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Quantitative trait loci controlling phenotypes related to the perennial versus annual habit in wild relatives of maize

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Abstract We used quantitative trait locus/loci (QTL) mapping to study the inheritance of traits associated with perennialism in a cross between an annual (*Zea mays* ssp. *parviglumis*) and a perennial (*Z. diploperennis*) species of teosinte. The most striking difference between these species is that *Z. diploperennis* forms rhizomes, whereas *Z. mays* ssp. *parviglumis* lacks these over-wintering underground stems. An F₂ population of 425 individuals was genotyped at 95 restriction fragment length polymorphism marker loci and the association between phenotype and genotype was analyzed by composite interval mapping. We detected a total of 38 QTL for eight traits. The number of QTL found for each trait ranged from two for rhizome formation to nine for tillering. QTL for six of the traits mapped near each other on chromosome 2, and QTL for four traits mapped near each other on chromosome 6, suggesting that these regions play an important role in the evolution of the perennial habit in teosinte. Most of the 38 QTL had small effects, and no single QTL showed a strikingly large effect. The map positions that we determined for rhizome formation and other traits in teosinte may help to locate corresponding QTL in pasture and turf grasses used as forage for cattle and for erosion control in agro-ecosystems.

Introduction

Perennial and annual plants can show striking differences in life history traits, reflecting different adaptive strategies. Annual plants multiply and disperse entirely through seeds, while many perennials multiply and spread both by seeds and by vegetative organs such as rhizomes. Rhizomes enable perennial plants to over-winter, allowing rapid growth at the beginning of the next growing season. By this means, rhizomes enable young perennial plants to compete effectively in an already established plant community (Horn and Rubenstein 1984). Vegetative propagation can be, however, a disadvantage in fluctuating and seasonally disturbed environments such as riverbanks and seashores.

Quantitative trait locus/loci (QTL) mapping is a powerful method for determining the genetic control of morphological differences between lines and species (Lander and Botstein 1989; Zeng 1994; Kao et al. 1999). This method enables the determination of the minimum number of QTL controlling a trait, the relative magnitudes of the effects of each QTL, their chromosomal location, and their mode of gene action. This method can be used to study the inheritance of agronomic traits in crops (e.g., Edwards et al. 1987; Paterson et al. 1988) and the evolution of adaptive traits in natural species (e.g., van Houten et al. 1994; Lin and Ritland 1997; Bradshaw et al. 1998; Kim and Rieseberg 1999; Westerbergh and Doebley 2002).

Here, we report the mapping of QTL affecting the differences in perennial versus annual habit between two wild species of teosinte that are close relatives of maize. The perennial species is *Zea diploperennis*, and the annual species is *Z. mays* ssp. *parviglumis*. These two species are easily distinguished by a number of morphological features, the most striking difference being that *Z. diploperennis* forms rhizomes (Fig. 1a), while *Z. mays* ssp. *parviglumis* lacks these over-wintering underground stems (Fig. 1b). *Z. diploperennis* is native to a small region in the state of Jalisco in southwestern Mexico. *Z. mays* ssp. *parviglumis* grows in diverse habitats (stream sides, hill

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sides, fallow fields) across much of southern and western Mexico.

Materials and methods

Plant materials

Z. diploperennis from a population in Jalisco, Mexico (Guzman no. 1120) was used as the maternal parent and crossed with a plant of the annual species *Z. mays* ssp. *parviglumis* from a population in Guerrero, Mexico (Iltis

and Cochrane No. 80). A single F_1 plant was grown and self-pollinated in isolation in a greenhouse. We germinated the F_2 seeds in small pots and after three weeks, the seedlings were transplanted to a field at the University of Hawaii Experiment Station in Waimanalo, Oahu. Because teosinte flowers only under a short-day photoperiod, Hawaii was chosen as the experimental site. 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 and a group of 20 cuttings of the F_1 hybrid. We carried out the morphological and molecular work on 425 F_2 plants. The large F_2 population was intended to reduce bias in estimating the number of QTL and the relative magnitude of their effects (Beavis 1994, 1998). We also studied the morphological traits on 20–30 plants from each parental population and the six surviving cloned F_1 plants.

Morphological analysis

Eight traits related to the difference in perennial versus annual habit between *Z. diploperennis* and *Z. mays* ssp. *parviglumis* were analyzed. We measured the number of tillers (NO-TL) and the number of side branches (NO-SB) on the main stem of 2-month-old plants. *Z. diploperennis* tends to have higher values for both of these traits. Four months after planting, the plants were checked for withered stems (WT-ST) on a scale from 1 (all stems green at the base and not withered) to 6 (all stems brown and withered). *Z. diploperennis* stems die back, while *Z. mays* ssp. *parviglumis* remain green under our conditions. In addition, at 4 months after planting, the plants were dug up, rinsed, dried, and then scored for the presence of rhizomes (RHIZ) from 0 (no rhizomes) to 4 (multiple well-developed rhizomes). We also scored the presence of elongated underground stems other than rhizomes (EL-ST) from 0 (no stems) to 4 (four or more stems). *Z. mays* ssp. *parviglumis* forms this type of stem at positions where *Z. diploperennis* forms rhizomes. We scored the presence of slender tillers (SL-TL), a tiller type seen only in *Z. diploperennis* from 0 (no slender tillers) to 4 (four or more slender tillers), and the looseness (*Z. diploperennis*-like) versus compactness (*Z. mays* ssp. *parviglumis*-like) of the tillers (LO-TL) from 0 (loose) to 4 (compact). Finally, we scored the presence of thin roots (TH-RO) as seen in the *Z. diploperennis* parent from 0 (no thin roots) to 4 (only thin roots). Unlike *Z. diploperennis*, *Z. mays* ssp. *parviglumis* forms relatively thick roots.

Genetic analysis

Total genomic DNA was isolated from leaves from each of the 425 F_2 plants. All plants were genotyped at 95 restriction fragment length polymorphism (RFLP) marker loci. The procedures for DNA extractions, restrictions, Southern transformations, and hybridizations followed Doebley and Stec (1991, 1993). We used maize plasmid



Fig. 1 a Roots of *Zea diploperennis* showing rhizomes (arrows). b Roots of *Z. mays* ssp. *parviglumis* showing underground stems without rhizomes (arrow)

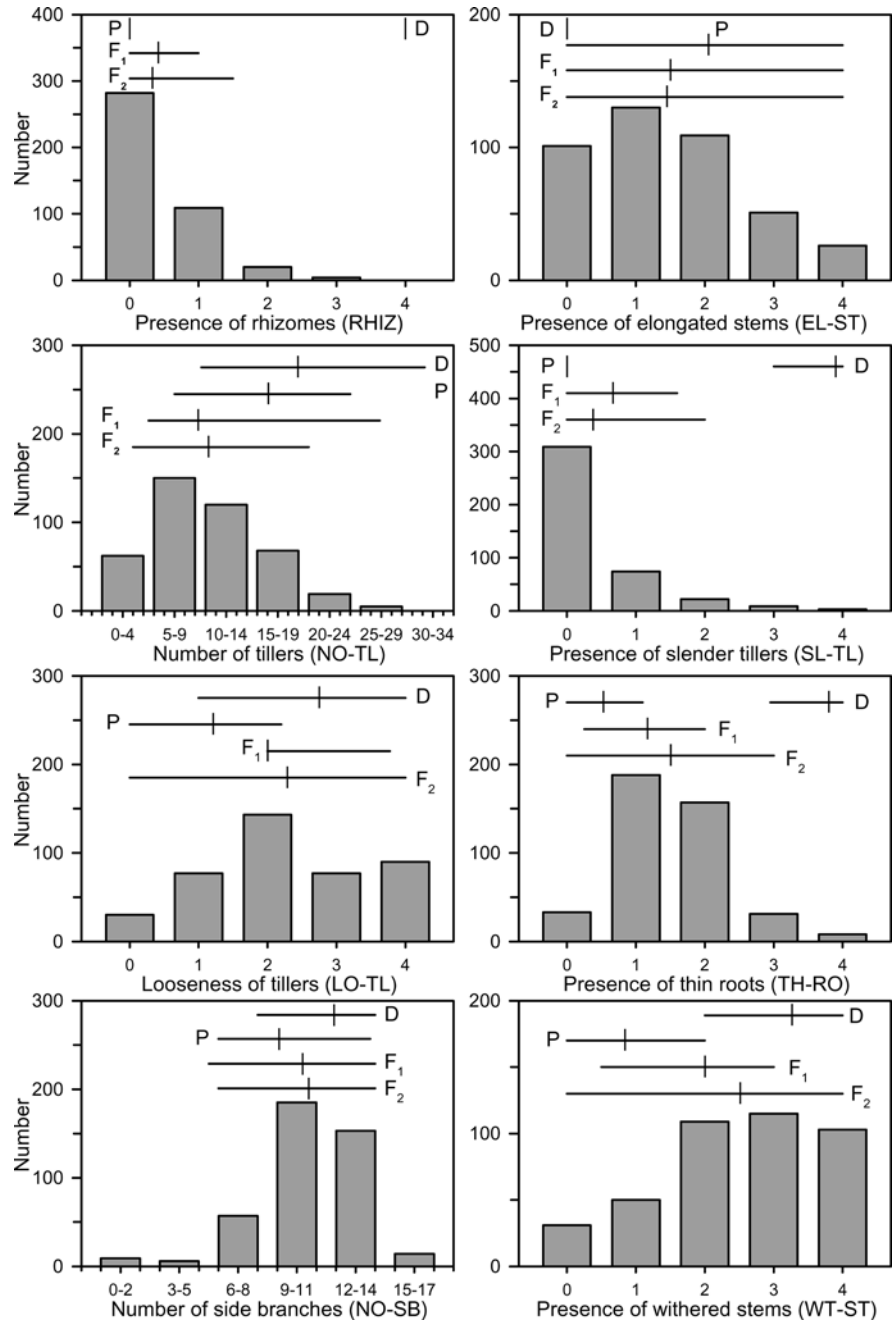
clones of low-copy-number nuclear sequences from the Brookhaven National Laboratory (Burr et al. 1988), Pioneer HI-Bred International (Beavis and Grant 1991), Native Plants (Helentjaris et al. 1988), and the University of Missouri–Columbia (Coe et al. 1990).

Statistical analysis

The construction of the linkage map for the F₂ population was presented in Westerbergh and Doebley (2002). Composite interval mapping of QTL was done using the computer program QTL Cartographer for Windows, version 1.21 (Basten et al. 1997). We used model 6 of

Basten et al. (1997), with the number of genetic background parameters set to five markers and the window size set to 10 cM. The strength of the data supporting a QTL is indicated by its LOD score, which is the log of the odds ratio. Empirical experiment-wise threshold values for significance ($P=0.05$) were estimated from 1,000 permutations of the data for each trait (Churchill and Doerge 1994; Doerge and Churchill 1996) as implemented by the program QTL Cartographer. The relative magnitude of the effect of each significant QTL was estimated as the proportion of phenotypic variance in the F₂ (R^2) explained by the QTL and as the number of phenotypic standard deviations (Orr 2001). In addition, we estimated the dominance and additive effects

Fig. 2 Distribution of trait values for the eight traits in the F₂ population and the mean values (vertical lines) and the interval between the 5.0 and 95.0 percentiles (horizontal lines) for *Z. diploperennis* (D), *Z. mays* ssp. *parviglumis* (P), clones of the F₁ hybrid, and the F₂ plants



of each QTL using QTL Cartographer. To test for epistatic interactions among QTL, we used two-way ANOVA.

Results

Morphological traits

When comparing the means for the parental populations of *Z. diploperennis* and *Z. mays* ssp. *parviglumis*, we found striking differences for five of the eight traits (RHIZ, EL-ST, SL-TL, TH-RO, and WT-ST). As expected, the *Z. diploperennis* plants all showed well-developed RHIZ but no other non-rhizomatous EL-ST (Fig. 2; Table 1). *Z. mays* ssp. *parviglumis* plants lacked rhizomes, but three

fourths of these plants showed EL-ST. Almost all *Z. diploperennis* plants possessed four or more SL-TL, while none of the *Z. mays* ssp. *parviglumis* plants produced this type of tiller. All *Z. diploperennis* plants had TH-RO, while most of the *Z. mays* ssp. *parviglumis* plants lacked thin roots. Four months after planting, all *Z. diploperennis* plants were brown and partly or completely withered down, while none of the *Z. mays* ssp. *parviglumis* plants had WT-ST, and about 50% of them still had green stems. The trait means for NO-TL, LO-TL, and NO-SB showed only moderate differences between the parental populations (Fig. 2; Table 1).

To describe the distribution and variation of phenotypes in the parental populations, the F₁ hybrid, and the F₂ population, we calculated histograms and the 5.0 and the

Table 1 Phenotypic means and standard deviations (*SD*) for perennial traits in *Zea diploperennis* (*diplo*), *Z. mays* ssp. *parviglumis* (*parvi*), and F₂ plants homozygous for the *diploperennis* (D) or the *parviglumis* (P) allele at each quantitative trait locus (*QTL*). The chromosome (*Chr*) and nearest of flanking molecular marker loci (*MML*) are given. QTL effect (σ) is expressed as the difference between the means of the two homozygous classes in terms of the number of phenotypic standard deviations for the two parental populations

Trait ^a	Mean (SD)		Chr	MML	Phenotypic mean (SD) DD / PP	σ_p
	<i>diplo</i>	<i>parvi</i>				
RHIZ	4.00 (0.00)	0.00 (0.00)	2	umc134/umc2b	0.47 (0.67) / 0.08 (0.31)	–
			6	umc132	0.22 (0.44) / 0.33 (0.67)	–
EL-ST	0.00 (0.00)	2.05 (1.70)	2	umc2b/umc5a	1.73 (1.20) / 1.05 (0.95)	0.82
			6	umc59	1.12 (0.96) / 1.32 (1.10)	0.24
			6	umc21/umc46	1.40 (1.18) / 1.16 (1.08)	0.29
NO-TL	18.95 (7.23)	16.08 (5.62)	1	umc29/bnl5.59	8.83 (4.97) / 10.26 (5.55)	0.22
			1	<i>tb1</i>	9.17 (5.49) / 9.39 (5.19)	0.03
			2	npi239	11.78 (5.93) / 7.91 (4.54)	0.60
			2	umc134/umc2b	12.88 (5.60) / 7.53 (4.38)	0.83
			2	phi20-0005/umc122	11.83 (5.34) / 7.26 (4.01)	0.71
			3	umc42b	9.96 (5.31) / 9.27 (5.04)	0.11
			3	umc16/npi425a	8.50 (4.85) / 10.55 (5.31)	0.32
			4	umc127/umc15	9.21 (5.03) / 10.61 (5.12)	0.22
			6	umc21/umc46	9.09 (4.82) / 9.63 (5.22)	0.08
			6	umc6/umc134	0.66 (0.99) / 0.16 (0.52)	3.29
SL-TL	3.90 (0.30)	0.00 (0.00)	2	umc6/umc134	0.66 (0.99) / 0.16 (0.52)	3.29
			3	phi20-0042a/umc92	0.65 (1.07) / 0.28 (0.62)	2.51
			7	phi20-0581a/bnl15.40	0.68 (0.91) / 0.28 (0.69)	2.65
LO-TL	2.75 (0.94)	1.21 (1.06)	1	bnl5.59	2.66 (1.13) / 2.30 (1.17)	0.37
			2	umc6/umc134	2.49 (1.07) / 2.49 (1.26)	0.00
			3	umc18a/umc60	2.05 (1.11) / 2.62 (1.23)	0.57
			4	umc15	2.62 (1.10) / 2.25 (1.20)	0.37
			6	umc59	2.59 (1.16) / 2.42 (1.20)	0.17
			10	umc64/bnl10.13	2.57 (1.17) / 2.0 (1.19)	0.56
			10	umc64/bnl10.13	2.57 (1.17) / 2.0 (1.19)	0.56
TH-RO	3.80 (0.53)	0.53 (0.57)	1	umc157	1.72 (0.82) / 1.40 (0.76)	0.57
			1	bnl5.59	1.81 (0.72) / 1.54 (0.87)	0.48
			2	umc134/umc2b	1.73 (0.80) / 1.41 (0.86)	0.59
			5	bnl7.71/npi412	1.86 (0.81) / 1.26 (0.80)	1.08
			5	umc21/umc46	1.71 (0.77) / 1.31 (0.76)	0.72
			6	umc21/umc46	1.83 (0.97) / 1.32 (0.73)	0.91
			7	bnl15.40	2.00 (0.87) / 1.49 (0.79)	0.93
			8	bnl9.44	1.09 (0.73) / 1.72 (0.82)	1.14
			8	bnl9.44	1.09 (0.73) / 1.72 (0.82)	1.14
			10	umc64/bnl10.13	2.57 (1.17) / 2.0 (1.19)	0.56
NO-SB	11.90 (1.85)	9.04 (2.28)	1	<i>tb1</i> /npi581a	10.65 (3.06) / 10.01 (2.66)	0.31
			2	umc6/umc134	10.67 (2.33) / 9.67 (3.61)	0.49
			6	umc38	10.33 (3.39) / 10.01 (2.74)	0.16
			7	umc91	11.45 (2.23) / 9.85 (3.22)	0.77
WT-ST	3.26 (0.64)	0.84 (0.74)	1	umc11/umc29	2.35 (1.31) / 2.77 (1.06)	0.61
			1	umc83a	2.91 (1.11) / 2.37 (1.15)	0.77
			3	umc16/npi425a	2.06 (1.28) / 2.82 (1.06)	1.10

^aRHIZ Rhizomes, EL-ST elongated underground stems other than rhizomes, NO-TL number of tillers, SL-TL slender tillers, LO-TL compactness of tillers, TH-RO thin roots, NO-SB number of side branches, WT-ST withered stems

95.0 percentiles for each trait (Fig. 2). Both the *Z. diploperennis* and the *Z. mays* ssp. *parviglumis* populations showed little or no phenotypic variation in RHIZ, SL-TL, and TH-RO, but considerable variation in NO-TL, NO-SB, LO-TL, and WT-ST. The *Z. mays* ssp. *parviglumis* population was also highly variable in EL-ST. The F₂ population showed more extensive variation than the parental populations for most traits except EL-ST and NO-TL. Novel gene combinations that do not exist in the parental populations may explain the larger phenotypic variation seen in the F₂ population.

The F₁ and F₂ hybrid means for seven of the eight traits (RHIZ, EL-ST, SL-TL, LO-TL, TH-RO, NO-SB, and WT-ST) were between the parental means (Fig. 2). For NO-TL, the means of the F₁ and F₂ hybrids were lower than both parental means. For four traits (RHIZ, EL-ST, SL-TL, and TH-RO), the means of the F₁ and F₂ hybrids were closer to the mean of *Z. mays* ssp. *parviglumis*, indicating some dominance for the *Z. mays* ssp. *parviglumis* phenotype.

The eight traits showed a significant degree of correlation with 15 of the 28 pair-wise comparisons having a significant ($P < 0.05$) correlation, using the non-parametric Kendall's Tau. Despite the large fraction of significant results, Tau was relatively small ($|\text{Tau}| < 0.20$) for 24 of the 28 pair-wise comparisons. The most strongly correlated traits were RHIZ and NO-TL (Tau = 0.21), TH-RO and LO-TL (Tau = 0.23), RHIZ and SL-TL (Tau = 0.24), EL-ST and NO-TL (Tau = 0.30).

Marker segregation

The linkage map of the *diploperennis* × *parviglumis* cross for the 95 RFLP markers spanning the ten teosinte chromosomes is shown in 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 located on eight chromosomal arms. The segregation of the marker loci in the population are more thoroughly discussed in Westerbergh and Doebley (2002).

Number of QTL and magnitudes of effect

Distorted segregation of markers in some regions of the genome caused aberrant results for the permutation tests. For some permuted data sets, the ECM algorithm “bails out” between pairs of distorted markers, resulting in extraordinarily high threshold values. To eliminate this problem, we dropped the four last markers on chromosome 8 and divided chromosome 7 into two linkage groups between marker umc5b and umc110. This prevented the ECM algorithm from bailing out for all traits except SL-TL. All markers on chromosome 8 had to be dropped in order to prevent the algorithm from bailing out for SL-TL. This may be due to non-normally distributed phenotypic data for this trait. Because of the impact of the distorted segregation on the permutation tests, we have here reported all QTL with LOD scores

above 2.5 based on Lander and Botstein (1989), and we indicate which of these QTL meet the threshold value given by the permutation tests (Table 2).

We detected a total of 38 QTL for eight traits (Table 2; Fig. 3). LOD scores for these QTL ranged from 2.6–9.1. The number of QTL found for each trait ranged from two for RHIZ to nine for NO-TL. For four traits, we found evidence for more than one QTL on the same chromo-

Table 2 QTL for perennial traits mapped in a *Z. diploperennis* × *Z. mays* ssp. *parviglumis* F₂ population. *d/a* Ratio of the dominance to additive effect, *a* additive effect, *d* dominance effect, *od* overdominance effect, *D* *diploperennis* allele, *P* *parviglumis* allele, *Mode* of gene action showing *a*, *d* or *od* and whether the *D* or the *P* allele was dominant, *Dir* direction of the effect (whether the *D* or the *P* allele contributed positively to the effect), *R*² proportion of phenotypic variance explained by a QTL

Trait	Chr	MML	LOD	<i>d/a</i>	Mode	Dir	<i>R</i> ²
RHIZ	2	umc134/umc2b	6.2 ^a	0.01	a	D	8.7
	6	umc132	3.0	-0.52	d, P	P	3.3
EL-ST	2	umc2b/umc5a	4.0 ^a	0.11	a	D	4.9
	6	umc59	3.2	-3.78	od	-	3.3
	6	umc21/umc46	3.1	2.96	od	-	4.1
NO-TL	1	umc29/bnl5.59	4.3 ^a	-5.05	od	-	5.6
	1	<i>tb1</i>	2.9	29.32	od	-	2.7
	2	npi239	3.3	0.11	a	D	2.8
	2	umc134/umc2b	9.1 ^a	-0.01	a	D	11.6
	2	phi20-0005/umc122	7.3 ^a	0.49	a	D	7.5
	3	umc42b	4.0 ^a	3.24	od	-	3.9
	3	umc16/npi425a	3.5 ^a	-1.29	od	P	4.2
	4	umc127/umc15	2.9	-2.40	od	P	2.9
	6	umc21/umc46	3.1	60.0	od	-	3.6
	6	umc6/umc134	5.4	-0.65	d, P	D	7.6
SL-TL	3	phi20-0042a/umc92	4.4	-1.05	d, P	D	6.9
	7	phi20-0581a/bnl15.40	4.7	-1.00	d, P	D	15.6
LO-TL	1	bnl5.59	3.3	-1.95	od	D	3.4
	2	umc6/umc134	2.6	-17.57	od	-	3.9
	3	umc18a/umc60	3.7 ^a	1.84	od	P	7.0
	4	umc15	3.5 ^a	-1.50	od	D	4.1
	6	umc59	2.9	-5.88	od	-	2.0
	10	umc64/bnl10.13	3.4 ^a	0.41	a	D	5.6
	10	umc64/bnl10.13	3.4 ^a	0.41	a	D	5.6
TH-RO	1	umc157	4.6 ^a	-0.71	d, P	D	4.6
	1	bnl5.59	5.0 ^a	-2.64	od	D	4.7
	2	umc134/umc2b	7.7 ^a	-1.24	d, P	D	10.7
	5	bnl7.71/npi412	8.5 ^a	-0.75	d, P	D	12.5
	5	umc104b	4.3 ^a	0.27	a	D	4.4
	6	umc21/umc46	5.2 ^a	-0.85	d, P	D	6.4
	7	bnl15.40	4.7 ^a	-1.10	d, P	D	4.2
	8	bnl9.44	5.4 ^a	-0.07	a	P	6.0
NO-SB	1	<i>tb1</i> /npi581a	2.6	3.64	od	-	4.6
	2	umc6/umc134	2.7	1.78	od	-	6.3
	6	umc38	2.9	7.7	od	-	2.9
WT-ST	7	umc91	4.1 ^a	0.22	a	D	4.2
	1	umc11/umc29	3.5 ^a	-0.02	a	P	5.4
	1	umc83a	4.3 ^a	-0.56	d, P	D	4.4
	3	umc16/npi425a	6.5 ^a	-0.19	a	P	9.3

^aSignificant QTL by permutation tests

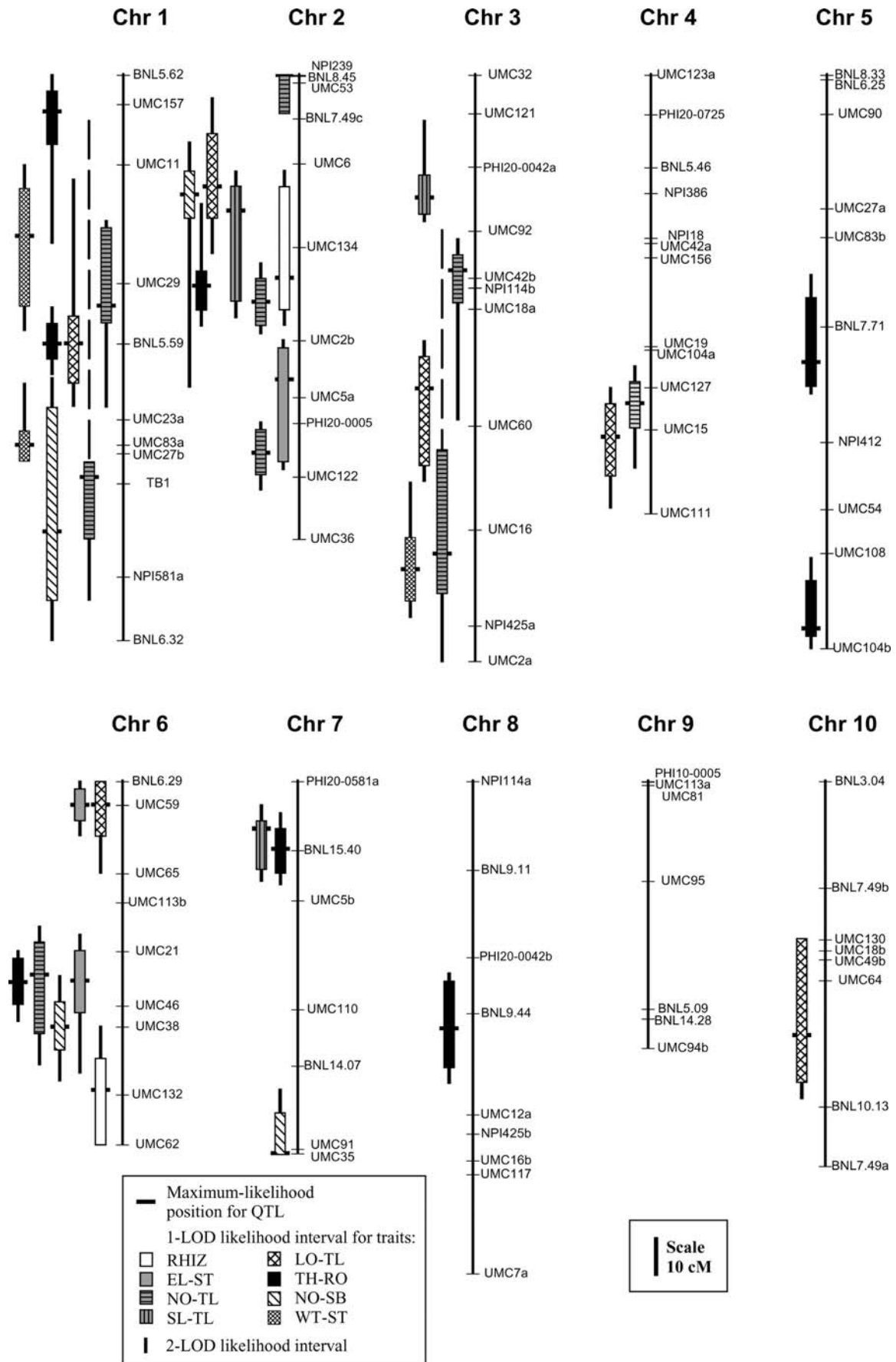


Fig. 3 Linkage map for *Z. diploperennis* and *Z. mays* ssp. *parviglumis* showing the positions of quantitative trait loci (*QTL*) associated with annual and perennial habit

some. These include two QTL on chromosome 6 for EL-ST, two QTL on chromosome 1 for WT-ST, two QTL on chromosomes 1 and 5 for TH-RO, and two QTL on chromosomes 1 and 3, as well as three QTL on chromosome 2 for NO-TL. The detection of more than one QTL per chromosome was probably not strongly biased, since all syntenic QTL were at least 40 cM apart.

Individual QTL explained 2.0% to 15.6% of the phenotypic variation (R^2) in the F_2 population (Table 2). Only four of these explained more than 10% of the variance. Two of these larger-effect QTL control TH-RO and are located on chromosomes 2 and 5. A QTL explaining 11.6% of the variance for NO-TL mapped near the larger-effect QTL found for TH-RO on chromosome 2. The QTL controlling the most variation (15.6%) is located on chromosome 7 and affects SL-TL.

We also estimated the magnitudes of QTL effect as the number of phenotypic standard deviations (σ_p) within the parental species (Orr 2001). Most QTL controlling the difference related to annual versus perennial habit for teosinte showed minor effects on this scale ($<1.0 \sigma_p$, Table 1). Such small QTL would not be noticed if they were segregating in a population of either *Z. diploperennis* or *Z. mays* ssp. *parviglumis*. One of three QTL for SL-TL was larger than $3 \sigma_p$, and the other two were near $2.5 \sigma_p$. This variation might stand out in a segregating population; however, our estimate of the standard deviation in *Z. mays* ssp. *parviglumis* for this trait was 0, which may cause a biased estimate of the pooled standard deviation for the parental species.

Genomic location of QTL

The 38 detected QTL occur on nine different chromosomes; however, most QTL are located on chromosomes 1, 2, and 6 (Table 2; Fig. 3). Eight QTL for five of the traits were distributed along chromosome 1. Nine QTL for six traits (RHIZ, NO-TL, SL-TL, LO-TL, TH-RO, and NO-SB) mapped along chromosome 2, with most near marker umc134. Seven QTL for six traits (RHIZ, EL-ST, NO-TL, LO-TL, TH-RO, and NO-SB) mapped to chromosome 6, with most of these near marker umc46. The clustering of QTL on chromosome 2 and 6 indicates that these regions play important roles in the control of perennialism in teosinte. Two or three closely mapping QTL for different traits were also found on chromosomes 1, 3, 4, and 7. The clustering of QTL could be explained by multiple adjacent QTL, each controlling one of the traits, or by a single QTL affecting these traits pleiotropically. Noor et al. (2001) has shown that the distribution of genes per centiMorgan in the *Drosophila melanogaster* genome is highly skewed, and that low recombination rates create high gene densities per centiMorgan in the centromeric regions. Low recombination rates in centromeric regions are also found in several plants (e.g., Tanksley et al. 1992; Copenhagen et al. 1998). The QTL for perennial traits in teosinte near umc134 on chromosome 2 are located close to the centromere. If this

region corresponds to an unusually gene-rich region, then the clustering of QTL near umc134 could be explained by multiple adjacent QTL.

Mode of gene action and direction of QTL effect

Of 38 QTL, 11 showed additive gene action ($-0.50 < d/a < 0.50$) and ten seem to be inherited in a dominant/recessive manner ($0.50 < |d/a| < 1.25$) (Table 2). The distribution of dominance between *Z. diploperennis* and *Z. mays* ssp. *parviglumis* alleles was completely skewed, with the *Z. mays* ssp. *parviglumis* allele being dominant in every case. The remaining 17 QTL showed apparent overdominance ($|d/a| > 1.25$).

There is an expectation that the effects of most *Z. diploperennis* alleles will be in the direction of the *Z. diploperennis* phenotype and the *Z. mays* ssp. *parviglumis* alleles in the direction of the *Z. mays* ssp. *parviglumis* phenotype (Rieseberg et al. 2002). For the 21 QTL with additive or dominance gene action (Table 2) plus six of the 17 overdominant QTL with significantly ($P=0.05$) different means for the two homozygous classes, there were 19 (of 27) cases for which the class means differed in the expected direction.

Epistatic effects

Two-way ANOVA was performed between 25 genomic locations harboring QTL. These locations covered 15 of the 20 chromosome arms. Each of these markers was the closest marker to a QTL; however, for three QTL between umc134 and umc2b on chromosome 2, only umc134 was used for the analysis. In addition, for two QTL near each other on chromosome 4, the most centrally located marker (umc15) was used. All pair-wise combinations gave 300 tests for each trait and a total of 2,400 tests for all eight traits. At a significance level of $P < 0.05$ for 300 tests, 15 significant interactions would be expected by chance alone, and for 2,400 tests, 120 would be expected. We found a total of 143 significant interactions or about 20% more than expected by chance alone given $P=0.05$. Of these, there were 26 significant interactions for NO-SB and 23 for TH-RO. Between 12 and 19 significant interactions were found for the other traits. Because the traits were all scored on discontinuous scales and the ANOVA test is a parametric test, the results from the statistical analysis have to be treated with caution.

To determine whether the epistatic effects were large relative to the main effects, we compared the R^2 values for these effects for the subset of the 2,400 ANOVA that presented a significant test ($P=0.05$) for either the main or the interaction effects. The main effects consistently presented larger R^2 values than the interactions (data not shown). Thus, while we observed more digenic epistatic interactions than expected by chance (143 vs 120), these interaction effects probably do not explain a particularly large portion of the variance.

Discussion

Perennial versus annual habit is an important component of plant reproductive strategy. The study of the inheritance of perennialism can contribute both to the understanding of how this trait evolves and to the identification of genes involved in specific features of perennialism such as rhizome formation. Perennialism also affects resource allocation in plants such that annuals, which lack underground perenniating organs, can allocate all available resources to seed production, while perennials typically partition available resources between seed production and vegetative perenniating organs. The study of the inheritance of perennialism can contribute to the understanding of the ecological consequences of these different life history strategies.

Genetic control of perennialism in *Zea*

We have detected a total of 38 QTL for morphological traits related to perennialism. One feature of the results is that the observed QTL do not explain a very large portion of the variance in the F_2 population for any of the traits. For example, for the presence of rhizomes, we detected only two QTL controlling 8.7% and 3.3% of the variation. For the presence of thin roots as seen in the *Z. diploperennis* parent, we detected eight QTL, but they still explain less than half the variation in the population. The failure to explain a very large portion of the variation for perennialism was also seen in a prior QTL study in *Sorghum* (Paterson et al. 1995) and could be explained in several ways. For example, if the phenotypes we studied are dependent on multilocus genotypes with epistatic interactions, then many QTL and the variance they explain could go undetected by our methods. The fact that the number of significant epistatic interactions that we observed is somewhat greater than expected by chance indicates that epistatic effects may be important in our F_2 population; however, our data also suggest that the observed epistatic effects are generally smaller than the main effects. Alternatively, if the traits we analyzed have low heritabilities, then the observed QTL would explain only a small portion of the phenotypic variation in the F_2 population. Paterson et al. (1995) reported low heritability (0.34) for perennialism in *Sorghum*.

While the presence of RHIZ is the key trait that confers perennialism on *Z. diploperennis*, we also studied several related traits. The underground buds, which make RHIZ in *Z. diploperennis*, form EL-ST in *Z. mays ssp. parviglumis*. We detected three QTL for EL-ST that map apart from the RHIZ QTL. *Zea diploperennis* forms distinctive SL-TL, while *Z. mays ssp. parviglumis* forms tillers that are more robust. We detected three QTL for this feature. The tillers of *Z. diploperennis* emerge from the ground at a distance of ~5 cm or more from the main stalk, while those of *Z. mays ssp. parviglumis* emerge from the soil adjacent to the main stalk (or another tiller). As a result, there is a

difference in the “looseness” of the tillers. We detected six QTL for this trait, which represents the extent to which the underground stems grow horizontally before switching to vertical growth. *Z. diploperennis* produces distinct slender roots that were not seen in *Z. mays ssp. parviglumis*. We detected eight QTL for this trait, although the relationship between this root type and perennialism (if any) is unknown.

Gene action and epistasis

Seventeen of the 38 QTL (44%) showed overdominance, which is a much higher percentage than seen in most QTL studies (Tanksley 1993). This result also contrasts with our earlier analysis of differences in tassel morphology for this same population, in which case most of 33 QTL showed additive gene action (Westerbergh and Doebley 2002). Paterson et al. (1995) report a high percentage (35%) of perennialism QTL with overdominance in *Sorghum*. One possible explanation for the high proportion of overdominant QTL is that there may be considerable heterosis for vegetative traits in these relatively wide interspecific crosses.

Most QTL studies that have performed tests of digenic epistasis report a frequency of significant test statistics near 5%, which is the frequency expected by chance alone given a $P=0.05$ significance threshold (Tanksley 1993). When more detailed analyses have been performed, however, convincing evidence for epistasis has been found (Mackay 2001). Our results show more significant tests of epistatic interaction than expected by chance; however, the epistatic effects are generally smaller than the main effects. It would be informative to study the relative importance of the epistatic and main effects in an isogenic background.

Comparison with other grasses and known maize genes

We mapped several QTL for traits associated with perennialism (RHIZ, NO-TL, SL-TL, LO-TL, and TH-RO) near marker umc134 on chromosome 2 in teosinte. The orthologous chromosomal region in *Sorghum* (near maize markers umc34 and csu40) on linkage group D and in rice (*Rhz3*) on chromosome 4 also possesses QTL for perennialism (Paterson et al. 1995; Hu et al. 2003). In rice and teosinte, this is the chromosomal region with the greatest importance for traits related to perennialism. Similarly, we have mapped QTL for multiple perennialism-related traits to chromosome 6 near umc38 and umc46. The orthologous region (*QRn5*) on rice chromosome 5 also possesses a cluster of perennialism QTL (Hu et al. 2003). Finally, QTL for NO-TL and NO-SB mapped near marker *tb1* on chromosome 1 in teosinte. The orthologous chromosomal region in *Sorghum* (near maize marker umc83) on linkage group C and in rice (*QRn3*) on chromosome 3 also possesses QTL for tillering (Paterson

et al. 1995; Hu et al. 2003). Once the underlying genes have been cloned, it will be of interest to determine whether orthologous genes control these traits in both teosinte, *Sorghum*, and rice.

There are also some apparent associations between the chromosomal locations of our QTL and known maize mutants that affect related phenotypes. A QTL for NO-SB and NO-TL mapped near marker *tb1* on chromosome 1. *tb1* is the locus for the maize mutant *teosinte branched1*, and this gene affects the number of tillers and the number and length of the lateral branches on the plant (Doebley et al. 1997). Other QTL for NO-SB and NO-TL mapped to chromosome 2 near *umc2b/umc134* and chromosome 3 near *umc18*. These two regions harbor the known maize mutants *barren stalk2* (*ba2*) and *barren stalk1* (*ba1*), respectively, both of which control the formation of lateral branches including tillers. *tb1*, *ba1*, and *ba2* represent potential candidates for the QTL that we have detected.

Perennials in agriculture

Pasture and turf grasses such as *Lolium perenne* (perennial ryegrass), *Phleum pratense*, and *Festuca pratensis* are important as forage for cattle and in erosion control of agro-ecosystems. The productivity of these grasses is enhanced by the ability to produce rhizomes and seedling tillers. Perennial traits may therefore have an impact on agricultural productivity. As gene order and gene content are highly conserved among diverse grass genera (Devos and Gale 1997), the map positions of QTL for RHIZ formation and other perennialism-related traits in teosinte may help to locate corresponding QTL in pasture grasses and other species.

Other advantages of perennials in agriculture may also be discussed. To ensure an adequate supply of food for the world, durable and highly productive plant varieties have to be developed for almost any production environment. In contrast to commercial farming in industrialized countries, small-scale farming is an important source of food for many people in developing countries. Growing perennial crops in these agro-systems would be less time consuming and less costly, as the time spent on seeding would be reduced, and the farmers would be less dependent on the purchase of high-quality seeds from commercial sources. In addition, there would be less loss of nutrients from the soil, as perennials store nutrients in their underground organs. However, perennialism would likely change resource allocation in maize and may come at a cost of seed production. The development of successful perennial maize or other cereals will depend on breaking any inverse linkage between rhizome and seed production. Marker-assisted selection may be useful for this purpose.

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