Title

The Origins of the Genomes of Triticum biunciale, T. ovatum, T. neglectum, T. columnare, and T. rectum (Poaceae) Based on Variation in Repeated Nucleotide Sequences

Journal

American Journal of Botany, 83(12)

ISSN

0002-9122

Authors

Resta, Paolo
Zhang, Hong-Bing
Dubcovsky, Jorge
et al.

Publication Date

1996-12-01

DOI

10.2307/2445829

Peer reviewed

American Journal of Botany 83(12): 1556–1565. 1996.

THE ORIGINS OF THE GENOMES OF *TRITICUM BIUNCIALE, T. OVATUM, T. NEGLECTUM, T. COLUMNARE,* AND *T. RECTUM* (POACEAE) BASED ON VARIATION IN REPEATED NUCLEOTIDE SEQUENCES[1]

PAOLO RESTA, HONG-BING ZHANG, JORGE DUBCOVSKY, AND JAN DVOŘÁK[2]

Department of Agronomy and Range Science, University of California, Davis, California 95616

The origins of the genomes of allotetraploid species *Triticum biunciale, T. ovatum, T. neglectum,* and *T. columnare,* and allohexaploid *T. rectum* were investigated by examining the presence of specific restriction fragments of repeated nucleotide sequences in DNAs of the polyploid species. The restriction fragments were detectable either in a single diploid *Triticum* species (unique characters) or a group of diploid species (unique shared characters). The analysis showed that *Triticum biunciale* and T. *ovatum* are closely related. In both species, one pair of genomes is closely related to the genome of *T. umbellulatum* and the other is a modified genome of *T. comosum.* The same genome formula, UUM°M°, is proposed for *T. biunciale* and *T. ovatum.* Potential reasons for the modification of the M° genome are discussed. *Triticum neglectum* and *T. columnare* are also closely related to each other and have the same genomes. They share the U genome with *T. biunciale* and *T. ovatum,* but their second pair of genomes is unrelated to the M° genome. No relationship was found of this genome to a genome of any extant diploid species of *Triticum* or any phylogenetic lineage leading to the extant diploid species. This unknown genome is designated X^t. The proposed genome formula for *T. neglectum* and *T. columnare* is UUX^tX^t. Hexaploid *T. rectum* originated from hybridization of one of the tetraploid species with the formula UUX^tX^t, likely *T. neglectum,* with *T. uniaristatum* (genome N), and its genome formula is UUX^tX^tNN.

Key words: *Aegilops*; genome analysis; phylogeny; polyploid; repeated nucleotide sequences; *Triticum.*

Triticum biunciale (Vis.) K. Richter, *Triticum ovatum* Raspail, *T. neglectum* Bowden, and *T. columnare* Bowden are allotetraploid species ($2n = 4x = 28$) and *T. rectum* (Zhuk.) Bowden is an allohexaploid species ($2n = 6x = 42$). They are morphologically similar (Kimber and Feldman, 1987). Their origin has been a subject of an extended debate because the classical approach to phylogenetic analysis of polyploid plants, the investigation of chromosome pairing in interspecific hybrids, has failed to determine unequivocally their origin.

Early chromosome pairing studies revealed that all five species have one pair of genomes closely related to the genome of *T. umbellulatum* (Zhuk.) Bowden (Kihara, 1963). The evidence was indirect, based largely on studies of hybrids between polyploid species of which one species had previously been shown to have the genome of *T. umbellulatum* (Berg, 1937; Kihara, 1937, 1940, 1949; Kimber, Sallee, and Feiner, 1988). More recently, hybrids were made between each of the four tetraploid species and an artificially produced autotetraploid *T. umbellulatum* (Kimber and Yen, 1989; Yen, 1990). Numerical analysis of metaphase I (MI) chromosome pairing in these hybrids showed directly that the tetraploid species have the genome of *T. umbellulatum.*

The origin of the second pair of genomes of the four tetraploid species and the second and third pairs of genomes of hexaploid *T. rectum* has not been satisfactorily determined. Kihara assumed that these genomes were closely related to a basic genome which he designated M (Kihara, 1963). The evidence for the existence of the M genome in these four tetraploid species is based on an observation of modal seven bivalents, five closed, in a hybrid between *T. comosum* (Sibth. & Smith) Richter ($2n = 2x = 14$, genomes MM) and *T. ovatum* (Kihara, 1949). However, a hybrid *T. uniaristatum* (Vis.) K. Richter ($2n = 2x = 14$; genomes M^uM^u according to Kihara, 1963) × *T. biunciale* showed only two to five bivalents per cell, a hybrid *T. uniaristatum* × *T. columnare* showed four to six bivalents per cell, none closed, a hybrid *T. comosum* × *T. columnare* showed three to seven bivalents per cell, a maximum one closed (Kihara, 1949), and a hybrid *T. comosum* × *T. neglectum* showed only two to three bivalents per cell (Tsuchiya, 1956). To resolve these conflicting findings, Kihara concluded that the M-genome taxa are in the state of active speciation and that the M genomes are modified relative to each other. According to Kihara (1963), the principal reason for the incomplete homology of the M genomes at the polyploid level was genome divergence associated with speciation at the diploid level and possible extinction of some of the diploid taxa.

Kihara (1963) believed that *T. comosum* and *T. uniaristatum* share a common genome, in spite of the fact that a hybrid he obtained between them showed a range of only four to six bivalents per cell (Kihara, 1937). A hybrid between *T. comosum* ssp. *heldreichii* Holzm. and *T. uniaristatum,* with higher pairing, ranging from five to seven bivalents, was reported by Percival (1932). Reinvestigation of the relationship between the genomes of *T. comosum* and *T. uniaristatum* revealed that they do not have the same basic genome (Kimber, Pignone, and Sallee, 1983) and their genomes were redesignated M for

[1] Manuscript received 26 October 1995; revision accepted 11 July 1996.

[2] Author for correspondence.

the genome of *T. comosum* and N for the genome of *T. uniaristatum.* Phylogenetic trees of the genus *Triticum* consistently show *T. comosum* and *T. uniaristatum* as sister taxa (Ogihara and Tsunewaki, 1988; Dvořák and Zhang, 1992).

The pivotal genome hybridization hypothesis of Zohary and Feldman (1962) is an alternative to Kihara's explanation of the M genome modification in these polyploid species. Zohary and Feldman based their hypothesis on the existence of natural hybridization among polyploid *Triticum* species (Feldman, 1965; Pazy and Zohary, 1965; Zohary and Feldman, 1965) and suggested that hybridization of tetraploid species sharing the genome of *T. umbellulatum* but differing in the second pair of genomes results in recombination of the differential genomes. The common genome was viewed as a pivot that ensures sufficient fertility of the hybrids and facilitates recombination of the differential genomes (Zohary and Feldman, 1962; Feldman, 1965). Thus, the second pairs of genomes of these species would have no counterparts among the genomes of the diploid species.

Triticum rectum is morphologically very similar to *T. neglectum* and was originally considered a hexaploid cytotype of it. A hybrid *T. neglectum* × *T. rectum* showed 14 closed bivalents and seven univalents at MI (Kihara, 1937). Kihara produced an artificial allotetraploid *T. umbellulatum* × *T. uniaristatum* and noted that it resembled more closely *T. rectum* than *T. neglectum.* He speculated, therefore, that the third genome pair of *T. rectum* was contributed by *T. uniaristatum.* However, since he believed that *T. uniaristatum* has a M genome, he designated the third pair of genomes of *T. rectum* as M^{t2} (Kihara, 1963).

A numerical analysis of hybrids between autotetraploid *T. uniaristatum* and *T. rectum* revealed that a genome of *T. rectum* must be related to the genome of *T. uniaristatum* (Yen and Kimber, 1992). However, since the analysis suggested that some differences may exist between the genomes, and since no data were available on the pairing of the *T. rectum* chromosomes with the *T. comosum* chromosomes, the evidence was considered equivocal (Yen and Kimber, 1992).

In the present work, variation in randomly selected families of repeated nucleotide sequences (RNSs) was employed to investigate the origins of the genomes of these five polyploid species. Repeated nucleotide sequences are a ubiquitous component of the nuclear plant genomes. They are particularly abundant in large plant genomes, such as those of the *Triticum* species. Most RNSs have no obvious function and may not code for any protein or RNA, whereas some may be important for the function of chromosomes. Some genes, e.g., those encoding ribosomal RNAs, are also extensively repeated in plant genomes, and may evolve in a similar manner as the main bulk of RNSs.

Individual sequences in RNS families show a tendency to evolve in concert by repeated cycles of homogenization, which lead to a gradual turnover of sequences within families (Dover, 1982). Concerted evolution is a conservative process because it tends to eliminate rare sequences from RNS families (Birky and Skavaril, 1976; Smith, 1976; Dvořák, Jue, and Lassner, 1987). Repeated nucleotide sequences within a RNS family maintain a high degree of homogeneity within a genome, in spite of being at a number of sites in a genome (Strachan, Webb, and Dover, 1985; Dvořák and Zhang, 1992; Dubcovsky and Dvořák, 1994b). At the interspecific level, divergence and amplification or deletion of sequences during species differentiation eventually result in sequence subfamilies differing in restriction sites (Dvořák, McGuire, and Cassidy, 1988; Dvořák and Zhang, 1990; Talbert et al., 1991; Zhang and Dvořák, 1991; Dvořák and Zhang, 1992; Zhang and Dvořák, 1992; Zhang, Dvořák, and Waines, 1992; Dvořák et al., 1993; Dubcovsky and Dvořák, 1994b). An analytical method based on variation in RNSs, described in detail earlier (Dubcovsky and Dvořák, 1994b; Dvořák and Dubcovsky, 1995), was employed to determine which of the extant diploid species, or their extinct ancestors, contributed the genomes of these five polyploid species.

MATERIALS AND METHODS

Plants—The materials used in this study, their accession numbers, and their origin are listed in Table 1. Seeds of all stocks used in the present study were stored in seed repository at the Department of Agronomy and Range Science, Davis, CA and are available on request.

DNA hybridization—Nuclear DNAs were isolated from leaves of single plants following the procedure of Dvořák, McGuire, and Cassidy (1988). Restriction endonuclease digested DNAs were electrophoretically fractionated in agarose gels and transferred to Hybond N+ nylon membrane (Amersham, IL) by capillary transfer. Prehybridization and hybridization were performed as described earlier (Dvořák, McGuire, and Cassidy, 1988; Zhang and Dvořák, 1991; Dubcovsky and Dvořák, 1994b). Probes were prepared from 64 pUC18 plasmids harboring random RNS fragments isolated from libraries of nuclear DNAs of *T. tauschii* (15 clones designated pTtUCD), *T. comosum* (12 clones designated pTcUCD), *T. longissimum* (12 clones designated pTlUCD), *T. speltoides* (six clones designated pTsUCD), *T. kotschyi* (Boiss.) Bowden (ten clones designated pTkUCD), *T. urartu* (six clones designated pTuUCD), and *T. monococcum* (one clone designated pTbUCD1) (Zhang and Dvořák, 1992; Zhang, Dvořák, and Waines, 1992; Dvořák et al., 1993; and this report). In addition, 5S RNA clone pTa794 and *T. tauschii* clone pAsKSU1 were employed. Inserted DNA fragments were excised by *Eco*RI-*Hind*III digestion and purified by electrophoresis or were amplified by polymerase chain reaction (PCR). M13/pUC sequencing primer (−20) 17-mer and M13/pUC reverse sequencing primer (−48) 24-mer (New England Biolabs, MA) were used for the PCR amplifications; PCR products were purified with the Magic PCR purification Kit (Promega, WI). The membranes were washed in 2 × SSC and 0.5% SDS for 30 min at 65°C, 1 × SSC and 0.5% SDS for 30 min at 65°C, and 0.2 × SSC and 0.5% SDS for 30 min at 65°C.

Data analysis—Marker bands for each diploid species were employed in the analysis (Dvořák and Zhang, 1990; for review see Dvořák and Dubcovsky, 1995). A marker band is defined as a restriction fragment that is observed in the restriction profiles produced by a specific probe × enzyme combination of all analyzed accessions of a diploid species but not in those of other diploid species. Note that the absence of a band in a restriction fragment profile of a particular species does not mean that the fragment is absolutely absent from the genome of the species. It means that it was not detected under conditions identical to those under which it was detected in the profile of a species for which it is a marker. 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 mass fraction. In those cases, the common bands were included only once in the analysis.

TABLE 1. Sources of DNAs employed in the analysis of variation of repeated nucleotide sequences.

Species	Accession	Origin	Source[a]
Triticum urartu Thum.	G2990	Armenia	J. G. Waines
Triticum urartu Thum.	G3135	Lebanon	J. G. Waines
T. monococcum ssp. *aegilopoides* L.	G3147	Lebanon	J. G. Waines
T. monococcum ssp. *aegilopoides* L.	G2528	Iran	J. G. Waines
T. speltoides (Tausch) Gren.	G1167	Turkey	J. G. Waines
T. speltoides (Tausch) Gren.	TS02	Israel	M. Feldman
T. speltoides (Tausch) Gren. ssp. *ligustica*	G1819	Turkey	J. G. Waines
T. speltoides (Tausch) Gren. ssp. *aucheri*	Sam-1	Unknown	Unknown
T. sharonense (syn. *Aegilops sharonensis* Eig.)	G946	Israel	J. G. Waines
T. sharonense (syn. *Aegilops sharonensis* Eig.)	TH01	Israel	M. Feldman
T. longissimum Schweinf. et Muschl.	G609	Israel	J. G. Waines
T. longissimum Schweinf. et Muschl.	TL17	Israel	M. Feldman
T. bicorne Forssk	G365	Unknown	J. G. Waines
T. bicorne Forssk	TB10	Israel	M. Feldman
T. searsii (syn. *A. searsii* Feldman et Kislev)	G3527	Israel	J. G. Waines
T. searsii (syn. *A. searsii* Feldman et Kislev)	TE27	Israel	M. Feldman
T. muticum (Bois.) Hackel	TK136-737	Turkey	R. J. Metzger
T. muticum (Bois.) Hackel	A-1	Unknown	Unknown
T. caudatum (L.) Godron et Gren.	Rub 78-751	Unknown	E. R. Sears
T. caudatum (L.) Godron et Gren.	Rub 74-751	Unknown	E. R. Sears
T. caudatum (L.) Godron et Gren.	PI551120	Greece	H. E. Bockelman
T. caudatum (L.) Godron et Gren.	PI254863	Iraq	H. E. Bockelman
T. caudatum (L.) Godron et Gren.	PI542197	Turkey	H. E. Bockelman
T. caudatum (L.) Godron et Gren.	PI263554	Turkey	H. E. Bockelman
T. comosum (Sibth. et Smith) Richter	G659	Unknown	J. G. Waines
T. comosum ssp. *heldreichii*	G603	Greece	J. G. Waines
T. comosum ssp. *heldreichii*	G1291	Greece	J. G. Waines
T. comosum ssp. *heldreichii*	G5034	Turkey	J. G. Waines
T. comosum ssp. *heldreichii*	G5035	Greece	J. G. Waines
T. comosum ssp. *heldreichii*	G5037	Greece	J. G. Waines
T. comosum ssp. *eucomosum*	G601	Unknown	J. G. Waines
T. comosum ssp. *thesalicum*	G1515	Greece	J. G. Waines
T. comosum ssp. *thesalicum*	G3566	Unknown	J. G. Waines
T. uniaristatum (Vis.) Richter	P68-33a-3	Unknown	E. R. Sears
T. uniaristatum (Vis.) Richter	PI276995	Turkey	H. E. Bockelman
T. uniaristatum (Vis.) Richter	G1439	Greece	J. G. Waines
T. uniaristatum (Vis.) Richter	G3586	Turkey	J. G. Waines
T. uniaristatum (Vis.) Richter	G3585	Turkey	J. G. Waines
T. uniaristatum (Vis.) Richter	G1297	Turkey	J. G. Waines
T. uniaristatum (Vis.) Richter	G1296	Greece	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G3584	Turkey	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G1164	Turkey	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G1210	Turkey	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	PI276994	Turkey	H. E. Bockelman
T. umbellulatum (Zhuk.) Bowden	G1060	Turkey	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G746	Turkey	J. G. Waines
T. umbellulatum (Zhuk.) Bowden	G3772	Unknown	J. G. Waines
T. tauschii (Coss.) Schmalh.	K901/75	Unknown	Unknown
T. tauschii (Coss.) Schmalh.	KU2073	Iran	Kyoto Univ.
T. tauschii (Coss.) Schmalh.	KU2025	Afghanistan	Kyoto Univ.
T. tauschii (Coss.) Schmalh.	KU2377	Iran	Kyoto Univ.
T. tauschii (Coss.) Schmalh.	KU2001	Pakistan	Kyoto Univ.
T. biunciale (Vis.) K. Richter	G596	Turkey	J. G. Waines
T. biunciale (Vis.) K. Richter	G597	Israel	J. G. Waines
T. biunciale (Vis.) K. Richter	G1033	Turkey	J. G. Waines
T. biunciale (Vis.) K. Richter	G3564	Iran	J. G. Waines
T. biunciale (Vis.) K. Richter	G3624	USSR	J. G. Waines
T. biunciale (Vis.) K. Richter	G4442	Syria	J. G. Waines
T. biunciale (Vis.) K. Richter	G4443	Syria	J. G. Waines
T. biunciale (Vis.) K. Richter	G1013	Unknown	J. G. Waines
T. columnare Bowden	G599	Turkey	J. G. Waines
T. columnare Bowden	G4435	Syria	J. G. Waines
T. columnare Bowden	P68-29-1	Unknown	E. R. Sears
T. columnare Bowden	Cambridge	Unknown	R. Johnson
T. ovatum Raspail	S1	Spain	Unknown
T. ovatum Raspail	G418	Turkey	J. G. Waines
T. ovatum Raspail	Cambridge	Unknown	R. Johnson
T. ovatum Raspail	G610	Turkey	J. G. Waines
T. ovatum Raspail	G611	Israel	J. G. Waines
T. ovatum Raspail	G612	Israel	J. G. Waines

TABLE 1. Continued.

Species	Accession	Origin	Source[a]
T. ovatum Raspail	G613	Turkey	J. G. Waines
T. ovatum Raspail	G1044	Turkey	J. G. Waines
T. ovatum Raspail	G3628	Rumania	J. G. Waines
T. neglectum Bowden	G621	Turkey	J. G. Waines
T. neglectum Bowden	G622	Turkey	J. G. Waines
T. neglectum Bowden	G1046	Turkey	J. G. Waines
T. neglectum Bowden	G1079	Turkey	J. G. Waines
T. neglectum Bowden	G1014	Turkey	J. G. Waines
T. rectum (Zhuk.) Bowden	G3562	Portugal	J. G. Waines
T. rectum (Zhuk.) Bowden	Cambr. 12	Unknown	J. G. Waines

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

The analysis of polyploid species involved either all marker bands found per probe × enzyme combination or only one marker band per probe × enzyme combination. If two marker bands per the same probe × enzyme combination showed different relationships with a polyploid, both were included because they represent different evolutionary events. The analysis employing single marker bands per probe × enzyme combination was used to reduce the possibility of counting the same evolutionary event several times, which potentially exists if all marker bands found in a single probe × enzyme combination are used.

Marker bands were also identified for groups of diploid species as done previously (Zhang, Dvořák, and Waines, 1992; Dubcovsky and Dvořák, 1994b). These marker bands are equivalent to unique shared characters in the cladistic terms. The diploid species were grouped according to their hypothetical phylogeny so that the groups represented clades in hypothetical phylogenetic tree of *Triticum* (Fig. 1). The tree was based on a phylogenetic analysis of variation in restriction fragments in RNSs (Dvořák and Zhang, 1992).

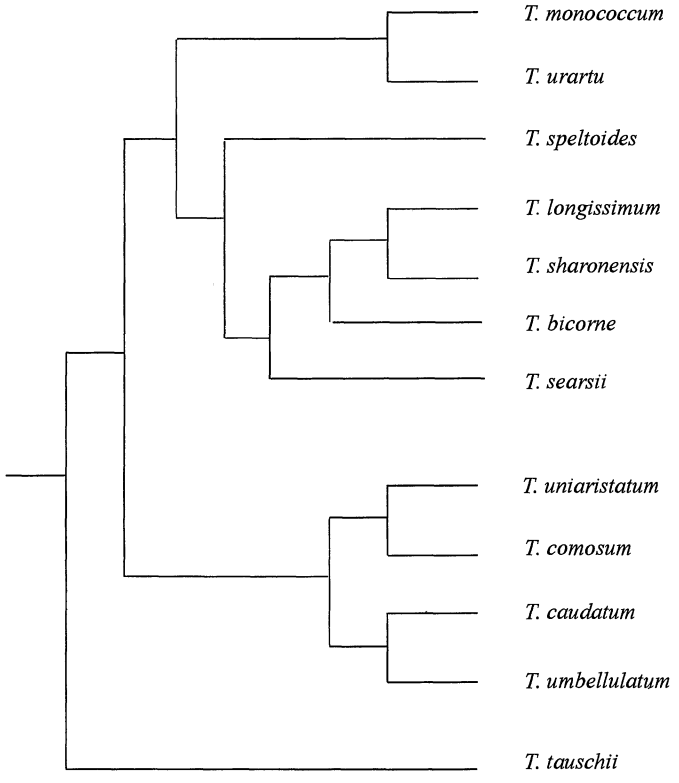

Fig. 1. A hypothetical phylogenetic tree of the genus *Triticum* based on variation in restriction fragments of repeated nucleotide sequences (Dvořák and Zhang, 1992). The position of *Triticum muticum* in the tree is not shown because of questionable authenticity of one of the *T. muticum* DNAs used by Dvořák and Zhang (1992).

The fraction of marker bands of a diploid shared with a polyploid is called repeated nucleotide sequence correspondence (RSC). 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 parallelisms (independent amplifications of the same RNS variant in investigated species) and convergence (different RNSs having by chance the same mobility) in the amplification or loss of sequence variants. Repeated nucleotide sequence families employed in the study are a sample of the repeated nucleotide sequence families present in the genomes of the investigated species, and RSC values based on them are estimates of some true correspondence of the repeated component of the genomes. RSC values are, therefore, subjected to sampling variation and whether observed differences among RSCs are real or not must be tested statistically. The RSCs were compared statistically with each other and with the expected RSC extremes, 1.00 and 0.00, using 2 × 2 contingency tables and Fisher's exact test.

RESULTS

Fifty-five probes and 119 probe × enzyme combinations were used in the investigation of the origin of the *T. biunciale, T. ovatum,* and *T. columnare* genomes, 64 probes and 158 probe × enzyme combinations were used in the investigation of the origin of the *T. neglectum* genomes, and 50 probes and 95 probe × enzyme combinations were used in the investigation of the origin of the *T. rectum* genomes (Table 2). Analyses were conducted using both all marker bands and a single marker band per probe × enzyme combination. Since both approaches yielded essentially identical results (Tables 3, 5), only data generated by the former approach will be described. Data generated by the latter approach can be found in Tables 3, 5.

T. biunciale—Sixteen of 17 marker bands of *T. umbellulatum* and 15 of 24 marker bands of *T. comosum* were found in *T. biunciale* hybridization profiles. A marker band for no other species was found. Repeated sequence correspondence of *T. umbellulatum* with *T. biunciale* was 0.94 and that of *T. comosum* was 0.63. Of 73 marker bands shared by groups of diploid species found in *T. biunciale* profiles, 72 were those shared by

TABLE 2. Probe × enzyme combinations that resulted in detection of marker bands.

Species and groups of species	Probe × enzyme combinations
A^{u}[a]	c2[b](T[c]1), u19(T1), t11(H1), pTb1(D3), s6(T1), k3(H3), l3(D2, T1), k10(M1, B1), l7(H1), l12(M1), k7(D2), k8(B1)
A^{m}	c1(A1), c2(H1), c3(T1), c6(H1), k4(T1), l1(M1), l3(T1), u19(T2), t1(T1), pTb1(D3, T2), u13(B1), s1(D1, T1), l4(M2)
S	C2(T1), c4(D1, H1), c6(H1), c13(H1), k1(D1), l1(Dr2), l2(T3, M1), l3(S1, H1, M1), l6(H1), t6(T1), t7(D2), t8(H3, A2), t9(T1), c7(A1), t11(H1), t2(H1, T1), u13(D1), u15(M2), s2(M1, H1), s6(H1, D1, M1), s4(D2), s5(T2), l8(B1), l5(S1, H1), l7(H1), l11(S2, T1), l3(T2), k2(A1, T1), k3(H1), k9(H1), u17(T1)
S^{sh}, S^{l} and $S^{sh} + S^{l}$	s2(M1), u13(T1), c2(T1), c7(T1), c11(H1), t2(H1, T1), t4(A1), t8(S1), t21(T1), l5(M1), l7(S3, B5), l11(S1), k3(H2), k1(H2), l6(D1)
S^{b}	c1(A1), c7(H1), c13(H1), t11(H1), s6(S1), l5(S1), l4(S1, M2), l7(S1), l3(A1, T1)
S^{se}	c1(A1), c4(Dr1), k2(A1), t2(T1), u14(H2), s3(H1), k3(H1), k4(D1), k7(A1), pTa794(D2), k8(D1)
T	16(H1), t2(H3), c3(T1), c4(H1), c5(H1), k3(H2), k9(H1), c10(H3), c11(H3), c12(H2), c13(H1)
C	c4(D1, H1), c5(A1, H2), c6(M1, H1), k4(H1, T1), c12(H2), k9(H3), t1(T1), t3(H1), k1(D2), t8(H2), t10(T1), t11(H5), t2(T2), l5(H1), l4(M1), k3(B1, S1), l3(A2, H1, T3), l9(T1), l6(D1, A3, H2), k2(A1, D1, T1), k8(D1, H1), u17(T1)
M	c2(H1, T1), k4(A1, D3), k3(B2, H2, T2, S1), k1(H1, B2), k7(D1), l1(M2), l3(A1), t10(T1), c7(A3), t2(H1), t1(T1), c11(H1)
N	11(Dr2, M1), l2(T1, M1), u19(H1), t4(A2), c7(A3), l7(B1), k3(S2, T2, H2), l3(T3), l6(H1)
U	12(T1), l14(H1), t4(A1), t12(A1), t2(T1), k3(S2, T1), u14(H1), l5(H1), l10(H1, B1), k4 (A1, D1), k2(T1, D1), k8(D1), c7(A2), c6(M1), c11(H1)
D	c3(T1), c4(H1), c6(H1, M1), k2(D2, T1, A3), k4(D2, H1), k10(T1), t2(H1, T1), t7(Dr2), t1(Dr1), t10(A2, E1), t4(A1), t11(H1), t20(T1), k3(S2, H3), pTb1(H1), pAsKSU1(D1, S2, T1, H1) u13(H1, T2), u15(T2), s2(H1), s4(D1), s5(T1), s2(T1), l5(S1, M1), k6(D1, A3), l7(S3, T3, H2, A3), l11(S3, T3), l10(S3, T1, D1, H1), l9(H1), l6(H3), k5(T1), l12(A1), k7(A5, D1), k8(B1), c14(H1)
$A^{u} + A^{m}$	11(T1), c4(H1), c5(H1), k2(A1), k4(A1), t2(H1), t5(T1), t6(T1), t7(Dr1), t9(T1), t21(T1), t22(T1), u19(T5, H2), pTb1(H2, S4, D3, T2), u14(T1), u13(H3, D3, M3, S2), s2(H1), s4(D1), l7(H2, A1), l11(S3, T1), k3(H4, T1), l3(H1), l6(A2, H1), u17(T4, U20(T1)
$S^{sh} + S^{b}$ or $S^{sh} + S^{l} + S^{b}$	11(A3, M2), l3(A1, T1), l4(M1), l6(A2, H1), l9(H1), l10(T2), k3(T1)
$S^{sh} + S^{b} + S^{se}$ or $S^{sh} + S^{l} + S^{b} + S^{se}$	c1(T1), c3(T1), k9(H1), t10(T1), k2(A1), t2(H1, T1), k3(T1), t11(H1), s4(D1, M2), s5(T1), l3(H1, T1), l7(H1), l10(S1, D2), k5(T1), l12(M2)
$S^{sh} + S^{b} + S^{se} + S$ or $S^{sh} + S^{l} + S^{b} + S^{se} + S$	c6(A1), k2(T1), k3(T1), l2(T1), s3(D1, H1), s4(D4, T1, M1), s5(T1, M1), l7(T1, H1), l11(T1), l3(A1, D1, T1), l10(T1), k1(D1), k8(D1, H1), t2(H2)
C + U	c6(A1), k9(H2), t1(T1), t2(H2, T1), t8(A1), l2(T1), l6(A2, H1), k3(H1), k4(B1), c3(T2, H1), c1(A1), s5(S2), l11(S2, T1), l10(H1, S1, T1), k5(T4, H1)
M + N	c2(T1), c5(A1, H1), pTb1(H2), l8(B1), k1(D1), c6(A1), c7(T1, A1), k10(M1), s4(D1), t2(H1, T1), t3(H1), t8(A1), c1(A1), k6(D1), l7(S1, T2, B4, H3), l11(S2, B1), k3(H1, T1), l3(T3), l10(S2), l6(D1, H1, A2, T1), k4(B1), u17(T1)
C + M + N + U	c2(H1), k2(A1), t10(T2), t2(H1), c7(A1), t4(A3), s6(S1), s4(T1), l5(S1), k6(S1), l7(S1, T2, H1), l11(S4), k3(H1), l3(B1), l6(D1), l10(H1)
novel bands[d]	t2(H1), k1(D1, T2), k9(H2), c2(H1), l1(M1), k3(T3, H1), c6(M1), t11(H1), t10(E1), l10(B1), l7(A1), l3(T1)

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

[b] A probe is designated by a lowercase letter identifying the species that was the source of a clone, *T. comosum* (c), *T. kostchyi* (k), *T. longissimum* (l), *T. speltoides* (s), *T. tauschii* (t), and *T. urartu* (u), and the number that follows designates the clone number. Thus c2 is clone pTcUCD2, etc. For clones designated in a different way, see Materials and Methods.

[c] The restriction endonucleases that produced marker bands are indicated in parentheses and are designated as follows: A = *Alu*I, B = *Bam*H1, D = *Dde*I, Dr = *Dra*I, E = *Eco*RV, H = *Hae*III, M = *Mbo*I, S = *Sst*I, T = *Taq*I. The number that follows specifies the number of marker bands revealed by a specific probe-enzyme combination. Thus, T1 indicates that one marker was observed in a *Taq*I digested DNA.

[d] These bands that were present in one or more of the five polyploid species and absent from all diploids (see Table 4).

groups involving *T. comosum, T. uniaristatum, T. caudatum,* and *T. umbellulatum* (Table 3). The RSCs of these groups with *T. biunciale* were 1.0 or close to 1.0. One shared marker band was shared by all species of *T.* sect. *Sitopsis* (*T. speltoides, T. searsii, T. bicorne, T. longissimum,* and *T. sharonense*) (RSC = 0.08). Only two of 119 profiles generated by hybridization of repeated nucleotide sequences with *T. biunciale* DNA showed a band unique to *T. biunciale* (Table 4).

T. ovatum—Fifteen of 17 marker bands of *T. umbellulatum* and 15 of 24 marker bands of *T. comosum* were found in *T. ovatum* hybridization profiles. A marker band for no other species was found. Repeated sequence correspondence of *T. umbellulatum* with *T. ovatum* was 0.88 and that of *T. comosum* was 0.63. Of 73 marker bands shared by groups of diploid species found in *T. ovatum* profiles, 72 were those shared by groups involving *T. comosum, T. uniaristatum, T. caudatum,* and *T. umbellulatum* (Table 3). The RSCs of these groups with *T. biunciale* were 1.0 or close to 1.0. One shared marker band was shared by all species of *T.* sect. *Sitopsis* (*T. speltoides, T. searsii, T. bicorne, T. longissimum,* and *T. sharonense*) (RSC = 0.08). Only one of 119 profiles gen-

TABLE 3. Repeated nucleotide sequence correspondence (RSC) of diploid *Triticum* species and groups of diploid species representing some of the clades in a *Triticum* phylogenetic tree (Dvořák and Zhang, 1992) with *Triticum biunciale* and *T. ovatum*. The denominator and numerator in the fractions are the numbers of marker bands that were examined and found in a polyploid, respectively. Fractions in parentheses are the numbers of marker bands examined and found in a polyploid using a single marker band per probe × enzyme combination.

Species or groups	*Triticum biunciale*		*Triticum ovatum*	
	Marker bands	RSC	Marker bands	RSC
A (*T. urartu*)	0/18 (0/12)	0.00	0/18 (0/12)	0.00
A (*T. monococcum*)	0/12 (0/8)	0.00	0/12 (0/8)	0.00
S (*T. speltoides*)	0/37 (0/26)	0.00	0/37 (0/26)	0.00
S^{sh} (*T. sharonensis*)	0/3	0.00	0/3	0.00
S^{l} (*T. longissimum*)	0/1	0.00	0/1	0.00
S^{b} (*T. bicorne*)	0/9 (0/8)	0.00	0/9 (0/8)	0.00
S^{se} (*T. searsii*)	0/10 (0/8)	0.00	0/10 (0/8)	0.00
T (*T. muticum*)	0/19 (0/11)	0.00	0/19 (0/11)	0.00
C (*T. caudatum*)	0/37 (0/23)	0.00	0/36 (0/23)	0.00
M (*T. comosum*)	15/24 (9/14)	0.63^{cd} $(0.64)^{cd}$	15/24 (8/14)	0.63^{cd} $(0.58)^{cd}$
N (*T. uniaristatum*)	0/16 (0/10)	0.00	0/16 (0/10)	0.00
U (*T. umbellulatum*)	16/17 (14/15)	0.94^{b} $(0.93)^{b}$	15/17 (13/15)	0.88^{b} $(0.87)^{b}$
D (*T. tauschii*)	0/65 (0/37)	0.00	0/65 (0/37)	0.00
$S + S^{sh} + S^{l} + S^{b} + S^{se}$	1/12 (1/8)	0.08^{a} $(0.13)^{a}$	0/12 (0/8)	0.00
$S^{sh} + S^{l} + S^{b} + S^{se}$	0/15 (0/11)	0.00	0/15 (0/11)	0.00
$S^{sh} + S^{l} + S^{b}$	0/7 (0/6)	0.00	0/7 (0/6)	0.00
$S^{sh} + S^{l}$	0/20 (0/11)	0.00	0/19 (0/11)	0.00
M + N	32/36 (20/22)	0.89^{b} $(0.91)^{b}$	33/35 (21/22)	0.94^{b} $(0.96)^{b}$
C + U	20/21 (13/13)	0.95^{b} (1.00)	19/21 (10/13)	0.91^{b} $(0.77)^{b}$
C + M + N + U	20/20 (14/14)	1.00	20/20 (14/14)	1.00
$A^{u} + A^{m}$	0/34 (0/20)	0.00	0/34 (0/20)	0.00

[a] Not significantly different from 0.0.
[b] Not significantly different from 1.0.
[c] Significantly different from 1.0.
[d] Significantly different from 0.0.

erated by hybridization of repeated nucleotide sequences with *T. ovatum* DNA showed a band unique to *T. ovatum* (Table 4). This band was different from the two unique bands found among the *T. biunciale* hybridization profiles.

TABLE 4. Probe × enzyme combinations detecting bands absent from diploid *Triticum* species and the presence or absence of the novel bands in the polyploids. (−) = absence, (− −) = absence of two bands, (+) = presence of a single band, (++) = presence of two bands, (n.i.) = not investigated.

Probe (enzyme)	*T. biunciale*	*T. ovatum*	*T. neglectum*	*T. rectum*	*T. columnare*
$l^{a}10(B1^{b})$	−	−	+	n.i.	+
l3(T1)	−	−	+	+	+
l7(A1)	+	−	+	n.i.	+
k1(T1)	−	−	+	n.i.	+
k1(T1)	+	−	−	n.i.	−
k3(T2)	−−	−−	++	++	++
k3(T1)	−	−	+	+	−
k3(H1)	−	−	+	+	+
c6(M1)	−	−	+	+	+
t11(H1)	−	−	+	+	+
t10(E1)	−	+	−	−	−
t2(H1)	n.i.	n.i.	+	+	n.i.
k1(D1)	n.i.	n.i.	+	+	n.i.
k9(H2)	n.i.	n.i.	++	++	n.i.
c2(H1)	n.i.	n.i.	+	+	n.i.
l1(M1)	n.i.	n.i.	+	+	n.i.

[a] Sources of clones were *T. comosum* (c), *T. kostchyi* (k), *T. longissimum* (l), and *T. tauschii* (t).
[b] Indicates the restriction endonuclease (A = *Alu*I, B = *Bam*H1, D = *Dde*I, E = *Eco*RV, H = *Hae*III, M = *Mbo*I, T = *Taq*I) and the number of marker bands detected.

T. neglectum—Nineteen of 21 *T. umbellulatum* marker bands (RSC = 0.91), two of 56 *T. speltoides* marker bands (RSC = 0.04), one of 51 *T. caudatum* marker bands (RSC = 0.02), and one of 22 *T. uniaristatum* marker bands (RSC = 0.05) were found in *T. neglectum* (Table 5). No RSC differed significantly from 0.0 except for that of *T. umbellulatum*. Of 57 marker bands shared by groups of diploid species found in *T. neglectum* hybridization profiles, 50 were marker bands shared by groups involving *T. umbellulatum* (C + U and C + M + N + U) (RSCs of 1.0 or close to 1.0 with *T. neglectum*), one was shared by all species of *T.* sect. *Sitopsis* (RSC = 0.04), three were shared by the species of the subsect. *Emarginata* of *T.* sect. *Sitopsis* ($S^{sh} + S^{l} + S^{b} + S^{se}$) (RSC = 0.14), one was shared by *T. sharonense, T. longissimum,* and *T. bicorne* (RSC = 0.07), and two were shared by *T. comosum* and *T. uniaristatum* (M + N) (RSC = 0.04). Fourteen of 158 *T. neglectum* hybridization profiles showed bands (a total of 16 bands, Table 4), which were not encountered in profiles of the diploid species (Table 4). One of these bands was found in *T. biunciale*.

T. columnare—Fifteen marker bands of *T. umbellulatum* (RSC = 0.88), two marker bands of *T. speltoides* (RSC = 0.05), and one marker band of *T. caudatum* (RSC = 0.03) were found in *T. columnare* (Table 5). Only the RSC of *T. umbellulatum* with *T. columnare* differed significantly from 0.0 (Table 5). Of a total of 165 marker bands shared by two or more diploid species, 46 were found in *T. columnare* hybridization profiles. Two were shared by all species of *T.* sect. *Sitopsis* (RSC = 0.17), two by the species of the subsect. *Emarginata* of

TABLE 5. Repeated nucleotide sequence correspondence (RSC) of diploid *Triticum* species and groups of diploid species representing some of the clades in a *Triticum* phylogenetic tree (Dvořák and Zhang, 1992) with *Triticum neglectum, T. columnare,* and *T. rectum.* The denominator and numerator in the fractions are the numbers of marker bands that were examined and found in a polyploid, respectively. Fractions in parentheses are the numbers of marker bands examined and found in a polyploid using a single marker band per probe × enzyme combination.

Species or groups	*Triticum neglectum*		*Triticum columnare*		*Triticum rectum*	
	Marker bands	RSC	Marker bands	RSC	Marker bands	RSC
A (*T. urartu*)	0/20 (0/14)	0.00	0/18 (0/12)	0.00	0/4 (0/4)	0.00
A (*T. monococcum*)	0/19 (0/14)	0.00	0/12 (0/8)	0.00	0/9 (0/8)	0.00
S (*T. speltoides*)	2/56 (2/44)	0.04[a] (0.05)[a]	2/37 (2/26)	0.05[a] (0.08)[a]	2/37 (2/26)	0.05[a] (0.08)[a]
S^{sh} (*T. sharonensis*)	0/3	0.00	0/3	0.00	0/2	0.00
S^{l} (*T. longissimum*)	0/1	0.00	0/1	0.00	—	—
S^{b} (*T. bicorne*)	0/12 (0/11)	0.00	0/9 (0/8)	0.00	0/7 (0/6)	0.00
S^{se} (*T. searsii*)	0/13 (0/11)	0.00	0/10 (0/8)	0.00	0/5 (0/4)	0.00
T (*T. muticum*)	0/19 (0/11)	0.00	0/19 (0/11)	0.00	0/19 (0/11)	0.00
C (*T. caudatum*)	1/51 (1/34)	0.02[a] (0.03)[a]	1/36 (1/23)	0.03[a] (0.04)[a]	1/37 (1/22)	0.03[a] (0.05)[a]
M (*T. comosum*)	0/28 (0/18)	0.00	0/24 (0/14)	0.00	0/15 (0/11)	0.00
N (*T. uniaristatum*)	1/22 (1/13)	0.05[a] (0.08)[a]	0/16 (0/10)	0.00	17/17 (12/12)	1.00
U (*T. umbellulatum*)	19/21 (17/19)	0.91[b] (0.90)[b]	15/17 (13/15)	0.88[b] (0.87)[b]	14/15 (12/13)	0.93[b] (0.92)[b]
D (*T. tauschii*)	0/88 (0/55)	0.00	0/65 (0/37)	0.00	0/31 (0/24)	0.00
S + S^{sh} + S^{l} + S^{b} + S^{se}	1/26 (1/22)	0.04[a] (0.05)[a]	2/12 (1/8)	0.17[d] (0.13)[a]	0/10 (0/7)	0.00
S^{sh} + S^{l} + S^{b} + S^{se}	3/22 (3/19)	0.14[a] (0.16)[a]	2/15 (2/11)	0.13[a] (0.18)[a]	1/13 (1/10)	0.08[a] (0.10)[a]
S^{sh} + S^{l} + S^{b}	1/15 (1/10)	0.07[a] (0.10)[a]	1/7 (1/6)	0.14[a] (0.17)[a]	0/7 (0/4)	0.00
S^{sh} + S^{l}	0/22 (0/13)	0.00	0/20 (0/11)	0.00	0/7 (0/6)	0.00
C + U	30/31 (21/22)	0.97[b] (0.96)[b]	21/21 (14/14)	1.00	19/20 (13/14)	0.95[b] (0.93)[b]
M + N	2/45 (2/38)	0.04[a] (0.06)[a]	0/36 (0/22)	0.00	17/18 (13/14)	0.94[b] (0.93)[b]
C + M + N + U	20/20 (18/18)	1.00	20/20 (14/14)	1.00	10/10 (7/7)	1.00
A^{u} + A^{m}	0/66 (0/37)	0.00	0/34 (0/20)	0.00	0/12 (0/8)	0.00

[a] Not significantly different from 0.0.
[b] Not significantly different from 1.0.
[c] Significantly different from 1.0.
[d] Significantly different from 0.0.

the *T.* sect. *Sitopsis* (RSC = 0.13), and one by *T. sharonense, T. longissimum,* and *T. bicorne* (RSC = 0.14). The remaining 41 were marker bands shared by species groups involving *T. umbellulatum* (C + U and C + M + N + U), and had RSCs of 1.0 (Table 5). Nine bands were found in the 119 hybridization profiles of *T. columnare* (in a total of eight profiles), which were not encountered in a profile of any diploid species (Table 4). All were in the profiles of *T. neglectum.* A band generated by hybridization of pTlUCD7 with *T. neglectum* DNA was observed in a profile of *T. biunciale,* but not in that of *T. ovatum* (Table 4).

T. rectum—Of 34 marker bands found in *T. rectum* hybridization profiles, two were *T. speltoides* marker bands (RSC = 0.05), one was *T. caudatum* marker band (RSC = 0.03), 14 were *T. umbellulatum* marker bands (RSC = 0.93), and 17 were *T. uniaristatum* marker bands (RSC = 1.00) (Table 5). The *T. umbellulatum, T. speltoides,* and *T. caudatum* marker bands found in *T. rectum* were the same as those found in *T. neglectum.* Of 47 marker bands shared by two or more diploid species found in *T. neglectum* hybridization profiles, one was a marker band shared by the species of the subsect. *Emarginata* of *T.* sect. *Sitopsis* (RSC = 0.08) and the rest were shared by the groups involving *T. comosum, T. uniaristatum, T. caudatum,* and *T. umbellulatum* (RSCs of 1.0 or close to 1.0, Table 5). A total of 13 bands in ten of the 95 *T. rectum* profiles investigated were not found in any diploid species (Table 4).

DISCUSSION

The four tetraploid species fall into two groups: *T. biunciale* plus *T. ovatum* and *T. neglectum* plus *T. columnare.* While there is high similarity between the species within each pair, there are considerable differences between the pairs. The first pair of species differed from the diploid species by three bands, two in *T. biunciale* and one in *T. ovatum.* Those in *T. biunciale* were absent from *T. ovatum* and the one in *T. ovatum* was absent from *T. biunciale.* This indicates that minor differences exist between the chromosome complements of *T ovatum* and *T. biunciale.* No divergence was found between *T. neglectum* and *T. columnare* and both differed by a large number of bands from all diploid species.

Chromosome pairing in hybrids *T. ovatum* × *T. biunciale* showed a modal number of 12 bivalents per MI cell (Kihara, 1937), as did hybrids *T. neglectum* × *T. columnare* (Kihara, 1949). Pollen viability in the latter hybrids was close to 20 %, indicating a very close relationship between the genomes of the two species (Kihara, 1949). This finding contrasts with chromosome pairing in hybrids between the species of the two groups: *T. biunciale* × *T. neglectum* showed a mode of eight bivalents per cell at MI (Kihara, 1937), *T. ovatum* × *T. neglectum* showed a mode of eight bivalents and a range from six to 12 per cell (Percival, 1932; Kihara, 1937), and *T. biunciale* × *T. columnare* showed a mode of seven to eight bivalents per cell at MI (Kihara, 1937). All hybrids between the two groups were sterile and showed very low pollen viability. Both chromosome pairing studies and those reported here agree and suggest that the species

within each group have two genome pairs in common, whereas the species between the groups have only a single pair of genomes in common. This is contrary to the conclusion of Kihara (1963) who assigned the same basic genome formula to all four species.

T. biunciale and T. ovatum—The observation that only two and one unique bands were found in the *T. biunciale* and *T. ovatum* RNSs profiles, respectively, indicates that their ancestors were present among the 13 diploid species investigated. One pair of the *T. biunciale* and *T. ovatum* genomes was contributed by *T. umbellulatum,* although the RSC was somewhat reduced, particularly for *T. ovatum.* The imperfect relationship between the genome of *T. umbellulatum* and the U genome of *T. ovatum* was also noted in chromosome pairing studies (Kimber, Sallee, and Feiner, 1988). It was suggested that introgression from *T. umbellulatum* into the U genome of *T. ovatum* occurs in sympatric populations. Lack of introgression in the peripheral populations was argued to cause some divergence of the *T. ovatum* U genome from the *T. umbellulatum* genome in the peripheral populations (Kimber and Yen, 1989; Yen, 1990). Since no direct evidence for diploid to polyploid introgression is available for these species, and since no differences were found in the restriction profiles of RNSs among the nine populations of *T. ovatum* investigated here, more work is needed to substantiate this hypothesis.

The second pair of genomes of *T. biunciale* and *T. ovatum* is closely related to that of *T. comosum.* The RSCs of *T. comosum* with either *T. biunciale* or *T. ovatum* were significantly lower than 1.0. For *T. biunciale,* the RSC with *T. comosum* was also significantly lower than the RSC with *T. umbelullatum.* Since all three subspecies of *T. comosum* were included into the present study, and since all showed similar RSCs with *T. biunciale* and *T. ovatum,* it seems rather unlikely that the low RSC reflects inadequate sampling of *T. comosum.*

Variation in the restriction sites of chloroplast DNA (ctDNA) suggested that the two tetraploids differ in their cytoplasms. Chloroplast DNA of *T. biunciale* is of the same type as that of *T. umbellulatum,* but that of *T. ovatum* is distinct from ctDNAs of the diploid *Triticum* species (Ogihara and Tsunewaki, 1988). *Triticum ovatum* ctDNA is the most closely related to the *T. muticum* and *T. umbellulatum* ctDNAs (Ogihara and Tsunewaki, 1988). Since RSCs of *T. muticum* with *T. biunciale* and *T. ovatum* were 0.0, it is unlikely that *T. muticum* was involved in the origin of *T. biunciale* and *T. ovatum. Triticum umbellulatum* appears, therefore, to be a species that is most closely related to both the nuclear U genome and the ctDNA of *T. ovatum.* The divergence of the nuclear genomes of modern *T. umbellulatum* and *T. ovatum* is, thus, paralleled by divergence of their cytoplasms.

Because of the close relationships between *T. biunciale* and *T. ovatum,* the same genome formula should be used for both of them. Genome formula UUM°M° is proposed for both species. The symbol M° indicates that this M genome exists only in *T. ovatum* and *T. biunciale.*

Modification of the M° genome—The finding that the correspondence between the genome of *T. umbellulatum* and the U genomes of *T. biunciale* and *T. ovatum* is higher than the correspondence between the genome of *T. comosum* and the M° genome of *T. biunciale* and *T. ovatum* could be attributed to pivotal hybridization of tetraploids sharing the U genomes but differing in the second genomes (Zohary and Feldman, 1962; Feldman, 1965). Since there is no obvious mechanism by which introgression could introduce variants of RNSs into the modified genome that are not present in the genomes of hybridizing species, introgression alone is an unlikely source of unique RNS variants in a polyploid. Hence, unique bands in a polyploid indicate absence (extinction) of an ancestor of a polyploid among the investigated diploids (Dubcovsky and Dvořák, 1994b). Since only two and one unique RNS variants were, respectively, found in the profiles of *T. biunciale* and *T. ovatum,* a pivotal genome hybridization would seem at the first glance a more likely reason for the M° genome modification than extinction. However, RNSs of both parental genomes should be found in the M° genome if it originated by recombination of two genomes, and that was not found. Hypothetically, this could be accounted for by assuming that the UUMM allotetraploid(s) ancestral to *T. biunciale* and *T. ovatum* hybridized with several allotetraploids that differed from each other in the second pair of genomes and each introgression event introduced only a limited number of RNSs, leaving their copy numbers below a level critical for their detection by DNA hybridization used in this study.

Another possible explanation of this dilemma is that genomes differentiate at different rates (Kihara, 1963). In general, the modified genomes in *Triticum* polyploids are invariably those that were contributed by diploids from the evolutionary lineages that are in an active state of evolutionary radiation, i.e., lineages that have differentiated several species or subspecies. In contrast, the so-called pivotal genomes are genomes of diploid species that appear to be evolutionarily stable. Kihara (1963) pointed out that the M and U genomes exemplify this dichotomy. While *T. umbellulatum* is morphologically uniform and has not differentiated any subspecies, *T. comosum* is morphologically variable and has differentiated several subspecies. Furthermore, variation in the RNS evolutionary rates could be related to the sizes of genomes. Large *Triticum* genomes tend to evolve new RNS subfamilies faster than small genomes (Dvořák and Zhang, 1992). In the genus *Triticum,* the genomes that appear to be "modified" in the polyploids are relatively large and the genomes that appear to be "pivotal" are relatively small. The U genome is small, whereas the M genome is large (Bennett, 1972). Obviously, additional work is needed to decide if the M° genome was contributed by an extinct species, or was contributed by *T. comosum* and was modified by pivotal hybridization or is intrinsically differentiating faster than the U genome.

T. neglectum and T. columnare—While one genome of each species matched closely the genome of *T. umbellulatum,* the other genome did not match any extant diploid species or group of species. Additionally, many bands were found in the RNS hybridization profiles of *T. neglectum* and *T. columnare* that were not found in those of any diploid *Triticum* species. These findings are expected if one of the pairs of the *T. neglectum* and *T.*

columnare genomes is a genome of a species that is now extinct (Dubcovsky and Dvořák, 1994b). Since none of the species groups, except for those involving *T. umbellulatum,* showed a RSC significantly different from zero, the present analysis has failed to identify a branch in the *Triticum* phylogenetic tree (Dvořák and Zhang, 1992) to which this hypothetical species belongs.

Kihara (1963) based his hypothesis of an M genome in *T. neglectum* and *T. columnare* on morphological comparisons. However, data based on chromosome pairing and on variation in RNSs do not agree with his hypothesis. Hybrids of *T. neglectum* with *T. comosum* obtained by Tsuchyia (1956) showed very low chromosome pairing. A numerical analysis of chromosome pairing in hybrids of autotetraploid *T. uniaristatum* with *T. neglectum* showed that the N genome of *T. uniaristatum* is not in *T. neglectum* (Yen, 1992). A hybrid *T. comosum* × *T. columnare* showed a range of three to seven bivalents per cell, zero to one of them closed (Kihara, 1949). This is the same or a lower level of pairing than that observed by Kihara (1949) in hybrids of *T. columnare* with *T. caudatum, T. uniaristatum,* and *T. speltoides.* Comparison of karyotypes also failed to find a genome resembling those of *T. comosum* or *T. uniaristatum* in *T. neglectum* and *T. columnare* (Chennaveeraiah, 1960). Chennaveeraiah (1960) stated that no genome in *Triticum* matches the second genome pair of *T. neglectum* and *T. columnare.* The cytoplasms of *T. neglectum* and *T. columnare* were both contributed by *T. umbellulatum* (Ogihara and Tsunewaki, 1988), which, unfortunately, does not help to identify the ancestor of the second pair of genomes.

Because the ancestor of the second pair of genomes of *T. neglectum* and *T. columnare* is absent from the diploid species included in this study, it is proposed to assign a genome formula UUX^tX^t to both species. The superscript t (derived from the synonym *Aegilops triaristata* Willd. for *T. neglectum*) indicates that this genome is different from the other unknown genome previously designated by X, the X^c genome that is present in the *T. crassum* species complex (Zhang and Dvořák, 1992; Dubcovsky and Dvořák, 1994a). The absence of marker bands of the X^c genome in the hybridization profiles of *T. neglectum* and *T. columnare* and the absence of marker bands of the X^t genome in the hybridization profiles of *T. crassum* shows that X^t and X^c genomes are different from each other (J. Dubcovsky, unpublished data).

T. rectum—Present data showed that all bands of *T. columnare* and *T. neglectum* were present in *T. rectum.* There is, therefore, little doubt that one of these species is the tetraploid parent of *T. rectum.* Hybrids between *T. neglectum* and *T. rectum* showed up to 14 ring bivalents and 48% pollen viability (Kihara, 1937). *Triticum neglectum* is morphologically very similar to *T. rectum* and is, therefore, a more likely tetraploid ancestor of *T. rectum* than *T. columnare.*

An artificial allotetraploid *T. umbellulatum* × *T. uniaristatum* was hybridized with *T. rectum* and their hybrid showed close to 14 bivalents. Since a hybrid from a cross of this artificial allotetraploid with *T. neglectum* showed only eight bivalents per cell, the high pairing in the former hybrid suggests that there is a N genome in *T. rectum* (Kihara, 1963). This is further evidenced by a high degree of morphological similarity between the artificial allotetraploid and *T. rectum* (Kihara, 1963). Kihara designated the third genome of *T. rectum* M^{t2}, because he believed that *T. uniaristatum* and *T. comosum* have the same basic genome, M, but recent data on chromosome pairing in interspecific hybrids do not agree with that hypothesis (Kimber, Pignone, and Sallee, 1983).

A numerical analysis of chromosome pairing in hybrids involving autotetraploid *T. uniaristatum* and *T. rectum* showed a close relationship between one genome of *T. rectum* and the genome of *T. uniaristatum* (Yen and Kimber, 1992). Yen and Kimber (1992) considered their study inconclusive since they did not investigate *T. comosum.* The relationship of *T. comosum* with *T. rectum* was investigated here, and it was shown that *T. comosum* did not contribute any of the three pairs of *T. rectum* genomes. The RSC of 1.0 between *T. uniaristatum* and *T. rectum* provides strong evidence that *T. uniaristatum* was the third parent of *T. rectum.* Thus, the most probable origin of *T. rectum* is hybridization of *T. neglectum* with *T. uniaristatum.* We therefore propose revising Kihara's (1963) formula $M^tM^tM^{t2}M^{t2}$ e^uc^u (Kihara's e^u is synonymous with U) for *T. rectum* to UUX^tX^tNN.

Utility of RNSs for phylogenetic studies of polyploids—Inferences obtained by the analyses of RNSs and those obtained by chromosome pairing studies remarkably agree. Both approaches showed that the five species have the U genome of *T. umbellulatum.* Both approaches also agree on the source of the third pair of genomes of *T. rectum.* Finally, both approaches failed to find a perfect homologue to the second pairs of genomes of the five polyploid species among the extant diploid *Triticum* species. These general agreements between the two approaches, as well as those reported previously for other polyploid *Triticum* species (Dubcovsky and Dvořák, 1994b), clearly show that the technique used here is a valid alternative to chromosome pairing studies in interspecific hybrids. Compared to chromosome pairing studies, the RNS technique is far less laborious and time consuming. In some situations, the technique may be also more informative than chromosome pairing studies, e.g., when a homologous genome is not found among the diploid relatives of a polyploid (Dubcovsky and Dvořák, 1994b; Dvořák and Dubcovsky, 1995). In the present study, the RNSs approach, in agreement with the chromosome pairing approach, identified *T. comosum* as an extant species that is the most closely related to the source of the second pair of genomes of *T. ovatum* and *T. biunciale* but, in disagreement with the chromosome pairing approach, showed that the almost universally accepted assumption that *T. neglectum, T. columnare,* and *T. rectum* have another version of the *T. comosum* genome is erroneous. The RNS technique of genome analysis is more informative than that employing variation in ctDNA because it provides information on the identity of all parents, not only one, as the ctDNA technique does.

LITERATURE CITED

BENNETT, M. D. 1972. Nuclear DNA content and minimum generation time in herbaceous plants. *Proceedings of the Royal Society of London* B 181: 109–135.

BERG, K. H. 1937. Beitrag zur Genomanalyse in der Getraide-Gruppe. *Zuchter* 9: 157–163.

BIRKY, C. W., AND R. V. SKAVARIL. 1976. Maintenance of genetic homogeneity in systems with multiple genomes. *Genetic Research* 27: 249–265.

CHENNAVEERAIAH, M. S. 1960. Karyomorpohologic and cytotaxonomic studies in Aegilops. *Acta Horti Gotoburgensis* 23: 85–178.

DOVER, G. 1982. Molecular drive: A cohesive mode of species evolution. *Nature* 299: 111–117.

DUBCOVSKY, J., AND J. DVOŘÁK. 1994a. Genome identification of the *Triticum crassum* complex with the restriction patterns of repeated nucleotide sequences. *American Journal of Botany* 82: 131–140.

———, AND ———. 1994b. Genome origin of *Triticum cylindricum, Triticum triunciale,* and *Triticum ventricosum* (Poaceae) inferred from variation in repeated nucleotide sequences: a methodological study. *American Journal of Botany* 81: 1327–1335.

DVOŘÁK, J., P. DI TERLIZZI, H. B. ZHANG, AND P. RESTA. 1993. The evolution of polyploid wheats: Identification of the A genome donor species. *Genome* 36: 21–31.

———, AND J. DUBCOVSKY. 1995. Genome analysis of polyploid species employing variation in repeated nucleotide sequences. *In* P. P. Jahar [ed.] Genome Analysis in Plants, 133–145. CRC Press, Boca Raton, FL.

———, D. JUE, AND M. LASSNER. 1987. Homogenization of tandemly repeated nucleotide sequences by distance-dependent nucleotide sequence conversion. *Genetics* 29: 34–40.

———, P. E. MCGUIRE, AND B. CASSIDY. 1988. Apparent sources of the A genomes of wheats inferred from the polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. *Genome* 30: 680–689.

———, AND H. B. ZHANG. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proceedings of the National Academy of Sciences,* USA 87: 9640–9644.

———, AND ———. 1992. Reconstruction of the phylogeny of the genus *Triticum* from variation in repeated nucleotide sequences. *Theoretical and Applied Genetics* 84: 419–429.

FELDMAN, M. 1965. Further evidence for natural hybridization between tetraploid species of *Aegilops* section *Pleionathera. Evolution* 19: 162–174.

KIHARA, H. 1937. Genomanalyse bei Triticum und Aegilops. VII. Kurze Ubersicht uber die Ergebnisse der Jahre 1934–36. *Memorial College of Agriculture, Kyoto Imperial University* 41: 1–46.

———. 1940. Verwandschaft der Aegilops-Arten in Lichte der Genomanalyse. Ein Uberblick. *Zuchter* 12: 49–62.

———. 1949. Genomanalyse bei Triticum und Aegilops. IX. Systematische Aufbau der Gattung Aegilops auf genomoanalytischer Grundlage. *Cytologia* 14:135–144.

———. 1963. Interspecific relationship in *Triticum* and *Aegilops. Seiken Ziho* 15: 1–12.

KIMBER, G., AND M. FELDMAN. 1987. Wild wheat: an introduction. *Special Report of the College of Agriculture, University of Missouri, Columbia* 353:1–142.

KIMBER, G., D. PIGNONE, AND P. J. SALLEE. 1983. The relationships of the M and M^u genomes of *Triticum. Canadian Jornal of Genetics and Cytology* 25: 509–512.

———, P. J. SALLEE, AND M. M. FEINER. 1988. The interspecific and evolutionary relationships of *Triticum ovatum. Genome* 30: 218–221.

———, AND Y. YEN. 1989. Hybrids involving wheat relatives and autotetraploid *Triticum umbellulatum. Genome* 32: 1–5.

OGIHARA, Y., AND K. TSUNEWAKI. 1988. Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. *Theoretical and Applied Genetics* 76: 321–332.

PAZY, B., AND D. ZOHARY. 1965. The process of introgression between *Aegilops* polyploids: natural hybridization between *A. variabilis, A. ovata,* and *A. biuncialis. Evolution* 19: 385–395.

PERCIVAL, J. 1932. Cytological studies of some wheat and *Aegilops* hybrids. *Annals of Botany* 46: 479–501.

SENJANINOVA-KORCHAGINA, M. 1932. Karyo-systematical investigation of the genus *Aegilops* L. *Trudi po Prikladnoi Botaniki, Genetiki i Selekci, Seria 2* 1: 1–90.

SMITH, G. P. 1976. Evolution of repeated DNA sequences by unequal crossing over. *Science* 191: 528–535.

STRACHAN, T., D. WEBB, AND G. DOVER. 1985. Transition stages of molecular drive in multiple-copy DNA families in Drosophila. *European Molecular Biology Organization Journal* 4: 1701–1708.

TALBERT, L. E., G. M. MAGYER, M. LAVIN, T. K. BLAKE, AND S. L. MOYLAN. 1991. Molecular evidence for the origin of the S-derived genomes of polyploid *Triticum* species. *American Journal of Botany* 78: 340–349.

TSUCHIYA, T. 1956. Hybrids between *Aegilops triaristata* (4×) and *A. comosa, Heldreichii* and *uniaristata. Wheat Information Service* 3: 22–23.

YEN, Y. 1990. The U genome in *Triticum ovatum* from Turkey. *Cereal Research Communications* 18: 13–17.

———, AND G. KIMBER. 1992. Genomic relationships of N-genome *Triticum* species. *Genome* 35: 962–966.

ZHANG, H. B., AND J. DVOŘÁK. 1991. Phylogeny of tetraploid species of *Leymus* (family Poaceae, tribe Triticeae) inferred from variation in repeated nucleotide sequences. *American Journal of Botany* 78: 871–884.

———, AND ———. 1992. The genome origin and evolution of hexaploid *Triticum crassum* and *Triticum syriacum* determined from variation in repeated nucleotide sequences. *Genome* 35: 806–914.

———, ———, AND J. G. WAINES. 1992. Evolutionn of tetraploid *Triticum peregrinum* and *T. kotschyi* examined with variation in repeated nucleotide sequences. *Genome* 35: 182–191.

ZOHARY, D., AND M. FELDMAN. 1962. Hybridization between amphiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. *Evolution* 16: 44–61.

———, AND ———. 1965. Colonizer species in the wheat group. *In* Genetics of colonizing species. Academic Press, New York, NY.