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Biological relevance of polyploidy: ecology to genomics
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Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons

DOUGLAS E. SOLTIS[1]*, PAMELA S. SOLTIS[2]*, J. CHRIS PIRES[3], ALES KOVARIK[4], JENNIFER A. TATE[2] and EVGENY MAVRODIEV[1]

[1]*Department of Botany, and* [2]*Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA*
[3]*Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA*
[4]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, CS61265 Brno, Czech Republic*

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Tragopogon mirus Ownbey and *T. miscellus* Ownbey are allopolyploids that formed repeatedly during the past 80 years following the introduction of three diploids (*T. dubius* Scop., *T. pratensis* L. and *T. porrifolius* L.) from Europe to western North America. These polyploid species of known parentage are useful for studying the consequences of recent and recurrent polyploidization. We summarize recent analyses of the cytogenetic, genomic and genetic consequences of polyploidy in *Tragopogon*. Analyses of rDNA ITS (internal transcribed spacer) + ETS (external transcribed spacer) sequence data indicate that the parental diploids are phylogenetically well separated within *Tragopogon* (a genus of perhaps 150 species), in agreement with isozymic and cpDNA data. Using Southern blot and cloning experiments on tissue from early herbarium collections of *T. mirus* and *T. miscellus* (from 1949) to represent the rDNA repeat condition closer to the time of polyploidization than samples collected today, we have demonstrated concerted evolution of rDNA. Concerted evolution is ongoing, but has not proceeded to completion in any polyploid population examined; rDNA repeats of the diploid *T. dubius* are typically lost or converted in both allopolyploids, including populations of independent origin. Molecular cytogenetic studies employing rDNA probes, as well as centromeric and subtelomeric repeats isolated from *Tragopogon*, distinguished all chromosomes among the diploid progenitors ($2n = 12$). The diploid chromosome complements are additive in both allopolyploids ($2n = 24$); there is no evidence of major chromosomal rearrangements in populations of either *T. mirus* or *T. miscellus*. cDNA-AFLP display revealed differences in gene expression between *T. miscellus* and its diploid parents, as well as between populations of *T. miscellus* of reciprocal origin. Approximately 5% of the genes examined in the allopolyploid populations have been silenced, and an additional 4% exhibit novel gene expression relative to their diploid parents. Some of the differences in gene expression represent maternal or paternal effects. Multiple origins of a polyploid species not only affect patterns of genetic variation in natural populations, but also contribute to differential patterns of gene expression and may therefore play a major role in the long-term evolution of polyploids. *Biological Journal of the Linnean Society*, 2004, **82**, 485–501.

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*Corresponding authors. E-mail: dsoltis@botany.ufl.edu; psoltis@flmnh.ufl.edu

RECENT POLYPLOIDY IN *TRAGOPOGON* (ASTERACEAE)

The immediate consequences of polyploidization can best be studied in polyploids of clear and recent ancestry. Only a few polyploid species are known to have arisen within the past 150 years: *Cardamine schulzii* (Urbanska *et al*., 1997), *Spartina anglica* (Huskins, 1931; Hubbard, 1965; Marchant, 1967, 1968; Raybould *et al*., 1991; Baumel, Ainouche & Levasseur, 2001; Ainouche, Baumel & Salmon, 2004 – this issue), *Senecio cambrensis* (Rosser, 1955; Ashton & Abbott, 1992), *Senecio eboracensis* (Abbott & Lowe, 2004 – this issue), and *Tragopogon mirus* and *T. miscellus* (Ownbey, 1950). All four genera have been the focus of recent investigation (see other papers in this issue).

Tragopogon (Asteraceae) comprises approximately 150 species native to Eurasia. Most species are diploid ($2n = 12$), but some polyploid species or cytotypes have been reported. Three diploid species, *T. dubius*, *T. porrifolius* and *T. pratensis*, were introduced from Europe into the Palouse region of eastern Washington and adjacent Idaho, USA, in the early 1900s (Ownbey, 1950). The introduction of these diploid species into the Palouse brought them into close contact, something that rarely occurs in the Old World where they are largely allopatric (but see Ownbey, 1950, for a list of reported hybrids). Using morphology and cytology, Ownbey (1950) demonstrated that *T. mirus* and *T. miscellus* are allotetraploids ($2n = 24$) whose diploid ($2n = 12$) parents are *T. dubius* and *T. porrifolius*, and *T. dubius* and *T. pratensis*, respectively (Fig. 1A). The allotetraploids have not formed in Europe, but are native to the Palouse, although their diploid parents are aliens in North America. Because the three diploids did not co-occur in the Palouse prior to 1928 (Ownbey, 1950), *T. mirus* and *T. miscellus* cannot be more than ~75–80 years old. In fact, the first collections of the allopolyploids were made in 1949. Given that these plants appear to be biennials, the timeframe involved may be fewer than 30 generations. The ancestries of both tetraploids were confirmed through flavonoid, isozymic and DNA studies (Ownbey & McCollum, 1953, 1954; Brehm & Ownbey, 1965; Kroschewsky *et al*., 1969; Roose & Gottlieb, 1976; D. Soltis & P. Soltis, 1989; P. Soltis & D. Soltis, 1991; P. Soltis *et al*., 1995; Cook *et al*., 1998).

MULTIPLE ORIGINS OF *TRAGOPOGON* ALLOTETRAPLOIDS

The diploid species of *Tragopogon* display an array of allozyme, cpDNA, rDNA and RAPD genotypes (Roose & Gottlieb, 1976; D. Soltis & P. Soltis, 1989; P. Soltis & D. Soltis, 1991; P. Soltis *et al*., 1995; Cook *et al*., 1998), making it possible to detect independent origins of each polyploid species from genetically distinct diploid parents. In 1949, Ownbey (1950) discovered two populations of each allotetraploid species and suggested that each was of independent origin. Subsequent morphological and cytological (Ownbey & McCollum, 1953, 1954), isozymic (Roose & Gottlieb, 1976; P. Soltis *et al*., 1995), and DNA evidence (D. Soltis & P. Soltis, 1989; P. Soltis & D. Soltis, 1991; Cook *et al*., 1998), when considered along with geographical distribution, suggests that there may be as many as 21 lineages of separate origin of *T. miscellus* and 11 of *T. mirus* just in the Palouse (P. Soltis & D. Soltis, 2000). On a larger geographical scale, both tetraploids have also formed

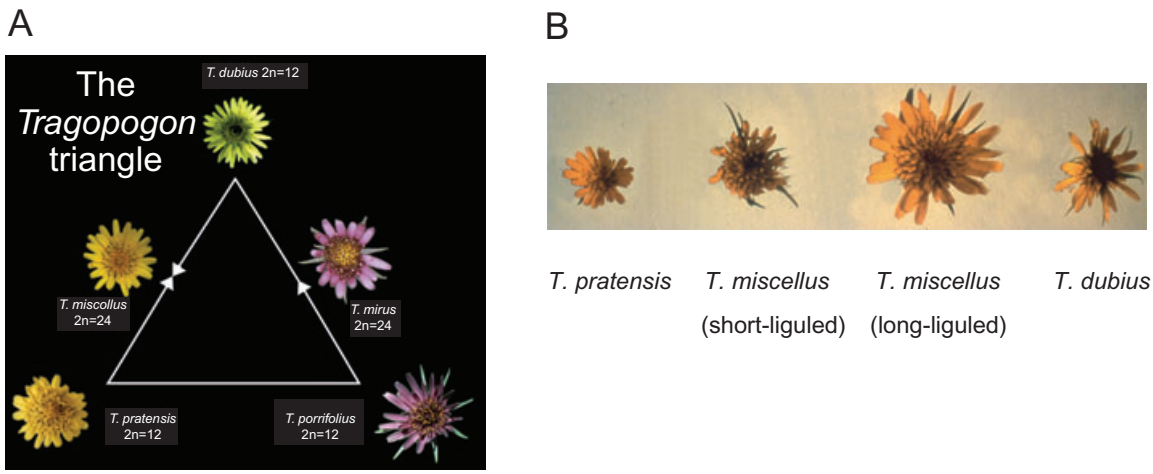


Figure 1. A, Parentage of tetraploid Tragopogons. Arrows indicate maternal parentage of polyploids. *T. miscellus* has formed reciprocally (see arrows); populations have either *T. dubius* or *T. pratensis* as the maternal parent. B, Populations of *T. miscellus* of separate origin differ in morphology. Those populations with *T. pratensis* as the maternal parent have short ligules; those with *T. dubius* as the maternal parent have long ligules.

in Flagstaff, AZ (Brown & Schaak, 1972); *T. miscellus* has formed in Gardiner, MT, and Sheridan, WY (Ownbey & McCollum, 1954; M. Ownbey, unpubl. data; D. Soltis & P. Soltis, pers. observ.).

In *T. mirus*, all of the recurrent origins involve *T. dubius* as the paternal parent and *T. porrifolius* as the maternal parent (D. Soltis & P. Soltis, 1989). However, *T. miscellus* has formed reciprocally (Ownbey & McCollum, 1953, 1954). Most of the recurrent origins of this tetraploid also involve *T. dubius* as the paternal parent (D. Soltis & P. Soltis, 1989), but plants from Pullman, WA, have formed with *T. dubius* as the maternal parent. This reciprocal formation of *T. miscellus* has resulted in a dramatic difference in inflorescence morphology (Fig. 1B). Ownbey & McCollum (1953) speculated that this morphological difference was the result of cytoplasmic inheritance.

Genetic consequences of multiple origins

Molecular data have revealed that most polyploid plant species are of multiple origin (D. Soltis & P. Soltis, 1993, 1999; P. Soltis & D. Soltis, 2000; Fig. 2). For example, in species of *Draba* (Brochmann, Soltis & Soltis, 1992a, b) and *Tragopogon* (P. Soltis *et al*., 1995; Cook *et al*., 1998), many populations are derived from separate origins, suggesting that polyploidization occurs frequently. D. Soltis & P. Soltis (1993) reviewed over 30 examples of polyploid plant species of recurrent origin and later (D. Soltis & P. Soltis, 1999) provided 15 additional examples. Indeed, there are few examples of polyploid plants for which a single origin is likely (e.g. Kochert *et al*., 1996; Ainouche *et al*., 2004). Polyploid animal species have also arisen recurrently (e.g. Little & Hebert, 1994; Ptacek, Gerhardt & Sage, 1994; Turgeon & Hebert, 1995). For polyploids studied extensively, the number of polyploid events is often high. Three origins of tetraploid *Heuchera grossulariifolia* (Saxifragaceae) were suggested using cpDNA restriction site data (Wolf, Soltis & Soltis, 1990); use of additional markers and populations raised that number to at least seven (Segraves *et al*., 1999). *Draba norvegica* (Brassicaceae) has formed at least 13 times in a small area of Scandinavia (Brochmann & Elven, 1992).

Recurrent polyploidy can create genetically distinct populations, among which subsequent gene flow, independent assortment and recombination may produce additional genotypes (Doyle, Doyle & Brown, 1999; D. Soltis & P. Soltis, 1999; P. Soltis & D. Soltis, 2000; Doyle *et al*., 2002, 2004a – this issue; Fig. 2). In species of *Draba*, genotypes of separate polyploid origin co-occur in the same populations, along with recombinants (Brochmann & Elven, 1992). Polyploid populations of *H. grossulariifolia* comprise a mosaic of genotypes of separate origin

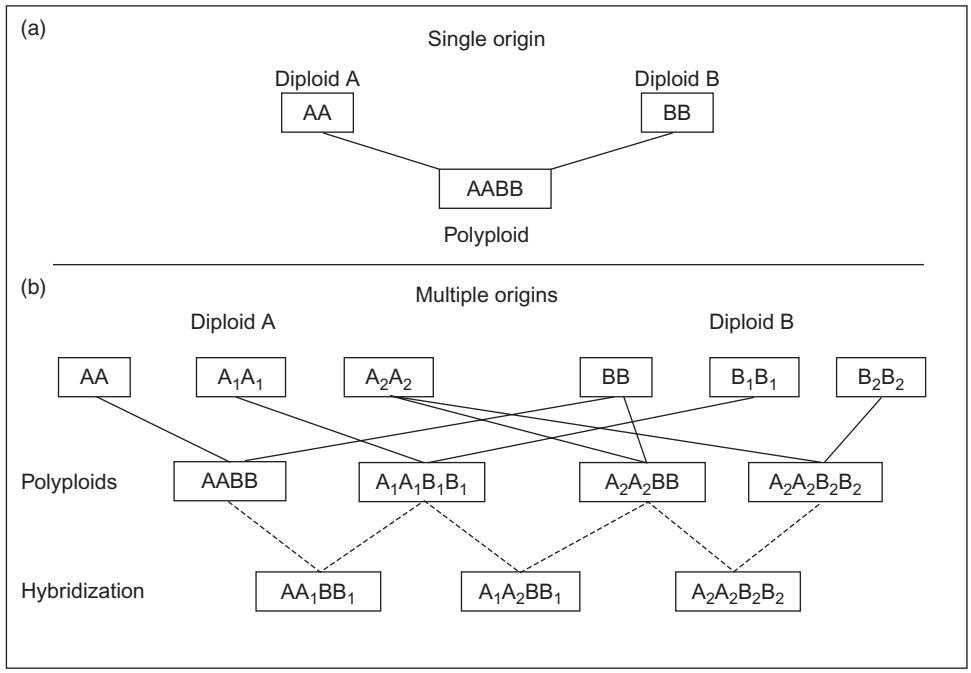


Figure 2. Traditional view of polyploid formation (a) in which each polyploid species formed once, resulting in a genetically uniform species. New view (b) of recurrent formation from different parental genotypes, generating an array of polyploid genotypes. Crossing, independent assortment and recombination result in additional variability [modified from D. Soltis & P. Soltis (1999)].

(Segraves *et al*., 1999). In *Tragopogon*, polyploid populations of separate origin come into contact (Cook *et al*., 1998). On a broad scale, recurrent polyploidy and subsequent interbreeding of genotypes are best seen in the arctic where diploid parents co-occur repeatedly on a circumboreal scale, thus explaining the well-known taxonomic uncertainty surrounding arctic polyploid complexes (D. Soltis & P. Soltis, 1999; Abbott & Brochmann, 2003; Brochmann *et al*., 2004 – this issue).

Polyploidization should therefore not be viewed as a rare event producing a species of unique origin and uniform genotype (Fig. 2; e.g. Stebbins, 1950; Wagner, 1970, 1983). Future studies of metapopulation dynamics (e.g. McCauley, Raveill & Antonovics, 1995; Husband & Barrett, 1996; Hanski, 1998) should consider the potential impact of recurrent polyploidy. Polyploid populations of independent origin are known to differ genetically, morphologically (Ownbey & McCollum, 1953; Lowe & Abbott, 1996) and physiologically; such differences could have important evolutionary consequences. In polyploid populations of separate origin, 'reciprocal silencing' of duplicated genes may lead to hybrid inviability, promoting reproductive isolation and speciation. Hence, the stochastic silencing of duplicated genes may play a major role in the 'passive origin' of new species (Werth & Windham, 1991; Lynch & Conery, 2000; Lynch & Force, 2000a; Taylor, Van de Peer & Mayer, 2001).

Spread of the *Tragopogon* allotetraploids

By 1949, the new allotetraploid species *T. mirus* and *T. miscellus* had not spread far from their points of origin. However, Ownbey (1950) stated that they had 'attained a degree of success' and appeared to be 'competing successfully' with their diploid parents. He reported two populations of each tetraploid in 1950, each with fewer than 100 individuals. Both allotetraploids have become extremely successful since their formation, with many new populations present and large populations with individuals numbering in the thousands (Novak, Soltis & Soltis, 1991). *Tragopogon miscellus* is now one of the most common weeds in waste areas in and around Spokane, WA. Both tetraploids form dense, monospecific stands and are now major weeds in the small towns of the Palouse. Multiple origins also have played an important role in the range expansion of both *T. miscellus* and *T. mirus* (Novak *et al*., 1991; P. Soltis *et al*., 1995; Cook *et al*., 1998).

Population dynamics and local extinctions

Ownbey (1950) described the early populations of the allopolyploid Tragopogons as 'small and precarious', a statement that summarizes well the population dynamics of both diploids and tetraploids on the Palouse. Local extinction has been a part of the population dynamics of the newly formed *Tragopogon* polyploids. Population numbers fluctuate greatly from year to year, and many small populations have gone extinct. Ownbey described a population of *T. mirus* from Oakesdale, WA, but extensive surveys of this area in the 1990s indicated that the population was no longer extant. D. and P. Soltis documented several extinctions of polyploid populations during 15 years of fieldwork. A population of *T. miscellus* discovered by D. and P. Soltis in Uniontown, WA, also disappeared a few years after its discovery, and a population of *T. mirus* comprising a single individual in Moscow, ID, in 1990 was extinct the following year (D. Soltis & P. Soltis, unpubl. data). The importance of local extinction in the dynamics of newly formed polyploids was similarly observed in *Senecio cambrensis* (Abbott & Forbes, 2002; Abbott & Lowe, 2004).

Ecology of the diploid and allotetraploid Tragopogons

Ecological data for the diploids (*T. dubius*, *T. pratensis* and *T. porrifolius*) and tetraploids (*T. mirus* and *T. miscellus*) remain largely anecdotal. However, based on field measurements, Sauber (2000) reported habitat differentiation among the diploid and allotetraploid species. These results and our own field observations support the idea that polyploidy in *Tragopogon* may have produced intermediate 'fill-in' taxa that contribute to a more intense partitioning of habitat space (Ehrendorfer, 1980; Bayer, Purdy & Lebedyx, 1991). Competition may also play a role in *Tragopogon*; the tetraploids appear to be replacing populations of the diploids (P. Soltis *et al*., 1995). It is noteworthy that populations of two of the parental diploids, *T. pratensis* and *T. porrifolius*, have become difficult to find in some areas of the Palouse. Some populations of *T. porrifolius* and *T. pratensis* that occur with *T. mirus* and *T. miscellus*, respectively, have steadily decreased in numbers, while the tetraploid populations at those same locations flourished (L. Cook, D. Soltis & P. Soltis, pers. observ.). Other historical data further support the view that the two tetraploids may be more successful in certain habitats than their diploid progenitors. The long-liguled genotype of *T. miscellus* formed in Pullman, WA, but the diploid parent *T. pratensis* is no longer present in Pullman. Similarly, *T. mirus* apparently formed in Palouse, WA; however, one of its parents, *T. porrifolius*, is no longer extant in Palouse.

Because the parentage and time of origin of *T. mirus* and *T. miscellus* are well known, they afford an

unusual opportunity for studying the genetic and evolutionary consequences of recent and recurrent allopolyploidy. Given that multiple polyploidizations are common, not only in plants, but in other organisms as well, *Tragopogon* represents in microcosm what occurs in other polyploid complexes over much larger geographical areas and much longer time frames. Here we present summaries of our recent investigations of *Tragopogon* in North America, focusing on phylogeny, molecular cytogenetics, concerted evolution and gene expression.

PHYLOGENY OF *TRAGOPOGON* AND RELATIONSHIPS OF THE DIPLOID PROGENITORS OF NORTH AMERICAN TETRAPLOIDS

Tragopogon is distributed from western Europe to central Asia. Many species occur in areas in which it is presently difficult to collect samples (e.g. Iran, Iraq, Kurdistan, Afghanistan, Russia). We have therefore assembled a large collection of leaf tissue of *Tragopogon* species, as well as other genera in subtribe Scorzonerinae, from herbarium specimens. Using ITS sequence data we have shown that *Tragopogon* is a well-supported monophyletic group within subtribe Scorzonerinae (Mavrodiev *et al*., in press a).

Using ITS and ETS sequences, Mavrodiev *et al*. (in press b) have also reconstructed phylogenetic relationships among diploid species of *Tragopogon*. Phylogenetic analyses provide support for the monophyly of many of the recognized sections within *Tragopogon*. The three diploid species (*T. dubius*, *T. pratensis* and *T. porrifolius*) that are progenitors of the North American allopolyploids have been placed in three different sections in taxonomic treatments and occur in three distinct clades, each of which receives moderate bootstrap support. *Tragopogon dubius* is part of a well-supported clade of species that mostly represent the recognized section *Majores*; *T. porrifolius* appears in a clade composed of species of section *Hebecarpus*; *T. pratensis* is a member of a clade corresponding to section *Tragopogon*. The clades that contain *T. dubius* and *T. porrifolius* are sister groups, a relationship that receives bootstrap support of >80%. A comparison of patristic distances shows greater similarity between *T. dubius* and *T. porrifolius* (0.01681) than between either species and *T. pratensis* (*T. dubius* vs. *T. pratensis*, 0.02832; *T. pratensis* vs. *T. porrifolius*, 0.03304). The phylogenetic distance among these three diploids is consistent with evidence of genetic distinctness at isozyme loci (Roose & Gottlieb, 1976; P. Soltis *et al*., 1995), in cpDNA restriction sites (D. Soltis & P. Soltis, 1989), and rDNA restriction sites (P. Soltis & D. Soltis, 1991) and sequences (see below). Thus, all evidence indicates that the new allopolyploids formed from diploids that are genetically well differentiated.

MOLECULAR CYTOGENETICS OF DIPLOID AND ALLOTETRAPLOID *TRAGOPOGON* SPECIES

Dramatic chromosomal changes have been identified subsequent to polyploidization in some species, such as *Nicotiana tabacum* L. (Kenton *et al*., 1993; Leitch & Bennett, 1997; Lim *et al*., 2004 – this issue). Did such chromosomal changes occur in one or both recently formed allotetraploid species of *Tragopogon*, and, if so, did the same chromosomal changes occur in populations of separate origin within both *T. mirus* and *T. miscellus*?

Given the utility of tandem repeats and molecular cytogenetics in providing insights into genome and chromosome evolution (e.g. Jiang & Gill, 1996; Heslop-Harrison, 2000), Pires *et al*. (2004) used fluorescent *in situ* hybridization (FISH) to investigate possible chromosomal repatterning in allopolyploid *Tragopogon* species. Five tandem repeats were characterized by Pires *et al*. and used as probes on *Tragopogon* chromosomes. Three of the five tandem repetitive sequences, 18S–26S rDNA, 5S rDNA and the telomeric repeat sequence (TRS), are commonly used in molecular cytogenetic studies. In addition, two repetitive sequences were specifically isolated for the study of *Tragopogon*: TPRMBO (unit size 160 bp) was isolated from *T. pratensis*, and TGP7 (unit size 532 bp) was isolated from *T. porrifolius*. FISH was carried out on the three diploid progenitor species of *T. dubius*, *T. pratensis* and *T. porrifolius*, and karyotypes were constructed following Ownbey & McCollum's (1954) assignment of letters A–F to the six pairs of chromosomes (from largest to smallest) in diploid species of *Tragopogon*. (Fig. 3). The same probes were then hybridized to the allotetraploid species to determine if chromosomal rearrangements had occurred subsequent to polyploidization.

rDNA LOCI IN DIPLOID *TRAGOPOGON*

The distributions of the rDNA loci on the chromosomes of the diploid species of *Tragopogon* are shown on the ideogram (Fig. 3: 18S–26S rDNA loci shown in red and the 5S rDNA loci shown in yellow). *Tragopogon dubius* has one pair of 18S–26S rDNA loci and one pair of 5S loci on the largest pair of chromosomes (chromosome pair A following Ownbey's karyotype nomenclature). *Tragopogon pratensis* also has one pair of 18S–26S rDNA loci and one pair of 5S loci on chromosome pair A. By contrast, *T. porrifolius* has two pairs of 18S–26S rDNA loci and two pairs of 5S loci.

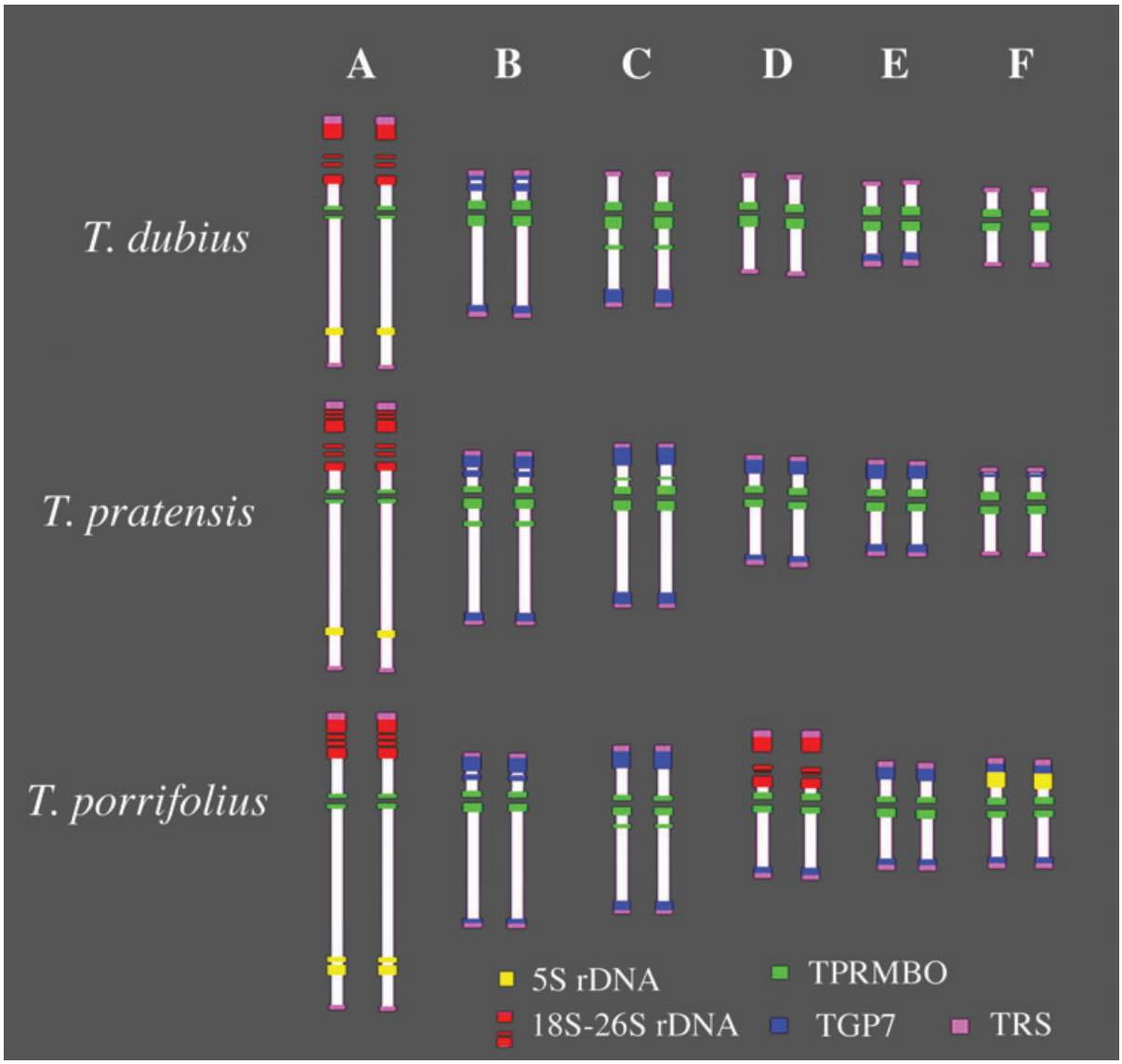


Figure 3. Ideograms of chromosomes for the three diploid *Tragopogon* species. The karyotypes have six pairs of chromosomes (A–F) arranged in size from largest to smallest. The distribution of five repetitive DNA elements as observed by fluorescent *in situ* hybridization is indicated on the karyotypes. Note that *T. porrifolius* has an extra 18S–26S rDNA locus on D and an extra 5S locus on F in comparison with the other two diploid species. *T. dubius* has fewer TGP7 subtelomeric repeats than the other two diploid species. 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 metaphase chromosomes (from Pires *et al*., 2004).

One pair of each type of rDNA locus is on chromosome A as in the other two diploids, the extra 18S–26S rDNA locus is on chromosome D, and the extra 5S locus is on chromosome F. The observation of an extra 18S–26S rDNA locus in *T. porrifolius* is consistent with the additional satellite seen on Ownbey & McCollum's (1954) karyotypes and with restriction site analyses of the 18S–26S cistron (P. Soltis & D. Soltis, 1991).

Centromeric and subtelomeric repeats in diploid *Tragopogon*

The distributions of the TPRMBO and TGP7 loci on the diploid species of *Tragopogon* are shown on the ideogram (Fig. 3: TPRMBO shown in green and TGP7 shown in blue). Fluorescent *in situ* hybridization to the diploids showed that TPRMBO is a predominantly centromeric repeat occurring on all 12 chromosomes of

the three diploid *Tragopogon* species studied. Some TPRMBO sites were dispersed a short distance away from the centromere with variation among the three species (Fig. 3: see chromosome pairs B and C). The probe TGP7 hybridized to subtelomeric regions of most, but not all, chromosome arms on all diploid *Tragopogon* species studied. The interpretation of these locations as subtelomeric was confirmed using the telomeric probe TRS (Fig. 3: TRS shown in pink). In *T. porrifolius* and *T. pratensis*, 18 of the 24 chromosome ends were labelled by TGP7. By contrast, *T. dubius* had only eight subtelomeric TGP7 sites (Fig. 3). In all species, the TGP7 signals varied in intensity among chromosome ends.

Molecular phylogenetic analyses indicate that *T. dubius* and *T. porrifolius* are in sister clades and are more closely related to each other than either is to *T. pratensis*. Molecular cytogenetic investigations point to the distinctness of each diploid, but do not indicate any clear pattern of relationships among the three diploids. *Tragopogon dubius* and *T. pratensis* share the same number of rDNA loci, but *T. pratensis* and *T. porrifolius* have similar patterns of TGP7 repeats. Without further cytogenetic data for other species of *Tragopogon*, these characters are not informative about phylogenetic relationships among these three diploid species. The TPRMBO repeats do not provide any information regarding relationships among the diploids.

The distribution of tandem repeats in *Tragopogon* allopolyploids

The distribution of the tandem repetitive DNA loci among the chromosome pairs allowed the construction of molecular cytogenetic karyotypes for the three diploid *Tragopogon* species (Fig. 3). Thus, the number, location and intensity of the FISH signals for all the mapped loci allowed for the identification of each of the diploid parental chromosomes in the polyploids. If rearrangements had taken place upon or immediately following polyploidization, we would expect to observe non-additive patterns in the polyploids. For example, the number of rDNA loci in a polyploid could be greater or fewer than the sum of those of the two diploid progenitors. Alternatively, rearrangements could move subtelomeric repeats found in the diploids to interstitial locations in the polyploids.

We found no evidence for major genomic rearrangements in the allopolyploid species of *Tragopogon* (Pires *et al*., 2004). The number and location of the 18S–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. Similarly, the tandem repetitive sequences TGP7 and TPRMBO also appear to be directly inherited in the allopolyploids from their corresponding diploid ancestors without organizational or distributional changes. The subtelomeric repeat TGP7 showed this most 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* (corresponding to nine loci). Each allotetraploid had 26 subtelomeric signals, suggesting that the subtelomeric regions of the parental chromosomes have remained intact. Thus, there is no evidence that the subtelomeric repeat has colonized new chromosomal locations, as may have occurred in *Nicotiana tabacum* (Kenton *et al*., 1993). In addition, no changes in the distribution or abundance of either the centromeric or subtelomeric repeats were detected even when we sampled polyploids of separate origins. Unlike *Nicotiana tabacum* (but similar to *N. rustica*, Lim *et al*., 2004), the number and location of the chromosomal loci examined were inherited without apparent changes in the allotetraploids *T. mirus* and *T. miscellus* because the hybridization signals were the sums of those observed in the diploids.

Although these data for allotetraploid *Tragopogon* species seem to suggest a lack of genome rearrangement relative to the parental karyotypes, it is possible that allopolyploidy-induced changes occurred in *Tragopogon*, but are located between the probes mapped. Furthermore, ribosomal RNA genes, whose sequence and copy numbers are supposedly under greater selective constraints than the non-transcribed satellite repeats, can undergo homogenization in allopolyploids (Wendel, Schnabel & Seelanan, 1995; Volkov *et al*., 1999; Lim *et al*., 2000; Álvarez & Wendel, 2003; Doyle *et al*., 2004a, b; Kovarik *et al*., 2004 – this issue). Future cytogenetic and fine-mapping studies will examine these unexplored areas of *Tragopogon* genome structure. However, based on the tandem repetitive sequences in the *Tragopogon* allopolyploids studied, no major changes in overall genome structure have occurred within the time frame of the past ~80 years since polyploid formation (Fig. 3).

CONCERTED EVOLUTION

Concerted evolution, resulting in the homogenization of gene sequences within species and heterogeneity among species, is a common aspect of multigene families, such as ribosomal RNA genes (Zimmer *et al*., 1980; Arnheim, 1983; see Hamby & Zimmer, 1992). In F_1 hybrids and allopolyploids, the rDNA repeat types of both parents are expected to co-occur and indeed do occur, for example, in polyploid species of *Paeonia* that are roughly one million years old (Sang, Crawford & Steussy, 1995). However, in some allopolyploid plants, only one parental rDNA repeat type is found, a result typically attributed to con-

certed evolution (e.g. Wendel *et al*., 1995; Franzke & Mummenhoff, 1999; Volkov *et al*., 1999; Lim *et al*., 2000). In several polyploid complexes, concerted evolution has been in the direction of one of the two parental genomes in some allotetraploids and in the direction of the second parental genome in other allotetraploids (i.e. 'bidirectional' concerted evolution, as in allotetraploid cottons; Wendel *et al*., 1995). Previous molecular studies of *T. mirus* and *T. miscellus* revealed the presence of both diploid parental rDNA types in the allotetraploids (P. Soltis & D. Soltis, 1991; P. Soltis, Doyle & D. Soltis, 1992). However, sequencing of rDNA internal transcribed spacers (ITS) cloned from both allotetraploid species indicated that the parental rDNA types were typically not present in equal frequency. A. Kovarik, J. C. Pires, A. Leitch, K. Y. Lim, A. Sherwood, R. Matyasek, J. Rocca, P. S. Soltis & D. E. Soltis (unpubl. data) then investigated concerted evolution in the recently formed allotetraploids, *T. mirus* and *T. miscellus,* using slot blot and Southern analysis, as well as sequence analysis of numerous sets of clones.

Slot blot experiments involving multiple populations of both polyploids and their diploid parents revealed that the diploids have roughly comparable numbers of rDNA repeats and that the two polyploids contain more rDNA repeats than do any of the diploid populations (Figs 4, 5C) (A. Kovarik *et al*., unpubl. data). However, the number of rDNA repeats in the polyploids was not double that found in the diploids, suggesting the possibility of some loss of rDNA repeats following polyploidization. Furthermore, A. Kovarik *et al*., (unpubl. data) observed variation in the number of rDNA repeats among populations within any given species (Figs 4, 5C), consistent with evidence for a four-fold level of variation in rDNA copy number within species (reviewed in Hamby & Zimmer, 1992) and a ten-fold difference among inbred lines of maize (Riven, Cullis & Walbot, 1986). This variation among diploid populations of *Tragopogon* species is an important consideration in the formation of new allotetraploids. A polyploid plant of *T. mirus* that was formed from a 'low rDNA copy' *T. dubius* parent (such as population 2614; Fig. 4) and a 'high rDNA copy' *T. porrifolius* (such as population 2607; Fig. 4) would yield a raw allopolyploid with more *T. porrifolius* rDNA repeats than *T. dubius* repeats. Conversely, a polyploidization event from a 'high rDNA copy' *T. dubius* parent (such as 2613) and a 'low copy' *T. porrifolius* parent (such as 2611) would result in the formation of a *T. mirus* with many more rDNA repeat units of *T. dubius* than of *T. porrifolius* (Fig. 4). Taking into account this variation in rDNA repeat number among diploid populations noted above (Fig. 4), the ratios of parental rDNA types in a newly formed allopolyploid might be as much as 1.5/1.

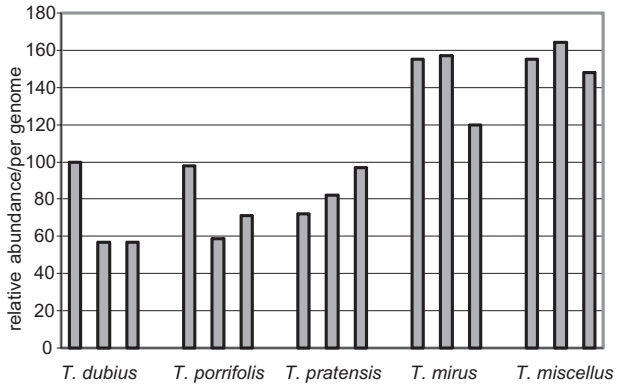


Figure 4. Summary of slot blot analyses of *Tragopogon* populations and species. The relative abundance of rDNA repeats is provided for diploid parents and allopolyploid derivatives.

Analyses of both Southerns and clones yielded results with identical interpretations (Fig. 5A, B; Table 1). In five of the six polyploid populations examined (except *T. mirus* 2602) there are far fewer rDNA copies of *T. dubius* than of the other diploid parent (Table 1). Using Southerns, it is also obvious that the ratios are highly skewed against *T. dubius* in all three populations of *T. miscellus*. The ratios are probably also skewed against *T. dubius* in two populations of *T. mirus* (2601, 2603) based on Southerns; however, exact ratios are difficult to determine due to minimal separation of parental *Eco*RV fragments on the blots (Fig. 5B). In any case, *T. mirus* 2602 is noteworthy in having a dominant 11.0-kb band with a mobility similar to *T. dubius* repeats. The small number of *T. dubius* repeats observed in five of six populations appears to be outside the limits of variation expected even if the polyploidy event involved a 'low rDNA repeat' *T. dubius* as parent (see above). For example, just considering clones (Table 1), the ratios of *T. pratensis* or *T. porrifolius* to *T. dubius* range from 2.1 : 1 to 12 : 1 with an average ratio of 5.6 : 1.

ITS was cloned and sequenced from additional populations not surveyed with a Southern blot approach. For these additional populations of *T. mirus* and *T. miscellus*, *T. dubius* was also the uncommon diploid ITS type (Kovarik *et al*., 2004). However, the population of *T. mirus* from Albion, WA, had comparable numbers of *T. dubius* and *T. porrifolius* ITS clones (Table 1) and may represent a recently formed population of *T. mirus*. We are now attempting to resynthesize the allotetraploids *T. mirus* and *T. miscellus*. Such plants would be ideal for assessing the 'starting point' ratio of ITS diploid types in a raw allopolyploid. As a substitute for resynthesized polyploids, A. Kovarik *et al*. (unpubl. data) used herbarium specimens (holotypes) collected by M. Ownbey in

Table 1. Results of ITS cloning for populations of *T. miscellus* and *T. mirus*

Population designation	No. of clones of:	
T. miscellus	*T. dubius* type	*T. pratensis* type
2604	6	14
2605	4	19
2606	1	12
From 1950, Moscow, ID	9	10
From 1953, Sheridan, WY	12	16
T. mirus	*T. dubius* type	*T. porrifolius* type
2601	9	19
2602	13	8
2603	3	20
Albion, WA	9	11
From 1950, Pullman, WA	8	7

1949; these specimens represent the first collections of the newly discovered allotetraploids. These two collections, one of *T. miscellus* from Moscow, ID, and one of *T. mirus* from Pullman, WA, represent a point in time very close to the origin of these allopolyploids in these two towns (Ownbey, 1950). A third early collection of *T. miscellus*, from Sheridan, WY, collected in 1953 was also used. Little is known about the origin of *T. miscellus* in Sheridan, but we assume that this time of collection is close to the time of polyploid formation in that area given that the diploid progenitors were only introduced into the western USA in the early 1900s. Cloning experiments demonstrated that all three of these 'new' allopolyploid populations have a ratio of diploid parental types that is close to 1 : 1 (Table 1). A. Kovarik *et al*. (unpubl. data) attempted to conduct Southern blot experiments on DNAs from the same three herbarium collections, but because the DNAs were slightly degraded, the results were not of suitable quality to quantify the diploid contributions.

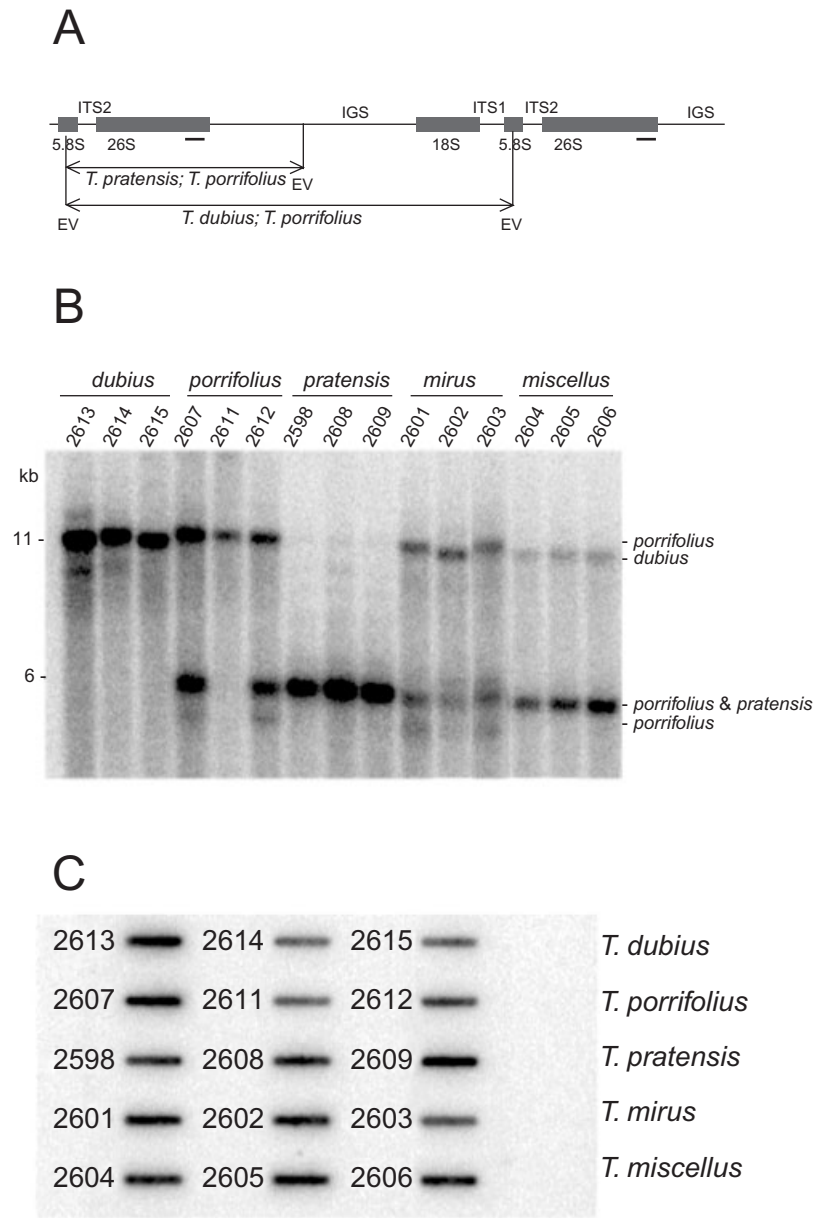
A. Kovarik *et al*. (unpubl. data) also conducted PCR experiments to ensure that the skewed clone ratios (typically away from *T. dubius*) were not the result of PCR bias. Mixing of diploid DNAs (*T. dubius* + *T. pratensis*; *T. dubius* + *T. porrifolius*) in precise ratios followed by PCR and digestion with species-specific restriction enzymes showed no evidence of PCR bias (Kovarik *et al*., 2004). Concerted evolution appears to be homogenizing the rDNA repeats in the allopolyploids. The results are significant in that A. Kovarik *et al*. (unpubl. data) have 'caught concerted evolution in the act', because the process has not gone to completion in any of the polyploid populations examined. Concerted evolution may therefore begin shortly after a polyploid is formed and occur rapidly in newly formed polyploids. For example, if the populations Ownbey described in 1950 formed in the 1940s, when the plants were first observed, concerted evolution has largely homogenized the rDNA repeats in less than 60 years, or approximately 30 generations. It will be of interest to follow these same populations during the next 10–20 years and continue to monitor the process of concerted evolution.

ANALYSIS OF GENE EXPRESSION IN *TRAGOPOGON MISCELLUS*

cDNA-amplified fragment length polymorphisms (cDNA-AFLP) (Bachem *et al*., 1996) have been applied to polyploid systems, including the model angiosperm *Arabidopsis thaliana* and its allopolyploid derivative *A. suecica* (Fries) Norrl. ex. O. E. Schulz (Comai *et al*., 2000; Lee & Chen, 2001), and wheat, *Triticum aestivum* L. (Kashkush, Feldman & Levy, 2002), to assess changes in gene expression between polyploids and their diploid progenitors. J. A. Tate, A. C. Scheen, Z. J. Chen, D. E. Soltis & P. S. Soltis (unpubl. data) used this genome-wide approach to examine differences in gene expression in reciprocally formed populations of *Tragopogon miscellus* and to assess the maternal and paternal contributions to the polyploid transcriptome. In these analyses, leaf tissue taken from seedlings grown under uniform conditions in a growth chamber served as a source of material.

The cDNA-AFLP data indicated that multiple origins are an important source of genic diversity in natural polyploid populations (J. A. Tate, A. C. Scheen, Z. J. Chen, D. E. Soltis & P. S. Soltis unpubl. data). Expression differences were observed not only in the Pullman, WA, and Moscow, ID, populations of *T. miscellus* relative to their diploid progenitors, but also between the populations of independent origin (Fig. 6). Approximately 5% of the cDNA-AFLP fragments were silenced in at least one polyploid, and 4.0% showed novel expression in the polyploids. Differential maternal and paternal contributions to the allopolyploid transcriptomes comprised 3.7% and 4.1% of the expression differences, respectively. BLAST similarity searches of the differentially expressed genes suggested that they belong to various functional classes (transcription factors, kinases, floral regulators, etc.) and may play a role in many cellular processes, including carbohydrate metabolism, signal transduction, protein transport and degradation, and cell division (Table 2).

The independently derived populations of *T. miscellus* showed further differential expression of homoeologous loci based on detailed characterization of selected cDNA-AFLP fragments (J. A. Tate *et al*., unpubl. data; Fig. 7). For a putative GTP-binding protein, *T. dubius* showed high expression in RT-PCR



analysis, whereas *T. pratensis* and both allopolyploid populations of *T. miscellus* showed low expression of this gene (Fig. 7A). Sequence data indicated that the expressed allele in both allopolyploid populations was identical to that of *T. pratensis*. Another cDNA fragment, similar to an unidentified expressed protein, was expressed in equal quantities in all individuals, but transcript sequencing demonstrated reciprocal expression of the maternal homoeologue in the allopolyploid populations (Fig. 7B).

The results of J. A. Tate *et al*. (unpubl. data) indicate that multiple origins may contribute significantly to the evolution of plant polyploids. Although only two populations were surveyed, the data demonstrate that expression of duplicated genes differs in populations of independent origin. Characterization of the underlying mechanisms responsible for regulating these genes should lend insight into genome evolution in these recent polyploids. It will also be useful to determine whether variation in expression occurs among polyploid populations of the same origin, or among other populations of independent origin.

Other recent studies have similarly demonstrated changes in gene expression in polyploids. Lee & Chen (2001) used cDNA-AFLP display to examine gene expression changes in natural *A. suecica*, an allotetraploid derived from *A. thaliana* and *A. arenosa* (L.) Lawalree, and found evidence of epigenetic gene

Figure 5. A, Restriction enzyme maps of the major *T. dubius*, *T. pratensis and T. porrifolius* rDNA units. The position of probe hybridization is indicated by a thick line. Restriction enzyme: EV = *Eco*RV. IGS, intergenic spacer; ITS, internal transcribed spacer; 18S, 5.8S, 26S genes coding ribosomal RNA molecules. Distances are approximately to scale. B, Restriction enzyme analysis of intergenic spacer (IGS) regions in diploid (*T. dubius*, *T. pratensis* and *T. porrifolius*) and tetraploid (*T. mirus* and *T. miscellus*) species. About 0.5 μg of total genomic DNA was isolated from a leaf of a single plant of each accession using the Qiagen kit. Purified DNAs were digested with *Eco*RV, separated by gel electrophoresis and blotted on to nylon membranes. The Southern blot hybridization was carried out in modified Church–Gilbert buffer (Lim *et al*., 2000) using the 26S rDNA labelled with [^{32}P] dCTP (ICN, USA). The probe was a ~280-bp PCR product derived from the 3′ end of the 26 rRNA gene (Lim *et al*., 2000). The bands were visualized by PhosphorImaging. There was a single hybridization band of about 11.2 kb in all populations of *T. dubius*, consistent with our previous report (P. Soltis & D. Soltis, 1991). All three populations of *T. pratensis* had a single 6.5-kb band. All populations of *T. miscellus* (allotetraploid of *T. dubius* and *T. pratensis*) inherited the 11.2- and 6.5-kb bands from their parents. Note substantial reduction of band intensity corresponding to *T. dubius* units. Populations 2607 and 2012 of *T. porrifolius* had bands of 11.5, 6.5 and 5.8 (weak) kb; population 2611 had an 11-kb band only. In lanes loaded with DNAs from *T. mirus* (allotetraploid of *T. dubius* and *T. porrifolius*), the repeats inherited from both parents were detected. In population 2602 of *T. mirus*, the strong 11.2-kb band in the high-molecular-weight fraction had a somewhat slower mobility than corresponding bands in populations 2601 and 2603 and could represent units of *T. dubius* origin. More detailed analysis of IGS among different populations of *T. mirus* is therefore warranted. C, Relative numbers of rDNA units in *Tragopogon* genomes. Quantification was performed by slot blot analysis. The blot was hybridized to the 26S rDNA probe. The signal in allotetraploids was nearly the same as in diploids. Considering equal amounts of DNA loaded into slots (0.2 μg) together with approximately twice the amount of DNA in the nuclei of tetraploids, it may be deduced that there is less than double the number of rDNA copies in the tetraploids as in their diploid progenitors. Information for populations used is given below. *Tragopogon dubius*, population 2613, Pullman, WA; population 2614, Rosalia, WA; population 2615, Spokane, WA. *Tragopogon porrifolius*, population 2607, Troy, ID; population 2611, Pullman, WA; population 2612, Troy, ID. *Tragopogon pratensis*, population 2598; population 2608, Moscow, ID; population 2609, Spangle, WA. *Tragopogon mirus*, population 2601; population 2602; population 2603. *Tragopogon miscellus*, population 2604, Moscow, ID; population 2605, Pullman, WA; population 2606, Spangle, WA.

Table 2. Putative identities for a subset of differentially expressed cDNA-AFLP fragments in allotetraploid *Tragopogon miscellus* populations (Tm, Tm′) and the diploid parents *T. dubius* (Td) and *T. pratensis* (Tp) as determined by BLAST similarity searches against the TIGR *Arabidopsis thaliana* database. Expression patterns are illustrated in the following order: Tp, Tm, Tm′ and Td where '+' and '–' indicate a cDNA-AFLP fragment that is present or absent, respectively. Tp is the maternal parent of Tm, and Td is the maternal parent of Tm′

Putative protein	Gene name	cDNA fragment ID	cDNA fragment size	Pattern (Tp, Tm, Tm′, Td)	Similarity and *E*-value
Auxin conjugate hydrolase	*ILL5*	T-62	255	+ – + –	63% 9.9 e-09
Basic leucine zipper transcription activator	*BZIP*	T-97	155	+ – + –	79% 1.4 e-11
Expressed protein	*EXPI*	T-53	370	– – + +	82% 1.4 e-10
GTP-binding protein	*GTPB*	T-90	176	– – – +	77% 3.0 e-08
Hypothetical protein	*HYPI*	T-75	300	– + + –	76% 1.7 e-13
Peroxidase	*PER*	T-13	327	– – + +	69% 1.5 e-21
Ruv DNA-helicase-like protein	*RDNA*	T-23	318	– + + –	71% 1.1 e-19
Ubiquitin-like protein	*UBQ12*	T-17.4	385	– + – +	78% 9.8 e-47

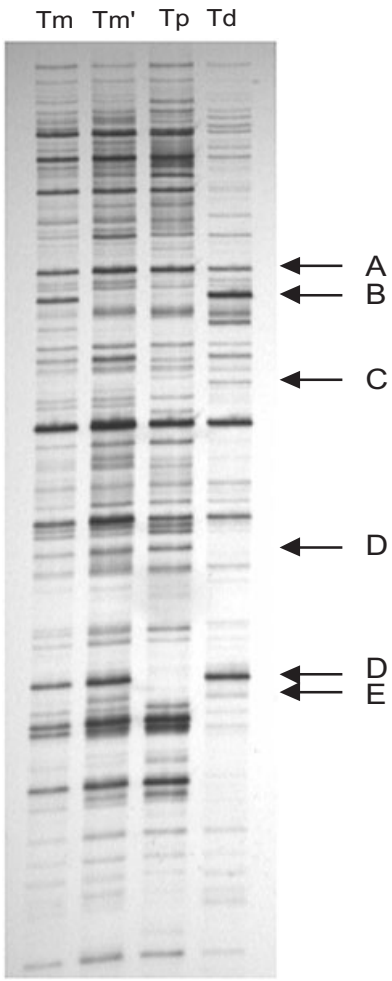


Figure 6. cDNA-AFLP gel showing differences in gene expression in reciprocally formed populations of *Tragopogon miscellus* (Tm & Tm′) and the diploid progenitors, *T. dubius* (Td) and *T. pratensis* (Tp). Tp and Td are the maternal parents of the Tm and Tm′ populations, respectively. The primer pair used in the amplification was *Eco*RI-AG/*Mse*I-CTT. Five expression patterns are highlighted here: A, monomorphic; B, paternal expression; C, gene silencing in the polyploids; D, coexpression of homoeologous loci and E, maternal expression.

silencing in the allotetraploid. Comai *et al*. (2000), in a study of resynthesized *A. suecica*, also determined that gene silencing plays an important role in regulation of duplicated genes. Functional diversification of genes following polyploid formation has also been documented in maize and wheat (Feldman *et al*., 1997; Gaut & Doebley, 1997; Zhang *et al*., 2001; Kashkush *et al*., 2002), and rapid subfunctionalization of duplicated genes has been reported in tetraploid cotton (Adams *et al*., 2003). Recent advances in microarray analysis are revealing global changes in gene expression patterns in polyploid *Arabidopsis* (Chen *et al.*, 2004), a method which promises significant advances in our understanding of polyploids.

FUTURE DIRECTIONS

Future work on *Tragopogon* will include mechanistic studies of the expression differences noted above, particularly the potentially silenced genes. Although genetic mutations can explain gene loss over evolutionary time, many silencing phenomena may be epigenetically controlled, especially in the early stages of polyploid formation (reviewed in Osborn *et al*., 2003). When two different genomes are combined in a single cell, they must respond to the consequences of genome duplication, particularly the presence of multiple copies of genes with similar or redundant functions. Thus, the expression of homoeologous genes must be reprogrammed through epigenetic mechanisms during the early process of polyploidization. 'Genomic shock', as predicted (McClintock, 1984), occurs rapidly in at least some polyploids, resulting in sequence elimination and rearrangement (Song *et al*., 1995; Feldman *et al*., 1997; Shaked *et al*., 2001; see also Chen *et al*., 2004 – this issue), demethylation of retroelements (O'Neill, O'Neill & Graves, 1998; Comai, 2000; Comai *et al*., 2000) and relaxation of imprinting (differential expression of the paternal and maternal alleles of a gene) (Vrana *et al*., 2000). In *A. suecica*, resynthesized allopolyploids were highly sensitive to the methylation inhibitor 5-aza-2′-deoxycytidine (Lee & Chen, 2001), whereas established *A. suecica* was not, again suggesting that changes in methylation play a critical role in the establishment of polyploids (Madlung *et al*., 2002). In *A. thaliana*, autotetraploidy was found to reactivate a silenced transgene (Mittelstein Scheid *et al*., 1996). Moreover, in interspecific hybrids or allopolyploids of some vertebrates, invertebrates and plants, one parental set of rRNA genes is subjected to silencing (Reeder, 1985; Pikaard & Chen, 1998). The genes are stochastically silenced within two generations after polyploid formation (Chen, Comai & Pikaard, 1998) and reactivated by blocking DNA methylation and histone deacetylation (Chen & Pikaard, 1997b) in floral tissues and organs (Chen & Pikaard, 1997a). Epigenetic change provides an effective and flexible means for a polyploid cell to respond to genome duplication or 'genomic shock', because it is established or erased relatively easily (Russo, Martienssen & Riggs, 1996). Indeed, silenced rRNA genes in vegetative tissues are reactivated during flower development (Chen & Pikaard, 1997a). Thus, an epigenetic silencing strategy could balance regulatory incompatibility with the advantages of having multiple copies of homoeologous genes or gene products (e.g. transcriptional factors) spontaneously produced in a polyploid cell.

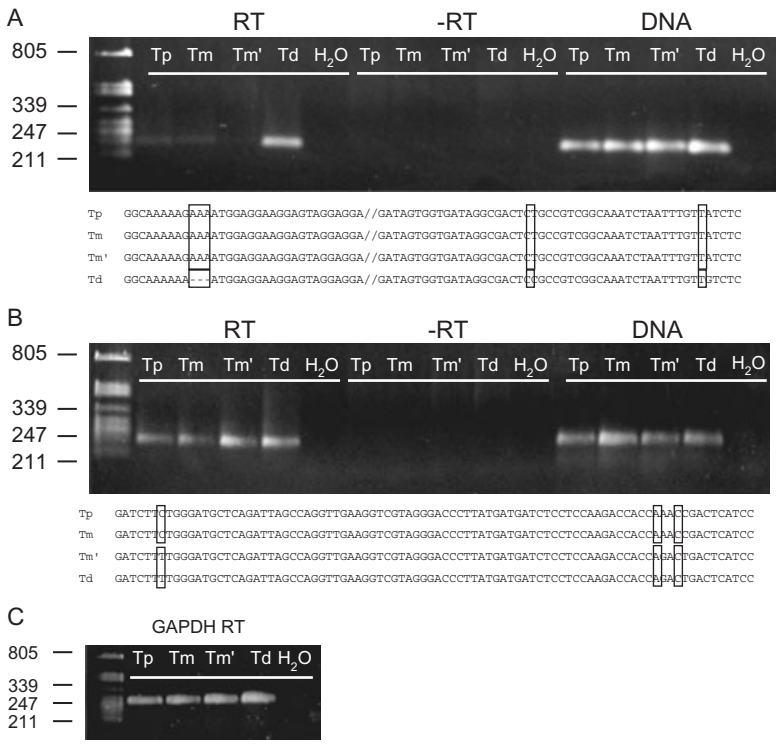


Figure 7. RT-PCR and transcript sequences in allotetraploid *Tragopogon miscellus* (Tm, Tm′) and its diploid progenitors *T. pratensis* (Tp) and *T. dubius* (Td). RT-PCR was performed with (RT) and without (–RT) reverse transcriptase. Genomic amplification (DNA) was performed as a positive control for each individual. Transcript sequences are shown below the RT-PCR panels. A, A gene (similar to a GTP-binding protein) showing strong expression in Td showed low expression in Tp and both polyploid populations. Sequence data indicate that the allele expressed in both polyploids was inherited from Tp. B, A maternally expressed transcript in AFLP-cDNA display was expressed in all taxa for RTPCR. Sequencing of these transcripts validates maternal expression of this gene in the polyploids. C, Expression of a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), in each taxon (modified from J. A. Tate *et al*., unpubl. data).

Heterologous proteins under epigenetic control may confer differential fitness under various environmental conditions and developmental programs. Epigenetic regulation may control the expression of many duplicate genes in polyploids and thus contribute to the molecular basis of natural variation. Differential epigenetic modification of homoeologous genes in polyploid populations, particularly among populations of separate origin, may play an important, and heretofore unrecognized, evolutionary role.

Major questions that remain in *Tragopogon* involve the implications of multiple polyploidizations in natural populations. For example, does reciprocal silencing of genes in polyploid populations of separate origin play a role in divergence and reproductive isolation (Werth & Windham, 1991; Levin, 2000; Lynch & Conery, 2000; Lynch & Force, 2000a, b; Taylor *et al*., 2001)? A straightforward initial experiment would involve analysis of the crossability of polyploid populations of separate origin, with analysis of hybrid fertility. To what extent does plant polyploidy affect pollination (Segraves & Thompson, 1999) and other plant–animal interactions? Field observations (Cook & Soltis, 1999) indicate that flower heads of polyploids remain open longer in natural populations than do those of diploids. What are the physiological implications of polyploidy (Warner & Edwards, 1989; Grisebach & Kamo, 1996)? What are the ecological and evolutionary implications of multiple polyploidizations? That is, are polyploid populations of independent origin locally adapted? Do these populations differ physiologically and ecologically? Is there gene flow between diploids and tetraploids (Bretagnolle & Lumaret, 1995; Husband & Schemske, 1997)? As this list indicates (and as is also evident from other papers in this issue), despite considerable recent progress on diverse aspects of polyploidy, many exciting questions remain.

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