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Evolution

Genetic and morphological analysis of a maize–teosinte F_2 population: Implications for the origin of maize

(molecular markers/evolution/*Zea*/restriction fragment length polymorphism/quantitative genetics)

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ABSTRACT **Genes controlling the dramatic morphological differences between maize and its presumed progenitor (teosinte) were investigated in a maize–teosinte F_2 population through the use of molecular markers. Results indicate that the key traits differentiating maize and teosinte are each under multigenic control, although for some traits, such as the number of ranks of cupules, the data are consistent with a mode of inheritance that would involve a single major locus plus several modifiers. For other traits, such as the presence/absence of the pedicellate spikelet, the data indicate multigenic inheritance with no single locus having a dramatically larger effect than the others. Results also indicate that the tunicate locus (*Tu*) had no major role in the origin of maize, despite previous opinion that it was involved. The major loci affecting the morphological differences between maize and teosinte are located on the first four chromosomes. The data suggest that the differences between teosinte and maize involve, in part, developmental modifications that enable (*i*) primary lateral inflorescences, which are programmed to develop into tassels (male) in teosinte, to become ears (female) in maize, and (*ii*) the expression of male secondary sex traits on a female background in maize. Similar changes were likely involved in the origin of maize.**

The origin of maize (*Zea mays* L. ssp. *mays*) has been the subject of intense debate (1, 2). In recent years, a large body of evidence has accumulated that supports the hypothesis that maize is a domesticated form of teosinte (*Zea* species) (3–5), and few authors still question this view (6). Biosystematic evidence indicates that the Mexican annual teosintes, *Z. mays* ssp. *mexicana* and ssp. *parviglumis*, exhibit closer genetic relationships to maize than other teosinte species and suggests that the latter of these two subspecies is the probable progenitor of maize (4). Despite growing acceptance of the view that Mexican annual teosinte is the ancestor of maize, there is no consensus concerning the genetic and morphological steps involved in the transformation of teosinte into maize. The fundamental problem is that maize and teosinte differ dramatically in their morphological characteristics (Figs. 1 and 2), and alternative views of the teosinte–maize transformation necessitate vastly different morphogenetic changes (7, 8).

In this paper, we report the results of a new approach to long-standing questions surrounding the origin of maize. We have analyzed segregation for both molecular marker loci (MMLs) and the key morphological traits distinguishing maize from teosinte in a maize–teosinte F_2 population. This approach has enabled us to (*i*) make the most precise available estimates of the number of genes controlling the key traits that distinguish maize and teosinte, (*ii*) characterize the chromosomal locations of these genes, and (*iii*) determine the relative contributions of different chromosomal regions to the key traits. The results also have important implications concerning the nature of the morphological–developmental changes involved in the origin of maize.

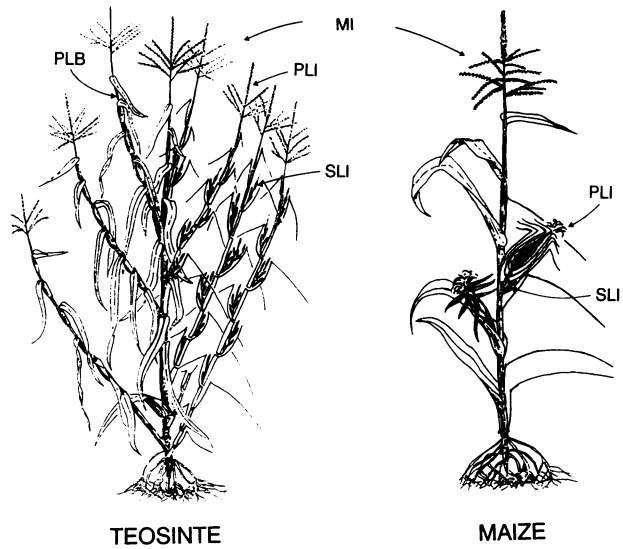

FIG. 1. Annual teosinte and maize plant architectures, adapted from Iltis (7). MI, main inflorescence; PLI, primary lateral inflorescence; SLI, secondary lateral inflorescence; PLB, primary lateral branch.

Morphological Characteristics of Maize and Teosinte

In both maize and teosinte, the inflorescence terminating the main culm (main inflorescence) is male and called the tassel (Fig. 1). In teosinte, the primary lateral branches are normally elongate, and the inflorescences terminating these branches (primary lateral inflorescences) are normally male (tassels). However, in typical maize, the lateral branch is short, and the primary lateral inflorescence is female—i.e., an ear (Fig. 1). The shorter lateral branches of maize result from the shortening of the internodes of the lateral branch rather than a reduction in internode number. In teosinte, there are also secondary lateral (and higher order) inflorescences that are female. In maize, female secondary lateral inflorescences may also be present, but only in some primitive races (Fig. 1).

The most dramatic difference between maize and teosinte concerns the architectures of their female inflorescences (Fig. 2). The teosinte ear is composed of 5–10 (or more) distichously (in two ranks) arranged cupulate fruitcases (Fig. 2 *A* and *C*). The cupule of the cupulate fruitcase is formed from the invaginated rachis internode. The cupule contains a single sessile spikelet whose outer glume seals the opening of

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Abbreviations: MML, molecular marker loci; MTL, molecular trait loci; abbreviations for traits are in Table 1.

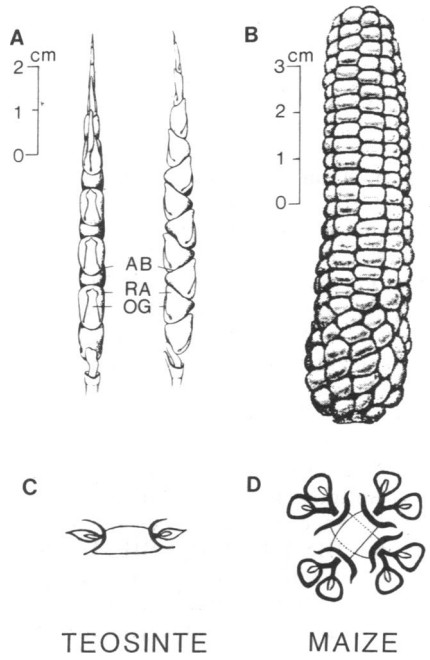

FIG. 2. Architecture of annual teosinte and maize ears (female inflorescences) adapted from Iltis (7). (*A*) Teosinte ear. AB, abscission layer; OG, outer glume; and RA, rachis internode. (*B*) Maize ear. (*C–D*) Schematic cross-sections of teosinte (*C*), showing two ranks of cupules with one spikelet per cupule, and of maize (*D*), showing four ranks of cupules with two spikelets per cupule.

the cupule, thus obscuring the kernel from view. Both the cupule and the outer glume are extremely indurate (hardened) in teosinte. The single spikelet of each cupulate fruitcase produces a single kernel (Fig. 2*C*). Cupulate fruitcases of a single ear are separated from one another by abscission layers that enable the fruitcases to separate (disarticulate) at maturity for dispersal.

The maize ear is also constructed from cupules (Fig. 2*D*). It is composed of generally 100 or more polystichously (in many ranks) arranged cupules that are hidden from view by the kernels (Fig. 2 *B* and *D*). In maize, there may be 4–10 or more ranks of cupules. Unlike teosinte, the cupules of maize do not envelop the kernels. Maize cupules may be somewhat indurate, but the outer glumes are always softer than the highly indurate glumes of the teosinte ear. In contrast to teosinte, two spikelets are associated with each cupule, one pedicellate and the other sessile. Thus, an ear with four ranks of cupules will have eight rows of kernels (Fig. 2*D*). The presence of the pedicellate spikelet (or two spikelets per cupule) in maize ears represents one of the key differences between maize and teosinte. Because sessile–pedicellate spikelet pairs are the common condition throughout the grass tribe Andropogoneae (to which *Zea* belongs), it is thought the pedicellate spikelet was lost during the evolution of the teosinte ear, and then restored to fertility during the transformation of teosinte into maize (ref. 8; compare ref. 7). Maize ears lack abscission layers as found in teosinte, so the ear remains intact at maturity.

The key morphological differences between teosinte and maize, respectively, are: (*i*) two ranks vs. four (or higher) ranks of cupules, (*ii*) single vs. paired spikelets, (*iii*) hard vs. soft outer glumes, (*iv*) shattering vs. nonshattering ears, (*v*) normally male vs. female primary lateral inflorescences, and (*vi*) normally long vs. short primary lateral branches. Other traits, such as the number of ears per plant or the number of cupules per ear, are presumably secondary effects of domestication, as opposed to primary morphogenetic changes involved in the transformation of teosinte into maize.

Theories on the Origin of the Maize Ear

Two principal models for the transformation of teosinte into maize have been proposed. One model provides that the maize ear originated by modification of the pistillate inflorescence of teosinte through a small number of key morphological changes controlled by an equally small number of major genes (3, 5, 8–11): the *tr* locus is said to control two-ranked (teosinte) vs. four-ranked (maize) cupules; the *pd* locus, single (teosinte) vs. paired (maize) spikelets; the *Ab* locus (or *Ph* and *Ri*), the presence (teosinte) vs. absence (maize) of abscission layers in the ear; and the *Tu* locus, soft vs. hard glumes. In addition to these major genes, Galinat (8) suggested that numerous modifier genes were involved in stabilizing the expression of the major genes.

An alternative view of the origin of the maize ear is that the central spike of the teosinte primary lateral inflorescence (Fig. 1) was transformed into the maize ear by sexual transmutation (7). According to this hypothesis, the principal event in the evolution of maize was a switch in sexuality from male to female at the tips of lateral branches (i.e., the primary lateral inflorescences). This theory proposes that, because the spikelets of the male inflorescences (tassels) are in sessile–pedicellate pairs and have soft glumes, paired spikelets and soft glumes are an automatic result of the feminization process. Therefore, *pd* and *Tu* are not necessary and had no role in the origin of the maize ear.

Materials and Methods

Plant Materials. Maize race Chapalote (Sin 2) was crossed as the female parent to Chalco teosinte, *Z. mays* ssp. *mexicana* (*Doebley 643*). A single F_1 plant was grown and self-pollinated. F_2 seeds were planted in a winter nursery on Molokai Island, Hawaii. The second primary lateral branch from the top of the plant (Fig. 1) was collected from each of 260 plants and used for the morphological measurements (Table 1). Cupules per rank (CUPR), ear disarticulation (DISA), glume induration and angle (GLUM), percentage of cupules without a pedicellate spikelet (PEDS) and RANK were measured on the basalmost secondary lateral inflorescence. The rank of a single ear can vary over its length. Accordingly, RANK was scored as the weighted sum of the ranks times the proportion of the ear

Table 1. List of principal traits distinguishing maize and teosinte

Trait	Description
CUPR (cupules per rank)	Number of cupules in a single rank
DISA (disarticulation score)	Tendency of ear to shatter (1–10 scale)
GLUM (glume score)	Hardness and angle of outer glume (1–10 scale)
LBIL (lateral branch internode)	Average length of internodes on the primary lateral branch
LIBN (branch number)	Number of branches in primary lateral inflorescence
PEDS (pedicellate spikelet score)	Percentage of cupules lacking the pedicellate spikelet
PROL (prolificacy)	Number of ears on the primary lateral branch
RANK (rank)	Number of rows of cupules
STAM (staminate score)	Percentage of male spikelets in primary lateral inflorescence

possessing each rank. The percentage of staminate (male) spikelets in the primary lateral inflorescence (STAM) and the number of branches in this inflorescence (LIBN) reflect the degree to which it is tassel-like (i.e., male, branched) or ear-like (i.e., female, unbranched).

Molecular Markers. Each of the 260 F_2 plants was assayed for its genotype with 58 MMLs (Fig. 3). Plasmid clones of low-copy-number nuclear DNA sequences of maize were available from Brookhaven National Laboratory (13) and the University of Missouri–Columbia (12). Isozyme loci were assayed according to published procedures (14).

Statistical Analyses. MMLs were checked for normal Mendelian segregation by using LINKAGE-1 version 3.50 (15). A linkage map for the MMLs was assembled using MAPMAKER version 1.01 (16). The ratio of dominant to additive effects and R^2-values for associations between MMLs and the morphological traits were calculated as outlined by Edwards *et al.* (17). In cases where a trait showed a significant R^2 for two adjacent MMLs, R^2 was recalculated for that chromosomal segment after excluding individuals that had detectable recombination events within that segment. This procedure should provide a more accurate estimate of the size of the effect associated with the chromosomal segment, as it reduces the influence of recombination between the two MMLs and the MTL on the estimate of R^2 (18).

Results

MMLs. Segregation ratios fit Mendelian expectations throughout the majority of the genome. Only regions of chromosomes 5 and 6 exhibited serious distortion (Fig. 3). The order of the MMLs along the chromosomes (Fig. 3) was the same as determined for the maize map (12, 13). The map distances between adjacent markers were generally less for the maize–teosinte map than for the maize map, with some segments showing less recombination by a factor of four.

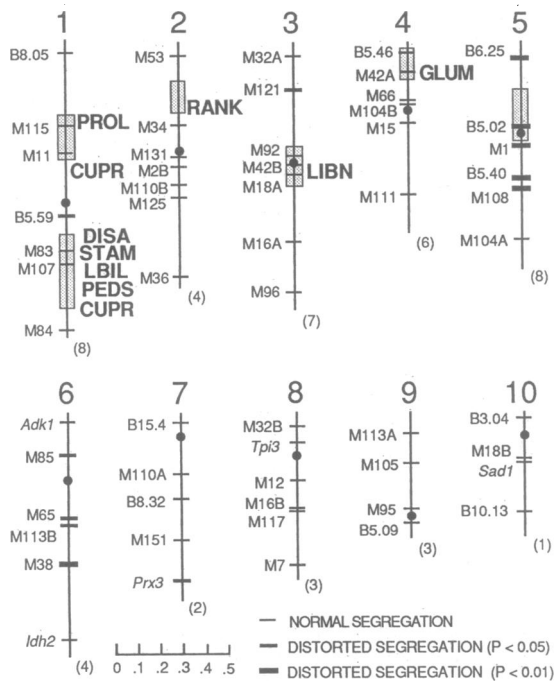

FIG. 3. Diagram of the 10 teosinte–maize chromosomes showing distribution of MMLs used in this study. Thickness of cross-lines for each MML indicates any departures from Mendelian segregation ratios (see scale). Distances between the MMLs are shown as *r*, the recombination fraction (see scale). Stippled blocks highlight six regions with major effects on the morphological differences between maize and teosinte. Positions of the largest effect for each morphological trait (Table 1) are shown. The estimated number of morphological trait loci (MTLs) (assuming no pleiotropy) on each chromosome is shown in parentheses. Prefixes indicate the source of cloned MMLs as either the University of Missouri–Columbia (M = UMC) or Brookhaven National Laboratory (B = BNL). Five isozyme loci (*Adk1*, *Idh2*, *Prx3*, *Sad1*, and *Tpi3*) are shown. Solid circles indicate the approximate positions of the centromeres (12).

Morphological Trait Analysis. Morphological trait data were checked for deviations from normality. Significance tests indicated that all traits except DISA, STAM, and CUPR were skewed ($P = 0.01$). DISA, RANK, STAM, and PEDS had significant kurtosis; however, only PEDS was severely kurtotic. Statistical theory indicates that skewness and moderate kurtosis have little effect on the reliability of the comparison of means tests such as we have performed (19). We performed square root or logarithmic transformations on some trait data. Transformations were successful in reducing both skewness and kurtosis. Magnitudes and significance levels of the R^2 values for the transformed data did not differ appreciably from those for the raw data.

A remarkable range of phenotypes was exhibited among the F_2 plants. For DISA, GLUM, LBIL, LIBN, STAM, and RANK, individuals with the parental phenotypes were commonly recovered. For example, many plants had either purely male or purely female primary lateral inflorescences, representing the teosinte and maize conditions of STAM, respectively. For CUPR, the parental phenotypes were recovered in only 1% of the plants; and for PEDS, the teosinte phenotype was recovered in less than 1% of the plants. No plants that closely resembled teosinte for all key traits were recovered. Two plants that possessed the maize key traits were recovered; however, these plants differed by many characters from the maize parent, most notably in their prolificacy and small ears.

Associations between Molecular Marker Loci and Morphological Traits. Table 2 lists all significant ($P = 0.01$) associations between MMLs and morphological traits. Morphological traits showed between four and eight significant independent associations with MMLs, and these associations had a broad range of R^2 values (Table 2). CUPR showed large significant effects with markers over 80 centimorgans apart on chromosome 1 and much smaller significant effects for two intermediate markers. This suggests two independent MTLs affecting this trait on either arm of chromosome 1. Similar situations were found for RANK on chromosome 5 and STAM on chromosome 3 (Table 2). In all other cases where a trait showed significant associations with multiple MMLs on a single chromosome, the distribution and relative magnitudes of the effects suggested either a single MTL or several tightly linked MTLs were present.

In general, the directions of the effects met the *a priori* expectation that maize alleles at MMLs should be associated with a maize-like phenotype, and that teosinte alleles, with a teosinte-like phenotype. The two exceptions are of interest. First, LBIL, LIBN, and STAM all showed factors near UMC65 on chromosome 6 for which the teosinte allele was associated with a maize-like phenotype. These three traits were highly correlated with one another, and it is reasonable to expect that a single MTL near UMC65 could be affecting these traits pleiotropically. Second, LIBN and PROL both had factors near BNL5.02 on chromosome 5 for which the teosinte allele was associated with a maize-like phenotype. Because both traits involve the proliferation of inflorescences or portions of inflorescences, this also suggests the pleiotropic effect of a single MTL.

Significant associations between MMLs and traits were found on all chromosomes (Table 2). However, the chromosomal locations with the largest significant R^2 values had a more narrow distribution, being found only on chromosomes 1–4 (Fig. 3). Many associations with large R^2 values are concentrated on the long arm of chromosome 1, which shows

Table 2. Significant associations ($P < 0.01$) between morphological traits and MMLs, the proportion of phenotypic variance explained by each association (R^2), the ratio of the dominance to additive effects (D/A), and the direction of the effect [i.e., whether maize (M) or teosinte (T) contributed positively to the effect, or apparent overdominance (O)]

Trait	MMLs*	R^2	D/A	Direction	Chromosome
CUPR	UMC15-*UMC11*	0.20	1.10	M	1
	UMC107-UMC84	0.20	0.48	M	1
	BNL5.02	0.04	0.61	M	5
	UMC85-*UMC65*	0.08	4.20	O	6
	BNL8.32	0.06	0.18	M	7
	UMC95-BNL5.09	0.06	0.38	M	9
DISA	UMC83-UMC107	0.26	0.36	T	1
	UMC53-UMC34	0.12	−0.07	T	2
	BNL5.46-*UMC42A*	0.09	1.16	T	4
	BNL6.25-*BNL5.02*	0.17	0.38	T	5
GLUM	*UMC107*-UMC84	0.08	0.53	T	1
	UMC34-UMC131	0.15	0.10	T	2
	UMC16A-UMC96	0.08	−0.43	T	3
	BNL5.46-*UMC42A*	0.42	0.13	T	4
	BNL5.02	0.06	0.12	T	5
LBIL	UMC107	0.31	−0.18	T	1
	UMC16A-UMC96	0.09	1.06	T	3
	BNL5.46-UMC42A	0.08	−0.53	T	4
	BNL5.02-UMC1	0.07	0.11	T	5
	UMC85-UMC65	0.09	0.07	M	6
LIBN	UMC42B-*UMC18A*	0.15	0.06	T	3
	BNL6.25-BNL5.02	0.07	0.05	M	5
	UMC65-UMC113B	0.10	−0.42	M	6
	Tpi3-*UMC12*	0.13	−0.26	T	8
	UMC95-BNL5.09	0.13	0.39	T	9
PEDS	BNL5.59-*UMC107*	0.24	−0.63	T	1
	UMC2B-UMC110B	0.05	−0.09	T	2
	UMC121-*UMC92*	0.13	−0.31	T	3
	BNL5.46-UMC42A	0.05	−1.33	T	4
PROL	*UMC115*-UMC11	0.20	0.52	T	1
	UMC42A	0.05	1.59	O	4
	BNL5.02-UMC1	0.06	−0.28	M	5
	BNL8.32-UMC151	0.10	0.65	T	7
RANK	UMC53-*UMC34*	0.42	−0.17	M	2
	UMC18A-UMC16A	0.08	0.69	M	3
	BNL5.46	0.07	0.97	M	4
	BNL6.25-*BNL5.02*	0.11	0.36	M	5
	BNL5.40-UMC104A	0.10	−0.06	M	5
	UMC12-UMC16B	0.06	−0.21	M	8
	UMC105-*UMC95*	0.10	0.23	M	9
	UMC10.13	0.04	−6.60	O	10
STAM	*UMC83*-UMC107	0.26	−0.07	T	1
	UMC121-UMC92	0.14	−0.01	T	3
	UMC16A	0.07	1.14	T	3
	UMC85-*UMC65*	0.08	−0.37	M	6
	UMC12-UMC16B	0.08	−0.26	T	8

*In cases where a trait was significantly associated with two adjacent MMLs, both are listed, and the MML with the larger associated effect is in italic type. If the trait showed roughly equal associations with both MMLs, then neither is in italics.

major effects for five of the nine traits. The short arm of chromosome 5, although not possessing any of the largest factors, had significant effects for seven of the nine traits. Chromosomes 7 and 10 had the smallest number of significant associations, two and one, respectively.

Discussion

Genetic Control of Morphological Traits. Our approach has several limitations in estimating the number of genes controlling a morphological trait. First, the ability to detect MTLs with small effects is limited by the size of the F_2 population, resulting in false negatives for some chromosomal regions. Second, recombination between MMLs and MTLs weakens the ability to detect MTLs. Third, several closely linked MTLs with small effects and a single MTL with a large effect often cannot be distinguished. Fourth, because of the large number of statistical tests performed, some false-positive associations are expected. Overall, these problems should result in an underestimate of the number of MTLs affecting the traits. Therefore, the observed number of significant associations for any single trait (Table 2) is probably best considered a minimal estimate of the number of MTLs controlling the trait. Taking these limitations into account, the data indicate that the key traits differentiating maize and teosinte are under multigenic control with minimally four to eight MTLs affecting each trait.

The data also provide estimates of the relative contributions of different chromosomal regions to the morphological differences between maize and teosinte. GLUM and RANK showed very large (0.42) R^2 values for single chromosomal regions and much smaller effects for several other regions (Table 2). LBIL and STAM showed a similar trend. These data are consistent with a model of genetic control involving a major locus plus modifiers. It is possible that the evolution of these traits was initiated by a major mutation with subsequent refinement through selection for modifier loci that either stabilized expression of the trait or enabled its expression earlier in development. In contrast, LIBN had five significant associations with MMLs, and the four largest effects (0.15, 0.13, 0.13, and 0.10) were approximately equal in magnitude. These data are consistent with LIBN being controlled by several loci, each with small effects. As such, LIBN is more likely to have evolved through a series of incremental mutations. DISA, PEDS, and PROL were intermediate between these two extreme patterns of inheritance, having effects that showed a continuous gradation from small to large (Table 2).

Correspondence of MTLs with Known Maize Genes. There are several notable associations between the MTLs detected in this study and previously described Mendelian loci in maize. The largest R^2 for LBIL is on the long arm of chromosome 1 where *tbl* (teosinte branched) is located. This gene has the same effect on the phenotype as our MTL from teosinte. The largest R^2 for STAM is also on the long arm of chromosome 1, as are *an1* (anther ear), *D8* (dwarf), and *Mpl1* (miniplant). These genes, like our MTL, affect the sex of the primary lateral inflorescence. The largest R^2 for LIBN is on chromosome 3 where *ra2* (ramosa) is located. This gene affects branching of the primary lateral inflorescence, which is how LIBN is defined.

Previous Analyses of Maize–Teosinte Hybrids. Some authors (20–22) reported that none of the key traits segregate in simple Mendelian ratios in a maize–teosinte F_2, agreeing with our results that the traits are under multigenic control. Conversely, Langham (11) concluded that paired spikelets and rank are each controlled by a single Mendelian locus. He defined *pd* and presented evidence of its linkage to *lg2* on the long arm of chromosome 3. In our population, PEDS was clearly a quantitative trait; however, one of the two largest effects for PEDS is on chromosome 3 (Table 2). Langham (11) also defined *tr* (two-ranked) as the single gene controlling RANK. In our population, RANK was clearly a quantitative trait. Our data agree with those of Galinat (23) in placing a major gene controlling RANK on the short arm of chromosome 2.

A factor controlling GLUM was mapped to chromosome 4 (linked to *su*) in several types of teosinte (24). Subsequently, some authors have suggested that *Tu* was the operative locus (3, 8). Our data show no linkage between the long arm of

chromosome 4, where *Tu* is found, and GLUM. Rather, our data would place the principal gene(s) controlling glume induration on the short arm of chromosome 4 in agreement with Rogers (24). Our interpretation is that *Tu* was not a major factor in the origin of soft glumes in maize, but rather that an undescribed gene(s) on the short arm of chromosome 4 was (were) involved. Galinat (25) defined *Ph* (pith abscission) and *Ri* (rind abscission), both on the short arm of chromosome 4. Our data suggest that DISA is under the control of MTLs on chromosomes 1, 2, 4, and 5. Our chromosome 4 location corresponds to the location of *Ri* and *Ph*, although our data suggest that this region has a smaller effect on DISA than do the chromosome 1, 2, and 5 locations.

Chromosomal Distribution of MTLs. Several authors (5, 21, 22, 26) have noted that maize-like and teosinte-like plants are recovered at relatively high frequencies (1 in 500) in F_2 populations, suggesting the involvement of four to five Mendelian factors (2, 5, 22). (It should be noted that the frequencies at which maize-like and teosinte-like plants are recovered is greatly influenced by how stringently one defines these parental phenotypes.) Our data revealed that the chromosomal regions with the largest R^2 values reside in five regions on the first four chromosomes (Fig. 3). The long arm of chromosome 1 showed the greatest concentration of factors, having the largest effect on five of the nine traits. The short arm of chromosome 2 has a major effect on RANK and smaller effects on several other traits. Chromosome 3 has a major effect on LIBN and smaller effects on five other traits. The short arm of chromosome 4 has a major effect on GLUM and smaller effects on several other traits. Additionally, chromosome 5, while not possessing any of the largest effects, has significant effects on seven of the nine traits. These data help explain the frequent recovery of maize-like and teosinte-like plants in F_2 populations.

Morphological Evolution of Maize. Morphological study of our F_2 plants provided some insights concerning the nature of the developmental differences between teosinte and maize. For example, lateral branch length and sex of the primary lateral inflorescence segregated dramatically in our population. In general, long lateral branches were terminated by branched, male inflorescences (tassels) and short lateral branches were terminated by unbranched female inflorescences (ears). Intermediate-length lateral branches were generally terminated by mixed-sex inflorescences. These observations agree with those of Iltis (7) and others (27, 28), who highlighted shortening of the primary lateral branch and changing of the primary lateral inflorescence from male to female as critical steps in the origin of maize.

Iltis (7) proposed that the soft glumes and paired spikelets of the maize ear are the automatic result of feminization of the primary lateral inflorescence. Results from our study do not agree with this model. Rather, our data would suggest that sex (feminization) and secondary sex traits (soft glumes or paired-spikelets) are largely independent. For example, our F_2 population contained three plants with long lateral branches terminated in tassels but on which the secondary lateral inflorescences were like typical maize ears (i.e., having paired spikelets, soft glumes, and four ranks of cupules). These plants indicate that it is not necessary to feminize the primary lateral inflorescence to create the maize ear and that maize ears can develop in morphological positions in which teosinte possesses female inflorescences. We also recovered several individuals in the F_2 that had short lateral branches terminated with female inflorescences with hard glumes and single spikelets (i.e., like teosinte ears). The recovery of such plants indicates that feminization of the primary lateral inflorescence will result in a typical teosinte ear in the absence of the genes that allow the expression of male secondary sex traits on a female background, as proposed by Galinat (10).

To the extent that segregation in a maize–teosinte F_2 population reflects the steps involved in the transformation of teosinte into maize, our results indicate that the key steps in this transformation include the following: (*i*) changes in the architecture of the primary lateral branch including shortening of its internodes, development of the primary lateral inflorescences into female rather than male structures, and suppression of branching in the primary lateral inflorescences; (*ii*) changes that allowed the expression of some male secondary sex traits on a female background in maize, including soft glumes and paired spikelets; (*iii*) a switch from two-ranked to four-ranked cupules, the developmental basis of which is not clearly understood (compare refs. 7 and 10); and (*iv*) changes to prevent the disarticulation of the ear, which has been a fundamental change in the evolution of all cereals. There are no available data to determine the evolutionary order in which these changes might have occurred (compare ref. 10).

To test these ideas further, the MTLs identified in this analysis should be isolated and characterized. Until such work is completed, the results of the present study must be viewed as preliminary.

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