

being conducted. It has been found that embryo preparations, purified as in previous experiments, contain a heat sensitive enzymatic activity which destroys a component of the reaction mixture, probably glucose-1-P. Thus, attention is now focused on the removal of this latter enzyme from the embryo pyrophosphorylase preparation in order to compare, in a definitive way, the pyrophosphorylases from the two tissues.

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Characterization of a gametophyte factor-like system on chromosome 5

A genetic system which leads to preferential fertilization by male gametes which contain bt-A on chromosome 5 has been discovered and is now being characterized. Given below is a summary of some features of this system. Seen initially in Dr. Oliver Nelson's laboratory, this system has several features in common with gametophyte factors and, because of this, the gene located on chromosome 5 will be given the symbol Ga. Because appropriate allelism tests with other known ga loci on chromosome 5 have not been completed, the allele carried on the chromosome favored in fertilization will be denoted Ga*-7001; the contrasting allele is termed ga*-7001. Initially, it was found that plants of the genotype Bt/bt-A gave rise to excess bt kernels when self-pollinated or when used as male parents in crosses with bt-A/bt-A plants. The reciprocal cross yielded the expected 50% frequency of bt kernels.

Furthermore, this system appears to select male gametes which carry Ga*-7001 for fertilization but not at the complete exclusion of ga*-7001 gametes. This is suggested from the following lines of evidence. First, among 43 F₂ families from the original F₂ progeny, 42 contained bt kernels at a frequency significantly greater than 25%. An average frequency of 39.5% was calculated from 17,570 kernels. If selection is complete for Ga*-7001, this gene must be approximately 21 map units from the bt locus. It follows then that 32% of the resulting F₂ Bt/bt-A kernels should be also Ga*-7001/Ga*-7001 and should give rise to 25% bt in the F₃ generation. Among 26 Bt/bt-A plants selected from 4 F₂ progenies, only one gave, upon self-pollination, a frequency not significantly greater than 25%. Again, 32%, or a total of 8, would be expected were the genetic distance equal to 21 map units. These latter results suggest, then, that Ga*-7001 and bt-A are much closer than 21 map units and this would suggest that some ga*-7001 pollen also effect fertilization. Secondly, there appears to be genetic modification of the extent of Ga*-7001 selection. This is from the following evidence.

Two F₂ families contained bt kernels at frequencies of 29.5% and 29.1%. These values are significantly greater than 25% but much lower than the overall average of 39.5%. Four Bt kernels, selected from each of these families and which proved to be Bt/bt-A, gave rise, upon self-pollination, to bt kernels at average frequencies of 27.8% and 30.8% respectively. Similarly, Bt/bt-A kernels selected from four other F₂ progenies which had bt frequencies of 53.8%, 39.5%, 49.5% and 40.7% produced F₃ progenies in which the average percent bt was 39.6%, 34.7%, 43.0% and 42.5%. The simplest explanation is that there exists a genetic system in which Ga*-7001 gametes are favored in fertilization but that the extent of Ga*-7001 selection is susceptible to genetic modification. Genetic heterogeneity for this modification existed in the original F₁ progeny.

In analogy with other ga systems, the simplest model to account for the observations thus far is that there exist two alleles of this locus; the allele Ga*-7001, when carried in the female, favors fertilization by pollen also carrying Ga*-7001. Data presented in Table 1 show, however, that this model cannot account for all the observations. It can be seen that plants heterozygous for other alleles of bt yield, upon self-pollination, the expected results. However, when these plants are used as males onto Bt/bt-A heterozygotes, an excess of bt kernels is observed.

Table 1. The percentage of brittle kernels resulting from self-pollinations and crosses of various bt heterozygotes.

Plant	Genotype	% <u>bt</u> kernels	
		Selfs	Onto <u>Bt/bt-A</u>
16-1	<u>Bt/bt-D</u>	23.2	35.9
16-3	<u>Bt/bt-D</u>	29.8	40.3
15-14	<u>Bt/bt-D</u>	22.2	35.9
6554-6	<u>Bt/bt-C</u>	28.2	44.9
6556-6	<u>Bt/bt-D</u>	25.7	40.2
6556-13	<u>Bt/bt-D</u>	28.9	41.0
6558-5	<u>Bt/bt-E</u>	24.2	46.8
6560-6	<u>Bt/bt-F</u>	22.2	31.8

Clearly, the one locus-two allele system is ruled out. At the next level of complexity are the following two models.

First, there may exist three alleles of this locus; the two alleles carried, for example, by plant 16-1 (Table 1) would lack female action (could not condition the ability to detect, in the incoming male gametes, the heterogeneity conditioned by this locus), but would have male action (Ga*-7001 in the female can differentiate between them). Secondly, there may be two loci involved. In its simplest form, this model would be the following. One gene would act in the male gametes to condition the differences detected and a second, independently acting, gene would condition in the female the ability to detect differences in the male gametes.

To distinguish between these possibilities, the following crosses were analyzed. Plant 8780-11, shown to be Bt ga*-7001/bt-A Ga*-7001 from appropriate crosses and, from pedigree analysis, homozygous for the presumed second gene, was crossed as female by plant 8777 (bt bv/bt bv). The resulting bt kernels were grown, self-pollinated and the resulting Bv progeny were tested for their ability to select Ga*-7001 pollen. Since bt and bv are 5 map units apart, and thus the maximum distance between Ga*-7001 and Bv is 26 units, a minimum of 85% of Bv plants would also carry Ga*-7001. Among 23 plants tested for female action, only 10 could select male gametes that contained Ga*-7001. Again, 20 of the 23 should have shown positive female action if Ga*-7001 alone can select Ga*-7001 pollen. Thus, the first alternative hypothesis appears unlikely. However, a second test, outlined below, suggests that Ga*-7001, or a recessive gene linked to it, is involved in selection. The Bt kernels from the 8780-11 x 8777 cross would lack Ga*-7001. These kernels were grown, self-pollinated, and the Bt progeny were tested for female action. Of 17 plants tested, only one gave rise to excess bt kernels in the cross. This would suggest that Ga*-7001 or a recessive gene linked to it is needed for female action. Future work will be directed at obtaining more definitive data concerning the proper mechanism.

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Attempts to induce cytoplasmic male sterility with chemical agents

During this past summer male sterile material that had been crossed with various inbreds (MNL 49:35) was grown to determine if any of the plants were