Molecular studies of pivotal-differential evolution of wild wheat
by Peng Wah Chee

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
The theory of pivotal-differential evolution states that one genome of polyploid wheats remains stable (i.e., pivotal) during evolution, while the other genome or genomes may become modified (i.e., differential). A proposed mechanism for apparent modification of the differential genome is that different polyploid species with only one genome in common may exchange genetic material. An alternative explanation is that, the differential genomes are variable due to variability imparted by diploid progenitors. In this study, we analyzed a set of sympatric and allopatric accessions of tetraploid wheats of genomic constitution UM and UC. The U genome of these species is from Triticum umbellulatum. and is considered to be the pivotal genome. The M and C genomes, from T. comosum and T. dichasians. respectively, are considered to be the differential genomes. Variation in structure among homologous low copy DNA fragments was analyzed using "sequence-tagged-site" primer sets in the polymerase chain reaction (PCR), followed by digestion with restriction enzymes. Genetic similarity matrices based on shared restriction fragments showed that sympatric accessions of different U genome tetraploid species did not tend to share more restriction fragments than allopatric accessions. Thus, no evidence for introgression was found. Analysis of the diploid progenitor species showed that the U genome was less variable than the M and C genomes. Additionally, comparison of diploid and tetraploid species with genome-specific primer sets suggested a possible multiple origin for T. triunciale and T. machrochaetum. respectively. Thus, our results suggest that the differential nature of the M and C genomes in these species may be due to variability introduced by the diploid progenitors, and not due to frequent introgression events.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The theory of pivotal-differential evolution states that one genome of polyploid wheats remains stable (i.e., pivotal) during evolution, while the other genome or genomes may become modified (i.e., differential). A proposed mechanism for apparent modification of the differential genome is that different polyploid species with only one genome in common may exchange genetic material. An alternative explanation is that the differential genomes are variable due to variability imparted by diploid progenitors. In this study, we analyzed a set of sympatric and allopatric accessions of tetraploid wheats of genomic constitution UM and UC. The U genome of these species is from Triticum umbellulatum, and is considered to be the pivotal genome. The M and C genomes, from T. comosum and T. dichasians, respectively, are considered to be the differential genomes. Variation in structure among homologous low copy DNA fragments was analyzed using "sequence-tagged-site" primer sets in the polymerase chain reaction (PCR), followed by digestion with restriction enzymes. Genetic similarity matrices based on shared restriction fragments showed that sympatric accessions of different U genome tetraploid species did not tend to share more restriction fragments than allopatric accessions. Thus, no evidence for introgression was found. Analysis of the diploid progenitor species showed that the U genome was less variable than the M and C genomes. Additionally, comparison of diploid and tetraploid species with genome-specific primer sets suggested a possible multiple origin for T. triunciale and T. machrochaetum, respectively. Thus, our results suggest that the differential nature of the M and C genomes in these species may be due to variability introduced by the diploid progenitors, and not due to frequent introgression events.

INTRODUCTION

Evolution of polyploid wild wheats in the U-genome cluster has been analyzed by morphological (Zohary and Feldman 1962; Feldman 1965a, 1965b) and cytological studies (Feldman 1965c; Kimber and Yen 1988a, 1988b; Yen and Kimber 1990). The theory of pivotal-differential evolution (Zohary and Feldman 1962) has been proposed to explain patterns of relationship for these species. The theory states that one genome of the polyploid wild wheats, the "pivotal genome", remains stable or unchanged during evolution. The other genome in the polyploids may become modified and is referred to as "differential".

The U genome cluster is composed of seven tetraploid species of UM, UC and US genome combinations (Kimber and Feldman 1987). Based on chromosome pairing analysis between tetraploids and putative diploid progenitors, Feldman (1965c) suggested that the U-genome from the diploid T. umbellulatum is the unchanged or pivotal genome. The M, C and S genomes from T. comosum, T. dichasians and T. speltoidies, respectively, were designated differential genomes. The frequency of chromosome pairing between tetraploid species and their putative genomic donors is less frequent within the differential genome group compared to the pivotal group (Feldman 1965c).

The theory of pivotal-differential evolution states that the unpaired genome has been modified since the original

polyploidization. The pivotal genome has acted as a buffer to ensure hybrid fertility while genetic exchange can occur between the differential genomes. This exchange of genetic information was proposed as a cause of the modified nature of the differential genome (Zohary and Feldman 1962). Observations that hybrids are often present where different species are found sympatrically supports the theory (Zohary and Feldman 1962; Feldman 1965a, 1965b). Also, morphological characters that are predominantly found in one species are sometimes found in other sympatric species, indicating the possibility of lateral gene flow (Zohary and Feldman 1962; Feldman 1965a). Therefore, gene flow among the U genome tetraploids is proposed to have resulted in a stable U genome and modified differential genomes.

Talbert et al. (1993) examined repetitive DNA sequences specific for the U and M genome, respectively, to address aspects of pivotal-differential evolution in the U genome cluster. In particular, investigation of sympatric and allopatric accessions of four UM tetraploid species did not show repetitive DNA similarities among sympatric accessions of different species. This result did not support the concept that continual exchange was occurring. Additionally, diploid T. umbellulatum (U genome) accessions were similar regarding hybridization patterns observed with repetitive DNA clones, while diploid T. comosum (M genome) accessions were much more variable. These results raised the possibility that the

apparent differential nature of the M genome in the tetraploid may be due to variability inherent in the diploid, rather than variation induced after polyploidization.

The development of sequence-tagged-site polymerase chain reaction (STS-PCR) in wheat offers an approach for wheat genome analysis targeting low copy DNA sequences. STS-PCR is an enzymatic amplification of a mapped DNA fragment flanked by a pair of oligonucleotide primers. This technique was first developed for human genome analysis (Olson et al. 1989) and has been successfully incorporated into various aspects of cereal research (Talbert et al. 1994, Chee et al. 1993, Tragroonrung et al. 1992).

For this paper, studies were conducted to test aspects of pivotal-differential evolution in U genome wild wheats. Specifically, we investigated the prevalence of introgression among different tetraploid species within the U genome cluster. Our hypothesis was that if introgression is a common event, accessions of different species from the same location (sympatric) should share more DNA fragments than the same species in different locations (allopatric). We also determined the relative levels of diversity among diploid accessions of T. umbellulatum, T. comosum and T. dichasians, and examined the possibility that diversity within the diploid species was analogous to that found in the tetraploids. Our results suggest an alternative explanation for the differential nature of the M and C genomes in the U-genome tetraploids.

LITERATURE REVIEW

Evolution of Wild Wheat

The current understanding of the phylogenetic history of Triticum (Aegilop) species is that a common diploid ancestor diverged into many diploid species, followed by subsequent hybridizations to form the polyploid species (Kimber and Feldman 1987). Meiotic pairing analysis demonstrates that almost every diploid species has a distinct genome and the polyploids consist of two or more different diploid genome combinations. The common ancestor of diploid Triticum species probably arose from a species with a basic chromosome number of seven, which may now be extinct.

The diploid Triticum species have diverged considerably from one another. As a result, homeologous chromosomes no longer pair regularly at meiosis in interspecific hybrids. Therefore, although natural hybridization between diploid species occurs, the hybrids are completely or almost completely sterile (Kimber and Feldman 1987). Over generations of divergent evolution, the diploids have become genetically isolated from each other.

The polyploids, in contrast, are genetically less restricted. Hybrids between different polyploid species are often encountered whenever they are found sympatrically (Zohary and Feldman 1962). A wide range of morphological intermediates between different polyploids growing together in

one mixed stand is a common phenomenon (Kimber and Feldman 1987). Based on morphological evidence, Zohary and Feldman (1962) have suggested that some polyploid species are genetically interconnected through genetic introgression and thus cannot be viewed as separate entities. Other researchers, however, suggest that introgression is of little importance in this plant group (Talbert et al. 1993). In either case there is no doubt that the ability to tolerate polyploidy in Triticum is a contributor to the evolutionary success of this species.

The advantages of polyploid over diploid species is reflected by the geographic and ecological distribution of the two groups. The diploid species have a narrow range of ecological niches. As a result, they each have their distinctive morphological characters such as spikelet shape and seed dispersal mechanisms due to different modes of adaptative specialization (Zohary and Feldman 1962). Conversely, the polyploids have a wide range of intermediate forms. They have a greater ability to invade and colonize new or disturbed habitats than the diploid progenitors.

One distinct feature of the polyploids is the presence of a common (unaltered) genome and one or more different (modified) genomes. Based on chromosome pairing analysis, the shared genome is determined to be the unaltered one and is easily identified by pairing with a known diploid species. The differential genomes, on the other hand, are unique to a

given polyploid species. They are altered and therefore do not pair well with the diploid progenitors. The evolutionary significance of such a genomic structure is explained by the pivotal-differential evolution theory.

Pivotal-differential Evolution

Based on morphological and cytogenetic studies the polyploid Triticum species are divided into three natural clusters (Zohary and Feldman 1962). The species of each cluster share one common genome and differ in their other genome or genomes. The three clusters include 1) species sharing the U genome of T. umbellulatum, 2) species sharing the D genome of T. tauschii, and 3) species sharing the A genome of T. monococcum.

The U-genome cluster has a wider distribution than the D- and A-genome clusters. This is hypothesized to be influenced by the ability of these species to exchange genetic material through interspecific hybridization (Zohary and Feldman 1962). Considerable evidence suggests the occurrence of hybridization between U genome tetraploids (Feldman 1965a; Zohary and Feldman 1962). For instance, hybrids were found in mixed population of T. peregrinus (UM genome) and T. kotschyi (US genome) in southern Judea in Israel. Also, T. peregrinus, T. ovatum, T. macrochaetum and T. triunciale were found growing together along with hybrids in northern Israel (Kimber and Feldman 1987).

Zohary and Feldman (1962) proposed the pivotal-differential evolutionary hypothesis for polyploid species in the U genome cluster. According to the theory, the pivotal and differential nature of the polyploid genomes are due to extensive hybridization between U genome tetraploids with a different second genome. For example, if T. macrochaetum (UM genome) is crossed with T. triunciale (UC genome), the offspring produced will have a common U genome and a modified genome due to the recombination of the M and C genomes. This type of genomic structure is thought to play an important role in the relatively high rate of successful hybridization within tetraploid species. The pivotal genome facilitates hybridization by acting as a genetic buffer to ensure fertility in the resulting hybrids, while recombination can occur between the differential genomes.

Introgression Studies

Introgression is considered to be important in providing plant populations with enhanced evolutionary flexibility, extending a species gene pool (Anderson 1948), producing novel gene combinations (Lewontin and Birh 1966), and a means of gene dispersal (Potts and Reid 1988). Attempts to detect introgression have been carried out by many researchers using various experimental techniques.

Taxonomic classification was first used to study introgression. This method involved observation of

distinctive morphological characters in one plant population and comparison of their intermediacy between one species and another (Anderson 1947). Morphological comparison has been quite successful in identifying introgression events. Anderson and Hubrieht (1938), studying intraspecific variation in herbarium material, have shown a strong introgression of Tradescantia canaliculata into T. occidentalis and T. bracteata. Introgression of morphological characters was observed in the Aegilops-Triticum group (Zohary and Feldman 1962; Feldman 1965a; Vardi 1973).

There are three potential problems associated with morphological comparison. Firstly, morphological characters can be affected by environmental fluctuations and stage of development (Monte et al. 1993). Secondly, it is difficult to determine if character intermediacy results from introgression or recent common ancestry. This is especially perplexing in species like Zea (Doebley 1984) and Triticum (Kimber and Feldman 1987) where a large amount of natural variation exists. Third, the occurrence of hybrids does not necessarily lead to genetic exchange, due to the fact that the hybrids produced may be noncompetitive with the parental types or reproductively sterile (Doebley et al. 1984).

Isozyme comparison has been a useful tool for some introgression studies. It has been employed to assess the prevalence of introgression in Zea (Doebley et al. 1948) and Cypripedium (Klier et al. 1991). This technique is not

informative for many introgression studies, because of limited numbers of isozyme loci and lack of polymorphism.

The best approach to study genetic introgression is through the analysis of the genetic material itself, the DNA molecule. The development of molecular techniques for DNA analysis provides a new means for evolutionary genetic studies. Molecular data obtained from RFLPs and DNA sequencing have proven to be powerful for investigation of introgession (Keim et al. 1989; Brubaker et al. 1993). Data from RFLPs is independent of environmental influences and has permitted clear documentation of interspecific gene flow among sympatric accessions of Gossypium species (Brubaker et al. 1993).

Chloroplast, mitochondria (Ogihara and Tsunewake 1988; Doyle et al. 1992) or nuclear DNA may be evaluated by RFLP analysis (Kleinhofs et al. 1993; Anamthawat-Jonsson and Helsop-Harrison 1992). Using chloroplast DNA, Murai and Tsunewaki (1986) found that T. triunciale originated at least twice, from reciprocal crosses of T. dichasians and T. umbellulatum. Keim et al. (1989), using nuclear DNA determined the unidirectional introgression of genes from a Populus species population in the Weber Canyon, Utah.

The development of sequence-targeted-site PCR (STS-PCR) primers in various plant species offers a simpler approach for genome analysis than RFLP analysis and allows a more thorough

investigation. STS-PCR is an enzymatic amplification of a mapped DNA fragment flanked by a pair of oligonucleotide primers (Saiki et al. 1985; Mullis and Faloona 1987). The amplification product is often a homogeneous DNA fragment that can be digested with restriction enzymes (Tragoonrung et al. 1992; Talbert et al. 1994) or sequenced (Wang et al. 1987) to detect polymorphisms. STS-PCR offers advantages over RFLP analysis. These include more rapid and simpler analysis, no need to use radioactive reagents and analysis of larger sample size at one time. The latter advantages permit analysis of more representative samples of the population being studied, increasing the reliability of the studies.

One misconception concerning molecular techniques, like RFLPs and PCR, is that they allow comparison of only a very small amount of DNA relative to the total genomic material (Kimber and Yen 1990; Wang 1992). This argument states that comparison involving a single locus in sequence analysis or a six base pair sequence in RFLP studies is not representative since only a very small proportion of the genome is being analyzed. Proponents of this argument fail to acknowledge that a RFLP characteristic of a single locus can be representative of a segment of the chromosome or even a chromosome arm. This is due to the fact that large chromosomal segments are likely to be inherited as a unit (Rick and Tanskley 1988; Rogowsky et al. 1991) due to linkage. For instance, two genes which lie close to one another on the

chromosome are seldom recombined and are transmitted together from a parent to its progeny - the principle behind a linkage map construction (Paterson et al. 1991). Thus, if many DNA markers are employed, provided they are widely spaced across the whole genome, analyzing small segments of the DNA sequences approximates analysis of the entire genome as a whole.

MATERIALS AND METHODS

Plant Materials

Species, accessions and collection sites of the plant materials used are listed in Table 1. Accessions of different species were collected from mixed stands in Turkey by Metzger and Kimber (Kimber and Yen 1988a). Availability of this collection allowed direct comparison between sympatric and allopatric accessions between and within species. Barley (Hordeum vulgare) was included in the analysis as an outgroup species. Diploid accessions used in the genetic diversity studies were either from the Kimber collection, University of Missouri, or from Harold Bockelman, USDA National Small Grain Collection, Aberdeen, Idaho (Table 2). All plants were grown in the greenhouse and total plant DNA was isolated from young leaves as previously described (Talbert et al. 1992).

DNA Sequencing

Genomic clones of the D genome T. tauschii that were kindly provided by Dr. B. Gill were used to develop the STS-PCR primers. The RFLP clones were sequenced at both ends by the dideoxy chain termination method (Sanger et al. 1977) using Sequenase (United States Biochemical, Cleveland, Ohio) following the manufacturer's protocol. Approximately 20 basepairs in length at both ends of sequenced clones were chosen based on GC content to design the oligo primers.

Table 1. List of wild wheat accessions analyzed for introgression studies

Location	Species	Genome	Accession
28	*T. macrochaetum*	UM	TP 15
	T. triunciale	UC	TW 103
30	*T. macrochaetum*	UM	TP 16
	T. triunciale	UC	TW 105
58	*T. columnare*	UM	TY 23
72	*T. macrochaetum*	UM	TP 19
	T. triunciale	UC	TW 116
157	*T. macrochaetum*	UM	TP 37
	T. triunciale	UC	TW 185
159	*T. macrochaetum*	UM	TP 38
	T. triunciale	UC	TW 187
	T. neglecta	UM	TN 76
208	*T. neglecta*	UM	TN 77
	T. ovatum	UM	TO 49
209	*T. columnare*	UM	TY 25
	T. ovatum	UM	TO 52
212	*T. macrochaetum*	UM	TP 48
	T. ovatum	UM	TO 53

Table 2. Diploid accessions analyzed for low copy DNA variation by STS-PCR.

accessions	*Triticum*	Origin	accessions	*Triticum*	Origin
T.umb1 = TU 35	*umbellulatum*	Turkey	T.com21= PI 542176	*comosum*	Turkey
T.umb2 = TU 37		Turkey	T.com22= PI 542175		Turkey
T.umb3 = TU 43		Turkey	T.dic23= PI 276970		Japan
T.umb4 = PI 227339		Iran	T.dic24= TF 5	*dichasians*	Crete
T.umb5 = PI 542370		Iran	T.dic25= TF 6		Crete
T.umb6 = PI 428569		USSR	T.dic26= TF 8		Turkey
T.umb7 = CI 29		Unknown	T.dic27= TF 11		Turkey
T.umb8 = PI 116294		Germany	T.dic28= TF 12		Turkey
T.umb9 = PI 226500		Iran	T.dic29= TF 19		Turkey
T.umb10= PI 226616		Iran	T.dic30= TF 20		Turkey
T.umb11= PI 298906		Iraq	T.dic31= TF 21		Turkey
T.umb12= PI 298907		USSR	T.dic32= TF 22		Turkey
T.com13= TC 2	*comosum*	Turkey	T.dic33= TF 24		Turkey
T.com14= TC 6		Turkey	T.dic34= TF 25		Turkey
T.com15= TC 9		Turkey	T.dic35= TF 26		Turkey
T.com16= TC 12		Turkey	T.dic36= PI 298887		Turkey
T.com17= TC 14		Turkey	T.dic37= PI 254863		Iraq
T.com18= TC 15		Turkey	T.dic38= PI 551121		Grace
T.com19= TC 16		Turkey	T.dic39= PI 564195		Turkey
T.com20= CI 38		Japan			

Note: PI, CI and TF accessions are from National Small Grains Collection, USDA-ARS, Aberdeen, Idaho. Additional accessions with TC and TU designations come from a collection of Triticum species collected by L. Metzger and Kimber(Kimber and Yen 1988a).

STS-PCR Primers and Reaction Conditions

STS-PCR primers used were from two sources: 1) primers developed from mapped RFLP clones of the D genome diploid T. tauschii (Talbert et al. 1994; Chen et al. 199-), and 2) primers developed from the Montana State University barley mapping project (Tragroonrung et al. 1992). Also, one additional primer set, primer set pTAG 546, was synthesized on the basis of published sequences of moderately repetitive, dispersed and highly variable genomic sequences in wheat (Liu et al. 1992). The sequences of the pTAG primers are (5′ TO 3′) TCTCATCCATGAACGCATG and ACTGCAACAGTACAGACTCC. PCR amplification was performed using the protocol of Chen et al. (199-).

Table 3. STS-PCR primer sets used for both diploids and tetraploids analysis.

Chromosome	Primer set
1	Pst 340, D14
2	D18, LPWG 178, G5, H9, F11
3	WG 110, G36
4	Pst 316, WG 464, G10, E9, TB 19-20, D21, B5, F8
5	G44, Pst 319, H8
6	ABG 458, G43, D17, ABG 20,
7	A1, D2, D15, WG 686
ND	F19, pTAG 546, KV 29-30

Chromosomal location were from either Talbert et al. 1994, Chen et al. 199-, Gill et al 1991, or Kleinhofs et al. 1993. Primer set sequences were previously published in Chen et al. (199-) and Talbert et al. (1994).
ND = Not Determined

Electrophoresis

PCR products of 25 ul were digested with 2 units of the four-base-cutter restriction enzymes HinfI and HhaI. Either RsaI, HaeIII or undigested products were also analyzed if results with HinfI or HhaI were unclear. Digested PCR products were separated on a 7% polyacrylamide gel at 200 V for 1.5 hour. The gel was stained with ethidium bromide and DNA fragments was visualized under UV light.

Statistical Analysis

Data was recorded as the presence (1) or absence (0) of restriction fragments for each accession. A genetic similarity (GS) matrix based on Jaccard's similarity coefficient was constructed for both the diploid and tetraploid species. Similarity coefficients were used to determine the number of restriction fragments shared between tetraploid accessions. The data matrices were then subjected to cluster analysis using the computer program NTSYS-PC (ver. 1.80 J. Rohlf 1993).

RESULTS

Introgression Among Tetraploid Species

A total of 50 primer sets were used for PCR amplification in 5 tetraploid species, consisting of 18 different accessions (Table 1). Although the primer sets were selected for their ability to amplify mapped low copy sequences in wheat (T. aestivum), only 33 primer sets produced readable results with the U genome wild wheats (Table 3). From those 33 primer sets, a total of 368 restriction fragments were scored. On the average, sympatric accessions of different species shared 51.7% of the restriction fragments while allopatric accessions of different species shared 51.3 % of the restriction fragments (Table 4). For example, Table 4 shows that sympatric accessions of T. macrochaetum (TP) and T. triunciale (TW) shared 50.2% of restriction fragments while allopatric accessions of these same species shared 50.5%. In no case were the differences between sympatric and allopatric accessions statistically significant ($P < .05$). Allopatric accessions of the same species shared 71.0 percent of restriction fragments (Table 4). Thus, genetic similarities based on shared low copy DNA sequences is not suggestive of frequent introgression among tetraploid species.

Table 4. Mean genetic similarities of sympatric and allopatric tetraploid accessions calculated from data matrix of shared restriction fragments.

Species compared	Sympatric	Allopatric	
	different species	different species	same species
TPs and TWs	.502	.505	-
TPs and TOs	.621	.610	-
TPs and TNs	.479	.485	-
TWs and TNs	.500	.493	-
TNs and TOs	.495	.487	-
TOs and TYs	.506	.497	-
TPs	-	-	.688
TWs	-	-	.759
TNs	-	-	.703
TOs	-	-	.793
TYs	-	-	.606
Mean genetic similarities	.517	.513	.710

Cluster Analysis

The greatest genetic similarity (GS) in pairwise comparisons among tetraploid species was 88.6% for accessions TO 49 and TO 52 and the lowest GS was 42.3% for accessions TN77 and TW103 (data not shown). Cluster analysis divided the 24 accessions into 8 distinct groups (Figure 1). Of the 8 groups, 4 groups were composed of the 5 tetraploid species *T. triunciale*, *T. machrochaetum*, *T. ovatum*, *T. columnare* and *T.neglecta*. Three groups were formed from diploid accessions

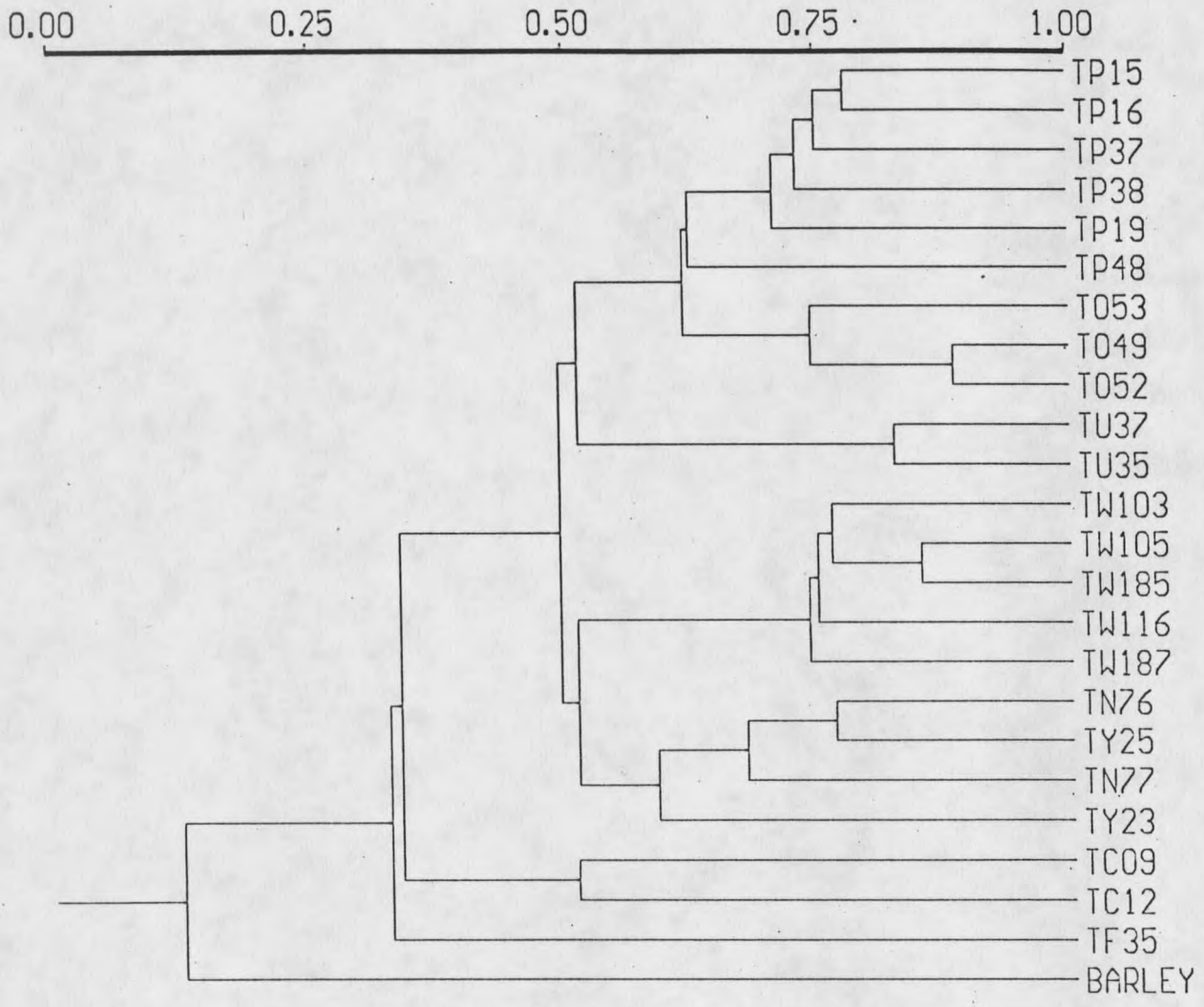

Figure 1. Cluster analysis of three diploid (TU, TC, TF) and five tetraploid (TP, TO, TW, TN, TY) Triticum species from Turkey collected by L. Metzger and Kimber (see Table 1). Barley was used as an outgroup. The phenogram has a cophenetic correlation (r) of 0.974.

of T. umbellulatum, T. comosum, and T. dichasians, respectively. Barley, which was used as a reference species in the analysis, represents the eighth group. With the exception of T. neglecta and T. columnare, all accessions maintained species integrity. Accessions did not group to location.

One primer set (primer set A1) indicated a possible introgression event whereby one accession of T. triunciale contained a fragment otherwise specific to T. macrochaetum. Figure 2 shows an 805 bp fragment which is absent in all the T. triunciale (UC genome) accessions except TW 116. Conversely, the fragment was present in all accessions of T. machrochaetum (UM genomes). As indicated in figure 1, for all other restriction fragments, TW 116 grouped consistently with T. triunciale. In that this type of observation is consistent with an introgression event (Kiem et al. 1989; Brubaker et al. 1993; Doebley 1990), we were interested in determining the origin of the anamolous fragment. Thus, we amplified DNA from a larger collection of U, M, and C diploid accessions with primer set A1. The presence of this fragment in some accessions of T. umbellulatum (U genome) and T. dichasians (C genome) indicated that the 805 basepairs fragment was not necessarily introgressed into T. triunciale. An alternative explanation is that the fragment was contributed by a diploid ancestor.

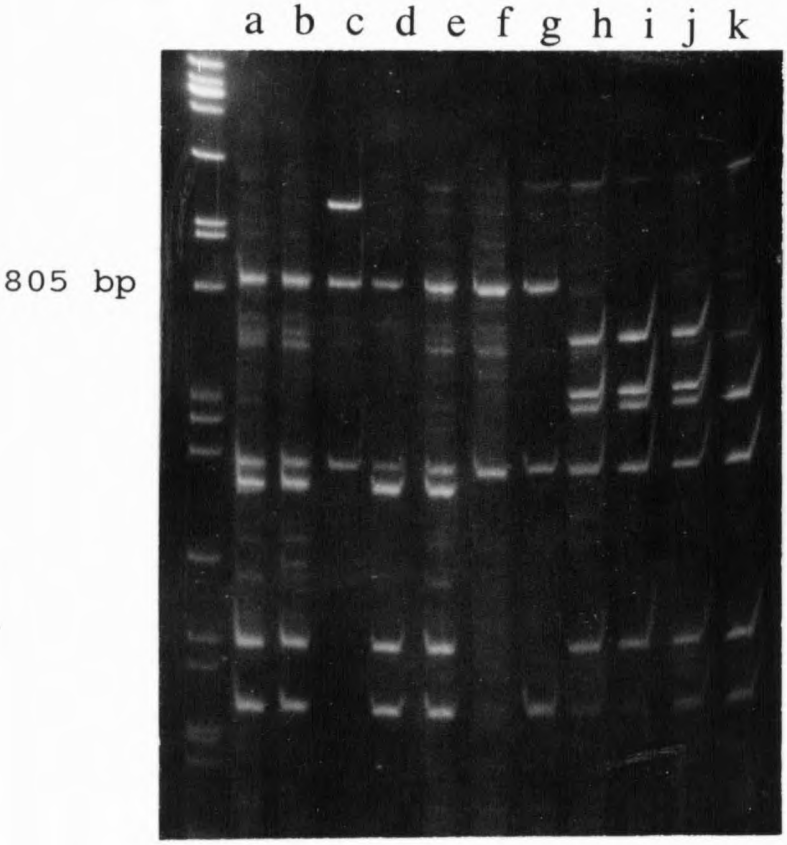

Figure 2. PCR products of primer set A1 separated on a 7% acrylamide gel. The samples were digested with Hha I restriction enzyme. Lanes a to f represent T. machrochaetum (a: TP15; b: TP16; c: TP19; d: TP37; e: TP38; f: TP48) and T. triunciale (g: TW116; h: TW103; i: TW105; j: TW185; k: TW187)

Genetic Diversity of the Diploid Progenitors

Results with the tetraploid species indicated that introgression may not explain the differential nature of the M and C genome in the UM and UC tetraploid. An alternative explanation is that the M and C genome diploids are themselves more variable than diploid U genome, and that multiple hybridization events would lead to variable genomes in the tetraploids. To test this hypothesis, we analyzed 39 accessions of the diploid species T. umbellulatum (U genome), T. comosum (M genome) and T. dichasians (C genome).

Figure 3 shows the phylogram constructed from a matrix based 344 restriction fragments. A cophenetic correlation (r) of 0.987 was obtained. All the 39 accessions of the three species maintain species integrity. Variation exist within accessions of the three species. T. umbellulatum has a mean GS of 0.80 with the standard deviation of 0.058. And T. dichasians has a mean GS of 0.71 with the standard deviation of 0.091. Whereas, T. comosum has a GS of 0.68 and a standard deviation of 0.190. These results indicate that there is less variation within accessions of T. umbellulatum as compared to T. comosum and T. dichasians. Paired t-test comparisons show that the differences in average genetic similarity among the three species are statistically significant ($P < .05$). Average genetic similarity for accessions within T. comosum and T. dichasians, respectively, were not significantly different. This result was clearly

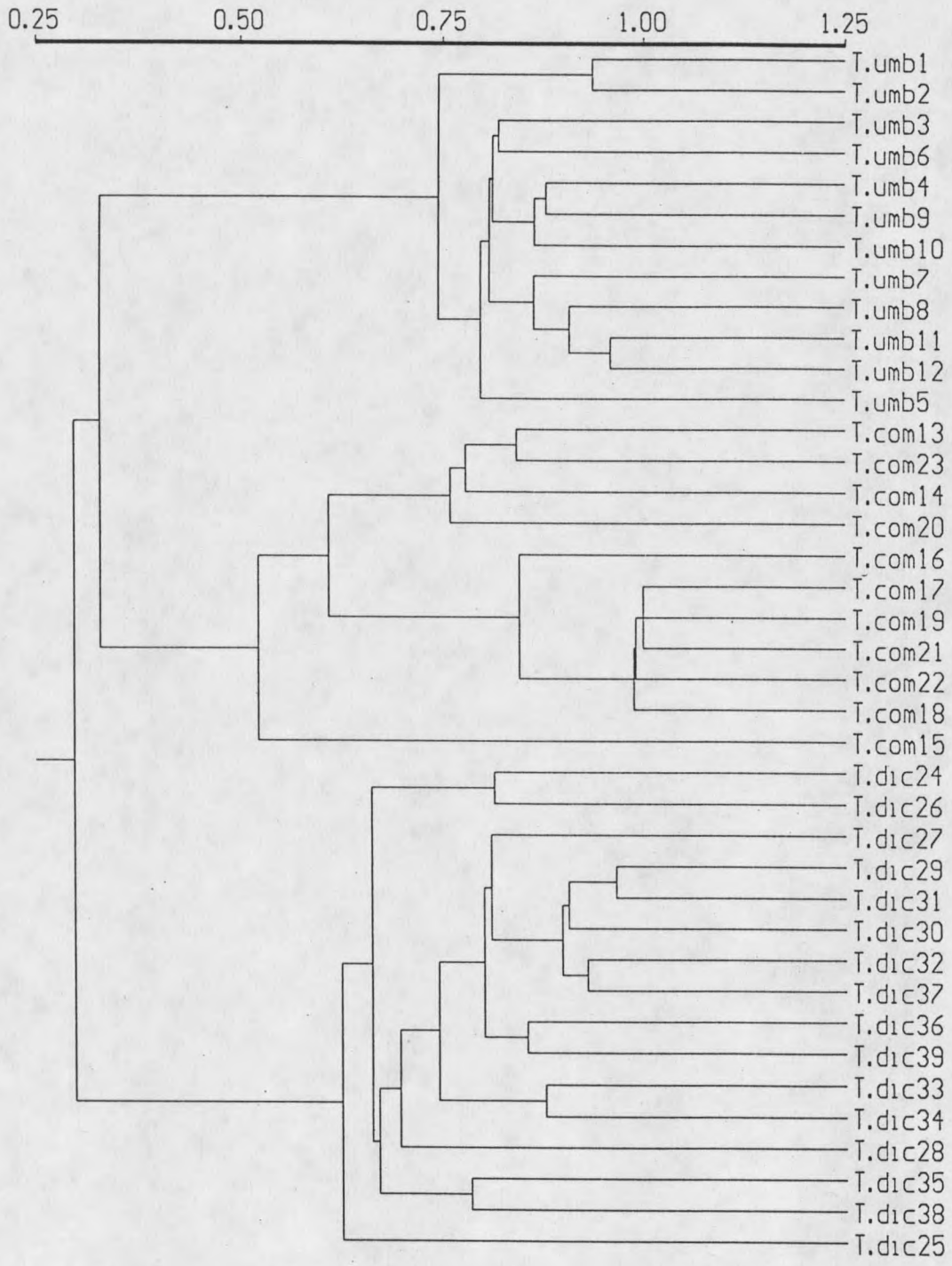

Figure 3. Phylogenetic tree showing the relationship among accessions of the three diploid Triticum species. The U, M and C genomes are represented by T. umbellulatum (T.umb), T. comosum (T.com) and T. dichasians (T.dic), respectively. The accessions in each lane are listed in Table 2. The phenogram has a cophenetic correlation (r) of 0.987.

reflected on the phylogram in that accessions of both T. comosum and T. dichasians were more widespread and point to the generalization that T. umbellulatum was the less diverse species of the three.

Evidence for Multiple Origin of the Tetraploids

Data with diploid and tetraploid species suggested that gene flow among tetraploids is low, and that diploid M and C accessions were more diverse than the U accessions. Thus, one explanation for the differential nature of M and C genomes in the tetraploids is multiple hybridization events leading to tetraploid UM and UC species, respectively. In search of evidence for multiple origin of the tetraploid wild wheats, we were fortunate to identify seven primer sets that preferentially amplified to only one or two of the three genomes. These primer sets allowed direct comparison between individual genomes in the tetraploids with those of the diploid (Figure 4). For instance, results obtained with the M-genome specific primer set D17 are shown in Figure 4, Panel 1. In this case, the restricted PCR products of T. macrochaetum (UM genomes) produced three patterns (lanes a - f) and two of those were present in accessions of diploid T. comosum (lanes g - q). Analogous results were obtained with primer set G43, which amplifies sequences in the U and M genomes. Amplification of T. triunciale (UC genome) with G43 produces only a U genome DNA fragment. Figure 4, Panel 2 has

a restriction analysis of PCR products for G43 showing three different banding patterns in T. triunciale (lanes a - e), two of which were present in T. umbellulatum (lanes f - n). Thus, results with primer sets G43 and D17 support a multiple origin for T. triunciale and T. macrochaetum, respectively. Of the other genome-specific primer sets tested, three did not show polymorphism within the tetraploids. For the other two primer sets, variation in the tetraploids did not correspond with that observed in any of the diploid accessions analyzed. This suggests divergence of the genome in the tetraploid and that of the diploid after hybridization.