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OVULE NUMBER PER FLOWER IN A WORLD OF UNPREDICTABLE POLLINATION[1]

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The number of ovules per flower varies over several orders of magnitude among angiosperms. Here we consider evidence that stochastic uncertainty in pollen receipt and ovule fertilization has been a selective factor in the evolution of ovule number per flower. We hypothesize that stochastic variation in floral mating success creates an advantage to producing many ovules per flower because a plant will often gain more fitness from occasional abundant seed production in randomly successful flowers than it loses in resource commitment to less successful flowers. Greater statistical dispersion in pollination and fertilization among flowers increases the frequency of windfall success, which should increase the strength of selection for greater ovule number per flower. We therefore looked for evidence of a positive relationship between ovule number per flower and the statistical dispersion of pollen receipt or seed number per flower in a comparative analysis involving 187 angiosperm species. We found strong evidence of such a relationship. Our results support the hypothesis that unpredictable variation in mating success at the floral level has been a factor in the evolution of ovule packaging in angiosperms.

Key words: bet hedging; ovule; pollination; reproductive evolution; seed set; stigmatic pollen load.

Ovule number per flower is analogous in many ways to clutch size in animals (May, 1978), yet our understanding of ovule number (for convenience, we omit hereafter the qualifier "per flower") is poor compared to the rich body of ideas and data on the evolution and ecology of clutch size in animals (e.g., Roff, 1992; Mock and Forbes, 1995). The ancestral condition and subsequent evolution of ovule number in the early history of the angiosperms is uncertain, due to variability of this trait among basal lineages (Doyle and Endress, 2000). Among extant angiosperms, a stereotyped ovule number is sometimes found throughout whole families (four per flower in Lamiaceae and Boraginaceae, for example), while considerable evolutionary lability occurs in other families, such as Rosaceae (Potter et al., 2007). Latitudinal trends in avian clutch size have been studied for decades (Ashmole, 1963; Ricklefs, 1980; Evans et al., 2005), but there is little equivalent knowledge about geographic patterns of variation in ovule number (May, 1978). Ovule number is an especially perplexing life-history trait in light of the widespread occurrence of pollen limitation of seed output among angiosperms (Burd, 1994b; Larson and Barrett, 2000; Ashman et al., 2004; Knight et al., 2005). Pollen limitation implies that ovule number exceeds, on average, the population of microgametophytes that have reached an ovary—not a strictly necessary implication (Harder and Routley, 2006), but seemingly true upon direct examination (Herrera, 2002, 2004).

Why would selection favor an ovule number greater than the average number of ovule fertilizations that flowers obtain? Among the many selective factors that could affect ovule number, we focus here on one feature of the pollination environment that is closely related to pollen limitation: random variation among flowers in pollen receipt and subsequent ovule fertilization. Variance in mating success is often quite pronounced, with substantial disparity in stylar microgametophyte populations found even among neighboring flowers on an inflorescence (Herrera, 2002, 2004).

Stochastic pollination success at the floral level seems to be inevitable for many flowering plants, due largely to the hazards of pollination service offered by animals and abiotic vectors. For example, Engel and Irwin (2003) used path analysis to show that variation in pollinator visitation had the largest direct effect on stigmatic pollen receipt (which ranged from 9 to 183 grains per stigma) in a population of *Ipomopsis aggregata*. Burd (1994a) dissected the wide variation in seed number per fruit in *Lobelia deckenii* even more finely. In this species, enormous disparity (interquartile range of 143–1061 seeds per fruit) is created by the compounded effects of random variation in

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pollinator arrival at inflorescences, in the number of flowers probed per inflorescence visit, and in the fertilization efficacy of individual probes into flowers by pollinators. Modeling of these processes indicated that longer exposure of flowers to pollinators exacerbated the degree of interfloral disparity (Burd, 1994a). If this is generally true, variation in fertilization success could be tied to floral longevity (Ashman and Schoen, 1994) or to the duration of stigma receptivity (Campbell et al., 1994). Pollinator behavior and floral mating success are also affected by traits such as corolla shape (Wilson, 1995), nectar quantity and quality (Burd, 1995b), stamen and stigma position (Galen, 1989), stigmatic area and diameter of stylar transmitting tissue (Matthews et al., 1999), pollen production (Ashman and Penet, 2007), pollen dispensing (Harder and Thomson, 1989; Castellanos et al., 2006), and inflorescence size (Dudash, 1991). Whether the variety of relevant traits restricts or enhances random differences in ovule fertilization among flowers is probably idiosyncratic to each species.

What effect would a highly uncertain pollination environment have on the evolution of ovule number? To address this question, it is worth noting initially that there are many instances in which environmental stochasticity creates selection on plant life history traits. For example, a constant environment should favor universal monocarpic reproduction (Cole, 1954), but the prevalence of polycarpy is perfectly explicable in light of random temporal variation in survival and fecundity (Goodman, 1984; Orzack and Tuljapurkar, 1989). Early flowering may evolve as a buffer against reproductive failure when the length of the growing season is uncertain, even if a later onset of flowering would, on average, allow greater annual seed output (Simons and Johnston, 2003). The degree of seed dormancy in desert annuals increases as the risks associated with survival, growth, and ultimate fecundity increase (Venable, 2007). These, and many other examples (Philippi and Seger, 1989), show that unpredictable environmental variation is a source of selection.

In a similar fashion, random variation in the pollination environment may affect the evolution of ovule number (Burd, 1995a). When disparity in pollen receipt is great, there are unpredictable winners and losers among a plant's flowers. Because ovule fertilization sets an upper limit for each flower's potential seed production (subject to resource availability, of course), a plant with a high ovule number has some winning flowers that make a large contribution to total female reproductive success, while the losers waste resources on unused ovules and associated structures. Had the plant's flowers contained fewer ovules, resources would seldom be wasted, but mating opportunities would be missed. The overall fitness consequence of any given ovule number depends on the resource cost of ovules and flowers, and on the relative frequency of winners and losers as reflected in the probability distribution of pollination or fertilization success. Burd (1995a) modeled these factors and showed that as the among-flower variance increases, the optimal ovule number per flower rises as well. This result echoed a similar finding by Koslowski and Stearns (1989). Although these models do not provide a full analysis of selection acting on reproductive investment (the interaction of ovule investment with male function in hermaphroditic plants is overlooked, for example), it seems reasonable as a working hypothesis to expect that high levels of fertilization and seed set will be favored in many circumstances.

Optimization of a trait value in the face of environmental stochasticity is often termed "bet hedging." With respect to ovule number, plants would "bet" on their fertilization success and "hedge" by erring on the side of too many rather than too few ovules (Koslowski and Stearns, 1989). However, Seger and Brockman (1987) prefer to limit the term to situations involving a reduction of both average fecundity and variance of fecundity, a combination that can be favored because selection is sensitive to both the first and second moments of reproductive success (Orr, 2007). Although we have previously described our ovule number hypothesis as "bet hedging" (Knight et al., 2005), this usage does not fall within the restricted definition of Seger and Brockman (1987).

In this article, we use a multispecies data set to test the prediction that the degree of disparity among flowers in pollination and fertilization will be associated with ovule number. The evolution of ovule number must be idiosyncratic in each species and subject to many selective factors, so we do not expect the hypothesized correlation to be the exclusive explanation of ovule packaging in angiosperms. However, the multispecies comparison we use provides a test of whether this correlation is a common theme in the evolution of flowering plants. We gathered our data from published studies and a few unpublished results of our own or provided by colleagues. As in any meta-analysis, sample sizes and other details of the measurements differed from study to study. Countering this heterogeneity is the advantage of comparing many species, allowing patterns to be detected at a scale unachievable in single studies (Gurevitch et al., 2001).

MATERIALS AND METHODS

Species and phylogenetic trees—The core of our data set comprised the 43 species used in a meta-analysis by Knight et al. (2005) for a preliminary test of the association of pollination variance with ovule number. To this core, we added data from other studies that measured variability in pollination and fertilization of naturally pollinated flowers in natural habitats. The final data set contained 187 species from 72 families recognized by Brummitt (1992) or 63 families recognized by the Angiosperm Phylogeny Group (2003). Of these, 114 species were herbaceous, 70 were trees or shrubs, and three were lianas. Single seeds from dehiscent fruits or one-seeded nondehiscent, dry fruits were the dispersal unit for 149 species; 38 species had uni- or multiseeded fleshy fruits or nondehiscent, dry fruits with multiple seeds. Information on plant habit (tree, shrub, herb, liana) and on seed or fruit dispersal unit was usually drawn from the species description given in the sources of the other data. Where necessary, we also drew on descriptions in taxonomic reference works for additional information. A list of the species and data sources is given in Appendix S1 (see Supplemental Data with the online version of this article).

We used two different estimates of the angiosperm phylogeny to provide family level trees and branch lengths for our data set. One was the analysis of Wikström et al. (2001), which used the topology of Soltis et al. (2000) with dating based on *rbcL*, *atpB*, and 18S rDNA sequence divergence, calibrated to fossil evidence for the split between Fagales and Cucurbitales. The other was the supertree constructed by Davies et al. (2004), with branch lengths based on *rbcL* divergence, also calibrated to the Fagales-Cucurbitales split. We mapped the families in our data set onto the Wikström et al. (2001) tree using the cladograms presented in their appendix, and onto the Davies et al. (2004) tree using the program Phylomatic (http://www.phylodiversity.net/phylomatic). Use of two trees provides a check on the robustness of our results.

Subfamilial topology and branch lengths for the taxa in our data set were added to the backbone of the family level trees in three ways. (1) Wikström et al. (2001) provided subfamilial structure and dating for many of the same genera and species contained in our data set. We incorporated this information into our phylogeny. (2) We used the phylogenetic analyses listed in Appendix S2 (see Supplemental Data with the online version of this article) to provide topologies or branch lengths for certain groups in our data. (3) We occasionally used membership in well-defined subfamilial taxa (e.g., Campanuloideae and Lobelioideae within Campanulaceae) to place species. Where no phylogenetic information was available, we left the topology as an unresolved polytomy. In the absence of information on branch lengths, we adopted Pagel's (1992) rule

for arbitrary branch lengths, scaled to the age of the family as specified in the backbone tree. The two trees are shown in Appendix S2.

Pollination—We used the mean, $\bar{X}_{pollen}$, and standard deviation, SD_{pollen}, of stigmatic pollen loads to quantify pollination patterns for individual species. (If a standard error of the mean was reported, we back-calculated the standard deviation from the standard error and the sample size.) Stigmatic pollen counts do not account for subsequent germination and pollen tube attrition, but stigmatic loads, stylar pollen tube number, and seed set are often well correlated (Cruzan, 1986; Guth and Weller, 1986; Herrera, 2002). Although higher moments of a distribution, such as the skew, may be relevant to selection on ovule number (Burd, 1995a), the standard deviation is the most commonly reported measure of statistical dispersion, and so we relied on SD_{pollen} as our measurement of among-flower variation for each species.

We obtained values of $\bar{X}_{pollen}$ and SD_{pollen} for 40 species (Appendix S1). Data were available from more than one population or year for 14 of the 40 species. In these cases, we calculated a weighted average of the mean and standard deviation, using sample sizes to weight the individual population or year estimates. The data on stigmatic pollen loads reported in these studies always treated individual flowers as the fundamental unit for calculation of sample statistics; thus, our values of SD_{pollen} measure variation among individual flowers. Sample sizes ranged from 5 to 529 flowers; 80% of the samples had between 20 and 116 flowers. There was no statistically significant relationship between sample size and $\bar{X}_{pollen}$ ($r = 0.007$, $P = 0.95$) or SD_{pollen} ($r = 0.081$, $P = 0.48$). Thus, differences in sample sizes among the studies from which we drew data do not appear to have introduced any systematic bias into the estimates of pollen load dispersion. Descriptive statistics for the pollination data from the 40 species are given in Table 1.

Ovule fertilization—We used the sample mean, $\bar{X}_{seed}$, and sample standard deviation, SD_{seed}, of seed number per fruit to characterize ovule fertilization patterns. Seed number has the advantage of being measured after pollen tube attrition in the style (Erbar, 2003), albeit possibly after ovule and fruit abortion as well. Seed number is only a surrogate for the number of fertilization opportunities (pollen tubes reaching the base of the style) obtained by any particular flower, but it has the advantage of being a very commonly reported measure of mating success. We obtained 382 measures of seed number per fruit for populations, years, or other sampling units from 169 species. As with pollen loads, weighted averages of these variables were calculated for the 69 species for which we had data from more than one sample.

Sample sizes for seed number per fruit ranged from 5 to 1275 fruits, with 80% of the sample sizes from 10 to 125 fruits. There was no statistically significant relationship between sample size and the value of $\bar{X}_{seed}$ ($r = -0.147$, $P = 0.06$) or SD_{seed} ($r = 0.079$, $P = 0.13$). The nearly significant relationship for $\bar{X}_{seed}$ occurred because sample sizes were greater than 100 fruits only for species with low seed number per fruit. This almost certainly reflects the labor-saving decisions of researchers, and not a tendency for large sample size to bias sample means toward low values. A bias in the sample mean due solely to sample size is unexpected on theoretical grounds, in any case (Quinn and Keough, 2002). Among populations with samples sizes under 100 fruits, the correlation was clearly nonsignificant ($r = 0.044$, $P = 0.61$). Thus, as with the pollen load data, sample size variation appears not to have distorted the estimates of $\bar{X}_{seed}$ and SD_{seed}. Descriptive statistics for the seed set variables are given in Table 1.

The modular structure of plants provides a hierarchy of possible sampling units for the calculation of $\bar{X}_{seed}$ and SD_{seed}. The data on seed number per fruit fell into two broad groups. For 113 species, individual fruits were the fundamental unit for calculation of the descriptive statistics, so that SD_{seed} for these species represents dispersion among individual fruits. For 56 species, average values of seed number per fruit within inflorescences or whole plants were used to determine SD_{seed}, which therefore represents the dispersion among inflorescence means or plant means. Because of this heterogeneity, we analyzed the seed number data as separate flower-level data or inflorescence- and plant-level data, and also as a combined data set. There was little difference in results between the two subsets, implying that variation among inflorescences and plants reflects, at least approximately, the degree of fertilization uncertainty faced by individual flowers. We report only the combined data here, and present the analyses of the separate subsets in online Appendix S3 (see Results).

Ovule number—For most of our species, we obtained the value of ovule number from the same published report that supplied data on stigmatic loads or seed set, or as a personal communication from the author of the report. Ovule number for some species was provided as a personal communication by another researcher, or obtained from a different publication or a botanical database. In two instances, we interpreted a published description of "hundreds" of ovules per flower as 300, and in one instance "a few thousand" as 2000. (We anticipated that with ovule number differing from one to thousands in the data set, these approximations would suffice for the comparative analysis.)

We used a single mean value of ovule number for each species in our analysis. Mean ovule number varied by about three orders of magnitude across the 187 species (Table 1). There were three species with only a single ovule per flower: *Daphne kamtchatica* (Thymelaeaceae), *Staberoha banksi* (Restionaceae), and *Viburnum lanatum* (Caprifoliaceae). A further 28 species from 12 families had a mean ovule number below five. At the other extreme, six species had over 1000 ovules per flower: *Stenocereus queretaroensis* (Cactaceae), 1264; *Lobelia deckenii* (Campanulaceae), 1524; *Pseudobombax munguba* (Malvaceae), 1876.5; *Drymonia rubra* (Gesneriaceae), 1678; *Titanotrichum oldhamii* (Gesneriaceae), 2000; and *Besleria triflora* (Gesneriaceae), 2975. Additional descriptive statistics are given in Table 1.

Ovule number can be variable within a species, of course. For 103 of the 187 species, we could calculate the intraspecific coefficient of variation (CV = standard deviation/mean) of ovule number, which ranged from a low of 0 (constant ovule number) for 12 species in seven families to a high of 0.61 in *Lycium cestroides* (Solanaceae). The median CV was 0.14, and 90% were below 0.37. The 10 species with the highest CV for ovule number came from nine different families. Thus, intraspecific variation in ovule number was sometimes present but appeared not to be severe, and, importantly for a comparative analysis, was not associated with particular taxa. Although intraspecific variation in ovule number may well be adaptive in its own right, we defer consideration of this aspect of ovule packaging to a future analysis.

Interpretation of the standard deviation of pollen load and seed number—We used SD_{pollen} and SD_{seed} to quantify the unpredictability of mating success of individual flowers. These two measures are surrogates that are meant to reflect the spread of the probability distribution for the number of pollen tubes reaching each ovary. It is important to note that these are not standard errors of the mean, and so do not vary automatically as a consequence of sample size, as a standard error does. (Also, as noted, there was no empirical correlation between SD_{pollen} or SD_{seed} and sample size among the species in the data sets.) But could either SD_{pollen} or SD_{seed} vary automatically as a consequence of ovule number, without the intervention of selection, so that any association between them would be an artifact?

Ovule number does not constrain any observation of stigmatic pollen load, which could be high or low in any flower regardless of how many ovules are present. Ovule number does, however, restrict the maximum observed value of seed number, so that a low ovule number could produce a low value of SD_{seed} even if variation in stigmatic loads and pollen tube numbers were large. How this affects our data depends on how often and how severely pollen tube arrivals exceed the available number of ovules—something we cannot know from the available information. If mean seed set per fruit is near the ovule number in any

TABLE 1. Descriptive statistics for ovule number, distribution of stigmatic pollen loads, and distribution of seed number per fruit among the species in the data set.

Statistic	Ovule no.	$\bar{X}_{pollen}$	SD_{pollen}	$\bar{X}_{seed}$	SD_{seed}
No. of species	187	40	40	169	169
Minimum	1	3	4.8	0.5	0
25th percentile	6.7	12.1	10.2	3.1	1.1
Median	22.8	67.8	45.0	7.2	3.1
75th percentile	99.1	300.8	144.6	40.1	19.0
Maximum	2975.0	3673.0	5669.7	1452.0	606.5
Mean	134.0	318.5	374.8	57.2	24.5
Standard deviation	361.0	710.1	1071.0	158.2	71.4
Skewness	5.0	3.6	4.1	5.9	6.0

Notes: The descriptive statistic at the left of each row was determined across the relevant species in the data set. For example, the 40 measurements of SD_{pollen} from the 40 species with pollen load data had a mean value of 374.8, and a standard deviation of 1071.0. Similarly, 7.2 was the median value of $\bar{X}_{seed}$ among the 169 species with data on seed number per fruit.

species, there is a danger that a good deal of the upper distribution of fertilization opportunities (pollen tube number) is truncated at the ovule number and therefore unobserved. Of the 169 species for which we have seed data, mean seed number per fruit is greater than two-thirds of the ovule number in 40 species. Thus, SD_{seed} may underestimate the true degree of flower-to-flower variation in potential mating success in some cases. We analyzed the seed number data despite this potential shortcoming because this is the most commonly reported type of data bearing on the question of fertilization opportunity per flower. Stigmatic load data are better for our purposes, but available for far fewer species. Comparing the analyses for both types of data is one way to assess the robustness of the results.

Phylogenetically independent contrasts—It is inappropriate to make multispecies comparisons without taking account of the phylogenetic structure of the species involved, so we used the technique of phylogenetically independent contrasts (PICs) (Felsenstein, 1985) for the analysis. All calculations were carried out with the PDTREE module of the program PDAP (Garland et al., 1993; Midford et al., 2005) implemented in the program Mesquite version 2.0 (Maddison and Maddison, 2007). The raw data showed a strong linear correlation between the logarithm of ovule number and the logarithm of SD_{pollen} ($r = 0.855$) or of SD_{seed} ($r = 0.919$). We therefore log-transformed all variables for the analysis and obtained standardized PICs for log(ovule number), $\log(SD_{pollen})$, $\log(SD_{seed})$, $\log(\bar{X}_{pollen})$ and $\log(\bar{X}_{seed})$. The contrasts were calculated using both the Wikström et al. (2001) and Davies et al. (2004) phylogenetic trees. To check the robustness of results with respect to branch lengths in the phylogenies, we transformed all branches on the two trees to unit length (equivalent to the assumption that phenotypic evolution occurs only at speciation) and repeated the analyses described later.

The PICs technique assumes a Brownian motion model of character evolution over all branches of the phylogeny (Diaz-Uriarte and Garland, 1996). We checked this assumption for each variable by testing the correlations between the standardized contrasts and their standard deviations, which are related to the branch lengths over which the contrast is made (Garland et al., 1992). Significantly nonzero correlations indicate a violation of the assumption. These tests were also conducted with PDTREE.

Statistical test of the hypothesis—PICs are substitutes (phylogenetically independent ones) for the original variables. The hypothesis under consideration is that ovule number will increase with the degree of random dispersion in pollination or fertilization success among flowers. We used multiple linear regression of the PICs to test this relationship. The regression equation for each analysis had the form $y = b_1x_1 + b_2x_2$, in which y represents the PICs for log(ovule number), x_1 the PICs for either $\log(SD_{pollen})$ or $\log(SD_{seed})$, and x_2 the PICs for either $\log(\bar{X}_{pollen})$ or $\log(\bar{X}_{seed})$. The regressions were forced through the origin, as required when dealing with PICs (Garland et al., 1992), and we examined the variance inflation factors to check for excessive collinearity of the independent variables. The partial regression coefficients in these analyses have the standard interpretation, i.e., the effect of each independent variable when holding the other constant. Thus, our expectation is that b_1, the coefficient for the $\log(SD_{pollen})$ term or the $\log(SD_{seed})$ term, will be significantly positive in each analysis.

RESULTS

Test of assumptions—PICs should be uncorrelated with their standard deviations (Diaz-Uriarte and Garland, 1996), and this was true for all variables in our analysis (the logarithms of ovule number, $\bar{X}_{pollen}$, SD_{pollen}, $\bar{X}_{seed}$, and SD_{seed}), for PICs calculated on either of the two phylogenetic trees, and with either their original branch lengths or unit branch lengths. Correlation coefficients ranged from 0.006 to 0.209 in absolute magnitude, and none was significantly nonzero (statistical significance ranged from $P = 0.93$ to $P = 0.10$). The diagnostic scatter plots available in PDAP further confirmed the lack of trends or aberrant points. Thus, the data conformed well to the assumptions of the PICs technique.

Does ovule number increase with increasing variability in pollen load?—There were strong, positive relationships between the PICs for log(ovule number) and for $\log(SD_{pollen})$, when using either phylogenetic tree to calculate the PICs (Fig. 1 A, B). These positive relationships were maintained when controlling statistically for the effect of the mean pollen load through multiple regression. The partial regression slopes represented by the coefficients b_1 in Table 2, rows a and b, were positive and statistically significant, as expected under our hypothesis. In contrast, the b_2 coefficients, representing the independent effect of mean pollen load, were not significant (Table 2, rows a and b).

Transformation of the phylogenetic trees so that all branches had unit length did not alter the fundamental pattern: PICs for log(ovule number) and for $\log(SD_{pollen})$ were positively correlated (Appendix S3, see Supplemental Data with the online version of this article). Statistical significance was retained for the overall regression models, but the independent variables were no longer individually significant (Table 2, rows c and d). Thus, when PICs are calculated on a tree with unit branch lengths, it is not possible to identify an independent effect of either SD_{pollen} or $\bar{X}_{pollen}$, even though the two variables together have a significant effect.

The variance inflation factor (VIF) measures the degree of collinearity between the independent variables in a regression. Perfectly orthogonal variables yield a VIF of unity, and values greater than 10 are usually taken as signals of excessive collinearity (Quinn and Keough, 2002). VIF values for the regressions in Table 2 were always less than 10, suggesting that collinearity was not a problem for these analyses (although the higher VIF values indicate greater collinearity in the analyses based on the transformed trees, and this collinearity could contribute to the loss of significant individual effects of the independent variables).

It is interesting to note which parts of the phylogenetic trees contributed the largest contrasts (those in the upper right-hand of the scatter of points in Fig. 1A, B). Two of the four largest contrasts involved groups within the Lamiales. Ovule number is highly variable in this order; for example, species in the Lamiaceae and Acanthaceae have only a few ovules per flower, while there are hundreds or thousands per flower in some species in the Gesneriaceae and Bignoniaceae. Another of the four largest contrasts occurred at the base of the Malvales, a diverse order containing families like Thymeliaceae, whose species have a single ovule per carpel, and Malvaceae, with species having hundreds of ovules per flower. A contrast in the monocots involving Iridaceae and related families is also one of the four largest. The species representing these families in our data set are listed in online Appendix S1 and their position in the phylogenetic trees is shown in online Appendix S2.

Does ovule number increase with increasing variability in seed number per fruit?—The results using the seed number data mirrored the results for pollen load. PICs for log(ovule number) were positively associated with PICs for $\log(SD_{seed})$, when using either phylogenetic tree in the analysis (Fig. 1C, D), and when the trees were transformed to unit branch lengths (online Appendix S3). The partial effect of SD_{seed} was statistically significant in all regression analyses (b_1 coefficients in Table 3). Indeed, the partial slopes for both independent variables were statistically significant in all the analyses in Table 3. Thus, each variable had a positive association with ovule number when the other was held constant. Variance inflation factors were well within tolerable limits (Table 3).

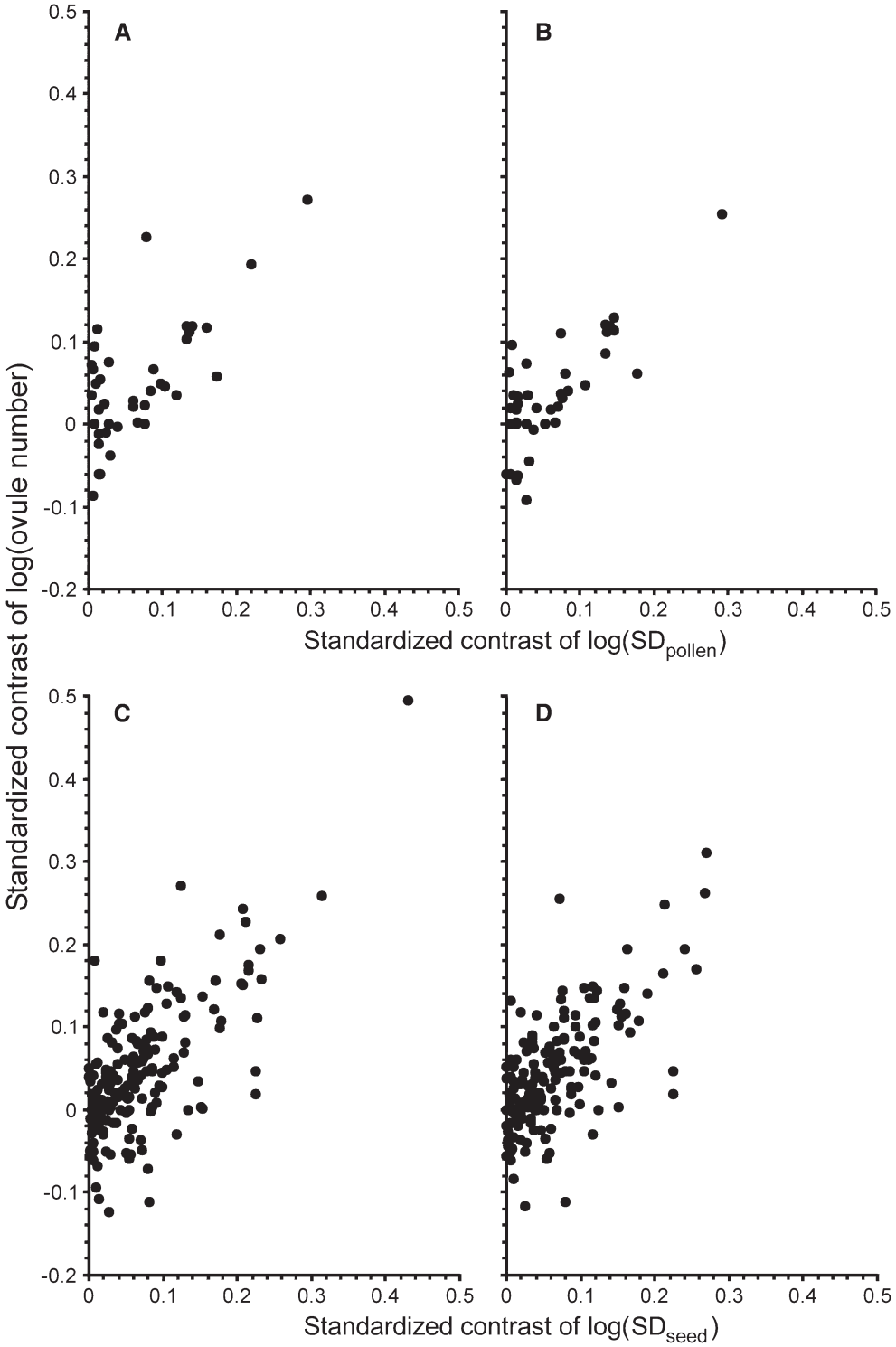

Fig. 1. Scatter plots of phylogenetically independent contrasts (PICs) for ovule number vs. two different measures of variation in floral mating success. PICs were calculated using the phylogenetic trees based on (a, c) Wikström et al. (2001) or (b, d) Davies et al. (2004).

The largest contrast for log(SD_{seed}) in both Fig. 1C and Fig. 1D is due to two congeners in the Fabaceae: *Genista tricanthos*, with an average of 22.8 ovules per flower and moderate variation among fruits in seed set, and *G. falcata*, with an average of 4.4 ovules per flower and invariably a single seed per fruit (Herrera, 1999). The difference between these species is not great, but it evolved over short branch lengths, and so the resulting contrast is large. Removal of this pair of species from the phylogeny scarcely changes the results from those shown in Table 3. In particular, the partial slopes represented by b_1 remain positive

TABLE 2. Regression analysis of phylogenetically independent contrasts involving the pollen load data.

	Regression model			Partial regression coefficients						
Tree	$F_{2,37}$	P	R^2	b_1	t	P	b_2	t	P	VIF
(a) W	36.5	<0.0001	0.66	0.50	2.89	0.006	0.29	1.71	0.094	3.69
(b) D	38.4	<0.0001	0.67	0.52	3.01	0.005	0.20	1.13	0.265	4.68
(c) W–ubl	19.1	<0.0001	0.51	0.18	0.62	0.539	0.52	1.92	0.062	6.10
(d) D–ubl	16.9	<0.0001	0.48	0.30	1.11	0.272	0.33	1.33	0.191	5.92

Notes: The regressions equations were $y = b_1x_1 + b_2x_2$, in which y represents the PICs for log(ovule number), x_1 represents the PICs for $\log(SD_{pollen})$, and x_2 the PICs for $\log(\bar{X}_{pollen})$. Contrasts were calculated on four phylogenetic trees: (a) W: Wikström et al. (2001); (b) D: Davies et al. (2004); (c) W–ubl: topology of Wikström et al. (2001) with unit branch lengths; (d) D–ubl: topology of Davies et al. (2004) with unit branch lengths. The t tests for the partial coefficients have df = 37. VIF is the variance inflation factor.

and statistically significant. Other large contrasts occurred between *Claytonia virginica* (Portulacaceae), with six ovules per flower, and *Stenocereus queretaroensis* (Cactaceae), with over one thousand; and between *Campsis radicans* (Bignoniaceae), with several hundred ovules per flower, and other taxa within the Lamiales (online Appendix S2).

When we repeated the analysis with only those species in which SD_{seed} was determined at the level of individual flowers, the b_1 coefficients in the multiple regressions retain their statistical significance (Table S3.2 in online Appendix S3). The b_1 coefficients are also significant for those species in which SD_{seed} was determined from inflorescence and plant means (Table S3.3 in online Appendix S3), although there are indications that these data violate an assumption of the PIC technique (Table S3.1 in online Appendix S3).

DISCUSSION

A strong, positive relationship exists between ovule number and stochastic interfloral variation in pollination and fertilization success for a large and taxonomically diverse sample of species. Two different estimates of the angiosperm phylogeny and two measures of floral mating success yielded very similar patterns of association between the variables (Fig. 1). The relationship between ovule number and interfloral variability remained statistically significant after controlling for variation among species in the mean level of pollination and fertilization (Tables 2 and 3). Transformation of the phylogenetic trees to unit branch length did eliminate the independent effect of variation in pollen loads (Table 2), but not of variation in seed number (Table 3). When we divided the seed number data set based on whether SD_{seed} was determined at the floral level or from means first determined for inflorescences or whole plants, we obtained essentially the same patterns that we found with the combined data set (online Appendix S3). Thus, the fundamental pattern we identified seems very robust, at least at the large phylogenetic scale of this analysis.

Taxonomic and life-history patterns—Our results emerge from a taxonomically diverse set of species. The diversity suggests that the evolutionary effect of uncertain mating on ovule number may be pervasive among angiosperms. However, our data set of fewer than 200 species necessarily leaves large phylogenetic gaps in the comparative analysis. It would certainly be useful to examine the hypothesis on a finer phylogenetic scale with a more complete representation of taxa. Large contrasts in Fig. 1 involving clades in the Lamiales and Malvales point to these orders as especially interesting groups in terms of ovule number evolution.

Although we do not report the results here, we used the phylogenetic ANCOVA technique of Garland et al. (1993) to test whether the relationships shown in Fig. 1 differed between groups with different life histories and reproductive traits. We examined woody species compared to herbaceous ones, trees vs. nontree species, annuals vs. perennials, and species that dispersed whole fruits vs. those that dispersed individual seeds from dehiscent fruits. For many of these analyses, we were handicapped by having very few species in a given category. In the pollen load data set, there were only three tree species, only three annuals, and only seven species that dispersed whole fruits rather than individual seeds from dehiscent fruits. In the seed number data set, there were eight tree species, 13 annuals, and 34 species that dispersed whole fruits. Thus, ANCOVAs testing for differences between groups may often have had limited power. Nonetheless, we did not find any evidence of substantial differences between any of these groups. The only minor exceptions were (1) a significant difference in the slope of the relationship of ovule number to SD_{pollen} between woody and herbaceous species (although both slopes were positive and the overlap of data in the scatter plot was substantial), and (2) a lack of a significantly positive slope between ovule number and SD_{seed} for annuals (although there were only 13 species in the analysis). The absence of pronounced difference between these groups with distinct life history and growth habits is consistent with a pervasive pattern of ovule number evolution. Future work on this topic should, however, address these and other important traits, such as self-compatibility and mating system.

TABLE 3. Regression analysis of phylogenetically independent contrasts involving the seed number data.

	Regression model			Partial regression coefficients						
Tree	$F_{2,165}$	P	R^2	b_1	t	P	b_2	t	P	VIF
(a) W	198.4	<0.0001	0.70	0.27	3.80	0.0002	0.51	6.77	<0.0001	3.80
(b) D	174.3	<0.0001	0.68	0.28	3.69	0.0003	0.48	5.80	<0.0001	4.13
(c) W–ubl	211.7	<0.0001	0.72	0.33	4.20	<0.0001	0.52	6.10	<0.0001	4.25
(d) D–ubl	223.8	<0.0001	0.73	0.35	4.36	<0.0001	0.52	5.99	<0.0001	4.44

Notes: Symbols have the same meanings as in Table 2, except that seed number data were used rather than pollen load data, and t tests of the partial coefficients have df = 165. Tree sources are also as in Table 2.

The morphological hierarchy of ovule packaging—ovules within flowers, flowers in inflorescences, and inflorescences on plants—adds further complexity to the issue because there are multiple ways in which ovule production can be adjusted. In particular, the evolutionary relationship between ovule number per flower and flower number per inflorescence seems worthy of future attention from plant reproductive ecologists. For example, certain inflorescence morphologies, such as the capitula of the Asteraceae, seem likely to encourage greater variation in pollination and fertilization success among inflorescences rather than among the flowers within them. Also, intraspecific variation in ovule number deserves attention. Late-blooming flowers often have fewer ovules and lower rates of fruit set than earlier ones, either because of intraplant resource competition (Dudash and Fenster, 1997) or architectural position itself (Diggle, 1995). Thus, the appropriate morphological level or temporal frame in which mating uncertainty has selective effects is likely to differ among species.

Theoretical implications—The association of higher ovule number with greater interfloral variance is broadly consistent with the ovule packaging model of Burd (1995a). The core idea of that model is simple: if plants undergo uncertain and highly disparate acquisition of pollen, they will generally make more seeds in total if every flower is stocked with enough ovules so that unpredictable windfalls of pollen receipt can be converted to large seed production. The more often such windfalls occur and the greater their magnitude, the greater is the ovule number that should be favored by selection. Statistical dispersion measures like SD_{pollen} and SD_{seed} reflect the likelihood of an extremely high pollen load or seed set, so ovule number should be positively related to these measures, and that is what we found. The number of pollen tubes at the base of a style is probably a better indicator of the potential for ovule fertilization in any given flower (Herrera, 2002, 2004), while the data in our analysis represent events before and after the growth of pollen tubes. Nonetheless, consistency in the results between the pollen load data and seed data (Fig. 1) lends some confidence that these two measures parallel the pattern of pollen tube success.

The results of our analysis help explain a common finding of plant reproductive ecologists: flowers that receive supplemental pollen through hand pollination very often have elevated seed production (Burd, 1994b; Ashman et al., 2004). The simplest explanation for this is that flowers must frequently have unfertilized ovules even after exposure to their natural pollination environment. Such overproduction of ovules makes evolutionary sense in the light of stochastic disparity in mating success, but not if flowers have equal success. Even if elevated seed set in these experiments is due in part to a plant's reallocation of resources toward abundantly hand-pollinated flowers (Zimmerman and Pyke, 1988), the implication remains that, on average, flowers contained ovules that would not have been fertilized in the absence of hand pollination.

Recent theoretical work on mating system evolution has tended to emphasize other causes of low seed-to-ovule ratios. Porcher and Lande (2005a, b) have argued that a high ovule number allows plants to absorb the effects of early acting lethal mutations because viable embryos can replace lost ones if there are more viable embryos than flowers could fully provision. Their model showed that this compensation for dead embryos allows the maintenance of intermediate levels of self-fertilization as well as lethal mutations in a population. Thus, the failure of flowers to convert all their ovules to seeds could be due to the poor quality rather than poor quantity of pollen they receive. Harder et al. (2008) have also incorporated this reproductive compensation for embryo mortality into a model of mating system evolution. They concluded that selection on the mating system should leave seed production constrained by either resources or by ovule number, but not by pollen limitation of fertilized ovules. The prevalence of the empirical pollen supplementation effect on seed set is an enigma under this model, unless hand pollination typically substitutes pollen of high genetic quality for low quality pollen (Harder et al., 2008).

Morgan and Wilson (2005) and Cheptou and Schoen (2007) have considered the evolution of rates of self-fertilization as a form of reproductive assurance in the face of pollination shortfalls (rather than embryo mortality) and concluded that stochastic pollination makes it easier for partial selfing to evolve despite the cost of inbreeding depression. Self-fertilization should tend to dampen interfloral variation in the number of ovule fertilizations, and thus the interplay of mixed mating and stochastic pollination may be important in ovule number evolution.

These theoretical analyses must, in the end, confront empirical evidence. Our results suggest that there is a pervasive effect of unpredictable pollination and fertilization on the evolution of ovule number at a broad phylogenetic scale. It is worth noting that none of the models cited incorporate stochasticity at the level of individual flowers. Random variation in ovule fertilization occurs only at the whole-plant level in the models of Morgan and Wilson (2005) and Cheptou and Schoen (2007). Harder et al. (2008) did not incorporate stochastic variation in the fraction of ovules fertilized (although they did allowed stochastic outcomes of competition between selfed and outcrossed embryos and of survival from dispersal to adulthood). The metameric hierarchy of reproductive modules in flowering plants may have important effects on fitness, such that random disparity among modules that make partial contributions to whole-plant female fitness is not adequately modeled by random variation at the whole-plant level. If, as our empirical results imply, random mating success at the floral level is important, it would be interesting to see how including this feature in future models affects predictions for the evolutionary stability of excess ovule production.

Ovule size must be another factor that influences female fitness along with ovule number. Greenway and Harder (2007) found that ovule volume varied more than 100-fold among 45 angiosperm species and that ovule volume was negatively related to ovule number but positively related to floral mass, in an interspecific comparison. Flower size and flower number per inflorescence seem to be subject to an evolutionary trade-off (Sargent et al., 2007) and because floral display size, in turn, affects pollinator behavior (Dudash, 1991), there must be a complex web of interactions creating selection on ovule number.

Conclusions—Overproduction of eggs and offspring (relative to the chances of fertilization or successful development) is a common feature of reproduction in many organisms, and explaining this apparent "parental optimism" is a principal task of life history analysis (Roff, 1992; Mock and Forbes, 1995). We have hypothesized that overproduction of ovules in flowering plants is a common adaptation to unpredictable fertilization opportunities at the floral level, and, consistent with the hypothesis, we have found statistically significant evidence of a positive association between ovule number and two surrogate measures of stochastic variation in ovule fertilization opportunity.

The consistency and clarity of the pattern is encouraging, but the evolution of ovule packaging strategies has not been widely investigated, and there is opportunity for much further work. Our findings suggest testable predictions for macroecological and macroevolutionary patterns in ovule number and seeds per fruit (Hampe, 2003). We expect that plants should have more ovules in habitats where pollination is more uncertain, such as high elevation environments where the coordinated timing between flowering and the emergence of pollinators is critical (Price et al., 2005) or climate is variable (Stone and Jenkins, 2008). Furthermore, variation among flowers in pollen receipt may be more prevalent for some species than others (e.g., among plants that typically occur in low densities or those that rely on high abundances of specialist pollinators that may vary in space and time). We would expect to find higher ovule number in species with these characteristics. Orchids, with their typically high ovule numbers, may provide an example of this. Because even small-scale variation in plant density may affect pollen receipt (Spigler and Chang, 2008), it may be possible to detect selection gradients on ovule number within populations, particularly if density or isolation has been altered through anthropogenic disturbance.

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