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MOLECULAR CYTOGENETIC ANALYSIS OF RECENTLY EVOLVED *TRAGOPOGON* (ASTERACEAE) ALLOPOLYPLOIDS REVEAL A KARYOTYPE THAT IS ADDITIVE OF THE DIPLOID PROGENITORS[1]

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Tragopogon mirus and *T. miscellus* (both $2n = 4x = 24$) are recent allotetraploids derived from *T. dubius* × *T. porrifolius* and *T. dubius* × *T. pratensis* (each $2n = 2x = 12$), respectively. The genome sizes of *T. mirus* are additive of those of its diploid parents, but at least some populations of *T. miscellus* have undergone genome downsizing. To survey for genomic rearrangements in the allopolyploids, four repetitive sequences were physically mapped. TPRMBO (unit size 160 base pairs [bp]) and TGP7 (532 bp) are tandemly organized satellite sequences isolated from *T. pratensis* and *T. porrifolius*, respectively. Fluorescent in situ hybridization to the diploids showed that TPRMBO is a predominantly centromeric repeat on all 12 chromosomes, while TGP7 is a subtelomeric sequence on most chromosome arms. The distribution of tandem repetitive DNA loci (TPRMBO, TGP7, 18S-5.8S-26S rDNA, and 5S rDNA) gave unique molecular karyotypes for the three diploid species, permitting the identification of the parental chromosomes in the polyploids. The location and number of these loci were inherited without apparent changes in the allotetraploids. There was no evidence for major genomic rearrangements in *Tragopogon* allopolyploids that have arisen multiple times in North America within the last 80 yr.

Key words: centromere; chromosomal evolution; fluorescent in situ hybridization (FISH); genome size; polyploidy; rDNA; subtelomere; *Tragopogon*.

Polyploidy, the process of genome doubling that gives rise to organisms with multiple sets of chromosomes, is a prominent feature in the evolution of flowering plants (Stebbins, 1971). Up to 70% of all species of flowering plants may have experienced one or more episodes of polyploidy in their evolutionary past, and many crops are of polyploid origin including maize, wheat, and cotton (e.g., Stebbins, 1971; Grant, 1981; Masterson, 1994). Polyploids often have novel phenotypes that are not present in their diploid progenitors or exceed the range of the parental species (reviewed in Levin, 1983, 2002; Ramsey and Schemske, 2002). The mechanisms by which polyploidy contributes to novel variation are not well understood, in part because polyploidization involves a number of genetic and epigenetic phenomena (reviewed in Soltis and Soltis, 1999, 2000; Matzke et al., 1999; Wendel, 2000; Liu and Wendel, 2002; Osborn et al., 2003; Soltis et al., 2004). The degree of genomic change in recent natural and synthetic allopolyploids seems to vary across taxa. For example, synthetic polyploids in *Gossypium* (Liu et al., 2001; Adams and Wendel, in press) and recently formed natural polyploids in *Spartina* (Baumel et al., 2001, 2002; Ainouche et al., in press) had few changes in overall genome structure. In contrast, *Arabidopsis* (Madlung et al., 2002; Chen et al., in press), *Brassica* (Song et al., 1995; Pires et al., in press), *Triticum* (Ozkan et al., 2001; Shaked et al., 2001; Levy and Feldman, in press), and *Nicotiana* (Skalicka et al., 2003; Kovarík et al., in press) demonstrate genomic change in association with allopolyploidy.

Another phenomenon that can accompany polyploidization is a change in genome size. One might expect a general pattern of additivity in genome size between progenitor diploids and derivative polyploids, as is seen in *Gossypium* (Liu and Wendel, 2002). However, there are also examples of non-additivity in polyploid genome size, as seen in polyploids reported to show decreased DNA amounts relative to diploids (e.g., *Vigna*, Parida et al., 1990), as well as polyploids with increased amounts of DNA (e.g., *Linum*, Cullis, 1979). In fact, the overall trend in polyploid evolution across the angiosperms is for ''genome downsizing'' (reviewed in Leitch and Bennett, in press). These and other recent studies have drawn attention to the potentially dynamic nature of polyploids (e.g., Soltis and Soltis, 1999). However, many aspects of the molecular consequences of polyploid formation and subsequent genome evolution remain to be elucidated.

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TABLE 1. Locality, accession, and genome size data for *Tragopogon* plants and seeds collected from Idaho (ID) and Washington (WA) in western North America. All accession numbers are from Soltis and Soltis herbarium specimens deposited in Pullman, Washington (WA). If population was not measured, then value is not applicable (na). If multiple individuals were measured in a population, then more than one value is shown. The last two columns indicate which accessions were used in molecular cytogenetic analyses.

Species	Collection number	Locality	4C DNA amount ± SD (pg)	TGP7, TPRMBO and TRS	18–26S and 5S
T. dubius	2613	Pullman, WA	10.83 ± 0.65	X	X
			11.76 ± 0.79		
T. dubius	2614	Rosalia, WA	10.80 ± 0.96		
T. dubius	2615	Spokane, WA	na	X	X
T. porrifolius	2612	Potlatch, ID	13.17 ± 0.83	TRS only	X
T. porrifolius	2611	Pullman, WA	12.50 ± 1.21	X	5S only
T. porrifolius	2607	Troy, ID	na	X	18S only
T. pratensis	2598	Colton, WA	11.59 ± 1.13	X	X
T. pratensis	2608	Moscow, ID	12.44 ± 0.87	X	X
T. pratensis	2609	Spangle, WA	11.08 ± 0.60	X	
T. mirus	2601	Finch's	24.33 ± 1.26		X
		Pullman, WA	24.32 ± 1.41		
			25.11 ± 1.75		
T. mirus	2603	Rosalia, WA	20.90 ± 1.23	X	5S only
			24.93 ± 1.55		
			25.64 ± 1.44		
T. mirus	2602	Palouse, WA	na		X
T. miscellus	2604	Moscow, ID	20.30 ± 1.50	X	18S only
T. miscellus	2605	Pullman, WA	20.99 ± 1.14		X
T. miscellus	2606	Spangle, WA	21.76 ± 0.87	X	5S only

The immediate consequences of polyploidization can best be studied in either synthetic polyploids or natural polyploids of recent ancestry. While evolutionary biologists can create synthetic polyploids from any number of taxa, only four plant genera have been documented to have generated natural polyploid species in the last 150 yr: *Cardamine* (Urbanska et al., 1997), *Spartina* (Baumel et al., 2001, 2002; reviewed in Ainouche et al., in press), *Senecio* (Ashton and Abbott, 1992; reviewed in Abbott and Lowe, in press), and *Tragopogon* (Ownbey, 1950; reviewed in Soltis et al., in press). *Tragopogon* (Asteraceae) has approximately 150 species native to Eurasia. Three diploid ($2n = 2x = 12$) species (*T. dubius*, *T. pratensis*, and *T. porrifolius*) that were introduced into eastern Washington State, USA, and adjacent Idaho in the early 1900s have recently and recurrently formed two allopolyploid ($2n = 4x = 24$) species, *T. mirus* (*T. dubius* × *T. porrifolius*) and *T. miscellus* (*T. dubius* × *T. pratensis*) in western North America (Ownbey, 1950; Ownbey and McCollum, 1954; Brown and Schaak, 1972; Soltis and Soltis, 1989, 1991, 1999; Soltis et al., 1995; Cook et al., 1998). Ownbey and McCollum (1954) used traditional cytogenetic methods to karyotype the six pairs of chromosomes in the introduced diploid species of *Tragopogon*. Along with morphological characters, Ownbey and McCollum (1954) observed sufficient chromosomal variation (e.g., terminal knobs and secondary constrictions) among the different populations of diploid species to infer the multiple origins of the two recently formed allopolyploids, *T. mirus* and *T. miscellus*. These recurrent formations were later confirmed with molecular methods (Soltis et al., 1995).

Our goal is to determine whether chromosomal rearrangements or changes in genome size have occurred in the *Tragopogon* allotetraploids since their recent formation. One way to survey for genomic rearrangements in polyploids is to locate repetitive DNA physically on chromosomes using the tools of molecular cytogenetics such as fluorescent in situ hybridization (FISH), a technique that has provided insights into genome and chromosome evolution (reviewed in Heslop-Harrison, 1991, 2000; Jiang and Gill, 1994, 1996; Leitch and Bennett, 1997; Schwarzacher and Heslop-Harrison, 2000; Singh, 2003). While some researchers have localized rDNA loci onto chromosomes of members of Asteraceae (reviewed in Torrell et al., 2003), few studies have isolated tandem repeats (satDNA) from representatives of the family for use in molecular cytological studies (*Crepis*, Jamilena et al., 1993, 1995; *Brachycome*, Houben et al., 2000). This is surprising given the historical interest in chromosome evolution and polyploidy in this large plant family (e.g., Tahara, 1915; Lawrence, 1929; Gustafsson, 1932; Babcock and Stebbins, 1938; Clausen et al., 1945; Carr, 1985; Baldwin, 1993). Because tandem repeats have provided insights into genome and chromosome evolution (Jiang and Gill, 1994, 1996; Heslop-Harrison, 2000), we characterized four tandem repeats in *Tragopogon*: two novel tandem repeats (TPRMBO and TGP7) that we isolated specifically for this study and two rDNA loci (18S-5.8S-26S and 5S). We used molecular cytogenetics (FISH) to determine the number and distribution of these four tandem repeats to investigate the genetic consequences of allopolyploidy in *Tragopogon*. The FISH technique was carried out on multiple populations of the three diploid species (*T. dubius*, *T. pratensis*, and *T. porrifolius*), and karyotypes were constructed. The same probes were then hybridized to multiple populations of the recently formed allotetraploid species (*T. mirus* and *T. miscellus*) to determine if chromosomal rearrangements had occurred subsequent to polyploidization. We also measured DNA *C* values for the same diploid and tetraploid *Tragopogon* species to determine the dynamics of genome size evolution in this polyploid complex.

MATERIALS AND METHODS

Plant material—Seeds of *Tragopogon* were collected from Idaho (ID) and Washington (WA) (USA) (Table 1) and were planted in the greenhouses at Washington State University. Plants were grown to maturity and allowed to

self-pollinate. Selfed seeds were collected and germinated in a greenhouse at RBG Kew; root tips and leaf tissue were harvested from young plants.

DNA C values—DNA *C* values for *Tragopogon* species were determined with Feulgen microdensitometry using methods described in Hanson et al. (2001) using *Pisum sativum* cv. Minerva Maple (4C = 19.46 pg) as a calibration standard. In brief, root tips from newly germinated *Tragopogon* seeds were fixed in a freshly prepared solution of 3 : 1 ethanol : glacial acetic acid at −20°C overnight. Roots were hydrolyzed in 5 mol/L hydrochloric acid at 25°C for 35 min, stained in the dark for 2 h in Feulgen reagent at 23°C, then washed three times each for 10 min in SO_2 water (per Hanson et al., 2001). Root tips were squashed in 45% acetic acid, and cover slips were temporarily sealed with rubber solution. Slides were kept in a refrigerator overnight, and readings were taken the following day on a Vickers M85a scanning microdensitometer (York, Yorkshire, UK). Thirty nuclei (10 nuclei judged to be at mid-prophase in mitosis on each of three slides) were measured for each *Tragopogon* species and for the calibration standard (*Pisum*). For each slide, the mean and standard deviation of readings were calculated and used to obtain the overall mean and standard deviation. Arbitrary values were converted to picograms from the ratio of the mean absorbency of the *Tragopogon* test species to that of the calibration standard. For *T. miscellus* populations 2604 and 2605, for which we also sampled populations representing their diploid ancestors (*T. dubius* 2613 and *T. pratensis* 2608), we compared the genome size value predicted by the sum of the parental genomes with the observed *C* values using a modified *t* test (Sokal and Rohlf, 1980, p. 231).

DNA extraction, Southern blotting, and DNA sequencing—Total genomic DNA was extracted from 1 g of fresh young leaves of each *Tragopogon* species using a cetyltrimethylammonium bromide (CTAB) method and ethanol and ammonium acetate precipitations (Doyle and Doyle, 1987) with minor modifications (Smith et al., 1991). Total genomic DNA was purified using spin columns in the QIAquick PCR purification kit (Qiagen, Valencia, California, USA). DNA was diluted to approximately 50–200 ng/mL. Methods for Southern blotting and methylation analysis follow standard procedures as described in Kovarík et al. (2000). DNA sequencing was carried out using the dideoxytermination method (Sequenase Version II DNA sequencing kit, USB, Amersham Biosciences, Cleveland, Ohio, USA). Sequence data were deposited, and the GenEMBL database was searched for similar sequences.

Cloning of tandem repeats from Tragopogon—*Isolation of TPRMBO tandem repeat*—DNA of *Tragopogon pratensis* was digested with an excess of *Mbo*I restriction endonuclease (10 units/g DNA) overnight and size-separated on a 7% polyacrylamide gel. A band of approximately 160 bp was excised, purified, and ligated into the *Bam*HI site of pZero vector (Invitrogen, Carlsbad, California, USA). The ligation mix was used to transform *E. coli* DH5 α cells, and clones were screened for recombinants that labeled strongly in Southern blot hybridization against the ^{32}P-labeled genomic DNA of *T. pratensis* and contained 160-bp inserts. Inserts from 14 candidate colonies were sequenced (GenEMBL #AJ307715-28) and aligned using Wisconsin GCG software. A consensus sequence was constructed because all colonies contained essentially the same sequence with only a few mutations.

Isolation of TGP7 tandem repeat—Because only low amounts of genomic DNA were obtained from *T. porrifolius*, we employed a novel strategy for the isolation of a repeat from this species. The method is based on the original AFLP methodology (Vos et al., 1995) and involved the ligation of an adapter to the *Sau*3AI-restricted genomic DNA from *T. porrifolius*. The *Sau*3AI adapter was prepared by annealing two single-stranded complementary oligonucleotides (5′-GACGATGAGTCCTGAG-3′, 5′-GATCCTCAGGACTCAT-3′)] and 50 pmols was used in a 50-μL ligation mixture containing 0.5 μg restricted genomic DNA, reaction buffer, and 1 unit T4 ligase (Roche, Mannheim, Germany). After ligation (2 h, 20°C), the DNA was purified using the QIAquick polymerase chain reaction (PCR) purification kit (Qiagen), and approximately 1/10 volume was amplified by PCR (0.5 μmol/L primer [5′-GATGAGTCCTGAGGATC-3′] in 0.2 mmol/L dNTPs, 2.5 mmol/L $MgCl_2$, enzyme buffer, and 1 unit of *Taq* polymerase; initial denaturation [3 min, 92°C] and 25 cycles of denaturation, 15 s at 92°C; annealing, 30 s at 50°C; extension, 30 s at 72°C). The PCR conditions were empirically adjusted to obtain ~100–200 ng of PCR products of <600 base pairs (bp) in length. Amplified DNA was purified using the QIAquick PCR purification kit (Qiagen), digested with *Sau*3AI, and ligated into the *Bam*HI site of pBluescript vector (Stratagene, La Jolla, California, USA). The ligation mix was used to transform *E. coli* DH5 α cells. Twenty randomly selected recombinant plasmids were digested with enzymes (*Bst*XI + *Pst*I) and screened by Southern hybridization with ^{32}P-labeled genomic DNA of *T. porrifolius*. The clone with the strongest signal was recovered (TGP7delta) and sequenced. On Southern blots, the cloned sequence appeared shorter than the monomeric unit of the satellite. The PCR primers were designed with a 2-bp overlap to obtain the full-length clone. The PCR products were cloned into the *Eco*RV site of pBluescript KS + vector (Stratagene, La Jolla, California, USA) and sequenced (GenEMBL #AF493256).

Analysis of chromatin structure—Nuclei of *T. pratensis* were prepared for micrococcal nuclease (MNase) treatments using a protocol modified from Espinás and Carballo (1993). All operations were performed at 0–4°C. Approximately 1.5 g of fresh leaves were ground in liquid nitrogen and transferred to 50 ml of buffer A (10 mmol/L NaCl, 10 mmol/L MES pH 6.0, 5 mmol/L EDTA, 0.15 mmol/L spermidine, 20 mmol/L beta-mercaptoethanol, 0.6% TRITON X-100, 0.2 mol/L sucrose, and 0.1 mmol/L PMSF) and quickly homogenized. The homogenate was filtered through silk and centrifuged at 2620 × g for 10 min. The pellet was resuspended in 15 mL of buffer A, centrifuged again, resuspended in buffer B (88% mass/mass PERCOL in buffer A), and centrifuged at 5150 × g for 10 min. Floating nuclei (0.4 mL) were removed, resuspended in 1 mL of MNase digestion buffer (10 mmol/L Tris-HCl pH 7.5, 100 mmol/L, ammonium sulfate, 5 mmol/L $MgCl_2$, 3 mmol/L $CaCl_2$, 5 mmol/L beta-mercaptoethanol), and treated with 30 units/mL of MNase at 37°C. Aliquots were removed from the reaction mixture at various times and the digestion stopped with equal volumes of stop buffer (1% sarcosyl, 0.25 mol/L EDTA pH 8, 5 mmol/L EGTA, and 0.5 mol/L NaCl). The samples were treated with proteinase K for several hours at 52°C. DNA was extracted with phenol-chloroform-isoamyl alcohol and ethanol precipitated. Five micrograms of DNA were loaded and fractionated on a 2% agarose gel. After subsequent hybridization of blots with TPRMBO, TGP7, and rDNA, the signal in the oligonucleosomal fraction (mononucleosome, dinucleosome, etc.) was quantitatively evaluated (using a Storm phosphorimager; Storm, Molecular Dynamics, Sunnyvale, California, USA). The average length of DNA was expressed in nucleosome multiples for each selected time interval of MNase digestion according to the method described in Fulnecek et al. (2002). The initial rate of MNase digestion was calculated and expressed as a decrease of fragment size per time interval. Only the nearly linear regions of curves (0–3 min) were considered for calculation of digestion rates.

Chromosome preparation and fluorescent in situ hybridization—Chromosome preparations and fluorescent in situ hybridization (FISH) were performed for multiple populations of the three parental diploid species (*T. dubius*, *T. pratensis*, and *T. porrifolius*) and two allopolyploid derivatives (*T. mirus* and *T. miscellus*) following Leitch et al. (1994) with slight modifications as in Lim et al. (2000b). Healthy root tips of germinating seeds were pretreated at 4°C for 18 h and fixed at room temperature in 3 : 1 ethanol : glacial acetic acid overnight. Root tips were digested in 0.2% (mass/volume) cellulase R10, 0.2% (mass/volume) pectolyase Y23, and 0.2% (mass/volume) drieselase for 30 min and then transferred to 1% citrate buffer for 3–4 h. The meristematic cells behind the root cap were dissected, macerated, and squashed on a glass slide in a drop of 60% acetic acid. Five probes were used in the FISH experiments: (1) TPRMBO, (2) TGP7, (3) TRS (telomere repeat sequence, Cox et al., 1993), (4) pTa71, a clone containing a 9-kb *Eco*RI fragment of 18S-5.8S-26S rDNA and the intergenic spacer from *Triticum aestivum* (Gerlach and Bedbrook, 1979); and (5) pTZ19-R, a 120-bp fragment of the 5S rDNA unit isolated from *Nicotiana rustica* (Venkateswarlu et al., 1991). Probes cut from plasmids were labeled using nick translation as follows: TPRMBO, TRS, and 5S rDNA were labeled with digoxigenin-11-dUTP (Roche Biochemicals, Sussex, UK) detected with antidigoxigenin-FITC;

TGP7 and 18S-5.8S-26S rDNA were labeled with biotin-16-dUTP (Sigma-Aldrich Company Ltd, Poole, Dorset, UK) detected with avidin-Cy3 (Amersham Pharmacia Biotech, Little Chalfont, UK). The probe mixture was prepared as in Lim et al. (2000b). After overnight hybridization at 37°C, the slides were given a stringent wash in 20% (volume/volume) formamide in 0.1% SSC at 40–42°C. Chromosomes were counterstained for DNA with 4,6-diamidine-2-phenylindole, dihydrochloride (DAPI). Images were taken with a Leica Aristoplan epifluorescent microscope, and photographs were scanned and processed using Adobe Photoshop (Adobe Systems, Edinburgh, UK). Only those functions that apply equally to all pixels in the image were used. For every set of observations, between five and 10 metaphases were examined from each of 1–3 slides.

RESULTS

C values in diploid and polyploid Tragopogon—Table 1 shows the DNA *C* values calculated from plants of different populations of diploids (*T. dubius*, *T. pratensis*, and *T. porrifolius*) and allotetraploids (*T. miscellus* and *T. mirus*). Genome size varied among different populations of the diploid species. Among the diploid species, *T. dubius* appears to have the smallest genome size and *T. porrifolius* the largest.

The DNA *C* values of *T. mirus* are not substantially different from the sum of the diploid parents. The genome size of *T. mirus* (population 2601 from Pullman, Washington, USA; mean 4C DNA value of 24.33) is additive of the genome sizes of its putative diploid progenitor populations, *T. dubius* (population 2613 from Pullman, Washington USA; mean 4C DNA value of 11.76) and *T. porrifolius* (population 2611 Pullman, Washington, USA; mean 4C DNA value of 12.5). Although we measured 4C DNA values for another population of *T. mirus* (2603 from Rosalia, Washington, USA), we cannot comment unequivocally on the additivity of its genome size because we were able to measure only the genome size of the *T. dubius* parent; plants of the second parent, *T. porrifolius*, are no longer present in Rosalia and are presumed extinct. Nonetheless, the two 4C DNA measurements for plants of *T. porrifolius* from other localities are very similar.

In contrast, the genome size of at least some populations of *T. miscellus* appears to have undergone downsizing when compared to its diploid progenitors. Populations 2604 (Moscow, Idaho, USA) and 2605 (Pullman, Washington, USA) have the same diploid parental genotypes (*T. pratensis* [2608 Moscow, Idaho, USA] and *T. dubius* [2613 Pullman, Washington, USA]), but are the result of reciprocal parentage (Soltis and Soltis, 1989). The genome size values for these two populations are 20.30 pg and 20.99 pg, respectively, which are lower than the value predicted (24.20 or 23.27 pg) by adding the values of the diploid parents ($t = 18.79958$, $P < 0.05$; $t = 13.88154$, $P < 0.05$, for predicted values of 24.20 and 23.27, respectively). This level of downsizing—approximately 15%—is similar to that reported in the allopolyploids *Brassica napus*, *B. juncea*, and *B. carinata* (Naryan, 1998). The genome size of a third population of *T. miscellus* (2606 from Spangle, Washington, USA) is 21.76 pg; the value for the parental *T. pratensis* from Spangle is 11.09 pg, but we lack genome size data for *T. dubius* from Spangle.

Detection, isolation, and characterization of the TPRMBO repeat—Total genomic DNA of *T. pratensis* digested with *Mbo*I yielded 160-bp bands after size fractionation on PAGE and ethidium-bromide staining (not shown). The 160-bp fragments were excised and cloned (GenEMBL #AJ307715-28). One clone, named TPRMBO4, was used as the probe for Southern hybridization. The ladder of bands generated using different restriction endonucleases in the Southern hybridization experiments indicates that TPRMBO is a tandem repetitive sequence (Fig. 1a). Regular ladders were found with *Sau*3AI and *Mbo*I restriction enzyme digestions and irregular ladders in higher multimer fractions using *Rsa*I, *Alu*I, *Mbo*II, *Ssp*I, and *Dra*I (Fig. 1a). Database searches revealed no sequence similarities to other known families of repetitive sequences. The sequenced TPRMBO monomers are 160 ± 2 bp in length and moderately A + T rich (67%). A search for internal structural motifs revealed no direct repeats, palindromes, or inverted repeats, although the sequence does have several oligoA and oligoT motifs in an arrangement typical for tandemly arranged and curved satellite sequences (Fitzerald et al., 1994).

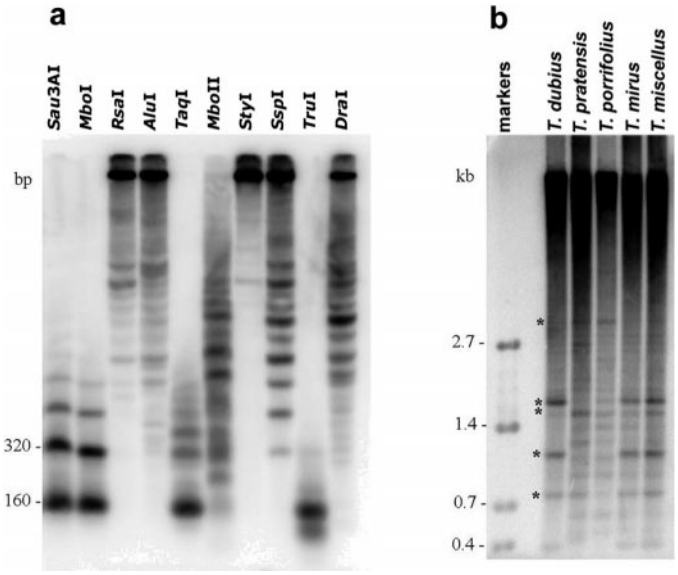

Fig. 1. Southern blot hybridization analysis of TPRMBO repetitive sequences in *Tragopogon* DNA using ^{32}P-labeled insert of TPRMBO clone 4. (a) Genomic organization in *T. pratensis*. The genomic DNA was digested with enzymes indicated. Both regular (*Sau*3AI, *Mbo*I digestions) and irregular (*Rsa*I, *Alu*I, *Mbo*II, *Ssp*I, *Dra*I digestions) ladders were revealed, indicating considerable heterogeneity in sequence and arrangement of repeats. A 160-bp fragment containing monomeric units was recovered after digestion with *Sau*3AI, *Mbo*I, and *Taq*I. *Sau*3AI digested DNA slightly less than *Mbo*I, probably due to methylation. (b) Distribution of TPRMBO repeats in diploid (*T. dubius*, *T. pratensis*, and *T. porrifolius*) and allotetraploid (*T. mirus*, *T. miscellus*) species. Genomic DNAs were digested with *Hind*III. All diploid and allotetraploid *Tragopogon* species have TPRMBO repeats. Note species-specific polymorphisms in the hybridization patterns among species in low-molecular-mass fraction (indicated by asterisks).

*Sau*3A1 is a methylation-sensitive isoschisomer of *Mbo*I and is unable to cut GAT^{m}C. A densitometric evaluation of the *Sau*3A1and *Mbo*I digestion patterns (Fig. 1a) revealed that the average numbers of restriction sites per monomeric unit are 0.68 and 0.59, respectively. Thus, at least 13% of the cytosines in sequences GATC are methylated. Inspection of GATC sites in TPRMBO sequences revealed that the cytosine occurred in asymmetrical sequence contexts (CAC and CTT) in the majority of cases. Asymmetrical sites are less methylated than the symmetrical CG and CNG sites in plant genomes (Meyer et al., 1994). Thus, the data show that TPRBMO contains some methylation at asymmetrical nucleotide sites.

To examine the occurrence of TPRMBO repeats in species

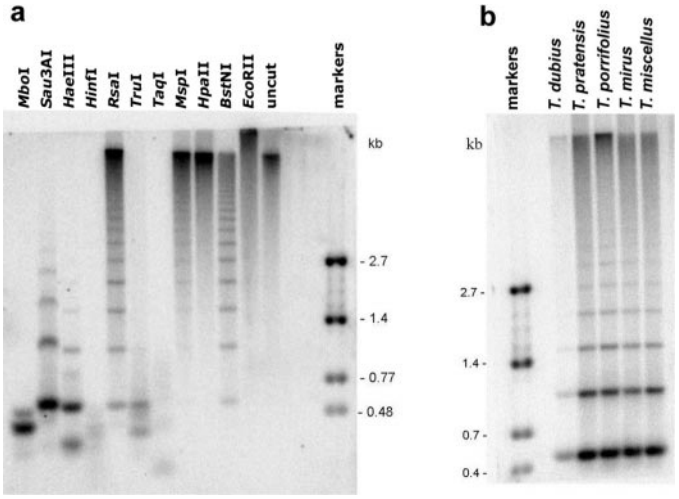

Fig. 2. Restriction analysis of TGP7 repetitive sequences in *Tragopogon* DNA using ^{32}P-labeled TGP7 in Southern hybridization reactions. (a) Genomic organization in *T. porrifolius*. The genomic DNA was digested with enzymes indicated. Regular ladders spaced at ~530 bp were revealed with most enzymes, indicating relative homogeneity of the repeats. The DNA was more efficiently digested with methylation-insensitive enzymes (*Mbo*I, *Bst*NI) compared to their methylation-sensitive isoschizomers (*Sau*3AI, *Eco*RII). (b) Distribution of TGP7 in diploid (*T. dubius*, *T. pratensis*, *T. porrifolius*) and allotetraploid (*T. mirus*, *T. miscellus*) species. Genomic DNAs were digested with *Hind*III. All diploid and allotetraploid *Tragopogon* species have TGP7 repeats. The signal in the lane with *T. dubius* DNA was significantly weaker than in lanes with DNAs of other species.

of *Tragopogon*, we digested genomic DNA from *T. dubius*, *T. pratensis*, *T. porrifolius*, *T. mirus*, and *T. miscellus* with *Hind*III and hybridized the blot with the TPRMBO4 probe (Fig. 1b). A hybridization signal of comparable intensity was found in all species. Band polymorphisms were revealed in low-molecular-mass fractions. The species-specific hybridization bands (indicated by asterisks, Fig. 1b) were additive in lanes loaded with DNAs from the allotetraploids *T. mirus* and *T. miscellus*. It is likely that *Tragopogon* allotetraploids inherited the genomic arrangement of TPRMBO repeats from their respective diploid ancestors.

Isolation and characterization of the TGP7 repeat—A novel isolation strategy was used to obtain a repeated sequence from *T. porrifolius*. The main advantage of this AFLP-based cloning strategy is the ability to isolate a sequence even when only low amounts of DNA or degraded DNA are available. While conventional band excision cloning requires at least 5000 ng of high-molecular-mass DNA, the described method enabled the successful cloning of a repeat from only 50 ng of genomic DNA. The only limitation of this method appears to be the requirement for a GATC site in the monomeric unit of a repeat. This limitation could be overcome by using multiple adapters to target sequences of frequent-cutting restriction enzymes (e.g., *Mse*I, *Rsa*I, *Taq*I).

We recovered a clone carrying a 532-bp insert that hybridized strongly with ^{32}P-labeled genomic DNA from *T. porrifolius*. This clone, TGP7, was used as a probe for Southern hybridization (Fig. 2). Highly regular ladders of bands were obtained using *Sau*3AI, *Hae*III, *Rsa*I, *Bst*NI (Fig. 2a), and *Hind*III (Fig. 2b). These restriction enzymes indicated a tandem arrangement of units. Most of the repeats were digested with *Hind*III into monomeric units of ~530 bp, suggesting a conserved *Hind*III site. *Mbo*I digested DNA more efficiently than its methylation-sensitive *Sau*3AI isoschisomer, suggesting cytosine methylation of some GATC motifs. Similar results were obtained with *Msp*I/*Hpa*II and *Bst*NI/*Eco*RII isoschisomeric pairs.

Two clones containing monomeric units were sequenced and found to be 95% similar within the region of overlap. One clone (clone 6) contained a 139-bp segment that represents a perfect duplication of the terminal region between nucleotides 384 and 532. Such duplications could have arisen during cloning procedures. The sequence of clone 8 that matched the size of the satellite monomer (determined by Southern hybridization) was deposited in the nucleotide database (GenEMBL #AF493256). The sequence was 532 bp long and moderately A + T rich (58%). A search for structural motifs revealed several oligo(dA) and oligo(dT) motifs that appeared to be clustered in one part of the sequence; however, there were no larger palindromes or inverted repeats. Computer database searches did not reveal any subrepeats or sequence similarities between TGP7 and other sequences.

To determine the occurrence of TGP7 repeats in other *Tragopogon* species, genomic DNA from each species was digested with *Hind*III and probed with TGP7 (Fig. 2b). The ladder of signals indicates that TGP7 is a tandem repetitive sequence found in both diploid and allotetraploid *Tragopogon* species. However, the signal in the lane loaded with DNA from *T. dubius* was significantly less (three- to four-fold), suggesting a lower content of TGP7 repeats in this species compared to the others.

Analysis of chromatin structure—There may be spatial control of DNA condensation as seen for repetitive subtelomeric and intercalary sequences of wheat and related species (Vershinin and Heslop-Harrison, 1998). Therefore, we compared the chromatin structure of TGP7 and TPRMBO in *Tragopogon* by using a micrococcal nuclease sensitivity assay. As expected, both probes revealed a pattern of bands with nucleosomal periodicity (Fig. 3a). However, the two probes differed in some aspects. First, the length of the nucleosomal unit containing TPRMBO sequence appeared shorter (by ~5–7 bp) than that of TGP7—based on length differences between corresponding neighboring bands (mononucleosome–dinucleosome, dinucleosome–trinucleosome, etc.). This feature of TPRMBO chromatin could be related to the relatively short (~160-bp) TPRMBO monomeric units. Second, the kinetics (rate) of digestion of the sequences were different (Fig. 3b). The rates of DNA shortening calculated from slopes of kinetic curves were −2.25 nucleosome/min for 18S rDNA (Southern blot data not shown), −2.21 nucleosome/min for TGP7, and −1.25 nucleosome/min for TPRMBO. Therefore, the TPRMBO chromatin appears substantially less accessible to digestion than TGP7 and 18S rDNA loci and is probably more compact. Third, while the basic MNase digestion profile for TPRMBO reveals a mononucleosomal periodicity, in TGP7 every third band is more prominent (Fig. 3c). It is probable that the higher order structure of TGP7 is of a basic unit with a trinucleosomal length (530–540 bp).

Chromosomal location of the two satellite repeats: TPRMBO and TGP7—The FISH technique was conducted on *T. dubius* (Fig. 4a–c), *T. pratensis* (Fig. 4d–g), *T. porrifolius* (Fig. 4h–k), *T. miscellus* (Fig. 4l–m), and *T. mirus* (Fig. 4n–o) using TPRMBO (Fig. 4b, c, d, g, k, l, n) and TGP7 (Fig.

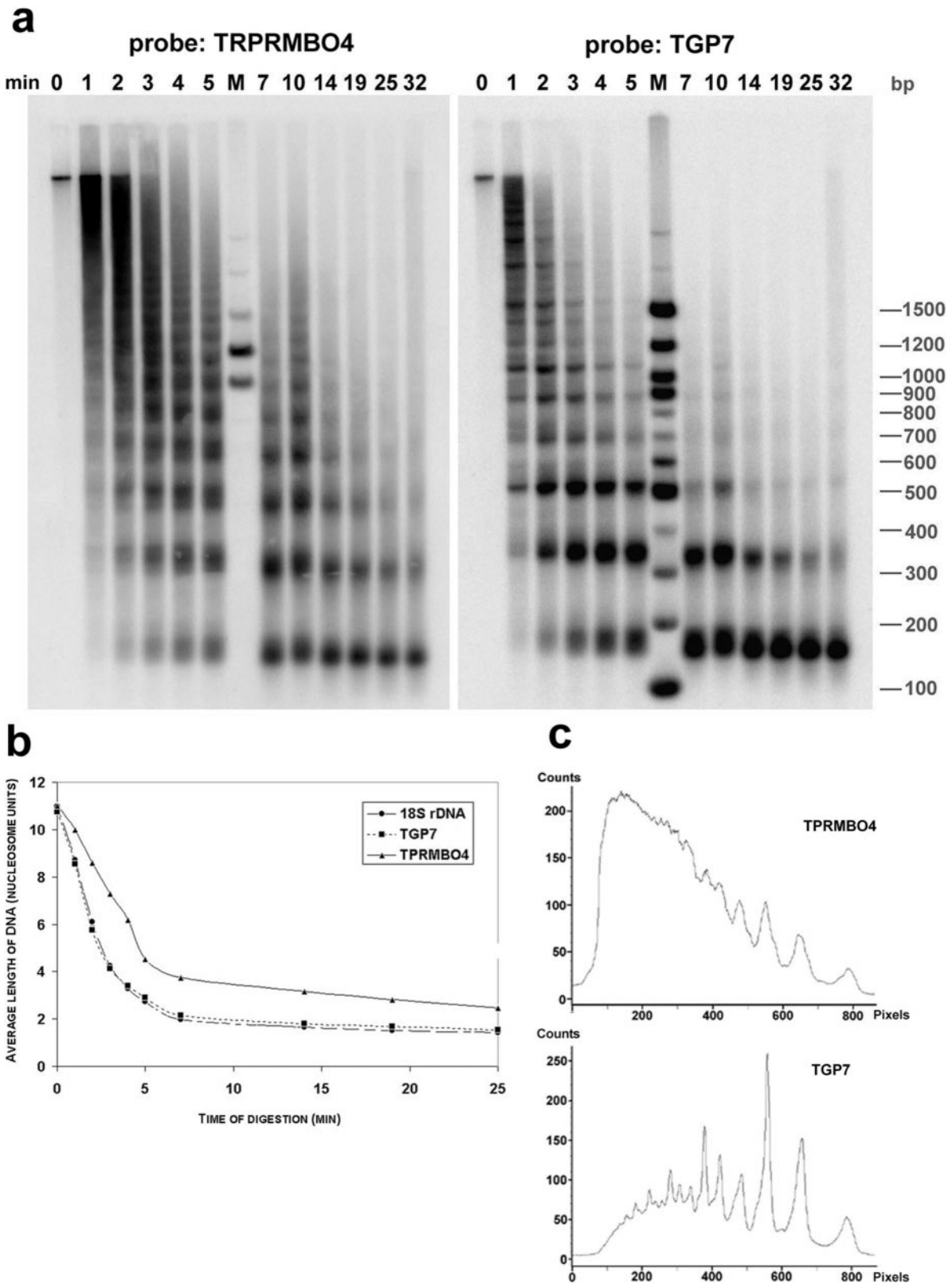

Fig. 3. Chromatin structure analysis of TPRMBO and TGP7 from *Tragopogon*. (a) Nucleosomal repeat analysis of chromatin containing TPRMBO and TGP7 sequences. Nuclei of *T. pratensis* were treated with MNase for the time intervals indicated and probed sequentially with TPRMBO and TGP7. M = 100 bp ladder. (b) After hybridization, the membrane was quantitatively evaluated (using a Storm phosphorimager) and the average length of DNA for each selected time interval of MNase digestion calculated from relative intensities of individual bands. (c) Densitometric scan of the line corresponding to 2 min of MNase digestion is shown for TPRMBO (top) and TGP7 (bottom) where the intensity of signal (in counts) is plotted against distance (in pixels) from electrophoretic start point.

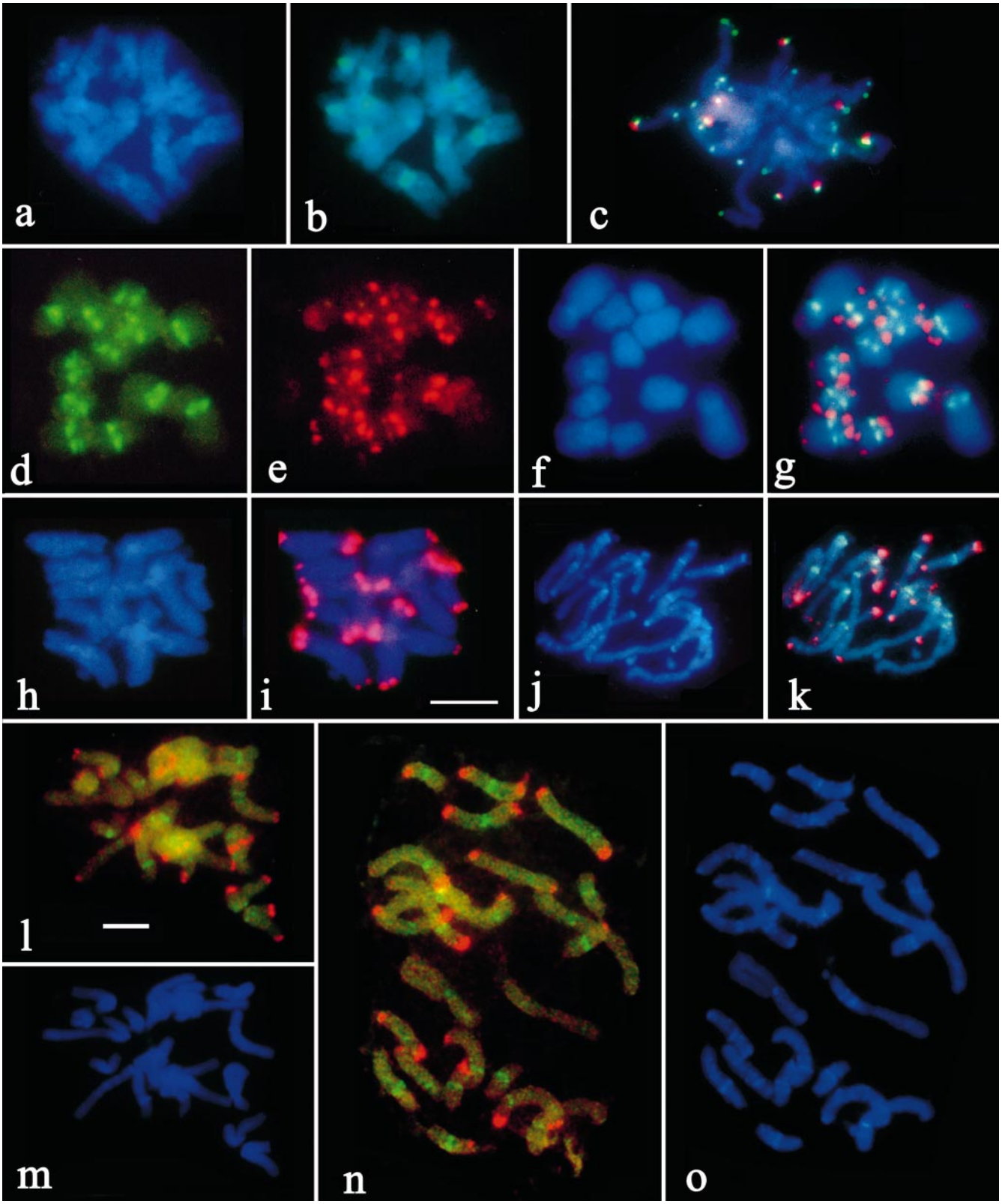

Fig. 4. Fluorescent in situ hybridization to *Tragopogon dubius* (a–c), *T. pratensis* (d–g), *T. porrifolius* (h–k), *T. miscellus* (l, m) and *T. mirus* (n, o). Metaphases of diploid *T. dubius*: (a) 4,6-diamidine-2-phenylindole, dihydrochloride (DAPI)-stained (blue) and (b) TPRMBO-labeled metaphase showing the probe hybridizes to centromeric repeats (green); (c) metaphase labeled with TGP7 (red, eight sub-telomeric signals) and TRS (green, at the telomeres of all chromosomes). Metaphase of diploid *T. pratensis* labeled with (d) TPRMBO, (e) TGP7, note the few weaker signals dispersed at pericentromeric regions, (f) DAPI-stained and d–f merged (g). Metaphases of diploid *T. porrifolius*: (h) DAPI-stained metaphase and (i) probed with TGP7; (j) DAPI-stained metaphase and (k) labeled with TGP7 (red) and TPRMBO (green). Metaphase of allopolyploids *T. miscellus* (l, m) and *T. mirus* (n, o): (l, n) FISH with TPRMBO (green) and TPG7 (red) and (m, o) DAPI stained (blue). Biotin-avidin-Cy3 detection, red fluorescence; digoxigenin-antidigoxigenin-FITC, green fluorescence; DAPI detection, blue fluorescence. Scale bars (a–i, j–o) = 10 μm.

4c, e, g, i, k, l, n) as probes. The distribution of signal on the diploid karyotypes is shown (Fig. 5g). For both diploid and polyploid species, TPRMBO hybridized to centromeric regions of all chromosomes, with FISH signals of similar intensity at each locus. Sometimes the denaturation conditions resulted in the formation of DAPI-positive bands; some of these bands coincide with TPRMBO signal (e.g., compare Fig. 4j, k).

The probe TGP7 hybridized to subtelomeric regions of most, but not all, chromosome arms. This location was confirmed using the telomeric probe TRS (shown only for *T. dubius*, Fig. 4c). In *T. porrifolius* and *T. pratensis*, 18 of 24 chromosome ends were labeled (Fig. 4e–g, i–k). In contrast, *T. dubius* had only eight subtelomeric TGP7 sites (Fig. 4c), which is consistent with the lower signal on the Southern blots for this species (Fig. 2b). In all species, the TGP7 signals varied in intensity between chromosome ends. The number and distribution of TPRMBO and TGP7 hybridization signals for *T. mirus* and *T. miscellus* (Fig. 4l–o) were the sum of those observed in the diploids (Fig. 4a–k). Thus, no changes in the distribution or abundance of either the centromeric or subtelomeric repeats were detected in the polyploids relative to their progenitor diploids.

Chromosomal location of rDNA loci—The FISH technique was conducted on *T. dubius* (Fig. 5a), *T. pratensis* (Fig. 5b), *T. porrifolius* (Fig. 5c), *T. miscellus* (Fig. 5d), and *T. mirus* (Fig. 5e–f) using 18S-5.8S-26S rDNA and 5S rDNA (Fig. 5a–f) as probes. The distributions of the rDNA loci on the diploid species of *Tragopogon* are shown on the karyotypes (Fig. 5g). *Tragopogon dubius* has one pair of 18S-5.8S-26S rDNA loci and one pair of 5S rDNA loci on the largest pair of chromosomes (Fig. 5a). This largest pair of chromosomes corresponds to chromosome pair A following Ownbey and McCollum's karyotype nomenclature (1954). *Tragopogon pratensis* also has one pair of 18S-5.8S-26S rDNA loci and one pair of 5S rDNA loci on chromosome pair A (Fig. 5b). In contrast, *T. porrifolius* has two pairs of 18S–5.8S–26S rDNA loci and two pairs of 5S rDNA loci (Fig. 5c). One pair of each type of rDNA locus is on chromosome A like the other two diploids; the extra 18S–5.8S–26S rDNA locus is on chromosome D, and the extra 5S rDNA locus is on chromosome F (Fig. 5g). The observation of an extra 18S–5.8S–26S locus on chromosome D in *T. porrifolius* is consistent with the additional satellite seen on Ownbey and McCollum's (1954) karyotypes and with restriction site studies of the 18S-5.8S-26S rDNA region (Soltis and Soltis, 1991). The rDNA hybridization signals in the polyploids *T. miscellus* (Fig. 5d) and *T. mirus* (Fig. 5e–f) were additive of those observed in the diploids (Fig. 5a–c, g). In sum, no changes in the distribution or abundance of either rDNA locus were detected in the polyploids relative to their diploid progenitors.

DISCUSSION

Because polyploidy has been one of the key elements involved in plant diversification (e.g., Stebbins, 1950, 1971; Grant, 1981; Levin, 1983), it is important to understand the range of molecular evolutionary phenomena associated with genome doubling. Genome doubling causes a range of responses that vary across taxa (reviewed in Soltis and Soltis, 1999; Matzke et al., 1999; Wendel, 2000; Liu and Wendel, 2002; Osborn et al., 2003; Soltis et al., 2004). With respect to chromosomal rearrangements, some polyploid genomes appear to be the sum of their parental diploid parts, such as *Gossypium* (Liu et al., 2001; Adams and Wendel, in press) and *Spartina* (Baumel et al., 2001, 2002; Ainouche et al., in press). Other polyploids such as *Brassica* (Song et al., 1995; Pires et al., in press), *Triticum* (Ozkan et al., 2001; Shaked et al., 2001; Levy and Feldman, in press), and *Nicotiana* (Lim et al., 2000a; Skalicka et al., 2003; Kovarík et al., in press) undergo rapid and dynamic genomic changes. Similarly, polyploid taxa can vary in genome size because some polyploids have a general pattern of additivity in genome size when compared to their progenitor diploids, while other polyploids do not (reviewed in Leitch and Bennett, in press). Here, we compare diploid and polyploid *Tragopogon* to determine whether rapid changes in genome size and chromosomal rearrangement may have accompanied recent polyploidization.

Genome size in diploid and polyploid Tragopogon—*Tragopogon* diploids vary in genome size, both within and among populations. Although measuring intrapopulational variation in genome size is technically difficult (Greilhuber, 1998), variation up to 40% has been confirmed in some species (e.g., *Zea mays*). In *Zea mays*, intrapopulation variation has been associated with changes in the heterochromatin knob number (Greilhuber, 1998). In addition, there are now reports correlating intraspecific variation in genome size of *Hordeum* with changes in copy number of retrotransposons (Vicient et al., 1999).

Tragopogon mirus does not show large departures from additivity of the genome sizes of its diploid progenitors (Table 1). This is consistent with genome size results for other polyploid taxa in the Asteraceae. Specifically, polyploids in *Artemisia* (Torrell and Valles, 2001), *Leucanthemum* (Asteraceae; Marchi et al., 1983), *Hypochaeris* (Cerbah et al., 1999), and Microseridinae (Asteraceae; Price and Bachmann, 1975) all show additivity of diploid genome sizes.

However, at least two populations of *T. miscellus* show evidence of genome downsizing. Thus, *T. miscellus* allopolyploids may be similar to polyploids in *Brassica* and Poaceae, and in angiosperms generally, in which both recent and ancient allopolyploids show signs of genome reduction (reviewed in Levy and Feldman, 2002; Leitch and Bennett, in press).

Repetitive DNA—To assess whether large-scale chromosomal rearrangements may have occurred, we used four tandem repetitive sequences (TPRMBO, TGP7, 18S–5.8S–26S rDNA, 5S rDNA) as molecular cytogenetic markers. Repetitive DNAs are useful molecular cytogenetic markers (Jiang and Gill, 1994, 1996; Heslop-Harrison, 2000) and can be used to detect chromosomal rearrangements in polyploids (Lim et al., 2000b; Mishima et al., 2002; Kotseruba et al., 2003). Repetitive DNA includes dispersed and tandem repeats, which can be further categorized into rDNA and satellite DNA (satDNA). Blocks of satDNA often form heterochromatin predominantly in the centromeric and/or subtelomeric regions of the chromosome (Ganal et al., 1991; Maluszynska and Heslop-Harrison, 1991; Hennig, 1999; Kishii et al., 1999, 2001; Guerra, 2000). The association of satDNA tandem repeats with heterochromatic regions, as well as their unit length similarity (150–180 bp) and nucleosomal periodicity, suggests a role in the maintenance of chromosomal structure (Martinez-Zapater et al., 1986; Heslop-Harrison and Bennett, 1990; Heslop-Harrison, 1991; Schmidt and Heslop-Harrison, 1998). Only a few

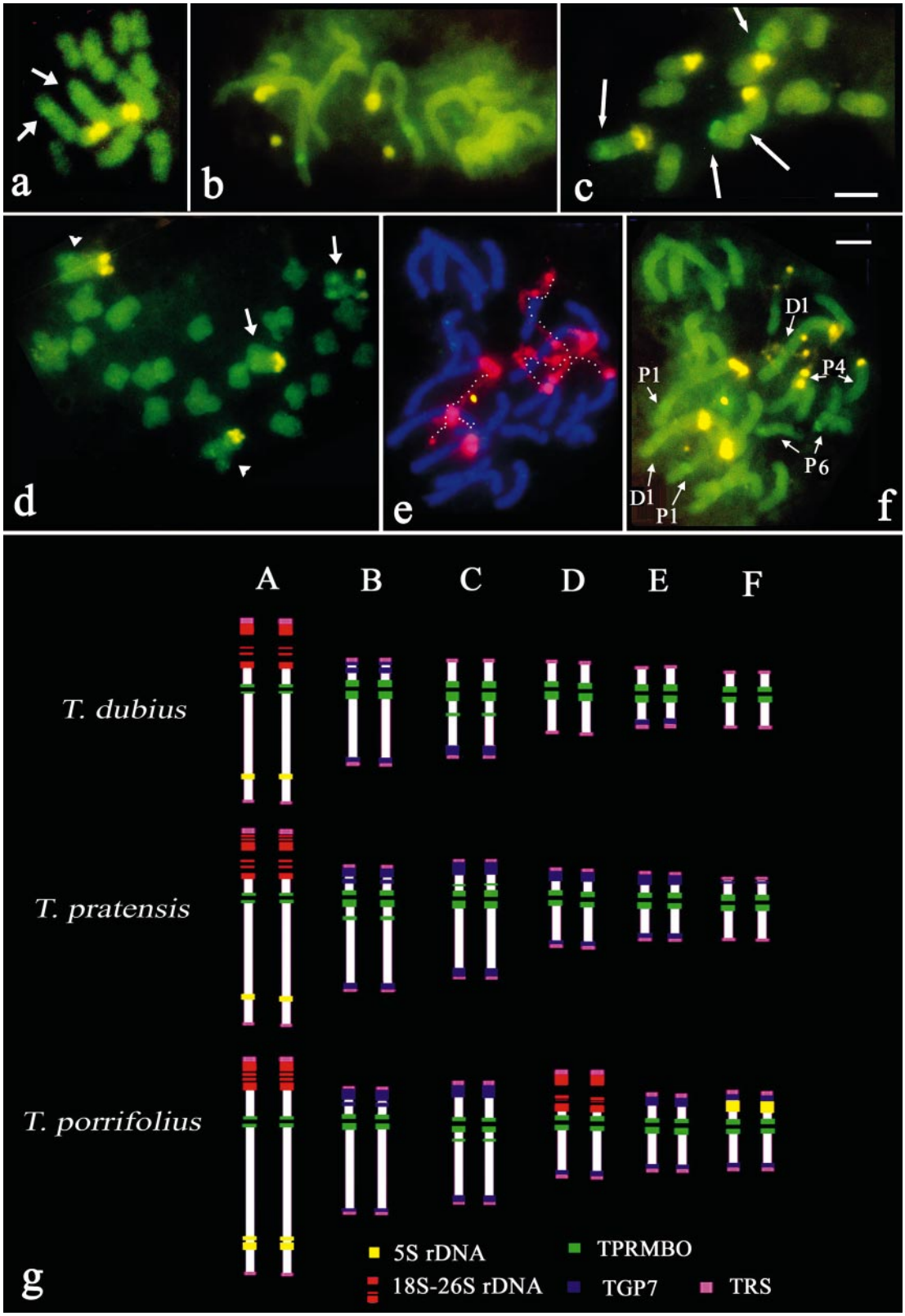

Fig. 5. Fluorescent in situ hybridization to *T. dubius* (a), *T. pratensis* (b), *T. porrifolius* (c), *T. miscellus* (d) and *T. mirus* (e–f) with 18S–5.8S–26S rDNA (red or yellow) and 5S rDNA (green). (a) Metaphase of *T. dubius* with a pair of 5S rDNA locus (green, arrow) on the same pair of largest chromosome with terminal 18S–5.8S–26S rDNA (yellow) on the short arm. (b) Early metaphase of *T. pratensis* with a pair of 5S rDNA locus on the same pair of largest chromosome with terminal 18S–5.8S–26S rDNA. (c) Metaphase of *T. porrifolius* with a pair of terminal 5S rDNA (green, arrow), a pair of terminal 18S–5.8S–26S rDNA (yellow), and an interstitial pair of 5S rDNA (green, arrow) on the largest chromosome pair with terminal 18S–5.8S–26S rDNA (yellow). (d) Metaphase of *T. miscellus* with two pairs of terminal 18S–5.8S–26S rDNA loci (yellow) on the largest chromosomes with interstitial 5S rDNA (green, arrow

tandem repeats have been isolated from species of Asteraceae (e.g., Jamilena et al., 1993, 1995; Houben et al., 2000), including those reported here. The *Tragopogon* genome has at least two kinds of satDNA repeats, TPRMBO and TGP7.

TPRMBO is a 160-bp centromeric tandem repeat—TPRMBO is a typical tandem repeat that has a predominantly centromeric localization to all chromosomes of all diploid *Tragopogon* species studied. It probably represents the main component of centromeric heterochromatin because the FISH signal corresponds to the DAPI-positive bands at the centromere (Fig. 4). TPRMBO has several characteristics typical of a centromeric repeat. First, the size of its monomeric unit (160 bp) appears to be similar to other satellites forming centromeric heterochromatin in plants (Martinez-Zapater et al., 1986; Crowhurst and Gardner, 1991; Rajagopal et al., 1999; Pedrosa et al., 2001) and animals (Mestrovic et al., 2000). Second, irregular ladders revealed by some enzymes (e.g., *Rsa*I, *Alu*I, *Mbo*II, *Ssp*I, *Dra*I in Fig. 1a) indicate considerable heterogeneity in sequence and arrangement of the TPRMBO repeats. The arrays of regular tandem repeats at the centromere could be intermingled with unrelated sequences (e.g., retrotransposon-related DNA sequences, Miller et al., 1998; Presting et al., 1998; Wiens and Sorger, 1998; Francki, 2001; Saunders and Houben, 2001; Zhang et al., 2001). Third, in most restriction enzyme digests, considerable undigested relic DNA was observed in the upper part of the gel, suggesting mutation of the restriction site in a number of units (Fig. 1a). Indeed, sequencing of 14 clones revealed relatively wide divergence among the TPRMBO units, with sequence similarities ranging from 82 to 93%. It is apparent that the relatively conserved regions (e.g., at the 5′ and 3′ terminus) were flanked by stretches of higher sequence divergence, suggesting functional constraints imposed on only some subregions. Chromosome-specific polymorphisms have been described in centromeric satellites of *Arabidopsis thaliana* (Heslop-Harrison et al., 1999) and *Ornithogalum longibracteatum* (Pedrosa et al., 2001). We speculate that TPRMBO sequence heterogeneity could reflect locus-specific differences in the *Tragopogon* genome. TPRMBO also showed some dispersion at pericentromeric locations (Fig. 4d, g), similar to satellite sequences in *Ornithogalum longibracteatum* (Pedrosa et al., 2001).

Mammalian centromeres contain an histone H3 variant called CENP-A (Palmer et al., 1991). Although it remains to be determined whether CENP-A is actually present in the form of a canonical nucleosome structure in vivo, it may be possible that binding of CENP-A could account for some of the distinct features of TPRMBO chromatin, including its relatively high degree of condensation and short nucleosomal periodicity.

TGP7 is a 532-bp subtelomeric tandem repeat—The FISH technique revealed that TGP7 is a typical subtelomeric tandem repeat in the chromosomes of the three diploid species of *Tragopogon* examined (Figs. 4, 5). The 532-bp monomeric length of TGP7 was conserved across the *Tragopogon* species studied (Fig. 2b). Monomers of similar lengths have been found for subtelomeric repeats in other genera (e.g., 570 bp in *Leymus*, Kishii et al., 1999, and *Secale*, Vershinin et al., 1996). The sequence is methylated, which is also typical of tandem repeats (Fig. 2a). In other satellite repeats, short sequences, such as oligo dA and CAAAA motifs, are over-represented (Macas et al., 2002). TGP7 also has several oligo dA tracts, and these can account for conformation polymorphisms observed in the satellite DNA (Fitzerald et al., 1994). In addition, six CAAAA motifs were found (five on the bottom strand) at a frequency that greatly exceeded expectation. It has been proposed that CAAAA pentanucleotides in heterochromatin might be involved in the breakage–reunion properties of repeated sequences (Macas et al., 2002) and can lead to sequence translocations and chromosomal rearrangements (Langdon et al., 2000). However, our analysis by FISH suggests no such evolution in the allopolyploids investigated. The restriction enzyme digestions of TGP7 repeats, using several enzymes, showed highly regular ladders in both diploids and allotetraploids (Fig. 2). Perhaps the TGP7 sequences in subtelomeric regions have experienced recent expansion and/or homogenization.

In MNase digests, the TGP7 bands revealed a periodicity of multiples of three basic TGP7 (3, 6, 9 . . . *n*) units (Fig. 3), suggesting that every third linker of a nucleosome fiber is more sensitive to nuclease cleavage. If this is the case, nucleosomal linker regions along the long, tandemly repeated TGP7 sequence might not be structurally equivalent. Such anisotropy could have several explanations. (1) There might be binding sites for proteins that prevent or enhance DNA cleavage. For example, HMGY protein binds to AT-rich tracts in mouse alphoid satellite DNA (Solomon et al., 1986). (2) There might be differential epigenetic modification of chromatin by methylation and/or acetylation occurring at a three-nucleosomal periodicity. Certainly heterogeneity in cytosine methylation occurs among units of the tobacco subtelomeric satellite repeat sequence HRS60 (Kovarík et al., 2000) and perhaps in other simple satellites as well (Saunders and Houben, 2001). (3) Nucleosome binding to a preferential structural motif could induce a conformation change in DNA, resulting in altered sensitivity of DNA toward nuclease. The prominent structural motifs in satellites are oligo dA tracts (Macas et al., 2002), known to induce DNA curvature (Goodsell et al., 1994). The dA_{4-6} tracts are clustered in approximately one half of the TGP7 monomer, while the second half is relatively devoid of these motifs. Such uneven distribution of oligo (dA) tracts has been observed in a 1.2-kb satellite of *Secale cereale* (Japanese rye, Nagaki et al., 1999) and could contribute to higher-order packing of chromatin.

Organization and distribution of TPRMBO and TGP7 in Tragopogon allopolyploids—The repetitive sequences TGP7

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on the long arms. (e) Early metaphase cell of *T. mirus* with six loci of 18S–5.8S–26S rDNA (red, decondensed rDNA indicated by dotted lines) and (f) with six 5S rDNA loci (green, arrow) identified as D1 from *T. dubius* and P1 and P6 from *T. porrifolius*. P4 identifies the pair of 18S-5.8S-26S rDNA locus from *T. porrifolius* (yellow, arrow). (g) Karyotypes of diploid *T. dubius*, *T. pratensis*, and *T. porrifolius* showing all the mapped repetitive sequences for 5S rDNA, 18S-5.8S-26S rDNA, TPRMBO, TPG7, and TRS. The names of the chromosome pairs follow Ownbey and McCollum's karyotype nomenclature (1954). The gaps on the short arm of chromosome A of all species and on chromosome D of *T. porrifolius* represent secondary constrictions observed on some metaphases associated with the 18S–5.8S–26S rDNA. Biotin-avidin-Cy3 detection, red fluorescence; digoxigenin-antidigoxigenin-FITC, green fluorescence; 4,6-diamidine-2-phenylindole, dihydrochloride (DAPI) detection, blue fluorescence. Scale bars (a–c, d–f,) = 10 μm.

and TPRMBO appear to be directly inherited in the allopolyploids without structural, organizational, or distributional changes. This inference is supported by additivity of both Southern hybridization bands (Figs. 1b, 2b) and FISH signals at metaphase (Fig. 4). The subtelomeric repeat TGP7 showed this more clearly because there were only eight subtelomeric FISH signals (corresponding to four genetic loci) in *T. dubius* and 18 in *T. porrifolius* and *T. pratensis*. The allotetraploids each had 26 subtelomeric signals, suggesting that the parental chromosome subtelomeric regions remained intact. In allotetraploid cotton, the two parental diploid genomes (A and D) have distinct repetitive elements. Following polyploidy, there has been substantial colonization of the D-genome by A-genome repetitive elements (Hanson et al., 1998; Zhao et al., 1998).

Organization and distribution of rDNA in Tragopogon diploids and allopolyploids—The distributions of the rDNA loci on the diploid species of *Tragopogon* were consistent with previous cytogenetic observations and restriction site studies (Ownbey and McCollum, 1954; Soltis and Soltis, 1991). *Tragopogon dubius* and *T. pratensis* each have one pair of 18S-5.8S-26S rDNA loci and one pair of 5S rDNA loci on the largest pair of chromosomes (Fig. 5a, b), while *T. porrifolius* has two pairs of 18S-5.8S-26S rDNA loci and two pairs of 5S rDNA loci (Fig. 5g). The number and distribution of the rDNA loci in the polyploids *T. mirus* (Fig. 5d) and *T. miscellus* (Fig. 5e–f) were additive of those observed in the diploids (Fig. 5a–c, g). This is similar to the situation in *Nicotiana tabacum*, where most cultivars have a number of rDNA loci that reflect the sum of the diploid progenitors (Kenton et al., 1993; Lim et al., 2000b; Kovarík et al., in press). This pattern contrasts with other allopolyploid systems, such as *Zingeria* (Poaceae; Kotseruba et al., 2003) and *Sanguisorba* (Rosaceae; Mishima et al., 2002), where rDNA loci were evidently lost after polyploidization.

Chromosome evolution in diploid and polyploid Tragopogon—Collectively, the distribution of the four tandem repetitive DNA loci (TPRMBO, TGP7, 18S-5.8S-26S rDNA, and 5SrDNA) among the chromosome pairs allowed the construction of molecular cytogenetic karyotypes for the three diploid species of *Tragopogon* (Fig. 5g). Thus, the number, location, and intensity of the FISH signals for all the mapped loci allowed for the identification of several of the diploid parental chromosomes in the polyploids. If rearrangements had taken place upon or immediately following polyploidization, we would expect to observe nonadditive patterns in the polyploids. For example, the number of rDNA loci in a polyploid could be greater or fewer than that found separately in the two diploid progenitors. Alternatively, rearrangements could move subtelomeric repeats found in the diploids to interstitial locations in the polyploids. Some synthetic allopolyploid species, including members of Triticeae (Shaked et al., 2001) and *Brassica* (Song et al., 1995), display rapid (within a few generations) evolution of polymorphic markers. However, other polyploid species show additive patterns, as we observed for *Tragopogon*; these include synthetic polyploids in *Gossypium* (Liu et al., 2001) and the natural *Nicotiana* polyploids (*N. rustica* and *N. arentsii*) compared with their putative diploid parents (Lim et al., 2004).

We found no evidence for major genomic rearrangements in the allopolyploids *T. mirus* and *T. miscellus*. The number and location of the tandem repetitive sequences TGP7 and TPRMBO appear to be directly inherited in the allopolyploids from their corresponding diploid ancestors without organizational or distributional changes. Similarly, the 18S-5.8S-26S and 5S rDNA loci in *T. miscellus* and *T. mirus* were exactly as predicted from the number and location of these loci in their diploid progenitors. There are several possible explanations for our inability to detect alterations in parental subgenomes in the allotetraploid species of *Tragopogon*. An obvious possibility is that perhaps no such rearrangements have occurred in *T. mirus* and *T. miscellus*. Alternatively, allopolyploid-induced changes may have occurred in *Tragopogon*, but are located in the middle- and low-repetitive fractions of the genome that have not been analyzed in this study. That is, we used a coarse approach; there may be microrearrangements that these methods were not able to detect. Similarly, ribosomal RNA genes, whose sequence and copy numbers are supposed to be under greater selective constraints than the nontranscribed satellite repeats, can undergo homogenization in allopolyploids (Wendel et al., 1995; Volkov et al., 1999; Lim et al., 2000a). In future cytogenetic studies and fine-scale mapping studies, these unexplored areas of genome structure in *Tragopogon* can be examined. However, based on the tandem repetitive sequences in the allopolyploids studied, we have been unable to detect major changes in overall genome structure that may have occurred within the time frame of 80 years since polyploidization.

While polyploid genomes may be quiescent in some respects, they can simultaneously be dynamic in other aspects. For example, polyploids in *Gossypium* and *Nicotiana* have an additive number of rDNA loci, but still exhibit concerted evolution between loci where repeats at the different loci become homogenized to the same sequence (reviewed in Adams and Wendel, in press; Kovarík et al., in press). In addition, rDNA loci may be additive in number, but then exhibit differences in gene expression. Interspecific hybrids often have rRNA genes of one parent functionally dominant over the rRNA of the other parent (nucleolar dominance, Navashin, 1934), and there are many examples of such regulation of rRNA gene activity in allopolyploids (reviewed in Pikaard, 2000). Ongoing studies suggest that concerted evolution and nucleolar dominance may be occurring in the recently formed polyploids *Tragopogon mirus* and *T. miscellus* (A. Kovarík et al., unpublished data). These phenomena could have profound impacts on attempts at phylogeny reconstruction based on rDNA loci such as ITS. Future studies in *Tragopogon* will put chromosome evolution into a broader phylogenetic context as is being done in other genera of the Asteraceae (*Hypochaeris*, Cerbah et al., 1999; Samuel et al., 2003; Weiss et al., 2003) and in other groups (*Sanguisorba*, Rosaceae, *Nicotiana*, Solanaceae, Lim et al., 2000b, 2004; Mishima et al., 2002).

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