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*THE TUNICATE LOCUS IN MAIZE DISSECTED
AND RECONSTITUTED*

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Tunicate maize, commonly known as pod corn, is a peculiar type in which the kernels are enclosed, as they are in wild grasses, in floral bracts called "glumes." Pod corn has been regarded as the ancestral form of cultivated corn by a number of nineteenth century students of maize and in this century by Mangelsdorf and Reeves^{1, 2} who have reviewed the extensive literature on the subject. A genetically reconstructed ancestral form of maize was developed by Mangelsdorf³ by crossing pod corn with popcorn, a type also regarded as primitive.

The principal characteristics of tunicate maize are controlled by a locus represented by the symbol, *Tu*, on the long arm of the fourth longest chromosome. In the course of our experiments this locus has several times mutated to a weak form of pod corn which we call "half tunicate" because its effects when homozygous in the genotype, $tu^h tu^h$, are about equal to those of the tunicate locus when heterozygous in the genotype, *Tutu*. This has led us to suspect that the tunicate locus may be a compound one and that the "mutations" which occur at this locus are, in fact, the product of crossing over which separates the components.

To test this possibility we crossed a uniform inbred strain, A158, into which the *Tu* locus had been incorporated by nine generations of crossing and backcrossing, with another inbred strain carrying two recessive marker genes on chromosome 4: *su* on one side and *gl*₃ on the other side of the *Tu* locus. The *F*₁ plants heterozygous for the three loci represented by the genotype, *SuTuGl*₃/*sutugl*₃, were then backcrossed to a second inbred strain homozygous for the three recessive loci. A total population of 10,248 plants of this backcross was grown over a period of three seasons, 1958-1960. Approximately half of these plants were expected to be heterozygous tunicate; 5,273 were found. There were, in addition, four heterozygous half tunicate plants, all of which proved by progeny tests to be crossovers, two being of the genotype *Sugl*₃/*sugl*₃, and two of the genotype *suGl*₃/*sugl*₃. This represents a "mutation" or crossover rate of one in 1,319.

Since the average percentage of crossing over between the loci *Su* and *Gl*₃ is 34 per cent,⁴ the chances of a mutation at the *Tu* locus being accompanied by a crossing over are approximately 1/3 if the two events are independent. The chances of four mutations being accompanied by crossing over are therefore 1/81. If this were the first case on record of a locus being separated by crossing over, we should not

regard these odds as proving a relationship between mutation and crossing over. However, since the components of other loci in both maize and *Drosophila* have been separated through crossing over, the probability that the four mutations occurring in this experiment were indeed crossovers seemed to us great enough to justify our proceeding to further steps.

The next step was to determine whether the two components were identical or different in their phenotypic effects. If identical, it could be concluded that the *Tu* locus is one which, like the classical case of the *Bar* locus in *Drosophila* described by Sturtevant,⁵ had arisen through a duplication without subsequent differentiation of function of the ancestral wild locus. This could have occurred at any time during the domestication of maize. However, if the two components proved to be different in their phenotypic effects as are the components of the pseudoallelic loci discussed by Lewis,⁶ it would suggest that divergence in function had occurred, and it seems unlikely that this degree of gene evolution could have taken place in the few thousand generations during which maize has been cultivated. Consequently, if the components proved to be identical, we would assume that the *Tu* locus is the product of unequal crossing over which occurred during domestication, and that the wild locus is the one producing the half tunicate effect. If, however, the two components proved to be different, we would assume either that the wild locus was *Tu* or that there had been two kinds of wild corn, one represented by the left-hand component tentatively designated *tu'* and the other by the right-hand component designated *tu^d*.

From the outset the two components appeared to differ slightly in their effects but whether this was actually the case or the product of differences in their residual heredity could only be determined with certainty by comparing them on the same genetic background. This was accomplished by incorporating both into the uniform inbred strain, A158, through repeated backcrossing. After the fourth backcross the difference between the components in their phenotypic effects was clear. The heterozygous genotype, *tu^dtu*, consistently had longer, more prominent glumes, both staminate and pistillate, than the genotype *tu'tu*, and its kernels were noticeably more difficult to remove from the cobs. Even more pronounced were the differences between homozygous genotypes produced by selfing heterozygotes after three generations of backcrossing. The genotype, *tu'tu'*, proved to be almost identical with one involving earlier mutations to half tunicate. The genotype, *tu^dtu^d*, however, produced monstrous inflorescences, both staminate and pistillate, of which the majority of the former and all of the latter were completely sterile.

Before the final proof of the differences between the two components of the *Tu* locus had been established, we had proceeded to an additional experiment—one designed to determine whether the *Tu* locus could be reconstituted by restoring its components to their original positions on the same chromosome. In 1961 the heterozygous genotypes, *tu'tu* and *tu^dtu*, were crossed. The progeny of one such cross was grown in 1962. It was expected that approximately one fourth of the progeny plants would be double heterozygotes in the *trans* configuration, *tu'tu/-tutu^d*. In a population of 133 plants, 24 proved to be of this type. Pollen from these plants was applied to plants of two inbred strains, A158 and NY16. It was assumed that the progeny of these test crosses would consist of the heterozygous genotypes, *tu'tu* and *tutu^d*, in approximately equal numbers and that the great

majority of the plants would fall into these two categories. It was assumed further, however, that there would be rare crossovers between the two components and that these would be of two complementary types, $tu^d tu^d$ and $tutu$. The occurrence of both types in approximately equal numbers would furnish virtually conclusive proof that mutations at the *Tu* locus are the product of crossing over between its components.

Because most modern corn, including the two inbreds used in this experiment, is of the genotype, $tutu$, extraordinary precautions were required to eliminate any possibility whatever of contamination. Consequently, stocks to be crossed were grown not in our regular experimental plots at Forest Hills but in a small garden in Cambridge completely isolated from all other maize and surrounded by University buildings. In the segregating progeny grown to provide the pollen parent genotype, $tu^d tu^d / tutu^d$, the plants of the remaining three genotypes, $tu^d tu^d / tutu$, $tutu^d / tutu$, and $tutu$, were removed as soon as they were identified and well before they had reached the pollen-shedding stage. Likewise, all staminate inflorescences were removed from the two inbred strains before their pollen had matured, and in addition their pistillate inflorescences were covered before their silks (styles) had emerged. It seemed certain that the only pollen to which the silks of the inbreds were exposed at any time was that of the selected double heterozygotes.

In the winter of 1962–1963, a population of hybrid plants resulting from the pollinations on the inbred, NY16, was grown near Homestead, Florida. When classified in April, three plants in a total of 2,333 proved to be similar to heterozygous tunicate *Tutu*, showing that the tunicate locus had been reconstituted. However, the other crossover class, $tutu$, did not occur. Its absence in a population of this size is not statistically significant but made it necessary to grow additional populations. This was done in the summer of 1963, a population of 956 additional plants involving NY16 as a parent and 6,801 plants involving A158 as a parent being grown. In the total population of 10,090 plants of these two crosses, eight were identified as *Tutu* and seven as $tutu$.

The rate of "mutation" involved in reconstituting the locus, 1 in 1,261, is of the same order as that, 1 in 1,319, which occurred in the experiment involving the dissection of the locus. This is further evidence that both types of "mutation" are the product of crossing over since reverse mutations are seldom as frequent as direct "point" mutations.

The experiment on reconstructing the tunicate locus shows why pod corn, which Weatherwax⁷ and others have assumed to be a mutant form, has never been reported in pedigreed cultures, although millions of ears of inbred strains and their first-generation hybrids have been studied by corn breeders. Pod corn, of the type represented by the *Tu* locus, can appear as a mutant only in stocks of half-tunicate maize. If our genetic analysis of its locus is valid, it cannot occur as a mutant in modern commercial nontunicate maize.

It now appears that there may have been two kinds of wild corn: one of the genotype $tu^d tu^d$, the other of the genotype $tu^d tu^d$. When these were brought together under domestication by the American Indians, hybridization would have produced—as it did in our experimental cultures—two new types: (1) an extreme form of pod corn which the Indians in parts of both South and Middle America preserved (and still do) for its supposed magical properties; (2) a nonpodded corn,

similar to modern corn in lacking conspicuous glumes, which is more productive and in other ways more useful than pod corn as a cultivated food plant.

The prehistoric wild corn, uncovered by archaeological excavations in the Tehuacán Valley of Southern Mexico, recently described by Mangelsdorf, MacNeish, and Galinat,⁸ appears to be a weak form of pod corn similar to that of the genotype, *tu'tu'* combined with an inhibiting factor, in our cultures. This wild corn is the progenitor of two still existing but somewhat primitive races of corn in Mexico, *Chapalote* and *Nal-Tel*. But the Tehuacán wild corn is quite distinct in a number of characteristics from a third primitive race, a Mexican popcorn known as *Palomero Toluqueño*. The Tehuacán wild corn lacks tillers (secondary stalks), has glabrous leaf sheaths and round kernels, brown or orange in color. *Palomero Toluqueño* has tillers, pilose leaf sheaths, and pointed white kernels, and probably stems from a different race of wild corn. If and when this prehistoric progenitor is uncovered by future archaeological excavations, it would not be surprising to find it resembling the genotype, *tu^dtu^d*, and having its kernels almost completely enclosed in glumes.

Summary.—The tunicate locus which is responsible for pod corn, a primitive type regarded as the ancestral form in which the kernels are enclosed in glumes, has been dissected and reconstituted by crossing over. The two components of the locus proved to be different in their phenotypic effects. The results are regarded as furnishing experimental support to the hypothesis that there were two kinds of wild corn which, when brought together under domestication by the American Indians, hybridized to produce an extreme form of pod corn, which was preserved for its supposed magical properties, and the nonpodded condition characteristic of modern corn, which was perpetuated because of its usefulness.

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³ Mangelsdorf, P. C., *Proc. Am. Phil. Soc.*, 102, 454–463 (1958).

⁴ Emerson, R. A., G. W. Beadle, and A. C. Fraser, *Cornell Univ. Agr. Exp. Sta. Mem.*, 180 (1935).

⁵ Sturtevant, A. H., *Genetics*, 10, 117–147 (1925).

⁶ Lewis, E. B., in *Genes and Mutations*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 16 (1951), pp. 159–174.

⁷ Weatherwax, P., *Indian Corn in Old America* (New York: Macmillan, 1954).

⁸ Mangelsdorf, P. C., R. S. MacNeish, and W. C. Galinat, *Science*, in press.