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Molecular analysis of crosses between *Tripsacum dactyloides* and *Zea diploperennis* (Poaceae)

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Abstract DNA fingerprinting verified hybrid plants obtained by crossing Eastern gamagrass, *Tripsacum dactyloides* L., and perennial teosinte, *Zea diploperennis* Iltis, Doebley & R. Guzmán. Pistillate inflorescences on these hybrids exhibit characteristics intermediate to the key morphological traits that differentiate domesticated maize from its wild relatives: (1) a pair of female spikelets in each cupule; (2) exposed kernels not completely covered by the cupule and outer glumes; (3) a rigid, non-shattering rachis; (4) a polystichous ear. RFLP analysis was employed to investigate the possibility that traits of domesticated maize were derived from hybridization between perennial teosinte and *Tripsacum*. Southern blots of restriction digested genomic DNA of parent plants, F_1, and F_2 progeny from two different crosses were probed with RFLP markers specifically associated with changes in pistillate inflorescence architecture that signal maize domestication. Pairwise analysis of restriction patterns showed traits considered missing links in the origin of maize correlate with alleles derived from *Tripsacum*, and the same alleles are stably inherited in second generation progeny from crosses between *Tripsacum* and perennial teosinte.

Key words Maize · Teosinte · *Zea* · *Tripsacum* · RFLP analysis

Introduction

Although progenitors of the major Old World cereal crops, wheat (*Triticum* L.), barley (*Hordeum* L.) and rye (*Secale* L.), are known, scientists have not agreed completely on the ancestry of maize (*Zea mays* L. subsp. *mays* Iltis & Doebley), the grain upon which New World civilizations were founded. Various speculations about the origin of maize abound in the literature. For reviews that provide details of different hypotheses and their advocates, see Mangelsdorf (1974); Randolph (1976); Doebley (1990), Goodman (1988); Wilkes and Goodman (1995). The "teosinte theory," popularized by Beadle (1939), is most widely accepted today (Doebley 1990), and the once prominent hypothesis that *Tripsacum* introgression was important in maize evolution (Mangelsdorf and Reeves 1939) is no longer favored. With the discovery of diploid perennial teosinte, *Zea diploperennis* Iltis, Doebley and Guzmán (Iltis et al. 1979), Mangelsdorf changed his earlier view and favored the "Wilkes hypothesis" (Mangelsdorf 1983, 1986), i.e., that annual teosinte derived from a cross between *Z. diploperennis* and extinct maize, and domesticated maize arose from subsequent introgressive hybridization between maize and the newly created annual teosintes (Wilkes 1979).

Zea and *Tripsacum* are monoecious grasses and the only exclusively New World members of the Andropogoneae (Kellogg 1993). The genus *Zea* includes maize and its closest wild relatives, four subspecies of annual teosinte, and two rhizomatous perennial species, a diploid and a tetraploid. Whereas maize is a major world crop, the teosintes are restricted to Mexico and Guatemala. *Zea* produces staminate inflorescences in tassels at the apex of primary culms, and pistillate inflorescences in leaf axils, i.e., ears enclosed in leaf sheaths or husks. The base chromosome number in *Zea* is $x = 10$, and all species except tetraploid *Zea perennis* are diploid ($2n = 20$).

Tripsacum species are rhizomatous perennials distributed geographically throughout North and South America. The base chromosome number is $x = 18$, and ploidy levels range from diploid ($2n = 36$) to hexaploid ($2n = 108$). *Tripsacum* bears flowers of both sexes on the same spike, with staminate above pistillate florets.

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Tripsacum sometimes produces two functional pistillate florets that result in paired caryopses in a single cupule (Farquharson 1954), superficially resembling the characteristic of paired kernels from paired spikelets that distinguishes domesticated maize from teosinte.

Crosses between species of *Tripsacum* and maize usually produce sterile hybrid plants, but attempts to produce hybrids between *Tripsacum* and annual teosintes have failed (Mangelsdorf and Reeves 1931; Randolph 1952; Tantravahi 1968; Mangelsdorf 1974). An unexpected breakthrough in crossability of the wild relatives of maize came when fertile F_1 hybrid plants were obtained from crossing *Z. diploperennis* Iltis, Doebley and Guzmán and *Tripsacum dactyloides* L. (Eubanks 1995), a development that revives feasibility of the hypothesis that *Tripsacum* played a role in the evolution of domesticated maize.

A striking feature of *Tripsacum*-teosinte hybrids is that the pistillate structures have fused cupules that do not disarticulate at maturity, and the cupules have paired kernels that are not completely enclosed by outer glumes (Fig. 1). The significant differences between domesticated maize and its wild relatives are respectively: (1) paired female spikelets in a cupule instead of single female spikelets; (2) four-ranked instead of two-ranked ears; (3) a non-shattering rachis instead of disarticulating fruitcases, and (4) kernels that are not completely enclosed by the cupule and outer glumes instead of being encased in hard fruitcases. The origin of maize is still a mystery because specimens that show the stepwise transition to the ear of cultivated maize have not been recovered in the archaeological record, and experiments to reconstruct the maize progenitor from crosses between maize and teosinte have failed to recover pure segregating parental phenotypes (Langham 1940; Mangelsdorf 1947; Galinat 1985; Rogers 1950). Because *Tripsacum*-teosinte hybrids exhibit intermediate forms resembling the missing link in the evolution of the maize ear in the first generation of crosses, molecular analysis was employed to investigate the possibility that these traits derived from genes introduced into teosinte from *Tripsacum*, rather than from a relatively short period of human selection for recombination between a few mutations in teosinte (Beadle 1939; Galinat 1971), or from cataclysmic transformation of the teosinte staminate inflorescence (Iltis 1983).

Mapping experiments combining quantitative trait loci (QTLs) and molecular marker loci (MMLs) have identified over 50 restriction fragment length polymorphisms (RFLPs) that distinguish advanced maize from its putative ancestor, annual teosinte (Doebley et al. 1990; Doebley and Stec 1991). Southern blots were probed with RFLPs associated specifically with changes in pistillate inflorescence architecture that would have been important to the domestication of the maize ear in order to investigate the hypothesis that those alleles are derived from *Tripsacum* in the *Tripsacum*-teosinte hybrids and, if so, to determine if they are stably inherited in F_2 progeny.

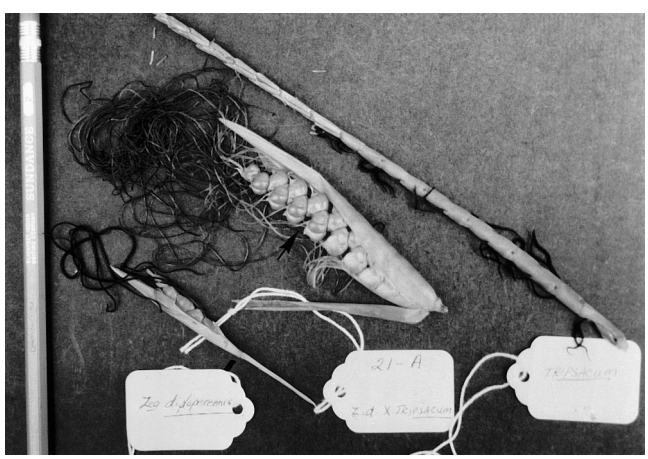

Fig. 1 *Z. diploperennis* × *T. dactyloides* F_1 hybrid ear in center; *Z. diploperennis* pistillate spike on left, with *T. dactyloides* spike bearing staminate and pistillate spikelets on right. *Arrow* indicates distinctive interspacing, a trait observed in archaeological maize thought to be controlled by genes from *Tripsacum* chromosome 5 (Galinat 1973)

Materials and methods

Hybrid plants in this study have been described elsewhere (Eubanks 1989, 1992, 1995). Specimens employed in the molecular assays include: (1) *T. dactyloides* (2n = 72), parent plant in all crosses reported here; (2) a *Z. diploperennis* plant, designated 3–7, that was a parent in one of the crosses; (3) an F_1 hybrid, designated Sun Dance, from a cross in which *Z. diploperennis* 3–7 was the female parent; (4) an F_1 hybrid, designated Tripsacorn, from a cross in which *T. dactyloides* was the female parent and a *Z. diploperennis* plant designated 3–3 was the pollen donor, and (5) F_2 Tripsacorn derived from selfing F_1 Tripsacorn. Voucher specimens have been deposited at the Duke University Herbarium.

DNA isolations, restriction-enzyme digests, transfers to Southern blots, and probings with RFLPs were conducted by Linkage Genetics, Salt Lake City, Utah. For DNA analysis, a minimum of 20 g of fresh leaf material was harvested from greenhouse grown plants and shipped on dry ice to Linkage Genetics. Methods for plant DNA preparation, Southern blots, hybridization (aqueous with 5 × SSC at 60°C) and washing (0.25 × SSC at 60°C) conditions followed Helentjaris et al. (1985, 1986). Genomic DNA from each specimen was digested with the restriction endonucleases *Eco*RI, *Hin*dIII, *Eco*RV, and *Bam*HI, transferred to Southern blots, and probed with the maize RFLP probes listed in Table 1. The amount of *Tripsacum* DNA loaded for gel electrophoresis was 1.5× that of *Zea*. As indicated by the prefix UMC, RFLP probes were from the University of Missouri-Columbia Maize RFLP Laboratory. Restriction fragment patterns shared between parent and hybrid plants were scored by pairwise analysis.

Results

The total number of bands scored on Southern blots probed with the 15 RFLP markers listed in Table 1 was

Table 1 Molecular markers used to characterize *Tripsacum*-teosinte hybrids

Marker	Maize chromosome	Map unit[a]	Trait[c]
UMC164[b]	1	4	Co-dominant
UMC11[b]	1	44	CUPR
UMC167[b]	1	98	Indeterminant
UMC83	1	190	DISA
UMC107[b]	1	223	DISA, CUPR[6], GLUM
UMC84	1	282	CUPR, GLUM
UMC53[b]	2	13	RANK, liguleless
UMC131[b]	2	94	RANK, GLUM
UMC2[b]	3	177	Not polymorphic in *Tripsacum*
UMC66[b]	4	107	Glossy
UMC54	5	119	Not polymorphic in *Tripsacum*
UMC85	6	2	CUPR
UMC65[b]	6	59	CUPR
UMC105	9	34	RANK
UMC95[b]	9	76	CUPR, RANK

[a]Map units according to Gardiner et al. (1993)
[b]Indicates restriction site in *Tripsacum*-teosinte hybrids uniquely derived from *Tripsacum*
[c]CUPR = cupules per rank; DISA = disarticulation; GLUM = glume score; RANK = number of rows of cupules

146 in *Z. diploperennis*, 110 in *Tripsacum*, 116 in Sun Dance, and 120 in Tripsacorn. Pairwise analysis revealed that *T. dactyloides* and *Z. diploperennis* have 34 restriction fragments in common; *Z. diploperennis* and Sun Dance have 75; *Z. diploperennis* and Tripsacorn have 78; *T. dactyloides* and Sun Dance have 30; *T. dactyloides* and Tripsacorn have 31, and Tripsacorn and Sun Dance have 104. The *Tripsacum*-teosinte hybrids have 14 bands associated with nine RFLP probes that correspond to unique bands in the *T. dactyloides* parent. Replicated DNA assays employing seven of nine RFLP markers associated with unique *Tripsacum* restriction fragments – UMC83, UMC11, UMC53, UMC2, UMC66, UMC65, and UMC95 – produced identical allelic patterns in *Tripsacum*-teosinte F_1 plants and F_2 progeny.

Unique *Tripsacum* loci in the *Tripsacum*-teosinte hybrids map to *Zea* chromosomes 1, 2, 3, 4, 6, and 9 (see Tables 1 and 2; Fig. 2). Of the four traits that distinguish ear morphology targeted by this investigation [cupules per rank (CUPR), disarticulation (DISA), glume score (GLUM), and number of rows of cupules (RANK)], markers associated with chromosome 1 correlate with CUPR, DISA, and GLUM; chromosome 2 with RANK and GLUM; chromosome 3 with GLUM; chromosome 6 with CUPR, and chromosome 9 with CUPR and RANK. Results of mapping investigations combining QTLs with molecular markers (Doebley et al. 1990; Doebley and Stec 1991) indicated that major effects on inflorescence architecture map to five regions of chromosomes 1, 2, 3, 4, and 5. Unique *Tripsacum* restriction fragments in the *Tripsacum*-teosinte hybrids correspond with, or are proximal to, those same regions on chromosomes 1, 2, 3, and 4. A fifth region corresponds to chromosome 6 rather than chromosome 5.

Discussion

Results suggest that changes in inflorescence architecture that distinguish maize from its wild relatives could be derived from hybridization between a perennial teosinte and *Tripsacum*. Although this finding is a departure from earlier hypotheses explaining the origin of maize, much of the evidence from previous research is congruent with this concept. The scenario that logically follows from the evidence presented here was predicted by Harshberger (1896), Collins (1912), and Kempton (1919) who postulated that maize originated from hybridization between teosinte and an unknown grass in the Andropogoneae. Signalling the involvement of *Tripsacum* and *Z. diploperennis* in maize evolution,

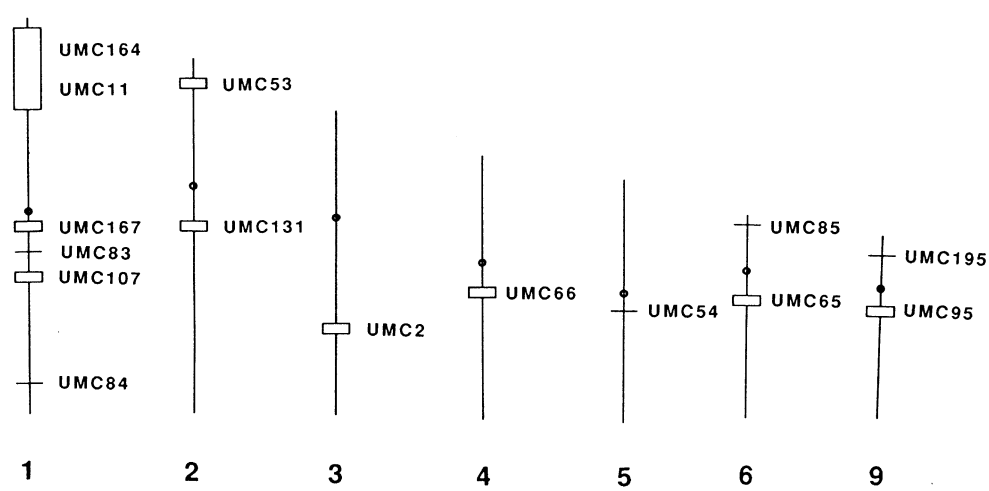

Fig. 2 Karyogram showing approximate locations of MMLs on the *Tripsacum*-teosinte chromosomes as reconstructed from *Z. diploperennis* karyotype analyses (Pasupuleti and Galinat 1982; Kato and Lopez 1990). *Blocked areas* indicate regions that correspond to loci uniquely derived from *Tripsacum* and are associated with traits for distinctive maize ear morphology. *Open circles* represent approximate postions of centromeres

Table 2 Genes, *Tripsacum* chromosomes, and linkage groups that correlate with molecular markers for unique *Tripsacum* alleles in *Tripsacum*-teosinte hybrids

Molecular marker	Gene	*Tripsacum* chromosome[a]	Linkage group[b]
Chromosome 1S			
UMC164-UMC11	*sr* (striate leaves)	?	I (?)
Chromosome 1L			
UMC167-UMC107	*bm*2 (brown midrib) *an*(anther ear) *br* (brachytic culms)	?	A
Chromosome 2S			
UMC53	*ws* (white sheath) *lg* (liguleless) *gl*2 (glossy) *b* (colored plant) *sk* (silkless ears) *fl* (floury endosperm)	Tr9	D
Chromosome 2L			
UMC131	*v* (virescent)	Tr14 or 18	N
Chromosome 3L			
UMC2	*a* (anthocyanin)	?	C
*Chromosome 4**L***			
UMC66	*gl*3 (glossy)	Tr13	F
Chromosome 6L			
UMC65	*sm* (salmon silks) *py* (pigmy plant)	Tr4	L
Chromosome 9L			
UMC95	*bm* (brown midrib)	Tr5	O

[a] Galinat 1973; ? = unconfirmed
[b] Blakey 1993

invokes aspects of both theories advocated by Mangelsdorf (Mangelsdorf and Reeves 1939; Mangelsdorf 1983, 1986).

Regions on chromosomes 1, 2, 3, 4, 6 and 9 match chromosomal regions which previous investigators have identified as having major effects on morphological differences between maize and teosinte inflorescence architecture (Mangelsdorf 1947; Rogers 1950; Doebley et al. 1990; Doebley and Stec 1991). Mangelsdorf (1947) mapped changes in the spikelet-rachis relationship of the ear of maize to the same regions on chromosomes 1, 3, and 4, and a region on chromosome 9. The teosinte segments from chromosomes 1, 4, and 9 affect the architecture of the spikelet-rachis relationship, as described and illustrated in detail (Galinat 1963). The teosinte chromosome-9 segment increases internode and ear length. A corresponding region on chromosome 9 in the *Tripsacum*-teosinte hybrids has a unique genetic component contributed by *Tripsacum*. Mangelsdorf (1947) and Rogers (1950) identified regions on chromosome 6 that affect inflorescence morphology also corresponding with unique *Tripsacum* loci in the *Tripsacum*-teosinte hybrids. Regions on chromosome 5 (Doebley et al. 1990; Doebley and Stec 1991) and chromosome 8 (Mangelsdorf 1947; Rogers 1950) have been linked to key traits in maize evolution, but molecular marker loci (MMLs) for chromosome 5 employed in this investigation, and probes to chromosome 5 (NPI1282) and chromosome 8 (UMC89) employed in preliminary screening, did not reveal unique *Tripsacum* bands associated with either chromosome.

Earlier studies of linkage relationships between maize and teosinte (Collins and Kempton 1920; Mangelsdorf and Reeves 1939; Rogers 1950) delineated four or five independently inherited regions affecting ear morphology and concluded that the cumulative data did not support the hypothesis that teosinte is a wild progenitor of maize. A region thought to be particularly important for changes in pistillate structure was mapped close to the *Su-su* locus on chromosome 4. An important trait in the *Tripsacum*-teosinte hybrids that distinguishes maize from its wild relatives is a reduction of the outer glume, which leaves the kernel partially exposed, a trait that would be selected for by humans because it facilitates threshing of the grain. Dorweiler et al. (1993) recently coined a symbol for this genetic locus, teosinte glume architecture 1 (*tga*1). Since it is the architecture of the whole spikelet (Galinat 1963), not just its outer glume that is involved, and since maize is the divergent form, perhaps a more appropriate symbol would be *msa* for maize spikelet architecture. *Tga*1 resembles the tunicate (*Tu*) allele that converts hard fruitcases into shallow cupules with elongate soft glumes that protect the kernels. *Tu* was characterized by Mangelsdorf and Galinat (1963) and Beadle (1972), and is proximal to *tga*1 on chromosome 4. The molecular marker UMC66, proximal to *tga*1 and *Tu*, revealed a unique *T. dactyloides* restriction

fragment in the *Tripsacum*-teosinte hybrids indicating that this trait, which represents a major step in maize evolution, could derive from *Tripsacum*.

In maize and *Tripsacum* intergenomic mapping, Galinat (1973) identified the maize-4 *su* locus on *Tripsacum* chromosome 7 (Tr7), and linked the maize-4 gl_3 locus to *Tripsacum* chromosome 13 (Tr13) with the conclusion that the teosinte chromosome 4 did not come from *Tripsacum*. He also found *Tripsacum* linkage with maize chromosomes 1, 2, 6, and 9, and showed two or more unidentified *Tripsacum* chromosomes have loci in common with maize chromosome 1. The homoeolog to the short arm of maize 2 was identified as *Tripsacum* chromosome 9 (Tr9), and the homoeolog to the long arm of maize 2 was either *Tripsacum* chromosome 14 or 18 (Tr14 or Tr18). Maguire (1961) and Galinat (1973) both noted that *Tripsacum* affects the expression of two-ranked vs four-ranked fruits and is linked to maize chromosome 2. The *Tripsacum*-teosinte hybrids also show this trait, i.e. RANK, connected with *Tripsacum* loci on chromosome 2. Galinat (1973) found *Tripsacum* chromosome 4 (Tr4) was the homeolog for maize 6, and *Tripsacum* 5 (Tr5) for maize 9. Since Tr5 has eight loci in common with maize chromosome 9, Galinat (1970, 1973) suggested Tr5 might contribute genes for the distinctive interspace between adjacent cupules to maize. The *Tripsacum*-teosinte ears strongly exhibit this interspace character (see Fig. 1).

Blakey (1993) conducted linkage analysis of *T. dactyloides* using RFLP markers and identified 16 homologous linkage groups in maize. The findings revealed tightly linked blocks of maize marker loci in *Tripsacum* that are conserved in maize in respect of the linear order of the loci and the genetic distances between them. Correspondence between Blakey's (1993) *Tripsacum* linkage groups and Galinat's (1973) intergenomic mapping data that correlate with unique *Tripsacum* alleles in *Tripsacum*-teosinte hybrids is as follows: linkage group A matches an unidentified *Tripsacum* chromosome that carries the gene *bm*2 (brown midrib), and probably *br* (brachytic culms) and *an* (anther ear), and maps to the long arm of *Zea* chromosome 1 (Z1L); I may correspond to another unidentified *Tripsacum* chromosome proximal to *sr* (striate leaves) and maps the short arm of *Zea* chromosome 1 (Z1S); D corresponds to *Tripsacum* chromosome 9 (Tr9) and maps to Z2S; N corresponds to Tr14 or 18 and maps to Z2L; C appears to correspond to another unidentified *Tripsacum* chromosome proximal to *a* (anthocyanin) and maps to Z3L; F corresponds to Tr13 and maps to Z 4L; L corresponds to Tr4 and maps to Z6L, and O corresponds to Tr5 and maps to Z9L. Genes proximal to unique *Tripsacum* alleles in the *Tripsacum*-teosinte hybrids that correlate with Galinat's (1973) findings include: *ws* (white sheath), *lg* (liguleless), *gl*2 (glossy), *b* (colored plant), *sk* (silkless), and *fl* (floury endosperm) on Z2S linked to Tr9 and linkage group D; *v* (virescent) on Z2L linked to Tr14 or Tr18 and associated with linkage group N; *gl*3 (glossy) on Z4L linked to Tr13 and linkage group F; *py* (pigmy plant) and *sm* (salmon silks) on Z6L linked to Tr4 near linkage group L, and *bm*4 (brown midrib) on Z9L linked to Tr5 within linkage group O (see Table 2). In summary, the close correspondence of the intergenomic map and the *Tripsacum* molecular map with unique *Tripsacum* restriction fragments that are stably inherited in *Tripsacum*-teosinte hybrids, combined with their association with key morphological changes in inflorescence architecture, lends credence to the hypothesis that hybridization between *Zea* and *Tripsacum* played a crucial role in the evolution of domesticated maize.

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