International Journal of Botany

ISSN: 1811-9700

International Journal of Botany 2 (1): 83-92, 2006
ISSN 1811-9700

Effects of Pollen Load Size and Maternal Plant on Pollen Performance and Seedling Vigor in *Clarkia unguiculata* (ONAGRACEAE)

M. Barbara Németh and Nancy L. Smith-Huerta
Department of Botany, Miami University, Oxford OH 45056, USA

Abstract: Pollen load size may vary both temporally and spatially in natural flower pollinations. This variation could lead to differences in the intensity of competition between pollen grains for access to ovules for fertilization. Increased competition for ovules could lead to increases in progeny vigor, if the fastest growing pollen tubes sire the most vigorous offspring. The maternal tissue supporting the germination and growth of pollen may also influence the competition between grains through the promotion of certain grain genotypes over others. This study examines the effects of pollen load size and maternal plant on pollen tube attrition, seed set and seedling vigor in chamber grown plants of the wildflower *Clarkia unguiculata* (*Lindl.*). Pollen load size significantly affected the number of pollen tubes in the style and number of seeds set. Pollen load size had no significant effect on attrition levels, seed weight, or any measures of seedling vigor (germination, cotyledon size, first foliar leaf size, seedling dry weight). In contrast, significant maternal effects were observed in all parameters measured with the exception of attrition levels and dry weight of seedlings.

Key words: Pollen load size, maternal effects, seedling vigor

INTRODUCTION

The size and composition of pollen loads deposited on stigmas may vary both temporally and spatially within and between populations of plants (Niesenbaum, 1994; Németh and Smith-Huerta, 2002). Variation in pollen load sizes could lead to differences in the performance of individual pollen grains. Pollen performance may be reflected in factors such as pollen germination, tube growth, attrition in the gynoecium and numbers of seeds set. Variation in these factors may, in turn, reflect the intensity of competition between microgametophytes. For example, pollen germination often increases with load size, as do numbers of pollen tubes in the style, attrition rates and seed set (Niesenbaum, 1999). These differences in the performance of microgametophytes could lead to increases in the overall quality of the resulting progeny if the best performing microgametophytes sire the most vigorous offspring.

Tests of this prediction have yielded mixed results. In a number of instances both pollen and seedling performance factors were significantly affected by the intensity of microgametophyte competition (Richardson and Stephenson, 1992; Janse and Verhaegh, 1993; Winsor *et al*., 2000). Other investigations have shown no such effects on pollen performance or seedling vigor (Smith *et al*., 1990; Snow, 1990, 1991; Németh and Smith-Huerta, 2002).

In a previous investigation of pollen deposition and seedling performance in natural populations of the wildflower *Clarkia unguiculata* (Lindl.), pollen load size was found to vary temporally (between blooming seasons) and spatially (between nearby populations) (Németh and Smith-Huerta, 2002). Variation in load size did not translate into differences in seedling vigor in these populations (Németh and Smith-Huerta, 2002). Perhaps field conditions such as heat or drought may have exerted such a strong influence on pollen performance or seed set that any effects of pollen load size were masked. Yet another possibility is that maternal plant effects were more important in determining seedling vigor than pollen load size. To further explore these ideas, a study of pollen performance and seedling vigor was conducted in chamber grown plants of *Clarkia unguiculata*. This study eliminates variation in environmental conditions, standardizes pollen load sizes and evaluates maternal effects on pollen performance factors and early seedling vigor. Specifically, the present study asks the questions: 1) what are the effects of pollen load size on the pollen performance factors of pollen tube growth, attrition and seed set? and 2) what effect does pollen load size have on the vigor of the progeny produced?

Corresponding Author: Nancy Smith-Huerta, Department of Botany, Miami University, Oxford OH, 45056, USA
Tel: (513) 529-4252 Fax: (513) 529-4243

MATERIALS AND METHODS

Seeds of *Clarkia unguiculata* were collected from a wild population at the University of California Stebbins Cold Canyon Reserve, in Solano County, 10 miles West of Winters, CA. Seeds were germinated in moist vermiculite at a constant temperature of 13 C, on a 12 h light/12 h dark cycle. Seedlings were transplanted 2 weeks after germination to 6 inch pots containing Metro Mix (Sierra Horticultural, Milpitas, CA) and grown in a growth chamber at 23 C on a 16 h light/8h dark cycle, at 350 μmol m^{-2} sec^{-1} of cool white fluorescent light. Humidity was maintained near 60%.

Clarkia unguiculata is a self-compatible but normally outcrossing species. Outcrossing is promoted by a physical and temporal separation of male and female functions within individual flowers. Flowers are protandrous, with anthers that mature two to three days before the female organs and most pollen grains are typically shed from the anthers before the stigma becomes receptive. The stigma is held away from the anthers and flowers within a plant mature acropetally. Self pollination is possible by movement of pollen grains from flowers located higher in the inflorescence to flowers lower in the inflorescence. The receptive stigma of *C. unguiculata* is four-lobed and fairly large, making it possible to consistently vary the size of pollen load treatments on a single stigma.

Pollen grains were applied to each stigma in one of two pollen load size treatments: Small or Large. The small load treatment received enough pollen grains to cover the surface of one stigmatic lobe and the large load treatment received enough pollen to cover the entire surface of the stigma (comprised of four lobes). Each of the two pollination treatments was performed on three flowers of the same maternal plant and repeated on a total of 18 plants. Plants with more than 1 missing flower in each treatment were removed from the analysis. This reduced the sample size to 16 plants with 3 flowers sampled in the small treatment and 3 flowers sampled for the large treatment, for a total of 6 flowers per plant. Flowers were emasculated at anthesis to prevent self-pollination. An additional set of two plants was used as pollen donors for the small and large load treatments. Pollen grains from an equal number of half-dehisced anthers from each pollen donor plant were collected and mixed before being applied to fully expanded, receptive stigmas. All pollinations were performed between 8 and 10 AM, at least one hour after the beginning of the light cycle.

Stigmas with attached styles were collected at 48 h post-pollination and fixed in 70% ethanol. Ovaries were allowed to mature on the plant before collection. Stigmas and styles were softened with 8 N NaOH and stained with aniline blue for viewing with fluorescence microscopy (Martin, 1959). An equal amount of 1% Saffranin Red stain was added 12 h prior to scoring of the tissue, in order to provide higher contrast between pollen grains and pollen tubes and between pollen tubes and style tissue.

The total number of pollen grains on a stigma, the number of pollen tubes in the upper style and lower style and the number of seeds produced were counted for all samples. Pollen tube attrition within the style was estimated for each treatment by calculating the proportion of pollen tubes that completed growth from the upper style to the lower-style. Seed production was further examined in two ways: as the proportion of viable seeds produced per pollen grain on the stigma and as the proportion of viable seeds produced per pollen tube that reached the lower-style. In a few cases, this last measure resulted in proportions of seeds per pollen tube greater than 100%. When this occurred the difference between pollen tube numbers and seed set was very low (less than 10).

The resulting data were analyzed with a series of two-way ANOVAs, Type III Sum of Squares, using the SuperAnova statistical analysis package (Abacus Concepts, 1998). Pollen Load Size (Small vs. Large) and Maternal Plant were used as fixed-effect independent variables. Interaction means were contrasted (Least square means, Superanova, 1998) and evaluated using a Bonferroni post-hoc test. The probability value cutoff for significance at a 5% confidence level was determined by dividing the 0.05 by the number of a priori comparisons.

The effects of pollen load size on seed germination and seedling vigor were also assessed in a separate experiment. Fruits from eight maternal plants, representing each pollen load size (small and large) were harvested. All seeds contained in each fruit were counted, weighed and planted in a 6 in. pot in Metromix (Milpitas, CA), covered with 1 cm of vermiculite and watered as needed to maintain moist soil conditions. A total of 48 pots were grown in a growth chamber at 22 C, on a 16 hr light/8 h dark cycle. Percent seed germination on the seventh and sixteenth day after sowing, cotyledon length on the ninth day after sowing and first foliar leaf length on the sixteenth day after sowing were measured. Plants were harvested and dried 19 days after planting. The resulting data were analyzed with a series of two-way ANOVAs (SuperAnova, Abacus Concepts, 1998) with pollen load size and maternal plant as independent variables. Because proportion data were not normally distributed, they were treated with arcsine square root transformations (Sokal and Rohlf, 1995). Seed weights and leaf lengths were log transformed. Interaction means were contrasted (Least

Square Means, SuperAnova, 1998) and evaluated using a Bonferroni post-hoc test. The probability value cutoff for significance at a 5% confidence level was determined by dividing the 0.05 by the number of a priori comparisons.

RESULTS

Significantly more pollen grains were deposited on stigmas in the large load treatment (approximately 340) than in the small load treatment (approximately 120) (Table 1 and 2). Number of pollen grains varied significantly from plant to plant (Table 2), ranging from 201 (plant 2) to 471 (plant 1) in the large load treatment and from 63 (plant 13) to 171 (plant 14) in the small load treatment (Fig. 1a).

Pollen load size and maternal plant had significant effects on the number of pollen tubes reaching the upper style (Table 1). On average, there were 42 pollen tubes in the upper style in the small load treatment and 67 pollen tubes in the large load treatment (Table 2). Pollen tube numbers varied significantly from plant to plant, ranging from 36 (plant 2) to 116 (plant 1) in the large load treatment and from 20 (plant 13) to 71 (plant 3) in the small load treatment (Fig. 1b).

Pollen load size and maternal plant had significant effects on the number of pollen tubes reaching the lower style (Table 1). On average, there were 30 pollen tubes were in the lower style in the small load treatment and 45 pollen tubes in the large load treatment (Table 2). Pollen tube numbers in the lower style varied significantly from plant to plant, ranging from 18 (plant 15) to 81 (plant 8) in the large load treatment and from 12 (plant 13) to 51 (plant 3) in the small load treatment (Fig. 1b).

Approximately 70% of pollen tubes in the upper style completed growth to the lower style within 48 h post-pollination for both the large and small load treatments (Fig. 1d, Table 2 and 3). Although the overall rate of attrition between the upper and lower styles varied considerably between the large and the small pollen load treatments, it was not significantly affected by either pollen load size or maternal plant (Table 3 and Fig. 1d).

Pollen load size and maternal plant had significant effects on the number of mature seeds produced per fruit (Table 4). On average, 19 seeds were produced in the small load treatment and 32 seeds were produced in the large load treatment (Table 2). Seed production varied significantly from plant to plant, ranging from 5 (plant 2) to 59 (plant 12) seeds per fruit in the large load treatment and from 3 (plant 15) to 49 (plant 6) seeds per fruit in the small load treatment (Fig. 2a).

Pollen load size and maternal plant had significant effects on the proportion of pollen grains on the stigma that resulted in mature seeds (Table 4). On average, 15.7% of pollen grains produced seeds in the small load treatment and 9.5% of pollen grains produced seeds in the large load treatment (Table 2). Percent seed set per pollen grain varied significantly from plant to plant, ranging from 1% (plant 2) to 21% (plant 12) seed set in the large load treatment and from 2.7% (plant 15) to 30% (plant 6) seed set in the small load treatment (Fig. 2b).

Table 1: ANOVA of the effects of pollen load size and maternal plant on the number of pollen grains deposited on the stigma, the numbers of pollen tubes reaching the upper style and the numbers of pollen tubes reaching the lower style. Residuals did not deviate significantly from a normal distribution

Source (df)	No. of grains on stigma	No. of tubes in upper style	No. of tubes in lower style
Pollen load size F (1, 64)	785.219**	72.351**	47.362**
Maternal plant F (15, 64)	6.238**	5.914**	5.149**
Load X maternal plant F (15, 64)	4.140**	4.168**	3.512**
Overall model R^2	0.936	0.777	0.735

*p<0.05, **p<0.01

Table 2: Summary of mean values (±SE) for pollen success and seedling performance in the pollination and seedling vigor experiments. The statistical significance of the pollen load size and maternal plant effects are reported at p<0.05

	Mean values		Significance	
Parameter	Small load	Large load	Pollen load size	Maternal plant
No. of pollen grains on stigma	119.81 (5.4)	341.27 (10.82)	*	*
Pollen tubes in the upper style	42.31 (2.35)	67.31 (3.78)	*	*
Pollen tubes in the lower style	29.65 (1.92)	45.31 (2.65)	*	*
Percent pollen tube growth in the style	69.40 (2.5)	67.50 (2.2)	NS	NS
Number of seeds per fruit	19.00 (2.23)	32.04 (2.69)	*	*
Percent pollen grains producing seeds	15.70 (1.6)	9.50 (0.8)	*	*
Percent pollen tubes producing seeds	62.80 (5.9)	69.00 (6.0)	NS	*
Seed weight (mg)	0.34 (0.03)	0.30 (0.01)	NS	*
Percent germination at 7 days	43.40 (4.8)	44.60 (4.3)	NS	*
Percent germination at 16 days	52.40 (5.0)	54.80 (4.2)	NS	*
Cotyledon length (mm)	8.07 (0.49)	7.72 (0.34)	NS	*
First foliar leaf length (mm)	17.77 (0.86)	16.02 (0.59)	NS	*
Dry weight (mg)	15.00 (1.0)	13.00 (1.0)	NS	NS

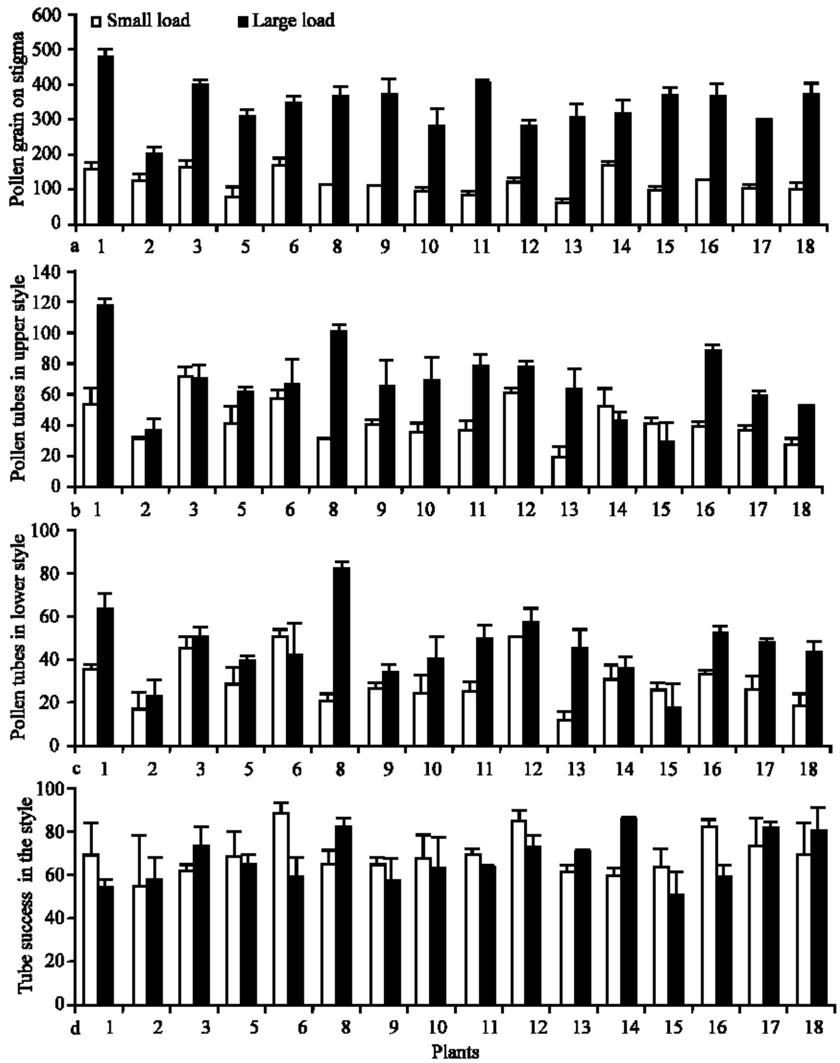

Fig. 1: Mean values (±SE) for a) number of pollen grains deposited on the stigma, b) number of pollen tubes reaching the upper style, c) number of pollen tubes reaching the lower style and d) percent pollen tubes completing growth from upper to lower style. Pollen load size X maternal plant interaction were significant in a, b and c, but not in d

Table 3: ANOVA of the effects of pollen load size and maternal plant on the proportion of pollen tubes completing growth from the upper-to the lower-style. Residuals did not deviate significantly from a normal distribution. Overall model $R^2 = 0.380$

Source	df	MS	F	p
Pollen load size	1	0.008	0.342	0.5607
Maternal plant	15	0.029	1.185	0.3065
Load X maternal plant	15	0.034	1.404	0.1728
Error	64	0.024		

The proportion of pollen tubes in the lower style that resulted in mature seeds was not significantly affected by Pollen Load Size (Table 4). Approximately 63-70% of pollen tubes reaching the lower style resulted in mature seeds in the small and large load treatments, respectively (Table 2). However the proportion of seeds produced per pollen tube varied significantly among individual maternal plants, ranging from 14% in plant 14 to 100% in plant 9 (Table 4 and Fig. 2c).

In our seedling vigor experiment mean seed weight was approximately 0.30 mg and did not differ significantly between the large and small load treatments (Table 2). However, mean seed weights varied significantly between individual maternal plants (Table 5 and Fig. 3a), ranging from 0.20 mg in Plants 3 and 17 to approximately 0.40 mg in Plants 16 and 18 (Fig. 3a). Significant differences observed between maternal plants resulted from differences in the mean seed weights between Plants 3 and 17, vs. 16 and 18 (LSM comparisons, $p<0.05$).

Approximately 41% of seeds germinated by the 7th day after sowing, with no significant difference observed between the large and small pollen load treatments

Table 4: ANOVA of the effects of pollen load size and maternal plant on the number of mature seeds produced per fruit, the proportion of pollen grains on the stigma that resulted in mature seeds and the proportion of pollen tubes at the lower style that resulted in mature seeds (arcsin sqrt. transformed). Residuals did not deviate significantly from a normal distribution

Source	No. of seeds/fruit	No. of seeds per pollen grain	No. of seeds per pollen tube
Pollen load size F (1, 61)	40.393**	2.644**	19.336
Maternal plant F (15, 61)	7.292**	4.751**	5.685**
Load X plant F (14,61)	2.267**	1.661**	2.445
Overall model R^2	0.738	0.605	0.703

*p<0.05, **p<0.01

Table 5: ANOVA of the effects of pollen load size and maternal plant on the mean weight of seeds used in the germination test (log transformed) and the proportion of germinated seeds seven and sixteen days after sowing (arcsine sqrt. transformed). Residuals did not deviate significantly from a normal distribution

Source	Mean seed weight	% Seed germination (7 days)	% Seed germination (16 days)
Pollen load size F (1, 32)	0.101	2.91	2.48
Maternal plant F (7, 32)	9.07**	7.73**	3.52**
Load X plant F (7, 32)	1.45	1.19	0.65
Overall model R2	0.697	0.671	0.498

*p<0.05, **p<0.01

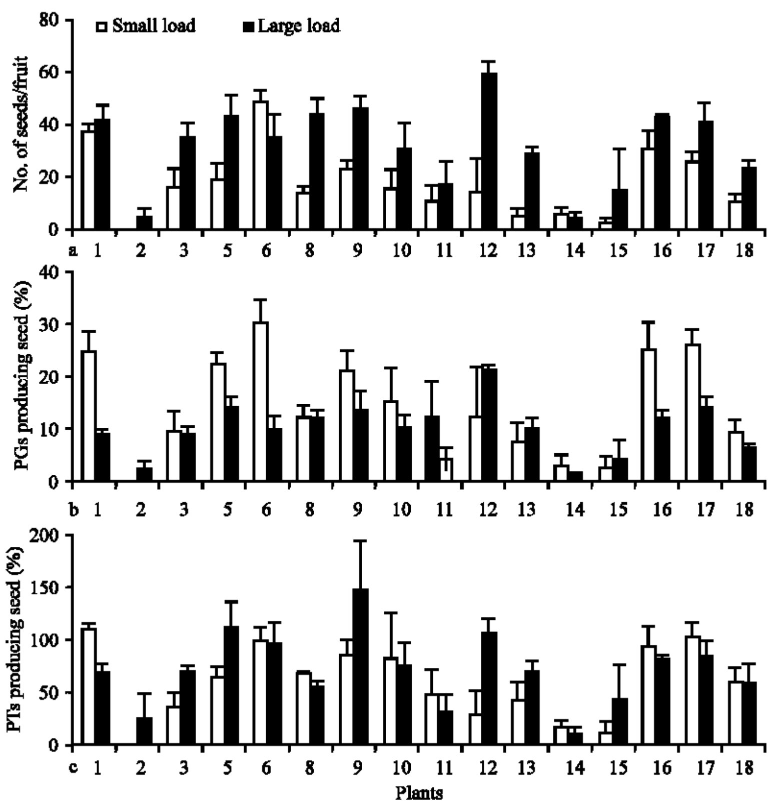

Fig. 2: Mean values (±SE) for a) the total number of seeds produced per fruit, b) percent seed production per pollen grain on the stigma and c) percent seed production per pollen tube in the lower style. All measures of seed production varied significantly by pollen load size and maternal plant

(Table 2 and 5). However, percent germination varied significantly between the progeny of individual maternal plants (Fig. 3b and Table 5), ranging from approximately 19% in Plant 17, to approximately 76% in Plant 16 (Fig. 3b). Significant differences in germination rates resulted from the differences between the two extremes observed in Plants 17 and 16 (LSM comparisons, p<0.05).

Approximately 50% of seeds germinated by the 16th day after planting (Table 2), with no significant difference observed between the large and small pollen load treatments (Table 5). However, percent germination varied significantly among the progeny of individual maternal plants (Table 5), ranging from approximately 27% in Plant 17 to approximately 79% in Plant 16 (Fig. 3c).

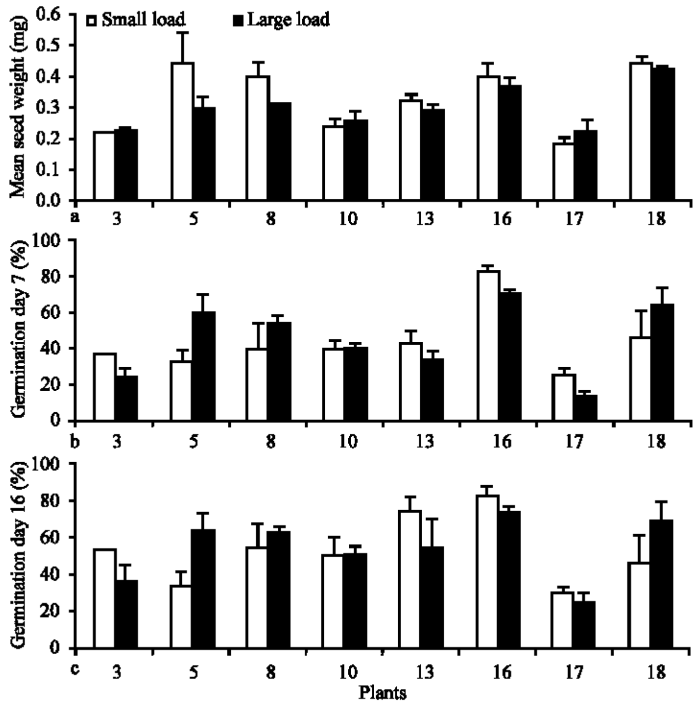

Fig. 3: Mean values (±SE) during the seedling vigor experiment, summarizing a) the average seed weight (mg) of the seeds participating in the seed germination trials, b) the percent seed germination by the 7th day after sowing and c) the percent seed germination by the 16th day after sowing. Only variations among individual maternal plants were statistically significant

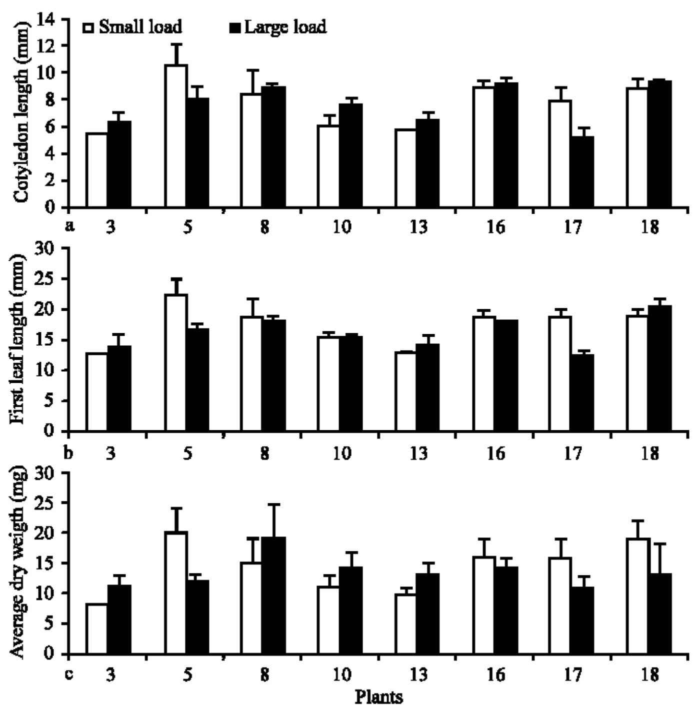

Fig. 4: Mean values (±SE) during the seedling vigor experiment, summarizing a) cotyledon length (mm) by the 9th day after sowing, b) first foliar leaf length (mm) by the 16th day and c) total dry plant weight (mg) by the 19th day after sowing. Cotyledon and foliar leaf length were significantly affected by maternal plant, but not pollen load size

Table 6: ANOVA of the effects of Maternal Plant and Pollen Load on the mean cotyledon length of seedlings by the 9th day after sowing, the first foliar leaf length by the 16th day after sowing and the dry weight of seedlings by the 19th day after sowing (log transformed). Residuals did not deviate significantly from a normal distribution

Source	Cotyledon length (9 days)	First foliar leaf length (16 days)	Dry weight (19 days)
Pollen load size F (1, 29)	0.03	1.39	0.48
Maternal plant F (7, 29)	5.09**	4.99**	0.61
Plant X load F (7, 29)	1.77	1.97	1.12
Overall model R2	0.623	0.64	0.305

*p<0.05, **p<0.01

Significant differences in germination rates resulted from the differences between the two extremes observed in Plants 17 and 16 (LSM comparisons, p<0.05).

Cotyledons reached an average length of 8 mm by the 9th day after sowing and did not differ significantly by pollen load size treatments (Table 2 and 6). However, cotyledon size varied significantly among the progeny of individual maternal plants (Table 6). Cotyledon lengths ranged from approximately 6 mm in plants 3 and 17, to approximately 9 mm in plants 16 and 18 (Fig. 4a). Significant differences in mean cotyledon lengths resulted from differences between the two extremes observed in Plants 3 and 17 vs. 16 and 18 (Least square means, p>0.05).

First foliar leaves reached a mean length of 16.8 mm by the 16th day after sowing and did not differ significantly by pollen load size treatment (Table 2 and 6). However, leaf length varied significantly among the progeny of individual maternal plants (Fig. 4b and Table 6). Sizes ranged from approximately 13 mm in Plants 3 and 13, to approximately 19 mm in Plants 5 and 18 (Fig. 4b). Significant differences in first foliar leaf lengths resulted from differences between the two extremes observed in Plants 3 and 13 vs. 5 and 18 (LSM comparisons, p<0.05).

Dry plant weights averaged approximately 14 mg on the 19th day after sowing (Fig. 4c). Neither pollen load size nor maternal plant had a significant effect on dry plant weight (Table 6).

DISCUSSION

Pollen load size: Increases in pollen load size resulted in greater numbers of pollen tubes in the style and higher seed set in our experiment. Pollen load size had no significant effect on attrition levels, seed weight, or any measures of seedling vigor (germination, cotyledon size, first foliar leaf size, seedling dry weight).

The pollen load sizes used in our current study reflect pollen load sizes found in nature for this species (Németh and Smith-Huerta, 2002). Pollen load sizes in four wild populations of *C. unguiculata* averaged 166 pollen grains after 6 h of exposure to pollinators and 323 after 48 h of exposure to pollinators (Németh and Smith-Huerta, 2002). These values are similar to our current small and large load treatments, which averaged 126 pollen grains and 330 pollen grains per stigma, respectively. The average number of ovules per ovary for this species is reported to be 84-120 (Bowman, 1987; Mazer and Dawson, 2001). In both the current study and our field study, stigmas received pollen approximately equal to the number of ovules (for 6 h exposures in natural populations or small load treatments) and three-times more pollen than the average number of ovules (for 48 h pollinator exposures or large load treatments). These low and high pollen treatments would appear to be sufficiently different from one another to create two distinct competitive environments for pollen. The pollen in smaller loads should experience little competition, the larger loads much more intense competition. However, examination of pollen germination and early pollen tube growth in the style demonstrated that pollen competition occurred in both treatments of our current study and in our past field studies (Németh and Smith-Huerta, 2002). This is evidenced by the fact that only a fraction of the pollen grains on the stigma eventually sired seeds (10% of pollen grains in the large load treatment and 15% of pollen grains in the small load treatment). This phenomenon was also observed in our previous field studies in which 17% of pollen grains in the 6 h treatment and 15% of grains on the stigma in the 48 h treatment produced seeds (Németh and Smith-Huerta, 2002). Therefore, all progeny in this study were produced under relatively similar levels of competition, even though some pollen loads were barely sufficient to fertilize all available ovules. Previous investigations have also observed that a considerable number of pollen grains may fail to achieve fertilization, even when pollen loads are insufficient to fertilize all available ovules (Motten, 1983; Snow, 1985; Bertin, 1990; Richardson and Stephenson, 1992; Németh and Smith-Huerta, 2002). These consistent attrition rates from stigma to ovary could be due to selective support or inhibition of pollen tube growth by maternal tissue (Hormaza and Herrero, 1996). The maintenance of a competitive environment for pollen tube growth by the maternal plant might result in fewer, but more vigorous progeny when pollen loads are small. In *Clarkia unguiculata* it appears that offspring quality is selected over offspring quantity and is likely facilitated by maternal control over pollen tube growth in the style.

There are only a few other studies that have failed to find an effect of pollen load size on progeny vigor (Smith *et al.*, 1990; Snow, 1990, 1991; Lassere *et al.*, 1996; Mitchell, 1997; Niesenbaum, 1999; Pasonen *et al.*, 2001). Far more investigations have found a positive effect of pollen load size on progeny vigor (Mulcahy and Mulcahy, 1975; Fingerett, 1979; Mulahy, 1979; Lee and Hartgerink, 1986; McKenna, 1986; Davis *et al.*, 1987; Winsor *et al.*, 1987; Bertin 1990; Richardson and Stephenson, 1992; Janse and Verhaegh, 1993; Quesada *et al.*, 1993; Palmer and Zimmerman, 1994; Björkman, 1995; Johannsson and Stephenson, 1997; Brown and Kephart 1999; Winsor *et al.*, 2000).

Mitchell (1997) examined and compared a number of the above investigations in an attempt to explain why not all studies demonstrated increasing progeny vigor with increasing load size. He concluded that in the studies that failed to detect increases in progeny vigor, the large and small pollen load treatments did not result in a different competitive environment for pollen. In his study of the desert mustard *Lesquerella*, only 3% of pollen grains sired offspring in the high load treatment, while 6% of grains sired seeds in his low pollen load treatment (Mitchell, 1997). Clearly pollen competition was intense in both treatments. This is comparable to our present study in which progeny in the large and small treatments were sired by approximately 10 and 15% of pollen grains on the stigma, respectively.

Maternal effects: Significant maternal effects were observed in all parameters measured with the exception of attrition levels and dry weight of seedlings. Other studies of pollen load size and progeny vigor have recognized maternal effects (Snow, 1990, 1991; Richardson and Stephenson, 1992; Niesenbaum, 1999). As discussed above, maternal tissue may significantly influence the numbers of pollen tubes germinating on the stigma and growing within the style. We also observed other interesting maternal effects. For example, differences in pollen load sizes occurred across maternal plants in spite of the extreme care taken when applying pollen loads. Although most plants received pollen loads similar to the average pollen load sizes reported above, one plant received significantly more pollen grains and one plant received significantly fewer pollen grains than the average pollen load size. Pollen load size was varied by either pollinating one stigma lobe for the small load treatment or all four lobes for the large load treatment. Variation in stigma lobe size between maternal plants was likely responsible for the within treatment variation in pollen load size. These maternal effects of pollen load size could in turn affect the numbers of pollen tubes in the style and ultimately the numbers of seed set per fruit. This is precisely what occurred in the present study. Maternal effects were also observed in seed weights. One plant produced seeds that were, on average, twice as heavy as other plants in the study. Larger seeds may produce larger, more vigorous offspring. Clearly, the potential for maternal influences on progeny vigor is present at many points in the life cycle and this maternal influence could have acted to mask the effects of pollen load size on progeny vigor. Many studies have documented the influence of maternal environment on seed size and seed size is known to be correlated with seed germination and seedling success (Roach and Wulff, 1987). These maternal effects can be strong enough to mask the effects f inbreeding depression early in the plant life cycle (Wolfe, 1993). For example, in *Hydrophyllum appendiculatum* inbreeding depression was much less pronounced early in the life cycle as measured in seed set, seed weight, percent germination and cotyledon length than later in the life cycle as measured in plant size at flowering, shoot number and flower number (Wolfe, 1993).

In conclusion, pollen load size significantly increased pollen tubes in the style and seed set in the wildflower *Clarkia unguiculata*. Intense competition occurred between pollen grains during germination and early tube growth in both pollen load size treatments. This resulted in far fewer seeds produced in both load size treatments than might be expected given the number of available ovules. It appears that a competitive environment for pollen performance is maintained in *Clarkia unguiculata*, regardless of pollen load size. This intense competition between pollen grains, facilitated by the maternal tissue, should maximize the vigor of the resulting progeny. This prediction held true in our investigation with no detectable differences between the progeny produced by small and large pollen loads. However this advantage of offspring quality comes at the cost of lower seed production. It appears that in *Clarkia unguiculata* offspring quality is selected over offspring quantity and is likely facilitated by maternal control over pollen tube growth in the style.

ACKNOWLEDGMENTS

We thank M. Hughes for assistance with statistical analysis. This article represents a portion of a Ph.D thesis submitted by M.B. Nemeth to the Department of Botany at Miami University in Oxford, Ohio. The research was supported by a Miami University Department of Botany

Academic Challenge Award and Willard Sherman Turrell Herbarium Grant.

REFERENCES

Abacus Concepts, 1998. Super Anova. Abacus Concepts, Berkeley, California.

Bertin, R.I., 1990. Effects of pollination intensity in *Campsis radicans*. Am. J. Bot., 77: 178-187.

Björkman,T., 1995. The effect of pollen load and pollen grain competition on fertilization success and progeny performance in *Fagopyrum esculentum*. Euphytica, 83: 47-52.

Bowman, R.N., 1987. Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). Am. J. Bot., 74: 471-476.

Brown, E. and S. Kephart, 1999. Variability in pollen load: Implications for reproduction and seedling vigor in a rare plant, *Silene douglasii* var. *Oraria*. Intl. J. Plant Sci., 160: 1145-1152.

Davis, L.E., A.G. Stephenson and J.A. Winsor, 1987. Pollen competition improves performance and reproductive output of the common zucchini squash under field conditions. J. Am. Soc. Hortic. Sci., 112: 712-716.

Fingerett, E.R., 1979. Pollen competition in a species of evening primrose, *Oenothera organensis*, Munz. M.Sc. Thesis. Washington State University, Pullman Washington.

Hormaza, J.I. and M. Herrero, 1996. Dynamics of pollen tube growth under different competition regimes. Sex Plant Reprod, 9: 153-160.

Janse, J. and J.J. Verhaegh, 1993. Effects of varying pollen load on fruit set, seed set and seedling performance in apple and pear. Sex Plant Reprod, 6: 122-126.

Johannsson, M.H. and A.G. Stephenson, 1997. Effects of pollination intensity on the vigor of the sporophytic and gametophytic generation of *Cucurbita texana*. Sex Plant Reprod., 10: 236-240.

Lassere, T.B., S.B. Carroll and D.L. Mulcahy, 1996. Effect of pollen competition on offspring quality at varying stages of the life cycle in *Silene latifolia* Poiret (*Caryophyllaceae*). Bull. Torr. Bot. Club., 123: 175-179.

Lee, T.D. and A.P. Hartgerink, 1986. Pollination intensity, fruit maturation pattern and offspring quality in *Cassia fasciculata* (*Leguminosae*). In: Mulcahy, D.L., G.B. Mulcahy and E. Ottaviano, Eds. Biotechnology and Ecology of Pollen. Springer-Verlag, New York, pp: 417-422.

Martin, F.M., 1959. Staining and observing pollen by means of fluorescence. Stain Technol., 34: 436-437.

Mazer, S.J. and K.A. Dawson, 2001. Size-dependent allocation within flowers of the annual herb *Clarkia unguiculata* (*Onagraceae*): Ontogenetic and among-plant variation. Am. J. Bot., 88: 819-831.

McKenna, M.A., 1986. Heterostyly and microgametophytic selection: The effect of pollen competition on sporophytic vigor in two distylous species. In: Mulcahy, D.L., G.B. Mulcahy and E. Ottaviano, Eds. Biotechnology and Ecology of Pollen. Springer-Verlag, New York, pp: 443-448.

Mitchell, R.J., 1997. Effects of pollen quantity on progeny vigor: evidence from the desert mustard *Lesquerella fendleri*. Evolution, 51: 1679-1684.

Motten, A.F., 1983. Reproduction of *Erythronium umbilicatum* (*Liliaceae*): Pollination and pollinator effectiveness. Oecologia, 59: 351-359.

Mulcahy, D.L. and G.B. Mulcahy, 1975. The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. Theor. Applied Genet., 46: 277-280.

Mulahy, 1979. The rise of the angiosperms: A genecological factor. Sci., 206: 20-23.

Németh, M.B. and N.L. Smith-Huerta, 2002. Effects of pollen load composition and deposition patterns on pollen performance in *Clarkia unguiculata* (Onagraceae). Intl. J. Plant Sci., 163: 795-802.

Niesenbaum, R.A., 1994. Spatial and temporal variation in pollen tube numbers in *Lindera benzoin* (*spicebush*). Can. J. Bot., 72: 268-271.

Niesenbaum, 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion and progeny vigor in *Mirabilis jalapa* (*Nyctaginaceae*). Am. J. Bot., 86: 261-268.

Palmer, T.M. and M. Zimmerman, 1994. Pollen competition and sporophyte fitness in *Brassica campestris*: Does intense pollen competition result in individuals with better pollen? Oikos, 69: 80-86.

Pasonen, H.-L., P. Pulkkinen and M. Käpylä, 2001. Do pollen donors with fastest-growing pollen tubes sire the best offspring in an anemophilous tree, *Betula pendula* (*Betulaceae*). Am. J. Bot., 88: 854-860.

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

Richardson, T.E. and A.G. Stephenson, 1992. Effects of parentage and size of the pollen load on progeny performance in *Campanula americana*. Evolution, 46: 1731-1739.

Roach, D.A. and R.D. Wulff, 1987. Maternal effects in plants. Ann. Rev. Ecol. Syst., 18: 209-235.

Smith, S.E., D.M. Contac and U. Benchers, 1990. Pollen load, seed position and agronomic performance in alfalfa. Crop Sci., 30: 561-565.

Snow, A.A., 1985. Evidence for and against pollen tube competition in natural populations. In: Mulcahy, D.L., G.B. Mulcahy and E Ottaviano, Eds. Biotechnology and Ecology of Pollen. Springer Verlag, New York, pp: 405-410.

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

Snow, 1991. Effects of pollen load size on sporophyte competitive ability in two *Epilobium* species. Am. Midl. Natl., 125: 348-355.

Sokal, R.R. and F.J. Rohlf, 1995. Biometry, 3rd Edn. WH Freeman, San Francisco, CA. USA.

Winsor, J.A., L.E. Davis and A.G. Stephenson, 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. Am. Natl., 129: 643-656.

Winsor, J.A., S. Peretz and A.G. Stephenson, 2000. Pollen competition in a natural population of *Cucurbita foetidissima*. Am. J. Bot., 87: 527-532.

Wolfe, L.M., 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: Role of maternal effects, crowding and parental mating history. Evolution, 47: 374-386.