RESEARCH ARTICLE

Tragopogon dubius: Multiple introductions to North America and the formation of the New World tetraploids

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DOI https://doi.org/10.1002/tax.12743

Abstract *Tragopogon* (Asteraceae) is an evolutionary model for the study of whole-genome duplication, with two recently and repeatedly formed allopolyploids, *T. mirus* and *T. miscellus*, and many additional polyploid species. *Tragopogon mirus* and *T. miscellus* formed in western North America following the introduction of three diploids from Europe: *T. dubius*, *T. porrifolius*, and *T. pratensis*. Of these diploids, *T. dubius* is a shared parent of both tetraploids and is broadly defined and widely distributed in Eurasia. Because human-mediated intercontinental introductions may lead to hybridization with local species, and associated polyploidization, the introduction history of *T. dubius* from Europe to North America provides further opportunity to investigate both the extent and consequences of plant introductions. Using ITS sequence data, we show that the morphologically diverse, broadly defined *T. dubius* comprises a complex of at least 10 different ITS types in its native range, six of which have been introduced from Europe into North America. Significantly, although the two allotetraploid species have each formed multiple times on geographical scales from local to regional, recurrent formation is the result of repeated hybridization involving only one of these ITS subtypes. These results reinforce earlier data suggesting that not all diploid genotypes can form allopolyploids. Several entities traditionally considered part of *T. dubius* s.l. are now recognized as distinct species (e.g., *T. lainzii*), and it is likely that other distinct ITS genotypes identified here may also mark unique lineages that ultimately merit recognition as separate species. However, more intensive phylogenetic analyses involving many more samples from across the geographic range of *T. dubius* are required, as are detailed assessments of taxonomy, morphology, and cytology.

Keywords allopolyploidy; intercontinental introductions; recurrent polyploidy; *Tragopogon*

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

There has been long-standing interest in the invasion genetics of organisms (reviewed in Barrett, 2015; Dlugosch & al., 2015). Several polyploids are noteworthy in having formed in the last several hundred years due to the introduction of one or both parents from the native range into a new region, mediated by human activity including native habitat alteration. These include *Tragopogon mirus* and *T. miscellus* (Soltis & al., 2012a), *Spartina anglica* (Ainouche & Gray, 2016), *Senecio cambrensis* (Hegarty & al., 2006, 2012), and *Cardamine schulzii* (Mandáková & al., 2013). As a result, knowledge of the introduction history of the parents of recently formed polyploids can provide important information crucial for a better understanding of polyploidization.

Tragopogon (Asteraceae) has emerged as an important evolutionary model for the study of polyploidy (whole-genome duplication; WGD), with well-studied examples of recent and recurrent allopolyploid speciation. Ownbey (1950) first discovered and described two new species of *Tragopogon*, *T. mirus* and *T. miscellus*, from the Palouse region of North America (eastern Washington and adjacent Idaho, U.S.A.) and determined that these species were allotetraploids: the diploid parents of *T. mirus* are *T. dubius* and *T. porrifolius*, and those of *T. miscellus* are *T. dubius* and *T. pratensis*. Thus, the two polyploids share one diploid parent, *T. dubius*. All three diploid species are widespread in Europe and some parts of Asia, but are isolated ecologically and geographically.

Ownbey (1950) first observed the two new allotetraploid species, *Tragopogon mirus* and *T. miscellus*, in 1949, and described the few polyploid populations (each consisting of fewer than 100 individuals) as "small and precarious", noting that they had "attained a degree of success" and that they appeared to be "competing successfully" with their diploid parents. Herbarium records for *T. dubius* (the shared diploid parent) in regions where the tetraploids have formed date back to 1926; even if there was a short lag following the introduction of *T. dubius* until a specimen was collected and deposited, it is

Article history: Received: 22 Sep 2021 | returned for (first) revision: 3 Nov 2021 | (last) revision received: 8 Mar 2022 | accepted: 9 Mar 2022
Associate Editor: Alfonso Susanna |

likely that the allotetraploid species could not have formed prior to approximately 1930, or ~90 years ago (~45 generations in these biennials; Soltis & al., 2004, 2012a).

Subsequent molecular studies not only confirmed the parentage of *Tragopogon mirus* and *T. miscellus*, but also showed that each allotetraploid formed repeatedly in the introduced range (e.g., Soltis & al., 2004, 2012a; Buggs & al., 2012). The tetraploids have not formed in Europe due largely to ecological and geographic isolation of the parents. Based on microsatellite markers and other lines of DNA evidence, there may be as many as 21 lineages of separate origin of *T. miscellus* and 11 of *T. mirus* just in the Palouse of Washington and Idaho (Soltis & al., 1995, 2004, 2008, 2012a; Symonds & al., 2010). The polyploids have also formed independently in Oregon, Arizona, Wyoming, and Montana (Ownbey & McCollum, 1954; Brown & Schaack, 1972; Soltis & al., 2012a,b). Multiple origins in some cases occurred on a very small geographic scale—polyploid populations in different parts of a single small city separated by only 1–2 kilometers originated independently (Soltis & Soltis, 1989; Symonds & al., 2010). Furthermore, although multiple microsatellite genotypes for the diploid parents occur throughout the Palouse region, only three multilocus genotypes corresponding to the *T. dubius* subgenome were detected in allopolyploid samples from throughout the range of the two recently formed polyploids (Symonds & al., 2010).

Given the degree of genetic differences among some of the genotypes of each diploid species, it is likely that there were multiple introductions of each species into the Palouse region alone (Symonds & al., 2010). This hypothesis is reasonable in that all three diploids are widespread weeds across North America (particularly *Tragopogon dubius* and *T. pratensis*) (Soltis & al., 2004, 2012a; Soltis, 2006) and likely were introduced multiple times by different groups of Europeans as they moved to and across North America.

Clarifying the introduction history of plants such as *Tragopogon* is crucial to obtaining a better understanding of polyploid formation in these plants and other polyploids formed via introduction from native to a new range. For example, are all genotypes of the introduced parental species equally likely to form polyploids? Are there regional differences of parental introduction that lead to genetically distinct polyploid subtypes? To understand introduction history from native to new range, it is critical to have a clear concept of what constitutes the native range in terms of component genotypes and their distribution. However, broad surveys of patterns of genetic variation across the extensive home ranges of *T. dubius, T. porrifolius*, and *T. pratensis* have not been conducted to date.

Tragopogon is native to Eurasia and comprises approximately 150 species. It has long been considered taxonomically complex; the variation within species and the strong similarities among species, as well as frequent hybridization and polyploidy, have resulted in extensive taxonomic confusion (reviewed in Mavrodiev & al., 2005, 2007, 2012).

Within its native range, *Tragopogon dubius* is one of the most geographically widespread species of *Tragopogon* (Alexeev & Mavrodiev, 2019), and it is morphologically highly variable, leading to a profusion of taxonomic names. At least 14 binomials are associated with the name *Tragopogon dubius* (Mavrodiev & al., unpub.), but, extending across much of Eurasia, *T. dubius* today is typically restricted to those collections that are yellow-flowered and have swollen peduncles. Suárez-Santiago & al. (2011) also stressed that the true extent of intraspecific morphological variation has long been underestimated in *T. dubius*. However, today no infraspecific taxonomic units are accepted within *T. dubius*. For example, *T. major* has sometimes been considered a distinct species, but also is frequently considered a synonym of *T. dubius* (reviewed in Montgomery, 1953; see also Richardson, 1976 and Soltis, 2006).

Phylogenetic analyses of DNA sequence data also demonstrate that *Tragopogon dubius* is highly diverse across its geographic distribution in Eurasia and potentially not monophyletic (Mavrodiev & al., 2008, 2012; Suárez-Santiago & al., 2011). For example, collections of what has been considered *T. dubius* from India are genetically, morphologically, and phylogenetically distinct from European populations of *T. dubius* (Mavrodiev & al., 2008), and a new species of *Tragopogon* from the Iberian Peninsula, *T. lainzii*, was segregated from *T. dubius* (Suárez-Santiago & al., 2011). Even with recent changes to the circumscription of *T. dubius*, it is likely that it remains non-monophyletic. Efforts to track the introduction history of *T. dubius* and determine the consequences of multiple introductions for allotetraploid formation with other introduced species of *Tragopogon* in North America will need to account for the extensive variation of *T. dubius* in its native range, as well as its likely polyphyly.

To track the introduction history of *Tragopogon dubius* into North America, we used DNA sequence variation for a broad sampling of herbarium specimens of *T. dubius* from Europe and North America and also included data from other species from a previous broad phylogenetic analysis of *Tragopogon* (Mavrodiev & al., 2005) and asked the following questions: (1) is *T. dubius* monophyletic, and if not, which lineages have been introduced into North America?; (2) how many introductions of *T. dubius* are detectable from Europe to North America?; (3) which genotypes of *T. dubius* contributed to the formation of the New World allopolyploids?

■ MATERIALS AND METHODS

Samples used. — We followed the circumscription of *Tragopogon dubius* as given in Ownbey (1950), Montgomery (1953), and *Flora Europaea* (Richardson, 1976) and employed the phylogenetic context of Mavrodiev & al. (2005, 2012). We began with a total of 182 ITS sequences; removing identical sequences resulted in a dataset of 72 sequences. To assess the monophyly of *T. dubius*, we used these 72 ITS sequences from 57 species of *Tragopogon* that comprehensively represent all the major clades of the genus (Mavrodiev & al., 2005), namely clades Angustissimi, Brevirostres, Tragopogon, Collini, the broadly defined Majores clade (including the Chromopappus and Hebecarpus clades, as defined in Mavrodiev & al., 2005), and also the Profundisulcati clade (Mavrodiev & al., 2005). One ITS sequence of *Podospermum jacquinianum* was used as the outgroup (based on Mavrodiev & al., 2004). Thus, the total number of sequence terminals in the final matrix was 73 (72 representing *Tragopogon* plus the single outgroup, *Podospermum*).

We included sequence data for five species previously described as closely related to, or part of *Tragopogon dubius*, namely *T. campestris* (one accession; see below), *T. capitatus* (three accessions), *T. kemulariae* (three accessions), *T. macropogon* (one accession), and *T. pseudomajor* (three accessions). Three of these species (*T. capitatus*, *T. kemulariae*, *T. pseudomajor*) were each non-monophyletic in our previous study (Mavrodiev & al., 2012). We therefore included more than one published ITS sequence (Mavrodiev & al., 2005, 2012) of each of these species in our current analyses to better assess their circumscription and relationships. The ITS sequence of *T. campestris* was generated for the first time as part of the current study using collections made by Besser (1822), the author of the species. The locations of vouchers and geographic distribution of all analyzed specimens are included in Appendix 1 (and as a csv file in suppl. Table S1).

Of the 182 sequences initially examined, 113 sequences represent *Tragopogon dubius*. Of these 113 sequences, 19 sequences were downloaded from GenBank, 73 sequences were generated as part of this study, and 21 sequences were generated previously (Soltis & al., 2012a) (Appendix 1; suppl. Table S1). Due to clear patterns in sequence variation and the fact that many sequences were identical (see below), not all of these 113 sequences of *T. dubius* were included in phylogenetic analyses. We identified 10 ITS ribotypes (sequence patterns) (Fig. 1, Appendix 1; suppl. Table S1) and included one sequence of each of these 10 genotypes (taken from Mavrodiev & al., 2005) as a placeholder in the phylogenetic analyses. Thus, the final ITS matrix includes: (a) placeholders of the 10 inferred genotypes of *T. dubius* (Fig. 1); (b) three previously generated sequences of *T. capitatus* from Mavrodiev & al. (2005, 2012); (c) three previously generated sequences of *T. kemulariae* from Mavrodiev & al. (2005, 2012); (d) three previously generated sequences of *T. pseudomajor* from Mavrodiev & al. (2012); (e) one previously generated sequence of *T. macropogon* (Mavrodiev & al., 2012); (f) one previously generated sequence of *T. campestris* (Mavrodiev & al., 2012); (g) one previously generated sequence of *T. lainzii* (Suárez-Santiago & al., 2011); (h) one previously generated sequence of *T. major* (Mavrodiev & al., 2005); (i) 49 previously generated ITS sequences of 49 species of *Tragopogon* that represent all of the major phylogenetic relationships within the genus (Mavrodiev & al., 2005, 2012); and (j) one previously generated sequence of *Podospermum jacquinianum* (outgroup) (Mavrodiev & al., 2004).

To include the direct parental *Tragopogon dubius* contribution to the recently formed polyploids, we also analyzed four "dubius-like" clones from the allopolyploids *T. mirus* and *T. miscellus*. Numerous clones had been generated earlier for the polyploids. Many clones were identical; these four sequences were chosen as being representative of the vast majority of cloned sequences given the narrow pool of variation in the polyploids (see also Kovarik & al., 2005). Considering these four clones, two clones were downloaded from GenBank (one representing the *T. dubius* contribution to *T. miscellus* from Moscow, Idaho, U.S.A., and the other the *T. dubius* contribution to *T. mirus*, from Palouse, Washington, U.S.A.). Two clones were sequenced during the study of Soltis & al. (2012b; *T. mirus* from Corvallis, Oregon, U.S.A., and Flagstaff, Arizona, U.S.A.) (Appendix 1; suppl. Table S1).

ITS amplification, sequencing, and alignment. — Methods of DNA extraction, as well as amplification and Sanger sequencing of the ITS region, follow Mavrodiev & al. (2012). The ITS region was amplified and sequenced using the same pair of primers employed in that study: ITS_LEU1 (GTCCACTGAACCTTATCATTTAG) (designed by B. Baldwin and published in Ballard & al., 1998) and ITS-4 (White & al., 1990).

As in Mavrodiev & al. (2012), the 72 ITS accessions of *Tragopogon* plus *Podospermum* (outgroup) selected for phylogenetic analysis were aligned using MAFFT v.7 following the L-INS-i alignment strategy (Katoh & al., 2002; Katoh & Standley, 2013), with default settings. Minor manual corrections of the MAFFT alignment (suppl. Appendix S1) were then made using Mesquite v.3.01 (Maddison & Maddison, 2021).

Parsimony-informative characters of the molecular alignment were extracted by PAUP* v.4.0a (Swofford, 2002). Molecular patterns of *Tragopogon dubius* were identified by simple manual comparison of the sets of parsimony-informative characters of all the included accessions of this species (Fig. 1). This comparison was performed using Mesquite v.3.01 (Maddison & Maddison, 2021).

Phylogenetic analyses of ITS sequence data. — The K80 + Γ model was selected by PhyML-SMS as the best choice for maximum likelihood (ML) analysis based on both Bayesian and Akaike information criteria. The ML analysis was conducted with PhyML v.3.0 (Guindon & al., 2010) following the automatic Smart Model Selection (SMS) option (Lefort & al., 2017). A BioNJ tree was used as a starting tree, and the strategy of tree improvement was specified as "Subtree-Pruning-Regrafting" (SPR) (Swofford, 2002; Guindon & al., 2010). The number of random starting trees was 10. As recommended by Guindon & al. (2010), branch support was calculated with the approximate likelihood-ratio test (aLRT) (reviewed in Guindon & al., 2010) instead of the ML bootstrap. We rooted the ML tree with *Podospermum jacquinianum* W.D.J.Koch, based on the results of Mavrodiev & al. (2004).

We will focus on the PhyML tree (above), but because RAxML is considered superior to PhyML and comparable to most other fast ML methods (Zhou & al., 2018), we also conducted analyses as implemented in RAxML v.8.00 (Stamatakis, 2014). However, RAxML does not have the chosen model, but because K80 + Γ is a special case of GTR + Γ, we used the latter. For RAxML, bootstrap analyses (Felsenstein, 1973) were conducted with 2500 replicates. Finally, we also ran PhyML using GTR + Γ to assess whether topological differences were due to the model used or the program. In all analyses, trees were visualized using FigTree v.1.4.2 (Rambaut & Drummond, 2012).

■ RESULTS

Patterns of variation of ITS sequence data in *Tragopogon dubius*. — The total number of characters in the final ITS alignment was 619, consisting of 427 invariant characters (proportion = 0.690) and 125 variable characters. Manually analyzing this matrix, we used variation at 24 nucleotide positions to identify 10 distinct sequence patterns that consistently differ from each other by 1–13 single nucleotide polymorphisms (SNPs) (Fig. 1). We use roman numerals for each of these major patterns (I–X) and also provide slight variants of the sequences from the same major ITS type; these minor variants differ from each other by one or a few double-peaks in the chromatograms. It is unclear what these double-peaks represent, either heterozygotes or multiple ITS types; both are possibilities (e.g., Álvarez & Wendel, 2003; Soltis & al., 2008).

Patterns VII (Canada), VIII (Kashmir, India) and IX (Germany), each contain only 1 sequence. Pattern I contains 6 sequences, pattern II – 6 sequences, pattern III – 16 sequences (plus one ITS sequence from the collections of what is now known as *T. lainzii*), pattern IV – 19 sequences, pattern V – 6 sequences, pattern VI – 51 sequences, and pattern X – 5 sequences (plus one ITS sequence derived from material of *T. campestris* collected by Besser in 1822) (Fig. 1). Patterns IV and VI are by far the most common.

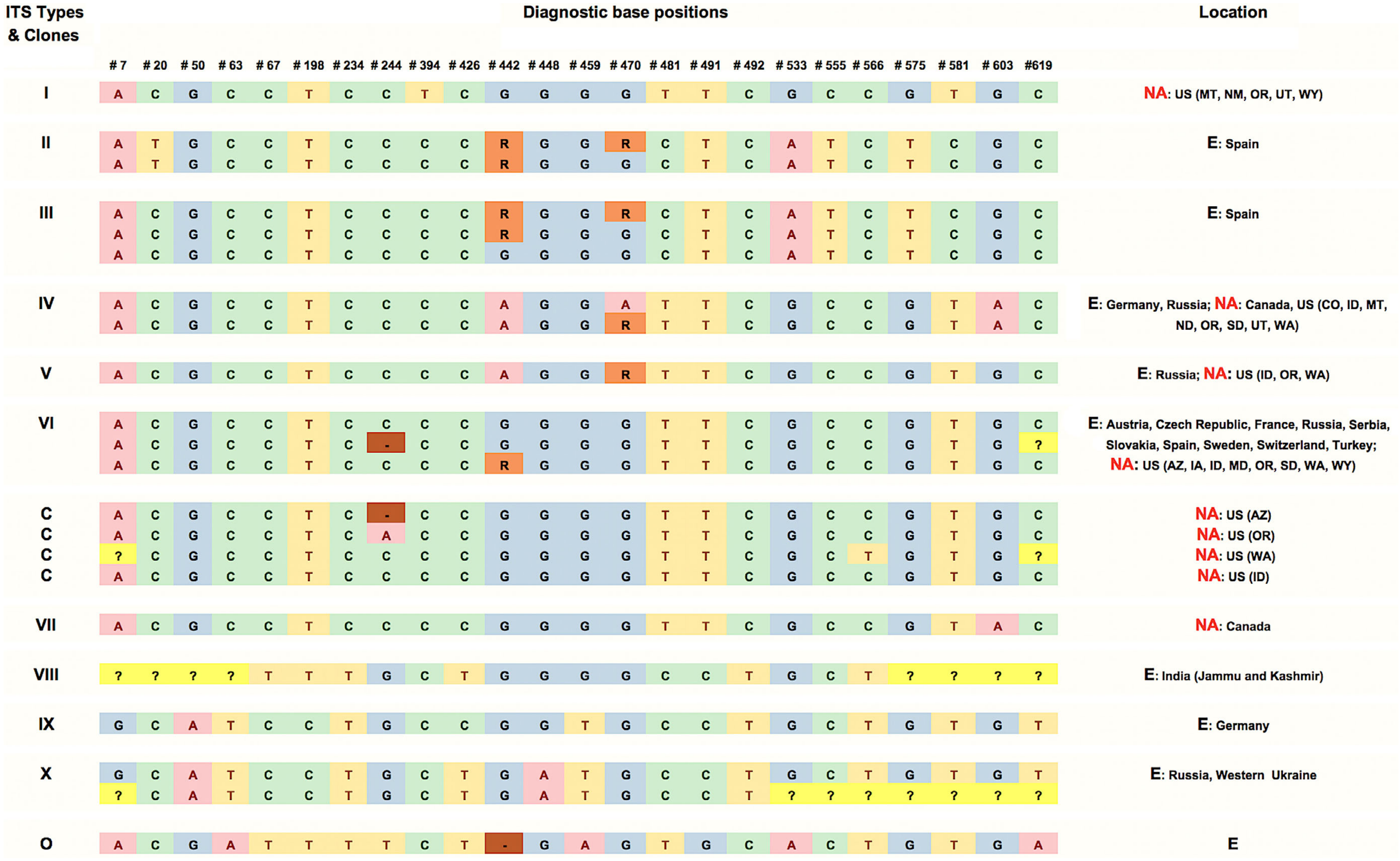
In terms of geographic distributions of these patterns (Figs. 1, 2), pattern I includes ITS sequences derived from individuals collected solely in the United States (in Montana, New Mexico, Oregon, Utah, and Wyoming). Patterns II and III include ITS sequences derived solely from plants from Spain; sequences from *T. lainzii* from Spain represent pattern III. Pattern IV includes sequences collected in Germany and Russia; these are also found in Canada and the U.S.A. Within Canada, plants with pattern IV have been collected from Vancouver Island and other parts of British Columbia in western Canada, as well as near Ontario in eastern Canada. In the U.S.A., pattern IV was detected in Oregon, Montana, Colorado, Utah, North Dakota, South Dakota, as well as Idaho and Washington. Pattern V contains ITS sequences from plants that have been collected in eastern Europe (Russia, Ryazan region, Kasimovsky District) and the U.S.A. (Oregon, Idaho, Washington). Pattern VI contains ITS sequences that are widely distributed in Europe, including Austria, Czech Republic, France, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, and Serbia (Figs. 1, 2), as well as from various parts of the western U.S.A. (Arizona, Idaho, Montana, South Dakota, Washington, Wyoming). The sequence representing *T. dubius* that was published in the first ITS phylogeny of the genus (collection *M. Ownbey, T-105-0*, June 30, 1956, WS274197; the Palouse area of Washington, U.S.A.) is of this pattern (see Mavrodiev & al., 2005). Pattern VII includes a single sequence that was obtained from a plant collected in Canada (Alberta). Pattern VIII also includes a single sequence, derived from a plant of *T. dubius* collected in Kashmir (Srinagar, Jammu and Kashmir, India). Pattern IX is the single sequence obtained from a plant from Germany. Finally, Pattern X contains the ITS sequences derived from two accessions from Russia, as well as from plants growing in Wyoming, U.S.A.; the ITS sequence of *T. campestris* also is pattern X (Figs. 1, 2). Patterns IV and VI are not only the most common, but are also distributed most widely (Figs. 1, 2). Some of the patterns are not only very similar (Fig. 1) but are also closely related to each other based on our phylogenetic analyses.

We focus on our PhyML K80 + Γ results. However, the PhyML K80 + Γ, PhyML GTR + Γ, and RAxML analyses all yielded very similar topologies with identical implications for the introduction history of *T. dubius* (Fig. 3; suppl. Figs. S1, S2). Interestingly, the PhyML GTR + Γ and RAxML topologies were not identical, indicating that differences were due to the program, not the model.

Seven Patterns (I, II, III, IV, V, VI, VII) are members of the Majores clade. Five of these (patterns I, IV, V, VI, VII) are part of one well-supported subclade of Majores with patterns V and IV sisters. Spanish patterns II and III form a clade with *T. lainzii* that is nested within a separate subclade of the Majores clade. Patterns VIII, IX, and X are all nested within the Tragopogon clade (see below; Fig. 3).

Introduction into North America and formation of *Tragopogon mirus* and *T. miscellus*. — The ITS sequences that have been obtained from the plants of *Tragopogon dubius* collected in the U.S.A. belong to patterns I, IV, V, VI, VII, and X (Appendix 1; suppl. Table S1; see also Figs. 1, 2). Thus, we have evidence for at least six introductions of '*T. dubius*' (as currently treated and circumscribed) to North America. Patterns IV, V, VI, and X were found in both the home range (Eurasia) and North America, but patterns I and VII were only detected in North America, indicating that our sampling of Eurasia was not broad enough to detect their area of origin or that they originated in North America.

Significantly, although there have been multiple introductions of what has been called *Tragopogon dubius* into the U.S.A., our data also suggest that only one of these patterns (namely, pattern VI) matches the parental ITS genotypes that gave rise to the repeated origins of the recently formed allopolyploids *Tragopogon mirus* and *T. miscellus* (Fig. 1). As noted in Soltis & al. (2012b), based on one change (at site 277 of the alignment of the ITS locus in that study), the recently discovered population of *T. mirus* from Oregon likely originated separately; it is distinct not only from the Arizona



ITS Types & Clones	# 7	# 20	# 50	# 63	# 67	# 198	# 234	# 244	# 394	# 426	# 442	# 448	# 459	# 470	# 481	# 491	# 492	# 533	# 555	# 566	# 575	# 581	# 603	#619	Location
I	A	C	G	C	C	T	C	C	T	C	G	G	G	G	T	T	C	G	C	C	G	T	G	C	NA: US (MT, NM, OR, UT, WY)
II	A	T	G	C	C	T	C	C	C	C	R	G	G	R	C	T	C	A	T	C	T	C	G	C	E: Spain
	A	T	G	C	C	T	C	C	C	C	R	G	G	G	C	T	C	A	T	C	T	C	G	C	
III	A	C	G	C	C	T	C	C	C	C	R	G	G	R	C	T	C	A	T	C	T	C	G	C	E: Spain
	A	C	G	C	C	T	C	C	C	C	R	G	G	G	C	T	C	A	T	C	T	C	G	C	
	A	C	G	C	C	T	C	C	C	C	G	G	G	G	C	T	C	A	T	C	T	C	G	C	
IV	A	C	G	C	C	T	C	C	C	C	A	G	G	A	T	T	C	G	C	C	G	T	A	C	E: Germany, Russia; NA: Canada, US (CO, ID, MT, ND, OR, SD, UT, WA)
	A	C	G	C	C	T	C	C	C	C	A	G	G	R	T	T	C	G	C	C	G	T	A	C	
V	A	C	G	C	C	T	C	C	C	C	A	G	G	R	T	T	C	G	C	C	G	T	G	C	E: Russia; NA: US (ID, OR, WA)
VI	A	C	G	C	C	T	C	C	C	C	G	G	G	G	T	T	C	G	C	C	G	T	G	C	E: Austria, Czech Republic, France, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Turkey; NA: US (AZ, IA, ID, MD, OR, SD, WA, WY)
	A	C	G	C	C	T	C	-	C	C	G	G	G	G	T	T	C	G	C	C	G	T	G	?	
	A	C	G	C	C	T	C	C	C	C	R	G	G	G	T	T	C	G	C	C	G	T	G	C	
C	A	C	G	C	C	T	C	-	C	C	G	G	G	G	T	T	C	G	C	C	G	T	G	C	NA: US (AZ)
C	A	C	G	C	C	T	C	A	C	C	G	G	G	G	T	T	C	G	C	C	G	T	G	C	NA: US (OR)
C	?	C	G	C	C	T	C	C	C	C	G	G	G	G	T	T	C	G	C	T	G	T	G	?	NA: US (WA)
C	A	C	G	C	C	T	C	C	C	C	G	G	G	G	T	T	C	G	C	C	G	T	G	C	NA: US (ID)
VII	A	C	G	C	C	T	C	C	C	C	G	G	G	G	T	T	C	G	C	C	G	T	A	C	NA: Canada
VIII	?	?	?	?	T	T	T	G	C	T	G	G	G	G	C	C	T	G	C	T	?	?	?	?	E: India (Jammu and Kashmir)
IX	G	C	A	T	C	C	T	G	C	C	G	G	T	G	C	C	T	G	C	T	G	T	G	T	E: Germany
X	G	C	A	T	C	C	T	G	C	T	G	A	T	G	C	C	T	G	C	T	G	T	G	T	E: Russia, Western Ukraine
	?	C	A	T	C	C	T	G	C	T	G	A	T	G	C	C	T	?	?	?	?	?	?	?	
O	A	C	G	A	T	T	T	T	C	T	-	G	A	G	T	G	C	A	C	T	G	T	G	A	E

Fig. 1. Ten patterns of variation in the ITS sequence data of multiple accessions of *Tragopogon dubius* and *Podospermum* (the outgroup). # at top refers to base position on the ITS sequence alignment; ? refers to uncertain nucleotides located near the end of the sequence read. Under ITS Types & Clones, roman numerals refer to the 10 ribotypes noted in the text, C = *T. dubius* clones from the two polyploids, and O = outgroup. Under Location, E = Eurasia and NA = North America, abbreviations are used for states in the United States.

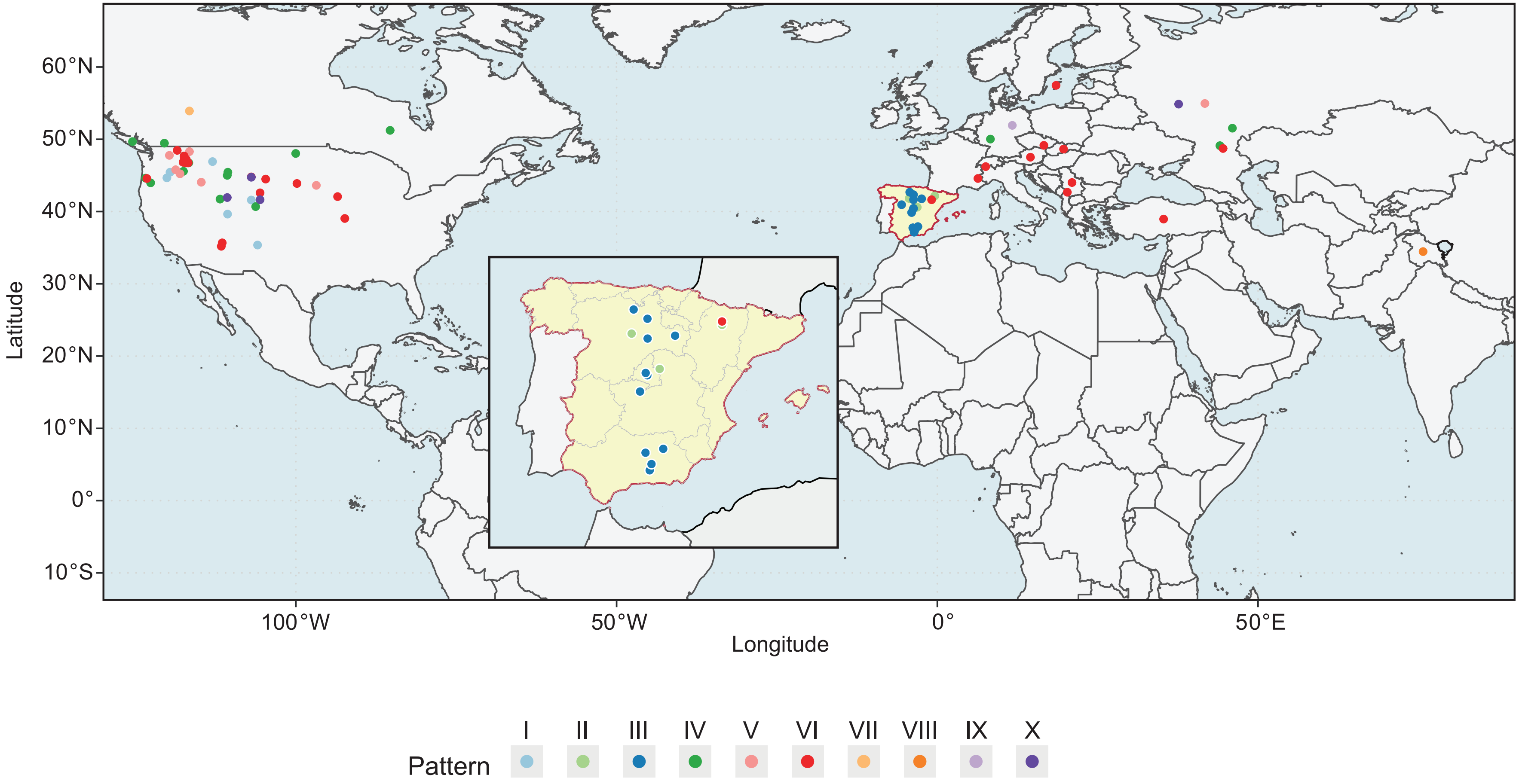


Fig. 2. Geographic distributions of ITS ribotypes estimated for *Tragopogon*. Ten ribotypes (patterns I–X) are shown as colored circles at 82 unique sampling localities, with patterns and localities within Spain highlighted as an inset. Mapped coordinates represent centroids of administrative areas identified from descriptive locality data (Appendix 1; suppl. Table S1).

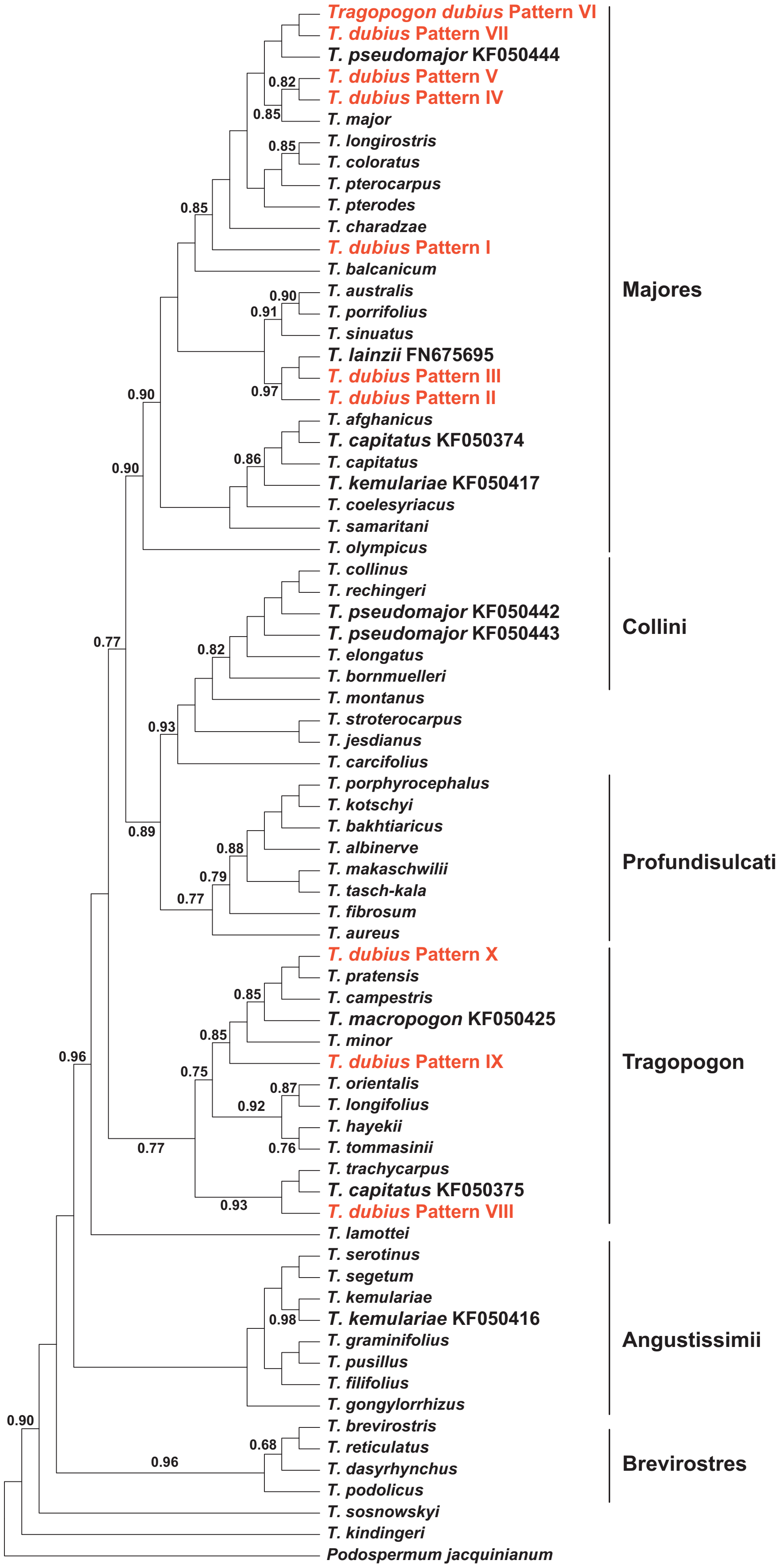


Fig. 3. The best tree (–log likelihood: –2431.218389) recovered from the ML analysis (PhyML with K80 + Γ) of the ITS sequence alignment of species of *Tragopogon* and outgroup (*Podospermum*). Numbers above or below branches indicate support values above 0.50 calculated with the approximate likelihood-ratio test (aLRT). Tree was a posteriori rooted relative to *Podospermum*. Clade names are to the right of vertical black lines. Patterns I–X of *T. dubius* are given in red and larger font. Species formerly considered part of *T. dubius* Scop. are given in larger black font and followed by GenBank numbers. Most of these species are non-monophyletic.

population, but also from populations of *T. mirus* analyzed previously from Washington. Soltis & al. (2012a,b) detected a double peak (G + A) in the Oregon sample at this position. This combination is not seen at that sequence position in any other population of *T. mirus* (Soltis & al., 2012b): all plants of *T. dubius* sampled for ITS have a C at this position, and all samples of *T. porrifolius* examined have a G (Soltis & al., 2012a,b). Our analyses indicate that the parental samples of *T. dubius* present in *T. mirus* from Oregon that were not included in Soltis & al. (2012b) while clearly pattern VI, must have an automorphy (A at position 277) to explain the G + A in the Oregon material. In other words, the sequence of the *T. dubius*-like clone of *T. mirus* from Oregon is identical to pattern VI, except for the A (site 277). Soltis & al. (2012b) suggested that the exact parental *T. dubius* genotype contributing to *T. mirus* from Oregon had still not been sampled. However, alternatively, the *T. mirus* sample from Oregon may have experienced a rapid base substitution at site 277 soon after polyploidization (Soltis & al., 2012b).

Our current comparison of the obtained dubius-like clone of *Tragopogon mirus* from Oregon (Fig. 1) with a much larger number of the ITS accessions of *T. dubius* than used in Soltis & al. (2012b) suggests that a point mutation in our alignment at site 244 (Fig. 1; which corresponds to site 277 in the alignment of Soltis & al., 2012b) is the best current explanation of the observed variation. With the exclusion of the unique A, the clone of *T. mirus* from Oregon is identical to pattern VI, which is the same pattern identified as a parent of all other polyploid populations and is distinct from all other patterns of *T. dubius*.

The presence of a unique deletion at the same nucleotide position (244) in our alignment of the dubius-like clone of *Tragopogon mirus* from Arizona and also of the ITS sequence of *T. dubius* that was also collected in Arizona (Fig. 1) also argues for the independent origin of the Arizonan population of *T. mirus* (Brown & Schaack, 1972; Soltis & al., 2012a,b) (see also Appendix 1; suppl. Table S1). All other samples of *T. dubius* analyzed (except the clone from *T. mirus* from Oregon noted above) have a C at site 244 (Fig. 1). Considering base substitutions, the sequence of the dubius-like clone of *T. mirus* from Arizona belongs to pattern VI (Fig. 1) but is a distinct variant of this pattern (Flagstaff, Arizona, *T. Ayers, 1748* (ASC); see also Soltis & al., 2012a,b) (Fig. 1), indicating a separate origin in Arizona (Brown & Schaack, 1972). A clone of *T. mirus* reported by Kovarik & al. (2005) is also part of pattern VI based on our analyses. A unique T at position 566 of this clone in our alignment (Fig. 1) is the clear result of a PCR-mediated recombination event (described in Kovarik & al., 2005) and does not reflect variation in nature.

Our newly obtained dubius-like clone of *Tragopogon miscellus* from Moscow, Idaho, as well as the previously published similar clone of the same species collected at the same locality (Kovarik & al., 2005) (Fig. 1), also matches pattern VI of *T. dubius*. In short, despite the presence of plants of *T. dubius* with four different ITS patterns (I, IV, V, VI) in Oregon, Arizona, Idaho, and Washington (Figs. 1, 2), only plants with one particular type of ITS sequence (pattern VI) have been involved in the repeated formation of the polyploids *T. mirus* and *T. miscellus*.

Defining *Tragopogon dubius*. — Our RAxML tree is sufficiently resolved to provide important insights into relationships (Fig. 3) (see also Mavrodiev & al., 2005, 2012). Based on our data, *Tragopogon dubius* as traditionally circumscribed is clearly non-monophyletic (Fig. 3; suppl. Figs. S1, S2). Samples of *T. dubius* occur in two phylogenetically distinct clades of *Tragopogon* (Majores clade; Tragopogon clade; see Mavrodiev & al., 2005) (Fig. 3), as reviewed in more detail below. However, no samples identified as *T. dubius* appear in the other subclades of *Tragopogon* (Angustissimi, Brevirostres, Collini, and Profundisulcati).

Based on our ML analysis, patterns I, IV, V, VI, and VII are all closely related to *Tragopogon charadzae*, *T. coloratus*, *T. longirostris*, *T. major*, *T. pseudomajor*, *T. pterocarpus*, and *T. pterodes* (Fig. 3). These patterns can be defined as part of the Majores clade as defined in Mavrodiev & al. (2005) (Fig. 3). The ITS trees are not well supported, so these differences must be viewed with that in mind. The ML analysis placed the clade (patterns IV, V) as sister to *T. major*.

Also in a separate subclade of the Majores clade are the collections from Spain. Materials from Spain are represented here by patterns II and III, and they form a clade together with *Tragopogon lainzii*, a segregate from *T. dubius* with pattern II (Fig. 3). Thus, in Spain, there are multiple distinct genetic entities: patterns II and III (along with *T. lainzii*) in one major subclade of the Majores clade and pattern VI from a different subclade of the Majores clade.

Three ITS patterns from *Tragopogon dubius* are recovered in the Tragopogon clade (patterns VIII, IX, X). The following collections form a clade that is well supported (Fig. 3) and well defined from a geographical standpoint: *T. capitatus* KF050375 (note that other collections of *T. capitatus* appear in the Majores clade) was collected near Almaty (southeastern Kazakhstan), *T. trachycarpus* is from the south of Eastern Siberia, and a collection of *T. dubius* of pattern VII was collected in Kashmir (Jammu and Kashmir, India). This clade is sister to the remainder of the Tragopogon clade (Mavrodiev & al., 2005) in the ML trees (Fig. 3).

Also appearing in the Tragopogon clade are samples with patterns IX and X. Pattern X forms a clade together with the Western Ukrainian *Tragopogon campestris*, *T. macropogon* from the Caucasus, and *T. pratensis* (Figs. 1, 3; suppl. Figs. S1, S2). Our analysis places *T. minor* and pattern IX as successive sisters to this subclade ((pattern X, *T. campestris*), (*T. macropogon*, *T. pratensis*)).

■ DISCUSSION

Introductions. — Our ITS sequence data from a broad sampling of populations together with previously published fine-scale microsatellite data for *Tragopogon dubius* from the Palouse region (Symonds & al., 2010) provide a picture of the invasion genetics of this species. As with other Eurasian species, there is evidence of multiple introductions from Eurasian source populations to North America (e.g., Meekins & al., 2001; Novak & Mack, 2001; Lee, 2002; Gaskin & al., 2005; Dlugosch & Parker, 2008; Platt & al., 2010; Novak, 2011; Lucardi & al., 2020). As with many other invasive species, these multiple introductions of *T. dubius* likely trace to the different human immigrant populations leaving Europe for North America from different regions at different times (Novak & Mack, 2001). This introduction was likely accidental, perhaps as part of contaminated grain. Importantly, previously published microsatellite data (Symonds & al., 2010) show clear evidence of admixture of distinct genotypes of *T. dubius* in eastern Washington and adjacent Idaho (Symonds & al., 2010). This pattern observed for *T. dubius* of repeated introductions from the home range and subsequent admixture in the new range follows a recurring pattern seen in other introductions of plants and animals on a worldwide basis (e.g., Lee, 2002; Facon & al., 2003, 2008; Kolbe & al., 2007; Dlugosch & Parker, 2008; Ward & al., 2008; Novak, 2011; Keller & al., 2014; Lucardi & al., 2020; Smith & al., 2020).

Polyploid formation: implications. — Broadly defined, *Tragopogn dubius* comprises morphologically similar, but molecularly different, entities (see below). It is essentially a complex of cryptic species that are currently circumscribed under the binomial '*Tragopogon dubius*'. This cryptic complexity of *T. dubius* becomes part of the explanation of the pattern of formation of the allopolyploids in North America. Regardless of the complexity surrounding *T. dubius*, it is clear that multiple ITS patterns—in some cases likely representing cryptic species—have indeed been introduced to areas of the U.S.A. where *T. mirus* and *T. miscellus* have formed: Arizona, Oregon, Idaho, and Washington. Significantly, however, only one (pattern VI; presumably corresponding to a narrowly defined *T. dubius*) contributed to the formation of the North American polyploids; the other genotypes we detected that were introduced into North America did not contribute to the formation of the polyploids. The minor variations of ITS pattern VI are clearly congruent with the independent formation of *T. mirus* and *T. miscellus* within the different states of the U.S.A., as confirmed using other types of molecular markers (e.g., Soltis & al., 2012a). That is, both *T. mirus* and *T. miscellus* have indeed formed multiple times on different geographical scales, from the local to regional (Soltis & al., 2012a), but that repeated formation is the result of repeated hybridization of only one subgroup of a broadly defined *T. dubius* (pattern VI).

These results based on a broad sampling of ITS sequences from across Eurasia and North America mirror those reported by Symonds & al. (2010) in a study of microsatellite markers on a small regional scale (the Palouse of Washington and Idaho). Symonds & al. (2010) detected several very distinct microsatellite types in samples of *Tragopogon dubius* from Washington and Idaho, suggesting multiple introductions to the area (but no comparisons were made to materials from Eurasia or other locations in North America). However, only a few microsatellite types of those detected actually were involved in the formation of the New World polyploids. Crossing studies aimed at resynthesizing the allotetraploids also support the hypothesis that not all diploid parental genotypes are capable of producing successful allopolyploids. Not all of the many diploid crosses made by Tate & al. (2009) when resynthesizing the polyploids were successful. Initially, we attributed these results to stochastic failure of crosses. We now propose that some combinations of diploid genotypes fail to produce polyploids—and that successful artificial crosses match the genotypes in nature that successfully formed the allopolyploids. We are now testing this hypothesis by reexamining all of the many samples of *T. dubius* used in the crossing studies and determining their ITS sequence type.

***Tragopogon dubius* circumscription. —** Two extreme views of the circumscription of *Tragopogon dubius* have been considered: (1) *T. dubius* is a broadly defined single species with *T. major* as the best-known synonym; this view of *T. dubius* still predominates among some modern botanists; (2) *T. dubius* is narrowly defined (as originally described, it corresponds only to the Italian and Slovenian plants); with this perspective, *T. major* as well as numerous other taxa that frequently have been circumscribed under the name *T. dubius* are separate species.

Our sequence data suggest a narrow delimitation of *Tragopogon dubius* and thus support the second option above (Figs. 1, 3). However, more samples from Europe require analysis to clearly delineate *T. dubius* in the narrow sense. Earlier DNA analyses revealed that *T. dubius* as well as several other of the geographically widespread species of *Tragopogon* (*T. porrifolius*, *T. pratensis*) comprise several distinct genotypes (e.g., Mavrodiev & al., 2007, 2008). A few of these entities are clearly distinct morphologically. Some populations from Spain traditionally considered part of *T. dubius* are now recognized as a distinct species (*T. lainzii*; Suárez-Santiago & al., 2011), but others have not been given recognition. Many of the other distinct genotypes we have identified here that are still considered part of '*T. dubius*' may ultimately merit recognition as distinct species. However, in most cases, there are no obvious morphological differences among specimens representing these genetically distinct lineages. Adding to the complexity, some of these lineages are only represented by a few collections. The current data suggest a complex situation with a number of cryptic species. There may be obscure morphological differences among these entities, and all certainly merit more detailed analysis. More intensive phylogenetic study using more markers, many more samples, coupled with detailed assessments of taxonomy, morphology, and cytology are all required to elucidate the tangle that is *T. dubius*.

■ AUTHOR CONTRIBUTIONS

DES, EVM, and PSS designed the research and conducted much of the research, discussion, and analyses. EVM obtained most of the

samples and did much of the lab work. MAG helped with analyses. GTG plotted collection distributions. YEA contributed plant material and discussion. DES wrote the initial manuscript; all authors contributed to its completion. — DES, https://orcid.org/0000-0001-8638-4137; EVM, https://orcid.org/0000-0002-9670-7662; MAG, https://orcid.org/0000-0002-7078-4336; GTG, https://orcid.org/0000-0003-2628-4729; PSS, https://orcid.org/0000-0001-9310-8659

■ ACKNOWLEDGMENTS

This work was supported in part by an OPUS grant from the National Science Foundation, DEB-2043478. We thank Ales Kovarik (Czech Academy of Sciences, Czech Republic), Gabriel Blanca (University of Granada, Spain), Pilar Catalan (University of Zaragoza, Spain), Alexey P. Laktionov (Astrachan State University, Russia), Riccardo M. Baldini (University of Florence, Italy), Víctor N. Suárez Santiago (University of Granada, Spain), Craig Freeman (University of Kansas, U.S.A.), Eric H. Roalson (Washington State University, U.S.A.), and Ralph Brooks for help in obtaining collections. Andre K. Calaminus is acknowledged for his assistance with some of the lab procedures related to this project.

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Appendix 1. Sample numbers, collection numbers, GenBank accession numbers, collectors, and details of the distribution of all samples of *Tragopogon dubius* and related species (*T. campestris*, *T. lainzii*, *T. mirus*, *T. miscellus*) analyzed in this study. * indicates new sequence. Samples from the United States are specified with state (using accepted abbreviations) and county, and some samples have additional locality information; samples from other countries are designated with country and other locality information. Some locations have been visited multiple times and hence multiple vouchers are listed. Collections followed by (+) represent several individual collections not associated with any herbarium.

Tragopogon campestris Besser, Ukraine, "Podolia-Volhynia", 1822, *W.S.J.G. von Besser, s.n*. (LE), *OL815012; ***T. dubius*** Scop., MT, Powell Co., *R.L. Lang, 326* (WS), *OL635186; OR, Wheeler Co., *A. Cronquist, 6696* (WS), *OL635187; OR, Morrow Co., *A.E. Grable, 4800* (WS), *OL635188; UT, Carbon Co., *N.H. Holmgren & al*. (WS), *OL635189; WY, Carbon Co., *J.M. Wirsing, 274* (WS), *OL635190; NM, Santa Fe Co., *B. & D. Halleck* (WS), *OL635191; Spain, Huesca, *P. Catalan, 1*, KF050397; Spain, Huesca, *P. Catalan, 2*, *OL639145; Spain, Huesca, *P. Catalan, 6*, *OL639146; Spain, Huesca, *P. Catalan, 4*, *OL639147; Spain, Castilla y León, *J. Algarra, G. Blanca, V. Suarez-Santiago, 266* (GDAC), *OL639148; Spain, Guadalajara, *V. Suarez-Santiago, S. Schiaffino, DU-AT1* (GDAC), *OL639149; Spain, Alar del Rey, Palencia, *V. Suarez-Santiago, DU-ALAR-9* (GDAC), *OL814561; Spain, *E.V. Mavrodiev (from seeds # 3, collected by V. Suarez-Santiago), 3* (FLAS), KF050394; Spain, Alar del Rey, Palencia, *V. Suarez-Santiago, DU-ALAR-7* (GDAC), *OL814562; Spain, Alar del Rey, Palencia, *V. Suarez-Santiago, DU-ALAR-8* (GDAC), *OL814563; Spain, Granada, *S. Ortiz & G. Blanca, 35315* (GDAC), *OL814564; Spain, Burgos, Castile de León, *V. Suarez-Santiago, S. Schiaffino, DU-LEN1* (GDAC), *OL814565; Spain, Jaén, El Oasis, *V. Suarez-Santiago, F. Lopez-Soriano, DU-OAS1* (GDAC), *OL814566; Spain, Toledo, Villatobas, *V. Suarez-Santiago, S. Schiaffino, DU-TOB1* (GDAC), *OL814567; Spain, Soria, El Burgo de Osma, *V. Suarez-Santiago, C. Abellán-López, 52777* (GDAC), FN675704; Spain, Burgos, Aranda de Duero, Castile y León, *V. Suarez-Santiago, C. Abellán-López*, *54996* (GDAC), FN675703; Spain, Jaén, El Oasis, *V. Suarez-Santiago, C. Abellán-López, 54996* (GDAC), FN675702; Spain, Burgos, Aldealengua, Castile y León, *V. Suarez-Santiago, C. Abellán-López, 52770* (GDAC), FN675701; Spain, Toledo, Villatobas, *V. Suarez-Santiago, S. Schiaffino, 54998* (GDAC), FN675700; Spain, Madrid, Somosierra, *V. Suarez-Santiago, S. Schiaffino, 54997* (GDAC), FN675699; Spain, Jaén, Cazorla, *V. Suarez-Santiago & al., 55948* (GDAC), FN675698; Spain, Alar del Rey, Palencia, *V. Suarez-Santiago, C. Abellán-López, 52776* (GDAC), FN675697; Russia, Volgograd region, Kachalino, *T.G. Yelymeeva, D.B. Sychova, Yu.E. Alexeev, 1* (MW), *OL814956; Canada, BC, Vancouver Island, *M. Ownbey, T-24-3* (WS), *OL814957; OR, Wallowa Co., *J.R. Murphy, 30* (WS), *OL814958; Germany, Mainz, *N. Schmalz, Indiv. 1* (+), *OL814959; WA, Spokane Co., *K.H. Gehrman, 202788* (WS), *OL814960; MT, Gardiner, Park Co., *D. Soltis, 2719* (WS), *OL814961; CO, Jackson Co., *C.C. Freeman, 22523* (KANU), *OL814962; Russia, Saratov, Balakovo, *Yu.E. Alexeev, 5* (MW), *OL814963; Russia, Volgograd region, Kachalino, *T.G. Yelymeeva, D.B. Sychova, Yu.E. Alexeev, 4* (MW), *OL814964; Canada, BC, Princeton, *D.R. Lindsay, 1154* (WS), *OL814965; ID, Troy, Latah Co., *L. Cook, P. Soltis, 49* (WS), *OL814966; OR, Benton Co., *R. Halse, 5978* (WS), *OL814967; Canada, ON, *M. Ownbey, T-47* (WS), *OL814968; OR, Lane Co., *G. Mason, 8651* (WS), *OL814969; UT, Logan, *B. Maguire, 21278* (WS), *OL814970; MT, Gardiner, Park Co., *P. Soltis, D. Soltis, 2564* (FLAS), *OL814971; WA, Whitman Co., *M. Ownbey, T-1* (WS), *OL814972; ND, Pierce Co., *M. Ownbey, T-22-1* (WS), *OL814973; SD, Minnehaha Co., *M. Ownbey, T-18-3* (WS), *OL814974; Russia, Ryazan region, Kasimovsky District, village Elatma, *T. Zorkind, 24* (MW), *OL814975; ID, Bonner Co., *J.H. Rumely, 101* (WS), *OL814976; ID, Idaho Co., *W.F. Barr* (WS), *OL814977; OR, Union Co., *A.L. Cook, 4* (WS), *OL814978; OR, Umatilla Co., *R.L. Lang, 367* (WS), *OL814979; WA, Douglas Co., *M. Ownbey, T-19* (WS), *OL814980; WA, Palouse Co., *M. Ownbey, 274197/T-105-0* (WS), AY645813; AZ, Flagstaff, Coconino Co., *T. Ayers, 1748* (ASC), OL826763; OR, Corvallis, Benton Co., *S. Meyers, 218783* (OSC), *OL826764; OR, Corvallis, Benton Co., *S. Meyers, 218784* (OSC), OL826765; OR, Corvallis, Benton Co., *S. Meyers, 219793* (OSC), OL814982; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-1* (FLAS), OL826766; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-2* (FLAS), OL826767; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-3* (FLAS), OL826768; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-4* (FLAS), OL826769; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-5* (FLAS), OL826770; WY, Sanger Ranch, Carbon Co., *D. Soltis, 2724-6* (FLAS), OL826771; ID, Moscow, Latah Co., *D. Soltis, P. Soltis, 2631-1, P. Soltis, X. Liu 3047* (FLAS), OL826772; WA, Oakesdale, Whitman Co., *D. Soltis, P. Soltis*, *2674-1, D. Soltis, S. Shan 3055* (FLAS), OL826773; WA, Oakesdale, Whitman Co., *D. Soltis, P. Soltis*, *2674-5, D. Soltis, S. Shan 3055* (FLAS), OL826774; WA, Palouse, Palouse Co., *D. Soltis, P. Soltis*, *2628-1, P. Soltis*, *S. Liu, 3030* (FLAS), OL826775; WA, Pullman, Whitman Co., *D. Soltis, P. Soltis*, *2679-3*, *P. Soltis*, *S. Liu, 3034* (FLAS), OL826776; WA, Pullman, Whitman Co., *D. Soltis, P. Soltis, 2613-23, P. Soltis, S. Liu, 3040* (FLAS), OL826777; WA, Rosalia, Whitman Co., *D. Soltis, P. Soltis*, *2666-10, D. Soltis, S. Shan*, *3072* (FLAS), OL826778; WA, Rosalia, Whitman Co., *D. Soltis, P. Soltis*, *2666-12, D. Soltis, S. Shan*, *3072* (FLAS), OL826779; WA, Spangle, Spokane Co., *D. Soltis, P. Soltis*, *2616-12A, D. Soltis, S. Shan*, *3050* (FLAS), OL826780; WA, Spokane, Spokane Co., *D. Soltis, P. Soltis*, *2665-8*, *D. Soltis, S. Shan*, *3065* (FLAS), OL826781; WA, Tekoa, Whitman Co., *D. Soltis, P. Soltis*, *2691-1, D. Soltis, S. Shan*, *2691, 3062* (FLAS), *OL826782; ID, Troy, Latah Co., *D. Soltis, P. Soltis*, *2683-1* (FLAS), *OL826783; Czech Republic, Brno, *R. Matyasek, A. Kovarik, CZ-6135*, *OL814983; IA, Story Co., *D.Q. Lewis, 2363-1* (ISU), *OL814984; WY, Corral Cr., North Fork Platte R., Carbon Co., *D. Soltis* (FLAS), *OL814985; Serbia, Pec, *M. Ownbey (from seeds collected 26 May 1953), T-163-0* (WS), *OL814986; Sweden, Gotland, *M. Ownbey (from B. Petterson's seed 5700)*, *T-117-0* (WS), *OL814987; MO, Boone Co., *Baadi Tadych, RSH-113* (UMO), *OL814988; SD, Lyman Co., *M. Ownbey (from seeds # 3217)*, *T-23* (WS), *OL814989; Serbia, *M. Ownbey (from seeds collected 13 June 1955)*, *T-255-0* (WS), *OL814990; Turkey *M. Ownbey (from seeds # 5220, collected by Baki Kasapligil)*, *T-106-0* (WS), *OL814991; Austria, Alpes Orientalis, *M. Ownbey, T-320-0* (WS), *OL814992; France, Hautes (Alpes), *M. Ownbey, T-283-0* (WS), *OL814993; WA, Ferry Co., *P.M. Peterson, C.R. Annable, 3582* (WS), *OL814994; Russia, Volgograd, *E.V. Mavrodiev, Vol-1\5*, KF050396; Slovakia, *Slovak & al. 2018*, KX643638; Switzerland, Kanton Wallis, *B. Gemeinholzer, K. Bachmann*, *bg216* (GAT), AJ633500; Spain, Huesca, *P. Catalan, 4* (+), *OL814995; Spain, Huesca, *P. Catalan, 5* (+), *OL814996; Spain, Huesca, *P. Catalan, 7* (+), *OL814997; Spain, Huesca, *P. Catalan, 9* (+), *OL814998; Spain, Huesca, *P. Catalan, 10* (+), *OL814999; Spain, Huesca, *P. Catalan, 11* (+), *OL815000; Spain, Huesca, *P. Catalan, 12* (+), *OL815001; Spain, Huesca, *P. Catalan, 13* (+), *OL815002; Spain, Huesca, *P. Catalan, 14* (+), *OL815003; Spain, Huesca, *P. Catalan, 15* (+), *OL815004; WY, Crook Co., *M. Ownbey (from seeds collected 28 Jul. 1947), T-20-2* (WS), *OL815005; WA, Whitman Co., *M. Bacon, 39576* (WS), OL815006; AZ, Flagstaff, Coconino Co., *E.B. Morrison, s.n*. (ASU), OL815007; Canada, AB, *A.J. Breitung, 15795* (WS), *OL815008; India, Jammu and Kashmir, the region of Drass, *I.A. Nawchoo, 211* (KASH), EU124010; Germany, Sachsen-Anhalt, *B. Gemeinholzer, K. Bachmann, bg236* (GAT), AJ633503; WY, Sheridan Co., *W. Fertig, J. Britt, 14781* (UWYO), *OL815010; WY, Lincoln Co., Southern Salt River Range and Vicinity, Hams Fork Plateau, *R.L. Hartman, 1356* (UWYO), KF050398; WY, Albany Co., *C.L. Porter, 8175* (UWYO), *OL815011; Russia, Moscow region, Serpukhov District, Pushchino-on-Oka, Prioksko-Terrasny Nature Reserve, Oka river, *Yu.E. Alexeev, 9579* (MW), KF050395; ***T. lainzii*** Suár.-Sant., P.S.Soltis, D.E.Soltis, C.Díaz & Blanca, Spain, Albacete, Riopar, *V. Suarez-Santiago & al., RIO* (GDAC), FN675695; ***T. mirus*** Ownbey, AZ, Flagstaff, Coconino Co., *T. Ayers, 1747* (ASC), OL819872; OR, *S. Meyers, 218787* (OSU), OL819873; WA, Palouse Co., *D. Soltis, P. Soltis, 2602* (FLAS), AY458586; ***T. miscellus*** Ownbey, ID, Moscow, Latah Co., *D. Soltis, P. Soltis, 2604* (FLAS), AY458588.