Genetic variation and ecological differentiation between two southern Utah endemics: U.S. federally threatened *Townsendia aprica* and a closely related congener, *T. jonesii* var. *lutea* (Asteraceae: Astereae)

by

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Abstract

Townsendia aprica Welsh & Reveal (Asteraceae) is one of 17 rare plants in Utah receiving protection under the U. S. Federal Endangered Species Act. *Townsendia aprica* (last chance Townsendia) is an herbaceous perennial, narrow endemic, occurring along the Colorado Plateau in southeastern Utah. Recent discoveries have increased its known range from roughly 301 square kilometers at the time it was first listed to 4186 square kilometers. Many of these populations comprise fewer than 100 individuals. It was presumed that small population sizes together with geographical isolation would lead to low levels of genetic variation and inbreeding within the different populations, as a result of genetic drift and restricted gene dispersal. With the use of enzyme electrophoresis, nine informative loci were resolved to gain insight into the level and pattern of genetic variation within *T. aprica's* range. These patterns were compared with those of another narrow endemic that is a close congener, *Townsendia jonesii* var. *lutea.* Analysis of inter- and intra-taxon genetic distances for *T. aprica* and *T. jonesii* var. *lutea* provides important insights into the consequences of rarity, as well as into the genetic and ecological distinctness of the two narrowly endemic taxa. The two taxa have very similar, relatively high values estimated for genetic variation parameters based on isozyme analysis of nine loci (%P > 70, A > 2.0, and $H_e \geq 0.30$), with much of the genetic variation being found within populations ($F_{ST} = 0.060$ for *T. jonesii* var. *lutea* and $F_{ST} = 0.100$ for *T. aprica*). No significant inbreeding found was in most populations of either taxon. Forty percent of the variation in genetic distance between populations of *T. jonesii* var. *lutea* could be explained by geographical distance, where as only about 10% of genetic variation in *T. aprica*'s could be explained by physical distance. Bottlenecks were detected in more than half of each taxon's populations. The results also reveal that populations of each taxon are more similar to one another ($F_{PT} = 0.14$), than to populations of the other taxon ($F_{ST} = 0.21$). Significant differences

were found in characteristics of the soils on which the two taxa occur, though so far, all measurable morphological characters are overlapping in these two taxa.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Chapter 1 Introduction

1.1 Rarity, Endemism and Utah

Roughly 250,000 plant species are thought to have existed in historical times, with almost 1000 becoming extinct in the last century, and there are estimates that sixty times that many could go extinct in the next fifty years (Raven, 1987). The International Union for Conservation for Nature and Natural Resources (IUCN) has been assessing the conservation status of species, subspecies and varieties on a global scale for the past four decades. The IUCN *Red List of Threatened Plants* has documented a 40% increase in the past decade of threatened plant species in North America (Walter and Gillett, 1998; Species, 2004). Utah's flora ranks as one of the top five among continental states in abundance of rare plant species and has one of the highest levels of endemism in North America (Welsh et al., 1975). It has been estimated that 225 to 240 plant species are endemic in Utah, with 200 considered threatened (Welsh et al., 1975; Shultz, 1993). *Townsendia aprica* Welsh & Reveal is one of 17 rare plant species in Utah that has been listed and given protection under the U. S. Federal Endangered Species Act.

More than 10% of the Utah's flora is endemic to the state, with the southeastern part of Utah, occupied by the Colorado Plateau, containing 50% of Utah's endemic flora (Shultz, 1993). It is speculated that the majority of these rare and endemic species are concentrated in this region of Utah because species have diverged and speciated in response to environmental stresses in this arid desert habitat (Shultz, 1993). Stebbins (1952) concluded that topography and soil features will have a greater effect on the character of vegetation where moisture is limiting, when compared to where water is in adequate supply. Shultz (1993) also pointed to similar factors to explain much of the diversity and the high occurrence of endemics found in Utah. Specifically, she points to habitat diversity of the Colorado Plateau region, with its islands of unique soil types, the severely arid climate, averaging 115-230 mm rain per year, and what is described as

the occurrence of contact zones between six phytogeographical regions, including that between the Great Basin and the Colorado Plateau which runs directly through the middle of the state.

There are many ways to define rarity. Specifically, rarity can describe a situation where few individuals are found, where a species is found in a very localized, area, or where the species only occurs in a rare habitat (Rabinowitz et al., 1986). Species may be rare either because they have a historically small range, or rare species may be remnants of much more widespread species (Welsh et al., 1975). In either case, threatened species are somehow not able to expand their numbers establishing new populations beyond current boundaries with possibly even declining numbers. Failure to expand beyond the current range could be due to limited seed dispersal, or lack of appropriate genetic variation to allow adaptation to new environments, perhaps because of a recent bottleneck.

Holsinger and Gottlieb report that the average rare and endangered species is usually defined as having few (1000-5000) total individuals, fewer than five populations, with many populations reduced by habitat loss (Fiedler, 1987), and inability to inhabit neighboring ecosystems (Drury, 1974). Drury (1974) gives the definition of a rare species as having small populations that are widely separated with interbreeding being seriously reduced between the subpopulations, or restricted to one population. Drury also explains that there are two types of extreme adaptive strategies seen commonly among plants and animals, either having a competitive or physiological means of advantage. The competitive strategy can be defined as the capacity for successful competition against other species at the sacrifice of the ability to handle extreme habitats. In the physiological strategy, individuals are able to withstand extreme habitat conditions at the expense of a competitive edge with other species under more favorable conditions.

Because of small range size and low numbers of populations and individuals, rare species are potentially at greater risk of extinction should they become threatened. The International

Union for the Conservation of Nature and Natural Resources (IUCN) *Red Book* (Nature, 1966) defines a threatened species as still abundant in parts of its range, but in threat due to declining numbers. Threats may include new competition (e.g. with non-native species), new pathogen or pest, habitat disturbance or loss (natural or anthropogenic), loss of pollinators or seed dispersers, small population size (<100), and low or fixed genetic diversity. Some of these factors have been identified as possible threats to the persistence of *T. aprica* populations. Because endemic species already have the physiological mechanisms in place to survive in Utah's desert conditions, most do not have to deal with competition of non-native species, which do not tend to persist under these conditions (Welsh et al., 1975; Shultz, 1993). Although there have been no studies of new pathogens or pests in *T. aprica*, there have also been no observations to suggest that these currently pose a threat to *T. aprica*. Habitat disturbance, both natural and anthropogenic, has been a noted factor in different reports about *T. aprica* (Welsh, 1978; Service, 1985; U. S. Fish and Wildlife Service, 1993; College, 1994; Robinson, 2002, 2003) and many of the other endemic species of Utah (Welsh et al., 1975). Specifically, industrial (mining), agricultural (cattle), and recreational activities have been noted as being a threat to *T. aprica*'s long term survival. Fluctuations in population size have been observed in this species, with some populations extirpated, have been attributed to environmental fluctuations, mainly in precipitation (Armstrong and Thorne, 1991; Robinson, 2002, 2003). And though no data have been collected, it has been suggested that *T. aprica* may have difficulty attracting density-dependent, nutrient-demanding pollinators, like bees (Tepedino et al., 2004). *Townsendia aprica* is known to have many populations with fewer than 100 individuals, which fluctuate in size. The effects of small population size on genetic variation have not been specifically been examined in this species.

1.2 Conservation Genetics

It is widely accepted that demographic instability is the most important factor affecting the long term survivorship of a rare species (Lande and Shannon, 1996). Because of the limited resources available for protecting federally endangered species, the value of conservation genetic data has been questioned in the past, but more recent reviews have shown how conservation genetics can be very useful in understanding rare species and improving management strategies for conserving a species (Ellstrand and Elam, 1993; Holsinger et al., 1999; Soltis and Gitzendanner, 1999; Amos and Balmford, 2001). Holsinger *et al.* (1999) explained how genetic assimilation via hybridization can pose a threat to a species' long-term persistence if there has been a critical loss of self-incompatibility alleles. Soltis and Gitzendanner (1999) agree that genetics can be used to detect the effects of hybridization on a rare species, but also argue that molecular systematics and population genetics can be used for clarification of species boundaries and identification of important lineages. Ellstrand and Elam (1993) show that small populations of rare plant species are at genetic risk from the effects of genetic drift and inbreeding. The risks of lower genetic variability, both through lowering fitness by inbreeding depression and through the loss of adaptive potential in the event of a severe bottleneck, have also been noted (Amos and Balmford, 2001).

Population size, the degree of isolation by pollination and seed dispersal mechanisms, geographic isolation, and bottlenecks are all traits that can affect the genetic diversity found within populations of a species (Richardson, 1986). Gene flow limitations can occur if a pollinator declines or is lost, due to human activities such as pesticide use or even a loss of secondary plant species facilitating the pollinator interaction (Thomson, 1978; Tepedino, 1979; Rathcke, 1983; Sipes and Tepedino, 1993). As Wright (1931) showed, the effect of random genetic drift and fixation of alleles occurs more quickly in smaller populations (less than 100

individuals). Stebbins (1942) also discussed the correlation between genetic variation within populations and the rarity or commonness of a plant species. He suggested that rare plants are genetically depauperate, though he later qualified this idea in his 1980 paper, citing examples of highly genetically variable rare species, and widespread species with low genetic variation (Stebbins, 1980; Gitzendanner and Soltis, 2000). Even so, many papers comparing average genetic variability in rare and widespread species have shown lower genetic variation in rare or endemic plants when compared with widespread species (Hamrick and Godt, 1989; Hamrick et al., 1991; Gitzendanner and Soltis, 2000; Cole, 2003). To better understand patterns of genetic variation in widespread and rare taxa, the current emphasis is on comparing close relatives, so that factors such as breeding system, dispersal mechanisms and phylogeny can be taken into account when comparing the two types of species (Karron, 1987; Gitzendanner and Soltis, 2000). Some more recent studies have concentrated on comparing genetic diversity of closely related endemics (Lopez-Pujol et al., 2001; McCraken, 2001) as I have done here.

Conservation studies are increasingly using genetics to better understand patterns of variation in endemic, rare or endangered plant species, which has helped inform government agency resource managers so that limited resources are used wisely. Two recent examples are studies of the narrow endemic species, *Artemisia molinieri* (Asteraceae) in southwestern Europe (Torrell et al., 1999) and one of the only known population of *Eriogonum ovalifolium* var. *williamsiae* (Polygonaceae) in Nevada (Archibald et al., 2001). In both studies, the species were found to have high genetic variability when compared to typical values for endemic species presented in Hamrick and Godt (1989). Because genetic diversity is often associated with long term fitness and adaptability of a species, finding high genetic variability in an endemic, rare or federally listed species will lead researchers to conclude that the species is not at genetic risk, as concluded in the above two cases. This enables management to concentrate resources and efforts

toward protecting the species in other ways such as limiting habitat destruction and increasing population sizes with translocations.

As pointed out by Holsinger and Gottlieb (1991), a key priority and first step in conservation of a rare species is knowledge of its breeding system. The first step towards understanding variation within *T. aprica* was to determine whether all populations are diploid, and have an outcrossing breeding system. This outcome would affect the assumptions of Hardy-Weinberg equilibrium (HWE), and would potentially affect the conservation status of populations because apomictic species are not necessarily eligible for federal protection under the US Endangered Species Act. With the use of isozyme electrophoresis, it is possible to detect recent polyploidy using observations of duplicate loci and different staining intensities of banding patterns (Gottlieb, 1982; Wendel and Weeden, 1989; Acquaah, 1992). It is also possible to detect an apomictic breeding system by observing fixed differences between homologous loci, so called "fixed heterozygosity" (Soltis and Rieseberg, 1986; Noyes and Soltis, 1996; Richards, 1997; Brown and Young, 2000). In *Erigeron compositus* (Noyes and Soltis, 1996) many individuals displayed identical multilocus genotypes, where a single heterozygous enzyme phenotype was found in most or all members of an apomictic population, while in diploid populations both alleles segregated and were found in heterozygous or homozygous states.

With the use of isozymes, I conducted an investigation into the distribution and patterns of genetic variation found within the geographical range of *T. aprica*. The results of this study will enable the United States Fish and Wildlife Service to manage the individual populations more carefully by taking into account which, if any, populations are apomictic, which populations are exchanging genetic information, and with so many small *T. aprica* populations, which ones would need to be monitored more closely if inbreeding is observed. This genetic information will also be useful in the implementation of any recovery plan for the translocation of *T. aprica* individuals into existing populations and for establishment of any new populations.

1.3 *Townsendia*

Townsendia was first described with one species, *T. sericea*, by William Jackson Hooker in 1834 (Larsen, 1927). The genus currently consists of about 30 taxa that occur in western North America, from the southern Yukon to central Mexico (Beaman, 1957; Welsh and Reveal, 1968). Utah represents an area of very high diversity for *Townsendia*, with sixteen native taxa, four of which are endemic to the state (Welsh, 1983; Welsh et al., 1987). John Beaman published a monograph of *Townsendia* (1957) that established much of the current taxonomic framework for the genus, and in which he shared his plethora of knowledge of *Townsendia*'s breeding system, distribution, morphology, and proposed relationships of the taxa. Two features of the genus are particularly relevant to this thesis.

First, *Townsendia* includes both sexual and asexual populations, with no apomictic diploids. Asexual reproduction in *Townsendia* occurs by diplosporous apomixis, in which the embryo develops parthenogenetically from an unreduced egg cell. Sexual diploids and asexual polyploids have been found in different populations of nearly half of the species of *Townsendia*, with the remaining species only exhibiting sexual diploids. Diploid *Townsendia* have 2n=2x=18 and apomictic plants are mostly tetraploids but some triploids and aneuploids are also found (2n = 27-36) (Beaman, 1954, 1957); Windham, unpublished).

Based on morphology and geographic distribution patterns, Beaman constructed an informal phylogenetic tree for *Townsendia* (Figure 1.1) and pointed out that apomictic taxa tend to be acaulescent (lacking a well developed stem, i.e. growing as a dense tuft of leaves), long-lived perennials. He also states that "without exception, the apomicts of *Townsendia* are concentrated at the higher elevations and higher latitudes relative to the total range of the species in which they occur" (Beaman 1957, p. 51).

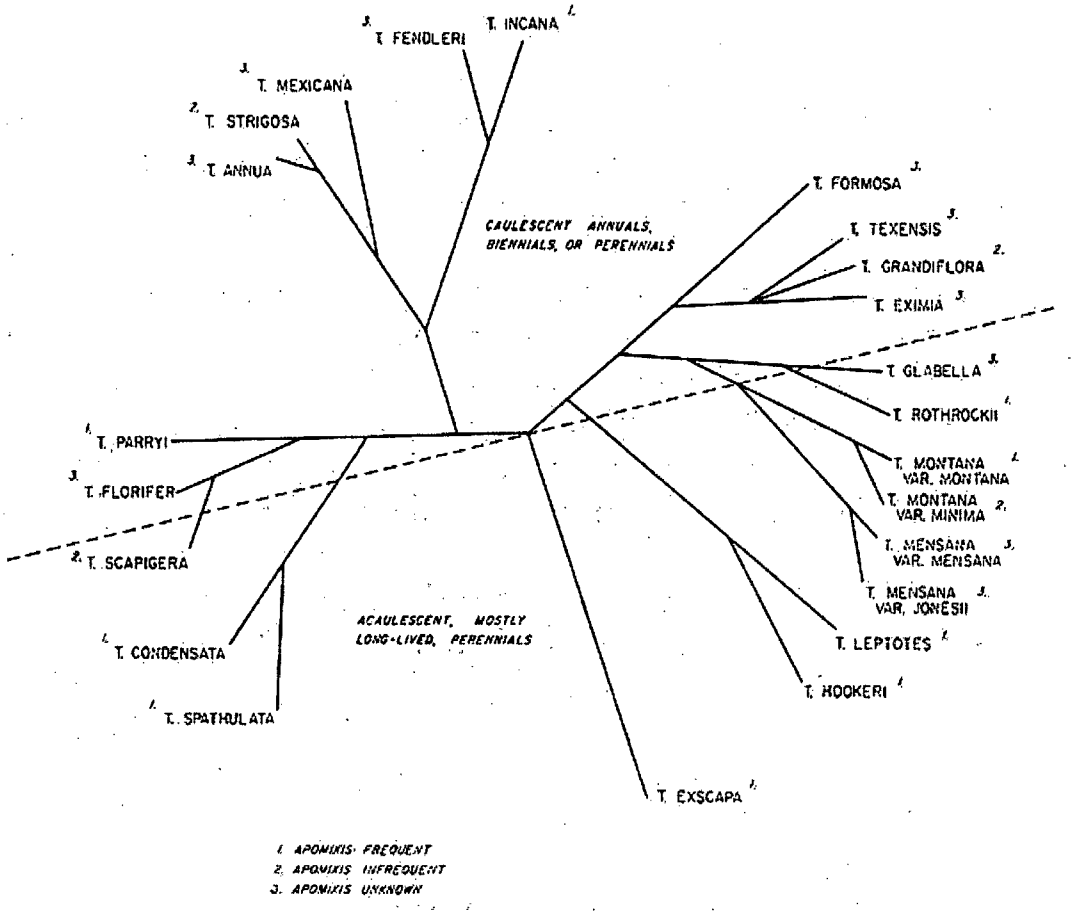

Figure 1.1. Phylogenetic relationships of the species of *Townsendia* and a summary of habit and the presence of apomixis in the species. From Beaman, 1957.

The second feature worth noting is that Beaman and others (Welsh et al., 1975; Welsh et al., 1987; Shultz, 1993) have stated that many taxa within *Townsendia* appear to be restricted to specific soil types. For example, *T. smithii* and *T. gypsophila* are restricted to soils that contain substantial amounts of gypsum (calcium sulfate). Beaman speculated that patterns of speciation in the genus have likely been influenced by edaphic factors, as well as by geographic barriers and climatic stresses. Narrow endemism is a common feature of species within *Townsendia*, and Beaman considered only three species to be widespread geographically (*T. excapa*, *T. florifer*, *T. incana*). Beaman also postulated a relatively recent divergence of the species, influenced by the retreat of Pleistocene glaciers, which lead to areas becoming available for rapid migration and the evolution of new types with the addition of isolation and time.

1.4 *Townsendia aprica* and *T. jonesii* var. *lutea*

Townsendia aprica was listed as a federally threatened species in 1985. This was due to *T. aprica*'s narrow geographical range of 301 km^2, from as few as 15 populations ranging from a reported 6-2000 individuals per population (very rough estimate), and compounded by the potential mining hazard and cattle activity threatening 95% of *T. aprica* populations (U. S. Fish and Wildlife Service, 1993). *Townsendia jonesii* (Beaman) Reveal var. *lutea* Welsh, which is also a narrow endemic in Utah is not eligible for federal protection because it does not have full species status. This is a grave concern because the taxon has a more restricted distribution, fewer populations and individuals, and has many populations that are threatened by active mining operations. As shown below, because of the shared feature of narrow endemism and the evidence of a close relationship between the taxa, they form a logical pair for inclusion in a joint study of conservation genetics.

Townsendia aprica had not been discovered when John Beaman conducted his studies. This inconspicuous, early flowering taxon appears to have been first collected by Welsh and Reveal in 1966. *Townsendia aprica* was later described by Welsh and Reveal (Welsh and Reveal, 1968) after the generic placement of the taxon was verified by Arthur Cronquist, John Beaman, and Arthur Holmgren. These experts were consulted in part because this new taxon had yellow ray ligules, a condition not previously known in *Townsendia*.

Townsendia aprica (last chance townsendia) is an herbaceous perennial, endemic to the Colorado Plateau region of southeastern Utah and has a narrow distribution of 4,186 km^2 which spans three counties in Utah (Sevier, Emery and Wayne; Figure 1.2). *Townsendia aprica* is acaulescent and grows woody stems with age, which may indicate that it is a long-lived perennial (Hamrick et al., 1991) although no lifespan studies have been done. No published chromosome counts exist for *T. aprica*, although a recent breeding system study of three populations determined that these populations are sexual (Tepedino et al., 2004) and thus likely diploid. Most

populations of *T. aprica* occur at middle elevations (1800m – 2350m), with one single, isolated, high elevation population (2500m) whose breeding system has not been characterized.

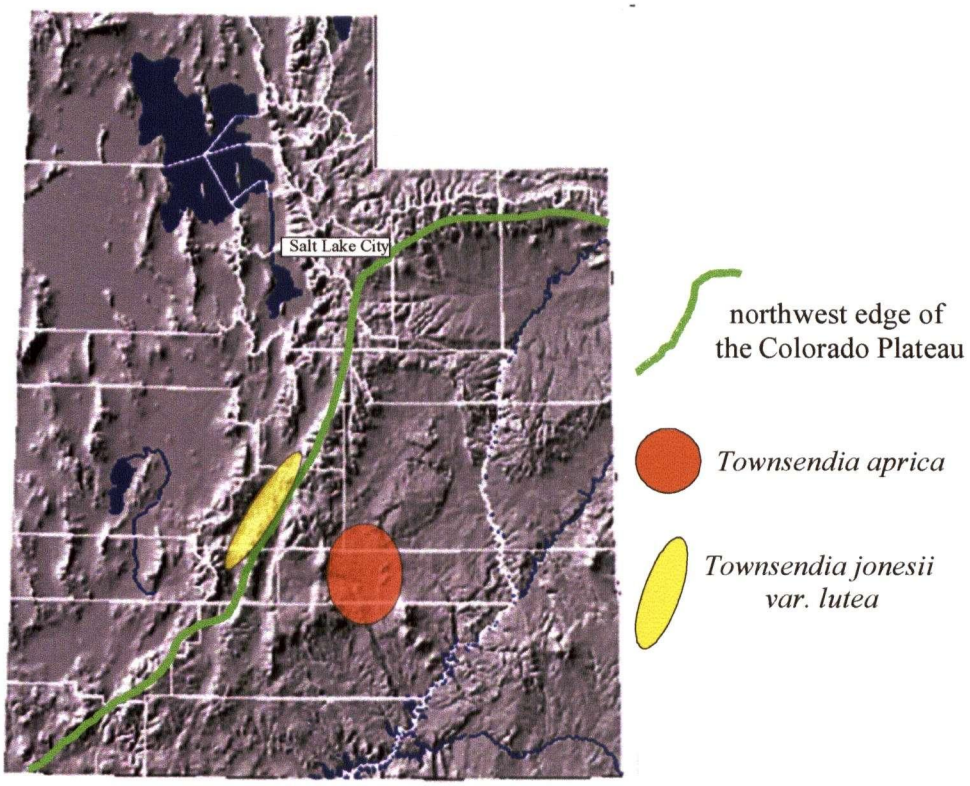

Figure 1.2. Map of Utah showing the geographic range of *T. aprica* and *T. jonesii* var. *lutea* in relation to the northwest boundary of the Colorado Plateau.

In their 1968 paper, Welsh and Reveal proposed that two taxa, *T. montana* specifically var. *minima* (recognized now as *T. minima* Eastw.) and *T. mensana* var. *jonesii* (classified now as *T. jonesii* var. *lutea*, *T. jonesii* var. *tumulosa*, and *T. jonesii* var. *jonesii*), were most closely allied to *T. aprica*. *Townsendia montana* differs from *T. aprica* by its larger size and less pulvinate (cushion-like) habit, longer and wider leaves, ray color (white or purple), and longer and less densely pubescent achenes. Ecologically, *T. minima* was described as being found only on red sandy soils, whereas *T. aprica* was thought to be restricted to heavy clay soils.

Townsendia jonesii differs from *T. aprica* in a less densely pulvinate habit, with a more open and spreading growth form, with somewhat larger heads having peduncles (stalk of inflorescences), and longer leaves, with less densely strigose (apprised hairs all in the same

direction) leaf bases. Interestingly, what was supposed to be the key character defining *T. aprica*, its unique yellow ligules, was later found to occur in the newly discovered *T. jonesii* var. *lutea* (Welsh, 1983b). This new variety was described in part from material originally assigned to *T. aprica*, but the long pappus of the ray florets (2-4.5 mm) suggested a closer affinity to the remainder of *T. jonesii*. This new taxon has an even more narrow distribution (311 km^2) than *T. aprica* and although the two taxa occur in nearby regions, their distributions are non-overlapping (Figure 1.2). *Townsendia jonesii* var. *lutea* is known from Sevier, Piute and possibly Juab counties, where it occurs most commonly on Arapian shale containing commercial deposits of gypsum.

As more surveys of both *T. aprica* and *T. jonesii* var. *lutea* have uncovered more populations of the two taxa, it has become apparent that the ligule colors in both taxa can range from white, to cream to yellow (Armstrong and Thorne, 1991; Clark, 1999, 2000, 2001). Welsh (1987; Welsh et al., 1993) uses yellow ray color in combination with ray pappus length to split these two taxa, even though both color and pappus length seem to overlap. The ray pappus length measurement for *T. aprica* has been recorded in the range of 0.7-1mm (Welsh et al., 1987; Welsh et al., 1993), with the latest reported measurements, based on many more individuals, given as between 0.5-2.5mm (Armstrong and Thorne, 1991). Pappus length in *T. jonesii* var. *lutea* reportedly ranges from 2-4.5mm (Welsh and Reveal, 1968; Welsh et al., 1987; Armstrong and Thorne, 1991; Welsh et al., 1993). Despite the overlap in these features, even the most recent *Townsendia* keys (Welsh et al., 1987; Welsh et al., 1993) use yellow ray color in combination with ray pappus length to distinguish *T. aprica* and *T. jonesii*.

A close relationship between *T. aprica* and *T. jonesii* var. *lutea* has more recently been confirmed through molecular phylogenetic studies of *Townsendia* based on ITS and ETS sequencing (Thompson *et. al.*, unpublished). Based on sequences from single individuals of *T. aprica* and the three varieties of *T. jonesii*, it is clear that the four taxa are very closely related.

Although there are substitutions that distinguish the ITS sequences of *T. aprica* from those of *T. jonesii*, whether these are shared among populations of the individual taxa is not currently known. It does, however, appear that *Townsendia aprica* and the three *T. jonesii* varieties form a moderately well-supported monophyletic sister group to one other Utah endemic, the more northern *T. mensana* (formally *T. mensana* var. *mensana*). Surprisingly, the other Utah endemic thought to be closely related to *T. aprica*, *T. minima*, does not appear to be closely related to this group. Interestingly, Beaman (1957) shows unknown apomixis in *T. mensana* var. *mensana* and *T. mensana* var. *jonesii*, but does show infrequent apomixis in *T. minima* (formally *T. montana* var. *minima*) (Figure 1.1). It is hoped that sequencing of additional regions for multiple populations of the *T. aprica-T. jonesii* clade, currently underway, will further resolve species boundaries and the relationships in this group.

With so much evidence of a close relationship between *T. aprica* and *T. jonesii* var. *lutea*, their overlapping flowering times and close geographical ranges, it was decided that *T. jonesii* var. *lutea* would be the best taxon to use for a genetic comparison with *T. aprica*. In addition, comparisons of *T. aprica* and *T. jonesii* var. *lutea* might aid in clarifying whether these two taxa are genetically distinct.

Previous Studies of *Townsendia aprica*

Owing to its designation as a threatened species, *T. aprica* has been the subject of a number of surveys and studies aimed at better understanding the biology of this species. *Townsendia aprica* grows on land administered by four different U.S. government agencies [(Bureau of Land Management (BLM), National Forest Service (NFS), National Park Service (NPS), and State Lands (SL)] who have carried out population monitoring studies and abiotic impact studies aimed at providing better protection for *T. aprica*.

A breeding system study was carried out by the USDA Forest Service on *T. aprica* (Tepedino et al., 2004). They experimentally determined the breeding system of three *T. aprica*

populations by testing for autogamy and apomixis by excluding pollinators, for geitonogamy (selfing) by pollinating receptive stigmas with fresh pollen from the same plant, and for xenogamy (outcrossing) by pollinating receptive stigmas with fresh pollen from a different plant. Based on the lack of seed when pollinators are excluded or florets are self-pollinated, they concluded that the individuals tested are neither apomictic nor self compatible but show an obligate outcrossing breeding system. The authors of this study report seeing mostly native solitary bees, such as *Osmia,* and ground-nesting species like *Synhalonia fulvitarsis*, nesting among *T. aprica* plants.

Over a 5 year period, the Utah Bureau of Land Management (BLM) monitored one plot of *T. aprica* at the type locality population (in this study classified as 9-TL) to observe fluctuations in survivorship of existing individuals and recruitment of seedlings. The populations were considered stable from 1996-1999, with a sharp decline in seedling and adult survivorship during 2000-2001, which was attributed to drought followed by an extended winter (Armstrong, pers. com.).

During this period, soil samples were also collected and soil composition and levels of micro and macronutrients were described from 17 sites and compared with soils data from five *T. jonesii* var. *lutea* sites (Armstrong and Thorne, 1991). Armstrong found that sites of the two taxa share many overlapping soil characteristics, with some large differences in %sand, %clay, $\%CaCo_3$, and %gypsum (Armstrong and Thorne, 1991). My project included a continuation of this study with additional data gathered from the southern *T. aprica* populations that were not included in the first study because they were unknown at the time or not on BLM land.

Another population monitoring project took place in 2002 and 2003 (Robinson, 2002, 2003) in which previously known northern sites of *T. aprica* were revisited over a two year period to see if they were extant and to look for evidence of population size fluctuations from year to year. The study found a 40% decrease in the number of standing populations from

previously documented sites (many at the edges of the northern range), with many of the known sites containing fewer individuals in 2003 than in the previous year. The conclusion, though not explicitly tested, was that heavy drought conditions during the two year study contributed to population decline.

Site visit reports were done for three years by CARE (Capitol Reef National Park Survey Team) (Clark, 1999, 2000, 2001) and during this time, four new populations (1-4 in this study) were found, extending *T. aprica*'s southern distribution limit by 20 km. The team raised concerns that these new populations showed different morphological and edaphic characteristics than the northern populations, and therefore sought out expertise to help confirm their identity.

With many new populations being found since its federal listing, representatives of Federal Fish and Wildlife were proposing to delist *T. aprica* if these new discoveries helped fulfill the criteria for minimal viable population size set out in *T. aprica*'s recovery plan (U. S. Fish and Wildlife Service, 1993). With the discovery of the southern populations, and the proposal for delisting, representatives of the various management agencies were eager to see a genetic study of *T. aprica* undertaken, and this was one of the reasons that management agencies cooperated by providing support and granting permits for this thesis.

Even if there were enough populations and individuals to propose delisting of *T. aprica*, conservation genetics can inform the managers about other factors that may cause species rarity to turn into species extinction. Genetic information will also be useful in the implementation of any recovery plan for the population augmentation of *T. aprica* individuals into existing populations and for any new establishment of populations. Knowledge of the pattern of among population variation will also inform strategies based on levels of genotypic and allelic diversity shared among the populations and will show how much those populations contribute to the species' diversity as a whole.

1.5 Objectives

The main objective of this study, as with many studies of rare endemics, was to estimate the level and pattern of genetic variation and inbreeding in *T. aprica*. Isozyme electrophoresis was employed to determine the spatial distribution of genetic variation among and within populations sampled throughout *T. aprica*'s known range. The genetic parameters obtained for *T. aprica* were compared to one sub-specific variant of its closest known congener, *T. jonesii* var. *lutea*, as well as to average levels found in other studies of rare endemic plants (Hamrick and Godt, 1989; Karron, 1991; Bayer, 1992; Gitzendanner and Soltis, 2000). With fewer than 15 known populations of *T. aprica*, many isolated from one another and containing fewer than 100 individuals, the expectation was of finding low overall genetic variation and inbreeding within populations of both taxa.

A number of *Townsendia* species have variable breeding systems, including both sexual diploid and apomictic polyploid populations, but the U.S. Endangered Species Act does not extend protection to apomictic species (United States, 1972) and would have to reconsider protection if this listed species was found to be exclusively apomictic. One of the first goals of this study was to confirm that the known populations of *T. aprica* are sexual outcrossers. By including experimentally confirmed outcrossing populations of *T. aprica* (Tepedino et al., 2004) as well as uncharacterized populations in this electrophoretic study, I will be able to make inferences about the breeding system of the uncharacterized populations.

The third goal of this study is to better understand any distinguishing differences and the degree of relatedness between *T. aprica* and *T. jonesii var. lutea* by comparing inter- and intra-taxon genetic distances, comparing their soil types, as well as determining whether pappus length is really overlapping in the two taxa.

Chapter 2 Materials and Methods

2.1 Field Sampling

Population localities for *T. aprica* were released through herbarium records from Utah's Natural Heritage Program that are deposited at BRY, NYBG, SJC, CRNP, OFSH (Table I.1-Appendix I). First-hand knowledge of *T. aprica* population locations, based on field surveys by different government agencies, was obtained from Debbie Clark, partner of Capitol Reef National Park. Populations of *Townsendia aprica* have patchy distributions and are isolated from one another throughout its range, and individuals within populations have clustered distributions. These populations were defined by D. Clark based on the assumption that the individuals at a given site are in close enough proximity to form an interbreeding group.

All *T. aprica* herbarium vouchers, as well as some representatives of *T. jonesii* var. *lutea*, were loaned to The University of British Columbia (UBC) Herbarium. These herbarium vouchers were used to examine morphological differences, as well as for pollen counts and pappus measurements.

In April of 2001 and May of 2002, samples were collected from 13 populations in three counties (Sevier, Wayne, Emery) representing the known range of *T. aprica* (Figure 2.1). We collected 25-30 individuals per sampled population, except for two populations, where too few individuals were found (Table 2.1). From each individual plant, 7-10 leaves and ten seeds were collected, along with measurements of whole plant diameter, number of rosettes, number of buds, number of flowering heads, number of seed heads, and ray ligule color, if available. Under the terms of the permit, I was not allowed to kill any *T. aprica* in the course of this study. This limitation included collection of herbarium vouchers, as well as progeny that might be raised from seed for a breeding system study or chromosome counts. Photographs of the habit and habitat for each taxa were taken, along with GPS coordinates.

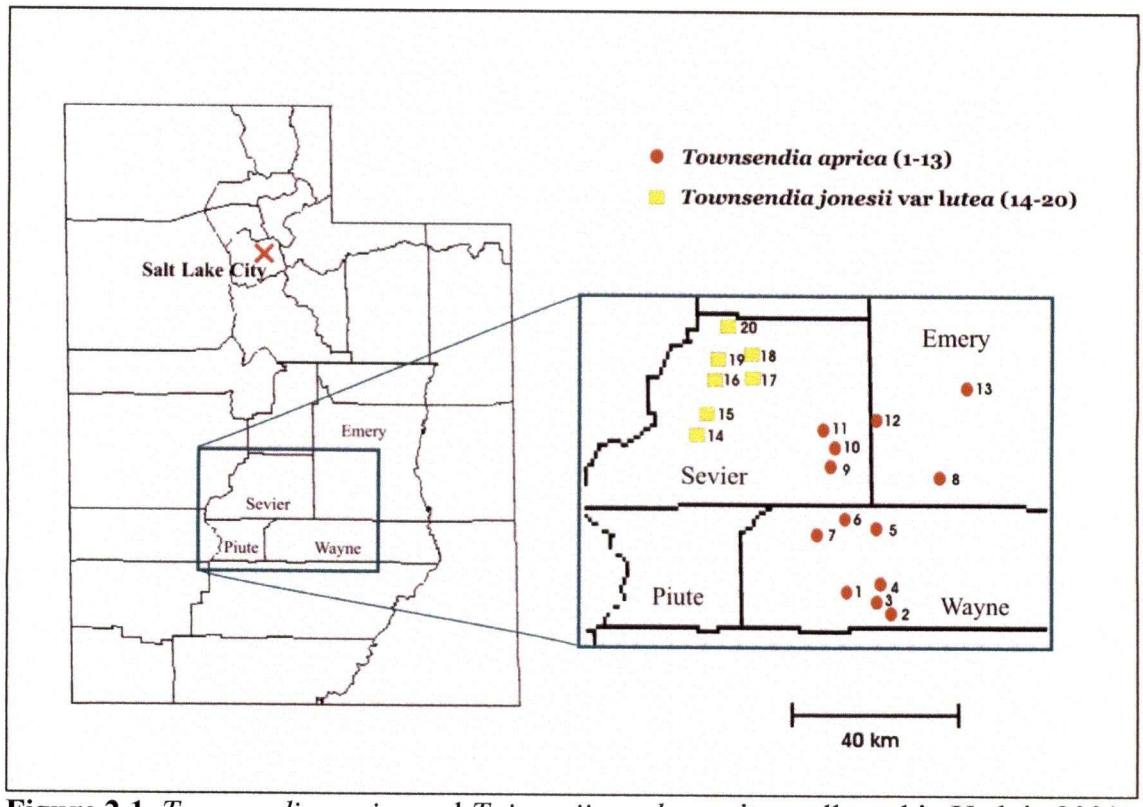

Figure 2.1. *Townsendia aprica* and *T. jonesii* var. *lutea* sites collected in Utah in 2001 and 2002.

During the 2001 and 2002 field seasons, *T. jonesii* var. *lutea* was also collected at eight sites, all in Sevier County (Table 2.2). Although *T. jonesii* var. *lutea* cannot be listed for protection due to its infraspecific taxonomic status, the same collecting guidelines used for *T. aprica* were followed for this rare, endemic variety to minimize any impact on these populations. When larger plants were found, extra leaves were collected to use for screening and optimizing isozymes protocols. The morphological measurements and descriptive characters noted for *T. aprica* were not documented for *T jonesii* var. *lutea* due to time constraints.

It should be noted that the geographic ranges of the two taxa are markedly different. The geographic range of *Townsendia aprica* is 82km from north to south and 43km from east to west with the closest pair of sampled populations being 3km apart (11-FJ & 12-FJR) and the furthest, 82km apart (2-MMD & 13-MCR) (Table II.1-Appendix II). The range for *Townsendia jonesii* var. *lutea* is 33km from north to south and 13km from east to west. The closest neighboring

populations sampled were <1 km apart (18-TME & 19-TMW) while the most distant were 33km apart (14-GH & 20-WC) (Table II.2-Appendix II).

Once collected, leaves were placed in individual cryogenic vials and held on ice for no less than one hour, until transferred to a portable liquid nitrogen tank while in the field. Upon return to UBC, the vials where transferred into a -80^{O}C freezer until they were analyzed.

Population density estimates were obtained for *T. aprica* by counting the number of individuals within a randomly chosen 10m x10m string quadrat (divided into four 5m x 5m plots) and then averaging the four counts from each sampled site (Table 2.1) (Kent and Ciocker, 1994).

In 2001, soil collections were made at six *T. aprica* sites and two *T. jonesii* var. *lutea* sites (Table 2.1 and Table 2.2). This was done to further an earlier survey by Armstrong and Thorne (1991), aimed at determining whether the two taxa occur on distinct soil types. Roughly two hundred grams of soil was collected from two spots at each site and deposited into a clean plastic bag with non-metal closures, sealed to avoid contamination and brought back to UBC for storage at room temperature in the Whitton lab.

Table 2.1. *Townsendia aprica* localities and collection information for this study, along with additional information available for each population.

Pop. number	Pop. acronym	Population name	Latitude & Longitude	Location	County	Elevation (m)	Collection year	Sample size	Soil samples	PC/BS/ CC/DNA	estimated density (100 m^2)	estimated size
1	MCC	Mini- Cockscomb	38° 15.165' N 111 ° 24.556' W	S. on Hwy 12, turn W. on road to Teasdale, Cockscomb	Wayne	2166	2001	21	J	PC	46	<100.
2	MMD	Miners Mt. Dixie	38° 11.399' N 111 ° 16.568' W	S. on Hwy 12, turn E. on to dirt road (Miners Mt. Rd.), drive S. 4.5 air miles to population on hill	Wayne	2330	2001	27	J		15	<100
3	WHF	Wide Hollow Fork	38° 13.208' N 111 ° 19.257' W	S. on Hwy 12, turn E. on to dirt road (Miners Mt. Road.), drive S. for 2 air miles then N. for 2 air miles	Wayne	2354	2001	27	J		22	>100
4	JM	Johnson's Mesa	38° 15.012' N 111 ° 17.586' W	S. on Hwy 12, turn E. on to dirt road (Miners Mt. Rd.), drive S. for 2 air miles then N. for 6 air miles	Wayne	2167	2002	27		PC	13	>100
5	HNE	Hartnet East	38° 27.237' N 111 ° 16.005' W	E. on Hwy 24, turn N. on dirt road, cross river into Hartnet, drive for 30 miles, population on mound	Wayne	1824	2001	25	J		17	<100
6	CV	Cathedral Valley	38° 28.354' N 111 ° 21.736' W	E. on Hwy 24, turn N. on to dirt road, cross river into Hartnet, drive to Cathedral Valley Campground	Wayne	2113	2002	27			48	>100
7	BCP	Bristlecone Pine	38° 26.385' N 111 ° 25.585' W	From Cathdral Valley Campground drive W. to Paradise Flats Campgroud, hike 3 miles to Billings Pass	Wayne	2749	2002	27		PC	36	>100
8	JHO	Johns Hole Overlook	38° 38.408' N 111 ° 02.213' W	E. on I-70, exit 97, head S. for 25 air miles, population on side of road	Emery	2030	2002	27			2	<100
9	TL	Type Locality	38° 40.544' N 111 ° 24.743' W	I-70, exit 72, drive for 15 miles, population on E. side of road	Sievier	2265	2001	15	A & T	BS	22	<100
10	FJ	Fremont Junction	38° 43.968' N 111 ° 23.844' W	I-70, exit 72, drive for 3 miles, population on W. side of road	Sievier	2200	2001	27	A & T	BS, DNA	43	>100
11	FJR	Fremont Junction Rest Stop	38° 45.191' N 111 ° 25.210' W	I-70, exit at Fremont Juction Rest Stop, just beyond fence behind rest stop	Sievier	2194	2002	27	A & T	BS	18	<100
12	DVM	Dog Valley Mine	38° 46.311' N 111 ° 16.043' W	E. I-70, exit on to dirt road in Canyon, NE of Windy Peak, drive E. for 4 miles, population on old mine	Emery	1967	2002	27	A & T		14	>100
13	MCR	Moore Cut Off Road	38° 51.419' N 110 ° 55.473' W	E. on I-70, exit off Moore Cut Off Road, drive for 1 mile N. to sand pit	Emery	1994	2002	27			23	>100.

Soil samples taken at each site are as follows: J - Jennings, (this thesis); A&T - Armstrong & Thorne (1999). Information on breeding system (BS) is from Tepedino et. al. (2004). DNA samples extracted (Thompson, *et al.*, unpublished). Pollen count (PC) (Jennings, this thesis) are from herbarium specimens (Appendix I- Table I.1).

Table 2.2. *Townsendia jonesii* var. *lutea* localities and collection information for this study, along with additional information available for each population.

Pop. number	Pop. acronym	Population name	Latitude & Longitude	Location	County	Elevation (m)	Year Collected	Sample size	Soil samples	PC/BS/ CC/DNA
14	GH	Gleenwood Hills	38°45.558' N 111 °55.278' W	E. on Hwy 119, NE of Glenwood in Rainbow Hills just before Hwy. 24	Sevier	1950	2001	27	J	
15	SFS	Sage Flat South	38°49.112' N 111 °56.011' W	S. on Hwy 24, head E. of Sigurd, turn N. on Sage Flat Road, population on S. side of road	Sevier	1683	2002	27		
16	SFN	Sage Flat North	38°53.291' N 111 °51.638' W	S. on Hwy 24, head E. of Sigurd, turn N. on Sage Flat Road, go 7.5 air miles, population on S. side of road	Sevier	1655	2002	27	A & T	
17	GBR	Gooseberry Road	38°49.380' N 111 °43.010' W	E. on I-70, take Soldier Canyon Road S. 9 air miles, population on S. side of dirt road	Sevier	2167	2002	27		
18	TME	Triangle Mountain East	38° 55.634' N 111 °48.754' W	E. on I-70, exit on Soldier Canyon Rd., on the nw side of Triangle Mountain	Sevier	1659	2001	27	J, A & T	CC
19	TMW	Triangle Mountain West	38° 55.675' N 111 °48.889' W	E. on I-70, exit S. on Soldier Canyon Road, W. across road from Triangle Mountain	Sevier	1659	2001	27		
20	WC	Willow Creek	39°01.247' N 111 °47.791' W	N. on Hwy. 89, E. of Redmond, head towards Willow Creek Reservoir, population on S. side of road	Sevier	1690	2002	27	A & T	

Soil samples taken at each site are as follows: J - Jennings, (this thesis); A&T - Armstrong & Thorne (1999). Chromosome counts (CC) are from (Windham, M., University of Utah, unpublished).

2.2 Lab Methods

2.2.1 Pollen size and stainability

Herbarium specimens were used to determine whether there was any evidence that *T. aprica* is apomictic by estimating pollen viability and size variation using aniline (cotton) blue staining (Beaman, 1957). This method is standard for determining polyploidy and apomixis in *Townsendia* (Beaman, 1957) and other groups (Hauser and Morrison, 1964; Torrell et al., 1999). Only those *T. aprica* populations whose breeding system were more in question (southern populations 1-4 and the highest elevation population 7-BCP), were sampled using pollen from herbarium vouchers (Table I.1-Appendix I). From each voucher, one floret from each individual plant was macerated on a slide with a drop of cotton blue stain, which viable pollen nuclei will take up within a few minutes of exposure. After staining, the slides were observed with a light microscope and pollen counts were done within ten minutes of dye exposure. Counts of fully stained (presumed viable) and unstained (not viable) pollen grains were made while also noting any size differences that might indicate polyploidy. Sample sizes per individual ranged from 79 to 268 pollen grains per floret.

2.2.2 Electrophoretic procedures

Designing and optimizing the isozyme protocol including extraction buffer, running buffers and enzymes was done with the extra leaf material of *T. jonesii* var. *lutea.* Looking for clear banding patterns and variability within each isozyme, I systematically tested six different extraction buffers with 2-5 leaves on three different running buffers and screened 21 enzymes to come up with the final isozyme protocols. Electrophoresis was performed with two electrode buffer systems, morpholine and lithium borate (Table 2.3).

Table 2.3. Electrode buffer systems including modifications in pH and run time.

Buffer system	Electrode buffer pH	Gel buffer pH	Typical run time and mA	Reference
Morpholine	8.0	8.4	7 hrs / 40 mA	Clayton & Tretiak, 1972
Lithium borate	8.0	8.0	4 hrs / 45 mA	Ridgeway *et al.*, 1970

Buffer recipes are listed in Appendix III (Table III.1).

Approximately 2-3 frozen leaves from each individual sample were ground to a slurry in individual vials on ice with 2-3 drops of germanium extraction buffer pH 7.1 (Table III.2-Appendix III), using a hand held drill fitted with a custom made plastic bit. Wicks (3mm x 15mm) made of Whatman chromatography paper were used to absorb the supernatant and were then briefly blotted on a paper towel to remove any excess material. The wicks were then inserted into a cut 11.5% starch gel chilling on ice. The gels were then placed onto eletrophoretic rigs, put into a refrigerator and run at a low voltage (160/35 V/mA) for the first twenty minutes. Once the dye indicator had moved into the starch matrix, the wicks were removed, and plastic spacers were inserted into the end of the tray to ensure good contact across the cut completing the circuit. The trays were placed back into the refrigerator with ice packs laid over the gels, and run for the appropriate time for adequate separation of alleles (Table 2.3). Once the running time had elapsed, gels were removed from the fridge, sliced into seven layers, and one slice was set into the appropriate staining tray for each enzyme. Trays were set in an incubator at 37^0 for approximately 30 minutes to complete the reaction. Seven enzymes resolved consistently for both taxa (Table 2.4). Staining protocols for resolving enzymes LAP and MDH followed Soltis *et al.* (1996) and AAT, PGM, IDH, 6PGDH followed Murphy *et al.* (Murphy et al., 1996).

Table 2.4. Resolved enzymes with each buffer system used.

Locus	Enzyme name	E.C. number	Buffer
6-PGDH	6-Phosphogluconate dehydrogenase	1.1.1.44	Morpholine
AAT	Aspartate aminotransferase	2.6.1.1	Morpholine
G6PGD	Glucose-6-phosphate dehydrogenase	1.1.1.49	Ridgeway
IDH	Isocitrate dehydrogenase	1.1.1.42	Ridgeway
LAP	Leucine aminopeptidase	3.4.11.1	Ridgeway
MDH	Malate dehydrogenase	1.1.1.37	Ridgeway
PGM	Phosphoglucomutase	5.4.2.2	Morpholine

E.C. number = Enzyme Commission number

As soon as bands were visible, each gel was scored by eye and documented with a digital image. The phenotypes were interpreted as genotypes with the most anodal (top of gel) isozyme numbered locus '1' and the fastest migrating allozyme designated allele 'a' with slower bands receiving the appropriate letter based on gel position.

2.2.3 Soil procedure

Once back in the lab, soil samples were air-dried, then sifted through a 2mm sieve. Paired samples from each site were then combined and sent to the Brigham Young University Soils Lab where the same procedures used in the 1991 survey (Armstrong and Thorne, 1991) were followed. Samples were analyzed for extractable forms of potassium, calcium, magnesium, phosphorus, sodium, phosphate, all in ppm (μg/ g of dry soil), pH, %sand, %clay, %silt, %calcium carbonate (free lime - $CaCO_3$), %calcium sulfate (gypsum - $CaSO_4$), sodium absorption ratio (SAR), %Moisture 1/3 bar, and %Moisture 15 bar, also known as soil moisture tension. This measurement indicates at lower (1/3 bar) and higher (15 bar) tensions to what degree the water adheres to the soil and the difference between the two should be an indication of available water to a plant.

2.2.4 Pappus measurement procedure

From each taxon, three to four ray and disk pappus length measurements were taken from at least one individual from each population to see if their length measurements were really non-overlapping as the flora of Utah suggests (Welsh et al., 1987; Welsh et al., 1993), or if they are overlapping, as the BLM report suggest (Armstrong and Thorne, 1991). Some of the herbarium vouchers were used for this part of the study (Table I.1-Appendix I) while the other pappus measurements were from seed collected in my 2002 field season.

2.3 Analysis

2.3.1 Isozyme analysis

The following genetic variability parameters were analyzed in POPGENE (Yeh, 1997): percent polymorphic loci (%P), mean number of alleles per locus (A), effective number of alleles (A_e), expected heterozygosity (H_e) and observed heterozygosity (H_o). Estimates of allele and expected genotype frequencies were generated in POPGENE from the sampled populations of *Townsendia aprica* and *T. jonesii* var. lutea, and used to test the null hypothesis that populations are in Hardy-Weinberg equilibrium (HWE) where $H_o = H_e$.

In this study, loci were considered polymorphic if more than one allele was observed, regardless of its frequency. Mean number of alleles per locus (A) (sometimes called allelic richness) is simply the total number of alleles observed at all loci divided by the total number of loci investigated. However, A does not take into consideration the frequency of the different alleles and is dependent on sample size, and therefore is only a very general indicator of diversity. The mean number of effective alleles per locus (A_e) (Kimura and Crow, 1964) as described by Berg and Hamrick (1997), is a measure of the evenness of the most common alleles, developed to account for the observation that rare alleles have a minor effect on genetic diversity (H_e) compared with the most common alleles. It is defined as

$$A_e = 1/(1 - H_e) \qquad 1 \leq A_e < \infty$$

Expected heterozygosity (H_e) is the expected proportion of heterozygous loci per individual assuming random union of gametes and for multiple alleles is defined as

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

where n is the number of alleles, and p_i^2 is the expected genotypic frequency of homozygotes for the i^{th} allele (Hedrick, 2000). Since H_o is observed, it is a biased diversity indicator because it

can be affected by any evolutionary processes that violates the assumptions of HWE such as non-diploid individuals, small populations size, overlapping generations, asexual reproduction, non-random mating, natural selection, migration, and mutation (Hartl, 1987; Hedrick, 2000). A significance test (t-test, $p = 0.05$) between H_o and H_e was carried out using SYSTAT (version 10.0) (Wilkinson et al., 1996) to see if H_o and H_e are significantly different. If dofferemces are significant, this could indicate that one or more of the HWE assumptions have been violated.

Expected and observed heterozygosity often differ due to violations of HWE. These deviations can be more closely measured with Wright's (1951) three F-statistics (F_{ST}, F_{IS}, F_{IT}). These are also known as *fixation indices* which describe the distribution of genetic diversity within and among sub-populations. F_{IS} and F_{IT} are defined as the probability of two gametes uniting (individual-I) relative to their frequency in the subpopulation (S) and total population (T), respectively. F_{ST} measures the degree of differentiation of these subpopulations by correlation of two randomly drawn gametes in each subpopulation (Nei, 1987). Their relationship is described as follows

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$$

F-statistics for both taxa and all loci were estimated in GENEPOP (Raymond and Rousset, 1995) using the modified coefficients of Weir and Cockerham (1984) which are considered better suited to small data sets than Nei's, because they do not make assumptions about the number of populations, number of samples, or heterozygote frequencies.

A significance test for linkage disequilibrium was also run in GENEPOP, using a Markov chain method. This tests the hypothesis that the genotypes at each locus are independent of other loci by looking for evidence of physical linkage or segregation distortion.

I also analyzed all populations for evidence of bottleneck effects using the program Bottleneck (version 1.2.02) (Cornuet and Luikart, 1997). The theory behind this test assumes that if a population has experienced a recent bottleneck, allele numbers will be reduced more than

expected heterozygosity (H_e) (Maruyama and Fuerst, 1985; Cornuet and Luikart, 1997; Piry et al., 1999). This is because the loss of low frequency alleles during a bottleneck event will have little initial impact on H_e. The program can detect a population bottleneck by comparing H_e with what is expected if the population was at mutation-drift equilibrium (H_{eq}) given the numbers of alleles. They defined this as heterozygosity excess, which is not to be confused with an observed excess of heterozygotes ($H_o > H_e$). The raw allele frequency data was inputed and analyzed following the Infinite Allele Model (IAM) of mutation, and significance was based on the Wilcoxon sign-rank test with 1000 iterations.

Nei's (1973) genetic diversity statistics (H_T, H_S, D_{ST}, G_{ST}) were assessed in FSTAT (Goudet, 2001) to allow comparison with other population genetic studies. The total genetic diversity (H_T) is estimated from average allele frequencies over all populations, H_S is the genetic diversity based on allele frequencies within populations, and D_{ST} is the genetic diversity found among populations with their relationship described as:

$$H_T = \overline{H_S} + D_{ST}$$

G_{ST} is described by Nei as the measure among-population differentiation, relative to total diversity, and is calculated as:

$$G_{ST} = \frac{D_{ST}}{H_T}$$

Genetic distances can reflect the relatedness of the sampled populations under the assumption that populations that are more closely related will share alleles at similar frequencies. A variety of algorithms exist to estimate genetic distances between populations, each with its own set of assumptions. Cavalli-Sforza and Edwards (1967) chord distances were used as the basis for tree construction using PHYLIP (version 3.63) (Felsenstein 1995). Cavalli-Sforza and Edwards (1967) chord distances were considered more appropriate when looking at relationships within taxa because differences found between the populations are more likely to be due to drift

alone rather than drift and mutation, as Nei's genetic distance assumes. Allele frequencies were input into GENEDIST program, and the distance matrix was placed into the tree joining program 'NEIGHBOR' in PHYLIP (Felsenstein 1995) where a tree was generated for populations of each taxon separately. The tree output from Phylip was input into TREEVIEW (Page, 1996) to generate a printable tree with a scale bar for genetic distance.

Bootstrapping was used in PHYLIP to evaluate the statistical support of the branching patterns. The allele frequency matrices were re-sampled 200 times in SEQBOOT and the re-sampled bootstrap data was then inserted into GENEDIST, again choosing Cavalli-Sforza and Edwards. The set of matrices was then entered into NEIGHBOR-joining to construct 200 unrooted trees which where then entered into CONSENSE (Margush and McMorris, 1981) to calculate the final bootstrap values based on the number of times that each branch was recovered in the bootstrap trees.

Correlation between genetic distance and geographic distance within *T. aprica* and *T. jonesii* var. *lutea* were examined using a Mantel's test (Mantel, 1967) in GENEPOP with 1000 permutations used to determine significance. The program ISOLDE follows Rousset (1997) who suggested that the use of F_{ST} instead of genetic identity is a more appropriate parameter to use for evaluating isolation by distance. Rousset argues that F_{ST} is relatively independent of mutation rate and mutational processes and is remarkably accurate at short distances. Since samples at small geographic distances are not expected to follow the general theory of isolation by distance, the ISOLDE program allows for input of the minimum geographical distance between populations and then only pairwise comparisons of larger distances are used to estimate the regression coefficient.

2.3.2 Genetic analysis between *T. aprica* and *T. jonesii* var. *lutea*

A genetic distance tree showing the relationship between the two taxa was constructed in PHYLIP and evaluated using bootstrapping following the methods described above. Nei's genetic distance (Nei, 1972) was used in comparisons of the two taxa because its assumption of mutation-drift equilibrium is more appropriate for inter-taxon comparisons (K. Ritland, personal communication).

2.3.3 Soil analysis

Available cations of Ca, Mg, K and Na were analyzed by the ammonium acetate method at pH 8.5 (Normandin et al., 1998). Phosphorus measurements, which is normally performed by extraction using $NaHCO_3$, instead followed Harper's protocol (University of Utah, unpublished), which involves extraction in 0.2N acetic acid. This protocol was used for samples analyzed in 1991, and we followed this protocol for the additional samples collected in 2003. Acid neutralizing potential or % calcium carbonate ($CaCO_3$) extraction followed the method of Allison and Moode (1965), while analysis of %calcium sulfate ($CaSO_4$) followed the method of Nelson (1982). Measurement of percent moisture at 1/3 bar and 15 bars, soil moisture tension, along with percent sand, clay and silt followed Day (1965).

Soil data were averaged and significance for each taxon was assessed using Kruskal-Wallis non-parametric test in SYSTAT (Wilkinson et al., 1996).

Chapter 3 Results

3.1 Breeding system in *Townsendia aprica*

Pollen grains were counted and percent pollen stainability estimated from one floret per flower head from a single herbarium voucher from each of three populations (Table 3.1). Counts were carried out on two (1-MCC & 2-MMD) of the four southern populations, which local field biologists have noted are of questionable identity (note that no voucher exist for 3-WHF and

there were no flowers on the voucher for 4-JM). I also looked at pollen from the one high elevation population (7-BCP) because apomixis and polyploidy tend to be found at higher elevations in other *Townsendia* species with narrow ranges.

Table 3.1. Estimated pollen stainability of *Townsendia aprica* individuals.

Population Name	Collection	Flower	Total Pollen Grains	Viable Pollen	% Viable Pollen
1-MCC	K. Heil/ D. Schles 7559	A	169	146	86.0
(southern)		B	no flowers		
		C	204	190	93.0
population mean					**89.5**
2-MMD	Flemming/ Romme 603	A	106	101	95.0
(southern)		B	268	260	97.0
		C	225	200	89.0
population mean					**93.7**
7-BCP	Mark Porter 3855	A	229	201	88.0
(high elevation)		B	no flowers		
		C	226	217	96.0
		D	79	35	44.0
		E	144	126	87.0
population mean					**78.8**
7-BCP	K.Heil 4376	A	148	78	53.0
(high elevation)		B	140	65	47.0
		C	231	164	71.0
population mean					**57.0**

All vouchers are from San Juan College (SJC).

All counts revealed pollen stainability above 20%, which is considered to be the cut off for low viability in *Townsendia* (Beaman, 1957). Also, there was no variation in pollen grain size observed in any of the samples. While pollen for the two southern populations is around 90%, viable, the stainability of pollen from population 7-BCP was significantly lower that that of the southern populations (t-test; $p < 0.05$).

Most of the enzymes resolved in this study show no indication of duplicate banding patterns or fixed differences between homologous loci across any of the populations. There were two loci resolved for the enzyme IDH, which has previously been documented in other genera to have only one locus (Wendel and Weeden, 1989), but as these authors noted, this is not consistent across all species.

3.2 Genetic diversity in *Townsendia aprica* and *T. jonesii* var. *lutea*

After screening 21 different enzymes, seven enzymes were chosen that resolved nine loci consistently for both taxa (Table 3.2). Other enzymes were also resolved during the screening process, but either had too many overlapping loci that made it too complex to score (EST), or did not stain consistently across all individuals in each population (ME, PGI), and therefore were excluded from this study. The preliminary screening showed that EST and PGI are polymorphic, while ME was monomorphic.

Table 3.2. Enzymes and loci number resolved.

Locus	Enzyme name	Resolved loci	Subunit structure
6-PGDH	6-Phosphogluconate dehydrogenase	1	dimer
AAT	Aspartate Aminotransferase	2	dimer
G6PGD	Glucose-6-phosphate dehydrogenase	1	dimer
IDH	Isocitrate dehydrogenase	2	dimer
LAP	Leucine aminopeptidase	1	monomer
MDH	Malate dehydrogenase	1	dimer
PGM	Phosphoglucomutase	1	monomer

Enzyme abbreviation, full name of the enzyme with the number of loci resolved and their subunit structure.

Seven of the nine isozymes were polymorphic across most populations, and AAT-1 and IDH-1 were monomorphic across all individuals. The known quaternary structures of PGM and LAP are monomeric, while the other five enzymes in this study are known to be dimeric. No significant disequilibrium was detected between any of the enzymes analyzed, showing that all genotypes are independent observations.

Both taxa share all alleles scored for in this study, but not all alleles are present within each population of *T. aprica*, nor are the alleles necessarily at similar frequencies in all populations (Table IV.1 and Table IV.2-Appendix IV).

Genotypes inferred from isozyme banding patterns were used to estimate genetic parameters for individual populations of *T. aprica* and *T. jonesii* var. *lutea*, as well as averages

for each taxon (Table 3.3 and Table 3.4). In this study, 7 of the 9 loci were considered polymorphic across all populations of *T. jonesii* var. *lutea*, and most populations of *T. aprica*, with percent polymorphic loci (%P) of 77.8 and 74.4, respectively.

The mean number of alleles (A) for *T. aprica* is 2.09 with only slight fluctuations among populations, while the mean for *T. jonesii* var. *lutea* is 2.22, with no variation among populations as every allele scored was found in every population. The effective number of alleles (A_e) varied among populations of both taxa, with a mean of 1.71 for *T. aprica* and mean of 1.65 for *T. jonesii* var. *lutea*. There were no significant differences found in these genetic parameters between the two taxa. The mean observed and expected heterozygosity values for *T. aprica* are 0.31 and 0.33, respectively, while *T. jonesii* var. *lutea* means are overall slightly lower at 0.25 and 0.30. There was no significant difference between H_o and H_e for either taxon.

The fixation index (F_{IS}) has a theoretical range between -1 and 1, where a significantly negative value reflects excess heterozygosity, and a significantly positive value reflects heterozygote deficiency, or inbreeding (Table 3.3 and Table 3.4). Although both excess and deficiency of heterozygotes relative to Hardy Weinberg expectations were found in *T. aprica* populations, in only one case was the deviation significant, with an excess of heterozygosity found in population 10-FJ (F_{IS} = -0.20). The majority of observations of excess heterozygosity were found in the northern populations (8 – 13). The highest positive F_{IS} values in *T. aprica* occurred in isolated, southern populations 2-MMD & 4-JM (0.14 and 0.17, respectively), although these were not significantly different from zero. While all but one *T. jonesii* var. *lutea* population had positive F_{IS} values, only two populations (18-TME & 19-TMW) had significant deficiency of heterozygotes (F_{IS} = 0.25 and 0.30, respectively), indicating possible inbreeding within these two geographically close, isolated populations.

Table 3.3. Genetic variability parameters for populations of *Townsendia aprica*.

Population name and number	Sample size	# of loci	# P	% P	mean A	mean A_e	mean Het $_o$	mean Het $_e$	F_{IS}
1 MCC	21	9	6	66.7	2.00 (0.19)	1.45 (0.13)	0.22 (0.06)	0.23 (0.05)	0.06
2 MMD	27	9	7	77.8	2.00 (0.14)	1.60 (0.13)	0.26 (0.05)	0.30 (0.05)	0.14
3 WHF	27	9	7	77.8	2.11 (0.15)	1.60 (0.13)	0.33 (0.06)	0.30 (0.05)	-0.09
4 JM	27	9	6	66.7	2.00 (0.17)	1.62 (0.13)	0.25 (0.05)	0.30 (0.05)	0.17
5 HNE	25	9	7	77.8	2.22 (0.19)	1.69 (0.15)	0.30 (0.06)	0.32 (0.05)	0.04
6 CV	27	9	7	77.8	2.11 (0.15)	1.50 (0.11)	0.30 (0.06)	0.27 (0.04)	-0.13
7 BCP	27	9	7	77.8	2.22 (0.19)	1.51 (0.10)	0.27 (0.05)	0.28 (0.04)	0.03
8 JHO	27	9	6	66.7	1.90 (0.15)	1.42 (0.12)	0.23 (0.06)	0.21 (0.05)	-0.12
9 TL	15	9	7	77.8	2.22 (0.25)	1.90 (0.20)	0.44 (0.09)	0.40 (0.07)	-0.14
10 FJ	27	9	7	77.8	2.11 (0.15)	1.75 (0.13)	0.43 (0.07)	0.36 (0.05)	-0.20*
11 FJR	27	9	7	77.8	2.11 (0.15)	1.75 (0.13)	0.38 (0.07)	0.36 (0.05)	-0.05
12 DVM	27	9	7	77.8	2.22 (0.19)	1.71 (0.12)	0.36 (0.06)	0.35 (0.05)	-0.02
13 MCR	27	9	7	66.7	1.90 (0.15)	1.60 (0.12)	0.30 (0.06)	0.29 (0.05)	-0.04
mean	25	9	7	74.4	2.09 (0.05)	1.71 (0.04)	0.31 (0.02)	0.33 (0.01)	-0.03

Percent polymorphic loci (%P), number of alleles per locus (A), effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F_{IS}). Standard errors are in parentheses.
* Significance level of ($p < 0.05$)

Table 3.4. Genetic variability parameters for populations of *Townsendia jonesii* var. *lutea*.

Population name and number	Sample size	# of loci	# P	% P	mean A	mean A_e	mean Het $_o$	mean Het $_e$	F_{IS}
1GH (14)	27	9	7	77.8	2.22 (0.19)	1.57 (0.12)	0.33 (0.06)	0.30 (0.04)	-0.13
2 SFS (15)	27	9	7	77.8	2.22 (0.19)	1.58 (0.14)	0.27 (0.06)	0.28 (0.05)	0.03
3 SFN (16)	27	9	7	77.8	2.22 (0.19)	1.50 (0.13)	0.24 (0.06)	0.25 (0.05)	0.02
4 GBR (17)	27	9	7	77.8	2.22 (0.19)	1.48 (0.11)	0.20 (0.05)	0.25 (0.05)	0.20
5 TME (18)	27	9	7	77.8	2.22 (0.19)	1.69 (0.14)	0.24 (0.05)	0.32 (0.05)	0.25*
6 TMW (19)	27	9	7	77.8	2.22 (0.19)	1.62 (0.12)	0.21 (0.05)	0.30 (0.05)	0.30*
7 WC (20)	27	9	7	77.8	2.22 (0.19)	1.69 (0.12)	0.28 (0.05)	0.33 (0.05)	0.15
mean	27	9	7	77.8	2.22 (0.07)	1.65 (0.05)	0.25 (0.02)	0.30 (0.02)	0.12

* Significance level of ($p < 0.05$)

Wright's F-statistics for each isozyme locus (Table 3.5) show that *T. aprica* has a slight excess of heterozygotes overall, while the mean F_{IS} values for *T. jonesii* var. *lutes* indicating 12% fewer heterozygotes than expected if populations are randomly mating.

Table 3.5. Wright's F-statistics (F_{IS}, F_{IT}, F_{ST}) for all isozyme loci examined and their means for *T. aprica* and *T. jonesii* var. *lutea*, respectively.

Locus	**F_{IS}**	**F_{ST}**	**F_{IT}**		**Locus**	**F_{IS}**	**F_{ST}**	**F_{IT}**
PGM	0.487	0.274	0.628		PGM	0.461	0.184	0.560
6PGDH	-0.210	0.050	-0.150		6PGDH	0.098	0.022	0.118
AAT-1	0.000	0.000	0.000		AAT	0.000	0.000	0.000
AAT-2	0.440	0.013	0.447		AAT-2	0.417	0.000	-0.282
MDH1	-0.272	0.033	-0.230		MDH1	-0.327	-0.002	-0.329
IDH1	0.000	0.000	0.000		IDH1	0.000	0.000	0.000
IDH2	0.153	0.150	0.281		IDH2	-0.124	0.022	-0.099
G6PDH	-0.274	0.128	-0.111		G6PDH	-0.043	0.001	-0.041
LAP	-0.217	0.051	-0.155		LAP	0.418	0.105	0.479
mean	**-0.027**	**0.100**	**0.075**		**mean**	**0.120**	**0.060**	**0.173**

The fixation index (F_{ST}) which gives an indication of how much divergence exists among populations, ranges between 0 (no differentiation among populations) and 1 (all genetic variation among populations, with no within-population variation). An $F_{ST} < 0.05$ is considered negligible, while $F_{ST} > 0.25$ shows great differentiation between the populations analyzed. The mean F_{ST} value for *T. aprica* (0.100) and *T. jonesii* var. *lutea* (0.060) indicates that most of the isozyme variation is found within populations and little structuring exists among the populations. Neither value is significantly different from zero.

The mean Cavalli-Sforza and Edwards chord distance (1967) between populations of *T. aprica* was 0.118 (range: 0.022-0.334) while the mean chord distance between *T. jonesii* var. *lutea* populations was 0.061 (range: 0.006-0.195), with a significant difference found between values in the two taxa ($p < 0.01$; Table 3.6 and Table 3.7). The neighbor-joining trees produced using the genetic distances within taxa reveal little support for structuring of groups of populations within taxa, with few exceptions. The two most southern populations in *T. aprica*'s range, 1-MCC and 2-MMD, clustered together and were somewhat distant from the rest of the

populations, with moderate bootstrap support of 63% (Figure 3.1). In *T. jonesii* var. *lutea* the neighbor-joining tree (Figure 3.2) showed three of the four branches with bootstrap values above 60%.

The bottleneck analysis revealed that the majority of populations of both taxa have gone through recent bottleneck events, with excess heterozygosity found ($H_e > H_{eq}$). Specifically, in *T. aprica*, the more southern populations (2-5) showed significance ($p < 0.05$) and the most northern populations (9-13) showed greater significance ($p < 0.01$) with more heterozygosity than expected under mutation-drift equilibrium (H_{eq}) (Table IV.1-Appendix IV). In *T. jonesii* var. *lutea* populations 1, 2, 5 and 7 also showed significant evidence of bottlenecks ($p < 0.05$) (Table IV.2-Appendix IV).

The Mantel test revealed a significant relationship between genetic and geographic distances within both taxa, suggesting that the populations show evidence of isolation by distance. *Townsendia aprica* showed a significant regression between genetic and geographic distance that explained roughly 11% of the variation in genetic distances ($r^2 = 0.112$; $p = 0.03$) (Figure 3.3), while geographic distances explain much more of the variation in genetic distances among *T. jonesii* var. *lutea* populations ($r^2 = 0.4125$; $p = 0.004$) (Figure 3.4), with the latter being sampled over a smaller geographical area.

Table 3.6. Cavalli-Sforza and Edwards (1967) chord distance pairwise comparisons of 13 populations of *T. aprica*.

	1 MCC	2 MMD	3 WHF	4 JM	5 HNE	6 CV	7 BCP	8 JHO	9 TL	10 FJ	11 FJR	12 DVM	13 MCR
1 MCC	*****												
2 MMD	0.045	*****											
3 WHF	0.135	0.097	*****										
4 JM	0.130	0.131	0.101	*****									
5 HNE	0.145	0.141	0.071	0.075	*****								
6 CV	0.142	0.124	0.031	0.066	0.045	*****							
7 BCP	0.166	0.136	0.061	0.105	0.025	0.022	*****						
8 JHO	0.217	0.209	0.073	0.167	0.156	0.079	0.133	*****					
9 TL	0.181	0.130	0.153	0.134	0.065	0.142	0.102	0.334	*****				
10 FJ	0.209	0.150	0.040	0.095	0.076	0.036	0.066	0.118	0.122	*****			
11 FJR	0.223	0.201	0.073	0.083	0.066	0.064	0.097	0.161	0.129	0.033	*****		
12 DVM	0.224	0.197	0.049	0.117	0.037	0.061	0.062	0.139	0.112	0.045	0.031	*****	
13 MCR	0.317	0.219	0.088	0.128	0.146	0.089	0.118	0.177	0.202	0.075	0.118	0.109	*****

Mean Cavalli-Sforza and Edwards chord distance for *T. aprica* = 0.118.

Table 3.7. Cavalli-Sforza and Edwards (1967) chord distance pairwise comparisons of 7 populations of *T. jonesii* var. *lutea*.

	14 GH	15 SFS	16 SFN	17 GBR	18 TME	19 TMW	20 WC
14 GH	*****						
15 SFS	0.067	*****					
16 SFN	0.099	0.021	*****				
17 GBR	0.195	0.070	0.048	*****			
18 TME	0.118	0.051	0.037	0.020	*****		
19 TMW	0.146	0.062	0.039	0.017	0.008	*****	
20 WC	0.112	0.066	0.055	0.031	0.006	0.012	*****

Mean Cavalli-Sforza and Edwards chord distance for *T. jonesii* var. *lutea* = 0.061.

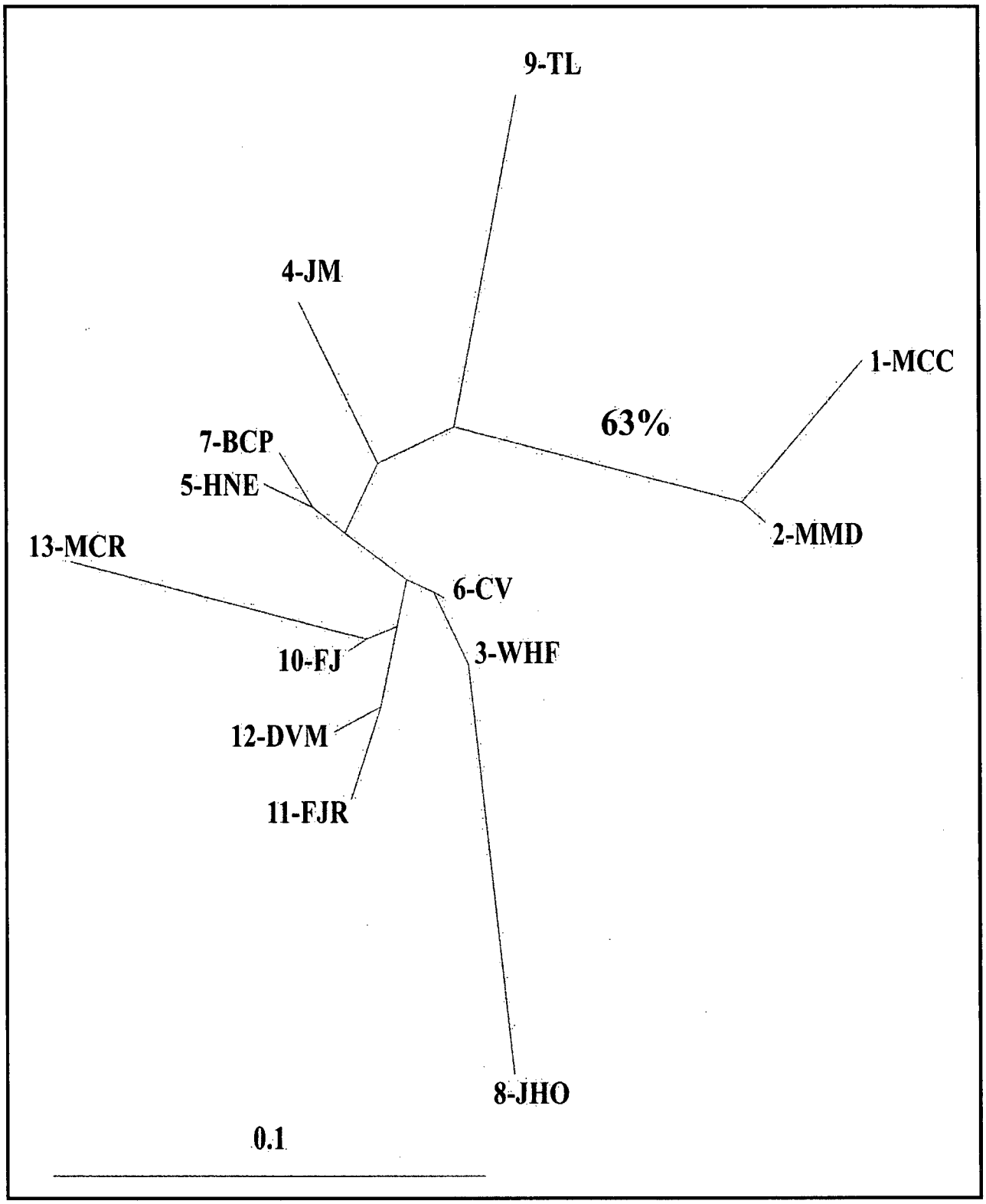

Figure 3.1. Neighbor-joining tree using Cavalli-Sforza and Edwards chord distances for *T. aprica*.
Bootstrap value above 60% is indicated along the branch.

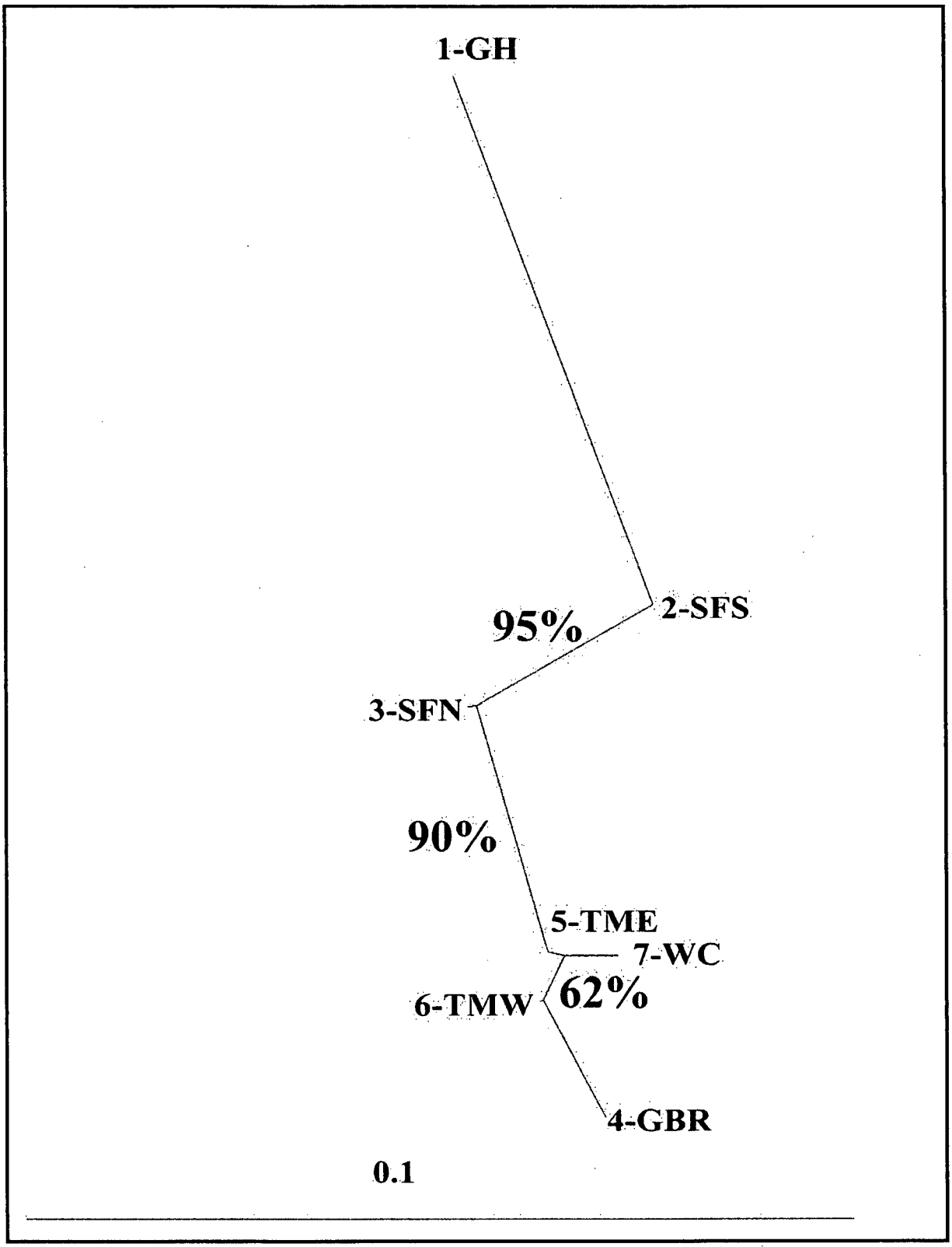

Figure 3.2. Neighbor-joining tree using Cavalli-Sforza and Edwards chord distances for *T. jonesii* var. *lutea*.
Bootstrap values above 60% are indicated along their branches.

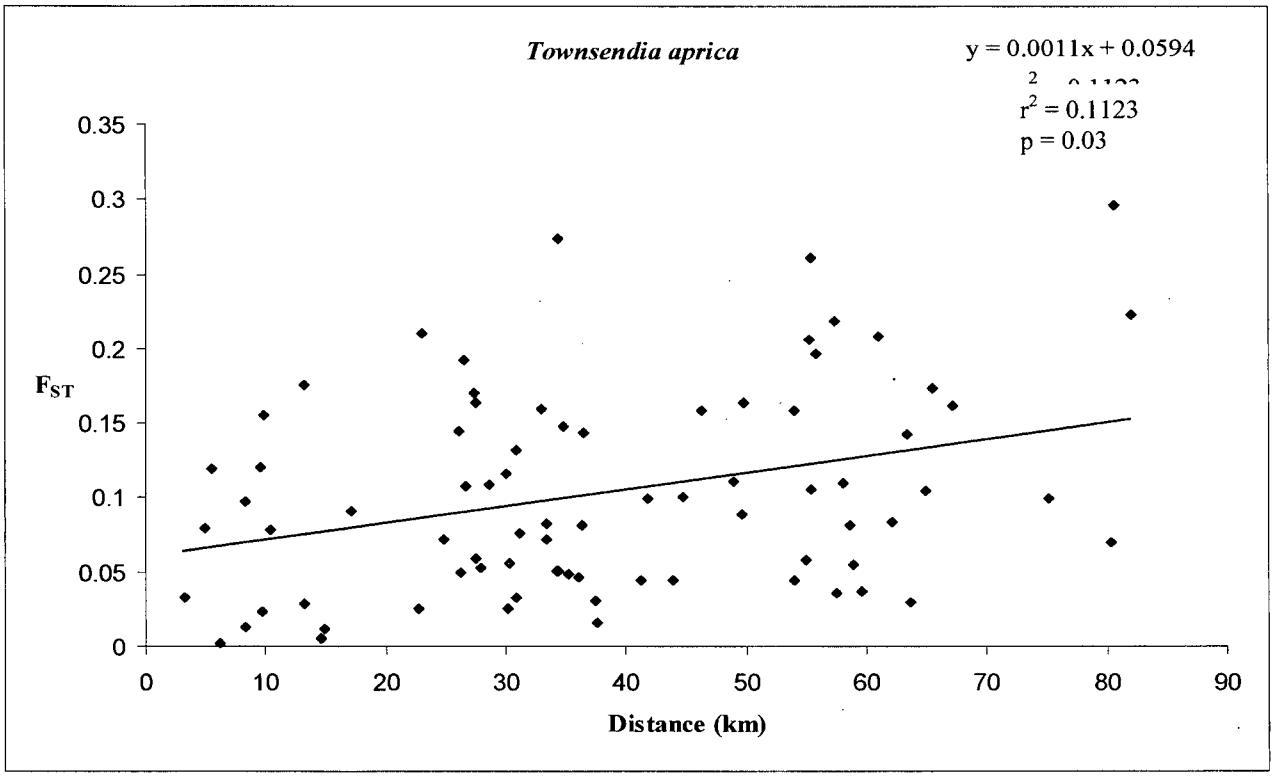

Figure 3.3. Graph of regression analysis between pairwise F_{ST} and geographical distance between populations for *T. aprica*.

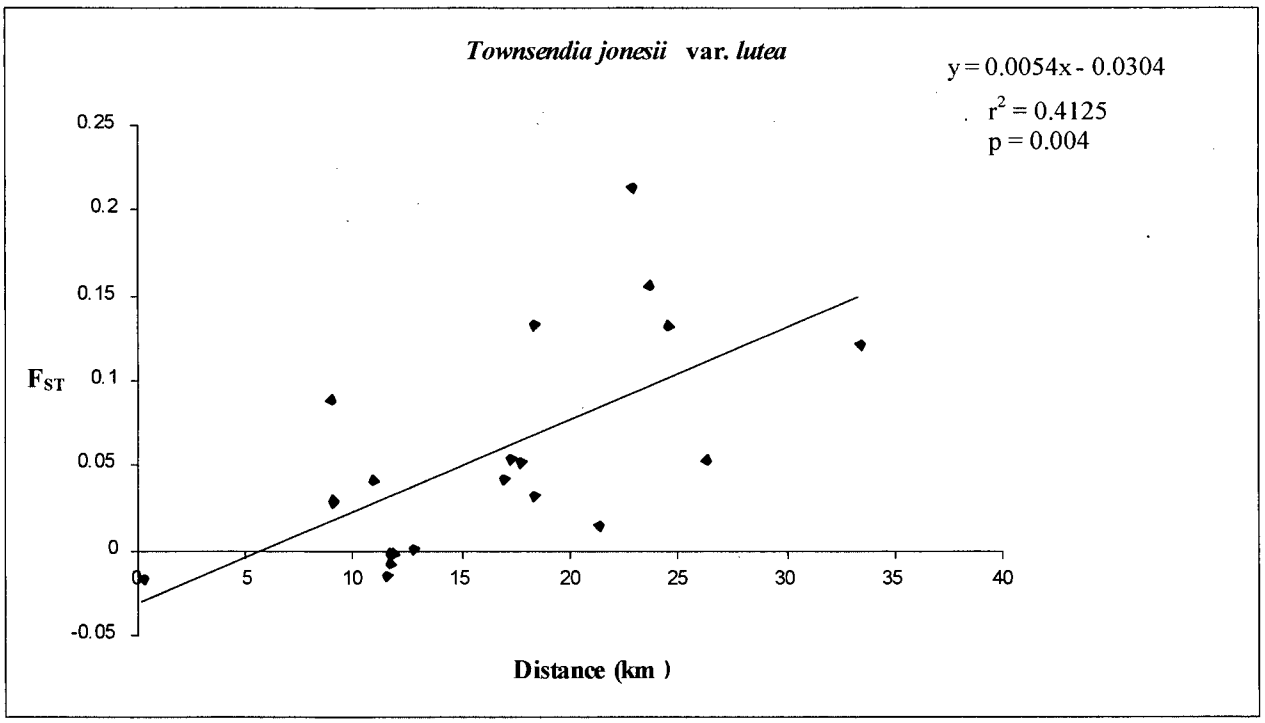

Figure 3.4. Graph of regression analysis between pairwise F_{ST} and geographical distance between populations for *T. jonesii* var. *lutea*.

3.3 Relatedness of *Townsendia aprica* and *T. jonesii* var. *lutea*

Nei's (1972) mean intra-taxon genetic distances within *T. aprica* and *T. jonesii* var. *lutea* are 0.055 and 0.031, respectively (Table 3.8). The mean inter-taxon genetic distance was significant ($p < 0.01$) and estimated as 0.119. Nei's genetic distance (1972) was used to construct a tree to study the relationship between *T. aprica* and *T. jonesii* var. *lutea.* The neighbor-joining tree revealed an interesting pattern (Table 3.5), with populations of *T. jonesii* var. *lutea* forming a moderately well-supported group (69% bootstrap). Support for two of the significant groupings obtained in analyses within taxa was again recovered in this combined tree. Populations 1 and 2 in *T. aprica* form a group with 68% bootstrap support and populations 17-20 of *Townsendia jonesii* var. *lutea* have 69% bootstrap support. Results from the hierarchical analysis confirm the distance results indicating structuring of genetic variation between *T. aprica* and *T. jonesii* var. *lutea* with more genetic differentiation being found between the two taxa ($F_{ST} = 0.211$) than within them ($F_{PT} = 0.137$).

There were three soil characters that were found to differ dignificantly between the two taxa, %sand, %clay, and $\%CaCo_3$ (Table 3.9). *Townsendia aprica* soils have significantly greater %sand and lower %clay than soils collected at *T. jonesii* var. *lutea* populations. *Townsendia jonesii* var. *lutea* soils have significantly higher average $\%CaCo_3$ at 47.8% than *T. aprica* with 13.7%, a difference that is significant ($p < 0.001$).

Table 3.8. Nei's genetic distance (1972) (below diagonal) and identity (above diagonal) for all pairwise comparisons between *T. aprica* (1-13) and *T. jonesii* var. *lutea* (14-20).

	1 MCC	2 MMD	3 WHF	4 JM	5 HNE	6 CV	7 BCP	8 JHO	9 TL	10 FJ	11 FJR	12 DVM	13 MCR	14 GH	15 SFS	16 SFN	17 GBR	18 TME	19 TMW	20 WC
1 MCC	****	0.992	0.920	0.930	0.919	0.917	0.905	0.901	0.917	0.888	0.880	0.889	0.847	0.829	0.830	0.808	0.791	0.822	0.831	0.825
2 MMD	0.009	****	0.939	0.937	0.928	0.928	0.919	0.918	0.934	0.916	0.895	0.905	0.877	0.833	0.848	0.824	0.817	0.844	0.846	0.848
3 WHF	0.083	0.063	****	0.960	0.968	0.987	0.974	0.984	0.903	0.979	0.953	0.982	0.966	0.828	0.892	0.869	0.878	0.879	0.883	0.875
4 JM	0.073	0.065	0.041	****	0.983	0.976	0.965	0.957	0.947	0.967	0.978	0.968	0.950	0.890	0.939	0.928	0.902	0.917	0.922	0.905
5 HNE	0.085	0.074	0.032	0.017	****	0.987	0.994	0.954	0.964	0.969	0.980	0.987	0.945	0.905	0.964	0.952	0.932	0.942	0.947	0.929
6 CV	0.086	0.075	0.013	0.024	0.013	****	0.996	0.983	0.921	0.984	0.963	0.976	0.963	0.837	0.908	0.894	0.877	0.882	0.889	0.867
7 BCP	0.100	0.084	0.026	0.036	0.007	0.004	****	0.963	0.940	0.973	0.957	0.976	0.950	0.846	0.926	0.918	0.903	0.905	0.914	0.889
8 JHO	0.104	0.086	0.016	0.044	0.047	0.017	0.037	****	0.861	0.968	0.937	0.956	0.935	0.780	0.858	0.830	0.829	0.830	0.833	0.814
9 TL	0.087	0.068	0.102	0.055	0.037	0.082	0.062	0.149	****	0.932	0.945	0.937	0.905	0.927	0.948	0.945	0.916	0.944	0.945	0.940
10 FJ	0.118	0.088	0.021	0.034	0.032	0.016	0.027	0.032	0.071	****	0.977	0.980	0.976	0.845	0.901	0.886	0.876	0.888	0.886	0.875
11 FJR	0.127	0.111	0.049	0.022	0.020	0.038	0.044	0.065	0.057	0.023	****	0.989	0.944	0.924	0.960	0.947	0.934	0.951	0.945	0.938
12 DVM	0.118	0.100	0.018	0.033	0.013	0.024	0.025	0.045	0.066	0.020	0.011	****	0.958	0.895	0.952	0.937	0.942	0.947	0.948	0.939
13 MCR	0.166	0.131	0.035	0.052	0.057	0.038	0.051	0.067	0.100	0.024	0.058	0.043	****	0.825	0.899	0.890	0.873	0.869	0.873	0.863
14 GH	0.188	0.182	0.189	0.117	0.100	0.178	0.168	0.248	0.076	0.169	0.079	0.111	0.192	****	0.958	0.940	0.896	0.929	0.917	0.933
15 SFS	0.187	0.165	0.114	0.063	0.037	0.097	0.077	0.153	0.054	0.104	0.041	0.049	0.107	0.043	****	0.997	0.976	0.981	0.977	0.971
16 SFN	0.213	0.193	0.140	0.075	0.049	0.112	0.086	0.186	0.057	0.121	0.054	0.065	0.117	0.061	0.003	****	0.981	0.984	0.984	0.973
17 GBR	0.234	0.202	0.130	0.103	0.070	0.132	0.103	0.187	0.088	0.133	0.069	0.060	0.135	0.110	0.024	0.019	****	0.996	0.995	0.990
18 TME	0.196	0.170	0.129	0.087	0.060	0.126	0.100	0.186	0.058	0.119	0.050	0.055	0.141	0.074	0.020	0.016	0.004	****	1.001	1.000
19 TMW	0.186	0.168	0.124	0.081	0.054	0.118	0.090	0.182	0.057	0.121	0.057	0.054	0.136	0.087	0.024	0.016	0.006	-0.001	****	0.997
20 WC	0.192	0.165	0.133	0.100	0.074	0.143	0.117	0.206	0.062	0.133	0.064	0.063	0.148	0.070	0.029	0.027	0.010	0.000	0.003	****

Nei's mean intra-taxon genetic distance for *T. aprica* (0.055) and *T. jonesii* var. *lutea* (0.031), and inter-taxon genetic distance 0.119.

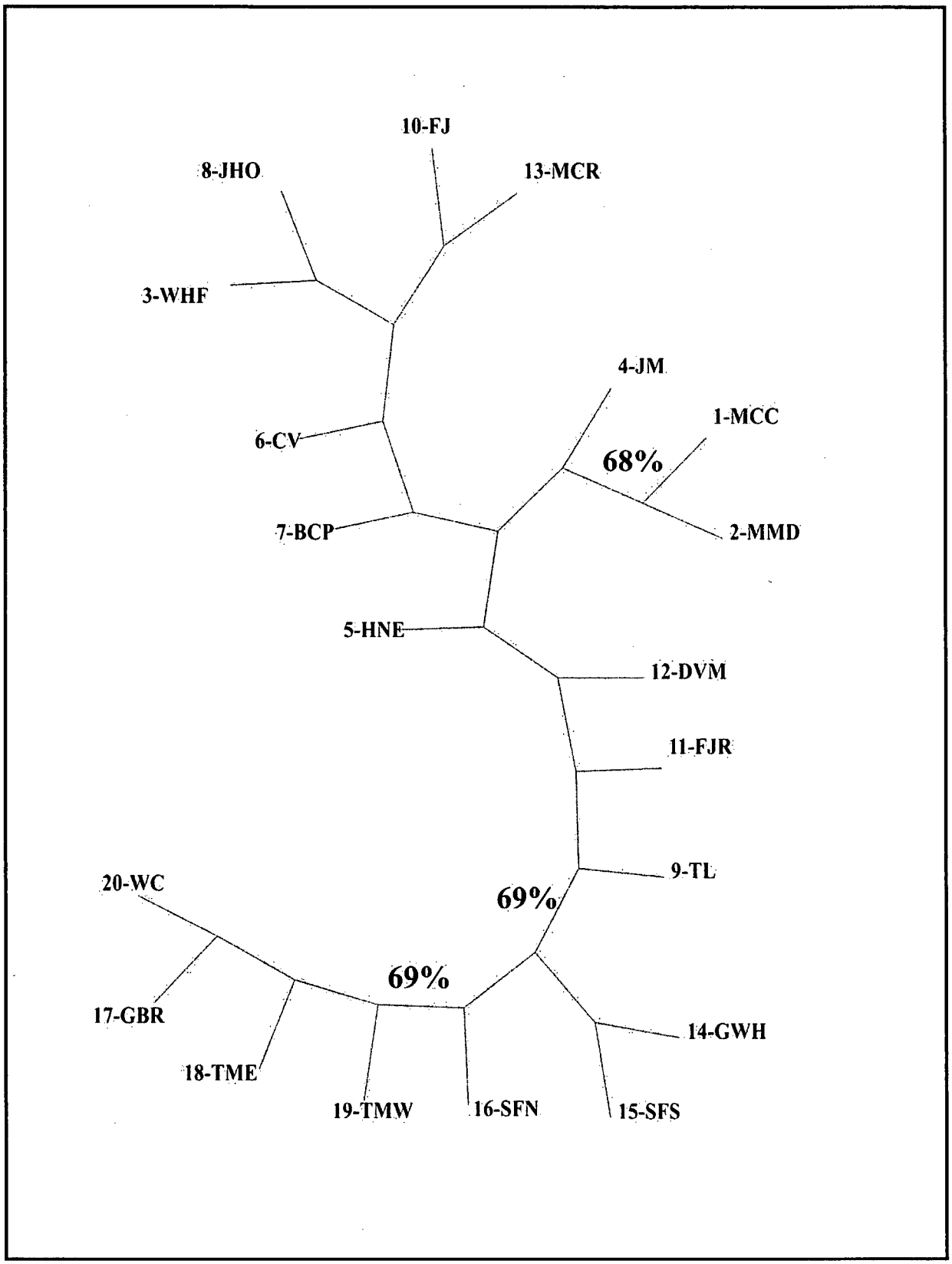

Figure 3.5. Neighbor-joining tree using Nei's distances (1978) between *T. aprica* (1-13) and *T. jonesii* var. *lutea* (14-20).
Bootstrap values above 60% are indicated along their branches.

Table 3.9. Soil characteristics comparing *T. aprica* (N = 23) and *T. jonesii* var. *lutea* (N = 7).

Feature	Taxa	Mean (SE)	Ranges
pH in $CaCl_2$	*T. aprica*	7.50 (0.06)	6.9 - 8.06
	T jonesii var. *lutea*	7.45 (0.08)	7.12 - 7.83
%Sand	*T. aprica*	46.0 (3.19)*	18.15 - 64.49
	T jonesii var. *lutea*	25.6 (5.58)*	2.2 - 39.11
%Clay	*T. aprica*	20.3 (1.55)**	8.24 - 35.08
	T jonesii var. *lutea*	35.4 (3.56)**	22.76 - 45.28
%Silt	*T. aprica*	33.4 (2.37)	19.37 - 57.16
	T jonesii var. *lutea*	38.9 (2.99)	28.46 -54.23
ppm K	*T. aprica*	6.8 (0.97)	7.36 -294.40
	T jonesii var. *lutea*	9.5 (2.32)	10.88 - 256.00
ppm Ca	*T. aprica*	19.2 (4.51)	25.28 - 669.50
	T jonesii var. *lutea*	22.8 (10.69)	19.20 - 512.00
ppm Mg	*T. aprica*	4.0 (0.66)	2.88 - 75.20
	T jonesii var. *lutea*	4.3 (1.26)	6.24 - 51.20
ppm Na	*T. aprica*	1.9 (0.69)	3.50 - 37.76
	T jonesii var. *lutea*	1.3 (0.22)	5.50 - 20.00
ppm P	*T. aprica*	2.7 (0.45)	1.95 - 89.15
	T jonesii var. *lutea*	1.9 (0.40)	12.34 - 51.58
%CaCo3	*T. aprica*	13.7 (2.20)***	1.38 - 48.75
	T jonesii var. *lutea*	47.8 (4.04)***	32.94 - 59.94
%Gypsum	*T. aprica*	4.0 (0.46)	0.93 - 10.65
	T jonesii var. *lutea*	5.8 (0.86)	3.81 - 10.60
SAR	*T. aprica*	0.25 (0.03)	0.09 - 0.58
	T jonesii var. *lutea*	0.37 (0.08)	0.06 - 0.64
%Moisture 1/3	*T. aprica*	13.7 (1.03)	7.25 - 25.00
	T jonesii var. *lutea*	17.7 (2.40)	9.04 - 25.88
%Moisture 15	*T. aprica*	6.9 (0.51)	0.96 - 11.40
	T jonesii var. *lutea*	9.0 (0.77)	4.76 - 10.64
EC (mS/cm)	*T. aprica*	0.95 (0.15)	0.37 - 2.80
	T jonesii var. *lutea*	1.0 (0.34)	0.46 - 2.40

EC = electrical conductivity, SAR = sodium adsorption ratio, ppm (µg/g soil)
p-values are as follows; $p < 0.05$*, $p < 0.01$**, $p < 0.001$**

The results from measurements of ray and disk pappus show that pappus length characters overlap between both taxa (Table 3.10). However, the overlap is found in the few extremes of the measurements (in parenthesis), while the majority of individuals in each taxa, has pappus length in both disk and ray that does not overlap (in bold). Since overlap was found between the two taxa pappus length, there was no need to count more then just the one individual from each population. If non-overlapping pappus length would have been found, then counts would have continued to get statistical strength for the difference.

Table 3.10. Ray and disk pappus measurements of *T. aprica* and *T. jonesii* var. *lutea.*

	N	N ray	ray (mm)	N disk	disk (mm)
T. aprica	17	52	(0.7) **1 - 2** (3)	26	(3) **4 - 5** (6)
T jonesii* var. *lutea	9	39	**2 - 3** (3.5)	24	(4) **5 - 6**

Majority of measurements for pappus length taken are in bold, and the few extremes of the pappus length found are in parenthesis.

Chapter 4 Discussion

This study was motivated in part by the fact that *Townsendia aprica* is being considered for delisting by the U.S. Federal Fish and Wildlife, yet many aspects of the biology of this rare, endemic species are still unknown. Although many new populations of *T. aprica* have been discovered since its original listing in 1980, many populations are small in size (<100 individuals) with estimates reporting as few as six individuals in some populations. The four more recently discovered southern populations (1-4) are disjunct from the rest of *T. aprica*'s known populations and also geographically isolated from one another. The identity of the southern populations has also been questioned by different government agencies who manage the species, because of difficulty in identifying these plants using the Flora of Utah (Welsh et al., 1987; Welsh et al., 1993). Notably, the ray pappus length appears to be shorter than originally described for *T. aprica*. Therefore, one reason to initiate a population genetic study was to clarify whether the southern populations are distinct from the remaining populations of *T. aprica*.

Endemic species are likely to be genetically depauperate (Stebbins, 1942), at least relative to their widespread congeners (Gitzendanner and Soltis, 2000; Cole, 2003), and are therefore theoretically less able to adapt to environmental fluctuations (Beardmore, 1983). It was important that patterns of genetic variation in *T. aprica* be examined before a decision on loss of federal protection occurred. Using isozyme electrophoresis, many populations throughout the range of *T. aprica* were sampled and I was able to more closely examine some of the agency's concerns and also test the hypothesis that this rare, endemic plant is genetically depauperate.

This study also adds to our general understanding of patterns of genetic variation in rare taxa. Karron (1987) and Gitzendanner and Soltis (2000) have pointed out that patterns of genetic variation in rare taxa are best considered in comparison with levels in a more widespread congener. In the absence of a suitable widespread taxon, genetic variation in *T. aprica* was

compared with that of a closely related endemic congener, *T. jonesii* var. *lutea*. Molecular phylogenetic data (ETS and ITS sequences) reveal that the three varieties of *T. jonesii* and *T. aprica* form a clade. I selected *T. jonesii* var. *lutea* for my comparison with *T. aprica* because both taxa share many characteristics, such as narrow endemism in Utah, along with similar ecology and mostly overlapping morphological features. In addition to comparing the patterns and levels of genetic variation between the two taxa, genetic relatedness was also assessed, along with discernable differences in soil types (Armstrong and Thorne, 1991) and a key morphological difference in ray pappus length (Welsh and Reveal, 1968; Welsh, 1983; Welsh et al., 1987; Armstrong and Thorne, 1991).

4.1 Breeding system

The first step towards a better understanding of *T. aprica* was to determine that all populations are diploid, and have an outcrossing breeding system. This outcome would not only affect the analysis of genetic variation in *T. aprica*, but the contrary outcome could result in the loss of its federal protection under the U.S. Endangered Species Act (United States, 1972). For example, if *T. aprica* were found to be an apomictic form of *T. jonesii*, then its taxonomic status could be called into question, which could result in delisting. When this study was initiated, no information was available about the ploidy level or breeding system of this taxon.

Previous evidence from a recent study of the breeding system of *T. aprica* indicates that populations studied to date are outcrossing diploids (Tepedino et al., 2004). I used two additional lines of evidence: pollen features and isozyme banding patterns, to assess whether there was evidence for variation in ploidy or breeding system (sexual versus apomictic) in *T. aprica*. Pollen stainability and size, as well as isozyme numbers and banding patterns were consistent with the populations being predominantly sexual and diploid. Furthermore, by extension of previous

studies of *Townsendia* (Beaman, 1957), I concluded that both *T. aprica* and *T. jonesii* var. *lutea* are most likely to have an outcrossing breeding system.

Apomictic plants produce asexual seed through a variety of developmental pathways. In *Townsendia*, apomixis is diplosporous (embryos develop from unreduced eggs) and autonomous (pollen is not needed to stimulate endosperm formation). In *Townsendia*, as in most cases, diplosporous apomixis occurs only in polyploid individuals. Apomicts of this sort tend to have high levels of pollen sterility, perhaps because of relaxed selection for viable pollen. Because of this, levels of pollen viability are taken as an indirect indication of apomixis. Beaman (1957) found that apomictic individuals in *Townsendia* typically had less than 20% stainable pollen (stainability in cotton blue is considered an indirect measure of viability), along with heteromorphic pollen sizes, with some large viable and some small in-viable grains. None of the twelve individuals from four populations that I examined were found to have less than 20% viability and all pollen was uniform in size. Therefore, these would be considered sexual diploids according to Beaman's criteria.

The mean pollen stainability that I observed was 80%, with the lowest levels (57 %) found at the Bristle Cone Pines population (7-BCP; Table 3.1). The reason for lower viability at 7-BCP is unclear, but the population showed no significant inbreeding (F_{IS} = 0.03; Table 3.3). Jones (1976) attributes differences in percent viable pollen in *Aster* to environmental conditions, such as cold temperatures or nutrient shortages. Considering that 7-BCP is the highest elevation population known for *T. aprica* (2750 m), and sets seed later in the season, it is possible that one of the vouchers examined (K. Heil 4376), was collected too early in the season for that year, and therefore all pollen had not fully developed. However, the other voucher examined (M. Porter 3855) was collected in mid June, which is typically a prime time to collect *T. aprica* seed at 7-BCP, and this collection had even lower stainability than the previous voucher. Given that at

least some of the florets from this population had high stainability, developmental timing seems like the most likely explanation for low viability found.

The second line of evidence used to describe *T. aprica*'s breeding system came from isozyme electrophoresis. By observing duplicate loci and different staining intensities of banding patterns, polyploidy can be detected, and an apomictic breeding system can be inferred if fixed differences between homoelogous loci (fixed genotypes) are found. This inference is strengthened when comparisons are made with known diploid and/ or outcrossing populations (Weeden and Wendel, 1989).

The subunit structure (monomer, dimer, tetramer) of each functional enzyme used in this survey is known to be highly conserved, and can be assumed to be constant among species under study (Weeden and Wendel, 1989). On the other hand, the number of isozymes (loci) and their subcellular distribution can vary among species, tissues or developmental stages, and can not be relied on to be consistent even with a species (Wendel and Weeden, 1989; Allphin, 2002). I compared banding patterns within both taxa using a single source of tissue (leaves), collected from tissues of similar age. Therefore, the assumption was made that isozyme number and subcellular distributions should not vary in either taxon if there is no variation in ploidy. In all sampled populations and all enzymes, only the enzyme IDH showed a different number of isozymes than typically found in diploid plants. Two isozymes were resolved in *Townsendia*, where typically only one cytosolic locus is inferred (Wolf, 1997; Archibald et al., 2001), though other studies (Richardson, 1986) have also resolved two IDH loci for diploid taxa. The remaining enzymes resolved in this study showed no sign of gene duplication and no variation in the number of loci detected among populations. These comparisons included the three known outcrossing populations of *T. aprica* (9-11; Table IV.1-Appendix). In addition, none of the populations showed evidence of apomixis. None displayed patterns of "fixed genotypes" across loci or individuals, and no significant linkage disequilibrium was detected. Although the focus of

this section was to look for variation in breeding system and ploidy level within *T. aprica*, it is worth noting that *T. jonesii* var. *lutea* also appears to be a diploid outcrosser. All populations share the same number of isozyme loci found in *T. aprica*, and the single reported chromosome count for *T. jonesii* var. *lutea* is 2n=2x=18 from population18-TME (Table 2.2), which was also surveyed in this study. Thus, with all this evidence taken together, it appears that both taxa are diploid outcrossers.

4.2 Levels and Patterns of Genetic Diversity

Overall findings

Townsendia aprica and *T. jonesii* var. *lutea* both showed higher levels of genetic diversity (as reflected in %P, A, Ae, H_o and H_e; Table 3.3 and Table 3.4) than expected on average in endemic taxa (Hamrick and Godt, 1989). Both taxa were found to have genotype frequencies in HWE, as expected for outcrossing species (Hedrick, 2000) and no significant inbreeding was detected in most populations of both taxa (Figure 3.5). The majority of genetic variation was found within populations in both taxa, with only weak evidence of divergence among populations (Table 3.5). Geographic distance between populations accounts for 11% of the among population genetic diversity found in *T. aprica*, and 40% in *T. jonesii* var. *lutea* (Figure 3.3 and Figure 3.4). All the loci and alleles screened in this study are shared between these two taxa, with many of the *T. aprica* populations lacking one or two low frequency alleles (Appendix IV- Table IV.1). This could be due to factors such as founder or bottleneck events or genetic drift. Indeed, results of the bottleneck test suggest that many of the populations in both taxa have gone through a bottleneck, although there was no obvious correlation between detection of a population bottleneck event and loss of alleles in those populations. Therefore, the overall pattern suggests that each taxon contains reasonable levels of genetic variation likely maintained by gene flow between populations, reflected by low levels of differentiation among populations.

Some of the limitations in the genetic part of this study have been encountered in many studies of federally listed species; few populations, few individuals sampled per population, and low numbers of total loci resolved. Berg and Hamrick (1997) state that for population genetic studies to have high statistical power (i.e. for high confidence in estimates of genetic diversity parameters), they should include sampling at least 30 individuals per population for at least 10 polymorphic loci. In this study, 13 *T. aprica* populations and all 7 known *T. jonesii* var. *lutea* populations were sampled, the average sample size per population was 27, and nine enzymes were resolved, with seven consistently polymorphic and scorable. The most significant deviation from the guidelines of Berg and Hamrick is in the number of populations sampled, although my sampling design did cover the known range of the two taxa, including the extreme edges of their ranges.

Genetic diversity in *T. aprica* and *T. jonesii* var. *lutea*

All populations of both taxa show similar levels genetic variability as reflected by the percent polymorphic loci (%P), mean alleles per locus (A) and mean effective alleles per locus (A_e), and expected heterozygosity (H_e) (Table 3.3,Table 3.4). For both taxa, levels of genetic diversity were found to be much higher than the means reported in Hamrick and Godt (1989) for locally endemic plant species (P = 40.0%, A = 1.80, A_e = 1.15, H_e = 0.01). Surprisingly, both taxa showed even higher levels of most genetic diversity parameters than reported for widespread species in Hamrick and Godt (1989) (widespread species, P = 58.9%, A = 2.29, A_e = 1.31, H_e = 0.20). Both taxa were also found to have higher total genetic diversity than endemic species (H_T = 0.26; Hamrick and Godt,, 1989) but interestingly, the total genetic diversity for *T. jonesii* var. *lutea* is H_T = 0.30, which is the same as the mean estimates for narrow species (H_T = 0.30), while *T. aprica* total genetic diversity (H_T = 0.35), falls more in line with mean estimates for widespread species (H_T = 0.35; Hamrick and Godt, (1989).

Because of the limited availability of leaf material and low enzyme activity in the extreme outer layers of the gels, I was unable to include additional non-polymorphic loci and %P can be taken to be an overestimate of variability because I targeted polymorphic isozymes. Also, %P, A, and A_e are considered strongly affected by population sample size because larger sample sizes will tend to pick up more low frequency alleles. Within this study, populations 1-MCC (21 individuals) and 9-TL (15 individuals) had the fewest individuals collected, but neither shows evidence of an effect on these three parameters when compared to all other populations (Table 3.3). Therefore, it may be more meaningful to compare the values of these parameters between the taxa and their populations within this study rather than drawing conclusions based on comparisons of different studies.

Townsendia jonesii var. *lutea* has slightly higher %P and A than *T. aprica*, while *T. aprica* shows a bit higher A_e and expected heterozygosity than *T. jonesii* var. *lutea*. Since all alleles were found in all populations of *Townsendia jonesii* var. *lutea* but not in all *T. aprica* populations, %P and A are higher for *Townsendia jonesii* var. *lutea*, but this is somewhat surprising since *Townsendia jonesii* var. *lutea* has a much more narrow range than *T. aprica*. The higher values of A_e are expected when higher heterozygosity is found, as in the case of *T. aprica*.

Surprisingly for a rare, endemic plant there was no significant deficiency of heterozygotes or inbreeding found in any populations of *T. aprica* (Table 3.3), while most of the *T. jonesii* var. *lutea* populations did have a deficiency of heterozygotes with the two most geographically near to each other but geographically isolated from the other sampled *T. jonesii* var. *lutea* populations having significant inbreeding (Table 3.4). The northern populations of *T. aprica* (9-12) show the greatest genetic diversity, showing slight excess of heterozygosity, on average with one population (10-FJ) showing significant excess heterozygosity (Table 3.3). The southern populations (1, 2, and 4) show the greatest deficiency of heterozygotes in *T. aprica*, indicating a tendency toward inbreeding. This pattern of genetic diversity and sub-structuring

can not be explained just by the physical distance between the three southern populations (13km), when compared to the similar physical distances in four northern populations (17km) (Table II.1-Appendix II), nor can it be explained by small population size (see below). One effect that could explain this pattern might be the greater degree of geographic isolation of southern populations from one another. The landscape in this region appears to be more fragmented, perhaps leading to stronger barriers to gene exchange between populations.

Although large populations are expected to maintain higher levels of genetic variability than small populations (Wright, 1931; Kimura and Crow, 1964), this was not the case with *T. aprica* (Table 3.3). There was no evidence found that random genetic drift had a stronger effect in small populations of *T. aprica* (<100) (Table 2.1) which did not show an overall tendency towards lower genetic diversity (Table 3.3) or more fixed alleles (Table IV.1-Appendix IV). By comparing the genetic diversity and allele frequencies of three small, geographically isolated populations (1-MCC, 2-MMD, and 8-JHO) to three other small populations which are not as isolated (5-HNE, 9-TL, and 11-FJR), it becomes apparent, that geographic isolation has a greater effect on genetic diversity patterns and allele fixation in *T. aprica* then the size of a population. Another explanation could be a recent reduction in population size or isolation which would not reflect the historic size of the population or effective population size (N_e) and not enough time has passed for a drop to occur in genetic diversity.

Similar high levels of genetic diversity have also been reported in narrow endemics of other genera such as *Dedeckera* (Polygonaceae) (Nickrent, 1989), *Abronia* (Nyctaginaceae) (Williamson and Werth, 1999), *Daviesia* (Brassicaceae) (Young and Brown, 1995), and endemic Asteraceae such as *Artemisia molinieri* (Torrell et al., 1999), and *Achillea millefolium* ssp. *megacephala* (Purdy and Bayer, 1996). Nickrent (1989) points to a strong correlation of breeding system with genetic variation, with the highest levels of polymorphism found in long-lived, outcrossing species. Purdy and Bayer (1996) also speculate that high levels of polymorphism in

Achillea millefolium ssp. *megacephala* relative to the widespread ssp. *lanulosa* may have to do with the greater reliance of ssp. *megacephala* on sexual reproduction. Williamson and Werth (1999) points to high genetic variation being found in the endemic *Abronia macrocarpa* as possibly the results of a recent speciation event involving divergence from a widespread species through long distance dispersal, or possibly an isolation event, where populations were once more widespread, with continuous distributions that have now been broken up.

The source of high genetic diversity in both taxa is not entirely clear, although a number of factors could be contributing. First, in the case of *T. jonesii* var. *lutea*, as its taxonomic rank assumes, is may still be capable of interbreeding with the other two *T. jonesii* varieties, most likely *T. jonesii* var. *jonesii,* whose distribution extends into the same counties as *T. jonesii* var. *lutea*. Collectively, these three varieties could be considered just one widespread species, or possibly part of a larger species complex, as suggested by John Strother (New York Botanical Garden, per. comm.). As for *T. aprica*, it is also possible that genetic diversity estimates are inflated as a result of a past hybridization event, or present-day hybridization occurring with one or both of the two physically close taxa, *T. jonesii* var. *lutea* or *T. incana*, which both have overlapping flowering times with *T. aprica*. *Townsendia incana* grows very close to many populations of *T. aprica* and was shown by Beaman to hybridize readily in the lab and in natural sympatric situations with other taxa, but preliminary screening of cpDNA has found no indication of shared maternal haplotypes between the two species (Chris Sears, per. comm.). Although *T. jonesii* var. *lutea* is thought to have a non-overlapping range with *T. aprica*, some of the mountainous terrain that separates the two could be hiding some unknown, sympatric or neighboring populations providing a bridge for the exchange of genetic material between the two taxa and contributing to maintenance of the high genetic variability. Another possible reason for the high genetic variation found could be due to a somewhat recent divergence of *T. aprica* from the *T. jonesii* group. There are very close similarities found in morphological features and

ecological attributes, as reflected by very similar ITS/ETS sequences. *Townsendia jonesii* var. *lutea* and *T. aprica* also share similar levels of genetic variability and patterning with little genetic distance found between the two taxa, but importantly, they do form distinct clusters in the neighbor-joining tree. A preliminary screening of cpDNA also has yet to detect differences between the two taxa.

Distribution of genetic variation

The fixation index (F_{ST}) (Table 3.5) shows evidence of modest levels of differentiation among populations within each taxon. The majority of genetic variation was found within populations, with only slight divergence found among populations of *T. aprica* ($F_{ST} = 0.10$) and *T. jonesii* var. *lutea* ($F_{ST} = 0.06$). The greater divergence found in *T. aprica* could be due to its larger range, with greater geographical and elevation distances found between many of the populations, including strong geological features that could drive isolation between different sets of populations, but there is not enough data to say. The results of the Mantel's tests support this notion, with a stronger relationship found between genetic diversity and geographic distances within the more restricted *T. jonesii* var. *lutea*, ($r^2 = 0.41$; Figure 3.4) than in the wider-ranging *T. aprica* ($r^2 = 0.11$; Figure 3.3). This more obvious geographic structuring of differentiation within *T. jonesii* var. *lutea*, may explain why relationships among populations within this taxon are better supported (Figure 3.2) than those within *T. aprica*, despite lower mean genetic distance among its *T. jonesii* var. *lutea* populations (0.061 versus 0.118 for *T. aprica*). Pollinators appear to be mainly native, solitary bees (Tepedino et al., 2004), which are unlikely to contribute significantly to long distance pollen flow. However, the pappus of *Townsendia* is barbed, which may contribute to possible animal dispersal of fruits. Hamrick and Godt (1996) found that animal dispersal is associated with lower levels of genetic differentiation than many other dispersal mechanisms. While pollen and seed dispersal distances have not been characterized in these or other townsendias, they appear to have some adaptations for long distance achene dispersal.

Patterns of differentiation and isolation by distance are not as clear within *T. aprica*, perhaps because differentiation between southern (populations 1-4) and northern (populations 8-13) populations obscures finer substructure (note that populations 5-7 have the largest range of elevation in *T. aprica* (Table 2.1), and it is unclear whether they belong to the northern or southern group). Mean genetic distance between southern populations is 0.107, compared with a mean of 0.127 between northern populations. In contrast, the mean genetic distance between northern and southern populations is 0.153, suggesting that northern and southern groups are somewhat differentiated. Although the northern and southern groups of populations show some evidence of differentiation, these trends do not show up clearly in the neighbor-joining tree (Figure 3.1).

Evidence of bottleneck events was detected in most *T. aprica* populations (2-5, $p< 0.05$ and 9-13, $p< 0.01$; Table IV.1-Appendix VI) and *T. jonesii* var. *lutea* populations (14, 15, 18, 20, $p < 0.05$; Table IV.2-Appendix VI). The interpretation of these findings is not straightforward, because all populations had very similar patterns and levels of genetic variation. The bottleneck test used here (Cornuet and Luikart, 1997) compares the expected heterozygosity (based on observed allele frequencies) with the heterozygosity expected if populations are at mutation-drift equilibrium. These patterns could be driven by periodic extinction and re-colonization dynamics, which have been reported to occur within *T. aprica*. M. Robinson (USFS) documented population sizes of *T. aprica* over two years (2002-2003; USFS, unpublished). She found both decreases in population sizes as well as some population extinctions within those two years. In fact, 43% of *T. aprica* locations showed a decrease between 2002 and 2003 with 18 of the 66 sites absent in 2003 and assumed to have died off. It would be helpful to have seed bank information about *T. aprica*, in order to understand how this might contribute to the observed patterns, though none is available at this time. It is possible that seed banks, as well as dispersal, contribute to re-colonization dynamics in this species.

In summary, I found that *T. aprica* and *T. jonesii* var. *lutea* both have overall levels of genetic variation considered high for rare, endemic taxa. The majority of the genetic variation was found within populations but results show relatively low levels of divergence among the populations. Much of the pattern of genetic variation between populations of *T. jonesii* var. *lutea* can be accounted for by geographic distances between populations. Patterns of differentiation within *T. aprica* are more complex, perhaps driven in part by differentiation between northern and southern groups of populations. As Gitzendanner and Soltis (2000) point out, rare or endemic species may have a tendency towards low genetic diversity, but this does not mean that all rare species have low genetic variation.

4.3 Relatedness conclusions

Townsendia aprica and *T. jonesii* var. *lutea* have shown very slight morphological (Welsh et al., 1987; Welsh et al., 1993) (Appendix V), ecological (Armstrong and Thorne, 1991) and genetic (Thompson et al., unpublished) differences in this and previous studies. However, I have found modest yet consistent genetic differentiation between these two taxa, along with further evidence of consistent ecological differences.

The neighbor-joining analysis based on Nei's genetic distance reveals a tendency for populations of *T. jonesii* var. *lutea* to cluster together, with weak bootstrap support of 69% (Figure 3.5). Stronger bootstrap support (73%) for grouping of *T. jonesii* var. *lutea* populations was obtained when I used Cavalli-Sforza chord distance (not shown). Looking closely at the branching pattern (Table 3.5) and the allele frequencies (Table IV.1, IV.2-Appendix I) it becomes apparent that the presence of the MDH-1*d* allele drives this attraction between the two taxa. The MDH-1*d* allele was found in all populations of *T. jonesii* var. *lutea* and in populations 5, 9, 11, and 12 of *T. aprica*. The effect of the MDH-1*d* allele was examined in more detail by removing MDH-1*d* and adding its frequency to the most common MDH allele. Constructing a

tree with Nei's genetic distance based on these new allele frequencies for MDH resulted in a tree in which *T. aprica* and *T. jonesii* var. *lutea* form two separate clusters with a bootstrap support of 72% along the branch that separates them. Therefore, the presence of this allele appears to emphasize the similarity between some populations of *T. aprica* and those of *T. jonesii* var. *lutea*, again underlining their close relationship, but still showing some distinct differences. These differences were also substantiated by the hierarchical analysis of genetic differentiation which revealed greater differentiation between the two taxa ($F_{ST} = 0.211$) than within each taxon ($F_{PT} = 0.137$).

Soil features provide additional evidence for a distinction between the two taxa. In general, % sand, % clay and % $CaCo_3$ distinguished the soil habitats of the two taxa, with larger ranges of most soil attributes found in *T. aprica*. It should also be noted, that *T. aprica* has been repeatedly described as being found on clay or clay-silt soils (Welsh and Reveal, 1968; Welsh et al., 1987; Welsh et al., 1993), where this and earlier studies (Armstrong and Thorne, 1991) show that *T. aprica* has a higher affinity for sandy soils (Table 3.9). As *T. aprica* was found to have higher genetic diversity and effective alleles than *T. jonesii* var. *lutea*, this could indicate that *T. aprica* has been able to adapt to a broader set of soil niches than *T. jonesii* var. *lutea*.

The fact that some *T. aprica* populations lack two alleles present in *T. jonesii* var. *lutea* populations (MDH-1*d* and PGM-1*c*) raises the possibility that *T. jonesii* var. *lutea* could be the progenitor of *T. aprica*, because progenitors will have a tendency to loose alleles during a founder event. Another explanation could be a buffering against loss of alleles in a bottleneck event in populations of *T. jonesii* var. *lutea* or that migrants replenish allele. However, additional lines of evidence, such as from DNA sequence data, would be needed to look more closely at the ancestral hypothesis (Gottlieb, 2004).

While the levels of differentiation detected could be used to support the continued recognition of distinct taxa, the genetic data are possibly biased toward higher levels of

differentiation because of the decision to focus on scoring polymorphic loci. If we considered these two distinct taxa, then we can be fairly certain that it has been a recent divergence, as postulated by Beaman for all of townsendias. Though the importance of the line of distinction between *T. aprica* and *T. jonesii* var. *lutea* remains open to debate, evidence has been presented of the existence of detectable and significant genetic and soil differences between the two taxa.

4.4 Future Directions

With such high genetic variation found, it is important to try to rule out hybridization as a possible contributing factor to the observed patterns. One step to ruling out hybridization is to continue studies of the chloroplast region of the genome, but possibly on a finer scale (more individuals sampled) or increasing the number of loci included in the screening. By using this maternally inherited genome, we can look for detectable differences between *T. aprica* and *T. jonesii* var. *lutea* and *T. incana* to see if there has been any recent or past hybrid interactions, or to look for evidence that *T. aprica* arose though a hybridization event. A hybrid origin for *T. aprica* may seem unlikely, given the absence of unique alleles in *T. aprica* that do not occur in *T. jonesii* var. *lutea*, but how strong this evidence is depends on whether *T. incana* also shares these alleles with either taxa.

In addition to looking in greater detail at phylogenetic data, I would suggest that crossing studies should be done between *T. aprica* and *T. jonesii* var. *lutea* and *T. incana* to see if they do readily hybridize and if they produce viable and fertile offspring. This would be valuable for a number of reasons. First, it would tell us whether breeding barriers exist between the various taxa, which is important for managers to know, especially in the case of *T. incana* which co-occurs with *T. aprica* in a number of localities and overlaps in flowering times. Second, this would be relevant to checking for the possibility that *T. aprica* originated via hybridization.

Another future priority would be to sample the very disjunct, and most southern population of *T. jonesii* var. *lutea* at Piute Reservoir. The distribution of *Townsendia jonesii* var. *lutea* was extended south in 1981 Piute County, (D. Atwood & E. Neese, 7794 NYBG). The original species identification of this disjunct population was as *T. aprica.* The herbarium voucher collection (NYBG 32226) loaned to the UBC herbarium from New York Botanical Garden is apparently being utilized for the Flora of North America project to write the taxonomic key for *T. aprica.* There is a note about the ray pappus length (1.5mm) and disk pappus length (4.5mm) on the herbarium specimen, but according to Welsh's description (Welsh et al., 1987; Welsh et al., 1993) that would mean that the ray pappus fits neither taxon, with the disk pappus suggesting it is *T. aprica.* According to Armstrong and Throne's measurements of *T. jonesii* var. *lutea* this specimen would be *T. aprica,* but they used these sites in their study in 1991 and had the herbarium specimen identified as *T. jonesii* var. *lutea*! During my field season, we searched this region extensively but we were unable to locate and collect from this important *Townsendia* population. Obviously, if this population could be found, and sampled, then electrophoresis data could help in its identification.

Also, with conflicting results in the bottleneck test, more information on pollen and seed movement, and a study to determine the existence and importance of a seed bank would help to fill in some of the missing pieces to this puzzle. In particular, it is important to understand how and when populations disappear and reappear with environmental fluctuations. The addition of chromosome counts from each population of *T. aprica* could also be done to confirm and extend my results and those of Tepedino et al.'s (2004) breeding system study.

4.5 Conservation considerations

In *T. aprica*'s recovery plan, there were three criteria would have to be met for delisting; maintaining 30,000 individuals total for five consecutive years, maintaining 20 populations with

a minimum of 5,000 individuals, and ensuring the long-term maintenance of undisturbed habitat. At this stage, I would estimate a maximum total of 4,000- 5,000 individuals, with no more than 10 stable populations and fewer than 500 individuals each. If *T. aprica* is a diploid, obligate outcrossing species, then describing six individuals or even 50 as a population could give the impression that more long-term stability is present than actually exists. Careful attention to how populations are defined could dramatically change the understanding and the long term recovery plan of this threatened species.

As demonstrated in this study, the overall genetic diversity of this species is not in immidiate peril, but careful attention to threats could affect the probability of survival of certain populations. As shown, it is not just the size of the population that affects overall genetic differentiation between all the populations of *T. aprica*, but the compound factor of geographic isolation. Many of the southern populations (1-MCC, 2-MMD, 4-JM) show signs of lower genetic variation and a deficiency of heterozygotes compared with all other *T. aprica* populations and should be monitored more closely for size fluctuations (bottlenecks) and more carefully protected from potential threats. Of special concern is 1-MCC because it is easily accessible from the roadside with indications of human threat with fires and camping near this site. It also seems to be the only population found on the unique substrate of Navajo Sandstone.

If a re-introduction program were initiated for *T. aprica*, my suggestion to management agencies at this stage of our understanding would be to use the highly genetically variable northern populations (9-12) for use in establishing new populations, although an initial transplant experiment should be carried out prior any transplanting projects to see if the northern individuals have the same amount of fecundity or vigor when transplanted to any southern population sites.

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Appendix I *Townsendia aprica* herbarium vouchers examined

Table I.1. All known herbarium vouchers for *Townsendia aprica*.

Pop	Site	Voucher number	Herbarium	Township, Range, Section	Year	Pollen Count	Ray Pappus	Disk Pappus
North	QCR	346172	BRY	22S-5E-31 SWQ	1991		1	4
MC	MCOR	330860	BRY	22S-9E-33 NEQ	1989		1	n/a
DV	DVM	199193	BRY	23S-6E-19 NWQ	1979		1-2.5	4
DV	ESCL	196940	BRY	23S-6E-19 SEQ/ SEQ	1979		1	4.5
DV	DVM	328952	BRY	23S-6E-33 SWQ	1989		1	4
DV	SRS	212698	BRY	24S-6E-4	1980		1	n/a
DV	ESCL	199196	BRY	24S-6E-5 NEQ	1979		1	4
DV	RC	196931	BRY	24S-6E-6	1979		1	5
FJ	WP	346181	BRY	23S-5E-35 SEQ/SWQ	1991		1.5-2.5	5.5
FJ	FJ	345948	BRY	24S-5E-5	1991			
FJ	EFJR	346167	BRY	24S-5E-6 NWQ	1991		1.5	5.5
FJ	SFJ	346168	BRY	24S-5E-7&8	1991		1.5-2	5
FJ	JFR	345949	BRY	24S-5E-8	1991			
FJ	NHJT	345950	BRY	24S-5E-16 NEQ/ SWQ	1991			
FJ	28.3	229536/ 119969	MSU /UTC	Isotype	1966			
FJ	28.3	67350	BRY	Holotype	1966			
FJ	28.3	84379/ 32228	BRY/ NYBG	28.3 miles North of Fremont	1969		2.5	5-Apr
FJ	28.3	76255/ 32227	UT/ NYBG	28.3 miles North of Fremont	1969			
FJ	FJ	12401	SJC	Fremont Junction	1988			
FJ	FJ	284769/ 32225	BRY/ NYBG	25S-5E-1	1985		1.5	5
TL	TL	281778	BRY	24S-4E-34 W-half	1985		1	5
TL	TL	346169	BRY	24S-5E-29 SWQ	1991		1.2	5
TL	TL	168719	BRY	24S-5E-30	1977		<1	4-5
TL	TL	346171	BRY	24S-5E-31	1991			
TL	SFJ	346170	BRY	24S-5E-31 SWQ	1991			
TL	LCC	346180	BRY	25S-5E-5 SEQ/ NWQ	1991			
TL	LCC	346176	BRY	25S-5E-6 NWQ	1991			
TL	TL	187456	UTC	S. of Fremont Junction	1984			
LCGF	OWB	328909	BRY	25S-7E-18 SEQ	1989		1-1.5	n/a
JH	JHO	330849	BRY	25S-8E-16 SEQ	1989			
Welsh	Welsh	95687	BRY	24S-7E-30	1971			
DC	BCP	1906	CRNP	27S-4E-25	1981			
DC	BCP	9585	SJC	27S-4E-25	1988	x		
DC	BCP	6131	SJC	27S-4E-25 E-half	1986	x		
HN	CVC	1907	CRNP	27S-5E-9	1981			
HN	CV	8032	SJC	27S-5E-10 SWQ	1987			
HN	CV	10801	SJC	27S-5E-10 SWQ	1987			
HN	CV	24361	SJC	27S-5E-10	1988			
HN	CVR	1905	CRNP	27S-6E-15	1981			
HN	SDO	3107	SJC	South Desert Overlook	1984		1	4
HN	SDO	269077	BRY	South Desert Overlook	1984			
HN	SDO	11569	SJC	South Desert Overlook	1987			
HN	SDO	9564	SJC	27S-6E-13	1988			
JM	JM	8027	SJC	29S-6E-29 SWQ	1987			
MM	MM/ NP	9633	SJC	30S-6E-11 SEQ	1988	x		
CC	MCC	19958	SJC	29S-5E-31 NWQ	1993	x		
CC	MCC	20190	SJC	(29S-5E-31 NWQ)	1994			
PR	PR	32226/ -	NYBG/ OFSH	29S-3W-3	1981		2	5

For full names of populations, sites, and herbaria please see Table I.2

Table I.2. List populations, sites and herbaria and their acronyms.

Herbarium		**Site Name**	
BRY	Brigham Young University	BCP	Bristle Comb Pine
CRNP	Capitol Reef National Park	CV	Cathedral Valley
MSU	Michigan State University	CVC	Cathedral Valley Campground
NYBG	New York Botanical Garden	CVR	Cathedral Valley Road
OFSH	Offical Forest Service Herbarium	DVM	Dog Valley Mine
SJC	San Juan College	EFJR	East of Fremont Junction Reststop
UTC	Utah State University	ESCL	East of Sevier County Line
UT	University of Utah	FJ	Fremont Junction
		JFR	Jim's Farm Road
Pop Name		JHO	John's Hole Overlook
CC	Cocks Comb	JM	Johnson's Mesa
DC	Deep Creek	LCC	Last Chance Creek
DV	Dog Valley	MCC	Mini-Cocks comb
FJ	Fremont Junction	MCOR	Moore Cut Off Road
HN	Hartnet	MM/NP	Miners Moutain / National Park
JH	John's Hole	NHJT	North Hollow Jeep Trail
JM	Johnson's Mesa	OWB	Oil Well Bench
LCGF	Last Chance Gas Field	PR	Piute Reservoir
MC	Moore Cut Off Road	QCR	Quitchupah Creek Road
MM	Miners Mountain	RC	Rock Canyon
TL	Type Locality	SDO	South Desert Overlook
		SFJ	South of Fremont Junction
		SRS	San Rafael Swell
		TL	Type Locality
		WSW	Willow Springs Wash
		WP	Windy Peak

Appendix II Geographical distance

Table II.1. Geographic distances (km) between the different sampled populations of *T. aprica* (1-13) and *T. jonesii* var. *lutea* (14-20).

	1- MCC	2- MMD	3- WHF	4- JM	5- HNE	6- CV	7- BCP	8- JHO	9- TL	10- FJ	11- FJR	12- DVM	13- MCR
1- MCC	****												
2- MMD	8	****											
3- WHF	13	5	****										
4- JM	10	10	5	****									
5- HNE	27	31	27	23	****								
6- CV	26	35	30	26	10	****							
7- BCP	23	33	28	25	15	6	****						
8- JHO	55	56	54	49	30	35	42	****					
9- TL	50	58	54	50	30	26	29	34	****				
10- FJ	55	63	60	55	34	31	34	33	8	****			
11- FJR	57	65	62	58	37	33	36	36	10	3	****		
12- DVM	61	67	64	59	38	36	41	27	17	13	15	****	
13- MCR	81	82	80	75	55	59	65	27	46	44	45	31	****
14- GH	76	83	88	82	69	64	59	82	48	49	46	60	94
15- SFS	80	86	86	86	71	64	63	82	49	48	46	59	90
16- SFN	83	96	90	87	72	65	64	78	46	45	42	53	81
17- GBR	70	78	81	74	57	51	51	64	31	29	27	40	67
18- TME	85	96	91	90	72	64	65	75	45	43	39	51	76
19- TMW	85	95	91	90	71	64	64	75	45	43	39	50	76
20- WC	93	100	105	97	75	72	72	80	51	48	44	54	77

Table II.2. Geographic distances (km) between the different sampled populations *T. jonesii* var. *lutea* (14-20).

	14- GH	15- SFS	16- SFN	17- GBR	18- TME	19- TMW	20- WC
14- GH	****						
15- SFS	9	****					
16- SFN	18	12	****				
17- GBR	23	18	11	****			
18- TME	24	18	9	12	****		
19- TMW	24	17	9	13	0	****	
20- WC	33	26	17	21	11	12	****

Appendix III Isozyme buffer recipes

Table III.1. Running buffer recipes used for both *T. aprica* and *T. jonesii* var. *lutea.*

Lithium-borate/ Tris-citrate	**pH 8.1/ 8.4**	**45mA / 175-300V / 7hrs**
Discontinuous	**Mol.**	**Chemical**
Electrode buffer	0.188M	Boric Acid (H3BO3)
titrate to pH 8.1 with	0.038M	Lithium hydroxide (LiOH)
Gel buffer	0.045M	Trizma Base (Tris)
titrate to pH 8.4 with	0.007M	Citric Acid anhydrous
1:9 mixture of EB:GB to make gel with 45ml EB with 405 ml GB		

Amine-Citrate (Morpholine)	**pH 8.0**	**50-60 mA / 160-175 V / 4hrs**
Continuous	**Mol.**	**Chemical**
Electrode buffer	0.04M	Citric Acid monohydrate
titrate to pH 8.0 with		N-(3-aminopropyl)-morpholine
Gel buffer	1:19 mixture of 22.5ml EB: 427.5 ml of ddH20	

Table III.2. Extraction buffer recipe used for leaf tissue of *T. aprica* and *T. jonesii* var. *lutea.*

Germanium	**Chemical**	**Amount**	**Molarity**
Heat up in microwav	dH2O	25 ml	
Add when H20 is hot	Germanium	0.028 g	0.005 M
Add once H20 has cooled	Diethydicarb acid	0.091 g	0.021 M
"	PVP 40	1.306 g	0.001 M
"	Borax	0.37 g	0.039 M
"	Sodium bisulfate (meta)	0.1 g	0.039 M
"	Asorbic Acid	1.3 g	0.262 M
"	Phophate buffer	2.67 ml	0.160 M
Add day of grinding	DMSO	2.67 ml	0.136 M
"	2-phemoxyethonol	0.17 ml	0.062 M
"	2-mercaptoethanol	2 drops	0.010 M

Appendix IV Allele Frequencies

Table IV.1. Allele frequencies of *Townsendia aprica.*

	Populations												
Locus/ allele	**1 MCC**	**2 MMD**	**3 WHF**	**4 JM**	**5 HNE**	**6 CV**	**7 BCP**	**8 JHO**	**9 TL**	**10 FJ**	**11 FJR**	**12 DVM**	**13 MCR**
Bottleneck significance		P< 0.05	P< 0.05	P< 0.05	P< 0.05				P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.05
N	21	27	27	27	25	27	27	27	15	27	27	27	27
PGM-1													
a	0.81	0.72	0.11	0.26	0.14	0.07	0.09		0.50	0.06	0.04	0.02	
b	0.12	0.28	0.80	0.65	0.70	0.83	0.87	0.91	0.40	0.78	0.61	0.74	1.00
c	0.07		0.09	0.09	0.16	0.09	0.04	0.09	0.10	0.17	0.35	0.24	
6PGDH-1													
a	0.48	0.52	0.28	0.26	0.44	0.44	0.57	0.39	0.53	0.37	0.19	0.22	0.20
b	0.29	0.15	0.37	0.44	0.34	0.44	0.35	0.46	0.17	0.44	0.50	0.43	0.32
c	0.24	0.33	0.35	0.30	0.22	0.11	0.07	0.15	0.30	0.19	0.32	0.35	0.48
AAT-1													
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b													
AAT-2													
a	0.93	0.83	0.85	0.80	0.88	0.87	0.87	0.96	0.73	0.78	0.85	0.87	0.69
b	0.07	0.17	0.15	0.20	0.12	0.13	0.13	0.04	0.27	0.22	0.15	0.13	0.32
MDH-1													
a	0.24	0.33	0.19	0.46	0.16	0.19	0.09	0.46	0.17	0.35	0.37	0.19	0.19
b	0.62	0.43	0.59	0.48	0.52	0.56	0.57	0.44	0.43	0.41	0.39	0.57	0.59
c	0.14	0.24	0.22	0.06	0.18	0.26	0.28	0.09	0.20	0.24	0.24	0.17	0.22
d					0.14		0.06		0.20			0.07	
IDH-1													
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b													
IDH-2													
a	0.91	0.89	0.91	0.59	0.52	0.78	0.63	1.00	0.33	0.72	0.46	0.57	0.74
b	0.10	0.11	0.09	0.41	0.48	0.22	0.37	0.00	0.67	0.28	0.54	0.43	0.26
G6PDH-1													
a	1.00	0.93	0.87	0.94	0.96	0.91	0.93	0.98	0.70	0.63	0.80	0.83	0.59
b		0.07	0.13	0.06	0.04	0.09	0.07	0.02	0.30	0.37	0.20	0.17	0.41
LAP-1													
a	0.88	0.78	0.70	1.00	0.86	0.91	0.83	0.80	0.83	0.78	0.82	0.67	0.96
b	0.12	0.22	0.30		0.14	0.09	0.17	0.20	0.17	0.22	0.19	0.33	0.04

Table IV.2. Allele frequencies of *Townsendia jonesii* var. *lutea.*

	Populations						
Locus/ allele	**14 (1) GH**	**15 (2) SFS**	**16 (3) SFN**	**17 (4) GBR**	**18 (5) TME**	**19 (6) TMW**	**20 (7) WC1**
Bottleneck significance	P< 0.05	P< 0.05			P< 0.05		P< 0.05
N	27	27	27	27	27	27	27
PGM-1							
a	0.13	0.04	0.09	0.15	0.24	0.28	0.30
b	0.13	0.69	0.74	0.83	0.61	0.65	0.54
c	0.74	0.28	0.17	0.02	0.15	0.07	0.17
6PGDH-1							
a	0.28	0.28	0.26	0.09	0.13	0.15	0.09
b	0.06	0.13	0.20	0.28	0.33	0.37	0.28
c	0.67	0.59	0.54	0.63	0.54	0.48	0.63
AAT-1							
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b							
AAT-2							
a	0.74	0.87	0.83	0.91	0.83	0.80	0.74
b	0.26	0.13	0.17	0.09	0.17	0.20	0.26
MDH-1							
a	0.20	0.19	0.13	0.13	0.17	0.11	0.13
b	0.48	0.46	0.54	0.52	0.44	0.57	0.50
c	0.20	0.20	0.26	0.32	0.32	0.24	0.32
d	0.11	0.15	0.07	0.04	0.07	0.07	0.06
IDH-1							
a	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b							
IDH-2							
a	0.19	0.15	0.02	0.06	0.07	0.07	0.11
b	0.82	0.85	0.98	0.94	0.93	0.93	0.89
G6PDH-1							
a	0.91	0.98	0.96	0.98	0.94	0.98	0.94
b	0.09	0.02	0.04	0.02	0.06	0.02	0.06
LAP-1							
a	0.91	0.87	0.91	0.59	0.61	0.63	0.56
b	0.09	0.13	0.09	0.41	0.39	0.37	0.44

Appendix V Representative photos of each taxon

T. aprica at 10-FJ yellow flowered.

T. aprica at 2-MMD cream flowered.

T. aprica at 2-MMD pink flowered.

T. aprica at 4-JM habit and typical size (2cm).

T. lutea var. *lutea* 14-GH cream flowered.

T. lutea var. *lutea* 18-TME pink flowered.

T. lutea var. *lutea* 18-TME cream flowered.

T. lutea var. *lutea* 20-WC disk pappus.