The Evolution of Ovule Number and Flower Size in Wind-Pollinated Plants

Jannice Friedman* and Spencer C. H. Barrett

Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

Submitted September 6, 2010; Accepted October 22, 2010; Electronically published January 11, 2011

ABSTRACT: In angiosperms, ovules are "packaged" within individual flowers, and an optimal strategy should occur depending on pollination and resource conditions. In animal-pollinated species, wide variation in ovule number per flower occurs, and this contrasts with wind-pollinated plants, where most species possess uniovulate flowers. This pattern is usually explained as an adaptive response to low pollen receipt in wind-pollinated species. Here, we develop a phenotypic model for the evolution of ovule number per flower that incorporates the aerodynamics of pollen capture and a fixed resource pool for provisioning of flowers, ovules, and seeds. Our results challenge the prevailing explanation for the association between uniovulate flowers and wind pollination. We demonstrate that when flowers are small and inexpensive, as they are in wind-pollinated species, ovule number should be minimized and lower than the average number of pollen tubes per style, even under stochastic pollination and fertilization regimes. The model predicts that plants benefit from producing many small inexpensive flowers, even though some flowers capture too few pollen grains to fertilize their ovules. Wind-pollinated plants with numerous flowers distributed throughout the inflorescence, each with a single ovule or a few ovules, sample more of the airstream, and this should maximize pollen capture and seed production.

Keywords: wind pollination, ovule number, resource allocation, flower size, pollen limitation, pollen-capture efficiency.

Introduction

Flowering plants are characterized by diverse reproductive structures indicating that the deployment of finite resources to reproduction can be optimized in many ways. The success of a plant as a seed parent depends on partitioning resources among flowers for ovule production, receiving an adequate supply of pollen, and maintaining resources for seed maturation (Lloyd 1980; Primack 1987). The number of ovules produced within a flower can reflect selection arising from any of these influences (Burd 1995;

* Corresponding author. Present address: Department of Biology, Duke University, Durham, North Carolina 27708; e-mail: jannice.friedman@duke.edu.

Burd et al. 2009). A striking feature of ovule packaging (the deployment and partitioning of resources to ovules within and between flowers of a plant) in angiosperms is the common occurrence in wind-pollinated (anemophilous) species of very low ovule numbers, including many species with uniovulate flowers. Comparative phylogenetic evidence indicates that ovule number usually declines after the evolution of anemophily, suggesting that it is an adaptation to wind pollination (Linder 1998; Friedman and Barrett 2008). The association between anemophily and low ovule number contrasts with the six orders of magnitude variation in angiosperms in general (Stebbins 1974) and suggests that optimal ovule packaging in wind-pollinated species may be highly constrained.

Various hypotheses have been proposed for the evolution of ovule packaging in angiosperms. The incidence of common paternity within a flower or plant (correlated mating) might affect ovule number. Kress (1981) argued that ovule number, pollen unit size, and pollen dispersal evolve together to regulate sibling competition among developing embryos within a fruit. In contrast, in a study of size-number trade-offs in ovule and seed size, Greenway and Harder (2007) suggested that ovule size and packaging might reflect maternal control in parent-offspring conflict. Microgametophyte competition to fertilize ovules might also select on variation in ovule number (Mulcahy 1975; Lee 1984). Finally, two hypotheses have been proposed to explain the overproduction of ovules in some species. Excess ovules might be adaptive if they enable plants to take advantage of stochastic pollination, particularly the random deposition of particularly high pollen loads (Burd 1995; Burd et al. 2009). Alternatively, the overproduction of ovules may allow maternal plants to screen embryos for viable offspring without compromising seed production (reproductive compensation; Harder et al. 2008). These hypotheses to explain optimal ovule packaging have largely considered animal-pollinated plants, and they generally predict the production of multiple, rather than single, ovules per flower.

The most frequently invoked hypothesis for the asso-

ciation between wind pollination and uniovulate flowers posits that stigmas of anemophilous species infrequently capture multiple pollen grains (Pohl 1929; Dowding 1987). According to this view, ovules would be wasted if they were concentrated within a few anemophilous flowers, and this has resulted in the production of many flowers, each with a single ovule. Unlike animal-pollinated plants, for which pollen grains are commonly transported in clumps (Harder and Johnson 2008), pollen of wind-dispersed species is usually transported in the air stream as individual pollen grains, so that grains are deposited independently.

The assumption that stigmas of wind-pollinated plants capture few pollen grains is based on the expectation that wind provides a random and relatively inefficient mode of pollen dispersal in comparison with animal pollination (Wessells and Hopson 1988, p. 560; Campbell et al. 1999, p. 734; Maseuth 2009, p. 205; but see Niklas 1985). In contrast, recent investigations of pollen loads in anemophilous species demonstrate that stigmas of wind-pollinated plants often capture amounts of pollen that far exceed ovule number. For example, Friedman and Barrett (2009) reported a mean stigmatic pollen load of 34.1 (SE = 3.8, median = 7) grains for 19 wind-pollinated herbaceous species from seven families. In addition, experimental studies involving the measurement of pollen loads of naturally pollinated Poaceae, Proteaceae, Ranunculaceae, Restionaceae, and Rosaceae (Honig et al. 1992; Linder and Midgley 1996; Davis 2004; Friedman and Harder 2004) report levels of pollen receipt well above ovule number (mean pollen grains per ovule range from 3 to 100). Thus, the current empirical data are not consistent with the proposal that low pollen loads lead to decreased ovule number in wind-pollinated species.

The aerodynamics of wind pollination provides insight into the functional advantage of uniovulate flowers for anemophilous taxa. In wind-pollinated plants, pollen capture is a function of the size of the female receptive surface, and there are significant aerodynamic constraints on stigma size (Niklas 1987). Furthermore, stigmas (and ovules) that are spatially separated throughout the inflorescence of wind-pollinated species will sample a greater portion of the airstream than stigmas and ovules that are concentrated in a few flowers. For animal-pollinated plants, the optimal resource allocation may favor grouping ovules within a flower and allocating to large, shared attractive structures. However, for wind-pollinated plants, the resource investment in individual flowers is considerably less, owing to the lack of showy floral parts or nectar, so that producing many uniovulate flowers may be more adaptive. Burd (1995) provided some support for this idea by showing theoretically that low floral costs generally favor fewer ovules per flower, although his model predicted uniovulate flowers only as an extreme case.

One of the defining features of the wind pollination syndrome is the occurrence of small flowers with highly reduced or no perianth parts (Faegri and van der Pijl 1979). Two lines of evidence provide support for this association. Comparative analyses indicate that wind pollination is statistically associated with small, unshowy flowers (Linder 1998; Weller et al. 2006; Friedman and Barrett 2008), and studies measuring biomass allocation have demonstrated that flowers of wind-pollinated species are substantially cheaper to produce than those of animal-pollinated species (Cruden and Lyon 1985). For example, comparative studies of 18 species of *Schiedea* indicate that wind-pollinated species have almost twice as many flowers that are four times smaller than those of animal-pollinated species (Weller et al. 2006; table 1). Finally, studies of the aerodynamics of pollen transfer (Niklas 1985) indicate that by having highly reduced or no perianth parts, the boundary layer effects near stigmas and anthers are reduced, facilitating more effective pollen release and capture.

Here, we develop a phenotypic model for the joint evolution of ovule number and flower size in wind-pollinated plants. We begin by considering the deterministic case in which pollen receipt is a function of flower size and outline

Table 1: Definitions of parameters, variables, and functions used in this study

Parameter/ variable/ function	Definition
β	Half-saturation constant for pollen capture function
B	No. flowers produced by a plant
c_f	Resource cost of producing one flower (excluding ovules)
c_o	Resource cost of producing one ovule
c_p	Resource cost of developing one fertilized ovule into a seed
k	Asymptote reflecting pollen concentration
ϕ	Proportion of resources spent on flowers and ovules
n	No. ovules per flower
R	Total resources available for flower, ovule, and seed production
F	Proportion of ovules that are fertilized
$g(\alpha, \beta)$	Negative binomial distribution with mean $$P = \alpha \frac{(\beta c_f)^2}{1 + (\beta c_f)^2}$$
$G(P, n)$	Cumulative probability function of $g(\alpha, \beta)$ evaluated at n ovules
P	No. pollen grains received by a stigma
W	Expected (mean) maternal fitness

Note: Values for the following parameters or variables were as follows: $\beta = 2.5$, $c_o = 0.001$, $c_p = 0.1$, $k = 10$, $R = 20$. The remaining variables or functions varied and were determined by the model.

the aerodynamic properties of pollen capture by stigmas. Next, we incorporate stochastic pollination and fertilization and use empirical data on stigmatic pollen loads in wind-pollinated species to parameterize the model. These analyses provide a novel explanation for the association between reduced ovule number and anemophily. Following the presentation of our results, we discuss some evolutionary consequences of reduced ovule number. We also consider how several recent hypotheses concerning variation in ovule number in animal-pollinated plants, including stochastic pollination and the occurrence of correlated mating (shared paternity within fruits), might also influence the evolution of ovule number in wind-pollinated species.

Model of the Evolution of Ovule Number and Flower Size

Maternal fitness in plants is generally measured through seed production, which depends on ovule production, the receipt of pollen, and fertilization of ovules. Thus, selection should favor a strategy in which the maximum number of ovules are fertilized and develop into seeds, given resource constraints. Our phenotypic model considers the allocation of resources to flower production, ovules, and seed provisioning (Lloyd 1980; Venable 1996). A proportion of reproductive resources is allocated to flower and ovule production, and the remaining proportion is allocated to seed (and fruit) provisioning.

Pollen Capture

For wind-pollinated plants, pollen capture is a function of the size of the female receptive surface (Niklas 1987). The physical models of Paw U and Hotton (1989) specifically describe total pollen deposited on stigmas as a function of stigma size, the pollen concentration in the airstream, and the impaction efficiency, or the ratio of the numbers of pollen grains captured to those that would have passed through the area if the stigma had not been present (Davies and Peetz 1956; Gregory 1973). Airflow streamlines near a stigma will be deflected around it in proportion to the boundary layer produced by the stigma, and large stigmas produce proportionally thicker boundary layers. Whether a pollen grain impacts a stigma depends on wind speed, stigma size, and pollen grain size. Large, heavy pollen grains are captured more often, whereas small, light grains are carried around the stigma by the airflow. In this model, we make the simplifying assumption that all pollen grains are of uniform size, so that the efficiency of capture depends only on stigma size.

The relation of capture efficiency (E) to receptor size is usually expressed in terms of Stokes's number:

$$S = \frac{V_{\text{set}}U}{Dg},   \tag{1}$$

where V_{set} is the settling velocity of a pollen grain (m s^{-1}), U is mean wind speed (m s^{-1}), D is the receptor diameter (m), and g is gravitational acceleration (9.8 m s^{-2}). The explicit relation of E to S is derived empirically (Gregory 1973) or by numerical integration over wind speed (Davies and Peetz 1956). The relation typically takes the form of an S-shaped curve, with pollen capture actually decreasing at very large receptor diameter sizes as a result of their thick boundary layers. We do not consider the decreasing part of the curve, since it is unrealistic that stigmas would evolve to be large enough to incur additional costs without receiving further benefits. To describe the number of pollen grains captured as a function of stigma size, we used a function that approximates the shape described above:

$$P = k\frac{(\beta c_{\text{f}})^2}{1 + (\beta c_{\text{f}})^2}   \tag{2}$$

(see Paw U and Hotton 1989), where k is a scaling parameter that reflects the concentration of pollen in the airstream, β affects the degree of saturation, and c_{f} is stigma size, as measured by its cost. We set $\beta = 2.5$ and $k = 10$; c_{f} can take any value from 0.001 to the maximum imposed by resource constraints.

Female Fitness and Resource Constraints

A plant's fitness through seed production (W) depends on its total flower production (B), the number of ovules in each flower (n), and their probability of fertilization (F):

$$W = BnF.   \tag{3}$$

Under the most simple deterministic case, the probability of fertilization is the ratio of the number of pollen grains received (from eq. [2]) to the number of ovules, $F = P/n$, with a maximum of 1. The proportion of reproductive resources allocated to flower and ovule production is defined as ϕ, and the proportion allocated to seed production is $1 - \phi$. Given a fixed resource pool for reproduction, flower and ovule production are constrained so that

$$\phi R = B(c_{\text{f}} + nc_{\text{o}}),   \tag{4}$$

where R is the amount of resources available, c_{f} is the cost of producing a flower (excluding ovules), c_{o} is the cost of an ovule, and n is the number of ovules in a flower. Because pollen capture is a saturating function of flower size (see eq. [2]), there will be a trade-off, whereby producing larger flowers that capture more pollen reduces flower production. Consequently, the optimal allocation will balance the

effects of larger flowers for pollen capture against their effects on flower number (eq. [4]).

Limits to Seed Production

Plants must allocate resources to flowers, ovules, and fruits in order to develop seeds (Primack 1987). To reflect these resource constraints on seed production (see Bloom et al. 1985), we use an approach that incorporates elements first outlined in a graphical model by Haig and Westoby (1988) and those of Harder et al. (2008). If a plant experiences abundant pollen capture and fertilization and has too few resources to mature all of its fertilized ovules, then final seed production will be depressed relative to the number of fertilized ovules (resource limitation). Alternatively, if a plant receives less pollen than the number of ovules produced, then all fertilized ovules are matured but there are resources remaining (pollen limitation). Ovule limitation occurs when the number of pollen tubes exceeds the number of ovules and there are resources remaining after seed provisioning (Harder et al. 2008). Evolutionarily, ovule limitation can be alleviated by increasing ϕ, the proportion of reproductive resources allocated to flower and ovule production, all else being equal.

Seed production will be constrained by how resources have been partitioned between ovule and flower production (ϕR) and seed production ($(1 - \phi)R$). When resources limit seed production, too few resources remain to provision the number of fertilized ovules. Resource limitation occurs when $(1 - \phi)R < BnFc_p$, where c_p is the cost of maturing a fertilized ovule into a seed, and seed production is

$$W_r = \frac{(1 - \phi)R}{c_p}. \tag{5}$$

In contrast, gamete limitation of reproduction occurs when resources remain at the end of reproduction, so that $(1 - \phi)R > BnFc_p$. If $F < 1$, pollen limitation occurs, in which case all fertilized ovules develop into seed, so that seed production is

$$W_p = BnF = \frac{R\phi - Bc_f}{c_o}F. \tag{6}$$

If $F = 1$, ovule limitation occurs, in which case seed production is

$$W_o = Bn = \frac{R\phi - Bc_f}{c_o}. \tag{7}$$

In the deterministic case, the optimal allocation strategy and greatest fitness will occur at the transition between resource, pollen, and ovule limitation (for an empirical example, see Lawrence 1993). Any other partitioning of

resources and allocation strategy will involve unused resources and decreased fitness (Maynard Smith 1978; Bloom et al. 1985; Haig and Westoby 1988). We explored the consequences of different parameter values numerically, by finding the values of n and c_f that maximize W.

Stochastic Pollination and Fertilization

After considering the deterministic case of $P = k\{(\beta c_f)^2/[1 + (\beta c_f)^2]\}$ and $F = P/n$, we add a stochastic component to the model. Pollen receipt is based on a negative binomial distribution with the mean equal to equation (2), using a skewed distribution with high variance. The probability of fertilization is then

$$F = (1 - G(P, n)), \tag{8}$$

where $G(P, n)$ is the cumulative probability function of the negative binomial distribution evaluated for n ovules, so that F represents the probability of capturing sufficient pollen to fertilize n ovules. Finally, we incorporate stochasticity into the fertilization process, so that not every pollen grain successfully fertilizes an ovule, even when ovule number exceeds pollen receipt. We represent fertilization as a Poisson process to account for the observation that plants typically require more pollen grains than ovules to achieve full fertilization and also to incorporate the assumption that pollen tubes fertilize ovules randomly. As proposed by Aizen and Harder (2007), if P is the total number of pollen grains captured by a stigma, then

$$F = 1 - e^{(-P/n)} \tag{9}$$

is the probability of an ovule being fertilized. In all cases, the optimal fitness is calculated similarly as above by finding the values of n and c_f that maximize W.

Parameterizing the Model

The parameters most relevant to maximizing fitness occur in equations (5)–(7). We arbitrarily set the amount of available resources, $R = 20$. Changing this parameter does not affect the optimal ovule number or flower size. However, it does change the number of flowers (B) and the number of seeds (W) produced in a linear manner. For example, for wind-pollinated trees, the total amount of resources (R) available to invest in reproduction will be considerably higher than for herbs and would therefore result in many more flowers and many more seeds, all else being equal. We chose to fix the cost of seed provisioning and the cost of ovules and focus on the joint evolution of flower cost and ovule packaging. Greenway and Harder (2007) found that ovule volume is a median 93-fold smaller than the seeds that develop. Thus, throughout the subsequent analyses, we set $c_o = 0.001$ and $c_p = 0.1$. After

we explore various pollination and fertilization scenarios with these fixed parameters, we show that changes in c_p do not affect the optimal ovule number or flower size but they do affect fitness, the number of flowers, and how resources are partitioned among flower costs and seed costs.

We investigated our model using empirical data on open-pollinated stigmatic pollen loads sampled from stigmas of 19 wind-pollinated herbaceous angiosperm species from seven families. From May to August 2004 and 2005, we sampled stigmatic pollen loads of 14 species—*Ambrosia artemisiifolia* (Asteraceae), *Chenopodium album* (Chenopodiaceae), *Carex arctata*, *Carex hirtifolia*, *Carex laxiflora*, *Carex pedunculata*, *Carex pensylvanica*, *Carex plantaginea*, *Carex scabrata* (Cyperaceae), *Scirpus microcarpus* (Cyperaceae), *Plantago lanceolata* (Plantaginaceae), *Rumex acetosella*, *Rumex crispus* (Polygonaceae), and *Thalictrum dioicum* (Ranunculaceae)—at the Koffler Scientific Reserve at Jokers Hill, Ontario (44°01′N, 79°32′W). During June–August 2001 and 2002, we collected equivalent data from five species—*Anthoxanthum nitens*, *Bromus inermis*, *Elymus repens*, *Festuca campestris*, and *Phleum pratense* (all Poaceae)—near the Barrier Lake Field Station of the Kananaskis Field Stations, Alberta (51°02′N, 115°03′W). All 19 species in the sample are herbs with uniovulate flowers. For each species, we selected 20–30 plants and collected stigmas from at least three (usually six) flowers per plant. Stigmas were stored in separate microcentrifuge tubes containing 1.5 mL of 70% ethanol. To assess pollen capture, we stained stigmas with 1% basic fuschin and counted pollen loads under a compound microscope (×20). We excluded any pollen that was obviously heterospecific (i.e., different size and sculpting), but our counts may include a small amount of pollen from other species within the same family. We are confident that this is not a major concern, since cofamilial species tended to flower at different times of the day or season at our sites.

Results

Ovule Number under Deterministic Pollination and Fertilization

We begin with the simplest scenario in which the probability of fertilization equals the ratio of the number of pollen grains to the number of ovules, until pollen receipt exceeds ovule number, in which case $F = 1$. When pollination and fertilization occur deterministically, the optimal ovule production occurs at the transition between pollen and ovule and resource limitation. Figure 1 shows the two-dimensional plots, holding either c_f (fig. 1a) or n (fig. 1b) constant, and the effect of changes in the other variable on gamete and resource limitation. Fitness is max-
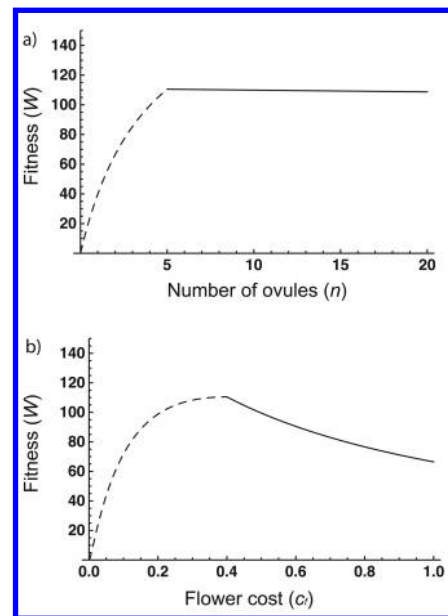
Figure 1: Relation of female fitness to ovule number when flower size is optimized at $c_f = 0.4$ (a) and to flower size when ovule number is optimized at $n = 5$ (b) under pollen limitation (*dashed lines*) and resource limitation (*solid lines*).

imized ($W = 110.5$) when $c_f = 0.4$ and $n = 5$ (table 2). Figure 2a illustrates the consequences of variation in flower cost and ovule number on fitness. When flowers are exceptionally cheap, fitness is reduced because of the S-shape relation of pollen capture to flower size. Figure 2a also illustrates that when flower cost is low, the highest fitness is achieved when ovule production per flower is low; as flowers become more expensive, ovule number per ovary increases. The reason for this is twofold. First, plants capture more pollen grains when they have larger stigmas, so that there is the potential for more ovules to be fertilized. Second, the relative cost of an ovule is considerably less than the cost of a flower, so plants pay little penalty for producing more ovules within each flower.

Ovule Number Using Observed Pollen Loads and Deterministic Fertilization

The negative binomial distribution of observed stigmatic pollen loads for the 19 anemophilous species is illustrated in figure 3. We calculated the distribution by estimating the parameters for a negative binomial distribution for each species and then identifying the average parameters over all species. The parameter values for this average distribution are $n = 0.979$ and $p = 0.093$. Further details of the observed stigmatic pollen loads per species can be found in table A1 in the appendix.

Table 2: Values for optimal fitness and key parameters under four pollination and fertilization scenarios used in our model of wind-pollinated plants

	Case 1: deterministic pollination and deterministic fertilization	Case 2: pollination from data and deterministic fertilization	Case 3: stochastic pollination and deterministic fertilization	Case 4: stochastic pollination and stochastic fertilization
Maximum fitness (W)	110.5	196.4	107.6	100.6
Ovule no. (n)	5	2	6	6
Flower cost (c_f)	.4	.001	.15	.134
No. flowers (B)	22.1	133.0	63.7	75.9
Proportion of resources on flowers and ovules (ϕ)	.45	.02	.49	.52

Rather than pollen capture being related to flower costs according to aerodynamic expectations, we now consider the case where the probability of a given pollen load is determined by the observed distribution of stigmatic pollen loads (fig. 3). In this case, maternal fitness is substantially reduced for plants with greater flower costs, because the number of flowers decreases without the benefit of increased stigmatic pollen loads (fig. 2*b*). Because in this scenario pollen capture is independent of c_f, we have the unrealistic result that the optimal flower size is the smallest possible. Interestingly, optimal ovule number is 2, even though average pollen capture is 9.5 grains. This result demonstrates the benefits of producing numerous flowers with few ovules that each sample the airstream with equivalent probabilities of pollen capture (table 2).

Ovule Number under Stochastic Pollination and Deterministic Fertilization

We now consider stochastic pollen receipt based on a negative binomial distribution with the mean equal to equation (2). Results are listed in table 2 and demonstrate that stochasticity in pollen receipt selects for more ovules per flower and substantially smaller flowers compared with the deterministic case (cf. cases 1, 3). The optimal allocation strategy shifts from producing fewer larger flowers to many smaller flowers. The increase in optimal ovule number occurs because plants are pollen limited (rather than resource limited) over a greater range of ovule numbers per flower, so that they pay little penalty for producing extra ovules.

Ovule Number under Stochastic Pollination and Stochastic Fertilization

Finally, we investigate the situation in which not every pollen grain successfully fertilizes an ovule, even when ovule number exceeds pollen receipt. The combined effects of stochastic pollination and stochastic fertilization are illustrated in figure 2*c* and table 2. Compared with deterministic pollination and fertilization, optimal ovule number is increased because there is always a finite possibility that an ovule will remain unfertilized. Optimal flower size is substantially reduced because plants producing more flowers offset stochasticity in pollen receipt and fertilization.

Testing Parameter Combinations

The relation between maximum fitness (W), flower cost (c_f), and ovule number (n) may be contingent on the choice of parameter values. To assess these dependencies, we examined the consequences of changes to c_p (the cost of maturing a fertilized ovule into a seed). Changes to c_p had no effect on optimal flower size or ovule number but do affect fitness. Moreover, varying c_p changes how resources are partitioned among flowers and seed provisioning (ϕ) and the number of flowers produced, B (fig. 4). Not surprisingly, when the cost of maturing a seed is low, plants should spend a smaller fraction of their resources on seed provisioning and more resources on making numerous flowers.

Discussion

Our model demonstrates that for wind-pollinated plants with limited resources, greatest fitness is achieved when plants produce relatively small flowers with very few ovules. When pollen receipt is a function of flower size, the benefits of producing larger flowers that capture more pollen are counterbalanced by the costs of producing fewer flowers (fig. 2*a*). However, when pollination and/or fertilization are stochastic, these benefits are diminished and fitness is reduced in comparison with plants that produce many inexpensive flowers (fig. 2*c*). Our model did not indicate that optimal ovule number equals 1, only that very few ovules were most beneficial in wind-pollinated plants. This result may be because our model incorporated only a few features of the reproductive biology of wind-pollinated plants—the aerodynamics of pollen capture with respect to flower size and pollen, ovule, and resource
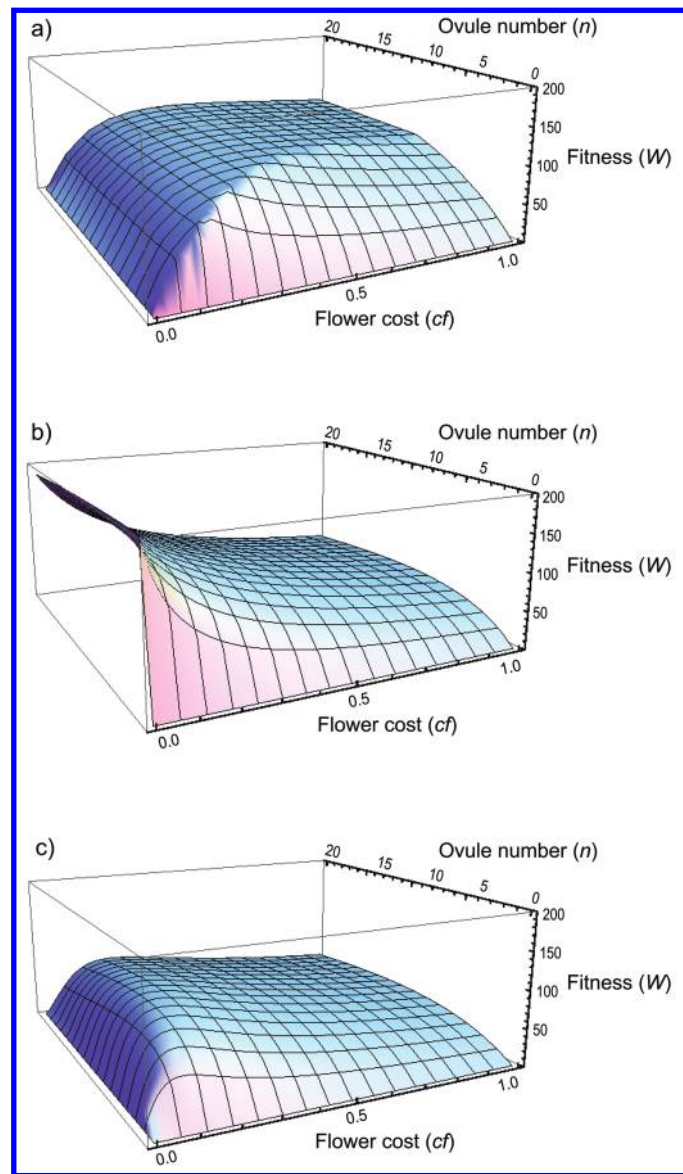
Figure 2: Relation of female fitness to ovule number and flower size under deterministic pollination and deterministic fertilization (*a*), stochastic pollination according to a distribution based on empirical values and deterministic fertilization (*b*), and stochastic pollination with pollen receipt varying according to a negative binomial distribution with the mean determined by equation (2) and stochastic fertilization (*c*).

limitation. Numerous other aspects of reproductive evolution are also likely to influence selection on ovule number, and we discuss some of these below.

Our results have several general implications for the allocation strategies of wind-pollinated plants. First, they suggest that plants that produce many inexpensive flowers might accrue greater benefits than those with more costly flowers, even if they capture less total pollen. Second, when pollination and/or fertilization are stochastic, plants that make many inexpensive flowers with few ovules suffer less than plants with fewer more costly flowers. We now discuss insights obtained from our model for several features of the allocation strategies of wind-pollinated species, including optimal ovule number per flower and seed provisioning. We also consider how several features of the reproductive biology of plants, including the incidence of correlated mating and pollen limitation, might influence the evolution of ovule number.
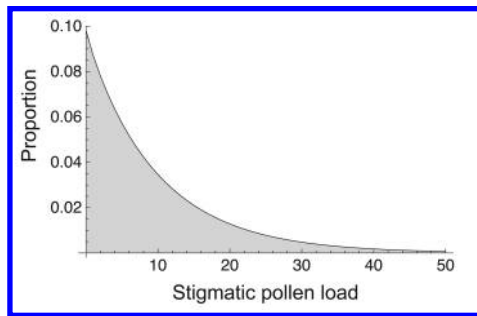
Figure 3: Negative binomial distribution of stigmatic pollen loads for 19 wind-pollinated herbaceous species with uniovulate flowers at the Koffler Scientific Reserve, Ontario, and Kananaskis Field Station, Alberta, sampled in May–August 2004 and 2005 and June–July 2001 and 2002. See text for a list of species and table A1 for samples sizes, means, and standard errors.

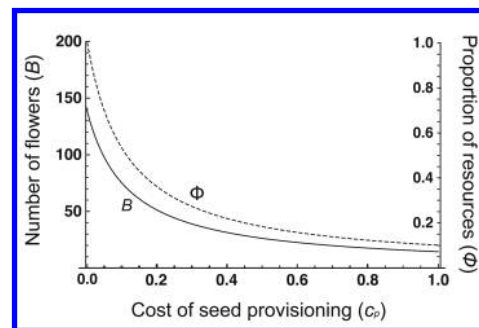### Ovule Size, Number, and Seed Provisioning

We have focused on how the pollination process of wind-pollinated plants may influence the evolution of ovule number per flower. However, ovule number variation could also be driven by selection on seed number per flower, which is constrained by trade-offs between seed number per flower and seed size (Harper et al. 1970; Willson 1983; Westoby et al. 1992). In species with many ovules per flower, the potential to abort developing embryos during seed development may mean that final seed number depends only partially on ovule number. However, in the uniovulate flowers that characterize many anemophilous species, seed set per flower will always be either 1 or 0. Thus, selection for relatively larger seeds could drive the evolution of ovule number. However, we can think of no adaptive explanation why wind-pollinated species, given their enormous range of life histories and the diversity of environments that they occupy, should collectively require larger seeds than plants with other pollination systems. Indeed, none of the reviews on seed size have suggested that pollination mode has contributed to the substantial variation in seed size (e.g., Harper et al. 1970; Baker 1972; Westoby et al. 1996; Leishman et al. 2000), other than indirectly through its effect on flower size (Primack 1987). Thus, factors related to pollination and mating seem more likely to govern ovule-number evolution.

The causes of variation in ovule size are largely unknown. Given the uncertainty that an ovule will be fertilized, allocation to ovules might be limited so that most investment is expended after fertilization when embryos develop into seeds (Lloyd 1980; Westoby and Rice 1982). Some support for this hypothesis is evident from the 93-fold conversion ratio of ovule volume into seed volume that occurs after fertilization on the basis of a survey of

45 species (Greenway and Harder 2007). However, plants appear not to produce the smallest possible ovules, because in this survey ovule volume correlated positively with flower mass and negatively with ovule number at the interspecific level. Ovule volume varied by two orders of magnitude among the species sampled. In contrast to the general size-number trade-off hypothesis, Greenway and Harder (2007) found that species with uniovulate flowers have the smallest ovules, although none of the species in their data set were wind pollinated. If parent-offspring conflict occurs primarily within flowers, species with one ovule per ovary have the least opportunity for conflict because optimal resource allocation should be similar for both the maternal plant and the developing seed. Thus, as proposed by Greenway and Harder (2007), uniovulate ovaries may eliminate the requirement of producing large ovules to allow maternal dominance in parent-offspring conflict.

Low Incidence of Correlated Mating

Wind-pollinated plants may have a low incidence of shared paternal parentage (correlated mating; Ritland 1988) at the plant level (when individuals are uniovulate) and at the fruit level (when individuals have more than one ovule per flower), because pollen grains in wind-pollinated species are sampled randomly from the airstream and arrive independently of one another. As a result, multiple paternity of the seed crops of anemophilous plants should be relatively common when maternal parents occur in neighborhoods with many potential mates. Particularly intense competition among unrelated embryos for maternal resources could select for the uniovulate condition in wind-pollinated plants (Kress 1981). Our model does not include the potential effects of paternal parentage and competition for maternal resources on seed provisioning

Figure 4: Effect of changing the cost of seed provisioning (c_p) on the number of flowers (B; *solid line*) and the proportion of resources spent on flowers and ovules (ϕ; *dashed line*) when flower size and ovule number are optimized for greatest fitness.

or seed viability; however, it is worth considering how these might influence the evolution of ovule number.

Opportunities for microgametophytic competition within a flower may be enhanced as a consequence of pollen receipt involving unrelated pollen grains. In general, when pollen arrives gradually rather than synchronously, the first pollen grains to be deposited on a stigma are more likely to be successful at fertilization (pollen precedence; Epperson and Clegg 1987), regardless of their competitive ability (Thomson 1989). However, anthesis in wind-pollinated plants tends to be highly synchronous within populations (Dowding 1987); for example, in grasses, pollen dispersal and stigma receptivity can be completed in <5 h (Beddows 1931). In this case, pollen grains may arrive during a brief period and competition to fertilize ovules may be quite severe. Intense postpollination competition can lead to gametophytic selection, and this could result in greater progeny vigor (Mulcahy and Mulcahy 1987). Studies that have attempted to demonstrate a relation between pollination intensity (pollen load size) and progeny vigor have been equivocal (e.g., Snow 1990; Quesada et al. 1993; Davis 2004; Marshall et al. 2007). Nonetheless, this influence may be of particular importance for wind-pollinated plants if they receive pollen from many potential paternal parents of varying quality.

The rapid germination of pollen grains on arrival on the stigma would be advantageous if they compete intensely for fertilization in uniovulate flowers. Several large wind-pollinated families, including Poaceae and Restionaceae, have trinucleate pollen, as does the uniovulate family Asteraceae, which also includes many wind-pollinated species (Brewbaker 1967). Trinucleate pollen, containing twin sperm cells and a vegetative cell, is a derived condition in the angiosperms (Brewbaker 1967). The pollen grains contain fully developed mitochondria at anther dehiscence, allowing for rapid germination on stigmas but very brief viability (Hoekstra 1979). A more detailed examination of the distribution of binucleate and trinucleate pollen with respect to ovule number and pollination mode may reveal interesting patterns and could provide novel insights on the consequences of the uniovulate condition on pollination and mating in wind-pollinated plants.

Pollen Quantity and Quality and the Evolution of Ovule Number

Our results demonstrate that stochastic pollination favors greater ovule production and that fitness is much reduced when flower costs are high (e.g., cf. fig. 2a, 2c). This finding has two implications for anemophilous species. First, if flower costs are low, then plants should benefit from producing numerous inexpensive flowers, even if some capture too few pollen grains to fertilize their ovules. Second,

the high incidence of uniovulate flowers in wind-pollinated plants suggests that stochastic pollination plays only a minor role in selection for optimal ovule number. However, it is not clear whether the pollination process is more or less variable for wind-pollinated plants than for animal-pollinated species.

Stochastic variation in pollen receipt has been proposed to select for increased ovule number, thus allowing plants to take advantage of unpredictably good pollination (Burd 1995, 2008). In a survey of 40 animal-pollinated species, Burd et al. (2009) found that ovule number varied positively with the statistical dispersion of pollen receipt (reflecting stochastic variation). Significantly, a comparison of the coefficient of variation (CV) reported in their study (CV = 441; calculated from table 1 in Burd et al. 2009) with that from pollen loads for the 19 wind-pollinated species examined in this study (CV = 167) indicates a striking difference, suggesting that pollen receipt is significantly less variable for wind-pollinated plants. Pollination in anemophilous species may be more predictable because of the higher synchrony of anthesis and the possibility that all stigmas on a plant will have approximately the same probability of pollen capture. On the other hand, wind pollination is at the mercy of uncertain atmospheric conditions, which could increase the stochasticity of pollen dispersal.

Pollen limitation appears to be common for animal-pollinated populations (Burd 1994; Larson and Barrett 2000; Ashman et al. 2004; Knight et al. 2005) and occurs when there are insufficient pollinators or a scarcity of mates. Of course, only the latter cause of pollen limitation is relevant for wind-pollinated plants, which might suggest that they are less often pollen limited, particularly when population densities are adequate. Several studies have demonstrated pollen limitation in wind-pollinated trees in fragmented habitats (Fox 1992; Knapp et al. 2001; Sork et al. 2002; Koenig and Ashley 2003). However, many wind-pollinated plants occur in dense populations, and studies in relatively undisturbed habitats that have compared seed set in open-pollinated versus supplemental pollinated flowers of anemophilous species have failed to demonstrate pollen limitation (Davis et al. 2004; Friedman and Barrett 2009).

In conclusion, this investigation suggests that the uniovulate condition that characterizes most wind-pollinated species most probably reflects the very small costs of individual flowers rather than low pollen capture, as most workers had previously assumed. The model we developed here demonstrates that making numerous inexpensive flowers favors few ovules in each, even when flower production does not trade-off directly with ovule number. This suggests that the observed reduction of ovule number

once wind pollination has evolved in angiosperm lineages (Friedman and Barrett 2008) results from selection on resource partitioning when flower cost is minimized for aerodynamic reasons. When pollination is stochastic, plants with fewer costly flowers suffer greater fitness consequences than plants with many inexpensive flowers, even though each small flower may capture too few pollen grains to fertilize all of its ovules. Plants that distribute flowers and ovules among multiple inflorescences sample more of the airstream, and this ovule packaging strategy should maximize fitness.

APPENDIX

Supplemental Table

Table A1: Details of sampling and stigmatic pollen loads for 19 wind-pollinated herbaceous species

Species	Location	N		Pollen load		
		Plants	Stigmas	Mean	SE	Maximum
Ambrosia artemiisifolia	44°01′N, 79°32′W	31	211	30.17	2.39	255
Anthoxanthum nitens	51°02′N, 114°51′W	36	160	6.28	.80	74
Bromus inermis	51°01′N, 115°02′W	25	230	48.03	2.57	196
Carex arctata	44°01′N, 79°31′W	30	99	7.46	1.48	94
Carex hirtifolia	44°01′N, 79°32′W	30	180	7.03	.73	78
Carex laxiflora	44°01′N, 79°31′W	27	113	4.31	.72	48
Carex pedunculata	44°01′N, 79°31′W	38	210	10.55	.93	70
Carex pensylvanica	44°01′N, 79°31′W	31	96	7.04	1.29	69
Carex plantaginea	44°01′N, 79°31′W	30	216	5.76	.61	70
Carex scabrata	44°01′N, 79°31′W	25	241	4.80	.60	79
Chenopodium album	44°01′N, 79°32′W	30	150	3.09	.30	20
Elymus repens	51°02′N, 115°03′W	28	160	66.11	2.99	159
Festuca campestris	51°02′N, 114°51′W	25	196	17.39	1.98	157
Phleum pratense	51°02′N, 115°03′W	40	249	29.60	1.61	115
Plantago lanceolata	44°01′N, 79°32′W	30	261	59.30	3.39	284
Rumex acetosella	44°02′N, 79°30′W	30	262	2.21	.16	17
Rumex crispus	44°01′N, 79°32′W	25	179	5.91	.60	59
Scirpus microcarpus	44°01′N, 79°32′W	30	268	5.16	.35	38
Thalictrum dioicum	44°01′N, 79°32′W	25	125	9.02	.68	46

Note: For all species, the minimum number of pollen grains was 0.

Literature Cited

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.

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.

Baker, H. G. 1972. Seed weight in relation to environmental conditions in California. Ecology 53:997–1010.

Beddows, A. R. 1931. Seed setting and flowering in various grasses. Bulletin of the Welsh Plant Breeding Station 12:5–99.

Bloom, A. J., F. S. Chapin III, and H. A. Mooney. 1985. Resource limitation in plants: an economic analogy. Annual Review of Ecology and Systematics 16:363–392.

Brewbaker, J. L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. American Journal of Botany 54:1069–1083.

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.

Burd, M., T.-L. Ashman, D. R. Campbell, M. R. Dudash, M. O. Johnston, T. M. Knight, S. J. Mazer, R. J. Mitchell, J. A. Steets, and J. C. Vamosi. 2009. Ovule number per flower in a world of unpredictable pollination. American Journal of Botany 96:1159–1167.

Campbell, N. A., J. B. Reece, and L. G. Mitchell. 1999. Biology. 5th ed. Benjamin Cummings, Menlo Park, CA.

Cruden, R. W., and D. Lyon. 1985. Patterns of biomass allocation to male and female functions in plants with different mating systems. Oecologia (Berlin) 66:299–306.

Davies, C. N., and C. V. Peetz. 1956. Impingement of particles on a transverse cylinder. Proceedings of the Royal Society A: Mathematical and Physical Sciences 234:269–295.

Davis, H. G., C. M. Taylor, J. C. Civille, and D. R. Strong. 2004. An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. Journal of Ecology 92:321–327.

Davis, S. L. 2004. Natural levels of pollination intensity and effects of pollen loads on offspring quality in females of *Thalictrum pubescens* (Ranunculaceae). Plant Systematics and Evolution 244:45–54.

Dowding, P. 1987. Wind pollination mechanisms and aerobiology. International Review of Cytology 107:421–437.

Epperson, B. K., and M. T. Clegg. 1987. First-pollination primacy and pollen selection in the morning glory, *Ipomoea purpurea*. Heredity 58:5–14.

Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology. 3rd ed. Pergamon, Oxford.

Fox, J. F. 1992. Pollen limitation of reproductive effort in willows. Oecologia (Berlin) 90:283–287.

Friedman, J., and S. C. H. Barrett. 2008. A phylogenetic analysis of the evolution of wind pollination in the angiosperms. International Journal of Plant Sciences 169:49–58.

———. 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. Annals of Botany 103:1515–1529.

Friedman, J., and L. D. Harder. 2004. Inflorescence architecture and wind pollination in six grass species. Functional Ecology 18:851–860.

Greenway, C. A., and L. D. Harder. 2007. Variation in ovule and seed size and associated size-number trade-offs in angiosperms. American Journal of Botany 94:840–846.

Gregory, P. H. 1973. The microbiology of the atmosphere. 2nd ed. Leonard Hill, England.

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

Harder, L. D., and S. D. Johnson. 2008. Function and evolution of aggregated pollen in angiosperms. International Journal of Plant Sciences 169:59–78.

Harder, L. D., S. A. Richards, and M. B. Routley. 2008. Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. Evolution 62:157–172.

Harper, J. L., P. H. Lovell, and K. G. Moore. 1970. The shapes and sizes of seeds. Annual Review of Ecology and Systematics 1:327–356.

Hoekstra, F. A. 1979. Mitochondrial development and activity of binucleate and trinucleate pollen during germination in vitro. Planta 145:25–36.

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.

Knapp, E. E., M. A. Goedde, and K. J. Rice. 2001. Pollen-limited reproduction in blue-oak: implications for wind pollination in fragmented populations. Oecologia (Berlin) 128:48–55.

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.

Koenig, W. D., and M. V. Ashley. 2003. Is pollen limited? the answer is blowin' in the wind. Trends in Ecology & Evolution 18:157–159.

Kress, W. J. 1981. Sibling competition and evolution of pollen unit, ovule number, and pollen vector in angiosperms. Systematic Botany 6:101–112.

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.

Lawrence, W. S. 1993. Resource and pollen limitation: plant size-dependent reproductive patterns in *Physalis longifolia*. American Naturalist 141:296–313.

Lee, T. D. 1984. Patterns of fruit maturation: a gametophyte competition hypothesis. American Naturalist 123:427–432.

Leishman, M., I. J. Wright, A. T. Moles, and M. Westoby. 2000. The evolutionary ecology of seed size. Pages 31–57 *in* M. Fenner, ed. Seeds: the ecology of regeneration in plant communities. 2nd ed. CABI, Wallingford.

Linder, H. P. 1998. Morphology and the evolution of wind pollination. Pages 123–135 *in* S. J. Owens and P. J. Rudall, eds. Reproductive biology in systematics, conservation and economic botany. Royal Botanic Gardens, Kew.

Linder, H. P., and J. Midgley. 1996. Anemophilous plants select pollen from their own species from the air. Oecologia (Berlin) 108:85–87.

Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytologist 86:69–79.

Marshall, D. L., M. G. M. Shaner, and J. P. Oliva. 2007. Effects of pollen load size on seed paternity in wild radish: the roles of pollen competition and mate choice. Evolution 61:1925–1937.

Maseuth, J. D. 2009. Botany: an introduction to plant biology. 4th ed. Jones & Bartlett, Sudbury, MA.

Maynard Smith, J. 1978. Optimization theory in evolution. Annual Review of Ecology and Systematics 9:31–56.

Mulcahy, D. L. 1975. Gamete competition in plants. North-Holland, Amsterdam.

Mulcahy, D. L., and G. B. Mulcahy. 1987. The effects of pollen competition. American Scientist 75:44–50.

Niklas, K. J. 1985. The aerodynamics of wind pollination. Botanical Review 51:328–386.

———. 1987. Pollen capture and wind-induced movement of compact and diffuse grass panicles: implications for pollination efficiency. American Journal of Botany 74:74–89.

Paw U, T. K., and C. Hotton. 1989. Optimum pollen and female

receptor size for anemophily. American Journal of Botany 76:445–453.

Pohl, F. 1929. Beziehungen zwischen Pollenbeschaffenheit, Bestäubungsart und Fruchtknotenbau. Beihefte des Botanischen Centralblatts 46:247–285.

Primack, R. B. 1987. Relationships among flowers, fruits and seeds. Annual Review of Ecology and Systematics 18:409–430.

Quesada, M. A., J. A. Winsor, and A. G. Stephenson. 1993. Effects of pollen competition on progeny performance in a heterozygous cucurbit. American Naturalist 142:694–706.

Ritland, K. 1988. The genetic-mating structure of subdivided populations. II. Correlated mating models. Theoretical Population Biology 34:320–346.

Snow, A. A. 1990. Effects of pollen-load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). American Naturalist 136:742–758.

Sork, V. L., F. W. Davis, P. E. Smouse, V. J. Apsit, R. J. Dyer, J. F. Fernandez, and B. Kuhn. 2002. Pollen movement in declining populations of California Valley oak, *Quercus lobata*: where have all the fathers gone? Molecular Ecology 11:1657–1668.

Stebbins, G. L. 1974. Flowering plants: evolution above the species level. Belknap, Cambridge, MA.

Thomson, J. D. 1989. Germination schedules of pollen grains: implications for pollen selection. Evolution 43:220–223.

Venable, D. L. 1996. Packaging and provisioning in plant reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1319–1329.

Weller, S. G., A. K. Sakai, T. M. Culley, D. R. Campbell, and A. K. Dunbar-Wallis. 2006. Predicting the pathway to wind pollination: heritabilities and genetic correlations of inflorescence traits associated with wind pollination in *Schiedea salicaria* (Caryophyllaceae). Journal of Evolutionary Biology 19:331–342.

Wessells, N. K., and J. L. Hopson. 1988. Biology. Random House, New York.

Westoby, M., and B. Rice. 1982. Evolution of the seed plants and inclusive fitness of plant tissues. Evolution 36:713–724.

Westoby, M., E. Jurado, and M. Leishman. 1992. Comparative evolutionary ecology of seed size. Trends in Ecology & Evolution 7:368–372.

Westoby, M., M. Leishman, and J. Lord. 1996. Comparative ecology of seed size and dispersal. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1309–1317.

Willson, M. F. 1983. Plant reproductive ecology. Wiley, New York.

Associate Editor: David D. Ackerly
Editor: Ruth G. Shaw

"On the 8th of June, 1867, several specimens of a new form of Ox-eye Daisy (Leucanthemum) were gathered in the fields of Hon. Matthew Vassar of Poughkeepsie, N.Y., two of which were kindly sent to me. ... Having sought in vain for any mention of this form in botanical works, and believing it to be new to science, I have ventured to name it *Leucanthemum tubuliforum.*" "Botany" by Sanborn Tenney (*American Naturalist*, 1867, 1:388–390).