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ECOLOGICAL AND EVOLUTIONARY MECHANISMS FOR LOW SEED : OVULE RATIOS: NEED FOR A PLURALISTIC APPROACH?

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Abstract. Central to the ecology and evolution of a broad range of plants is understanding why they routinely have submaximal reproduction manifested as low seed : ovule and fruit : flower ratios. We know much less about the processes responsible for low seed : ovule ratios than we do for fruit : flower ratios. Current hypotheses for low seed : ovule ratios are largely drawn from those for fruit : flower ratios, including proximate (ecological) causes of pollen limitation, resource limitation, and pollen quality, as well as the ultimate (evolutionary) hypothesis of "bet hedging" on stochastic pollination. Yet, such mechanisms operating on fruit : flower ratios at the whole-plant level may not best explain low seed : ovule ratios at the individual-flower level. We tested each of these proximate and ultimate causes for low seed : ovule ratios using the specialized pollination mutualism between senita cacti (*Pachycereus schottii*) and senita moths (*Upiga virescens*). Seed : ovule ratios were consistently low (~0.61). Such excess ovule production by senita likely has a strong genetic component given the significant differences among plants in ovule number and the consistency in ovule production by plants within and among flowering seasons. Excess ovule production and low seed : ovule ratios could not be explained by pollen limitation, resource limitation, pollen quality, or bet hedging. Nevertheless, phenotypic selection analyses did show significant selection gradients for increased ovule number, suggesting that other evolutionary processes may be responsible for excess ovule production and low seed : ovule ratios. In contrast, low fruit : flower ratios at the whole-plant level were explained by an apparent equilibrium between pollen and resource limitation. Thus, mechanisms responsible for low fruit : flower ratios at the whole-plant level are not necessarily in accord with those of low seed : ovule ratios at the individual-flower level. This suggests that we may need to adopt a more pluralistic approach to seed : ovule ratios and consider alternative hypotheses, including a greater array of proximate and ultimate causes. Initial results of this study suggest that floral allometry, selection on correlated floral traits, stigma clogging with pollen grains, and style clogging with pollen tubes may provide promising avenues for understanding low seed : ovule ratios.

Key words: bet hedging; Cactaceae; floral allometry; fruit : flower ratio; Pachycerus schottii; *phenotypic selection; pollen limitation; pollen load; pollen quality; resource limitation; seed : ovule ratio; senita.*

Introduction

Not all plants in nature mature all their ovules and flowers into seeds and fruit. In fact, many plants produce substantially more ovules and flowers than mature seeds and fruit, resulting in reduced reproduction manifested as low seed to ovule and fruit to flower ratios (Sutherland and Delph 1984, Wiens 1984, Ayre and Whelan 1989, Charlesworth 1989). For example, typical seed : ovule ratios of perennials and annuals are 0.50 and 0.85, respectively, while among perennials, seed : ovule ratios of outcrossing species tend to be lower than selfing species (Wiens 1984). Similar patterns occur for low fruit : flower ratios among hermaphroditic plants (Sutherland and Delph 1984, Sutherland 1986). In recent years, increased attention has been given to the potential role of limited seed production (resulting from low seed : ovule and fruit : flower ratios) on the recruitment and dynamics of plant populations (e.g., Ashman et al. 2004, Morgan et al. 2005). Gaining a better understanding of the ecological and evolutionary mechanisms responsible for such low seed : ovule and fruit : flower ratios may thus yield insights into the structure and dynamics of plant populations and communities.

While there is still much to learn about low fruit : flower ratios, some general mechanisms responsible for them have been recognized, including both proximate (ecological) and ultimate (evolutionary) causes (Stephenson 1981, Sutherland and Delph 1984, Ayre and Whelan 1989). Proximate causes involve pollen limitation, resource limitation, and poor pollen quality, each of which reduces fruit set, thereby leading to low fruit : flower ratios. Ultimate causes of low fruit : flower ratios may include selection for increased flower number for pollinator attraction, pollen donation, and/or bet hedging for stochastic pollination. Low fruit : flower ratios result from

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resource (sex) allocation trade-offs between flower and fruit production associated with selection on female vs. male fitness functions of flowers (Haig and Westoby 1988, Brunet 1992, Klinkhamer et al. 1997, Campbell 2000, Galen 2000, Knight et al. 2005).

Presently, we know much less about the processes responsible for low seed : ovule ratios than we do of fruit : flower ratios. Current hypotheses for low seed : ovule ratios are largely drawn from hypotheses for low fruit : flower ratios, including pollen limitation, resource limitation, pollen quality, and bet hedging on stochastic pollination. Little attention has been given to the proximate and ultimate nature of these mechanisms for low seed : ovule ratios. Pollen limitation, resource limitation, and pollen quality (genetic diversity and genetic load) are largely proximate mechanisms, while bet hedging for stochastic pollination is currently the leading ultimate (evolutionary) cause for low seed : ovule ratios. Pollen limitation results in low seed : ovule ratios due to an insufficient quantity of pollen deposited on stigmas for all ovules to be fertilized and matured into seeds. Resource limitation results in low seed : ovule ratios due to an insufficient quantity of resources for all fertilized ovules to mature into seeds. Potentially correlated with resources, plant size may contribute to the number of ovules produced by plants, and hence low seed : ovule ratios. Pollen quality, that is the general compatibility between pollen and ovule genotypes, may reduce seed : ovule ratios due to unsuccessful fertilization or abortion of incompatibly fertilized ovules (Wiens et al. 1987, 1989, Charlesworth 1989). Increased genotypic diversity through multiple pollen donors may minimize such genetic issues, thereby increasing seed : ovule ratios. Alternative to these proximate causes, plants may have evolved a bet-hedging strategy of producing excess ovules to capitalize on stochastic variation in pollen loads or genetic diversity of pollen deposited on stigmas (Burd 1995).

While pollen limitation, resource limitation, pollen quality, and bet hedging are each feasible mechanisms that may contribute to low seed : ovule ratios, such processes operating on fruit : flower ratios at the whole-plant level may not necessarily extend to low seed : ovule ratios at the individual-flower level. For example, unlike excess flower production at the whole-plant level, which can increase pollinator attraction and male fitness, excess ovule production at the individual-flower level may not contribute directly to plant fitness. It may well be that alternative processes to those operating on fruit : flower ratios contribute to low seed : ovule ratios. Excess ovule production and low seed : ovule ratios may occur due to allometric correlations between ovule number and other floral traits and selection on floral traits correlated with ovule number.

In this study, we tested leading hypotheses for low seed : ovule ratios, including pollen limitation, resource limitation, pollen quality, and bet hedging on stochastic pollination. We also examined the less recognized mechanisms of floral allometry and phenotypic selection on floral traits correlated with ovule number. We tested these hypotheses using the specialized pollination mutualism between senita cacti and senita moths. The senita system presents a paradox for understanding the mechanisms responsible for low seed : ovule ratios. On the one hand, evolution has favored specialization on a pollinator. Senita moths are reliable, providing sufficient pollination at the whole-plant level so that routinely low fruit : flower ratios typically do not result from pollen limitation, but instead result from excess flower production for pollinator attraction (and pollen donation) and resource (sex) allocation trade-offs between flower and fruit production (Holland 2002, Holland and Fleming 2002, Holland et al. 2004*a, b*). Yet, despite moths being effective pollinators at the whole-plant level, preliminary data indicated that senita still produce excess ovules and have low seed : ovule ratios at the level of individual flowers.

METHODS

The study system.—We studied senita cacti (*Pachycerus schottii*) at Organ Pipe Cactus National Monument in Arizona, USA in 2002 and 2005. At this site, senita cacti produce 10–40 flowers per night from April through July. Senita cacti produce hermaphroditic flowers, with an inferior ovary containing one pistil, many ovules, and hundreds of anthers. Only 50% of flowers produce any nectar at all, with an average 0.5 μL in nectar-producing flowers. At sunset, flowers open and anthers dehisce; flowers close within 12 h of their opening but typically close prior to sunrise, thereby excluding diurnal pollinators. Senita cacti rely on pollination by an obligate seed-eating moth *Upiga virescens* (Pyralidae), as plants are entirely self-incompatible and copollinating bees are typically unimportant. Female moths actively pollinate by rubbing pollen-covered abdomens directly onto stigmas and then lay a single egg on the open corolla. Before departing a flower, but after active pollination, moths collect pollen by rubbing their abdomen among anthers. Low fruit : flower ratios and fruit set (the fraction of flowers initiating fruit maturation) at the whole-plant level result from trade-offs in resource (water) allocation between flower production and fruit set, though pollen limitation can occur. Fruit abortion occurs within 6 d after anthesis and fruits mature within 30 d. Fruit abortion does not appear to be selectively based on pollen quality, pollen quantity, or oviposition. Differential resource allocation does not appear to occur among open- and hand-pollinated flowers (Zimmerman and Pyke 1988). Senita is ideal for this study, as they have a specialized pollinator that rarely limits fruit set at the whole-plant level, but excess ovule production occurs at the level of flowers. These and other descriptions of the senita system are found elsewhere (Holland and Fleming 1999, 2002, Holland 2002, Holland et al. 2004).

Ovules, seeds, and seed : ovule ratios.—We quantified ovule production, seed production, and seed : ovule ratios for senita cacti both within and among flowering seasons. In 2002 and May and June 2005, we quantified within- and among-plant variation in ovules per flower, seeds per fruit, and seed : ovule ratios for 25–35 plants. We could not count both ovules and seeds from the same flowers because sampling was destructive, so from the same plants we collected 3–7 flowers to count ovules and ~30 d later 3–7 ripe fruit to count seeds. Ovaries were preserved in FAA (formalin : acetic acid : alcohol) and later ovules were counted using a dissecting microscope (Kearns and Inouye 1993). We tested for differences among plants in ovule number and seed number using ANOVA, with multiple flowers or fruit sampled within plants as replicates. We examined if differences occurred for ovule number and for seed number between 2002 and 2005 and if ovule number differed between May and June 2005 using paired-difference *t* tests. We examined if seed : ovule ratios differed from the null hypothesis of an expected mean of 1.0 using a *t* test. For these latter two analyses (and others below), we used averages of within-plant subsamples of flowers and fruit (Gotelli and Ellison 2004). Analyses here and below were performed using SAS (version 8.02) or JMP (version 5.1.2).

Pollen quantity.—We tested the pollen limitation hypothesis by examining whether pollen limits fruit : flower ratios at the level of whole plants (fruit set) and whether pollen limits seed : ovule ratios at the level of individual flowers (seeds per fruit) using pollen supplementation experiments. We conducted three trials, one in June 2002 and two (May and June) in 2005. We measured fruit set and seed production for 8–15 open-pollination control (OP) and 6–15 pollen-supplementation (HP) flowers on 11 plants in 2002 (274 flowers) and 19 and 21 plants in May and June of 2005 (890 flowers). Stigmas of HP flowers were given excess pollen from ~50 anthers of a fresh flower from another plant. We examined fruit set and mean seed production per fruit between OP and HP flowers with individual plants as replicates using two-tailed paired-sample *t* tests. Sample sizes varied among response variables because not all flowers set fruit and some fruit were lost to natural forces. These experiments allowed us simultaneously to examine pollen limitation of fruit : flower ratios at the level of whole plants via measures of fruit set (the fraction of flowers initiating fruit) and seed : ovule ratios at the level of individual flowers.

Stigma pollen loads.—We further examined the pollen limitation hypothesis for low seed : ovule ratios by quantifying pollen loads (number of pollen grains) deposited on stigmas by senita moths. In both May and June 2005, we examined whether pollen loads were large enough to fertilize all ovules. In May we sampled five flowers from each of 24 plants; in June we sampled one flower from each of 26 plants. Pollinated flowers were identified by inspecting stigmas with a hand lens for pollen and corollas for an egg. Flowers were assumed to be pollinated once, as flowers with multiple eggs (pollinations) are statistically rare events (Holland and Fleming 1999). False positive pollinations via selfing were unlikely because stigma height of herkogamous flowers exceeds anther height. Before collecting each flower, we removed the portion of the pistil containing the stigma. Stigmas were placed in vials of 70% ethanol to later count pollen grains. Ovaries were preserved in FAA. Pollen loads were estimated by staining pollen grains on stigmas with Calberla's fluid (Surveillance Data, Plymouth Meeting, Pennsylvania, USA) and then counting with a microscope (Kearns and Inouye 1993). The number of pollen grains remaining in vials was estimated by counting six 0.1 mL aliquot subsamples and extrapolating based on ethanol volume. We compared pollen loads with ovule number in both May and June using *t* tests. We used means of the five subsamples of flowers per plant for the May trial (Gotelli and Ellison 2004). Bet-hedging analyses examined within-plant variation in pollen loads of the five subsamples.

We were unsuccessful in manipulating pollen loads, in particular small loads less than ovule number, which prevented us from experimentally examining how seed production varied with the amount of pollen. However, we were able to examine if seed production varied with pollen quantity by studying natural variation in seed production with natural variation in pollen loads. We haphazardly chose 1–7 pollinated flowers on each of 20 plants. We emasculated flowers after pollination to avoid confounding effects of within-flower pollen transfer to stigmas during corolla wilting. Within-flower pollen transfer prior to flower closing is highly unlikely, as stigma height of herkogamous flowers exceeds anther height and the stereotyped active pollination behavior of moths entails first pollinating flowers upon their arrival and then no subsequent interactions with stigmas. Two days after flowers closed, we clipped the top 15 mm of wilting corollas to collect pollinated stigmas. Approximately 25 days later, we collected the mature fruit and counted seeds. Corolla clipping did not alter seed number relative to unclipped flowers (paired difference *t* test, df = 17, $t = 0.487$, $P = 0.684$). We examined if seed : ovule ratios and seed number varied with pollen loads using regression after averaging within plant subsamples (Gotelli and Ellison 2004).

Bet hedging.—We conducted multiple analyses of predictions and assumptions of bet-hedging theory for stochastic variation in pollination. First, we tested the prediction that ovule number increases as the cost of producing an ovule (C_o) decreases relative to the costs of a flower (C_f): that is C_f/C_o (Burd 1995). For this desert plant for which water is known to limit reproduction (Holland 2002), we estimated C_f and C_o based on the wet mass of flowers (to 0.01 g) and ovules (to 0.001 mg) from 36 plants. Ovules were counted, and the cost of an ovule was calculated by dividing the mass of all ovules by ovule number. We examined if ovule number varied with C_f/C_o using regression after a log transformation. We further examined if a trade-off occurred between flowers and ovules by regressing mean ovule production on flower number of individual plants.

Second, we tested the bet-hedging hypothesis that ovule number exceeds mean pollen loads using t tests and the above data on stigma pollen loads. Third, we tested the prediction that if plants are bet hedging on stochastic variation in pollen loads, then variance in seed production should increase with variance in pollen loads. Using seed number and pollen loads from the above described studies, we examined if the intraplant coefficient of variation (CV) for seed production increased with the intraplant CV for pollen load using regression. Fourth, using regression analyses and our previously described studies on seed and ovule numbers, we tested the prediction that if the pollination environment is stochastic, then the intraplant CV for seed number should increase with ovule number (Knight et al. 2005). Lastly, we tested the hypothesis that if plants are bet hedging on stochastic pollen loads, then the difference in seed production between HP and OP should increase with ovule production (Burd 1995, Ashman et al. 2004, Knight et al. 2005). We used regression to examine if HP – OP seed number increased with ovule number for pollen-supplementation experiments of both May and June 2005.

Plant size and resource limitation.—We examined whether plant size contributes to low seed : ovule ratios by examining if ovules per flower ($n = 34$ plants), seeds per fruit ($n = 30$ plants), and seed : ovule ratios ($n = 29$ plants) varied with plant size. We measured all stem lengths on each plant to 0.03 m and used cumulative length for a measure of plant size (range: 17–179 m). Measures of stem girth do not reflect plant size, as cacti do not have secondary cambium. We examined if ovule number, seed number, and seed : ovule ratios varied with plant size using regression.

If resources, rather than pollen, are limiting plants, then small plants with few resources may have fewer ovules per flower, fewer seeds per fruit, and lower seed : ovule ratios than large plants with more resources (Griffin and Barrett 2002). Thus, analyses of plant size may provide some insight into resource limitation. Because such an analysis is not a particularly strong test of resource limitation, we also examined results of a prior experiment on a different senita population that directly manipulated water resources (Holland 2002). In that study, senita cacti were randomly assigned to water addition and control (unwatered) treatments. Treatments were maintained for two years (1999, 2000). In each year, flowers on plants of both treatments were hand pollinated and seed production analyzed using a repeated-measures ANOVA. A complete description of that study can be found in Holland (2002).

Pollen quality.—We tested whether pollen quality (genotypic pollen donors) alters seed production using two separate pollen supplementation experiments. In both experiments, we held pollen quantity constant and manipulated the number of outcross pollen donors. For each treatment flower, we applied pollen from ~100 anthers to each stigma but varied the number of pollen donors. For the first experiment conducted in 2002, we used an unreplicated randomized block design in which each of five pollen donor treatments (one, two, three, four, and five pollen donors) were applied per block ($n = 11$ plants). Identities of pollen donors were held constant for each treatment. We used a general linear model to analyze pollen donor and plant block effects on seed production for this experiment. In the second experiment conducted in 2005, we used a fully randomized design in which 32 plants were randomly assigned pollen donor treatments (one, two, three, and four pollen donors). Five flowers were treated per plant. Identities of pollen donors varied haphazardly among replicate plants. We used one-way ANOVA to analyze pollen donor effects on seed production, using mean seeds per fruit for replicate plants.

Floral allometry and phenotypic selection.—In 2005, we collected 3–7 flowers from 38 plants to assess if allometry occurred among the floral traits of ovule number, anther number, flower wet mass (to 0.01 g), corolla width, and corolla length. We measured corolla width and length with calipers to 0.1 mm. Corolla width was the distance from the opposing tips of the perianth. Corolla length was from the top of the inferior ovary to the anthers within the corolla. We examined allometry among floral traits with Pearson's product-moment correlation after log transformation (Niklas 1994). We used means of within-plant measures of floral traits.

We examined phenotypic selection gradients on ovule number per flower in 2002 ($n = 20$ plants) and 2005 ($n = 29$ plants). We quantified seeds per fruit and ovules per flower for individual plants as described above. In 2005 we also measured other floral traits listed above. We examined selection on ovule number in 2002 and multivariate selection on ovules and correlated floral traits in 2005 by regressing relative fitness ($W_i/\bar{W}$) on trait values standardized to a mean of zero and a standard deviation of 1.0 (Lande and Arnold 1983). Sample size was not sufficient to test nonlinear selection. For multivariate selection of 2005, all variance inflation factors (VIF) were <3.0 indicating no confounding effects of multicollinearity. Flower mass did have a VIF twice that of other traits, so we performed analyses with and without this variable; qualitative results of the models did not differ. We also performed analyses with log-transformed data, which did not alter model results. Selection gradients are the partial regression coefficients.

RESULTS

Ovules, seeds, and seed : ovule ratios.—On average, senita cacti produced 250 ovules per flower, 150 seeds per fruit, and a seed : ovule ratio of 0.61 (Table 1). Ovule number varied more among plants than within plants.

TABLE 1. Mean (±SE), range, and coefficient of variation (CV) for ovules per flower, seeds per fruit, and seed : ovule (S:O) ratios for senita cacti in years 2002 and 2005.

	2002			2005		
	$\bar{X}$ ± SE	Range	CV	$\bar{X}$ ± SE	Range	CV
Ovules	250 ± 12	125–395	25.5	241 ± 13	135–363	25.3
Seeds	140 ± 10	70–225	34.2	161 ± 8	72–242	26.7
S:O	0.58 ± 0.04	0.30–0.98	31.9	0.64 ± 0.03	0.32–0.90	23.5

Coefficients of variation (CV) in ovule number among plants for 2002 and 2005 were 25.5 and 25.3, respectively, whereas mean CV for ovule number within plants were 12.7 and 11.7, respectively. There were significant differences among plants in ovule number for both 2002 ($F_{25,104} = 22.7$, $P < 0.0001$) and 2005 ($F_{24,76} = 14.6$, $P < 0.0001$), but there was no difference in ovule number between 2002 and 2005 ($t_{22} = -1.53$, $P = 0.145$) or between May and June 2005 ($t_{21} = -0.958$, $P = 0.349$). Compared with ovules, seeds per fruit did not vary as much among plants as within plants. Coefficients of variation in seed number among plants for 2002 and 2005 were 34.2 and 26.7, respectively, whereas mean CV for seed number within plants were 28.2 and 26.0. As with ovules, there were significant differences among plants in seeds per fruit for both 2002 ($F_{22,124} = 5.3$, $P < 0.0001$) and 2005 ($F_{29,113} = 4.37$, $P < 0.0001$), but there was no difference in seed production between 2002 and 2005 ($t_{16} = -0.074$, $P = 0.942$).

Just over half of ovules matured into seeds, resulting in low seed : ovule ratios (~0.61; Table 1) in both 2002 ($t_{20} = -11.1$, $P < 0.0001$) and 2005 ($t_{28} = -12.8$, $P < 0.0001$). Seed : ovule ratios did not differ between 2002 and 2005 ($t_{14} = -0.021$, $P = 0.983$). These results indicate that seed : ovule ratios of senita are substantially small and that there is likely a strong genetic component to ovule production given the significant differences among plants in ovule number and the consistency in ovule production by plants both within and among flowering seasons.

Pollen quantity.—In June 2002 and May and June 2005, we examined if pollen quantity limited plant reproduction, both at the level of whole plants (fruit set; Fig. 1A) and at the level of ovules within flowers (seeds; Fig. 1B). In 2002, fruit set was not pollen limited; no difference in fruit set occurred between open-pollinated (OP) and pollen-supplemented flowers (HP; HP – OP; $t_{10} = 0.641$, $P = 0.536$). In May 2005, fruit set was pollen limited (HP – OP; $t_{18} = 6.22$, $P < 0.001$), but in June 2005, it was only marginally limited by pollen (HP – OP; $t_{19} = 2.24$, $P = 0.037$). Seed production was not pollen limited in any of the trials (2002: HP – OP, $t_8 = 0.69$, $P = 0.508$; May 2005: HP – OP, $t_{13} = -0.88$, $P = 0.393$; June 2005: HP – OP, $t_{12} = -2.05$, $P = 0.063$). Thus, at the individual-flower level, pollen limitation did not result in low seed : ovule ratios, but at the whole-plant level, both pollen and resource limitation contributed to reduced fruit set and hence low fruit : flower ratios.

Stigma pollen loads.—We further tested the pollen-limitation hypothesis for low seed : ovule ratios by examining pollen loads deposited on stigmas by senita moths. First, we quantified pollen loads deposited on stigmas immediately following moth pollination. In May and June 2005, mean (±SE) pollen deposition on stigmas was 2753 ± 186 pollen grains (range: 1567–5559; $n = 25$ plants) and 2449 ± 153 pollen grains (range: 1430–4354; $n = 26$ plants). Pollen loads deposited on stigmas of flowers did not differ between trials of May and June 2005 ($t_{16} = 0.6115$, $P = 0.550$). These pollen loads deposited on stigmas by moths were significantly

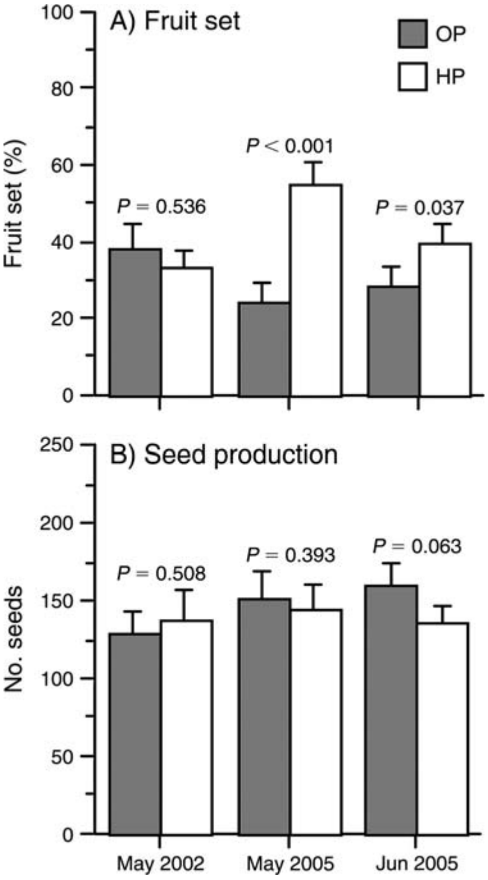

FIG. 1. Mean (+SE) fruit set (percentage of flowers) and seeds per fruit for open-pollinated (OP) and pollen-supplemented (HP) flowers for 2002 and May and June 2005 trials. *P* values for paired-sample *t* tests are for differences in fruit set or seed number between OP and HP.

greater than ovule number in both May ($t_{24} = 13.9$, $P < 0.001$) and June ($t_{25} = 14.8$, $P < 0.001$). Second, we quantified whether seed production and seed : ovule ratios were limited by natural pollen loads deposited by moths. Seed number per fruit did not vary with pollen load ($r^2 = 0.111$, $F_{1,18} = 2.237$, $P = 0.152$; $y = 0.027x + 104$) nor did seed : ovule ratios vary with pollen load ($r^2 = 0.003$, $F_{1,16} = 0.0403$, $P = 0.844$; $y = 0.000x + 0.589$). These results on stigma pollen loads indicate that pollen did not limit seed number or reduce seed : ovule ratios of senita cacti.

Our analyses and results of such large pollen loads (~2600 grains) compared with ovule numbers (~250) were not confounded by within-flower pollen transfer to stigmas, as we emasculated flowers prior to their closing to avoid self pollen during flower wilting. Also, stigma height of herkogamous flowers exceeds anther height, thereby preventing within-flower pollen transfer during anthesis. In addition, senita moths have evolved stereotyped active pollination behaviors. On arrival at a flower, moths first pollinate, only interacting with the stigma. Behaviors following active pollination, including oviposition and pollen collection, do not include interactions with stigmas. Moreover, because senita cacti are entirely self-incompatible, selection has likely favored outcross pollination by moths and avoidance of self pollen, otherwise eggs deposited by moths on flowers would die due to abortion of selfed flowers.

Bet hedging.—We conducted multiple analyses of assumptions and predictions of bet hedging for stochastic variation in pollination. Theory predicts that for such a strategy to be favored, ovule number should increase as the cost of producing an ovule (C_o) decreases relative to the costs of a flower's production (C_f); that is, ovule number should increase with C_f/C_o. Consistent with bet-hedging theory, ovule number did increase with C_f/C_o ($r^2 = 0.14$, $F_{1,35} = 5.69$, $P = 0.023$; $y = 0.339x + 0.950$), suggesting that excess ovule production per flower may be favored given the small costs of ovules relative to flowers. In addition, trade-offs did not occur between ovule number and flower number ($r^2 = 0.001$, $F_{1,22} = 0.014$, $P = 0.908$; $y = 0.0834x + 245$). Empirical support for these two assumptions of bet-hedging theory indicate that such a strategy is feasible in senita. However, all other analyses of bet-hedging indicate that senita do not employ a bet-hedging strategy of excess ovule production to increase seeds under stochastic pollination.

First, if plants are employing a bet-hedging strategy of producing excess ovules to take advantage of large pollen loads, then ovule number should exceed mean pollen loads deposited on stigmas of flowers. Yet, pollen loads were consistently and substantially greater than ovule number. Stigmatic pollen loads were a mean of 2600 grains (minimum of 1400 grains), while ovule number was a mean of 250 per flower (maximum of 450 ovules). For no case was pollen load less than ovule number. Second, if plants bet hedge on stochastic variation in pollen loads, then variance in seed production should increase with variance in pollen load. Yet, intraplant coefficient of variation (CV) in seed number was unrelated to intraplant CV in pollen load ($r^2 = 0.108$, $F_{1,17} = 2.06$, $P = 0.169$; $y = 0.262x + 16.94$). Third, if the pollination environment is stochastic, then the intraplant CV for seed number should also increase with ovule number. Yet, no significant relationship was observed between intraplant CV for seed number and ovule number per plant ($r^2 = 0.00$, $F_{1,17} = 0.001$, $P = 0.971$; $y = 0.0016x + 23.95$). Lastly, if plants are bet hedging on stochastic pollen loads, then the difference between HP and OP seed production of pollen-supplementation experiments is predicted to correlate positively with ovule production. Yet, no relationship was observed between HP – OP seed number and ovule number in either May ($r^2 = 0.020$, $F_{1,12} = 0.248$, $P = 0.627$; $y = -0.07x + 9.5$) or June ($r^2 = 0.109$, $F_{1,11} = 1.345$, $P = 0.271$; $y = 0.22x - 83.2$) trials of 2005. Despite its feasibility, producing excess ovules as a bet-hedging strategy for large pollen loads did not explain low seed : ovule ratios of senita.

Plant size and resource limitation.—No significant relationship occurred between plant size and ovule number ($r^2 = 0.039$, $F_{1,32} = 1.31$, $P = 0.261$; $y = 0.402x + 228$), seeds per fruit ($r^2 = 0.062$, $F_{1,28} = 2.237$, $P = 0.186$; $y = -0.416x + 190$), or seed : ovule ratios ($r^2 = 0.101$, $F_{1,27} = 3.04$, $P = 0.093$; $y = -0.002x + 0.730$), even though pollen loads were large enough for the maturation of all ovules into seeds. Assuming plant size is a reasonable surrogate for resources, small plants with few resources were predicted to have fewer ovules per flower, fewer seeds per fruit, and lower seed : ovule ratios than large plants with more resources (Griffin and Barrett 2002), then it appears that resource limitation is at least not a strong determinant of low seed : ovule ratios in senita. We further addressed resource limitation of seed production by examining results of a prior experiment that manipulated water resources (Holland 2002). Hand-pollinated flowers on control, unwatered plants had a mean (±SE) of 125 ± 9 and 137 ± 8 seeds per fruit in 1999 and 2000, respectively. Hand-pollinated flowers on water-addition plants produced 154 ± 8 and 167 ± 11 seeds per fruit in 1999 and 2000, respectively. Watered plants did produce significantly more seeds per fruit than control, unwatered plants (repeated-measures ANOVA, $F_{1,48} = 6.98$, $P < 0.02$). If ovule production in that senita population was at all similar to that of this study population (~250 ovules per fruit), then seed : ovule ratios still remained substantially low. Collectively, these analyses suggest resources can contribute modestly to low seed : ovule ratios, but that resource limitation alone cannot explain routinely low seed : ovule ratios in senita cacti.

Pollen quality.—We tested the hypothesis that pollen quality and genetic load alters seed production using two separate pollen supplementation experiments in 2002 and 2005. If pollen quality and genetic load reduce seed : ovule ratios, then increasing the number of genotypic pollen

TABLE 2. Allometric relationships and mean values (±SE) of six traits of senita cactus flowers.

		Allometric correlations among phenotypic traits			
Trait	$\bar{X} \pm$ SE	No. anthers	Flower mass	Corolla length	Corolla width
Ovule number	256 ± 10	0.305† (34)	0.475** (38)	0.355* (38)	0.108 (38)
No. anthers	123 ± 3		0.420* (34)	0.330† (34)	0.120 (34)
Flower mass (g)	1.8 ± 0.07			0.608*** (38)	0.673*** (38)
Corolla length (mm)	20.8 ± 0.36				0.318† (38)
Corolla width (mm)	37.9 ± 0.54				

Notes: Values for pairwise relationships are Pearson product-moment correlations of log–log analyses. Sample sizes (number of plants) are in parentheses.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

donors should lead to a greater number of compatible pollen donors and hence increase seed : ovule ratios. For the unreplicated randomized block design in 2002, seed production did not vary with the number of genotypic pollen donors ($F_{4,17} = 0.472$, $P = 0.756$) or plant block ($F_{10,17} = 1.059$, $P = 0.441$). For the fully randomized experimental design in 2005, seed production also did not vary with the number of genotypic pollen donors ($F_{3,24} = 0.9413$, $P = 0.436$). Even though the number of pollen donors varied from one to five genotypes, seed production remained relatively constant at ~150 seeds per fruit, which corresponded with seed numbers from pollen quantity experiments (Fig. 1B) and natural variation studies (Table 1). Thus, pollen quality and genetic load did not appear to contribute to low seed : ovule ratios of senita cacti.

Floral allometry and phenotypic selection.—Low seed : ovule ratios may occur because ovule number is constrained to a value based on its allometric relationships with other floral traits. Significant correlations occurred among multiple traits of senita flowers (Table 2). Ovule number correlated with anther number, flower mass, and corolla length. As flower mass and corolla length increased, so did ovule number. If ovule number is correlated with another floral trait upon which selection is acting, then low seed : ovule ratios may result from selection on correlated floral traits. Regression models of phenotypic selection were significant in both 2002 ($r^2 = 0.253$, $F_{1,19} = 6.397$, $P = 0.0204$) and 2005 ($r^2 = 0.454$, $F_{5,24} = 3.99$, $P = 0.0089$), with positive selection gradients on ovule number in both years (Table 3). However, no significant relationship occurred between seed : ovule ratio and ovule number in either 2002 ($r^2 = 0.120$, $F_{1,19} = 2.582$, $P > 0.05$) or 2005 ($r^2 = 0.253$, $F_{1,26} = 4.186$, $P > 0.05$). Furthermore, multivariate selection analyses of the few correlated floral traits measured in 2005 showed no significant selection on other floral traits, including anther number, flower mass, corolla length, or corolla width (Table 3). Despite the lack of support for pollen limitation, resource limitation, pollen quality, and bet-hedging, phenotypic selection analyses indicate that plants producing more ovules per flower also produced more seeds per fruit. This suggests that even though not all ovules mature into seeds (i.e., low seed : ovule ratios), producing more ovules leads to more seeds.

DISCUSSION

Current hypotheses for low seed : ovule ratios are largely based on a subset of those processes proposed to explain low fruit : flower ratios, including pollen limitation, resource limitation, pollen quality, and bet hedging for stochastic pollination. Processes contributing to low fruit : flower ratios at the whole-plant level, however, are not necessarily operating on seed : ovule ratios at the individual-flower level. Little attention has been given to alternative ecological (proximate) and evolutionary (ultimate) causes of low seed : ovule ratios. In this study, we tested the leading proximate and ultimate hypotheses for low seed : ovule ratios in the specialized pollination mutualism between senita cacti and senita moths. As predicted by theory (Haig and Westoby 1988, Ashman et al. 2004), low fruit : flower ratios at the whole-plant level appeared to fluctuate around an equilibrium between pollen and resource limitation (Fig. 1A; Holland and Fleming 2002). This pollen/resource equilibrium and ensuing low fruit : flower ratios result from excess flower production for pollinator attraction and pollen donation and associated resource (sex) allocation trade-offs between flower and fruit production (Holland 2002, Holland and Fleming 2002, Holland et al. 2004*a, b*). On the other hand, at the individual-flower level we found little to no empirical support for pollen limitation, resource limitation, pollen quality, or bet hedging to explain excess ovule production and low seed : ovule ratios (~0.61). In discussing these results below, we suggest that a more pluralistic approach may

TABLE 3. Standardized selection gradients for ovules in 2002 ($n = 21$ plants) and for ovules and other floral traits (number of anthers, flower mass, corolla length, and corolla width) in 2005 ($n = 30$ plants), based on relative fitness of mean seed production per fruit per plant.

Trait	β	SE	*P*
2002			
No. ovules	0.168	0.066	0.0204
2005			
No. ovules	0.260	0.065	0.0005
No. anthers	0.046	0.057	0.4245
Flower mass	−0.111	0.091	0.2301
Corolla length	0.029	0.061	0.6413
Corolla width	−0.031	0.073	0.6726

need to be taken to better understand mechanisms resulting in low seed : ovule ratios including, in particular, the expansion of hypotheses to incorporate alternative ecological and evolutionary processes.

We found little to no empirical support for either pollen quantity or quality to be a proximate cause of low seed : ovule ratios in senita. Pollen loads deposited on stigmas never limited seeds per fruit or seed : ovule ratios. Pollen loads never fell below ovule number, and were routinely 10 times greater than ovule number. These results for natural variation in pollen loads are consistent with experimental results, in which pollen quantity also did not limit seeds per fruit or seed : ovule ratios. In addition to pollen quantity, pollen quality could not explain low seed : ovule ratios. If senita had low seed : ovule ratios due to poor matches between pollen and ovule genotypes, then increasing the genotypic diversity of pollen deposited on stigmas should have increased seed : ovule ratios. However, seeds per fruit and seed : ovule ratios did not increase with the number of pollen donors in either of our experimental designs varying pollen donors. Among the multiple methods used, including studies of natural variation, hand-pollination, and genotypic pollen donors, remarkably little variation occurred in seeds per fruit and seed : ovule ratios. Such consistency in results between natural variation and experimental studies indicates that confounding effects of hand-pollination techniques were not occurring. While pollen limitation is increasingly recognized to be more common than previously thought (Larson and Barrett 2000, Ashman et al. 2004, Knight et al. 2005, 2006), results of this study are consistent with others in that aspects of pollen quantity and quality could not explain routinely low seed : ovule ratios (Griffin and Barrett 2002).

Resource limitation and plant size also did not explain low seed : ovule ratios of senita. If resources cause low seed : ovule ratios, then small plants may be predicted to have fewer seeds than large plants with more resources (Griffin and Barrett 2002). Yet, plant size did not influence ovules per flower, seeds per fruit, or seed : ovule ratios. This was a fairly weak test for a large plant with thousands of flowers like senita. A stronger test was provided by our analyses of seeds per fruit from a prior experimental study that manipulated water resources. Those analyses did show a significant increase in seeds per fruit between control, unwatered, and water-addition plants. However, seeds per fruit of watered plants still remained low (~160), despite having hand-pollinated flowers. If ovule production in that population was at all similar to that of this study population, then seed : ovule ratios of watered plants also remained substantially low (~0.64). Thus, while resource limitation can make a modest contribution to low seed : ovule ratios, it alone cannot explain routinely low seed : ovule ratios of senita.

Bet hedging for stochastic pollination is currently the leading ultimate cause for excess ovule production and low seed : ovule ratios. Indeed, great temporal and spatial variation occurs in pollinator availability and pollen deposition on stigmas, making bet hedging a central evolutionary hypothesis. Results of this study support assumptions of Burd's (1995) ovule packaging hypothesis in that plants may package more ovules in flowers if their costs are low relative to a flower (C_f/C_o). However, we found no empirical support for bet hedging on stochastic pollination to explain excess ovule production and low seed : ovule ratios in senita. In fact, contrary to meta-analyses and predictions of bet-hedging theory (Burd 1995, Ashman et al. 2004, Knight et al. 2005), mean ovule production was never greater than mean pollen receipt; variation in pollen loads was not correlated with variation in seed production; within plant variation in seed production was not correlated with ovule production; and pollen limitation (HP − OP seed production) from hand-pollination experiments did not increase with ovule number.

In addition to bet hedging on stochastic pollen loads, plants may produce excess ovules to bet hedge on stochastic variation in pollen quality, rates of predispersal seed predation, or environmental resources. If plants are bet hedging for pollen with high genetic diversity, then our pollen donor experiments should have increased seeds per fruit and seed : ovule ratios, which they did not. Senita cannot bet hedge on rates of seed predation by producing extra ovules per flower because senita moth larvae necessarily induce abscission of immature fruit as they exit them to pupate in cactus stems, regardless of the number of ovules produced. Lastly, a long-lived desert plant with resource-storage capacity, such as water storage by senita, is unlikely to bet hedge on resources. If senita were bet-hedging on resources, then seeds per fruit should have increased more than they did in the water-addition experiment (Holland 2002).

Despite lack of support for current proximate and ultimate causes of low seed : ovule ratios, analyses of phenotypic selection did show significant selection gradients for increased ovule production. Plants that produced more ovules per flower also produced more seeds per fruit, but not as a trade-off with flower number. Such excess ovule production by senita likely has a strong genetic component given the significant differences among plants in ovule number and the consistency in ovule production by plants within- and between-flowering seasons. Even though evolution has favored plant specialization on senita moths, and moths are effective pollinators providing sufficient pollen quantity and quality for all ovules to mature seeds, senita nevertheless still produces excess ovules and selection appears to favor more ovules than can be matured into seeds. Thus, the senita system presents a paradox for current hypotheses for low seed : ovule ratios, as the mechanism responsible for low seed : ovule ratios remains elusive. Lack of support for current hypotheses suggests that processes explaining low fruit : flower ratios at the whole-plant level are not necessarily in accord with those explaining low seed : ovule ratios at the individual-flower level. Unlike excess flower production, which can increase male fitness at the whole-plant level, excess ovule production may not contribute directly to plant fitness at the individual-flower level. Current theory and mechanisms may be only part of the story explaining routinely low seed : ovule ratios among plants (Charlesworth 1989).

At the risk of being redundant, why then does a plant with a reliable, effective pollinator produce excess ovules and have low seed : ovule ratios when it would be expected not to do so? We suggest that a more pluralistic approach may need to be taken by expanding hypotheses on low seed : ovule ratios to incorporate alternative ecological and evolutionary processes. We propose several alternative proximate and ultimate causes that have general applicability. First, stigma clogging with pollen grains or style clogging with pollen tubes may be proximate causes of low seed : ovule ratios. Stigma clogging due to heterospecific pollen or incompatible (self) pollen can certainly be important in many systems, but we emphasize that low seed : ovule ratios may arise from the clogging of stigmas and styles simply due to the numerical abundance of pollen grains deposited onto stigmas. For example, senita moths are unlikely to deposit heterospecific pollen, as they are host-specific to senita cacti, and they are also unlikely to deposit self pollen as discussed previously. Yet, senita moths did deposit thousands more pollen grains than flowers had ovules, which could have resulted in stigmas clogging with pollen grains and/or styles clogging with pollen tubes, both of which could be an alternative ecological mechanism for low seed : ovule ratios among many species of plants.

Second, floral allometry and constraints on floral development may be ultimate causes that lead to correlated selection on floral traits, including ovule number. Allometry and developmental constraints abound among plants (Niklas 1994). In this study, phenotypic correlations did occur among multiple floral traits, but selection did not appear to be acting on the particular traits we measured. Nevertheless, selection may be acting on some other trait correlated with ovule production. In particular, selection may favor a floral morphology that reduces stigma or style clogging due to the numerical abundance of pollen, including dimensions of stigmas and styles not measured in this study. In doing so, selection may also lead to increases in ovule number due to genetic correlations with other such floral traits, thereby increasing seed production by maintaining low seed : ovule ratios.

Although our study is of only one plant species, our results do highlight the need for alternative hypotheses and mechanisms that build upon or possibly diverge from contemporary hypotheses for low seed : ovule ratios. In particular, we suggest that floral allometry, development constraints, stigma clogging, style clogging, and phenotypic selection on correlated floral traits are possible promising avenues of study. We hope that future studies will begin to include such hypotheses and adopt a more pluralistic view in evaluating their feasibility.

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