Influence of Environmental Quality on Pollen Competitive Ability in Wild Radish

HELEN J. YOUNG* AND MAUREEN L. STANTON

Pollen of *Raphanus raphanistrum* produced under low nutrient conditions sired fewer seeds than pollen produced under better conditions when the two types were applied on a stigma together. No difference was seen in single-donor crosses. Male mating success can be strongly influenced by the environmental conditions of pollen-bearing plants, a factor overlooked in studies of plant reproductive biology and in standard quantitative genetic crossing designs, where effects of male parent are equated with heritable genetic variation.

ENVIRONMENTAL EFFECTS ON FEmale reproductive success in plants are well documented (*1*). Environmental conditions can also affect pollen characters that may, in turn, influence paternal success. The microenvironment of pollen-producing plants has been shown to affect pollen production (*2*), pollen size (*3*), pollen germination (*4*), and pollen tube growth rate (*5*), but the relations between each of these pollen traits and male success remain unknown. In general, environmental effects on pollen traits are ignored in studies of variation in male success, sexual selection, and classical crossing designs (*6*). The assumption of no paternal environmental effects overestimates genetic variance and response to selection in the evolution of characters that influence paternity, if the environmental variance for these traits is not zero.

We show that pollen quality, measured as the number of seeds sired, is influenced by environmental conditions during pollen development. We performed two types of crosses to compare seed production resulting from low levels of pollen competition (single-donor crosses) versus a higher degree of pollen competition (multiple-donor crosses).

Raphanus raphanistrum (Brassicaceae) was chosen for this study because of the simple pattern of flower color inheritance (one locus, two alleles), which we used as a genetic marker to determine the paternity of seeds. Yellow petal coloration is recessive, white is dominant (*7*). Seeds from wild-grown plants were collected near Hamden, Connecticut. Lines homozygous for petal color were created through three generations of hand pollinations (*8*).

We used pairs of full sibs to minimize genetic differences between plants grown in different conditions. Paired full sibs were divided among the high nutrient ("control") and low nutrient ("stress") treatments when the first floral buds appeared (*9*). Pollen recipients had the recessive yellow allele. These plants were unrelated to the yellow-petaled individuals used as pollen donors and were grown under control conditions.

Mature flower buds were collected nine times during the flowering period from each pollen donor to determine the number and size of pollen grains being produced (*10*). Groups of four plants (full sibs of white-petaled plants: one control and one stressed, and full sibs of yellow-petaled plants: one control and one stressed) were chosen on the basis of approximately equal numbers of pollen grains per flower, so hand pollinations would result in approximately equal pollen deposition within each group of donors. Pollinations were performed on two recipient plants for each set of these four pollen donors (Fig. 1); a total of four recipients and four donor families was used (*11*).

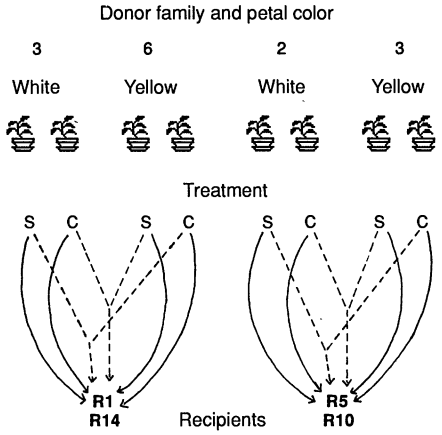

Fig. 1. Experimental design testing for environmental effects on paternity in wild radish. Full sibs of each family were split among nutrient treatments: S for stress (low nutrient) or C for control. Solid arrow, single-donor pollination; dashed arrow, mixed-donor pollination.

For the single-donor crosses, pollen from one freshly dehisced anther from each donor was applied to the stigma of a freshly opened flower on the recipient. At least 16 pollinations were performed from each donor on each of its assigned recipients. Mixed-donor pollinations were performed by applying pollen from two donors grown in different environments to each half of a

Table 1. Analysis of variance results for treatment, recipient, and donor effects on (A) fruit set (arcsine-transformed) and seed number per fruit from single-donor pollinations and (B) number of seeds sired by pollen of different treatments in the mixed-donor pollinations. For all analyses, type III sums of squares were used to calculate *F*-values, and the residuals were normally distributed.

(A) Single-donor pollinations

Source of variation	df	Fruit set		Seed number per fruit	
		F	*P*	*F*	*P*
Recipient set	1	12.8	0.012	29.1	<0.001
Recipient (recipient set)	2	4.3	0.063	41.2	<0.001
Donor environment (recipient set)	7	2.0	0.215	1.2	0.33
Total model	9,6	3.7	0.061	8.1	<0.001

(B) Mixed-donor pollinations: analysis on number of seeds sired

Source of variation	df	*F*	*P*
Recipient set	1	0.13	0.72
Recipient (recipient set)	2	0.37	0.69
Donor environment	1	37.68	<0.001
Donor environment × recipient set	1	8.95	0.003
Donor environment × recipient (recipient set)	2	1.83	0.162
Total model	7236	7.01	<0.001

Department of Botany, University of California, Davis, CA 95616.

*To whom correspondence should be addressed at Biology Department, Barnard College, 3009 Broadway, New York, NY 10027.

stigma on a recipient flower (white control + yellow stress; white stress + yellow control). Pollen from the different donors was applied less than 1 min apart, and the order of placement of the two pollen types was randomized across flowers on each plant. Between 20 and 40 pollinations of each type were made on each recipient. All pollinations were performed in a random order among flowers on recipients.

The single-donor crosses allowed us to determine the fertilization potential (that is, the number of fruits matured and seeds sired) for pollen developed under control and stress conditions in the absence of between-donor pollen competition. For these, mature fruits were counted, and the number of seeds per fruit was determined. The mixed-donor crosses allowed pollen from stressed and control plants to compete for fertilization of ovules. Mature fruits from these pollinations were collected and all seeds were planted singly in pots (710 seeds planted of which 290 germinated). Petal color of the offspring indicated paternity. For each seed planted, the dates of germination and flowering were recorded.

Conditions during pollen development (donor environment in Table 1A) did not have significant effects on fruit set or seed set after single-donor pollinations. There were, however, significant differences in response between the two recipient sets in fruit and seed set, due to the high fecundity of recipient 10.

In mixed pollinations, pollen from control plants sired significantly more seeds than competing pollen from stressed plants (Table 1B and Fig. 2). This inequality was statistically significant for three of the four recipients. The same trend, while not significant, was found for recipient 5 and for a recipient not included in the analysis because it produced so few seeds (recipient 7).

Analysis of progeny growth under greenhouse conditions showed that the environmentally caused differences in pollen competitive ability had no effect on offspring performance. There were no significant differences in progeny produced from pollen from stressed and control plants with respect to the number of days to germination [mean ($\bar{x}$) ± SD 6.8 ± 1.81 and 6.6 ± 1.38, respectively; $F(1,270) = 1.73$, $P = 0.19$] or the number of days to flowering [$\bar{x}$ ± SD = 32.3 ± 4.69, 32.3 ± 4.42, respectively; $F(1,270) = 0.05$, $P = 0.82$]. Treatment-based differences in progeny quality might become evident only in older individuals (*12*) or for individuals grown in competition (*13*).

There were significant differences in pollen size and number among the four sets of full sib donors; however, there was no consistent relation with nutrient treatment (Fig. 3). Family differences for both traits were larger than treatment differences.

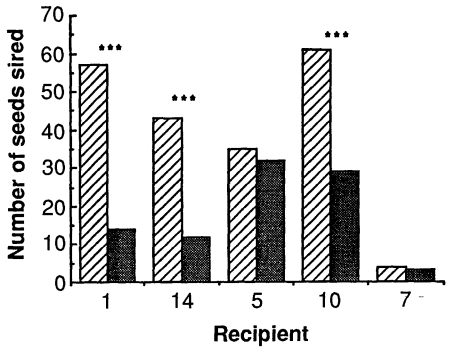

Fig. 2. Number of seeds sired by pollen from "stress" (low nutrient conditions) (shaded bars) and control plants (hatched bars) in mixed pollinations (ANOVA results in Table 1B). *P* values above bars refer to results of chi-square analysis on the number of seeds sired by pollen of each treatment on each recipient (***$P < 0.001$). Overall, control pollen sired 200 seeds, stress pollen sired 90 seeds; $\chi^2 = 32.5$, $P < 0.0001$.

When no between-donor pollen tube competition is occurring (single-donor crosses), pollen produced by plants grown under low nutrient conditions performs as well as pollen from control plants. Pollen grains developed under conditions of environmental stress are capable of germinating, growing down the style, and fertilizing ovules. It is possible that pollen tubes from unsupplemented plants grow more slowly than pollen tubes from control plants and thus reach ovules later, but because pollen from different parents were not competing in this experiment, seed production was equal for the two treatments. In contrast, when pollen from stress and control plants were applied to stigmas simultaneously, pollen from control plants sired more seeds. This may be due to differential pollen tube growth rates of pollen from the two treatments, but differential abortion of seeds sired by pollen of the two treatments cannot be excluded as a cause. Whatever the cause, plants grown under poor conditions experienced reduced male success relative to plants grown in better conditions.

Our results shed light on previous studies of pollen fertilizing ability. Snow and Mazer (*14*) found no heritable variation for pollen competitive ability in *R. raphanistrum*, suggesting that pollen genotype is less important than other factors (such as environment) in determining seed-siring ability under competitive conditions. Sexual selection models assume that pollen competition is mediated primarily by pollen genotype (*15, 16*), not by the environment in which pollen development occurs (but suggested in *17*). The quality of progeny (as measured by seedling dry weight and corm weight) resulting from multiple-donor pollinations has been shown to be higher than that of progeny of single-donor crosses (*18*). Because genetic expression of the sporophyte and male gametophytes overlap substantially (*19*), the implication is that under conditions of pollen competition, pollen of superior genetic quality successfully fertilizes ovules, resulting in higher quality offspring. An alternative explanation is that favorable growth conditions of pollen-bearing plants give rise to more competitive pollen, which sire larger seeds (because early fertilized ovules may garner more resources than those fertilized later), which will produce larger and probably more competitive seedlings (*16, 20*).

Pollen size and number did not vary consistently with environmental conditions, therefore variation in paternity of plants grown in different treatments cannot be attributed to variation in these two pollen traits. Knowledge of pollen production or pollen size reveals little about realized male reproductive success if microenvironmental variation exists within the panmictic population. These results extend to sex allocation studies, which use allocation patterns to male and female reproductive structures as estimates of relative success as male and female parents (*21*). If paternity is more strongly influenced by less visible pollen traits (pollen nutrients), then measuring pollen number and size is not sufficient for estimating male fitness.

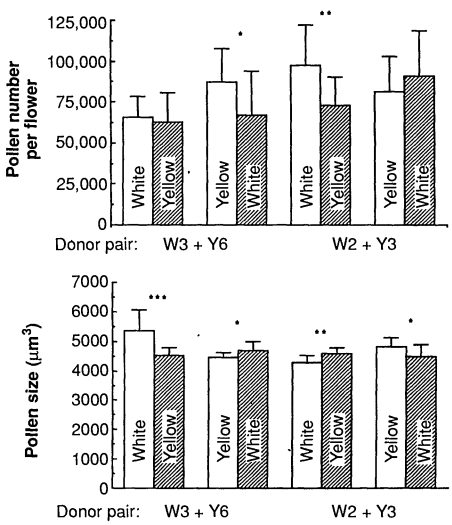

Fig. 3. Mean (+ SD) of pollen traits of donors, presented in pairs of donors are used for hand pollinations on recipients; open bars, pollen from control plants; hatched bars, pollen from stressed plants (low nutrient conditions). ANOVA was used to detect differences in pollen number per flower and pollen size between the plants of each pair; significant differences are indicated by asterisks above the bars (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Sample sizes for each bar range from 18 to 20.

If we had performed only single-donor crosses, we would have concluded that environmental conditions during pollen development are unimportant in determining fruit set and seed set. Single-donor pollinations are appropriate only when it is known that, under natural conditions, stigmas receive pollen from just one donor. Multiple paternity is common in wild *Raphanus* populations (*22*). Potential pollen performance in mixtures need not parallel performance in isolation, just as competition between species in mixtures is difficult to predict from growth characteristics when species are grown alone (*23*). It is therefore important to determine the types of pollen loads occurring under natural conditions to understand the potential effect of environmental variation on male mating success.

Typically, analyses of phenotypic variation consider variance due to nuclear genetic, maternal genetic (cytoplasmic), maternal environment, and environmental variation. Any differences between paternal half sibs are considered to result solely from additive genetic variation (*24*). We suggest that paternal environment effects may have important fitness consequences, especially with regard to mating success. Paternal success variation may have a genetic component, but our study demonstrates the existence of strong nongenetic components. It is thus essential to control conditions of pollen development in experiments testing for genetic variation in paternity.

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9. Four to eight full sib individuals from four families each of white and yellow-petal homozygotes were grown from seed to act as pollen donors. These were planted in 10-cm pots in a University of California, Davis, greenhouse. When flower development was just beginning, full sib pairs were divided between two treatments: half were transplanted into 18-cm pots of a mixture of clay, loam, sand, and nutrients, and fertilized with Hoagland's solution two to three times weekly (control treatment); half were transplanted into 18-cm pots of pure loam and never fertilized ("stress" treatment). All plants were placed randomly within the greenhouse and positions were rotated biweekly.
10. Pollen was counted and size distributions were determined using an electronic particle analyzer (Elzone 180+; Particle Data, Inc., Elmhurst, IL), as described in H. J. Young and M. L. Stanton, *Can. J. Bot.* **68**, 480 (1990).
11. We originally used eight recipients, but three showed incompatibility with at least one of the donor lines and one produced very few seeds from mixed pollinations. Therefore, results are reported for only four recipients.
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Light-Evoked Changes in the Interphotoreceptor Matrix

FUMIYUKI UEHARA, MICHAEL T. MATTHES, DOUGLAS YASUMURA, MATTHEW M. LAVAIL*

The normal function of vertebrate photoreceptor cells depends on multiple interactions and transfer of substances between the photoreceptors and the retinal pigment epithelium (RPE), but the mechanisms of these interactions are poorly understood. Many are thought to be mediated by the interphotoreceptor matrix (IPM), a complex extracellular matrix that surrounds the photoreceptors and lies between them and the RPE. Histochemical, immunocytochemical, and lectin probes for several IPM constituents revealed that components of the IPM in the rat undergo a major shift in distribution or molecular conformation after the transition between light and dark. In the light, various IPM constituents concentrated in bands at the apical and basal regions of the outer segment zone; in the dark, they distributed much more uniformly throughout the zone. The change in IPM distribution was triggered by the light-dark transition; it was not a circadian event, and it was not driven by a systemic factor. The light-evoked change in IPM distribution may facilitate the transfer of substances between the photoreceptors and the RPE.

THE NORMAL FUNCTION AND METABolism of vertebrate photoreceptor cells depend on numerous interactions with the RPE. These interactions include exchange of metabolites and catabolic by-products (*1*), water and ion transport, retinoid transfer between photoreceptors and RPE during the visual pigment cycle, control of the proper ionic composition in the external milieu, alignment and adhesion of photoreceptor outer segments to the RPE, and possible signaling from the retina to the RPE for the regulation of outer segment disk shedding (*2–7*). Several of these events follow the environmental light-dark transition or follow a light-entrained circadian rhythm (*2–4*). Because there are no direct intercellular connections between

Departments of Anatomy and Ophthalmology and Beckman Vision Center, University of California, San Francisco, CA 94143.

*To whom correspondence should be addressed at the Beckman Vision Center.