Genetic Variation for Phenotypically Invariant Traits Detected in Teosinte: Implications for the Evolution of Novel Forms

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ABSTRACT

How new discrete states of morphological traits evolve is poorly understood. One possibility is that single-gene changes underlie the evolution of new discrete character states and that evolution is dependent on the occurrence of new single-gene mutations. Another possibility is that multiple-gene changes are required to elevate an individual or population above a threshold required to produce the new character state. A prediction of the latter model is that genetic variation for the traits should exist in natural populations in the absence of phenotypic variation. To test this idea, we studied traits that are phenotypically invariant within teosinte and for which teosinte is discretely different from its near relative, maize. By employing a QTL mapping strategy to analyze the progeny of a testcross between an F_1 of two teosintes and a maize inbred line, we identified cryptic genetic variation in teosinte for traits that are invariant in teosinte. We argue that such cryptic genetic variation can contribute to the evolution of novelty when reconfigured to exceed the threshold necessary for phenotypic expression or by acting to modify or stabilize the effects of major mutations.

HIGHER taxonomic groups of plants typically differ from one another for traits that show discrete differences between groups while being invariant within groups, *e.g.*, four *vs.* five petals or an inferior *vs.* superior ovary. For 100 years, genes that act as switches between character states for such discrete traits have been known as horticultural or laboratory mutants. This situation invites the view that the evolution of higher taxa was driven by changes in single major genes that acted as switches between such alternate forms (Hilu 1983). Recent cloning of several genes that control traits distinguishing plant families has added to the enthusiasm for this view (Shannon and Meeks-Wagner 1991; Luo *et al.* 1996; Running and Meyerowitz 1996). An implication of this model is that evolution is dependent on rare mutations that create novel morphologies in a single step. While the simplicity of this "one gene-one trait" model has a strong appeal, it has yet to be tested rigorously since higher taxa are not cross-compatible and are thus not amenable to genetic analysis.

An alternative view is that the discrete traits distinguishing higher taxonomic groups are threshold traits whose underlying mode of inheritance is multifactorial although not necessarily highly polygenic. Under this model, selection would favor new multigenic combinations that would switch the trajectory of development to another path and thereby create a discrete shift in morphology. An implication of this model is that evolution is dependent on multiple genes that segregate in populations without a visible effect on the phenotype, since they exist only in combinations below the threshold required to shift the trajectory of development. Concordant with this model, substantial genetic variation for normally invariant traits can be uncovered in populations upon experimental disruption of the genetic background or molecular mechanisms that buffer such traits (Waddington 1942; Dun and Fraser 1958; Huether 1968; Wade *et al.* 1997; Polaczyk *et al.* 1998; Rutherford and Lindquist 1998; Gibson *et al.* 1999). These experiments imply that populations contain substantial cryptic genetic variation, which, if reconfigured, could produce a discrete shift in morphology and thereby a novel phenotype. Thus, evolution would not be dependent on rare mutations, but on standing, albeit cryptic, genetic variation.

Research in our laboratory has focused on the inheritance of the differences in inflorescence and plant architecture between maize and its wild ancestor, teosinte (Doebley 1992). Three of the morphological differences between maize and teosinte are like the differences between higher taxa in that they are discretely different between maize and teosinte and invariant within these taxa. These traits are inflorescence phyllotaxy, which is always distichous (two ranked) in teosinte and always polystichous (many ranked) in maize, the presence of single spikelets in the teosinte ear *vs.* paired spikelets in the maize ear, and inflorescence disarticulation, which is complete in teosinte but absent in maize. Since maize and teosinte are cross-compatible and differ for these variant-between-taxa and invariant-within-taxon

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traits, maize and teosinte offer the opportunity to study the evolution of such traits using a genetic approach.

In this article, we report the detection of genetic variation for phenotypically invariant traits in teosinte. This was accomplished by using a maize-teosinte hybrid genetic background to uncover genetic variation that is not normally expressed at the phenotypic level in a pure teosinte background. We mapped and characterized the quantitative trait loci (QTL) underlying the phenotypic variation that we observed. Since the traits involved are those that are discretely different between maize and teosinte, our results indicate that teosinte contains a pool of cryptic genetic variation upon which selection could have acted during maize domestication. Interestingly, the detected QTL map to many of the same regions of the genome as QTL involved in the evolution of maize from teosinte. We discuss the implications of our results for the evolution of discrete traits in general as well as for the evolution of maize. We also examine genetic variation in teosinte for several quantitative traits that differentiate maize and teosinte.

MATERIALS AND METHODS

Plant materials: An F_1 hybrid of two forms of teosinte (*Zea mays* ssp. *parviglumis* and *Z. mays* ssp. *mexicana*) was testcrossed to maize inbred A158 (*Z. mays* ssp. *mays*). The resultant testcross (TC_1) plants were grown in a winter nursery (1996–1997) on Molokai Island, Hawaii. The *Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *mexicana* parents of the F_1 were grown from seed collected near Teloloapan, Mexico (H. Iltis and T. Cochrane, collection 81) and Chalco, Mexico (H. Iltis, B. Benz and M. Burd, collection 28622), respectively.

To assess the mean phenotypes for these two teosinte subspecies and the maize inbred, we grew and phenotyped 21 plants each of ssp. *mexicana* and ssp. *parviglumis* and 23 maize A158 plants in a winter nursery (1999–2000) on Molokai Island, Hawaii. So that these values should represent means for the two teosinte taxa, we grew a variety of accessions of each subspecies. The accessions and the number of plants analyzed from each accession are the following: for ssp. *mexicana*: CIMMYT-8749 (two), CIMMYT-11400 (four), CIMMYT-11409 (one), Iltis-28620 (three), USDA-PI 566682 (six), and USDA-PI 566685 (five); for ssp. *parviglumis*: CIMMYT-8762 (one), CIMMYT-8780 (two), CIMMYT-8782 (one), CIMMYT-11353 (two), CIMMYT-11407 (three), USDA-PI 331783 (two), USDA-PI 331785 (one), USDA-PI 384064 (five), and USDA-PI 566688 (four).

Experimental design logic: To assay the teosinte genome for genetic variation affecting traits that are phenotypically invariant within teosinte, we made a hybrid between two types of teosinte (*Z. mays* ssp. *mexicana* and ssp. *parviglumis*) and subsequently testcrossed this hybrid to a maize inbred line. The resultant testcross (TC_1) progeny varied considerably for the traits that distinguish maize and teosinte, despite the fact that they all possess a genome that is 50% maize and 50% teosinte. Since the maize portion of each TC_1 plant's genome came from an inbred line, any genetic variation among the plants for the traits must result from QTL differences between the two teosinte parents that contributed equally to the teosinte portion of their genomes. Thus, we are relying upon a differential interaction of teosinte alleles with the maize (A158) genome to detect QTL polymorphisms within teosinte.

TABLE 1

List of primary lateral branch morphologies analyzed

Trait	Description
DISA	The extent to which the ear falls apart at maturity (disarticulation)
LBIL	Average length of primary lateral-branch internodes
LIBN	No. of branches in the primary lateral inflorescence
PEDS	Percentage of female internodes lacking the pedicellate spikelet
PROL	No. of inflorescences on the primary lateral branch (prolificacy)
RANK	No. of internode columns (ranks) on the primary lateral inflorescence
STAM	Percentage of inflorescence internodes that are male (staminate)

Genotyping: Genomic DNA was extracted from 290 TC_1 plants, digested with restriction endonucleases, and transferred onto nylon membranes for Southern hybridizations with DNA probes as described by Doebley and Stec (1991). The Maize Core Marker Set from the 1998 UMC map and a few additional restriction fragment length polymorphism (RFLP) probes were obtained from the University of Missouri-Columbia Maize RFLP Laboratory (http://www.agron.missouri.edu). Genotypes at 98 loci for which an RFLP exists between ssp. *parviglumis* and ssp. *mexicana* were determined for each TC_1 plant. Unscorable bands on autoradiograms resulted in 3.4% missing genotype data.

Phenotyping: Seven traits were measured 2–3 weeks postanthesis on the uppermost primary lateral branch and the inflorescence at its tip (Table 1). These traits were chosen because they define the key morphological differences between maize and teosinte (Doebley and Stec 1993). Because teosinte has a long lateral branch and maize a short one, we measured the lateral branch length and divided this by the number of internodes in the branch to determine the lateral branch internode length (LBIL; Figure 1A). Because teosinte has many secondary inflorescences (ears) along the lateral branch and maize typically has none, we counted the number of ears borne in the axils of the leaves (husks in maize) along the lateral branch and recorded this as the prolificacy score (PROL).

The inflorescence at the tip of the primary lateral branch is typically a branched male inflorescence (tassel) in teosinte and an unbranched female inflorescence (ear) in maize (Figure 1A). To record variation in the sex of this inflorescence, we counted the number of inflorescence internodes with male spikelets and the number with female spikelets and computed the percentage of the total that were male or staminate (STAM). To record variation in inflorescence branching, we counted the number of branches on the primary lateral inflorescence (LIBN).

The ears of maize bear a pair of spikelets on each internode, one sessile and one pedicellate, while the ears of teosinte bear only single sessile spikelets on each internode since the pedicellate spikelet is aborted early in development (Figure 1, B and C). To record variation in this trait, the percentage of female internodes lacking the pedicellate spikelet was computed and termed PEDS. The internodes (and associated spikelets) of the teosinte ear are borne in two ranks on opposite sides of the ear, while the internodes (and associated

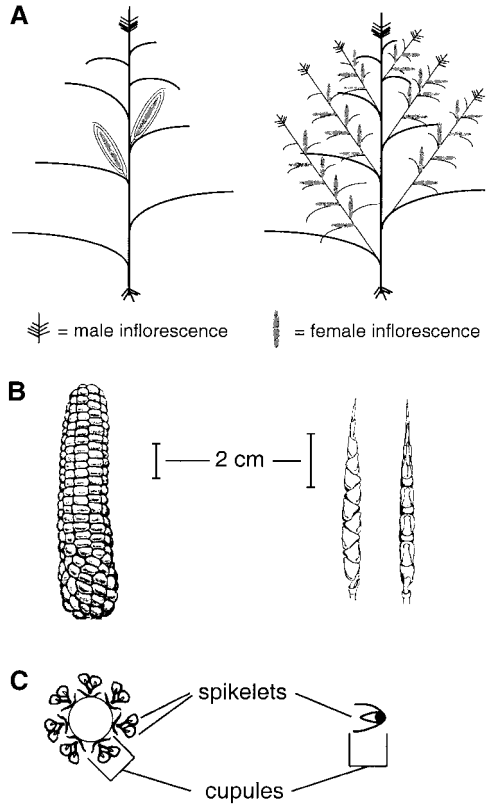

FIGURE 1.—Schematic drawings of maize (left) and teosinte (right). (A) Plants: The maize plant has short lateral branches tipped by female inflorescences (ears) and the teosinte plant has long lateral branches tipped by male inflorescences (tassels). Teosinte has multiple ears borne along each of the lateral branches in the axils of the leaves. (B) Ears: The maize ear has eight or more rows of kernels borne around the circumference of the ear. The teosinte ear has two rows of kernels borne on opposite sides of the ear, each kernel hidden inside a triangular-shaped cupulate-fruitcase. (C) Ear cross sections: In maize, the spikelets are arranged in pairs, one pedicellate and one sessile. Each spikelet produces a single kernel. Each pair of spikelets (kernels) is associated with an internode of the axis of the ear. The internodes have invaginations that are termed cupules. In teosinte, the pedicellate spikelet is aborted early in development so that there is only a single spikelet (kernel) associated with each cupule.

spikelet pairs) of the maize ear are borne in four or more ranks around the entire circumference of the ear (Figure 1, B and C). To record variation in this trait, the number of ranks of internodes was counted for the inflorescence terminating the uppermost primary lateral branch. Since RANK can vary over the length of the inflorescence in teosinte-maize hybrids, it was calculated as a weighted sum. Thus, if half of the inflorescence were two ranked and the other half were four ranked, then RANK would equal three for that plant ($0.5 \times 2 + 0.5 \times 4$). Finally, the teosinte ear breaks apart at maturity due to abscission layers that form at the nodes, so that no two kernels remain attached to one another as they do on the maize cob. To record this variation, the female portion of maize-teosinte hybrid inflorescences was given a qualitative disarticulation (DISA) score of two, one, or zero, according to whether it disarticulated completely, partially, or not at all.

Linkage map: MAPMAKER version 2.0 (LANDER *et al.* 1987) was used to construct a linkage map of the 10 gametic *Z. mays* chromosomes. The minimum LOD score was set at 3.0 for the determination of linkage group composition as well as for three-point analysis of marker order within linkage groups. Chi-square statistics were calculated to test for deviations from the expectation of normal Mendelian segregation at each marker locus. Statistically significant deviation at a locus was deemed to indicate distorted segregation.

QTL mapping: QTL Cartographer v1.14 was used for QTL mapping (BASTEN *et al.* 1994, 2000). The cross type for the Rmap input file was designated as a first generation backcross (B_1) with the ssp. *parviglumis*/maize genotype specified as A and the ssp. *mexicana*/maize genotype specified as H. Use of the B_1 cross type is appropriate since only two genotypic classes can exist for a locus and only the additive effects of QTL were under consideration. We employed composite interval mapping, which uses multivariate regression to incorporate the effects of background QTL into the interval mapping model. Background markers for model six of Zmap were picked by forward and backward elimination in SRmap. In the analyses for all seven morphological traits, up to 10 markers were used to control for background effects, and the blockout window on either side of the test site was set to 10 cM. LOD scores were computed at 2-cM intervals along the chromosomes. The chromosomal location, magnitude, and direction of the additive effect and the proportion of the phenotypic variance explained (PVE) for each detected QTL were obtained from the composite interval mapping output.

To control for experimentwise errors in determining whether or not a LOD peak should be deemed a QTL, composite interval mapping was performed on 1000 permutations of the data for each trait. The 50th highest LOD score observed for the permuted data sets was used to establish the significance threshold ($P = 0.05$) under the null hypothesis of no QTL at each position along the chromosomes (CHURCHILL and DOERGE 1994). QTL were deemed to exist only at positions where a LOD score exceeded the corresponding significance threshold.

RESULTS

Linkage and segregation: All 98 genetic markers could be placed in linkage groups and ordered unambiguously (Figure 2). Coverage of the genome is quite complete with all 20 telomeric bins of the Maize Database Map represented (DAVIS *et al.* 1999). The average intermarker distance is 12.3 cM, with only three gaps of >30 cM. In general, the order of the markers was consistent with the Maize Database Map; however, probes for NPI409, UMC49, and UMC161 all mapped to positions that differ from their placement on the Maize Database Map (DAVIS *et al.* 1999). In addition to its normal position, UMC66 also mapped to two previously undescribed positions.

Of the 98 marker loci, 29 have segregation ratios that deviate significantly from the 1:1 Mendelian expectation for a testcross (Table 2). The distorted segregation detected on chromosomes 2, 7, 9, and 10 is weak; one marker in each of these cases narrowly meets the $P < 0.05$ significance criterion. However, the segregation distortion seen on chromosomes 3, 4, 5, and 6 is much stronger. On both chromosomes 3 and 4, 9 of the 12 markers show distorted ratios with an excess of the ssp.

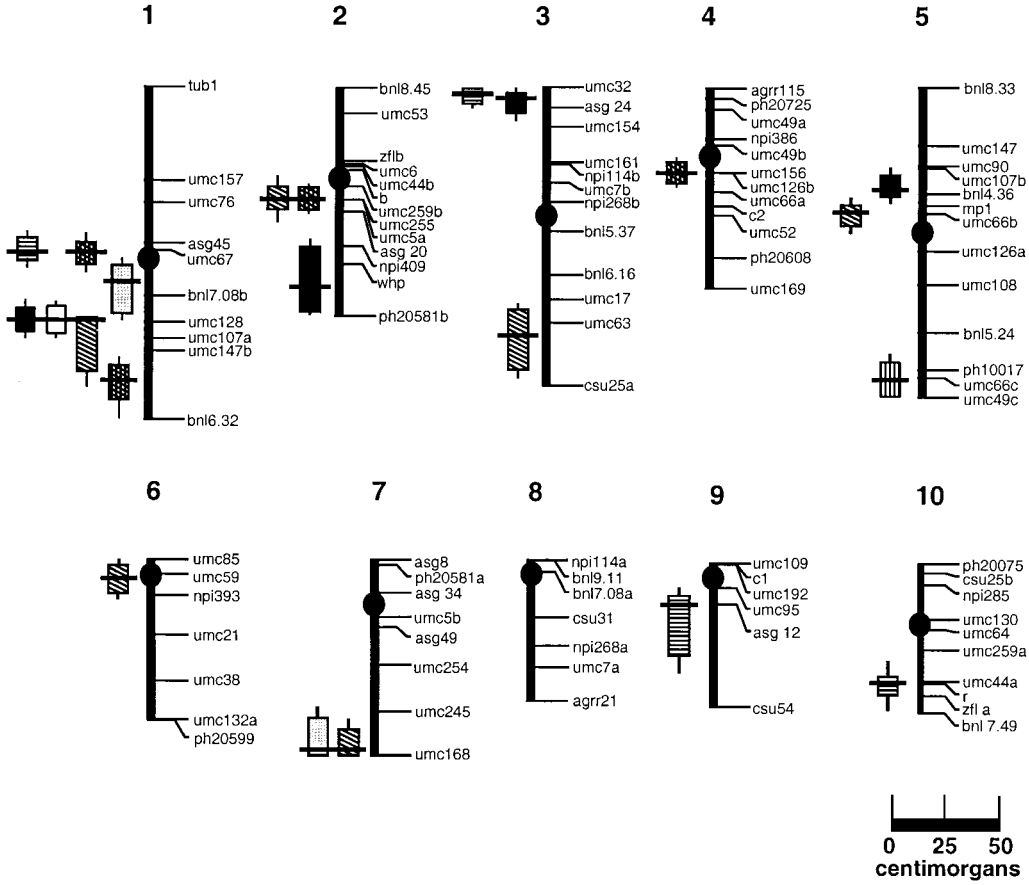

FIGURE 2.—Genetic linkage map of the 10 gametic maize-teosinte chromosomes showing QTL positions for primary lateral branch and primary lateral inflorescence traits. Each QTL (LOD score peak) is shown as a horizontal line surrounded by a box and whiskers. The box surrounding the LOD peak delineates the one-LOD support interval and the whiskers of each box delineate the two-LOD support interval. The positions of RFLP markers are indicated by horizontal hatch marks along the chromosomes. Each chromosome is depicted with its cytologically defined short arm on top. Centromeres are represented by bulges on the chromosomes. (■) DISA, (▧) LBIL, (□) LIBN, (▤) PEDS, (▩) PROL, (▥) RANK, and (▨) STAM.

parviglumis alleles. Chromosomes 5 and 6 both show strong deviations from 1:1, but over less extensive portions of those chromosomes. An excess of the ssp. *mexicana* alleles is seen at distorted loci on chromosome 5 and an excess of the ssp. *parviglumis* alleles on chromosome 6. Of the 29 distorted loci, 25 show excesses of the ssp. *parviglumis* allele, which may be indicative of the close genetic relationship that this subspecies shares with maize and thus with inbred A158, as compared to a somewhat more distant relationship between maize and ssp. *mexicana* (DOEBLEY *et al.* 1984; WANG *et al.* 1999). The most severe distortion in this population had the less abundant marker-genotype class represented by 77 of the 290 individuals, a sufficient proportion to obtain reasonable estimates of QTL effects.

Morphological traits: We first measured trait means for ssp. *mexicana* and ssp. *parviglumis*. There is no variation within or between these subspecies for RANK, DISA, and PEDS (Table 3). These traits are discrete, with teosinte inflorescences always being two ranked and fully disarticulating into single fruitcase segments that always lack the pedicellate spikelet. The remaining four traits are quantitative, rather than discrete, but still the two subspecies show equivalent mean phenotypic values and there is only a modest amount of variation within either subspecies as compared to the difference between the mean values of maize and teosinte (Table 3). Both teosinte subspecies typically have elongated primary lateral branches with ears in the axils of the leaves along these branches and a branched, nearly pure male inflorescence at the tips of the branches. *t*-tests ($P = 0.05$) indicated that there are no significant differences between the subspecies for these four traits.

For comparison, we also phenotyped the inbred line used for making the testcross. Like most maize lines, A158 has no quantitative variation for four of the seven traits (Table 3). Unlike teosinte, the uppermost primary lateral branch is tipped by a nonbranching (LIBN = 0) exclusively female inflorescence or ear (STAM = 0%) and there are no secondary ears along the primary branch (PROL = 0). The A158 ear always has paired spikelets, one sessile and one pedicellate (PEDS = 0%). Although A158 maize always has compressed internodes in the primary lateral branch (shank) and polystichous inflorescences, there is minimal quantitative variation for LBIL and RANK (Table 3). Since A158 is an inbred, this variation is likely to be largely, if not exclusively, environmental in origin.

Despite the fact that ssp. *mexicana* and ssp. *parviglumis* are morphologically either identical or equivalent for these seven plant and inflorescence traits, the cross between their F_1 hybrid and the maize inbred line produced offspring that varied widely for all of them (Figure 3). The TC_1 trait value averages for DISA, LIBN, PEDS,

TABLE 2

Loci showing segregation distortion

Chromosome	Locus	Genotype	
		parviglumis	*mexicana*
2	*b**	128	162
3	UMC154*	166	124
3	UMC161**	171	118
3	NPI114b**	168	120
3	UMC7b**	163	119
3	NPI268b*	127	91
3	BNL5.37**	172	117
3	BNL6.16**	167	123
3	UMC17*	162	127
3	UMC63**	169	121
4	NPI386*	163	127
4	UMC49b*	146	108
4	UMC156**	167	122
4	UMC126b**	167	122
4	UMC66a**	172	117
4	*c2***	173	115
4	UMC52**	170	116
4	PH20608**	171	113
4	UMC169*	162	127
5	UMC66c*	92	129
5	UMC49c**	113	166
6	UMC85**	185	104
6	UMC59**	191	98
6	NPI393**	213	77
6	UMC21**	186	93
6	UMC38**	168	122
7	UMC168*	162	127
9	UMC95*	163	127
10	UMC130*	128	162

The chromosome and name of the genetic locus at which the segregation distortion was observed are listed along with the numbers of testcross individuals carrying the ssp. *mexicana* and ssp. *parviglumis* alleles, respectively. $*P < 0.05$; $**P < 0.01$.

and STAM are maize like, being <1 of their respective standard deviations from the mean for A158 (Table 3, Figure 3). The TC_1 trait value averages for LBIL, PROL, and RANK, however, are more intermediate between the teosinte and maize phenotypic values (Table 3, Figure 3). For six of the seven traits, the mean trait values of the TC_1 population deviate from the maize-teosinte midpoint value in the direction of maize, while for PROL, the mean of the TC_1 population is equivalent to the midpoint value. These data suggest that the domestication of maize from a teosinte ancestor largely involved selection for dominant or semidominant alleles.

QTL numbers: A total of 22 QTL affecting the seven traits were detected in the testcross population (Figure 2 and Table 4). For PEDS, RANK, and DISA, the three traits that are invariant among all teosinte populations, we detected 4, 1, and 4 QTL, respectively. The remaining 13 QTL demarcate allelic variation within teosinte at loci affecting quantitative traits for which ssp. *mexicana* and ssp. *parviglumis* have equivalent phenotypes. Among these four traits, we detected 6 QTL affecting LBIL, while 1, 2, and 4 QTL were detected for LIBN, PROL, and STAM, respectively. In addition to the 22 QTL reported, there were 11 LOD peaks that narrowly failed to meet the experimentwise significance thresholds, suggesting that we may be underestimating the number of QTL differences that existed between the two teosinte parents (data not shown).

There are four sites on the linkage map where QTL for multiple traits have LOD peaks that map exactly on top of one another, suggesting either pleiotropy or linkage of multiple QTL (Figure 2 and Table 5). If all four of these locations harbored a single QTL with pleiotropic effects on several traits, the total number of detected loci affecting these traits would be reduced from 22 to 17.

QTL effects: The magnitudes of the additive effects of the QTL ranged from medium to small, relative to the trait differences between maize and teosinte (Tables 3 and 4). No single QTL accounts for >14% of the phenotypic variance in the testcross population or for >28% of the mean difference between maize and teosinte. Aside from DISA, which has QTL accounting for 26 and 28% of the mean difference between maize and teosinte, most of the QTL have smaller additive effects. Of the 22 QTL effects, 12 account for between 10 and 19% of the mean difference between maize and teosinte, and the remaining 8 QTL effects each account for 6–9% of a trait's mean difference between maize and teosinte. There is a clear correspondence between the number of QTL detected for a trait and the total amount of phenotypic variance explained, indicating that the QTL detected by this study have relatively equal and similarly small effects (Table 6).

QTL locations: We considered whether or not the QTL detected in this experiment are the same as those controlling differences between maize and teosinte. Doebley and Stec (1993) dissected the inheritance of all seven traits in two different maize-teosinte F_2 populations, derived from maize race Chapalote by ssp. *mexicana* (C × M) and maize race Reventador by ssp. *parviglumis* (R × P) crosses. The LOD peaks for 11 of the 22 QTL reported in this study map near a LOD peak of a previously detected QTL affecting the same trait (Table 4). These coincident LOD peaks represent 11 putatively orthologous QTL. Of these 11, 4 were detected in both the R × P and C × M populations. The remaining 7 cases were detected only in one or the other.

To test whether or not the tendency of our QTL to map near QTL detected by Doebley and Stec (1993) exceeds random expectations, we employed permutation tests. For each trait, we created 10,000 permuted data sets in which our QTL were randomly assigned to positions in the genome. The probability that the number of coincident QTL (QTL whose 1-LOD support intervals extend into the chromosomal bin) was greater

TABLE 3

Average parental and testcross progeny phenotypes

Trait	A158 maize (mean ± SE)	*mexicana*[a,b] (mean ± SE)	*parviglumis*[b,c] (mean ± SE)	TC_1 progeny (mean ± SE)
DISA	0	2	2	0.76 ± 0.04
LBIL	0.43 ± 0.02	13.38 ± 0.77	15.16 ± 0.68	4.17 ± 0.15
LIBN	0	3.33 ± 0.42	5.09 ± 0.94	0.27 ± 0.05
PEDS	0%	100%	100%	21% ± 1.4%
PROL	0	11.04 ± 0.57	11.24 ± 0.50	5.84 ± 0.11
RANK	6.56 ± 0.14	2	2	3.52 ± 0.05
STAM	0%	99% ± 0.5%	97% ± 1.4%	23% ± 0.2%

The average phenotypes are listed in trait units (see text). A158 maize was the isogenic maize line to which the ssp. *mexicana* by ssp. *parviglumis* hybrid plant was crossed to create the testcross population.

[a] Twenty-one plants representing seven ssp. *mexicana* accessions were analyzed.

[b] Subspecies by phenotype *t*-tests ($P = 0.05$) revealed that ssp. *mexicana* and ssp. *parviglumis* are phenotypically equivalent for each of the four traits for which they are variable.

[c] Twenty-one plants representing seven ssp. *parviglumis* accessions were analyzed.

than expected by chance was calculated as the proportion of permuted data sets in which there were x or more coincident QTL, where x is the number of coincident QTL actually observed. Only LBIL showed a greater number of coincident QTL than expected by chance ($P = 0.04$). Thus, there is limited statistical evidence that some of our QTL are the same as those previously identified by Doebley and Stec (1993).

Directions of QTL effects: For four of the seven traits, both the ssp. *mexicana* and ssp. *parviglumis* parents contributed QTL alleles that make the TC_1 plants more maize like (Table 4). For three traits (LIBN, PROL, and RANK), it was only the ssp. *mexicana* QTL alleles that made the plants more maize like. In total, the ssp. *mexicana* allele at 15 of the 22 QTL makes the phenotype of a plant more maize like. A chi-square test revealed that this is not significantly different from an even split ($\chi^2 = 2.98$; $P = 0.08$).

Given that ssp. *mexicana* and ssp. *parviglumis* both contribute alleles that lead toward more maize-like phenotypes, it is noteworthy that at each of the four locations where QTL for multiple traits map, the allele for the more maize-like phenotype for each trait comes from the same parent (Table 5). This is the result that one would expect if each of these locations harbored a single QTL with pleiotropic effects on several traits. If we were to assume pleiotropy in all four of these cases, there would be a total of 17 QTL and the ssp. *mexicana* allele would contribute to the more maize-like phenotype for 11 of these. This would make the disparity in direction of allelic effects between the two parents even less substantial ($\chi^2 = 1.49$; $P = 0.22$).

DISCUSSION

Linkage and segregation: Our testcross population reveals the degree of recombination in a teosinte (ssp. *parviglumis*) by teosinte (ssp. *mexicana*) cross. Genetic distances between adjacent molecular markers in our testcross population are roughly comparable to those in maize by maize populations (data not shown) except on the short arm of chromosome 9, where teosinte is known to be polymorphic for a chromosomal inversion (Ting 1958). Recombination on the short arm of chromosome 9 in the teosinte parent of the testcross population was likely limited by the presence of this inversion. The resultant collapse of ~70 cM of this chromosome arm into <1 cM (Figure 2) has no consequence for this study, since the limited recombination affects QTL localizing ability, but not detection power, and the short arm of chromosome 9 does not contain QTL for any of the traits.

Segregation distortion for molecular markers in populations derived from wide crosses involving crop plants and their wild relatives is a common phenomenon (Doebley and Stec 1991; Harushima *et al.* 1996; Boyko *et al.* 1999). Our data show a comparable degree of distorted segregation with our teosinte by teosinte cross (Table 2). The departure from the 1:1 expectation at 9 of the 12 markers on chromosome 4 can reasonably be attributed to the presence of multiple incompatibility loci in this linkage group (Kermicle and Allen 1990); however, the distortion seen in the other linkage groups lacks a clear explanation.

Cryptic genetic variation in teosinte: The primary purpose of employing QTL analysis was to determine if and to what extent the broad phenotypic variability among TC_1 plants was due to genetic differences between the teosinte parents of the testcross population. Twenty-two QTL were detected, indicating that the phenotypic variability for each of these traits in the TC_1 population is at least in part attributable to natural genetic polymorphisms within teosinte. By extension, this demonstrates that a lack of phenotypic variation within or between teosinte populations does not necessitate a corresponding lack of underlying genetic variation.

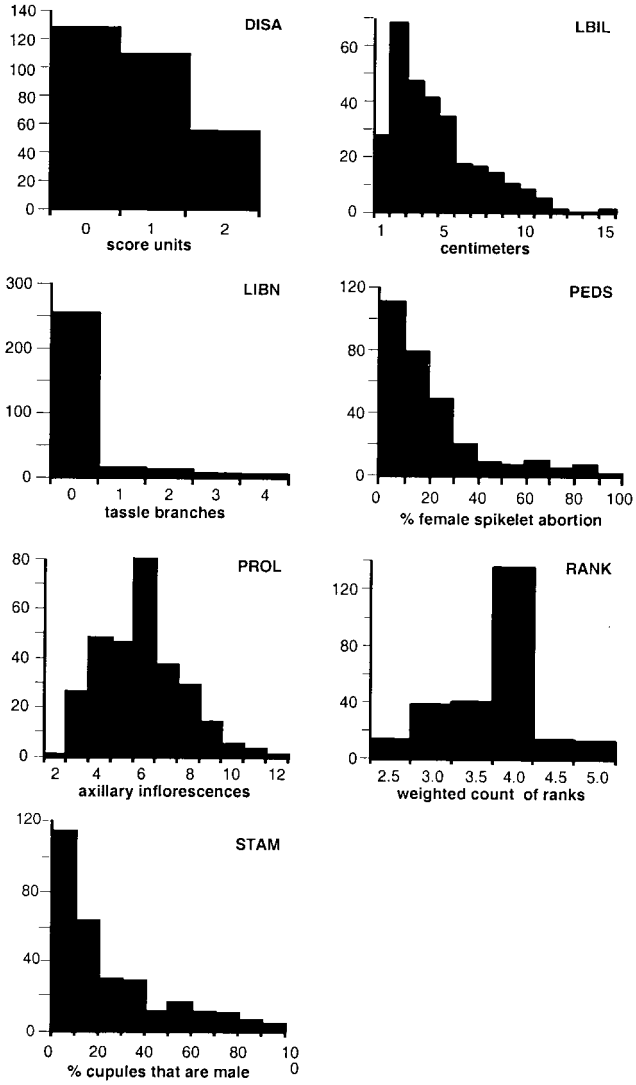

FIGURE 3.—Histograms showing frequency distributions of trait values in the TC_1 population. Column heights represent the number of TC_1 plants in each frequency class. Trait values defining the frequency classes are given on the *x*-axes. Table 3 contains phenotypic data for A158 maize, ssp. *mexicana* and ssp. *parviglumis*.

It is unlikely that spurious trait by locus associations in the data have appreciably exaggerated the actual number of QTL for cryptic variation, since the LOD thresholds were adjusted to give an experimentwise type I error rate of $P = 0.05$. In fact, the per trait number of QTL reported is likely an underestimate because there were 11 strong LOD peaks that fell just below the threshold for statistical significance and were not considered *bona fide* QTL.

While a portion of the variation in the testcross population is explained by the observed QTL, there remains much variation for which the source is unknown. For the seven traits, the total R^2 values for the QTL effects range from 6.9 to 26.6%, so the source of most variation is unknown (Table 6). Typically, a QTL mapper would interpret the unexplained variance to be due to environmental effects and/or multiple small-effect QTL that are below the level of detection because of limited statistical power. In the current context, there is another potential explanation. The hybrid background of the testcross may have caused a decanalization of the traits, adding considerably to the variation in the population.

Distribution of cryptic genetic variation in teosinte: Our results demonstrate that the ssp. *mexicana* and ssp. *parviglumis* parents of our testcross population possessed allelic differences at multiple QTL that represent cryptic genetic variation. Two lines of evidence suggest that this type of cryptic genetic variation not only occurs between these subspecies but also exists within them. First, 12 F_1 families derived from crosses between maize inbred W22 and teosinte plants from different natural populations of both ssp. *mexicana* and ssp. *parviglumis* were scored for LBIL, LIBN, PEDS, RANK, and STAM (LAUTER and DOEBLEY 2002). There was considerable variation among F_1 families within the subspecies, suggesting that the type of cryptic variation observed in our ssp. *mexicana* by ssp. *parviglumis* testcross population also exists among populations within these subspecies. Second, both isoenzymatic and sequence data have established that ssp. *mexicana* and ssp. *parviglumis* populations are better distinguished by allele frequency differences than by the presence or absence of individual alleles (DOEBLEY *et al.* 1984; WANG *et al.* 1999). We have no reason to believe that the distributions of alleles at our QTL would deviate from this trend, especially since polymorphisms at these loci are likely to be phenotypically neutral.

Cryptic variation and the evolution of discrete traits: The evolution of discrete traits that distinguish higher taxa could be driven either by allelic differences in single major genes or by allelic differences at multiple genes, each making a partial contribution to the phenotype. Laboratory and horticultural mutants suggest that single-gene changes are sufficient to create novel discrete phenotypes (HILU 1983). There are known examples of major or qualitative mutations for discrete traits arising in natural populations (FORD and GOTTLIEB 1992; CUBAS *et al.* 1999). On the other hand, results from several QTL dissections of discrete trait differences between species indicate that the evolution of such traits can involve multiple QTL with a range of large to small effects (VLOT *et al.* 1992; DOEBLEY and STEC 1993; BRADSHAW *et al.* 1995; GAILING *et al.* 1999). In some of these QTL studies, the inheritance of discrete or qualitative traits is indistinguishable from that of quantitative traits.

How can cryptic genetic variation such as we have detected in teosinte contribute to the evolution of discrete traits? In cases where qualitative mutations largely control the inheritance of the trait, the qualitative locus difference could arise first and subsequently be stabilized by modifier loci that were preexistent in the population but had no effect on the phenotype except in the presence of the qualitative gene. Since the use of

TABLE 4

QTL detected by composite interval mapping

Trait	Chromosome	Marker	Additive effect	Allele	PVE	Coincident QTL[a]
DISA	1L	UMC128	−0.33 score units	*m*	4.4	C × M only
	2L	*whp*	−0.37 score units	*m*	6.0	
	3S	UMC32	−0.56 score units	*p*	13.5	
	5S	BNL4.36	−0.52 score units	*m*	11.8	C × M only
LBIL	1L	UMC128	−1.48 cm	*m*	7.7	C × M and R × P
	2L	UMC259b	−1.39 cm	*p*	6.9	
	3L	UMC17	−1.06 cm	*m*	3.9	C × M only
	5S	UMC66b	−1.44 cm	*m*	7.5	C × M and R × P
	6S	UMC59	−1.34 cm	*p*	5.7	C × M only
	7L	UMC168	−1.17 cm	*m*	4.9	
LIBN	1L	UMC128	−0.46 branches	*m*	6.9	R × P only
PEDS	1S	UMC67	−12.55% points	*m*	6.9	C × M and R × P
	3S	UMC32	−11.20% points	*m*	5.8	
	9L	ASG12	−8.10% points	*p*	3.0	
	10L	UMC44a	−12.87% points	*p*	7.4	R × P only
PROL	1L	BNL7.08	−1.09 inflorescences	*m*	8.8	
	7L	UMC168	−0.79 inflorescences	*m*	4.6	
RANK	5L	UMC66c	0.41 ranks	*m*	7.2	
STAM	1S	UMC67	−13.45% points	*m*	7.1	
	1L	UMC147b	−16.80% points	*m*	11.9	C × M and R × P
	2L	UMC259b	−16.06% points	*p*	6.6	R × P only
	4L	UMC156	−11.35% points	*p*	5.3	

For each trait, the chromosome (with S and L indicating short and long arms) and the nearest marker to the LOD peak are listed for all statistically significant QTL. The additive effect of each QTL is shown as a trait unit contribution toward the more maize-like phenotype. The allele contributing to the more maize-like phenotype is indicated by *m* for ssp. *mexicana* and *p* for ssp. *parviglumis*. The percentage of the PVE by each QTL is given.

[a] Doebley and Stec (1993) dissected the inheritance of all seven of these traits in two distinct maize-teosinte F_2 populations, C × M and R × P (see text). This column lists the previous studies that contained a QTL peak for a trait near a QTL peak for the same trait detected in the current study.

existing variation seems more plausible than the occurrence of multiple timely new mutations, cryptic genetic variation in populations provides a plausible source of genetic variation for stabilizing or enhancing the effects of qualitative mutations. Results of the present study suggest that the trait RANK may have evolved during maize domestication by this means since it is controlled by a major QTL plus several QTL of smaller effect (Doebley and Stec 1993) and since we have uncovered cryptic genetic variation for this trait in teosinte (Table 4).

TABLE 5

Evidence for pleiotropic QTL

Chromosome	Traits involved	Alleles[a]
1S	PEDS, STAM	*m*, *m*
1L	DISA, LBIL, LIBN	*m*, *m*, *m*
2L	LBIL, STAM	*p*, *p*
7L	LBIL, PROL	*m*, *m*

For each case where LOD peaks of QTL for multiple traits map within 1 cM of one another, the chromosomal location (with S and L for short and long arms) and traits involved are listed.

[a] The alleles contributing to a more maize-like phenotype are listed for each trait involved. In all five cases of putative pleiotropy, the additive effects of the QTL alleles involved are in the same direction, *m* for ssp. *mexicana* and *p* for ssp. *parviglumis* (see Table 4).

Cryptic variation may also contribute to the evolution of discrete traits even when a qualitative locus is not involved. At first glance, cryptic variation would seem inaccessible to the force of selection since it has no effect on the phenotype. However, if discrete traits are threshold traits, then one could imagine that allelic variation at the multiple QTL could be reconfigured such that an individual or population would rise above the threshold and thereby switch the trajectory of development so that a discrete adult phenotype is produced. We find this an attractive model since evolution would not be constrained to "wait" for new major mutations to arise in populations. This model would also be consistent with QTL mapping studies that have shown that discrete traits can have multifactorial inheritance with strong epistatic interactions (Doebley *et al.* 1995; Gailing *et al.* 1999).

Two of the traits we studied (DISA and PEDS) may have evolved during maize domestication as threshold traits by selection for novel combinations of cryptic QTL.

TABLE 6

Percentage of phenotypic variance explained by QTL

Trait	No. of QTL	Average PVE	Highest PVE	Total R^2
DISA	4	8.9	13.5	26.4
LBIL	6	6.1	7.7	26.6
LIBN	1	6.9	6.9	6.9
PEDS	4	5.8	7.4	24.1
PROL	2	6.7	8.8	13.6
RANK	1	7.2	7.2	7.2
STAM	4	7.7	11.9	23.2

The average and highest PVE values for the QTL are summarized for each trait. Since the collective effect of all of the QTL alleles together cannot be calculated simply by adding the PVE values of the individual QTL, we used multiple regression to obtain R^2 estimates of the total phenotypic variance that was explainable for each trait using a model that included the genotypes at the markers closest to each of the QTL.

DISA and PEDS are both discretely different between maize and teosinte and invariant within these taxa. Neither trait is controlled by a major locus; rather, DISA and PEDS are controlled by 9 and 10 QTL, respectively (Doebley and Stec 1993). In this article, we report 4 QTL for cryptic variation in teosinte for DISA and 4 for PEDS (Table 4). When unmasked in the hybrid genetic background, the combined effects of these QTL are substantial. The 4 QTL for DISA have a combined effect of 80% of the maize-teosinte difference when the mean phenotype of plants possessing all four low alleles is subtracted from the mean phenotype of plants possessing all four high alleles. The 4 PEDS QTL have a combined effect of 43% of the maize-teosinte difference when computed the same way. These results suggest that teosinte populations have sufficient cryptic variation, which if reconfigured, could bring the genotype above a threshold and allow a novel discrete phenotype to arise.

A final argument in support of a role for cryptic genetic variation in the evolution of discrete traits comes from the effect that stabilizing selection will have on genetic variation for discrete traits. Stabilizing selection will efficiently remove variation from the population; however, cryptic variation will escape the force of selection and can thus remain within the population until reconfigured as described above (Gibson *et al.* 1999).

Implications for maize evolution: The results of our search for cryptic genetic variation in teosinte have some implications for the evolution of maize under domestication since the traits that we analyzed are those that distinguish maize from its progenitor, teosinte. First, some traits (DISA and PEDS) may have evolved as threshold traits rather than as simply inherited mono- or digenic traits (see Galinat 1978). Second, the existence of cryptic variation for many traits implies that maize domestication may have been based significantly upon reconfiguring existing polymorphisms into novel combinations rather than upon selecting for new mutations. If this is the case, then domestication may have proceeded quite rapidly. Our results also raise the possibility that the genetic variation that enabled early farmers to create maize may be extant within modern teosinte populations.

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