

Hybridization between the invasives *Tragopogon dubius* and *T. pratensis* in the Gunnison Valley

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Advanced Independent Research

Summer 2011

Introduction

Hybridization can have several interesting effects on species that interbreed. First, hybridization can produce offspring that are the same ploidy level as their parents, which allows for possible interbreeding with the parent species (Whitney *et al*, 2010). This can lead to the development of a coalescent complex and the merging of two species into a single species (Ellstrand *et al*, 2006), or, alternately, the production of a third (new) species. Hybridization can also generate offspring of a different ploidy level as opposed to their parents, generating a new third species if the hybrid is fit (Whitney *et al*, 2010). Most importantly, hybridization allows for the transfer of genes between species, thus potentially increasing fitness (Whitney *et al*, 2010). In the case of invasive plants, it can increase their invasiveness by novel genotypes through recombination of genes from both parent species; by promoting genetic variation via freely recombining “coalescent complexes”; by fixed heterosis; or by allowing “gene dumping” in isolated populations that have accumulated deleterious mutations and alleles (Ellstrand *et al*, 2006). In Ellstrand’s (2006) study, 28 cases were found in which a hybridization event preceded the invasiveness of a species.

In the Gunnison Valley, there are two closely related species of exotic (sometimes deemed invasive) herbaceous plants in the family Asteraceae, *Tragopogon dubius* and *Tragopogon pratensis*. Recently (it is difficult to determine how recently) the two species have been apparently interbreeding and producing hybrid offspring, given the presence of morphological intermediates (K.D. Whitney, unpubl. data). While interspecific hybridization between these two species of *Tragopogon* has been documented for over sixty years in other locations (Ownbey, 1950), the most heavily and, to my knowledge, only reported hybrid, known as *T. miscellus*, is an allotetraploid (Tate *et al*, 2009) which occurs in eastern Washington and northern Idaho (Buggs *et al*, 2009). However, putative hybrids discovered in the Gothic area have been found to be diploid, bearing the same number of chromosomes as their diploid parent species (K.D. Whitney and E.J. Baack, unpublished flow cytometry data). The consequences of this recent hybridization, and the reasons behind differing ploidy levels of the Gunnison Valley vs. western hybrids, are unknown.

Two main questions were addressed in this project: first, what are the altitudinal distributions of *Tragopogon* species and putative hybrids in the Gunnison Valley?; and second, do *Tragopogon* species and putative hybrids perform better at different altitudes, and do hybrids outperform parents?

In order to better understand the dynamics of *Tragopogon* populations in Gunnison County, a survey was conducted. Before this study there were conflicting accounts of the altitudinal distributions of populations of the two species: according to Harrington’s (1954) *Manual of Plants of Colorado*, *T. dubius* is found in areas in the western two thirds of Colorado at elevations ranging from 4500-10,500 feet, while *T. pratensis* is found within the 5,000-8,000 ft. range. In a conflicting account from *Colorado Flora: Western Slope*, Weber and Wittman (2001) state that *T. pratensis* replaces *T. dubius* at higher altitudes. Evidently, the literature lacks a consensus on the distribution of the taxa in question, necessitating a survey. In particular, the survey helped to tell us if hybrid individuals appear to be more common at, and thus potentially more adapted to, certain elevations.

Whether the hybrids have greater performance compared to their parents is an important question to consider; if the hybrids are more fit at certain elevations than either parent, this means that hybridization may be responsible for generating aggressively invasive weeds with more advantageous genotypes than either parent. In order to test the fitness of the hybrids and the two parents relative to one another, a pilot common garden experiment was conducted with two plots at two elevations.

Methods

Survey of altitudinal distribution of *Tragopogon* species and putative hybrids in the Gunnison Valley

Forty-one *Tragopogon* populations were collected from the Gunnison Valley, the first ten being collected in the summer of 2010 and the remaining in the summer of 2011. One fruiting head was collected from up to thirty individuals per population (a mean of 27.33 individuals were collected per population), and a distance of $\approx$ 3-4 miles was maintained between population sites for collection. Heads were collected at a stage when the seeds were mature or nearly mature, but prior to the stage when the head opens into a puff. The latitude, longitude and elevation of each population was recorded. In order to classify them as *Tragopogon pratensis*, *T. dubius* or a hybrid, morphological characters were recorded. The two parent taxa have distinct morphology which makes them easily identifiable: *Tragopogon dubius* has a head which is tall and thin, with phyllaries normally numbering from 10-13, while *T. pratensis* is shorter, fatter and has phyllaries that number around 8, which are edged with purple. Hybrids have intermediate morphology and can bear a mix of characters from both parents. Measurements for each individual head were taken of key morphological characters, which were stem diameter, head diameter, phyllary length and width, width of purple on phyllary edge, seed length and width, and number of phyllaries. Using these characters, ratios were calculated, which were Phyllary Ratio (phyllary length divided by width), Seed Ratio (seed length divided by width), Purple Ratio (width of purple along phyllaries divided by phyllary width) and Head Fatness (head diameter divided by phyllary length).

To putatively classify individuals, data on Phyllary Ratio, Purple Ratio, and Head Fatness (described above) were used in a Cluster Analysis (SAS Proc CLUSTER, method=Ward, SAS v. 9.2). Five classes were specified, with Class 1 being most *T. dubius*-like, Class 5 being most *T. pratensis*-like, with intermediates bearing characteristics from both parents and thus being potential hybrids. To explicitly test the relationship between elevation and population composition, we used regression (SAS Proc REG). The number of individuals from each taxon (*T. pratensis*, *T. dubius*, or hybrid from Class 2-4) in a population was divided by total individuals sampled in a population to yield the fraction represented by each taxon. These fractions were then regressed on elevation.

Each population was plotted on a map of the Gunnison Valley using ArcMap; the makeup of each population (*T. pratensis*, *T. dubius*, or hybrid from Class 2-4) was displayed on the map using pie charts.

Common Garden Experiment at high and low elevation plots

Using seeds collected by Ken Whitney last summer (2010), I planted *Tragopogon* seedlings in two plots at different elevations of $\approx$ 8500 and $\approx$ 9800 feet. The lower elevation plot was located in Crested Butte South (backyard of J. Reithel and I. Billick), and the higher elevation plot was within the Gothic townsite, on a slope in front of Barclay cabin. Roughly 150 plants were planted in each plot. The seedlings were classified according to cluster analysis on the mother plants' morphology (see above) into five classes, Class 1 being most *T. dubius*-like, Class 5 most *T. pratensis*-like, with intermediate classes being more hybrid-like. Seeds were germinated in wet paper towels in plastic ziploc bags (rough time to germination = 4-7 days) and then transferred to peat pellets (36 mm, Jiffy International, Kentucky, USA); they were kept indoors for a few days to a week until they had enough chlorophyll in their leaves to be transferred to a weatherport for one to two weeks before planting. The seedlings were then transplanted in either the high-elevation plot or the low-elevation plot.

In the high-elevation plot, a 14x14 meter plot was staked out, and the seedlings were planted a distance of 0.5 meters apart on Thursday, July 14, 2011. They were watered on the day of planting; on Friday, July 15; and on Saturday, July 16. In the low-elevation plot, a more spread-out plot was created due to the sagebrush and other vegetation present in the site, with seedlings planted at about 0.5-1 meters apart on

Monday, July 18, 2011. Because of a shortage of individuals from Class 5, three of the 152 seedlings were not planted, meaning that 149 individuals were planted in the low-elevation plot. The plot was watered on the day of planting; on Wednesday, July 20; Friday, July 22; and on Monday, August 1.

The day after planting each plot, measurements for each individual were taken of the number of leaves and length in centimeters of the longest leaf. Weekly censuses were taken in each plot to record mortality of individuals. On Monday, August 8, final measurements were taken of individual leaf number and longest leaf length for both plots. Leaf number was multiplied by longest leaf length to calculate a measurement of size, and the initial size (July) was subtracted from final size (August) for each individual to determine its growth.

To test whether individuals of the five morphological classes differed in survival or growth, we used ANOVAs (SAS Proc GLM). Survival (coded as 0 or 1) and growth were the dependent variables in separate ANOVAs (run separately for each plot; four ANOVAs total). Class and initial size (included as a covariate) were the independent variables. Tukey tests were used to determine which classes differed significantly from each other, and least-square means were plotted. Note that individual seedlings that died before the final size measurements of 8 Aug were excluded from the analyses of growth.

Results

*Survey of altitudinal distribution of *Tragopogon* species and putative hybrids in the Gunnison Valley*

We found no evidence of strong relationships between elevation and the frequencies of individuals in different morphological classes (*dubius*, *pratensis*, and hybrid), with regressions showing nonsignificant p-values and r^2 values < 0.05 in all three cases (Figs. 1-3). However, trends were observed that suggest *T. dubius* may increase in frequency with elevation (Fig. 1), while hybrids may decrease in frequency (Fig. 2). *T. pratensis* was rare relative to the other taxa (many populations contained no *pratensis* individuals), with no evidence of a response to elevation (Fig. 3). Overall abundance of the classes from all populations showed that *pratensis*-like individuals were the least abundant at 19.5% of individuals, with *dubius*-like individuals being the next most abundant at 24.7%, and hybrid-like individuals from Classes 2, 3, and 4 being the most abundant at 55.8% (Fig. 4).

While sampling coverage could be improved, there did seem to be spatial differences in the frequencies of the *Tragopogon* taxa (Fig. 5). *T. dubius* seemed to be most common in the Ohio Creek Valley, while *T. pratensis* was most common in parts of the East River Valley. Putative hybrids were found in nearly all populations (they were completely absent from only a single population) but were especially dominant in the area south of Blue Mesa Reservoir.

Common Garden Experiment at high and low elevation plots

There were significant differences in growth among morphological classes in both plots ($P < 0.001$ in both plots); initial size was also important at the upper plot ($P < 0.001$) but not the lower ($P = 0.9649$). In both the high and low elevation plots, Classes 4 and 5 (*T. pratensis*-like individuals) had significantly lower growth than Class 3 putative hybrids (Figs. 6,7). In both plots, there was no significant difference between the growth of Classes 1, 2 or 3, but there was a trend in the lower elevation plot for Classes 1 and 2 to have lower growth than Class 3. The lower elevation plot also showed no significant difference between the growth of Classes 1 and 2 and Classes 4 and 5.

Morphological classes of *Tragopogon* did not differ in survival rates in either garden ($P = 0.1172$ at higher elevation, $P = 0.7083$ at lower elevation). Initial plant size of the seedlings did have a

significant effect on survival in both gardens ($P=0.0012$ at high elevation, $P<0.0001$ at low elevation), with larger plants surviving at higher rates.

Discussion

The results of both the survey and common garden experiments suggest that there is no significant relationship between elevation and fitness of the three *Tragopogon* taxa (though the survey shows trends of increasing frequency of *T. dubius* and *T. pratensis*, and decreasing frequency of hybrids, with increasing elevation). Both experiments, however, suggest that *T.pratensis*-like individuals have decreased fitness compared with other taxa, having lower frequency in populations in the survey and having lower growth compared with other classes in the common garden experiment. These results did not conform with expectations, as we predicted that the taxa would display different frequencies and different performance according to elevation. However, because only two plots at two different elevations were used in this preliminary experiment, it could be that the taxa perform differently at intermediate elevations and elevations lower than 8500 feet (e.g. *T. pratensis* could outperform *T. dubius*). And it could also be that further sampling of populations in the survey could strengthen the trends seen in the elevation regressions and significant relationships may be seen.

It was also not predicted that one of the parent species would have lower fitness than the other, yet the largest difference in fitness is seen to be between *T. pratensis*-like individuals and the remaining taxa. Hybrid Class 3 in the common garden experiment displays equal fitness compared to *T. dubius*. Interestingly, the Hybrid Class 3 does best in the lower elevation plot, though its growth was not significantly greater than that of Classes 1 or 2. Since the data from these common garden plots is only from the first month of growth, trends may become significant as measurements continue to be taken. It will also be extremely revealing to measure fitness via seed production next year in the coming summer.

Increased invasiveness as a result of hybridization between the two *Tragopogon* parent species could indeed be a concern given the results of this study. Hybrids are very abundant relative to their parent species, and more abundant than *T. pratensis*. The results at the lower elevation common garden plot also show a trend that Class 3 hybrids perform better than any other class. Given the low abundance of *T. pratensis* and its poor performance in the garden plots, this taxon seems to be the least fit of the *T. dubius* and hybrid species. It is possible that backcrossing with *T. pratensis* could cause the extinction of this less fit parent, or that hybrids and *T. dubius* could eventually outcompete *T. pratensis*, leading to its disappearance in the Gunnison Valley area.

Literature Cited

- Buggs R.J.A., Doust A.N., Tate J.A., Koh J., Soltis K., Feltus F.A., et al. 2009. "Gene loss and silencing in *Tragopogon miscellus* (Asteraceae): Comparison of natural and synthetic allotetraploids". *Heredity*. 103 (1): 73-81
- Campbell DR, NM Waser, and GT Pederson. 2002. "Predicting patterns of mating and potential hybridization from pollinator behavior". *The American Naturalist*. 159 (5): 438-50.
- Ellstrand, N. C., and K. A. Schierenbeck. 2006. "Hybridization as a stimulus for the evolution of invasiveness in plants?" *EUPHYTICA*. 148 (1-2): 35-46.
- Harrington, H.D. *Manual of Plants of Colorado*. 1954.
- Ownbey, M. 1950. *Natural hybridization and amphiploidy in the genus Tragopogon*. S.l: [Botanical Society of America].

Tate J.A., Symonds V.V., Doust A.N., Buggs R.J.A., et al. 2009. "Synthetic polyploids of *Tragopogon miscellus* and *T. mirus* (Asteraceae): 60 Years after Ownbey's discovery". *American Journal of Botany*. 96 (5): 979-988.

Weber, A., R.C. Whitman. *Colorado Flora: Western Slope*. Revised ed.

Whitney K.D., Ahern J.R., Campbell L.G., Albert L.P., and King M.S. 2010. "Patterns of hybridization in plants". *Perspectives in Plant Ecology, Evolution and Systematics*. 12 (3): 175-182.

Figures

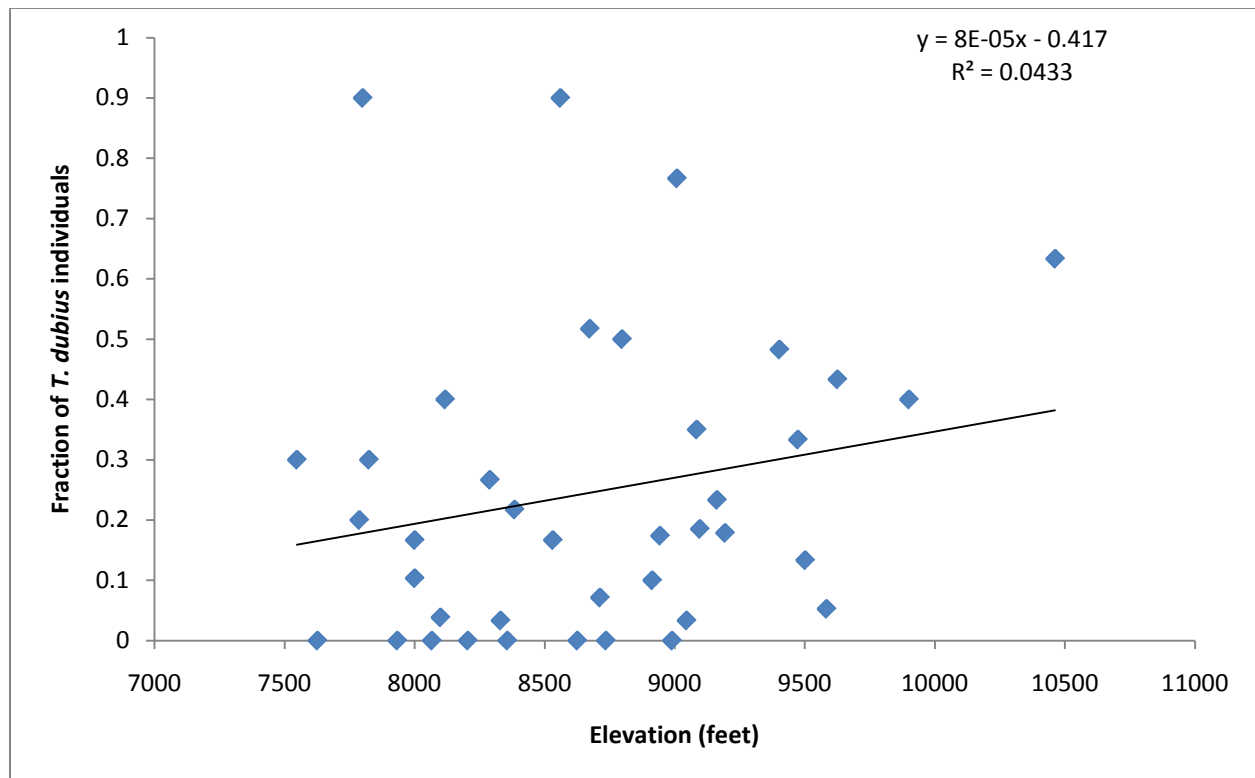


Fig. 1 Fraction of *Tragopogon dubius* in each population with respect to elevation ($P = 0.2034$). Each point represents a population. Though the relationship is not significant, there is a trend for an increase in the fraction of *T. dubius* individuals with increasing elevation.

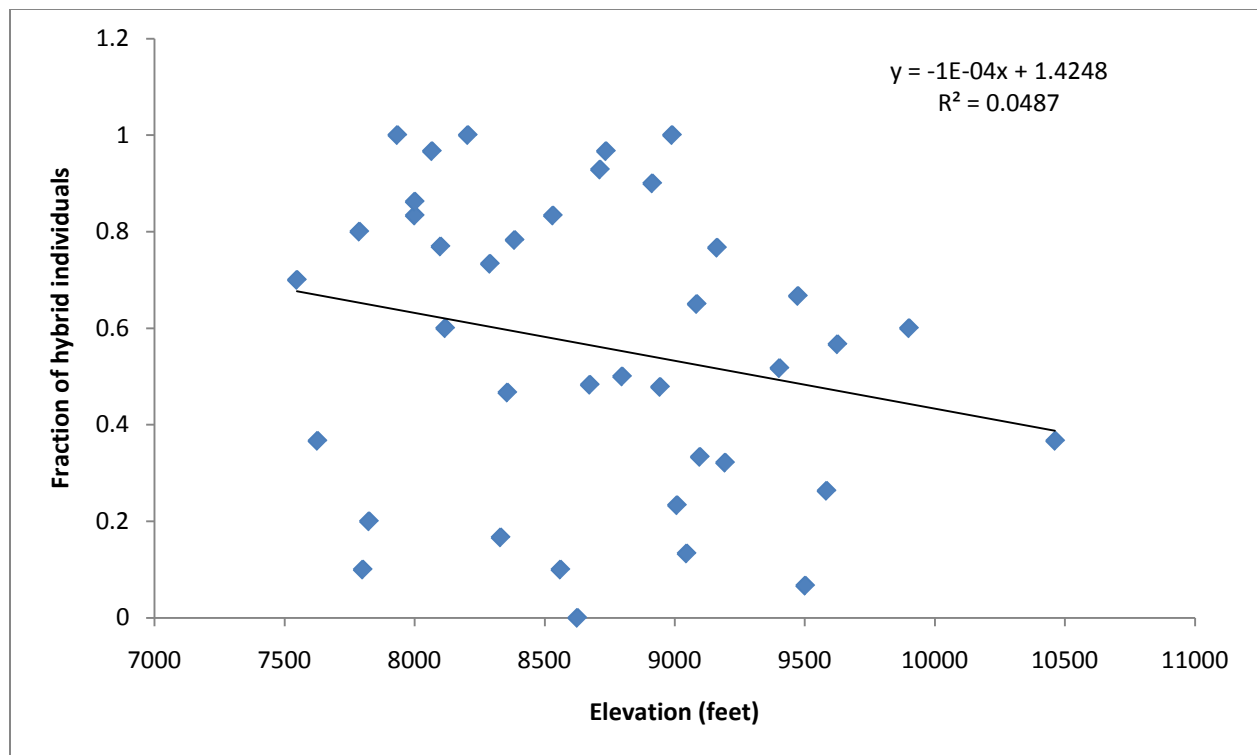


Fig 2. Fraction of putative *T. dubius* x *T. pratensis* hybrids (from Classes 2, 3, & 4) in each population with respect to elevation ($P = 0.1769$). There is a trend towards a decrease in the fraction of hybrids with increasing elevation.

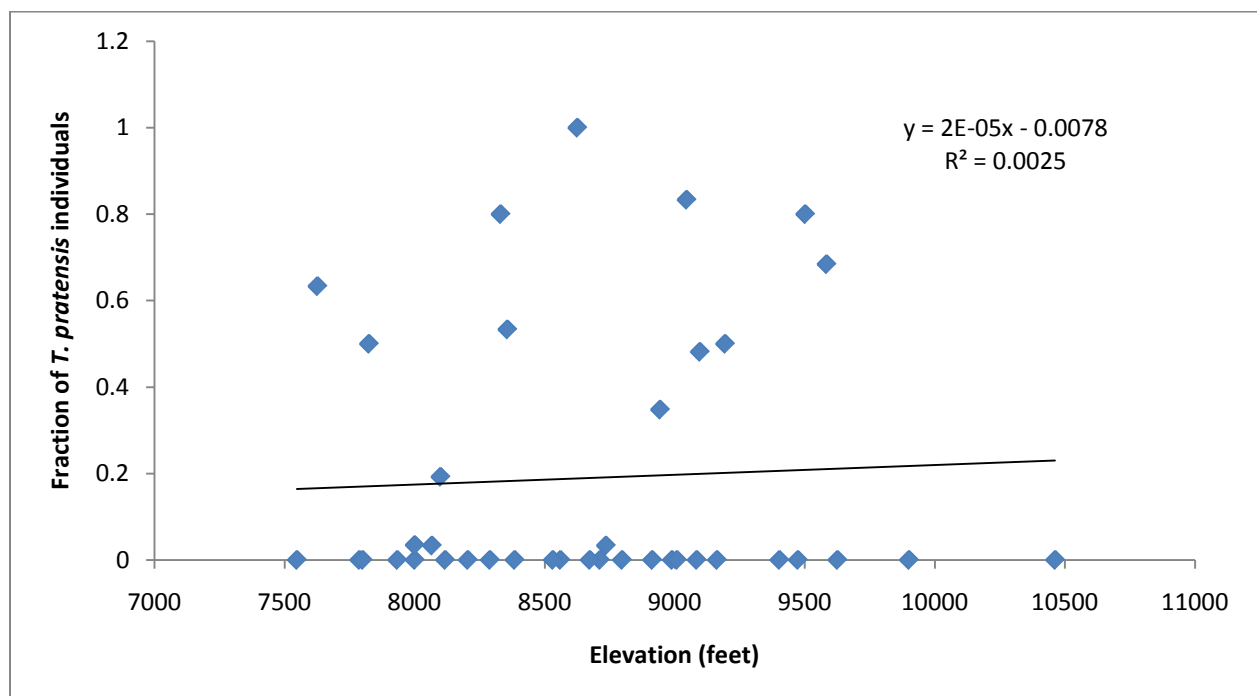


Fig. 3 Fraction of *Tragopogon pratensis* individuals in each population with respect to elevation ($P = 0.7621$). The relationship is essentially flat

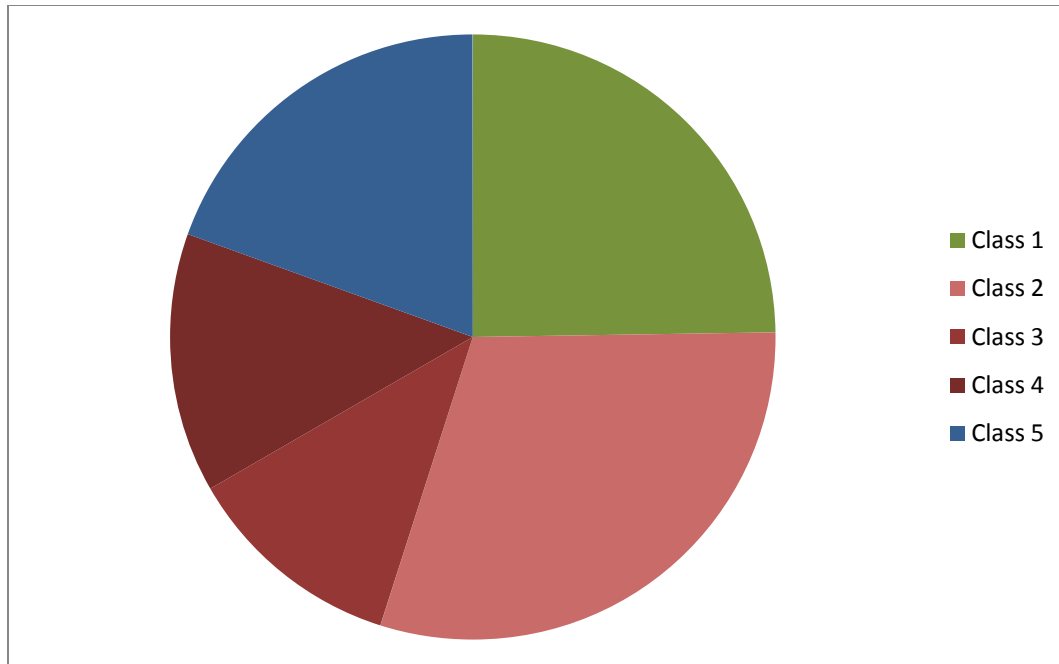


Fig. 4. Pie chart of the fractions of each class from all populations in survey. Class 1 (*T. dubius*) was 24.7%, Class 2 (hybrid) was 30.2%, Class 3 (hybrid) was 11.7%, Class 4 (hybrid) was 13.9%, and Class 5 (*T. pratensis*) was 19.5%.

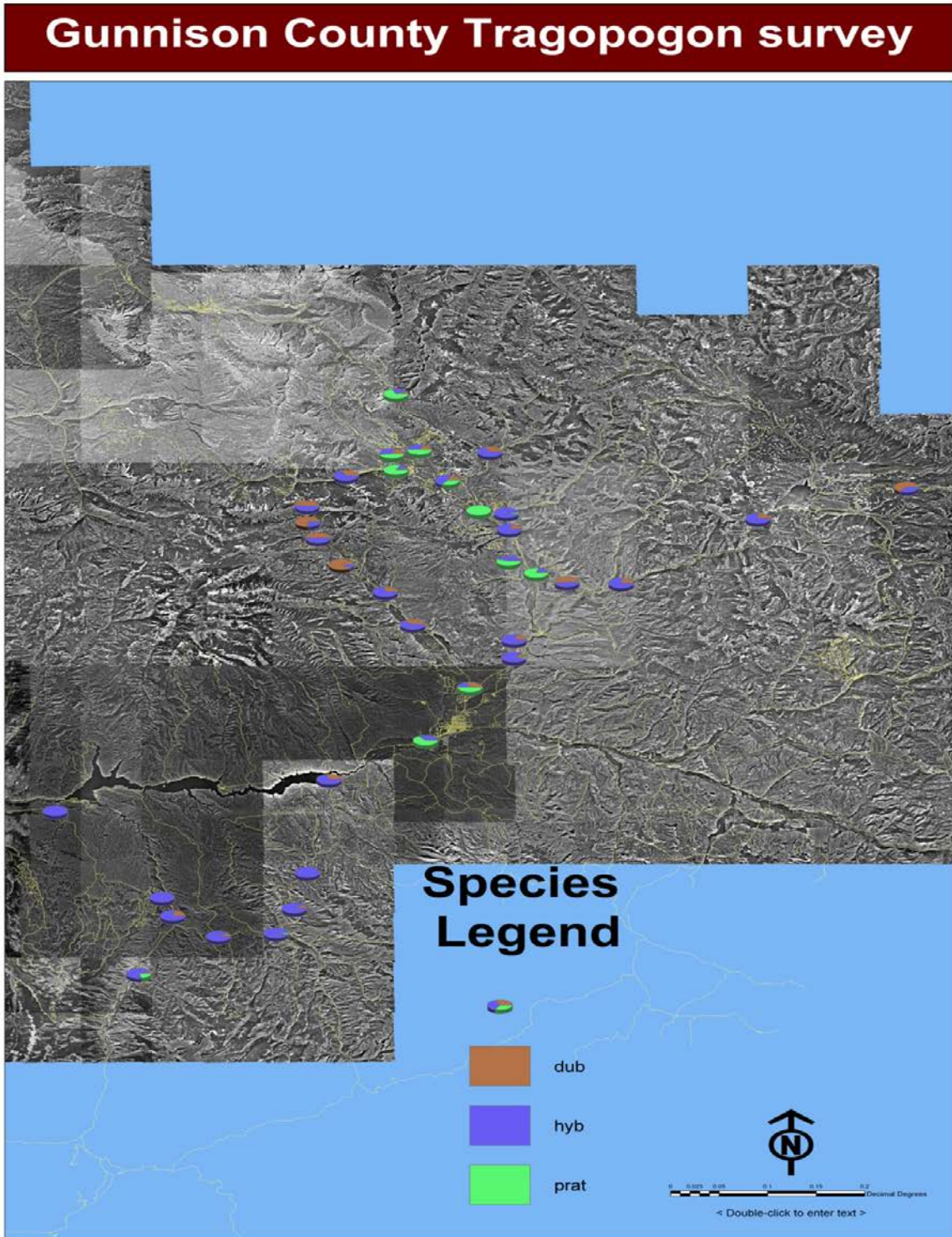


Fig. 5 Map of *Tragopogon* population survey, with pie charts for each population showing fraction of each taxon. *T. dubius* is Class 1, Classes 3, 4 and 5 are grouped as "hyb", and *T. pratensis* is Class 5.

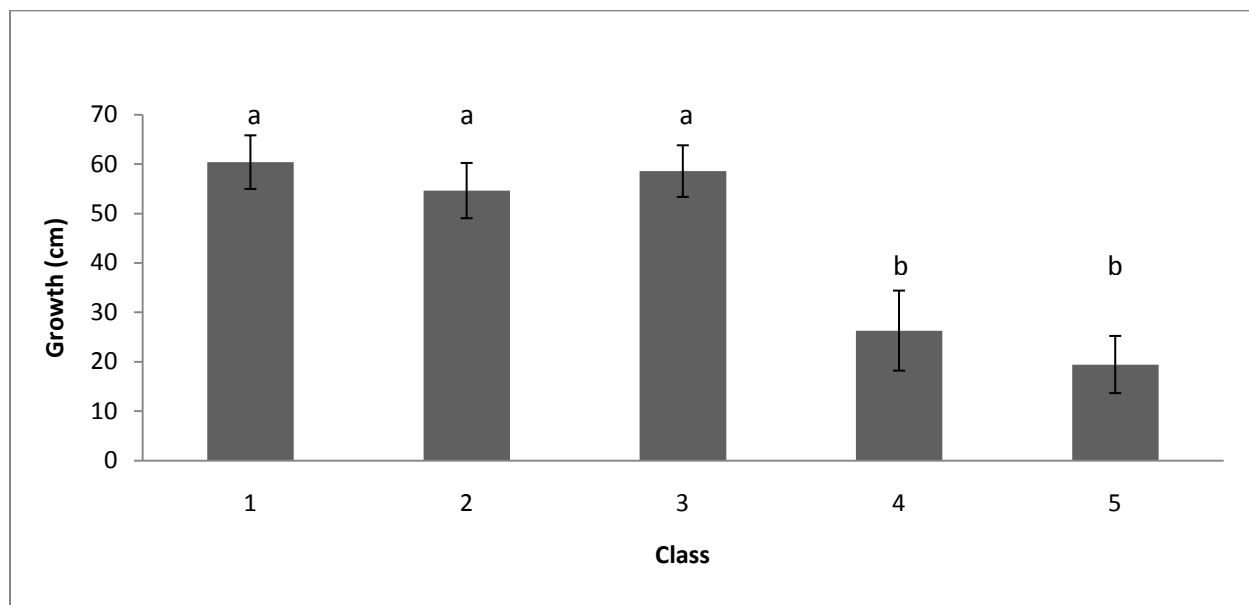


Fig. 6 Mean growth according to class in the upper elevation common garden site in Gothic townsite (elevation ~9500 ft); bars not sharing a letter differ significantly ($P < 0.05$) from one another. There was no significant difference between the growth of Classes 1, 2 and 3, nor between 4 and 5. Classes 1, 2 and 3 were different from Classes 4 and 5. Classes 4 and 5 (*T. pratensis*-like) had lower growth rates than Classes 1, 2 (*T. dubius*-like) and 3 (hybrid).

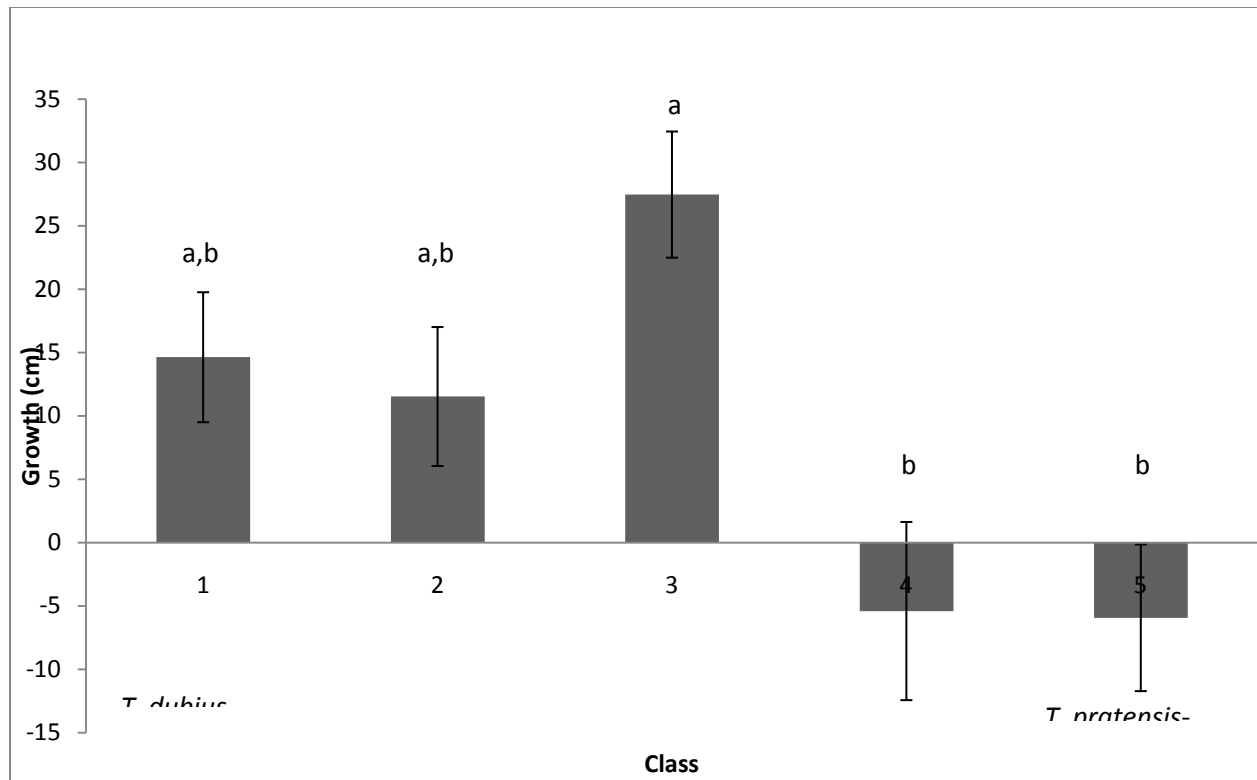


Fig. 7 Mean growth according to class in the lower elevation common garden site in South Crested Butte (elevation ~8500 ft)); bars not sharing a letter differ significantly ($P < 0.05$) from one another. Classes 1 and 2 (*T. dubius*-like) were not significantly different from Classes 3, 4 and 5. Classes 4 and 5 (*T. pratensis*-like) had significantly lower growth than Class 3.