New Phytologist

Tissue-specific silencing of homeologs in natural populations of the recent allopolyploid Tragopogon mirus

Journal:	*New Phytologist*
Manuscript ID:	Draft
Manuscript Type:	MS - Regular Manuscript
Date Submitted by the Author:	
Complete List of Authors:	Buggs, Richard; University of Florida, Florida Museum of Natural History; University of Florida, Department of Biology Elliott, Natalie; University of Florida, Department of Biology Zhang, Linjing; University of Florida, Florida Museum of Natural History; University of Florida, Department of Biology Koh, Jin; University of Florida, Florida Museum of Natural History; University of Florida, Department of Biology Viccini, Lyderson; University of Florida, Florida Museum of Natural History; University of Florida, Department of Biology Soltis, Pam; FLORIDA MUSEUM OF NATURAL HISTORY, Molecular Systematics & Evolutionary Genetics Soltis, Doug; University of Florida, Department of Biology; University of Florida, Genetics Institute
Key Words:	Polyploidy, homoeolog, whole genome duplication, gene expression, subfunctionalization, Tragopogon (Goatsbeard)

Tissue-specific silencing of homeologs in natural populations of the recent allopolyploid *Tragopogon mirus*

Richard J. A. Buggs[1,2], Natalie M. Elliott[1,2], Linjing Zhang[1,2], Jin Koh[1,2], Lyderson F. Viccini[1,2,3], Douglas E. Soltis[1,4], and Pamela S. Soltis[2,4]

[1]Department of Biology, University of Florida, Gainesville, Florida 32611.

[2]Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611.

[3] Departamento de Biologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, 36036-900 Juiz de Fora, Minas Gerais, Brazil

[4]Genetics Institute, University of Florida, Gainesville, Florida 32610.

Correspondence: Dr Richard Buggs, Department of Biology, University of Florida, Gainesville, Florida 32611, USA.

Email: buggs@ufl.edu

Telephone: 352-273-1976

Key words: polyploidy, whole-genome duplication, *Tragopogon mirus*, homeolog evolution, tissue-specific expression

Running title: Tissue-specific homeolog expression

Total word count: 3647

Abstract: 195

Introduction: 483

Materials and Methods: 554

Results: 1043

Discussion: 1483

Acknowledgements: 37

Number of figures: 1

Number of tables: 3

Abstract

- Recent years have seen rapid advances in our knowledge of the transcriptomic consequences of allopolyploidy, primarily through the study of polyploid crops and model systems. However, few studies have distinguished between homeologs and between tissues, and still fewer have examined young natural allopolyploid populations of independent origin, whose parental species are still present in the same location.
- We examined the expression of 13 homeolog pairs in 7 tissues of 10 plants of allotetraploid *T. mirus* from two natural populations formed by independent polyploidizations between *T. dubius* and *T. porrifolius* ~ 40 generations ago. We compare these with patterns of expression in the diploid parental species from the same locality.
- Of the 910 assays in *T. mirus*, 576 (63%) showed expression of both homeologs, 63 (7%) showed no expression of either homeolog, 186 (20%) showed non-expression of one homeolog across all tissues of a plant, and 72 (8%) showed non-expression of a homeolog in a particular tissue within a plant. We found two cases of reciprocal tissue-specific expression between homeologs, potentially indicative of subfunctionalization.
- Our study shows that tissue-specific silencing, and even apparent subfunctionalization, can arise very rapidly in the early generations of natural allopolyploidy.

Key Words

Polyploidy; *Tragopogon* (Goatsbeard); homoeolog; whole genome duplication; gene expression; subfunctionalization

Introduction

Whole-genome duplication (WGD), or polyploidy, has long been considered an important factor in the evolution of plants (e.g. Darlington, 1937; Stebbins, 1950; Grant, 1981; Doyle *et al.*, 2008). For many decades researchers have sought to understand the causes and consequences of WGD and the reason for polyploid success. Recent studies at the molecular level have demonstrated that polyploidy may have profound consequences on the genome and transcriptome (reviewed in Soltis & Soltis, 1999; Wendel, 2000; Osborn *et al.*, 2003; Leitch *et al.*, 2004; Soltis *et al.*, 2004b; Adams, K. L. & Wendel, J. F., 2005b; Adams, Keith L. & Wendel, Jonathan F., 2005; Rapp & Wendel, 2005; Chen & Ni, 2006; Doyle *et al.*, 2008; Hegarty & Hiscock, 2008; Leitch & Leitch, 2008; Ha *et al.*, 2009). However, relatively few studies (e.g. Adams *et al.*, 2003; Chaudhary *et al.*, 2009) have examined an aspect of gene expression that is likely to be involved in evolution after WGD: changes in the relative expression of the duplicate genes (homeologs). One way in which homeologs may diverge in expression is through differential expression among tissues of a polyploid plant; this may contribute to phenotypic variation in polyploids.

Among-tissue divergence of duplicate gene expression patterns has long been regarded as a precursor of future evolution (Ohno, 1970), hence studies of expression may allow us to predict the final fates of duplicated genes. Expression of a gene duplicate in a tissue where the progenitor copy was not expressed may indicate neofunctionalization (Ohno, 1970; Duarte *et al.*, 2006); division of ancestral patterns of tissue-specific expression among duplicates suggests subfunctionalization (Lynch & Conery, 2000; Rodin & Riggs,

2003; Duarte *et al.*, 2006); and silencing of a gene duplicate in all tissues points to nonfunctionalization (Duarte *et al.*, 2006). Both neo- and sub-functionalization will lead to long-term retention of duplicated genes, whereas silencing/nonfunctionalization will generally lead to loss of a duplicate. Tissue-specific expression of duplicated genes has been studied in older gene duplicates in model organisms and crops (Adams *et al.*, 2003; Duarte *et al.*, 2006; Ganko *et al.*, 2007; Semon & Wolfe, 2008; Chaudhary *et al.*, 2009), but in these species the ancestral patterns of gene expression and the age of duplicates are not known precisely. Cases of tissue-specific expression patterns of very young gene duplicates are restricted to a few synthetic polyploids (Adams *et al.*, 2003; Adams *et al.*, 2004; Wang *et al.*, 2004; Chaudhary *et al.*, 2009).

Here, we examine tissue-specific expression of 13 duplicate gene pairs (homeologs) in the young natural allotetraploid species *Tragopogon mirus* (Asteraceae), which formed ~80 years ago (~40 generations, given that these are biennials) (Ownbey, 1950; Soltis *et al.*, 2004a). *Tragopogon mirus* as well as *T. miscellus* have become textbook examples of recent and recurrent allopolyploidy. We analyzed 10 individuals from two populations of reciprocal origin, with the null hypothesis that patterns of expression would be additive of those of the parental species *T. dubius* and *T. porrifolius*. Tissue-specific homeolog silencing was detected frequently in the ~40 generations after WGD.

Materials and Methods

Seeds were collected from natural populations (Table 1) of *T. mirus* of independent origin (Symonds et al., submitted): Palouse WA, USA (Soltis and Soltis collection number 2602) and Pullman, WA, USA (collection number 2601). Samples of *T. dubius* were obtained from Pullman, WA, USA (2613), and samples of *T. porrifolius* were obtained from Palouse WA, USA (2626) and Pullman, WA, USA (2611). The *T. mirus* seeds were grown in the greenhouse (at Washington State University) and allowed to self-fertilize for one generation. The selfed seeds, together with seed of the diploid parent species, *T. dubius* and *T. pratensis*, were germinated and grown under controlled conditions in a greenhouse at the University of Florida (Gainesville, FL, USA). We analyzed six plants from the Pullman *T. mirus* population (individual nos. 2601-45, -47, -7, -5, -2, -14), four plants from the Palouse *T. mirus* population (2602-1, -2, -25, -4), two plants from the *T. dubius* Pullman population (2613-35, -21), one plant from the *T. porrifolius* Pullman population (2611-2) and one plant from the *T. porrifolius* Palouse population (2626-4).

Leaves and inflorescences were collected from all plants and flash frozen in liquid nitrogen (Tate *et al.*, 2006). Inflorescences were dissected on dry ice into six tissue types: corolla, pappus, ovary, stigma, style, and phyllary. Tissues were ground with a mortar and pestle in liquid nitrogen, and RNA was extracted using the RNeasy kit with on-column DNAse digestion from Qiagen (Valencia, CA, USA). First-strand cDNA synthesis was carried out on 500 ng of RNA using Superscript II reverse-transcriptase

(Invitrogen, Carlsbad, CA, USA) and polyT primers, following the manufacturer's protocol.

Homeolog expression of 13 gene pairs was analyzed using cleaved amplified polymorphic sequence (CAPS) markers. Homeologous gene pairs were amplified by PCR from the cDNA samples using primer sets from Koh *et al.* (in press), shown in Table 2. Genomic and cDNA fragments were amplified in 25 µl volume with 50–100 ng template, 20% final volume Promega 5 × sequencing buffer (Promega, Madison, WI, USA), 1mM $MgCl_2$, 0.4mM dNTPs, 0.2 mM of each primer and 0.4 units of Promega Taq polymerase. Most of the primers were designed with annealing temperatures close to 60 °C; a representative set of thermocycling conditions is as follows: 94 °C for 2 min, followed by two cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, followed by three further sets of two cycles in which the annealing temperature was dropped to 58, 56 and 54 °C for each set, followed by 27 cycles with an annealing temperature of 52 °C. Putative identities of these genes, found by nucleotide blast against *Arabidopsis thaliana*, are provided in Table 2.

One to two micro-liters of non-purified PCR products were digested using the enzymes specified in Table 2, according to manufacturer's protocols (New England Biolabs, Ipswich, MA, USA), with a total reaction volume of 10 µl. Digestion products were visualized on high-resolution 4% Metaphor agarose (Lonza, Allendale, NJ, USA) gels. Where one homeolog appeared to be silenced in the gel, the PCR product was also sequenced using the Applied Biosystems Model 3130 Genetic Analyzer at the

Interdisciplinary Center for Biotechnology Research, University of Florida. Where clear sequence was obtained, the sequence from the allopolyploid was compared with sequences from the two parental species to double-check which homeolog(s) were present.

Results

Homeologous gene expression was analyzed in 7 tissues of 10 allopolyploid plants of *T. mirus* using 13 cleaved amplified polymorphic sequence (CAPS) markers. Results for each gene are described below and summarized in Figure 1. Raw data are shown in Figure S1. All gene functions are putative. One cDNA sample, leaf tissue of *T. mirus* plant 2602-4, failed to amplify any of the genes successfully, and the RNA could not be re-extracted due to loss of the sample.

Beta-glucosidase (LEC3)

This gene showed several cases of tissue-specific homeolog expression in *T. mirus*, including one case where the two homeologs were reciprocally silenced (Figure S1a,c). In diploids the gene was expressed in all tissues examined, except leaf of 2626-4 (a band does not appear in Figure S1a for ovary of 2613-21, but expression of the gene here was confirmed by another PCR reaction). In 15 of the 69 *T. mirus* samples, tissue-specific homeolog silencing was seen; nine of these were confirmed by sequencing. Homeolog silencing was found in leaf tissue of three plants, three times in pappus and stigma tissue, and twice in ovary, phyllary and style tissue. In plant 2601-5, the *T. dubius* homeolog

was not expressed in the leaf, and the *T. porrifolius* homeolog was not expressed in the ovary, pappus and style. Expression in the leaf and pappus of this plant was confirmed by sequencing.

Rab GTPase homolog (GTPB)

This gene exhibited no expression of the *T. porrifolius* homeolog in any *T. mirus* plant or tissue (Figure S1a,c). The gene was expressed in all tissues of the diploid parents examined. The gel of the AciI digest (Figure S1) shows faint bands of undigested PCR product in some *T. mirus* tissues that appear to indicate the presence of the *T. porrifolius* homeolog. We tested this by sequencing of the PCR products of five tissues that showed more intense undigested bands. The chromatograms showed that the *T. porrifolius* homeolog was absent and the faint bands were therefore due to incomplete digestion by the enzyme.

DNA binding/transcription factor (Cry1)

There were no cases of homeolog silencing for this gene (Figure S1a,c).

Nuclear ribosomal RNA (nrDNA)

This gene showed expression of both homeologs in most *T. mirus* plants and tissues (Figure S1a,c). Digestion showed three clear cases of homeolog silencing: the *T. porrifolius* homeolog in the leaf of 2601-45 and 2601-14, and the *T. dubius* homeolog in the leaf of 2602-25. The two cases of *T. porrifolius* silencing were confirmed by sequencing.

Glyceraldehyde-3-phosphate dehydrogenase (G3PDH)

This gene was expressed in all diploid tissues tested (Figure S1a,c). The *T. porrifolius* homeolog was not expressed in any tissues of *T. mirus* 2602-1. The *T. dubius* homeolog was not expressed in the leaf tissue of 2602-25; sequencing confirmed this. The gel suggested that the *T. porrifolius* homeolog might be unexpressed in the ovary of 2602-4, but sequencing showed clear double peaks at two diagnostic loci, showing that both homeologs were present in cDNA.

Gibberelic Acid Insensitive transcription factor (RGA)

There were no cases of homeolog silencing for this gene (Figure S1a).

Thioredoxin M-type (THIOR)

There were no cases of homeolog silencing for this gene (Figure S1a).

Beta-fructofuranosidase homolog (BFRUCT4)

The only case of homeolog silencing of this gene was of the *T dubius* homeolog in the leaf tissue of plant 2602-25 (see Figure S1a,c).

Heat shock protein 2 (HS)

This gene was expressed in all plants and tissues of *T. dubius* and *T. porrifolius*. Both homeologs were expressed in all samples from *T. mirus*, except ovary tissue of 2601-2

which expressed only the *T. dubius* homeolog (Figure S1a). This was confirmed by sequencing.

Transducin family protein (TDRC)

This gene was expressed in all diploid samples. Six plants from *T. mirus* population 2601 showed no expression of the *T. dubius* homeolog in any tissue tested (Figure S1a,c). Both homeologs were expressed in all tissues of three *T. mirus* plants from *T. mirus* population 2602. In the leaf of plant 2602-2 the digest banding was very faint, and only the *T. porrifolius* homeolog was detected by sequencing. This plant expressed both homeologs in all other tissues tested, as shown by the enzyme digest.

Ascorbate peroxidase 2 (AP2)

The CAPS analysis for this gene appeared to show only *T. dubius* homeolog expression in all *T. mirus* samples (Figure S1a). However, the *T. porrifolius* homeolog band was close to one of the *T. dubius* homeolog bands, so we sequenced the PCR product for 52 of the *T. mirus* tissues and confirmed that they expressed only the *T. dubius* homeolog, confirming that *T. porrifolius* was silenced in all tissues.

Peroxidase (PA)

This gene was expressed in a tissue-specific manner in the diploid plants (Figure S1b). In *T. porrifolius* 2626-4, it was not expressed in the stigma, and in 2611-2, it was not expressed in the leaf, corolla or style. In *T. dubius* 2613-35 it was not expressed in the corolla, style or stigma. In *T. mirus*, all plants showed no expression of either homeolog

in at least one tissue: 7 times the gene was not expressed in leaf tissue, 6 times in corolla tissue, once in ovary tissue, once in phyllary tissue, twice in pappus tissue, six times in stigma tissue and four times in style tissue. There were seven additional cases where only one homeolog was expressed in a tissue. The *T. dubius* homeolog was not expressed in the stigma of 2601-47, the stigma and style of 2601-7, the corolla and style of 2602-2 and the corolla of 2602-4. The *T. porrifolius* homeolog was not expressed in the stigma of 2601-2. These seven cases were confirmed by sequencing.

Peroxisomal NAD-malate dehydrogenase (MD)

This gene was expressed in all tissues of *T. dubius* diploids (Figure S1b) but was not expressed in the ovary, phyllary, pappus or stigma of *T. porrifolius* 2626-4 and in the leaf and style of 2611-2. In *T. mirus*, expression of the gene was occasional: the gene was not expressed in the leaf (7 times), corolla (4 times), ovary (3 times), phyllary (once), pappus (twice), stigma (4 times) and style (once). Where the gene was expressed in *T. mirus*, it was the *T. dubius* homeolog that was expressed in all tissues, except the stigma of 2602-4 where the *T. porrifolius* homeolog was expressed, and the leaf of 2601-7 where both homeologs were expressed.

Discussion

We examined the expression of 13 homeolog pairs in 7 tissues of 10 plants of *T. mirus* from two natural populations formed by independent crosses between *T. dubius* and *T.*

porrifolius. Of the 910 assays, 576 (63 %) showed expression of both homeologs (Table 3), 63 (7 %) showed no expression of either homeolog, 186 (20%) showed non-expression of one homeolog across all tissues of a plant, and 72 (8%) showed non-expression of a homeolog in a particular tissue within a plant. The 13 assays failed in the leaf tissue of 2602-4 (1.4 %). We discuss these different categories of results below.

Expression of both homeologs in all tissues was our null expectation for ten of the 13 genes, as they were expressed in all tissues of the parental diploids. This was therefore the *additive* pattern of expression in the allopolyploids for these genes. This additive pattern was found in the majority (69 %) of assays for these ten genes. In contrast, two genes, MD and PA, did not show expression in all tissues of the diploids. A putative peroxisomal NAD-malate dehydrogenase (MD) was expressed in all tissues of the diploid *T. dubius*, but was not expressed in the ovary, phyllary or pappus of one *T. porrifolius* plant and was not expressed in the leaf or style of the other *T. porrifolius* plant. A putative peroxidase (PA) was not expressed in the corolla, style or stigma of a *T. dubius* plant and was not expressed in the style of one *T. porrifolius* plant and the leaf, corolla or stigma of the other. For these two genes, therefore, an additive pattern of expression would involve some tissue-specific silencing. For gene MD, expression of both homeologs was only found in one assay, and in PA it was found in 37 of 70 assays. Over all, 30 % of assays in PA and MD showed expression of both homeologs, compared to 69 % in the other 11 genes.

Lack of expression of both homeologs in *T. mirus* was found most commonly in genes MD and PA: genes that were not expressed in certain tissues in the diploid parents (see above and Table 3). Eighteen other assays showed no expression of either homeolog. These cases are unlikely to be due to failure of PCR reactions, as several attempts were made to amplify the genes, with different annealing temperatures and concentrations of magnesium chloride.

Non-expression of one homeolog in all sampled tissues of a plant occurred for at least one gene in all plants. Silencing in all tissues may indicate loss of the homeolog from the genome (Duarte *et al.*, 2006) – a process already known to be common in *Tragopogon* polyploids (Tate *et al.*, 2006; Buggs *et al.*, 2009; Tate *et al.*, 2009) – or an epigenetic mechanism of gene silencing in all tissues (Galili & Feldman, 1984; Wang *et al.*, 2004). In all, there were 36 cases of possible genomic loss: 28% of 130 gene pairs examined (13 gene pairs × 10 plants). These occurred in five genes. The *T. porrifolius* homeolog of G3PDH was not expressed in any tissues of 2602-1, suggesting a recent homeolog loss. Putative gene loss occurred for the *T. dubius* homeolog of TDRC in all plants from the 2601 (Pullman) population. This could be due to a loss of that gene in a common ancestor of all *T. mirus* plants in that population. All plants showed putative loss of the *T. porrifolius* homeolog of genes GTPB and AP2. Eight plants showed putative loss of MD, apart from 2602-4 which appeared to express the *T. porrifolius* homeolog in the stigma, and 2601-7 which expressed both homeologs in the leaf. The fact that we did not find any plants expressing both of these genes at the same time, in two independent populations, suggests that this is not simply due to random gene loss but could indicate that it is

somehow deleterious for both homeologs to be expressed together (Paterson *et al.*, 2006; Thomas *et al.*, 2006).

Eight per cent of assays showed tissue-specific homeolog non-expression. This was most common in the putative beta-glucosidase gene (LEC3), where eight plants showed lack of expression of one homeolog in at least one tissue. The causes of these cases of non-expression appear to have arisen recently within the allopolyploids, because the gene was expressed in all tissues of the diploids, and each allopolyploid plant shows a different pattern of expression. Of particular interest is *T. mirus* plant 2601-5, which shows reciprocal silencing: the *T. dubius* homeolog of LEC3 was silent in leaf tissue, but the *T. porrifolius* homeolog was silent in ovary, pappus and style tissue. Such a pattern of expression may be indicative of – or a step toward the evolution of – subfunctionalization, whereby the functioning of a gene is partitioned between two duplicates (Force *et al.*, 1999; Lynch & Force, 2000; Adams *et al.*, 2003).

Other genes showed occasional tissue-specific expression patterns. In the ribosomal RNA gene (nrDNA) examined, there was some repetition of pattern: the *T. porrifolius* homeolog was silent in two leaf tissues from population 2601, and the *T. dubius* homeolog was silent in two leaf tissues from population 2602. In other genes, most cases of tissue-specific silencing were found in the leaf tissues of plants 2602-25 which showed silencing of the *T. dubius* homeolog in several genes. Genes MD and PA, which were not expressed in all tissues of diploid plants (see above), showed more frequent tissue-specific expression in *T. mirus* than the other genes (Table 3), including one case of

reciprocal silencing. Such patterns of expression may be additive of diploid expression, or may be due to disruption of expression when two differentially regulated orthologs are brought together as homeologs.

Loss or silencing of *T. porrifolius* homeologs was more common than loss or silencing of *T. dubius* homeologs. Two per cent of assays showed tissue-specific silencing of the *T. dubius* homeolog but 6 % showed tissue-specific silencing of the *T. porrifolius* homeolog (Table 4). Six plants showed silencing of a *T. dubius* homeolog in all tissues, but 30 showed silencing of a *T. porrifolius* homeolog in all tissues. *Tragopogon porrifolius* is the maternal parent of the *T. mirus* allopolyploids studied. In a study of rRNA expression (Matyasek *et al.*, 2007), rRNA derived from *T. dubius* was found to be more highly expressed than rRNA derived from *T. porrifolius* in *T. mirus* plants from population 2601 and three other populations, but the opposite was true in plants from population 2602.

Tragopogon mirus is one of only a few systems that afford the opportunity to examine evolution in the early generations following allopolyploidization in natural populations. We have also studied tissue-specific silencing in the closely related species *T. miscellus*, which originated in the same geographical areas and at the same time as *T. mirus* via hynridization between *T. dubius* and *T. pratensis* (Buggs *et al.* in prep). In *T. miscellus*, we examined 18 genes using CAPS analysis and 120 genes using Sequenom MassArray allelotyping. In natural *T. miscellus* populations we found similar patterns of tissue-specific homeolog expression to those reported here for *T. mirus*, though we found silencing of the *T. dubius* homeolog to be more common in both reciprocal formations of

this allopolyploid. In *T. miscellus* (Buggs *et al.* in prep.) species we also examined F_1 hybrids and first-generation synthetic allopolyploids, finding tissue-specific expression to be much rarer in these generations than in the ~40-generation-old natural populations.

Tissue-specific homeolog expression changes have also been found during allopolyploid evolution in cotton (Adams *et al.*, 2003; Adams *et al.*, 2004; Adams & Wendel, 2005a; Flagel *et al.*, 2008; Chaudhary *et al.*, 2009). In allopolyploid *G. hirsutum* there was a general tendency for homeologs from the "D" genome to be silenced more often than those from the "A" genome, as was also found in F_1 hybrids and synthetic allopolyploids (Chaudhary *et al.*, 2009). Studies not distinguishing between homeologs have shown alterations in gene expression upon allopolyploidization in *Arabidopsis* (Comai *et al.*, 2000; Lee & Chen, 2001; Wang *et al.*, 2004; Wang *et al.*, 2006), wheat (Kashkush *et al.*, 2002; He *et al.*, 2003; Levy & Feldman, 2004; Mochida *et al.*, 2004; Bottley *et al.*, 2006), *Senecio* (Hegarty *et al.*, 2006) and *Brassica* (Xu *et al.*, 2009). These studies show the importance of hybridization on gene expression, but none examine early natural generations of allopolyploidy.

Our studies in *Tragopogon mirus* (this paper) and *T. miscellus* (Buggs *et al.*, in prep) are unusual in that they: distinguish between homeologs and among tissues; they examine very young naturally occurring polyploids; and they examine patterns of expression in ancestral diploid species found in the same geographical location. Our studies caution us against attributing all patterns of tissue-specific expression in allopolyploids to processes arising after cross-fertilization between the parental diploids, if the diploids are not

known or not examined: genes that show tissue-specific expression in diploids may be likely to do the same in polyploids. Significantly, data for *Tragopogon* polyploids provide new insights into the early generations of natural allopolyploidy, showing that tissue-specific silencing, and even apparent subfunctionalization, can arise very rapidly.

Acknowledgments

Funding for this research was provided by the University of Florida and NSF grants DEB-0614421 to Doug and Pam Soltis, MCB-0346437 to Doug and Pam Soltis and Jennifer Tate, and by a CNPq/Brazil Fellowship to L.F.V (200733/2009-5).

Footnotes

Author contributions: R.J.A.B., J.K., P.S.S and D.S.S. designed research; R.J.A.B., N.E., L.Z., and L. F. V. performed research; R.J.A.B., P.S.S and D.S.S. analyzed data; and R.J.A.B., P.S.S and D.S.S. wrote the paper.

The authors declare no conflict of interest.

References

Adams KL, Cronn R, Percifield R, Wendel JF. 2003. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy of Sciences of the United States of America* **100**(8): 4649-4654.

Adams KL, Percifield R, Wendel JF. 2004. Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid. *Genetics* **168**(4): 2217-2226.

Adams KL, Wendel JF. 2005a. Allele-specific, bidirectional silencing of an alcohol dehydrogenase gene in different organs of interspecific diploid cotton hybrids. *Genetics* **171**(4): 2139-2142.

Adams KL, Wendel JF. 2005. Novel patterns of gene expression in polyploid plants. *Trends in Genetics* **21**(10): 539-543.

Adams KL, Wendel JF. 2005b. Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* **8**(2): 135-141.

Bottley A, Xia GM, Koebner RMD. 2006. Homoeologous gene silencing in hexaploid wheat. *Plant Journal* **47**(6): 897-906.

Buggs RJA, Doust AN, Tate JA, Koh J, Soltis K, Feltus FA, Paterson AH, Soltis PS, Soltis DE. 2009. Gene loss and silencing in *Tragopogon miscellus* (Asteraceae): comparison of natural and synthetic allotetraploids. *Heredity* **103**: 73-81.

Chaudhary B, Flagel L, Stupar RM, Udall JA, Verma N, Springer NM, Wendel JF. 2009. Reciprocal silencing, transcriptional bias and functional divergence of homeologs in polyploid cotton (*Gossypium*). *Genetics* **182**(2): 503-517.

Chen ZJ, Ni Z. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. *Bioessays* **28**(3): 240-252.

Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B. 2000. Phenotypic instability and rapid gene silencing in newly formed *Arabidopsis* allotetraploids. *Plant Cell* **12**(9): 1551-1567.

Darlington CD. 1937. *Recent Advances in Cytology*. Philadelphia: Blakiston.

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF. 2008. Evolutionary genetics of genome merger and doubling in plants. *Annual Review of Genetics* **42**: 443-461.

Duarte JM, Cui L, Wall PK, Zhang Q, Zhang X, Leebens-Mack J, Ma H, Altman N, dePamphilis CW. 2006. Expression pattern shifts following duplication indicative of subfunctionalization and neofunctionalization in regulatory genes of *Arabidopsis*. *Molecular Biology and Evolution* **23**(2): 469-478.

Flagel LE, Udall J, Nettleton D, Wendel J. 2008. Duplicate gene expression in allopolyploid *Gossypium* reveals two temporally distinct phases of expression evolution. *BMC Biology* **6**: 16.

Force A, Lynch M, Postlethwait J. 1999. Preservation of duplicate genes by subfunctionalization. *American Zoologist* **39**(5): 460.

Galili G, Feldman M. 1984. Intergenomic suppression of endosperm protein genes in common wheat. *Genome* **26**(6): 651-656.

Ganko EW, Meyers BC, Vision TJ. 2007. Divergence in expression between duplicated genes in *Arabidopsis*. *Molecular Biology and Evolution* **24**(10): 2298-2309.

Grant V. 1981. *Plant Speciation*. New York: Columbia University Press.

Ha M, Kim E-D, Chen ZJ. 2009. Duplicate genes increase expression diversity in closely related species and allopolyploids. *Proceedings of the National Academy of Sciences* **106**(7): 2295-2300.

He P, Friebe BR, Gill BS, Zhou JM. 2003. Allopolyploidy alters gene expression in the highly stable hexaploid wheat. *Plant Molecular Biology* **52**(2): 401-414.

Hegarty M, Barker G, Wilson I, Abbott RJ, Edwards KJ, Hiscock SJ. 2006. Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Current Biology* **16**: 1652-1659.

Hegarty MJ, Hiscock SJ. 2008. Genomic clues to the evolutionary success of polyploid plants. *Current Biology* **18**(10): R435-R444.

Kashkush K, Feldman M, Levy AA. 2002. Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. *Genetics* **160**(4): 1651-1659.

Lee HS, Chen ZJ. 2001. Protein-coding genes are epigenetically regulated in Arabidopsis polyploids. *Proceedings of the National Academy of Sciences of the United States of America* **98**(12): 6753-6758.

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**(5875): 481-483.

Leitch AR, Soltis DE, Soltis PS, Leitch IJ, Pires JC, eds. 2004. *Biological Relevance of Polyploidy: Ecology to Genomics*. *Biological Journal of the Linnean Society 82:4 (Special issue).*

Levy AA, Feldman M. 2004. Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biological Journal of the Linnean Society* **82**(4): 607-613.

Lynch M, Conery JS. 2000. The evolutionary fate and consequences of duplicate genes. *Science* **290**(5494): 1151-1155.

Lynch M, Force A. 2000. The probability of duplicate gene preservation by subfunctionalization. *Genetics* **154**(1): 459-473.

Matyasek R, Tate JA, Lim YK, Srubarova H, Koh J, Leitch AR, Soltis DE, Soltis PS, Kovarik A. 2007. Concerted evolution of rDNA in recently formed tragopogon allotetraploids is typically associated with an inverse correlation between gene copy number and expression. *Genetics* **176**(4): 2509-2519.

Mochida K, Yamazaki Y, Ogihara Y. 2004. Discrimination of homoeologous gene expression in hexaploid wheat by SNP analysis of contigs grouped from a large number of expressed sequence tags. *Molecular Genetics and Genomics* **270**(5): 371-377.

Ohno S. 1970. *Evolution by Gene Duplication*. New York: Springer-Verlag.

Osborn TC, Chris Pires J, Birchler JA, Auger DL, Jeffery Chen Z, Lee H-S, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* **19**(3): 141-147.

Ownbey M. 1950. Natural hybridization and amphiploidy in the genus *Tragopogon*. *American Journal of Botany* **37**(7): 487-499.

Paterson AH, Chapman BA, Kissinger JC, Bowers JE, Feltus FA, Estill JC. 2006. Many gene and domain families have convergent fates following independent whole-genome duplication events in *Arabidopsis*, *Oryza*, *Saccharomyces* and *Tetraodon*. *Trends in Genetics* **22**(11): 597-602.

Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. *New Phytologist* **168**(1): 81-91.

Rodin SN, Riggs AD. 2003. Epigenetic silencing may aid evolution by gene duplication. *Journal of Molecular Evolution* **56**(6): 718-729.

Semon M, Wolfe KH. 2008. Preferential subfunctionalization of slow-evolving genes after allopolyploidization in *Xenopus laevis*. *Proceedings of the National Academy of Sciences, USA* **105**(24): 8333-8338.

Soltis DE, Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology & Evolution* **14**(9): 348-352.

Soltis DE, Soltis PS, Pires JC, Kovarik A, Tate JA, Mavrodiev E. 2004a. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biological Journal of the Linnean Society* **82**(4): 485-501.

Soltis DE, Soltis PS, Tate JA. 2004b. Advances in the study of polyploidy since Plant Speciation. *New Phytologist* **161**(1): 173-191.

Stebbins GL. 1950. *Variation and Evolution in Plants*. New York: Columbia University Press.

Tate J, Joshi P, Soltis K, Soltis P, Soltis D. 2009. On the road to diploidization? Homoeolog loss in independently formed populations of the allopolyploid *Tragopogon miscellus* (Asteraceae). *BMC Plant Biology* **9**(1): 80.

Tate JA, Ni ZF, Scheen AC, Koh J, Gilbert CA, Lefkowitz D, Chen ZJ, Soltis PS, Soltis DE. 2006. Evolution and expression of homeologous loci in *Tragopogon miscellus* (Asteraceae), a recent and reciprocally formed allopolyploid. *Genetics* **173**(3): 1599-1611.

Thomas BC, Pedersen B, Freeling M. 2006. Following tetraploidy in an *Arabidopsis* ancestor, genes were removed preferentially from one homeolog leaving clusters enriched in dose-sensitive genes. *Genome Research* **16**(7): 934-946.

Wang J, Tian L, Lee H-S, Wei NE, Jiang H, Watson B, Madlung A, Osborn TC, Doerge RW, Comai L, Chen ZJ. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. *Genetics* **172**(1): 507-517.

Wang JL, Tian L, Madlung A, Lee HS, Chen M, Lee JJ, Watson B, Kagochi T, Comai L, Chen ZJ. 2004. Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. *Genetics* **167**(4): 1961-1973.

Wendel JF. 2000. Genome evolution in polyploids. *Plant Molecular Biology* **42**(1): 225-249.

Xu Y, Zhong L, Wu X, Fang X, Wang J. 2009. Rapid alterations of gene expression and cytosine methylation in newly synthesized *Brassica napus* allopolyploids. *Planta* **229**(3): 471-483.

Table 1 List of plants used

T. dubius
2613-35, -21 (Pullman, WA)
T. porrifolius
2611-2 (Pullman, WA)
2626-4 (Palouse WA)
T. mirus
2601-45, -47, -7, -5, -2, -14 (Pullman, WA)
2602-1, -2, -25, -4 (Palouse, WA)

Table 2 Homeologous genes investigated by CAPS analysis

Name	Ortholog in *Arabidopsis thaliana* (expect value in parentheses)	Primer sequences	Diagnostic restriction enzyme	Fragment sizes (bp)
LEC3	No ortholog found (E<0.05)	F: GAGCTCACCAAGATTAGTGG R: ATACAGACGAGTTGGCACAC	BsmAI	Td: 133/198 Tp: 67/130/131
GTPB	Rab GTPase homolog (3e-58)	F: CCTCTCTCTACAATTCCGGC R: TACGGCGACTGATGTCGTAA	AciI	Td: 2/10/33/44/85/141 Tp: 2/10/33/44/229
Cry1	No ortholog found (E<0.05)	F: CTAAAACTCGTCCCACTAGAAG R: GGAATGGAAGAAGGACTCGG	ApoI	Td: 362 Tp: 69/293
nrDNA	Nuclear ribosomal RNA (2e-52)	F: GCATCGATGAAGAACGCAGC R: CTTTTCCTCCGCTTATTGATATGC	HincII	Td: 164/241 Tp: 405
G3PDH	Glyceraldehyde-3-phosphate dehydrogenase (3e-76)	F: CAACTGCCTTGCTCCTTTAG R: AACACGTGAACTGTAACCCC	$Taq^{\alpha}1$	Td: 54/88/369 Tp: 10/44/88/369
RGA	Gibberelic Acid Insensitive transcription factor (1e-24)	F: CGTCCACGTCGTTGATTTCA R: GATTCGATTCCTGTTCCACG	PleI	Td: 404 Tp: 190/214
THIOR	Thioredoxin M-type (1e-04)	F: AATCAGAAGCATCCCGACTG R: CACAATCTTTTTGTGAAATGCAA	DdeI	Td: 72/87 Tp: 160
BFRUCT4	beta-fructofuranosidase (2e-10)	F: GGAAGACCTTGATTGATCGG R: AAGGATGTTGTGGTGGAAGC	MwoI	Td: 121/182 Tp: 22/121/160
HS	Heat-shock protein (5e-100)	F: GATAGTCTACCCTTGTCGTTAG R: GCACTTAATGTGCATGTGATCC	DdeI	Td: 15/33/55/92/147/163 Tp: 55/92/163/195
TDRC	Transducin family protein (5e-06)	F: GGAAAGAAAGCCGAGGGTAA R: CATGTTTTCATGAACATCGAGC	NlaIII	Td: 8/51/54/88 Tp: 8/51/142
AP2	Ascorbate peroxidase 2 (2e-14)	F: TCTTCCTGATGCAACCAAGG R: GAAAGATAATGCAGCACCCC	MboII	Td: 172/76/90/71 Tp: 160/17/166/71
PA	Putative Peroxidase (1e-04)	F: GGTAGTGCTGCATTTGCATG R: TCTTGTTGCGAATGAGACCC	BstNI	Td: 455 Tp: 195/262
MD	Peroxisomal NAD-malate dehydrogenase (1e-61)	F: TTACAGGGATGGACCTTGTG R: ACTTAACTGCTGCATACGCC	SpeI	Td: 505 Tp: 138/368

Table 3 Patterns of homeolog expression in tissues of natural allopolyploid *T. mirus* plants compared for 11 genes showing expression in all diploid tissues tested, versus two genes that showed tissue-specific silencing in diploids.

	Genes expressed in all tissues of diploids (11 genes)	Genes showing tissue-specific silencing in diploids (2 genes)	Total (13 genes)
Number of assays	770	140	910
Both homeologs expressed	69.4 %	30.0 %	63.3 %
Neither homeolog expressed	2.6 %	30.7 %	6.9 %
T. dubius homeolog silent in whole plant	5.5 %	0.0 %	4.6 %
T. porrifolius homeolog silent in whole plant	17.9 %	4.3 %	15.8 %
T. dubius homeolog tissue-specific silencing	1.4 %	5.0 %	2.0 %
T. porrifolius homeolog tissue-specific silencing	1.8 %	28.6 %	5.9 %
Failed assays	1.4 %	1.4 %	1.4 %

Figure 1 Expression of 13 homeologous gene pairs in 7 tissues of 10 *Tragopogon mirus* plants grown in a common greenhouse from seed sourced from two natural populations of independent origin.