DIVERSIFICATION AND EVOLUTION OF TOWNSEND'S DAISIES (*TOWNSENDIA*-ASTERACEAE): A PHYLOGENETIC AND NICHE MODELLING PERSPECTIVE

by

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ABSTRACT

Townsendia is a genus of the sunflower family (Asteraceae) flush with species that frequently specialize in marginal habitats limited by various abiotic factors (e.g., soil, temperature, precipitation), particularly in higher elevation habitats. Although species are well defined geographically and morphologically, the evolutionary relationships in the genus remain unclear. This thesis investigates the evolutionary relationships of *Townsendia* species through phylogenetic inference, and also uses an ecological niche modelling (ENM) approach to better understand the processes that have lead to inter- and intra-specific variation of the genus. Phylogenetic analysis of plastid DNA regions and the internal transcribed spacer (ITS) establishes the monophyly of the group, and also recovers a large core polytomy in *Townsendia* and two clades sister to the rest of the genus. The presence of this core polytomy suggests that the diversity in *Townsendia* may be the result of a recent and rapid process of adaptive radiation. Further evidence for an adaptive radiation in the genus may appear as greater rates of ecological niche divergence between related species, relative to expectations based on a process of random diversification under background environmental conditions. Looking at interspecific variation, ENMs presented varying levels of niche divergence between related species, suggesting that a combination of niche conservatism and divergence played a role in the evolutionary history of the genus. Niche modelling is also used to better understand the variation present within one species, particularly one with discrete population subsets such as diploid sexuals and polyploid asexuals. Polyploid asexual populations of *T. hookeri* tend to have a wider and more northerly distribution than their diploid counterparts, though the role of this pattern on the speciation of *Townsendia* is unclear. Such unequal distributions may arise from differences in dispersal ability, or in abiotic preferences between reproductive types. Comparisons of

ENMs between these groups find evidence for intraspecific niche variation, and also predict the role of competitive exclusion, as factors that drive and maintain this distributional pattern. Overall, the combined use of phylogenetic analysis and ecological niche modelling in this thesis improves our understanding regarding the distribution patterns, evolutionary history and speciation pathways of *Townsendia.*

PREFACE

All of the research presented in this thesis was conducted in the field or in the Whitton Lab at the University of British Columbia, Point Grey campus.

I was the lead investigator for all of the projects in Chapters 2 to 4, where I was primarily responsible for the formation of concepts, data collection and analysis, as well as for the composition of the manuscripts. Undergraduate students, Scott Black, Beryl Zhuang, Erica Li-Leger and Alberto Ruiz-Larrera, each contributed in the georeferencing of species localities used in Chapter 2 and 3. Then undergraduates, Kate McGrath and Ryan Godfrey were involved in a portion of the sequencing results in Chapter 2 as part of their lab skills training. Alice Garani (graduate student), Alberto Ruiz-Larrera, Erica Li-Leger and Ryan Godrey contributed many of the ploidy determinations via flow cytometry or pollen counting that were used in Chapter 4. Sean Graham, Loren Rieseberg and Wayne Maddison contributed through manuscript edits and in data interpretation. Jeannette Whitton was the supervisory author on this thesis and was involved in the project from the early stages of concept formation, through data analysis and interpretation, to manuscript edits.

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I'd also like to thank all my lab mates in the Whitton Lab. In particular, Chris Sears and Gina Choe helped me immensely in getting my thesis project started, and have been invaluable sources of advice and guidance. Also, Kate McGrath, Alice Garani, Jamie Leatham, Evan Hersh, Jamie Fenneman and Michael Scott have all taught me a lot as we helped each other through our respective research projects. Thank you also to all the undergraduate students that have helped me with various projects in the lab. The students of the Graham Lab have also been incredibly helpful. Will Iles has given endless advice regarding all topics, but with phylogenetics in particular. Also, Marybel Soto Gomez has discussed a huge variety of topics with me and has helped me become a better teacher and ethnobotanist. I'd also like to thank Jenny McCune for her endless optimism, which has helped me so much academically and personally. Matt Strimas-Mackey has been an amazing help for all things R related, and was a fantastic travel companion.

I also thank Adam Wilkinson, Elizabeth Kleynhans and Bill Harrower for their irreplaceable companionship as we explored the mountains of Vancouver while discussing botany, ecology, evolution and more. I'd also like to thank Paul Johnson, Valerie Jerome, Randy Paterson and Geoff Bannoff for their support and for encouraging me to finish my thesis. Thanks also to my parents, Alex and Angel Lee, who have always supported my academic decisions even though I never became a "real" doctor. Lastly, thank you to Luke Halpin who has been my constant companion and has instilled in me an even greater love for nature by teaching me all about birds while I taught him about plants.

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DEDICATION

For every mountain that rises, and erodes away,
And for each tree, shrub or herb, wherever they may lie.
For all the places I've been, or have yet to find,
And for those who preserve it for future minds.

CHAPTER 1: Introduction

General Introduction:

The mid 19th century was a turning point in the botanical world due to the rapid discovery of new species and the improved dissemination of botanical research. This period was rich with naturalists, such as Asa Gray and Sir Joseph Dalton Hooker, who endured trans-oceanic voyages to document through collections or illustrations the flora of distant lands (Gribbin and Gribbin, 2008). In this age of exploration, new plants were rapidly being discovered and incorporated into the Linnaean classification system presented in the *Systema Naturae* (Linnaeus, 1735). This artificial system of plant classification was known as the 'Sexual System' and was based on the number of stamens in a flower rather than on perceived natural relationships. Despite its simplicity of use and its popularity, Linnaeus's sexual system began to fall into disuse around the 1840s. For the first time, the format of floras became recognizably 'modern' (Frodin, 2001) as botanical floras shifted to focused on organizing plant families, genera and species based on taxonomic similarities within groups.

One early example of the 'modern' flora is the *Flora Boreali-Americana* (Hooker, 1840), the first flora of the northern British Americas (now including much of Canada and north-western USA). Based primarily on the field collections of Dr. John Richardson and Thomas Drummond who accompanied Captain Sir John Franklin's northern expeditions, the flora was compiled in 1840 by William Jackson Hooker, then director of the Royal Botanic Gardens, Kew. This flora brought the botanical diversity of northern British Americas to the forefront of British botanists' minds, and described for the first time many northern American taxa. Among these is our charismatic *Townsendia,* first described as a monotypic genus of subtribe Astereae in the Asteraceae (Figure 1.1). Prior to the publication of the

Flora Boreali-Americana, the first voucher of *Townsendia* had been tentatively named *Aster (?) exscapus* by Dr. Richardson. However, due to the breadth of knowledge he gained from examining the plant vouchers deposited at Kew Gardens, Hooker determined that the specimen represented a new genus, and named it *Townsendia sericea* (Hooker, 1840).

The years following the publication of the *Flora Boreali-Americana* (Hooker, 1840) marked the beginnings of the second industrial revolution. Although the first industrial revolution saw many fortuitous innovations, there was a distinct lack in scientific understanding behind these advancements, which led, for example, to a "chemical industry without chemistry, and an iron industry without metallurgy" (Mokyr, 1990). In contrast, the second industrial revolution was facilitated by breakthroughs that underscored a deeper understanding of the fundamental concepts underlying innovations. For example, the discovery of the Bessemer process was the beginning of a series of metallurgical discoveries that vastly improved the quality and affordability of steel production (Adams and Dirlam, 1966). Buoyed in part by the improvements in steel production, the railway networks expanded dramatically, and in combination with the development of macadam (and later tarmac) road surfaces, and vulcanized rubber for tire production, overland transportation vastly improved.

Improvements in transportation facilitated and fuelled expeditions into the new world for purposes of resource extraction and colonization, but also enabled additional research on the natural history of newly explored territories, including North America. For example, Nuttall (1841) added four species (*T. incana*, *T. spathulata*, *T. strigosa* and *T. grandiflora*) to *Townsendia*, and this was further expanded by various botanists until Gray's treatment (1888) recognized a full seventeen species and four subspecies in the genus (Table 1.1). Over the

next forty years, additional species were discovered and named until Larsen (1927) produced the next complete treatment of the genus as a whole, in which she consolidated the genus into 19 species (Table 1.1). At the time, Larsen combined species that she believed represented regional variation rather than distinct species, such as *T. pinetorum*, now *T. formosa*, and *T. vreelandii*, now *T. eximia*. Larsen also recognized for the first time that *T. sericea* as described by Hooker comprised two species, *T. exscapa* as well as *T. sericea*. Following this discovery, the original *Aster (?) exscapus* voucher became the type for *T. exscapa*, and a later collection by Drummond (1830) became the new type specimen for *T. sericea.* Synthesizing previous work, Beaman's monograph (1957) included 21 species and 4 varieties (Table 1.1). Since then, additional species such as *T. microcephala* (Dorn, 1992), *T. smithii* (Shultz, Holmgren, and Jun, 1980) and *T. gypsophila* (Lowrey *et al.*, 1994) have been discovered, which led to their incorporation in the *Flora of North America* (Strother, 2006). In this study I recognize 28 species and 3 varieties of *Townsendia* (Table 1.1).

Just as the greater understanding of scientific principals allowed the vast improvement of many industrial processes, concurrent advances in biology improved our understanding of the natural world. In particular, Darwin's theory of natural selection (1859) altered the fundamental understanding and study of the origin and evolution of species. The most recent treatment of *Townsendia* (Strother, 2006) in the *Flora of North America*, and the earlier revision of the genus (Larsen, 1927) used morphological measurements alone to summarize the circumscription and relationships of species known at the time of their writing. In contrast, Beaman (1954, 1957) was the first to examine the entire genus in an evolutionary framework, and generally followed a biosystematics approach by combining field work, herbarium studies, cytological work and crossing experiments. His work included

extensive field study, which instilled in him an understanding and appreciation for the importance of geography and edaphic specialization on the origins and distribution of *Townsendia*. Beaman's work in the field was accompanied by an abundance of morphological study of herbarium specimens, and in combination, allowed Beaman to make inferences regarding the phylogenetic relationships within the genus. In his monograph, Beaman was also able to finally clarify the confusion surrounding the circumscription of *T. exscapa* and *T. sericea* by recognizing that the type specimen for *T. exscapa* was in fact a mixed voucher that also included a specimen of *T. sericea*. Thus, the rules of botanical nomenclature (ICBN) invalidated the species epithet '*sericea'*, and Beaman then renamed this species *T. hookeri* in honour of William Hooker.

Through his fieldwork and herbarium studies of *Townsendia* vouchers, Beaman noticed the presence of plants with intermediate or atypical morphology at species range margins. Because of their presence between species ranges and their relative rarity on the landscape, Beaman took these populations to be putative natural hybrids. To gather evidence for potential hybridization in *Townsendia*, Beaman conducted preliminary crossing experiments mostly between species that he considered closely related. Through these experiments, Beaman found that hybridization occurred readily in many experimental crosses, even between apparently distant species. The ease of artificial crossing suggested that intrinsic genetic reproductive barriers were less important than factors such as geographic distribution, ecological specialization and phenology in maintaining barriers between species.

To better understand relationships in *Townsendia*, Beaman also conducted a number of cytological studies looking at pollen and ovule development, pollen viability and root

chromosome squashes (Beaman, 1957). Extensive cytological work confirmed to Beaman the occurrence of polyploidy and asexual seed production via apomixis in many *Townsendia* species. Furthermore, hybridization appeared to have no role in the occurrence of these traits, and polyploids were almost always inferred to be of purely autopolyploid origin. Beaman did notice, however, that polyploidy and apomixis are linked in *Townsendia*, and also found evidence for multiple, independent origins of these traits throughout the genus. Multiple origins were later confirmed in one species, *T. hookeri*, based on analysis of chloroplast haplotype variation (Thompson and Whitton, 2006). Considering the distribution of polyploids and apomicts across the landscape, Beaman concluded that polyploidy and apomixis granted a plant improved dispersal ability and contributed to intraspecific variation. However, he asserted that these factors seemed unlikely to be important drivers of speciation because no species is solely polyploid. In addition, Beaman noticed the tendency for apomictic polyploid populations to inhabit higher latitudes and elevations than their diploid progenitors, which formed a pattern known as geographical parthenogenesis (Vandel, 1928). Based upon his body of work, Beaman believed that geographic isolation, edaphic specialization and unique climate preferences were the driving forces behind speciation in *Townsendia*.

The striking landscapes on which many of the species of Townsendia grow are so distinctive that they naturally evoke questions about speciation, biogeographic history, species relationships, dispersal, reproduction, abiotic limitations and edaphic specialization (Figure 1.2). Beaman (1954, 1957) saw the landscapes and distributional patterns common to the genus, and began forming hypotheses to address broader evolutionary questions such as how the landscapes promoted reproductive isolation and speciation in the group. In doing so,

he laid down a solid foundation for future investigation in the group. For example, Beaman's cytological work found that many species of *Townsendia* had polyploid populations, which were inferred to be mostly autopolyploid due to species distributions. Beaman also found that polyploidy was typically associated with obligate apomixis, in fact, there are no known sexual polyploids in *Townsendia*. Apomixis, the production of seeds without fertilization of the egg, is a well documented occurrence in plants (Whitton, *et al.*, 2008), especially in Asteraceae (Noyes, 2007). When coupled with field and herbarium studies, Beaman (1957) characterized for the first time the distribution of polypoid and diploid populations of the species of *Townsendia*. From this work, different patterns of distribution became apparent in the two reproductive types. Species such as *T. hookeri*, *T. parryi*, and *T. leptotes* had much more widespread apomictic polyploid populations when compared to sexual diploid populations, which remained in a small core distribution. Additionally, the asexuals were generally found in more northerly localities and at higher elevations than their diploid progenitors. Even though these distributional patterns are likely determined by an array of multivariate factors ranging from ecological preferences to dispersal ability, it is notable that the outcome is repeatedly a broader and more northern distribution of apomicts. Do asexual polyploids and sexual diploids of the same species have different ecological niches? If so, under what set of ecological conditions do asexuals hold an advantage over sexuals, and vice versa? These questions are further examined within one species, *T. hookeri*, in Chapter 2.

Another outcome of Beaman's work was a preliminary phylogeny for *Townsendia* based on the correlation of morphological traits and geographical distribution. Although Beaman's tree (Figure 1.3) provides an initial hypothesis, new species of *Townsendia* have been added since Beaman's treatment (Table 1.1). Most importantly, our understanding of

speciation, evolution, and "primitive" (ancestral) and derived character traits, has changed over time. Furthermore, the expansion of molecular data and phylogenetic inference methods provides additional characters and robust methods with which to infer the phylogenetic history of *Townsendia*. In Chapter 3, I use chloroplast and nuclear ribosomal data to infer relationships in *Townsendia*, allowing new insights into the origin and diversification of the genus.

Beaman's work on *Townsendia* culminated in his belief that the process of speciation and the factors maintaining species boundaries in the genus were based on geography, climate and niche specialization. One example where climate appears especially important is *T. rothrockii,* a high elevation species present in the southwestern quadrant of Colorado. *Townsendia rothrockii* is found at elevations as low as 2500 m, but more frequently inhabits the high mountain crests, ridges and summits of the Continental Divide up to 3900 m. At these cold, high elevation sites, plants overwinter under snow banks and flower when overtopping snow melts away. Another example of niche specialization in the genus is *T. glabella*, a species restricted to the Mancos Shale formations of southwestern Colorado. Soils of the Mancos Shale formation are sticky and binding when wet, but hard and impenetrable when dry (Cannon, 1986). These water-binding qualities create an impermeable layer that prevents water absorption into the deeper regions of the soil. Additionally, the chemical properties of Mancos Shale include high salinity, and high concentrations of selenium (Welsh, 1978), both of which are detrimental to plant growth (Broyer, Lee, and Asher, 1966; Bernstein, 1975). These physical and chemical properties of the Mancos Shale contribute to osmotic stress, and likely have a role in the evolution of this species.

Townsendia rothrockii and *T. glabella* are two examples in a genus replete with species that appear to specialize in specific climatic or edaphic conditions, and these factors appear to have a role in speciation of *Townsendia* as a whole. In Chapter 4, I develop ecological niche models for a set of species of *Townsendia* to explore the association of climatic variables with the distribution of species. I compare models between putative sister species (inferred from the phylogeny produced in Chapter 3) to assess niche overlap, conservatism and divergence, and use these metrics to gain insight into the role of niche shifts in speciation.

In any study of ecological shifts, the interpretation of data necessitates an understanding of patterns of variation in abiotic features, including current and past climatic conditions, and the glacial and geological history of the landscape. Although the biogeographic history of *Townsendia* is not fully understood, the genus appears to have diversified rapidly in North America following the Pleistocene (Beaman, 1957). For example, Beaman believed that the widespread cooling and increased aridity during the Pleistocene, including areas where *Townsendia* are currently found, likely had a large impact on the evolution of the genus. The impact of changing climates on the evolutionary history of plants during and after the Pleistocene is evident in many taxa and habitats (Comes and Kadereit, 1998). Associated with these climatic changes, the cycle of retreating glaciers following the Last Glacial Maximum (LGM) approximately 22,000 years ago, also opened up new habitats into which many groups, including *Townsendia,* could diversify (Shafer *et al.*, 2010). The geographical distributions of the species of *Townsendia* sister to the rest of genus and of outgroup taxa, *Astranthium* and *Dichaetophora*, suggest a Mexican origin for these groups. Mostly a genus of Mexico and the southern USA, *Astranthium* appears to have

diversified in the foothills between the Sierra Madre Occidental and Oriental mountain ranges of Mexico. Although, glaciers had little presence in Mexico, the Pleistocene nonetheless represented a period of dying and cooling that seemed to have spurred diversification in *Astranthium* (DeJong, 1965). In contrast, *Townsendia* apparently diversified northward, approximately following the Rocky Mountain regions through the U.S. and into Canada (Beaman, 1957).

Climate is inextricably linked to and reciprocally affected by the surrounding landscapes, soils and plant species. Broadly speaking it can be argued that geomorphological features such as oceans and mountains play the largest role in shaping the regional climate (Kruckeberg, 2004). The formation of mountains ranges such as the Sierra Madres, for example, results in a rain shadow that contributes to regional drying. In return, however, mountain ranges are affected by climate as wind and water erode the soil, which may create sheltered crevices or other microhabitats that promote plant growth (Kruckeberg, 2004). Similarly, plant communities can affect soil by promoting soil development, or by preventing erosion, which affects the experienced microclimate (e.g., through soil water retention) (Rees *et al.*, 2008). Overall, though, regional climates denote the absolute limits of water and temperature stress on a plant. For example, the lack of overall precipitation in a region limits water availability regardless of localized features like shaded crevices or water retaining soils. The availability of suitable climatic conditions, then, allows species range shifts that have the potential to be later cemented into speciation events based on adaptation to specific soil characteristics. Even though it is only one component of the interrelated factors contributing to plant growth and speciation, the study of climate preferences indirectly relates the factors of geomorphology and soil in the evolutionary history of a taxon.

Thesis Outline and Objectives:

This thesis aims to contribute to our understanding of how ploidy, asexuality, climatic variation and geographic distribution affect, and are affected by the processes of speciation in *Townsendia* in three studies.

In Chapter 2, I detail the geographic distribution of diploid sexual and polyploid apomictic populations *T. hookeri*, and apply ecological niche models to test hypotheses for their classical pattern of geographical parthenogenesis. Significant differences in the niche models of sexuals and asexuals would lend support to a role for ecological diversification in the distinct distribution of reproductive types.

In Chapter 3, I include all currently recognized species of *Townsendia* in a molecular phylogenetic study based on regions of the plastid and nuclear ribosomal DNA to clarify relationships among species in the group. The resulting phylogeny is used to explore the evolution of various taxonomic traits in certain clades, and also reveals a potential adaptive radiation in *Townsendia*.

In Chapter 4, I investigate the potential role of ecological diversification, a key component of adaptive radiation, in the diversification of *Townsendia* through the use of ecological niche modelling. The niche models of phylogenetically supported clades are compared to investigate whether inferred niches diverge more, less or as expected under neutral divergence between sister groups. Detection of greater than neutral diversification would support a role for adaptive divergence in *Townsendia*, as expected under a model of adaptive radiation.

Table 1.1 - List of *Townsendia* species recognized in other treatments and in this thesis

Species	Current Name (*if different*)	Authority	Treatment*				
			Gray	Larsen	Beaman	Strother	Lee
T. annua		Beaman 1957			+	+	+
T. aprica		Welsh & Reveal 1968				+	+
T. arizonica	*T. incana*	Nuttall 1840	+	+			
T. condensata		Parry 1874	+		+	+	+
T. eximia		A. Gray 1849	+	+	+	+	+
T. exscapa		(Richardson) Porter 1894		+	+	+	+
T. fendleri		A. Gray 1894	+		+	+	+
T. florifer		(Hooker) A. Gray 1880	+	+	+	+	+
T. formosa		Greene 1906		+	+	+	+
T. glabella		A. Gray 1881	+	+	+	+	+
T. grandiflora		Nuttall 1840	+	+	+	+	+
T. gypsophila		Lowrey & P.J. Knight 1994				+	+
T. hookeri		Beaman 1957			+	+	+
T. incana		Nuttall 1840	+	+	+	+	+
T. jonesii var. *jonesii*		(Beaman) Reveal 1970				+	+
T. jonesii var. *tumulosa*		Reveal 1970					+
T. jonesii var. *lutea*		S.L. Welsh 1983					+
T. leptotes		(A. Gray) Osterhout 1908		+	+	+	+
T. mensana		M.E. Jones 1910			+	+	+
T. mensana var. *jonesii*	*T. jonesii*	(Beaman) Reveal 1970			+		
T. mexicana			+	+	+	+	+
T. microcephala		Dorn 1992				+	+
T. minima		Eastwood 1936				+	+
T. montana		M.E. Jones 1895		+	+	+	+

*Treatments include Synoptical Flora of North America: The Gamopetalae (Gray, 1888), A Revision of the Genus *Townsendia* (Larsen, 1927), The Systematics and Evolution of *Townsendia* (Beaman, 1957), and The Flora of North America: *Townsendia* (Strother, 2006).

Table 1.1 (cont.) - List of *Townsendia* species recognized in other treatments and this thesis

Species	Current Name (*if different*)	Authority	Treatment*				
			Gray	Larsen	Beaman	Strother	Lee
T. montana var. *minima*	*T. minima*	Eastwood 1936			+		
T. parryi		D.C. Eaton 1874	+	+	+	+	+
T. parryi var. *alpina*	*T. spathulata*	Nuttall 1840	+				
T. rothrockii		A. Gray ex Rothrock 1879	+	+	+	+	+
T. scapigera		D.C. Eason 1871	+	+	+	+	+
T. scapigera var. *ambigua*	*T. parryi*	D.C. Eaton 1874	+				
T. scapigera var. *caulescens*	*T. scapigera*	D.C. Eason 1871	+				
T. sericea	*T. exscapa*	(Richardson) Porter 1894	+	+			
T. sericea var. leptotes	*T. leptotes*	(A. Gray) Osterhout 1908	+				
T. smithii		L.M. Shultz & A.H. Holmgren 1980				+	+
T. spathulata		Nuttall 1840	+	+	+	+	+
T. strigosa		Nuttall 1840	+	+	+	+	+
T. texensis		Larsen 1927		+	+	+	+
T. watsonii	*T. florifer*		+	+			
T. wilcoxiana	*T. exscapa*	(Richardson) Porter 1894	+				

*Treatments include Synoptical Flora of North America: The Gamopetalae (Gray, 1888), A Revision of the Genus *Townsendia* (Larsen, 1927), The Systematics and Evolution of *Townsendia* (Beaman, 1957), and The Flora of North America: *Townsendia* (Strother, 2006).

Figure 1.1 - Images of representative species of *Townsendia*. A) *T. parryi*. B) *T. hookeri*. C) *T. condensata*. D) *T. annua*

Figure 1.2 – Selected images of Townsendia habitats. A) In Capitol Reef National Park near Torrey, Wayne County, Utah. A typical locality of *T. aprica*. B) Disturbed roadside near Datil, Socorro County, New Mexico. *Townsendia annua* was present here. C) Rocky, exposed ridgetops in the Gros Ventre Wilderness near Jackson, Teton County, Wyoming. A typical locality of *T. parryi*.

Figure 1.2 (cont.) - Habitats of *Townsendia*. D) Rocky outcrop on mixed sagebrush-grass hillside near road in Wilsall, Park County, Montana. *Townsendia hookeri* was present here. E) Steep, eroding outlook in the Absaroka Range between Yellowstone National Park and Cody, Park County, Wyoming. The exposed habitat is typical of *T. condensata*. F) Cushion plant community on exposed mesa rim of Cedar Mountain near Lyman, Uinta County, Wyoming. This is the only known locality of *T. microcephala*.

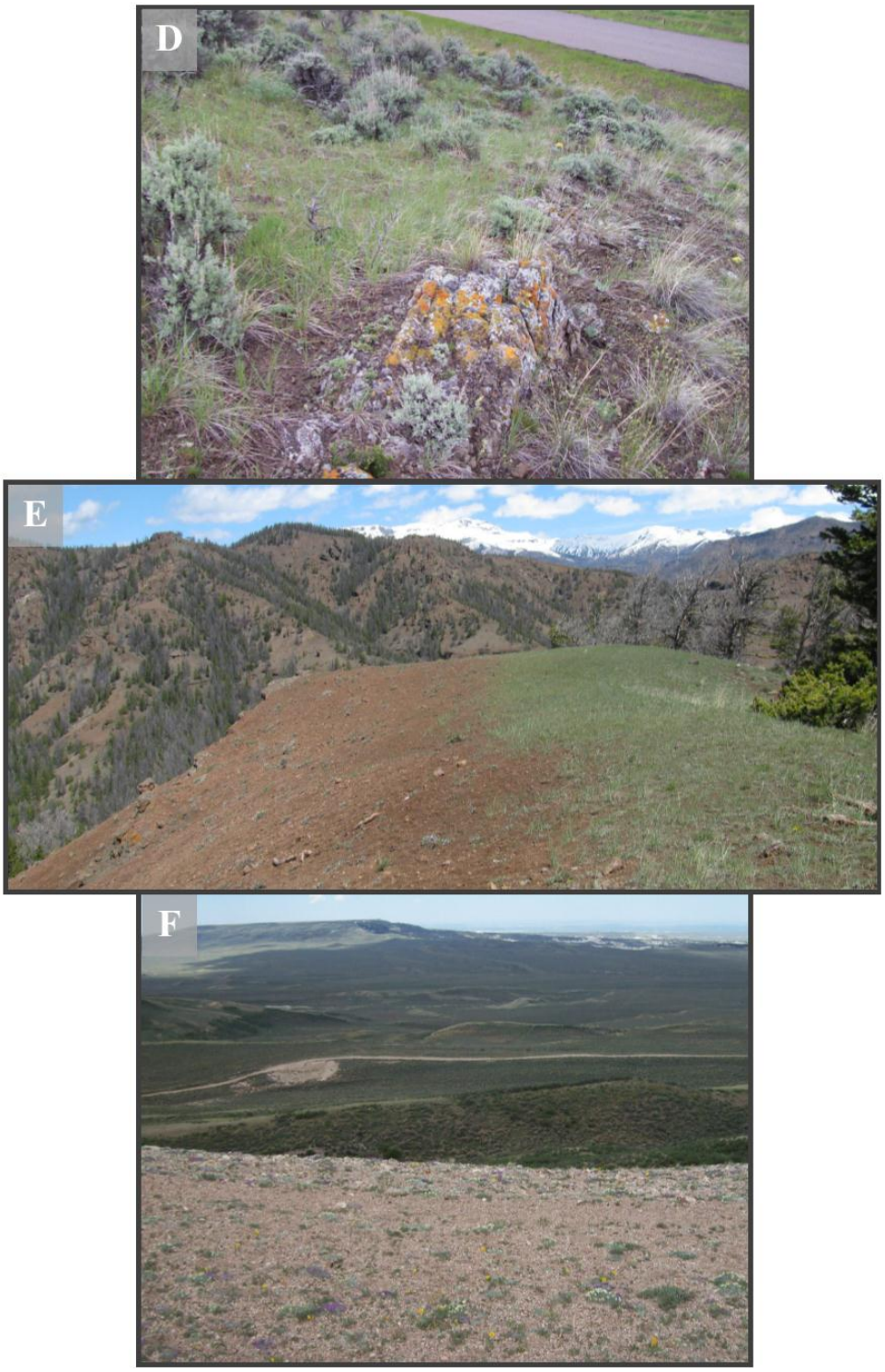
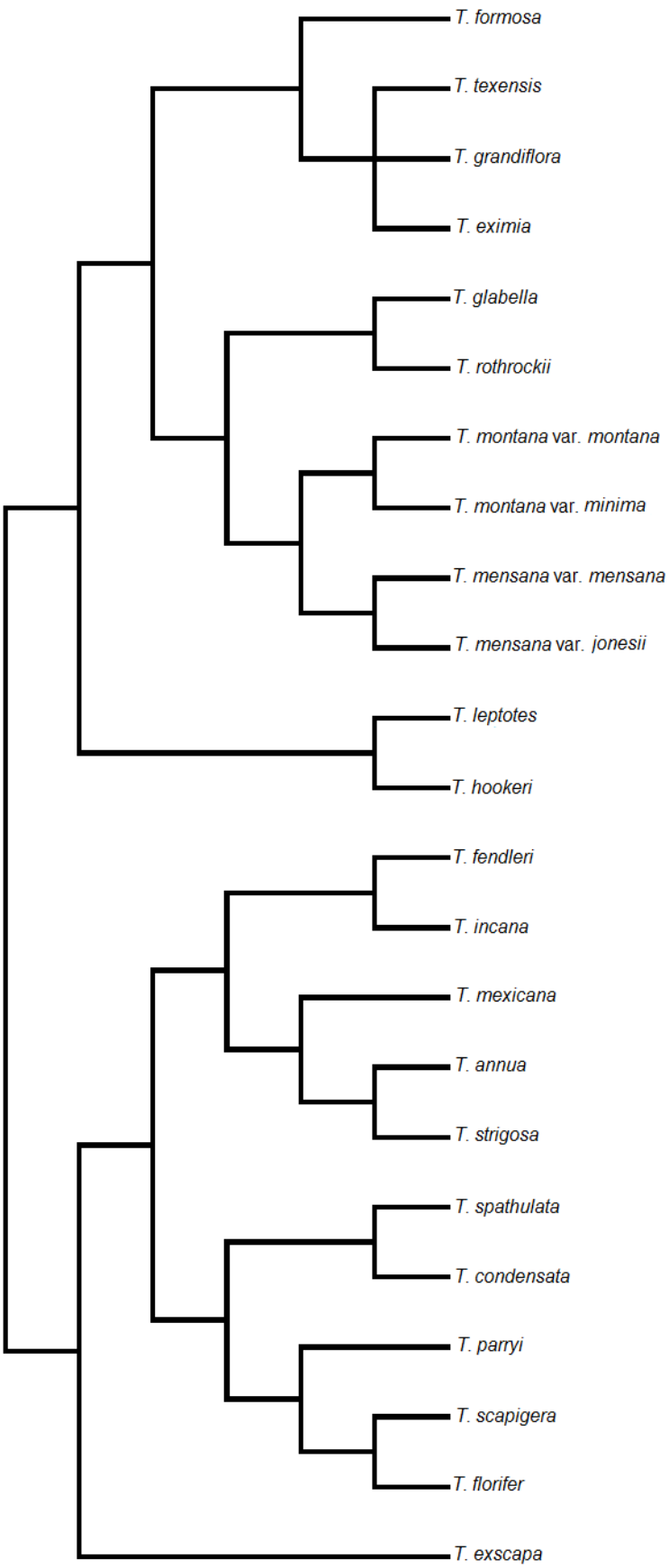


Figure 1.3 - Redrawn and unrooted tree representing Beaman's hypothesis of the phylogenetic relationships within *Townsendia* based on a biosystematic approach with cytological, field, taxonomic and hybridization studies (1957)


T. formosa
T. texensis
T. grandiflora
T. eximia
T. glabella
T. rothrockii
T. montana var. montana
T. montana var. minima
T. mensana var. mensana
T. mensana var. jonesii
T. leptotes
T. hookeri
T. fendleri
T. incana
T. mexicana
T. annua
T. strigosa
T. spathulata
T. condensata
T. parryi
T. scapigera
T. florifer
T. exscapa

CHAPTER 2: Testing the Role of Niche Divergence in Geographical Parthenogenesis within *Townsendia hookeri* (Asteraceae)

Summary:

Townsendia is a charismatic genus of the Asteraceae native to the Rocky Mountain regions of North America. Comprised of many narrowly endemic edaphic specialists, *Townsendia* also contains several widespread taxa. Within this latter group, *T. hookeri* ranges from the Front Range and Sangre de Cristo Mountains of south-central Colorado to Fort Saint John in British Columbia. Disjunct populations of *T. hookeri* bypass the remainder of northern British Columbia and appear again in the Watson Plateau of Yukon Territory, and near the Porcupine River of northern Alaska. Within the southern distribution, populations of *T. hookeri* are found as far east as Saskatchewan and the Dakotas, and to the west in Idaho and Utah. Two distinct types of populations occur over this range: outcrossing sexual diploids and apomictic polyploids (triploids and tetraploids), with both types occurring in the northern and southern parts of the range. The focus of this study is on the populations of the southern range where apomictic polyploid populations are known from southern British Columbia and Alberta to southern Wyoming. Apomict and sexual ranges overlap in southern Wyoming, but sexual populations extend further southward into central Colorado. This pattern of widely distributed, more northerly apomicts with relatively restricted, southern sexuals is known as geographical parthenogenesis and has been widely documented in plants and other groups (Vandel, 1928; Republic, 2007; Cosendai and Hörandl, 2010; Tarkhnishvili *et al.*, 2010). Several hypotheses have been proposed to explain the phenomenon of geographical parthenogenesis, each emphasising to varying degrees the role of dispersal ability, and ecological distinctness in establishing such a pattern. This study measures niche

divergence between sexual and asexual *T. hookeri* test ecologically based hypotheses of geographical parthenogenesis including the general-purpose genotype (Lynch, 1984) and frozen niche variation hypotheses (Vrijenhoek, 1984). I used climate data and species occurrence records to assess the degree of climatic niche overlap between *T. hookeri* sexuals and apomicts. The principal bioclimatic variable (Hijmans *et al.*, 2005) associated with the distribution of sexual diploids is precipitation seasonality, whereas for apomicts it is the mean temperature of the driest quarter. Furthermore, niche overlap statistics, identity tests and background tests show evidence of niche differentiation between sexuals and apomicts. Niche models predict the occurrence of apomictic polyploids is largely in agreement with its current distribution. In comparison, the model for sexual diploids predicts a larger range than observed that encompasses a portion of the apomictic range. These results suggest a role for niche divergence as part of the explanation for geographical parthenogenesis, but also suggest that factors such as minority cytotype exclusion prevent the northern expansion of sexual diploids into apomictic polyploid habitat.

Introduction:

Townsendia hookeri is one of the most widely distributed species of *Townsendia* (Figure 2.1), with disjunct northern (Yukon and Alaska) and southern (Colorado to Alberta and British Columbia) ranges. In each of these regions, both apomictic polyploid and sexual diploid populations are known (Beaman, 1957; Thompson and Whitton, 2006; Garani, 2014); however, in both the southern and northern parts of the range, sexual populations are geographically restricted relative to their more widespread apomictic counterparts. This distribution pattern of restricted, sexual diploids and more widely distributed, northern, asexual polyploids exemplify a pattern of geographical parthenogenesis (Vandel, 1928) that

is well documented in groups that include sexual and asexual reproductive types (Bierzychudek 1985; Cosendai, *et al*, 2013; Verduijn, *et al*, 2004; Vorburger, 2006). Although theories to explain the pattern of geographical parthenogenesis often include a role for ecological divergence between sexual and asexual forms, tests of these differences are uncommon. However, a number of studies have recently explored the evidence for ecological divergence between close relatives with different reproductive strategies (Lovell *et al.*, 2014; Thompson, Husband, and Maherali, 2014; Dalrymple, Buswell, and Moles, 2015; Murakami *et al.*, 2015) using ecological niche models (ENMs), plant trait measurements and genetic markers, such as microsatellites and single-nucleotide polymorphisms. Here, I use ecological niche models (Peterson, 2001) to look for evidence of climate related niche divergence between sexual and apomictic populations of *T. hookeri*.

In the southern range, sexual diploid populations occur predominantly in the Front Range of northern Colorado around Boulder, and can occasionally be found to the south in the foothills around Colorado Springs, Fremont and Gunnison (Beaman, 1957; Garani, 2014). Sexual populations of *T. hookeri* extend into the Wyoming Basin and Southern Rocky Mountain ecoregions (Western Ecology Division, 2011) around Laramie and Cheyenne, Wyoming, and populations in this part of the range are among the largest known, averaging approximately 60-80 plants in a population, with some populations being much larger (J. Whitton, pers. obs.). The frequency and size of sexual diploid populations decreases in the Northwestern Great Plains and the High Plains and are replaced by apomictic populations. The Wyoming Basin and Middle Rocky regions of Western Wyoming hold many small apomictic populations, many containing approximately 10-15 plants per population. Sparse

apomictic populations of *T. hookeri* also occur along the spine of the Rocky Mountains through Montana, and into southern British Columbia and Alberta.

The eastern range limit of *T. hookeri* occurs in western North Dakota and Saskatchewan, where populations appear to be apomictic polyploids. Scattered populations in and around the Little Missouri National Grasslands near Dickinson, North Dakota may represent the remnants of a much larger grassland range prior to the intensive agricultural development of the prairies beginning in the late 1880s (Hurt, 2002). In this part of the range, grassland populations of *T. hookeri* are frequently found mixed with *T. exscapa* (Lee and Garani, 2012). Despite individual plants growing as near as 50 metres to each other, the species show evidence of differences in ecological tolerances, and both appear to remain genetically distinct from each other (Lee and Garani, 2012). Whereas *T. exscapa* apparently thrives on fertile grassy plains, *T. hookeri* specializes on the exposed clinker outcrops and buttes, known locally as "scoria". Clinker is a class of rock composed of baked shale, limestone and sandstone produced as a by-product of burning underground coal beds (Heffern and Coates, 2004). Presently, the coal beds of North Dakota are often ignited through human activities such as mining. However, over the past four million years and prior to human intervention, natural causes such as lightning strikes periodically ignited underground coal deposits (Whitman and Hanson, 1939a). Heat from these past coal fires baked the sedimentary layers of rock to form new, hard, brick-like, erosion resistant rock, which over geological time has resulted in the characteristic clinker knobs and outcrops of the North Dakota Badlands. These geological features are hard and difficult to penetrate, which may hinder seedling establishment. Clinker outcrops are also highly alkaline and relatively impermeable, contributing to water stress by limiting water absorption into the

underlying soil (Whitman and Hanson, 1939). This combination of traits causes clinker to be a challenging substrate on which to survive and establish, and adaptation to this substrate may contribute to maintaining species distinctness in this region. In addition to habitat specialization, there is also evidence of different flowering time between taxa, further reducing the likelihood of hybridization.

The northern range of *T. hookeri* is separated by more than 1000 km from the nearest population in British Columbia. There are approximately 14 disjunct populations restricted to a small area in Yukon Territory (YT) encompassing parts of Kluane National Park and in the hillsides approximately 160 km north and 400 km west of Whitehorse. A single Alaskan (AK) locality is known, near the segment of the Porcupine River within the Arctic National Wildlife Refuge (Museum of the North Herbarium, 2013), more than 1300 km northwest of the Yukon populations. This disjunction between the southern and northern (including YT and AK) portions of the species distribution is thought to have arisen by vicariance (Thompson and Whitton, 2006), and may have been initiated during Pleistocene glaciation events that began more than 2.5 MYA (Yokoyama et al., 2000) (Figure 2.1). This long separation of the northern populations of *T. hookeri* roughly corresponds to a distinct plastid lineage (haplotypes g, h and j, in Thompson and Whitton, 2006) that is shared by the two YT and the northern apomictic populations. Even though the northern and southern lineages of *T. hookeri* share a common origin, their spatial and temporal separation likely mean that each has achieved its current distribution under independent and distinct circumstances, and should therefore be modelled separately. However, the low number of sexual occurrences in the north (two to three) precluded analysis of niche divergence in this part of the range. For these reasons, the sub-arctic populations are not included in my niche modelling and

analysis, and unless otherwise noted the remainder of this chapter refers to the southern range of *T. hookeri*.

In the southern range, sexual diploid populations of *T. hookeri* occur only in unglaciated regions, *i.e*. beyond the southernmost extent of the glacial sheets at the last glacial maximum (LGM). Although the distribution and extent of apomictic polyploids during the LGM is unclear, their current distribution suggests that asexual populations expanded into deglaciated landscapes following the retreat of the Cordilleran ice sheet. This general pattern of asexuals recolonizing previously glaciated landscapes is a hallmark of geographical parthenogenesis (Vandel, 1928), a hypothesis that attempts to account for the recurring pattern of broadly distributed northern asexuals and comparatively restricted southern sexuals.

In most groups with sexual and asexual members, these reproductive types evolve largely independently, and therefore research on geographical parthenogenesis can be situated in a broader research framework that aims to understand the factors that contribute to the existence of distinct geographical ranges in closely related taxa. Disparate ranges between related taxa may be the product of multiple factors such as physical barriers and environmental gradients (Rieseberg and Willis, 2007). These factors may drive speciation as organisms develop distinct habitat preferences, an important pre-zygotic mechanism of reproductive isolation between species (e.g., Rieseberg and Willis, 2007; Schluter, 2009), and between cytotypes (Felber-Girard, Felber, and Buttler, 1996; Thompson, Husband, and Maherali, 2014). The prevalence of recent and ancient whole genome duplication events heightens the importance of understanding speciation via polyploidy, including the role of ecological divergence and other pre-zygotic mechanisms of isolation. Prezygotic

reproductive isolation, including ecological divergence, is believed to be critical for the early establishment of sexual polyploids as they would otherwise suffer from minority cytotype disadvantage caused by the low fitness of inter-cytotype hybrids (Levin, 1975). Few studies have sought evidence for ecological divergence between cytotypes in sexual polyploids, with some studies finding little evidence for ecological divergence (Baack and Stanton, 2005), and others finding evidence for segregation, such as along environmental gradients (Schönswetter *et al.*, 2007).

Because apomixes protects new apomictic polyploids from gene flow, minority cytotype disadvantage is not expected to drive ecological divergence (assuming new apomictic polyploids are mostly or obligately apomictic) (Kao, 2007), therefore, the existence of geographical parthenogenesis in a diversity of unrelated taxa requires explanation. However, the factors that contribute to the origin and maintenance of this pattern are not necessarily identical. Asexual reproduction, for example, can result in a colonization advantage when compared to outcrossing. According to Baker's Law (1955), a single propagule can establish a new population of asexuals, but at least two propagules would be required in an obligately outcrossing species. However, this does not explain why sexual types cannot catch up or invade a site where asexuals are already established (Lynch, 1984; Peck, Yearsley, and Waxman, 1998). As this example illustrates, although numerous studies have aimed to explain the pattern of geographical parthenogenesis, the distinction between explaining the origins and maintenance of the pattern is not always clear.

Hypotheses to explain geographical parthenogenesis fall under two broad categories: ecological and demographic (Figure 2.2) (Bierzychudek, 1985; Haag and Ebert, 2004; Kearney, 2005; Vrijenhoek and Parker Jr., 2009). Ecological explanations focus on

differences in abiotic tolerances and competitive abilities of sexuals and asexual types. For example, the general-purpose genotype (GPG) hypothesis suggests that a polyploidization event results in a duplicated genotype that confers the ability to survive in a wide range of common ecological conditions. However, they may be comparatively poorer at surviving in the extreme or specialized habitats where their diploid counterparts reside (Lynch, 1984). In this way, geographical parthenogenesis can arise because general-purpose polyploids disperse to a variety of new, less extreme habitats. In contrast, diploids that have specialized in a specific habitat type may have decreased fitness in these new habitats, therefore both initiating and maintaining the pattern of geographical parthenogenesis. To further maintain this distributional pattern, apomixis serves as a barrier to introgression with diploid sexuals and also prevents segregation within polyploids, both of which could lead to fixation of more specialized genotypes in apomicts. Thus, general-purpose polyploids are able to disperse and colonize available habitats with little chance of losing their 'broad' ecological advantage, or of developing the narrower tolerances of the specialized sexuals. Implicit in the GPG hypothesis is a low rate of origination of asexual polyploids.

Related to the GPG hypothesis, the frozen niche variation (FNV) hypothesis suggests that a history of polyploidization events has led to numerous extant (and extinct) polyploid lineages (Vrijenhoek, 1984). Instead of the broadly adapted polyploid genotype of the GPG hypothesis, in the FNV hypothesis each polyploid lineage specializes in one particular set of habitat conditions. Only when all the extant polyploid lineages are taken together do they achieve a wide polyploid range (Vrijenhoek and Parker Jr., 2009). This situation may be more likely in our system because of the evidence for multiple asexual polyploid lineages in *T. hookeri* (Thompson and Whitton, 2006).

Whether the GPG and FVN hypotheses account for a pattern of geographical parthenogenesis, both suggest that ecological processes may be the dominant force behind the distribution seen in asexual and sexual individuals of some species. If dispersal barriers or sufficient time do not pose a limitation, then the current distributions of asexuals and sexuals are determined primarily by the specific ecological niche tolerances of each reproductive type (Figure 2.2). For example, sexual diploids may have very specific ecological tolerances that limit successful establishment to the restricted range where they currently exist. Under an ecological model, cytotypes and their offspring should have decreased fitness when a dispersal event takes them into the range of an opposing cytotype. Decreased fitness, combined with competitive exclusion by opposing cytotypes would then serve to prevent the long-term establishment of cytotypes outside their range.

Aside from ecological considerations, another set of explanations lie in plant demography. As noted above, uniparental reproduction provides a colonization advantage relative to outcrossing (Baker, 1955). In addition, some asexuals have been shown to have different dispersal features relative to their sexual counterparts, which may further increase the potential rate of range expansion of the asexuals (Eriksson, 1992; Roff and Fairbairn, 2001). Apomicts also differ from sexuals in that the offspring of apomicts are genetic clones of the maternal plant. However, the manner in which they attain asexuality varies and this has implications for the genetic makeup of their offspring, their dispersal ability and more (Ozias-Akins, 2006; Whitton *et al.*, 2008). In *Townsendia*, apomixis has been categorized as diplosporous, which is a form of apomixis where abnormalities during female meiosis cause the megaspore mother cell to remain unreduced, resulting in an unreduced megagametophyte. Notably, depending on how far meiosis I proceeds, diplosporous

apomicts may be able to generate genetic variation through crossing over in prophase I (Whitton *et al.*, 2008).

Another key feature of diplosporous apomicts such as *Townsendia* (Beaman, 1957) is that they typically generate endosperm tissue autonomously, without the need for pollination. This release from pollen and pollinator dependence has been hypothesized to be especially favoured in areas with scarce pollinators such as open habitats of recently glaciated areas (Bierzychudek, 1985). Furthermore, successful long distance dispersal (LDD) events (*i.e.*, dispersal beyond 100m) (Cain, Milligan, and Strand, 2000) are exceedingly rare. For example, it is estimated that in an average plant species a single 34 km dispersal might occur yearly, while a single 415 km dispersal might happen each decade (Nathan, 2006). Long-lived, perennial species such as *T. hookeri* have the opportunity to reproduce over a number of years, which may mediate the low annual rates of successful LDD events. Regardless of their frequency, rates of population establishment following LDD will be lower for obligate outcrossers, as they require at least two LDD events to occur in near vicinity to make pollen transfer possible between individuals. Taken together, this suite of demographic factors could drive the establishment of a broader distribution of asexuals than sexuals within a species and may explain the initial process that gives ride to a pattern of geographical parthenogenesis.

In a simplified example where demographic factors are the predominant drivers of geographical parthenogenesis, then ecological tolerances and limits of sexuals and asexuals could be assumed to be more or less equivalent (Figure 2.2). Given their similar ecological niches, more time and additional LDD events may allow sexuals to fill their fundamental niche and invade areas where apomicts have already colonized. However, the arrival of sexuals does not necessarily denote their successful establishment. For example, factors such

as resource competition could hinder establishment by sexual individuals if earlier arriving asexuals already occupy prime habitat (e.g., sheltered areas near rock outcrops). This is one of many factors that may inhibit the movement of sexual plants, as discussed below.

Studies that propose hypothese to explain geographical parthenogenesis typically assume that sexuals and asexuals exist in a state of equilibrium; however, they do not explicitly state why this should be the case. Competitive exclusion is probably the most common hypothesis for why close relatives do not co-occur (Hardin, 1960; Grime, 1973). However, destabilizing hybridization may also act to limit the co-occurrence of sexual diploids and apomictic polyploids, and could be the driver of niche divergence. Destabilizing hybridization may also explain why co-occurrence might not be expected even in the absence of niche divergence (Lynch, 1984). In an apomictic population, viable pollen can be considered a vestige of sexuality, which may explain the common observation of reduced pollen viability in polyploid apomicts (Kostoff, 1938). Nonetheless, apomictic plants typically retain their ability to attract pollinators, and therefore continue to export and receive pollen, which serves no function in strictly apomictic populations. In populations where sexuals and apomicts co-occur, the transfer of pollen from apomictic polyploids to sexual diploid mothers results in decreased fitness of sexual diploids because the diploids produce a higher proportion of odd-ploidy offspring (Lynch, 1984). This may lead to decreased offspring fitness, and may also spread apomixis genes to the offspring of interploidy crosses (Smith, 1841; Mogie, 1992; Whitton *et al.*, 2008). Thus, if destabilizing hybridization is a factor, diploid sexuals may not be able to stably co-occur with apomicts, and may only grow in places where they have a clear selective advantage over apomicts (Lynch, 1984; Kearney, 2005).

Although destabilizing hybridization focuses on unequal pollen acceptance between cytotypes, the phenomenon of minority cytotype exclusion is centered on plant population dynamics (Levin, 1975). More specifically, minority cytotype exclusion can occur when two cytotypes occur in the same location so that the cytotype in lesser abundance is at a disadvantage and is more likely to die out, even if it has otherwise similar fitness. Exceptions can occur if each cytotype has distinct ecological requirements or if prezygotic barriers are present to exclude mating between cytotypes. For example, apomictic polyploids such as *T. hookeri* are unlikely to be affected by minority cytotype disadvantage when present in sexual diploid populations because they only rarely accept diploid pollen (Kao, 2007). In addition, the relative immunity of asexual polyploids to diploid pollen has been shown to cause eventual polyploidization of co-occurring diploids in mixed ploidy populations, and eventual extinction of diploids, unless the relative fitness of the polyploids is very low (Yamauchi *et al.*, 2004). Our field surveys have yet to find evidence for mixed ploidy populations of *T. hookeri*, and crossing studies have indicated that the pollen of apomicts can reduce the fitness of sexual diploids (Garani, 2014).

I focus here on assessing evidence for ecological divergence as a factor contributing to the pattern of geographical parthenogenesis seen in *T. hookeri*. Using ecological niche models (ENMs; Peterson, 2001), I examine differences in the pattern of occurrence of *T. hookeri* polyploid apomict and diploid sexual populations. If differences in abiotic tolerances play a significant role in the distribution of *T. hookeri*, then we would expect to see non-identity between the niche models of each reproductive type, particularly in areas of overlap. Conversely, if niche models are statistically indistinguishable, then it is more likely that

relative to demographic factors, ecological processes only play a small role in the pattern of geographical parthenogenesis in *T. hookeri*.

Ecological Niche Modelling: Model Building

Ecological niche modelling, or predictive species distribution modelling, is a method that incorporates the ever-growing abundance of high quality GIS data and detailed occurrence records to create probability of presence distributions for the taxa in question (Peterson, 2001; Phillips and Dudık, 2008). The method has risen in popularity in recent years and has been applied to problems ranging from cryptic species discrimination (Raxworthy *et al.*, 2007; Blair *et al.*, 2013; Lipsen, Lee, and Whitton, 2013), past and future distribution mapping (Perkins *et al.*, 2007; Alsos *et al.*, 2009; Fouquet *et al.*, 2010), to phylogeography in plants and animals (Carstens and Richards, 2007; Jakob *et al.*, 2007). Plants are well suited to niche modelling approaches because they are sessile and must either endure abiotic conditions or die out. Thus, the persistence of a plant population over successive years, as indicated by field studies or herbarium records, suggests that the local climate is at least tolerable (Guisan and Thuiller, 2005).

Despite the rising popularity of niche modelling approaches, there are a number of limitations when applying the methods and interpreting results. One of the largest concerns is the assumption that the distributions of the taxa in question are at equilibrium (Kidd and Ritchie, 2006). This is an important assumption because, if true, then the area where a species is currently found is representative of its abiotic tolerances, mediated by factors such as herbivory, inter- and intra-specific competition and geographical barriers. In other words, the species distribution reflects the realized niche (Hutchinson, 1965). Most niche modelling approaches, however, are unable to capture the effect of limiting factors such as competition,

and only take into account abiotic data. Thus, if a species is currently at equilibrium, niche models based on current distributions should be a prediction of their fundamental niche, potentially encompassing areas where limiting factors preclude establishment or persistence. Lack of distributional equilibrium, as in a newly derived species, may confound results because the observed occurrences may not include all of the abiotic conditions in which the species will ultimately occur. However, this may be partially addressed by restricting study areas to a range where both taxa can be assumed to be stable. Furthermore, the questions involving niche modelling are diverse and not all require that organisms be at equilibrium. For example, studies regarding invasive species, which may have distributions that are rapidly changing, may utilize the native range of a species to predict its spread into invaded habitats (Mandle *et al.*, 2010; Lozier and Mills, 2011; Gallien *et al.*, 2012).

Another consideration is the effect of sampling bias on the ecological niche model (Syfert, Smith, and Coomes, 2013). Herbarium collections are notoriously biased toward easily accessible areas such as sites near populated areas and within hiking distance from roadsides (Schmidt-Lebuhn, Knerr, and Kessler, 2013). Together, these biases result in niche models that inadvertently include factors such as human accessibility as an abiotic character that contributes to the distribution of a species. This results in oversampling in accessible areas and reduced sampling in isolated areas, which may give the appearance that the organism prefers unrelated abiotic features. A bias toward human settlements and roads, for example might appear as a tendency toward moderate climates and gentle grades, and a tendency to be near bodies of water.

In addition to unintentionally biasing the results in this way, it is also necessary to take into account the potential for adding irrelevant data, or missing key abiotic data in the

analysis. The addition of irrelevant data has the effect of over parameterizing models, making ENMs more complex than is biologically plausible and losing predictive ability in areas that have not been sampled (Peterson, 2011). These data may also be geographically structured, which would confound the analysis of results, especially in taxa with differing range sizes such as the polyploids and diploids of *T. hookeri*. In contrast, variables like soil characteristics are conspicuously lacking in many ENM analyses due to low data resolution of such data. Although soils data (Hengl *et al.*, 2014) have recently been made available at a resolution similar to that of climate data, the manner in which these datasets are related to each other and how they are used in conjunction to produce ENMs is unexplored. Even though both climate and soil data are currently available at the same resolution, the effect of soil characteristics on plant growth is much more localized than that of climate data. North Dakota populations of *T. hookeri,* for example, occur only on small outcrops of specialized clinker soil that were surrounded by grasslands, and such a localized feature may not be captured in soils data (Lee and Garani, 2012). Furthermore, preliminary analyses of soils from collection sites have thus far failed to show significant differences between soils where sexual diploids and asexual polyploids occur (A. Garani, UBC, unpublished data). Nonetheless, such exclusion may lead to ecological niche models that show incomplete pictures of the fundamental niches of the study taxa, particularly if essential variables such as soil characteristics are lacking in the ENMs developed for species that are considered edaphic specialists. My assumption is that the omission of soils data would make it less likely to detect a signal of niche divergence between cytotypes, and therefore, a lack of evidence for niche divergence based on models from climatic factors should be interpreted with caution.

In addition to the choice of variable to use in building niche models, other methodological considerations can affect a variety of aspects of the models. One method of assessing the fit of a niche model developed for a species is with the measurement of the Area under the Curve (AUC) (Phillips and Dudık, 2008; Warren and Seifert, 2010). However, the AUC is affected by the delimitation of the study area, and an overly generous delimitation of the accessible area will result in false confidence in the results (Barve *et al.*, 2011). Programs such as Maxent (Phillips and Dudık, 2008) measure AUC by comparing how well ENMs predict species occurrence relative to a random points drawn from the entire study area. As a result, to avoid inflating our confidence in an ENM, it is important to restrict the study area to a small and biologically meaningful area around the known occurrences (Barve *et al.*, 2011).

Finally, the choice of modelling method can have a large effect on the final modelling results. One of the earliest modelling methods named BIOCLIM, which uses the environmental distance from occurrence points to develop climate envelopes (Nix, 1986). Bioclim has since fallen out of use as it tends to perform worse than other modelling methods and is especially poor at making predictions in light of climate change (Graham and Hijmans, 2006). Other methods such as Generalized Linear Models (GLM) and Generalized Additive Models (GAM) use regression techniques to predict species niches (Nelder and Wedderburn, 1972; Hastie and Tibshirani, 1990). Some algorithms use only presence data, but others require a combination of presence and absence data, which is challenging to obtain and a limiting factor for many projects. Algorithms such as GLMs produce relaxed niche models that are less prone to over-fitting, which may be useful for identifying potential habitat and finding new occurrences for a rare species. Meanwhile, models such as Random Forests (RF)

fit niche predictions tightly to currently known species occurrence points, which produces highly accurate models (Breiman, 2001). However, this implies that researchers have a thorough understanding of the entire distribution of species, and may be prone to over -fitting data such that there is little prediction or extrapolation beyond current occurrences. Most recently, methods have been implemented that amalgamate various modelling methodologies into a consensus result (Thuiller *et al.*, 2009; Georges and Thuiller, 2012); however, one of the problems here lies in weighting and comparing the individual results of each algorithm. Differences in the approach to modelling of each algorithm may cause their individual outputs to be nonequivalent and difficult to meaningfully compare. Considering the wide range of available tools, Maxent is a modelling approach that has consistently proven to be reliable in many situations by using presence data alone (Phillips and Dudık, 2008), and this is the approach that I have chosen to use here.

Ecological Niche Modelling: Niche Overlap Comparisons

Niche models can be further explored through comparisons of ENMs at various taxonomic levels such as between species, subspecies or cytotypes within a species. A set of test statistics has been developed to facilitate comparisons of ENMs. The simplest of these implemented in ENMTools V1.3 (Warren, Glor, and Turelli, 2010) are the three niche overlap statistics: Hellinger's *I* (Warren, Glor, and Turelli, 2008), Schoener's D (1968) and relative ranks (RR) (Warren, Glor, and Turelli, 2010). These values estimate the degree of similarity between two ENMs and are derived from the comparison of geographically overlapping cells in the probability of presence grids for the taxa being compared. Hellinger's *I* and Schoener's D are different methods of measuring quantitative differences between niche suitability scores whereas RR considers the overall, qualitative agreement in the differences

between grid value pairs (Warren, Glor, and Turelli, 2010). Although these three statistics vary in absolute value, they typically show similar trends between taxa (e.g., high, medium or low niche overlap) (Warren, Glor, and Turelli, 2010).

The significance of niche overlap between taxa can be examined in ENMTools by comparing against a randomized set of background points. These tests include the niche identity and the niche background tests, and both compare the ENMs of randomly drawn points from throughout the study area, in replicate, to observed data. A result of niche identity between asexual and sexual populations of *T. hookeri* suggests that there are no significant differences in the climatic tolerances of cytotypes. However, niche identity is only expected when taxa tolerate an indistinguishable set of abiotic conditions and also have the same geographic and climatic conditions available to them, thus, entirely overlapping taxon ranges are required for a meaningful test of niche identity. Within a study area composed of smaller, overlapping portions of ranges, the distribution of taxa can more reasonably be assumed to be at equilibrium, and thus the restricted niche identity test can mitigate the effects of potential distributional disequilibrium. In contrast, the background test looks at taxa with non-overlapping ranges and considers whether niche overlap between taxa reflects significant similarities or differences in abiotic preferences, or whether they represent inherent differences in the geographic range where the taxa are found (*i.e.*, are the abiotic variables geographically autocorrelated?). Less niche overlap than expected from the null distribution would suggest that differences in climatic tolerances between cytotypes of *T. hookeri* exceed the differences predicted by their non-overlapping ranges, and therefore provide a signal of ecological divergence of cytotypes (Warren, Glor, and Turelli, 2010). However, a null result would suggest that any differences in climatic tolerances between

cytotypes can be explained by geographic structure and variable autocorrelation due to differences in range size.

Thus, careful decision-making and data clean up can help address many of the issues and assumptions inherent in ecological niche modelling. Ecological niche modelling remains a powerful tool that can provide evidence to support or refute hypotheses to explain the causes of distributional patterns that we see in the natural landscape. Our choice to examine intraspecific differences in ploidy and reproductive strategy in *T. hookeri* as a framework from which to compare ecological niches is one that is novel and addresses fundamental questions in our understanding of apomixis.

Materials and Methods:

Species occurrence data were taken from two sources: personal field collections, and herbarium specimen data (Chavan; ALA; ALTA; CSU; LEA; RHM; UBC; USU-UTC). Many herbarium records did not include accurate latitude and longitude data. These were georeferenced based on locality and habitat information found on herbarium labels, and discarded if the descriptions were ambiguous. I made personal field collections over four years, and sampled in areas with previous herbarium collection data, and in new areas that appeared underrepresented. For each record, plant identifications were confirmed using various floras (Beaman, 1957; Dorn and Dorn, 2001; Strother, 2006)

Ploidy

Ploidy was determined for plant populations via flow cytometry and/or pollen measurements. Vouchers with freshly collected leaf material from recent fieldwork were used for flow cytometry. When intact florets were available on freshly collected material and

on herbarium vouchers, pollen measurements were conducted. I performed the initial flow cytometry and pollen measurements, but many ploidy determinations were later completed by Alicia Garani (Whitton lab, UBC) and numerous undergraduate assistants, as part of her thesis work (Garani, 2014). I performed flow cytometry on silica dried and frozen (-80ºC) leaf material by using both the chopping method utilized by Sears (2011) and later switching to the bead-beater protocol implemented by Garani (2014). Both methods returned similar results, but the latter methodology was quicker and more efficient to perform.

Pollen measurements were made by removing six to eight florets from inflorescences and combining them with 20µL of lactophenol (cotton blue) and 10µL distilled H_2O in a 1.5 mL microcentrifuge tube. Tubes were agitated on a mini-vortexer (VWR International, Radnor, Pennsylvania, USA) for one minute to release pollen into suspension. Fifteen µL of the resultant suspension was placed on a slide and viewed with a Nikon Eclipse 80i microscope. Slides were scanned under the microscope and pollen units were counted as viable or inviable until a total of 100 pollen units were counted. Viable pollen grains stained a dark blue colour, and were regularly shaped, while inviable pollen stained light blue and were often oddly shaped. The diameter of twenty randomly chosen viable pollen units were also measured to determine average pollen size. Plants with a high proportion of viable pollen and with smaller average pollen size were considered sexual diploids, while plants with many inviable and larger sized pollen units were considered asexual polyploids.

Ecological Niche Modelling

Various types of data were prepared for use in predicting ENMs including soil (STATSGO2, 2011), ecoregion (Western Ecology Division, 2011), elevation and climate data. The climate data included 19 bioclim climate variables from WorldClim.org. These

widely used global climate grids are available at ~1km resolution (Hijmans *et al.* 2005) and consist of biologically relevant variables derived from precipitation and temperature data. Each data layer was formatted in ArcGIS 10 (ESRI Inc., 2012) by drawing 20km buffers around *T. hookeri* occurrence points and extracting only the data within the buffered area. These restricted ranges were used to predict ENMs and estimate model fit, and were later projected onto a broader study area formed by drawing a 100km buffered convex hull polygon encompassing all occurrence points (Figure 2.1).

I used an approach similar to Syfert, Smith and Coomes (2013) to account for potential bias that arises from differences in collecting effort. A bias file was compiled from herbarium records provided by the major herbaria of the Rocky Mountain region. To create this file, the number of vascular plants recorded in each cell of our study area was summed and log-transformed, producing a new raster file representing plants per cell in our study area. This bias file was used to estimate relative search effort at any given point in our study area. Although this is an approximate method to determine bias, incorporating an estimate of this can greatly improve the predictive ability of ecological niche models (Syfert, Smith and Coomes, 2013), and some studies suggest that the incorporation of bias is an essential step in producing robust and defensible ecological niche models (Costa *et al.*, 2009; Phillips *et al.*, 2009; Syfert *et al.*, 2013).

For each bioclimatic variable, the climate values for individual species occurrence points were extracted via the "species with data" function in Maxent (Phillips and Dudík 2008). Principal component analysis was performed using MyStat V12 (SYSTAT Software Inc. 2007), to identify the bioclimatic variables contributing most to the first three component axes. Internal Jackknife statistics of variable contribution implemented in Maxent were also

taken into account when choosing key variables (Phillips and Dudík 2008). Pairwise correlations among selected variables were then examined using a Pearson correlation matrix. Variables that showed correlation with the highest number of other variables were discarded from analysis to avoid overweighting similar traits (Rissler and Apodaca 2007).

I used Maxent version 3.3.3k (Phillips and Dudık 2008; Phillips *et al.* 2006, 2004) to estimate niche models because its maximum entropy algorithm consistently exhibits better performance than other modelling programs such as GARP (Graham and Hijmans 2006; Townsend *et al.* 2007). Additionally, Maxent is well suited for presence only datasets such as mine and does well with both small and large datasets (Phillips and Dudík, 2008). I ran the analysis with Maxent defaults, with modifications as follows: I opted to create response curves, ran 100 bootstrap replicates with the random seed option, and used 20and of our data for testing purposes, and the remainder for training the model. I used the area under the curve (AUC) statistic to assess the fit of our models. The ENMs were output as logistic and raw probability grids. Due to the number of herbarium collections and the nature of our sampling strategy, many of our collection sites were in close vicinity to other sites. As a result, all but one of the occurrences occupying the same pixel in the abiotic data was automatically discarded by Maxent to avoid duplication and overweighting. After accounting for overlapping data points, the final analysis included 36 unique diploid and 62 polyploid occurrences.

Niche Comparisons

After producing the models, I measured niche overlap between asexual and sexual ENMs using ENMTools 1.3 (Warren, Glor, and Turelli, 2010). I calculated Hellinger's *I* (Warren, Glor, and Turelli, 2008), Schoener's D (1968) and relative ranks (RR) (Warren,

Glor, and Turelli, 2010) between the two cytotypes. Each comparative statistic returned a value ranging from zero (completely non-overlapping models) to one (identical niche models). I also tested for niche identity in areas where cytotypes had overlapping ranges. Niche identity discerns whether the observed level of niche similarity is significant and therefore due to differences in abiotic forces, or if they are simply a result of stochastic variation (Warren, Glor, and Turelli, 2010). I ran niche identity tests in ENMTools (Warren, Glor, and Turelli, 2010) to create null distributions of the data, where population localities were randomly assigned a reproductive type until the simulated datasets were distributed in the same numbers as empirical data. One thousand randomizations were done and niche models were produced for each simulated dataset. I then measured niche overlap for each randomized simulation and compared overlap scores to the results from the observed data. To gauge the sensitivity of the test to study area delimitation and to limit artifacts from potential disequilibrium, I measured niche identity in two manners. First, I measured niche identity across the full range of asexual and sexual populations, and then I performed a "restricted test" that utilized a restricted range covering only areas of cytotype overlap, drawn with a 30 km buffer and with a 200 km buffer (Figure 2.1). The identity test assesses whether ENMs are statistically identical, and therefore it is a one-sided test (using $\alpha = 0.05$). An observed overlap value falling within the bottom 5% of the null distribution is considered evidence that the niches are non-identical in the area of overlap. The restricted identity test also addressed the potential for distributional disequilibrium, as cytotypes could reasonably be assumed to be at distributional equilibrium within the overlapping range.

I used the niche background test (Glor and Warren, 2011) to decouple the effect of geographic range and structure from the factors that influence niche overlap. As with the

identity test, I implemented the background test in ENMTools through simulation. Unlike the niche identity test, the background test does not require strictly overlapping ranges and I performed the background test on the respective ranges of both sexual diploids and asexual polyploids. Whereas the function of the niche identity is to measure similarity between the niches of two species, the background test measures the niche overlap between a species and the abiotic variability present within the range of a second species. Using ENMTools, I simulated species distributions by randomly selecting points from within the sexual range and the asexual range, equal to the number of observed occurrences of the cytotype in that range. I ran one thousand simulations for the range of each cytotype and created an ENM for each simulated dataset. Next, I compared the niche overlap between the ENM from observed data and the ENM produced from each simulation. For example, the sexual niche model was compared to the randomized asexual climate background model, and asexual niche model compared to the randomized sexual climate background model. This resulted in a null distribution of overlap values to compare against observed niche overlap. The background test is two-sided and I used an alpha level of 0.05 to assess whether the observed overlap value between the species ENMs was within the 95% confidence interval of the null distribution of overlap values. If the observed value was significantly greater than expected from the null distribution, then cytotypes had more conserved ENMs than expected and factors other than ecology may explain their distribution. Conversely, I interpreted observed values with significantly lower overlap values than expected from the null distribution as support for ecological divergence between cytotypes. The background test results may also be bi-directional, in which case demographic factors may disproportionately affect one cytotype but not the other. Observed overlap values that fell within the 95% confidence

interval suggest that niche similarity between cytotypes can be explained by the effect of geographic structure on the abiotic variables in their respective range.

Results:

Ploidy Assessment

Beaman's cytological work (1957) established the diploid chromosome number of *T. hookeri* to be 2n = 18, and his description of the geographical distribution of cytotypes was based on series of roots squashes, microsporogenesis and megasporogenesis studies, and pollen staining. With the addition of flow cytometry, this study found that the average DNA content in *T. hookeri* sexual diploids was 14.02 pg, and in asexual polyploids was 21.34 pg. Although I did not obtain direct chromosome counts to validate that the lower values correspond to diploids, their distribution coincides generally and in specific cases with the locations where Beaman mapped diploids. The DNA content of the polyploids suggests that polyploids are triploids, and their localities also correspond with the 37 polyploid populations observed by Beaman (1957). As with previous pollen studies (Thompson and Whitton, 2006), pollen data followed a trend where small, regularly shaped pollen (~22.7µm diameter) were more likely to have a higher proportion of viable pollen (average=79.58%). These vouchers corresponded to samples that were found to have lower DNA content through flow cytometry, further supporting that these are sexual diploid occurrences. In contrast, larger, irregularly shaped pollen (33.72µm) with low viability (average=20.95%) corresponded to samples that had higher DNA content measured through flow cytometry and were presumed to be polyploid. Overall, 567 collections of *T. hookeri* were available from various herbaria, of which 526 included latitude and longitude data or sufficiently detailed habitat and locality information for georeferencing. Of these, flow cytometry or pollen count data were obtained

for 132 collections. After removing duplicate populations, 62 polyploid and 36 diploid populations were available for niche modelling analysis.

Ecological Niche Modelling

The variable used in the ENM predictions included a reduced set of eight climatic variables: Bioclim 2, 4, 5, 7, 9, 11, 15, and 16. Variable response curves and a jackknife test of variable importance (Figure 2.3. Table 2.1) show that the diploid ENM placed heavy emphasis on temperature seasonality (Bioclim 4), maximum temperature of warmest month (Bioclim 5) and precipitation of wettest quarter (Bioclim 16). Whereas, the most important variables for the polyploid ENM partially overlaps that of the diploid ENM and included mean temperature of driest quarter (Bioclim 9), precipitation seasonality (Bioclim 15) and precipitation of wettest quarter (Bioclim 16). The niche models for sexual diploids and asexual polyploids had an AUC of 0.970 (std 0.011) and 0.869 (std 0.021), respectively, which indicates a good fit to the given data (Phillips and Dudık, 2008). After incorporating the bias file into the modelling, AUC scores decreased and the models predicted high probability of presence in wide swathes of habitat where plants have never been observed. High level of overprediction led me to abandon the models that incorporated the bias files in subsequent analyses.

Maxent models place the diploid niche in a restricted area that closely circumscribes the known distribution of *T. hookeri* diploids (Figure 2.4). Additionally, the projected diploid model extends further into southern Colorado, and into portions of the polyploid range; however, these additional areas exhibited lower probability of presence than in the known range. As with the diploid sexuals, the ENM of polyploid asexual populations showed the areas of highest suitability in its currently described range. In addition, the polyploid niche

model also extends niche suitability into a much broader area, particularly into the Rocky Mountains of British Columbia, where no populations of *T. hookeri* are currently known to exist. Despite this extended area of suitability, there is weak overlap in areas predicted to be highly suitable for diploid populations. Broadly speaking, diploid sexuals showed stronger responses to the climatic variables, as indicated by more clearly defined peaks in their response curves (Figure 2.3), whereas polyploid response curves included a mix of sharply and shallowly peaked responses to climatic variables.

The results of niche overlap and identity, and the background test are summarized in Table 2.2. Based on empirical data, polyploid and diploid ENMs exhibited moderate to strong niche overlap based on *I*, D and RR measures. However, the full range and the restricted identity tests show that at all levels of comparison: full range, overlap range with 200 km buffer and overlap range with 30 km buffer, niche overlap is much lower than expected by the null prediction in all but the RR statistic. The background tests suggest that the polyploid ENM overlaps significantly less than expected when compared to simulated diploid data, suggesting ecological differentiation. In the other direction, the background test suggests that the diploid ENM overlaps significantly more than expected when compared to simulated polyploid data, suggesting ecological conservatism.

Discussion:

The flow cytometry and pollen data agree with previously published data on the distribution of sexual diploids and apomictic polyploids within *T. hookeri* (Beaman, 1957; Thompson and Whitton, 2006). Thus far, no evidence has been found for mixed ploidy populations, which suggests that newly formed polyploids derived from diploid mothers must either colonize new habitats or wipe out their local sexual progenitors. Results from niche

models reveal differences in the niche preference of the sexual and asexual populations (Figure 2.4), which indirectly makes sense of the failure to detect mixed populations. In sexual *T. hookeri*, the geographic projection of the sexual niche model and the tendency for more sharply peaked response curves (Figure 2.3, e.g., Bio 2,5,17) suggest that sexuals are more tightly bound to a narrow set of climatic conditions, consistent with their smaller geographic range. In contrast, the asexual model projections display moderate suitability over a wider geographical area and the response curves range from strong to shallow peaks. It is tempting to interpret these results as indicating narrow versus broader ecological tolerances of sexuals and apomicts, respectively, further discussed below.

Overall, high niche overlap scores (Table 2.2a) suggest that asexuals and sexuals populations share a similar suite of abiotic niche preferences, despite striking visual differences in the projected niche models. Even so, high levels of niche overlap are expected based on years of collective field experience in the Whitton lab that have always suggested a distinct habitat type for *T. hookeri* as a whole. Given that this study compares ENMs of cytotypes within a species, we would expect much finer scale similarities and differences between groups. However, the niche identity test and background test can probe deeper to address the question of whether these similarities are more or less than expected given the suite of available climatic niches that exist within the geographic range of the two cytotypes.

Niche identity tests for the full range indicate significantly less overlap than expected by chance, which suggests that ENMs between cytotypes are not identical and that ecological niche preferences may indeed contribute to the distributional pattern of *T. hookeri* sexuals and asexuals (Table 2.2b). The fact that asexuals have a larger distribution that does not overlap with the sexual distribution could in and of itself account for the significant identity

result (Warren, Glor, and Turelli, 2010). However, additional identity tests including only the overlapping portions of the polyploid and diploid range maintained the same pattern of non-identity of cytotype ENMs. As expected, the overlap between models increased in these geographically restricted tests; however, two of the three overlap metrics still exhibited significantly less overlap than expected by chance, based on the randomizations (Table 2.2b). Thus, despite the strong niche overlap measures, asexuals and sexuals are more distinct in their climatic niche models than expected by chance. In the restricted test, the study range shrank from a large and climatically diverse area to a small overlap zone that excluded 74% of the polyploid occurrence data. Even if one or both cytotypes are in a state of disequilibrium, within the narrower bounds of the restricted test both cytotypes are more likely to have reached colonization equilibrium and the results still strongly suggest niche differentiation between cytotypes.

The background test allowed an examination of the distributional space of asexual polyploid populations that are present outside the area of overlap with the sexual diploids of *T. hookeri*. When compared to the initial overlap statistics, the simulated results of the background test, in both directions, predict significantly different levels of niche overlap than the empirical observations (Table 2.2c). Overall, a significant result in the background test indicates that the observed niche trends are not simply a product of the available habitat present in the regions where diploids or polyploids occur. Rather, the niche models appear to be the result of divergent abiotic tolerances between the two reproductive types found in *T. hookeri*.

Results from the background test can be further divided into its individual components: the comparison of the asexual occurrences against the environmental

background of the sexual range, and the comparison of the sexual occurrences against the environmental background of the asexual range. The background test indicates that the sexual niche model shows markedly more overlap with simulated asexual background than expected by chance. This suggests that the climatic conditions in certain areas where polyploids occur may be suitable for the sexual cytotype. Conversely, the asexual niche model exhibits significantly less overlap with the sexual background than expected by chance. Although seemingly counterintuitive, it is possible for overlap to be less than expected in one direction, and greater than expected in the other direction. One possibility is that the diploids may not currently be at distributional equilibrium and may have the potential to continue to disperse through the upcoming centuries. While I cannot fully address the longer-term dynamics of species dispersal, the restricted identity test examines the smaller area where both reproductive types are most likely to have reached distributional equilibrium, and found significant ecological differences between the ENMs of both cytotypes. Furthermore, herbarium records and years of extensive fieldwork on *T. hookeri* suggest that its distribution is not rapidly shifting. Even if the background tests indicate that diploids have a strong affinity for portions of polyploid habitat and may be optimally suited for a distribution wider than where they currently reside, the apparent absence of mixed-ploidy populations suggests that barriers exist that prevent diploid transgression into the polyploid range. For example, greater dispersal ability would allow asexuals to establish more rapidly than diploid sexuals, and barriers such as inter-cytotype competition and genetic swamping could act to prevent later invasion of polyploid populations by diploids. In contrast, there is weaker than expected overlap by polyploid asexuals on the diploid range, which suggests that extant polyploids have specialized in habitat unique from diploid habitat,

and would have low fitness in a diploid habitat. In other words, the polyploid range may offer more ideal habitat for both cytotypes, while the diploid range is suitable only for diploids. Given the opportunity, these results suggest that diploids would do well in portions of the polyploid range, and could potentially invade the polyploid populations, but in the opposite direction, polyploids are unlikely to encroach on diploid habitat

Conclusion:

These results lend support for hypotheses that posit ecological diversification as an important component in the distribution of sexual and asexual populations found in *T. hookeri* (*i.e.*, the GPG and FNV hypotheses), and also indicate the importance of dispersal dynamics. Ecological specificity appears to partially constrain the current distribution of sexual diploids, but ENMs also suggest that diploids could possibly grow well in limited parts of the polyploid range. However, it is likely that the prior establishment of polyploids, possibly combined with competitive and/or reproductive barriers, prevent diploid incursion. For example, cross pollination experiments in *Townsendia* show the reproductive interference of asexual polyploid pollen on sexual diploid mothers (Garani, 2014). In contrast, asexual polyploids have more generalist tendencies that allow them to take advantage of a wider range of habitats, exclusive from the more specialized habitat of the diploids. Lower resolution soil data, as was available when this study was initiated, was found wanting in the development of ENMs; higher resolution soil data that have recently become available (Hengl *et al.*, 2014) are worth closer inspection. However, preliminary univariate soils analyses (A. Garani, UBC, unpublished) do not shown significant soil differences between cytotypes, suggesting that the incorporation of higher resolution soil datasets may not significantly alter the results reported here. Furthermore, future work should

take into account the potential for lineage specific variation within polyploid lineages. Given finer scale population sampling and a better understanding of polyploidization events, future work may be able to disentangle frozen niche variation from the general-purpose genotype hypothesis.

For good reason, niche modelling often comes under fire for its burdensome caveats and considerations. Beyond asserting the relatively longstanding presence of *T. hookeri* in the landscape relative to contemporary shifts in distribution of a recently introduced weed, there is little I can do to empirically address the assumption of geographic stability. In applying the methods of niche modeling, I have taken care to reduce the number of chosen abiotic variables to decrease the chance of overfitting the data, and increase the likelihood that my results return more realistic statements about the confidence in the niche models. Furthermore, the background tests that I have performed serve to decouple the confounding effects of unequal range sizes and habitat availability from the niche modelling process. In addition, the identity test on the restricted range alleviates concerns of distributional disequilibrium and shows that the observed niche differences in the full data remain significant even when much of the data is excluded from analysis. These multiple checks give me confidence in the observed association of abiotic climate variables with the distribution of sexual and asexual *T. hookeri.* Nonetheless, it is clear that the forces driving species distribution are multifaceted and varied. In fact, recent morphology work on *T. hookeri* fruits (cypselae) lends evidence to the theory of improved dispersal ability in the asexual polyploid populations of *T. hookeri* (A. Ruiz-Larrea, UBC, unpublished). These conclusions are not at odds, and only enforce the idea that all these biotic and abiotic concepts of dispersal, ecology, competition, reproductive interference, niche specificity,

asexuality and sexuality interact in complex manners to bring about the observed species distributions.

Table 2.1 - Predictive ability of Maxent models for *Townsendia hookeri* at varying ranges used in this study

Cytotype	Range	N*	AUC**	std	Bioclim Variables Used***
Diploid	Full	36	0.97	0.011	4, 5, 16, 7, 15, 11, 9, 2
Polyploid	Full	62	0.869	0.021	9, 16, 15, 2, 5, 4, 7, 11
	30 km Buffer	16	0.948	0.023	"
	200 km buffer	24	0.898	0.026	"

*N = Number of presence points used to build model

**Area under curve (AUC) = highest area under the receiver-operating characteristic curve

***Bioclim variables are listed in order from most to least important in the Maxent model.

The numbers refer to:

1.Annual Mean Temperature
2. Mean Diurnal Range
3.Isothermality
4. Temperature Seasonality
5. Max Temperature of Warmest Month
6. Min Temperature of Coldest Month
7. Temperature Annual Range
8. Mean Temperature of Wettest Quarter
9. Mean Temperature of Driest Quarter
10. Mean Temperature of Warmest Quarter
11. Mean Temperature of Coldest Quarter
12. Annual Precipitation
13. Precipitation of Wettest Month
14. Precipitation of Driest Month
15. Precipitation Seasonality
16. Precipitation of Wettest Quarter
17. Precipitation of Driest Quarter
18. Precipitation of Warmest Quarter
19. Precipitation of Coldest Quarter

Table 2.2 - Results from ENMTools analysis of niche overlap, identity tests (niche equivalency) and background tests (niche similarity) between cytotypes at varying ranges.

	a. Niche Overlap			**b.** Identity Test (*P*)		
Range	*I*	D	RR	*I*	D	RR
Full	0.715	0.426	0.715	<0.001*	<0.001*	0.01*
30 km Buffer	0.766	0.477	0.769	0.019*	0.01*	0.157
200 km Buffer	0.901	0.672	0.813	<0.001*	<0.001*	0.008*

	c. Niche Overlap			Background Test (*P*)			
	I	D	RR	*I*	D	RR	Inference
Diploid x background	0.706	0.426	0.714	**<0.001***	**<0.001***	**<0.001***	Conserved
Polyploid x background				<0.001*	<0.001*	0.168	Divergent

* = significant *P* value, overlap less than expected
Bold* = significant *P* value, overlap more than expected

Figure 2.1 - *Townsendia hookeri* collections examined, with glacial extent at the Last Glacial Maximum, and outlines of study areas used.

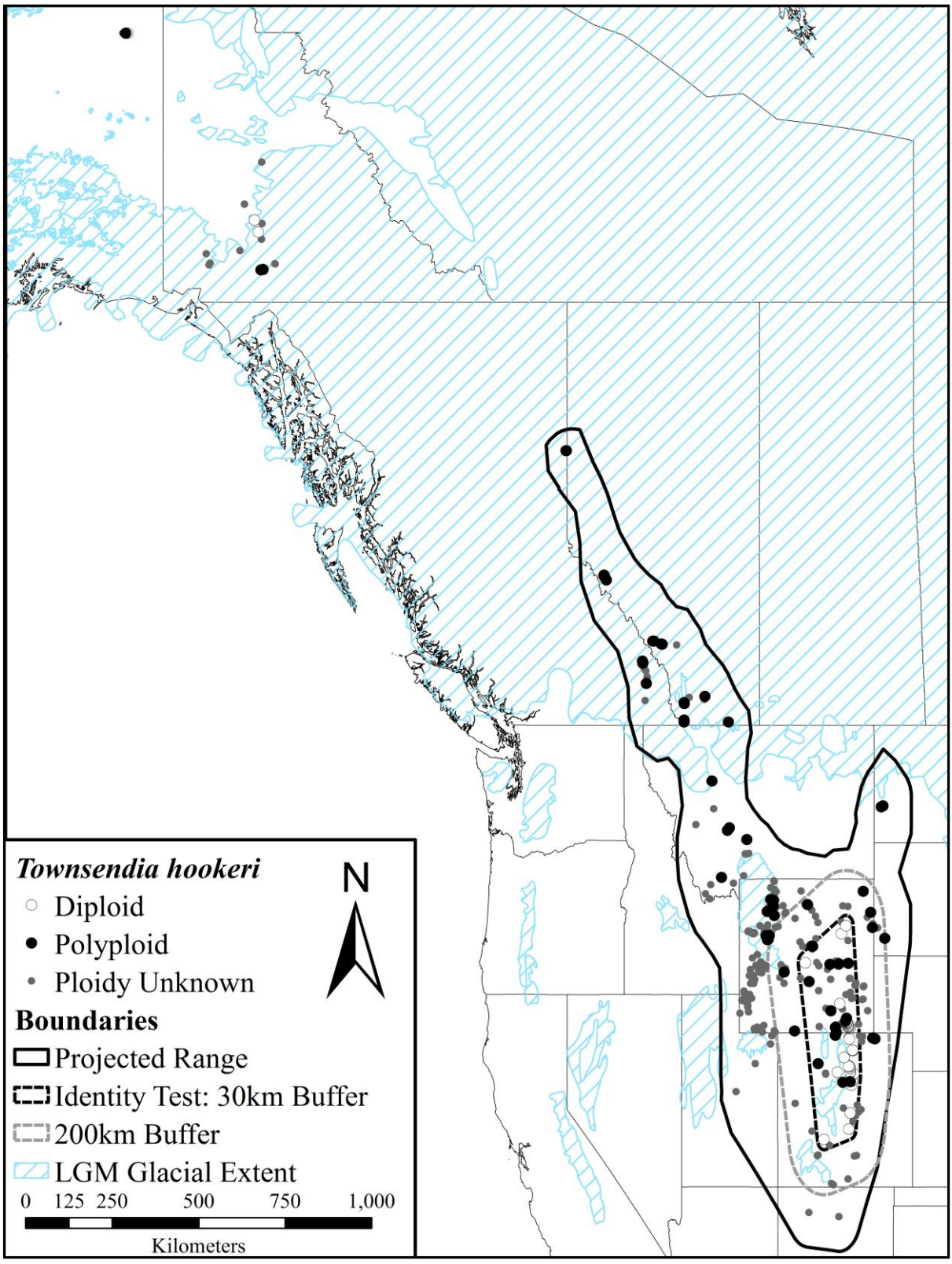


Figure 2.2. - Hypothetical results of niche differences under ecological and demographic explanations for observed distribution patterns

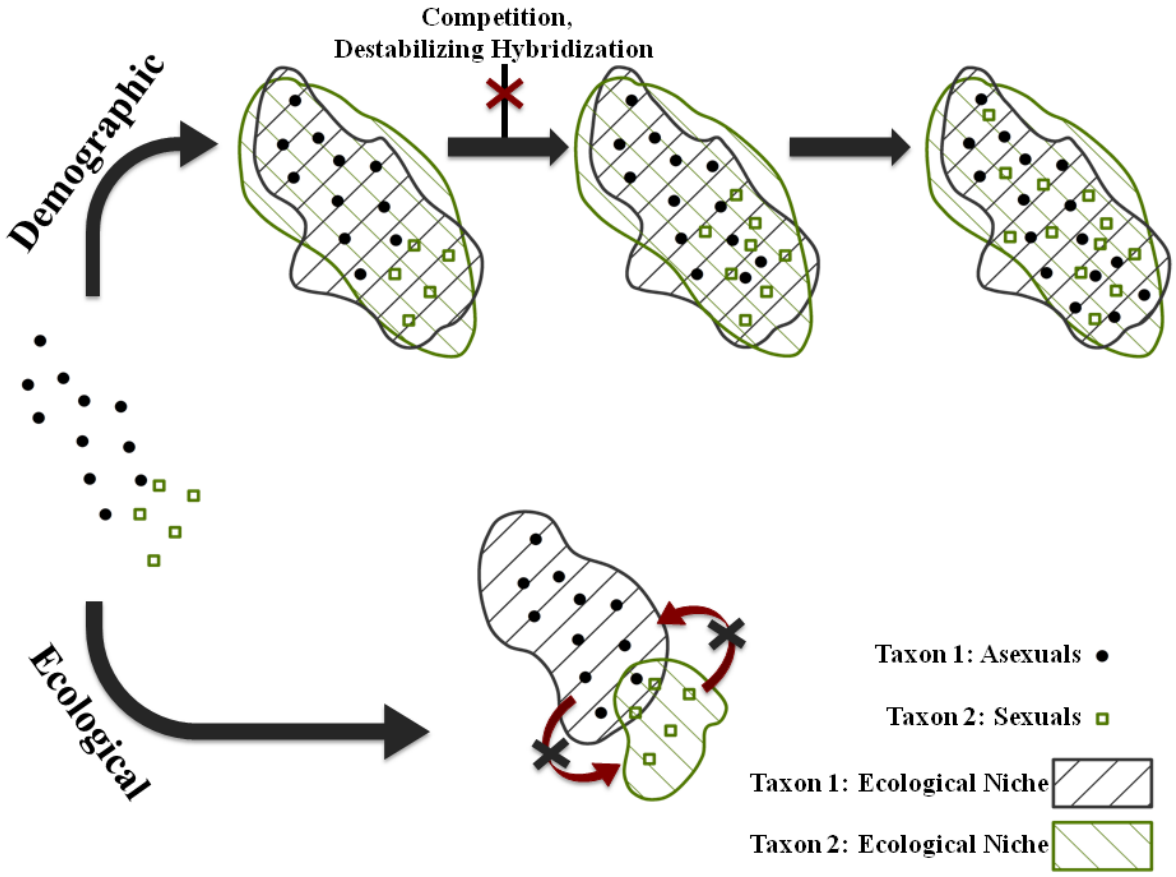


Figure 2.3 – Predicted response of diploids and polyploids to climate variables

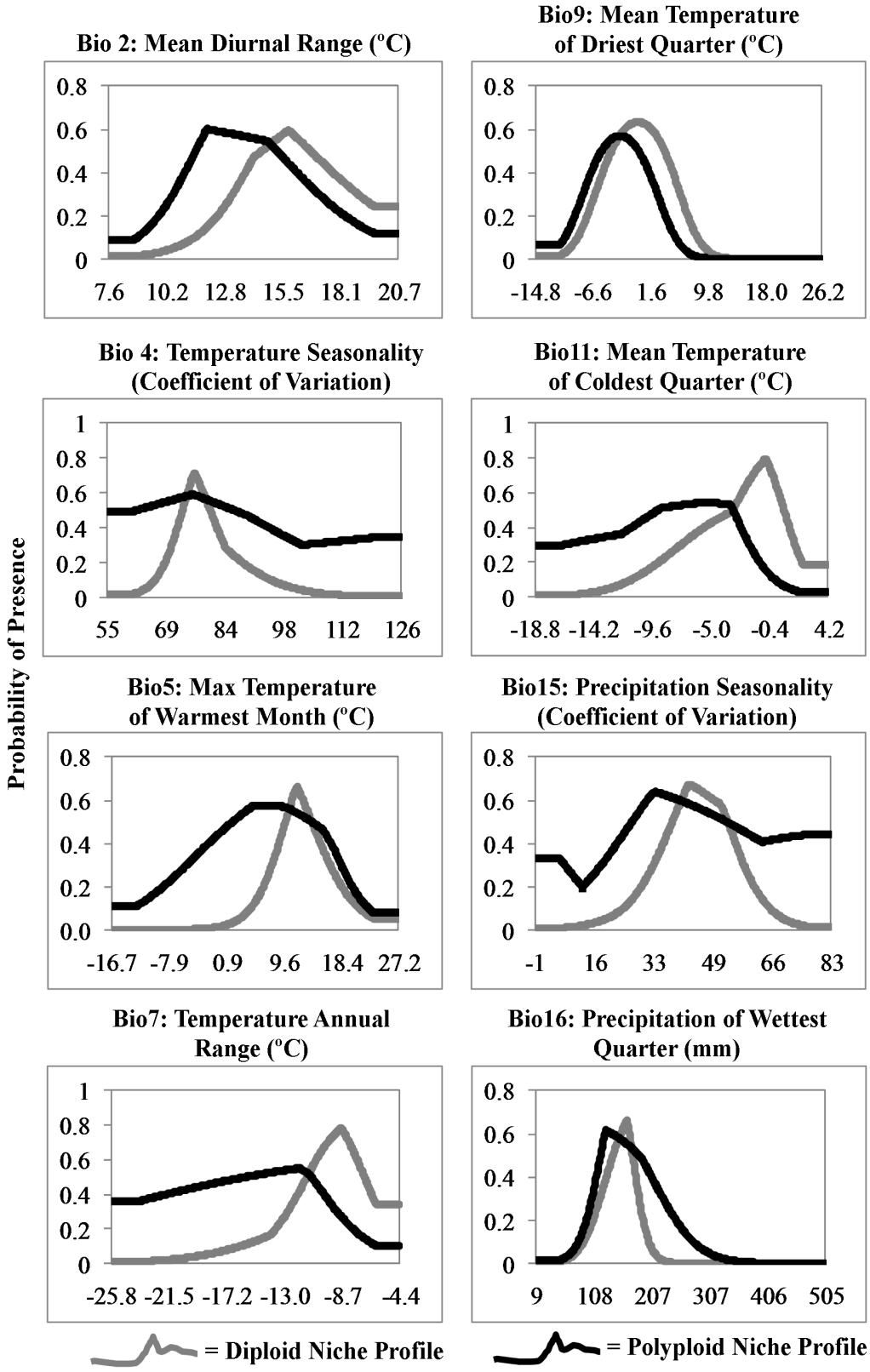
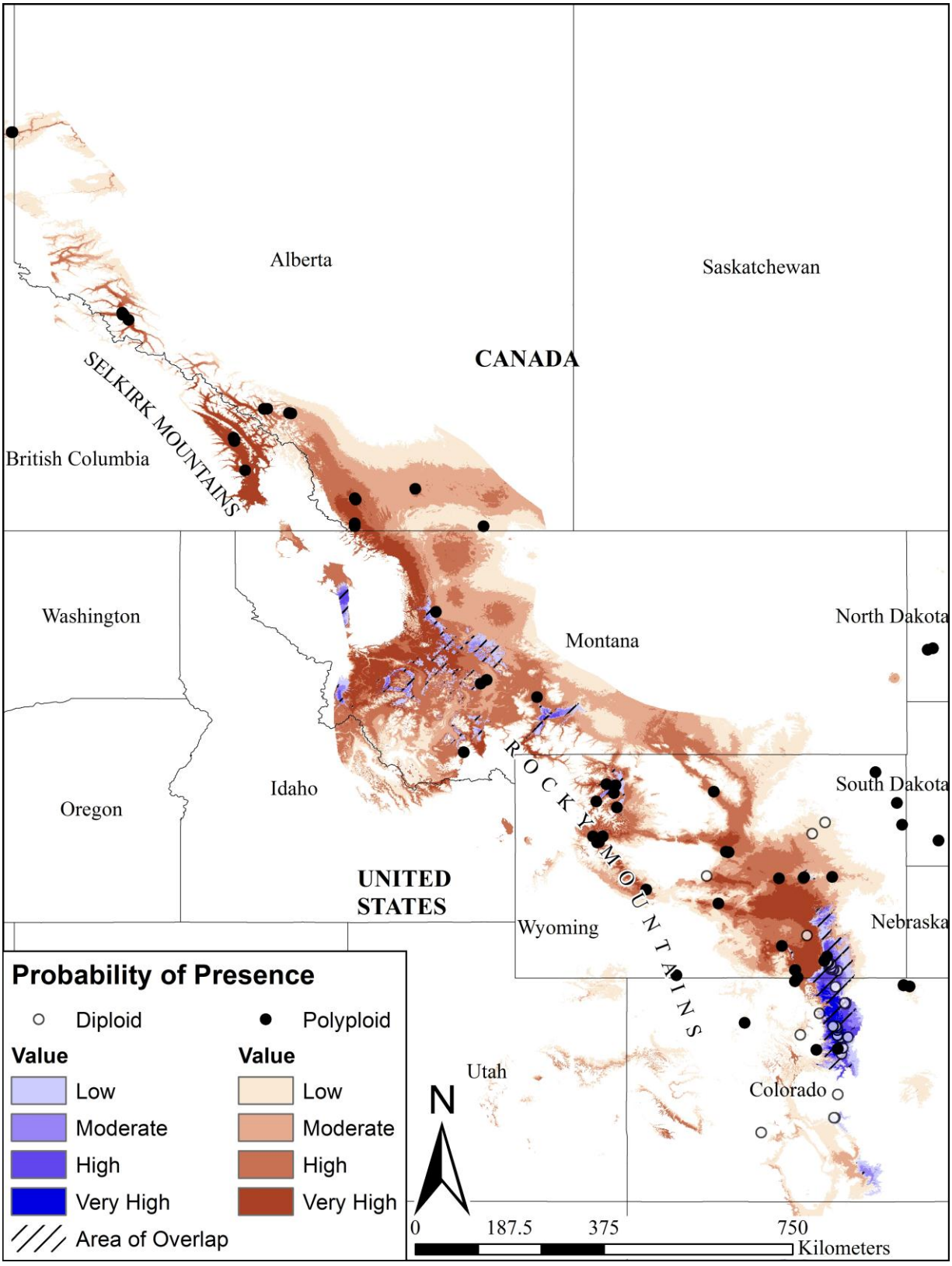


Figure 2.4 - Geographic distribution of occurrence records for *T. hookeri* diploids and polyploids, overlaid with ecological niche models averaged over 100 replicate runs and projected onto the entire study area. Predicted species suitability scores were as follows: Low=0.3-0.45, Moderate=0.45-0.6, High=0.6-0.85, Very High=0.85-1.


Alberta
Saskatchewan
CANADA
SELKIRK MOUNTAINS
British Columbia
Washington
Montana
North Dakota
Idaho
Oregon
South Dakota
ROCKY MOUNTAINS
UNITED STATES
Wyoming
Nebraska
Utah
Colorado
Probability of Presence
Diploid
Polyploid
Value
Low
Moderate
High
Very High
Area of Overlap
Value
Low
Moderate
High
Very High
N
0
187.5
375
750
Kilometers

CHAPTER 3: Phylogenetic, Life History and Morphological Evidence for Rapid Diversification of *Townsendia* (Asteraceae)

Introduction:

Townsendia is a North American genus in the large and highly variable tribe Astereae (Asteraceae). Since its initial description in the Flora Boreali-Americana (Hooker, 1840), *Townsendia* has been examined and revised in multiple taxonomic treatments, and currently includes ~30 taxa at or below the species level (Gray, 1888; Larsen, 1927; Beaman, 1957; Strother, 2006). Although we have a fairly clear understanding of the taxonomic boundaries of *Townsendia*, and of the circumscription of individual species, phylogenetic relationships among species are not clear. Only a preliminary and unpublished phylogenetic study of *Townsendia* exists (Thompson, 2006). This included currently recognized taxa but was limited by two major issues. First, low sampling within species (the majority of taxa sampled only once), which is likely to be especially problematic for widespread taxa. Second, limited sampling of the genome: Thompson (2006) used a single genomic region, the external and internal transcribed spacer (ETS and ITS) regions of nuclear ribosomal DNA (nrDNA). Additional sampling of taxa and genetic regions is needed to improve phylogenetic resolution, and to clarify interspecific relationships in *Townsendia*.

Although not as species-rich as many other Astereae genera, *Townsendia* nonetheless covers much of western North America. Its northernmost representative (*T. hookeri*) occurs in isolated patches of central-northern Alaska, and its southernmost taxon *T. mexicana* grows roughly forty degrees of latitude to the south in the Mexican states of Nuevo Leon, Durango and San Luis Potosi. Other than two Alaskan occurrences of *T. hookeri*, *T. florifer* has the westernmost distribution of the genus, with populations scattered throughout the plains of

central Oregon and Washington. The easternmost species of *Townsendia* includes *T. exscapa*, which runs from Saskatchewan and North Dakota to Texas. Generally, it is typical for species of *Townsendia* to be restricted within 4-8 degrees of latitude or less. Despite this general trend, much of the North American latitudinal range of *Townsendia* is covered by just two widespread taxa, *T. hookeri* and *T. exscapa*, each scattered across a latitudinal range of approximately 30 degrees.

The wide geographical range of the genus encompasses a variety of habitats to which the species of *Townsendia* have become adapted. For example, the genus occurs over an extreme elevation gradient beginning with *T. florifer*, which can be found on river plains at elevations as low as 83 m. In contrast, *T. rothrockii* commonly reaches up to 3899 m along parts of the Continental Divide in Colorado, and is only exceeded by a solitary population of *T. condensata* found at 4145 m in the White Mountains of California. Considering all occurrences of *Townsendia*, the range in elevation between the lowest and highest population span 380-3180 m; however, the majority of populations occur at lower-middle elevations between 2000-2500 m, and the range of elevations of an individual taxon is generally more restricted. Even with these observations of the elevational trends in *Townsendia*, the role of elevation in plant distributions is complex and often confounds the effects of variables such as moisture, wind, UV exposure, temperature and atmospheric pressure (Körner, 2007). Variables of precipitation and temperature have been found to be more important than elevation in driving adaptation (Manel *et al.*, 2012); thus, elevation may serve to maintain precipitation and temperature within a limited range, regardless of the latitude.

A diversity of forms of *Townsendia* has evolved and apparently adapted to localized regions within this geographic distribution. Among them are rare species such as *T.*

gypsophila, which thrives on gypsum rich soils in New Mexico, and *T. rothrockii*, which lives in cold, high elevation habitats. Environmental variation in *Townsendia* habitats appears to be correlated with differences in growth form. For example, Beaman (1957) suggested that the species can be sorted into three broad categories: prostrate, tall-stemmed and cushioned. The cushion form is by far the most common, occurring in 17 taxa, more than half of the genus, and always in association with perenniality. These plants have a thick, subterranean caudex (stem), with a deep taproot that is topped by a dense cushion of leaves from which many showy flowering heads emerge. The thick caudices likely help *Townsendia* persist through harsh conditions, as in other genera (Porembski, 2007; Ogburn and Edwards, 2010). Species with caudices can persist without leaves during periods of drought, cold or fire, with new leaves emerging when conditions improve (Sisson, 1983; Brown and Mies, 2012). As a result, this feature may facilitate the establishment and survival of *Townsendia* in otherwise barren landscapes. The tall-stemmed, erect growth form occurs in eight species, including biennials and perennials. Biennials have large taproots and basal rosettes in their first year, and produce showy, tall-stemmed, and sparsely leaved flowering stalks the following year. Among the erect species, *T. formosa* and occasionally *T. eximia* are perennial, but they achieve this through a rhizomatous or stoloniferous form rather than the dense, cushion form. Whereas, perennial *T. smithii* bears a combination of features with tall stemmed, leafless inflorescences rising above the basal leaves of a caespitose mat (Table 3.1). Lastly, there are four sprawling taxa, which can grow as annuals or short-lived perennials depending on seasonal conditions. They have relatively long, branched, leafy stems that lie prostrate on the soil, terminating in small inflorescences (capitula).

Previous taxonomic work has also highlighted variation in the form of the pappus bristles, which range from hairs to bristles (Macloskie, 1883; Larsen, 1927; Beaman, 1957). Most *Townsendia* species have barbellate, plurisetose pappus hairs that loosely interlock among the fruits (cypselae) of individual heads to form aggregate spheres at maturity. Wind gusts can pick up and roll these loose aggregates for short distances across the landscape, before obstructions break them up into individual fruits (C. Lee, pers. obs.). Although this is the main dispersal method, a subset of taxa have greatly reduced, stiff pappus bristles. In these species the mature fruits are loosely attached to the dry capitulum and disperse by gravity, dropping to the ground when the plants are disturbed (C. Lee, pers. obs.).

Beaman (1957) used the character-state distribution of characters of interest in the close relatives of *Townsendia* to propose various evolutionary hypotheses for the genus. He considered *Boltonia*, *Astranthium* and the monotypic genus *Dichaetophora* to be close relatives of *Townsendia*. However, molecular systematic analyses (Noyes and Rieseberg, 1999) and a taxonomic study by Nesom (2000) both support *Boltonia* as more distantly related to *Townsendia*. Nesom's work (2000) also points to the monotypic genus *Geissolepis* as closely related to *Dichaetophora*, *Astranthium* and *Townsendia*. Many species of *Astranthium* and *Dichaetophora campestris* have gravity-dispersed seeds and short, stiff pappus bristles, conical receptacles and tall, erect stems. In addition, they are found in more southern and eastern localities than *Townsendia*. Nine of twelve *Astranthium* species are found in Mexico, with the remainder located in Texas, Oklahoma, Kansas, Missouri and Arkansas. Monotypic *Dichaetophora* is found only in Texas and parts of Mexico. Because of morphological similarities among these closely related genera, Beaman predicted that primitive characters in *Townsendia* include the form of perenniality associated with fibrous-

roots and a rhizomatous habit, in addition to the presence of a small, unelaborate pappus, a conical receptacle, and an erect, monocephalous stem. He used the presence of these characters to suggest that *T. formosa* is a "primitive" lineage within the genus. *Townsendia formosa* is found in both Arizona and New Mexico. If this species is indeed sister to the remainder of the genus as suggested by Beaman, its distribution may be consistent with a scenario in which the common ancestor of *Townsendia* originated in Mexico. Following this hypothesis, diversification in *Townsendia* would have generally proceeded in a northerly fashion along the Rocky Mountains, while the outgroup taxa diversified in the northern Sierra Madre ranges of Mexico and in the semi-arid prairies of Texas and neighbouring states. Throughout this process, it is possible that features such as the pappus of *Townsendia* may have increased in complexity from short, stiff barbs to long, setose hairs, whereas others traits potentially represent a reduction in form from tall, leafy stems to nearly stemless, subterranean caudices.

Although diversification in *Townsendia* has been accompanied by specialization of taxa into specific ecological niches, most species retain a characteristic appearance that is quite distinct from other genera in Astereae. The genus is readily identifiable by features such as the density and hue of its strigose pubescence, and the overall appearance of the rows of imbricate, pointed involucral bracts on its capitula. Since its formal description by Hooker (1840), the taxonomic history of *Townsendia* has included changes in the circumscription of species within the genus, with the discovery of new taxa, or the recircumscription of existing ones into previously described taxa. Some of the new species may simply be regional variants, as appears to be the case for *T. nuttallii*, now recognized as part of *T. hookeri* (Strother, 2006). Only one species (*Xylorhiza wrightii*, formerly *Aster wrightii*) has been

included in *Townsendia* and later removed (Gray, 1888; Beaman, 1957). In comparison, deep divisions have been found in other Astereae such as *Aster*, which has been reclassified into multiple new genera, including *Symphyotrichum*, *Eucephalus* and *Eurybia* (Semple and Brouillet, 1980; Xiang, 1994; Noyes and Rieseberg, 1999). The recognition of closely related genera such as *Astranthium* have also been questioned, and various botanists consider *Astranthium* to be congeneric with *Bellis* (Hooker, 1840). *Astranthium* is currently recognized as distinct from *Bellis*, and has been shown to be sister to *Townsendia* in molecular analyses (DeJong, 1965; Noyes and Rieseberg, 1999; Nesom, 2000). Considering the inconstancy of species placement within many other genera of Astereae, the taxonomic stability of *Townsendia* is notable.

Townsendia may represent a group that radiated rapidly early in its phylogenetic history like many genera of Asteraceae (Baldwin and Sanderson, 1998; Gruenstaeudl, Santos-Guerra, and Jansen, 2013; Ramsey, Robertson, and Husband, 2008; Wagstaff and Breitwieser, 2004). The challenge in these taxa is to find sufficient sequence variation to resolve species-level phylogenetic trees. Through a comparative analysis of the *Lactuca* and *Helianthus* plastid genomes, Timme *et al.* (2007) identified three of the most variable Asteraceae specific intergenic spacer regions (*ndh*C-*trn*V, *trn*L-*rpl*32, and *trn*Y-*rpo*B) and suggested that they would be useful to resolve relationships within genera. Additional candidate loci for plant species-level descrimination include the nuclear ITS region (Baldwin *et al.*, 1995), and various candidate plant DNA barcoding regions: the *mat*K, *rpo*C1, *rpo*B, *acc*D, YCF5 and *ndh*J genes, and the plastid intergenic spacer region *psb*A-*trn*H (Kress *et al.*, 2005; Fazekas *et al.*, 2008). These regions have not been universally adopted as markers for species discrimination, and many combinations of gene regions have

been applied to phylogenetic or DNA barcoding studies, with mixed results. Researchers have noted low phylogenetic resolution in lineages such as *Crocus* (Seberg and Petersen, 2009), *Salix* (Percy *et al.*, 2014) and *Berberis* (Roy *et al.*, 2010), and in many tropical tree species (Gonzalez *et al.*, 2009). However, some studies have found success using these candidate markers, and they have yielded sufficient variation to resolve relationships in genera that include *Tolpis* (Park *et al.*, 2001), *Nannoglottis* (Liu *et al.*, 2002) and *Centipeda* (Nylinder *et al.*, 2013), and have also been found to be broadly applicable in the Rosaceae as a whole (Pang *et al.*, 2011). The goal of the current study this is to infer phylogenetic relationships within *Townsendia* using expanded intraspecific sampling, and screening a wide selection of plastid sequence regions in addition to the nuclear ITS region.

Materials and Methods:

Sampling Strategy

The range of each currently recognized taxon was established with collection data from herbarium records in UBC, including loans from RMH, ARIZ, TEX-LL, ASU, UTC, CSU, UNM, ALTA, ALA, IE-XAL, UNLV. These were supplemented with digital records from the Global Biodiversity Information Facility (GBIF), and the Southwest Environmental Information Network (SEINet). Available herbarium vouchers were confirmed using a combination of taxonomic treatments, including Beaman (1957), the Flora of North America (Strother, 2006), and Dorn and Dorn (2001), which I also used to verify the identity of field-collected samples. Digital records were assumed to be correctly identified unless they appeared to be geographical outliers or disjunct populations, in which case I gave them additional scrutiny. I excluded from further consideration outliers that did not have additional collections in the vicinity made by different collectors or in different years, because species

level identification is somewhat error prone in *Townsendia*, and these outliers generally fell within the range of another taxon within the genus. I georeferenced specimens lacking geographic coordinates using Google Earth (Google, 2010) to look up the habitat and locality data found on herbarium labels, discarding records when the data were vague. I consolidated the remaining occurrences to remove duplicates and plotted them on a map using ArcMap (ESRI Inc., 2012).

These distribution maps were used to design a targeted sampling strategy for *Townsendia* species, which took place over four field seasons (2008-2012) using several criteria: to maximize the chances of detecting variation within species, to survey underrepresented portions of species ranges, and to sample in the vicinity of type localities. For widespread taxa such as *T. hookeri*, I included as many as eight distinct samples that represent central, range-edge and disjunct populations. Whenever possible, I included two vouchers for taxa with small ranges. I also chose four closely related Astereae (Noyes and Rieseberg, 1999) as outgroup taxa: *Astranthium integrifolium, A. purpurascens*, *Dichaetophora campestris*, and *Boltonia asteroides*. *Geissolepis suaedifolia* was not included as an outgroup due to the difficulty of obtaining material from this Mexican endemic species.

DNA Extraction, Amplification and Sequencing

Whole plants were collected in the field and stored in a cooler. I removed 10-15 leaves from plants within eight hours and dried them in silica gel. The remainder of the plant was pressed as a voucher specimen and deposited at the UBC Herbarium. Upon returning to the lab, 3-35 days later, leaf material was removed from silica and transferred into a -80C freezer. For herbarium specimens, a small piece green leaf material was removed from a

single plant and this was often suitable for DNA extraction (Rogers and Bendich, 1985). The quality and yield of DNA for amplification and sequencing generally decreased with the age and quality of preservation of the material, and so most of the utilized extractions came from recently collected field materials. However, herbarium vouchers were used to fill in sampling gaps, particularly for species of conservation concern (e.g., *T. aprica*) or that were difficult to access (*T. smithii*). In total I extracted DNA from 114 plants representing all 30 species and varieties, and obtained sequenced data from 107 of these (representing all taxa) for the analysis (source information is provided in Table 3.3).

I extracted DNA using a protocol that involves a modified reciprocating saw, CTAB extraction and silica-column cleanup (Alexander *et al.* 2007). This involved a customized Mastercraft 8.5A reciprocating saw (Canadian Tire, Toronto, Ontario, Canada) and used 5 mm ceramic beads (BioSpec Products, Inc., Bartlesville, Oklahoma, USA) to grind approximately 20mg of dried, green leaf material per sample. I removed dense, long,wooly trichomes with a razor blade prior to weighing and grinding for a subset of species. The reciprocating saw was set to power level 4 and samples were ground for 60 s. I added 700 µL of 10% CTAB at 65 ºC directly to the pulverized leaf material, incubated the mixture at 65 ºC for 1-3 hours and occassionally agitated it before adding 500µL of 24:1, chloroform:isoamyl alcohol. Samples were agitated and centrifuged at 13,000 G for 15 minutes. The upper aqueous phase was removed and added to 750µL of 2M guanadine hydrochloride binding buffer before being applied to the filter in an Epoch spin column (Epoch Lifescience, Inc, Sugar Land, Texas, USA). The DNA was rinsed with 70% ethanol and centrifuged at 13,000 G for 2 minutes to dry the filter membrane. The extracted DNA was suspended in milli-Q H_2O and stored in a -20°C freezer until needed for amplification.

The yield and quality of the extractions was assessed using a DyNA Quant 200 flourometer (Hoefer Parmacia Biotec, San Francisco, California, USA) and a NanoDrop 1000 Spectrophotometer (ThermoScientific, Wilmington, Delaware, USA). The quality of extracted DNA was further assessed by running 3 µL aliquots of the extractions on a 1% agarose gel in 0.5 X Tris/Borate/EDTA (TBE) buffer at 80 V for 20 minutes. The gels were stained in ethidium bromide and visualized on an AlphaImager EC (Cell Biosciences Inc., Santa Clara, California, USA).

Target regions were amplified using the polymerase chain reaction (PCR) on a PTC-100 Programmable Thermal Controller (MJResearch Inc., Waltham, Massachusetts). The thermal cycling conditions for the three Asteraceae specific primers for three intergenic spacer regions (*ndh*C-*trn*V, *trn*L-*rpl*32, and *trn*Y-*rpo*B), were as follows: initialization at 94°C for 3 min; 35 X (denaturation at 94°C for 30s; annealing at 53°C for 30s; extension at 72°C for 2 min); followed by a final extension at 72°C for 10 minutes. For the nuclear ITS region and for *matK*, thermal cycling conditions were similar, except with a higher annealing temperature at 57°C. For the DNA barcoding regions (*mat*K, *rpo*C1, *rpo*B, *acc*D, YCF5 and *ndh*J, and plastid intergenic spacer *psb*A-*trn*H), the annealing temperature was set to 53°C for 40s. Each PCR reaction included a mix of 12.5 pmoles of each primer, 2X Tris buffer, 3mM $MgCl_2$, 1.6mM dNTPs, 0.6 units of Taq polymerase, 20-80 ng of template DNA in a total volume of 25µL made up with milli-Q water. Amplified products were viewed on EtBr stained gels with a AlphaImager EC (Cell Biosciences Inc., Santa Clara, California, USA). Products that showed a single, bright, solid band in the approximate predicted size range of the region were considered successfull reactions. When regions failed to amplify cleanly, I adjusted the annealing temperature and attempted the PCR again. When multiple DNA bands

were observed, I repeated the amplifications by increasing the annealing temperature by 1°C until only a single band was produced. I decreased the annealing temperature when no bands were produced.

Amplification products were cleaned using a column clean-up protocol. I added 1M guanidine hydrochloride to each PCR reaction at a 5:1 ratio to facilitate the binding of the amplified regions to the silica membrane of a mini-spin column (Epoch, Missouri City, Texas, USA). Solutions were run through the silica membrane with a vacuum manifold, and then thoroughly dried to the membrane in a microcentrifuge spun at 6000 G. The DNA left on the membrane was washed with 70% ethanol, redried in the microcentrifuge, and eluted with 50 µL of sterile milli-Q water warmed to 60°C. The approximate DNA concentration of the cleaned PCR products was quantified using a NanoDrop 1000 Spectrophotometer (ThermoScientific, Wilmington, Delaware, USA). Products above 10 ng/µL were sequenced in forward and reverse directions using a BigDye® Terminator v3.1 (Applied Biosystems, South San Francisco, California, USA) sequencing kit, with 20 ng of template and following manufacturer instructions. The resulting Sanger sequencing reactions were cleaned using a Sephadex G-50 column cleanup and then sequenced at the UBC NAPS DNA sequencing facility using a 3730S 48-capillary DNA Analyzer (Applied Biosystems, South San Francisco, California, USA).

Of the three Asteraceae specific intergenic spacer regions developed by Timme *et al.* (2007), preliminary sequencing results showed potentially useful levels of variation for species-level phylogenetics in *Townsendia* in both *ndh*C-*trn*V and *trn*Y. However, *trn*L-*rpl*32 failed to amplify consistently, possibly due to an inversion found within *Townsendia* and related genera in subtribe Astereae (Rick Noyes, *pers comm*). The ribosomal ITS region,

as well as the proposed DNA barcoding regions *mat*K, *rpo*C1, *rpo*B, *acc*D, YCF5 and *ndh*J, and plastid intergenic spacer *psb*A-*trn*H (Kress *et al.* 2005) all amplified consistently and cleanly. Of these, ITS, *mat*K, and *psb*A-*trn*H contained the highest levels of parsimony informative characters and were used in conjunction with *ndh*C-*trn*V and *trn*Y-*rpo*B in subsequent phylogenetic inference for *Townsendia*.

Sequence Assembly and Phylogenetic Analysis

I edited and base-called raw sequences using Sequencher (version 4.8; Gene Codes Corporation; Ann Arbor, MI). When the complementary sequences had conflicting base pair assignments these were called manually by considering the quality and clarity of the sequence chromatogram. A minority of sites was left polymorphic and coded as uncertain. The consensus sequences were exported and compiled as Nexus files and loaded into Mesquite (Maddison and Maddison, 2011). I aligned sequences using Opal and MAFFT (Wheeler and Kececioglu, 2007; Katoh and Standley, 2013) and adjusted them manually by visually scanning the sequences and also by examining areas highlighted as "slightly misaligned" by Mesquite, making changes following Graham *et. al.* (2000). The four plastid regions were concatenated in Mesquite, and the concatenated plastid and ITS regions were analyzed separately with maximum likelihood (ML) and Bayesian inference methods.

I used jModeltest2 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012) to find the nucleotide substitution model that best fit each data set. For ML analysis of the plastid data matrix, I found the HKY+G+I model (Hasegawa, Kishino, and Yano, 1985) of nucleotide substitutions best fit the data, and the TIM3ef+G model of nucleotide substitutions best fit the ITS matrix. I used GARLI V2.0 (Zwickl, 2006) on the CIPRES Portal (Miller, Pfeiffer, and Schwartz, 2010) and on a personal computer to perform separate ML analysis on

concatenated plastid data and on the nuclear ITS data set. Each analysis was run until no significantly better likelihood score was found for 10,000 generations. I also estimated branch support using a bootstrap analysis (Felsenstein, 1985) with 500 replicates. Bootstrap values less than 70% were considered to indicate weak support for a particular branch, values greater than 90% were considered strong and everything in between was considered to have moderate support.

I also analyzed the data using the Bayesian inference (BI) method implemented in MrBayes V3.2.2 (Huelsenbeck and Ronquist, 2001). For both the ITS and plastid matrices, I ran the analysis with four chains for ten million generations and sampled every one thousand generations. The plastid DNA analysis was also run with the HKY+G+I model of nucleotide substitution, and the ITS analysis used the GTR+G model of nucleotide substitution. For these Bayesian results, I used Tracer V1.6 (Rambaut, Suchard, and Drummond, 2003) to test for chain convergence. Majority rule consensus trees were constructed, edited and viewed in Mesquite (Maddison and Maddison, 2011). Posterior probability values less than 90% were considered to indicate weak support for a particular node and values greater than or equal to 90% were considered strong. Within large polytomies, samples are displayed together, despite the presence of autapomorphies that distinguish them.

Haplotype Network Estimation

After removing samples or regions with missing data, haplotypes were identified in the plastid DNA dataset using DnaSP (Librado and Rozas, 2009). The *trn*Y region was excluded from this analysis because it was missing data in a number of important taxa with small ranges. An ML tree was inferred from this reduced set of data using a TVM model of sequence evolution in GARLI; a haplotype genealogy was constructed from this tree using

Haploviewer (Salzburger *et al.*, 2011). Patterns of haplotype variation were explored by labeling the network by species, ecoregion (Western Ecology Division, 2011) or geographic cluster. Geographic clusters were determined using ArcMap (ESRI Inc., 2012), grouping samples found within 100 km of at least one other sample, drawing polygons around the connected individuals.

Results:

Sequencing

Sequence data were obtained for 107 samples (Table 3.3), and in the plastid data, 60 samples were sequenced for all regions, 20 lacked a single region, seven lacked two regions, and 16 lacked three regions. The ITS region was not recovered for 13 samples. Problems with amplification occurred when extracted DNA was poor quality, which tended to be the case when leaf tissue originated from older herbarium vouchers. Single sequencing reads were usually between 500-1000 bp, except for the *psbA-trnH*, which had a short read (~300 bp). When concatenated and aligned, the total length of the plastid data matrix was 4629 bp and the ITS alignment was 768 bp in length.

Phylogenetic Analysis: ITS Region

I present an ML tree for each type of analysis here (Figures 3.1-3.4). The ITS region's aligned length of 768 characters included 237 variable characters, of which 104 were parsimony informative. Using Tracer, I found the Bayesian inference had an effective sample size (ESS) of 2760. The ML and Bayesian trees (Figures 3.1-3.2) were generally topologically congruent. Both analyses support the monophyly of *Townsendia* relative to the outgroups. Both ML and Bayesian analyses find strong support for a clade comprising *T.*

annua and *T. mexicana* as the sister group to the rest of *Townsendia*. The next well supported early split leaves *T. formosa* and *T. smithii* unresolved, but distinct from a clade including the remainder of *Townsendia*. Much of the remainder of *Townsendia* forms a core polytomy with little resolution or structure. Within this grouping, a number of conspecific samples form weakly-to-moderately supported clades individually, such as *T. condensata*, *T. glabella*, northern *T. hookeri* (from Alaska and Yukon), and *T. minima*. Beyond these conspecific groupings, a subset of the *T. eximia*, *T. texensis* and *T. grandiflora* samples are weakly resolved in a clade (marked as "Erect biennials" in Figures 3.1-3.2). There is also moderate support for a clade that includes seven of ten samples of *T. exscapa* and all but one of the southern samples of *T. hookeri* (marked as "Hookeri/Exscapa" in Figures 3.1-3.2). Although these *T. hookeri* samples resolve into their own moderately supported clade, the relationship of the seven *T. exscapa* samples relative to *T. hookeri* samples is unclear. In addition, the Bayesian analysis recovered a clade with strong support that was found without support in the best tree of the ML analysis, which consists of *T. condensata, T. florifer, T. scapigera, T. parryi*, and two subspecies of the *T. jonesii* complex.

Phylogenetic Analysis: Plastid DNA

The plastid data matrix included 4629 characters, of which 482 are variable but uninformative characters, and 195 are parsimony-informative characters. Using Tracer, I found the Bayesian inference of plastid data had an ESS of 3642. As with the ITS phylogeny, the plastid phylogeny inferred from ML analysis supports the monophyly of *Townsendia* relative to the outgroup taxa (Figure 3.3). The plastid phylogeny places most of *Townsendia* in a large polytomy with minimal resolution. Samples within several taxa of this group, including *T. hookeri* (subsets), *T. minima*, *T. mexicana, T. montana* (subsets) and *T. eximia*

(subsets), form weakly supported individual clades. There is moderate support for a clade that includes *T. annua, T. aprica, T. incana, T. jonesii* and its varieties, *T. mensana, T. rothrockii* and a subset of *T. exscapa*, *T. strigosa* and *T. nuttallii*/*T. hookeri* representatives.

Haplotype Network Analysis

Haplotype analysis revealed 24 haplotypes clustered into five major and two minor haplotype groups (Figure 3.5) in the combined analysis of three plastid regions. Different samples of the same species did not always belong to the same haplotype group. For example, *T. exscapa* samples were present in four of the five major haplotype groups, although samples of the similarly widespread *T. hookeri* were only found within two of the five major groups. Some samples, including the *T. jonesii* varieties, *T. condensata, T. scapigera* and *T. minima,* shared haplotypes with conspecifics, but these haplotypes were typically shared with additional taxa. Overall, the individual species do not appear to carry unique haplotypes, and the largest haplotype included 17 individuals of 10 different species all with the same haplotype variant. Although haplotypes did not clearly track species taxonomy, there was nominal evidence of spatial clustering present in haplotype group 1 and haplotype 5 (Figure 3.6A).

Discussion:

Despite the overall lack of phylogenetic resolution within the genus and frequent sharing of haplotypes among species, phylogenetic analyses of both ITS and plastid sequence variation provide strong support for the monophyly of *Townsendia*. Because the ITS phylogeny provides the most resolution within *Townsendia,* I will focus the following discussion of inferred relationships primarily on results of analysis of this region, and highlight differences with the plastid DNA results.

As noted, species of *Astranthium* (12 species) and *Dichaetophora* (monotypic) share many features, including terminal inflorescences on tall, leafy stems, involucres with conical receptacles, cypselae with a short, stiff and reduced, or absent pappus, and fibrous root systems. The bulk of these species occur in relatively moist, montane habitats (DeJong, 1965). Because many of these morphological and ecological features are shared with *T. formosa*, and in part with *T. smithii*, Beaman (1957) believed that *T. formosa* was a "primitive" species in *Townsendia* and that it retained many shared ancestral features. *Townsendia smithii* was not yet known at the time of Beaman's monograph, however, it shares several features with *T. formosa* (Shultz, Holmgren, and Jun, 1980) and both species are exclusively known as sexual diploids and possess a perennial habit. Both species also have unspecialized, short, scale-like pappus hairs, which is a rare character in *Townsendia*, and suggests a close relationship between *T. formosa* and *T. smithii*. Although the placement of *T. smithii* remains unresolved in this clade, there is strong support for a clade comprising these two species. Together, they represent the second, and later diverging, of the two lineages that form at the base of *Townsendia*, which is consistent with their possible close relationship. In contrast, the plastid phylogeny does not recover *T. smithii* and *T. formosa* as a clade. Instead, both species remain unresolved within a large polytomy of the inferred plastid phylogeny. These weakly supported plastid results belie the distinct geographic boundaries, and morphology of these two species, when compared to the remainder of the genus.

A clade comprising two species, *T. annua* and *T. mexicana* (marked as "Prostrate" in Figures 3.1-3.2), is inferred to be the sister group of the remaining species in the ITS tree. These two species share a number of features that are uncommon in the remainder of

Townsendia, including an annual to scarcely perennial life history, and the presence of delicate, leafy stems with a predominantly prostrate habit. In noting these differences, Beaman interpreted *T. mexicana* as the most "derived" species of *Townsendia*, stating that its diminutive size, two series of phyllaries, short ray and disk corollas, and short pappi and small achenes were all signs of evolutionary reduction. However, the inference of derived and ancestral features should be done with caution and skepticism, as any lineage may hold a mixture of both derived and ancestral character traits. Formal ancestral state reconstructions would be needed to assess these characters on a well-resolved species tree. *Townsendia annua, T. mexicana, T. formosa* and *T. smithii* all share several characters with *Astranthium* and *Dichaetophora* that are not present in the remainder of *Townsendia*, such as a reduced pappus, which may indicate that these are ancestral features. Also, these four townsendias are exclusively sexual and diploid, and are all found in the southern portion of the range of the genus, in Arizona, New Mexico and Mexico. Their distribution is nearer to the core distribution of the outgroup taxa in Mexico and southeastern U.S., which may support a southern origin for *Townsendia* and its relatives. Although the present data are not sufficient to resolve ancestral character states, the remainder of *Townsendia* (the 'core' polytomy) possess a number of characters that are unique within *Townsendia* and not found in the outgroups, and which I interpret as derived for the core.

Core Polytomy

The remaining 26 species of *Townsendia* comprise a poorly resolved grouping that I refer to as the 'core polytomy'. This core includes six species of tall-stemmed biennials such as *T. parryi* and *T. florifer*, which have ranges predominantly in the northwestern Rockies and the Oregon river plains. Included among these biennial species is a clade including *T.*

eximia, T. texensis and *T. grandiflora,* that is recovered with weak support in the ML ITS tree, and with strong support in the Bayesian ITS and plastid trees (in part). However, the most distinctive feature found in the majority of taxa in the core polytomy is the perennial cushion habit, with a branched, subterranean caudex. These features are found in taxa such as *T. leptotes*, a species that is widely distributed in the Rockies, and *T. scapigera*, a species that is predominantly found in California and Nevada. This robust growth form may have been associated with the movement of *Townsendia* into more seasonally arid habitats, away from the mesic habitat of *T. formosa* and of *Astranthium*. Beyond an increased tolerance of dry conditions, these cushion plant species have apparently been able to specialize in a variety of rare or restricted habitats. For example, *T. gypsophila* lives only in the gypsophilous hills around Albuquerque, New Mexico, and *T. microcephala* is a rare endemic present only on two small mesas in southwestern Wyoming. With the degree of geographic and ecological isolation, and the morphological distinctness of many taxa, the general lack of phylogenetic resolution and the discord found between species of the core polytomy are unexpected, but could arise from hybridization and introgression, low levels of genetic variation among lineages, or problems with lineage sorting and ancestral polymorphisms.

Genetic Differentiation

Amounts of genetic divergence and variation within the sequenced regions of the sampled taxa in this study were low, and in the plastid data only 10.4% of the aligned sites were variable across taxa, and 4.2% parsimony informative. The ITS region showed higher levels of variation (30.86% variable, 13.54% parsimony informative), but this is still relatively low when contrasted with other Asteraceae studies within genera. For example, previous studies have found 55% parsimony informative characters in *Aster* (Li *et al.*, 2012),

15% in *Nannoglottis* (Liu *et al.*, 2002), and 44% in *Espeletia* (Rauscher, 2002). Furthermore, the initial screening of eleven regions uniformly detected low levels of sequence variation in *Townsendia* (data not shown). These regions have been successfully used to resolve species-level phylogenies in a number of groups within the Asteraceae (Denda *et al.*, 1999; Wagstaff and Breitwieser, 2004; Gruenstaeudl *et al.*, 2009). This suggests that in *Townsendia*, plastid DNA as a whole harbors low levels of variation for species level phylogenetic resolution. Sampling of low copy nuclear markers may yield better results, in addition to revealing both the maternal and paternal evolutionary history of the genus. However, it seems most likely that the observed variation may indicate that the genus diversified recently.

Hybridization

Incidences of hybridization and introgression could contribute to the scattered distribution of species in the inferred ITS- or plastid-based phylogenies. However, in order for hybridization to contribute to the lack of resolution in the phylogeny, a plastid haplotype would have needed to have rapidly swept through the core polytomy after the group's speciation. *Townsendia* is considered a relatively young genus of Asteraceae, and hybridization is known to occur among species in this group. Based on field studies, and his understanding of the cytology, geography and morphology in the group, Beaman (1957) believed that 15 of the 21 species that he recognized formed putative natural hybrids with at least one other species, and that experimental crosses of non-overlapping taxa were often successful (Beaman, 1957). Gene flow through hybridization transfers alleles between otherwise independently evolving taxa, and although introgressive hybridization can facilitate speciation (Anderson, 1953; Rieseberg, Archer, and Wayne, 1999; Rieseberg *et al.*, 2003), the short-term effect is to share genes between taxa and obfuscate the relationship

between gene trees and species trees. This problem is especially highlighted and long lasting in plastid DNA because of maternal inheritance, which could potentially maintain a signal from a rare hybridization event whereas recombination in nuclear DNA would dilute evidence for hybridization. Overall, this may result in increased periods of time and reproductive separation before gene trees begin to match species trees. Despite the potential for hybridization between many *Townsendia* species, natural hybrids are thought to rarely occur between these species: *T. parryi* and *T. florifer*, and among *T. leptotes*, *T. hookeri* and *T. exscapa* (Beaman, 1957). Hybridization in these species is thought to be restricted only to the narrow areas where species ranges overlap or are adjacent (Beaman, 1957). Furthermore, marked differences in traits such as flowering time and habitat preference make it likely that hybridization is overall a rare occurrence occurring only in outlying populations, and seems likely to minimally contribute to the overall low phylogenetic resolution. Of all taxa in the genus, *T. hookeri* and *T. exscapa* have broad distributions that bring them into contact zones with many other species, which suggests that the pair appear to have the highest potential for hybridization (Beaman, 1957, C. Lee, pers. obs.). Yet, they are one of the few pairs for which the inferred ITS phylogeny supports their taxonomic relatedness. In contrast, even in examples where no hybridization has been observed to occur (Beaman, 1954, 1957), species such as *T. glabella*, *T. rothrockii*, and *T. scapigera* remain unresolved phylogenetically.

Even when natural hybrids apparently form between species, they appear to retain unique groups of morphological features, suggesting that partial reproductive isolation may maintain species cohesion. For example, although *T. parryi* and *T. florifer* are thought to hybridize in peripheral populations (Beaman, 1957), the two species are readily distinguished in all parts of their range based on, respectively, blue vs. pink ray corollas, large few-

stemmed vs. slender many-stemmed plants, and a montane vs. plains habitat. The suggestion here is that *Townsendia* consists of good species, and the genus is not an example of few widespread taxa, that have been taxonomically oversplit based on regional differences. However, despite the morphological stability of individual species, it is clear that members of a species often share haplotypes found in other species (Table 3.3). Only Haplogroup 1 showed a moderate sign of haplotype sharing between related taxa by connecting the three varieties of *T. jonesii* with *T. aprica* and *T. mensana* (Table 3.3, Figure 3.6B). Haplogroup 1 and 5 also indicate the potential for a geographic basis for shared haplotypes (Figure 3.6B). The majority of the taxa in these haplogroups are locally restricted, and even widely distributed species such as *T. exscapa* are not fixed in adopting the haplogroup identity of nearby geographic neighbours. Overall, haplogroup membership is weakly suggestive of species relatedness, but the lack of geographic correlation of haplogroups suggest that hybridization is a rare event in *Townsendia*.

Lineage Sorting and Ancestral Polymorphism

Discordance between species boundaries and the inferred phylogeny of *Townsendia* may be a result of incomplete lineage sorting. Because the genus is thought to be relatively young, insufficient evolutionary time may have passed for species to achieve reciprocal monophyly, resulting in shared genetic polymorphisms in sequence data across species. These shared polymorphisms may be part of a transitory phase that leads to monophyly via gene flow in plesiospecies as suggested by Olmstead (1995).

Taking all of this into account, the divergence in growth habit, morphology, life history and ecological tolerances appear to have developed relatively rapidly and recently, such that the genetic variation of the plastid DNA is not yet representative of species trees in

Townsendia. These genetic data, and the presence of a large core polytomy, support Beaman's species concept regarding *Townsendia,* which suggested that genetic barriers had a weak influence on diversification in the genus, as evident by the presence of putative, natural hybrids and the ease of experimental crossing. Rather, it appears that geographic barriers, by which he meant distance, habitat and climate, were the foremost factors in the isolation of species (Beaman, 1957).

Low sequence variation within the core polytomy has produced a pattern where only a limited number of samples resolve into clades with varying levels of support in the phylogeny that correspond to species fully, or in part. Examples include *T. condensata* (ITS ML/ITS BI/Plastid ML/ Plastid BI; 57/95/-/-) found at high elevation sites in northwest Wyoming, *T. minima* (83/100/85/90) restricted to specific iron-rich, talus slopes in Utah, and the Arizona *T. exscapa* samples (93/97/-/-) where they overlap with *T. mensana*. Among these resolved clades are also examples of extremely disjunct populations such as *T. hookeri*'s sub-arctic contingent (64/96/-/-) from Alaska and the Yukon, and highly specialized species like *T. glabella* (51/73/-/-) from the Mancos Shale formation in southwestern Colorado. As examples of strong geographic and ecological separation, the latter two examples could potentially be interpreted as lineages found in unique scenarios that promote greater genetic isolation, and this could potentially result in higher levels of sequence variation within a taxon. However, this seems unlikely because other unresolved taxa of the core polytomy are similarly replete with rare endemics, niche specialists and disjunct taxa.

Two weakly supported, but interesting groupings of species are recovered within the third clade of the ITS tree (Figures 3.1-3.2). One labeled the Hookeri/Exscapa group

comprises the southern populations of *T. hookeri*, and the majority of the *T. exscapa* samples. These two are the most widespread species of *Townsendia*, and each includes both sexual diploid and apomictic polyploid populations. *Townsendia hookeri* occurs predominantly in the foothills of the Colorado and Wyoming Front Range, and similar areas in Montana, British Columbia and Alberta. Sexual diploids are known from Colorado and southern Wyoming, while apomictic polyploids are found from northern Colorado northward (Beaman, 1957). *Townsendia exscapa* occurs further south and east than *T. hookeri*, and is widespread across lower elevation plains from Mexico to southern Saskatchewan; however, it is most common in the southern or eastern states of Arizona, New Mexico, Colorado, Nebraska and Oklahoma, where the majority of populations are sexual diploids. Apomictic polyploids are less common, but are known especially from western Colorado, Montana, the Dakotas and Canada; however, the overall distribution of the two reproductive types is less well known than in *T. hookeri*. Certain portions of the two species' ranges overlap, forming local contact zones where putative hybrids may arise (Beaman, 1957), especially where at least one of the taxa is represented by sexual diploids. These hybrids further confound the circumscription of species that are already morphologically similar, and commonly misidentified by field botanists. However, the degree to which hybridization contributes to morphological variation between species is not clear, as latitudinal gradients and regional conditions may also play a role in shaping variation within species (SantamarÍa *et al.*, 2003; Molina-Montenegro and Naya, 2012). Useful characteristics for distinguishing pure *T. hookeri* and *T. exscapa* include, respectively: shorter pappus bristles approximately the same height as disk corollas (4-6mm) vs. long pappus bristles markedly longer than disk corollas (7-10mm); smaller inflorescences (~1.5cm wide) with linear involucral bracts vs. larger

inflorescences (~2.2cm wide) with lanceolate involucral bracts; and leaves linear with fine, silvery, sericeous hairs vs. leaves oblanceolate with strigose hairs (C. Lee, pers. obs.). Certain plants found in sympatry may have a combination of characters from both taxa, suggesting a zone of hybrid contact. Nonetheless, differences in flowering time and in the ecological requirements of each species may reduce the potential for gene flow (Rieseberg and Willis, 2007). As predicted by their morphological similarity, the results of the genetic analysis suggest that *T. hookeri* and *T. exscapa* are closely related, but nonetheless each contain some genetic differentiation. Although *T. hookeri* and *T. exscapa* samples are scattered throughout the inferred plastid phylogeny (Figure 3.3-3.4), there is at least a substantial portion of both species that resolve in a clade of the ITS phylogeny (Figure 3.1-3.2). Although the placement of *T. exscapa* remains unresolved in this clade, there is at least enough differentiation to underscore its separation from *T. hookeri*.

Additional support for the separation of *T. exscapa* and *T. hookeri* can be found in overlap zones where observed genetic differences between taxa nonetheless outweigh geographic proximity. A suitable test case can be seen in North Dakota where co-occurring populations of *T. hookeri* and *T. exscapa* from the Little Missouri National Grasslands were sampled and included in this analysis. Even though as few as 20 metres separated these samples, they uniformly grouped more closely with conspecifics from populations both near and far (Lee and Garani, 2012) (Figure 3.1, H3-H5 and E1-E7 are North Dakota samples). At these sites, flowering time was later in *T. exscapa*, and there were marked differences in habitat with *T. exscapa* found on grassy plains, and *T. hookeri* found on bare and exposed scoria mounds. Morphologically, both species appeared more similar to conspecifics from

distant sites than to each other. All these observations suggest that it is likely that *T. hookeri* is a distinct species from *T. exscapa*.

Another informative, though weakly supported clade (ITS 51% ML bootstrap support, 92% BPP; plastid 99%BPP in part), consists of *T. eximia*, *T. texensis*, and *T. grandiflora* (marked as "Erect Biennials" in Figures 3.1-3.2). This trio of taxa was previously identified as a distinct group by Beaman based on their shared appearance as tall, erect plants with bristly involucral bracts. Despite general similarities, each species is well defined by distinct morphological features and by largely non-overlapping distributions. *Townsendia eximia* tends to be distributed at higher elevations (2000-3500m), and is scattered throughout the Southern Rockies and the New Mexico Mountains ecoregions located in north and north-central New Mexico. Beaman considered it one of the more "primitive" species because of its similarity to *T. formosa*, particularly its biennial life history that borders on rhizomatous at high elevation sites, and its short, stiff pappus bristles. *Townsendia texensis* occupies the limestone slopes in the High Plains of the Texas Panhandle and in adjacent regions of Oklahoma. Bienniality and blue corollas mark its relationship with *T. eximia*; however, it generally has attenuate rather than acuminate involucral bracts, as well as longer, barbellate pappus bristles. Lastly, the distribution of *T. grandiflora* is sandwiched between those of *T. eximia* and *T. texensis* in the Southwestern Tablelands in New Mexico, extending northward into the High Plains of Colorado and Wyoming, and into the northwestern Great Plains of the Dakotas. This species has long, barbellate pappus bristles and white-cream ray corollas, and also shows no evidence of the rhizomatous perenniality found in *T. eximia.*

Overall, the circumscriptions of *T. eximia*, *T. texensis*, and *T. grandiflora* are clear and unchallenged, but they are nonetheless collectively held together by key features such as

their generally southern distribution, and their predominantly biennial life history, with tall, erect flowering stems and cauline leaves. These types of features were enough for Beaman to infer an evolutionary relationship among them, as well as between *T. hookeri* and *T. exscapa*, in a manner that is consistent with the results of my phylogenetic analyses. A few other species groupings revealed through phylogenetic inference here were also reflected in Beaman's monograph. However, these groups had low support values, or were recovered in only one tree (for example, the grouping of *T. scapigera, T. spathulata, T. condensata, T. florifer* and *T. parryi* in the Bayesian ITS tree, Figure 3.2).

Adaptive Radiation

The apparently rapid divergence of species in *Townsendia* may be the result of adaptive radiation (Schluter, 1996). Rapid diversification of a lineage into a set of new taxa may be triggered by three potential events: release from competition, niche specialization, and evolution of a key adaptation/innovation (Schluter, 1996). Classical examples of a release from competition include the colonization of a new island, or a mass extinction; such events provide taxa with a multitude of empty niches. However, there is no evidence for these events here, and *Townsendia* already occurs in relatively species-depauperate landscapes with a seemingly limited number of biotic interactions (C. Lee, pers. obs.). It is likely the ability of many *Townsendia* to specialize in such marginal habitats, often with unusual edaphic conditions, has contributed to its rapid speciation. In particular, the evolution of several key adaptations may have spurred further diversification in *Townsendia*. Features such as a subterranean caudex, a cushion-plant form, and an ability to reproduce via apomixis are known to be adaptive traits (Billings, 1974; Porembski, 2007; Lodé, 2012). The compact cushions of leaves sitting atop an underground caudex, all attached to a deep

taproot, may have allowed *Townsendia* to persist in marginal habitats that would have been unavailable to annuals, rhizomatous or stoloniferous plants. This could potentially allow *Townsendia* with cushion plant forms to persist during suboptimal seasons, which present the opportunity for speciation to occur in the core polytomy through adaption to additional climate variation. Polyploid asexuality, found in 14 species in the genus, could also have profound effects on the proliferation and speciation of *Townsendia* by providing all the benefits of improved colonization ability through geographical parthenogenesis (Bierzychudek, 1985), as well as presenting extra chromosomes that may potentially alter species traits via neo- or subfunctionalization (Comai, 2005; Porembski, 2007; Lodé, 2012). However, sexual diploids are present in all species, and their distributions suggest that all species originate as sexual diploids. Asexuality may play a role in the spread of a species, and it will affect levels of intraspecific variation, but other factors such as the caudex and cushion-plant form, or ecological specialization likely have a larger role in the diversification of the genus.

The core polytomy (Figures 3.1-3.2) contains all of the taxa with underground caudices and a cushion growth form. Outside this core polytomy, the non-core species of *Townsendia* tend to be sprawling, or rhizomatous/stoloniferous species that occur in low elevation plains, or in more mesic habitats. Furthermore, these *Townsendia* species, in addition to the outgroup taxa, all show comparatively longer branch lengths in well support clades, whereas there is an overall lack of internal branches in the core polytomy of *Townsendia*. Taken as a whole, this suggests a relatively rapid and recent divergence of species in the core polytomy when compared to the outgroups and early-diverging *Townsendia*.

Conclusion:

Townsendia appears to be a case where the scarcity of resolution and low support values from phylogenetic inference informs our understanding of the evolutionary history of the group. The patterns observed here suggest a rapid radiation of species in *Townsendia*, and the complex pattern of allele or haplotype sharing make the estimation of species trees and species boundaries from molecular data difficult, even with a larger number of gene regions. Although factors such as hybridization may explain the scattering of species across the inferred phylogeny, and may partly contribute to the reduction of phylogenetic resolution, the low occurrence of hybrids, and their restriction to range edge populations, suggests its relatively low importance in this group. Lack of phylogenetic resolution may also be the outcome of poorly chosen plastid gene regions rather than low genetic variability within the genus. However, this seems unlikely due to the broad, initial sampling of sequence regions, all of which have been found to be rapidly evolving in many groups (Baldwin *et al.*, 1995; Chase *et al.*, 2005; Kress *et al.*, 2005; Timme *et al.*, 2007). Nonetheless, future examination of more rapidly evolving genetic regions in low-copy nuclear genes may open the possibility of further disentangling the species relationships in *Townsendia*. Taken in combination with the current knowledge about the biology, ecology and life history of this genus, the evidence from this phylogenetic study tell a story of rapid diversification via adaptive radiation.

Table 3.1 - *Townsendia* taxa with morphological traits

		Characteristics		
Species	**Abbreviation**	**Stem**	**Inflorescence**	**Receptacle**
T. annua	ANN	Prostrate, leafy	Terminal	Convex
T. aprica	APR	Cushion	Sessile	Convex
T. condensata	CND	Cushion	Sessile	Convex
T. eximia	EXI	Basal and leafy	Erect	Convex
T. exscapa	EXS	Cushion	Sessile	Convex
T. fendleri	FND	Prostrate-cushion	Sessile	Convex
T. florifer	FLR	Basal rosette	Erect	Convex
T. formosa	FRM	Stolons and leafy	Erect	Conic
T. glabella	GLB	Dense cespitose	Pedunculate	Convex
T. grandiflora	GRN	Basal rosette	Erect	Convex
T. gypsophila	GYP	Dense cespitose	Terminal	Convex
T. hookeri	HOK	Cushion	Sessile	Convex
T. incana	INC	Dense cespitose	Sessile	Convex
T. jonesii	JON	Cushion	Sessile	Convex
T. jonesii var. *lutea*	JLT	Cushion	Sessile	Convex
T. jonesii var. *tumulosa*	JTM	Cushion	Sessile	Convex
T. leptotes	LEP	Cushion	Sessile	Convex
T. mensana	MEN	Cushion	Sessile	Convex
T. mexicana	MEX	Prostrate, leafy	Terminal	Convex
T. microcephala	MIC	Cushion	Pedunculate	Convex
T. minima	MIN	Cushion	Sessile	Convex
T. montana	MON	Cushion	Pedunculate	Convex
T. nuttallii	NUT	Cushion	Sessile	Convex
T. parryi	PAR	Basal rosette	Erect	Convex
T. rothrockii	ROT	Cushion	Sessile	Convex
T. scapigera	SCP	Dense cespitose	Pedunculate	Convex
T. smithii	SMI	Dense cespitose	Erect	Convex
T. spathulata	SPA	Cushion	Sessile	Convex
T. strigosa	STR	Basal rosette	Erect	Convex
T. texensis	TEX	Basal rosette	Erect	Convex
Astranthium	ASTR	Basal and leafy	Erect	Conic
Dichaetophora	DIC	Basal rosette	Erect	Conic
Boltonia	BOL	Stolons and leafy	Erect	Conic

Table 3.1 (cont.) - *Townsendia* taxa with morphological traits

	Characteristics		
Species	**Pappus**	**Roots**	**Life History**
T. annua	Plurisetose, long	Taproot	Annual
T. aprica	Plurisetose, long	Taproot	Perennial
T. condensata	Plurisetose, dehiscent	Taproot	Perennial
T. eximia	Stiff, unelaborate, short	Taproot or fibrous	Biennial
T. exscapa	Plurisetose, long	Taproot	Perennial
T. fendleri	Plurisetose, long	Taproot	Perennial
T. florifer	Plurisetose, long	Taproot	Biennial
T. formosa	Stiff, unelaborate, short	Fibrous	Perennial
T. glabella	Plurisetose, long	Taproot	Perennial
T. grandiflora	Plurisetose, long	Taproot	Biennial
T. gypsophila	Plurisetose, long	Taproot	Perennial
T. hookeri	Plurisetose, long	Taproot	Perennial
T. incana	Plurisetose, long	Taproot	Perennial
T. jonesii	Plurisetose, long	Taproot	Perennial
T. jonesii var. *lutea*	Plurisetose, long	Taproot	Perennial
T. jonesii var. *tumulosa*	Plurisetose, long	Taproot	Perennial
T. leptotes	Plurisetose, long	Taproot	Perennial
T. mensana	Plurisetose, long	Taproot	Perennial
T. mexicana	Plurisetose, long	Taproot	Perennial
T. microcephala	Plurisetose, dehiscent	Taproot	Perennial
T. minima	Plurisetose, long	Taproot	Perennial
T. montana	Plurisetose, long	Taproot	Perennial
T. nuttallii	Plurisetose, long	Taproot	Perennial
T. parryi	Plurisetose, long	Taproot	Biennial
T. rothrockii	Plurisetose, long	Taproot	Perennial
T. scapigera	Plurisetose, long	Taproot	Biennial
T. smithii	Reduced, short squamellae	Taproot	Perennial
T. spathulata	Plurisetose, dehiscent	Taproot	Perennial
T. strigosa	Plurisetose, long	Taproot	Biennial
T. texensis	Plurisetose, short	Taproot	Biennial
Astranthium	Lacking or greatly reduced	Fibrous or taproot	Ann/Perennial
Dichaetophora	Stiff, unelaborate, short	Taproot	Annual
Boltonia	Stiff, unelaborate, short	Fibrous	Perennial

Table 3.1 (cont.) - *Townsendia* taxa with morphological traits

	Characteristics		
Species	**Phyllaries**	**Sexuality**	**2n**
T. annua	3, elliptical/obovate-acute	Sexual	18
T. aprica	3-4, lanceolate, acute	Sexual	18
T. condensata	3-5, lanceolate-linear, acuminate	Apomictic/Sexual	18,36
T. eximia	4-6, lanceolate, acuminate	Sexual	18
T. exscapa	4-7, lanceolate-linear, acute	Apomictic	18,27-36
T. fendleri	4-5, lanceolate, acute	Sexual	18
T. florifer	3-4, lanceolate	Sexual	18
T. formosa	4-6, ovate, acute	Sexual	18
T. glabella	4, oblanceolate, acute	Sexual	18
T. grandiflora	4-7, lanceolate, acuminate	Sexual/Apomictic	18,27-36
T. gypsophila	3-4, lanceolate, acute.		
T. hookeri	5-7, linear, acute	Apomictic/Sexual	18,27-36
T. incana	3-4, lanceolate, acute	Apomictic/Sexual	18,27-30,36
T. jonesii	4-5, broadly lanceolate-obovate	Sexual	18
T. jonesii var. *lutea*	4-5, broadly lanceolate-obovate		
T. jonesii var. *tumulosa*	4-5, broadly lanceolate-obovate		
T. leptotes	4-7, lanceolate-linear, acute	Apomictic/Sexual	18,27-36
T. mensana	4-5, lanceolate	Sexual	
T. mexicana	2-3, elliptic-obovate	Sexual	18
T. microcephala	3-4, lanceolate, acute		
T. minima	3-6, obovate, obtuse	Sexual/Apomictic	18,27
T. montana	3-6, obovate, obtuse	Apomictic/Sexual	18,36
T. nuttallii	5-7, linear, acute		
T. parryi	4-7, lanceolate, acuminate	Apomictic/Sexual	18,36
T. rothrockii	4-6, ovate, acute	Apomictic	36
T. scapigera	3-4, lanceolate, acute	Sexual/Apomictic	18,36
T. smithii	2-3, lanceolate	Sexual	18
T. spathulata	3-4, lanceolate, acute	Apomictic/Sexual	18, 36
T. strigosa	3-4, elliptic-lanceolate, acute	Sexual/Apomictic	18, 36
T. texensis	4-6, lanceolate	Sexual	18
Astranthium	2 ovate to linear-lanceolate	Sexual/Selfing	6,8,10,16, 18,20
Dichaetophora	2, obtuse	Sexual	6
Boltonia	3-6, linear	Sexual	18

Table 3.2 - List of sampled *Townsendia*

Sample	Species	Herbarium†	Accession or Collector* Number	Latitude	Longitude
APR1	*aprica*	SJNM	9633	38.2169	-111.2380
APR2	*aprica*	pers-CL	0027A*	38.2131	-111.2378
ANN1	*annua*	pers-CL	1032B*	34.0999	-107.5978
ANN2	*annua*	ARIZ	363550	32.1966	-112.9022
ASTR1	*Astranthium integrifolium*	RM	561196	37.0178	-96.1725
AST3	*Astranthium integrifolium*	IEB	139853	21.4030	-99.0848
BOL1	*Boltonia asteroides*				
CND1	*condensata*	pers-CL	0004C*	44.4661	-109.8612
CND2	*condensata*	pers-CL	0005*	44.4556	-109.6282
DIC1	*Dichaetophora campestris*	IEB	425595		
E1	*exscapa*	pers-CL	3003A*	48.0944	-102.8682
E2	*exscapa*	pers-CL	3004A*	48.0997	-102.8716
E3	*exscapa*	pers-CL	3006A*	48.1065	-102.8743
E5	*exscapa*	pers-CL	3009A*	47.4058	-103.9661
E6	*exscapa*	pers-CL	3011C*	47.2563	-103.4977
E7	*exscapa*	pers-CL	3012A*	46.3049	-104.0237
EXI1	*eximia*	pers-CL	1033B*	34.6675	-106.4040
EXI2	*eximia*	pers-CL	1014A*	35.5813	-105.7927
EXI3	*eximia*	pers-CL	1021A*	37.5761	-105.1711
EXI4	*eximia*	pers-CL	1034*	35.2264	-106.4055
EXI5	*eximia*	pers-CL	1033*	34.6675	-106.4040
EXS1	*exscapa*	MSC	156009	37.9932	-107.2855
EXS2	*exscapa*	pers-CL	1012A*	35.1928	-107.7252
EXS3	*exscapa*	pers-CL	1008E*	35.9240	-111.9427
EXS4	*exscapa*	COLO	352751	34.3724	-111.2467
EXS5	*exscapa*	pers-CL	1022D*	37.8240	-106.9402
EXS6	*exscapa*	pers-CL	2022*	40.9124	-104.7859
FLR1	*florifer*	pers-CL	0028A*	43.6064	-113.1634
FLR2	*florifer*	MSC	289268	44.8489	-112.5213
FLR3	*florifer*	pers-CL	2006*	43.2270	-116.5641
FND1	*fendleri*	pers-CL	1035B*	35.6902	-105.9316
FND2	*fendleri*	pers-JW	221*		
FRM1	*formosa*	MSC	162672		

† = "pers" indicates a persional collection by Chris Lee (CL) or Jeannette Whitton (JW)

***** = record is a personal collection number and voucher has not been accessioned

Table 3.2 (cont.)- List of sampled *Townsendia*

Sample	Species	Herbarium†	Accession or Collector* Number	Latitude	Longitude
FRM2	*formosa*	ARIZ	396452	34.0377	-109.4595
FRM3	*formosa*	ARIZ	100675	33.3767	-107.8993
GLB1	*glabella*	pers-CL	1042A*	37.1899	-106.9400
GLB2	*glabella*	pers-CL	1027A*	37.3284	-108.1082
GLB3	*glabella*	pers-CL	1026*	37.2026	-107.2615
GRN1	*grandiflora*	pers-CL	1019A*	36.2732	-104.2592
GRN2	*grandiflora*	pers-CL	1041A*	36.6741	-106.7291
GRN3	*grandiflora*	MSC	297698	42.7074	-104.2401
GRN4	*grandiflora*	pers-JW	220*		
GYP1	*gypsophila*	pers-CL	1031A*	35.5305	-106.6939
GYP2	*gypsophila*	MSC	345690	35.5192	-106.7907
H1	*hookeri*	pers-CL	3001A*	51.1870	-115.5375
H2	*hookier*	pers-CL	3002A*	49.5599	-113.8922
H3	*hookeri*	pers-CL	3008A*	46.9021	-103.5754
H4	*hookeri*	pers-CL	3010A*	46.8788	-103.6716
H5	*hookeri*	pers-CL	3011A*	46.8742	-103.6706
HOK1	*hookeri*	UBC	76257	66.9833	-142.8500
HOK2	*hookeri*	pers-CL	0002A*	46.0225	-110.6505
HOK3	*hookeri*	pers-CL	0006A*	44.3057	-109.2757
HOK4	*exscapa*	pers-CL	1022A*	37.8240	-106.9402
HOK6	*hookeri*	pers-CL	2047*	50.6057	-116.0595
INC1	*incana*	pers-CL	1010A*	35.5483	-108.6044
INC2	*incana*	MSC	156235	38.4700	-107.2749
INC4	*incana*	pers-CL	2009*	43.2373	-108.0930
JON1	*jonesii* var. *jonesii*	pers-CL	1002A*	39.4769	-111.8791
JON3	*jonesii* var. *jonesii*	pers-CL	0026*	38.2116	-111.2283
JTM1	*jonesii* var. *tumulosa*	pers-CL	1005C*	36.3136	-115.6262
JTM2	*jonesii* var. *tumulosa*	UNLV	42773	36.6299	-115.1840
LEP1	*leptotes*	pers-CL	0020A*	40.1191	-106.4294
LEP2	*incana*	IEB	566896	36.5310	-107.2760
LEP3	*leptotes*	IEB	542149	39.4445	-116.0023
LEP4	*leptotes*	IEB	660332	43.5764	-110.2776

† = "pers" indicates a persional collection by Chris Lee (CL) or Jeannette Whitton (JW)

***** = record is a personal collection number and voucher has not been accessioned

Table 3.2 (cont.)- List of sampled *Townsendia*

Sample	Species	Herbarium†	Accession or Collector* Number	Latitude	Longitude
MEN1	*mensana*	UTC	161822	39.6706	-109.2908
MEN2	*mensana*	pers-JW	225*		
MEX1	*mexicana*	MEX	201168	20.1642	-98.9076
MEX2	*mexicana*	TEX	542149		
MIC1	*microcephala*	pers-CL	0016D*	41.1155	-110.0268
MIC2	*microcephala*	RM	602530	41.1349	-110.1339
MIN1	*minima*	pers-CL	1004A*	37.5003	-112.5867
MIN2	*minima*	pers-CL	1003*	37.7487	-112.3185
MON1	*montana*	pers-CL	0029A*	44.1353	-113.8101
MON2	*montana*	pers-CL	1044B*	40.5993	-111.6118
MON3	*montana*	pers-CL	0019*	40.7049	-109.5608
NUT1	*incana*	pers-CL	0007A*	43.5293	-109.4785
NUT2	*nutallii*	pers-CL	0014A*	41.1458	-110.1101
PAR2	*parryi*	pers-CL	0010A*	43.5738	-110.2693
ROT1	*rothrockii*	pers-CL	1023A*	37.9934	-107.2049
ROT2	*rothrockii*	pers-CL	0024A*	39.0294	-108.2216
SCP1	*scapigera*	RM	367834	41.5258	-119.3155
SCP2	*scapigera*	RM	396446	39.0770	-119.3721
SCP3	*scapigera*	pers-CL	2003*	37.2729	-118.1508
SMI1	*smithii*	MSC	267804	36.7955	-113.7693
SPA1	*spathulata*	pers-CL	0012B*	42.7066	-109.9465
SPA2	*spathulata*	MSC	306144	46.3017	-111.5869
STR1	*grandiflora*	pers-CL	1030A*	35.9823	-106.9172
STR2	*strigosa*	RMH	84065	42.5490	-110.0793
TEX1	*texensis*	IEB	172963	36.0275	-99.8728
TEX2	*texensis*	IEB	197360	34.9339	-101.6312
TEX3	*texensis*	IEB	443385	35.6379	-101.5625

† = "pers" indicates a persional collection by Chris Lee (CL) or Jeannette Whitton (JW)

***** = record is a personal collection number and voucher has not been accessioned

Table 3.3 - Gene regions sequenced and haplogroup assignment

Sample	Species	Region					Haplo-group*
		ndhC	trnY	matK	psbA	ITS	
APR1	*aprica*	+	+	+	+	+	**1**
APR2	*aprica*	+	+	+	+	+	**1**
ANN1	*annua*	+	+	+	+	+	**2**
ANN2	*annua*	-	-	-	+	+	
ASTR1	*Astranthium*	+	+	+	+	+	
AST3	*Astranthium*	+	+	-	+	+	
BOL1	*Boltonia asteroides*	-	-	-	+	+	
CND1	*condensata*	+	+	+	+	+	**3**
CND2	*condensata*	+	-	+	+	+	**3**
DIC1	*Dichaetophora*	+	+	-	+	+	
E1	*exscapa*	+	-	-	-	+	
E2	*exscapa*	+	-	-	-	+	
E3	*exscapa*	+	-	-	-	+	
E5	*exscapa*	+	-	-	-	+	
E6	*exscapa*	+	-	-	-	+	
E7	*exscapa*	+	+	+	+	-	**2**
EXI1	*eximia*	+	+	+	+	+	**5**
EXI2	*eximia*	+	+	+	+	+	**3**
EXI3	*eximia*	+	+	+	+	+	**5**
EXI4	*eximia*	+	-	+	+	+	**6**
EXI5	*eximia*	+	+	+	+	+	**5**
EXS1	*exscapa*	+	+	+	+	+	**4**
EXS2	*exscapa*	+	+	+	+	+	**5**
EXS3	*exscapa*	+	-	+	+	+	**1**
EXS4	*exscapa*	+	-	+	+	+	**4**
EXS5	*exscapa*	+	+	+	+	+	**4**
EXS6	*exscapa*	+	+	+	+	+	**4**
FLR1	*florifer*	+	+	+	+	+	**3**
FLR2	*florifer*	+	-	+	+	+	**4**
FLR2a	*florifer*	+	-	-	-	-	
FLR3	*florifer*	+	+	+	+	+	**3**
FND1	*fendleri*	+	+	+	+	+	**3**
FND2	*fendleri*	+	+	-	+	+	
FRM1	*formosa*	-	-	-	+	+	
FRM2	*formosa*	+	+	-	+	+	

*ITS and trnY data not included in haplotype assignment.

Table 3.3 (cont.) - Gene regions sequenced and haplogroup assignment

Sample	Species	Region					Haplo-group*
		ndhC	trnY	matK	psbA	ITS	
FRM3	*formosa*	-	-	-	+	+	
GLB1	*glabella*	+	+	+	+	+	**5**
GLB2	*glabella*	+	+	+	+	+	**4**
GLB3	*glabella*	+	+	+	+	+	**4**
GRN1	*grandiflora*	+	+	+	+	+	**4**
GRN2	*grandiflora*	+	+	+	+	+	**5**
GRN3	*grandiflora*	+	+	+	+	+	**7**
GRN4	*grandiflora*	+	+	+	+	+	
GYP1	*gypsophila*	+	+	+	+	+	**5**
GYP2	*gypsophila*	+	+	-	+	+	
H1	*hookeri*	+	+	+	+	-	**4**
H2	*hookier*	+	-	-	-	+	
H3	*hookeri*	+	+	+	+	-	
H4	*hookeri*	+	-	-	-	+	
H5	*hookeri*	+	-	-	-	+	
HOK1	*hookeri*	+	+	+	+	+	**4**
HOK2	*hookeri*	+	+	+	+	+	
HOK3	*hookeri*	+	+	+	+	+	**5**
HOK4	*exscapa*	+	+	+	+	+	**4**
HOK5	*hookeri*	-	+	+	+	+	
HOK6	*hookeri*	+	+	+	+	+	**4**
HOK-x5	*hookeri*	+	-	-	-	-	
INC1	*incana*	+	+	+	+	+	**2**
INC2	*incana*	+	+	-	+	+	
INC3	*incana*	+	+	+	+	+	**1**
INC4	*incana*	+	+	+	+	+	
INC-6	*incana*	+	+	-	-	-	
JLT1	*jonesii var. lutea*	+	+	+	+	+	**1**
JLT32F	*jonesii var. Lutea*	+	-	-	-	-	
JLT-7	*jonesii var. lutea*	+	+	-	-	-	
JON1	*jonesii var. jonesii*	+	+	+	+	+	**1**
JON2	*jonesii var. jonesii*	+	+	+	+	+	**1**
JON3	*jonesii var. jonesii*	+	+	-	-	-	
JON-8	*jonesii var. jonesii*	+	+	-	-	-	
JTM1	*jonesii var. tumulosa*	+	+	+	+	+	**1**
JTM2	*jonesii var. tumulosa*	+	-	+	+	+	**1**
LEP1	*leptotes*	+	+	+	+	+	**4**

*ITS and trnY data not included in haplotype assignment.

Table 3.3 (cont.) - Gene regions sequenced and haplogroup assignment

Sample	Species	Region					Haplo-group*
		ndhC	trnY	matK	psbA	ITS	
LEP2	*Incana*	+	+	+	+	+	**5**
LEP3	*leptotes*	+	-	-	+	+	
LEP4	*leptotes*	-	+	-	+	+	
MEN1	*mensana*	+	+	+	+	+	**1**
MEN2	*mensana*	+	-	+	+	+	**1**
MEX1	*mexicana*	+	+	+	+	+	**3**
MEX2	*mexicana*	-	+	-	+	+	
MIC1	*microcephala*	+	+	+	+	-	**3**
MIC2	*microcephala*	+	+	+	+	+	**3**
MIN1	*minima*	+	+	+	+	+	**4**
MIN2	*minima*	+	-	+	+	+	**4**
MON1	*montana*	+	+	+	+	+	**4**
MON2	*montana*	+	+	+	+	+	**3**
MON3	*montana*	+	+	+	+	+	**3**
NUT1	*incana*	+	+	+	+	+	**1**
NUT1a	*incana*	+	-	-	-	-	
NUT2	*nutallii*	+	+	+	+	+	**4**
PAR1	*parryi*	+	+	+	+	+	**4**
PAR2	*parryi*	+	+	+	+	+	**4**
PAR3	*parryi*	+	+	-	+	+	
ROT1	*rothrockii*	+	+	+	+	+	**2**
ROT2	*rothrockii*	+	+	+	+	+	**2**
SCP1	*scapigera*	+	+	+	+	+	**3**
SCP2	*scapigera*	+	-	+	+	+	**3**
SCP3	*scapigera*	+	+	+	+	+	**3**
SMI1	*smithii*	+	-	+	+	+	**1**
SPA1	*spathulata*	+	+	+	+	+	**4**
SPA2	*spathulata*	+	-	+	+	+	**4**
SPA3	*spathulata*	+	+	+	+	+	**3**
STR1	*grandiflora*	+	-	+	+	+	**5**
STR2	*strigosa*	-	-	-	+	+	
STR3	*strigosa*	+	-	+	+	+	**1**
TEX1	*texensis*	+	+	+	+	+	**6**
TEX1A	*texensis*	+	-	-	-	-	
TEX2	*texensis*	-	+	+	+	+	
TEX3	*texensis*	+	+	+	+	+	**5**

*ITS and trnY data not included in haplotype assignment.

Figure 3.1 - ITS-based phylogeny of *Townsendia* with *Astranthium, Dichaetophora* and *Boltonia* outgroups. Inferred using ML analysis under a TIM3ef+G model of sequence evolution. The ML tree (-*ln*L=-3027.271034) is depicted as a phylogram. Clades with long branches were scaled back as indicated with '\\' markings. Values below internal branches represent ML bootstrap values. Polytomies were drawn with triangles, and samples have different numbers of nucleotide substitutions per site. Bar indicates number of nucleotide substitutions per site. Labels are abbreviated from taxon list (Table 3.2).

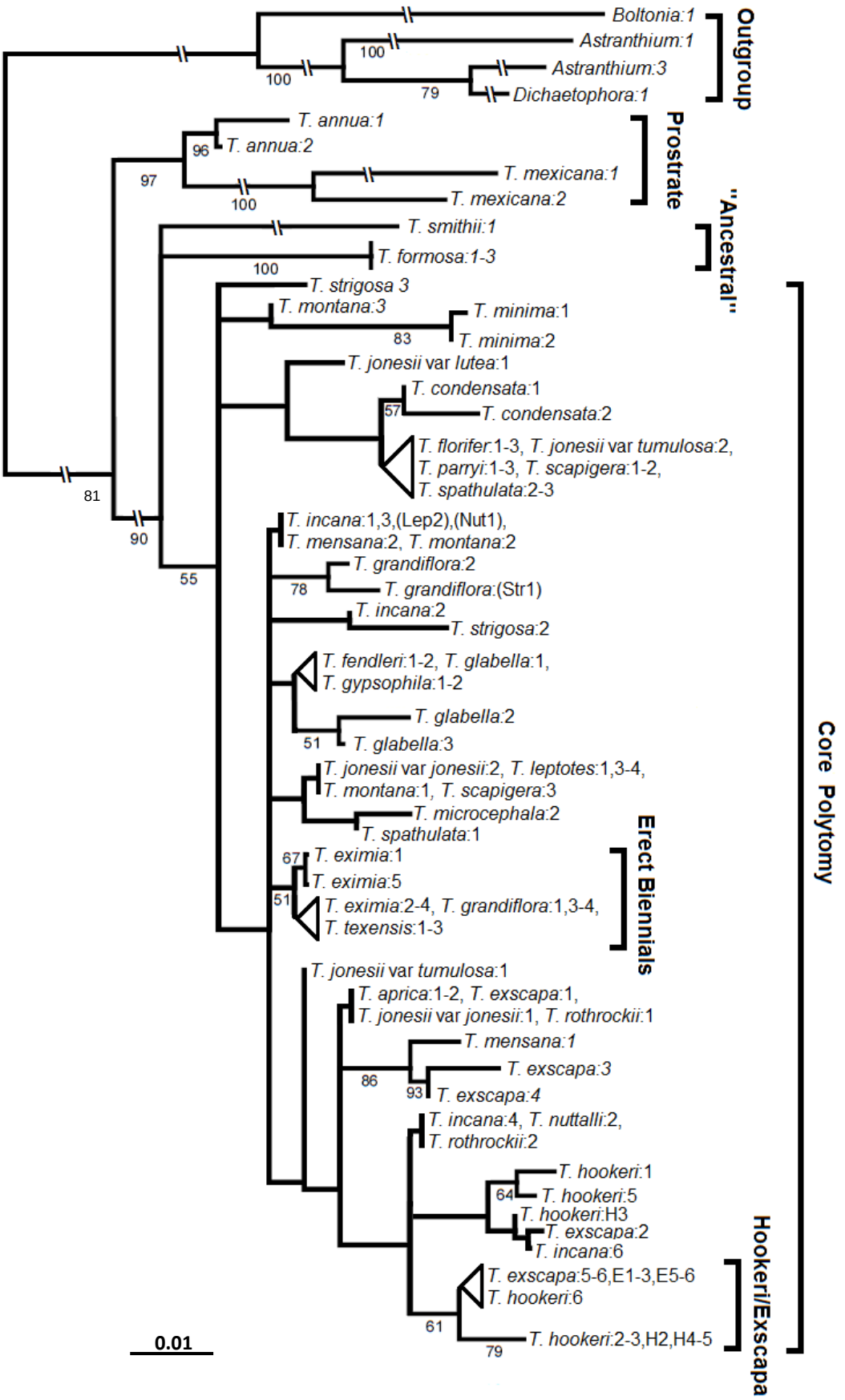

Boltonia:1
Astranthium:1
Astranthium:3
Dichaetophora:1
Outgroup
T. annua:1
T. annua:2
T. mexicana:1
T. mexicana:2
Prostrate
T. smithii:1
T. formosa:1-3
"Ancestral"
T. strigosa 3
T. montana:3
T. minima:1
T. minima:2
T. jonesii var lutea:1
T. condensata:1
T. condensata:2
T. florifer:1-3, T. jonesii var tumulosa:2,
T. parryi:1-3, T. scapigera:1-2,
T. spathulata:2-3
T. incana:1,3,(Lep2),(Nut1),
T. mensana:2, T. montana:2
T. grandiflora:2
T. grandiflora:(Str1)
T. incana:2
T. strigosa:2
T. fendleri:1-2, T. glabella:1,
T. gypsophila:1-2
T. glabella:2
T. glabella:3
T. jonesii var jonesii:2, T. leptotes:1,3-4,
T. montana:1, T. scapigera:3
T. microcephala:2
T. spathulata:1
T. eximia:1
T. eximia:5
T. eximia:2-4, T. grandiflora:1,3-4,
T. texensis:1-3
Erect Biennials
T. jonesii var tumulosa:1
T. aprica:1-2, T. exscapa:1,
T. jonesii var jonesii:1, T. rothrockii:1
T. mensana:1
T. exscapa:3
T. exscapa:4
T. incana:4, T. nuttalli:2,
T. rothrockii:2
T. hookeri:1
T. hookeri:5
T. hookeri:H3
T. exscapa:2
T. incana:6
T. exscapa:5-6,E1-3,E5-6
T. hookeri:6
T. hookeri:2-3,H2,H4-5
Hookeri/Exscapa
Core Polytomy
100
100
79
96
97
100
100
83
57
81
90
55
78
51
67
51
86
93
64
61
79
0.01

Figure 3.2 - ITS-based phylogeny (ESS=2760) of *Townsendia* with *Astranthium*, *Dichaetophora* and *Boltonia* outgroups. Inferred using Bayesian analysis under a GTR+G model of sequence evolution. Values below internal branches represent Bayesian posterior probabilities. Polytomies were drawn with triangles, and samples have different numbers of nucleotide substitutions per site. Bar indicates number of nucleotide substitutions per site. Labels are abbreviated from taxon list (Table 3.2).

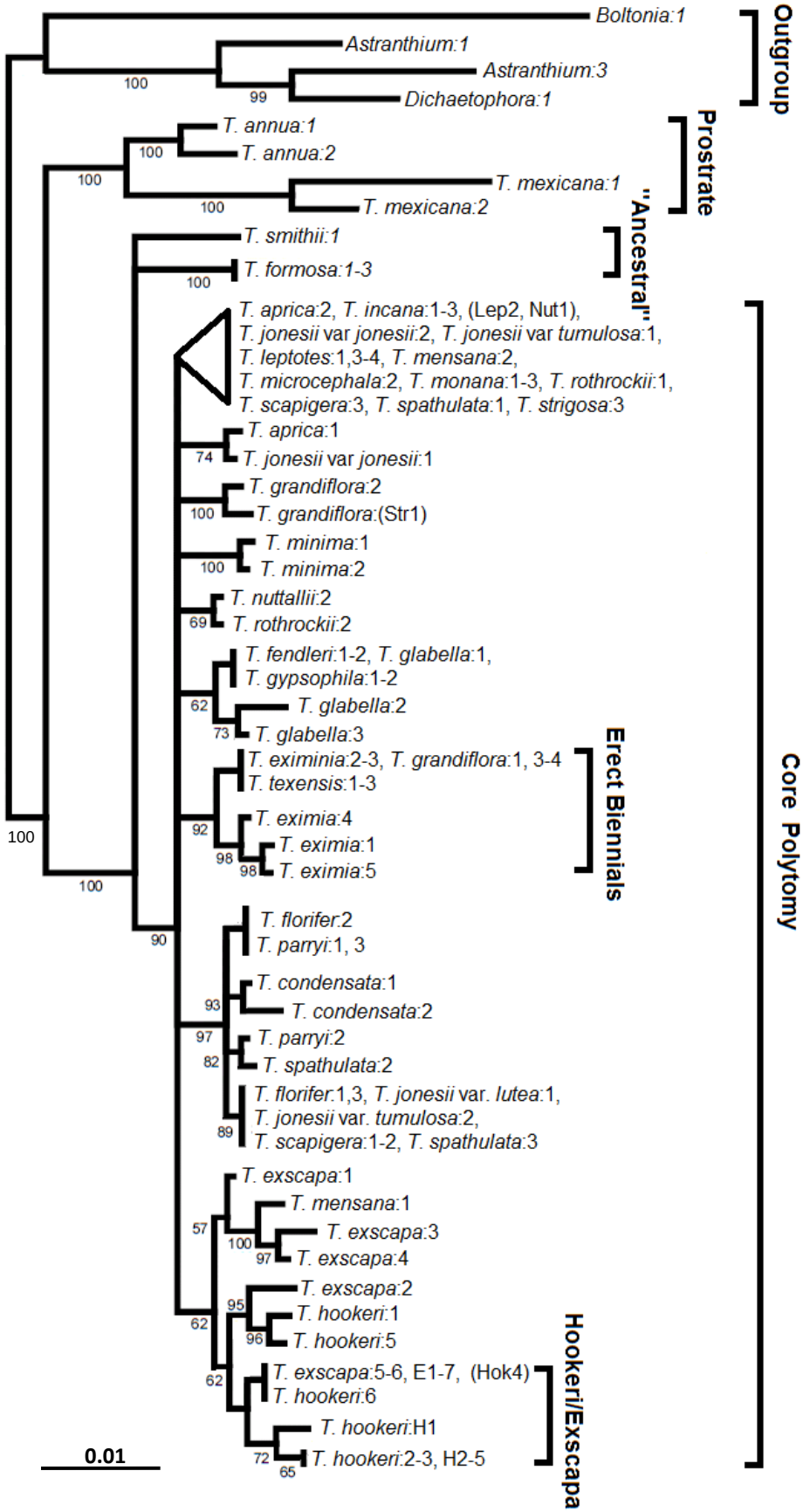

Boltonia:1
Astranthium:1
Astranthium:3
Dichaetophora:1
Outgroup
T. annua:1
T. annua:2
T. mexicana:1
T. mexicana:2
Prostrate
T. smithii:1
T. formosa:1-3
"Ancestral"
T. aprica:2, T. incana:1-3, (Lep2, Nut1),
T. jonesii var jonesii:2, T. jonesii var tumulosa:1,
T. leptotes:1,3-4, T. mensana:2,
T. microcephala:2, T. monana:1-3, T. rothrockii:1,
T. scapigera:3, T. spathulata:1, T. strigosa:3
T. aprica:1
T. jonesii var jonesii:1
T. grandiflora:2
T. grandiflora:(Str1)
T. minima:1
T. minima:2
T. nuttallii:2
T. rothrockii:2
T. fendleri:1-2, T. glabella:1,
T. gypsophila:1-2
T. glabella:2
T. glabella:3
T. eximinia:2-3, T. grandiflora:1, 3-4
T. texensis:1-3
T. eximia:4
T. eximia:1
T. eximia:5
Erect Biennials
T. florifer:2
T. parryi:1, 3
T. condensata:1
T. condensata:2
T. parryi:2
T. spathulata:2
T. florifer:1,3, T. jonesii var. lutea:1,
T. jonesii var. tumulosa:2,
T. scapigera:1-2, T. spathulata:3
T. exscapa:1
T. mensana:1
T. exscapa:3
T. exscapa:4
T. exscapa:2
T. hookeri:1
T. hookeri:5
T. exscapa:5-6, E1-7, (Hok4)
T. hookeri:6
T. hookeri:H1
T. hookeri:2-3, H2-5
Hookeri/Exscapa
Core Polytomy
0.01

Figure 3.3. - Plastid DNA-based phylogeny of Townsendia with *Astranthium, Dichaetophora* and *Boltonia* outgroups. Inferred using ML analysis under an HKY+G+I model of sequence evolution. The ML tree (-*ln*L=-12808.84366) is depicted as a phylogram. Clades with long branches were scaled back as indicated with '\\' markings. Values below internal branches are ML bootstrap values. Polytomies were drawn with triangles, and samples have different numbers of nucleotide substitutions per site. Bar indicates number of nucleotide substitutions per site. Labels are abbreviated from taxon list (Table 3.2).

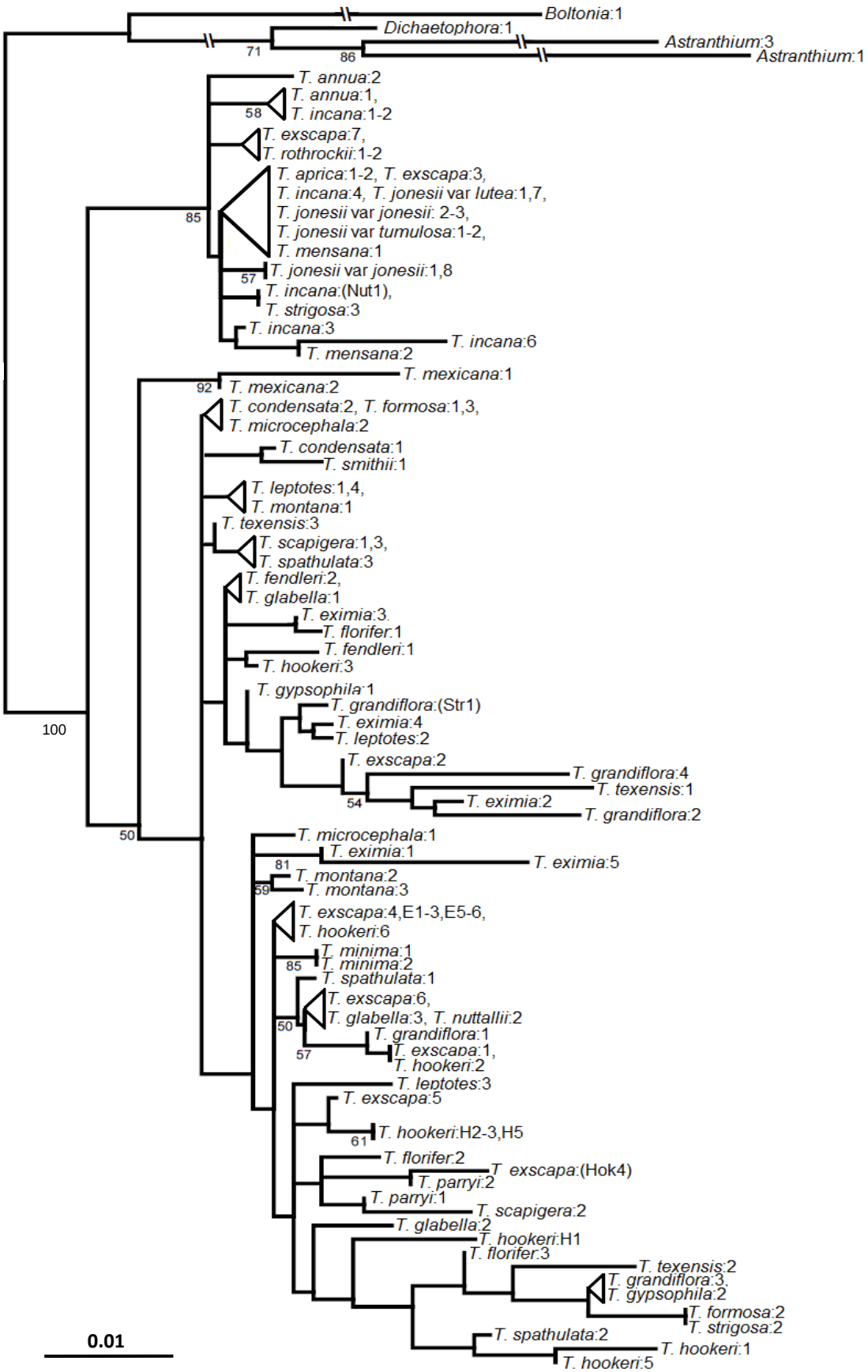

Boltonia:1
Dichaetophora:1
Astranthium:3
Astranthium:1
71
86
T. annua:2
T. annua:1,
T. incana:1-2
58
T. exscapa:7,
T. rothrockii:1-2
T. aprica:1-2, T. exscapa:3,
T. incana:4, T. jonesii var lutea:1,7,
T. jonesii var jonesii: 2-3,
T. jonesii var tumulosa:1-2,
T. mensana:1
85
T. jonesii var jonesii:1,8
57
T. incana:(Nut1),
T. strigosa:3
T. incana:3
T. incana:6
T. mensana:2
T. mexicana:1
92
T. mexicana:2
T. condensata:2, T. formosa:1,3,
T. microcephala:2
T. condensata:1
T. smithii:1
T. leptotes:1,4,
T. montana:1
T. texensis:3
T. scapigera:1,3,
T. spathulata:3
T. fendleri:2,
T. glabella:1
T. eximia:3.
T. florifer:1
T. fendleri:1
T. hookeri:3
T. gypsophila:1
T. grandiflora:(Str1)
T. eximia:4
T. leptotes:2
100
T. exscapa:2
T. grandiflora:4
T. texensis:1
54
T. eximia:2
T. grandiflora:2
50
T. microcephala:1
T. eximia:1
T. eximia:5
81
T. montana:2
59
T. montana:3
T. exscapa:4,E1-3,E5-6,
T. hookeri:6
T. minima:1
T. minima:2
85
T. spathulata:1
T. exscapa:6,
T. glabella:3, T. nuttallii:2
50
T. grandiflora:1
T. exscapa:1,
T. hookeri:2
57
T. leptotes:3
T. exscapa:5
T. hookeri:H2-3,H5
61
T. florifer:2
T. exscapa:(Hok4)
T. parryi:2
T. parryi:1
T. scapigera:2
T. glabella:2
T. hookeri:H1
T. florifer:3
T. texensis:2
T. grandiflora:3,
T. gypsophila:2
T. formosa:2
T. strigosa:2
T. spathulata:2
T. hookeri:1
T. hookeri:5
0.01

Figure 3.4 - Plastid DNA-based phylogeny(ESS=3642) of *Townsendia* with *Astranthium, Dichaetophora* and *Boltonia* outgroups. Inferred using Bayesian analysis under a HKY+G+I model of sequence evolution. Clades with long branches were scaled back as indicated with '\\' markings. Values below internal branches are Bayesian posterior probabilities. Polytomies were drawn with triangles, and samples have different numbers of nucleotide substitutions per site. Bar indicates number of nucleotide substitutions per site. Labels are abbreviated from taxon list (Table 3.2)

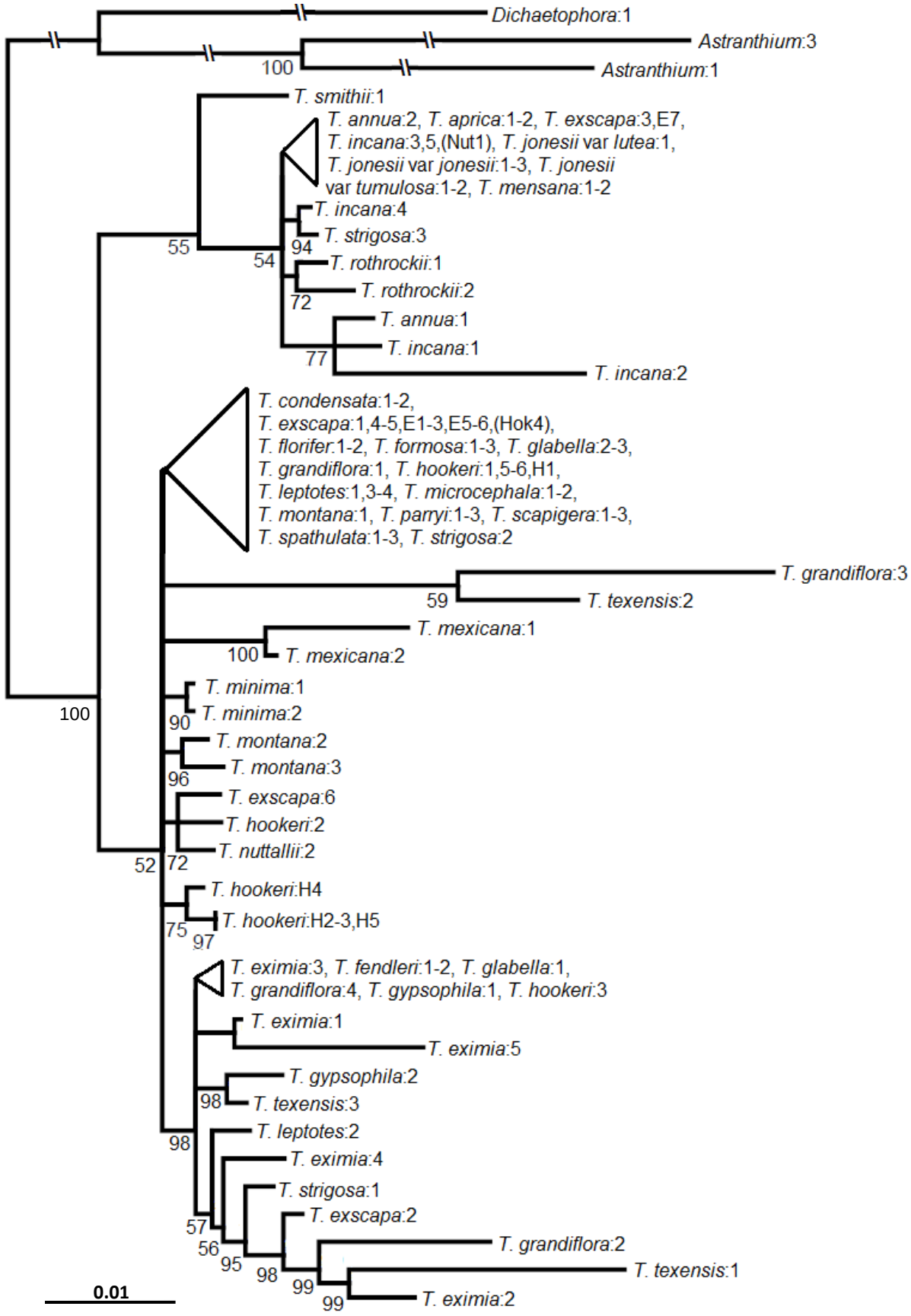

Dichaetophora:1
Astranthium:3
100
Astranthium:1
T. smithii:1
T. annua:2, T. aprica:1-2, T. exscapa:3,E7,
T. incana:3,5,(Nut1), T. jonesii var lutea:1,
T. jonesii var jonesii:1-3, T. jonesii
var tumulosa:1-2, T. mensana:1-2
T. incana:4
T. strigosa:3
55
94
54
T. rothrockii:1
T. rothrockii:2
72
T. annua:1
T. incana:1
77
T. incana:2
T. condensata:1-2,
T. exscapa:1,4-5,E1-3,E5-6,(Hok4),
T. florifer:1-2, T. formosa:1-3, T. glabella:2-3,
T. grandiflora:1, T. hookeri:1,5-6,H1,
T. leptotes:1,3-4, T. microcephala:1-2,
T. montana:1, T. parryi:1-3, T. scapigera:1-3,
T. spathulata:1-3, T. strigosa:2
T. grandiflora:3
59
T. texensis:2
T. mexicana:1
100
T. mexicana:2
T. minima:1
100
T. minima:2
90
T. montana:2
T. montana:3
96
T. exscapa:6
T. hookeri:2
T. nuttallii:2
52
72
T. hookeri:H4
T. hookeri:H2-3,H5
75
97
T. eximia:3, T. fendleri:1-2, T. glabella:1,
T. grandiflora:4, T. gypsophila:1, T. hookeri:3
T. eximia:1
T. eximia:5
T. gypsophila:2
98
T. texensis:3
98
T. leptotes:2
T. eximia:4
T. strigosa:1
T. exscapa:2
57
56
95
T. grandiflora:2
98
T. texensis:1
99
99
T. eximia:2
0.01

Figure 3.5 - Haplotype network diagram of the *ndh*C, *psb*A and *mat*K data set.

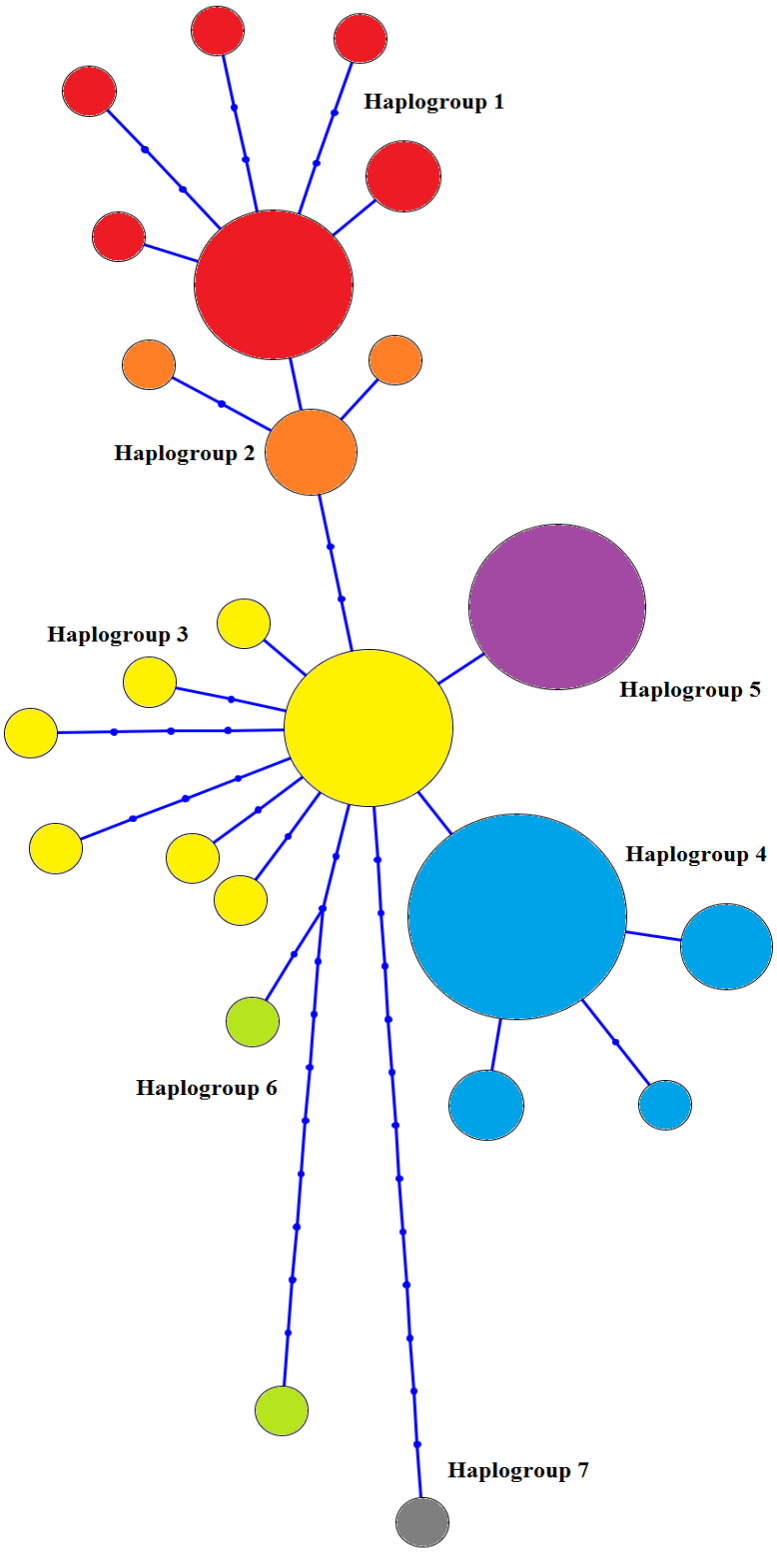
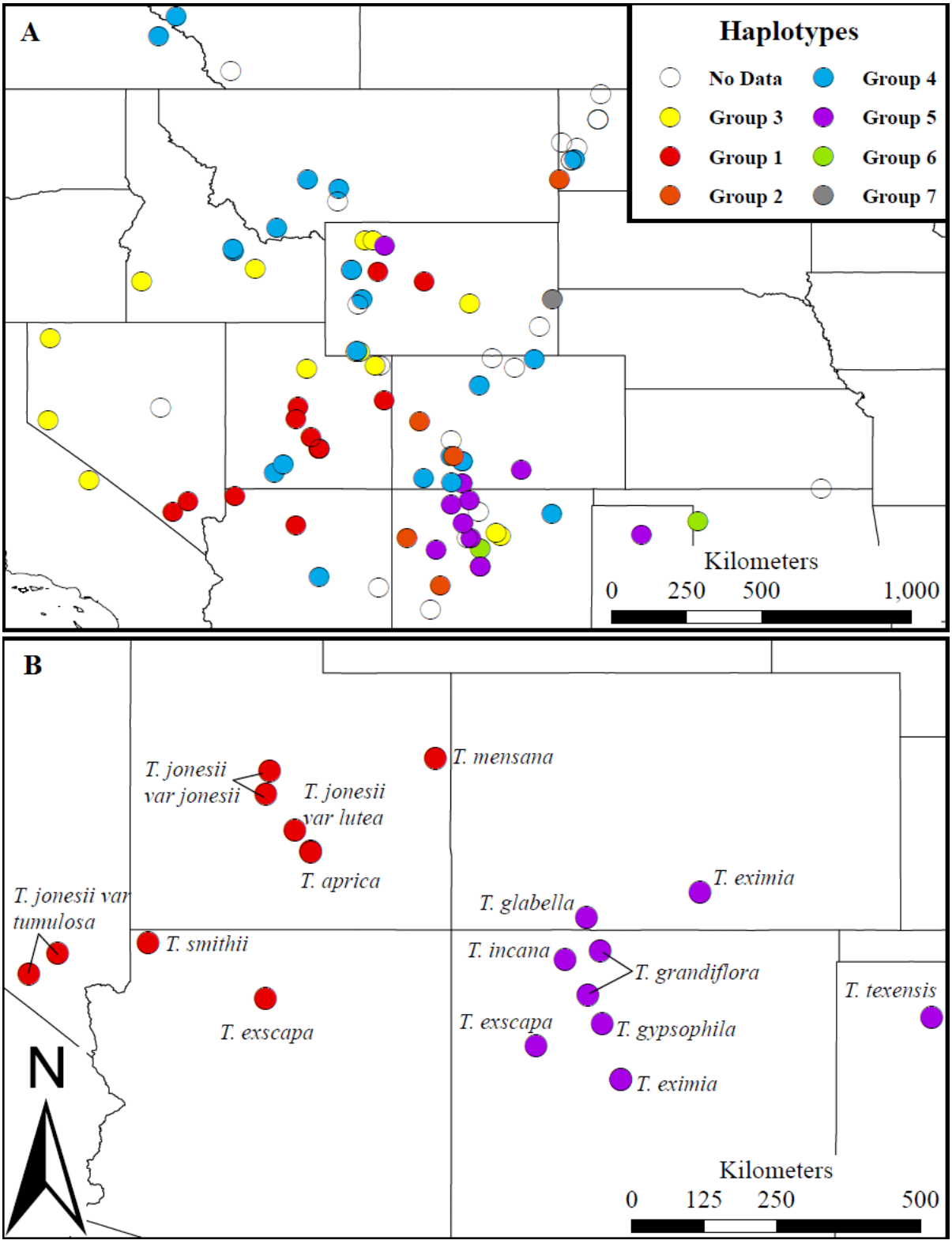


Figure 3.6 - A) Collection localities and distribution of the seven chloroplast haplogroups. B) Collection localities and species identity highlighted in Haplogroup 1 and 5.



CHAPTER 4: Patterns of Niche Evolution and Speciation in *Townsendia*

Introduction:

Phylogenies are useful for testing hypotheses about the role of climatic niche divergence in the divergence of lineages (Graham, Ron, Santos, Schneider, and Moritz, 2004). The phylogeny that I obtained (Chapter 3), although based on regions selected for their high levels of variation in other Asteraceae genera (Timme *et al.*, 2007), in addition to plant DNA barcoding regions (Kress and Erickson, 2008; Hollingsworth *et al.*, 2011), was nonetheless relatively poorly resolved. Relationships among species within other genera such as *Tolpis* (Park *et al.*, 2001) and *Centipeda* (Nylinder *et al.*, 2013) have been resolved successfully using a similar combination of DNA regions, but were more poorly resolved in other cases like *Berberis* (Roy *et al.*, 2010) and *Scalesia* (Blaschke and Sanders, 2009). The low level of genetic variation found in *Townsendia* may seem contrary to the distinct differences among species in their morphological features, life history characteristics, geographical distributions, and ecological requirements, with species often found associated with distinct geological formations. These features may play a role in the diversification of *Townsendia*, and this study infers and compares the ecological requirements of sister species to explore potential speciation pathways in *Townsendia*

The combination of low phylogenetic resolution among species in the core polytomy (Figures 3.1 -3.4) and their expansion into diverse ecological settings suggest the possibility that speciation in *Townsendia* is mediated by the process of adaptive radiation. Defined as the rapid diversification and adaptation of a lineage into numerous species that each specialize in a particular niche, adaptive radiations are typically triggered by release from competition, evolution of a novel innovation/adaptation, or niche specialization (Schluter, 1996). Phylogenetic

evidence for adaptive radiation manifests as a burst of diversification, e.g., a cluster of species with short internal branch lengths, relative to species outside the radiation, and this pattern is especially well documented in island habitats such as volcanic islands or coral atolls (Baldwin, 2007; Blackburn *et al.*, 2013; Kapralov, Votintseva, and Filatov, 2013). Islands are geographically isolated and species depauperate, especially when recently emergent, which results in habitats with many empty niches into which colonizing species that arrive by rare long distance dispersal events can readily diversify (Losos and Ricklefs, 2009). Hawaii is perhaps the most notable example, with numerous groups exhibiting evidence for adaptive radiation, including the silverswords (*Argyroxiphium*, *Dubautia* and *Wilkesia*; Baldwin and Sanderson, 1998) and lobeliads (*Lobelia*, *Trematolobelia*, *Brighamia*, *Delissea*, *Cyanea* and *Clermontia*; Givnish *et al.*, 2009).

In comparison, mainland habitats are more likely to feature higher species densities, and are generally less geographically isolated than island habitats. Although species radiations may be more common on islands, the same mechanisms that promote rapid speciation on islands may also operate in mainland habitats (Sanderson, 1998; Losos and Ricklefs, 2009). For example, the Great Basin floristic province where much of the diversity of *Townsendia* lies, includes the Great Basin and Great Basin Desert, the Central Basin and Range, the Great Salt Lake Desert and Escalante Desert, and the Madrean Sky Islands regions. These regions are typically characterized as mountainous regions surrounded by desert, and this has led to a range of habitats from alpine to xeric, separated by an intermediate zone of more mesic woodlands. This assortment and arrangement of varied habitat types has resulted in climatic gradients and ecologically isolated areas that may drive speciation via ecological specialization (Axelrod and Raven, 1985). Overall, habitats within the Great Basin floristic province seem to be associated with high speciation rates

in many genera, including *Astragalus*, *Eriogonum*, *Penstemon*, *Lomatium*, and *Castilleja* (Axelrod and Raven, 1985) and the causes for speciation in these groups may vary. For example, *Penstemon* is thought to have diversified as a result of novel innovations in floral characters that direct plant-pollinator interactions (Wolfe *et al.*, 2006), whereas other genera like *Astragalus* appear to be driven by less apparent ecological or physiological processes (Sanderson and Wojciechowski, 1996).

Novel traits may speed diversification and lead to reproductive isolation through a variety of mechanisms, such as through the co-evolution of unique floral and pollinator morphology as in the Hawaiian lobeliads (Givnish et al., 2009). These traits may also be associated with ecological specialization. For example, the caudex found in many *Townsendia* species is a stem structure that is often subterranean, branched and woody, and can vary in girth from relatively thick to thin. Subterranean caudices have been shown to provide insulation from temperature extremes or to increase water storage capacity (Porembski, 2007; Calonje *et al.*, 2010), and such characteristics may contribute to a plant's ability to persevere through periods of drought or freezing. However, just as novel innovations may result in reproductive isolation without a change in ecology, ecological specialization is possible in the absence of a novel innovation.

For species to diverge ecologically, differences in their abiotic tolerances must arise such that some form of reproductive isolation emerges. Incidences of ecological speciation may occur regardless of the species inherent distributions, whether allopatric, sympatric or paraptric (Rundle and Nosil, 2005). Allopatric species are inherently reproductively isolated by the nature of their distribution and by geography. If ecological differences arise in allopatric species, these differences may be a result of pleiotropy or by drift. Comparatively, species in parapatry may have a more direct connection to ecological changes as parapatric speciation is often linked to

gradual divergence along a cline. In parapatry, species accumulate ecological differences overtime, resulting in the hybrids that are maladapted to both parental habitats (Campbell, 2003). This type of ecological specialization in parapatry serves to enforce reproductive isolation mechanisms and can lead to speciation. While different distributional patterns may all display ecological specialization, it is the degree to which differentiation occurs (*i.e.* more or less than predicted) that supports or contradicts the potential presence of adaptive radiation.

There is evidence for ecological specialization in *Townsendia* as many species are found in habitats that present especially challenging physical limitations and resource restrictions to plant growth and establishment. One such taxon is *T. gypsophila*, which grows only in the gypsum rich soils found near Albuquerque, New Mexico. Gypsum soil is understood to be a challenging condition for plants because it acts as a physical barrier to plant establishment and root penetration by forming hard surface crusts (Meyer, 1986) and mechanically unstable soil (Bridges and Burnham, 1980). In addition to these physical soil properties, high salinity and sulphur levels are also associated with gypsum, and these pose additional challenges for water-soil relations and mineral toxicity (Parsons, 1976). Adaptation to gypsum appears to have arisen twice in *Townsendia*, with *T. jonesii* var. *lutea* also occurring on gypsum, in Utah (Lipsen, Lee and Whitton 2013). Although not as apparent as edaphic specialization, climatic variation can also directly and indirectly impose limitations on species' ranges. Direct climate limitations may include factors such as low precipitation limiting the overall growth of vegetation, which may produce indirect climate limitations such as decreased soil development and increased prevalence of exposed sites. In this manner, multivariate aspects of climate variation may influence plant diversification, and they have been studied in both plants (Evans *et al.*, 2009; Mandle *et al.*, 2010) and animals (Blair *et al.*, 2013).

To explore the processes of ecological specialization in *Townsendia* and how they relate to adaptive radiation, I used ecological niche models (ENMs). Ecological niche modelling is a method to estimate the ecological requirements of species, based on species locality data, and on a combination of abiotic data such as precipitation, temperature, altitude and soil. Subsequent projection of an ENM onto a landscape of climate data produces an estimate of the probability of presence for a taxon at any given point on that landscape in the past, present or future (Perkins *et al.*, 2007). By focusing on the use of abiotic data, ENMs approximate the concept of a fundamental niche, as proposed by Hutchinson (1965), and lend insight to the abiotic tolerances of a taxon. Even though there are various methods to derive ENMs, here I used a maximum entropy approach to modelling (Phillips and Dudık, 2008), as in Chapter 2.

Taken alone, a predicted ENM is similar to distinct taxon characteristics, and can be compared between taxa just as characters such as leaf morphology, or DNA sequences. The degree of overlap between the ENMs of species pairs, also known as niche overlap, can be studied quantitatively and qualitatively using tools such as ENMTools (Warren, Glor, and Turelli, 2010). Comparing the relative amount of niche overlap between the ENMs of closely related species provides insight into the degree of niche differentiation since speciation. These ENM comparisons further facilitate the exploration of niche changes through evolutionary time, and potentially show how environment and climate can affect diversification. However, the interpretation of these comparisons can be challenging as species can have a variety of distributions ranging from allopatry to full sympatry, each with different implications for the overlap results. For example, species with sympatric distributions are more likely to be affected by, and have access to, a similar set of abiotic conditions. As such, a comparison of niche overlap between sympatric species is more likely to be representative of true differences or

similarities in the fundamental niche of both species. Rather than fully sympatric distributions, many taxon pairs have allopatric, parapatric, or partially sympatric distributions, as is seen in many species in *Townsendia*. Species with parapatric or allopatric distributions are unlikely to face identical abiotic conditions, as abiotic conditions can vary over space. When comparing ENMs, it is important to attempt to control for the heterogeneity of abiotic variables on the spatial landscape across the ranges of allopatric and parapatric species. In this study, I follow the approach of Warren (2010) and use simulation to attempt to account for climate variation across spatial landscapes.

The degree and direction of niche overlap compared to the background abiotic conditions informs us about niche divergence between species. Niche preferences of sister species may diverge more, less, or neutrally based on their phylogenetic separation. Within such a framework, niche conservatism can be used to describe species that are more ecologically similar than expected based on their phylogenetic relationship (Losos, 2008). As such, the pattern of niche conservatism may appear in species as the retention of ecological traits similar to those of their shared ancestors (Wiens and Graham, 2005). Because of these ecological similarities, additional factors such as differential phenology or geographic barriers may be required to prevent gene flow during speciation. Niche conservatism may help maintain separation of allopatric species, by discouraging dispersal on and across the landscape barrier that separates them (Wiens, 2004). Given sufficient evolutionary time, species can also diverge following a Brownian motion-like pattern of ecological differentiation so that species can remain distinct even during secondary contact. As a result, both niche conservatism and random divergence may result in similar patterns of ecological differentiation, or lack thereof, and both may be considered indicative of non-ecological speciation (Blair *et al.*, 2013).

Alternately, closely related species can also present a pattern of non-neutral ecological divergence, which may be suggestive of ecological speciation (Rundle and Nosil, 2005). Ecological speciation can occur between species with parapatric or sympatric distributions, and is a process through which species diverge into different environments (Schluter and Rambaut, 1996). Because of their adjacent or overlapping ranges, ecological divergence is important in limiting gene flow between diverging species, and many cases have been studied in both animals (Schluter and Rambaut, 1996; Knox *et al.*, 2001; Blair *et al.*, 2013) and plants (Pillon *et al.*, 2007; Evans *et al.*, 2009b; Nakazato *et al.*, 2010). Unlike allopatric speciation, where an unsuitable habitat between taxon ranges prevents contact, ecological speciation depends on the reciprocal decreased ecological suitability of adjacent ranges. Organisms crossing into these adjacent ranges would do worse than locally adapted species, and hybrids would also do worse than non-hybrids in either habitat (Hatfield and Schluter, 1999; Campbell, 2003). Under this process of speciation, ecological niches of sister species should overlap less than expected based on background ecological conditions.

When taken together with other lines of evidence, niche divergence may be indicative of adaptive radiation. For example, the molecular phylogeny of *Townsendia* (Chapter 3) is consistent with speciation by adaptive radiation because it hosts a core polytomy of species with short internal branch lengths and low phylogenetic resolution, suggestive of rapid divergence among lineages. Although physiological studies have not been performed to quantify the abiotic tolerances of each species, differences inferred from ENMs offer preliminary insight. Interspecific comparison of climate-based ENMs may then provide evidence for the role abiotic limitations in diversification of the genus. If ecological specialization contributes to diversification in *Townsendia*, then supporting evidence would appear as a trend toward niche

divergence rather than niche conservatism or random drift among species in the core polytomy.

In my phylogenetic analysis of *Townsendia* (Chapter 3), I recovered four distinct groups: species with a prostrate habit, species bearing Beaman's hypothesized "ancestral" characteristics, and the erect biennials and acaulescent perennial groups of the core polytomy. Despite low levels of resolution, four clusters of species emerge within these groups: *T. annua* and *T. mexicana* (prostrate group), *T. formosa* and *T. smithii* (Beaman's "ancestral" group), T. *hookeri* and *T. exscapa* (acaulescent perennials), and *T. texensis*, *T. grandiflora* and *T. eximia* (erect biennials). Although three of these groups have low bootstrap support values, morphological and geographical data support the distinctness of them, and also suggest a close relationships among species in each group (Beaman, 1957). In this chapter, I will use comparisons of climatic niche characteristics between species in these groups of *Townsendia* to assess evidence for ecological niche divergence as a driver of diversification within the core polytomy. I predict that species groups within the core polytomy should have significantly greater than neutral levels of niche divergence, whereas species groups outside the core polytomy should have lower or neutral levels of niche divergence.

The four groupings of species evident in the ITS phylogeny were each considered for their utility in ENM comparison. Taken together, these four lineages bear characters that represent a diverse sampling of the traits in *Townsendia*. Of these groups, the clade consisting of *T. mexicana* and *T. annua* appear to be sister to the remainder of *Townsendia*, and both have the strongest bootstrap support values of the genus, while *T. mexicana* also has some of the longest terminal branch lengths. Both species have similar, prostrate growth habit and have clearly defined allopatric distributions that are separated by a distinct physical barrier, the Chihuahuan Desert. Their distributions are restricted to a few states in the southern range of the genus, with

T. annua found in New Mexico, Arizona, Utah and Colorado in the USA, and *T. mexicana* in Coahuila, Nuevo Léon, Hidalgo, Querétaro, and San Luis Potosi of Mexico. The other early diverging lineages, *T. formosa* and *T. smithii* share features such as reduced pappus hairs, which Beaman (1957) considered an "ancestral" trait. Although *T. formosa* is present in numerous sampling localities in New Mexico and Arizona, *T. smithii* is known only from two sites in the northwest corner of Arizona.

Biennial species, *T. texensis*, *T. eximia*, and *T. grandiflora*, also tend to be found in the southern part of the range, but in more restricted and closely adjacent, or partially overlapping areas. *Townsendia texensis* is found only in the Texas panhandle and adjacent Oklahoma, whereas *T. eximia* is predominantly of New Mexico and south-central Colorado. *Townsendia grandiflora* is found in the grasslands between the distribution of *T. texensis* and *T. eximia* in New Mexico, and extends narrowly north into Colorado, Wyoming and the Dakotas. A small, southern portion of the range of *T. grandiflora* overlaps with the northern part of the range of *T. eximia*; however, there is a stark contrast, between their respective grassland and mountain habitats. Although the two are geographically close, the difference in their habitats reflects their parapatric distribution, rather than one of sympatry. Overall, the species circumscriptions of *T. grandiflora, T. eximia* and *T. texensis* remain uncontested (Beaman, 1957; Strother, 2006), even if the relationships between species remain phylogenetically unresolved. Nonetheless, morphological and geographic clues led Beaman to infer that *T. grandiflora*, and *T. texensis* both diverged from *T. eximia* at approximately the same time (Beaman, 1957).

The final sister pair is composed of two perennial species, *T. hookeri* and *T. exscapa*, which areexemplars of the cushion-plant habit and the subterranean caudex common throughout the genus. Also part of the 'core' polytomy, a clade comprising these two species has greater

bootstrap support than the biennial trio, but have similarly short terminal branch lengths. *Townsendia hookeri* and *T. exscapa* also have the widest ranges of all species and are scattered, respectively, across 11 and 16 states, provinces or territories in western North America. With their wide distributions, a portion of their ranges overlap and the species can be found in sympatry. Additionally, a portion of the range of *T. hookeri* is represented by 10-15 disjunct populations found in Alaska and the Yukon Territory. These populations are thought to have originated from ancestors that survived in Pleistocene refugia (Thompson and Whitton, 2006) and form a clade separate from *T. exscapa* and the southern *T. hookeri*. Taken together, evidence of long temporal and spatial separation of the northern populations from the rest of *T. hookeri* suggests their independent adaptation over time, and warrants their removal from this study.

This study aims to test the hypothesis that adaptive radiation has played a role in the ecological speciation of the core polytomy of *Townsendia* through the use of niche modelling. If rapid climatic niche divergence has played a role in speciation, I expect to find decreased niche overlap values relative to a model of null distribution between closely related species of the core polytomy (*i.e., T. grandiflora-T. eximia-T. texensis*, and *T. hookeri-T. exscapa*). Outside of the core polytomy (*i.e., T. annua-T. mexicana*), I expect species to have rates of niche diversification lower than, or as expected in a null model, which would support a model of niche conservatism or gradual, undirected change following allopatric separation. Ecological niche models for all study species were developed from climate data, and are used to examine the evolutionary processes of niche divergence or conservatism that may have driven speciation in *Townsendia*.

Materials and Methods:

Selection of Species

Species were selected for pairwise comparison wherever their monophyly was supported by the inferred ITS phylogeny for *Townsendia* (Figure 3.1), or where, despite weak bootstrap support, taxonomy and distribution supported their status as closely related species (Beaman, 1957). *Townsendia formosa* and *T. smithii* were removed from the study because *T. smithii* is known only from two distinct localities, which is an insufficient number of occurrences for reliable niche modelling. Divergence within the biennial species was unclear. However, morphological, molecular and distribution evidence suggest that *T. grandiflora* and *T. texensis* diverged from *T. eximia* concurrently, and species comparisons assume that this is correct.

Occurrence Data

I gathered distribution data from herbarium loans, online databases, and field collections, as in Chapter 2 and 3. Unless they appeared to be geographical outliers or disjunct populations, I assumed that digital records were assumed correctly identified. However, I excluded from the dataset outliers that lacked additional collections made in the vicinity by different collectors or in different years. When specimens lacked geographic coordinates, I georeferenced them using habitat and locality data found on herbarium labels. Where these data were vague (*i.e.* failure to ascertain locality within 1 km^2), the record was discarded. The number of samples per species ranged from 22 to 419 unique localities.

Ecological Niche Modelling

I used Maxent v3.3.3k (Phillips, Dudık, and Schapire, 2004; Phillips, Anderson, and Schapire, 2006) and bioclim climate data from WorldClim.org (Hijmans *et al.*, 2005) to

construct ecological niche models for each species. The 19 climatic layers consisted of raster data that is a worldwide grid of continuous numerical data for each climate variable. These data were downloaded at approximately 1 km^2 resolution, and all data preparation was done in ArcMap 10 (ESRI Inc., 2012) using the ArcGIS Cylindrical Equal Area datum. For each of these species, I extracted climate data from each sample locality and compared them using a Pearson's correlation matrix to identify potentially correlated variables (Warren, Glor, and Turelli, 2010; ESRI Inc., 2012). I then constructed preliminary ENMs for each species using Maxent's default settings, all climate variables, and 20 bootstrap replicates. The results from Maxent include a jackknife output that ranks the overall contribution of each climate variable to the accuracy of the model. To reduce overfitting of the data and to optimize the Maxent models, I discarded climate layers in a manner that minimized the use of layers with a correlation coefficient greater than |0.8|, and maximized the number of important climate layers used.

Niche models for individual species were constructed in Maxent using the restricted set of climate variables, whereas ENMs for species pair comparisons used the combined set of restricted climate variables for each species. I defined different geographic areas for the purpose of predicting the niche model for individual species, and for interspecific model comparisons as follows. For individual species, I restricted climate data using a polygon formed from the combination of 20 km buffered points around the occurrence points of an individual taxon. These data were used to construct ENMs for individual species (Figure 4.1A). I then projected these results onto a larger study area formed from a 20 km buffered convex hull polygon drawn to encompass all localities of the species being compared (Figure 4.1B). All analyses were run with Maxent defaults, with the following changes: I created response curves, ran 100 bootstrap replicates with the random seed option, and used 20% of the data for testing purposes, and the

remainder for training the model. I used the area under curve (AUC) statistic to assess the fit of my models (Phillips and Dudık, 2008) and the ENM projections were output as logistic and raw probability grids. Collection sites within the same grid squares were functionally identical and automatically discarded to avoid data duplication and collection bias.

Niche Comparisons

I used ENMTools v1.3 (Warren, Glor, and Turelli, 2010) to compare the ENMs in each species group: prostrate species (*T. annua* and *T. mexicana*), caudex species (*T. hookeri* and *T. exscapa*), and biennial species (*T. texensis*, *T. grandiflora*, and *T. eximia*). With ENMTools, I calculated three measures of niche overlap, Hellinger's *I* (Warren, Glor, and Turelli, 2008), Schoener's D (Schoener, 1968) and relative ranks (Warren, Glor, and Turelli, 2010), between each pair of species within each group. Each of these measures ranges from 0 (completely non-overlapping models), to 1 (identical niche models), and I provide more detailed descriptions of each measure in Chapter 2.

I used niche background tests to assess the significance and direction of niche overlap between species of each group, while controlling for the spatial separation of species ranges. In this test, the background climate in the range of a taxon is estimated by scattering the same number of occurrences to random grid cells within the range of taxon A (Fig. 4.1). An ENM is then constructed based on this new set of occurrences, and this process is repeated 1000 times, producing a null distribution of ENMs. Each of these background ENMs for taxon A is then compared to the empirical ENM of taxon B, and niche overlap scores are determined for each replicate. The background test is then reproduced in the opposite direction with 1000 randomly drawn points from taxon B to compare against taxon A.

The background test is two-sided, and I used an alpha level of 0.05 to assess whether the observed overlap value between the species ENMs is within the 95% confidence interval of the null distribution of overlap values. If the observed value was significantly greater than expected from the null distribution, this was interpreted as support for niche conservation. Alternatively, I interpreted observed values with significantly lower overlap values than expected from the null distribution as support for niche divergence between species. Observed overlap values that fell within the 95% confidence interval suggest that niche similarity between species can be explained by geographically structured climate space in their respective ranges.

An additional test of niche similarity was performed only for the comparison of *T. hookeri* and *T. exscapa*. These two species have a large portion of partially overlapping range, and thus met the requirement for an identity test (Warren, Glor, and Turelli, 2010). For this test, I extracted climate data only from the overlapping portion of their ranges (Fig 4.1B). I then used ENMTools to randomize the species identity of each locality in the overlapping range, with each pseudospecies population equal in size to the original samples. From these new distributions of occurrences, I constructed ENMs and calculated the *I*, D and RR niche overlap values between models. This procedure was repeated 1000 times, and from it I produced a null distribution of niche overlaps values. The identity test assesses whether ENMs are statistically identical, and therefore is a one-sided test (using $\alpha = 0.05$). An observed overlap value falling within the lower 5% of the null distribution is considered evidence that the niches are non-identical in the area of overlap.

Results:

Five to seven Bioclim variables were used to produce ENMs for individual species, with

AUC values ranging from 0.823 to 0.910 (Table 4.1). Niche models for species comparisons used the combined set of Bioclim variables from each species in the comparison group, resulting in the use of ten or eleven climate layers that allow the comparison of equivalently constructed ENMs. Overall, the importance of precipitation variables outweighed the importance of temperature variables in the most number of ENMs. In particular, the precipitation during the driest (Bio17) and warmest (Bio18) quarter were positioned as variables of high importance in the most number of species ENMs. Other variables that were commonly incorporated in ENM construction include temperature seasonality (Bio4), temperature of driest quarter (Bio9) and precipitation seasonality (Bio15). Variables of least importance included temperature of the warmest quarter (Bio10), and precipitation of the wettest (Bio16) and coldest quarters (Bio19), none of which was used in the construction of any ENMs.

Three of the five pairwise species comparisons yielded low levels of niche overlap (Table 4.2A), while the remaining two comparisons returned either moderate or strong niche overlap values. The prostrate species pair (*T. annua*-*T. mexicana*) exhibited the lowest levels of overlap across *I*, D and RR. Comparisons involving *T. texensis*, when paired with either *T. eximia* or *T. grandiflora* returned low overlap levels in *I* and D, and moderate overlap in RR. However, the comparison of *T. eximia* and *T. grandiflora* yielded intermediate levels of overlap. The final comparison between *T. exscapa* and *T. hookeri* showed unequivocally high levels of niche overlap throughout their full range (Table 4.2A), as well as in their shared range (Table 4.2C), though to a lesser degree. Despite high niche overlap values in this final pairing, the identity test produced a null distribution of ENMS that had significantly higher overlap values than empirical data, suggesting that the species have non-equivalent ecological niche models.

Background tests returned *I*, D, and RR values for each taxon per pair (*e.g.*, *I*, D and RR

for taxon 1 vs. taxon 2 background, and for taxon 2 vs. taxon 1 background), (Table 4.2B). In the prostrate species pairing (Figure 4.2), niche overlap was found to be higher than expected in three overlap values, supportive of a null distribution in two values, and marginally lower in one value. Taken together, these results indicate a pattern of niche conservatism between species. The biennial group comparisons also reject the null hypothesis, but in the opposite direction, and are indicative of niche divergence. More specifically, the *T. eximia-T. grandiflora* background test (Figure 4.3) shows that empirical values overlap significantly less than expected in *I* and D in both directions, and are neutral in RR. In contrast, *T. eximia-T. texensis* values are neutral for *I* and DD, but lower than expected for RR, but in the opposite direction, *T. texensis-T. eximia* experiences lower than expected niche overlap values in all tests. The *T. texensis-T. grandiflora* overlap is lower than expected in the *I* and D tests, and higher in RR; however, in the opposite direction the comparison is only lower in D, and otherwise neutral in *I* and RR. Lastly, *T. exscapa-T. hookeri* background tests (Figure 4.4) show significantly greater levels of niche overlap than expected for *I*, D and RR in both directions, which is indicative of niche conservatism.

Discussion:

Combining ecological niche modelling with phylogenetic inference has provided evidence for the role of both niche divergence and niche conservatism in the evolution of *Townsendia*. The sampling and testing methodology used in this study produced ENMs with AUC values between 0.823-0.910 (Table 4.1), which suggest good predictive ability of the models (Phillips and Dudık, 2008). Many other niche modelling studies report higher AUC values than those presented here, with values typically found at 0.9 or higher (Smith and Donoghue, 2010; Kalkvik *et al.*, 2012; Blair *et al.*, 2013). However, by using a conservatives

approach with buffered points rather than a convex hull around occurrence points, the predictive ability of ENMs was compared against a smaller area of spatially autocorrelated land. In effect, this better examines the subtle nuances between nearby localities that potentially contain plant populations, instead of comparing vastly divergent habitats that are clearly unsuitable for *Townsendia* species. Overall, I suggest that the conservative approach for selecting the study areas utilized in this study resulted in lower but more accurate AUC values (VanDerWal *et al.*, 2009; Anderson and Raza, 2010; Merow *et al.*, 2013).

Sister species *T. annua* and *T. mexicana* share the lowest predicted overlap values of all the species comparisons in this study (Table 4.2A). Their predicted ENMs emphasize different variables, with the distribution of *T. annua* predominantly dictated by climate extremes such as the maximum temperature of the warmest month (Bio5), the minimum temperature of the coldest month (Bio6), and the precipitation of the warmest quarter (Table 4.1). In contrast, the ENM of *T. mexicana* is influenced by factors such as isothermality (*i.e.* annual and diurnal variation in temperature), and precipitation seasonality. These differences in climate variables may be a result of different life history strategies, as strict annuals like *T. annua* need only invest in one growing season. In contrast, facultative perennials like *T. mexicana* might benefit from the ability to persist through exceptionally bad years, and set seed when conditions are more conducive to reproduction. Despite differences in the models, three of six background tests returned higher overlap than expected, and two tests showed neutral levels of differentiation. As a whole, the results of the background test are consistent with a pattern of niche conservatism for this species pairing, and suggest divergence in allopatry.

Geographically, *T. annua* and *T. mexicana* have the largest geographic distance between species compared in this study. *Townsendia annua* is predominantly found in the Colorado

Plateau and the San Francisco Plateau, frequently in lower elevation areas sloping toward nearby major rivers. To the south, *T. mexicana* is found in the lower elevation foothills leading into the Sierra Madre Occidental and the Sierra Madre Oriental of Mexico, and in the mountains that are isolated by surrounding deserts to form sky islands with cooler and wetter climatic conditions. Currently, these sister species are separated by the Chihuahuan Desert, a young desert formed approximately 8000 years ago by the drying effect of the rain shadow caused by the surrounding Sierra Madre Occidental, Sierra Madre Oriental, and Rocky Mountains from the north (Buck and Monger, 1999; Cartron *et al.*, 2005) . Despite its recent origin, the drying and desertification of the Chihuahuan Desert began during the Miocene (15-8MYA) and continues in present day (Upchurch, McGowan, and Slater, 2011). The evidence for niche conservatism suggests that the ancestral stock of these species may have previously occupied portions of both current day ranges, and intermediate areas. Inability to adapt to the process of desertification may have driven the species into more suitable habitats in the north and south, explaining their current allopatric distribution. Over time, independent adaptation to their new distributions could have resulted in the unique, non-overlapping niches predicted by the ENMs.

Species of the biennial group show greater geographic and genetic closeness among species than do *T. annua* and *T. mexicana*. The individual ENMs for *T. texensis*, *T. grandiflora*, and *T. eximia* seem to predict species presence well, although there is over-prediction of *T. texensis* in adjacent areas located within the same ecoregions (Western Ecology Division, 2011), and under-prediction in the southernmost range of *T. grandiflora* (Figure 4.3). Overall, the important predictive climate variables for *T. eximia* include precipitation variables, whereas models for *T. texensis* and *T. grandiflora* were more influenced by temperature variables (Table 4.1). Comparing *T. eximia* and *T. grandiflora*, each of the quantitative measures of niche overlap

(*i.e. I* and D) suggest lower than average niche overlap, while the qualitative measure (RR) provides a null result. Although these species apparently have a contact zone in southern Colorado, both species produce distinct ENMs that overlap only in a small area just north of the Nacimiento Mountains. In the remainder of the range, ENMs for both species fit together like puzzle pieces but rarely overlap, suggesting a fundamental difference in their respective predicted climatic tolerances. These differences are reflected in the distribution of the species, and *T. eximia* is found mostly in higher elevation, slightly more mesic sites in the Sangre de Cristo, Nacimiento and Manzano mountains of New Mexico. In contrast, *T. grandiflora* is predominantly found in the more arid, rolling hills east of the Front Range. Differences in predicted niche likely represent a shift in habitat preference, as would be predicted under parapatric speciation along an ecotone, and are in line with the hypothesis of niche diversification within the core polytomy.

In contrast, *Townsendia eximia* and *T. texensis* have allopatric distributions separated by a patch of unsuitably arid land of the New Mexican Southwestern Tablelands. Background tests indicate that the *T. texensis* ENM overlaps much less than expected with a null distribution drawn from the range of *T. eximia* (Table 4.2B). However, in the opposite direction, *T. eximia* overlaps as expected from a null distribution in *I* and D, but less than expected in the qualitative (RR) test. This seemingly contradictory result suggests that niche divergence was integral to the separation of *T. texensis* from *T. eximia*, while niche conservatism characterizes the separation of *T. eximia* from *T. texensis*. During the Pleistocene, the High Plains and Southwestern Tablelands that currently separate *T. eximia* and *T. texensis* were generally cooler and wetter than in the present day (Holliday, 2000), and it is possible that the ancestral stock of *T. eximia* was distributed across portions of this area. Given the evidence for niche conservatism in *T. eximia*,

the current distribution of the taxon in the Nacimiento, Manzano and Sangre de Cristo Mountains might be explained by changing climatic conditions. As glaciers retreated following the last glacial maximum, a trend for drying and warming occurred across the High Plains and Southwestern Tablelands (Holliday, 2000). The western portion of the precursor to *T. eximia* may have tracked these climatic changes, retreating with the cooler, wetter habitats, and more Pleistocene-like conditions into the higher elevation, montane regions, and eventually becoming modern *T. eximia*. During this period of climatic change, the range of the ancestral stock was greatly reduced. Through niche divergence it is possible that the easternmost populations adapted to the drier, silty, mineral rich, and river-eroded canyon sides of the Texan tablelands and are now recognized as *T. texensis*.

Townsendia hookeri and *T. exscapa* have the widest distributions of all species in the genus. Both species have a patchy distribution across their ranges, and although local habitats tend to be unique and specialized (e.g., an exposed rocky outcrop against otherwise grass dominated habitat), they display greater site variation than any other species of *Townsendia*. The combination of a wide range and high between-site variation likely explains the high degree of over-prediction in the ENMS, particularly in western North America (Figure 4.4). Much of this over predicted area, including the Nechako Plateau, Harney Basin, Fraser Plateau, and Thompson Plateau, has similar dry habitats and great temperature extremes as the current distribution. This climate similarity suggests that future colonization of these areas may occur; however, the distinct habitats along the spine of the Rocky Mountains, and in the Great Basin likely serve as barriers to dispersal. Under-prediction is also noticeable at the range periphery of both species, particularly in more northern populations and in putative polyploid populations. Nonetheless, the ENMs successfully predict the predominantly diploid centres of diversity for

both species, and the area of overlap in Colorado's Front Range.

Identity tests on the overlapping portion of the ranges of *T. hookeri* and *T. exscapa* suggest that these species have nonequivalent niches despite living in partial sympatry (Table 4.2C), and cannot readily exchange habitat. For example, predicted ENMs tend to emphasize temperature-related variables in *T. hookeri*, and precipitation-related variables in *T. exscapa.* Habitat specificity acts as a prezygotic barrier to interspecific gene flow, and thus may limit the incidences of hybridization between species. Background tests (Table 4.2B) show significantly higher niche overlap than expected when compared to the available background habitat, which is indicative of niche conservatism. Because of their partially sympatric distribution and the evidence for non-identical niches, and for niche conservatism, it is unlikely that this species pair diverged by ecological speciation. Such a divergence in this species pair would necessitate some form of immediate reproductive isolation mechanism (Turelli, Barton, and Coyne, 2001). Instead, current day partial sympatry of *T. hookeri* and *T. exscapa* may be a product of secondary contact after an allopatric speciation event initially caused by Pleistocene glaciation events.

Overall, the comparison of ENMs between species provided some support for ecological divergence that would support a hypothesis of adaptive radiation within the core polytomy of *Townsendia*. Background tests on the ENMs of the biennial species show evidence for niche divergence in half of the niche overlap comparisons, and weak niche conservatism in the other half. Taken together, these results suggest that adaptive radiation by ecological niche divergence played a role in the evolution and divergence of the erect biennial species that include *T. eximia*, *T. texensis*, and *T. grandiflora*. In contrast, the background tests for *T. hookeri* and *T. exscapa* strongly indicate niche conservatism between the species. Unlike in the biennial clade, this result in *T. hookeri* and *T. exscapa* does not provide evidence for adaptive radiation in the evolution of

the species. Considering the evidence for niche conservatism, it may seem contradictory that the identity test comparing ENMs between *T. hookeri* and *T. exscapa* returns significant differences in areas of sympatry. However, it is possible that these differences arose earlier as a result of specialization to their allopatric ranges, and subsequent post-glacial range shifts brought the species together in secondary contact. Outside the core polytomy of *Townsendia*, species such as *T. annua* and *T. mexicana* were not expected to provide evidence for adaptive radiation. Instead, the comparatively long, internal branch lengths suggest a distant and slow origin of these species, perhaps facilitated by niche conservatism as found in comparisons of niche overlap.

Despite evidence for ecological speciation in the biennial species, my results suggest that ecological specialization, in regards to adaptive radiation, is not equally important across all species of the core polytomy. Eventhough *T. hookeri* and *T. exscapa* exhibit significant differences in their abiotic tolerances, these differences were less than expected under a scenario of rapid adaptive radiation. Indirectly, these results may also suggest that the caudex is unlikely to have contributed to the rapid diversification of *Townsendia*.

Conclusion:

Overall, niche comparisons of closely related species within *Townsendia* provide support for different processes of speciation and divergence in Townsend's Rocky Mountain daisies. The comparisons between *T. annua* and *T. mexicana*, and *T. hookeri* and *T. exscapa* provide evidence for niche conservatism in *Townsendia*. This finding of niche conservatism was expected in *T. annua-T. mexicana* because they form a clade that is sister to the rest of *Townsendia* that has the longest internal and terminal branch lengths of all species. However, *T. hookeri* and *T. exscapa* have diverged within the core polytomy of *Townsendia*, and their speciation and distribution

pattern may be best described by post-speciation secondary contact. Strong to moderate evidence for niche diversification was found in the final pairing between *T. eximia* and *T. grandiflora*, and *T. eximia* and *T. texensis*, and this may be supportive of the hypothesis of rapid speciation by adaptive radiation in the core polytomy of *Townsendia*. Considering the mixed results in this study, further sister species comparisons are necessary to gain additional support for the role of adaptive radiation in the diversification of speciation processes in *Townsendia*. These would be reliant on a clarification of species boundaries (e.g., in species that appear in multiple clades throughout the inferred phylogeny), as well as a more robust and well-resolved phylogeny using additional molecular markers. A more robust phylogeny would also enable additional comparisons between sister species within the core polytomy, thus expanding the scope with which we can infer the importance of ecological specialization in the diversification of *Townsendia.* Use of additional abiotic characters such as soil characters may also improve results as the ENMs become better inferences of the fundamental niche. With the understanding that soil characteristics are important in this genus, the finding of meaningful results based only on climate variables in this study are indicative of the utility of ecological niche modelling in studying the evolutionary processes in plants. As expected of adaptive radiations, species outside the core polytomy of *Townsendia* appear to have evolved through a process of niche conservatism. Within the core polytomy, the clade comprised of compact perennials apparently evolved through niche conservatism, which is not indicative of adaptive radiation. However, the clade of biennial species apparently evolved through niche divergence, and this is taken as evidence for adaptive radiation in *Townsendia*.

Table 4.1 - Predictive ability of Maxent models for *Townsendia* species in this study.

Species	N*	AUC**	std	Bioclim Variables used***
T. eximia	180	0.91	0.007	14, 18, 15, 6, 11, 2, 9
T. grandiflora	203	0.899	0.01	7, 9, 18, 5, 12, 4
T. texensis	22	0.838	0.036	9, 1, 17, 18, 14, 8, 4
T. annua	216	0.835	0.013	6, 18, 5, 11, 2, 17, 3
T. mexicana	64	0.823	0.026	15, 13, 3, 14, 2
T. exscapa	419	0.824	0.011	8, 17, 6, 4, 15, 18
T. hookeri	309	0.841	0.013	4, 17, 13, 7, 5, 9, 15

*N = Number of presence points used to build model

**Area under curve (AUC) = highest area under the receiver-operating characteristic

***Bioclim variables are listed in order from most to least important in the Maxent

The numbers refer to:

1.Annual Mean Temperature
2. Mean Diurnal Range
3. Isothermality
4. Temperature Seasonality
5. Max Temperature of Warmest Month
6. Min Temperature of Coldest Month
7. Temperature Annual Range
8. Mean Temperature of Wettest Quarter
9. Mean Temperature of Driest Quarter
10. Mean Temperature of Warmest Quarter
11. Mean Temperature of Coldest Quarter
12. Annual Precipitation
13. Precipitation of Wettest Month
14. Precipitation of Driest Month
15. Precipitation Seasonality
16. Precipitation of Wettest Quarter
17. Precipitation of Driest Quarter
18. Precipitation of Warmest Quarter
19. Precipitation of Coldest Quarter

Table 4.2 - Results from the ENMTools analysis of niche overlap, and background tests (niche similarity) and identity tests (niche equivalency) between sister species.

A.	Niche Overlap			**B.**	Background Test**			
Sister species	*I*	D	RR		*I*	D	RR	Inference
T. annua X	0.13	0.03	0.32		**0.025***	0.047*	**<0.001***	Conserved
T. mexicana					0.407	0.338	**0.011***	Null
T. eximia X	0.48	0.22	0.69		<0.001*	0.006*	0.458	Divergent
T. grandiflora					<0.001*	<0.001*	0.258	Divergent
T. eximia X	0.08	0.01	0.55		0.372	0.169	0.004*	Null
T. texensis					0.002*	<0.001*	0.007*	Divergent
T. texensis X	0.06	0.01	0.47		.009*	0.004*	**0.006***	Divergent
T. grandiflora					0.073	<0.001*	0.066	Null
T. hookeri X	0.93	0.71	0.64		**<0.001***	**<0.001***	**<0.001***	Conserved
T. exscapa					**<0.001***	**<0.001***	**<0.001***	Conserved

C.	Niche Overlap			Identity Test		
	I	D	RR	*I*	D	RR
T. hookeri X *T. exscapa*	0.86	0.57	0.63	<0.001*	<0.001*	<0.001*

* = significant *P* value, overlap less than expected

Bold* = significant *P* value, overlap more than expected

**=Entries in the first row represent results from the first species x background. Following row represents results from the second species x background (e.g., first row = *T. annua* X *T. mexicana* background, second row = *T. mexicana* X *T. annua* background*)*

Figure 4.1 – A) Example of study area selection by buffered points for hypothetical species used for niche model inference. B) Example of study area selection by buffered convex hull and intersecting range for hypothetical species used for niche overlap, identity test and background test.

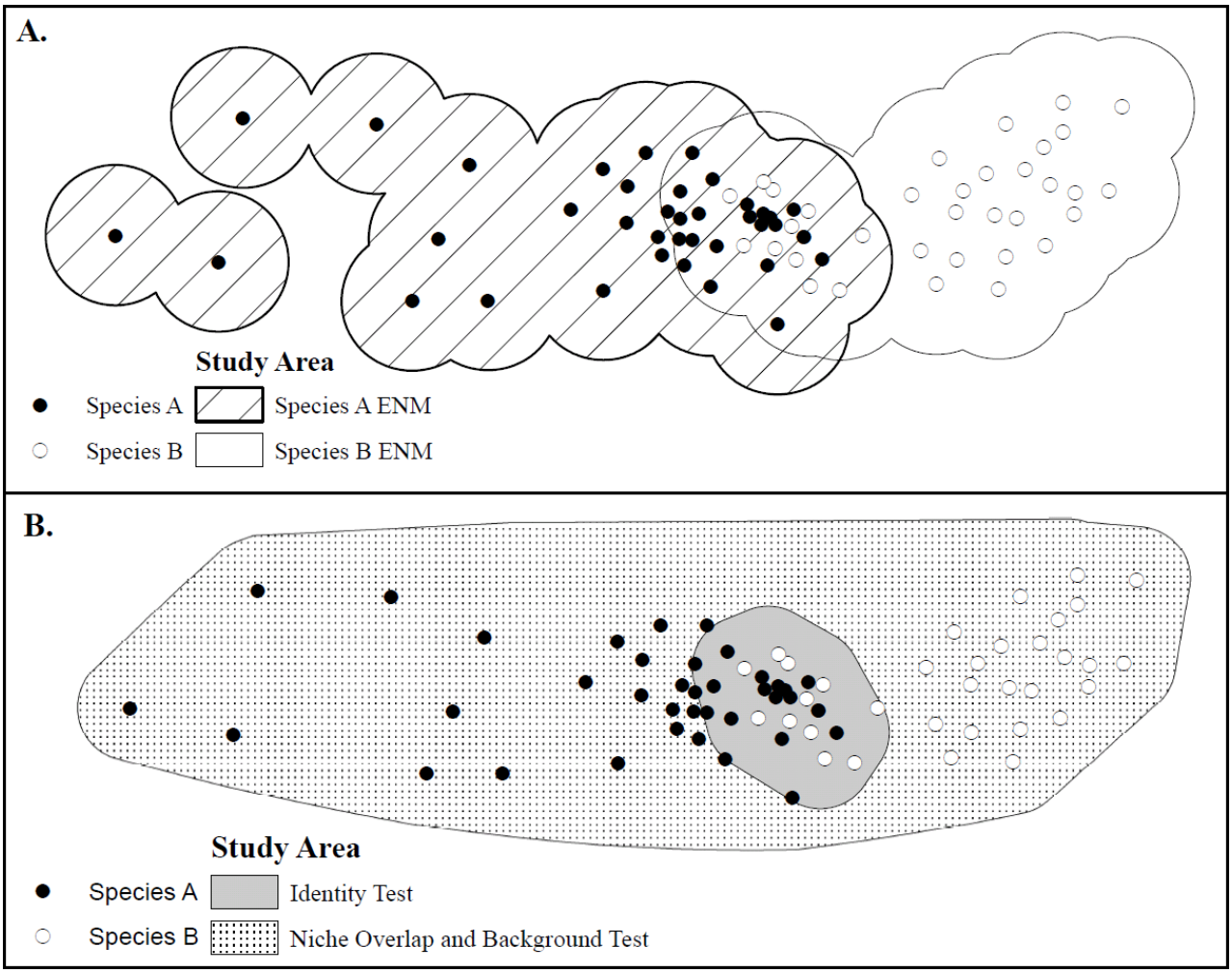


Figure 4.2 - Geographic distribution of occurrence records for *T. annua-T. mexicana,* overlaid with ecological niche models averaged over 100 replicate runs and projected onto the entire study area. Predicted species suitability was scored in Figures 4.2-4.4 as follows: Low=0.3-0.45, Moderate=0.45-0.6, High=0.6-0.85, Very High=0.85-1.

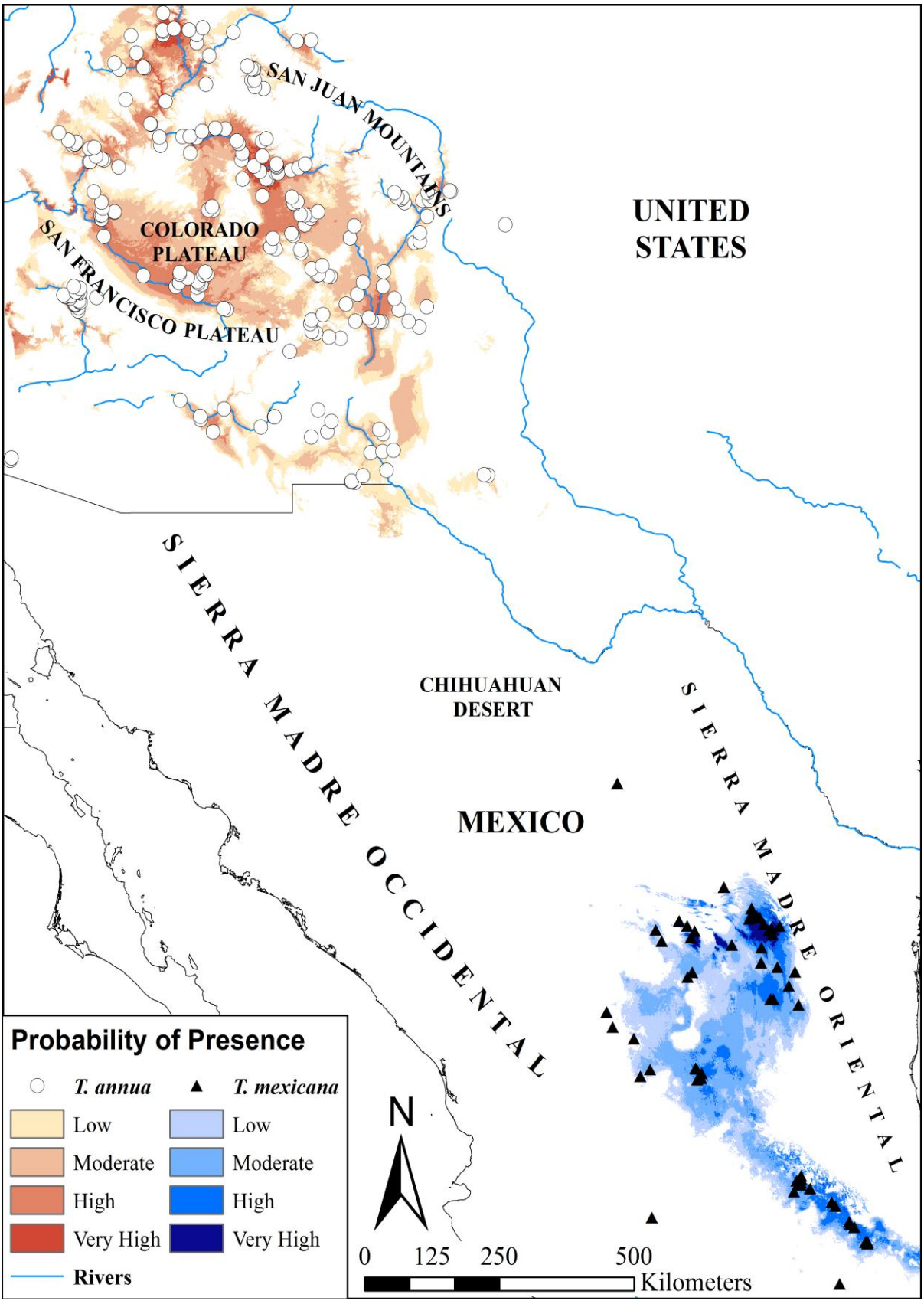

SAN JUAN MOUNTAINS
COLORADO PLATEAU
SAN FRANCISCO PLATEAU
UNITED STATES
SIERRA MADRE OCCIDENTAL
CHIHUAHUAN DESERT
SIERRA MADRE ORIENTAL
MEXICO
Probability of Presence
T. annua
T. mexicana
Low
Moderate
High
Very High
Rivers
N
0 125 250 500
Kilometers

Figure 4.3 - Geographic distribution of occurrence records for *T. eximia-T. grandiflora-T. texensis*, overlaid with ecological niche models averaged over 100 replicate runs and projected onto the entire study area.

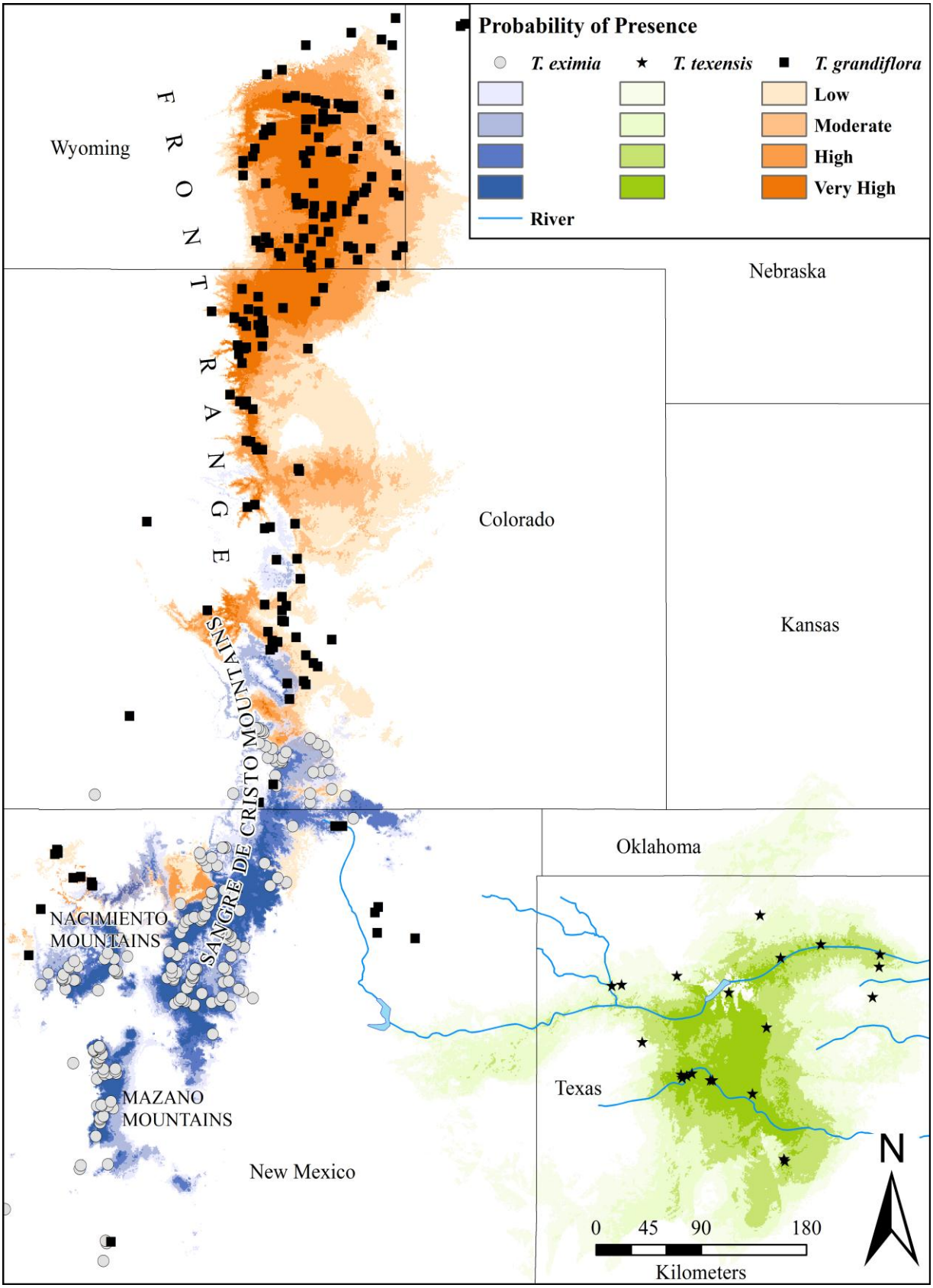
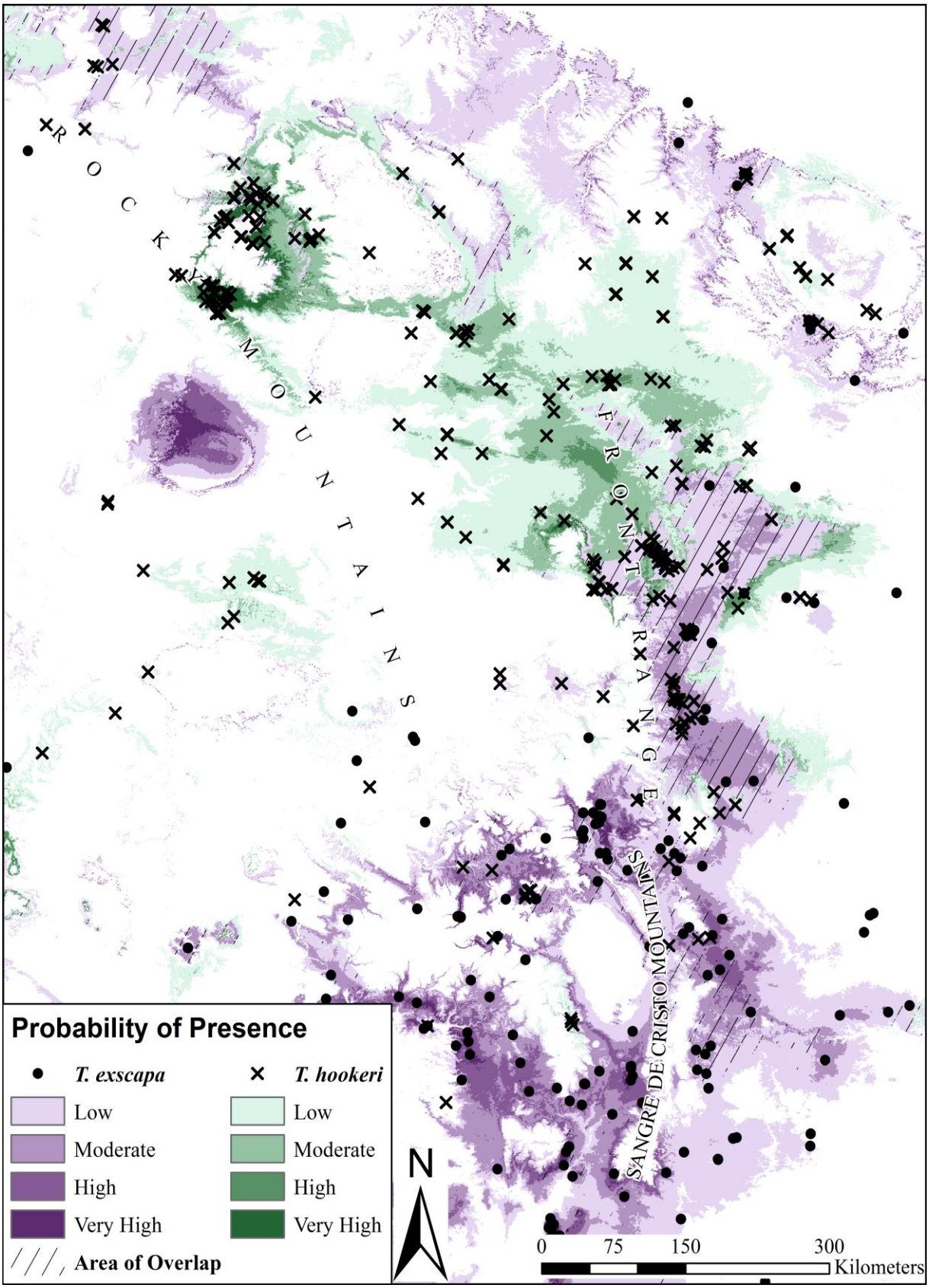

Probability of Presence
T. eximia
T. texensis
T. grandiflora
Low
Moderate
High
Very High
River
Wyoming
FRONT RANGE
Nebraska
Colorado
Kansas
SANGRE DE CRISTO MOUNTAINS
Oklahoma
NACIMIENTO MOUNTAINS
MAZANO MOUNTAINS
New Mexico
Texas
N
0 45 90 180
Kilometers

Figure 4.4 - Geographic distribution of occurrence records for *T. hookeri-T. exscapa*, overlaid with ecological niche models averaged over 100 replicate runs and projected onto the entire study area.


ROCKY MOUNTAINS
FRONT RANGE
SANGRE DE CRISTO MOUNTAINS
Probability of Presence
T. exscapa
Low
Moderate
High
Very High
T. hookeri
Low
Moderate
High
Very High
Area of Overlap
N
0
75
150
300
Kilometers

CHAPTER 5: Conclusion

Characterizing *Townsendia* as a Rocky Mountain genus packages together (or obscures) many factors that contribute to the diversification and distribution of its component species, and of distinct elements (e.g., cytotypes) within species. Although the species are clearly circumscribed and their distributions are well known, many questions remain unresolved, and addressing these questions can potentially shed light on broader issues related to the factors that relate range size, species occurrence and diversification. Whether species are localized in a small area, or distributed across the entirety of the Rocky Mountains of North America, across their ranges, *Townsendia* species are confined to unique habitats scattered, rather than continuous, across the landscape. Populations of *Townsendia* species are frequently found at relatively open and exposed sites such as rocky outcrops, but populations of some species may also persist at disturbed sites such as along road cuts. Add to this the rich and recent geological and glacial history of the Rocky Mountain regions, and the value of *Townsendia* in studying plant distributions is clear.

Following the Last Glacial Maximum (LGM) approximately 19,000 years ago (Yokoyama *et al.*, 2000), the retreat of the glaciers exposed swathes of uninhabited land, and caused a gradual shift toward drier and warmer climatic conditions in many areas (Holliday, 2000). New groups of plants, including *Townsendia*, would have spread into these newly ice-free landscapes. However, as habitats became crowded with other forms of vegetation, *Townsendia* may have shifted into areas that remained open, especially in cooler, drier and more isolated sites at higher elevations. This fragmentation of open space may have facilitated diversification within *Townsendia*. In marginal landscapes such as subalpine habitats where many species of *Townsendia* are found, abiotic limitations, rather than biotic

interactions (e.g., competition), are often thought to limit plant growth and fecundity (Bertness and Callaway, 1994). In fact, the presence of other species in these barren landscapes may facilitate the establishment of other plants (Bruno, Stachowicz, and Bertness, 2003). For example, in alpine regions, certain plants will produce above ground biomass that creates a more hospitable microclimate serving to insulate seedlings from the effects of wind and cold (Choler, Michalet, and Callaway, 2001). A move into harsher climates may have resulted in a sort of release from competition that in turn led to greater rates of speciation in *Townsendia*.

Within a species, cytotypes may inhabit differing habitats such as in the pattern of geographical parthenogenesis where asexuals are found at higher latitudes and elevations than sexuals (Vandel, 1928; Bierzychudek, 1985). To further study this phenomenon, Chapter 2 defines the current distribution cytotypes in one of the most widespread species, *T. hookeri*. The distribution of *T. hookeri* is a classic example of geographical parthenogenesis as it includes geographically restricted sexual diploids and far ranging, more northern apomictic polyploids. Despite differences in distribution, cytotypes appear to share sensitivity to a similar set of abiotic variables. Even so, the ecological niche models (ENMs) of the cytotypes are significantly different from one another and the response of sexual diploids to climate variables appears to be much sharper and narrower than the asexual polyploids. In contrast, asexuals appear to be suited for a broader range of climatic conditions, and together, these findings align with the general-purpose genotype and frozen niche variation hypotheses.

Further insight into the factors that may govern the distribution of the cytotypes is gained from background tests, which indicate that the diploid ENM is a closer match to the

polyploid ENM than expected by the null prediction, whereas polyploid ENMs are more divergent from diploid climatic niche than expected. Taken together, these results suggest that diploids could be well suited for specific portions of the polyploid range, but polyploids would not do well in the area currently occupied by diploids. The absence of mixed-ploidy populations implies that competition (*i.e.* higher polyploid fitness in polyploid range) and other factors such as diploid cytotype exclusion (via reproductive interference) and dispersal barriers may also work to maintain the current distribution of geographical parthenogenesis. Cross pollination experiments in *Townsendia* (Garani, 2014) have shown the negative effect of apomictic polyploid pollen on the reproductive output of sexual diploid mothers, which could account for the lack of mixed-ploidy populations. Such patterns are not isolated to *Townsendia* and evidence for geographical parthenogenesis has been found in cytotypes of genera such as *Antennaria* (Bierzychudek, 1989). Although a sexual system, cytotypes of *Larrea tridentata* exhibit a combination of ecological specialization and niche overlap that is similar to the pattern found in *Townsendia*, and indicate the role of inter-cytotype competition in maintaining the distribution of *Larrea* (Laport *et al.*, 2013).

This research project leads to predictions about the relative fitness of each cytotype in the range of the other type, and a set of common garden experiments is underway (by Ph. D. student Evan Hersh) to assess the fitness of cytotypes across the range. Disentangling the mechanisms that produce the broader range of polyploids, whether achieved via general-purpose genotype or frozen niche variation also remains. Future work on *T. hookeri* could better delimit the various haplotype lineages of asexual polyploids, and evaluate their individual abiotic preferences through niche modelling and greenhouse experiments aimed at assessing tolerance breadth. Evidence of significant ecological differences between asexual

lineages would provide strong support for the frozen niche variation hypothesis and would provide a more nuanced view of polyploidy. Rather than being regarded as a single monolithic entity, polyploids may consist of multiple lineages that diversify uniquely and independently, each with varying chances of long-time survival. While these differences may be difficult to detect, and their contributions to polyploid success may be obscured in sexual polyploid systems, the presence of apomixis may facilitate an understanding of the contributions of multiple polyploid origins to the ecological success of polyploids.

Related to the factors contributing to broad versus narrow distributions, the initial aim of my Ph. D. was to explore via niche modelling the role of ecological divergence in the origins and spread of *Townsendia*. An assumption of my initial study design was that I would be able to produce a well-resolved phylogeny for the genus that would identify clades that could be used for phylogenetically supported hypothesis testing. To this point, Chapter 3 explores between species relationships by sampling chloroplast and nuclear ribosomal gene regions to infer a phylogeny for *Townsendia*. Overall, the phylogeny was poorly resolved and the likeliest cause appeared to be low sequence variation in the group. Despite clearly circumscribed species boundaries, genetic variation was low, which suggests that the more important factors to the delimitation of species in *Townsendia* may be geographic barriers, ecological specialization and phenology. The four species that split early from the rest of *Townsendia* are well resolved and tend to have longer internal and terminal branch lengths, which is suggestive of greater time since divergence. In contrast, the remaining 26 species and varieties resolve into a polytomy with generally short terminal branch lengths and poor taxon resolution. Taken together, this pattern is suggestive of rapid diversification, which raises the possibility that adaptive radiation may have contributed to diversification in

Townsendia. Furthermore, shared morphological traits found in some groups of the polytomy suggest that a novel innovation may have arisen to drive speciation in the genus. The low variation in normally highly variable regions of the plastid DNA (Shaw *et al.*, 2005; Timme *et al.*, 2007) make it unlikely that the selection of regions is at the root of poor resolution. As such, it seems unlikely that incrementally adding plastid DNA regions would improve phylogenetic resolution in *Townsendia*. However, the addition of more rapidly evolving low copy nuclear genes may result in a better-resolved phylogeny, which would allow for more insight in the potential for adaptive radiation. For example, a fully resolved phylogeny would enable more confident mapping of traits such as habitat and morphology, and this may highlight the components that have potential to contribute to adaptive radiation in *Townsendia*.

Although the phylogeny produced in Chapter 3 is not fully resolved, I used a niche modelling approach to compare phylogenetically supported sister species in Chapter 4 to seek evidence for niche divergence among close relatives in the core polytomy of *Townsendia*, which could provide additional support for a hypothesis of adaptive radiation. Within the core polytomy, the ENMs of sister species were compared and their significance tested through randomization. I found evidence for niche divergence, which would be consistent with adaptive radiation in the group of erect biennials, and evidence for niche conservatism in the species pair with cushion-plant morphology. The cushion-plant morphology is unique to the core polytomy and these results suggest that its evolution may not have been a contributor to adaptive radiation in *Townsendia*. Further work on characterizing the anatomical and physiological properties of each species may also further our understanding of potentially adaptive traits in *Townsendia* as has been found in the

Hawaiian lobeliads (Givnish, Montgomery, and Goldstein, 2004). As expected of species outside of the core polytomy, I found evidence for niche conservatism in the first split of the genus. With a better resolved phylogeny, additional sister species comparisons can be used to test for niche divergence, which may lend support to the role of adaptive radiation in the diversification of *Townsendia*.

The interpretation of these niche modelling results provides some evidence for adaptive radiation for a single, relatively small genus of the North American Asteraceae. Even so, the factors affecting the evolution and distribution of *Townsendia* are common and shared across plant life. Factors such as ecological specialization, dispersal barriers, modes of speciation, and inter- and intra-specific competition all have the potential to affect the distribution of a plant whether they are generalist or specialist species. Utilizing a relatively new technique such as ecological niche modelling to study *Townsendia*, then, has broad applications in understanding the components that come together to affect or determine the occurrence, range size and diversification of other genera.

Improvements in transport technology and in the infrastructure in North America facilitate travel across the continent in our research and field studies. Although these advances have doubtlessly made fieldwork in North America more efficient and affordable, they also introduce additional challenges to research. The road is a path of least resistance, and the focus of many field collection trips on sites easily accessible by road makes it possible to introduce bias into future studies. In a similar manner, new research tools and techniques such as ecological niche modelling add more ways to study the natural world, but are also restricted by new caveats and limitations. Methodological approaches to inferring ENMs are widespread and varied, and my work attempts to address the issues that I found

most pressing. More specifically, sampling bias, inflated model support due to inappropriate study areas and plant voucher misidentification were addressed in this study. Even though the incorporation of a bias file did not appear to improve model performance, limiting the study area seems to result in ENMs with more realistic AUC values. Furthermore, the sampling of *Townsendia* has been the collective work of many past botanists, and together builds a good backbone on which to base the research presented here. The additional crosschecking procedure to exclude potentially misidentified vouchers, combined with these other conservative measures lead me to have good confidence in the models produced for this study. Continued collection of plant voucher specimens is required especially for future work as the climate and landscape changes through natural and anthropogenic processes.

Notably, the availability of relatively high-resolution soil data was lacking until recently (Hengl *et al.*, 2014), and their addition may enhance the accuracy of ENMs for a genus where edaphic conditions appear to be important for the distribution of species. However, early soil analysis found little difference in soil preferences between cytotypes of *T. hookeri* (Garani, unpublished), though edaphic differences maybe more salient between species. Furthermore, the addition of soil data, which doubtlessly affects plant growth on a much more localized scale than climatic variables, needs to be further studied to understand how to incorporate them with climate variables. In *Townsendia*, ENMs based solely on climatic data already appear to perform well because they closely track current species distributions. Although additional data on edaphic variables could refine ENMs, it seems likely that more refined models would only strengthen the observed differences and similarities already found between sister species and between cytotypes of *Townsendia*.

In closing, *Townsendia* offers a multitude of research opportunities. A relatively

small genus, the species of *Townsendia* nonetheless inhabit a wide range of unique habitats across western North America. Some species may be rare and restricted to lone mountainsides, or distributed widely, but in discrete sites. These same species may also be highly specialized for habitats that present varying degrees of physiological challenges to germination, growth and persistence. Added to all this, many species exhibit varying cytotypes with the ability for sexual or asexual reproduction. In combination, all these features naturally evoke questions on how ecological specificity, dispersal dynamics, and inter- and intra-specific competition affect the distribution of species. This thesis looks predominantly at the role of climate data on the distribution of species, and future work will go further to quantify the specific abiotic requirements of species in theory and on the landscape.

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