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The effect of pollen load and pollen grain competition on fertilization success and progeny performance in *Fagopyrum esculentum*

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Summary

Flowers of cultivated buckwheat (*Fagopyrum esculentum* Moench) often receive natural pollen loads of fewer than 10 pollen grains. The cultivated varieties also have high genetic variability. These observations raise the question of whether seed production in buckwheat is often limited by pollen delivery, and whether small increases in pollen load could result in gametophytic selection through pollen grain competition. In greenhouse-grown buckwheat plants, embryo sac penetration by pollen tubes was universal with 10 or more pollen grains. However, seed production increased with pollen load up to 30 grains per flower. Larger pollen loads, which intensify selection among gametophytes, resulted in more vigorous progeny. Seedlings produced from high pollen load (15–20 pollen grains) were larger (40% by weight) than those from low pollen load (5 pollen grains). These results are evidence that pollen grain competition can occur in buckwheat with benefits for progeny performance.

Introduction

Competition among pollen grains to fertilize each egg can result in selection for those gametophytes that sire the strongest sporophytic offspring. There are several aspects of the reproductive biology of cultivated buckwheat, *Fagopyrum esculentum*, that predict an important role for pollen grain competition. Buckwheat has a few, fast-growing pollen, many of which carry detrimental alleles, that compete for a single egg cell. The pollen load necessary to obtain beneficial competition has not been previously determined for buckwheat.

The number of pollen grains reaching the stigma of a flower influences seed production in several ways. Pollen tubes can act cooperatively during growth in the style to increase fertilization success (Esser, 1953). They can also compete with each other to fertilize the available egg cells (Mulcahy & Mulcahy, 1975). That competition determines the paternity of the progeny and can affect the performance of the progeny. Pollen-grain competition selects for superior gametophytic genotypes on the basis of rapid pollen-tube growth (Ter-Avanesian, 1949; Mulcahy & Mulcahy, 1975). Pollen grains are genetically different, with some genotypes having faster-growing pollen tubes and some producing more vigorous progeny. Haploidy assures that all alleles are expressed. For competition among pollen grains to work, the traits of fast pollen growth and vigorous seedling growth must be closely associated (Mulcahy, 1971; Mulcahy & Mulcahy, 1975), so that the fast-growing pollen will also produce better progeny.

The pollen load on naturally-pollinated buckwheat varies greatly and is frequently less than 10 per flower (Namai, 1990; Björkman, 1995). Although there is good pollen-tube growth in most flowers, a large proportion of fertile flowers fail to develop (Adachi et al., 1983; Namai & Ohsawa, 1986). Research to date has not shown whether the pollen load influences which flowers fail to develop; either because of an insufficient quantity of pollen to fertilize each ovule, or because of insufficient quality of pollen to produce successful progeny. Low seed set can be due to the pre-zygotic failure of the small number of pollen tubes to grow well enough to fertilize the ovule, or to the post-zygotic abortion of weak embryos resulting from low pre-zygotic selection pressure when there are not enough maternal resources to mature all zygotes. It is more likely that a pollen tube will enter the micropyle if more pollen are on the stigma because several pollen tubes grow through the style better than single pollen tubes (Ter-Avanesian, 1978; Cruzan, 1986). Alternatively, with a low intensity of pollen-grain competition, even pollen grains carrying detrimental recessive alleles may be successful. Some of the resulting embryos will then be weak, and be aborted early in development (Willson & Burley, 1983). These possibilities can be distinguished: if pollen-tube growth is the limiting factor, the effect of pollen load will be similar on pollen-tube growth and seed set; if abortion due to low pollen-grain competition is limiting, the seed set should increase substantially only at higher pollen loads.

Buckwheat differs from other species in which pollen-grain competition has been studied in that the fruits contain a single ovule. Therefore, in each flower the competition among male gametes has no second place. Pollen-tube growth is also so rapid in buckwheat that there is little opportunity for pollen from many deposits to compete. The interval between pollinator visits can easily be long enough to give the first pollen delivered an insurmountable head start. The effective pollen load may, from the respect of pollen-grain competition, be the number delivered in the first bee visit rather than the daily total. This restriction should further sensitize buckwheat to naturally-occurring variation in pollen load.

Buckwheat should have substantial variation in pollen quality because the number of detrimental recessive genes is high. Estimates are available for the proportion of gametes carrying certain deleterious recessive genes: 22% chlorophyll-deficient cotyledons, 6% chlorophyll-deficient leaves (Ohnishi, 1988); 12% female sterility and 8% male sterility (Ohnishi, 1985). Furthermore, in an experiment using inbred lines to express deleterious recessive alleles, inbred progeny had a dry weight less than half that of sibs sired by other inbred lines (Marshall, 1979). With such a high incidence of deleterious genes and relatively few pollen per ovule, the intensity of competition among pollen grains is especially responsive to variation in the pollen load. Environmental factors causing variation in the pollen load would therefore be expected to affect the growth and reproductive success of subsequent generations.

This paper examines whether low pollen delivery limits seed set through unsuccessful pollen-tube growth or through abortion of weak progeny. It further examines whether pollen-grain competition can improve the performance of those progeny that do become mature seeds.

Methods and materials

Plant culture

Seeds of Manor buckwheat were sown in 15 cm pots containing peat moss-vermiculite (1:1), adjusted to pH 6.8 with dolomitic lime. Plants were fertilized weekly with soluble 20-20-20 fertilizer with micronutrients (Grace-Sierra, Allentown, PA, USA). Plants were grown in a greenhouse with minimum temperatures of 15°C night and 20°C day. On cloudy days, supplemental light (500 μmol cm^{-2} s^{-1}) was provided with 1000 W metal-halide lamps.

Greenhouse pollination

Unless noted, all crosses were made by transferring pollen from short-styled plants to long-styled plants. This is a far easier cross to make, and the success rate is the same in the two compatible crosses (Morris, 1951). Pollination was made by touching the open anther sac of the donor to one stigma of each recipient flower. For experiments in which pollen grains were counted, a cat's whisker was used to place pollen grains individually on the stigma. All pollinations were made during the first two weeks of flowering when female fertility was high.

Seed set was scored 10 days after pollination, at which time the ovule is swollen and accumulating starch in fruits that will mature (Horobowicz & Obendorf, 1992). In buckwheat, abortion generally occurs 1 to 3 days after pollination (Adachi, 1990). Therefore successful seed set could be predicted based on observation at 10 days after pollination. In the experiments reported here, very few (0–10%) of the flowers produced empty fruits. The number was too low to be meaningfully analyzed, so these fruits were not counted as seeds.

Pollen-tube growth was measured by collecting flowers 1 h after pollinations and placing them immediately in 1 N NaOH. The flowers were cleared in the NaOH for 1 h, then incubated in 0.01%. Aniline blue overnight. The ovaries were then excised and observed with fluorescence microscopy to observe whether any pollen tube had reached the micropyle. The formation of a brightly fluorescent swelling of the pollen tube at the micropyle made this determination unambiguous.

Pollen-tube growth and seed set were determined with simultaneous pollination on one set of 8 recipient plants. All pollen loads were done on each plant on each day to avoid bias due to maternal variation. Identical pollinations were done on two flowers at a time, with one sampled for pollen-tube growth, the other for seed set.

The effect of load on seed set was measured four additional times because the maximal seed set varied among plantings, though the same seed lot was used and the growing conditions were similar. Flowers were pollinated with one of four pollen loads: 3, 10, 30 or 90 pollen grains. The actual number of pollen grains for the four loads was 2–4, 7–14, 25–35 and 80–120 respectively, based on counts made on stigmas in test pollinations. Each of the tree stigmas can hold a maximum of 40 pollen grains. The three lower loads were applied to a single stigma, the highest load was intended to place the greatest possible number of pollen grains on the flower. For each experiment, 50 to 100 flowers were pollinated at each pollen load, distributed over 8 recipient plants. The seed set was reported as the proportion of fertile female flowers, with the standard error of the mean determined with the χ^2 test for proportions.

The effect of load on progeny performance was determined in two experiments. In the first, pollen loads that either had no competition (2–5 pollen grains per flower) or moderate competition (12–18 pollen grains) were applied to individual flowers on 11 recipient plants. Both high and low pollen load treatments were done on each plant so that the intensity of competition between developing seeds was similar. All the seeds produced from these pollinations were planted (26 from low load, 60 from high load), after 25 days, 10 low-load and 22 high-load plants were sampled and weighed. Twenty-five flowers on each of the remaining plants were abundantly pollinated to assess seed production. The data were analyzed using Student's t-test for differences in quantitative traits and the χ^2 test for proportions.

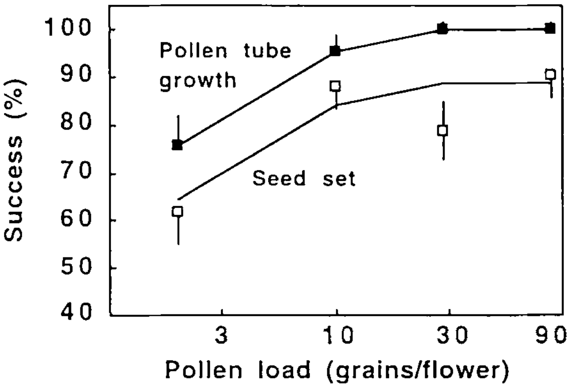

Fig. 1. Effect of pollen load on pollen tube growth and seed set. These measurements were made from simultaneous pollinations on the same plants. Pollen tube growth was scored as succesful if a pollen tube had entered the micropyle one hour after pollination. Note the logarithmic x-scale. The vertical bars are the standard errors calculated from the binomial distribution.

Results

Pollen limitation of pollen-tube growth

Pollen tubes entered the micropyle in virtually all flowers receiving 10 or more pollen grains (Fig. 1). There was evidence of some lack of success only when just 2 pollen grains were placed on one stigma. Seed set showed the same dependence on pollen load as did the pollen-tube growth.

Pollen limitation of seed set

The pollen load that effectively saturated seed set was between 10 and 30 pollen grains per flower (Fig. 2). Although there was a difference in the maximal seed set obtained in different plantings, there was a similar sensitivity to pollen load.

The flowers that failed to produce seed even with $\geq$ 90 pollen grains were defective in a pollen-independent process; we regard this as inherent female sterility. The sterility varied from 4 to 39% among the four experiments. Only about 20 flowers per plant were pollinated, less than the plant's seed-filling capacity.

Pollen-grain competition: effect of pollen load on progeny performance

The performance of progeny from crosses varying in the degree of pollen-grain competition varied as pre-

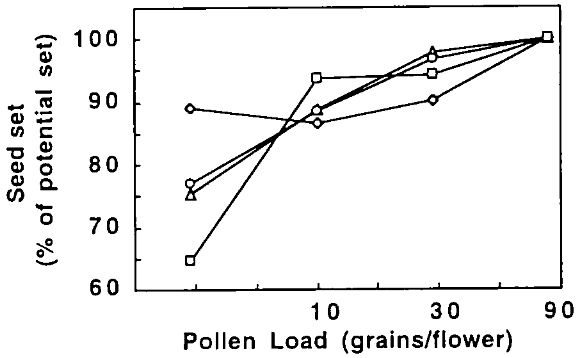

Fig. 2. The effect of pollen load on seed set. Each point is the proportion of 50 to 100 flowers showing normal seed development; the corresponding standard errors are about ± 5%. The data are the results of four different replications done over three months. The stigmas of flowers with 90 pollen grains were pollen-saturated, the balance of the flowers did not develop for reasons unrelated to the pollen load. This proportion varied between experiments for reasons that remain unclear. The seed set at pollen-saturation for the four replications was 89% (triangles), 61% (circles), 82% (diamonds), 96% (squares).

Table 1. Effect of pollen load on reproduction and progeny quality. A difference in seed set indicates that seed set was limited by the number of pollen grains; a difference in offspring vigor indicates that the pollen grains were competing for ovules

	Pollen load		P[1]	
	High (15)	Low (5)		
Seed set[2] (%)	55.0	28.8	< 0.005	($X^2 = 8.90$, 1 df)
Seed wt. (mg)	40.1	41.5	N.S.	(t = 0.93, 84 df)
Progeny performance				
Fresh Weight[3] (g)	3.4	2.3	0.001	(t = - 3.62, 29 df)
Seed number	19.9	16.0	0.12	(t = - 1.71, 9 df)

[1] Probability that difference is due to chance.
[2] 109 and 90 flowers pollinated at each load.
[3] Measured 21 days after planting.

dicted if faster growing pollen also produce more vigorous progeny. Low competition (5 pollen grains) or high competition (15 pollen grains) mainly differed in the early growth of the progeny, with high competition producing plants which grew again half as fast as the progeny of low competition (Table 1).

Seed size is often a measure of offspring quality, and one which can predict seedling vigor. However, in these experiments, the seed size was constant (41 mg) in all treatments (Table 1).

Discussion

Pollen limitation occurs when the pollen load is insufficient to produce maximal seed set (Snow, 1986). Pollen limitation has also been defined as fewer than one pollen per ovule (Stephenson & Bertin, 1983). Frequently, flowers are pollen-limited in Snow's sense even when they receive more pollen grains than there are ovules. When the pollen load is larger than that necesssary to overcome limitation, competition occurs among pollen grains for the opportunity to fertilize (Snow, 1986). This paper examines why seed set is limited by pollen number even when there are more pollen grains than ovules. If maternal resources are adequate, seed set may yet be limiting either through pollen-tube attrition or through abortion of weak progeny. The paper further examines whether pollen-grain competition improves the performance of the offspring.

Pollen limitation

Each buckwheat flower has only one ovule, so a single pollen grain could in principle be sufficient for maximal seed set. However, pollen load was limiting (*sensu* Snow, 1986) even with more pollen than ovules. As the pollen load was increased, pollen tubes more frequently reached the embryo sac. In buckwheat the primary obstacle to pollen-tube growth appears to be physical rather than nutritional or hormonal (Schoch-Bodmer, 1934). The improved growth with several pollen tubes fits the model that the pollen tubes cooperatively open a path between the stylar cells more efficiently than opening individual paths for each pollen tube (Esser, 1953; Ter-Avanesian, 1978). Only a few pollen tubes are needed for this cooperativity, so pollen-tube attrition through the transmitting tissue is less severe in buckwheat than, for instance, *Nicotiana glauca* (Cruzan, 1986).

The saturating pollen load for both embryo sac penetration and fruit initiation occurred at about 10 pollen per flower. This relationship indicates that pollen-tube growth is the main reason that 2 to 10 pollen grains do not give maximum seed set. There was no evidence that the failure was due to selective abortion of weak embryos, or those fertilized by non-preferred gametes (Willson & Burley, 1983), because all the failed flowers at low pollen loads can be accounted for by female sterility and unsuccessful pollen-tube growth. Distinguishing between these possibilities can be done clearly with buckwheat because it has only one ovule per flower, whereas similar results with multi-ovule fruits can be confounded by intra-fruit competition (Shore & Barrett, 1984; Wiens et al., 1987; Herrera, 1990; O'Donnell & Bawa, 1993). Perhaps the differences among developing zygotes are less important when they are in separate fruits.

In nature, pollination occurs over 1–2 h with total pollen delivery of one to 75 compatible pollen per flower (Björkman, 1994; Björkman, 1995). This is enough that successful pollen-tube growth is nearly universal, as has been determined by direct examination (Morton, 1966).

Pollen-grain competition

If there is pollen-grain competition at higher pollen loads, that competition should result in selection for more vigorous embryos. Differences in embryo vigor can be expressed in two measurable ways: abortion of weaker embryos (Sutherland, 1987) and differences in growth of the resulting plants (McKenna, 1986). We used the latter measure, producing seeds in pollinations with or without pollen-grain competition. The seeds were the same size, but pollen competition resulted in seedlings that were 40% heavier. Their plants were grown well-spaced in the greenhouse. In field plantings, they are very close, so small differences in early growth result in substantial shading of the smaller plant. This difference in seedling performance should confer a substantial fitness advantage. The effect of pollen-grain competition on growth that was seen here may have been large because the original seeds were produced under conditions with weak pollen selection (Charlesworth & Charlesworth, 1992). The previous weak selection has left residual differences in quality that can be detected when stronger selection is applied. The better performance of progeny from non-limiting pollen loads shows that pollen-grain competition can occur in buckwheat.

The number of pollen grains per ovule required for maximal seed set in buckwheat is lower than for many other species in which pollen limitation has been studied. Seed set is maximal with as few as 10 pollen grains per flower (Namai & Ohsawa, 1986) in buckwheat. It is comparable to *Turnera ulmifolia* (Shore & Barrett, 1984) in the limiting pollen load, but in that species there are about 40 ovules per flower. Many other species require a much higher gamete ratio for maximal seed set. Cucumber requires hundreds of pollen per ovule (Winsor et al., 1987), cotton and trumpet creeper require thousands (Ter-Avanesian, 1978; Bertin, 1990). The relatively low saturating pollen load in buckwheat should also make it more sensitive to variation in the amount of pollen delivered.

Having determined that pollen-grain competition can occur in buckwheat, the question remains to what extent it occurs in nature: that depends on natural pollen loads. Bees and syrphid flies are the most common pollinators and they typically leave only about 5 compatible pollen in the first visit (Namai, 1990; Björkman, 1995). Only if all the initial pollen fail to fertilize will pollen grains from subsequent deliveries experience competition. The accumulation of pollen relative to that needed for full set was similar to that observed in *Hibiscus moscheutos* (Spira et al., 1992) and *Geranium maculatum* (Mulcahy et al., 1983), but the rapid pollen-tube growth in buckwheat makes pollen-grain competition less likely than it is in those species. In the long-styled flowers, the pollen tube reaches the base of the style in about 20 min (Schoch-Bodmer, 1930); in short-styled flowers it takes even less time. The pollination system in buckwheat seems to favor low pollen-grain competition because fewer pollen are delivered than can produce good competition, and the speed of pollen-tube growth makes increased competition difficult with the existing pollinators.

This research has distinct implications for agricultural production. Modest pollen loads are sufficient to avoid pollen limitation of the seed yield and to produce large seeds. However, the extent of male competition in a population, whether prezygotic or postzygotic, will affect the fitness of that population. Therefore, ensuring delivery of more pollen than is necessary for maximal seed yield ought to result in higher quality seed. Cultivated varieties of buckwheat have been selected for good performance through recurrent selection (Campbell, 1983) to maintain genetic variation, while increasing the frequency of desirable genes and reducing the frequency of undesirable ones. Keeping undesirable genes rare may require ongoing selection in the gametophytic generation. When a buckwheat crop is grown for seed to be planted, achieving a high pollen load will increase the quality of the seed lot. With honeybees providing only small pollen loads, it remains to be discovered how to obtain the pollen delivery needed to increase pollen-grain competition in the field.

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