NOTES AND COMMENTS

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GERMINATION SCHEDULES OF POLLEN GRAINS: IMPLICATIONS FOR POLLEN SELECTION

JAMES D. THOMSON
Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY 11794 and Rocky Mountain Biological Laboratory, Crested Butte, CO 81224

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Because many of the genes expressed during pollen-tube growth are also expressed in the resulting sporophyte (Tanksley et al., 1981; Willing and Mascarenhas, 1984; Sari-Gorla et al., 1986; Weeden, 1986), selection during tube growth has been proposed as a technique for rapid screening of desired plant characteristics, such as tolerance to extreme physical conditions, heavy metals, and herbicides (den Nijs et al., 1986; Searcy and Mulcahy, 1986; Simon and Sanford, 1986). More generally, intense "gametophytic competition" among growing tubes may produce more vigorous or more competitive progeny, apparently because faster-growing pollen genotypes fertilize ovules first and transmit their metabolic superiority to the seeds (Mulcahy, 1974; Mulcahy and Mulcahy, 1975; McKenna and Mulcahy, 1983). Mulcahy (1979) has proposed that such processes are fundamental to the evolutionary success of the angiosperms. For such a "pollen-tube race" to allow strong selection among pollen tubes based on small growth-rate differentials, however, the race must have a fair start, i.e., a cohort of grains must reach the stigma together, then hydrate (Heslop-Harrison, 1979) and germinate in approximate synchrony. Existing models of the pollen-tube race have concentrated on such factors as the arrival schedule of multiple depositions of pollen (Mulcahy et al., 1983; Snow, 1986) or on sources of variation among flowers (e.g., age) that affect whole cohorts of grains equally (Galen et al., 1986). This paper, in contrast, specifically considers germination synchrony.

It is usually "tacitly assumed" (Stead et al., 1979) that hydration and germination occur quickly after pollination, such that intergrain differences in starting time would be negligible in comparison to growth-rate variation. However, gametophytic competition appears to be intensified when tubes grow through longer styles, implying that there is some initial random variation in germination time that is countered by a longer growing phase (Mulcahy and Mulcahy, 1975; McKenna and Mulcahy, 1983). Although individual grains are known to begin tube growth very quickly in some systems (Heslop-Harrison, 1979; Stead et al., 1979), no data are available on the distribution of germination times for a cohort of simultaneously applied grains in vivo. Here, I use a natural visual marker to study: 1) variation in germination times in undisturbed conditions, 2) effects of the spatial arrangement of grains on germination time, and 3) the relation between pollen density and mean germination rate.

Some plants of *Erythronium grandiflorum* (Liliaceae) produce red pollen grains (see Thomson, 1986) that contrast sharply with the whitish stigma until hydration/germination, at which point they quickly become colorless. When single grains are removed from stigmas for examination, fully colored ones have never hydrated and remain furrowed and ellipsoidal; in contrast, newly decolored ones are fully swollen, unfurrowed, and nearly spheroidal and almost invariably have a short pollen tube. Where lacking, pollen tubes may have broken off during removal of the grain, and I conclude that color loss 1) is a prerequisite for germination and 2) roughly coincides with germination.

In June 1986, I collected flower buds near the Rocky Mountain Biological Laboratory, at Gothic, Colorado (2,900 m altitude), and kept them indoors in vases until the stigmas were receptive. Red outcross pollen from a flower with freshly dehisced anthers was applied to clean stigmas with a brush (flowers 1–2 in Table 1) or by a feeding bumble bee queen (*Bombus occidentalis*, an important pollinator in nature) (flowers 3–8 in Table 1). Whether applied by hand or by bee, pollen-load sizes and distributions were similar to those resulting from natural pollination, including the frequent occurrence of clumps of 5–15 grains. To determine whether these clumps affected germination rate, I gently spread the clumped grains evenly over the receptive surface on one of the three distinct stigma lobes, using a fine insect pin (flowers 1–2) or an eyelash (flowers 3–8). The remaining two lobes were left as controls; on all except the first flower, I chose the lobes with the highest and the lowest numbers of grains for the controls. The mean (standard deviation) initial grain numbers were 75.6 (12.3) for the treatment lobe, 71.1 (11.7) for the low control, and 105.5 (9.4) for the high control ($N = 8$ for each). Within flowers, the number of grains on the high control lobe exceeded that on the low control lobe by a factor ranging from 1.21 to 1.69 ($\bar{x} = 1.51$, SD = 0.16). The treatment: low-control ratio of initial loads ranged from 0.74 (flower 1) to 1.20 ($\bar{x} = 1.07$, SD = 0.15), and the treatment : high-control ratio ranged from 0.54 to 0.86 ($\bar{x} = 0.72$, SD = 0.10). I counted colored grains at intervals over the next 29 hours. Laboratory conditions (20°C and 30% RH) approximated field conditions during the day, but nights were much colder in the field.

Grain germination rates are summarized as a "survivorship curve" in Figure 1. On both treatment and control lobes, some grains decolored within five min-

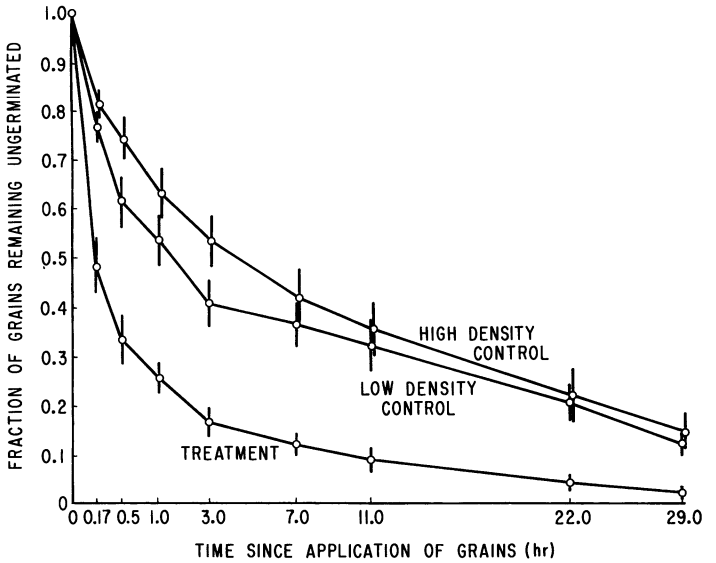

FIG. 1. A "survivorship curve" for *Erythronium grandiflorum* pollen, giving the mean (±SE; $N = 8$ for each point) fraction of grains remaining ungerminated as a function of the time since their placement on the stigma. The time scale is nonlinearly expanded for clarity.

utes. These were typically grains that were deposited in the fluid-filled cleft separating the two rows of stigmatic papillae. This cleft is continuous with the fluid-filled lumen of the style, down which the tubes grow. Most grains lodged on the papillae, and these took considerably longer, not germinating until they contacted the stigmatic exudate that appeared to collect near them. Grains on the tops of clumps, especially clumps along the outer fringes of papillae, germinated last. Stigmatic exudate eventually reached these grains, as if by capillary action, and the prominent clumps appeared to "melt" into the stigmatic surface as the lower grains hydrated and germinated. Germination was never complete within 22 hours, and red grains remained on most flowers at the end of the experiment, especially on the control lobes. Some of these may not have been capable of germination, but most were, as indicated by the near-complete germination on the treated lobes (97%, on average). On all lobes, many competent grains experienced long delays.

Because the age of a stigma is known to affect overall pollen germination rate in *Erythronium* (unpubl.) and other Liliaceae (e.g., *Clintonia* [Galen et al., 1986]), I compared treatments statistically only within stigmas. I followed Kruskal-Wallis tests for an overall treatment effect by pairwise comparisons using Mann-Whitney tests (Table 1). The removal of clumps (treatment) yielded faster germination than either of the controls for all eight stigmas, a result confirmed by significant Mann-Whitney tests of germination rank for 15 of 16 treatment-control comparisons. Comparing the two control lobes of each stigma, mean germination time is shorter in the low-density lobe in five of eight cases. Where germination on the high-density control is faster than on the low-density control (flowers 2, 4, and 8), the two values are very close, and the Mann-Whitney comparisons are all statistically nonsignificant. In two of the other flowers (1 and 5), the Mann-Whitney tests indicate that the difference is statistically significant. Thus, clumping very strongly delayed the germination of a set of grains. I tentatively conclude that high densities of grains also delay germination but that the effect is smaller than that due to clumping. The mechanism for the density effect probably also involves clumping; lobes that receive large numbers of grains probably do so because they receive clumps. Unfortunately, the "degree of clumping" is hard to assess objectively. The heterogeneity among the eight high-density vs. low-density control comparisons is probably due to variation in clumping; it does not appear to be related to the magnitude of the difference between the high and low load sizes.

Some of the variance in germination times may be due to intrinsic differences in hydration or germination ability within a cohort of grains. However, the strong role of clumping indicates that extrinsic, chance events of positioning contribute strongly to this variance. The pollen-tube race does not have a fair start, and a fast-growing grain that lands on top of a clump may give up a head start of several hours to a slow-grower that immediately lodges in stigmatic exudate.

Obviously, germination delays will weaken selection based on tube growth rates. It is impossible to tell how important this weakening is, because we lack the necessary parameter estimates to measure the selection, with or without delays. However, it is possible to model the effect of the observed germination delays for a range of parameters, by comparing the intensity of selection for growth rate (sensu Arnold and Wade, 1984) for two hypothetical cohorts of grains—one with the observed germination delays and one with instantaneous germination (unpublished simulations are available on request). As one would expect, delays weaken selection most when the mean growth rate is fast, the variance

TABLE 1. Statistical comparisons of pollen-germination times for the three stigma-lobe treatments within stigmas of *Erythronium grandiflorum*. Treatment = treatment lobe (grains spread to reduce clumping); low = low-density control lobe; high = high-density control lobe. Distributions of germination intervals (with each grain assigned to one of the intervals shown in Fig. 1) were compared by Kruskal-Wallis (K-W) tests to yield a test of an overall effect of the three treatments (Sokal and Rohlf, 1981 pp. 429–432). All stigmas showed a significant effect at $P \doteq 0.001$, except stigma 4 ($P < 0.005$). Multiple pairwise comparisons were made using Mann-Whitney tests (Sokal and Rohlf, 1981 pp. 432–436). Corrections for tied variates were applied, and the t approximation for large sample sizes was calculated. Significance tests for the t statistics are based on Sĭdák's multiplicative inequality (Sokal and Rohlf, 1981 pp. 240–241): a single asterisk indicates an experimentwise error rate of 0.05; two asterisks indicate an experimentwise error rate of 0.01. All statistics follow Sokal and Rohlf (1981).

Flower	H statistic for K-W test (adjusted for ties)	T_S approximation for Mann-Whitney test (two-tailed probability)		
		Treatment vs. low	Treatment vs. high	Low vs. high
1	35.1	3.37**	5.36**	2.55*
2	38.0	4.80**	4.95**	0.47
3	43.6	5.41**	7.70**	1.82
4	12.5	4.04**	3.41**	0.72
5	96.4	5.87**	8.23**	3.64**
6	63.2	4.55**	7.00**	1.70
7	32.6	3.35**	5.60**	1.69
8	15.4	1.99	3.65**	1.08

in growth rate is low, and the ratio of competing tubes to available ovules is small (see Mulcahy, 1983). For some parameter combinations (e.g., a mean tube-growth rate of 0.2 style lengths hr^{-1} with a coefficient of variation of 5% and a tube : ovule ratio of 2:1), the observed germination delays are sufficient to cancel selection entirely. Do delays affect selection this strongly in nature? I know of no system in which the relevant parameters have been measured. Virtually all reported distributions of tube growth rates are calculated with the implicit assumption of instantaneous germination.

Although it is easy to see how pollen selection might produce substantial responses following novel stresses, such as heavy metals, the continuing importance of gametophytic competition is harder to understand. In its simplest formulation, it would seem that selection would quickly eliminate any heritable variation in pollen-tube growth rate. However, if selection based on tube growth does confer fitness advantages, as proposed by Mulcahy and coauthors, irremediable germination delays should favor longer styles (to slow the mean speed in terms of style lengths per unit time), and larger stigmas or fewer ovules (to decrease the ovule : pollen ratio). Selection for larger depositions of pollen would also operate, but it would lose strength as load sizes began to exceed the amount necessary to coat the stigma evenly. Alternatively, plants with unfavorable speeds and ovule : pollen ratios might be under pressure to reduce germination delays, perhaps by maintaining "wetter" stigmas (Heslop-Harrison and Shivanna, 1977) or otherwise promoting the rapid and uniform attachment and hydration of a monolayer of pollen (cf. Ganeshaiah et al., 1986). Variations in stigma morphology may be important in this respect. For applied purposes, pollen selection could be considerably strengthened by prior hydration (Shivanna and Heslop-Harrison, 1981) and careful application of grains to reduce and equalize germination delays.

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LITERATURE CITED

ARNOLD, S. J., AND M. J. WADE. 1984. On the measurement of natural and sexual selection: Theory. Evolution 38:709–719.

GALEN, C., J. A. SHYKOFF, AND R. C. PLOWRIGHT. 1986. Consequences of stigma receptivity in schedules for sexual selection in flowering plants. Amer. Natur. 127:462–476.

GANESHAIAH, K. N., R. U. SHAANKER, AND G. SHIVASHANKER. 1986. Stigmatic inhibition of pollen grain germination—Its implication for frequency distribution of seed number in pods of *Leucaena leucocephala* (Lam) de Wit. Oecologia 70:568–572.

HESLOP-HARRISON, J. 1979. An interpretation of the hydrodynamics of pollen. Amer. J. Bot. 66:737–743.

HESLOP-HARRISON, J., AND K. R. SHIVANNA. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. 41:1233–1258.

MCKENNA, M., AND D. MULCAHY. 1983. Ecological aspects of gametophytic competition in *Dianthus chinensis*: Effects on sporophytic competitive ability, pp. 419–424. *In* D. L. Mulcahy and E. Ottaviano (eds.), Pollen: Biology and Implications for Plant Breeding. Elsevier, N.Y.

MULCAHY, D. 1974. Correlation between speed of pollen tube growth and seedling height in *Zea mays*. Nature 249:491–493.

———. 1979. The rise of angiosperms: A genecological factor. Science 206:20–23.

———. 1983. Models of pollen tube competition in *Geranium maculatum*, pp. 151–161. *In* L. Real (ed.), Pollination Biology. Academic Press, Orlando, FL.

MULCAHY, D., P. S. CURTIS, AND A. A. SNOW. 1983. Pollen competition in a natural population, pp. 330–337. *In* C. E. Jones and R. J. Little (eds.), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, N.Y.

MULCAHY, D., AND G. B. MULCAHY 1975. The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. Theoret. Appl. Genet. 46:277–280.

DEN NIJS, A. P. M., B. MAISONNEUVE, AND G.

HOGENBOOM. 1986. Pollen selection in breeding glasshouse tomatoes for low energy conditions, pp. 125–130. *In* D. Mulcahy, G. Bergamini Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, N.Y.

SARI-GORLA, M. C. FROVA, AND R. REDAELLI. 1986. Extent of gene expression at the gametophytic phase in maize, pp. 27–32. *In* D. Mulcahy, G. Bergamini Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, N.Y.

SEARCY, K., AND D. MULCAHY. 1986. Gametophytic expression of heavy metal tolerance, pp. 159–164. *In* D. Mulcahy, G. Bergamini Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, N.Y.

SHIVANNA, K. R., AND J. HESLOP-HARRISON. 1981. Membrane state and pollen viability. J. Ann. Bot. 47:759–766.

SIMON, J., AND J. C. SANFORD. 1986. Induction of gametic selection *in situ* by stylar application of selective agents, pp. 107–112. *In* D. Mulcahy, G. Bergamini Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, N.Y.

SNOW, A. A. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): Consequences for gametophytic selection. Amer. J. Bot. 73:139–151.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd Ed. Freeman, San Francisco, CA.

STEAD, A. D., I. N. ROBERTS, AND H. G. DICKINSON. 1979. Pollen-pistil interactions in *Brassica oleracea*: Events prior to pollen germination. Planta 146: 211–216.

TANKSLEY, S., D. ZAMIR, AND C. M. RICK. 1981. Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon esculentum*. Science 213:453–455.

THOMSON, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. J. Ecol. 74:329–341.

WEEDEN, N. F. 1986. Identification of duplicate loci and evidence for post-meiotic gene expression in pollen, pp. 9–14. *In* D. Mulcahy, G. Bergamini Mulcahy, and E. Ottaviano (eds.), Biotechnology and Ecology of Pollen. Springer-Verlag, N.Y.

WILLING, R. P., AND J. P. MASCARENHAS. 1984. Analysis of the complexity and diversity of mRNAs from pollen and shoots of *Tradescantia palludosa*. Plant Physiol. 75:865–868.

Corresponding Editor: A. G. Stephenson

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ANALYZING TABLES OF STATISTICAL TESTS

WILLIAM R. RICE
Department of Biology, University of New Mexico, Albuquerque, NM 87131

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Tables of statistical tests are commonly analyzed in evolutionary studies. These include analysis-of-variance and regression tables as well as tables of correlation coefficients, chi-square values, G values, Student's t values, etc. To see the prevalence of such tables, one need only refer to a recent issue of *Evolution* (e.g., Evolution 41(6), November 1987, where such tables appeared in 14 of 22 empirical articles). Here, I point out that testing for the statistical significance of component tests is routinely carried out in a biased fashion that liberally judges far too many tests to be significant. I then describe a nonparametric technique, originally proposed by Holm (1979), to eliminate this bias.

So as not to single out any one person unfairly and use his published results as a straw man, consider a hypothetical correlation table examining five variables. The procedure standardly used to evaluate such a table is to carry out an individual significance test on each of the ten correlation coefficients and then denote those significant at the 5% level with an asterisk, those significant at the 1% level with two asterisks, etc. Suppose that two of the ten correlation coefficients were found to be individually significant ($P < 0.05$). Using the "individual significance method," a researcher might spend several journal pages explaining the evolutionary ramifications of the two individually significant correlations observed in the table. Yet there may be insufficient evidence to be 95% confident that there are any nonzero correlations. Appropriate probability values must adjust for the number of simultaneous tests.

One can solve for the probability of observing at least one individually significant correlation (P value less than 0.05) in the above, hypothetical correlation table on the composite null hypothesis ($H_{0,c}$) that all the component correlations are zero. In computer simulations (Appendix), this probability is approximately 40%. Moreover, the probability of observing two or more individual P values less than or equal to 0.05 is about 7%. If a dozen variables were correlated, we would be more than 95% certain, on $H_{0,c}$, that at least one correlation would be judged individually significant by chance alone. Even very small P values are expected in moderately large correlation tables. With a dozen variables, chance alone would produce a P value less than or equal to 0.001 about 7% of the time. The marking of component tests as statistically signif-