Inheritance of Kernel Weight in Two Maize–Teosinte Hybrid Populations: Implications for Crop Evolution

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A QTL-mapping strategy was employed to determine the number of quantitative trait loci (QTLs) that control the difference in kernel weight between maize (*Zea mays* L. ssp. *mays*) and its probable progenitor, teosinte [*Z. mays* ssp. *parviglumis* Iltis and Doebley, or ssp. *mexicana* (Schrader) Iltis]. Two maize-teosinte F_2 populations were analyzed. In the first population, we detected six QTLs, each controlling 4%–34% of the phenotypic variance and, in the second, four QTLs, each controlling 9%–31% of the phenotypic variance. The QTL of largest effect, which maps to chromosome 3 in both populations, adds more than 30 mg to the average kernel weight, nearly doubling the weight of a teosinte kernel. This QTL may represent a major step in the early evolution of maize. The relatively small number of QTLs detected and the large magnitude of some of their effects suggest that the difference in kernel weight between maize and teosinte is more aptly described as oligogenic rather than polygenic. The inferred role of genes of large effect in the evolution of kernel weight in maize implies that there may have been evolutionary periods during which the fixation of these genes brought about relatively rapid change in a reasonably short period of time.

The evolution of all cereal crops from their wild relatives involved changes in numerous morphological traits. Among these is kernel weight, for which a cereal crop may exceed its wild relatives by a factor of 10 or more. This dramatic increase in kernel weight is the result of human selection for higher yields over the millenniums since cereal domestication first began some 10,000 years ago. Although there is little direct evidence concerning the mode and tempo of the evolution of kernel weight in the cereals, a common perception is that the evolution of such quantitative traits typically involves numerous loci with individually small effects (but see Gottlieb 1984; Orr and Coyne 1992). In this paper, we report the results of a study of the inheritance of kernel weight in two hybrid populations of maize (*Zea mays* L. ssp. *mays*) and its probable wild progenitor, teosinte [*Z. mays* ssp. *parviglumis* Iltis and Doebley and ssp. *mexicana* (Schrader) Iltis]. Our results show that while there are multiple QTLs (quantitative trait loci) controlling the difference in kernel weight between maize and teosinte, some of these QTLs have large effects relative to others. This result suggests that the evolution of kernel weight in maize, and perhaps other crops, involved some reasonably large evolutionary steps and that the early evolution of this trait in maize might be more aptly described as oligogenic rather than polygenic.

Materials and Methods

We studied the inheritance of kernel weight in two F_2 populations. One population (C × M) was derived from a cross of Race Chapalote maize by *Zea mays* ssp. *mexicana* teosinte (Doebley and Stec 1991) and the other population (R × P) from a cross of Race Reventador maize by *Zea mays* ssp. *parviglumis* teosinte (Doebley and Stec 1993). The C × M population included 260 plants, and the R × P population included 290. Both populations were grown in nurseries in Hawaii. These populations have been previously studied for traits other than kernel weight, and fuller descriptions of the populations are presented elsewhere (Doebley and Stec 1991, 1993).

After harvesting the plants, all ears were dried for 3 days in an oven at 45°C. Subsequently, the ears were stored in cabinets in the University of Minnesota Herbarium for 2–3 years before being measured for kernel weight. Ten kernels were taken from each plant and weighed on a standard laboratory analytic balance. Only 10 kernels

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per plant were weighed because the ears of the F_2 plants are often quite small (like teosinte), and a larger number of kernels could not be obtained from all individuals. Kernel weight for the parents of each F_2 population was also determined by weighing 10 kernels from each of 10 plants.

For the C × M population, 58 molecular marker loci covering the entire genome were analyzed, while 82 marker loci were analyzed in the R × P population (Figure 1). Most marker loci were low-copy-number nuclear probes used to detect restriction fragment length polymorphisms; however, the C × M population included a few isozyme loci. All details concerning genotyping of the molecular marker loci in these two populations have been published elsewhere (Doebley and Stec 1991, 1993). Both populations were derived from single F_1 plants; thus, there were only two alleles per locus in each population and we did not have to pool marker locus classes.

To determine the chromosomal locations and phenotypic effects of the QTLs controlling kernel weight in the two F_2 populations, we employed interval mapping using the computer program MAPMAKER-QTL version 0.9 (Lander and Botstein 1989). Interval mapping involves calculating the ratio of the likelihood (odds) that there is a QTL to the likelihood that there is no QTL at each position along the length of a chromosome. These likelihoods are normally reported as the $\log_{10}$ of the "odds" ratio or LOD-score. Based on the genetic length of the maize-teosinte linkage map and the number of marker loci we analyzed, LOD-scores above 2.4 indicate the presence of a QTL. The LOD-score threshold of 2.4 provides an overall probability of a Type I error of .05 in each population (Lander and Botstein 1989). Interval mapping also provides an estimate of the percentage of the phenotypic variance explained (PVE) by a QTL or a group of QTLs. Interval mapping was used to assess the probability of two linked QTLs on a single chromosome such that a LOD-score increase of 2.0 or more upon adding a second QTL to the model was regarded as evidence for a second QTL (Stuber et al. 1992). We also used MAPMAKER-QTL to examine the modes of gene action for each QTL as explained by Paterson et al. (1991). The LOD-scores for dominant, recessive, and additive gene action were calculated for each QTL. If one mode had a LOD-score that exceeded that of the others by 1.0 or more, then it was judged to be the most likely mode of gene action. Finally,

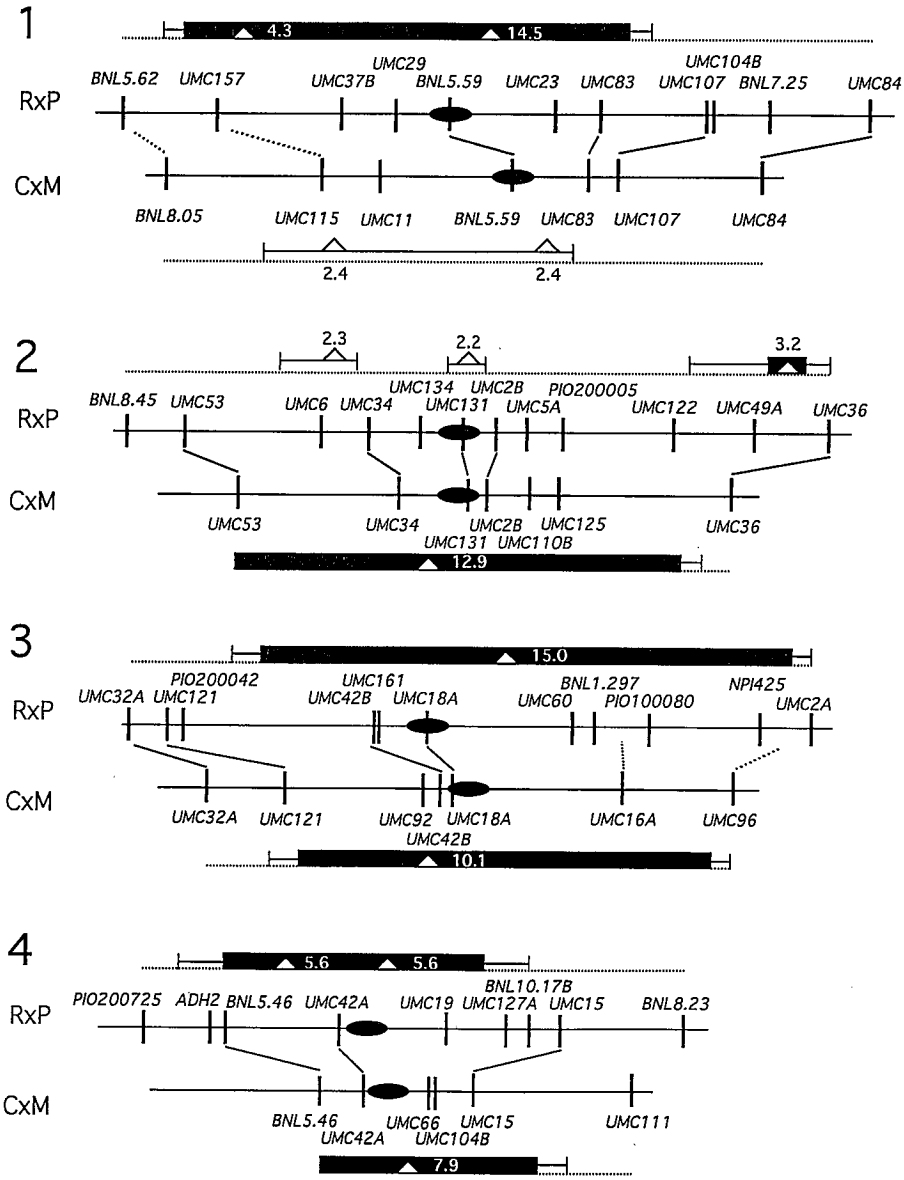

Figure 1. Chromosome maps showing LOD-scores and the positions of QTLs detected in the R × P and C × M F_2 populations. The black bars indicate LOD > 3.0 and the whiskers LOD > 2.0, and triangles show the positions of the peak LOD-scores on each chromosome. Where the LOD-score exceeds 2.4, we infer the presence of a QTL. The names of the molecular marker loci and the positions of the centromeres (black ovals) are shown on the chromosomes. Diagonal lines show exact (solid diagonal line) and approximate (dashed diagonal line) points of alignment between the chromosome maps for the two populations. The cytologically defined short and long arms of each chromosome are on the left and right, respectively. Scale is recombination units.

to test for digenic epistatic interactions, mean kernel weights for the nine possible two-locus genotypic classes at the marker loci were subjected to two-factor analysis of variance. A significant ($P = .05$) interaction term was interpreted as evidence of epistasis. To reduce the large number of possible tests of epistasis that could be performed to a manageable number, tests of epistasis were performed only for marker loci flanking QTLs that explained 10% or more of the phenotypic variance for kernel weight.

Results and Discussion

The maize and teosinte parents of the two F_2 populations differ widely in mean kernel weight (Table 1). For the C × M population, the means of the two parents differ by more than five standard deviations, and for the R × P population this difference is more than six standard deviations. The distribution of kernel weights among the F_2 plants was approximately normal with a slight positive skewing (Figure 2). The means of both F_2 populations are closer to teosinte parent values, suggesting a

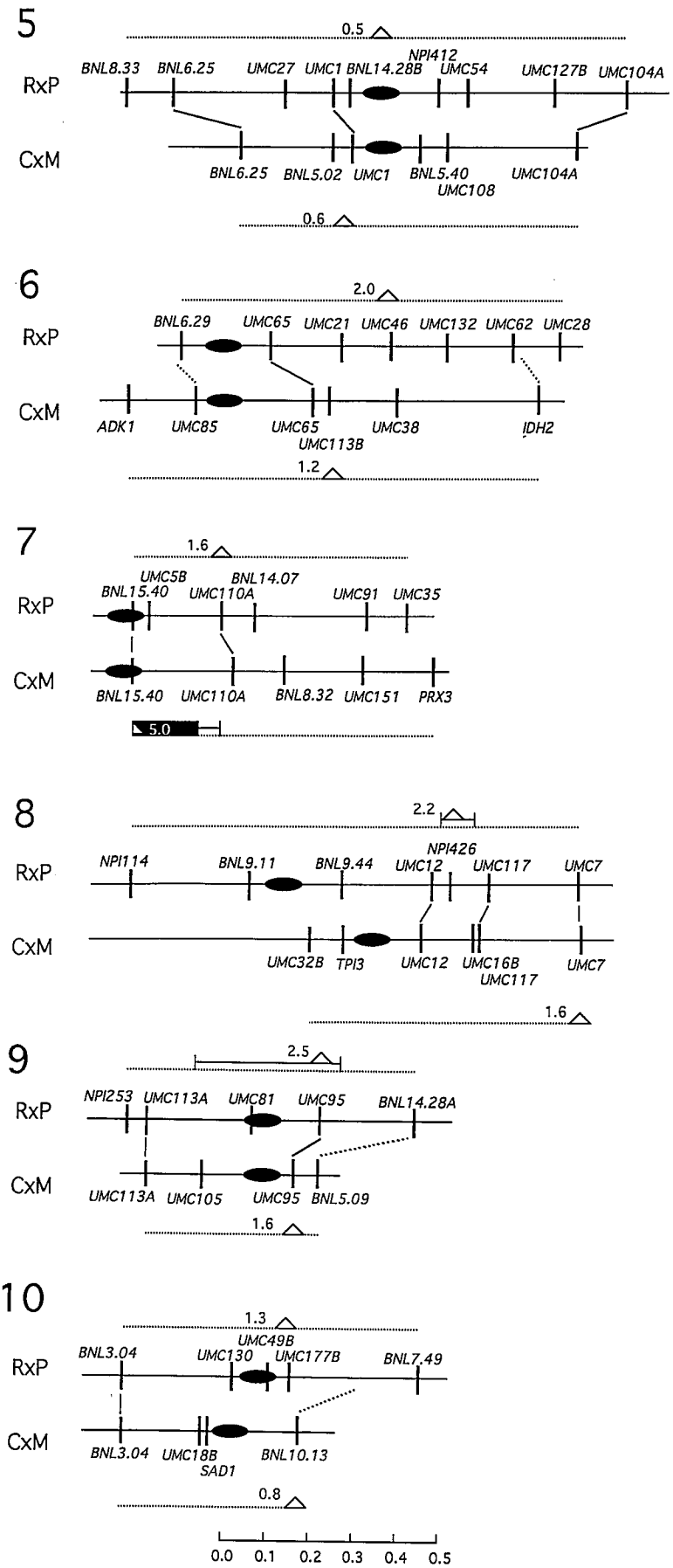

Figure 1. Continued.

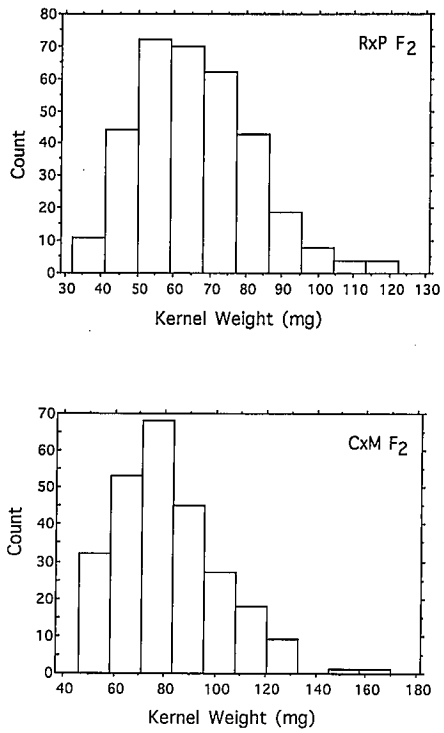

Figure 2. Histograms for kernel weight in the two maize-teosinte F_2 populations.

slight dominance of the teosinte phenotype. There is no evidence for transgressive segregation; i.e., all F_2 plants have kernel weights within the range of the parents.

Figure 1 shows the LOD plots for each of the 10 *Zea* chromosomes in both populations. LOD-scores above 3.0 are indicated by the heavy black bars, LODs between 2.0 and 3.0 by the whiskers on these black bars, and LODs below 2.0 by the dashed lines. The peak LOD(s) on each chromosome is indicated by a triangle(s). Based on these LOD plots, we inferred the presence of six QTLs in the R × P population, and four in the C × M population where the peak LOD exceeds the threshold value of 2.4 (Table 2). On chromosomes 1 and 4 in the R × P population, there are two distinct LOD peaks. Comparison of one- versus two-QTL models enabled us to reject the one-QTL model for the peaks on chromosome 1 but not for those on chromosome 4. The magnitudes of the QTLs vary considerably, controlling 4%–34% of the phenotypic variance in the R × P population and 9%–31% in the C × M population (Table 2). The percentage of the phenotypic variation explained by all QTLs was 69% in the C × M population and 70% in the R × P population.

The directions of the effects of all observed QTLs were such that the maize allele acts to increase kernel weight relative

Table 1. Mean kernel weight for the maize and teosinte parents, and the two F_2 populations

Taxon	Kernel weight (mg)	Standard deviation	Range
Reventador maize	135.6	26.8	104–198
Subspecies *parviglumis* teosinte	33.4	5.0	27–40
R × P F_2 population	65.8	16.4	32–122
Chapalote maize	170.3	40.7	115–236
Subspecies *mexicana* teosinte	43.3	5.8	34–53
C × M F_2 population	81.1	20.6	46–169

Table 2. Chromosomal location and magnitude of the effect of QTLs affecting kernel weight in two maize × teosinte F_2 populations

Population	Chromosome	Flanking markers[a]	Gene LOD	PVE[b]	Action[c]	Effect[d]
R × P	1	UMC157–UMC37B	4.3	11.6	AD	13
	1	BNL5.59–UMC23	14.5	28.8	A	24
	2	UMC49A–UMC36	3.2	5.9	AD	13
	3	UMC18A–UMC60	15.0	34.4	A	31
	4	BNL5.46–UMC42A	5.6	12.8	AR	15
	9	UMC95–BNL14.28A	2.5	4.0	AD	12
C × M	2	UMC34–UMC131	12.9	23.6	A	29
	3	UMC18A–UMC16A	10.1	31.1	A	37
	4	UMC42A–UMC66	7.9	15.7	AR	19
	7	BNL15.40–UMC110A	5.0	8.8	AR	11

[a] Molecular marker loci flanking the QTL.

[b] Percentage of phenotypic variance for kernel weight explained by the QTL.

[c] A = additive; D = maize allele dominant; R = maize allele recessive.

[d] The effect of substituting two maize alleles for two teosinte alleles at the QTL, expressed as the increase in kernel weight (mg).

to the teosinte allele. This was the expected result given that maize has been under continual selection for increased kernel weight since its domestication. This outcome also explains why we did not observe transgressive segregation for kernel weight. With some other traits such as plant height in the C × M population, Doebley and Stec (1991) observed QTLs with effects in the unexpected direction, e.g., QTLs from the short parent that contributed positively to plant height. Most QTLs exhibit additive gene action, although dominant or recessive action could not be excluded in some cases (Table 2). We also performed a total of 11 tests of epistasis with no significant results. The lack of detectable epistasis is consistent with what has typically been found in other QTL-mapping experiments (Cowen et al. 1992; Doebley and Stec 1991; Edwards et al. 1987; Paterson et al. 1991).

Examination of Figure 1 reveals that genetic control of kernel weight shows some similarities and differences between the two F_2 populations. In both populations, the QTL of largest effect is near the centromere on chromosome 3. Both populations also possess a QTL of moderate effect near the centromere on chromosome 4. Because these two QTLs differentiate both types of teosinte from both types of maize, it is likely that they were involved in the early evolution of the large grains of maize from the small grains of teosinte. The R × P population possesses two QTLs on chromosome 1 that were not detected in the C × M population; however, in the latter population, there are effects in the same regions of chromosome 1 that fall just below the level of statistical significance. Similarly, the C × M population possesses a QTL of large effect near the centromere on chromosome 2 that was not detected in the R × P population; however, in the latter population, there are effects in this region that again fall just below the level of statistical significance. There are three other QTLs of small effect on chromosomes 2 (near the end of the long arm), 7, and 9 that were found in only one population.

There are a variety of reasons why a QTL may be detected in one population and not the other (Doebley and Stec 1993; Paterson et al. 1991). First, our two teosinte parents have different kernel weights, as do our two maize parents (Table 1). Thus, QTLs not found in both populations may represent genes responsible for the natural variation that occurs within maize and teosinte. Second, detection of QTLs, especially those with small effects, is affected by sample size and environmental variation. A sample size of 250–300 F_2 plants may provide a high probability of detecting major QTLs but is unlikely to guarantee detection of all QTLs of small effect.

Evidence from allozymes and chloroplast DNA suggests that *Zea mays* ssp. *parviglumis* is most likely the original progenitor of maize, but that *Zea mays* ssp. *mexicana* may have contributed to present day variation in maize via introgressive hybridization after the original domestication event (Doebley 1990). It is also known that ssp. *parviglumis* has smaller kernels than ssp. *mexicana* (Table 1; Doebley 1984; Wilkes 1967). Given this information, the QTL of large effect on the long arm of chromosome 1 may represent a gene involved in the evolution in nature of the difference in kernel sizes between ssp. *parviglumis* and ssp. *mexicana,* conferring a larger kernel size on the latter. After maize was domesticated from ssp. *parviglumis,* the ssp. *mexicana* allele at this QTL may have been transferred into maize via introgression, making the kernels of early maize larger. Thus, we detect this QTL in the maize-*parviglumis* population (R × P) but not in the maize-*mexicana* population (C × M) because the parents of the latter population would be homoallelic for this QTL. If this interpretation is correct, then this QTL on chromosome arm 1L should segregate in a ssp. *parviglumis* × ssp. *mexicana* population.

The QTL on chromosome 3 accounts for more than 30% of the phenotypic variance for kernel weight in both F_2 populations. Substitution of two maize alleles for the teosinte alleles at this QTL adds 37 mg to the weight of a kernel in the C × M population and 31 mg in the R × P population (Table 2). Thus, this QTL nearly doubles the weight of the teosinte kernel (Table 1), clearly a large effect relative to the weight of the teosinte kernel. If this QTL represents a single gene, then the evolution of kernel weight may have involved at least one reasonably large step that brought this putative gene to fixation in a relatively short period of time. However, at this time, it is not known whether this QTL represents a single gene or multiple linked genes.

Our results demonstrate that genetic control of the difference in kernel weight between maize and teosinte involves a relatively small number of QTLs. Some of these have large effects and others relatively small effects. Our results are not consistent with a mode of evolution involving many QTLs with equal and small effects. Thus, our results suggest that evo-

lution of large kernels in maize may not have involved a continuous and gradual increase in kernel weight over thousands of years, but rather a process in which mutations of reasonably large effect arose and were rapidly brought to fixation within the population, causing oscillation between periods of rapid and slow change or stasis. A similar scenario may also apply to the evolution of fruit weight in tomato (Paterson et al. 1991), and seed weight in cowpea and mung bean (Fatokun et al. 1992).

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