

Minn A158T (T)  $\underline{rf_1 rf_1 Rf_2 Rf_2 rf_3 rf_3}$   
 R273N (N)  $\underline{Rf_1 Rf_1 rf_2 rf_2}$

Fifty  $F_1$  kernels each of the crosses Minn A158T x Ionia and R273N x Ionia were planted on the experimental field of the Faculty of Agriculture, University of Cantho. Of these, 42 and 48 germinated, respectively, and were grown during the dry season of 1971. Anthers were examined for male fertility or sterility in 42 and 46 individuals, respectively.

All of the anthers of the  $F_1$  hybrids involving Minn 158T were small in size, slender in shape, brownish in color, and empty; that is, practically no pollen grains were observed. On the contrary, all of the  $F_1$  hybrids involving R273 had normal anthers with fertile pollen grains.

From the above observation it can be concluded that the genotype of Ionia is  $\underline{rf_1 rf_1 rf_3 rf_3}$ . It was not possible, however, to identify the genotype for  $\underline{Rf_2}$  from the present experiment.

Yasuo Ohta  
Phan van Chuong

CORNELL UNIVERSITY  
Ithaca, New York  
and  
FAIRCHILD TROPICAL GARDEN  
Miami, Florida

1. Retention of Euchlaena as a genus separate from Zea.

During the 3 decades since Reeves and Mangelsdorf cited cytogenetic evidence in support of the transfer of the genus Euchlaena to the genus Zea, as originally proposed by Kuntze in 1904 and subsequently ignored by Hitchcock and other grass taxonomists, much additional botanical and archeological information has become available that does, in my opinion, fully justify the retention of Euchlaena as an autonomous genus. Reeves and Mangelsdorf conceded that Zea and Euchlaena are morphologically distinct, but argued that their cross-fertility, the similarity of their chromosomes and linkage relations, and the observed

prevalence of natural hybrids in certain areas of sympatric association with indigenous races of Mexican and Guatemalan maize, justified a congeneric status. Retention of Tripsacum as a separate genus was recommended by Reeves and Mangelsdorf although admitting that it was very similar to Euchlaena with respect to various morphological characteristics. They had also produced maize-tripsacum hybrids and obtained fertile progeny from backcrosses to maize, as others have done in conducting cytogenetic studies of their homeologous chromosomes.

The occurrence of fertile teosinte-maize hybrids would be a more convincing argument in favor of the congeneric status of Zea and Euchlaena if it were not obvious from field observations of populations where sympatric associations are prevalent that teosinte has retained all of the more important morphological characteristics by which its generic status was first recognized by Hooker, the famous English taxonomist, nearly 100 years ago.

In evaluating the taxonomic status of Euchlaena, Tripsacum and Zea, very thoroughly reviewed by Wilkes in 1967, it is noteworthy that they were first classified as members of the tribe Maydeae by Hackel in 1890, and less closely related genera such as Manisuris and Elionurus were placed by him in the Andropogoneae. Members of this latter tribe have perfect flowers and differ chiefly in this respect from the Maydeae which have staminate and pistillate flowers arranged either in different positions in the plant, or in different parts of the same inflorescence--the former occurring in Zea and Euchlaena and the latter in Tripsacum. Subsequent retention of Hackel's treatment by Bentham and Hooker, Hitchcock, and other grass taxonomists has continued to meet with the approval of horticulturists, agronomists and plant breeders who are, like many other plantsmen, dependent on readily recognizable morphological differences to identify plants they are working with in the field and garden--as well as in the herbarium.

It is well known that teosinte and maize have the same number of chromosomes and the same frequencies of genetic crossing-over that have been tested. However, the significant differences in reported frequencies of occurrence of intercalary and terminal chromosome knobs, especially

in Guatemalan races of teosinte and maize, suggest that the homology of their chromosomes is not as intimate as generally assumed; or, perhaps in areas where teosinte-maize hybrids are most prevalent, as in neglected corn fields near Chalco, Mexico, such differences in chromosome morphology do not exist.

Emphasis on chromosome homologies in evolutionary studies of natural relationships is well deserved, but many obvious limitations to their usefulness that have been experienced by taxonomists concerned primarily with the naming of plants is coming to be more generally recognized. Now it is becoming increasingly apparent that archeology and anthropology also are making important contributions to existing knowledge of the evolutionary history of maize and teosinte.

Very recent archeological discoveries in the Valley of Mexico, which I have been privileged to discuss with Dr. Peter Lorenzo, director of the Department of Prehistory in Mexico City, have revealed the existence at Tlapacaya near Chalco of well preserved teosinte kernels of an age contemporary with that of the oldest Tehuacan cobs, established as approximately 7,000 B.P. Also, in the Oaxaca area of southwestern Mexico kernels of teosinte, much like those of triangular shape characteristic of existing populations in the neighboring state of Guerrero, have been discovered recently by Dr. Kent Flannery, professor of anthropology at University of Michigan who, in personal conversations a few months ago, estimated their age as dating from about 1500 A.D. Thus, it appears from these discoveries and other less relevant archeological evidence that teosinte has retained for at least 7,000 years its unique and highly efficient method of seed production and dispersal, comprising caryopsis and seed case structures wholly different from the caryopsis and cob morphology evolved under domestication by the races of maize with which it was closely associated for prolonged periods of time.

The possibility, suggested by Reeves and Mangelsdorf, that several of the characteristics of survival value possessed by teosinte and not by maize probably would be the first to be selected against if the domestication of teosinte were undertaken, appears at the present time to be of relatively little significance in relation to questions

concerning the taxonomic status of Euchlaena as congeneric with Zea; now it seems more relevant as having been a portent of the current interest among maizologists in exploring more thoroughly than in the past the possibility that teosinte was the progenitor of domesticated maize.

In the accompanying table are listed contrasting morphological and ecological characteristics of major significance in differentiating modern maize and teosinte, and in this same table there is also a separate listing of similarly contrasting traits of archeological maize and teosinte that have been identified in the well preserved oldest Tehuacan maize cobs, possibly in early stages of domestication and dating from about 7,000 B.P., and in the equally well preserved seed cases and enclosed kernels of teosinte of contemporary age from Tlapacaya in the Valley of Mexico. Of the 14 traits distinguishing modern maize and teosinte, seven (nos. 1 to 4, 6, 7 and 10) similarly distinguish existing races of Mexican maize and all known species of tripsacum. In addition several species of tripsacum have culms as slender or more slender than those of teosinte, equally strong responses to photoperiod, climatic and edaphic tolerances, and even more restricted distributions in Mesoamerica. But these many similarities of teosinte and tripsacum were not considered by Reeves and Mangelsdorf to be ample justification for treating both Euchlaena and Tripsacum as congeneric with Zea, perhaps because the Tripsacum karyotype is more dissimilar to that of Zea than is the Euchlaena karyotype and Tripsacum-maize hybrids are less fertile than those of teosinte and maize.

From comparisons of the above mentioned differences separating Zea, Euchlaena and Tripsacum with those appearing in descriptions of related genera of the Andropogoneae, there is ample justification for the retention of Euchlaena as a separate genus. The partial similarity of maize and teosinte chromosomes, exclusive of their knob morphology, their similar linkage relations and the fertility of their hybrids should not be considered of primary significance in evaluating the taxonomic status of Zea and Euchlaena. It is now clearly apparent from field studies of teosinte populations, including those in which intimate sympatric associations with maize have produced apparently significant

Table 1. Characteristics of Taxonomic Significance Differentiating Zea and Euchlaena

Maize	Teosinte
<u>Comparisons of modern maize and teosinte applicable to their representative existing races and cultivars</u>	
<ol style="list-style-type: none"> <li>1. Paired pistillate spikelets</li> <li>2. Pedicellate pistillate spikelets</li> <li>3. Outer glume of pistillate spikelet membranous</li> <li>4. Rachis (cob) intact at maturity and non-corneous</li> <li>5. Cobs borne singly at one or few nodes</li> <li>6. Cobs of large size bearing many kernels</li> <li>7. Large kernels adhering to surface of mature cob at maturity</li> <li>8. Mature ears enclosed by numerous husks</li> <li>9. Culm diameter and leaf width exceeding those of teosinte</li> <li>10. Culms unbranched at upper nodes</li> <li>11. Terminal staminate inflorescence has central spike</li> <li>12. Weak response to photoperiod</li> <li>13. Broad climatic and edaphic tolerances</li> <li>14. Worldwide distribution in temperate and tropical climates</li> </ol>	<ol style="list-style-type: none"> <li>1. Unpaired pistillate spikelets</li> <li>2. Sessile pistillate spikelets</li> <li>3. Outer glume of pistillate spikelet corneous</li> <li>4. Rachis disarticulating at maturity</li> <li>5. Bundles of cobs ordinarily produced at many nodes</li> <li>6. Cobs of miniature size bearing few kernels</li> <li>7. Small kernels within seed case shattering at maturity</li> <li>8. Mature ears rarely fully enclosed by single husk at maturity</li> <li>9. Culms more slender and leaves narrower than in maize</li> <li>10. Culms often branched at upper nodes</li> <li>11. Terminal staminate inflorescence typically has no central spike</li> <li>12. Strong response to photoperiod</li> <li>13. Narrow climatic and edaphic tolerances</li> <li>14. Limited distribution in Mesoamerica</li> </ol>

Table 1 (Continued)

Maize	Teosinte
<u>Comparisons of archeological maize and teosinte including oldest Tehuacan maize cobs and contemporary Tlapacaya teosinte kernels of the Valley of Mexico dated from about 7,000 B.P.</u>	
<ol style="list-style-type: none"> <li>1. Paired pistillate spikelets</li> <li>2. Pedicellate pistillate spikelets</li> <li>3. Outer glume of pistillate spikelet membranous</li> <li>4. Kernels not completely enclosed by glumes of pistillate spikelet at maturity</li> <li>5. Cobs not disarticulating at maturity</li> <li>6. Cobs at maturity of chiefly noncorneous tissue</li> <li>7. Glumes of pistillate spikelet chaffy and nonfunctional at maturity</li> <li>8. Effective method of seed dispersal lacking</li> </ol>	<ol style="list-style-type: none"> <li>1. Unpaired pistillate spikelets</li> <li>2. Sessile pistillate spikelets</li> <li>3. Outer glume of pistillate spikelet corneous</li> <li>4. Kernels completely enclosed by outer glume of pistillate spikelet at maturity</li> <li>5. Cob disarticulating at maturity</li> <li>6. Cobs at maturity corneous</li> <li>7. Kernels at maturity enclosed within seed case by outer glume</li> <li>8. Shattering of seed cases at maturity provides effective method of seed dispersal</li> </ol>

numbers of fertile hybrids and backcrosses to one or both parents, that teosinte has retained its status as a good species having most, if not all, of the identifying characteristics listed in the table as differentiating modern races of maize and teosinte. This retention of its identity in recent years is now known from reliable archeological evidence to have extended into the remote past when the domestication of maize was being initiated. It is equally apparent from comparisons of the oldest Tehuacan cobs with those of modern maize that there is an equivalent similarity between archeological and modern maize with respect to the 8 characteristics listed in Part II of the table. The possible significance of such apparent genetic stability over prolonged periods of time in related species, of which one is cultivated and the other not, is of current interest in relation to unsolved problems concerning the origin of maize that are amenable to experimental verification and will be discussed in more detail later.

Participation in a teosinte mutation hunt in November, 1971 at the invitation of Dr. George Beadle, president of the Chicago Horticultural Society, provided a favorable opportunity to examine closely large populations of teosinte growing in intimate sympatric association with indigenous races of maize in southwestern Mexico. Near Mazatlan in the state of Guerrero, between 20 and 30 km south of Chilpancingo, there are large populations of teosinte adjoining corn fields and in neighboring areas not being actively utilized for farming purposes. The group of participants with which I was associated examined large numbers of plants at four separately located sites in this area where teosinte was most abundant. Large populations at three additional sites on canyon slopes where altitudes ranged from 1100 to 1350 meters approximately 37, 45 and 69 km southwest of Valle de Bravo in the state of Mexico also were inspected for maize-like mutants and naturally occurring hybrids of maize and teosinte. At two of the Mazatlan sites Tripsacum dactyloides was growing together with teosinte at the edge of a corn-field, where earlier in the season it had been noted that all three were silking and shedding pollen at the same time. But a diligent search in the surrounding area within range of their windblown pollen for hybrids

either of tripsacum and maize, tripsacum and teosinte, or teosinte and maize resulted in the detection only of 6 teosinte-maize hybrids.

The following procedure was found to be effective in examining individual plants for mutants (including sectors as well as whole-plant mutants) and hybrids not readily identifiable except from cob and kernel characteristics--especially those resulting from first and second backcrosses to teosinte. When first entering a site of maximum plant density within a teosinte population usually near one or more corn fields, where densities of 20 to 30 teosinte plants per  $\text{cm}_2$  were not unusual, an overall inspection of nearby plants was made to detect exceptional phenotypes--especially  $F_1$  teosinte-maize hybrids which often could be spotted in this manner. A more careful inspection was then made of individual teosinte and off-type corn plants. This included the examination among teosinte plants of the clusters of distichous spikes (ears), preferably from at least two successive midculm nodes to minimize the possibility of overlooking sectorial mutants involving not more than one or a few ears, such mutant sectors having been identified previously in tripsacum. The number of plants examined individually in this manner was recorded by placing a seed from each plant in a "Beadle bottle" supplied by the ingenious organizer of the expedition. The kinds of mutants of special interest, which would make teosinte a more acceptable food plant included: intact nonshattering cobs, cobs of softer noncorneous tissue, paired pistillate spikelets, spikelets with noncorneous outer glumes, pedicellate rather than sessile spikelets, and increased caryopsis size and/or number per individual ear. Since the oldest Tehuacan maize had most of these characteristics it must have been a more attractive food plant than teosinte even at that early stage of its domestication, and its spontaneous hybrids with teosinte would have had appreciably more food value than either parent if they were at all like the  $F_1$  hybrids seen during the mutation hunt.

During  $5\frac{1}{2}$  days in the field at Mazatlan and in the Valle de Bravo area, approximately 4500 plants were examined individually for mutant and hybrid characteristics. During  $1\frac{1}{2}$  days at Mazatlan Site IV, my tally count was 1568 but somewhat lower numbers were examined daily at



less accessible sites elsewhere in these areas. A total of 8 teosinte-maize hybrids were identified at 3 of the 4 sites in the Mazatlan area, but none was discovered in an equally thorough inspection of 3 sites at Valle de Bravo. Two of these 8 hybrids were classed as  $F_1$ 's, one as resulting from a first or second backcross to maize, and 5 as being from first or second backcrosses to teosinte. These classifications were made with the assistance of Dr. Walton Galinat, who has been especially interested in the comparative morphology of teosinte, maize and tripsacum. The 8 plants classed as hybrids, with one exception, exhibited 2 or more of the parental characteristics of cobs, kernels and spikelets differentiating maize and teosinte; in addition there were intermediate expressions of cob and outer glume induration, husk development, culm diameter and leaf width. The exceptional plant appeared to differ from normal teosinte plants only with respect to such an extreme modification of the seed case in which the caryopsis ordinarily is enclosed that more than a single gene difference was suspected of being involved; for this reason it was tentatively classed as a hybrid rather than as a mutant.

It was not possible definitely to identify, in the teosinte populations, progenies from successive backcrosses to teosinte beyond the first and second generations, although it was obvious from the fertility of the backcrossed plants previously identified in these populations that such backcrossing might be taking place. However, none of the various maize characteristics readily identifiable in the  $F_1$  maize-teosinte hybrids and backcrosses to teosinte was detected among the many otherwise typical teosinte plants of these same populations. Perhaps the negative survival value of such apparently nonrecoverable characteristics explained their absence in these wild populations actively competing with other rank-growing tropical plants, farm animals and a climate in which the morphologically less well protected seeds of these hybrids may fail to survive from one growing season to the next. There was, however, in all of the populations inspected during the mutation hunt ample evidence of residual heterozygosity for traits such as extreme amounts of tassel branching and tillering, secondary branching

at upper nodes, many conspicuous variations in seed case color and differences in seed case size and shape ranging from triangular to trapezoidal. Very few, if any, of these variants plausibly could be attributed to introgression from the nontillering maize with sparsely branched tassels being grown in nearby fields, or to environmental effects.

Although the search for variants definitely identifiable as whole-plant mutants was unsuccessful, interesting sectorial mutants were found with no more than one or a few ears of individual axillary bundles being involved. Among these were ears with intact rachis and delayed shattering of seed cases, extremes of seed case condensation and multiplication, and one noteworthy occurrence of reduced seed case development, partly exposed kernels and more pronounced glume development like that of the ancient indigenous race of Chapalote popcorn described by Wellhausen et al. as being of very limited distribution in northwestern Mexico. As possible examples of weak penetrance at least some of these variants may prove to be heritable and, as more prominently developed phenotypes, serve as encouraging indicators of the mutability of these and other loci that might have made teosinte a more attractive food plant in pre-historic times.

Unfortunately, there are conflicting reports concerning the mode of inheritance of the various characteristics differentiating modern and archeological maize and teosinte. From currently available data including his own, Dr. Galinat told me recently that at least two genes probably are involved in the expression of the first 4 and possibly other characteristics listed in the accompanying table as applicable to modern maize. Only for alleles at the tunicate locus, described by Mangelsdorf et al. as having pleiotropic effects on rachis, seed case and cupule development, is there convincing evidence of monogenic inheritance. With respect to dominance, our inspection of several  $F_1$  teosinte-maize hybrids during the mutation hunt showed that the 1st and 4th characteristics listed in the table as applicable to modern maize are inherited as dominants, the 2nd, 5th, 6th and 10th as recessives and the other 4 of the first 10 as intermediate expressions of differences

between maize and teosinte. Thus, it appears that mutations in both directions--from recessive to dominant as well as from dominant to recessive states--and at more than 4 loci, would be required to transform teosinte into a primitive maize-like plant with respect only to the first 4 of these items. Also, the mutation frequencies observed by L. J. Stadler for dominant to recessive endosperm characters, ranging from about 1:150,000 to 1:2,000,000 or more, might be much less frequent for the loci under consideration since at some of these loci reverse mutations from recessive to dominant would be required and all would involve reproductive structures which have been shown by the archeological record of the past 7,000 years not to have changed significantly during that time. However, after having accumulated the required mutations it could be assumed that teosinte would have a bright future as an important food plant.

L. F. Randolph

CORNNUTS, INCORPORATED  
Salinas, California  
Plant Breeding Department

1. Non-liguleless liguleless-1.

A narrow-based Cuzco flour corn synthetic was found to be segregating for upright leaves as a discrete phenotypic class. Plants having the extreme upright leaves were sib pollinated and outcrossed to 26 standard inbred lines. The sibbed progeny was uniformly extreme upright-leaved, and have bred true for this phenotype in two further successive generations. The 26  $F_1$ 's involving standard inbred lines were each backcrossed to the respective inbred parent, and then selfed once. The combined  $BC_1I_1$  populations segregated cleanly in the 7:1 ratio expected if the trait were monogenically inherited. In all 26 backgrounds, the gene was clearly expressed and always produced the extremely upright leaf condition. Development of the ligule was normal or nearly normal in all inbred backgrounds.