

THE POTENTIAL FOR GENETIC ASSIMILATION OF A  
NATIVE DANDELION SPECIES, *TARAXACUM CERATOPHORUM*  
(ASTERACEAE), BY THE EXOTIC CONGENER  
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Exotic plant species can threaten closely related native congeners through asymmetric hybridization and subsequent backcrossing, the process known as genetic assimilation. I explore the initial stages of this process in *Taraxacum ceratophorum* (Asteraceae), the native alpine dandelion, and the invasive apomict *T. officinale*. In central Colorado, seven *T. ceratophorum* populations all occur in sympatry with *T. officinale*. In one large population on Pennsylvania Mountain, surveys further revealed that flowering phenologies and visiting insect taxa overlap almost completely for both *Taraxacum* species. Together these results indicated that heterospecific pollen transfer is likely. Crossing experiments showed that *T. ceratophorum* is an obligate outcrosser, and interspecific hand pollinations resulted in 37.3% seed set. However, molecular analysis of the F1 offspring indicated that only 33.2% of germinating seeds were hybrids; the remainder were selfed offspring produced from a breakdown in self-incompatibility (the mentor effect). Although the mentor effect helps reduce the production of hybrids, the asymmetrical direction of hybridization creates the potential for genetic assimilation of *T. ceratophorum* by *T. officinale*.

**Key words:** Asteraceae; exotic species; genetic assimilation; hybridization; *Taraxacum*.

For species that have diverged in allopatry, secondary contact between congeners may result in interspecific hybridization (Stebbins, 1950; Arnold, 1997). The resulting hybrid zones can be stable due to habitat-specific hybrid superiority (bounded hybrid superiority; Moore, 1977) or a balance between selection against hybrids and parental dispersal (dispersal/selection balance; Key, 1968; Barton and Hewitt, 1985). Alternatively, hybrid zones can be transient, and parental populations enter into a race with two possible finish lines: separation or fusion (Howard, 1993). If hybrids are sterile or of low fitness, the reinforcement of stronger prezygotic reproductive barriers between congeners should be favored by natural selection (Dobzhansky, 1940). Although still controversial, “reinforcement” of species boundaries can theoretically reduce hybridization and eventually dissolve the hybrid zone (Howard, 1993; Cain et al., 1999). Alternatively, production of fertile hybrids represents an initial step in the merging of two populations to form a hybrid swarm. If one or a few hybrid genotypes are competitively superior, this swarm may give rise to a novel “species” (evolutionary novelty; Arnold, 1997). However, hybrids may backcross with one or both parental species resulting in introgressive hybridization (Anderson, 1949; Rieseberg and Wendel, 1993). Numerical superiority (the minority principle), a fitness advantage, or unidirectional reproductive barriers favoring one species over the other can lead to asymmetrical introgression (genetic assimilation)

resulting in one species genetically “swamping out” the other (Levin, 1975; Ellstrand and Elam, 1993; Rhymer and Simberloff, 1996). Hybrid zones have traditionally been associated with local disturbance events (Stebbins, 1950). One such man-made “disturbance” is the introduction of non-indigenous species (Vitousek et al., 1997). It follows that exotic plant species may offer unique opportunities to examine the beginning stages of hybrid zones, which often otherwise lack an historical context and can easily go unnoticed because they are transient.

If fertile hybrids are formed and backcross with parental species, native populations may be directly threatened by introgression (Ellstrand and Elam, 1993). Interspecific gene flow can result in the formation of native–exotic hybrid swarms and the loss of “pure” native populations (Rhymer and Simberloff, 1996). Ultimately if introgression is asymmetrical, favoring the exotic species, genetic assimilation can result in the extinction of the native species (Levin et al., 1996; Huxel, 1999; Wolf et al., 2001). Here I examine several factors influencing interspecific hybridization and hybrid zone formation between native and exotic congeners. First, for heterospecific pollen transfer to occur, native and exotic species must exhibit sympatry and overlap in flowering phenology. Next, one or both of the interacting species must reproduce sexually and receive heterospecific pollination to produce fertile hybrid offspring. Third, pollen limitation of plants receiving mixed or heterospecific pollen loads also may enhance the rate of hybridization by relaxing pre-zygotic reproductive barriers, which often limit overall hybridization rates (e.g., pollen competition; Carney et al., 1994, 1996).

The genus *Taraxacum*, dandelions, is a useful system for the study of hybrid zones because of its past evolutionary history as well as interactions between extant native and exotic species. Although various breeding systems occur in *Taraxacum*, most species in the genus are obligate agamosperms, producing seed asexually and endosperm autonomously (Richards, 1986; Asker and Jerling, 1992). Agamospermous dandelions are typically triploid ( $3x = 24$ ) but higher ploidy levels

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are also apomictic. The diploid ( $2n = 16$ ) *Taraxacum* species that have been surveyed reproduce sexually, and this reproductive mode is commonly coupled with self-incompatibility (Richards, 1973, 1986). True hybrids are produced in both diploid–diploid and triploid–diploid crosses (Richards, 1970; Morita et al., 1990a; Tas and van Dijk, 1999), although crossing apomictic triploid *Taraxacum* species with sexual diploid species can also produce high percentages of self-fertilized seed via the mentor effect (Morita et al., 1990a; Tas and van Dijk, 1999). Richards (1973) theorized that the genus *Taraxacum* evolved in the Himalayan region, where primitive sexual species are common. After the last glacial period, northern advanced apomicts (possibly sections *Arctica* and *Borealia*) met advancing hybrid swarms that formed from sexual species meeting in the tailings of receding glaciers. Hybridization between apomictics and sexuals led to the vast array of current apomictic lineages. Here I investigate an analogous situation, where anthropogenic introduction, rather than glacial retreat, provides the basis for interspecific contact.

*Taraxacum ceratophorum* (Asteraceae), the alpine dandelion, is a circumboreal species found throughout the North American Rocky Mountains (Scott, 1995). Fossils of *T. ceratophorum*, estimated to be 100 000 yr old, have been discovered in Alaska, indicating a native range in North America (Chaney and Mason, 1936; Richards, 1973). The range of this indigenous dandelion overlaps with *Taraxacum officinale*, an exotic species introduced early during European settlement of North America (Josselyn, 1672; Solbrig, 1971; Mack, 2003). Both species are perennial herbs and from a radial rosette of leaves produce solitary heads composed of yellow ligulate florets. Although inflorescence diameter and height overlap (Cronquist et al., 1994; Brock, 2003), *T. ceratophorum* and *T. officinale* are distinguishable by the position of bracts in the outer involucre whorl (appressed and reflexed, respectively) and the tips of bracts in the inner involucre whorl (corniculate and not corniculate, respectively; Cronquist et al., 1994). North American populations of *T. officinale* are composed of triploid apomictic individuals (King, 1993; Lyman and Ellstrand, 1998). Both tetraploid ( $4x = 32$ ) and diploid chromosome counts have been reported for *T. ceratophorum* (Scott, 1995).

Species status in *Taraxacum* varies along a spectrum from the predominantly European “microspecies” concept to the North American “macrospecies” concept (Hughes and Richards, 1989). In its extreme form, different genets of asexual dandelions could be described as different microspecies, while the more conservative approach combines variants under broader species boundaries. Due to the lack of microspecies descriptions in North America, I follow the current species designations (Harrington, 1954; Cronquist et al., 1994; Scott, 1995; for sections see Kirschner and Stepanek, 1987); *Taraxacum ceratophorum* Ledeb. (section *Borealia*) and *T. officinale* Weber (section *Ruderalia*).

The goal of this study was to examine the initial biotic conditions required for genetic assimilation of native plant populations by exotic congeners. I address the following specific questions: (1) Do *T. ceratophorum* and *T. officinale* occur in sympatry in the Colorado Rocky Mountains? (2) To what extent do their flowering phenologies and insect visitors overlap? (3) Do populations of *T. ceratophorum* reproduce sexually? (4) If so, are they pollen limited? (5) Does heterospecific pollination result in fruit set in *T. ceratophorum*? (6) If so, what proportion of germinating seeds are hybrids?

## MATERIALS AND METHODS

**Population survey for chromosome number in *T. ceratophorum* and sympatry with *T. officinale***—I surveyed 10 alpine sites in Park and Summit Counties, Colorado, USA, to locate *T. ceratophorum* populations. In each native population, the presence or absence of sympatric *T. officinale* was noted. Because sexual reproduction is, with rare exception, found only in diploid *Taraxacum* species, I used chromosome counts (ploidy) as a proxy for breeding system in these *T. ceratophorum* populations (Richards, 1973). The assumption that diploid populations are sexual was also tested directly in a *T. ceratophorum* population on Pennsylvania Mountain (see Experimental analysis of the *T. ceratophorum* breeding system: Test for sexual reproduction).

To conduct chromosome counts, I sampled mature infructescences from four randomly chosen *T. ceratophorum* plants along a 50-m transect in each population. Seeds from each plant were germinated and the seedlings grown for 3 mo in the greenhouse at University of Missouri-Columbia, at which time the smaller branch roots were pruned to induce new root growth. Five days later, young roots from each plant were removed and placed separately into moist microcentrifuge tubes. After piercing the lid, tubes were placed in an air-sealed iron chamber and subjected to  $N_2O$  gas (1013 kPa) for 1 h at room temperature (Kato, 1999). Root tips were fixed in 90% acetic acid (5 min) and then washed in  $dH_2O$  (5 min). Following Kato (1997), root tips were enzymatically macerated for 50 min at 37°C, and meristematic tips were removed. I spread the root cells on microscope slides, which were then placed in a humidity chamber for 15 min. Chromosomes were stained with 2.5% acetic orcein and visualized with a light microscope at 1000× magnification.

**Experimental analysis of hybridization potential between *T. ceratophorum* and *T. officinale***—**Study site**—Experiments took place on Pennsylvania Mountain (Park County, Colorado, USA, 39°15' N, 106°07' W) in the Mosquito Range of the central Rocky Mountains. At this site, *T. ceratophorum* grows from the tree line (~3505 m) upwards into the open alpine tundra. *Taraxacum officinale* is found within the same elevation range, where it often (though not exclusively) grows along road cuts, trails, and in areas of natural disturbance. I studied two spatially distinct subpopulations of each species. For *T. officinale*, I examined a subpopulation (To1) at the lower edge of the transition zone between timberline (krummholz) and alpine communities and a subpopulation (To2) at the upper edge of the zone. These subpopulations were separated by 780 m. The two subpopulations of *T. ceratophorum* (Tc1 and Tc2) were both at the upper limit of the krummholz and were separated by 310 m. The upper *T. officinale* subpopulation was separated by about 250 m from each of the *T. ceratophorum* subpopulations.

**Flowering phenology and insect visitation**—In June 1999, I randomly located 10 0.25-m<sup>2</sup> quadrats along a 50-m transect within each of the four subpopulations, subject to the constraint that each quadrat contained at least two dandelions with flower buds. The initial flowering date was missed at the lower elevation subpopulation (To1). As a result, censusing started during initial flowering at higher elevations and continued until flowering was complete in all subpopulations. Plots were censused every fourth day, and the number of inflorescences with open florets in each was recorded. Flowering phenology was surveyed again in the summer of 2000, using the same permanently marked quadrats. In 1999, the density of flowering plants per quadrat in Tc1 and Tc2 subpopulations averaged  $13.2 \pm 1.68$  plants/m<sup>2</sup> (mean  $\pm$  95% confidence limits [CL]) and  $10.8 \pm 1.2$  plants/m<sup>2</sup>, respectively. For quadrats in To1 and To2 subpopulations, flowering plants averaged  $12.4 \pm 1.84$  plants/m<sup>2</sup> and  $14.4 \pm 3.56$  plants/m<sup>2</sup>, respectively. In 2000, the average density of reproductive plants per quadrat decreased for both *T. ceratophorum* subpopulations (Tc1,  $7.2 \pm 2.8$  plants/m<sup>2</sup>; Tc2,  $8.8 \pm 2.28$  plants/m<sup>2</sup>) and *T. officinale* subpopulations (To1,  $11.6 \pm 1.4$  plants/m<sup>2</sup>; To2,  $10.8 \pm 3.32$  plants/m<sup>2</sup>). Inflorescence counts were averaged for the 10 plots within each subpopulation and normalized by dividing daily means by the peak flowering density. During peak flowering of 1999 (20 July–1 August), insects were collected daily (0800–1400 hours) from inflorescences of *T. ceratophorum* and *T. officinale* to examine the overlap in insect visitor taxa between species.

**Experimental analysis of the *T. ceratophorum* breeding system—Test for sexual reproduction**—In each of the two *T. ceratophorum* subpopulations, 16 spatial blocks were arranged haphazardly within a 200-m<sup>2</sup> plot. Individual blocks were established by selecting four dandelions within 2 m of one another. Two dandelions per block were randomly assigned to treatments intended to elucidate the breeding system of *T. ceratophorum*. To prevent insect visitation, both plants were individually surrounded by fine mesh (1-mm<sup>2</sup> bridal veil) that was held away from the inflorescence by a supporting frame (14 cm height × 11.5 cm diameter). I performed a conspecific hand pollination on florets of the first plant, while florets of the remaining plant were not pollinated. For conspecific pollination, I cross-pollinated inflorescences daily by gently brushing four florets from a pollen donor across the stigmatic lobes of recipient florets. *Taraxacum* florets are protandrous; the style emerges through a ring of five dehiscing anthers picking up pollen, which is then presented to visiting insects. As each floret matures, the stigma lobes reflex, allowing for self-pollination (Richards, 1970). The absence of fruit production in the unpollinated individual would indicate self-incompatibility. Alternatively, fruit set in both treatments would indicate either self-compatibility or apomixis. To distinguish between these reproductive modes I conducted a genetic analysis of progeny produced from the hand pollination treatment (described below).

Seeds of agamosperous dandelions are (barring mutation and somatic recombination) genetically identical to the maternal parent (Richards, 1996). In the hand pollination treatment, I performed crosses between and among different genotypes at the polymorphic isozyme locus malate dehydrogenase (MDH) to produce F1 progeny with expected Mendelian segregation ratios. Crosses were performed between the following homozygote and heterozygote genotypes using Fast (F) and Slow (S) allele designations; FF × SS, FS × SS, FS × FS. Sexual reproduction would be confirmed in FF × SS crosses by the production of all FS offspring and in FS × SS crosses by the production of FS and SS offspring in a 1 : 1 ratio. Sexual reproduction (but not necessarily outcrossing) would be confirmed in FS × FS crosses by a 1 : 2 : 1 progeny ratio of FF : FS : SS genotypes.

To genotype parents, I took leaf tissue from each pollen recipient and from randomly sampled pollen donors at least 15 m away. Before anthesis tissue from each parent plant was placed in a plastic bag on ice for transport to the laboratory. Plants were screened at the MDH locus, so that donors could be assigned to specific crosses designed to yield progeny genotype frequencies for tests of expected Mendelian segregation ratios. Leaf tissue from donor and recipient plants was ground in an extraction buffer consisting of 0.1 mol/L Tris (pH 7.0), 0.132 mol/L ascorbic acid, 0.001 mol/L MgCl<sub>2</sub>, 0.01 mol/L KCl, 0.001 mol/L EDTA, and 0.1% (v/v) 2-mercaptoethanol (Hughes and Richards, 1985). Following recipes established by Hughes and Richards (1985), Dowex chloride (mass equivalent to sample tissue) was added to each extraction to reduce the protease activity of latex. Using filter paper wicks, proteins were loaded onto horizontal starch (11%) gels. I used a histidine-citrate (pH 5.7) buffer system and then stained gels for resolution at the MDH locus (protocols in Wendel and Weeden, 1989).

Fruits generated by these and other pollination treatments (described below) were bagged with lightweight cloth to prevent loss of the seed and collected in September when mature. The percentage of fruits containing seeds (percent seed set) was assayed by distinguishing fertilized *T. ceratophorum* achenes (straw to dark brown colored) from the flat white to yellow empty fruits (Richards, 1970). To determine whether progeny genotypes conformed to predicted Mendelian ratios at the polymorphic MDH locus, seeds from five randomly chosen families in each subpopulation were planted in trays of peat growing medium (Pro-Mix BX Professional General Purpose Growing Medium; Premier Horticulture, Red Hill, Pennsylvania, USA) in the greenhouse at the University of Missouri-Columbia. Leaf tissue from at least 16 individuals in each progeny array (mean *N* = 19) was screened at the MDH locus.

**Test for pollen limitation on seed set rates**—To test for pollen limitation, inflorescences of two additional randomly selected *T. ceratophorum* plants in each of the 16 blocks per subpopulation were left open to natural pollinators. One individual was assigned at random to receive additional pollen, while the other plant was not manipulated (control). Again, four florets from a randomly

selected *T. ceratophorum* donor were brushed across the stigmas of each plant daily in the pollen addition treatment. Seed set in the control treatment, when compared with that of plants receiving conspecific hand pollination, also provided a measure of hand pollination efficiency.

**Test for interspecific compatibility, seed viability, and parentage**—In 2001, I crossed *T. officinale* pollen donors onto recipient *T. ceratophorum* plants to test for interspecific cross-compatibility. In the *T. ceratophorum* Tc1 population I caged 18 randomly selected plants to receive heterospecific pollination (cages described in: Experimental analysis of the *T. ceratophorum* breeding system: Test for sexual reproduction). A day prior to use as pollen donors, *T. officinale* inflorescences from the To1 population were individually placed in florist “aquatics” and then housed at the field site in a mesh-covered insect exclusion box. Due to low fertility in the 1999 conspecific hand pollinations (Results: Test for pollen limitation on seed set rates), I performed interspecific crosses in 2001 by daily brushing the receptive stigmas of each *T. ceratophorum* inflorescence with a fresh randomly selected *T. officinale* inflorescence. Each donor inflorescence was used only once to prevent pollen contamination between crosses. Mature seeds were collected in August and percent seed set determined. To estimate seed viability and provide cotyledon tissue for paternity analysis, I planted the seeds in the greenhouse at the University of Missouri-Columbia in trays of Pro-Mix peat medium and monitored germination over 30 d. Due to variation in fruit set and damage by Hemipteran seed predators, only a subset of seeds produced by each of the 18 crosses was included in the germination trial (mean *N* = 10, range 4–13).

Seedlings were screened at the species-specific microsatellite locus, MSTA 64 (described below), to determine if they were true hybrids or alternatively, if they matched *T. ceratophorum* genotypes. Individuals with *T. ceratophorum* genotypes presumably represent selfed seedlings from a breakdown in self-incompatibility (the mentor effect) and not outcrossed individuals since caging effectively prevents seed set (Results: Test for sexual reproduction). For each seedling, cotyledon tissue was removed and DNA was extracted using the method of Wang et al. (1993). I followed protocols for polymerase chain reaction (PCR) and product resolution on acrylamide gels developed while identifying the species-specific marker (below); however I increased the amount of template DNA to 2 μL in each PCR reaction.

MSTA 64 was identified as a species-specific microsatellite marker by sampling *T. ceratophorum* and *T. officinale* leaf tissue from three populations in Park County, Colorado, USA (Weston Pass, Pennsylvania Mountain, and Bross Mountain), and two populations in Summit County, Colorado, USA (Hoosier Pass and Boreas Pass). Within each population I found patches composed entirely of *T. ceratophorum* or *T. officinale*. From each of the species-specific patches, I randomly sampled leaf tissue of five individuals along a 50-m transect. Tissue was placed on ice in damp plastic bags and was later flash frozen in liquid nitrogen.

I extracted DNA from each sample using a protocol developed by Paterson et al. (1993) and modified for use in a microcentrifuge tube. Sample tissue (0.04 g) was placed in a 1.5-mL microcentrifuge tube and ground in liquid nitrogen. Protocols described by Paterson et al. (1993) were followed thereafter but 700 μL of lysis buffer, 700 μL of chloroform-isoamyl alcohol (24 : 1), and 1 mL of 70% ethanol were substituted for original volumes in the protocol. The DNA pellet was resuspended in 50 μL of TE buffer (pH 8.0) for storage and diluted 1 : 8 with dH<sub>2</sub>O for use in PCR reactions. Primers for the locus MSTA 64, developed by Falque et al. (1998), were screened for species-specific products. The PCR reactions contained 1× buffer, 2.5 μmol/L MgCl<sub>2</sub>, 200 nmol/L of each primer (A/B), 200 μmol/L of each dNTP, 0.625 units of Taq, and 1 μL of template DNA. Reactions were run on a Hybaid thermocycler (Thermo Electron Corporation) with the following program: 1 cycle of 94°C (5 min), followed by 40 cycles of 94°C (30 s), 52°C (45 s), and 72°C (1 min), and a final elongation step at 72°C (5 min). The PCR products were separated on 8% polyacrylamide gels using 1× TBE buffer and then resolved with ethidium bromide stain. Using this microsatellite marker, *T. ceratophorum* and *T. officinale* samples from all five populations could be taxonomically resolved based on species-specific banding patterns.

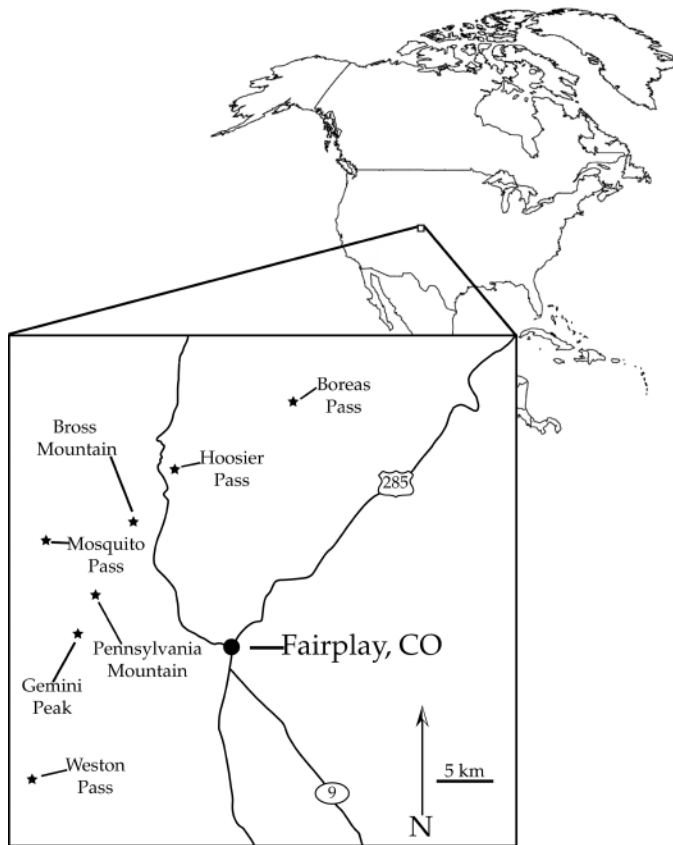


Fig. 1. Map of *Taraxacum ceratophorum* populations surveyed in Park and Summit counties of Colorado, USA. Native plants examined from each population were diploid ( $2n = 16$ ). Sympatric exotic *T. officinale* populations were also present at all sites.

**Data analysis**—I used Hurlbert’s Index to quantify the degree of overlap in flowering phenology of the four subpopulations (Ludwig and Reynolds, 1988). Hurlbert’s index is:

$$HO_{1,2} = (p_{1j})(p_{2j})/c_j$$

where  $p_1$  and  $p_2$  are the proportions of species 1 and species 2, respectively, flowering at the  $j$ th time and  $c$  is the proportion of open inflorescences (of the total) at the  $j$ th time. This index varies from 0 to 1, with a value of 1 indicating complete synchrony. To directly test for sexual reproduction in *T. ceratophorum*, I performed a goodness-of-fit test ( $G$  test) on progeny genotypes at the MDH locus, comparing observed genotypic ratios in progeny to expected Mendelian ratios. Results from crosses yielding the same expected segregation ratio were further subjected to a  $G$  test of heterogeneity ( $G_H$ ) to ensure that each array pooled into the overall  $G$  test individually met Mendelian expectations. Variation in percent seed set among conspecific crosses, pollen addition, and open treatments was analyzed using a mixed model analysis of variance (ANOVA) with treatment and subpopulation designated as fixed effects and the nested blocking term defined as a random effect (GLM procedure, Statistical Analysis System, version 6.12; SAS Institute, Cary, North Carolina, USA). The nonsignificant blocking term ( $P \geq 0.25$ ) was subsequently dropped from the model. Data were angular transformed prior to analysis to fit assumptions of ANOVA. As plants that were caged from insects and left unpollinated set no seed, this treatment was not included in the ANOVA. Planned comparisons were performed to test for significant differences between treatments. Categorical analysis of variance (Catmod procedure, SAS version 6.12) was used to test if maternal plants of *T. ceratophorum* varied in their likelihood of producing hybrid or selfed seed (the binomial response variable) when pollinated by *T. officinale* donors.

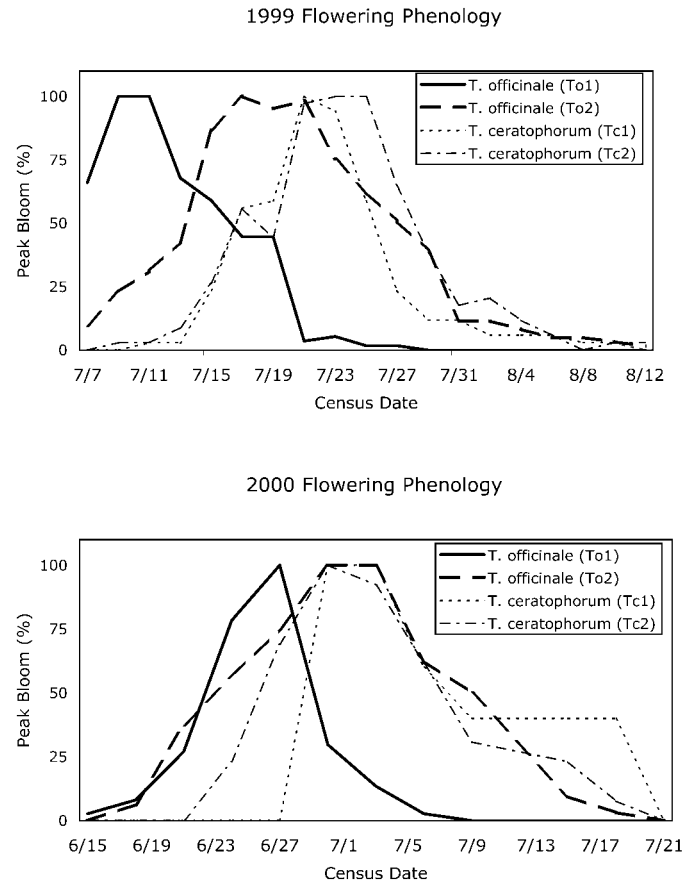


Fig. 2. Flowering phenologies for *Taraxacum ceratophorum* and *T. officinale* on Pennsylvania Mountain during 1999 and 2000.

RESULTS

**Geographical survey of chromosome number in *T. ceratophorum* and sympatry with *T. officinale***—I located seven geographically isolated *Taraxacum ceratophorum* populations in Park County, Colorado, USA: Weston Pass, Gemini Peak, Pennsylvania Mountain, Mosquito Pass, and Bross Mountain; and in Summit County, Colorado, USA: Hoosier Pass and Boreas Pass (Fig. 1). *Taraxacum officinale* at each site occurred within 50 m of *T. ceratophorum*. All chromosome counts from *T. ceratophorum* populations had a diploid complement ( $2n = 16$ ).

**Flowering phenology and insect visitation**—In 1999, the *T. ceratophorum* subpopulations Tc1 and Tc2 had lower inflorescence production ( $17.2 \pm 6.48$  plants/m<sup>2</sup> [means  $\pm$  95% CL] and  $18 \pm 6.36$  plants/m<sup>2</sup>, respectively, at peak bloom) than To1 ( $30.24 \pm 9.72$  plants/m<sup>2</sup>) and To2 ( $35.6 \pm 18.84$  plants/m<sup>2</sup>) subpopulations. Inflorescence density in *T. ceratophorum* subpopulations (Tc1,  $4 \pm 1.92$  plants/m<sup>2</sup> and Tc2,  $7.6 \pm 3.16$  plants/m<sup>2</sup>) and *T. officinale* (To1,  $16 \pm 6.88$  plants/m<sup>2</sup> and To2,  $17.2 \pm 11.84$  plants/m<sup>2</sup>) was observed in 2000. In both years flowering in *T. officinale* began before flowering in *T. ceratophorum*, but extensive overlap characterized the flowering phenologies of the two species (Fig. 2). *Taraxacum ceratophorum* (Tc1 and Tc2) and *T. officinale* (To2) subpopulations in close proximity had high overlap in flowering for 1999 and 2000 (Table 1), however the more distant To1 subpopu-

TABLE 1. Hurlbert's Index for overlap in flowering phonologies among all subpopulations of *Taraxacum ceratophorum* and *T. officinale*.

Species	<i>T. officinale</i>		<i>T. ceratophorum</i>	
	To1	To2	Tc1	Tc2
A) 1999				
<i>T. officinale</i>	To1	0.62	0.35	0.33
	To2		0.91	0.90
<i>T. ceratophorum</i>	Tc1			0.95
	Tc2			
B) 2000				
<i>T. officinale</i>	To1	0.77	0.24	0.60
	To2		0.76	0.93
<i>T. ceratophorum</i>	Tc1			0.84
	Tc2			

lation showed reduced synchrony with all other subpopulations (Table 1). These results indicate that temporal segregation does not limit heterospecific pollen transfer. Furthermore, inflorescences of *T. ceratophorum* and *T. officinale* were visited by similar arrays of insects in 1999, with 58% of visitor taxa found on both species. The insect visitors sampled from inflorescences of both *Taraxacum* species included: Diptera (Muscidae and Syrphidae), Hymenoptera (Apidae *Bombus* sp.), and Lepidoptera (Hesperiidae *Polites* sp., Nymphalidae *Occidryas* sp., Papilionidae *Parnassius* sp., Pieridae *Colias* sp.). Insects only found on *T. officinale* were Diptera (Anthomyiidae and Tachinidae) and Hymenoptera (Megachillidae and Tentredinidae), while Coleoptera (Melyridae) was only sampled from *T. ceratophorum*.

**Test for sexual reproduction**—Results from isozyme analysis of conspecific crosses demonstrate sexual reproduction in *T. ceratophorum*. Two FF × SS crosses produced heterozygous seedlings. Progeny of seven FS × SS crosses exhibited segregation ratios that conformed to the 1 : 1 expectation ( $N = 7$ ,  $G_T = 3.41$ ,  $df = 7$ ,  $P = 0.84$ ,  $G_H = 3.29$ ,  $df = 6$ ,  $P = 0.77$ ). Progeny from the single FS × FS cross exhibited a genotype ratio conforming to the 1 : 2 : 1 expectation ( $N = 1$ ,  $G_T = 0.66$ ,  $df = 2$ ,  $P = 0.72$ ). Results of the FF × SS and FS × SS crosses also indicate an absence of selfing (mentor effect) with compatible cross pollination. The interpretation of sexual reproduction via outcrossing is consistent with the diploid chromosome number of *T. ceratophorum* and with results from insect exclusion. When flowers were screened from insect visits, 23 of the 28 plants (82.1%) produced no seed, and the remaining five plants exhibited extremely low mean seed set ( $0.45 \pm 0.31\%$  [ $\pm 95\%$  CL]), suggesting a low level of pollen contamination. Because self-pollination results from reflexing stigmas, the absence of seed production in the insect exclusion treatment also suggests self-incompatibility.

**Test for pollen limitation on seed set rates**—Pollen addition significantly affected seed set in *T. ceratophorum* ( $F_{2, 72} = 12.50$ ,  $P \leq 0.0001$ ) (Fig. 3). Variation in seed set among subpopulations and in the effect of pollen addition among subpopulations (site by treatment interaction) were not significant ( $P = 0.22$ ). Planned comparisons revealed that plants receiving only conspecific hand pollination were not as fertile ( $46.2 \pm 10.6\%$ ) as plants open to insect visitation ( $70.1 \pm 8.4\%$ ) (control) ( $t = 3.12$ ,  $P = 0.0026$ ). The reduced seed set of hand-pollinated flowers is likely due to the low number of

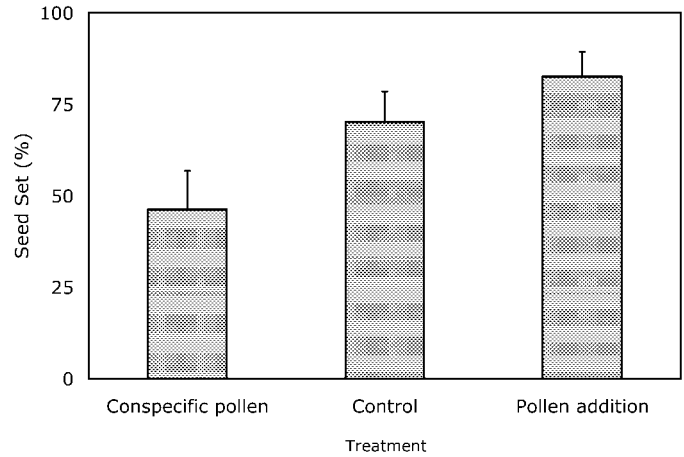


Fig. 3. Mean percent seed set (+95% confidence limits) in *Taraxacum ceratophorum* following experimental pollination treatments. Plants in the conspecific pollen treatment were caged (preventing insect visitation) and hand-pollinated using conspecific florets. The remaining two treatments were open to visiting insects; however, plants in the pollen addition treatment also received hand pollinations with conspecific pollen. Planned comparisons indicate that plants receiving conspecific pollination were less fertile than control plants ( $P = 0.0026$ ) and that pollen addition significantly increased seed set ( $P = 0.0527$ ).

donor florets (four) used. Pollen addition increased seed production by 17.8% compared to that of open controls ( $82.6 \pm 6.8\%$  vs.  $70.1 \pm 8.4\%$ ,  $t = 1.97$ ,  $P = 0.0527$ ), indicating pollen limitation of seed set (Fig. 3).

**Test for interspecific compatibility, seed viability, and parentage**—Interspecific crosses in 2001 yielded seed set of  $37.3 \pm 11.1\%$ . On average  $68.1 \pm 8.6\%$  of seeds planted in the greenhouse germinated. Subsequent analysis with species-specific microsatellite primers revealed that only  $33.2 \pm 10.6\%$  of the resultant seedlings were true hybrids. The remaining seedlings had *T. ceratophorum* genotypes consistent with self-fertilization by the maternal plant. Maternal families did not vary significantly in percentage of hybrid seed produced ( $\chi^2 = 18.31$ ,  $df = 17$ ,  $P = 0.37$ ).

## DISCUSSION

In the Colorado Rocky Mountains, heterospecific transfer of pollen between *T. officinale* and *T. ceratophorum* is not limited by habitat isolation, phenological isolation, or insect taxa visiting *Taraxacum* inflorescences. All populations of *Taraxacum ceratophorum* examined in this study reproduced sexually, and conspecific pollen addition significantly increased seed set, indicating ovule fertilization was pollen limited. Interspecific crosses, using apomictic *T. officinale* as a pollen donor, resulted in seed set that was comprised of a mixture of hybrid and selfed offspring genotypes. Taken together, these results suggest that initial conditions promoting hybridization occur in sympatric populations of sexual *T. ceratophorum* and apomictic *T. officinale*. Although the mentor effect from heterospecific pollen reduces the hybridization rate, evidence from other *Taraxacum* systems indicates that hybrids are frequently apomictic and fertile. If hybrids between *T. ceratophorum* and *T. officinale* also possess these characteristics, then asymmetrical introgression could eventually occur in *T. ceratophorum*.

Ecological conditions for the initial stages of genetic assimilation are met in populations of *Taraxacum ceratophorum* and *T. officinale*. Both spatial and phenological overlaps in flowering occur in this system. *Taraxacum officinale* co-occurs with *T. ceratophorum* at all sites surveyed in this study. Flowering phenology during 2 yr at Pennsylvania Mountain also showed considerable interspecific overlap. These findings indicate that spatial and temporal barriers will not limit heterospecific pollen transfer between *T. officinale* and *T. ceratophorum*. Furthermore, insect visitors to inflorescences of *T. ceratophorum* and *T. officinale* overlapped extensively and observations in mixed arrays of *T. ceratophorum* and *T. officinale* inflorescences indicated that insects (primarily dipterans) indiscriminately visit both *Taraxacum* species (Brock, 2003). *Taraxacum ceratophorum* and *T. officinale* produce morphologically similar florets (e.g., color and stylar pollen presentation) and inflorescences (e.g., height and diameter) and conform to the generalist pollination syndrome exhibited in the Asteraceae (Torres and Galetto, 2002) and genus *Taraxacum* (Mosquin, 1971). These factors favor the incidence of heterospecific pollen transfer.

Three lines of evidence demonstrate that *T. ceratophorum* reproduces sexually. Allele segregation at the MDH locus in progeny of known crosses conformed to predicted Mendelian ratios under outcrossing. The absence of seed production in the pollinator exclusion treatment also supports a sexual breeding system with self-incompatibility. Third, all populations surveyed in this study contained only diploid individuals ( $2n = 16$ ). Although sexual reproduction has occasionally been demonstrated in higher ploidy levels (Kirschner et al., 1994; Lyman and Ellstrand, 1998), all diploid *Taraxacum* species that have been tested are reported as sexual (Richards, 1973). Sexual dandelions are thought to be infrequent outside of Europe and Asia (Richards, 1973, 1986). However, other sexual dandelion species have been reported in North America (e.g., *T. californicum* and *T. pumilum*) (Richards, 1986; Lyman and Ellstrand, 1998).

Heterospecific pollination of *T. ceratophorum* with pollen from *T. officinale* resulted in moderate levels (37.3%) of seed production in 2001. Other reports of diploid–triploid interspecific crosses, in which entire capitula were used as pollen donors, yielded similar levels of seed set (mean 18–28%) (Richards, 1970; Morita et al., 1990a; Tas and van Dijk, 1999). The moderate to low seed production from interspecific diploid–triploid crosses is most likely due to inviability or reduced performance of aneuploid pollen at the prezygotic and/or postzygotic level (Richards, 1973, 1986; Tas and van Dijk, 1999).

Under greenhouse conditions, seed germination was high (68.1%) for seeds resulting from interspecific crosses. However, only 33% of seedlings were of hybrid origin, with the remaining seedlings derived from self-fertilization by the maternal *T. ceratophorum* plant. It should be noted that this is not a direct measurement of the interspecific hybridization rate, since seeds failing to germinate may be a nonrandom sample of the total. Maternal families did not vary significantly in the production of hybrid seed, suggesting that all of the maternal genotypes sampled are equally at risk of interspecific mating. Other studies on *Taraxacum* have also demonstrated high seed germination rates following hybridization (Richards, 1970; Morita et al., 1990a; Tas and van Dijk, 1999) and a mentor effect in triploid–diploid crosses (Morita et al., 1990a; Tas and van Dijk, 1999). However the proportion of true hybrids in the F1 generation is much higher in this study than

in studies of other sexual taxa (12.5% and 11%, Morita et al., 1990b; Tas and Van Dijk, 1999, respectively). It is possible that my hand pollination technique transferred more heterospecific pollen to *T. ceratophorum*, resulting in increases in both seed set and the proportion of hybrids in the F1 generation. Alternatively, euploid *T. officinale* pollen grains may have higher tube growth rates in *T. ceratophorum* styles, competing against conspecific pollen more effectively than in other diploid–triploid species crosses in *Taraxacum*.

Together these results indicate that sexual populations of the native *T. ceratophorum* may face the threat of genetic assimilation by the non-indigenous *T. officinale*. Several factors make this scenario possible. First, the breeding systems in this case allow for extreme asymmetrical introgression. Hybrids will only be formed in sexual *T. ceratophorum* and possible sexual hybrids, resulting in unidirectional interspecific gene flow. Second, *T. ceratophorum* exhibited pollen limitation of seed set in 1999, although the reduction in fertility is likely to vary among years. Because pollen competition is often cited as a barrier to hybridization (Arnold et al., 1993; Carney and Arnold, 1997; Wang and Cruzan, 1998), pollen-limited populations should experience reduced native–exotic competition for ovules favoring paternity of heterospecific donors. Third, although the mean hybridization rate with hand pollination was relatively low, breeding systems in hybrids can, depending upon their ploidy level, range from sexual to the more frequent apomictic (Richards, 1970; Morita et al., 1990a, b). Any apomictic hybrid individuals that become established could represent sources for large numbers of genetically identical hybrid offspring, inflating the future contribution of current hybridization to the gene pool.

On the other hand, the likelihood of genetic assimilation depends not only on early stages in the hybrid life cycle, but also on the establishment of fertile hybrid plants. Crosses between other diploid–triploid *Taraxacum* species have shown that, relative to sexual diploids, both sexual and apomictic F1 hybrids exhibit reductions in seed set (Tas and van Dijk, 1999; van Dijk et al., 1999). Yet despite reduced fertility, greenhouse-raised hybrids from sexual–apomictic crosses are commonly reported as vigorous (Richards, 1970; Morita et al., 1990b) and a recent study found that 82% ( $N = 225$ ) of plants with morphological characteristics of *T. officinale* sampled in Japan were hybrids between *T. officinale* and sexual Japanese dandelions (primarily *T. platycarpum*) (Shibaike et al., 2002 and references therein). Although these other studies suggest that *Taraxacum* hybrids are vigorous and fertile, these later stages in the life cycle of interspecific hybrids between *T. ceratophorum* and *T. officinale* must be investigated.

The net production of hybrid seed by *T. ceratophorum* would be higher if not for the mentor effect, which mitigates hybridization through self-fertilization. Although the mentor effect should theoretically reduce the rate of extinction due to hybridization (Wolf et al., 2001), self-fertilization in an otherwise obligately outcrossing species, like *T. ceratophorum*, results in inbreeding depression for the maternal plant (Daehler, 1999; Stephenson et al., 2000). As a result, *T. officinale* may not only be directly altering *T. ceratophorum* offspring through hybridization, but might also indirectly alter the genetic and demographic viability of native congeners by promoting inbreeding depression.

In simulating extinction due to hybridization, Wolf et al. (2001) and Huxel (1999) indicate that factors contributing to the asymmetry of the species relationship can lead to rapid

loss of the native species. In *Taraxacum*, asymmetrical hybridization, reduced prezygotic reproductive barriers, and potentially vigorous fertile hybrids may encourage the loss of the native gene pool. Similar genetic threats may face *Taraxacum californicum*, a federally endangered native species. Despite its uncommon aneuploid number of chromosomes ( $2n = 31$ ), this species also appears to be sexual and in frequent sympatry with *T. officinale* (Lyman and Ellstrand, 1998). Threats of hybridization with *T. officinale* are compounded by small population sizes of endemic species, which also contributes to an asymmetrical advantage for the exotic *T. officinale*. Taken together, these results illustrate that when exotics expand their range, native congeners face not only threats to demographic parameters but genetic threats as well.

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