changes in allocations to pollen attraction and seed maturation (e.g. Fig. S4a–c), as plants redirected the freed-up resources; thus, changes in pollen limitation reflect the combined influences of these changes. As ovule number increased (in this case, moving right to left along the *x*-axis) in response to decreased ovule cost, pollen limitation decreased in the base parameter set and in 14 of the 16 additional scenarios examined in the sensitivity analyses (Figs S4–S7). As was observed in the ovule supplementation experiment, the effect size was generally

modest: on average across all scenarios explored, increasing ovule number was associated with a 14.8% drop in the magnitude of pollen limitation (range of effect sizes: −43.4 to + 8.7%).

Finally, ovule oversupply was again associated with decreased pollen limitation when plants could continue to produce resources used for seed maturation after pollination was completed (income breeding; Fig. S8). Decreases in pollen limitation associated with ovule oversupply were modest (30.4% on average; range: 17.6–43.9%).

## Discussion

Contrary to the ovule oversupply hypothesis (Burd, 1995), increasing the oversupply of ovules does not increase the degree to which pollen receipt limits the lifetime seed production of plants. Greater numbers of ovules clearly do elevate the amount of pollen that must be received to achieve full ovule fertilization, and as ovule oversupply increases, the proportion of flowers experiencing a shortfall of pollen receipt grows (Fig. 3f). Thus, zygote production becomes predominantly pollen limited as ovule oversupply increases. But, as suggested by our cartoon model, another offsetting process is also at work: increasing ovule number per flower increases pollen-use efficiency. Abundant ovules mean that

even those rare flowers that receive exceptionally high amounts of pollen still have sufficient ovules to match each pollen packet with an ovule, generating an abundant supply of zygotes. In a fully physiologically integrated plant or plant module, this abundant supply of zygotes makes it more likely that plant reproduction will be limited by the availability of resources needed to mature zygotes into seeds, rather than by either of the prezygotic components of reproduction (ovules or pollen). Thus, an escalation of pollen limitation is averted. In sum, the degree to which pollen availability limits zygote production ('prezygotic pollen limitation'; Alonso *et al.*, 2013; Arceo-Gómez & Ashman, 2014) appears not to be a reliable indicator of the degree to which pollen availability limits seed production. The simple models that we present here suggest that the oversupply of ovules is part of a plant's 'evolutionary solution' to the problem of pollen limitation, rather than the cause of that problem.

## Why does ovule oversupply reduce pollen limitation?

Not only does ovule oversupply fail to emerge as a key positive driver of pollen limitation, but our model suggests that ovule oversupply acts consistently in the opposite direction, modestly reducing the impact of pollen limitation. This result was observed in all scenarios explored in our ovule supplementation experiment, which changed ovule number without imposing any cost on the plant and while holding all other parameters constant. Can we understand why? We have defined pollen limitation as [(seeds produced with supplemental pollen) − (seeds produced under natural pollination)]/(seeds produced under natural pollination). How will an increase in ovule supply affect the two quantities contained in this formula? Begin with the first quantity: the number of seeds produced by plants receiving supplemental pollen. Under pollen supplementation, every ovule produced by the plant will be fertilized. An optimal plant life history will never involve producing fewer ovules than the number of zygotes the plant can provision to produce mature seeds, because such a strategy would necessarily waste resources available for seed maturation. Thus, the fitness of plants receiving supplemental pollen can never be ovule limited, and adding ovules cannot therefore increase the fitness of such plants. Adding ovules cannot cause a decrease in total seed production either, as the plant is getting the ovules for free. Thus, adding ovules cannot change seed production under pollen supplementation. Examine now the second quantity: the number of seeds produced under natural pollination. As before, adding ovules cannot decrease total seed production. But we can make a stronger statement: adding ovules to plants under open pollination must increase mean whole-plant seed production, because if ovules were never limiting to the fitness of open-pollinated plants, then the plants would evolve reduced ovule numbers to save the costs of building excess ovules. This is an example of the general result that optimal life histories always balance nonzero risks of having fitness constrained by each of the essential, nonsubstitutable factors required for reproductive success (Rosenheim *et al.*, 2010). Thus, if adding ovules does not change seed production under pollen supplementation, but always increases seed production under

open pollination, then adding ovules must decrease pollen limitation. Thus, it is not surprising that we confirm this expectation in our *in silico* ovule supplementation experiment. The ovule supplementation experiment is still instructive in demonstrating that the magnitude of the effect is generally modest. We suggest that the rare exceptions to this expectation observed in our experiment that manipulated ovule number through changes in ovule cost (Figs S4–S8) must, therefore, stem from the concurrent changes in allocations to pollen attraction and seed maturation.

## The empirical record: ovule number per flower versus pollen limitation

Our result that ovule oversupply lessens the impact of pollen limitation may still seem counterintuitive to some readers. Grounding this result in empirical observations would, therefore, be welcome. To our knowledge, only two comparative studies have examined the relationship between ovule number per flower and pollen limitation, and they produced seemingly opposite results (Knight *et al.*, 2005; Lázaro *et al.*, 2015). (Note that other studies frequently cited as supporting the ovule oversupply hypothesis, such as Burd *et al.* (2009), tested the relationship between pollen receipt uncertainty and ovule number per flower (arrow 2 in Fig. 1), but not the relationship between ovule number and pollen limitation (arrow 3 in Fig. 1), which is our focus here.) These two studies, however, measured pollen limitation in different ways, and their different methodologies probably shaped their apparently opposing results.

Knight *et al.* (2005) found that ovule number per flower was significantly positively related to pollen limitation, both when the data were and were not corrected for phylogenetic relationships among taxa. This result seems to support the ovule oversupply hypothesis and to be inconsistent with our model predictions. However, Knight *et al.* (2005) included in their meta-analysis only studies that measured pollen limitation by supplementing pollen to a subset of flowers produced by a plant during a single reproductive season ('partial plant level'). They did this because pollen supplementation at the partial plant level was the most common type of supplementation experiment reported in the literature, and they explicitly recognized that partial plant-level studies probably overestimate the effects of pollen limitation on lifetime seed production, because plants can reallocate resources for seed maturation from nonsupplemented flowers to supplemented flowers, both within and between breeding seasons (Zimmerman & Pyke, 1988; Obeso, 2002; Knight *et al.*, 2006; Wesselingh, 2007). However, pollen limitation estimates based on partial plant-level pollen supplementation are likely to be heavily influenced by pollen limitation at the flower level (i.e. prezygotic pollen limitation), which our model predicts will consistently increase as ovule number per flower increases (Fig. 3f). In the limit, strong reallocation of resources to pollen-supplemented flowers from other reproductive structures on a plant can ensure that all ovules available on those flowers are fertilized and matured to seeds. Thus, it is unsurprising that a positive relationship emerged in the Knight *et al.* (2005) analysis.

By contrast, Lázaro *et al.* (2015) examined the relationship between ovule number per flower and pollen limitation using almost exclusively pollen supplementation at the whole-plant level, with the explicit goal of minimizing the degree to which resource reallocation would distort the resulting estimates of pollen limitation. Although most of their species were polycarpic perennials, and thus their pollen limitation estimates were still probably inflated by resource reallocations across breeding seasons, their estimates were probably less strongly influenced by pollen limitation at the flower level, and more reflective of effects of pollen limitation on plant fitness. Lázaro *et al.* (2015) quantified pollen limitation for 24 species in each of two plant communities. In the first community ('Ryghsetra'), mean ovule number per flower did not predict pollen limitation; in the second community ('Finse'), increasing ovule number was associated with declining pollen limitation. Lázaro *et al.* (2015) noted that their results are the opposite of that expected under the ovule oversupply hypothesis. A decline in pollen limitation with increasing ovule number is instead consistent with the predictions of our model. Our model does, however, predict sufficiently small effect sizes that a failure to resolve statistically significant relationships in some communities (i.e. Ryghsetra) is unsurprising.

In sum, although there is clearly a need for additional empirical tests of the relationship between ovule number and pollen limitation, the existing studies suggest that moving away from pollen limitation estimates heavily influenced by pollen limitation at the flower level ('prezygotic pollen limitation') and towards estimates more strongly influenced by effects of pollen limitation on plant fitness produces a shift from a positive to a neutral or negative relationship between ovule number and pollen limitation, as predicted by our model.

## Alleviating pollen limitation

Pollen limitation of plant reproduction is a growing concern world-wide, as shortfalls of pollination are thought to represent a threat to the long-term viability of some native plant populations (Anderson *et al.*, 2011; Pauw & Hawkins, 2011). But, before we can develop rational strategies for ameliorating perceived pollination shortfalls, we need to understand their causes. In particular, we need to be able to identify cases where negative anthropogenic impacts on plant populations (pollen donors) or pollinator populations (pollen vectors) have increased the magnitude of pollen limitation substantially beyond that which should be expected in healthy communities coping with natural levels of unpredictable variation in pollen receipt.

Pollen limitation does not appear to be an inexorable consequence of plants producing abundant ovules. Nevertheless, some pollen limitation is still expected under an optimal life history (Figs 2j, S1–S8). Empirical and theoretical results suggest that pollen limitation should vary strongly across plant species, depending on the degree to which plants rely on outcrossing (Burd, 1994; Alonso *et al.*, 2010; Lázaro *et al.*, 2015) or specialized pollinators (Lázaro *et al.*, 2015), the relative magnitudes of prepollination versus postpollination costs of seed production (Rosenheim *et al.*, 2014; Schreiber *et al.*, 2015), and the

underlying intrinsic levels of unpredictability in pollen receipt (Burd *et al.*, 2009; Schreiber *et al.*, 2015). Pollen limitation that greatly exceeds that expected under an optimal plant life history can serve as a clear signal of anthropogenic interruptions in pollination and set the stage for effective counter-measures.

## Conclusions

Faced with uncertain pollen receipt, plants are favored to produce an oversupply of ovules so that they can capitalize on flowers that receive abundant pollen. Here, we ask whether this optimal oversupply of ovules is a primary cause of pollen limitation. That is: does ovule oversupply cause pollen receipt shortfalls to limit lifetime seed production by plants? We find that the answer is no. And, not only does ovule oversupply not create pollen limitation, it has the reverse effect, decreasing the magnitude of pollen limitation. Ovule oversupply does increase the likelihood of pollen being the primary constraint to the number of zygotes produced ('prezygotic pollen limitation'), but abundant ovules also allow plants to use the pollen that they do receive more efficiently, converting a larger proportion of pollen received into zygotes. This makes pollen limitation less likely. Thus, ovule oversupply is part of the evolutionary solution to the problem of pollen limitation, rather than the cause of the problem.

## Author contributions

J.A.R., S.J.S. and N.M.W. designed the research; J.A.R. and S.J.S. performed the modeling; J.A.R., S.J.S. and N.M.W. wrote the manuscript.

## References

**Alonso C, Herrera CM, Ashman T-L. 2011.** A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. *New Phytologist* **193**: 532–542.

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

**Alonso C, Vamosi JC, Knight TM, Steets JA, Ashman T-L. 2010.** Is reproduction of endemic plant species particularly pollen limited in biodiversity hotspots? *Oikos* **119**: 1192–1200.

**Anderson SH, Kelly D, Ladley JJ, Molloy S, Terry J. 2011.** Cascading effects of bird functional extinction reduce pollination and plant density. *Science* **331**: 1068–1071.

**Arceo-Gómez G, Ashman T-L. 2014.** Patterns of pollen quantity and quality limitation of pre-zygotic reproduction in *Mimulus guttatus* vary with co-flowering community context. *Oikos* **123**: 1261–1269.

**Ashman T-L, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ *et al.* 2004.** Pollen

limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.

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

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

Burd M. 2008. The Haig-Westoby model revisited. *American Naturalist* 171: 400–404.

Burd M, Ashman T-L, Campbell DR, Dudash MR, Johnston MO, Knight TM, Mazer SJ, Mitchell RJ, Steets JA, Vamosi JC. 2009. Ovule number per flower in a world of unpredictable pollination. *American Journal of Botany* 96: 1159–1167.

Burkle LA, Marlin JC, Knight TM. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339: 1611–1615.

Charlesworth D, Morgan MT. 1991. Allocations of resources to sex functions in flowering plants. *Philosophical Transactions of the Royal Society of London B* 332: 91–102.

Friedman J, Barrett SCH. 2011. The evolution of ovule number and flower size in wind-pollinated plants. *American Naturalist* 177: 246–257.

Fulkerson JR, Whittall JB, Carlson ML. 2012. Reproductive ecology and severe pollen limitation in the polychromic tundra plant, *Parrya nudicaulis* (Brassicaceae). *PLoS ONE* 7: e32790.

Greenway CA, Harder LD. 2007. Variation in ovule and seed size and associated size-number trade-offs in angiosperms. *American Journal of Botany* 94: 840–846.

Hadley AS, Frey SJK, Robinson WD, Kress WJ, Betts MG. 2014. Tropical forest fragmentation limits pollination of a keystone understory herb. *Ecology* 95: 2202–2212.

Klein A-M, Vaissière BE, Cane JH, Steffen-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings Royal Society London B* 274: 303–313.

Knight TM, Steets JA, Ashman T-L. 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 TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T-L. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology and Systematics* 36: 467–497.

Larson BMH, Barrett SCH. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69: 503–520.

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

Obeso JR. 2002. The costs of reproduction in plants. *New Phytologist* 155: 321–348.

Pauw A, Hawkins JA. 2011. Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. *Oikos* 120: 344–349.

R Core Team. 2014. *R: a language and environment for statistical computing.* [WWW document] URL http://www.R-project.org/ [accessed 2 July 2014].

Rosenheim JA, Alon U, Shinar G. 2010. Evolutionary balancing of fitness-limiting factors. *American Naturalist* 175: 662–674.

Rosenheim JA, Williams NM, Schreiber SJ. 2014. Parental optimism versus parental pessimism in plants: how common should we expect pollen limitation to be? *American Naturalist* 184: 75–90.

Rosenheim JA, Williams NM, Schreiber SJ, Rapp JM. 2016. Modest pollen limitation of lifetime seed production is in good agreement with modest uncertainty in whole-plant pollen receipt. *American Naturalist* 167 (in press).

Schreiber SJ, Rosenheim JA, Harder LD, Williams NM. 2015. Evolutionary and ecological consequences of multiscale variation in pollen receipt on plant reproduction. *American Naturalist* 185: E14–E29.

Vamosi JC, Steets JA, Ashman T-L. 2013. Drivers of pollen limitation: macroecological interactions between breeding system, rarity, and diversity. *Plant Ecology & Diversity* 6: 171–180.

Wesselingh RA. 2007. Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist* 174: 26–37.

Zimmerman M, Pyke GH. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* 131: 723–727.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** *In silico* ovule supplementation experiment: sensitivity analysis examining the influence of different values for $k$, the number of flowers per plant.

**Fig. S2** *In silico* ovule supplementation experiment: sensitivity analysis examining the influence of different values for $c_s$, the seed cost.

**Fig. S3** *In silico* ovule supplementation experiment: sensitivity analysis examining the influence of different values for $CV_{plant}$, the coefficient of variation in pollen receipt across whole plants, and $CV_{flower}$, the coefficient of variation in pollen receipt within plants, across flowers.

**Fig. S4** *In silico* experiment manipulating ovule number by changing the cost of producing an ovule: baseline parameter values.

**Fig. S5** *In silico* experiment manipulating ovule number by changing the cost of producing an ovule: sensitivity analysis examining the influence of different values for $k$, the number of flowers per plant.

**Fig. S6** *In silico* experiment manipulating ovule number by changing the cost of producing an ovule: sensitivity analysis examining the influence of different values for $c_s$, the seed cost.

**Fig. S7** *In silico* experiment manipulating ovule number by changing the cost of producing an ovule: sensitivity analysis examining the influence of different values for $CV_{plant}$, the coefficient of variation in pollen receipt across whole plants, and $CV_{flower}$, the coefficient of variation in pollen receipt within plants, across flowers.

**Fig. S8** *In silico* experiment manipulating ovule number by changing the cost of producing an ovule: sensitivity analysis examining the influence of different mean expected values for *Inc*, the amount of new resources available for seed maturation as a result of the plant's ability to gather key nutrients via root uptake or photosynthesis occurring postpollination.

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