Title

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GENOME ORIGINS OF *TRITICUM CYLINDRICUM*, *TRITICUM TRIUNCIALE*, AND *TRITICUM VENTRICOSUM* (POACEAE) INFERRED FROM VARIATION IN RESTRICTION PATTERNS OF REPEATED NUCLEOTIDE SEQUENCES: A METHODOLOGICAL STUDY[1]

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Three methods of phylogenetic inferences on polyploid plants employing variation in restriction sites in repeated nucleotide sequences were compared. Allotetraploid *Triticum* species of well-established origin were used as a model. Methods based on determination of the proportion of restriction fragments shared between a polyploid and its diploid relatives generated biased results because of uneven numbers of restriction fragments among diploid species and presence of common bands in phylogenetically related diploid species. A method employing restriction fragments unique to a diploid species (marker bands) was not affected by either factor and generated results consistent with cytogenetic inferences. It is shown that the latter method can be used to investigate the origin of a polyploid species even when one of its progenitors is extinct or when the polyploid and its diploid progenitors have diverged.

In this paper variation in the restriction sites in repeated nucleotide sequences is employed to investigate the origin of three tetraploid species in the genus *Triticum* L.—*T. cylindricum* (Host) Ces., Pass. & Gib., *T. triunciale* (L.) Raspail, and *T. ventricosum* (Tausch) Ces., Pass. & Gib. The origin of these allotetraploid species has been investigated by cytogenetic techniques, and the origin of each species is reasonably well understood. These species are used here as a model to scrutinize the methodology of inferring origin of allopolyploid taxa from variation in the sizes of restriction fragments of repeated nucleotide sequences.

Triticum cylindricum originated from hybridization of *T. caudatum* (L.) Godron et Gren. (C genome) with *T. tauschii* (Coss.) Schmalh (D genome) (Fig. 1). The presence of the genomes of these species in *T. cylindricum* was shown by investigation of chromosome pairing in interspecific hybrids (Kihara and Matsumura, 1941; Kimber and Zhao, 1983), karyotype analysis (Chennaveeraiah, 1960), variation in isozymes (Jaaska, 1981; Nakai, 1981) and gliadins (Masci et al., 1992), and hybridization of a repeated nucleotide sequence that preferentially hybridizes with the C genome (Baldauf, Schubert, and Metzlaff, 1992). Analysis of the chloroplast DNA (ctDNA) showed that *T. tauschii* contributed the cytoplasm of *T. cylindricum* (Tsunewaki, 1989).

The origin of *T. triunciale* from hybridization of *T. caudatum* with *T. umbellulatum* (Zhuk.) Bowden (U genome) (Fig. 1) was indicated by chromosome pairing in interspecific hybrids (Kihara, 1949; Kimber and Yen, 1989), karyotype analysis (Chennaveeraiah, 1960), isozyme variation (Fleischmann, 1990), and hybridization of a repeated nucleotide sequence preferentially hybridizing with the C genome (Baldauf, Schubert, and Metzlaff, 1992). Cytoplasms of both *T. caudatum* and *T. umbellulatum* were detected in different accessions of *T. triunciale* (Tsunewaki, 1989).

The evidence on the origin of *T. ventricosum* is less unambiguous. Chromosome pairing studies in interspecific hybrids clearly established the presence of the D genome in *T. ventricosum* (McFadden and Sears, 1946; Kimber and Zhao, 1983). The other genome was first considered a modified C genome (Kihara, 1940) and then a modified genome of the M group that includes *T. comosum* (Sibth. et Smith) Richter (M genome) and *T. uniaristatum* (Vis.) Richter (N genome) (Kihara, 1954). Kimber, Pignone, and Sallee (1983) and Yen and Kimber (1992) showed that the genome is closely related to the genome of *T. uniaristatum* (N genome). Karyotype analysis (Chennaveeraiah, 1960) also suggested the presence of a *T. tauschii* genome and a slightly modified *T. uniaristatum* genome. Analysis of the ctDNA of *T. ventricosum* showed that *T. tauschii* contributed its cytoplasm (Tsunewaki, 1989).

Amphiploids including the putative progenitors of *T. cylindricum* (McFadden and Sears, 1946), *T. triunciale* (Kihara and Kondo, 1943), and *T. ventricosum* (Matsumoto, Shimotsuma, and Nezu, 1957) have been synthesized. The resulting allotetraploids closely resembled the natural species and showed regular chromosome pairing when hybridized with the respective natural tetraploid species.

Variation in repeated nucleotide sequences is a potentially powerful tool to scrutinize the origin of allopolyploid taxa because of the particular way of evolution of repeated nucleotide sequences. Repeated nucleotide sequence families evolve in concert by repeated cycles of homogenization that result in a gradual turnover of sequences within families (Dover, 1982). Concerted evolution is a conservative process because it tends to eliminate rare sequences from the families (Birky and Skavaril, 1976; Smith, 1976; Dvorak, Jue, and Lassner, 1987). Via homogenization and recombination repeated nucleotide sequence families

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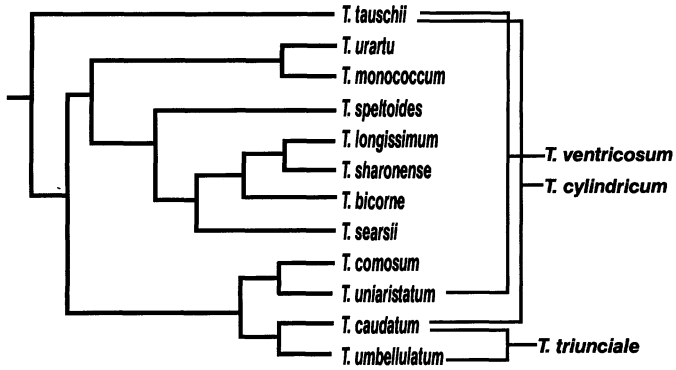

Fig. 1. Phylogenetic tree of the diploid species of *Triticum* based on variation in the restriction patterns of repeated nucleotide sequences (Dvorak and Zhang, 1992) and postulated origin of *T. cylindricum, T. triunciale,* and *T. ventricosum.*

maintain a high degree of homogeneity within species (Strachan, Webb, and Dover, 1985; Dvorak and Zhang, 1992).

At the interspecific level, divergence and amplification or deletions of sequences eventually result in an emergence of repeated nucleotide sequence families that hybridize with only a single species or a group of closely related species (Bedbrook et al., 1980; Prosnyak, Kartel, and Ryskov, 1985; Rayburn and Gill, 1986; Dvorak, McGuire, and Cassidy, 1988; Zhao et al., 1989; Crowhurst and Gardner, 1991; Raz, Puigdomènech, and Martínez-Izquierdo, 1991; Iwabuchi, Itoh, and Shimamoto, 1991; Hueros, Monte, and Ferrer, 1990; Zhang and Dvorak, 1990; Talbert et al., 1991, 1993; Baldauf, Schubert, and Metzlaff, 1992; Zentgraf, King, and Hemleben, 1992). Interspecific variation resulting in preferential hybridization of repeated nucleotide sequences with DNA of a specific genome represents only a fraction of variation that exists among species. A large amount of variation resides in the differences in restriction sites in nucleotide sequences which readily cross-hybridize among different species (Dvorak and Zhang, 1990, 1992; Talbert et al., 1991; Zhang and Dvorak, 1991, 1992; Zhang, Dvorak, and Waines, 1992; Dvorak et al., 1993). High degree of homogeneity within species and variation among species makes variation in the restriction patterns of repeated nucleotide sequences a potentially valuable resource for taxonomic studies, particularly for polyploid species.

MATERIALS AND METHODS

Plants—The materials used in this study, their accession numbers, and origins are listed in Table 1. *Triticum longissimum* (Sweinf. et Muschl.) Bowden was not included in this study because previous studies (Zhang and Dvorak, 1992; Zhang, Dvorak, and Waines, 1992) have failed to detect any bands that would unequivocally discriminate between this species and the closely related *T. sharonense* (syn. *Aegilops sharonensis* Eig.).

DNA hybridization—Nuclear DNAs were isolated from leaves of single plants following the procedure of Dvorak, McGuire, and Cassidy (1988). Restriction endonuclease digested DNAs were electrophoretically fractionated in 1.7% agarose gel and transferred to Hybond N+ nylon membrane (Amersham) by capillary transfer. Prehybridization and hybridization were performed in a rotary hybridization oven (National Labnet Company) at 65 C in a solution containing 1% sodium dodecyl sulphate (SDS), 2.5× SSC buffer, 0.1% polyethylensulfonic acid, and 0.01% sodium pyrophosphate. The immobilized DNAs were hybridized overnight with [α-^{32}P]-labeled probes by random primer method (Feinberg and Vogelstein, 1983). Probes were prepared from 35 pUC18 plasmids harboring fragments of repeated sequences isolated from libraries of nuclear DNA of *T. tauschii* (ten clones designated pTtUCD), *T. comosum* (seven clones designated pTcUCD), *T. longissimum* (six clones designated pTlUCD), *T. speltoides* (Tausch.) Gren. (three clones designated pTsUCD), *T. kostchyi* (Boiss.) Bouden (eight clones designated pTkUCD), and *T. urartu* Thum. (one clone designated pTuUCD) (Zhang and Dvorak, 1992; Zhang, Dvorak, and Waines, 1992; Dvorak et al., 1993; P. Resta and J. Dvorak, unpublished data). Inserted DNA fragments were excised with *Eco*RI-*Hind*III and isolated by electrophoresis or amplified using PCR. M13/pUC sequencing primer (−20) 17-mer and M13/pUC reverse sequencing primer (−48) 24-mer (New England Biolabs) were used for the amplifications; PCR products were purified with the Magic PCR purification Kit (Promega). The membranes were washed in 2× SSC and 0.5% SDS for 30 min, 1× SSC and 0.5% SDS for 30 min, and 0.2× SSC and 0.5% SDS for 30 min at 65 C. All accessions listed in Table 1 were hybridized with each probe. Generally, two enzyme-probe combinations were used per probe (70 enzyme-probe combinations).

Data analysis—*Polyploid-to-diploid direction*—Southern blots including 11 diploid species of *Triticum* and three tetraploid species—*T. cylindricum, T. triunciale,* and *T. ventricosum*—were hybridized with cloned repeated nucleotide sequences. All restriction fragments present in each polyploid were analyzed. The proportions of restriction fragments observed in a polyploid shared with each diploid were calculated by dividing the number of bands of a polyploid shared with a diploid by the total number of bands of the polyploid. The values were compared statistically using χ^2 test corrected for continuity.

Diploid-to-polyploid direction—Two methods of analysis in the diploid-to-polyploid direction were investigated. In the first method, the proportion of restriction fragments of a diploid shared with a polyploid was calculated by dividing the number of shared restriction fragments by the total number of restriction fragments of the diploid. Proportions of shared fragments obtained for different diploid species were compared by χ^2 test corrected for continuity.

In the second method, only marker bands for each diploid species were employed in the analysis (Dvorak and Zhang, 1990; Zhang and Dvorak, 1991; Zhang, Dvorak, and Waines, 1992). A marker band is defined as a restriction fragment that is observed in the restriction profiles of all analyzed accessions of a diploid species but not in those of other diploid species. Note that absence of a band in the restriction fragment profile of a particular species does not mean that the fragment is absolutely absent from the profile of that species. It means that it

TABLE 1. Sources of the plant materials used in the study.

Species	Accession	Origin	Source[a]
Triticum urartu Thum.	G3135	Lebanon	J. G. Waines
T. monococcum ssp. *aegilopoides* L.	G2528	Iran	J. G. Waines
T. speltoides (Tausch) Gren.	TS02	Israel	M. Feldman
T. sharonense nom. nud.	TH01	Israel	M. Feldman
T. bicorne Forssk	TB10	Israel	M. Feldman
T. searsii nom. nud.	TE27	Israel	M. Feldman
T. caudatum (L.) Godron et Gren.	Rub 74	Unknown	E. R. Sears
	PI551120	Greece	H. E. Bockelman
	PI254863	Iraq	H. E. Bockelman
	PI542197	Turkey	H. E. Bockelman
	PI263554	Turkey	H. E. Bockelman
T. comosum (Sibth. et Smith) Richter	G659	Unknown	J. G. Waines
ssp. *eucomosum*	G601	Unknown	J. G. Waines
ssp. *heldreichii*	G5037	Greece	J. G. Waines
ssp. *heldreichii*	G5034	Turkey	J. G. Waines
var. *thesalicum*	G1515	Greece	J. G. Waines
var. *thesalicum*	G3566	Unknown	J. G. Waines
T. uniaristatum (Vis.) Richter	G3586	Turkey	J. G. Waines
	G1439	Greece	J. G. Waines
	G3585	Turkey	J. G. Waines
	G1297	Turkey	J. G. Waines
	G1296	Greece	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G1164	Turkey	J. G. Waines
	G1210	Turkey	J. G. Waines
	G3772	Unknown	J. G. Waines
T. tauschii (Coss.) Schmalh.	KU2075	Iran	Kyoto Univ.
	KU2025	Afghanistan	Kyoto Univ.
	KU2377	Iran	Kyoto Univ.
	KU2001	Pakistan	Kyoto Univ.
T. cylindricum (Host) Ces., Pass. & Gib.	PI374380	Yugoslavia	H. E. Bockelman
	PI172357	Turkey	H. E. Bockelman
	PI298893	Afghanistan	H. E. Bockelman
	PI276977	Israel	H. E. Bockelman
T. triunciale (L.) Raspail	1565	Unknown	K. Tsunewaki
var. *eu-triuncialis*	1563	Unknown	K. Tsunewaki
	A-Cam 3	Unknown	R. Johnson
	1568	Unknown	K. Tsunewaki
var. *orientalis*	1572	Afghanistan	K. Tsunewaki
T. ventricosum (Tausch) Ces., Pass. & Gib.	PI369658	Unknown	H. E. Bockelman
	PI276999	Unknown	H. E. Bockelman
	PI330493	Unknown	H. E. Bockelman
	PI266823	Unknown	H. E. Bockelman
	PI388753	Morocco	H. E. Bockelman

[a] M. Feldman, Weizmann Inst. Science, Rehovot, Israel; E. R. Sears, University of Missouri, Columbia, MO; H. E. Bockelman, National Small Grain Collection, USDA-ARS, Aberdeen, ID; J. G. Waines, University of California, Riverside, CA; K. Tsunewaki, Mitsui Plant Biotechnology Research Institute, Tsukuba, Japan; R. Johnson, John Innes Institute, Norwich, UK.

was not detected in the autoradiograms of the same Southern blot which revealed the band in the profiles of a species for which it is a marker. When autoradiograms are overexposed, faint bands may occasionally appear in other diploid species. The marker band was still employed in the analysis if its intensity, measured by a laser densitometer, was at least one order of magnitude higher than that of the faint bands.

Marker bands were also identified for groups of diploid species as done previously by Zhang, Dvorak, and Waines (1992). The species were grouped according to their hypothetical phylogeny so that the groups represented each internode in the phylogenetic tree of *Triticum* based on variation in repeated nucleotide sequence restriction fragments (Dvorak and Zhang, 1992).

The fraction of marker bands of a diploid shared with a polyploid is called repeated nucleotide sequence correspondence (RSC, synonymous with the term RSI, repeated nucleotide sequence identity [Dvorak and Zhang, 1990; Zhang and Dvorak, 1991; Zhang, Dvorak, and Waines, 1992]). The RSC varies from 0.00, if no marker band of a diploid or a group of diploids is encountered in a polyploid, to 1.00, if all are. The relationship between RSC and the presence of a genome in a polyploid is not absolute because of the possibility of evolutionary reversals and parallelisms in the amplification or loss of sequence variants. The RSCs were compared statistically with each other and with the extremes, 1.00 and 0.00, using 2×2 contingency tables. Test of χ^2 corrected for continuity gives an approximate solution. Fisher's exact test (SAS Institute, Inc., 1986) is more appropriate when the total number of bands is less than 25 or some cell has values lower than five. Since this was the case for most RSC values, Fisher's exact test was employed.

The autoradiograms of the restriction profiles obtained with different clones were compared with each other to

eliminate duplicates. In some cases only a subset of bands in two profiles was common, usually involving the high molecular weight fraction. In that case the common bands were included only once in the analysis.

To express intraspecific variation in the bands obtained in the Southern blots, average band diversity (Bd) was calculated according to equation 1:

$$\mathrm{Bd} = \frac{\sum_{i=1}^{N} [1 - (p/K)^2 - (a/K)^2]}{N}, \quad (1)$$

where N is the total number of bands analyzed, K is the number of DNAs analyzed, p is the number of DNAs where the band is present, and a is the number of DNAs where the band is absent. Bd varies from 0 (all bands monomorphic) to 0.5 (all bands present in half of the accessions). Bd gives the probability that two accessions were different for a randomly chosen band. Equation [1] is equivalent to that proposed by Nei (1973) for gene diversity. However, the repeated sequence bands are not alleles. Repeated nucleotide sequences are usually interspersed, and each band generally represents more than one locus in the genome. Presence or absence of a band in a particular accession indicate either presence or absence of that fragment in the specific genome or more likely, that the copy number of that fragment is above or below the detection threshold for the hybridization conditions used. The distribution of individual band diversities is usually L-shaped with most bands being monomorphic and having zero band diversity. Since t-tests are not appropriate for this asymmetric distribution (Archie, 1985), Bds were compared using Kolmogorov-Smirnov nonparametric test for two samples (SAS Institute, Inc., 1986).

RESULTS

Of 753 bands analyzed, 233 were present in the profiles of all diploid species and were discarded as noninformative. The remaining 520 bands were present in a subset of diploid species or in a single diploid species. The numbers of bands varied from 127 bands in *T. tauschii* to 236 bands in *T. caudatum* and *T. umbellulatum* among the diploid species. The total number of bands in *T. tauschii* was significantly lower than that found in other diploid species (χ^2, $P < 0.01$, except for *T. urartu* $P = 0.06$).

Polyploid-to-diploid direction—Of 266 bands found in *T. triunciale* (CU genomes), the highest proportion was shared with *T. caudatum* (C genome) and *T. umbellulatum* (U genome), followed by *T. uniaristatum* (N genome) and *T. comosum* (M genome). The lowest proportion was found in *T. tauschii* (D genome) (Fig. 2).

A similar distribution was found for 265 bands present in *T. cylindricum* (CD genomes), except that the proportion of bands of this species shared with *T. umbellulatum* diminished and the number of bands shared with *T. tauschii* increased (Fig. 2). However, the proportion of shared bands with *T. tauschii* was still lower than with *T. umbellulatum* and several other diploid species (Fig. 2).

Of 250 bands found in *T. ventricosum* (DN genomes) the highest proportion was found in *T. uniaristatum* followed by *T. comosum* (Fig. 2). The proportion of *T. ventricosum* bands shared with *T. tauschii* was similar to the proportion of bands of *T. cylindricum* shared with *T. tauschii* and was lower than the proportion of *T. ventricosum* bands shared with the majority of the diploid species.

All bands found in *T. cylindricum* and *T. triunciale* were found in their diploid ancestors. All bands found in *T. ventricosum* except one were found in its diploid progenitors. The exception was a pTlUCD2 *Taq*I band which was not present in any diploid species.

Diploid-to-polyploid direction: Shared bands—Different results were obtained when the analysis was reversed and performed in the diploid-to-polyploid direction. When the numbers of shared bands between each diploid species and *T. triunciale* were divided by the total number of bands in each diploid, *T. caudatum* and *T. umbellulatum* showed significantly higher proportions of bands shared with *T. triunciale* ($P < 0.01$) than other diploid species (Fig. 2).

The proportions of bands of *T. caudatum* and *T. tauschii* shared with *T. cylindricum* were higher than for the rest of the diploid species ($P < 0.01$). The proportion of *T. umbellulatum* bands shared with *T. cylindricum* was lower than those from the two previous species ($P < 0.01$) but still higher ($P < 0.05$) than the proportions of shared bands of the remaining diploid species with *T. cylindricum* (Fig. 2).

Triticum uniaristatum and *T. tauschii* exhibited the highest proportions of bands shared with *T. ventricosum* ($P < 0.01$), followed by *T. comosum.*

Diploid-to-polyploid direction: Marker bands—In this analysis only marker bands for each diploid were used. Of the 520 informative bands, 132 were marker bands (Table 3). Since scoring more than one marker band per probe (Table 2) could result in scoring the same evolutionary event more than once, two versions of this analysis were performed. In version one, all marker bands were considered, and in the other, only a single marker band per probe was considered (Table 2, numbers in parentheses). However, if a probe showed two marker bands in a diploid species but only one in a polyploid, both marker bands were considered since they must have resulted from independent evolutionary events. Although the use of single marker bands per probe reduced the numbers of marker bands by 30%, none of the RSCs differed significantly ($P > 0.05$) from RSCs obtained by using all marker bands.

All marker bands of *T. caudatum* and *T. tauschii* were found in *T. cylindricum* (Table 3; Figs. 2–4), while a marker band for no other diploid was found (Fig. 2). A perfect correspondence was also found between *T. umbellulatum* and *T. caudatum* and *T. triunciale* (Figs. 2, 4) while a marker band for no other diploid was found (Fig. 2). All but one marker band of *T. tauschii* and all but one marker band of *T. uniaristatum* were found in *T. ventricosum* (Fig. 3). As in the previous polyploids, a marker band for no other diploid was found.

Diploid-to-polyploid analysis using marker bands was further performed for groups of species. The diploid *Triticum* species were grouped according to their position in

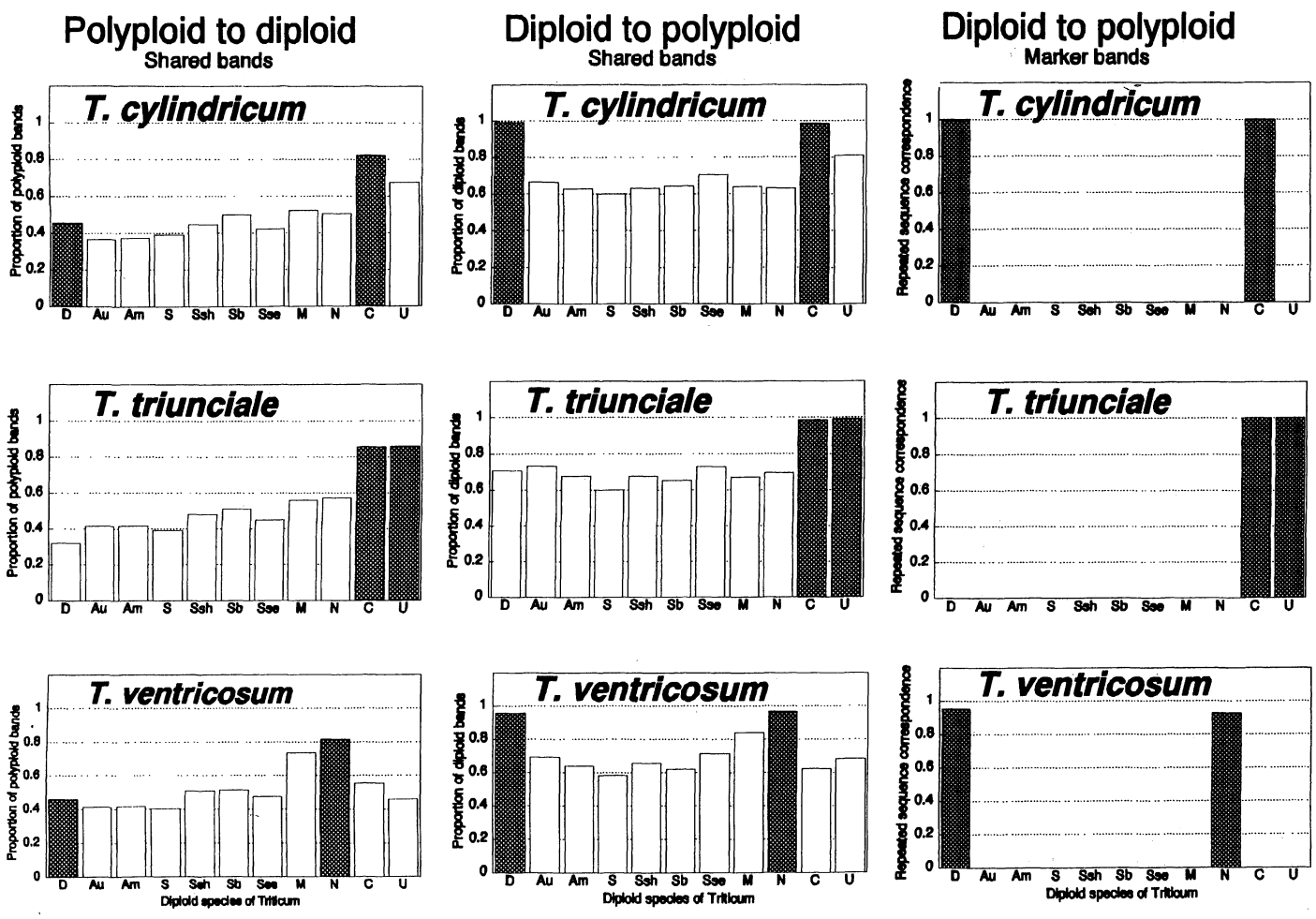

Fig. 2. Left—Proportion of restriction fragments from *T. cylindricum, T. triunciale,* and *T. ventricosum* shared with each diploid species of *Triticum.* Middle—Proportion of restriction fragments from each diploid species shared with *T. cylindricum, T. triunciale,* and *T. ventricosum.* Right—Proportion of marker bands from each diploid species (RSC) shared with *T. cylindricum, T. triunciale,* and *T. ventricosum.* The diploid species were *Triticum tauschii* (D), *T. urartu* (A^u), *T. monococcum* (A^m), *T. speltoides* (S), *T. sharonense* (S^{sh}), *T. bicorne* (S^b), *T. searsii* (S^{se}), *T. comosum* (M), *T. uniaristatum* (N), *T. caudatum* (C), and *T. umbellulatum* (U). Cross-hatched bars represent the known diploid ancestors of teraploid species based on cytogenetic and isozyme evidence.

the phylogenetic tree of *Triticum* reconstructed from variation in repeated nucleotide sequences (Fig. 1).

Marker bands for the groups of species representing internodes in the branches leading to *T. caudatum,* C + U, M + N + C + U, and all *Triticum* species including or excluding *T. tauschii,* which represents the basal lateral branch in the phylogenetic tree (Fig. 1), were all found in *T. cylindricum* and showed RSCs of 1.0 or close to 1.0 with it (Table 3). Marker bands for groups representing internodes of branches not leading to *T. caudatum* were not found in *T. cylindricum,* and their RSCs with *T. cylindricum* were zero.

The same findings were made for *T. triunciale.* Most marker bands for the groups of species representing internodes of the branch leading to *T. uniaristatum* showed complete correspondence with *T. ventricosum* (Table 3). Marker bands for the groups of species representing internodes in branches that did not lead to *T. uniaristatum* were all absent from *T. ventricosum,* and their RSC values with *T. ventricosum* were universally zero.

Extinction of a diploid progenitor—The effects of the absence of a progenitor from the repertoire of diploid relatives available for analysis or the extinction of a diploid progenitor of a polyploid were investigated by eliminating individually *T. tauschii, T. caudatum, T. umbellulatum, T. uniaristatum,* and *T. comosum* from the analysis (Table 4).

When all bands were analyzed (polyploid-to-diploid direction), extinction (absence) of a diploid progenitor of a polyploid resulted in an increase in the number of bands found in the polyploid that were not found in any diploid species. When only marker bands were considered (diploid-to-polyploid direction), extinction (absence) of a progenitor resulted in finding only a single diploid species with an RSC of 1.00 or near 1.00. Thus, the absence of *T. caudatum* in the analysis of *T. cylindricum* resulted in its nearest relative, *T. umbellulatum,* appearing to be one of the putative ancestors of *T. cylindricum.* We propose to call this erroneously identified progenitor, defined by the above criteria, as pseudoprogenitor. While RSC of *T.*

TABLE 2. Probe-enzyme combinations that resulted ln detection of marker bands.

Species or group of species	Enzyme-probe combinations
A^{u} [a]	c^{b}2(T1[c]), u19(T1)
A^{m}	c1(A1), c2(H1,T2), c6(H1), k4(T1), l1(M1), t1(T1), u19(T2)
S	c2(T1), c4(D1), c6(H2), k1(D1), k3(T1), k9(H1), l1(Dr2), l2(T1,M1), l3(T1), l4(D2,M1), l6(H1), s2(M1), s6(D1), t1(T2), t6(T1), t7(D2), t8(H2,A2), u19(H1)
S^{s} or S^{s} + S^{l}	c2(T1), c7(T1), k3(T1), t8(A1)
S^{b}	c1(A1), c7(H1), l1(A1), l3(A1), l4(M2), t8(H1)
S^{se}	c1(A1), c4(Dr1), k2(A1)
C	c4(D1), c5(A1,H2), c6(H1), k4(H1), k9(Dr2), l3(A2), l4(M1), l6(A2,H2), t1(T1), t3(H1), t8(H2), t10(T1), u19(T1)
M	c2(H1), c4(Dr2), k3(H1), k4(A1), l1(M3), l2(T1), l3(A1), t10(T2)
N	c2(H1), k3(H3,T1), l1(Dr3,M1), l2(T2,M1), t3(H1), u19(H1)
U	c2(T1), c4(Dr1), k3(T1), k4(A1), l2(T1), l10(H1), t10(A2), u19(T1)
D	c4(Dr1), c6(H1), k2(D2,T1,A2), k4(H1), k6(A1), k10(T1), l4(D2), 16(H3), l10(D1), s2(A1), t7(Dr2), t1(Dr1), t10(A2)
A^{u} + A^{m}	k2(A1), k3(H1,T2), k4(A1,T1), s4(D1), t5(T1), t7(Dr1), u19(T5,H2)
S^{s} + S^{b} + S^{se}	c1(T1), c3(T1), 16(H1), l10(D2), k9(H1), s4(D1), t10(T1)
S + S^{s} + S^{b} + S^{se}	c6(A1), k1(D1), k2(A1,T1), k3(T2), k8(H1), l2(T1), l3(A1), s4(D1), t2(H1)
C + U	c3(T2,H1), c6(A1), k2(D1), k3(T1), k9(H2), l3(T1), l4(D3), l6(H1), l10(H1), t2(H2,T1), t8(A2)
M + N	c2(T1), c3(T1), c5(A1), c6(A1), c7(T1), k10(M2), l6(A3,H1), s4(D1), t2(H1,T1), t3(H1), t6(T1), t8(A1)
C + M + N + U	c2(H1), c7(H1), k2(A1), t10(T2)
All sp. − D	c2(H1), c3(T1), c6(H1), k2(A1,T1), k3(H1,T1), k4(T1), k5(A1), k6(A1), k8(T1), k10(T1), t5(A1), t6(A1), t9(T1)

[a] *Triticum urartu* (A^{u}), *T. monococcum* (A^{m}), *T. speltoides* (S), *T. sharonense* (S^{s}), *T. longissimum* (S^{l}), *T. bicorne* (S^{b}), *T. searsii* (S^{se}), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), *T. tauschii* (D).

[b] Sources of clones were *T. comosum* (c), *T. kostchyi* (k), *T. longissimum* (l), *T. speltoides* (s), *T. tauschii* (t), and *T. urartu* (u).

[c] Indicates restriction endonuclease and number of marker bands detected. A = *Alu*I, D = *Dde*I, Dr = *Dra*I, H = *Hae*III, M = *Mbo*I, T = *Taq*I.

tauschii was still 1.00, that of the pseudoprogenitor, *T. umbellulatum,* was only 0.64. Similarly, the absence of *T. uniaristatum* from the diploid species in the analysis of *T. ventricosum* indicated that *T. comosum* was one of the progenitors of *T. ventricosum.* However, while RSC of *T. tauschii* was 0.95, RSC of *T. comosum* was only 0.58 (Table 4).

A situation where the diploid-to-polyploid analysis failed to indicate the absence of one of the progenitors by low RSC was when the parents of a polyploid were species that evolved by a terminal dichotomy, as in *T. triunciale* (Table 4). In that case the progenitor available for analysis was potentially both the progenitor and pseudoprogenitor. Theoretically, the absence of one of such progenitors of an allotetraploid could be detected by double intensity of marker bands corresponding to the internode preceding the dichotomy. Thus, in the case of *T. triunciale* the marker bands for the internode preceding the divergence of *T. caudatum* and *T. umbellulatum* (Fig. 1) should be present in double dose in *T. triunciale* since they are

TABLE 3. Repeated nucleotide sequence correspondences (RSCs) of *T. cylindricum, T. triunciale,* and *T. ventricosum* with each diploid species and group of species of *Triticum* representing internodes in the phylogenetic tree (Dvorak and Zhang, 1992). Number of marker bands and RSCs obtained when only a single marker band per probe was used are in parentheses.

Species or group of species	No. of marker bands	*Triticum cylindricum*		*Triticum triunciale*		*Triticum ventricosum*	
		No.	RSC	No.	RSC	No.	RSC
A^{u}	2 (2)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
A^{m}	10 (7)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S	28 (18)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S^{s}	4 (4)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S^{b}	7 (6)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S^{se}	3 (3)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
C	21 (13)	21 (13)	1.00 (1.00)	21 (13)	1.00 (1.00)	0 (0)	0.00 (0.00)
M	12 (8)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
N	14 (6)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	13 (5)	0.93[a] (0.83)[a]
U	5 (5)	0 (0)	0.00 (0.00)	5 (5)	1.00 (1.00)	0 (0)	0.00 (0.00)
D	22 (14)	22 (14)	1.00 (1.00)	0 (0)	0.00 (0.00)	21 (13)	0.95[a] (0.93)[a]
A^{u} + A^{m}	16 (7)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S^{s} + S^{b} + S^{se}	8 (7)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
S + S^{s} + S^{b} + S^{se}	11 (9)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)
C + U	19 (11)	18 (10)	0.95 (0.91)	19 (11)	1.00 (1.00)	0 (0)	0.00 (0.00)
M + N	17 (12)	0 (0)	0.00 (0.00)	0 (0)	0.00 (0.00)	17 (12)	1.00 (1.00)
C + M + N + U	5 (4)	5 (4)	1.00 (1.00)	5 (4)	1.00 (1.00)	5 (4)	1.00 (1.00)
All − D	15 (13)	15 (13)	1.00 (1.00)	15 (13)	1.00 (1.00)	15 (13)	1.00 (1.00)
All *Triticum*	213 (33)	213 (33)	1.00 (1.00)	213 (33)	1.00 (1.00)	213 (33)	1.00 (1.00)

[a] Significantly different from 0 but not from 1 ($P < 0.01$). *Triticum urartu* (A^{u}), *T. monococcum* (A^{m}), *T. speltoides* (S), *T. sharonense* (S^{s}), *T. bicorne* (S^{b}), *T. searsii* (S^{se}), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), *T. tauschii* (D).

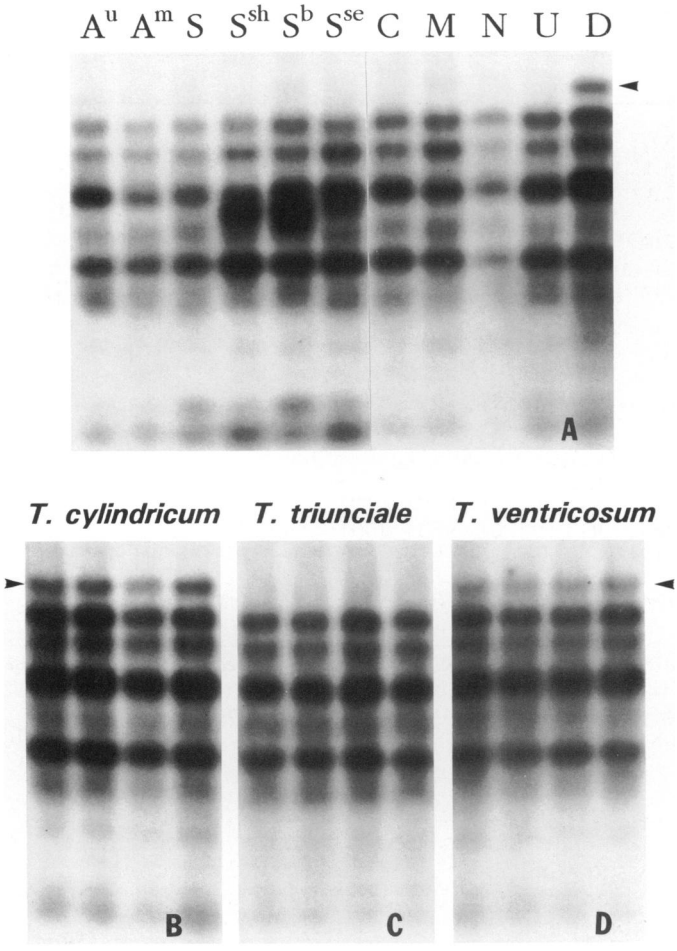

Fig. 3. Probe pTlUCD10, restriction enzyme *Dde*I. A. *Triticum tauschii* (genome D) marker band (arrowhead) in a screening panel including *Triticum urartu* (A^u), *T. monococcum* (A^m), *T. speltoides* (S), *T. sharonense* (S^{sh}), *T. bicorne* (S^b), *T. searsii* (S^{se}), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), and *T. tauschii* (D). B. Presence of the *T. tauschii* marker band in all *T. cylindricum* (genomes CD) accessions. C. Absence of the *T. tauschii* marker band in all *T. triunciale* (genomes CU) accessions. D. Presence of the *T. tauschii* marker band in all *T. ventricosum* (genomes DN) accessions.

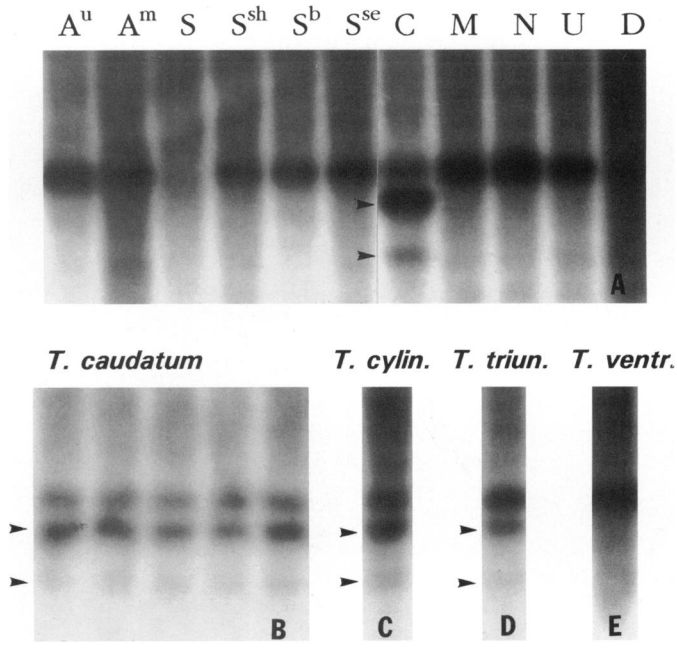

Fig. 4. Probe pTkUCD9, restriction enzyme *Dra*I. A. *Triticum caudatum* (genome C) marker bands (arrowhead) in a screening panel including *Triticum urartu* (A^u), *T. monococcum* (A^m), *T. speltoides* (S), *T. sharonense* (S^{sh}), *T. bicorne* (S^b), *T. searsii* (S^{se}), *T. caudatum* (C), *T. comosum* (M), *T. uniaristatum* (N), *T. umbellulatum* (U), and *T. tauschii* (D). B. Presence of the marker bands in all *T. caudatum* accessions. C. Presence of *T. caudatum* marker bands in *T. cylindricum* (genomes CD). D. Presence of *T. caudatum* marker bands in *T. triunciale* (genomes CU). E. Absence of *T. caudatum* marker bands in *T. ventricosum* (genomes DN).

present in the genomes of both parents. This will, however, only be true if the numbers of copies of the specific DNA fragment were similar in the two progenitors and have not changed during the evolution of the polyploid. In the example of *T. triunciale* most bands that mark the progenitor of *T. umbellulatum* and *T. caudatum* do not satisfy these requirements, and inferences based on quantification of band intensities are likely to be of a questionable value.

Intraspecific variation of the bands—Bd calculated for five diploid species of *Triticum* ranged from 0.0028 in *T. tauschii* to 0.0084 in *T. comosum* (*T. uniaristatum* Bd = 0.0038, *T. umbellulatum* Bd = 0.0057, and *T. caudatum* Bd = 0.0058). Differences among species were not significant (Kolmogorov-Smirnov $P > 0.05$).

Since the origins of marker bands that differentiate diploid species represent sequence variants that are of relatively recent origin, marker bands might be expected to

TABLE 4. Effect of the absence of a progenitor of a polyploid in the population of investigated diploids on the RSC of a species most closely related to the progenitor (pseudoprogenitor), on the numbers of progenitors detected, and the numbers of bands in polyploids not shared with any diploid species.

Absent putative ancestor	Closest relative (pseudoprogenitor)	RSC of closest relative (no. of progenitors inferred)			No. polyploid bands absent in diploids		
		T. cyl. DC	*T. tri.* CU	*T. ven.* *DN*	*T. cyl.* DC	*T. tri.* CU	*T. ven.* DN
T. tauschii (D)	None[a]	— (1)	— (1)	— (1)	22	0	22
T. caudatum (C)	*T. umbellulatum* (U)	0.64[b] (2)	1.00 (1)	0.00 (2)	21	21	0
T. umbellulatum (U)	*T. caudatum* (C)	0.97 (2)	1.00 (1)	0.00 (2)	0	9	0
T. uniaristatum (N)	*T. comosum* (M)	0.00 (2)	0.00 (2)	0.58[b] (2)	0	0	14

[a] When *T. tauschii* was eliminated from the sample no diploid species appeared as a pseudoprogenitor because *T. tauschii* diverged from the rest of the genus at the basal internode of the phylogenetic tree.

[b] Significantly different from 0 and 1 ($P < 0.05$).

be more variable than other bands. Out of 134 bands that appeared to be marker bands on the basis of investigation of a single accession, only one *T. tauschii* and one *T. caudatum* band were found variable and discarded after screening the remaining accessions of the species. Thus, the marker bands in this study did not appear more variable than the rest of the bands employed.

DISCUSSION

The levels of intraspecific variation in repeated nucleotide sequences in five diploid *Triticum* species analyzed here and in another group of diploid *Triticum* species analyzed by Dvorak and Zhang (1992) were universally low. This high homogeneity within species and ease of obtaining large quantities of data make the restriction profiles of repeated nucleotide sequences an excellent new tool for phylogenetic studies in *Triticum* and other genera of the tribe *Triticeae.*

The method employing marker bands generated a clear picture of the phylogeny for all three allotetraploids investigated here. *Triticum cylindricum* genomes appeared to be contributed by *T. tauschii* and *T. caudatum,* those of *T. triunciale* by *T. caudatum* and *T. umbellulatum,* and those of *T. ventricosum* by *T. tauschii* and *T. uniaristatum.* These inferences fully agree with evidence obtained by analysis of chromosome pairing in artificial hybrids, karyotypes, isozymes, and ctDNA.

While the method employing marker bands yielded a clear picture, the other methods investigated here generated less unambiguous results. The proportion of bands of *T. cylindricum* and *T. ventricosum* shared with the diploid species failed to provide evidence that *T. tauschii* was one of the progenitors of these two species. While one genome of *T. cylindricum* and *T. ventricosum* was correctly identified, the other (D genome) would have been misidentified as the genomes of *T. umbellulatum* and *T. comosum,* respectively. The reason for these false inferences is in the uneven numbers of repeated nucleotide sequence bands among the diploid species and in the presence of common bands among phylogenetically related diploids. The same problems were encountered when the relationships were investigated using the formula of Nei and Li (1979) to estimate the proportion of shared bands (data not shown).

When the numbers of shared bands between a diploid and a polyploid were expressed as a proportion of the total numbers of bands per diploid, the bias due to uneven number of bands per diploid was eliminated. The putative ancestral diploid species showed significantly higher proportions of shared bands with the polyploids than the remaining diploid species. However, the bias due to phylogenetic relationships among the diploid species was not eliminated. When the frequencies of shared bands between the diploids and polyploids were compared with the putative phylogeny of the diploids the proportions of shared bands decreased with increasing phylogenetic distance of the diploids from the actual source of a genome of a polyploid. For example, although *T. uniaristatum* and *T. tauschii* showed the highest proportion of shared bands with *T. ventricosum, T. comosum* also showed high proportions of shared bands. This clearly follows the degree of relatedness of this species with *T. uniaristatum,* as indicated by the phylogenetic tree of *Triticum* (see Fig. 1). Since bands observed in a diploid include not only those that are unique to the diploid but also those that are common with its relatives, this method is likely to provide misleading pictures.

Neither of these factors affected the analysis based on marker bands since this analysis employs only bands that are unique to a diploid species; the bands shared among diploid species are not considered to be informative in the analysis of extant diploid species.

The technique based on marker bands can be used to examine the relationships between a polyploid and extinct diploid species, provided that the phylogeny of the extant diploid relatives of the polyploid has been reconstructed. Assume that an extinct species representing an internode in the phylogenetic tree was the progenitor of a polyploid. It seems reasonable that the DNA variants that are common to all extant diploid species that evolved from this species, but are absent in all other species, already were present in this extinct species; hence, they can be considered to be markers for this extinct species. Both present data and those reported by Zhang, Dvorak, and Waines (1992) showed that most marker bands for the internodes of the branch leading to an extant diploid ancestor of an investigated polyploid were encountered in the polyploid, whereas marker bands for other internodes in the phylogenetic tree were not encountered in the polyploid. Thus, if a diploid ancestor of a polyploid is extinct, marker bands for the internode from which it branched off, or which it represented, will be present in the polyploid. Hence, the most recent internode in a branch showing a high RSC with the polyploid is expected to be in the greatest proximity of the source of one of the genomes of the polyploid.

In most cases, the absence of a progenitor is indicated by finding an RSC for the nearest relative of the absent progenitor that is significantly different from either extreme, 0.00 and 1.00. A reduced RSC will not be observed if only a few repeated sequence markers were scored for the nearest relative or if two species that evolved by a terminal dichotomy were the actual progenitors of an allotetraploid (the case of *T. triunciale*). In the latter case, only one progenitor showing a high degree of correspondence would be detected. Since the absence of an ancestral diploid results in the presence of bands in a polyploid that are not found in any of the investigated diploid species, phylogeny should be investigated in both directions to achieve a maximal resolution.

Finally, methodology based on marker bands makes it possible to discriminate between a failure to identify both progenitors of an allotetraploid because of extinction (absence) of one of its diploid progenitors and a failure caused by evolutionary divergence of the genomes reflecting ancient origin of the polyploid. If the former alternative is the case, only one of the RSCs with the putative progenitors will be low, whereas if the latter alternative is the case both will be low. The latter situation has been described in the *T. crassum* Aitch. & Hemsl. complex (Zhang and Dvorak, 1992). In the present investigation, the only tetraploid that showed some divergence of its genomes from the genomes of its progenitors is *T. ventricosum.* One marker band of each of its progenitors was absent from *T. ventricosum,* and one band from this polyploid was not present in any of the diploid *Triticum* species.

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