MORPHOLOGICAL TRAITS DEFINING SPECIES DIFFERENCES IN WILD RELATIVES OF MAIZE ARE CONTROLLED BY MULTIPLE QUANTITATIVE TRAIT LOCI

Anna Westerbergh[1,2] and John Doebley[3,4]

[1]*Department of Plant Biology, Swedish University of Agricultural Sciences, Box 7080, S-750 07 Uppsala, Sweden*
[2]*E-mail: Anna.Westerbergh@vbiol.slu.se*
[3]*Genetics Department, 445 Henry Mall, University of Wisconsin, Madison, Wisconsin 53706*
[4]*E-mail: jdoebley@facstaff.wisc.edu*

Abstract.—We analyzed the genetic basis of morphological differences between two wild species of teosinte (*Zea diploperennis* and *Z. mays* ssp. *parviglumis*), which are relatives of maize. These two species differ in a number of taxonomically important traits including the structure of the tassel (male inflorescence), which is the focus of this report. To investigate the genetic inheritance of six tassel traits, quantitative trait locus (QTL) mapping with 95 RFLP markers was employed on a population of 425 F_2 plants. Each trait was analyzed by interval mapping (IM) and composite interval mapping (CIM) to identify and characterize the QTL controlling the differences in tassel morphology. We detected two to eight QTL for each trait. In total, 30 QTL with IM and 33 QTL with CIM were found for tassel morphology. QTL for several of the traits mapped near each other, suggesting pleiotropy and/or linkage of QTL. The QTL showed small to moderate magnitudes of effect. No QTL of exceptionally large effect were found as seen under domestication and in the case of some other natural species. Thus, the model involving major QTL of large effect seems not to apply to the traits and species analyzed. A mixture of QTL with positive and negative allelic effects was found for most tassel traits and may suggest a history of periodic changes in the direction of selection during the divergence of *Z. diploperennis* and *Z. mays* ssp. *parviglumis* or fixation of QTL alleles by random genetic drift rather than selection.

Key words.—Morphological evolution, multiple quantitative trait loci, quantitative trait locus mapping, tassel morphology, teosinte.

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The genetics of morphological differentiation among species is poorly understood, and since the early 1900s there has been an interest in discerning the numbers of genes involved in the evolution of new morphological traits. One view has been that major morphological differentiation, whether rapid or gradual, is the result of changes in numerous genes, each of slight phenotypic effect (e.g., Dobzhansky 1937; Stebbins 1974). Alternatively, it has been suggested that morphological divergence may involve only a few genetic changes of large effect (e.g., Maynard Smith 1983; Gottlieb 1984). Orr and Coyne (1992) reviewed the evidence regarding the genetic basis of the evolution of adaptations and found that neither of these views has compelling support. Among the many important implications of these contrasting models for the inheritance of species differences are the differing responses to selection for highly polygenic versus oligogenic traits (Mitchell-Olds and Rutledge 1986) and the movement of adaptive alleles across subdivided populations (Rieseberg and Burke 2001).

The most powerful means of determining the number of genes controlling a trait is quantitative trait locus (QTL) mapping with genetic markers (Lander and Botstein 1989; Tanksley 1993). This method is built upon the ability to construct saturated linkage maps using molecular markers and statistical methods for mapping and characterizing quantitative trait loci (e.g., Lander and Botstein 1989; Zeng 1994; Kao et al. 1999). QTL mapping enables one to determine the minimum number of QTL controlling a trait, the relative magnitudes of their individual effects, their chromosomal location, and their mode of gene action.

Most published studies that have used QTL mapping to examine the genetic basis of morphological differences have involved domesticated plants, although there are a growing number of examples with natural species (van Houten et al. 1994; Bradshaw et al. 1998; Long et al. 1995; Laurie et al. 1997; True et al. 1997; Kim and Rieseberg 1999). Investigators employing QTL mapping have demonstrated that numerous traits that distinguish crop plants from their wild relatives are often controlled by a relatively small number of loci with unequal magnitudes of effect (Paterson et al. 1988; Doebley et al. 1990; Li et al. 1995; Lin et al. 1995; Grandillo and Tanksley 1996). In several cases, the inheritance of a domestication trait involved a single QTL of large effect plus others of more modest effect. However, the evolution of domesticated plants is a result of artificial selection and it may not provide an appropriate model for evolution under natural selection.

To test whether the mode of inheritance controlling differences between crops and their wild relatives applies broadly to natural plant species, we have chosen to analyze the genetic control of morphological differences between two wild species of teosinte (*Zea diploperennis* and *Z. mays* ssp. *parviglumis*) that are close relatives of maize. *Zea diploperennis* is a perennial species with a restricted distribution in the state of Jalisco, southwestern Mexico, and *Z. mays* ssp. *parviglumis* is widespread across southwestern Mexico. These two species differ in a number of traits, but this report focuses on differences in the tassel morphology, which were employed as the basis for a taxonomic treatment of the genus (Doebley 1983). The most evident differences are that the tassels of *Z. mays* ssp. *parviglumis* typically possess a large number of tassel branches (usually more than 50) and bear small male spikelets (~ 6 mm long), whereas the tassels of *Z. diploperennis* possess few branches (usually less than 10) and large spikelets (~ 10 mm long; Fig. 1).

FIG. 1.   Tassels of *Zea diploperennis* (left) and *Z. mays* ssp. *parviglumis* (right).

MATERIALS AND METHODS

Plant Materials

We used a plant of the perennial species *Z. diploperennis* (Guzman no. 1120) as the female and crossed it with a plant of the annual species *Z. mays* ssp. *parviglumis* (Iltis and Cochrane no. 80). A single F_1 hybrid was grown and self-pollinated in a greenhouse. In August 1995, F_2 seeds were germinated in small pots and after three weeks the seedlings were transplanted to a field at the University of Hawaii Experiment Station in Waimanalo, Oahu. Hawaii was chosen as the field site because these plants need a short-day environment to
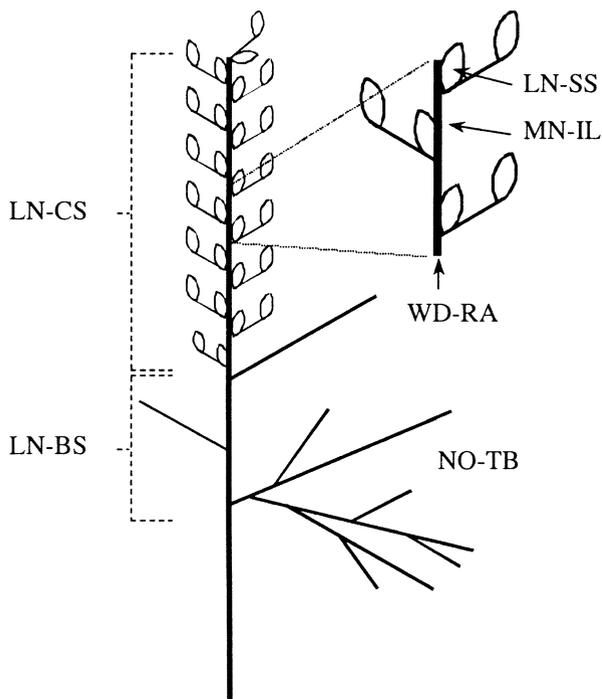

FIG. 2.   Drawing of a teosinte tassel showing the traits analyzed in this study. NO-TB, number of tassel branches; LN-CS, length of central spike; LN-BS, length of branching space; LN-SS, length of sessile spikelet; WD-RA, width of rachis on the central spike; and MN-IL, mean internode length of the central spike.

induce flowering. We planted 550 F_2 seedlings in one block, of which 425 survived. We also planted 60 seedlings of each parental population in two different blocks. Because the parents and F_2 offspring were planted in separate blocks, differences between these groups could be due to among-block environmental differences in addition to genetics. In addition, we planted a group of 20 cuttings of the F_1 hybrid, which was possible because the F_1 behaved as a perennial. Because simulations have shown that QTL mapping analyses of small F_2 populations might overestimate the effects of QTL (Beavis 1994), we used a relatively large F_2 population of 425 plants. We also studied the morphological traits on 20 to 30 plants from each parental population and six surviving plants from the cloned F_1 hybrid.

Morphological Analysis

The tassels on the apex of the main stem of the plants were collected approximately 10 weeks after planting We measured the number of tassel branches (NO-TB), length of central spike (LN-CS), length of branching space (LN-BS), length of sessile spikelet (LN-SS), width of rachis on the central spike (WD-RA), and mean internode length of the central spike (MN-IL, see Fig. 2).

Genetic Marker Analysis

Leaves were collected from 425 plants of the F_2 population and genomic DNA was isolated from each. The procedures for DNA extractions, restrictions, Southern blots, and hybridizations followed Doebley and Stec (1991, 1993). Each of the 425 F_2 plants was genotyped at 95 restriction-fragment-length polymorphism (RFLP) marker loci. We used maize plasmid clones of low-copy-number nuclear sequences of maize from Brookhaven National Laboratory (Burr et al. 1988), Pioneer HI-Bred International (Beavis and Grant 1991), Native Plants Incorporated (Helentjaris et al. 1988), and University of Missouri–Columbia (Coe et al. 1990).

Statistical Analysis

We constructed a linkage map based on the 95 RFLP markers using the program MAPMAKER version 3.0 (Lander et

al. 1987). The order of closely located markers was verified by permuting the order of neighboring loci, and the order with the highest LOD score was then selected. The Kosambi mapping function was used for calculating the map distances. We tested each of the markers for normal Mendelian segregation using χ^2-tests with a significance level of 0.05 corrected according to the Bonferroni-Holm sequential method (Rice 1989). The trait data were checked for deviations from normality, and nonnormally distributed traits were transformed to improve normality.

Interval mapping (IM) and composite interval mapping (CIM) were performed using the computer program QTL Cartographer version 1.13a (Basten et al. 1997). Standard IM calculates the ratio of the likelihood that there is a QTL to the likelihood that there is not a QTL at any position in the interval between two markers or at the markers themselves (Lander and Botstein 1989). CIM combines interval mapping with multiple regression so that the most significant markers outside the test interval will be included in the model (Zeng 1993, 1994). For CIM, we used model 6 of Basten et al. (1997) with the five most significant markers as genetic background parameters and a window size of 10 cM on either side of the markers flanking the test site. The strength of the data supporting a QTL is indicated by a LOD score, which is the log of the odds ratio. In the IM and CIM analyses, we considered a LOD score above 2.5 as significant based on Lander and Botstein (1989). This is the criterion of establishing a threshold level for statistical significance used in most crop-wild relative QTL studies to which we wish to compare our results. The proportion of the phenotypic variance explained by a QTL was estimated using MAPMAKER/ QTL version 1.1 (Lander and Botstein 1989). When multiple QTL were found to affect a trait, we also estimated the total proportion of the phenotypic variance explained by all observed QTL. In addition, we estimated the dominance and additive effects of each QTL using QTL Cartographer. To test for two linked QTL on the same chromosome, we calculated the likelihood of a second QTL on a chromosome given that there is already a QTL at a given position on that chromosome. The two-QTL model was tested using MAPMAKER/QTL version 1.1. Finally, we tested whether there are significant epistatic interactions between QTL using two-way ANOVA.

RESULTS

Segregation of Markers Loci

The 95 RFLP markers were spaced at an average of 13 cM apart throughout the genome (Fig. 3). After checking for normal Mendelian segregation (1:2:1 for codominant loci, $P > 0.05$), we found distorted segregation for 27 of the 95 markers in eight chromosomal arms (where S denotes the short and L denotes the long arm): five markers on chromosome arm 2S, three markers on 3S, one marker at the end of 3L, five markers on 4S, two markers on 5L, four markers on 7S, six markers on 8L, and one marker on 10S (Table 1). The strongest distortion was found on chromosome arm 8L, where only one out of 425 plants was homozygous for the *diploperennis* allele for umc117. Most of the markers, except the ones on 2S and 10S, showed a deficiency of homozygotes

for the *diploperennis* allele and an excess of homozygotes for the *parviglumis* allele. The region on 2S had a deficiency of homozygotes for the *parviglumis* allele and an excess of the homozygotes for the *diploperennis* allele. Marker bnl7.49b on 10S showed an excess of heterozygotes. Some of the regions with distorted segregation, such as 4S and 5L, also exhibited segregation distortion in a cross between maize and *parviglumis* (Doebley and Stec 1993). At least one of these regions (4S) is known to harbor a cross incompatibility gene (*Ga1*), which can contribute to segregation distortion (Kermicle and Allen 1990). Because most of the F_2 seeds from our *diploperennis* × *parviglumis* cross germinated and produced seedlings, the distorted segregation for 4S is most likely explained by pollen incompatibility as controlled by *Ga1*. Distorted segregation is a common phenomenon in plants and has been found in crosses of several crops and their wild relatives (e.g., Bonierbale et al. 1988; Paterson et al. 1988).

Morphological Variation within and among Populations

The means of the six tassel characters of the parental population of *Z. diploperennis* differed significantly (95% confidence interval) from the means of the *Z. mays* ssp. *parviglumis* population (Fig. 4). The tassel of *Z. mays* ssp. *parviglumis* possessed, on average, 10 times more tassel branches (NO-TB) than *Z. diploperennis*, with a branching space (LN-BS) more than four times longer than *Z. diploperennis*. On average, the *Z. diploperennis* population produced tassels with central spikes (LN-CS) twice as long as those of *Z. mays* ssp. *parviglumis*. In addition, the rachis (WD-RA) for *Z. diploperennis* was twice as wide as that for *Z. mays* ssp. *parviglumis*. Mean internode length in the central spike (MN-IL) was smaller in *Z. diploperennis* than in *Z. mays* ssp. *parviglumis*, and *Z. diploperennis* bore 50% longer sessile spikelets (LN-SS) than *Z. mays* ssp. *parviglumis*.

For four of the traits (NO-TB, LN-BS, LN-SS, and WD-RA), the mean of the F_1 hybrids lay in between the means of *Z. diploperennis* and *Z. mays* ssp. *parviglumis* (Fig. 4). The F_1 hybrids showed a significantly higher mean for MN-IL than the two parental populations, indicating hybrid vigor for this trait. For LN-CS, the F_1 hybrids have about the same mean as *Z. diploperennis*, indicating dominance for the *Z. diploperennis* phenotype.

The trait means of the F_2 population showed the same pattern as trait means for the F_1 hybrids. The F_2 population means of NO-TB, LN-BS, LN-SS, and WD-RA were intermediate between the parents and significantly different from the means of the two parental populations (Fig. 4). The F_2 population showed a higher mean for MN-IL compared to the means of the parental populations. The F_2 mean of LN-CS differed from the mean of the *Z. mays* ssp. *parviglumis* population but not from the *Z. diploperennis* population, suggesting dominance for the *Z. diploperennis* phenotype. For four of six traits, the F_2 mean was below the F_1 mean, suggesting some loss of vigor with inbreeding. Transgressive F_2 segregant plants were observed only for LN-CS and MN-IL. The rarity of transgressive individuals may reflect a high level of environmental variation and/or genetic variation in the parental populations.
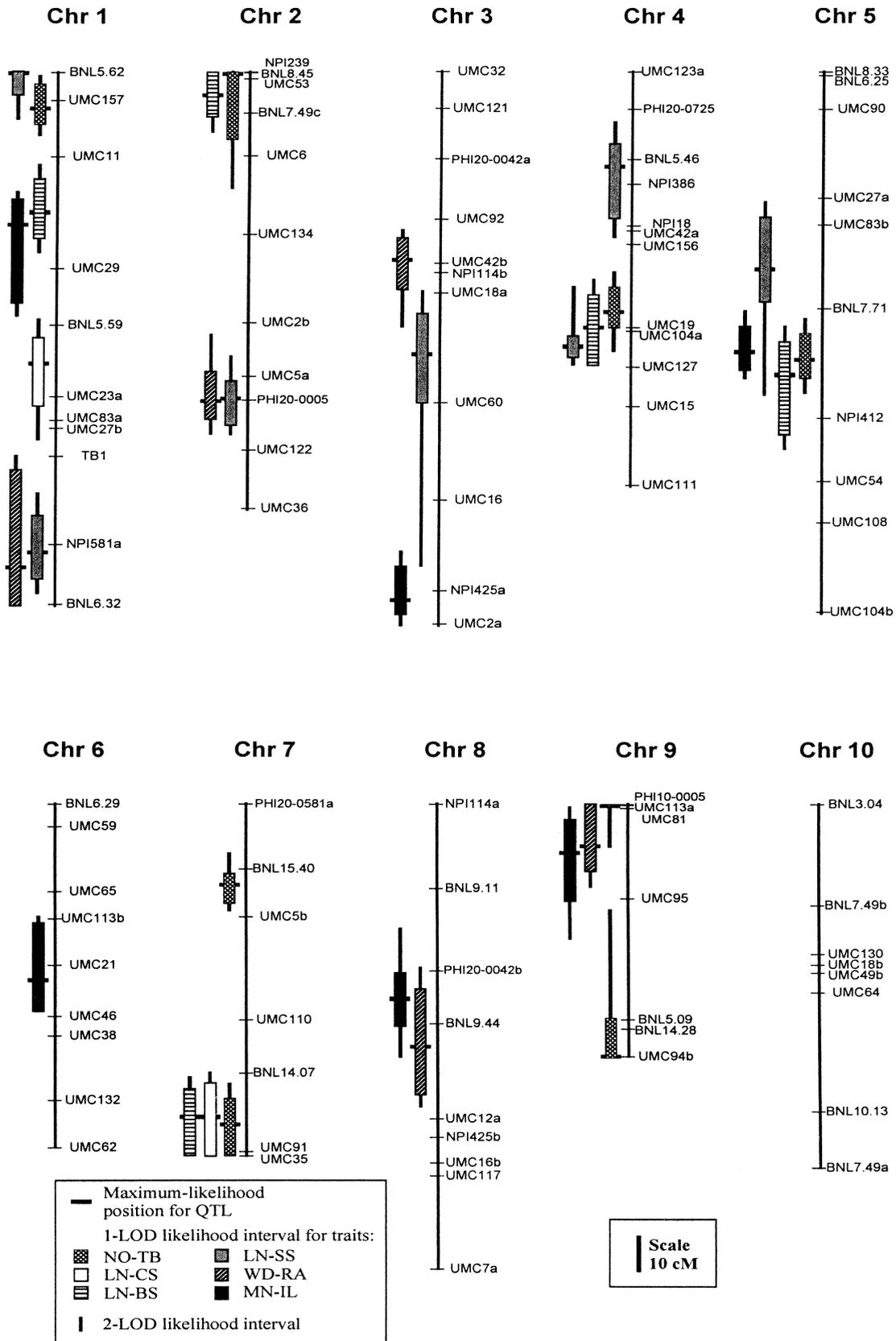
FIG. 3. Linkage map for *Zea diploperennis* and *Z. mays* ssp. *parviglumis* showing the positions of quantitative trait loci found with composite interval mapping.

TABLE 1. Loci showing segregation distortion. D, *diploperennis* allele; P, *parviglumis* allele.

Chromosome	Marker loci	Genotype frequency (%)		
		DD	DP	PP
2S	npi239*	32.6	48.9	18.5
2S	bnl8.45*	33.2	48.2	18.6
2S	umc53*	32.7	48.7	18.6
2S	bnl7.49b*	31.5	51.3	17.2
2S	umc6***	35.1	50.5	14.4
3S	umc42b*	16.7	52.6	30.7
3S	npi114b*	17.3	53.2	29.5
3S	umc18a**	15.6	51.1	33.3
3L	umc2a***	6.9	64.6	28.5
4S	bnl5.46**	15.6	53.9	30.5
4S	npi386***	15.6	52.6	32.8
4S	npi18***	15.8	49.7	34.5
4S	umc42a***	16.2	48.3	35.5
4S	umc156***	17.7	46.5	35.8
5L	umc108***	12.5	49.0	38.5
5L	umc104b***	17.8	45.7	36.5
7S	phi20-0581a***	11.2	50.2	38.6
7S	bnl15.40***	5.9	44.0	50.1
7S	umc5b***	9.6	46.6	43.8
7S	umc110**	16.1	54.1	29.8
8L	phi20-0042b***	5.8	64.2	30.0
8L	bnl9.44***	15.5	49.3	35.2
8L	umc12a***	6.1	57.0	36.9
8L	npi425b***	3.3	58.5	38.2
8L	umc117***	0.2	62.0	37.8
8L	umc7a***	14.8	53.6	31.6
10S	bnl7.49b***	18.6	67.4	14.0

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The significance levels are corrected according to the Bonferroni-Holm sequential method (Rice 1989).

As a rough measure of the frequency of plants with relatively normal parental phenotypes in the F_2 population, we counted the number of F_2 plants that lay within the 2.5 to the 97.5 percentiles of the variation of the phenotypic values of either of the two parental populations. The phenotypes of F_2 plants showed extensive overlap with the parental populations for most individual traits (Table 2). For LN-CS, LN-BS, and MN-IL, the phenotypic variation of the parental populations overlapped each other, and thus some F_2 individuals showed a phenotype that lay within the phenotypic variation for both *diploperennis* and *parviglumis*. If we consider all traits collectively, the frequency of parental types is much smaller. We found only five of 425 F_2 plants that resembled the *parviglumis* parent for all studied tassel characters and 16 F_2 plants that possessed the *parviglumis*-like phenotype for five of six traits. Only one F_2 plant resembled the *diploperennis* parent for all traits, whereas 44 plants showed a *diploperennis*-like phenotype for five of the six tassel characters.

Quantitative Trait Loci for Tassel Morphology

We detected a total of 30 QTL with IM and 33 QTL with CIM (Table 3). All QTL detected by IM, except one on chromosome 7 for the length of branching space, seemed to correspond to a QTL found by CIM (overlap in their 1-LOD support intervals). Four additional associations were detected by CIM. The five QTL detected by only one of the mapping methods had LOD scores ranging from 2.7 to 4.4. The number of QTL found for each trait ranged from two for LN-CS to

eight for LN-SS (CIM, Table 3). For some traits, we found two QTL on the same chromosome. These include two QTL each for NO-TB and LN-BS on chromosome 7 and two QTL for LN-SS on both chromosomes 1 and 4. The syntenic QTL are sufficiently far apart (48 cM or more) that the detection of one was not strongly biased by the other. In addition, the pairs of adjacent QTL on chromosome 7 for NO-TB and LN-BS had effects with opposite polarity, that is, the *parviglumis* allele of one QTL of each pair had a positive effect on the trait, whereas for the other QTL the *parviglumis* allele contributed negatively to the trait.

Prior to the development of QTL mapping with molecular markers, Wright's (1968) biometrical method was used to estimate the number of loci (n_e) controlling a trait. Based on Wright's assumption of additive gene action at all loci involved, one would expect the mean for F_1 and F_2 to be about the same and these means to be intermediate between the parents. Four of our traits fit these expectations, and thus, we have estimated n_e for these traits: NO-TB = 5.7, LN-BS = 4.0, LN-SS = 1.5, and WD-RA = 1.7. Wright's model is based on some additional assumptions. For example, one parent contains all alleles contributing positively to a trait and the other parent contains all alleles contributing negatively to a trait. It also assumes equal allelic effects at all loci. None of the traits seem to fit these assumptions, and in general these assumptions are not met by real data (Zeng et al. 1990). Nevertheless, the numbers of QTL estimated by Wright's method for NO-TB and LN-BS, the two traits that best fit the assumptions, are close to the numbers found by IM and CIM (Table 3).

LOD scores for the 30 QTL found by IM ranged from 2.8 to 11.7 and the 33 QTL found by CIM ranged from 2.7 to 15.4 (Table 3). All traits were controlled by two or more QTL that explained as little as 3.4% or as much as 19.9% of the phenotypic variation in the F_2 population. Only seven of 34 QTL explained more than 10% of the variance, and only one QTL explained more than 13% of the variance. The QTL controlling 10% or more of the variation were located on five different chromosomes. Three of these are involved in the inheritance of LN-SS. The QTL controlling the most variation (19.9%) is located on chromosome 5 and affects MN-IL. The amount of variation controlled by all detected QTL for a single trait ranged from 16.5% to 48.1% (Table 3).

We have also calculated the effect of each QTL as the percentage of the species difference for which the QTL accounts (Table 4). When viewed this way, most QTL explain 15% or less of the species difference. The sum of the effects of the QTL for most traits falls far short of the difference between the means of the parental species. For example, the QTL for NO-TB collectively explain only 35% of the species difference. This result suggests that either there are many other QTL that have gone undetected or that interactions between QTL contribute appreciably to the species difference. The results with two traits appear aberrant. First, the two QTL detected for LN-CS both act in the ''wrong'' direction with the *parviglumis* allele contributing positively to the trait. This trait was also unusual in that the F_1 and F_2 means were equivalent to the mean of the *diploperennis*. Second, for MN-IL, the QTL collectively account for 170% of the species difference, which may indicate that the two plants
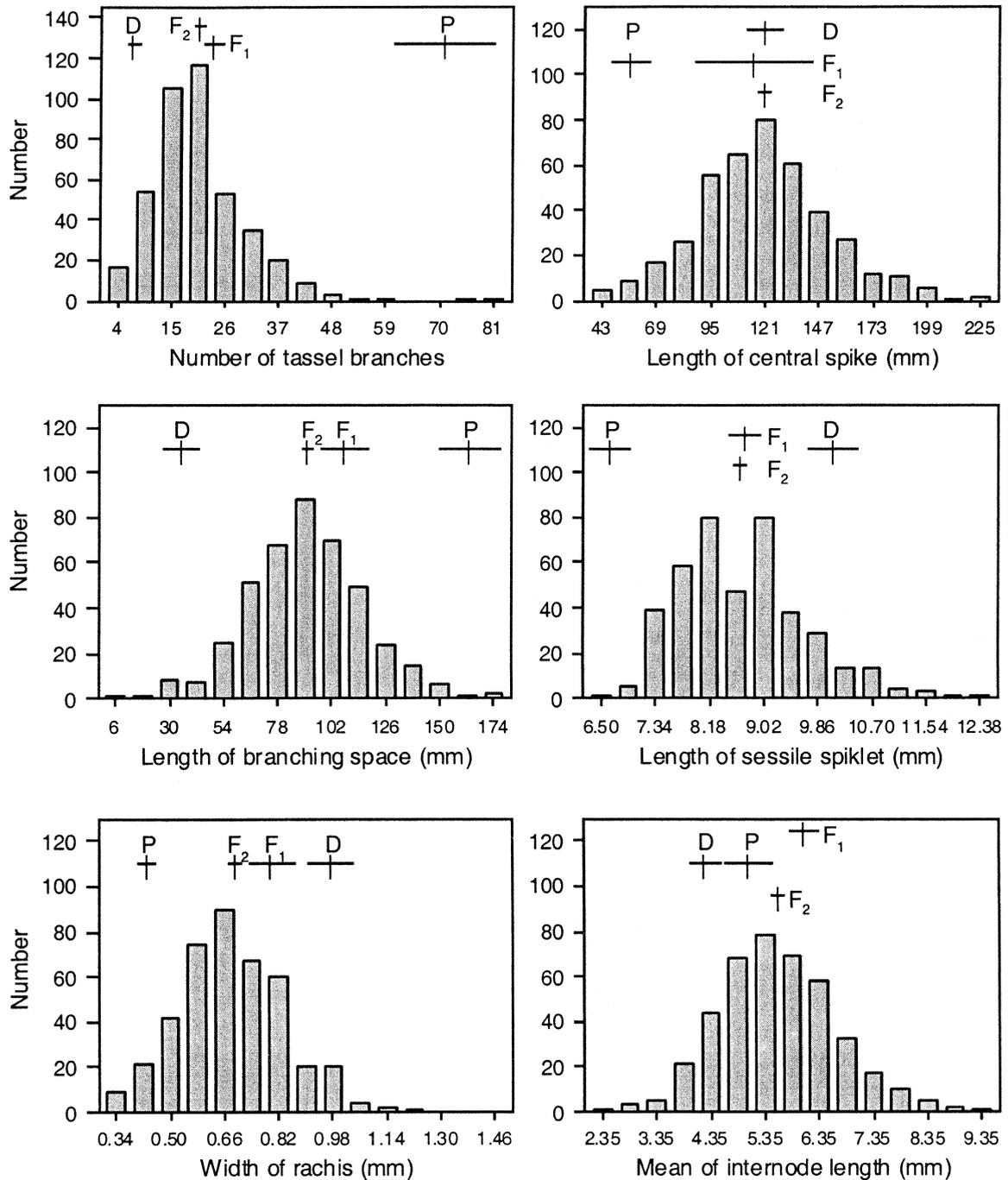
FIG. 4. Distribution of trait values for six tassel traits in the F_2 population, and the mean values (vertical lines) and 95% confidence intervals (horizontal lines) for *Zea diploperennis* (D), *Z. mays* ssp. *parviglumis* (P), the F_1 hybrid clones, and the F_2 population.

used to create the F_2 were more dissimilar genetically than the parent species overall. This trait was also unusual in that the F_1 and F_2 means were outside the range of the means of the two parental populations (Fig. 4).

The QTL found by IM and CIM were distributed on nine different chromosomes (Table 3, Fig. 3). However, QTL for several of the tassel traits mapped near each other (their 1-LOD support intervals overlapped), and the chromosomal regions with significant effects are therefore more concen-

trated. Two cases are of interest. First, QTL for four traits (NO-TB, LN-BS, LN-SS, and MN-IL) all mapped near each other on chromosome 5 close to bnl7.71. This could either be explained by adjacent multiple QTL on chromosome 5, each controlling one of the traits, or by a single QTL affecting these traits pleiotropically. The large number of significant associations on chromosome 5 suggests that this region has an important impact on tassel morphology. Second, five of six QTL involved in the inheritance of NO-TB mapped near

TABLE 2. Percent of parentlike F_2 plants. *diplo, Zea diploperennis*; *parvi, Z. mays* ssp. *parviglumis*. Plants were considered parentlike if the phenotype lay within the 2.5 to 97.5 percentiles of the phenotypic variation of either of the two parental populations.

Trait	*diplo*-like	*parvi*-like	*diplo*- and *parvi*-like
NO-TB	17.0	25.2	0.0
LN-CS	49.4	3.1	34.5
LN-BS	23.0	70.7	6.2
LN-SS	55.8	25.4	0.0
WD-RA	64.2	17.8	0.0
MN-IL	1.4	39.1	55.4

QTL for LN-BS on chromosome 1, 2, 4, 5, and 7. These two traits are highly correlated ($r = 0.715$, $P = 0.0001$). It seems likely that there is a single QTL in each chromosomal region having a pleiotropic effect on these two traits rather than a separate QTL in each region controlling the expression of each trait.

If the traits under study were adaptive and subject to consistent directional selection during the evolution of Z. *diploperennis* and Z. *mays* ssp. *parviglumis*, then one would expect the direction of the effects of all Z. *diploperennis* alleles to be consistent with a Z. *diploperennis* phenotype and Z. *mays* ssp. *parviglumis* alleles with a *parviglumis* phenotype. About one-third of the QTL (10 of 30 detected with IM and 12 of 33 detected with CIM) showed alleles with opposite effects than expected (Table 3). To compare a model of consistent directional selection with one of random genetic drift (or oscillating directional selection) in the evolution of tassel morphology, we used the method of Orr (1998). This test calculates the probability that the number of QTL with effects in the same direction is more extreme than expected by chance given the phenotypic difference between the taxa, the number of observed QTL, and the direction and magnitude of QTL effects. The probability of finding five of seven QTL of the same sign for NO-TB by chance alone was $P = 0.70$, the probability for two of two QTL for LN-CS was 0.75, four of five QTL for LN-BS was $P = 0.60$, five of eight for LN-SS was $P = 0.81$, three of five QTL for WD-RA was $P = 0.89$, and four of six for MN-IL was $P = 0.81$.

The ratio of the dominance to additive effects (d/a) for the QTL ranged from -1.54 to 0.92 for IM and -1.90 to 0.71

TABLE 3. Quantitative trait loci (QTL) for tassel traits, their chromosomal location (Chr), nearest or flanking molecular marker loci (MMLs), LOD score, dominance/additive ratio (d/a), mode of gene action (a, additive; d, dominant; or od, overdominant), direction of their effect (Dir, whether the *diploperennis* [D] or the *parviglumis* [P] allele contributed positively to the effect), proportion of phenotypic variance explained by a QTL (PVE), and by all QTL for a trait (multi QTL PVE). All values are from composite interval mapping, except for the QTL for LN-BS near bnl15.40, which was only detected by interval mapping. PVE values for QTL were also calculated under IM with Mapmaker/QTL, except for the four QTL detected only by CIM. The multiple QTL PVE values were computed with Mapmaker/QTL and are based on QTL found by IM.

Trait	Chr	MMLs	LOD	d/a	Mode	Dir	PVE	Multi QTL PVE
NO-TB (log)	1	umc157	10.6	-0.29	a	P	8.9	43.9
	2	bnl8.45	2.6	-0.44	a	P	4.9	
	4	umc156-umc19	8.1	-0.63	d, P	P	7.1	
	5	bnl7.71-npi412	8.5	-0.76	d, P	P	10.8	
	7	bnl15.40-umc5b	9.3	-0.12	a	P	9.3	
	7	bnl14.07-umc91	8.4	-0.05	a	D	8.4	
	9	umc94b	2.7	0.30	a	D	2.1	
LN-CS	1	bnl15.59-umc23a	7.2	-0.28	a	P	8.6	16.5
	7	bnl14.07-umc91	5.2	-0.46	a	P	6.9	
LN-BS	1	umc11-umc29	8.8	-0.58	d, P	P	12.5	42.0
	2	umc53-bnl7.49c	8.3	-0.12	a	P	8.2	
	4	umc19	5.8	-0.56	d, P	P	7.1	
	5	bnl7.71-npi412	4.9	-1.90	od, P	P	6.5	
	7	bnl15.40	3.1	-0.32	a	P	3.4	
	7	bnl14.07-umc91	5.7	-0.45	a	D	7.8	
LN-SS (log)	1	bnl5.62	4.9	0.42	a	P	4.3	48.1
	1	npi581a-bn16.32	4.0	0.33	a	P	4.5	
	2	phi20-0005	6.0	0.71	d, D	D	4.9	
	3	umc18a-umc60	4.9	0.06	a	D	11.3	
	4	bnl5.46-npi386	2.8	0.00	a	D	3.2	
	4	umc104a-umc127	15.4	-0.16	a	D	11.2	
	5	umc83b-bnl7.71	6.3	-0.86	d, P	P	8.8	
	9	phi10-0005	14.6	0.00	a	D	12.3	
WD-RA	1	npi581a-bnl6.32	4.1	0.45	a	D	4.6	26.5
	2	phi20-0005	3.1	-1.20	d, P	P	4.9	
	3	umc42b	8.9	-1.39	od, P	P	9.6	
	8	bnl9.44-umc12a	3.1	-0.46	a	D	5.4	
	9	umc81-umc95	7.7	0.37	a	D	8.7	
MN-IL	1	umc11-umc29	7.4	-0.45	a	P	6.3	39.7
	3	npi425a-umc2a	5.1	0.01	a	P	5.0	
	5	bnl7.71-npi412	14.5	0.20	a	P	19.9	
	6	umc21-umc46	3.7	0.27	a	D	3.5	
	8	phi20-0042b-bnl9.44	4.4	-0.52	d, P	P	11.0	
	9	umc81-umc95	4.4	-0.32	a	D	7.8	

TABLE 4. Mean (95% confidence interval) for tassel traits studied in *Zea diploperennis* (*diplo*) and *Z. mays* ssp. *parviglumis* (*parvi*). Phenotypic mean (95% confidence interval) of F$_2$ plants homozygous for the *diploperennis* (D) or the *parviglumis* (P) allele at each quantitative trait locus (QTL) observed with composite interval mapping. QTL effects are given as the difference between the two homozygotes expressed as a percentage of the difference between the two parental populations and the sum of QTL effects for each trait.

Trait	Mean (CI) *diplo*	Mean (CI) *parvi*	Chr	MMLs	Phenotypic mean (CI) DD/PP	QTL effect (%)	Sum of QTL effects (%)
NO-TB	7.0 (1.1)	71.1 (10.2)	1	umc157	16.1 (1.5)/23.9 (2.0)	12.2	35.1
			2	bnl8.45	17.7 (1.3)/23.2 (2.6)	8.6	
			4	umc156-umc19	16.4 (1.4)/23.0 (2.0)	10.3	
			5	bnl7.71-npi412	17.9 (1.5)/21.1 (1.8)	5.0	
			7	bnl15.40-umc5b	13.9 (2.4)/23.0 (1.6)	14.3	
			7	bnl14.07-umc91	25.0 (2.1)/17.5 (1.6)	−11.6	
			9	umc94b	20.8 (1.9)/18.4 (1.6)	−3.7	
LN-CS (mm)	120.9 (8.5)	56.6 (9.3)	1	bnl5.59-umc23a	107.8 (6.2)/130.2 (6.2)	−34.8	−63.6
			7	bnl14.07-umc91	109.4 (5.4)/127.9 (6.6)	−28.8	
LN-BS (mm)	35.1 (8.0)	163.3 (13.6)	1	umc11-umc29	80.2 (5.2)/97.9 (4.8)	13.8	38.4
			2	umc53-bnl7.49c	82.4 (3.5)/100.9 (6.4)	14.4	
			4	umc19	80.2 (4.3)/96.8 (4.5)	12.9	
			5	bnl7.71-npi412	82.4 (5.0)/94.0 (5.6)	9.1	
			7	bnl14.07-umc91	100.0 (4.7)/84.9 (5.5)	−11.8	
LN-SS (mm)	10.1 (0.4)	6.6 (0.3)	1	bnl5.62	8.4 (0.2)/8.9 (0.2)	−14.2	52.6
			1	npi581a-bnl6.32	8.4 (0.2)/8.9 (0.2)	−15.7	
			2	phi20-0005	8.8 (0.2)/8.3 (0.2)	15.4	
			3	umc18a-umc60	9.0 (0.2)/8.4 (0.2)	15.9	
			4	bnl5.46-npi386	9.0 (0.3)/8.4 (0.2)	18.7	
			4	umc104a-umc127	9.0 (0.2)/8.3 (0.2)	21.3	
			5	umc83b-bnl7.71	8.4 (0.2)/8.9 (0.2)	−17.2	
			9	phi10-0005	9.2 (0.2)/8.2 (0.1)	28.4	
WD-RA (mm)	1.0 (0.1)	0.4 (0.0)	1	npi581a-bnl6.32	0.7 (0.0)/0.6 (0.0)	13.2	18.0
			2	phi20-0005	0.6 (0.0)/0.7 (0.0)	−9.2	
			3	umc42b	0.6 (0.0)/0.7(0.0)	−20.9	
			8	bnl9.44-umc12a	0.8 (0.0)/0.7 (0.0)	15.7	
			9	umc81-umc95	0.7 (0.0)/0.6 (0.0)	19.2	
MN-IL (mm)	4.2 (0.3)	5.1 (0.4)	1	umc11-umc29	5.2 (0.2)/5.9 (0.2)	80.0	170.8
			3	npi425a-umc2a	5.3 (0.2)/6.0 (0.2)	82.7	
			5	bnl7.71-npi412	5.1 (0.2)/6.0 (0.2)	108.2	
			6	umc21-umc46	5.9 (0.2)/5.3 (0.2)	−70.6	
			8	phi20-00426-bnl9.44	5.3 (0.3)/5.7 (0.2)	51.6	
			9	umc81-umc95	6.0 (0.3)/5.4 (0.2)	−81.0	

for CIM (Table 3). About two-thirds of the QTL showed additive gene action ($-0.50 <$ d/a < 0.50). The remaining QTL showed dominance or overdominance. For most of these QTL, the *parviglumis* allele was dominant over the *diploperennis* allele. Overdominance ($-1.25 >$ d/a > 1.25) was found for two QTL, one on chromosome 3 for WD-RA and one on chromosome 5 for LN-BS.

By using a two-way ANOVA, we found 55 significant interaction effects ($P < 0.05$) when looking at all combinations of 19 genomic locations that harbor QTL, given a total of 1026 tests. At a significance level of $P < 0.05$ for 1026 tests, one would expect 51 significant interactions by chance alone. Because the number of significant interactions does not differ much from what is expected by chance we did not find strong evidence for epistasis underlying the differences in tassel morphology between teosinte taxa. A more comprehensive analysis of all 26,790 possible pairwise epistatic interactions among markers may reveal a different picture. This seems unlikely, however, because the 19 loci tested represent 16 of 20 chromosome arms.

DISCUSSION

The genetic basis of morphological trait evolution in natural plant species is poorly understood (Orr and Coyne 1992).

Recent QTL mapping studies in crop plants have indicated that QTL of large effect were commonly involved in crop plant domestication (e.g., Doebley and Stec 1991; de Vicente and Tanksley 1993; Lin et al. 1995). In the present study, we wished to determine if the genetic basis of natural species differences would follow the model of crop species, involving relatively few QTL including some of large effect. Our data suggest that the crop species model does not apply fully to the evolution of tassel morphology in natural teosinte species. Although the present study identified some loci of moderate effects, no QTL of very large effect ($>$ 20% of the phenotypic variance) were found as seen under domestication.

The association between genetic markers and traits can be studied by IM and CIM. IM estimates the most likely position and effect of a QTL within an interval between two markers. The estimates of position and effect may be biased because the test statistic on a marker interval is not corrected for other QTL located outside the interval. CIM combines IM with multiple regression so that the effects of QTL outside the test interval are included in the model. For this reason, the estimates of position and effect of the QTL within the test interval should be more precise with CIM (Zeng 1993, 1994). In our study, the number of QTL found was similar for IM and CIM. Thirty QTL were found by IM and 33 QTL were

found by CIM (Table 3). CIM appears to have somewhat greater power to detect QTL with our data; however, the two methods gave very similar results overall.

Our results can be compared to other QTL studies of differences between natural species. Some studies, like ours, have found only QTL of small to moderate effect. For example, Laurie et al. (1997) found that multiple QTL of moderate effects (10–15% of the species difference) control morphological differences in male genitalia between *Drosophila simulans* and *D. mauritiana*. Other studies of natural species have detected QTL of large effect. Major QTL explaining up to 54% of the species difference were detected in the analysis of some male sexual traits in *D. simulans* and *D. mauritiana* (True et al. 1997). Bradshaw et al. (1998) found that floral traits associated with reproductive isolation in *Mimulus lewisii* and *M. cardinalis* are controlled by a few QTL, some explaining a large fraction of the phenotypic variance. The difference in plant mating system between *M. guttatus* and *M. platycalyx* is controlled by multiple QTL, with some explaining 29% of the phenotypic variance (Lin and Ritland 1997). The evolution of microsporangia number in *Microseris* also involves a QTL of large effect (Gailing et al. 1999). Additional QTL studies will be required to accurately document the full range of genetic architectures underlying trait differences between natural species.

One concern with QTL mapping as done by us and others is that there can be inbreeding depression in an F_2 derived from a single F_1 plant when the parents are predominantly outcrossing as our parental species are. As a result, F_2 plants homozygous at loci contributing to inbreeding depression may have a loss of vigor and a drop in trait values (e.g., fewer tassel branches). These inbreeding loci could be detected as QTL for tassel traits. If this were the case, one would expect such QTL to exhibit either dominant or overdominant gene action. Although we cannot exclude the possibility that inbreeding has affected our results, two observations suggest that it has not had an overarching effect. First, we did not observe strong differences between the F_1 and F_2 as expected with inbreeding. Second, two-thirds of the QTL detected show additive gene action.

The tassel traits that we studied are important in the taxonomic discrimination of the species; however, they have no known adaptive significance, although it is likely that some of our traits, which are related to overall flower number in the tassel, impact male fitness. Among the QTL that we detected, fully one-third had effects in the wrong direction (e.g., a *parviglumis* allele that confers a *diploperennis* phenotype). An analysis using the method of Orr (1998) failed to support the hypothesis that the tassel traits have been under consistent directional selection since the divergence of *parviglumis* and *diploperennis.* Thus, it is possible that fixation of alleles by either random drift or oscillating directional selection has governed the divergence in tassel morphology between these two species. Because crop evolution is expected to involve strong and consistent directional selection, the lack of such a selective regime during the divergence of *diploperennis* and *parviglumis* may explain why we failed to detect QTL of large effect.

In our F_2 population, the QTL for any one trait collectively explained at most about 48% of the variation. This is much lower than observed in QTL mapping studies of maize-teosinte F_2 populations in which this percentage ranged from 50% to 87% (Doebley and Stec 1993). The lower percentages in the present study may be due to low heritability of the traits or additional undetected QTL. The heritability of some traits may be lower in natural populations than in domesticated populations as breeders select for environmental stability. In contrast, natural plants often respond to local conditions by varying the number and sizes of the organs they produce and are thus under selection for phenotypic plasticity. For example, Lukens and Doebley (1999) provide evidence that the maize allele of the *tb1* gene has a less plastic response to environment than does the teosinte allele. Several of the tassel traits that we studied measure organ size or number, and plasticity for these traits may be particularly high. For example, teosinte plants in cultivated fields with deep well-watered soil are usually tall with large highly branched tassels, whereas plants growing in rocky soil on dry hillsides are shorter with fewer tassel branches. If our traits are particularly plastic in teosinte, then the QTL we detected will explain a much larger percentage of the genetic variance in the F_2 populations than they do of the phenotypic variance.

For model organisms and species closely related to them, QTL can be associated with candidate loci of known genomic location. For the present data, several QTL for NO-TB are located near candidate genes. First, the QTL between bnl15.40 and umc5b on chromosome 7 maps near *ramosa1,* a mutant that produces a larger number of tassel branches (Neuffer et al. 1997). Other studies in maize have identified QTL for NO-TB in this region (Berke and Rocheford 1999). Second, the QTL near bnl7.49c on chromosome 2 maps near the maize homolog of the *Arabidopsis Leafy* gene. *Leafy* has been proposed to control inflorescence branching in grasses (Kyozuka et al. 1998). Third, the QTL near bnl14.28 on chromosome 9 maps near *fascicled ear,* which causes an increase in the number of tassel branches (Neuffer et al. 1997). Because these genes have been or will likely be molecularly cloned, it will be possible to investigate these QTL further and perhaps to demonstrate whether they correspond to these candidates.

Transgressive segregation was observed for two tassel traits (LN-CS and MN-IL). LN-CS and MN-IL are developmentally related because longer internodes (MN-IL) will produce a longer central spike (LN-CS). Transgressive segregation can be explained by overdominance, epistasis or the presence of QTL of opposite effect in each parental species. We have found little or no evidence for epistasis, however, our results clearly indicate that each parent contains a mixture of positive and negative QTL for most traits. Thus, the transgressive individuals most likely have a combination of complementary positive or negative alleles from both parents causing them to have more extreme phenotypes than the parents. Transgressive segregation due to complementary alleles has been reported for other plant species such as tomato (de Vicente and Tanksley 1993) and rice (Li et al. 1995).

Overdominance may also contribute to transgression for MN-IL. In contrast to the other tassel traits, MN-IL showed a significantly higher mean for the F_1 plants than for the parental populations (Fig. 4). Thus, overdominance could

also contribute to transgression if the transgressive F_2 plants are those plants that are heterozygous at QTL for MN-IL. However, the observation that none of the individual QTL for MN-IL showed overdominance is inconsistent with this interpretation.

In conclusion, our results indicate that a modest number of QTL of small to moderate effect were involved in the evolution of the divergent tassel morphologies of *Z. diploperennis* and *Z. mays* ssp. *parviglumis*. Unlike evolution under domestication, we detected no single QTL of strikingly large effect and almost all of the 33 QTL detected had relatively similar magnitudes of effect. Thus, the model involving major QTL in crop evolution seems not to apply to the traits and species analyzed. In other cases, QTL of large effect have been found to distinguish natural species. One would therefore expect plant evolution to have involved the full range of genetic mechanisms from a few genes of large effect to more numerous genes of small effect. To determine the importance of these contrasting modes of evolution, more genetic analyses of natural populations are still needed.

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Corresponding Editor: J. Conner