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Species and Genetic Diversity of *Draba*: Phylogeny and Phylogeography

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Abstract

This thesis summarizes my work on the phylogeography and phylogeny of the largest genus in the Brassicaceae, *Draba*. There over 370 *Draba* species, distributed globally (except Australia, all lowland southern Asia, and all Africa minus the Atlas Mountains) in extreme ecosystems which include high altitudes and latitudes. *Draba* is an excellent model for studying the global migration of species in alpine/arctic habitats which occur in currently or historically relatively continuous paths of major mountain areas and polar regions. Species richness data was collected and species distribution patterns were compared. Here I present the first geographical distribution compilation of the entire genus on a global scale using a presence/absence matrix and maps generated with ArcView 9. Chloroplast and nuclear sequences were obtained for 580 individuals from leaf material collected from Herbaria. Molecular evolutionary methods for inferring phylogenies and gene genealogies were used. This thesis provides the first successful genus–wide phylogeny of *Draba*, and was able to identify three major cores of *Draba* which correspond to geographic regions. I also identified species which have historically been *Draba*, but are not part of the Core and are proposing them to be renamed. I have explored under what types of circumstances a species develops, by discussing the affects of environmental factors on the lineages' migration patterns over time. I explored why the genus *Draba* appears to be more species-rich than others, and concluded that its preferred habitat of high altitude and latitude have influenced its ability to adapt, and affected its speciation and polyploidization rate. Its speciation rates have been calculated, and genetic mutation rates have been correlated with time in which I was able to make an estimate of the age of the core *Draba* species to be 2.3 million years old, and last diverged from their closest ancestor between 11 and 18 mya. Its migration routes have followed numerous global patterns, but specifically can be correlated with glaciation cycles during the Pleistocene. *Draba* has adapted to survive on the margins of these ecosystems by developing such reproductive behaviors as asexual seed development (apomixis), polyploidization, self–fertilization, and hybridization. In addition, a long-standing hypothesis about the origin of a genus is that the location of highest diversity, both in numbers of species in an area and in the corresponding genetic diversity, is also the same as its origin. In the case of *Draba*, I have concluded that the area of highest genetic diversity is correlated with the region of origin, which has been identified here to be between the Caucasus and Central Asian Mountains, but not the area of highest species (alpha) diversity or richness, which was identified as the Central Rocky Mountains. This is contrary to the accepted theory that the identification of diversity hot–spots in order to target conservation efforts is by species numbers, when here we show the genetic diversity is also crucial for the maintenance of a species.

Chapter 1

Introduction

1.1 Evolutionary Biology: Why plants? Why *Draba*?

At the base of all scientific query in biology lies evolution. It is through evolution that we venture to understand at a deeper level what we cannot see with our bare eyes, the historical processes that have shaped the present life on Earth. Evolutionary studies of organisms bridge experimental with theoretical biology in a way which other aspects of biology must not necessarily do. In order to study evolutionary processes we need time; how much time is the question. Depending on the organism, we could either need time enough for a viral population to undergo succession, mutate, and develop new strains in the matter of days or weeks. *Drosophila* needs weeks or months in order to interpret something meaningful from the traits which you are trying to follow. However, with plants it is a different story. This depends on the type of plant: photosynthetic algae, the floating aquatic *Lemna minor*, or the long–lived *Quercus rubra*, or the possibly a fast–growing *Arabidopsis thaliana*. Most importantly for my research presented here is to grasp the time that is needed in order to follow natural evolution within the natural ecosystems, by only observing present species patterns, incidence of genome aberrations, and mutations within short sequences of re–hydrated DNA. Regardless of the feeling of hopelessness in the reality of being able to observe such historical changes with what appears to be such little resources, it is very possible and I have succeeded in doing just that. In a way, studies of plant evolution are a unique type of evolutionary research because plants are often difficult to get to, and are also so extremely dependent upon their environment, that you must consider more aspects of its direct interactions than say with animals. Virtually all plants require a rooting medium, proper nutrients, adequate water, and protection from the elements, which animals can more easily provide for themselves. Yet, despite this seemly daunting task, so many plant species are able to adapt and develop ways to continue their adaptation processes in order to ensure survival. A plant cannot simply get up and walk away to find a more suitable environment. It is most likely for this reason why plants have developed unique ways to adapt to their surroundings, by increasing the ability to speciate without given the real opportunity to choose a mate or locality which may better provide a more stable existence. In particular interest to my research with *Draba*, is its ability to adapt to harsh environments such as alpine and arctic types, in rock crevices, areas of possible sea spray, and open, dry, exposed, rock above the tree line. *Draba* has developed, like many other plant species, the ability to duplicate its genome possibly in hopes to maintain its species. However, at the same time staying in

relatively small populations (compared to say bacteria in a lake). It is this exact subject which drives the research within this thesis. I ultimately strive to understand what is driving *Draba* speciation, how much has it speciated, for how long, and where. I also want to understand what environmental processes or stresses leads to further adaptation through natural selection.

Draba is the largest genus in the Brassicaceae (mustard family) and there are possibly 400 species worldwide, (the current count is about 390, with more being described, Al–Shehbaz, personal communication). The Brassicaceae has been a strong focus for plant evolutionary research for some time and much progress has been made regarding the age of the family (Franzke et al. 2009; Couvreur et al. 2009), genome evolution (e.g. Koch and Kiefer 2005), molecular systematics and phylogeny (Koch et al. 2001; Koch and Al-Shehbaz 2002; Koch et al. 2003; Bailey et al. 2006; Koch and Al-Shehbaz 2008), polyploidy (e.g. Brochmann et al. 1992d; Brochmann and Elven 1992; Brochmann et al. 1992a,b,c; Brochmann 1993; Brochmann et al. 2004; Jordon-Thaden and Koch 2008), and phylogeography (Koch and Kiefer 2006; Koch et al. 2006; Lihová et al. 2004; Widmer and Baltisberger 1999a, e.g.). The study herein literally combines all of these aspects, short of completed work on *Draba* genome evolution, as well as the addition of species richness compilation, and represents the first comprehensive, globally–distributed genus in the Brassicaceae studied at such a deep level. Previous work in *Draba* specifically has been extensive (refer to the references within each Chapter). However, most studies have only dealt with a narrow distribution range. I argue that in order to study the evolution of a highly global, and migrating genus and its species and lineages, it is essential to consider the whole picture.

1.2 Stresses that increase diversity and speciation

The initial step to understanding the migration, speciation, and evolution of *Draba*, is to access the current distribution of species. In Chapter 2, I not only combined this information on a global scale, but have also discussed the many types of distribution patterns which *Draba* appears to possess. Despite the extensive work by Barthlott and colleagues (Kier et al. 2005) on mapping, predicting, and estimating the causes of highly diverse ecosystems, such a method cannot be used for all plant types. The diversity hot–spots identified in Barthlott's map indicate that the arctic and most alpine regions are relatively low in species diversity in comparison to tropical forests. However, Grundt et al. (2006) eloquently points out that due to high incidence of cryptic species in the arctic, there actually is high species diversity in the arctic flora. *Draba* has both high species diversity, and as we show within, high genetic diversity, and at the same time grows specifically in habitats that are considered high in stress. However, environmental stress is an understudied, yet important driver of speciation, especially in plants (reviewed in Lexer and Fay 2005). Exogenous environmental stress can lead to ecological divergences via environmental–dependent selection which is directly connected to a habitat that has diverged or a newly formed niche has been created. During the glaciation cycles of the Pleistocene, the time which *Draba* advanced in its speciation, new niche development was frequent. Glaciation patterns is particular of interest in *Draba* research due to its close proximity to the snow line and modern glaciers which it prefers even today, and should be assumed also preferred during its evolution over the past 2 million to 3 million years. The ability of *Draba* adhere to this glacial boundary specifically in mountainous climates is illustrated in Figure 1.1. I will illustrate in this thesis the direct correlation between the Pleistocene glacial boundaries and the current distribution, its genetic structure, and gene genealogies.

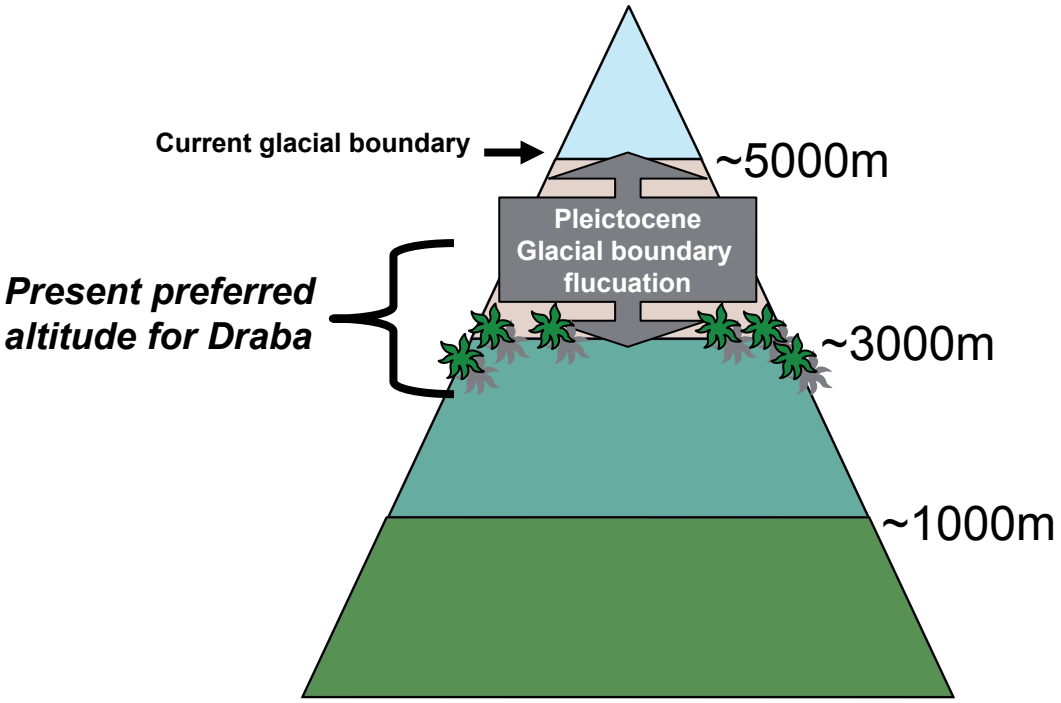


Figure 1.1: Illustration of the relationship between Pleistocenic and present glacial boundaries to current *Draba* mountainous habitat zones.

Speciation is also accelerated by genomic stresses which are often caused by chromosomal changes or polyploidization. This allows the species to undergo genomic re-organizations which make them directly more adapted to the stresses they have experienced. Unfortunately, studies with *Draba* with this exact goal are limited. The most exciting new studies of Skrede et al. (2008) attempt to identify genes in the arctic *Draba nivalis*, by developing a QTL, which are directly involved reproductive isolation. They concluded there are many different genetic mechanisms which have contributed to the rapid evolution of the reproductive isolation seen in the arctic species *Draba nivalis*. This can help us understand why so many *Draba* species have evolved and seem to have relatively overlapping niches in many cases, while others are highly endemic to a specific mountain range or even a peak. They are apparently able to reproductively isolate themselves as well as adapt to new ecological niches. As mentioned above, however, the multiple origins of arctic polyploid *Draba* species indicates that *Draba* also uses polyploidization as a method for dealing with genomic stress (see Grundt (2003), and the many publications from Brochmann). Regardless of the many studies of these arctic species, it is still not fully understood what drives this sort of genomic stress to occur, and why so many *Draba* species seem to have undergone polyploidization. *Draba* polyploids have been shown to often self–fertilize more than their diploid relatives, but more studies are needed (Barringer 2007). Understanding these aspects is another focus within my thesis.

1.3 Species Radiations and Migrations

By using chloroplast genome sequence information, lineages of *Draba* can be traced and migration patterns can be inferred. As I will show in Chapter 5, *Draba* migration shows a similar pattern to that of *Hordeum* (Jakob and Blattner 2006; Blattner 2006). Both *Draba* and *Hordeum* have apparently undergone a rapid radiation of species in recent times (less than 10 million years, with most of its radiation in the past 6 to 2 years). They are both globally distributed species, consist of diploids and polyploids, and have experienced vicarience events and long-distance dispersal. They also have the same hypothetical origin in Central Asia. They even show similar haplotype network structure with incomplete lineage sorting, and difficult to place connections between the haplotypes. However, they are different in a few very important ways. First, the phylogenetic reconstruction of *Hordeum* with three nuclear genes resulted in a highly resolved tree. As I show in chapter 4, that is not the case for *Draba*, making the parental inheritance more difficult to understand. Second, is the fact that even though *Draba* seems to have successfully migrated all over the globe since its origin in the Caucasus to Central Asian Mountains, it is not necessarily biologically suited to long–distance dispersal, and seems to inhabit niches that most other plants would find uninhabitable. *Hordeum* on the other hand (a grass) is wind pollinated and has much larger effective population sizes, and would seem to be able to migrate across large tracts of land with little trouble at all. However, Jakob and Blattner (2006) also ties seed dispersal with bird migrations. Most *Draba* species are cushion plants, with dehiscent siliques (fruits), and do not have a winged seed or fruiting body, and are relatively small. Therefore, within this thesis I discuss how a species that seems to not be able to 'move' well on its own has had little problem becoming such a global genus.

1.4 Overview of the Chapters

In the second chapter I begin by discussing the areas of distribution of the taxon (i.e. taxon biogeography) and finish with the the areas of endemism of *Draba* (i.e. area biogeography). In the third chapter I correlate these biogeographic groups at a large scale with the patterns of polyploidy in the genus, as well as discuss the concept of environmental factors such as glaciation cycles that could cause an increase in speciation and polyploidization. In the fourth chapter I discuss the taxonomic and phylogenetic circumscription of the entire genus and its closest relatives. This leads directly into the fifth chapter, where I discuss the phylogeography of the genus from investigation of the chloroplast genome, keeping in mind the biogeographic regions and phylogenetic relationships that have been determined by this research. Within this last chapter, I attempt to summarize what has been explored about *Draba* speciation and migration over the past 2.3 million years. This overall circumscription of the genus *Draba* is unique due to many factors. These include the large number of species, the wide range of distribution, the wide variety of distribution types, the stable polyploids and diploids, the perennial to infrequently annual habit, the tendency for the species to be isolated to cliff sides and to have little opportunity to evolve into a population.

Chapter 2

Species Richness Patterns of *Draba*

2.1 Introduction

Earth history determines historical events which modify species distribution areas and their aggregation into an area resulting in areas of endemism, and these areas aggregate and result in biogeographical regions. This is the definition and mode of historical biogeography. On the other hand, ecological biogeography creates what is referred to as a ecoregion from two different major influences. First, ecological niches begin to aggregate and therefore create functional types of niches, and they in turn result in what is called an ecoregion. Second, the Earth's environment generates constraints which also ultimately determine the ecoregions. By combining the biogeographical regions and the ecoregions, we can analyze the distribution patterns of a species or group of species, like *Draba*. We can then go further to speculate on what sort of influences or effects cause changes in those distribution patterns. For example, what historical events or environmental constraints have affected the species distribution within each ecoregion? It is the aim of this research to determine *Draba*'s biogeographical regions and ecoregions, to identify the center of species and genetic diversity of the genus and to eventually correlate the historical events or environmental influences which have shaped the distribution and evolution of *Draba*. Within this Chapter, we focus on identifying *Draba*'s regions and the areas of highest species richness or diversity. Historical research done regarding geographical patterns of species and the migration of those species has long since pointed out the routes between North and South America (Raven 1963), and other worldwide disjunctions of species (Wood Jr. 1972). Current studies have been addressing the distribution of species between North and South America (e.g. Simpson et al. 2005; Lia et al. 2001). With the current *Draba* dataset, we are able, on a genus level, to explore these relationships on a global scale.

This collection of *Draba* species richness data is the beginning point for our exploration of the genus *Draba*. Even without getting into the genetic data as discussed in the later chapters of this dissertation, we can observe patterns regarding *Draba* speciation. First of all, there is high amount of endemism throughout *Draba*'s entire distribution, with the South American species having the narrowest distribution patterns. On the other hand, there is an extremely uniform distribution of arctic species and other species which are found throughout large continuous mountain ranges such as the Greater Rocky Mountains or the Central Asian Mountains. In addition, there is a distinct divide between the western North American

and eastern North American species (later, in chapter 4 of the phylogeny we will see that these eastern North American species may not even be *Draba* after all). These basic patterns have been seen in countless other genera and family-wide studies. Due to the best estimation of the age of Brassicaceae (Couvreur et al. 2009) we can estimate that *Draba* could be between 11 million and 18 million years old, but the majority of the currently observed species are as young as 2 million to 5 million years old (see discussion of age estimation in section 5.1). This evidence directly places older *Draba* species at the earliest in the Pliocene and the newer *Draba* species evolving during the glaciation cycles of the Pleistocene in the last 2 million years. This time frame puts *Draba* speciation after major continental movements, and therefore *Draba* has migrated across the globe via other means (see section 5.1).

The preferred ecosystem or ecotype of *Draba* habitat is on coastal shores, ice fields and glacier edges, high alpine and rocky places, inactive volcanoes, wasteland roadsides and ditches. This indicates that *Draba* is truly a species that lives on the margins of ecosystems. A pioneering species, one which can easily take advantage of recently exposed habitat, is typical for these types of niches. However, unique to most pioneering species, *Draba* is mostly a long-lived perennial, creating a relatively strong tap root with supporting fibrous roots that can reach deep between rocks, pebbles, or ice, for example, to find a source of soil (e.g., Pérez 2002). In fact Pérez (2002) measured a 100–plant population of the Andean *Draba chionophila* to have a maximum radius of lateral roots up to 2.5 m^2 and a tap root of 5-10 cm. It was also determined that *Draba* greatly prefers pebble–filled troughs which are known to be less susceptible to frost-heaving. In addition, the majority of *Draba* have a cushion habit which allows it to stay close to the surface, leaving it more sheltered from high winds that would normally blow a weakly held annual species from its root or rooting. This is a typical growth habit for most alpine and rock-dwelling species. Two examples of its micro-habitat can be seen in Figure 2.1 which shows *D. densifolia* growing on the surface of a large rock above the tree line in Snowbird, Utah, and *D. aizoides* in a rock crevice of the Slovakian High Tatras. It is still uncertain whether *Draba* is truly a pioneering species, able to colonize rapidly under harsh circumstances or insufficient nutrition. It can only be inferred from the habitats where it has been seen growing, but very few comparative studies have been made with seed set, germination, and seedling success rates. The exception is a study by Hodgson (1987) which showed that pioneering species preferred newly opened areas and were often polyploids.

(a) *Draba densifolia* (Snowbird, Utah, notice the *Pinus* sp. needle)

(b) *Draba aizoides* (High Tatras, Slovakia)

Figure 2.1: Two species shown growing in their rocky habitat. Photos by Ingrid Jordon-Thaden

The compilation of the literature regarding the distribution of *Draba* has been a monumental task. For each major region of the world the following questions were asked: Where do the different species occur?, How many different species occur in the different regions? and How is a region defined in the Floras or checklists used? Herein we defined the similar groups of species which are reported to be found in a similar ecotype. When the compilation of the lists and ecoregions was complete, the patterns of distribution and range were analyzed. Unfortunately, it is not possible to say for certain which species are endemic to a small location and which are not. This is because the literature does not thoroughly document this information in all of the checklists that have consulted in this study. Therefore, it is still uncertain to as how many endemic *Draba* occur worldwide due to the fact that some endemic species most likely are yet to be discovered or others which may be considered endemic have other populations that have not been found to otherwise contradict its status. *Draba* is often found at the highest elevation of a mountain, which may or may not have been summited by a naturalist, and is often found on rocky cliffs that are not directly on a safe passage for a hiker. Therefore, in contrast to conventional discussion of levels of endemism with the study of vicariance biogeography (see Humphries and Parenti 1986) for a species in particular regions, we have chosen to refer to four levels of distribution: I, II, III, and IV. These regions are defined further in the Materials and Methods section and were modeled after the Ecoregion Map of North America four–level system (see United States Enviromental Protection Agency 2009) which uses the same shape files. It was not possible to compare the distributions between groups as in traditional biogeography due to the fact the areas to compare vary so greatly.

It must be noted that the species list here is a compilation of the most accepted lists which are currently available. A further daunting task would be to compile a complete worldwide taxonomic treatment of *Draba*. Here we attempt to bring together the 'west' and the 'east' and their taxonomic bridges. Central European *Draba* has been reviewed by both the Flora Europea and the Atlas Florae Europaeae. The taxonomy was essentially established by Schulz (1927), and revisited by Buttler and Elven (1996) in the Atlas Florae Europaeae. We chose the Atlas Florae Europaeae for this study because it has better distribution maps and because the comments made within the text of the treatment stated where Flora Europea had been mistaken. European *Draba* are well–established in their taxonomy and distribution, and further work is not immediately needed.

Prior to the work of Ihsan Al–Shehbaz, North American *Draba* was circumscribed by Mulligan (1976) for Canada and Alaska and Fernald (1934) and Rollins (1993) for the remainder of North America. As for South American *Draba*, no previous comprehensive work has been done outside of the work of Al–Shehbaz. Ihsan Al–Shehbaz, with the help the many major herbariums and his close colleges Beilstein and Windham and others, who have either provided loans for him (or hosted his visits) to investigate the morphological characters all together at the Missouri Botanic Gardens, have been able to compare and contrast the species from all of North and South America, China, Japan, Iran, Iraq, Afghanistan, Pakistan, and Turkey in order to completely rewrite the treatment of *Draba* for all of these regions. In his monumental feat of observing such a wide range of species from these diverse regions, he has been able to describe many new species, as well as reduce many to the same species or synomony. The North American, South American, and Central Asian *Draba* are more understood today than they ever have been in the past even with the previous separate taxonomical treatments in Al-Shehbaz et al. (2010); Cheo et al. (2001), and the unpublished species lists from personal communication with Al–Shehbaz.

The arctic *Draba* treatment has been compiled from various angles, either from the view of North Amer-

ican botanists from USA or Canada like Mulligan, Rollins, Scoggan, and Fernald, or from the European side by Schulz, Pohl, Lid, Hulten, and Elven, to name the most prominent observers of *Draba*. Reidar Elven, with assistance of Petrovsky, and Murray, have created the most comprehensive taxonomic description and distribution of panarctic *Draba* (as well as many other genera) Elven (2007). Within this panarctic study, great care was shown in investigating the historical herbarium specimens of *Draba* and from newer recent collections and field observations to assist in solving some of the taxonomic confusions of arctic *Draba*. Chromosome counts are also well discussed in this panarctic treatment of *Draba*. The Russian botanists, Tolmachev, in the Flora of U.S.S.R. and Nikiforova (1994) in the Flora of Siberia, have created the latest circumscription of the *Draba* of Siberia, and the Caucasus. In the Flora of Siberia with the *Draba* treatment by Nikiforova it is uncertain how much has been simply reiterated or whether the species described were fully reanalyzed from the initial *Draba* treatment in the Flora of U.S.S.R. by Tolmachev (1939). Therefore, these regions require another look in comparison to the newly circumscribed *Draba* from Elven and/or Al-Shehbaz, due to the possibility more species that could be either newly described or combined in the *Draba*–rich regions of the Russian provinces and former U.S.S.R. countries of Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, which are not included in the panarctic checklist (Elven 2007).

Lastly, perhaps the region which requires the most immediate attention is the *Draba* of the Caucasus, Turkey, and Iranica (Iran/Iraq) regions. A brief discussion of the *Draba* of Turkey was recently made by Al-Shehbaz et al. (2007), with the last full treatments being in the Flora of Turkey , with the *Draba* treatment by Coode and Cullen (1965), and Flora Iranica, with the *Draba* treatment by Hedge (1968), and Flora of U.S.S.R. (only for the Caucasus species). This region has been historically isolated from comparative analysis and also must be re-circumscribed with full collaboration of *Draba* experts.

2.2 Materials and Methods

2.2.1 Compilation of species for alpha diversity

Species distribution was entered into a spreadsheet for all of the different regions and Floras or checklists available for *Draba* (Appendix A). A presence/absence matrix was constructed to observe species distribution within, between, and among regions (Supplementary Materials Species Matrix). In order to determine the overlap of the information, the descriptions in the literature were consulted in detail and the distribution ranges of the Floras and checklists, and the individual species ranges within each species description were compared. These ecoregions are based on WWF Ecoregion 200 map (Olson et al. 2001) from ESRI ArcView 9 (California), and is also available from the WWF Terrestrial Ecoregions of the World web site (The National Geographic Society 2009). The ecoregions that had similar ecotypes and consist of similar *Draba* species defined to them were then identified by us to be *Draba* Ecoregions. The details from each species description were used to determine which administrative boundary best correlated with the *Draba* distribution and the boundary of the WWF ecoregions. This is how we were able to put *Draba* in certain regions specifically that had previously been defined only by its larger, unnatural administrative boundaries. With these *Draba* Ecoregions, we were able to analyze the data in ways that would otherwise not have been possible. These maps were then used throughout the remainder of this research and can be seen within this thesis.

For comparison, an initial study was made between species distribution with the Floristic regions laid out initially by Takhtajan et al. (1986) and those which we had defined from the treatments of *Draba*. The *Draba* ecoregions distributions did not correlate well with Takhtajan's boundaries, and therefore, the WWF200 Ecoregions map was far superior in defining *Draba*'s ecological boundaries.

2.3 Results and Discussion

2.3.1 Levels of Distribution

The largest division of the *Draba* Ecoregions (mostly continental is **Level I** with 8 regions (Figure 2.2). Which consist of the Arctic (71 species), Central Europe (29), Eastern USA (7), Greater Asian Mountains (97), North American Cordillera (102), Mediterranean (32), South America (71), and Turkey Caucasus Iran region (69). The division of the **Level II** into 15 smaller regions consist of AlaiPamirTienShan (33), AltaiSayanBaikalMongolian (27), AmphiAtlantic (28), Arctic-NorthAmerica/Asia (52), Beringia (55), CentralAndean (23), CentralCordillera (57), Chile/Patagonian (6), EastHimalaya (28), EasternUSA (7), European/Alps/Carpathians (29), Mediterranean (32), NorthAndean (47), NorthernCordillera (26), SouthernCordillera (41), Tibet/Central/SWChineseForests (52), Turkey/Caucasus/Iran (29), and WHimalaya/Karakorum/AfgPakMts (45) (Figure 2.3). **Level III** comprises 27 regions which are Alai/Pamir/TianShan (33), AltaiSayanNMongoliaMts (27), AmphiAtlantic (28), ArcticAsia (37), ArcticNorthAmerica (40), BeringiaNorthAmerica (38), BeringianAsia (39), CentralAndes (23), CentralAmericanMountains/Forests (9), CentralEurope (29), Central/East/CoastalCanada (14), EastMediterranean (27), EastNAmerHardwoodForests (5), GreaterRockyMountains (57), Himalaya/Karakorum/Kashmir/HinduKush (45), NorthAndean (47), NorthCentralCanadianTiaga, NorthernRockyMountains (22), PacificNorthwestCoasts/Forests (5), SWAmericanDeserts (8), SierraNevada/GrtBasin/SWArizona (41), SouthernSouthAmerica (6), SouthernUSCoastalForests (0), TheGreatPlains (3), Tibet/ChineseForests (52), Turkey/Caucasus/Iran (29), WestMediterranean (10) (Figure 2.4). The final **Level IV** regions, have 64 ecoregions and are best seen in the Figure 2.5 and the following figures of species richness and in Table 2.1.

These species numbers for Levels I, II, III, and IV were plotted onto the maps by increasing number of species in a region being shown in red, less species going to orange, and fewer species in yellow (Figure 2.6). When observing these four figures together, it nicely illustrates directly those regions which have higher number of *Draba* species compared to their neighboring regions. It can be seen here that in Level IV, the region with the highest species richness is in the Central Rocky Mountains. Also, it can easily be seen that *Draba* species richness is globally concentrated within the mountainous and extreme habitats.

In order to further discuss the patterns seen within these four levels, the following categories were developed. Narrow distribution is defined here as occurring in one of the Level IV regions, medium is defined as occurring in one of the Level II or III regions but in more than one Level IV region, and wide is defined as occurring in one of the Level I regions, but not in more than one Level II or III region. Large distribution is defined as the occurring in more than one Level I region. Each of these groups will then be defined by the regions which they share. For example, if a species group only grows in circumpolar regions, then it will be a called a circumpolar group. By extension, if a group of species occurs in many Cordilleran regions as well as arctic ones then it will be a Cordilleran/Arctic group. This allows us to

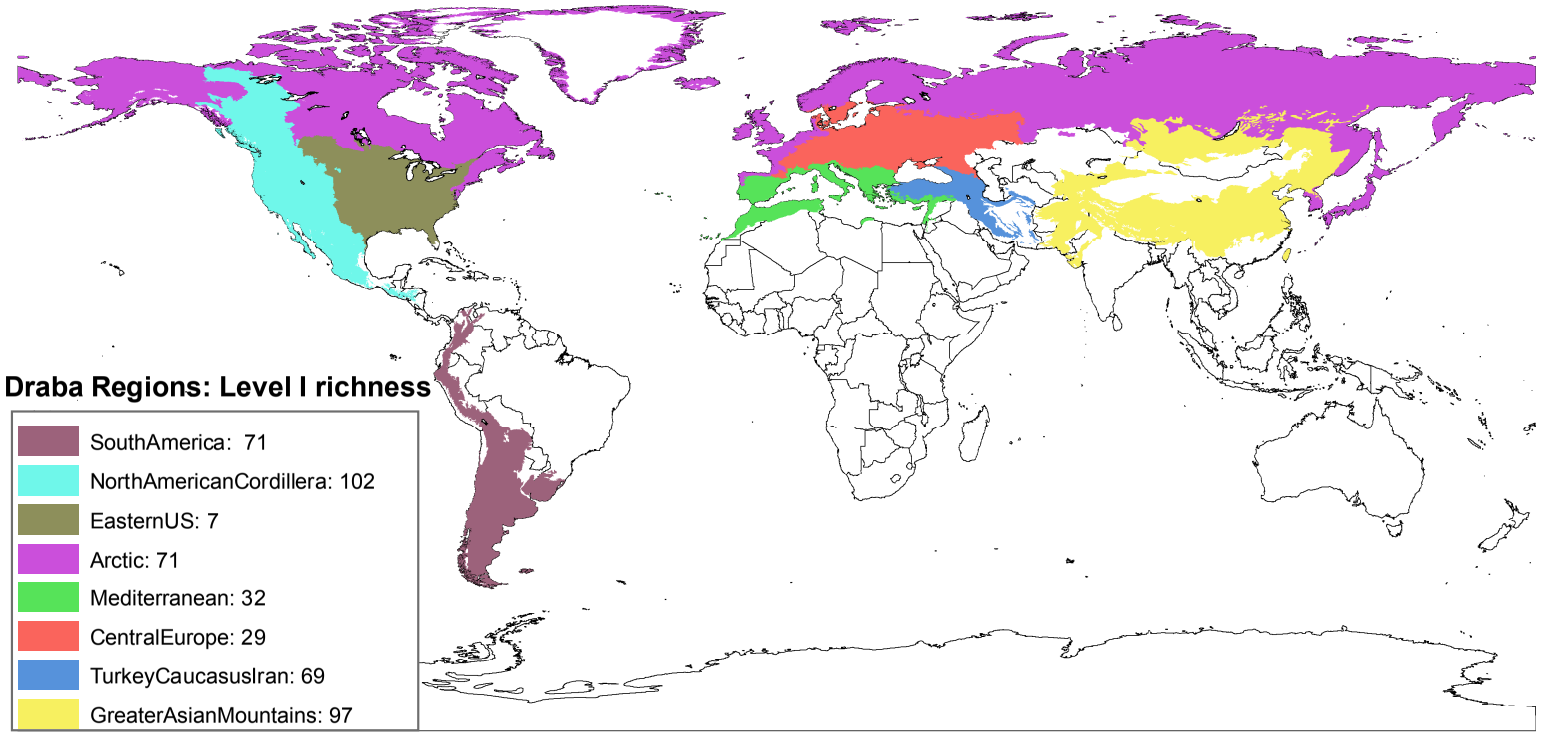


Figure 2.2: Map illustrating the Level I *Draba* Ecoregions defined herein.

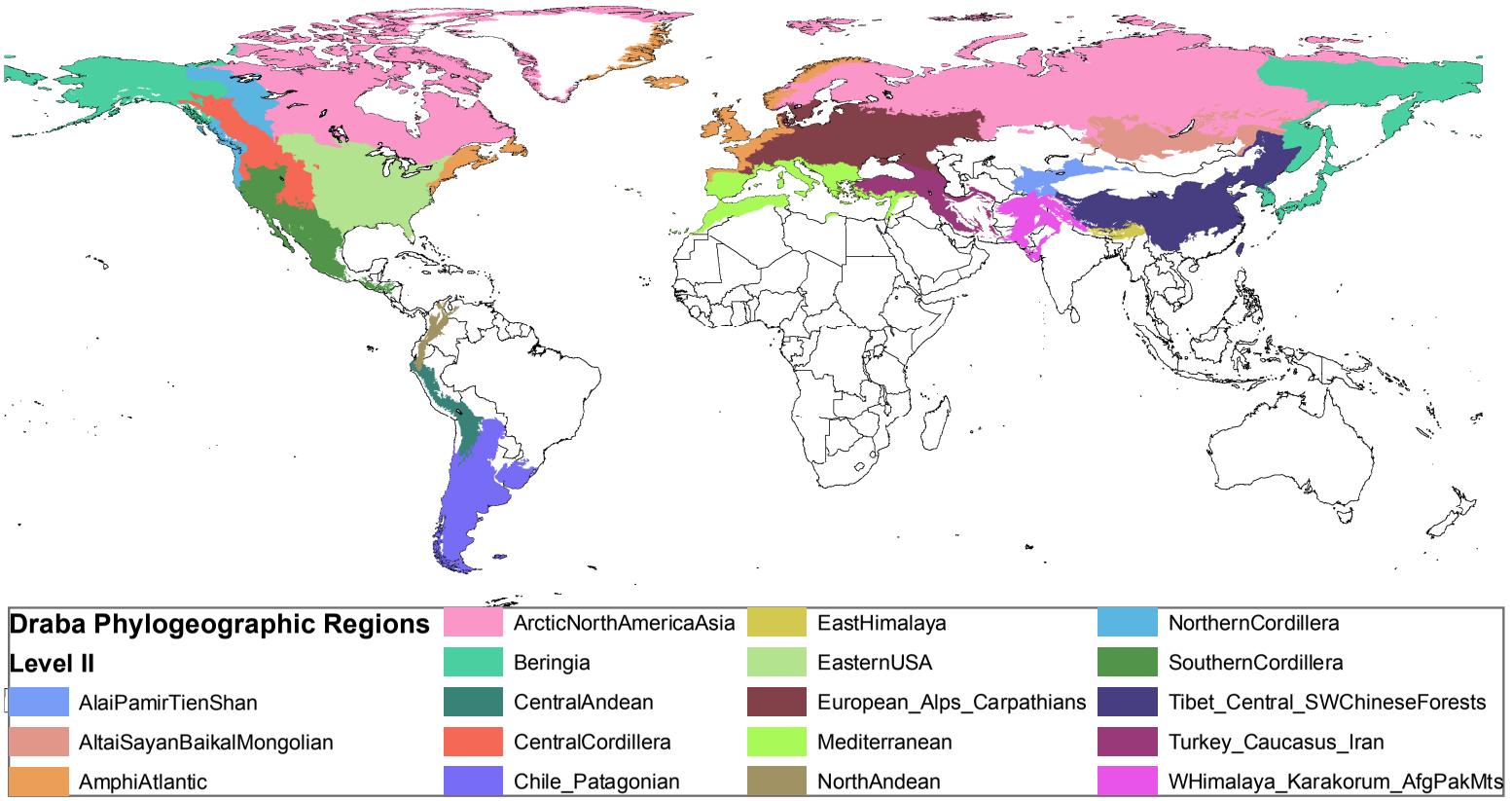


Figure 2.3: Map illustrating the Level II *Draba* Ecoregions defined herein.

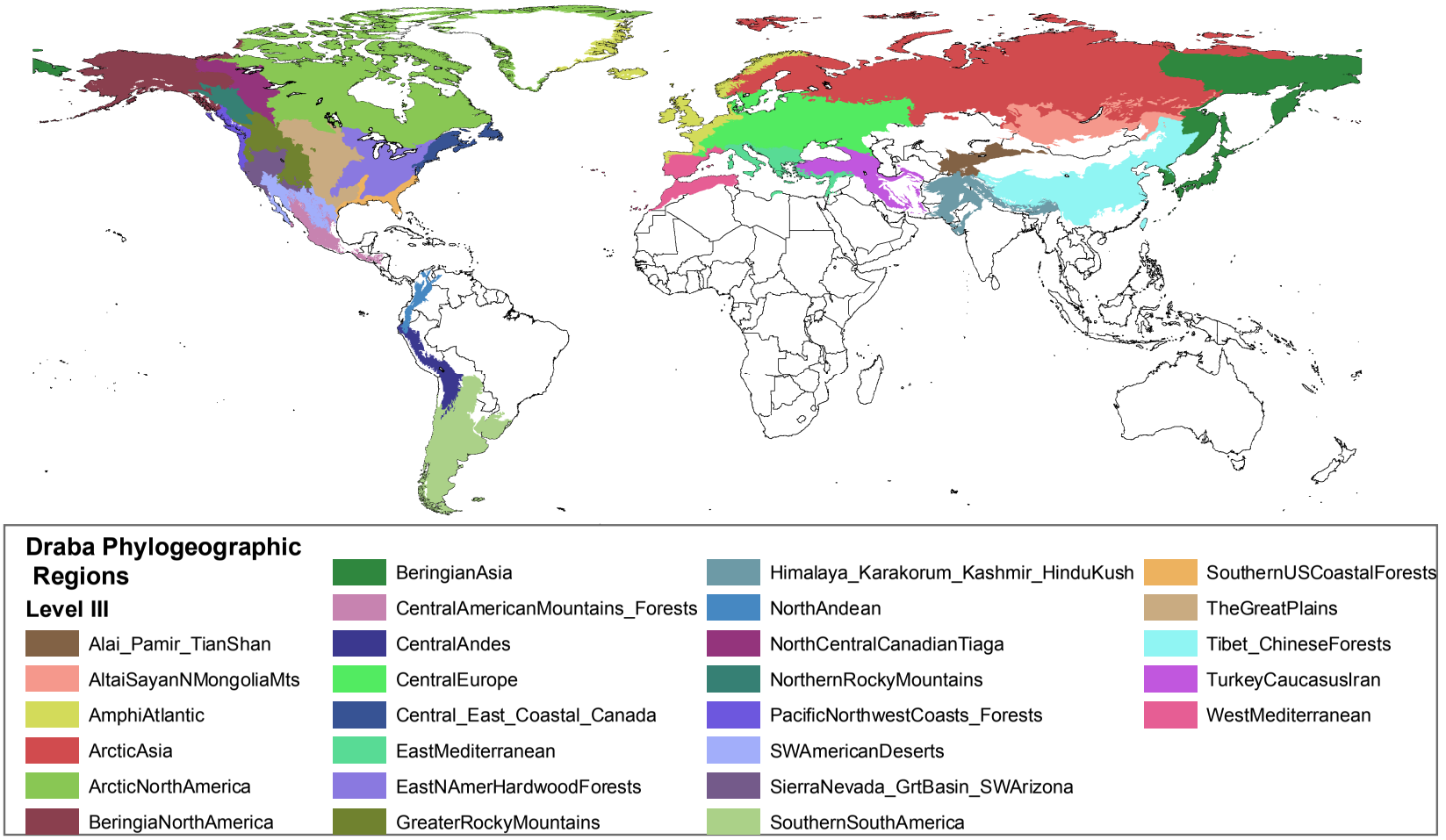
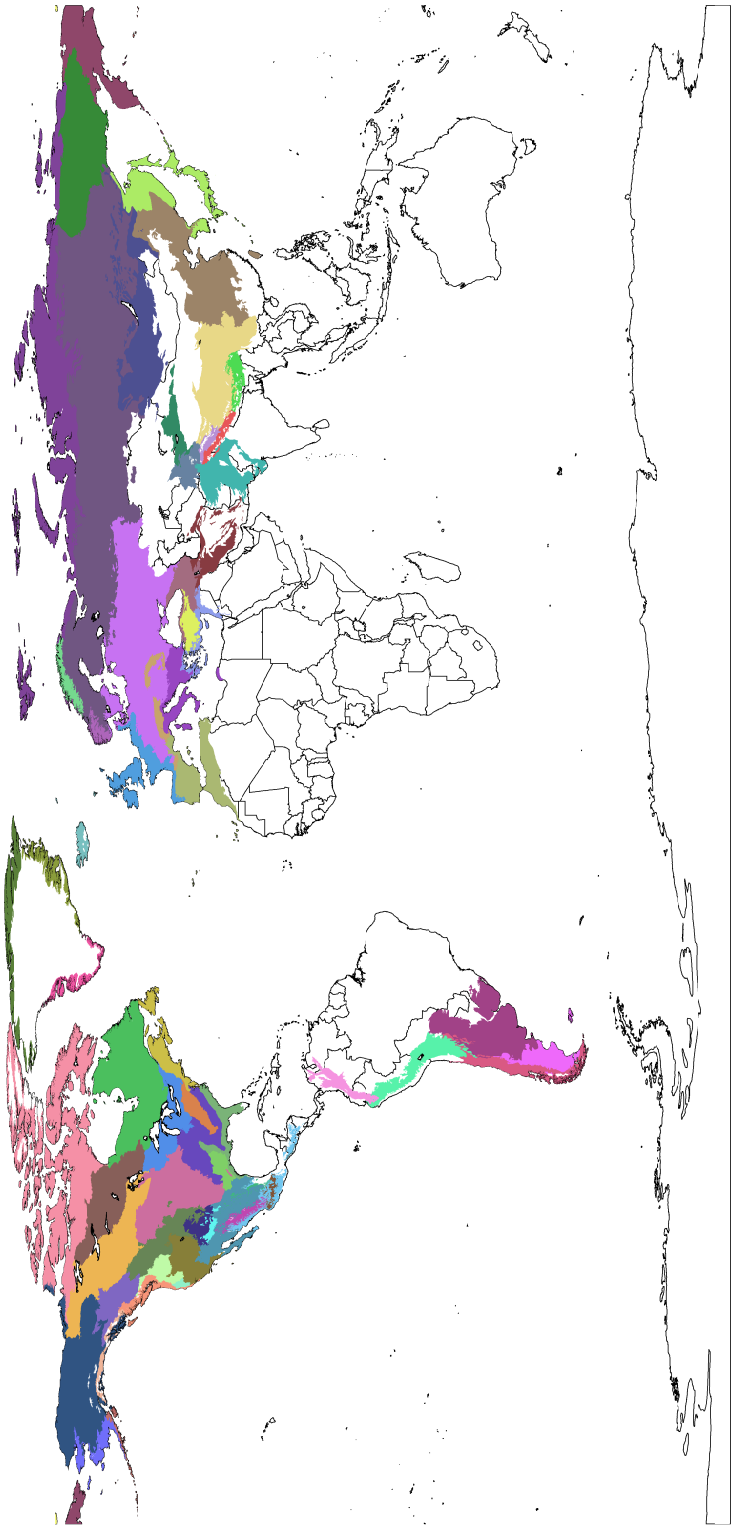


Figure 2.4: Map illustrating the Level III *Draba* Ecoregions defined herein.

Level IV Ecoregion	**# of species**	**Level IV Ecoregion**	**# of species**
Aegean W Turkey S Anatolia	17	Grt Sierra Nevadas / Grt Basin / Snake Col Shrub Steppe	32
Afghanistan Pakistan Mts / Highlands	22	Iraq / Iran Mts	10
Alai / Pamir	27	Japan / Korea / Kuriles / Okhotsk	15
Alaska Central / Yukon	34	Karakoram	23
Alaska West Coast	28	Lappland	18
Aleutian / Alaska Penn	31	North Andean	47
Altai Sayan N Mongolia Mts	27	North Atlantic Islands	9
Appalachian Mixed Forests	1	North Central Canadian Taiga	22
Arctic Coastal Tundra Asia	31	Northeastern Atlantic Coastal Forests	7
Atlantic European Forests Grasslands	7	Northern California Forests	5
Canadian Arctic Archipelagos	27	Northern Cordillera	26
Cascades / Columbia River Valley / Palouse	14	Northern Rocky Mountains	21
Caucasus / NE Turkey	26	Norway Coastal	10
Central American Xeric Matorral Forests	6	Pacific Coastal Ice / Tundra	0
Central Andes	23	Pacific Northwest Coasts / Forests	0
Central Canadian Forests	5	Patagonia Steppe	4
Central Great Plains	3	Scandinavian Russian Tundra Taiga	20
Central Rocky Mountains	48	Sierra Madre DeChiapas Moist Forests	1
Central US Hardwood Forests	1	Sierra Madre Occidental	4
Cherskii / Kolyma Mountain Tundra	12	Sierra Madre Oriental	4
Chilean Mountains	6	South American Southern Dry Shrubs Grasslands	1
Chukchi / Kamchatka	25	South Centr N Amer Grass Shrub	0
Colorado Plateau	4	Southern US Coastal Forests	0
East Chinese Forests	9	SW American Deserts	8
Eastern Canada / Hudson Bay Taiga / Forests	14	SW Arizona Forests	13
East Himalaya	28	Tian Shan	26
East Mediterranean / Balkan / Italy	14	Tibet / Central / SW Chinese Forests	50
European Alpine Forests	25	Trans Mexican Volcanic Belt	3
European NonAlpine Forests	12	Turkey / Central	7
Great Lakes Forests	4	West Himalaya	31
Greenland Eastern	22	West Mediterranean	10
Greenland Northern	22	Wrangel Island	18
Greenland SW	22		

Table 2.1: The number of species that is found within the *Draba* Ecoregion Level IV.



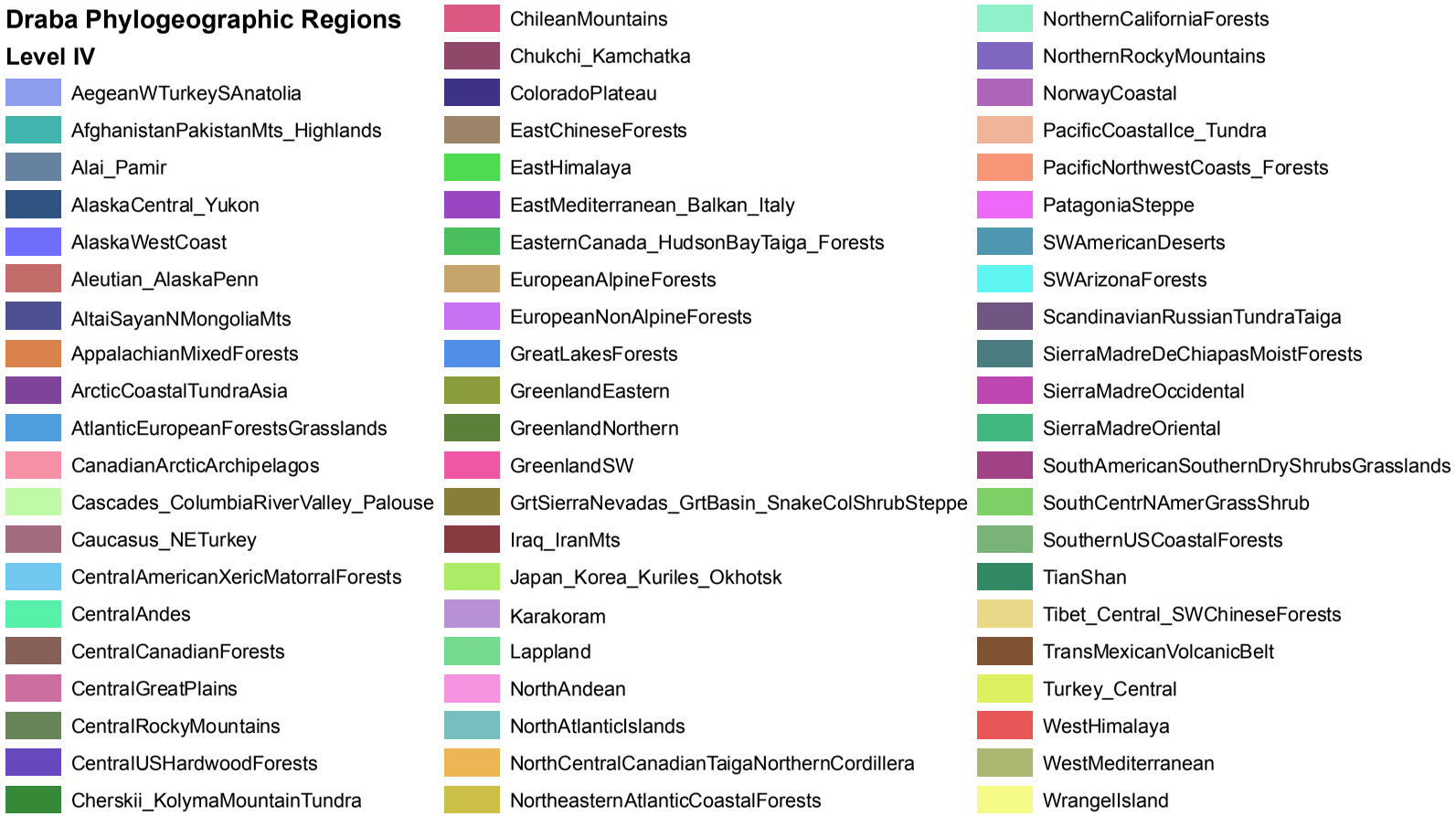
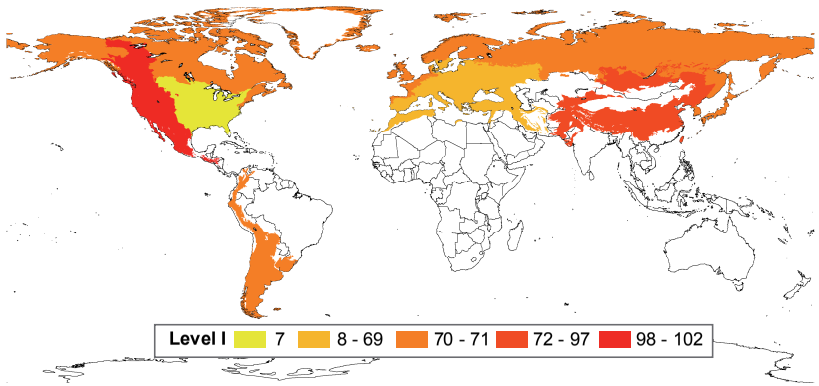
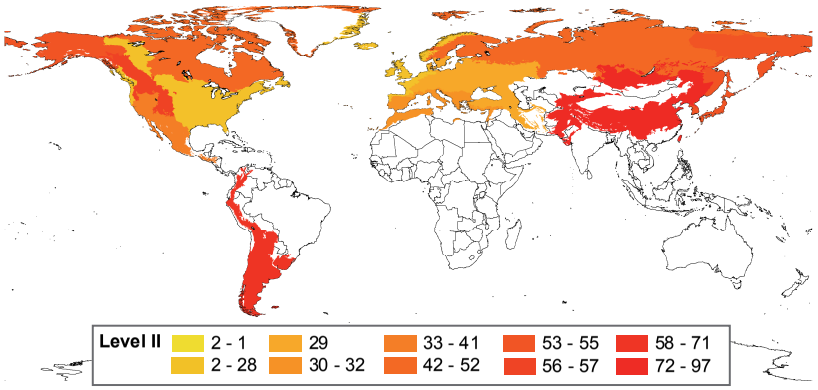


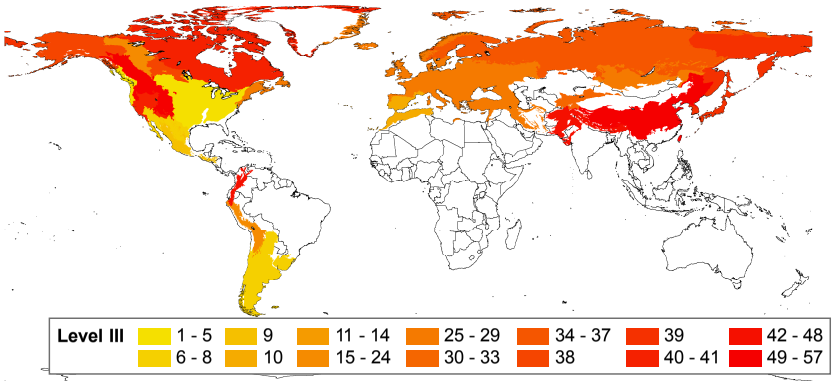
Figure 2.5: Map illustrating the Level IV *Draba* Ecoregions defined herein.



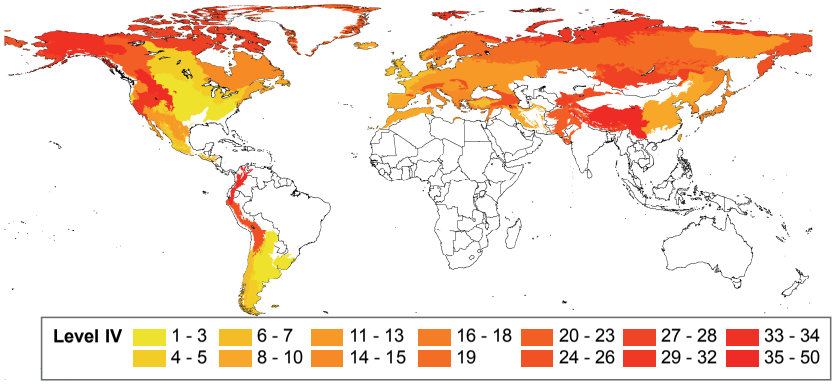
(a) Level I



(b) Level II



(c) Level III



(d) Level IV

Figure 2.6: Biodiversity of *Draba* species at four separate levels of ecological divisions in order to illustrate which regions and sub–regions have high species richness: a. Level I b. II c. III d. IV. Red areas are high in different species number, range to yellow, which means fewer, and white areas are where *Draba* does not exist. Numbers were obtained from literature search through Floras, checklists, and Herbaria. Map was made with ESRI ArcView 9 mapping software.

compare directly trends about species distribution. The remainder of this results and discussion section will discuss each of the major regions and their *Draba* species distributions.

2.3.2 North America

North American *Draba* were analyzed completely from the arctic into Guatemala. Eastern and Western North America show a significant distribution difference, and as we will see in chapter 4, the phylogeny shows that most of the Eastern North American species are not true *Draba*. The Eastern North American region is defined as all areas east of the Rocky Mountains in Canada and United States to the Atlantic coast. This region has 21 species combined, with only 7 of them being true *Draba* and was divided into five large regions, each with subdivided fine regions.

Eastern North America

Eastern North America be divided into two major regions, those of Canadian and New England ecotype, and those south of that in United States. The Eastern Canadian species are more akin to the species in the arctic and are therefore discussed together. The first eastern group mostly in the United States is the eastern North American hardwood forests and has 5 species. This region was divided into three smaller regions: the Great Lakes forests (4), Appalachian mixed forests (1), and the remainder of the central U.S. hardwood forests (1). The remainder of the Eastern North American groups represent very few species: the Great Plains (3) and southern U.S. coastal forests (0). The Great Plains are divided into two major groups: the Great Plains proper (3) and south central North American grasslands and shrubs (0) which includes part of Texas and neighboring states. The area surrounding the Hudson Bay and other eastern Canadian forests and taiga form three larger groups: the Central Canadian Forests (3), Eastern Canada and Hudson Bay taiga and forests (14), and the Northeastern Atlantic Coastal Forests (7). The northern Eastern North American region is subdivided into two smaller groups: eastern Canada and Hudson Bay taiga (14) and eastern Canadian forests (11). Northeastern Atlantic Coastal Forests, is divided into two smaller groups which are the New England and North Atlantic coasts (5) and the forests of Newfoundland (7).

Species distribution in the eastern portion of North America can be described quite easily. The northern half share species with arctic regions while only one is specifically found in Eastern United States, *D. ramosissima*. It is important to mention here many of the species which were formally thought to be Draba are in the eastern United States and some of those also are shared with the western side of North America. However, these species which are soon to be recognized as *Abdra* and *Tomostima* species (*Draba/Abdra aprica*, *Draba/Abdra brachycarpa*, *Draba/Tomostima reptans*, and *Draba/Tomostima platycarpa*) and are not considered in the species richness data within this Chapter (see chapter 4). Regardless, there distribution will be outlined here. *Draba/Abdra brachycarpa* and *Draba/Tomostima reptans* are the only species which are common to both non-arctic Cordilleran and all of Eastern North America, and *Draba/Tomostima platycarpa* which has a large distribution and is almost exclusively found throughout the United States. *Draba/Tomostima platycarpa* is found from the southern United States coastal forests, Appalachians and neighboring forests, the Great Plains, Rocky Mountains and the Cascades and Sierra Nevadas, as well as into the southwest American Deserts and the Sierra Madre Oriental of Mexico.

Draba arabisans is found only in the Central Canadian forests and in the area around the Great Lakes. *Draba aurea*, a strongly Canadian species is also found in the High Great Plains. As well as *D. cana*, except it is also more circumpolar in its distribution and can also be found in Siberia. *Draba ruaxes* is also found in the Great Plains, but only again found in Alaska, apparently skipping most of Canada. The mostly circumpolar species, *D. incana* and *D. glabella*, reach into the Great Lakes region, but go no further south. This leaves *D. ramosissima* as the only *Draba* species to not be found in the arctic and exclusively found in the central forests of the United States.

Western North America

Draba cuneifolia (also soon to be recognized as *Tomostima cuneifolia*), *D. aurea*, and *D. crassifolia* are distributed throughout North America, including both western and eastern regions. *Draba/Tomostima cuneifolia* is not arctic, but is reported from the Aleutian islands to the Sierra Madres of Mexico. On the other hand, *D. aurea* and *D. crassifolia* are the most widely distributed *Draba* species in North America. Their distribution includes arctic Canada, Greenland, and Beringia, and as far south as the southwestern forests of Arizona, excluding Mexico and Eastern United States.

The Western portion of North America includes the Cordillera, which is considered the Northern Cordillera of the Western Hemisphere, the Southern Cordillera is in South America and consists of the Andes. This Northern Cordillera is mainly described as the Greater Rocky Mountains and neighboring ecosystems and are illustrated in Figure 2.2. Within this thesis the Northern Cordillera is divided into three major regions: Northern North American Cordillera (26), Central North American Cordillera (57), and Southern North American Cordillera (41). It stretches to the volcanic fields of Central America in Guatemala from Alaska. This region has 102 *Draba* species, most of which have a narrow distribution within the different mountain ranges. This region has been divided into seven large groups. In the Northern North American Cordillera is divided into the North/Central Canadian forests and taiga (22), the Pacific Northwest Coast and Forests (5) (subdivided into Northern California Forests (5), and the Pacific Northwest coasts and forests (0)). The Central North American Cordillera is divided into the Greater Rocky Mountains (57), which consists of the Central Rocky Mountains (48) and has the most number of narrowly distributed species in the entire distribution of *Draba* worldwide, Northern Rocky Mountains (21), the Cascades/Columbia River Valley (14), and the Colorado Plateau (4). The Southern North American Cordillera is divided into the Sierra Nevadas, Great Basin, Snake and Columbian River Valleys (32), the southwest Arizona forests (13), the southwest American deserts (8), the Central American mountains and forests (10). The southwestern American deserts which straddle the border between the U.S. and Mexico and have a unique ecosystem type that divides it from the neighboring mountains have 8 species and were not divided into smaller groups. The Central American mountains and forests is described here to include all mountainous regions that are not tropical in Mexico and other Mesoamerican countries. Guatemala is the only other country in this region besides Mexico that has *Draba* species. Mexico has five main ecotypes where *Draba* has been described. These are the Sierra Madre Occidental (4), Sierra Madre Oriental (4), Central American Xeric and Matorral forests (6), the Trans–Mexican Volcanic Belt (3), and the Sierra Madre/De Chiapas moist forests (1). Most North American Cordilleran species share distribution ranges with either other parts of North America, Arctic Canada, or Siberia. Some North American Cordilleran species like, *D. praealta*, *D. albertina*, *D. densifolia*, *D. grandis*, *D. porsildii*, *D. macounii*, *D. oligosperma*, *D. stenoloba*, *D. ruaxes*, and *D. lonchocarpa*, have more in common

with Beringia than other areas of North America, also differentiating between East and West Beringia. *Draba albertina*, *D. densifolia*, *D. porsildii*, and *D. grandis* are Cordilleran and only on the western side of Beringia. Similarly, *D. macounii*, *D. oligosperma*, *D. stenoloba*, and *D. incerta*, also are Cordilleran and stay on the western side of Beringia, but continue into the Canadian Arctic Archipelagos. As mentioned above, a unique distribution is for *D. ruaxes*, which occurs from Western Beringia, throughout the North American Cordillera, and into the Great Plains. *Draba lonchocarpa* is similar to other Cordilleran and Western Beringian species, except that it is also found in Eastern Beringia and partly into continental Siberia.

Two species, *D. aureola* and *D. howellii*, have a distribution that includes the forests of Northern California, into the Cascades and Columbia River Valley, only 2 other species, *D. carnosula* and *D. pterosperma*, have a narrow distribution in Northern California only. *Draba cusickii* has a medium range distribution that includes the Oregon Cascades and the Snake and Columbian River valleys and steppe. *Draba cyclomorpha* is the only species with a narrow distribution in northeastern forests of Oregon.

The Central North American Cordillera has only three species, *D. novolympica*, *D. brachystylis*, and *D. rectifructa*, that occur outside of the Central Rocky Mountains. All other species occurring in the Rockies are only found within the Rockies. These three species have a wide distribution and grow from the Sierra Nevadas, Great Basin, Snake River Valley, Cascades, and Colorado Plateau, Northern and Central Rocky Mountains, and into the southwestern Arizona forests. There are 26 species that have a narrow distribution within the Central Rocky Mountains. Refer to Supplementary Materials for the Species Matrix for a list of these species. The southwestern forests of Arizona have 4 narrowly distributed species: *D. petrophila*, *D. mogollonica*, *D. asperella*, and *D. bifurcata*. *Draba spectabilis* is found both in the southwestern forests of Arizona and the Central Rocky Mountains. While *D. abojoensis* is similar to *D. spectabilis*, except is not found in the Rockies, but the Colorado Plateau instead. Five species are found in the unique ecosystem of the Colorado Plateau: *D. rectifructa*, *D. aurera*, *D/Tomostima. reptans*, *D. albertina*, and *D. abajoensis*.

Nine species have a narrow distribution in the Sierra Nevadas: *D. asterophora*, *D. sierrae*, *D. scharsmithii*, *D. breweri*, *D. monoensis*, *D. longisquamosa*, *D. lemmonii*, *D. incrassata*, and *D. cruciata*. Seven species, *D. arida*, *D. pedicellata*, *D. sphaeroides*, *D. pennellii*, *D. paucifructa*, *D. kassii*, and *D. incrassata*, are narrowly distributed in the Great Basin. Only 2 species, *D. subumbellata* and *D. californica*, are found in both the Sierra Nevadas and the Great Basin.

Draba helleriana and *D. viridis* have a distribution that is Southern Cordilleran, which includes the deserts of southwestern North America, the southern portion of the Central Rocky Mountains, and into Mexico in the xeric matorral forests and Sierra Madre Oriental. Two species, *D. corrugata* and *D. saxosa*, have a narrow distribution in the southwest American deserts and do not grow outside of these regions. *Draba nivicola* and *D. hidalgensis* are exclusively Central American species; they are found throughout the dry forests of Mexico and small areas in Guatemala, including the Sierra Madre Oriental and the Trans Mexican volcanic Belt. Four species, *D. standlei*, *D. jorullensis*, *D. implexa*, and *D. rubicaulis*, are found in the broadly defined Mexican and Central American dry mountains which include the southwestern deserts straddling the border of Mexico. These mountain ranges include the Sierra Madre Occidental and Oriental, and the Trans Mexican volcanic belt.

2.3.3 South America

South American *Draba* can be described as occurring in the entire Andes chain from Western Venezuela to Chile, the Patagonian Steppe and the dry grasslands of Eastern Argentina. There are 69 species currently described in all of South America. The Northern Andes has 47 species, the Central Andes with 23, and the Southern Andes with 6. Not a single species is distributed throughout the entire South American continent. However, a few species are shared between the regions. Below is described the species distribution for the three major regions.

Northern Andes

The Northern Andes, which consists of the high mountains of Venezuela, Colombia and Ecuador, was not divided into any smaller groups for this analysis. There are 41 species which are growing only in the Northern Andes. Eighteen species occurring in this region are extremely narrow to endemic, while the others occur in only two to five neighboring states within the Northern Andes. None of the Northern Andes species occur throughout the entire region. *Draba hallii* has the widest distribution and occurs in numerous administrative states in the Northern Andes. The region near Quito has the most number of species, with 12 species occurring there. There are 9 species which occur in the southern part of the Ecuadorian Andes, most of which are in either Cañar or Azuay. This southern region has no Quaternary volcanoes in contrast to the central and northern portions of the Ecuadorian Andes.

Central Andes

The Central Andes region, with 23 species, is described here as most of Peru, the western portion of Bolivia, and the very northern parts of Chile and Argentina. This region has been divided into three smaller groups. The first is the small Central Andean paramo (13 species), second is the non-tropical neighboring forests of the Central Andes (22), and third is the Central Andean puna (23). Thirteen species occur in all three of the ecoregions defined here. Compared to the Northern Andes, there are fewer endemic species. Only 1 species occurs in only a single administrative state. Most other species occur in multiple administrative states. *Draba brackenridgei* is the most commonly distributed species in the Central Andes. Sixteen species have a distribution that is only in the Central Andes and neighboring forests. The Northern and Central Andes share 7 species: *D. depressa*, *D. schusteri*, *D. wurdackii*, *D. alyssoides*, *D. matthioloides*, and *D. hallii*. One species, *D. tucumanensis*, is shared between the Central and Southern Andes. *Draba araboides* is soon to be considered a *Tomostima* and is not included here in these counts.

Southern Andes and Patagonia

Southern South America was divided into the following groups, but altogether the species distribution is quite similar. There are 7 species in the southern portion of South America. These are, *D. magellanica*, *D. pusilla*, *D. funiculosa*, *D. thalspiformis*, *D. gillesii*, and *D. tucumanensis*. The Southern Andes with 6 species, were divided into 4 smaller groups. There are 4 species, *D. gilliesii*, *D. pusilla*, *D. thlaspiformis*, and *D. tucumanensis*, in the Southern Andes yungas, steppe, and neighboring forests. Continuing into

the unique neighboring regions of Chile, the Valdivian temperate (3) and the Magellanic subpolar forests (4), together have 4 species, *D. pusilla*, *D. gilliesii*, *D. magellanica*, and *D. thlaspiformis*. The Patagonian region was divided into two smaller groups: mainland Patagonia and the Falkland Islands. The mainland Patagonia has 4 species which are distributed there: *D. pusilla*, *D. magellanica*, *D. gilliesii*, and *D. funiculosa*. Only two species, *D. magellanica* and *D. funiculosa*, are recorded from the Falkland Islands. A unique ecosystem is defined here as the southern South American dry shrubs and grasslands region, in the dry areas of northern Argentina, southern Paraguay and most of Uruguay. It has one species, *D. pusilla*, which also continue into Patagonia and the Southern Andes. None of the species in southern South America are found in the Northern Andes. *Draba australis* is the last species to soon be recognized as a *Tomostima* species, and is distributed in the entire southern portion of South America where other *Draba* species are also growing.

2.3.4 Europe to Iran

European Distributions

Central Europe is defined here to include Europe proper and the Russian provinces that have forested ecosystems similar to most of Europe. It also includes most of southern Sweden, but the Amphi-Atlantic region is considered partially separate and more related to the arctic group. Further discussion about the Amphi–Atlantic species is in the arctic section of the results.

There are 29 species in the Central European region as defined here. Most of those species occur in the major alpine regions (25) which are the Alps (19), Carpathians (14), and Pyreenes (11). The remainder of the forested and non-Mediterranean species of Europe are distributed from Eastern France to the western border of the Southern Ural Mountains (12). *Draba cuspidata*, *D. lactea*, *D. sibirica* and *D. simonkaiana* are the only European species that do not occur in alpine regions as well. Very few other European species have a very narrow distribution. *Draba cuspidata* is known only to be found on Crimea. *Draba simokaiana* is known only from the non-alpine regions of Romania. Only 4 of the most common out of the 12 species continue all the way to the Urals; these are *D. lactea*, *D. nemorosa*, *D. sibirica*, and *D. verna*.

Nine species have a narrow distribution in the alpine forests of Europe: *D. haynaldii*, *D. stellata*, *D. dolomitica*, *D. sauteri*, *D. hoppeana*, *D. pacheri*, *D. ladina*, *D. kotschyi*, and *D. dorneri*. Five species, *D. dolomitica*, *D. hoppeana*, *D. ladina*, *D. sauteri*, and *D. stellata*, are only found in the Alps, but 19 species can be found within the boundaries, with only 3 being found only in the Austrian Alps: *D. dolomitica*, *D. sauteri*, and *D. stellata*. *Draba ladina* is the only species endemic to the Swiss Alps. *Draba hoppeana* is found in all of the Alps, but not elsewhere. The Carpathians have 14 species and three are endemic: *D. dorneri*, *D. haynaldii*, and *D. kotschyi*. One species is found only in the Alps and the Carpathians: *D. pacheri*. The Pyreenes have no narrowly distributed species, but have 11 which are also found in either the other European alpine, non-alpine or Mediterranean regions. Eleven European species grow both in and outside the alpine regions, and have rather large distributions and are discussed either below in relation to neighboring regions or in the arctic section of the results. These are: *D. aizoides*, *D. dubia*, *D. fladnizensis*, *D. muralis*[1], *D. nemorosa*, *D. verna*, *D. lasiocarpa*, *D. siliquosa*, *D. tomentosa*, *D. aspera*,

[1]After the analysis from the phylogeny in chapter 4, *Draba muralis* will soon be recognized as its own genus outside of *Draba*. However, further work needs to be done in the Arabidae to determine whether it is a monotypic genus.

and *D. incana*.

Ties between Iranica, Mediterranean and Europe

Only one species has been able to colonize almost the entire distribution range of *Draba* except for South America, and that is true annual *D. verna*. It is considered only naturalized in North America and was therefore not included in the counts for any North American regions. *Draba verna* is currently thought to originate somewhere between the European and Caucasus regions. There are other European species that have varied distribution patterns into the Mediterranean, Caucasus, and Iranica regions. Three species, *D. bruniifolia*, *D. siliquosa*, and *D. heterocoma*, are found from Eastern Mediterranean to the mountains of Iran and Iraq. *Draba siliquosa* is also found in the alpine forests of Europe, as well as being distributed in East Mediterranean and Iranica. *Draba muralis* is largely a European species that is distributed from the Mediterranean, Anatolia, Caucasus, and into the alpine and non-alpine forests of Europe and Scandinavia, including the Atlantic coastal line. *Draba dubia*, *D. lasiocarpa*, *D. aspera*, and *D. tomentosa* are exclusively European and Mediterranean, similar to *D. muralis*, but they do not go into Scandinavia, Iranica, Caucasus or the Atlantic coastal regions. *Draba aizoides* and *D. cantabriae* are distributed throughout Europe and the Mediterranean regions, including the Atlantic European forests, non-alpine and alpine Europe, and the West and East Mediterranean regions. *Draba compacta* has a wide distribution that ranges from the Balkan and Italian peninsulas and continues into other alpine regions of Europe, while *D. subnivalis* and *D. thomasii* are distributed in alpine Europe and the Caucasus and northeastern Anatolia regions.

Mediterranean: East vs. West

There are 32 species throughout the entire Mediterranean region, and most are in the eastern part. There are 27 species in the Eastern Mediterranean region with 14 in the Italian and Balkan peninsulas and 17 from Greece, Aegean Sea, western Turkey, and southern Anatolia. Only 10 species are in the Western Mediterranean region.

Draba lacaitae and *D. korabensis* are exclusively found in the Eastern Mediterranean. Eastern Mediterranean regions defined here include West Anatolia, the Taurus Mountains, the islands of the Aegean Sea, and the Balkan and Italian peninsulas. Two species have a narrow distribution within the Balkan and Italian peninsulas: *D. bertiscea* and *D. loiseleurii*. Six species, *D. strasseri*, *D. parnassica*, *D. acualis*, *D. elegans*, *D. cretica*, and *D. haradjianii*, have a narrow distribution in the Aegean Sea and South Anatolia regions. The high mountains of Morocco, Algeria and Tunisia have 6 of the 10 Western Mediterranean species. These species are *D. hederifolia*, *D. hispanica*, *D. lutescens*, *D. muralis*, *D. oreadum*, and *D. verna*. *Draba hederifolia* and *D. oreadum* have a narrow distribution in the Moroccan Atlas Mountains, while *D. lutescens* is in both the Moroccan Atlas and Spain. *Draba hispanica* is the probably the most characteristic Western Mediterranean species and is known from the Moroccan, Algerian, and Tunisian Atlas Mountains and woodlands and the whole of Spain, including the Pyreenes. *Draba aizoides* and *D. cantabriae* (European to Arctic), *D. dedeana* (Amphi–Atlantic), and *D. dubia* (Mediterranean and Europe) are the remaining Western Mediterranean species and occur in Spain and beyond, but not Africa. The only species that occur in Portugal, are the most common European species: *D. muralis* and *D. verna*.

Iranica and Caucasus

The Caucasus regions includes the Caucasus mountain range, of course, but here we also have added the Eastern Anatolian and Pontic Mountains of Turkey. The Iranica region is defined here as including the Zagros and Elburz Mountains, which grow mostly in Iran and the northern part of Iraq. The region has been divided into three smaller regions: Caucasus and Northeastern Turkey (26 species), Central Turkey (7), and the Zagros and Elburz Mountains of Iran and Iraq (10). There are 13 unique distribution patterns for the 29 species that are in the entire region.

Except for the 14 narrow species within these regions, only one species, *D. rosularis* occurs entirely within the Caucasus and Iranica defined boundaries. *Draba bruniifolia* also covers this region completely, but continues into the Eastern Mediterranean. *Draba siliquosa* and *D. thomasii* cover the same region as *D. bruniifolia*, but also continue into the alpine mountains of Europe. *Draba subnivalis* has a disjunct distribution that is reported from the Caucasus and then again in alpine Europe. Three species are exclusively found in Caucasus to Central Turkey: *D. cappadocica*, *D. polytricha*, and *D. rigida*. Ten species have a narrow distribution in the Caucasus and northeastern Turkey, including the northern Anatolian mountains. These are *D. mollissima*, *D. araratica*, *D. elisabethae*, *D. hispida*, *D. imeretica*, *D. longisiliqua*, *D. ossetica*, *D. thylacocarpa*, *D. subsecunda*, and *D. scabra*. Only one species, *D. pulchella*, is narrowly distributed in the Zagros and Elburz Mountains. *Draba heterocoma* has a large distribution that goes from the Caucasus to all of the Mediterranean.

Three species have relationships with the Central Asian Mountains. *Draba aucheri* is found in the Zagros, Elburz, Alai, Pamir, and Tian Shan. *Draba nuda* has a larger distribution starting from the Eastern Mediterranean, through the Zagros and Elburz, and in most of the Central Asian Mountains. *Draba huetii* is similar, but it also grows into Caucasus and only in the Tian Shan, as well as the Zagros and Elburz. *Draba nemorosa*, *D. sibirica*, and *D. verna* grow throughout the entire region as well as all neighboring regions. Except for not growing in the Zagros or Elburz, *D. muralis* has a similar distribution as these three other common species.

Transition from Europe to Russia and the Arctic

These species are found throughout European Russian regions, but mostly are shared with circumpolar species: *D. verna*, *D. lactea*, *D. incana*, *D. glabella*, *D. cinerea*, *D. muralis*, *D. nemorosa*, *D. alpina*, *D. nivalis*, *D. norvegica*, *D. oxycarpa*, *D. insularis*, and *D. sibirica*. *Draba insularis* is the only species that has a narrow distribution in the northern central Russian Tundra, which includes the non-arctic portion of the Kola peninsula and Karelia regions.

2.3.5 Central Asian Mountains

Large and/or Disjunct Distributions

Draba lanceolata has a large distribution that occurs from the Afghanistan and Pakistan highlands continuously into the Russian side of Beringia, including all the major mountain ranges in Central Asia. A disjunctive distribution is seen for *D. koeiei* where it grows in the mountains of Yunnan, then in the Pamir and into the Afghanistan and Pakistan highlands. *Draba nuda* has a unique distribution that goes from

the eastern Mediterranean, through Iranica, excluding Caucasus, and continuously into the Himalayas and the Tian Shan. *Draba huetii* is recorded from the Aegean Sea continuously into the Tian Shan, but stays north of Karakorum and the Himalayas. *Draba aucheri* has a large distribution that spans from the Tian Shan, Pamir, and Alai of Central Asia and also in Iran and Iraq mountainous areas. *Draba alajica* stays south of the Altai and alternatively grows in the Alai, Pamir, Tian Shan into Tibet and the southwestern mountains of China, but excludes the Himalayas. *Draba tibetica* also is distributed throughout Central Asia, including the Himalayas and Afghanistan and Pakistan highlands, but omitting the Altai, Sayan, and Mongolian mountains and the forests of eastern China. One species, *D. turczaninowii*, has a very large distribution in Central Asia that includes most regions above the Himalayas and continues into the Okhotsk region.

Circum–Central Asiatic with Exceptions

Draba stenocarpa is distributed throughout the entire mountain range that creates a circle within Central Asia that surrounds the dry ecosystems such as the Gobi desert. *Draba eriopoda* is distributed almost similar to *D. stenocarpa*, but is not in the Alai, Pamir, and Tian Shan. Similar to *Draba stenocarpa* and *D. eriopoda*, *D. parviflora* is distributed throughout the entire mountain range circle of Central Asia, but excludes the Himalayas. *Draba oreades* is distributed throughout all of Central Asian mountains, including Afghanistan and Pakistan highlands. *Draba lasiophylla* grows in the entire Greater Asian mountain region, including eastern China, but does not venture into the highlands of Afghanistan and Pakistan. Similar to *D. lasiophylla*, *D. olgae* also is distributed throughout most of Central Asian mountains, but on the other hand it excludes eastern China and is found in the Afghanistan and Pakistan highlands. *Draba alticola* grows continuously from the highlands of Afghanistan and Pakistan into the Alai, Pamir, and Tian Shan, across Tibet and into southwestern China.

The Related Tian Shan, Pamir, Alai and Highlands of Pakistan and Afghanistan

Four species, *D. pamirica*, *D. hissarica*, *D. darwasica*, and *D. odudiana*, are exclusively found in the Alai and Pamir mountain ranges. While two other species also are found in the Tian Shan as well: *D. albertii* and *D. physocarpa*. Continuing south into the highlands of Afghanistan and Pakistan, *D. melanopus* is also found in all four regions. *Draba arseniewii* is distributed exclusively from the Alai all the way to the Sayan mountains. The highlands of Afghanistan and Pakistan have one species with a narrow distribution, *D. hystrix*[2]. The Tian Shan has three species that are narrowly distributed: *D. lipskyi*, *D. fedtschenkoi*, and *D. talassica*.

Eastern Himalaya, Eastern Chinese Forests, and Tibet

Surprisingly enough, only two species, *D. bagmatiensis* and *D. sherriffii*, are exclusively found in the Eastern Himalaya. *Draba altaica* has a wide distribution throughout the entire Central Asian mountains, but does not grow into eastern China. *Draba sekiyana* is the only species that has a narrow distribution for the eastern Chinese forests, and is restricted to the high elevations in Taiwan. Six species have a wide distribution of eastern Himalaya, Tibet and the southwestern mountains of China: *D. elata*, *D.*

[2]However, this species is also more related to Arabis and will not be recognized as Draba, see chapter 4

bhutanica, *D. polyphylla*, *D. lichiangensis*, *D. cholaensis*, and *D. humillima*. Although the distribution region for *D. ussuriensis* seems relatively small compared to the above mentioned super regions, it is the only species which shares the distribution range only from the eastern Chinese forests to the Russian province of Okhotsk.

The Tibetan Plateau has 11 species in common with the southwestern Chinese forests. These 11 species are not found in the Himalayas or other mountains neighboring Tibet. One species in particular, *D. ladyginii*, covers the entire range of Chinese forests as well as Tibet. *Draba mongolica* has an even wider distribution as it also grows into the Altai, Sayan and Mongolian mountains, as well as Tibet and the Chinese forests. *Draba subamplexicaulis* is yet even wider with its distribution going into the Tian Shan as well. Tibet has six species with a narrow distribution: *D. nylamensis*, *D. mieheorum*, *D. jucunda*, *D. linearifolia*, *D. kongboiana*, and *D. sunhangiana*.

Himalayas and its Neighbors

Draba glomerata also has a wide distribution growing from the southwestern Chinese mountains, Tibet, completely in the Himalayas and Karakorum and continuing into the highlands of Afghanistan and Pakistan. *Draba korschinskyi* has similar distribution to *D. glomerata*, but also continues into the Alai and Pamir. *Draba gracillima* and *D. ellipsoidea* share a distribution from the southwestern mountains of China, through Tibet, the Himalayas and Karakoram. Three species, *D. affghanica*, *D. stenobotrys*, and *D. falconeri*, occur from the Himalayas, Karakoram, expanding into greater Kashmir, and into the Hindu Kush and other Afghanistan and Pakistan highland mountains. Four species are exclusively distributed only in the Himalayas proper: *D. amoena*, *D. staintonii*, *D. polunininana*, and *D. macbeathiana*, while *D. radicans* is the only species that grows exclusively only in the Himalayas and Karakoram. *Draba sikkimensis* is the only species which exclusively grows in the Himalayas, Tibet, and the southwestern mountains of China. The ecosystem that includes the western Himalayas and Karakoram often shares the distribution range with the highlands of Afghanistan and Pakistan. For example, *D. trinervis* has this distribution, while *D. setosa* and *D. winterbottomii* also continue into Tibet and southwestern China, but not in the eastern Himalaya. Alternatively, *D. involucrata* and *D. cachemirica* have a similar distribution, but excluding the highlands of Afghanistan and Pakistan. Three species are exclusively found only in the Western Himalaya and Karakoram: *D. himachalensis*, *D. aubrietiodes*, and *D. tenerrima*.

Altai, Sayan and Mongolian Mountains and Going North

The Altai and Sayan mountain ranges seem to be a mixing point for Central Asian species and Arctic and Siberian ones (see arctic section). *Draba chamissonis* uniquely grows in a large distribution from the Altai and Sayan into central Siberia then continuously into Chukchi and into Alaska. Two species, *D. kusnetzowii* (or *D. kuznetsovii*) and *D. pygmaea*, have a large distribution from the Altai and Sayan and Mongolian mountains into central Siberia. *Draba cardaminiflora* has a narrow distribution growing only in the Okhotsk region. *Draba hyperborea* is currently only recorded from Kamchatka and Okhotsk regions. *Draba ochroleuca* has a large distribution range that spans from the Tian Shan, into Siberia, both arctic and non-arctic, across the Altai, Sayan and Mongolian mountains and into the Chukchi peninsula. Four species have a narrow distribution from the Altai, Sayan and into the Mongolian mountains: *D. dasycarpa*, *D. baicalensis*, *D. primuloides*, and *D. kodarica*. It is interesting to observe these species

distribution for *Draba*, especially noting the species with narrow distributions and how they overlap with the neighboring mountain systems (Figure 2.7). One can almost see the path of species migration purely by the distribution of species and which regions share which species.

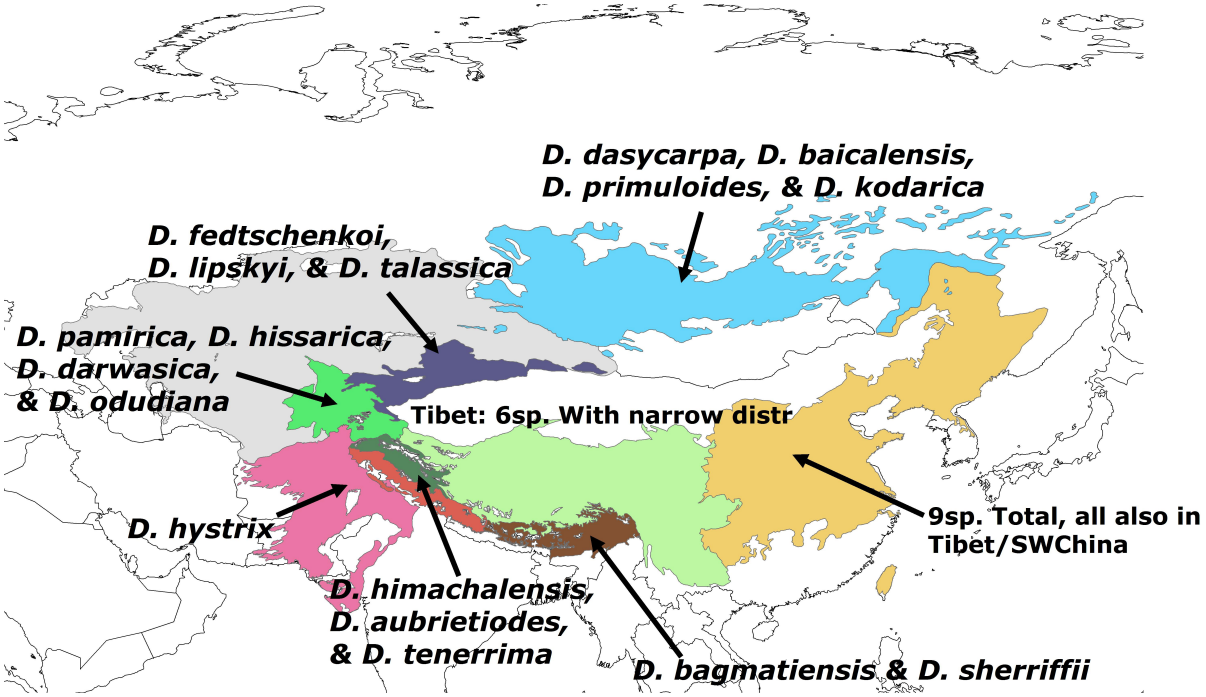


Figure 2.7: Map illustrating some of the regions which encompass narrowly distributed species occurring in the Central Asian Mountains. There are a total of 97 different species occurring throughout this region.

2.3.6 CircumNorth

The largest distribution region for *Draba* is circumpolar or arctic (71 species). The terms circumpolar and arctic must be briefly explained here. For the purposes of this analysis, it was decided that arctic meant any regions that are above the 60 degree latitude. Circumpolar was used to describe a region if a species was distributed throughout this entire arctic region. The arctic *Draba* have been divided into four major regions. These are the Arctic Coastal Tundra, non-arctic Siberia and Scandinavia, Beringia, and Amphi–Atlantic (Figure 2.8). These sections will be discussed in the following paragraphs.

Arctic Coastal Tundra

This region essentially covers all regions bordering and above the Arctic Circle. The number of ecological niches is high in this region, but still enough uniformity exists that it can be grouped into one region. There was a significant difference between the Asian and North American species distribution. The Asian portion, which includes the Kola Peninsula Tundra (7 species), Arctic Svalbard and neighboring islands (14), and Arctic Siberia (30), has 31 species combined. The North American portion, which includes Arctic Alaska, Brooks Range, and Arctic Yukon (29), Canadian Arctic Archipelagos (27), and most

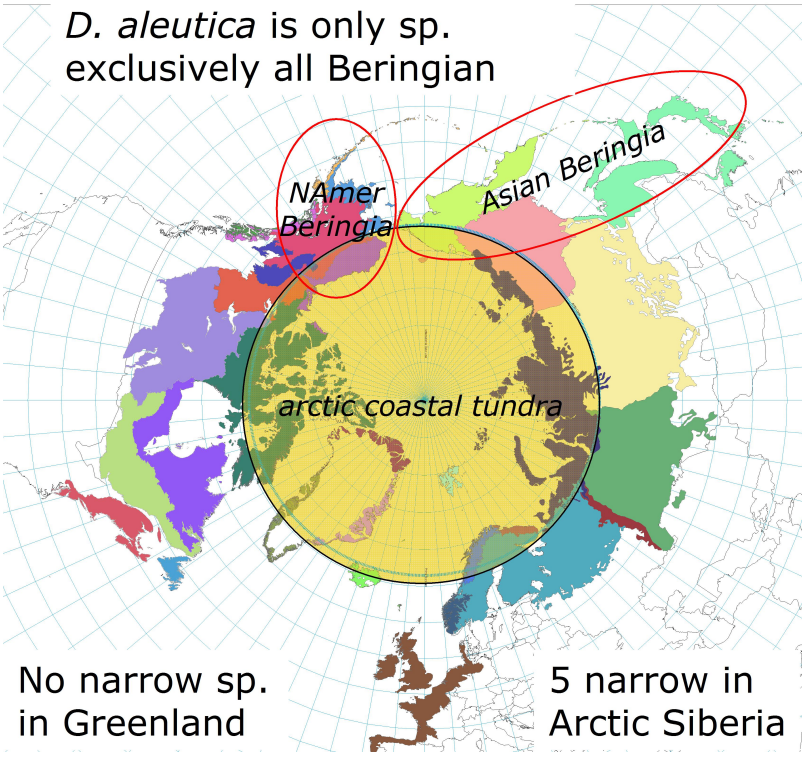


Figure 2.8: Map illustrating the region defined here as Circum-North/Arctic, where similar *Draba* species co-occur. Regardless of the arctic coastal tundra environment being unique, arctic *Draba* also occur further south as shown here. There are a total of 71 different species occurring in this large region.

of Greenland (22), also has 39 species when combined. The total number of species when Asian and North American are combined is 52. Within the Arctic Siberia group there are four subgroups. These groups are: Novaya Zemlya, Kanin Peninsula, and all other islands north of this region including Franz Josef Land (16); Yamal and Gydan Peninsulas to the Polar Urals (18); Taymyr Peninsula to the Lena River (26); and from the Lena River to the Kolyma Lowland (23). Some Siberian Arctic species have narrow ranges within a large area, but do not grow in continental Siberia. These include, *D. glacialis*, *D. prozorovskii*, *D. pohlei*, *D. taimyrensis*, and *D. sambukii*. *Draba parvisiliquosa* is the only species which grows only throughout the entire central Siberian area, including both arctic and continental Siberia.

Non-Arctic Siberia and Scandinavia

This group is mostly circumpolar in similarity of species, but has enough unique patterns to separate it from the other Arctic regions despite the fact it is not a true arctic ecological region. This groups shares species with the Arctic Coastal Tundra, Europe, circumpolar, Central Asian Mountains, Iranica and Caucasus regions. There are 19 different distribution patterns seen within this region, mostly described in the circumpolar, Central Asian Mountains, European, and North American sections of the results. It seems to be a mixing point for arctic and non-arctic *Draba*. The regions within this area are divided into four groups. The first is the Scandinavian and Russian Tundra and Taiga (12 species), second are the Ural Mountains (7), third is the Western Siberian Taiga and Forests (4), and fourth is the Central

Siberian Taiga (13). Combined the region has 20 species. *Draba cinerea*, *D. nemorosa* and *D. sibirica* are common throughout this entire region. *Draba parvisiliquosa* is the only species that is found only from the arctic to non-arctic Central Siberia. As discussed in the European section, *Draba insularis* is the only species that has a narrow distribution in the northern central Russian Tundra, which includes the non-arctic portion of the Kola peninsula and Karelia regions.

Beringia

The *Draba* species of Beringia also show a significant difference between the North American and Asian areas (refer to Figure 2.8). The Asian side of Beringia is divided into four main groups: one which includes Japan, part of Korea, the Kuriles, and Okhotsk region (15 species); a second that includes the Chukchi and Kamchatka Peninsulas (25), Wrangel Island (18), and the Cherskii and Kolyma Mountain tundra (12); all together, the Asian portion has 39 species. The North American portion is divided into three groups: the Aleutian Islands and Alaskan Peninsula (31) and western coast of Alaska (28), and Central and Southern Alaska and the Yukon taiga and tundra (34). Together the North American Beringia region has 38 species. The Beringian region in its entirety has 55 species, making it a region of high species richness. However, it is rather large in area compared to the area of highest species richness, the Central Rocky Mountains, with 57 species.

Draba aleutica is the only species that is exclusively Beringian, growing both on the North American and Asian sides. The distribution of *D. kamtschatica* is reported from Central Alaska, throughout Beringia and into Arctic Siberia, but the Alaskan plants most often represent misidentification for *D. chamissonis*. *Draba kamtschatica* does not continue into continental Siberia or Europe. Four species, *D. japonica*, *D. sakuraii*, *D. shiroumana*, and *D. kitadekensis*, have a narrow distribution on the islands of Japan. *Draba sachalinensis* is found both in Japan and on the Kruiles. Japan has a unique group of species that are endemic to the high mountains of the islands. Their chloroplast haplotypes group more closely with the other Beringian species rather than the Chinese species (see section 5.1 of this thesis). This is expected because this group of islands, also called the "Ring of Fire," shares the same geological history of Kamchatka and the Aleutian Islands, and are more likely to share species distribution.

Despite the high number of ecosystem types in Alaska, there are still four species, *D. ogilviensis*, *D. murrayi*, *D. stenopetala*, and *D. palanderiana*, which are distributed throughout the region. *Draba ogilviensis* and *D. murrayi* also continue into the Yukon, while *D. stenopetala* is also covering the entire Beringian area. *Draba palanderiana* covers most of northern North America going from Alaska, Yukon and the acrtic of Canada. There are two species known only from Kluanei National Park in the Yukon: *D. kluanei* and *D. yukonensis*. *Draba ventosa* has a wide distribution that is mainly Cordilleran, which continues from the Yukon to the Central Rocky Mountains. *Draba scotteri* has an extremely disjunct distribution, being reported only from the Yukon and again in the Great Basin.

The species distribution between the North American and Asian Beringia is also an interesting area of lineage mixing (see section 5.1). The mixing of lineages had ample opportunity during the at least two (or three) land bridges that existed in the area. It is now known that the entire Beringian area had an herbaceous tundra with enough plant cover to support the habitat for many large mammals such as mammoth. Ager (2003) proved among many other tundra herbs, Brassicaceae pollen was also found, we can assume *Draba* may be one of them. This habitat is also called a Mammoth-Steppe, and since the

introduction of its theory (Guthrie 1990), has been well correlated with paleobotanical and paleofaunal evidence (Zazula et al. 2002, 2005; Vetter 2000). The divide of *Draba* species distribution between these two regions is quite interesting. With *D. aleutica* being the only true Beringian species, as mentioned above, and on the other hand, other species are found to be mostly arctic or mostly North American Cordilleran. Further analysis of the genetic story in this region may lead us to more conclusions about this "*Draba* highway" between North America and Asia.

Amphi–Atlantic

The Amphi–Atlantic region includes the following smaller regions: Greenland (22 species), North Atlantic Islands (9), Atlantic European Forests (which includes the British Isles) (7), coastal Norway (10), and Lappland (18). The entire Amphi–Atlantic region has 28 Draba species. This is different from Hultén (1958)'s inclusion of 6 *Draba* species in his treatment of Amphi–Atlantic plants. However, here I include species which also have other distribution areas outside of the Amphi–Atlantic 'borders'. Since Hultén (1958)'s first description of the Amphi–Atlantic flora, much work has been done and the region is far better understood (see Brochmann et al. 2003, 2004) – especially due to the strong geological evidence of the Amphi–Atlantic land bridge (reviewed in Brochmann et al. 2003). However, fossil evidence indicating that the current lineages of plants have survived there *in situ* has not been found. Brochmann et al. (2003) explains that this region has been migrated into and across much more recently from the neighboring areas even when the mode has not been identified (i.e. dispersal across bodies of water). In fact the region seems to be better defined as one of constantly changing habitats containing highly hybridizing, self–fertilizing polyploids, and apomictic or highly asexual species that have survived in one place or another.

Within this data analysis, there are 15 different distribution patterns for the *Draba* species which occur in the Amphi–Atlantic region. Most of these species also have circumpolar distribution, but some are also distributed in either western or eastern North America, and others are also in Central and Southern Europe. These patterns are similar to most Amphi–Atlantic plants. This group has been identified as unique due to the comparison of the genetic haplotype information from cpDNA (see section 5.1 of this thesis). There are no *Draba* species that have a narrow distribution in the Amphi–Atlantic region. However, there are some unique cpDNA haplotypes. *Draba incana* and *D. lactea* are largely Amphi–Atlantic and circumpolar species with a range that continues into Siberia and Europe and the northeastern portions of North America. But there are many other Amphi–Atlantic species, including 12 other species (*D. alpina*, *D. arctica*, *D. arctogena*, *D. cinerea*, *D. corymbosa*, *D. crassifolia*, *D. nivalis*, *D. micropetala*, *D. norvegica*, *D. oxycarpa*, *D. pauciflora*, and *D. subcapitata*), of which some extends their ranges into the Russian Far East (and therefore circumpolar; see below), whereas others have ranges only into Greenland and northeastern Canada. *Draba cacuminum* has a narrow distribution to the Lappland and Norwegian coastline. The differences in distribution between the Atlantic coastal forests of Europe and the British Isles is slight. *Draba aizoides*, *D. muralis*, and *D. verna* are known on the British Isles and also in mainland Europe and the Mediterranean. *Draba aizoides* is also a commonly found alpine and sub-alpine European species found in all major mountain ranges within and as far west as the Carpathians, and also into the Mediterranean. The Amphi–Atlantic species have an interesting distribution which is a merging point between arctic, European and western Mediterranean groups. For example, *D. dedeana* is known from both the Atlantic coast of Europe and West Mediterranean. The Atlantic Ocean European coast

is home to *D. hispanica* which is found throughout the western Mediterranean, including the Atlas and neighboring mountains of Africa as well as in the Pyreenes. However, unlike *D. aizoides* and *D. dedeana*, *D. hispanica* does not venture out of its Mediterranean climates further into the Amphi–Atlantic region. The Pyreenes often share species with other European Alpine species, and not arctic ones. The other 21 species that are in this region are discussed within the following section due to their greater distribution patterns within the arctic and beyond.

Comments on Circum–North and Beyond

Six species, *D. arctica*, *D. arctogena*, *D. micropetala*, *D. oblongata*, *D. pauciflora*, and *D. subcapitata*, are exclusively circumpolar. Other circumpolar species have further distributions into neighboring regions. There are 10 distinct distribution patterns for 16 different species that are part of the circumpolar region. *Draba oxycarpa* has an extremely large distribution that ranges from the Aegean and southern Anatolian mountains, throughout arctic and non-arctic Siberia, most Amphi–Atlantic regions including Greenland, and across Beringia into Central Alaska. While *D. norvegica* has similar distribution range as *D. oxycarpa*, it does not continue south beyond Scotland, but instead reaches the majority of the Canadian forests. Three species, *D. corymbosa*, *D. borealis*, and *D. juvenilis*, are completely circumpolar and Cordilleran. *Draba fladnizensis*, has a similar distribution but goes even further into Siberia and as far south as the Tian Shan, and the alpine regions of Europe. Similar to *D. fladnizensis*, *D. nivalis* is found throughout the circumpolar, Cordilleran, and Siberian regions, but only goes as far south as the Altai and Sayan mountains and instead of the Tian Shan. It also covers most of the Canadian forests. Another extremely large distribution is that of *D. nemorosa* and *D. sibirica*. These two species range from most regions in Iranica, Anatolia, Caucasus, and the mountains of Central Asia, into Siberia and Europe and Scandinavia and ultimately circumpolar and Beringian. One could draw the conclusion that these species could map the progression of the genera from the center of origin in the Caucasus or Central Asian Mountains into the arctic. *Draba pilosa* is the only species occurs in all of the circumpolar regions, but it does not occur in Greenland. Four species occur in all areas except South America, Europe, and the Iranica and Caucasus regions: *D. alpina*, *D. cana*, *D. cinerea*, and *D. glabella*.

2.4 Conclusions

We also have fully illustrated the numerous patterns seen in *Draba* species on a global scale which has never been compiled before. In this Chapter, we now have a full description of the distribution of *Draba* as well as the identification of the highest center of species richness to be in the Central Rocky Mountains, and other areas with similar high numbers of species, such as Beringia, the arctic coastal tundra of North American and the mountains of Central Asia, and the Northern Andes. In addition, we can now see that the North American Cordillera has three distinct species patterns: northern, central, and southern. In the later Chapters of this thesis we will see the importance of these lateral divisions along the entire Cordillera. The uniqueness of the *Draba* found within the boundaries defined as Amphi–Atlantic have been shown to share species with almost all other regions where *Draba* is found. The species found within the northern part of the Central Asian Mountains, Altai, Sayan, and Mongolian Mountains seem to be a cross–roads between the *Draba* from further south and those from Siberia and the arctic. Arctic

Draba species have the widest distribution of all the regions which we will show in further chapters to be directly correlated with hybridizing lineages and species with higher ploidy levels. The arctic species have numerous representatives that also grow south into almost parts of *Draba* distribution (Cordilleran, European, and Asian), indicating the possible migration of arctic species further south during its most recent history (i.e. glacial cycles). The continent with the most unique set of species distributions is South America where no single *Draba* species occurs throughout the entire continent, despite the almost continuous mountain range of the Andes. Are these South American species more isolated than those from other major regions? This lays the foundation for all further work in studying the biogeographical, phylogenetic, and phylogeographical aspects of *Draba* species evolution, as well as eluding to the areas of *Draba*'s distribution that requires further attention like the Caucasus, for example.

Chapter 3

Species Richness in comparison to polyploid patterns in the genus *Draba* [1]

3.1 Introduction

Two major questions regarding polyploid evolution are: Do polyploids have an advantage over their diploid ancestors, and what causes high species richness to be positively correlated with high levels of polyploidy? Since these and related questions about polyploid evolution were first discussed by Stebbins and colleagues (e.g. Stebbins 1938, 1950, 1971, 1980), many studies have explored relationships between polyploids and diploids and examined their respective evolutionary advantages or disadvantages. Some studies posit that an evolutionary advantage to polyploids might not be necessary to bring about an increase in chromosome number. For example, Meyers and Levin (2006) demonstrated that as a species evolves, it eventually will undergo generally irreversible polyploidisation and therefore more polyploids will develop over a long period of time, and that ultimately all derived species will be polyploids at some point in the future. However, regardless of such inevitable increases in ploidy level, there seem also to be evolutionary or adaptive advantages to the process of polyploidy, as explained in the classic review by Levin (1983) and demonstrated in numerous studies since (reviewed in Soltis et al. 2004, 2007). Nonetheless, the question remains as to which specific processes or parameters favour an increase in ploidy level, and therefore what sort of environmental pressures might be responsible for such increases.

Increased polyploidy has been positively correlated with species richness by Petit and Thompson (1999) and Otto and Whitton (2000), however few other studies have explored this association. In a recent analysis of genera in the Rosaceae, where a positive correlation of polyploidy and high species richness was found, it was concluded that the polyploidy events themselves created high species diversity due to

[1] This chapter was published virtually in exact form: Jordon-Thaden, I. and Koch, M. (2008) Species richness and polyploid patterns in the genus *Draba* (Brassicaceae): a first global perspective. Plant Ecology and Diversity Vol.1, No.2, 255-263. Minor changes have been made to update the results after the completion of the phylogeny, but conclusions did not change.

polyploids being reproductively isolated from their parents, and thus recognised as new species (Vamosi and Dickinson 2006). However, it was not possible to explain why polyploidy and high species richness were correlated; for example, there was no association with growth habit. Certainly, it is not always the case that a correlation is found. For example, Nie et al. (2005) reported that in the Hengduan Mountains of south-western China, polyploids represent only 22% of the flora, and therefore polyploidy is not a cause of increased species richness in this area. However, as chromosome counts were known for approximately only 6% of all plant species in the region, the data available may have provided misleading conclusions. Here, we present an analysis of polyploidy in comparison to species richness within the globally distributed, environmentally extreme, arctic-alpine genus *Draba*, which may be classified as a young polyploid complex (Stebbins 1971; Grundt 2003; Grundt et al. 2004, 2005a; Marhold and Lihová 2006).

Draba currently is placed in the newly revised Arabideae tribe of the Brassicaceae (Al-Shehbaz et al. 2006; Koch and Al-Shehbaz 2008) and has a chiefly arctic-montane distribution throughout the world, being absent from extreme sandy deserts and tropical regions. While most common *Draba* species were described in the nineteenth and early twentieth centuries (e.g. Schulz 1927; Fernald 1934; Tolmachev 1939), new species are still being discovered from high alpine areas of the world (e.g Al-Shehbaz 2006, 2007). However, most taxonomic treatments of *Draba* are finalized (North America, Arctic, and China (see Appendix A) and many taxonomic uncertainties have been resolved.

We have generated a global map of species richness in *Draba* as a prerequisite for future studies on *Draba* evolution and systematics. Here, we provide a first examination of this species richness map and use it to illustrate patterns of ploidy level distribution in *Draba* to elucidate general aspects of species richness and polyploid evolution. A more detailed analysis of species richness in *Draba* will be discussed elsewhere.[2] Here we focus on presenting general patterns between species richness and ploidy level distribution, and also rates of speciation and polyploidisation in the genus.

3.2 Materials and Methods

3.2.1 Distribution of Species Richness

Species names, distributions and ploidy levels were compiled from Floras, checklists, and personal consultation with Ihsan Al-Shehbaz (see Appendix A). The status of the accepted names was cross-checked with Warwick et al. (2006), the *Draba* treatment in the Panarctic Checklist (Elven 2007), and FNA-Flora of North America (Al-Shehbaz et al. 2010). A total of 355 species are currently accepted, with approximately 10 or more species still in question (personal communication, Ihsan Al-Shehbaz). Maps were created and analysed with ArcView 9.1 (ESRI; California, USA) using shape files within the program. In particular, the WWF Ecoregions 200 Map shape file (Olson et al. 2001, and http://www.nationalgeographic.com/wildworld/terrestrial.html), which is a set of polygons that include ecological regions defined by a complex set of satellite imagery and known vegetation types, was used to generate the map images presented here. Species richness was assessed and grouped biogeographically into 75 regions largely corresponding to ecoregions defined by shared species, ecological similarities, and common phylogeographic patterns (Avise 2000; Hewitt 2004). In order to simplify the comparison to

[2]Refer to chapter 2 of this thesis where species richness is discussed in detail.

ploidy levels, these ecoregions were further combined into 5 major ecoregions based on observed species distribution patterns as described in each treatment of the species in Floras. The species occurrence matrix (presence/absence in of other levels of ecoregions can be found in chapter 2 and the Supplementary Materials Species Matrix. Extreme care was taken to correlate species distribution, ecoregion, and administrative or floristic boundaries provided in Floras and checklists (countries, counties, provinces, cities, mountain ranges, etc.). Here we focus on species richness and polyploidy in *Draba* within five major ecoregions of the world.

3.2.2 Ploidy distribution patterns

Chromosome counts and associated ploidy levels have been reported for 42.5% of the genus (151 of the 355 accepted species). Fourteen species are reported to have more than one ploidy level, giving the total number of ploidy levels recorded to be 165 out of 151 species. Base chromosome number is usually x = 8 (except for many North American Cordilleran species, see Table 3.3), and ploidy level varies from diploid to octadecaploid, with the most common polyploids being aneuploids (23.0%), tetraploids (20.6%), hexaploids (9.1%), and octaploids (6.7%) (Table 3.1). Diploids and polyploids comprise 22.4% and 77.6%, respectively, of the 165 ploidy levels reported. Ploidy levels for species in each of the five ecoregions recognised were incorporated into the *Draba* ecoregion shape file for an analysis of associations between distributions of species richness and ploidy levels.

3.2.3 Rates of speciation and polyploidisation

To calculate speciation (s) and polyploidisation (p) rates using the polyploid ratchet model of Meyers and Levin (2006), all ploidy levels of all species in a genus should ideally be known. However, due to the fact that chromosome numbers have not been recorded for all *Draba* species (approx. 204 out of 355 have no records), we conducted an initial analysis, also described by Meyers and Levin (2006), to estimate the number of diploids and polyploids expected in this 5 million-year-old genus (Koch et al. 2001). Estimates of rates of speciation and polyploidisation were based on the following assumptions. First, it was assumed that diploids are ancestral, meaning that a genus evolves from a single diploid ancestor and that it is possible to track changes in ploidy level as the genus evolves further through time. Second, a species is more likely to increase rather than decrease in ploidy level given the theory and evidence that indicate polyploid reversals are evolutionary dead ends (Stebbins 1980; Grant 1981; Ramsey and Schemske 2002). Third, that species with different ploidy levels all speciate at the same rate. Fourth, all species are sexual, and uneven chromosome numbers are excluded (see below). Fifth, autopolyploidy is ignored. This last assumption, of course, is unlikely to hold true and we expect at least some autopolyploids to be present in *Draba*, simply because of parallel observations in many other genera of crucifers (*Biscutella*, *Arabis*, *Cardamine*). However, it is evident from studies on material from Scandinavia by Christian Brochmann and colleagues (summarised in Koch et al. 2003; Koch and Kiefer 2006), from Central Europe (Widmer and Baltisberger 1999a), and from America (Koch and Al-Shehbaz 2002; Beilstein and Windham 2003), that allopolyploidy has led to reticulate evolution, and hybridisation has played an important and perhaps even dominant role during *Draba* speciation.

Even ploidy level	#	(%)
Diploid	37	(22.4%)
Tetraploid	34	(20.6%)
Hexaploid	15	(9.1%)
Octaploid	11	(6.7%)
Decaploid	6	(3.6%)
Dodecaploid	3	(1.8%)
Tetradecaploid	1	(0.6%)
Hexadecaploid	1	(0.6%)
Octadecaploid	1	(0.6%)
Total	109	(66.06%)
Uneven ploidy level	**#**	**(%)**
Triploid	7	(4.2%)
Pentaploid	6	(3.6%)
Heptaploid	2	(1.2%)
Pentadecaploid	2	(1.2%)
Enneploid	1	(0.6%)
n=Ì¸8 Aneuploid	38	(23.0%)
Total uneven + aneuploid	56	(33.94%)

Table 3.1: The number (#) and percentage (%) of *Draba* species at each ploidy level is listed. Percentages are out of the total number of different estimates for which chromosome counts are available (165 different counts out of 151 species).

We calculated the expected number of diploids ($\hat{X}_{d_n}$) in a genus (g) of age n, using the equation:

$$\hat{X}_{d_n} = y_{2,g} = \frac{o_{k,g}}{N_g} G_g \tag{3.1}$$

and the expected number of total polyploids ($\hat{X}_{p_n}$) in a genus (g) at age n, with the equation:

$$\hat{X}_{p_n} = \sum_{k=4,6,8\ldots} y_{k,g} \tag{3.2}$$

where for *Draba*, $n = 5$ million years. The symbol $y_{k,g}$ represents the number of species at a given ploidy level k, estimated by scaling the observed number of polyploid species ($o_{k,g}$) by the ratio of the estimated number of species ($G_g = 355$) to the observed number of even polyploidy species ($N_g = 109$). Note that in calculating $\hat{X}_{p_n}$ all even ploidy levels above diploid are included ($k = 4, 6, 8,$). In this data set, eight different ploidy levels ($4n, 6n, 8n, 10n, 12n, 14n, 16n, and 18n$) are above diploid and are used to calculate $\hat{X}_{p_n}$.

These estimates were, in turn, used to calculate the speciation rate (s) of the genus, in number of lineages

per million years, using the equation:

$$s = \sqrt[n]{\hat{X}_{d_n}} - 1 \tag{3.3}$$

Next when calculating the net rate of polyploidisation we assume that polyploid evolution is a result of a combination of speciation and polyploidy events. Therefore the speciation rate is used to calculate the rate of polyploidisation (p) as the mean increase in ploidy diversity per million years by the equation:

$$p = \sqrt[n]{\hat{X}_{p_n} + (1+s)^n} - (1-s) \tag{3.4}$$

As mentioned above, the analytical model of Meyers and Levin (2006) excludes the presence of uneven or unusual ploidy levels (aneuploids, triploids etc.) even when they exist in a genus. This exclusion follows from the assumption that all species are sexual. The polyploidy ratchet model was developed to estimate rates of speciation and polyploidisation in different genera over long periods of time (˜50 my). The large number of uneven ploidy levels present in *Draba* were necessarily excluded from estimates of speciation and polyploidisation in our study, and consequently our analysis provides a test of the versatility of the polyploid ratchet model in genera containing a high percentage of aneuploids and uneven ploidy levels.

3.3 Results

3.3.1 Species richness

The number of species of *Draba* recorded in each of five major ecoregions recognised is shown in Figure 3.1 and was as follows: Circum-North (71 species), Europe to Iran (69), the Greater Asian Mts (97), North America Cordillera(102), and South America (69). Major hotspots of richness are the North American Cordilleran Mts (102), which also includes Mexico, Beringia (55) in the Circum–North region), the circumpolar arctic coastal tundra (52 in the Circum-North region), the Northern Andes Mts (47 in the South American region), and several areas within the Greater Asian Mts region, i.e. the Tibetan and South-west Mts (52), the Himalaya to Afghan highlands (45), and the Alai, Pamir, and Tian Shan mountain ranges (33).

Approximately half (i.e. 175) of the 355 *Draba* species have a narrow to endemic distribution, while the other 180 species have medium to wide distributions. The most widely distributed species are those found within the arctic, which can be explained by the occurrence of large geographic areas without major geographic barriers (Grundt et al. 2006).

3.3.2 Ploidy distribution patterns

Tetraploids and hexaploids are the most common even-numbered polyploids in *Draba*, and are found almost throughout the entire range of the genus (Table 3.1). Octaploids (11 species) and decaploids (6) are mostly Circum-North, and North American Cordilleran species within the non-arctic North America region. *Draba* also has a large percentage of uneven ploidy levels (10.9%) aside from aneuploids ($x = 8$). However, considering the similarity in alternative reproductive modes (i.e. apomixis, alternative meiosis) between uneven chromosome levels and aneuploids, we have considered them together, giving a combined value of 33.9% of all levels reported.

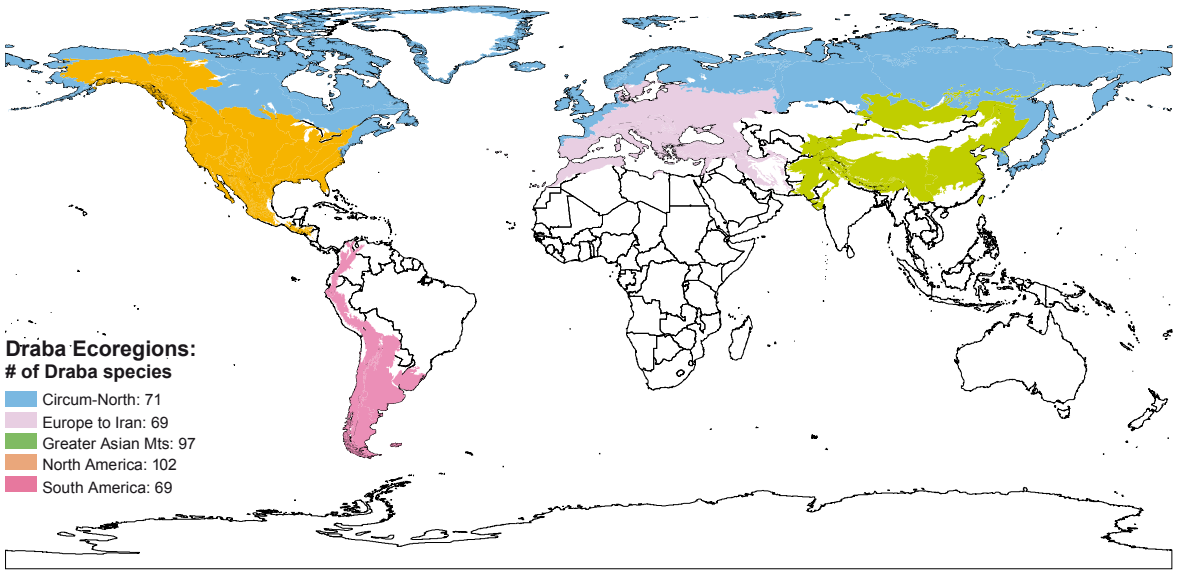


Figure 3.1: *Draba* species richness within five major ecoregions.

The relationships between ploidy level and species richness within ecoregions are presented in Table 3.1. Circum-North (73%) and North American Cordillera (84%) have the highest percentage of species with reported ploidy levels. In the Europe to Iran region, the ploidy of 62% of species has been estimated, while in the Central Asian Mts and South America there are chromosome counts for only 25% and 13% of *Draba* species, respectively. Ploidy levels have been plotted using the same shape files of ecoregion definitions as were used for species richness

Diploid and tetraploid species (the most common even polyploids) are dominant in the Europe to Iran region, where 51% and 33% of *Draba* species are diploid or tetraploid, respectively (Figure 3.2a-b; Table 3.2). When only polyploids are examined within each region, it is seen that the frequency of uneven to even ploidy levels varies drastically between regions (Figure 3.2c-d; Table 3.2). For example, of the 78 polyploids counted in North American Cordillera region, 60% exhibit an uneven ploidy level; these are found mostly in the Greater Rocky Mt area and surrounding areas of western North America. In contrast, in the Circum-North region, 80% of polyploids examined have an even ploidy level, while in the Europe to Iran region, 92% of the polyploids are even and mostly tetraploid. The Greater Central Asian Mts also contain a high proportion of even polyploid species (72%), but the data here are insufficient to draw firm conclusions. In this region, there are no chromosome counts available for the majority of narrowly distributed species. According to Table 3.2 (and also apparent from Figure 3.2d), South America has a very high percentage (89%) of even polyploids, but only nine different ploidy estimates have been reported for the 69 species occurring on this continent, and therefore the estimated percentage needs to be treated with caution.

A closer look at the distribution of species with uneven chromosome numbers, such as triploids, pentaploids, heptaploids, enneploids, and pentadecaploids shows that they are found mainly in high alpine regions (Figure 3.2c). Most triploid species occur in the North American Cordillera: *Draba albertina*-northern and central Cordillera; *D. rectifructa*-central to southern Cordillera; *D. crassa* and *D. grayana*-

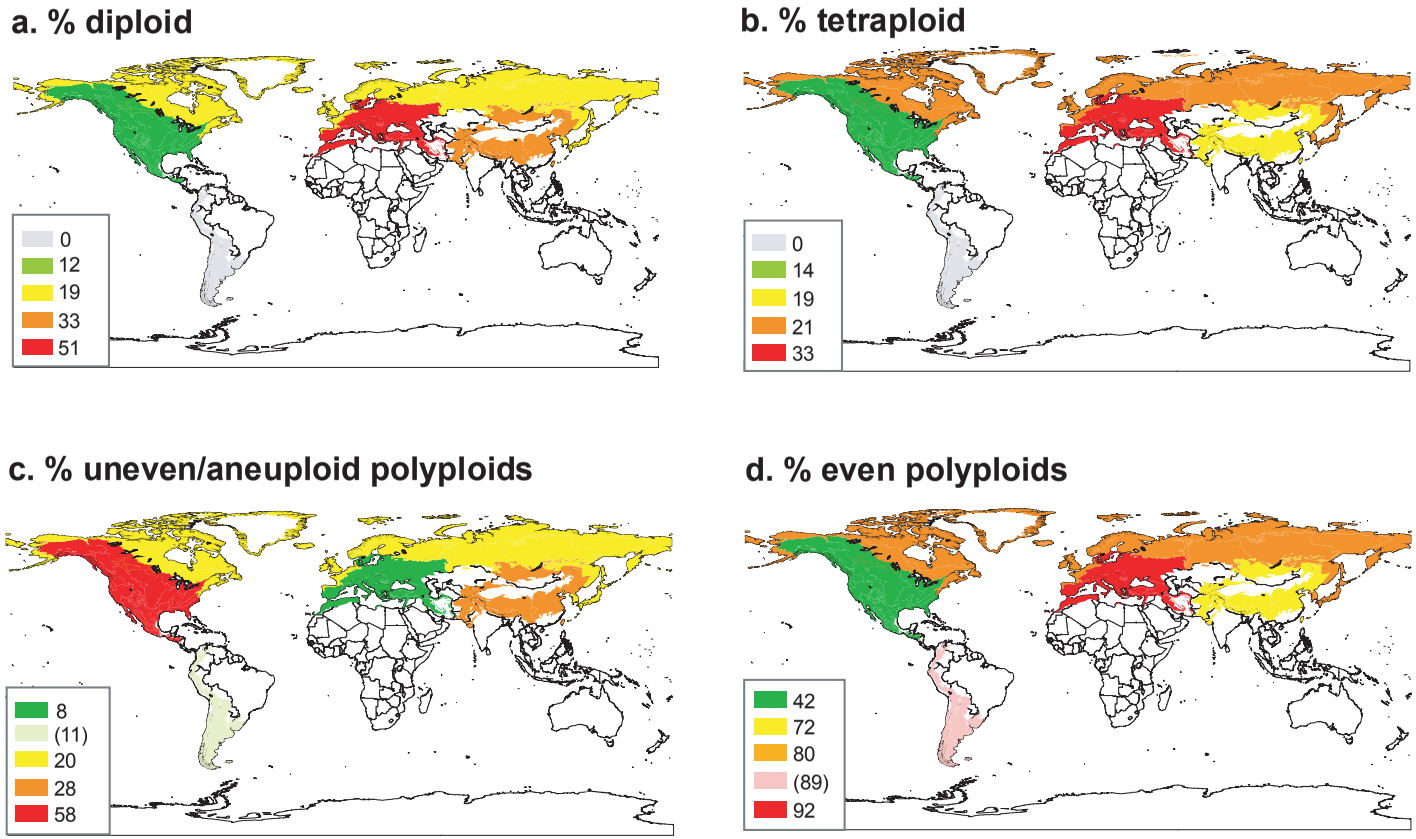


Figure 3.2: Percentages of (a) diploids, (b) tetraploids, (c) uneven/aneuploids, and (d) even polyploids within five major ecoregions. Percentages for South America in (c) and (d) are in parentheses due to them being based on a low number of chromosome counts in this region.

Ecoregion	(# sp. counted / # sp. in ecoregion). % sp. counted	Ploidy levels of total counts in region*			Regional comparisons of polyploids only**			
		# and % diploid	# and % tetraploid	# and % polyploid	uneven vs. even	$\% \leq 6x$	$\% > 6x$	$\% > 10x$
Circum-North	(52/71) 73%	(13/67) 19%	(14/67) 21%	(54/67) 81%	20%/80%	52%	48%	13%
North American Cordillera	(86/102) 84%	(8/86)9.3%	(10/86)12%	(78/86) 91%	60%/40%	69%	31%	6%
Europe to Iran	(43/69) 62%	(25/49) 51%	(16/49) 33%	(24/49) 49%	8%/92%	83%	17%	0%
Greater Asian Mts	(24/97) 25%	(9/27) 33%	(5/27) 19%	(18/27) 67%	28%/72%	67%	33%	6%
South America	(9/69) 13%	(0/9) 0%	(0/9) 0%	(9/9) 100%	11%/89%	89%	11%	0%

* A number of species have more than one type of ploidy level reported; therefore, 'total counts' does not usually equal the total number of species counted.

** The percentages in these four columns are of the total polyploid counts for each region (numbers in bold), and exclude diploid counts.$\% \leq 6x$ are those with chromosome numbers equal tor are less than 48, $\% > 6x$ are those with chromosome numbers greater than 48, $\% > 10x$ are those with chromosome numbers greater than 80

Table 3.2: Number (#) and percentage (%) of Draba species (sp.) for which chromosome numbers have been recorded in each of the five ecoregions (# sp. counted / # sp. in region), ploidy levels reported in each region (# / # of counts in region), and comparison of polyploids within each region, i.e. uneven vs. even, high vs. low levels.

Central Rocky Mts; *D. arida*-Great Basin mountain ranges; and *D. incrassata*-Sweetwater Mts of California, while one triploid species, *D. pickeringii*, is endemic to the Central Andes. There are five pentaploids: *D. oreades*, which is distributed widely in the Greater Asian Mts; *D. crassifolia* and *D. stenoloba*, which are generally North American Cordilleran and Canadian arctic species; the narrowly endemic species *D. streptocarpa*, from the Central Rocky Mts; *D. asterophora* from the Sierra Nevada and Great Basin mountain ranges; and *D. paucifructa* from the Great Basin mountain ranges. Other infrequent uneven-numbered polyploids are the enneploid-*D. ruaxes*, a northern North American Cordilleran species, and heptaploids *D. praealta*, which spans the entire North American Cordillera, and *D. exungiculata*, which is narrowly distributed in the Central Rocky Mts. Lastly is the pentadecaploid report of *D. alpina* from the Altai and Sayan Mountain ranges of Central Asia (Malyschev 1994). Most other reports of *D. alpina* are decaploid in its Circum-North distribution (Elven 2007; Al-Shehbaz et al. 2010).

Aneuploid *Draba* (38 species) are mostly restricted to mountainous regions (Table 3.3 and Figure 3.2c). [3]North America has 33 (+) aneuploid species comprising 40.7% of polyploids in this region. Three of the 33 in this region, also occur in the Circum-North region: *D. densifolia*, *D. aurea*, and *D. grandis*. Four more aneuploids are *D. verna*, an Asian and European annual species that is invasive in North America; Western Mediterranean *D. hederifolia*; *D. draboides* of the Tibetan and Central Chinese Mts; and *D. olgae*, a species occurring throughout the Greater Central Asian Mts. Lastly, *D. corymbosa* is an exclusively Circum-North species, and has been reported as either aneuploid, pentadecaploid, hexadecaploid, or octadecaploid (Elven 2007).

In South America (Figure 3.2c-d) *Draba pamploensis*, *D. pulvinata*, *D. chionophila*, *D. arbuscula*, *D. bellardii*, and *D. cryophila* are hexaploid species with narrow distributions in the Northern Andes. One other hexaploid species has been recorded in South America, *D. gilliesii*, while *D. magellanica* is an octaploid. The ploidy counts for these latter two species are from Patagonia and the Falkland Islands, respectively.

Also presented in Table 3.2 are the percentages of polyploids with ploidy levels equal to or lower than hexaploid ($6x = 48$), greater than hexaploid to octadecaploid ($> 6x = 48$), and greater than decaploid to octadecaploid ($> 10x = 80$). The Europe to Iran and North American Cordilleran regions are dominated by polyploid species of low ploidy level with 83% and 70%, respectively, having a ploidy level equal to or less than hexaploid (Figure 3.3a). In contrast, within the Circum-North region polyploid species of high ploidy level are common, but coexist with those of lower ploidy level, with 52% from diploid

[3]After the publication of this chapter, it was brought to my attention by M. Windham of the following corrections in this table (Windham 2000, 2003). My definition for an aneuploid was any species with an unusual chromosome level that is either not base x = 8, or an uneven level of number of chromosomes in the somatic phase. However, I had erroneously stated a number of these species as having a base of x = 8 when in fact meiotic counts have been made to be otherwise. For most of these alternative base chromosome numbered species, a mitotic chromosome count has not been made and it is assumed they are acting as diploids, even when this may not be the case. The following species are x = 10: D. burkei (C.L. Hitchc.) Windham & Beilstein, D. pedicellata (Rollins & Price) Windham (with one 2n = 20 count), D. spectabilis Greene var. spectabilis, D. sphaerocarpa J.F. Macbr. & Payson, D. sphaeroides Payson; x = 11: D. juniperina Dorn, D. kassii Welsh; x = 12: D. albertina Greene, D. arida C.L. Hitchc. with 2n = 24, D. rectifructa C.L. Hitchc.; x = 13: D. cusickii B.L. Robins. ex O.E. Schulz, D. sobolifera Rydb., D. subalpina Goodman & C.L. Hitchc.; x = 15: D. asperella Greene var. stelligera O.E. Schulz with 2n = 30; x = 16: D. maguirei C.L. Hitchc. var. maguirei, D. oreibata J.F. Macbr. & Payson, D. pennellii Rollins; n = 18: D. argyrea Rydb.; n = 20: D. asterophora Payson var. asterophora, D. crassifolia Graham, D. paucifructa Clokey & C.L. Hitchc; n = 27: D. hitchcockii Rollins, D. jaegeri Munz & Johnston with 2n = 54. The patterns inferred within this paper are not significantly changed. In fact, these counts add to the list of species in the North American Cordillera that have alternative chromosome patterns.

Chromosome #*	Species	Region
12	*D. olgae*†	Greater Central Asian Mts
18	*D. graminea*	Central Rocky Mts: SW Colorado
18	*D. helleriana*	Central to Southern Cordilleran
20	*D. abajoensis*	Colorado Plateau, SW Arizona and New Mexico Forests
20	*D. aureola*	Cascade Mts
20	*D. burkei*	Central Rocky Mts: Utah
20	*D. pedicellata*	Great Basin Mt Ranges: Nevada and Utah
20	*D. santaquinensis*	Central Rocky Mts: Utah
20	*D. spectabilis*	Central to Southern Cordilleran
20	*D. sphaerocarpa*	Central Rocky Mts: Idaho
20	*D. sphaeroides*	Great Basin Mountain Ranges: Nevada
22	*D. kassii*	Great Basin Mt Ranges: Utah
22	*D. mogollonica*	Mogollon and neighbouring Mts in New Mexico
22	*D. pectinipila*†	Central Rocky Mts
24	*D. jorullensis*	Southern Cordilleran: Arizona and Mexico
26	*D. cusickii*	Cascade Mts and SE Oregon
26	*D. sobolifera*	Central Rocky Mts: Utah
26	*D. subalpina*	Central Rocky Mts: Utah
26	*D. zionensis*	Central Rocky Mts: Utah
30	*D. asprella*	SW Arizona Forests
30	*D. hederifolia*† † †	Western Mediterranean
36	*D. argyrea*	Central Rocky Mts: Idaho
36	*D. densifolia*	Cordilleran and Arctic Alaska
36	*D. grandis*	Northern Cordilleran and Beringian
36	*D. sharsmithii*	Eastern California Mountain Ranges
36	*D. ventosa*	Northern and Central Cordilleran
42	*D. draboides*†	Tibetan and Central Chinese Mts
42	*D. novolympica*	Northern and Central Cordilleran
42	*D. paysonii*†	Central Rocky Mts: Wyoming and Montana
44	*D. brachystylis*	Utah and Nevada
50	*D. lemmonii*	Eastern California Mountain Ranges
54	*D. hitchockii*	Central Rocky Mts: Idaho
54	*D. jaegeri*	Great Basin Mountain Ranges: Nevada
54	*D. serpentina*	Great Basin Mountain Ranges: Nevada
56±5	*D. exunguiculata*	Central Rocky Mts: Colorado
74	*D. aurea*	Cordilleran and North American Arctic
120, 120±10, 128, 128±10, 128, 135, 144	*D. corymbosa*††	Circumpolar
14, 28, 30, 32,36, 38, 39, 52, 58, 64	*D. verna*††	Asian, European, Circum-North, (North American)

* Most counts are referenced in Al-Shehbaz et al. 2009 except for those marked otherwise.

† Warwick and Al-Shehbaz 2006

†† Elven 2007

† † † Tattou 1999

Table 3.3: Chromosome numbers and locations of aneuploid *Draba* species (i.e. those with alternative base chromosome numbers or uneven ploidy levels.

to hexaploid, 48% above hexaploid to octadecaploid and more exclusively with 13% above decaploid to octadecaploid (Figure 3.3b-c). These highest ploidy levels (Figure 3.3c) refer to the Circum-North species *D. corymbosa* (aneuploid, pentadeca, hexadeca, or octadecaploid) and *D. pilosa* (decaploid to dodecaploid), the Cordilleran *D. incerta* (tetradecaploid), and *D. arabisans* an Eastern Canadian species (dodecaploid). The Greater Asian Mts region shows a greater percentage of diploids to hexaploids (67%), but also contains a substantial percentage of higher polyploids (Table 3.2). From the data presented here, therefore, it can be seen that as ploidy level increases, the distribution becomes increasingly restricted to species-rich regions, i.e. montane areas and ultimately into the arctic.

3.3.3 Rates of speciation and polyploidy

The expected number of diploid species was estimated to be 120, while the expected number of polyploid species was calculated to be 234 (i.e. with even ploidy level only). The expected number of species at each even polyploid level were: $4n = 111, 6n = 49, 8n = 36, 10n = 20, 12n = 10, 14n = 3, 16n = 3, and 18n = 3$ (refer to Table 3.1 for observed values). These estimates for 5 million year old *Draba* yielded a rate of speciation of s = 1.61, and a rate of polyploidisation of p = 0.627. These estimates are extremely high compared to generalised rates for flowering plants. Thus, according to Levin and Wilson (1976): for herbs s = 1.05, p = 0.05; for shrubs s = 0.24, p = 0.01; and for trees s = 0.09, p = 0.001. The high rates of polyploidisation which are estimated here indicate that polyploidisation has consistently contributed to the evolution of new lineages and species in the genus *Draba*.

3.4 Discussion

3.4.1 Species richness and the distribution of polyploids

Draba diploids (22.4%) coexist with polyploids (77.6%) throughout the geographic distribution of the genus, and thus *Draba* can be classified as a young polyploid complex (see Stebbins 1971). High percentages of diploids and tetraploids in the Europe to Iran ecoregion suggest the occurrence of ancestral *Draba* lineages there, and that long-term speciation in this region was less influenced by the origin of higher ploidy polyploids than was the case in other ecoregions (Figure 3.3). However, a phylogenetic analysis of the entire genus will allow more precise conclusions to be drawn on this point.

Greatest species diversity in *Draba* was found in the Circum-North region and the North American Cordillera. The presence in the North American Cordillera of a high percentage of aneuploids and species with uneven ploidy might be explained in different ways. It could reflect the consequences of a suture zone between two major clades (Koch and Al-Shehbaz 2002) in North America, with putative cytogenetic imbalance of hybrids stabilized by subsequent asexual propagation via apomixis. Alternatively, apomixis may have been selected during periods of environmental change (Koch and Al-Shehbaz 2002) resulting in the creation of species of both uneven and high ploidy. The situation is comparable with that in the North American genus *Boechera* (Brassicaceae) where swarms of aneuploids and apomicts were formed many times independently during the Pleistocene (Koch et al. 2003; Dobeš et al. 2004; Schranz et al. 2005, 2006) resulting in the creation of approximately 102 species. The high percentage of polyploids recorded in the North America Cordillera that are either less than or equal to hexaploid indicates a

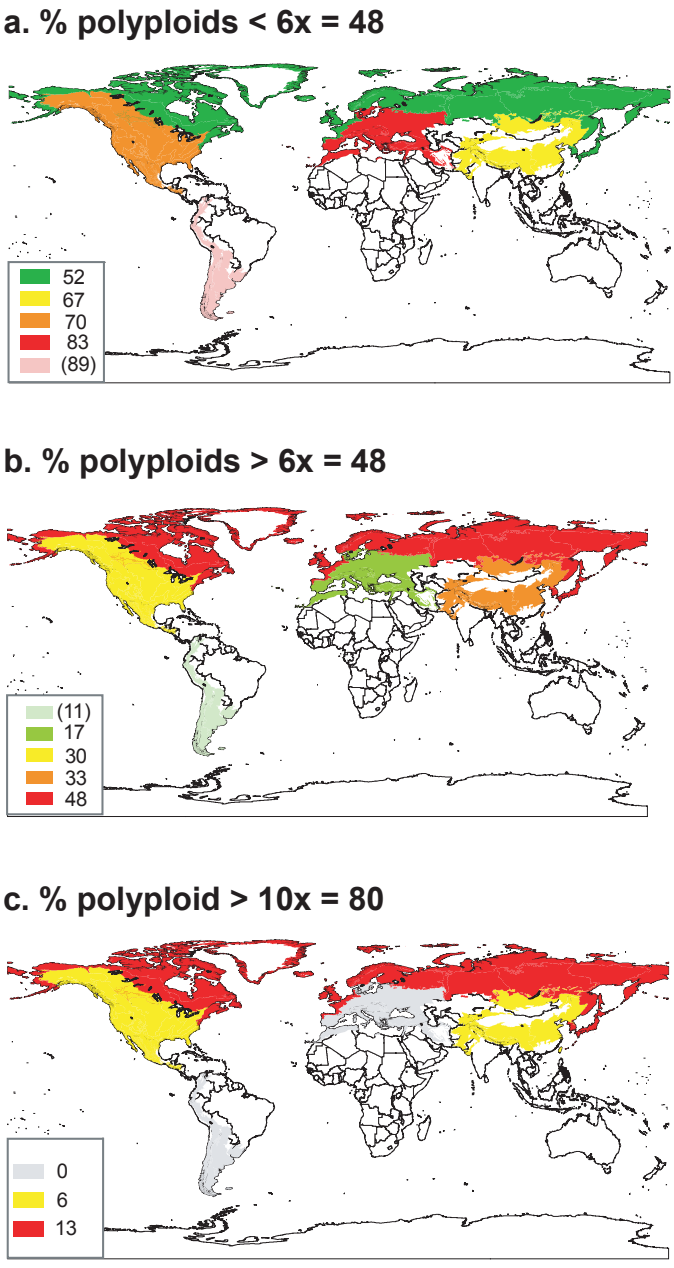


Figure 3.3: Percentages of polyploid levels from low to high in five ecoregions show an altitudinal and latitudinal gradient. (a) Polyploids with $6x = 48$; (b) Polyploids $> 6x = 48$; (c) Polyploids $> 10x = 80$.

younger polyploid complex in comparison to that present in the Circum-North region. This, together with the presence of abnormal ploidy levels suggests that speciation events in the region are relatively recent and likely to have coincided with recent glaciation cycles. It is possible that a similar scenario holds for the Greater Asian Mts; however, a lack of data from this region means that caution is currently required in drawing this conclusion.

The Circum-North species, which are predominately above hexaploid and reach as high as octadecaploid, coexist with diploids, indicating that there has been considerable migration throughout this region. Brochmann et al. (2003) hypothesised that species which survived in ice-free regions during glaciations, would have been subjected to repeated migration into and out of such refugia during glaciation cycles, thus encouraging polyploid formation. This may have occurred frequently in *Draba* in the most extreme north of its range and resulted in the formation of polyploids of high ploidy level.

Inspection of the distribution of species ploidy levels together with species richness, allows some conclusions to be drawn about causes of high diversity in *Draba*. The data presented here are in agreement with Stebbins (1984) conclusion that degree of glaciation is ultimately more correlated with an increased occurrence of polyploidy than latitude per se. Interestingly, a previous analysis of only arctic species (Brochmann et al. 2004) showed that degree of glaciation was not correlated with polyploidy. However, from the present study it seems that when patterns of polyploidy are observed on a worldwide scale, and at the genus-level, a more complete picture is obtained indicating a trend of increased ploidy level in regions affected by glaciation cycles, most notably in mountainous and northern regions (Figure 3.3). As pointed out by Tribsch (2004), glaciation cycles are the most important factor contributing to areas with high levels of endemism, which are significantly correlated with low amounts of glacial ice. Moreover, Thompson and Whitton (2006) have shown that in the North American species, *Townsendia hookeri*, asexual polyploids are exclusively found in formally glaciated areas, while diploids are confined to unglaciated areas. In regard to *Draba*, we hypothesize that it has been the ability of the genus to speciate frequently via polyploidy that has resulted in high species richness, and that this has been promoted in the Circum-North and the North American Cordillera regions during glacial cycles through adaptation to a wide range of extreme ecosystems and environmental fluctuations.

3.4.2 Speciation and polyploidy rates

The rates of speciation (s) and polyploidisation (p) that we estimated for *Draba* are much higher relative to rates estimated for plant groups by Levin and Wilson (1976). The results of Meyers and Levin (2006) confirmed that the accumulation of polyploids in a genus need not result from greater fitness of polyploids relative to diploids. However, we cannot eliminate this possibility in *Draba*, where polyploids might show increased adaptation to environmental variation and change. The combination of high speciation and polyploidisation rates in *Draba* suggest that the genus has developed a large number of polyploids which has accelerated speciation rate. It is likely that the late Pleistocene glaciation cycles were a major cause of accelerated speciation in *Draba*, given that highest species richness and high levels of polyploids occur within and around glacial boundaries and at high altitudes.

Chapter 4

Molecular Phylogeny and systematics of the genus *Draba* (Brassicaceae) and identification of its closest related genera [1]

4.1 Introduction

Draba L., the largest genus in the family Brassicaceae and a member of the tribe Arabideae (Al-Shehbaz et al. 2006; Bailey et al. 2006; Koch et al. 2007), is taxonomically diverse and comprises over 370[2] species (Warwick et al. 2006). It is distributed in the arctic, subarctic, alpine, and most mountainous regions of the world. *Draba* is mostly perennial, with some annuals and biennials. Unique to this study, we propose that many of the lowland and coastal temperate species in Eurasia and North America are not within the genus *Draba*. The genus is well defined morphologically, and the existing sectional classification of (Schulz 1927, 1936) is based solely on convergent morphological characters such as the presence vs. absence of cauline leaves, petal color, and style length. Previous molecular studies (Koch and Al-Shehbaz 2002; Beilstein and Windham 2003; Grundt 2003) demonstrated that most of Schulz's sections are polyphyletic. Furthermore, Tolmachev's (Tolmachev 1939) classification, which assigns the 91 species of the Former Soviet Union to 29 series, is largely incongruent with both Schulz's system and the preliminary molecular phylogenetic investigations of *Draba* by Koch and Al-Shehbaz (2002).

Draba was subjected to many studies covering several aspects of its systematics, includingpolyploid speciation (Brochmann 1992, 1993; Brochmann and Elven 1992; Brochmann et al. 1992a,b,c,d; Widmer and Baltisberger 1999b; Grundt et al. 2004, 2005b; Jordon-Thaden and Koch 2008; Skrede et al. 2009,

[1] This chapter was published virtually in exact form: Jordon-Thaden, I., Hase, I., Al-Shehbaz, I., and Koch, M. (2010) Molecular Phylogeny and systematics of the genus *Draba* (Brassicaceae) and identification of its closest related genera. Molecular Phylogenetics and Evolution, in press.

[2] An estimate of *Draba* species is approximately 390, but have not all been fully described (personal communication, Ihsan Al–Shehbaz.)

2008), cytology (Brochmann et al. 1993; Grundt et al. 2005a; Mulligan 1966, 1970b,a, 1971a,b, 1972, 1974; Mulligan and Porsild 1969; Windham 2000, 2004), breeding systems (Mulligan and Findlay 1970; Grundt et al. 2005a), molecular phylogeny (Koch and Al-Shehbaz 2002; Beilstein and Windham 2003), phylogeography (Widmer and Baltisberger 1999a), and taxonomy of selected species (Al-Shehbaz 1989, 1990, 1991, 1992, 1994, 2002, 2004b,a, 2007; Al-Shehbaz and Koch 2003; Al-Shehbaz and Windham 2007; Elven and Al-Shehbaz 2008; Price and Rollins 1991; Rollins 1984; Rollins and Price 1988; Scheen et al. 2002). In addition, Skrede et al. (2008) have constructed a newly developed QTL-mapping population of arctic *Draba nivalis* to investigate quantitative traits meaningful for understanding the evolution of arctic *Draba* species.

Although the above studies showed the potential of cytological and molecular investigations for a better understanding of the systematics and phylogeny of this complex genus, the picture remains largely incomplete. Koch and Al-Shehbaz (2002) provided what was then the most comprehensive phylogenetic analysis of *Draba* using molecular markers, but that study dealt with a limited number of North and South American species and did not address the monophyly of the genus. Even after this study, a worldwide taxon sampling, including the most divergent species and possible closest relatives, is needed. Their study demonstrated that *Arabis* L. s.str. is more closely related to *Draba* than believed in previous classifications of the Brassicaceae (von Hayek 1911; Schulz 1927, 1936; Janchen 1942), which assigned the two genera to different tribes based on fruit morphology. The most recent classification of the family (Al-Shehbaz et al. 2006; Bailey et al. 2006; Koch et al. 2007) placed the two genera in one tribe, Arabideae. *Arabis* is clearly distinct morphologically from *Draba* and, therefore, serves as an ideal outgroup in phylogenetic studies on *Draba*. The Eastern North American *Draba* were found to be genetically distinct and may form independent genera (Koch and Al-Shehbaz 2002). We are able to confirm herein most of what was hypothesized but on a global scale. Most importantly, the addition of annual Arabideae outgroups (e.g., *Heterodraba* Greene and *Athysanus* Greene) to this dataset allowed a better assignment of the annual *Draba* species and delimitation of *Draba*'s core groups.

The frequent hybridization events, often followed by polyploidization (e.g., Brochmann 1992) and sometimes apomixis (Koch, unpublished), complicate the evolutionary history of *Draba* and make the reconstruction of a comprehensive phylogeny a challenging task. Despite the striking alpha diversity and well-defined biogeographic patterns (Jordon-Thaden and Koch 2008), few molecular phylogenetic studies have been performed on this genus. In addition, the phylogenetic position of the segregated genera *Abdra* Greene, *Athysanus*, *Drabopsis* K. Koch, *Erophila* DC., *Graellsia* Boiss., *Heterodraba*, *Schivereckia* Andrz. ex DC., and *Tomostima* Raf. needs to be addressed in relation to *Draba*.

Abdra and *Tomostima* were segregated from *Draba* by Greene (1900) and Rafinesque (1825), respectively, and were recognized by Schulz (1927, 1936) as sections (*Abdra* (Greene) O. E. Schulz and *Tomostima* (Raf.) O. E. Schulz) and were reduced to synonymy of *Draba* by all other contributing authors (e.g., Fernald 1934; Rollins 1993). Both *Abdra* and *Tomostima* comprise annual species with apetalous, cleistogamous, late-season flowers. *Drabopsis* was long recognized as a monotypic genus, but Nagshi and Javied (1984) added *Drabopsis brevisiliqua*, which was reduced by Al-Shehbaz and Koch (2003) to synonymy of *Draba nuda* (Bél.). de Candolle (1821) established *Erophila* as a genus originally described by Linnaeus in 1753 as *Draba verna*. Hyam and Jury (1990) revised the Southwest Asian *Graellsia* and expanded it to include the Moroccan *Draba* sect. *Helicodraba* O. E. Schulz. Finally, Berkutenko (1995) showed that the type of *D. hyperborea* (L.) Desv. belongs to *Schivereckia*, an eastern European genus. The species

was erroneously assigned by Schulz (1927, 1936) to *Draba* sect. *Nesodraba* (Greene) N. Busch and also was erroneously recognized by Hitchcock (1941) and Rollins (1993) as a North American and Russian Far Eastern species, instead of accepting the correct name *D. grandis* Langsd. *Heterodraba* and *Athysanus* were established by Greene (1885) as monospecific genera based on species originally described in *Draba* and *Thysanocarpus* Hook., respectively. Both of Greene's genera were recognized by Rollins (1993) in his alphabetical arrangement of taxa, but Schulz (1936) placed *Athysanus* in the tribe Alysseae and *Heterodraba* in the tribe Drabeae, whereas Al-Shehbaz et al. (2010) united them in one genus, *Athysanus*, in the tribe Arabideae.

The aim of this study is to investigate the classification of *Draba* using a diverse sample of geographically and morphologically very distinct species. To achieve that, we reconstructed phylogenies based on sequence polymorphisms of two unlinked gene regions, the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) and the combined trnL intron and spacer of the chloroplast DNA (cpDNA) region trnL-trnF (collectively trnL-F). The combined use of these independently inherited markers facilitates the independent assessment of hypotheses on interspecific evolution and can reveal cases in which introgression or incomplete lineage sorting has caused incongruence among phylogenies. Specifically, this study aims to: (1) test the monophyly of *Draba*, as suggested by Koch and Al-Shehbaz (2002), (2) provide a genus-wide robust hypotheses on the evolutionary relationships among *Draba* species, (3) test the monophyly of the infrageneric classifications of Schulz's (Schulz 1927) sections and Tolmachev's (Tolmachev 1939) series, and (4) survey the phylogenetic relationships of *Abdra*, *Athysanus*, *Drabopsis*, *Erophila*, *Graellsia*, *Heterodraba*, *Schivereckia*, and *Tomostima* to *Draba*.

4.2 Materials and Methods

4.2.1 Introductory comment on methodology

Herbarium vouchers were sampled for leaf tissue from 15 different herbaria (see Accession List and Herbarium Photos in Supplementary Materials). Live material was collected for only a few accessions (deposited in HEID and O). Because of the varying quality of the accessible leaf material and the observation that the success of the PCR was directly related to the purity of the DNA prior to the PCR reaction, we used in various modifications of the protocols (e.g., purification of the DNA by using NucleoSpin©Plant, silica columns in tubes, following the manufacturer's instructions, Macherey-Nagel, after the traditional CTAB method). Strong PCR bands and full length sequences were obtained from cleaned DNA without any additional PCR additives or special adjustments to the PCR reaction times and temperatures. However, it was not necessary to clean all samples with a column in order to obtain good PCR and sequencing results. By contrast, samples difficult to access were successfully amplified after cleaning with a column (see above) in most cases and regardless of the age of the herbarium material.

4.2.2 Molecular Markers

The molecular markers selected for this analysis were previously shown to be useful in resolving species-level phylogenetic relationships in other groups (e.g., Guo et al. 2004; Woods et al. 2005; Koch et al. 1999; Samuel et al. 2003). Rapidly evolving intron and intergenic spacer regions were amplified from both the

chloroplast (trnL introns and trnL-F intergenic spacer: hereafter trnL-F) and the nuclear genome (internal transcribed spacer region of nuclear ribosomal DNA including ITS1, 5.8S, and ITS2; hereafter ITS). The trnL intron was amplified using the forward primer 5'-CGA AAT CGG TAG ACG CTA CG-3' and the reverse primer 5'-GGG GAT AGA GGG ACT TGA AC-3', referring to primers c and d according to Taberlet et al. (1991) Taberlet et al. (1991), which anneals in the first and second exon of the trnL gene, respectively. For amplification of the trnL/F IGS (hereafter trnF), we used primers 5'-GGT TCA AGT CCC TCT ATC CC-3'f forward (Taberlet et al. 1991, primer e according to) and 5'-GAT TTT CAG TCC TCT GCT CTA-3' reverse (Dobeš et al. 2004), annealing in the second exon of the trnL gene and the trnF gene, respectively. Sequences comprised the complete intron and the second exon of the trnL gene. The primer used to amplify the complete ITS region (including ITS2, ITS1, and the 5.8 S rDNA region) was the ITS 18 forward 5'-GCA TGT TTT CCC AGT CAC GAC GGA - AGG AGA AGT CGT AAC AAG G-3‘ which includes an M13 extension (the last 19 bases). The reverse ITS-25 primer was 5'-ACT TCA GGA AAG AGC TAT GAC GGG TAA TCC CGC CTG ACC TGG-3' which also includes an M13 extension (the first 21 bases). Internal primers ITS2A-int (GCA ATT CAC ACC AAG TAT CG) and ITS3-int (GCA TCG ATG AAG AAC GTA GC) were used in the beginning of the project when amplification in one step of the whole ITS region failed. However, this step was not necessary after it was realized that cleaning the DNA resulted in better PCR products for all markers. For sequencing ITS, the M13 extension primer alone was used for the PCR products that had been generated with the M13 extension attached to the ITS 18 for and ITS 25 rev primers. The M13 extension for forward primer is 5'-GCA TGT TTT CCC AGT CAC GAC-3' and reverse is 5'-ACT TCA GGA AAC AGC TAT GAC-3'.

4.2.3 DNA extraction, amplification, and sequencing

DNA extractions were carried out using a modified protocol of the CTAB extraction method of Doyle and Doyle (1987) that involved grinding in 2 ml round-bottomed Eppendorf tubes with glass beads and using a Retsch swing mill (MM 200), and addition of 2 units of ribonuclease per extraction tube to the isolation buffer. For each extraction, approx. 50-75 mg of herbarium leaf material was used. Before further use, DNA was diluted 1:3 in TE-buffer. The PCR reactions were performed in a total volume of 30μl containing ProMega colorless GoTaq buffer (including 3mM MgCl2), 0.4 μM of each primer, 0.2 mM of each dNTP, one unit GoTaq DNA polymerase (ProMega), and approximately 100 ng of template DNA using a PTC200 (MJ Research) thermal cycler. For the PCR cycling scheme of the ITS region, a ẗouch-down PCRẅas applied to amplify of the target sequence only. However, later it was determined that the touch-down PCR was not needed if the DNA was cleaned prior to PCR. The amplification program for ITS included a denaturing step for 2 min at 95°C, followed by 29 cycles of 30 s denaturing at 95°C, 30 s annealing at 56.8°C, 30 s elongation at 72°C; then a final elongation of 5 min at 72°C. For cpDNA markers the thermal cycling was exactly the same as for ITS except the annealing temperature was 54.6°C for trnF and 48.4°C for trnL. PCR products were checked for length and concentrations on 1.5% agarose gels, staining with ethidium bromide, but later switched to non-toxic Sypro Ruby Red. Before sequencing, the obtained PCR products were purified using a PCR product purification kit (Macherey-Nagel). Multiple random repeats of sequences were made as well as negative controls to ensure there was no contamination between samples. Cycle sequencing was performed using the Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) and the original amplification primers following the manufacturer's protocol. Products were analyzed on a MegaBACE 500 capillary sequencer

(Amersham Biosciences). Cycle sequencing was performed on both strands; in the majority of cases, each reaction spanned the complete sequence. Accessions that did not give quality results in the first PCR or sequencing attempt were filtered as mentioned above in this section of the materials and methods. Sequences that were poor even after filtering were excluded from the phylogenetic analyses.

4.2.4 Sampling of species

The species nomenclature was adapted from the Brassicaceae checklists and current flora treatments (Warwick et al. 2006; Elven 2007; Al-Shehbaz et al. 2010). Taxon sampling was designed to cover most of the sections of Schulz (1927, 1936) and the series of Tolmachev (1939). Furthermore, the sampling included the full range of morphological and biogeographical diversity in the genus. A total of 384 accessions, representing 164 *Draba* species and six segregate genera (*Abdra*, *Drabopsis*, *Erophila*, *Graellsia*, *Schivereckia*, and *Tomostima*), were used in this analysis. The sampling also comprised 11 outgroup accessions, including four of *Athysanus*, three of *Arabis hirsuta*, and one each of *A. rimarum*, *A. alaschanica*, *Pseudoturritis turrita*, and *Heterodraba unilateralis*. The sample included about 45% of the species in a given global region in which *Draba* occurs. The complete accession list, including taxonomic and geographic affiliations, is shown in Accession List in Supplementary Materials. Samples were obtained from herbarium material (B, BM, CAS, DAO, E, GH, JEPS, K, LE, MO, UC, US, and WHB), but some additional accessions were collected as silica-gel dried leaf material from natural populations in various circumarctic regions (provided by Dr. C. Brochmann and group, vouchers in O) and the Slovakian Carpathians (vouchers in HEID). Other ITS sequences were used with permission from Beilstein and Windham (2003), and the vouchers are deposited in NMC and UT. DNA from these accessions was also shared with us to generate trnL-F sequences. All accessions were geo-referenced using Google Earth and plotted on maps with ArcView-ArcGIS 9.1 (ESRI, California) (Figure 4.4). Geographic abbreviations following species names in the phylogenetic trees indicated in the Supplementary Materials.

4.2.5 Sequence selection and compilation of alignment

Sequences were assembled automatically and subsequently adjusted by hand using SeqMan (DNAStar; Madison, Wisconsin). In proof-reading the sequences, several ambiguous base-callings were encountered, and these were coded using IUPAC (International Union of Pure and Applied Chemistry) ambiguity codes. However, whenever possible, sequences with too many ambiguities were omitted from the dataset because the sequence quality was too poor and the ambiguities were most likely unreal. After sufficient species and geographic representations of the genus were in the sequence dataset, an alignment was made by hand within GeneDoc (Nicholas et al. 1997, Ver. 2.6.002). The boundaries of the ITS spacer regions and the 5.8S gene were estimated in comparison with previously published sequences (Lihová et al. 2004; Bailey et al. 2006; Koch and Al-Shehbaz 2002). In more than half the total accessions, each species was represented by at least two different accessions (i.e. two separate herbaria sheets) for comparison within the alignment. When two sequences of the same species were questionable, a third (and often fourth) accession of the same species was added to the alignment. Also, if a species occurs frequently in many different geographical regions, accessions from all the necessary locations were attempted. For this analysis, it was imperative that both the trnL-F and ITS sequences were of high quality in order to use the accession. Quality was judged by sequence length, low number of missing data, and low

number of ambiguous sites. A total of 384 accessions were compiled (alignments in Supplementary Materials), which included all of the above mentioned repeated species. Detailed observation of the resulting phylogenetic analysis of the combined gene alignment was done to assure the sequences truly represented each species to avoid the following potential errors: First, this eliminated those accessions which were obviously misidentified herbarium vouchers and led to subsequent correction of the vouchers. Second, the placement of the species within the tree was confirmed by the addition of more accessions for each taxon. Third, species which have had a complicated history of hybridization could be easily recognized because numerous accessions from representative regions for each of these species were added. All molecular sequences are archived in GenBank with accession numbers listed in the Accession List in the Supplementary Materials.

4.2.6 Phylogenetic analyses

Parsimony analyses

Models of nucleotide evolution were selected with hLRT and AIC using ModelTest version 3.7 (Posada and Crandall 1998; Akaike 1974). Parsimony analysis was done in PAUP* 4.0 beta10 (Swofford 2002). Parsimony analysis was done with the branch-swapping algorithm5 (TBR) and was set to reach a maximum of 10,000 trees, with random addition of sequences, 10000 replicates, using the nchuck (number of chucks) of 25 with a chuck score greater than the distance of each tree (trnL-F = 400, ITS = 700, combined = 1200). Bootstrap analysis for 1000 replicates was done in PAUP* but never finished after six months and stopped after 165 replicates. Tree statistics and results can be seen in Table 4.1 and Supplementary Materials.

Bayesian analyses

Gaps in the sequence data were coded as missing values (N) with no additional coding. Bayesian analysis, using Monte Carlo Markov Chain Metropolis Coupling (MC3), was carried out with the MPI (i.e. for parallel processing) version of MrBayes version 3.4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). In all phylogenetic analyses, trees were rooted with *Pseudoturritis turrita* and other Arabideae genera (*Arabis* spp., *Athysanus*, *Heterodraba*). Bayesian MC3 analyses of nucleotide characters from each of the loci (ITS and trnL-F) and a combined analysis of all nucleotide characters were performed with MrBayes using the most basic models available (i.e. equal rates), since using the suggested models via ModelTest gave the same topology and only increased the analysis time dramatically (data not shown but available upon request). The trnL-F and ITS runs were carried out with 20 million generations, using six simultaneous runs, with a temperature set to t = 0.11, with a run time of 40 hours on a parallel processing machine of four processors, running in GNOME with 4 GB RAM. The tree of the combined gene analysis was created after 400 million generations using four simultaneous runs, with the temperature set to 0.01 (t = 0.01). The final analysis of the combined gene alignment took 976 hours (40 days). The temperature of the hot chains was reduced from the default of 0.2 after tests to ensure efficient chain swapping (data not shown). For each run, 1000 trees were sampled and 25% of the trees were discarded (burnin=250) when creating the consensus trees (see Supplementary Materials). Tree statistics are shown in Table 4.1. Cartoon trees were drawn to relative scale

for each marker separately and the combined analysis using the program MEGA4 (Tamura et al. 2007; Kumar et al. 2008). Completion of the Bayesian runs was accessed using the online program AWTY (Are We There Yet, Nylander et al. 2008). Cumulative split frequency plots and the comparison of the split frequencies between runs 1 and 2 were generated (Supplementary Materials).

4.3 Results

4.3.1 ITS sequences

The full nrITS1-5.8S-ITS2 alignment for the 384 sampled accessions, including the outgroup taxa, consisted of 629 characters, of which 337 were constant and 214 were potentially parsimony informative (Table 4.1). No evidence of paralogous ITS sequences was found because all PCR products were resolved as a single band, and no double peaks were encountered. Using ModelTest 3.7 (Posada and Crandall 1998), the GTR + I + G model with a proportion of invariable sites of 0.2748 and a discrete gamma rate (gamma shape parameter 0.7256) was chosen as the model of sequence evolution for the ITS dataset by nested hierarchical Likelihood Ratio Test (hLRT). The model chosen with the Akaike Information Criterion (AIC) was SYM + I + G; however, their results were not significantly different from hLRT. Therefore, the GTR was used. The models were only used for the Parsimony analysis in PAUP* and not in the Bayesian analysis in MrBayes. Maximum parsimony analyses of the full nrDNA ITS dataset converged a large set of trees with a length of 877 steps (CI = 0.50; RI = 0.88; see also Table 4.1) and a strict consensus tree was calculated (Supplementary Materials). The Bayesian analysis with the ITS dataset resulted in an average likelihood score of -7696.51 and an average harmonic mean of -7676.87, with the final average standard deviation of split frequencies of 0.039615 after six simultaneous runs and 20 million generations (cartoon of ITS tree Figure 4.1, full tree in Supplementary Materials).

4.3.2 trnL-F sequences

The final aligned trnL-F data matrix for the 384 sampled accessions consisted of 942 nucleotide positions of which 852 were used in the analysis. In this dataset 568 characters were constant, and 174 were variable and parsimony informative (Table 4.1). Using ModelTest, the F81 + G model of sequence evolution with a proportion of invariable sites at zero and a gamma-distributed rate variation (gamma shape parameter 0.5167) was chosen by hLRT. The AIC test gave a different model of TVM + I + G sequence evolution. The TVM model gave a significantly better lnL and was therefore selected for maximum parsimony analysis. Parsimony analysis of the chloroplast DNA sequence data resulted in a strict consensus tree with 550 steps (CI = 0.63; RI = 0.92) and a strict consensus tree was calculated (Supplementary Materials for full Heuristic tree). Bootstrap analysis were done, but never completed and therefore are not shown. Bayesian analysis of the trnL-F region gave an average log likelihood of -5764.40 and an average harmonic mean of -5883.82. The final average standard deviation of the split frequencies was 0.033846 for six simultaneous runs, after 20 million generations (cartoon of tree in Figure 4.2, full Bayesian tree in Supplementary Materials).

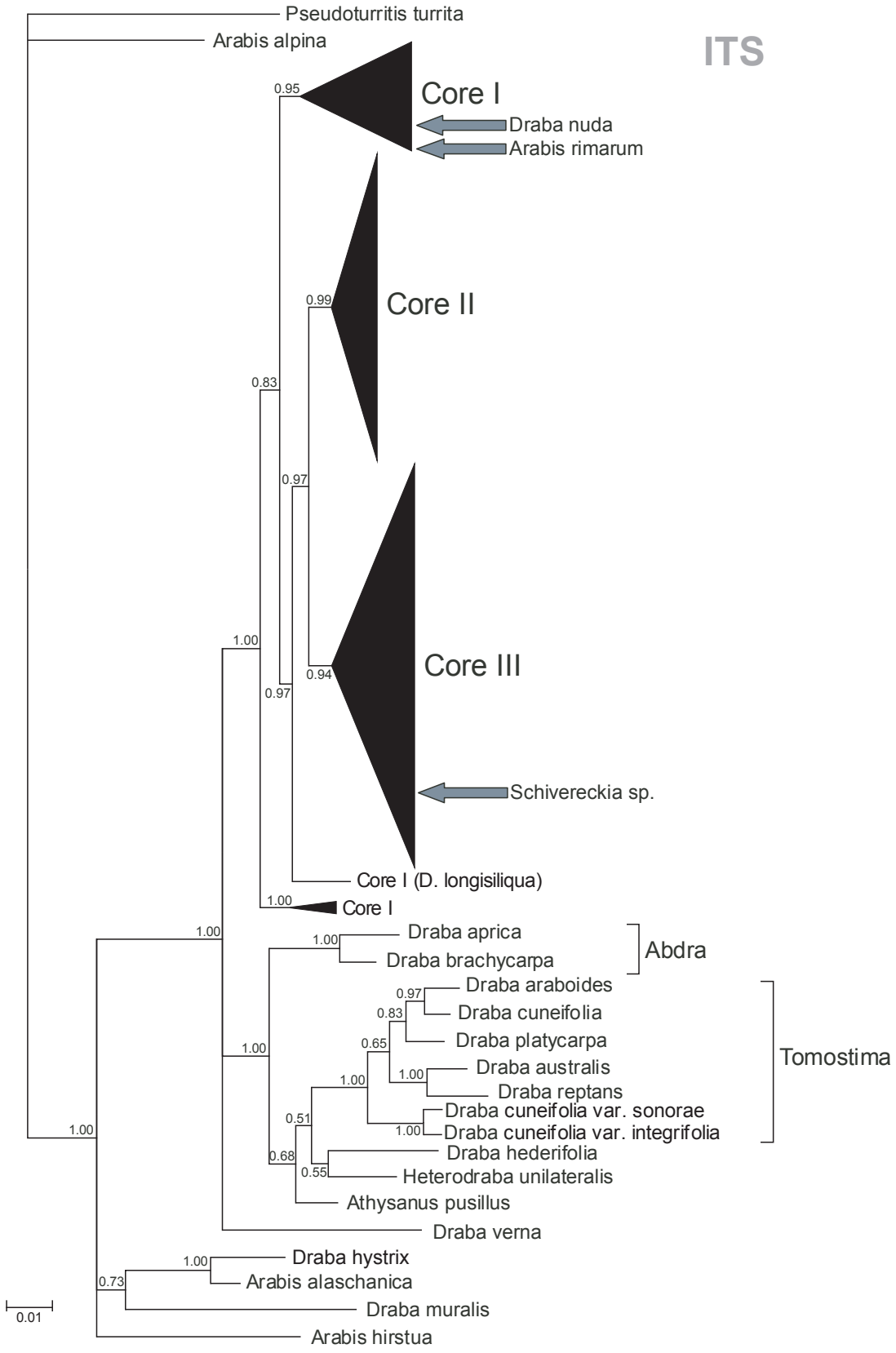


Figure 4.1: Cartoon illustrating the phylogenetic framework from the ITS dataset.

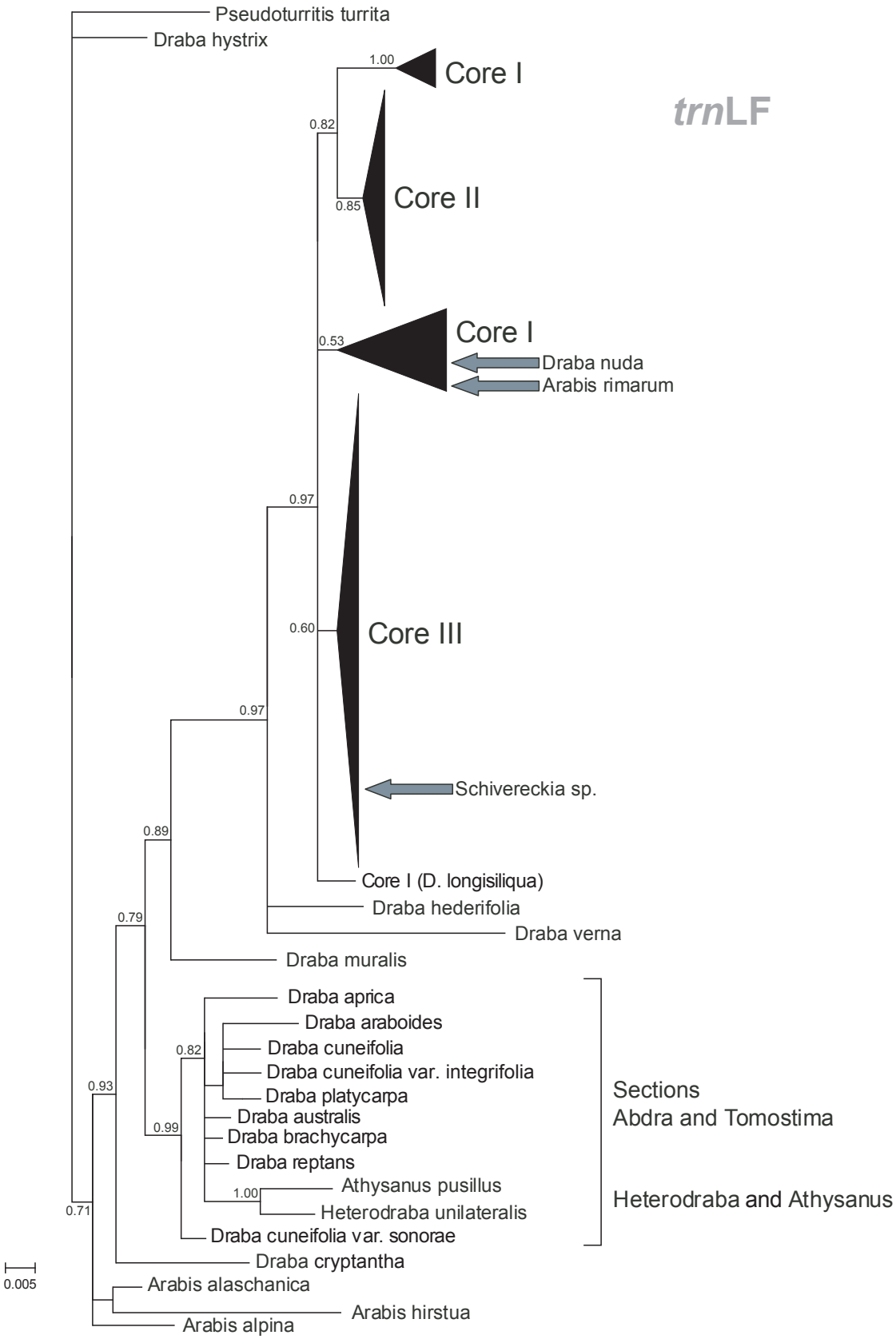


Figure 4.2: Cartoon illustrating the phylogenetic framework from the trnLF dataset.

	Nuclear ITS	**Plastid *trn*L-F**	**Combined (cp+nr)**
No. ingroup accessions	383	383	383
No. outgroup accessions	1	1	1
Sequence length	629	942	1571
Aligned length used in analysis	629	852	1429
No. variable characters	292	284	559
No. of parsimony-informative characters	214	174	374
Tree length (steps) (parsimony)	877	550	1566
Consistency index (CI) (parsimony)	0.50	0.63	0.49
Retention index (RI) (parsimony)	0.88	0.92	0.86
Avg. log Likelihood (Bayesian)	-7696.51	-5764.40	-14,424.65
Avg. harmonic mean (Bayesian)	-7676.87	-5883.82	-14,737.95
Final Avg. std dev of split frequencies (Bayesian)	0.039615	0.033846	0.029220
No. trees sampled/run (Bayesian)	1000	1000	1000
Number of generations (Bayesian)	20 million	20 million	400 million
Number of simultaneous runs (Bayesian)	6	6	4
Temperature setting (Bayesian, t = #)	0.11	0.11	0.01

Table 4.1: Values, statistics and settings from the phylogenetic analyses (parsimony and Bayesian) of separate and combined data matrices

4.3.3 Combined ITS and trnL-F sequences

The full nrITS1-5.8S-ITS2 and trnL-F alignment for the 384 sampled accessions, including the outgroup taxa, consisted of 1571 characters, and of the 1429 used, 870 were constant and 374 potentially parsimony informative characters (Table 4.1). Using ModelTest the GTR + I + G model was selected with both hLRT and AIC with the proportion of invariable sites 0.3303 and the variable sites give a gamma distribution shape parameter of 0.7341. Here again, these model settings were only used for the parsimony analysis. Maximum parsimony analysis of the full dataset gave a tree length of 1566 with a consistency index of 0.49 and a retention index of 0.86 (see Supplementary Materials for full Heuristic tree). Bayesian analysis resulted in an average log likelihood of -14,424.65 and an average harmonic mean of -14,737.95 for four simultaneous runs for 400 million generations (cartoon of tree in Figure 4.3, full tree in Supplementary Materials). The final average standard deviation of split frequencies was 0.029220. In addition, the harmonic mean for run 1 was -14,572.20, run 2 was -14,591.97, run 3 was -14,575.51 and run 3 was -14,739.26 which gives an average difference of 86.3 between the runs. This indicates a relatively significant convergence considering the number of internal polytomies that were not resolvable with these markers.

Analysis of the ITS, trnL-F and combined Bayesian tree files with AWTY showed that convergence was mostly reached and further generations may not achieve greater results with this dataset (see Supplementary Materials for AWTY plots for each Bayesian run). The backbone and general topology of the trees generated from Bayesian analysis of the ITS, trnL-F and combined data (Figs. 4.1, 4.2, and 4.3 and Supplementary Materials for the full trees) are highly congruent with the topology of the strict consen-

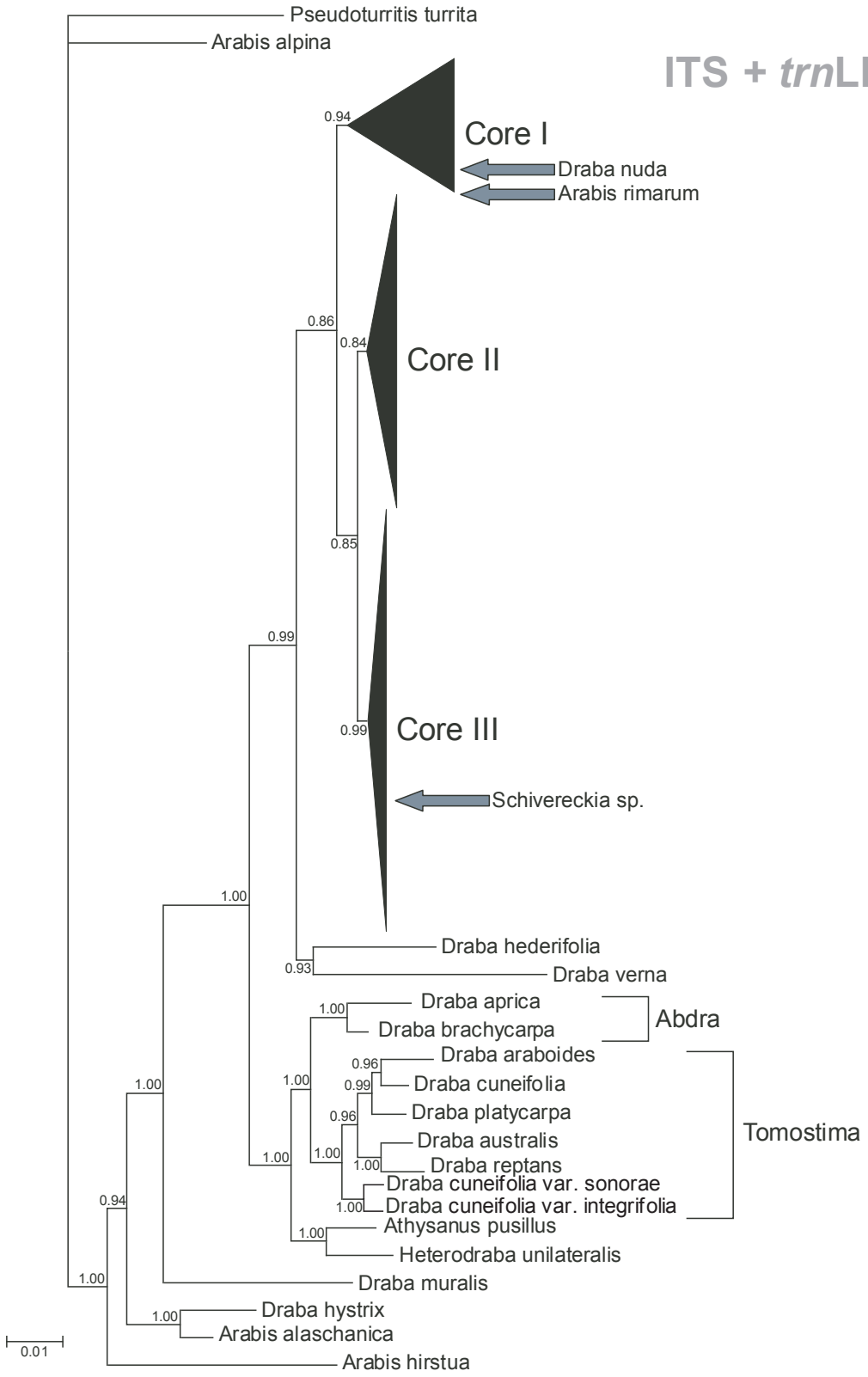


Figure 4.3: Cartoon illustrating the phylogenetic framework from the ITS and trnLF combined dataset.

Group	# Acc	# sp	
Core I	52	24	
Core II	121	66	
Core III	163	68	
Basal	9	2	
Out	39	6	outgroup sp
		9	Draba
Totals	384	175	Total
		169	Draba
			$\sim$ 45% of the genus

(a) Composition of tree shown in Supplementary Materials of the combined analysis (*trn*L-F and ITS sequences) indicating number of accessions and species per group.

Species	Shared Cores
D. aurea	II & III
D. eriopoda	I & III
D. micropetala	II & III
D. oligosperma	II & III
D. oreades	I & III
D. surculosa	II & III
D. alpina	II & III

(b) Possible hybridizing species that appear in more than one Core (i.e. I, II, or III)(marked with pink in Supplementary Materials trees).

Table 4.2: Species composition of Bayesian phylogenetic tree throughout the three identified Cores for the combined analysis of ITS and trnLF. See Supplementary Materials for full tree.

sus tree from the parsimony analysis (Supplementary Materials for the full trees), showing three major "Cores" and basal annual species and genera. In the trnL-F analysis, Cores II and III do not separate as well as they did in the ITS and combined analysis. In general, most major branches were supported with moderate to high posterior probability values (Figs. 4.1, 4.2, and 4.3). For all three parsimony analyses, the bootstrap support values after 165 replicates were mostly insignificant, and are therefore not shown.

4.3.4 Description of the three Cores of *Draba*

A total of 384 accessions was analyzed, with 52 fell in *Draba* Core I (24 species), 121 in Core II (66 sp.), 163 in Core III (68 sp.), nine in the basal group (2 sp.), and 39 outside of *Draba* (6 outgroup species and 9 *Draba* species) (Figure 4.4 and Table 4.2a).

Out of approximately 370 species in the genus, this represents $\tilde{4}5\%$ of the genus (169 species). In the trnL-F analyses, 26 species (16%) are found in a different core as is in the ITS and combined analyses (marked as such in Supplementary Materials). The remainder of the species (84%) are stably found in the same Core in all three markers. Most taxa appeared within the same larger Core (I, II, or III), but sometimes occurred in different places within the Core. However, seven species consistently appeared in more than one Core (Table 4.2b): *D. alpina*, *D. aurea*, *D. eriopoda*, *D. micropetala*, *D. oligosperma*, *D. oreades*, and *D. surculosa*. These seven species, which may be subject to a complex pattern of hybridization, are highlighted with pink in the full Bayesian trees (see Supplementary Materials). Extensive analysis with tree building still does not resolve the phylogeny fully, and alternative methods of data analysis will be done on a larger scale.

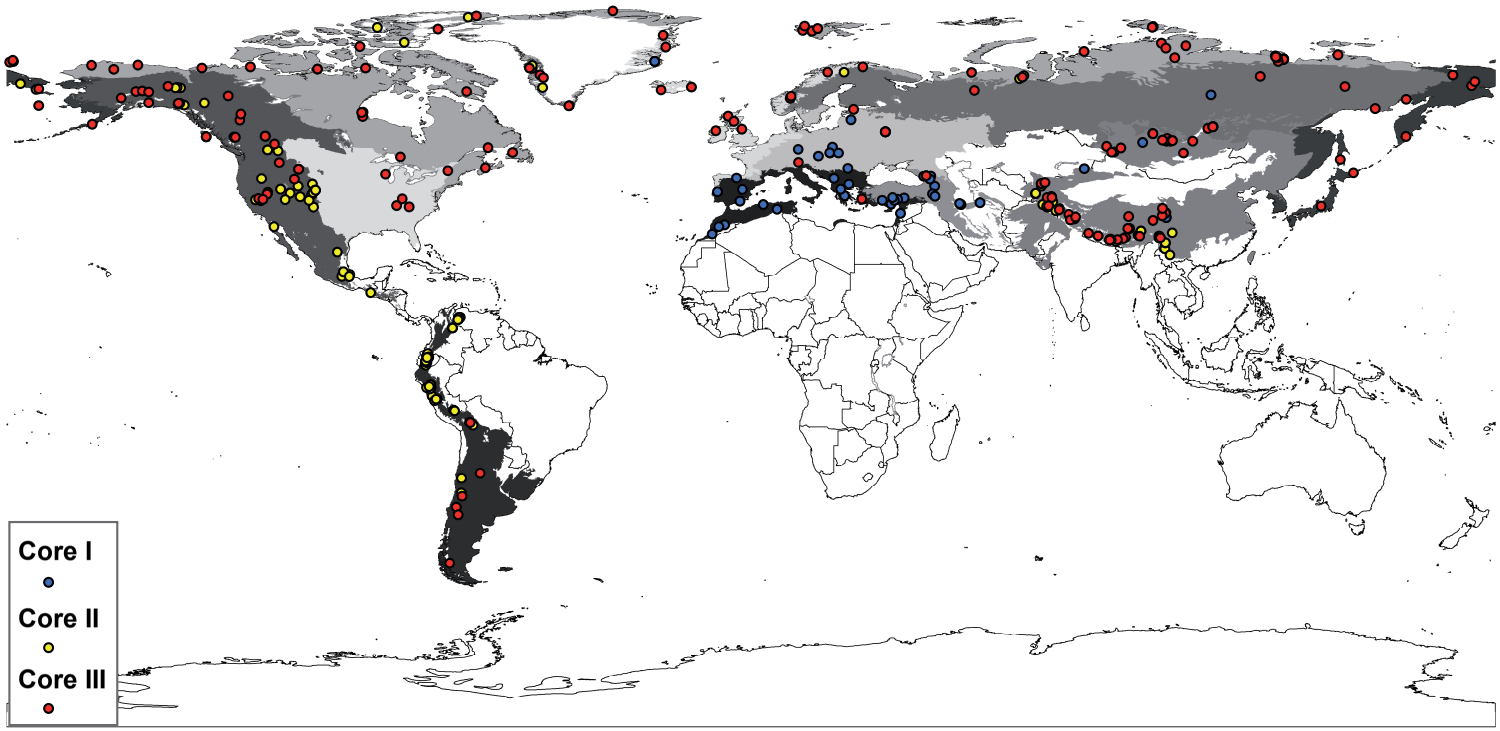


Figure 4.4: Map of 384 georeferenced accessions for the phylogenetic analysis of the genus *Draba*, with the three cores indicated.

Draba Core I

Core I consists mostly of species occurring in a region defined by Jordon-Thaden and Koch (2008) to extend from Europe into Iran (Figs. 4.1, 4.2, and 4.3 and Supplementary Materials) and includes non-arctic alpine Europe, Mediterranean ecosystems, Turkey, Caucasus, and Iran. Central Asian species make up a small part of this core and are highly supported as monophyletic clade. The ITS and combined analyses show there are virtually no arctic, North and South American *Draba* in this Core. The few exceptions are the movement of *D. inquisiviana* and *D. macleanii*, two South American species, from Core II to Core I in the trnL-F trees. *Draba oreades* and *D. eriopoda* are the only species which also showed up Core III. The Caucasian *D. longisiliqua* is the basal species of this core. *Draba nemorosa*, once thought to be a basal species that grouped with *D. verna* in Koch and Al-Shehbaz (2002) and placed in sect. Drabella by Schulz (1927, 1936), is well supported within Core I and is a sister to *Draba nuda* (formerly *Drabopsis nuda*). *Arabis rimarum*, added to expand the outgroup, unexpectedly appeared as a perfectly good *Draba* within Core I (see below).

Draba Core II

Core II consists mostly of North and South American Cordillera *Draba*. Again, central Asian species represent a small portion of this core, with one group positioned at the center of the core and the other at its base. Six species (*D. stenopetala*, *D. ogliviensis*, *D. crassifolia*, *D. albertina*, *D. ventosa* and *D. eschscholtzii*), occurring in the northern edges of the North American Cordillera and/or the Russian Far East (i.e. Beringia), and three arctic species (*D. micropetala*, *D. pauciflora*, *D. aurea*) also belong to Core II. Aside from these mentioned species, the core is predominantly North and South American. Four species (*D. aurea*, *D. micropetala*, *D. oligosperma*, and *D. surculosa*) of this Core (highlighted in pink in Supplementary Materials) also appeared in Core III.

Draba Core III

Core III is the most heterogeneous in *Draba*, though it is largely represented by 50 Central Asian, circumarctic, and Beringian species. However, this core has nine western North American (*D. murrayi*, *D. ruaxes*, *D. praealta*, *D. porsildii*, *D. grandis*, *D. incerta*, *D. paysonii*, *D. subumbellata*, and *D. breweri*) and four South American (*D. scopulorum*, *D. magellanica*, *D. gilliesii*, and *D. tucumanensis*), and four European and Caucasus species (*D. elegans*, *D. heterocoma*, *D. tomentosa*, and *D. supranivalis*). Core III also includes two eastern North American species (*D. arabisans* and *D. ramosissima*) together with six amphi- Atlantic species (*D. incana*, *D. alpina*, *D. subcapitata*, *D. arctica*, *D. cana*, and *D. nivalis*).

Schivereckia podolica and *S. doerfleri* are found with Central Asian, Siberian, and northern North American species as a well supported clade at the base of Core III in the ITS and combined analysis. This clade is not well resolved in the trnL-F analysis, but is still well within Core III. Therefore, *Schivereckia* species (highlighted in yellow in Supplementary Materials) ought to be placed in *Draba*.

4.3.5 The fate of the fallen *Draba* species

Perhaps the most significant finding shown in the present data is the exclusion of basal annual *Draba* from the genus after the inclusion *Heterodraba unilateralis* and *Athysanus pusillus* in the outgroup. The last two taxa, thought to be closely related to *Draba*, are monophyletic to annual basal *Draba*. Schulz (1927, 1936) placed most of these annual *Draba* into sections *Abdra* and *Tomostima* (Table 4.3). Therefore, we conclude that *D. aprica* and *D. brachycarpa* should be recognized as species of *Abdra* and that *D. cuneifolia*, *D. araboides*, *D. platycarpa*, *D. australis*, and *D. reptans* should all be assigned to the genus *Tomostima*. Considering the significance of such a drastic change for so many species, we included as many accessions as possible for each species to ensure the proper placement of these excluded species within the phylogenetic analysis (see Supplementary Materials). The three varieties of *D. cuneifolia* did not occur exactly on the same branch and did not form a monophyletic clade. It is therefore concluded that *D. cuneifolia* var. *sonorae* and *D. cuneifolia* var. *integrifolia*, already considered distinct species, should both be recognized as species of *Tomostima*.

The other surprising finding is the placement of *Draba muralis* and *D. hystrix* (Figs. 4.1, 4.2, and 4.3, and Supplementary Materials) outside of *Draba*. *Draba muralis* was recognized as the type species of the genus *Drabella* (DC.) Fourr. over 140 years ago, a position we fully support. *Drabella* was not recognized as a genus by almost all authors, though it was treated by Schulz (1927, 1936) as a section of *Draba*. Additionally, *Draba hystrix* fell in a clade one more branch out from *Drabella*, but most significantly it was well resolved within *Arabis* (Koch et al., unpubl.). Morphological comparisons of these species and nomenclatural changes are been prepared by these authors in a separate publication. Further studies are in progress to incorporate the molecular data with the morphological data of *Draba* and *Arabis*. tod

4.3.6 Basal (?) *Draba* species

Draba hederifolia and *D. verna* are strongly supported to be basal species to the genus *Draba* in the trnL-F and combined analysis (Figs. 4.2 and 4.3). However, their ITS placement is intermingled with the annual group (Figure 4.1). Their exact relationship with other groups remains unclear (See Discussion).

4.4 Discussion

Despite its enormous variability in every conceivable character, *Draba* is a morphologically well-circumscribed genus that can be separated from other genera of the Brassicaceae by a combination of latiseptate (flattened parallel to the septum) silicles (or rarely siliques), biseriate seeds four to numerous per fruit, accumbent cotyledons, and branched trichomes (Schulz 1927; Hitchcock 1941; Al-Shehbaz 1988; Appel and Al-Shehbaz 2003). Extensive work has been done to revise parts of the genus (Elven 2007; Al-Shehbaz et al. 2010; Cheo et al. 2001). However, previous revisions and monographic studies on the classification of *Draba* (Pohle 1925; Schulz 1927; Tolmachev 1939; Hitchcock 1941) were either narrow in scope or largely outdated and do not meet modern standards. Numerous new species have since been described (150), and there is urgent need to fully understand the phylogeny of this challenging genus. Although the sectional synopsis of Schulz (1927, 1936) offers a rich source of information on the ca. 210 of the currently recognized 370-plus species (Al-Shehbaz, unpublished), it clearly falls short of providing

data on the relationships among closely related species and genera or among sections within *Draba*.

The present sampling and phylogenetic analyses, including that of Koch and Al-Shehbaz (2002), form a solid basis for proposing a future infrageneric classification of *Draba*. As indicated above, 16 of the 17 sections of Schulz (1927, 1936) and 26 of 29 series of Tolmachev (1939) were sampled herein for the combined DNA analysis. They are displayed according to their placement in the three new Cores in Table 4.3 and Table 4.4, where their petal color is also indicated. In order to represent the geographic range of *Draba*, over 45% of the species occurring in each region (i.e. Circum-North, western North America, South America, eastern North America, Europe to Iran, and Greater Central Asian Mountains; see Jordon-Thaden and Koch 2008) were included to give a solid view of the entire genus. The molecular data strongly supports uniting *Draba* with the previously recognized *Drabopsis* and *Schivereckia*, moderately supports the inclusion of *Erophila* and Moroccan *Graellsia* in *Draba*, and strongly supports the exclusion of several annual *Draba* species into *Abdra*, *Drabella*, and *Tomostima*, as well as the placement of *D. hystrix* possibly in *Arabis* s.l. or a separate genus. Further discussions on each of these groups are given below.

This study is based on sampling 45% of *Draba*, and includes multiple accessions (50% of the dataset) for most species. Therefore, we were able to elucidate many relationships. All of the multiple accessions are presented in the large trees in the Supplementary Materials. This enabled us to identify those species that had complicated evolutionary histories. Several new, well-defined, major evolutionary lineages in *Draba* are apparent, but other species appear to have complex hybridizing histories which merit further discussion elsewhere. These groups are discussed in the order which they appear in the trees starting from the outgroup: *Draba hystrix*, *Draba muralis*, *Heterodraba* and *Athysanus*, *Tomostima*, *Abdra*, *Draba/Graellsia hederifolia*, *Draba/Erophila verna*, and Core *Draba*.

4.4.1 *Draba hystrix*

Schulz (1927, 1936) placed *Draba hystrix* (endemic in Afghanistan and Pakistan) in sect. Aizopsis DC., which also included 15 primarily European and Caucasian species. The species is morphologically anomalous in *Draba* because it has strongly thickened leaf midveins that persist as spines, acicular (needle-shaped) leaves, and leaf margins ciliate-pectinate with trichomes 1.5-2.5 mm long. The ITS and trnL-F trees agree in the separation of *D. hystrix* from the rest of *Draba* (Figs. 4.1 and 4.2; Supplementary Materials), and the Bayesian posterior probability support was 94% in the combined tree (Figure 4.3). The present study included eight other species of sect. Aizopsis sensu Schulz (1927, 1936), and they fell in Cores I and III (see below). *Draba hystrix* forms a distinct evolutionary line that should be recognized as a genus independent of *Draba*. Preliminary analysis shows its close relationship with *Arabis alaschanica*, which may or may not be a true *Arabis* (Koch et al., unpubl.). Further studies, however, are needed to establish its generic affiliation within the tribe Arabideae.

4.4.2 *Draba muralis* becomes *Drabella muralis*

Draba muralis formed a monospecific lineage with high (100%) posterior probability support in the combined tree (Figure 4.3). The incongruence between the ITS and trnL-F datasets suggests that diversification or introgression took place while at the same time conserving ancient haplotypes as in plastid

capture (Figs. 4.1 and 4.2). Regardless to this incongruence, the species clearly falls outside of *Draba* in both markers. Although the *D. muralis* lineage resembles *Tomostima* in being an annual with white petals and in occupying a basal position in the ITS, trnL-F, and combined trees, it differs from section *Tomostima* and the rest of *Draba* in having a combination of amplexicaul stem leaves and four, instead of six, stamens. Therefore, the *D. muralis* lineage merits the recognition as a monospecific genus. The species is distributed in southern and central Europe, northwestern Africa, Turkey, and Caucasus (Jalas et al. 1996). Schulz (1927, 1936) placed *D. muralis* in sect. *Drabella* DC., along with very heterogeneous assemblage of white- and yellow-flowered annuals and perennials distributed on all continents but Antarctia. As indicated above, *Drabella*, a genus proposed more than 140 years ago to accommodate *Draba muralis*, should be restored, and our data strongly support that.

4.4.3 *Heterodraba* and *Athysanus*

The inclusion of *Heterodraba unilateralis* (M. E. Jones) Greene and *Athysanus pusillus* (Hook.) Greene (sequences provided by Donovan Bailey), both as outgroup members of the tribe Arabideae, solidly influenced the outcome of the present analysis and forced the exclusion of several annual *Draba* into the segregate genera *Tomostima* and *Abdra* (Figure 4.3). Their addition also helped in resolving the complicated generic history of *Draba*/*Graellsia hederifolia* (see below). This study also confirms the distinctness of *Athysanus* and *Heterodraba* within the rest of the Arabideae. It is a matter of opinion to treat both monospecific genera or unite them in one, as done by Al-Shehbaz et al. (2010).

4.4.4 *Tomostima*

Schulz (1927, 1936) recognized *Tomostima* as a New World section of five species, including the North American *D. cuneifolia* Nutt., *D. platycarpa* Torrey and A. Gray and *D. reptans* (Lam.) Fern., and the South American *D. araboides* Wedd. and *D. australis* R.Br. They are annuals that produce early-season chasmogamous flowers with white petals and late-season cleistogamous flowers without petals. *Draba reptans* and *D. australis* are in a highly supported clade, 99% and 100%, in the ITS and combined analysis, respectively (Figs. 4.1 and 4.3). However, in the chloroplast analysis they form a polytomy with the other species in the *Tomostima* and *Abdra* (Figure 4.2). This indicates the close and possible historical connection between *Tomostima* and *Abdra*.

The *Tomostima* species formed a well-supported clade with 100% in both the ITS and the combined trees and always appeared basal in relation to the rest of *Draba* (Figs. 4.1, 4.2 and 4.3). However, in the trnL-F analysis they, together with *Abdra*, *Athysanus*, and *Heterodraba*, formed a monophyletic clade (99% support) although without a clear demarcation of generic lines. When all analyses of these markers are considered, the best solution is to recognize *Tomostima* as a distinct genus. Formal transfers will be done in a subsequent paper. From a phylogeographic point of view, it is interesting to note the central Andean *Draba araboides* is completely separate in the trees from all other Northern and Central Andean *Draba* species. Also, *D. australis*, known only from southernmost South America, is also separate from all other *Draba* in that region. All other high mountainous South American *Draba* are well within Core *Draba*. The transfer of these two species, with the three North American ones (see Discussion above) to *Tomostima* would eliminate the lowland annual *Draba* species of the New World from *Draba*. This strengthens the observation that Core *Draba* species have ecological preference for high altitudes, alpine

areas, and circumpolar regions.

4.4.5 *Abdra*

Greene (1885) segregated *Draba brachycarpa*, which is a widespread annual east of the Great Plains of the United States, into the monotypic genus *Abdra*. Schulz (1927, 1936) recognized *Abdra* as a section of two species that included *D. aprica* Beadle (restricted to the U.S.: Arkansas, Georgia, Missouri, Oklahoma, and South Carolina). The position of *D. brachycarpa* was strongly resolved in the ITS and combined trees (Figure 4.1 and Figure 4.3), whereas in the chloroplast tree (Figure 4.2) it forms a polytomy with *Tomostima* species (mentioned above). Interestingly, in the ITS analysis, *Abdra* appears as a well supported clade (100%) below *Athysanus*, *Heterodraba*, *Draba*/*Graellsia*, and the *Tomostima* clade. It is best to recognize *Abdra* as a distinct genus, although its exact placement with regards to *Tomostima*, *Heterodraba*, and *Athysanus* is not fully certain.

4.4.6 *Graellsia* (vs. *Draba*) *hederifolia*

The Moroccan perennial *Draba hederifolia* Coss. was placed by Schulz (1927, 1936) in sect. *Helicodraba* O. E. Schulz and was transferred by Hyam and Jury (1990) to the otherwise exclusively Southwest Asian *Graellsia* Boiss. (as *G. hederifolia* (Coss.) Hyam and Jury). However, in Beilstein et al. (2006) plastidic ndhF phylogeny, the Asian *G. saxifragifolia* (generic type species) showed closest relation to *Thlaspi* and others, much further away from *Draba* or any member of the tribe Arabideae. Our analyses clearly show that *D. hederifolia* should either be maintained in *Draba* or placed in a genus different *Graellsia*. The transfer by Hyam and Jury (1990) of *D. hederifolia* to *Graellsia* is not supported by the present molecular data. However, in contrast to the trnL-F and combined (93%) data, where *D. hederifolia* appears with *D. verna* at the base of Core *Draba*, the ITS data place it in a clade with *Heterodraba unilateralis*, making its true placement difficult. To complicate the picture further, *D. hederifolia* is a Mediterranean mountain species with morphological characters not found in any other *Draba* (e.g., plamately lobed and palmately veined leaves). In the chloroplast and combined data, the species is placed sister to *Draba verna* at the base of *Draba*, inside of the *Tomostima* and *Abdra* group (Figs. 4.1 and 4.3). The species is polyploid with 2n = 30 (Galland 1988) and this might indicate an old hybrid origin explaining incongruencies among the two phylogenetic hypothesis. Further studies are needed to establish the evolutionary history of this species.

4.4.7 *Erophila* (vs. *Draba*) *verna*

Draba verna was segregated by de Candolle (1821) into the genus *Erophila* because it has deeply bifid petals, a feature not found elsewhere in *Draba*. Schulz (1927, 1936) maintained both genera and placed them in the tribe Drabeae. Analyses of the combined data (Figure 4.3) reveal that *D. verna* is strongly supported (93%) at the base of the Core *Draba*. However, the ITS analyses show it on a polytomy with the *Abdra, Tomostima, Heterodraba, Athysanus, D. hederifolia* clades as mentioned above (Figure 4.1). The chloroplast analyses show both *D. verna* and *D. hederifolia* inside the four genera above and at the base of Core *Draba* (Figure 4.2). In conjunction with the above-mentioned situation of *D. hederifolia*, the exact position of *D. verna* is still uncertain. If we are to maintain *D. hederifolia* into the genus, the

same would apply to *D. verna*. Further studies are needed to strongly support the generic placement of the latter species. The highly variable chromosome counts (2n = 14, 28, 30, 32, 36, 38, 39, 52, 58, and 64; compiled in Warwick and Al-Shehbaz (2006)) in *D. verna* indicate a complicated evolutionary history of this widespread species. It is the only *Draba*-related species naturalized worldwide, and its weedy tendencies could have contributed to its relatively inconclusive genetic inheritance and multiple ploidy level reports. One may further speculate that *D. verna* or its ancestor was involved in a putative hybrid origin of *Draba hederifolia*, but this hypothesis requires additional studies for verification.

4.4.8 Core *Draba*

With the exclusion of the species discussed in the seven items above, the remaining 158 species of this study form one clade designated herein as Core *Draba*. Less than 10% of all *Draba* are either annual or biennial. The vast majority of perennials are caespitose or pulvinate, but several South American species that occupy paramo vegetations have woody stems, and *D. lindenii* (Hook.) Planch. (Venezuela) is a shrub or subshrub to 1.5 m tall. Under favorable conditions, some of the annuals or biennials (e.g., *D. albertina*, *D. helleriana*, *D. jorullensis*, *D. stenoloba*) become perennials, and *D. jorullensis* (Central America) sometimes grows to become a densely caespitose, long-lived perennial.

According to the basal positions of the *Drabella muralis*, *Draba verna*, *Tomostima*, *Abdra*, *Heterodraba*, and *Athysanus* (Figure 4.3) lineages, it appears that the annual habit in *Draba* is ancestral and that the currently dominant perennial habit is derived. Although the annuals are found in other lineages of the genus (asterisked species in Supplementary Materials), these evidently represent reversals within Core *Draba*. Despite our comprehensive sampling, not all annual to biennial or short-lived perennial *Draba* were included. Eighteen out of 21 annual species and seven out of 13 biennials to short-lived perennials were included in this study. In order to estimate the extent of the annuals in the Core, a preliminary phylogenetic analysis was done with four other ITS sequences from *D. aureola*, *D. brachystylis*, *D. mogollonica*, and *D. mongolica* (results not shown). These four annuals were found well within the Core *Draba* and there is no reason to believe, that if trnL-F data were available, that they would be at the base with the other basal annuals. The present ITS and trnL-F data show that the remaining annual species (e.g., *D. ellipsoidea*, *D. eriopoda*, *D. huetii*, *D. nemorosa*, *D. nuda*, *D. praealta*, and *D. stenocarpa*) were found well within the Core *Draba*. Yet to be sequenced are the remaining annuals or biennials not studied here (e.g., the North American *D. bifurcata*, *D. corrugata*, *D. rectifructa*, and *D. santaquiensis*, and the Moroccan *D. lutescens*). These may well fall also within the Core or be at the base. The addition of these five taxa may shed additional light at the base of *Draba* and its related genera.

Core *Draba* is strongly supported in the ITS tree (100%), the trnL-F tree (97%), and in the combined tree (86%) (Figs. 4.1, 4.2, and 4.3 and Supplementary Materials). This Core is divided below into three moderately supported but stable groups.

Core I: European, Caucasus, Iranian and Asian

This Core has a 94% support in the combined analysis (Figure 4.3) but is partially separated in the ITS and the trnL-F analyses. Regardless of the separation seen in the ITS and trnL-F analysis (Figs. 4.1 and

4.2), the species within this Core do not roam around the tree and are mostly found together in all three analyses, except for the above mentioned species which move in the trnL-F analyses. This Core includes 24 species, and all except two consistently occur together. Most species have yellow flowers and a base chromosome number of 8 (see Warwick and Al-Shehbaz 2006; Jordon-Thaden and Koch 2008). Both *D. eriopoda* and *D. oreades* appeared in Cores I and III in all three trees and, therefore, it is assumed that this sequence dislocation reflects true phylogenetic history and a product of introgression events. Core I is the most well-resolved of the three Cores, with high support for many of its subclades. This can be explained by the fact that it is the oldest *Draba* core lineage (see all figures). Also found in this Core are the annuals *D. nemorosa* and *D. nuda*, which formed a well-supported (98%) clade in the ITS tree (Figure 4.1), but are found on a polytomy within Core I in the trnL-F (Figure 4.2). Because of the very high supported number of nodes between this subclade and sister taxa to *Draba*, its generic placement in *Draba* is well-supported. These findings fully agree with Al-Shehbaz and Koch (2003), who reduced *Drabopsis* to synonymy of *Draba*. Also, new with this study, is the occurrence of the Iranian *Arabis rimarum* Rech.f. well within the Core I and formation with two other Iranian species, *D. araratica* and *D. pulchella* (Figs. 4.1, 4.2, and 4.3), a well-supported subclade. The examination by one of us (Al-Shehbaz) of the type and other collections of *Arabis rimarum* leave no doubt that it is a synonym of *D. aucheri* Boiss., a species widespread in Afghanistan, Iran, Kazakhstan, and Tajikistan. Rechinger (1951) placed the species in *Arabis* because it has linear fruits 1-2.5 cm long, but he overlooked the scapose habit and subbiseriate seeds, both of which are characteristic of *Draba*. Furthermore, several *Draba* have linear fruits, and in some (e.g., *D. nuda*) the fruits are up to 4 cm long.

At the base of Core I in the combined analysis is Caucasian *D. longisiliqua*. It appears well supported to be ancestral to Cores II and III in the ITS and on a polytomy of all three Cores in the trnL-F. The placement of this species is unusual, but the sequence indicates it is a true *Draba*. Unfortunately, there were no repeat sequences available; however, the quality of the sequence and its position as an ancestral perennial *Draba* species is promising. Could this possibly be the most ancestral perennial *Draba*? Further studies and additional sampling may help resolve that question.

Cores II and III: North and South American and Siberian, Central Asian, Beringian and Arctic

Core II is predominately North and South American, though it also includes four widespread arctic and central Asian mountain species (4.2b, highlighted in pink in Supplementary Materials). It is the youngest of the three Cores by its position in all phylogenetic trees. Core III is mostly Siberian, central Asian, Beringian, and Arctic. Only 18 species which were found in Core II in the ITS and combined analyses, are found in Core III in the trnL-F (Supplementary Materials). In addition, the four species that occur in both Cores II and III suggest much migration and hybridization of arctic, Beringian, and Cordilleran species, but they still maintain separate stable lineages differing in ploidy patterns. As discussed in Jordon-Thaden and Koch (2008), Core II is comprised of mostly polyploids with odd and aneuploid chromosome levels. Core III, also rich in polyploids, has more species with higher ploidy levels (i.e. > 10x). Another distinction between the two is petal color (Table 4.3). As shown by Beilstein and Windham (2003) in their ITS study of 15 North American *Draba*, their A2 corresponds with Core II and are mostly yellow-flowered (also with many aneuploids) and their A1 corresponds with Core III and are mostly white-flowered (and with base of $x = 8$).

The present data show that both *Schivereckia podolica* (Besser) Andrz. and *S. doerfleri* (Wettst.) Bornm. are perfectly good species of *Draba* Core III. The first species was shown to be nested in *Draba* (Bailey et al. 2006), but its correct name is *D. hyperborea* (L.) Desv., a name initially proposed in 1753 as *Alyssum hyperboreum* L. and transferred to *Draba* some 194 years ago. Although Berkutenko (1995) correctly interpreted the species status, she erred in transferring the name to *Schivereckia* (as *S. hyperborea* (L.) Berkutenko) instead of maintaining it in *Draba*. All North American authors (Hitchcock 1941; Rollins 1993, e.g.,) misapplied the name *D. hyperborea* for a Beringian species (distributed in Alaska, British Columbia, Russian Far East), the correct name of which is *D. grandis* Langsd. (Al-Shehbaz et al. 2010). The second species of *Schivereckia* was originally and correctly described as *D. doerfleri* Wettst. (distributed in Albania, Bulgaria), where it should stay.

A curious observation can be made for the species *D. cryptantha*. In the ITS, its position is strongly within Core II and even sits with other Andean species. However, in the trnL-F tree, it falls completely out of *Draba* (Figure 4.2 and Supplementary Materials). Due to the high quality of the sequence, this information is kept within the dataset as a real indication of an ancient and ancestral chloroplast haplotype that dates further back than the origin of *Draba*.

Two central Asian samples (one each from Afghanistan Pamir and Indian Himalaya) were used in this study to resolve the occurrence of the arctic *D. alpina* in central Asia. The data do not show that central Asian samples represent a separate species because all arctic *D. alpina* sequences were also scattered throughout Core III, as well as being found in Core II. Therefore, we can only conclude that this species must have a high propensity to hybridize and has a complex history. A more likely alternative explanation is that *D. alpina* was commonly misidentified and could possibly represent three or even four distinct species.

4.4.9 Comparison to Previous taxonomical treatment: Sections and Series

While Schulz's (Schulz 1927, 1936) and Tolmachev's (Tolmachev 1939) subdivisions are artificial classifications useful in the identification of taxa, both are inadequate in subdividing *Draba* into infrageneric monophyletic groups. However, some species which were classified into sections appear within the same *Draba* Core (Table 4.3). For example, Schulz's sect. *Aizopsis* included 28 species of which nine were sampled, and of these, seven are in Core I, *D. heterocoma* in Core III, and *D. hystrix* falling out of *Draba* completely (see below). In addition, sect. *Tylodraba* included eight species, and four of the six species sampled appeared in Core II. Four of the ten species in the sect. *Rhabdodraba* are in Core II. Section *Calodraba* included 14 species, and eight of the nine sampled also appear in Core II. Similarly, three of the four species sampled in sect. *Adenodraba* fell in Core II. The section which shows the most congruence with the genetic data is *Leucodraba* (30 out of 43 sampled), where all species were in Core III. Three of Schulz (1927, 1936) sections are quite artificial. Species of his sect. *Chrysodraba*. were distributed throughout all three of the Cores (Core I: ten spp., II: 17 spp., III: 14 spp.) and similarly those of sect. *Phyllodraba* (Core II: nine spp., III: five spp.). Section *Drabella* was also completely artificial, with the species sampled appearing in each of the three Cores, as well as *Draba muralis* having fallen out of the genus (see above under *Drabella*). There is no justification here for the separation of the smaller sections from the rest of the *Draba* Cores. *Draba oreadum* from sect. *Acrodraba*, for example, is nested well within other Core I species which are mostly represented by Schulz's sections *Aizopsis* and *Chrysodraba*. Also,

D. lindenii of sect. *Dolichostylis* and *D. funckiana* of sect. *Chamaegongyle* are both within most of the other Core II species. *Draba grandis* from sect. *Nesodraba* is also well within Core III and should not be separated as its own section. Any division of the Cores I, II and III may only be possible with an increasing sequence and morphological data of unsampled species.

Tolmachev's Tolmachev (1939) series represent a limited number of species from a portion of genus geographic area (Former Soviet Union) and, therefore, it is to be expected that they do not correlate with any of the three Cores (Table 4.4). Little can be said here, except that most of the species of Tolmachev's series are represented in Core III, which comprises most of the Siberian, Asian, and arctic species.

4.4.10 Comparison to previous molecular and cytological analysis

Mulligan (1976) divided the 40 Canadian and Alaskan *Draba* into three groups based on petal color and ploidy levels: white-flowered with a chromosome base of $x = 8$, yellow-flowered with $x = 8$, and yellow-flowered aneuploids with $x \neq 8$. Beilstein and Windham (2003) examined 14 North American and four Eurasian *Draba* species and basically supported some of Mulligan's groupings. The present study sheds light on the most representative set of data to date and also recognizes three groups: Core I, mostly yellow-flowered with $x = 8$; Core II, mostly yellow-flowered with $x \neq 8$; and Core III, mostly white-flowered with $x = 8$. Koch and Al-Shehbaz (2002) suggested that the yellow-flowered species are basal, but the present data show that white-flowered lineages most likely gave rise to *Draba*. It was shown that *D. nemorosa* was basal to *Draba* and close to *D. verna* (Koch and Al-Shehbaz 2002). However, both are shown herein to be well within Core I. Lastly, the hybrid origin demonstrated for a considerable number of *Draba* species (Widmer and Baltisberger 1999a,b; Brochmann et al. 1992b,c) was also seen in the present data (4.2b).

4.4.11 The origin of *Draba*

Important to note is the presence of a highly supported clade of central Asian species in each of the three cores. This indicates stable migration routes from this vast mountainous region in all three lineages. These clades are mostly at the base of all three cores, indicating once more that *Draba* possibly originated somewhere within the regions between the central Asian and Caucasian Mountains. This includes most of the floristic region referred to as Irano Turanian by Takhtajan et al. (1986).

4.4.12 Character evolution

The present findings suggest that the annual life form is plesiomorphic in *Draba* s.l., and that the reversals from the perennial to biennial and annual life forms in some species (e.g., *D. melanopus*, *D. jorullensis*, *D. helleriana*, and *D. incana*) took place independently in the genus. The white petal color seems to be plesiomorphic, though reversals from yellow to white and vice versa was common during the evolutionary history of the genus (see Table 4.3). The basal groups of annuals have cauline leaves, and the absence of cauline leaves, and consequently the evolution of scapose habit is derived, although reversal in these character states also was common. The presence vs. absence of cauline leaves was emphasized in previous studies (Pohle 1925; Schulz 1927, 1936; Fernald 1934; Tolmachev 1939; Hitchcock 1941;

Sections by Schulz 1927	$\frac{\#spdataset}{\#sp.Schulz}$	Draba Core I	Draba Core II	Draba Core III	Basal Draba	Outside Draba
I. Aizopsis	**9 / 15**	*D.aizoidesY,* *D.brunifoliaY,* *D.dedeanaW,* *D.hispanicaY,* *D.lasiocarpaY,* *D.oxycarpaY,* *D.parnassicaY*		*D.heterocomaY*		*D.hystrixY*
II. Lindodraba	**0 / 1**					
III. Chrysodraba	**38 / 54**	*D. acaulis, D.araratica,* *D.cappadocica, D.hispida,* *D.jucunda,* *D.longisiliqua, D.oreades,* *D.pulchella, D.rosularis,* *D.sibirica (all yellow)*	*D.albertii, D.alpina,* *D.amplexicaulis,* *D.cruciata, D.densifolia,* *D.eschscholtzii,* *D.korschinskyi,* *D.lemonii, D.micropetala,* *D.odudiana, D.olgae,* *D.oligosperma,* *D.pauciflora,* *D.sphareroides,* *D.stenopetalaY/V,* *D.trinervis, D.ventosa* *(all but one are yellow)*	*D.alpinaY,* *D.corymbosaY,* *D.elegansY, D.glacialisY,* *D.incertaY,* *D.oblongataW,* *D.ochroleucaW,* *D.oligospermaY,* *D.oreadesY, D.paysoniiY,* *D.micropetalaY,* *D.pilosaY,* *D.subcapitataW,* *D.supranivalisW*	
IV. Rhabdodraba	**5 / 8**		*D.hookeri, D.macleani,* *D. pickeringii,* *D.schusteri (all white)*	*D.scopulorumW*		
V. Tylodraba	**4 / 6**		*D.aretoidesY/W,* *D.cryptanthaW,* *D.depressaW,* *D.obovataY*			

Table 4.3: Schulz's classification compared to the results of the phylogenetic analysis.

Sections by Schulz 1927	$\frac{\#spdataset}{\#sp.Schulz}$	Draba Core I	Draba Core II	Draba Core III	Basal Draba	Outside Draba
VI. Acrodraba	**1 / 1**	*D.oreadumW*				
VII. Helicodraba	**1 / 1**				*D.hederifoliaW*	
VIII. Calodraba	**9 / 13**		*D.alyssoidesW/V,* *D.confertifoliaW,* *D.cuzcoensisY,* *D.farsetioidesY,* *D.halliiW,* *D.matthioloidesO,* *D.splendensW,* *D.violaceaeV*	*D.gillesiiW*		
IX. Dolichostylis	**1 / 3**		*D.lindeniiY*			
X. Adenodraba	**3 / 4**		*D.discoideaW,* *D.jourlensisY/V,* *D.nivicolaW*			
XI. Chamaegongyle	**1 / 5**		*D.funckianaY*			
XII. Phyllodraba	**12 / 23**		*D.amplexicaulis,* *D.aurea, D.crassa,* *D.helleriana,* *D.matangensis,* *D.polyphylla,* *D.spectabilis,* *D.surculosa, D. yunnanensis (all yellow)*	*D.aureaY, D.amoeanaV,* *D.ramossisimaW,* *D.surculosaY,* *D.ussuriensisW*		
XIII. Nesodraba	**1 / 1**			*D.grandisY*		

Table 4.3: Schulz's classification compared to the results of the phylogenetic analysis. (continued)

Sections by Schulz 1927	$\frac{\#spdataset}{\#sp.Schulz}$	Draba Core I	Draba Core II	Draba Core III	Basal Draba	Outside Draba
XIV. Leucodraba	**30 / 43**			*D.altaica, D.arabisans, D.arctica, D.borealis, D.breweri, D.cana, D. cinerea, D.fladnizensis, D.glabella, D.glomerata, D.incana, D.juvenilisY, D.kamtschatica, D.kuznetsovii, D.lactea, D.lanceolata, D.lasiophylla, D.lichiangensis, D.lonchocarpa, D.magellanica, D.nivalis, D.norvegica, D.palanderiana, D.sachalinensis, D.sikkimensis, D.stenobotrys, D.subamplexicaulis, D.tibeticaY, D.tomentosa, D.turzaninovii, D.winterbottomii. (all but two are white)*		

Table 4.3: Schulz's classification compared to the results of the phylogenetic analysis. (continued)

Sections by Schulz 1927	$\frac{\#spdataset}{\#sp.Schulz}$	Draba Core I	Draba Core II	Draba Core III	Basal Draba	Outside Draba
XV. Drabella	**12 / 18**	*D.ellipsoideaW, D.eriopodaY, D.heutiiY, D.nemorosaY*	*D.albertina, D.crassifolia, D.melanopus, D.pusilla, D.stenocarpa, D.stenoloba (all yellow)*	*D.eriopodaY, D.praealtaW*		*D.muralisW*
XVI. Tomostima	**5 / 5**					*D.araboides, D.australis, D.cuneifolia, D.platycarpa, D.reptans (all white)*
XVII. Abdra	**2 / 2**					*D.aprica, D.brachycarpa (white)*
Erophila sp.	**1 / 7**				*D.vernaW*	
Described after Schulz 1927, and other genera included in this study.	**n/a**	*D.nudaY (was Drabopsis nuda), Arabis rimarumW*	*D.boyacanaW, D.confertifoliaW, D.cuzcoensisY, D.demareeiY, D.exunguiculataY, D.hidalgensisY, D.inquisivianaW, D.kassiiY, D.kongboianaY, D.lapazianaW, D.novolympicaY, D.ogiliviensisY, D.pulvinataY, D.streptobrachiaY, D.subalpinaW, D.wurdackiiY*	*D.arseniewii*, D.murrayiW, D.nylamensisY, D.pohleiY, D.porsildiiW, D.prozorovskiiW, D.ruaxesY, D.subumbellataY, D.tucumanensisW, D.zangbeiensisY, Schivereckia sp.*		*Athysanus pusillus , Heterdraba unilateralis (both white)*

Table 4.3: Schulz's classification compared to the results of the phylogenetic analysis. (continued)

Tolmachev series	$\frac{\#spdataset}{\#sp.Tolm.}$	Core I	Core II	Core III	out of Draba
1. Cuspidatae	**0 / 1**				
2. Bryoideae	**2 / 6**	*D.brunifoliaY*		*D.heterocomaY*	
3. Pilosae	**3 / 4**		*D.stenopetalaY/V*	*D.pilosaY*, *D.subcapitataW*	
4. Polytrichae	**1 / 2**	*D.araraticaY*			
5. Pamiricae	**1 / 2**		*D.korschinskyiY*		
6. Oblongatae	**4 / 4**	*D.lasiocarpaY*	*D.pauciflora Y*	*D.oblongataW*, *D.micropetalaY*	
7. Alpinae	**6 / 11**	*D.oreadesY*	*D.alpinaY*, *D.olgaeY*	*D.alpinaY*, *D.corymbosaY*, *D.pohleiY*, *D.ochroleucaY*, *D.oreadesY*	
8. Lacteae	**3 / 7**		*D.pusillaY*	*D.fladnizensisW*, *D.lacteaW*	
9. Supernivalis	**1 / 1**			*D.supranivalisW*	
10. Physocarpae	**1 / 3**		*D.albertiiY*		
11. Darwasicae	**2 / 5**		*D.odudianaY*	*D.arseniewi**	
12. Tibeticae	**1 / 3**			*D.tibeticaY*	
13. Imereticae	**0 / 1**				
14. Mollissimae	**1 / 3**	*D.longisiliquaY*			
15. Nivales	**3 / 4**			*D.kamtschatica*, *D.nivalis*, *D.turzaninovii (white)*	
16. Cinereae	**2 / 2**			*D.arctica*, *D.cinerea (white)*	
17. Rupestris	**3 / 5**			*D.altaica*, *D.kuznetsovii*, *D.norvegica (white)*	
18. Hirtae	**4 / 4**			*D.glabellaW*, *D.juvenilisY*, *D.prozorvskiiW*, *D.subamplexicaulisW*	
19. Lasiophyllae	**1 / 1**			*D.lasiophyllaW*	
20. Incanae	**3 / 4**			*D.cana*, *D.incana*, *D.lanceolata (white)*	
21. Cardaminiflorae	**1 / 2**			*D.ussuriensisW*	
22. Boreales	**2 / 3**			*D.borealis*, *D.sachalinensis (white)*	

Table 4.4: Comparison between Tolmachev (1939) series classification of Draba to the current results from the genetic data within this project. Refer to 4.3 to see those not included in Tolmachev's series.

Tolmachev series	$\frac{\#spdataset}{\#sp.Tolm.}$	Core I	Core II	Core III	out of Draba
23. Hyperboreae	**1 / 1**			*D.hyperboreaY (Schivereckia)*	
24. Hispidae	**1 / 1**	*D.hispidaY*			
25. Subsecundae	**0 / 2**				
26. Repentes	**1 / 3**	*D.sibiricaY*			
27. Eriopodae	**2 / 2**	*D.eriopodaY, D.huetiiY*		*D.eriopodaY*	
28. Nemorosae	**3 / 3**	*D.nemorosaY*	*D.stenocarpaY*		*D.muralisW*
29. Lineares	**1 / 3**		*D.melanopusY*		

Table 4.4: Comparison between Tolmachev (1939) series classification of Draba to the current results from the genetic data within this project. Refer to 4.3 to see those not included in Tolmachev's series. (continued)

Mulligan 1976; Rollins 1984, 1993) as an important trait to distinguish *Draba* species. The reduction of seed numbers per fruits, the evolution of numerous-rayed stellate trichomes, the absence of simple trichomes, the development of woody stems, the evolution of purple flowers, the origin of fully bracteate racemes, the development of broad seed wings, the origin of narrowly linear siliques, the deep division of petals into lobes, the evolution of high ploidy chromosome numbers, the establishment of apomixis, and the development of very long styles all appear to be derived within the genus. However, in the absence of thorough studies that involve multiple accessions from as many species as possible and that utilize as many informative markers as possible, it would be premature to advance any solid hypotheses on the evolution of various character states in the genus. It would also be premature to present a broad-scaled infrageneric classification of *Draba*.

4.4.13 Polyploidy evolution

Polyploidy and diploidy are found throughout each of the Cores. Patterns in polyploidy within *Draba* have been discussed in detail by Jordon-Thaden and Koch (2008); Grundt (2003); Grundt et al. (2004, 2005b). It was concluded that European to Iranian species (here represented by Core I) are high in ancestral diploid and tetraploid species with some polyploids; North and South American species (here mostly represented by Core II) have numerous polyploids and aneuploids along with diploids; and the remainder of Circum-North, Beringian, Asian, and Siberian species (here represented by Core III) are rich in higher level polyploids (> 10x), as well as diploids and tetraploids. It was also found that *Draba* has an unusually high rate of speciation and polyploidization, and it was proposed that the high amount of environmental fluctuation (i.e. glaciation cycles) has accelerated these factors in *Draba* (Jordon-Thaden and Koch 2008). Considering that about 50% of *Draba* species are known cytologically, the above correlations agree well with the present phylogenetic analysis.

4.4.14 Comments on the Bayesian analysis

Due to *Draba*'s highly diverse set of sequences with relatively short genetic distances between species, a deeper method for searching tree space was desired. For this reason, we used Bayesian analysis and

explored the notion of a "complete" analysis. With the help of AWTY (Nylander et al. 2008) and much discussion with colleagues, it was concluded that such a dataset of 384 sequences with approximately 1500 base pairs that have minor but many differences, a very high number of generations was needed in order to obtain significant results (at least 20 or more million generations for the trnL-F and ITS analyses, and 400 million or more generations for the combined analysis). However, with modern computing power, analyses such as these are now feasible. Many adjustments, tests and assessments were carried out in order to determine the success of each run (for example, see Supplementary Materials AWTY plots). The resulting trees published here are the best of approximately 50 separate runs (with increasing numbers of generations), where adjustments were made to the alignments, the number of repeats, exclusions of sequences with missing data, and/or the settings of the program MrBayes. In addition, with such a complex set of data, it was also concluded that using ModelTest to determine the "best" model only obviously made the analyses go slower but did not affect the topology of the trees.

4.5 Conclusions

The present study showed that the previously recognized infrageneric subdivisions of *Draba* are artificial. This study also clearly demonstrates that several previously recognized segregate species (*Drabopsis nuda*, *Schivereckia podolica*, and *S. doerfleri*) are more appropriately united with *Draba*. *Arabis rimarum* is a synonym of the earlier published *D. aucheri*. *Erophila*/*Draba verna* and the Moroccan *Graellsia*/*Draba hederifolia* need further analysis to fully resolve their relationship with *Draba* and its close relatives. *Abdra* and *Tomostima*, treated as sections in Schulz (1927, 1936), are indeed genera outside of *Draba*. *Heterodraba* and *Athysanus* are distinct genera that solidify the relationship of the *Tomostima* and *Abdra* groups. *Draba muralis* forms a monospecific genus (*Drabella*) that lies between the annual ancestors of *Draba* and *Arabis*. *Draba hystrix* is also possibly a monospecific genus that is more closely related to *A. alaschanica* than to *Draba*.

4.5.1 Alternative taxonomic solution

The alternative and conservative solution to handling the taxonomy of *Draba* would involve the following: First, to exclude *D. hystrix* from *Draba* and eventually place it in a monospecific genus closely related to another that would contain *Arabis alaschanica*. Second, to unite the monospecific *Athysanus* and *Heterodraba* with *Draba*, only one formal transfer is needed for placing *Thysanocapus (Athysanus) pusillus* in *Draba*. Finally, to maintain all of the other segregates (*Abdra*, *Drabella*, *Tomostima*), treated by Schulz (1927, 1936) as sections, as members *Draba*. This would also mean to bring *Graellisa hederifolia* back to its original name of *Draba hederifolia*, as well as *Erophila verna* back to *Draba verna*. Although such approach is most conservative nomenclaturally, it faces only one difficulty regarding the position of *Draba muralis*: The species fell in the ITS analysis outside of *Draba* s.l. and sister to *Arabis alaschanica* and *D. hystrix*, but in the trnL-F analysis is was basal to all Core *Draba* plus *Graellsia hederifolia*/*Erophila verna* and inside the white-flowered annual complex (*Abdra*, *Athysanus*, *Heterodraba*, *Tomostima*), and in the combined tree it was basal to all of the above minus *D. hystrix*.

Chapter 5

Phylogeography of *Draba*

5.1 Introduction

In this chapter, we now come to the cumulation of all the presented data regarding *Draba* speciation in this thesis. We have shown that *Draba* is widely distributed in arctic–alpine habitats across the Northern Hemisphere and South America, has multiple areas of high species richness, elevated rates of speciation and polyploidization, and a complex phylogeny indicating strong hybridization and recent radiation. The initial estimation of the age of the split from its closest relatives gave an age of *Draba* to be approximately 5 million years old (Koch et al. 2001). However, after the completion of the phylogeny of *Draba* (chapter 4), we can show that the resulting core *Draba* species are possibly even younger. Within this chapter, we show that the core *Draba* species are approximately 2.3 million years old, which puts extant *Draba* in the geological time frame at the base of the Pleistocene epoch of the Quaternary. However, since the circumscription of the tribes of the Brassicaceae (Al-Shehbaz et al. 2006; Bailey et al. 2006; Koch et al. 2007) and further work done on the age of the Arabidae (of which *Draba* and *Arabis* and others are part of), and with the addition of more markers, recent dating of the family itself is estimated to be as young as approximately 37.6 mya (Couvreur et al. 2009). From these estimates we can extrapolate the split of *Draba* from other Arabidae genera to be approximately 11 mya and core *Draba* to be 7 million years old. In this study, however, we estimated this split to be 18 mya and core *Draba* to be 2.3 million years old. Meaning that old *Draba* lineages (which are most likely extinct) must have established themselves for some time, 16 million years during the Miocene, and maintained constant before the development of the currently seen radiating lineages of Cores I, II and III.

It also must be cautioned that even with the advances in clade age estimations from sequence data, the error bars for the age of each node can be larger than the time scale we are considering, especially with a young genus such as *Draba* which only diversified to its present state on a time frame of 100,000's of years. Here we can show that for the radiation of *Draba*, considering the previous discussion (chapter 3) on *Draba*'s accelerated speciation and polyploidization rates, the recent radiation of *Draba* species has not only been continuously linear during the Pleistocene, but has experienced periods of exponential growth and most likely due to environmental factors such as glacial coverage and retreat cycles.

Regardless of our ability or inability to accurately date these migration and speciation events, phylogeo-

graphic inferences can be made from the correlation between gene diversity (h) and nucleotide diversity (p) as discussed in Avise (2000, pages 59–60), which gives us clues to as what has shaped the present day lineages. In this study, we are using groups of individual accessions from herbaria collections that are not true populations and have chosen to treat them as such for the purpose of investigating global patterns. We are using microevolutionary principles and theory to explore macroevolutionary questions, which is an integral facet of phylogeography (Avise 2000, pages 3–9). The scale of a global study such as this can only be achieved in this manner with individuals from such collections of material and is otherwise impractical for such a large genus. In continuation of what was illustrated in chapter 4, we show the results of haplotype networks which indicate hybridization, chloroplast capture, and recent radiation as a result of these possible factors which may have influenced population structure. We aim to determine if the current distribution of *Draba* was the result of a series of vicariance and/or dispersal events. Even on such a global scale as this, we are able to identify populations that have experienced one or the other. Most likely vicariance events that could have effected *Draba* are the formations and retreats of glaciers, as discussed in the Introduction of this thesis. *Draba*'s ability to adhere to this slowly moving barrier also creates many opportunities for permanent separation of a population and subsequent speciation. The notion of the ability of mammalian fauna in the Mammoth Tundra of the Pleistocene (Guthrie 1990, 2001) such as the woolly mammoth (Zazula et al. 2002; van Geel et al. 2008) or even a Beringian 'packrat' (Zazula et al. 2005) is explored. Bird migrations either coastal or mainland believed to have assisted in the dispersion of *Draba* lineages is discussed.

Consequently, *Draba* is an exceptional model for studying recent continent–wide macroevolution processes like historic migration, adaptation, and speciation. We will show here that its migration is most likely a combination of ancestral, diversification events from even older lineages during the early cooling stages of the Pleistocene in the Galasian and more recent radiations due to the numerous glaciation and deglaciation cycles during the remainder of Pleistocene. This study will continue the investigation of the glacial survival theory, glacial refuge areas, and geographical barriers such as water, glaciers, deserts, tropical forests which relegate *Draba* to extreme arctic and alpine habitats resulting in accelerated radiation of lineages. We will explore these facets by correlating lineage and genetic diversity and species richness. This study of the phylogeographic history of *Draba* includes 57% of the genus on a worldwide scale. Sequence information from *Draba* was obtained for the plastidic trnLF for this phylogeographic analysis (see chapter 4) for phylogeny from the combination of the trnLF and ITS sequences). In conjunction with the explanation of the historical migration patterns of *Draba*, one main question we seek to finally explore is whether the origin of a genus can be inferred from the identification of the area of highest species richness and genetic diversity, and if these two centers of diversity are located in the same general area.

5.2 Materials And Methods

5.2.1 Selection of regions and samples

Sequences of the non–coding chloroplast trnLF region were generated as stated in chapter 4 of this thesis. Sequences were given a geographic identification that correlates with the *Draba* regions as discussed in . There were 21 regions defined here and each group/population

has 20 to 30 sequences (except for 1–ChilePatagonian with only 11 and 9–AmphiAtlantic having 55, see below). The regions were defined as: 1–ChilePatagonian, 2–PeruvianAndes, 3a–ColombianVenezuelanAndes, 3b–EcuadorianAndes, 4–SouthernCordilleraSCD, 5–CentralCordilleraCCD, 6–NorthernCordilleraNCD, 7a–BeringiaNorthAmerica, 7b–BeringiaAsia, 8a–ArcticNorthAmerica, 8b–ArcticAsia, 9–AmphiAtlantic, 10–EasternNorthAmerica, 11–Turkey–Caucasus–Iran, 12–Mediterranean, 13–EuropeanAlpsCarpathians, 14a–AltaiSayanBaikalMongolianMts, 14b–AlaiPamirTienShan, 15a–WHimalaya–Karakoram–AfgPakMts, 15b–EastHimalaya and 16–Tibet–ChineseMts (Figure 5.1). Refer to the Accession List in the Appendix B for sample information and haplotype results on all sequences. In most cases, the same sequences that were used for the phylogeny were used for the phylogeographic analysis. However, approximately 180 more trnLF sequences were added to the phylogeographic study in order to create a larger geographic sample and to include an approximately even number of sequences per region. Adjustments to the alignments were done with PhyDE (Müller et al. 2007) (see Supplementary Materials for alignments).

5.2.2 Phylogenetic analysis

Bayesian inference was carried out for the alignment of 580 sequences with 799 characters (gaps not coded) for 40 million generations with two simultaneous runs with a sample frequency of 800,000 in order to obtain $\sim$ 500 trees for each run (i.e. 25% were discarded). The analysis was done with the MPI parallel version of MrBayes version 3.4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). The temperature of the hot chain was set to t = 0.01 in order to ensure proper chain swapping for this dataset of short sequence divergence. Convergence was assessed using the online program AWTY (plots not shown) (Are We There Yet?, Nylander et al. 2008). Due to the complex nature of the resulting phylogenetic relationships and the inevitable polytomies using trnLF, as determined in chapter 4, SplitsTree4 (Huson and Bryant 2006) was used to create a consensus network from the resulting saved trees from the Bayesian inference analysis. This network was used to confirm similar topology from that which was calculated in the phylogenetic analysis in chapter 4 after the addition of the 180 new sequences, and to compare to the other phylogeographic analyses herein. The consensus network used the tree transformation with the threshold set to 0.33 and the split transformation using equal angle with the 'run Convex Hull' option activated. Consensus networks have been proposed as a method to help the illustration of conflicting evidence in phylogenies either due to using unrelated loci or when hybridization made simple lineages difficult to detect (Holland et al. 2004). Accessions were labeled with colored dots which correspond to eight geographic regions which group the 21 populations (see below).

5.2.3 Phylogeographic analysis

The phylogeographic alignment in the analysis consists of 558 sequences and 712 characters after coding of the indels. Twenty indels were coded in the alignment using the simple indel coding (SIC) method (Simmons and Ochoterena 2000), i.e. presence vs. absence. We chose SIC over the newer MCIC (multiple complex indel coding) because we disagree with proposing excessive inferences of the evolutionary changes within one indel (Simmons et al. 2007). Four separate analyses were done with this dataset: neighbor–net network, TCS haplotype network, genetic population structure measures with gene and nucleotide diversity, and AMOVA. Due to the sensitivity to missing data when constructing networks (e.g.

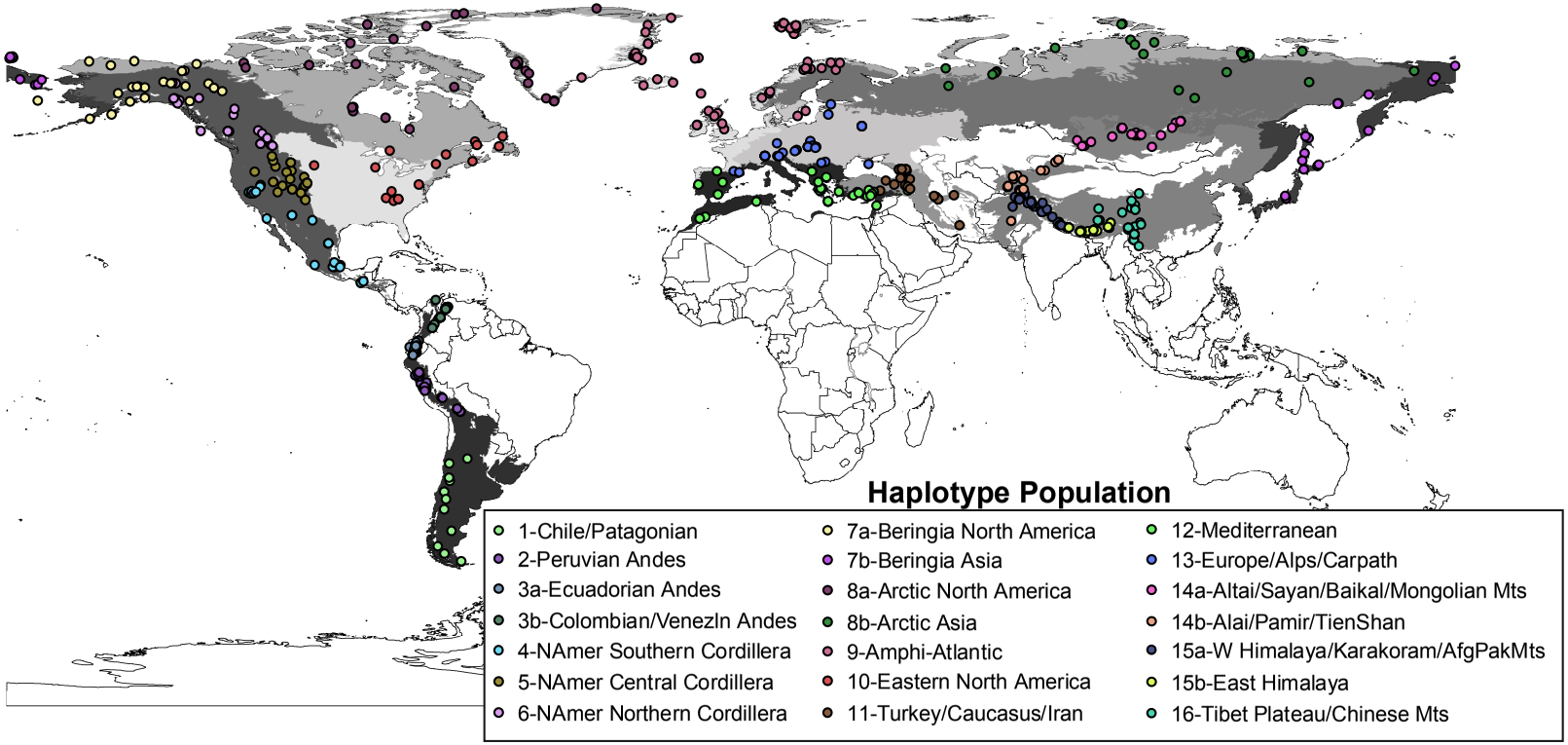


Figure 5.1: Map of 580 georeferenced accessions for the phylogeographic analysis of the genus *Draba*, with the populations indicated.

Joly et al. 2007; Kearney and Clark 2003), the following accessions were excluded from these analyses which were included in the phylogenetic analysis in this chapter: L488–alyssoides–3b, B28–aurea–8a, L33–borealis–7b, MO26–brackenridgei–2, 5–cryophila–3a, 46–pycnophylla–3b, B87—huetii–12, NH20–incana–9, L478–jorullensis–4, M12–kassii–5, L119–kongboiana–15b, L188–korshinskyi–15a, NH3–lasiocarpa–12, MO159–ramosissima–10, D47–surculosa–16 and O33–ventosa–6. The following sequences also were removed due to the long-branch effect that most certainly results in false haplotype network connections and biased elevated genetic structure that cannot be trusted: MO37–cryptantha–2, MO8–jorullensis–4 and MO167–nuda–14b. In addition, as the outgroup accession, MO100–brachycarpa–10, and the two non-core *Draba* species, L241–hederifolia–12 and NH32–verna–13 which were removed because there are too many mutation steps between them and the core *Draba* species.

Neighbor-net network analysis was done in SplitsTree4, due to its ability to show better resolution, in order to illustrate those lineages which normally are shown as polytomies in a tree (Bryant and Moulton 2004). This distance–based method is similar to the neighbor–joining (nj) tree algorithm, but allows for representation of both the groupings in the data and evolutionary distances instead of only the distances as in a tree. It generates weighted splits (which are bipartitions of the set of taxa and is a process also called splits transformation) from a distance matrix and creates a splits graph, which is fundamentally different from a tree (Dress and Huson 2004). A splits network of all 558 accessions with 712 characters was constructed from genetic distances using this neighbor–net distance transformation algorithm. The splits transformation was equal angle with the 'run Convex Hull' option activated (see Huson and Bryant 2006). The network was calculated using the recommended model for this dataset, which was calculated with ModelTest 3.7 (Posada and Crandall 1998; Akaike 1974). The model parameters determined were F81+I+G with the substitution model for rates being equal and the among site variation having a gamma distribution shape parameter for variable sites with an alpha parameter of 0.7574 and the proportion of invariable sites of 0.3465. The base frequencies for the dataset were A = 0.3303, C = 0.1578, G = 0.1847 and T = 0.3272. Accessions in the network were colored in the same manner as the consensus network.

In conjunction with the tip–oriented analysis in SplitsTree4, TCS 1.21 (Clement et al. 2000) was used to parsimoniously identify the unique haplotypes present in the dataset and to create gene genealogies using the method established by Templeton et al. (1992). The haplotype network of all 558 accessions with 712 characters was calculated with gaps coded and otherwise considered missing data. The physical appearance of the network figure was improved in Adobe Illustrator CS4, and the full network is relegated to the Supplementary Materials. Therefore, a reduced image was constructed in order to illustrate general patterns of the results. The haplotypes were colored with the same color scheme as the phylogenetic consensus tree and the neighbor–net analysis.

Phylogeographic statistical analyses of genetic diversity measures were calculated in MEGA4.1 beta3 (Tamura et al. 2007) and in Arlequin 3.01 (Excoffier et al. 2005) for the entire dataset and for the inferred structure within and between the defined 'populations'. First, the haplotype or gene diversity (h) is defined as:

$$h = \frac{n}{n-1}(1 - \sum_{i=1}^{k} f_i^2) \tag{5.1}$$

where n is the number of haplotype copies in the sample (population), k is the number of haplotypes, and f_i is the frequency of the i^{th} haplotype (Nei 1987, p. 180). Second, the mean number of pairwise

differences ($\hat{\pi}$), which is defined as F_{st}–based genetic distance measurement for populations with short divergence times, is the mean number of differences between all pairs of haplotypes within the sample. This is defined as:

$$\hat{\pi} = \frac{n}{n-1} \sum_{i=1}^{k} \sum_{j<i} f_i f_j d_{ij} \tag{5.2}$$

where d_{ij} is the sequence divergence (number of mutations) between the i^{th} and j^{th} haplotypes (Tajima 1983). The mean pairwise differences among samples was then calculated and resulted in a distance matrix, which is shown as a UPGMA tree (constructed in MEGA4.1 beta3). Third, nucleotide or sequence diversity (p) over L loci (number of bases in the sequence = 712) was calculated and is defined as:

$$p = \hat{\pi}_n = \frac{\sum_{i=1}^{k} \sum_{j<i} f_i f_j d_{ij}}{L} \tag{5.3}$$

and was plotted on maps in ESRI ArcView 9 in relation to h for phylogeographic inferences. These measures were then used for further interpretation of the genetic structure of the inferred populations. AMOVA (hierarchical **A**nalysis **O**f **M**olecular **V**ariance) was completed, which goes through a permutation of fixation indices to evaluate the amount of genetic population structure due to the variation within and among populations (Excoffier et al. 1992; Weir and Cockerham 1984; Weir 1996). In essence, this allows one to simultaneously compare the gene diversity (h), and the nucleotide diversity between the haplotypes (p) within and among the populations in the dataset. Please refer to the Arlequin manual for a full description of the calculations completed for AMOVA. AMOVA was calculated for the grouping of the populations into eight groups.

Core age analysis was done in MEGA4.1 beta3 with the unique coded haplotype alignment. A UPGMA tree was created, with pairwise deletion and unequal rates (gamma parameter 0.7), and linearized assuming clock–like behavior. The mutation rate selected for trnLF was 8.24×10^{-9}, which was calculated as a calibrated rate of the relatively young (0.3 mya) perennial *Aichryson*, (Crassulaceae) that has a minimum age a plant of 1 to 2 years by Richardson et al. (2001). A lineage through time plot (LTT) was constructed with this UPGMA–linearized tree in Genie 3.0 (Pybus et al. 2000, 2001; Strimmer and Pybus 2001). Geological events were placed upon the time scale of these data from the freely available summary of the geological time scale over the last 2.7 million years from the IUGS (Gibbard et al. 2005). Age estimation was also done with the addition of outgroup of *Abdra brachycarpa* and the closest non-core *Draba verna* in order to estimate the age of the Core *Draba* species (plot not shown).

5.3 Results and Conclusions

All networks resulting from these analyses do not fully separate the 21 regions which correspond to the origin of the samples. Therefore, the 21 regions were combined into Groups A to H for further comparison. Group A is South America (red dots) and it includes: 1–ChilePatagonian, 2–PeruvianAndes, 3a–ColombianVenezuelanAndes and 3b–EcuadorianAndes. Group B is North American Cordillera (orange dots) which includes: 4–SouthernCordilleraSCD, 5–CentralCordilleraCCD and

6–NorthernCordilleraNCD. Group C is Circum-North or arctic (green dots) which combines: 7a–BeringiaNorthAmerica, 7b–BeringiaAsia, 8a–ArcticNorthAmerica and 8b–ArcticAsia. Group D stands alone for clarity of its subtle differences from other arctic haplotypes and represents only the Amphi-Atlantic accessions (light green dots): 9–AmphiAtlantic. Group E is only represented by those accessions from Eastern North America (dark green dots): 10–EasternNorthAmerica. Group F represents the most isolated group, the region corresponding to Caucasus mountains, eastern Turkey and Iran (yellow dots): 11–Turkey–Caucasus–Iran. Group G combines all European and Mediterranean accessions (blue dots): 12–Mediterranean and 13–EuropeanAlpsCarpathians. Lastly, Group H combines all Central Asian Mountain accessions (purple dots): 14a–AltaiSayanBaikalMongolianMts, 14b–AlaiPamirTienShan, 15a–WHimalaya–Karakoram–AfgPakMts, 15b–EastHimalaya and 16–Tibet–ChineseMts. Despite the initial confusing nature of the resulting networks, there are some very intriguing conclusions and overall statements that can be made. First, the three Cores as defined in chapter 4 of this thesis (phylogeny) also are visible in all three networks. Second, each of the main clusters in the networks have the presence of Central Asian haplotypes, also corresponding with the previous phylogenetic study. Third, the networks indicate a large amount of haplotype sharing, chloroplast capture and evidence of hybridization, as shown by the immense amount of network connections in each network. Fourth, a remarkable amount of evidence indicates that this genus has undergone radiation events in all regions in which it is currently distributed.

5.3.1 Phylogenetic consensus network

After the addition of the approximately 180 sequences in this phylogeographic study in comparison to the phylogeny study in chapter 4, the general structure of the three Cores is unchanged. Most of the species added in this dataset grouped well with the other accessions of the same species or same regions (see the Accession List in Supplementary Materials for which Core each new accession appeared). In chapter 4 it was determined that the trnLF sequences were not able to fully resolve the branches within the Cores as well as not able to place certain accessions in the same Cores as the nuclear ITS data allowed. Knowing this, we chose to display the chloroplast haplotypes as a consensus network constructed from Bayesian trees which better illustrates this radiation. A reduced image of this network with only colored geographical dots can be seen in Figure 5.2 and the full network with accession names can be seen in the Supplementary Materials. A few unusual placements must be discussed. First is the presence of two old chloroplast types grouping with the outgroup, the Eastern North American genus *D. brachycarpa* (soon to be recognized as *Abdra brachycarpa*). Due to the fact that the Mexican MO8–jorullensis–4 and Northern Andean MO37–crypthantha–2 are well within the Core II in the ITS analysis from the phylogeny, the presence of this chloroplast type in the trnLF network must be an indication that these are relics of chloroplast capture events. Therefore, for the neighbor–net and TCS haplotype analyses these two chloroplast types were removed to avoid erroneous haplotype connections.

5.3.2 Neighbor–Net analysis

Again, a reduced image of this network with only colored geographical dots can be seen in Figure 5.3 and the full network with accession names can be seen in the Supplementary Materials. It is important to note that a neighbor–net analysis can be superior to other network methods in its ability to show the

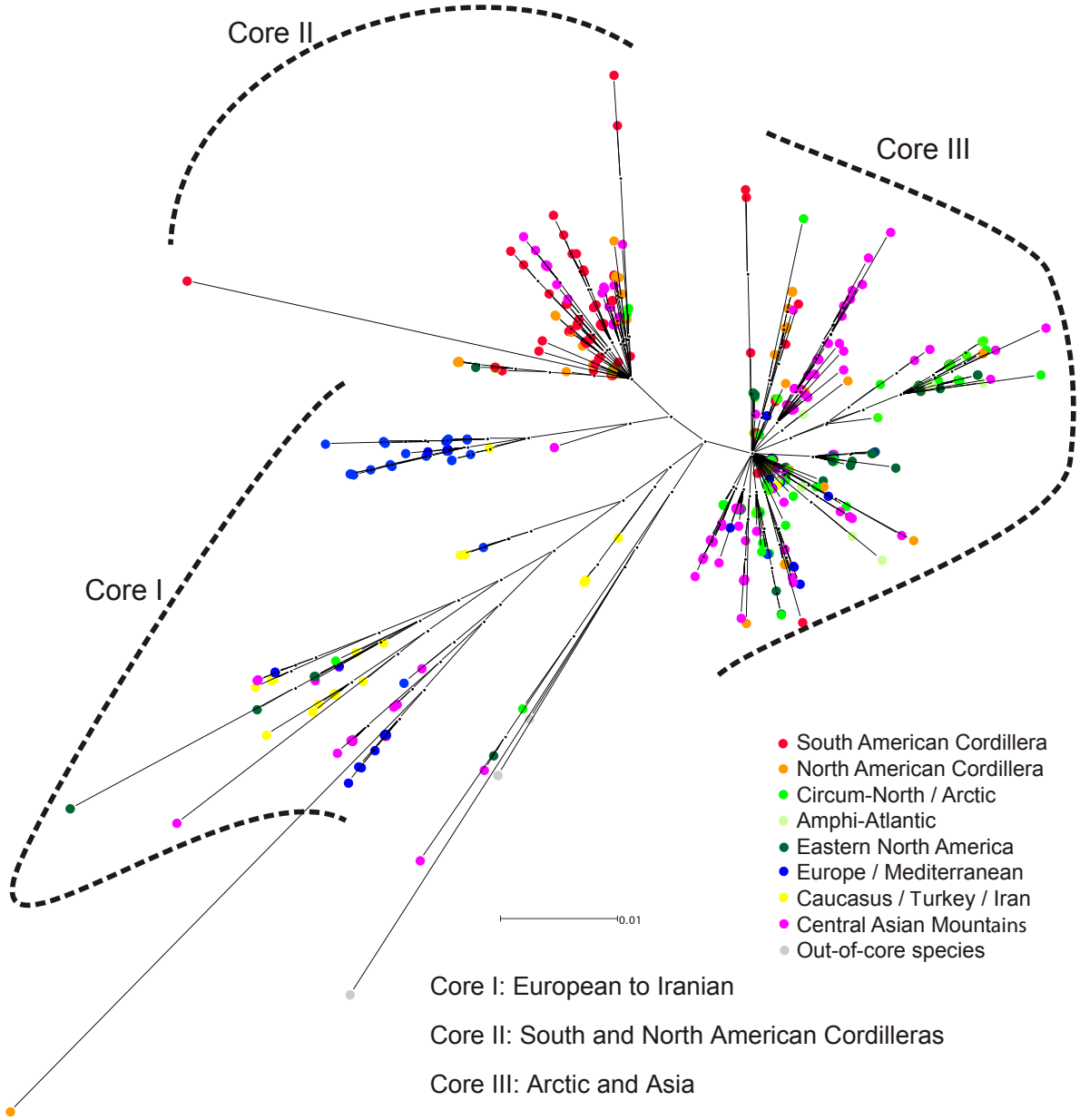


Figure 5.2: Consensus network calculated from Bayesian inference trees in Splitstree4. Regions are labeled by colored dots and Cores I, II, and III refer to those identified in chapter 4. See Supplementary Materials for full network with names.

relatedness of the haplotypes within the network. Each parallel line between the branches indicate that the branches have a shared character. Given that *Draba* shows a high amount of haplotype sharing, it is appropriate to use this neighbor–net network. A few things can be said about the chloroplast types that are seen in this network. First, present in the types grouped together with most of the South American accessions, is one from Sweden, 15a, 15b, 14b, and 16, and nearby many others from Central Asia. This reflects a similar composition of Core II in the phylogeny. These Central Asian and arctic accessions show that there was migration from these locations into the North American Cordillera and down into South American Cordillera, most likely via the Beringian Land bridge. Therefore, these chloroplast types show a distinct global migration path. Second, is the Mediterranean and European cluster, which coincides with Core I and is grouped together, and has no other chloroplast types from other geographic locations. Third, is the majority of the group from Caucasus, Turkey to Iran region, are found together in the network, but also are found connected to multiple other geographical regions. Those species include Central Asian representatives such as *D. nuda* and *D. nemorosa* and *D. pulchella* and other Asian species. This group of species, including *D. sibirica* is mostly also represented by Core I in the phylogeny, and they share a common set of indels in their trnLF sequences. *Draba longisiliqua* and *D. mollissima* are found together and were declared as the most basal lineage types for Core *Draba* in the phylogeny analysis. This network analysis yet again contributes to the hypothesis of this region being the origin of *Draba*. Fourth, is the massive radiation of haplotypes found on the lower right part of the network. This radiation consists of many species from all over the world, and is mostly represented by Core III, which is dominated by arctic and Asian species. We can therefore state that these would be some of the youngest haplotypes or have the least amount of time for diversification, and would be seen as evidence of recent and massive migration of lineages throughout the world.

5.3.3 TCS haplotype network

A total of 238 unique haplotypes were identified out of 558 accessions used for the TCS network analyses (Figure 5.4). The full network with accession names can be seen in the Supplementary Materials. There are a total of 202 species in the dataset. Haplotype singletons made up 73.5% of the haplotypes; with shared haplotypes making up the remaining 26.5% (62 types are shared). Of the shared haplotypes, 66.6% are represented by more than one species and 33.3% are of the same species. In addition, 55.5% of the shared haplotypes are found in more than one of 21 regions, while 44.4% are only found in one region. However, when considering only the eight regions, 65.1% of the shared haplotypes are found in only one region and 34.9% of the shared haplotypes are in more than one region. Each of the 8 regions have a high number of singletons compared to shared haplotypes. In A–South America, 78% are singletons; and B–North American Cordillera has 65%. The C–Circum–North–Arctic has 65%, D–Amphi–Atlantic has 61%, and E–Eastern North America has the highest percentage of singletons with 92%. F–Turkey–Caucasus–Iran has 71% and the G–Europe to Mediterranean region has 72%. Lastly, the H–Central Asian Mountains consist of 75% singletons. There are numerous interesting (i.e. ones that represent distant geographic locations) shared haplotypes that are worth discussing further. Most of these haplotypes are corresponding to Cores II and III, while those which are shared in Core I are mostly of the same species and are in the same geographical region. There are of course some haplotypes that are from Cores II and III that also are of the same species and region, but I refer you to the Accession List in the Supplementary Materials for these haplotype types. The most striking feature of this TCS

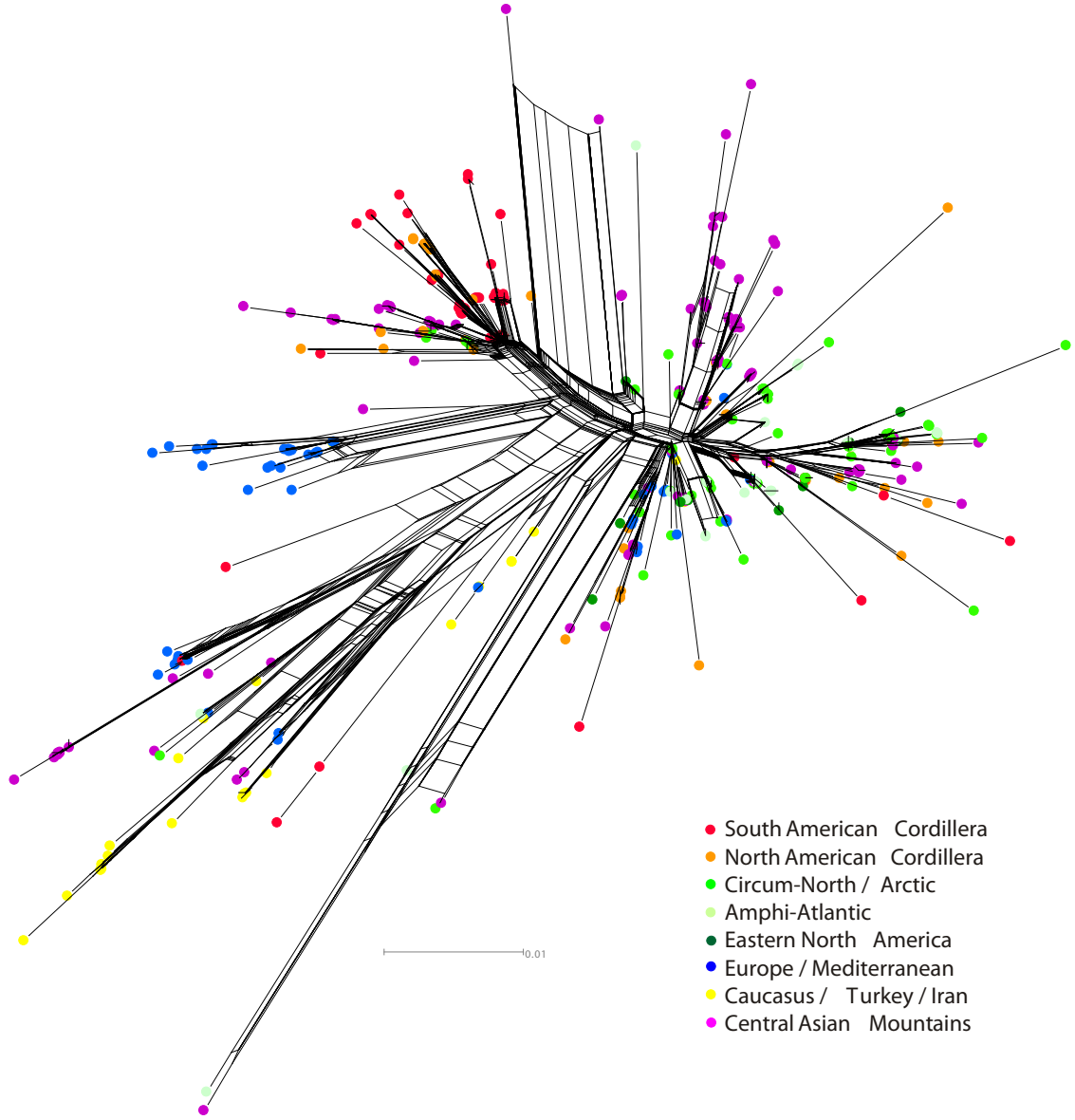


Figure 5.3: Neighbor–net network from sequences in constructed Splitstree4. Regions are labeled by colored dots, as in Figure 5.2. The three Cores can also be seen (orientation of network is the same). See Supplementary Materials for full network with names.

haplotype network is the obvious complicated connections that create the polytomy that we see in Cores II and III in the phylogeny, and the ever–present Asian lineages throughout the entire network.

The most common type of intriguing geographical sharing that can be observed is those which possibly have originated in the H–Central Asian Mountains and pass through northeastern Siberia, through Beringia, and either down into the B–North American Cordillera or eastward across arctic Canada and some also covering the D–Amphi–Atlantic region. There are 10 shared haplotypes that have approximately these same collection of geographical locations. These are types 10, 11, 12, 13, 18, 24, 33, 37, 43, and 47 (see Accession List in Supplementary Materials). With 4 of these being the most global haplotypes: types 10, 18, 24, and 33. Haplotype 37 is unique out of this group in that it is the only one that reaches as far south as Mexico as well as covering the arctic. There are also a number of haplotype groups that have a unique circumpolar trend and do not appear to venture south. These are types 7, 20, 30, 32, 34, 41, and 49, with the most common locations these share being Svalbard, the Taymyr Peninsula and Greenland. Haplotype 18 in particular is quite unique in having accessions from the Alai, Pamir, Tien Shan region, throughout North American Beringia and Cordillera, across arctic Canada, then as far south as Chile and Argentina. However, type 18 totally skips Mexico and the Northern and Central Andes. The only other haplotype group that does connect the B–North American Cordillera with the northern part of the Andes is type 21, but is distant from type 18 in the TCS graph. Type 21 is found in North American Beringia, Mexico, and northern and central Andes, however, not going into Chile or Patagonia. However, one mutation step from type 21 is type 38, which is solely northern and central Andean. There are many South American singletons that are directly connected to types 21 and 38, and therefore, this is the most geographically isolated and genetically similar group of the entire dataset. Also interesting is type 23 with three individuals from southwestern Greenland and one other from the Chilean Andes (MO62–magellanica–1), on the Pacific coastal side, and is one mutation step from type 21 in the TCS graph. The last piece to this migration puzzle may be type 46 with one individual in Mexico (56–jorullensis–4) and the other in Patagonia (41–magellanica–1), this is the only haplotype that holds both of these regions, but is in close proximity to other arctic and Beringian haplotypes in the TCS graph. Lastly is the strange type 4 with individuals from the Swiss Alps, the Slovakian Carpathians, and one from the Colombian Andes (1–cuatrecasana–3a). Also similar to these are two other Andean individuals, MO53–inquisiviana–2 and MO59–macleanii–2 from Peru, which are close to other arctic and Asian species in the networks. These connections can only be explained as chloroplast capture events where the Andean lineages seemed to survive the first radiation of *Draba* and currently coexists with the younger lineages in the region which are strongly grouping in Core II. The oldest haplotypes are from the European (most), Mediterranean, Turkey, Caucasus and Iranian regions (yellow and blue), and again are mostly grouping together in the right hands side of the graph. This is also supported by the phylogenetic analysis, being closest to the outgroups of that analysis. There are the few European accessions, NH1–aizoides–12 and B51–dedeana–12 from Spain, NH15–dubia–13 from the French Pyrenees, AL-DT1–tomentosa–13 from the Swiss Alps, B181–tomentosa–13 from the Italian Alps, and B115–lasiocarpa–13 from Montenegro that are separated from all the other European and Mediterranean accessions. These are grouping quite differently with mostly arctic and Asian individuals compared to the other European and Mediterranean individuals. These could be a result of recent speciation due to the LGM, compared to other European and Mediterranean lineages, leaving arctic lineages behind as the glaciers retreated back to the north.

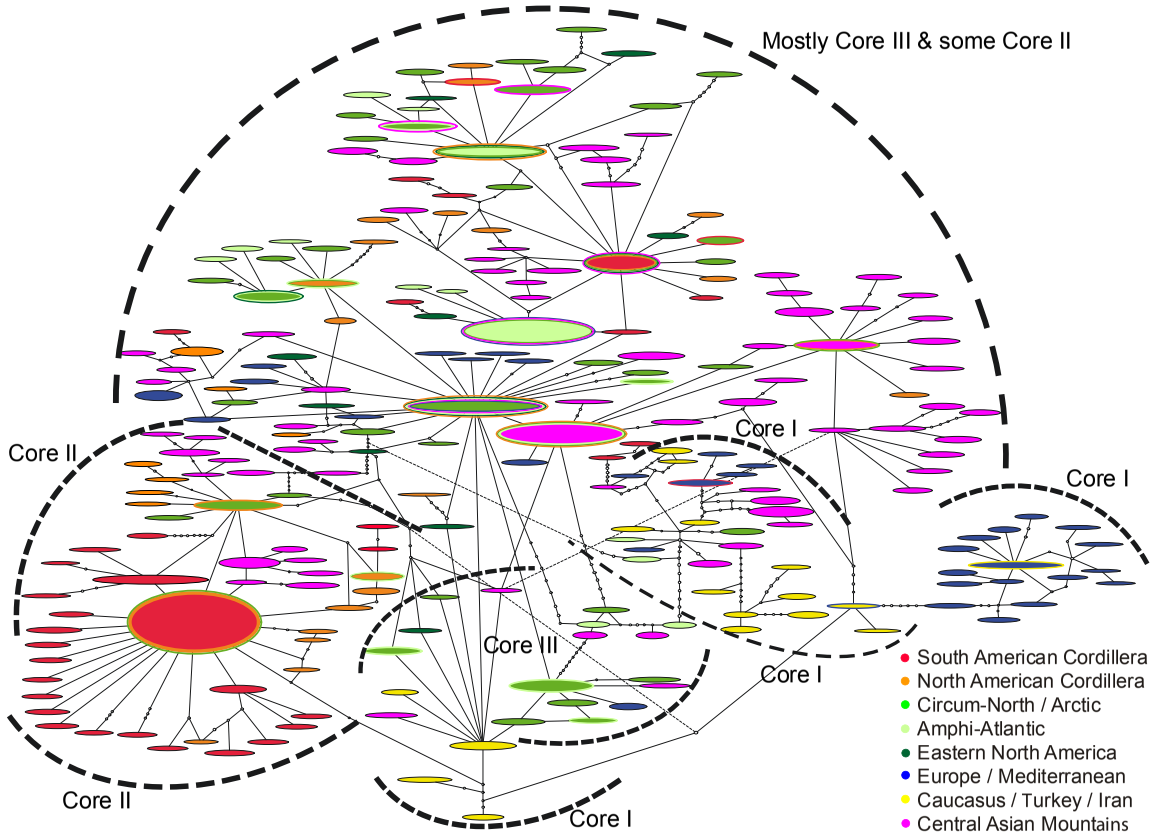


Figure 5.4: TCS network which has been reduced in size and the names have been deleted. Regions are labeled by colored bubbles and sizes of the bubbles correspond approximately to the relative number of accessions present in each haplotype. Bubbles with colored margins are those with shared haplotypes that represent more than one geographic region. The main color of the bubble means the majority of haplotypes are from that region. Thin dashed lines between haplotype groups are those which seem to have an unusual connection between geographic regions. White dots between the colored bubbles represent a mutation step between the haplotypes. General groups of the three Cores are marked. See Supplementary Materials for full network with names and the Accession List for all information about each accession.

5.3.4 Genetic diversity statistics

In order to analyze the genetic structure of the dataset, our predefined populations of 21 groups were subject to population genetic theory. The haplotype or gene diversity h has a narrow range of 0.8148 to 0.9872 and mean value being 0.9163 (see Table 5.1, Figure 5.5a). On the other hand, the nucleotide diversity p has values with a wider range, compared to the values for h, from 0.0067 to 0.0768, and a mean of 0.0314 (see Figure 5.5b). Since we had already pointed out that, on average, 70% of all haplotypes are singletons for each region, it is not surprising that the gene diversity is relatively constant between the populations. However, the nucleotide diversity shows a large difference between the regions and much can be said about these values. In addition the p–values were significant for both h and p, and therefore we can reject the null hypothesis and conclude there is statistically significant genetic structure in the dataset. The populations with the lowest p are the South and North American Cordilleras, the arctic (excluding the Asian Arctic), Eastern North America, and the Western Himlaya, Karakoram, Afgahn and Pakistan mountains. The population with the lowest p is the 3b–Ecuadorian Andes with a value of 0.0067. The populations with the highest p are the Asian Arctic, Amphi–Atlantic, the Caucasus, Turkey, and Iran group, the Mediterranean and European groups, and the remainder of the Central Asian Mountains. The population with the highest p is the 11–Turkey–Caucasus–Iran region with a value of 0.0768. The values for mean number of pairwise differences within the population or sample ($\hat{\pi}$) gave essentially the same trend as those for p and are therefore not shown in the Table.

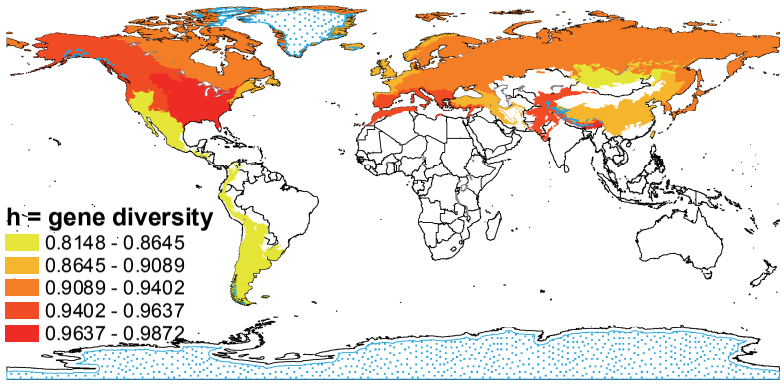
In the comparison of h and p, I refer to Table 5.2 which summarizes Avise's phylogeographic inferences made from these values (Avise 2000). Those values which are below the average were considered 'low' and those above were considered 'high' from Table 5.1 and a best illustrated on the maps seen in Figure 5.5c. We can then conclude that the populations 1–ChilePatagonian, 2–PeruvianAndes, 3a–ColombianVenezuelanAndes, 3b–EcuadorianAndes and 4–SouthernCordilleraSCD all could have experienced severe or prolonged bottlenecks in recent history or a selective sweep as a result of recent and strong positive natural selection (i.e. low h and p). These populations show that their migration and diversification have been isolated to a relatively continuous corridor of mountain ranges stretching mostly from north to south. As mentioned in the results of TCS, haplotypes are shared from the arctic to this region indicating the possibility of a once–continuous effective population that has since gone extinct and what we are now seeing is the relict of this path (see Dvornyk et al. 2002) for an example with *Pinus sylvestris*). Next, the populations 5–CentralCordilleraCCD, 6–NorthernCordilleraNCD, 7a–BeringiaNorthAmerica, 7b–BeringiaAsia, 8a–ArcticNorthAmerica, 10–EasternNorthAmerica and 15a–WHimalaya–Karakoram–AfgPakMts could all possibly have experienced rapid population growth from an ancestral population with a relatively small effective population size (N_e), and had enough time for variation to accumulate, but not long enough for major differentiation (i.e. high h and low p). These regions are also those which where the most affected by the glacier cover during the last glacial maximum (LGM), and is illustrated in Figure 5.5d with a proposed N_e, which would have shrunken their N_e, severed populations, and what we see today is an expansion from these resulting lineages. Their similarity leads us to conclude that their post–vicarience dispersal events may have been similar as well (i.e. shared with bird habitat and migration patterns). Populations 8b–ArcticAsia, 12–Mediterranean, 13–EuropeanAlpsCarpathians, 14b–AlaiPamirTienShan, and 15b–EastHimalaya could be the result of a stable population with a large, long–term N_e or a collection of admixed individuals from historically separated older populations (i.e. high h and p). It is not surprising that the 8b–ArcticAsia group does not share a history similar to other

arctic regions, due to the fact it was not glaciated during the LGM as most other arctic regions. This is also the case for the less affected regions of 13–EuropeanAlpsCarpathians, 14b–AlaiPamirTienShan, and 15b–EastHimalaya in comparison to the large ice sheet in the north, and the numerous refugia that have been identified in these mountains, allowing for relatively uninterrupted speciation over shorter geographical distances. Lastly, the remaining groups 9–AmphiAtlantic, 11–Turkey–Caucasus–Iran, 14a–AltaiSayanBaikalMongolianMts and 16–Tibet–ChineseMts could have experienced a transient bottleneck in a large ancestral population which resulted in fewer haplotypes, but does not effect p or they are geographically subdivided populations (i.e. low h and high p). These groups allow us to conclude the they are the closest to the ancestral populations that are still occurring today given they have had the most time for continual divergence seen from their high nucleotide diversity.

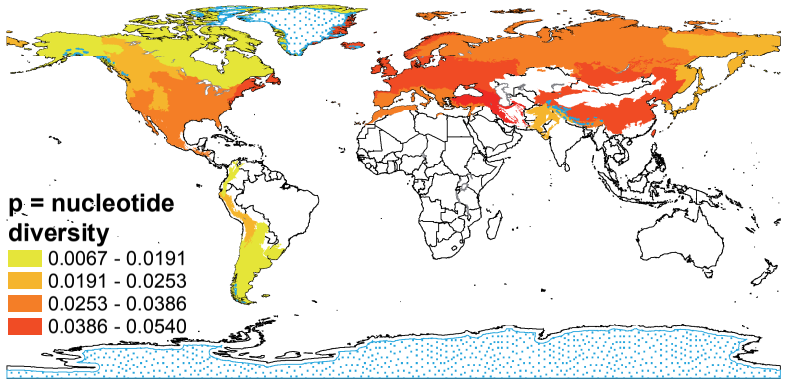
In the calculation of the pairwise differences between populations, the matrix computed gives a zero value for identical sequences, a value of 1 for sequences which are totally unrelated, and a value of 0.5 for a distinct genetic differentiation. These matrix is displayed as a UPGMA tree for the eight regions

21 Regions	h	p
1–Chile Patagonian	0.8182 ± 0.1191	0.0191 ± 0.0107
2–Peruvian Andes	0.8433 ± 0.0592	0.0205 ± 0.0108
3a–Colombian Venezuelan Andes	0.8874 ± 0.0540	0.0134 ± 0.0073
3b–Ecuadorian Andes	0.8148 ± 0.0529	0.0067 ± 0.0039
4–Southern CordilleraSCD	0.8571 ± 0.0637	0.0287 ± 0.0148
5–Central CordilleraCCD	0.9610 ± 0.0240	0.0226 ± 0.0119
6–Northern CordilleraNCD	0.9526 ± 0.0279	0.0217 ± 0.0115
7a–Beringia North America	0.9600 ± 0.0233	0.0169 ± 0.0090
7b–Beringia Asia	0.9384 ± 0.0340	0.0211 ± 0.0110
8a–Arctic North America	0.9261 ± 0.0232	0.0178 ± 0.0094
8b–Arctic Asia	0.9402 ± 0.0202	0.0326 ± 0.0166
9–Amphi–Atlantic	0.8929 ± 0.0313	0.0477 ± 0.0236
10–Eastern North America	0.9872 ± 0.0354	0.0272 ± 0.0146
11–Turkey–Caucasus–Iran	0.8893 ± 0.0344	0.0768 ± 0.0378
12–Mediterranean	0.9630 ± 0.0200	0.0386 ± 0.0196
13–Europe–Alps–Carpathians	0.9379 ± 0.0319	0.0479 ± 0.0241
14a–Altai–Sayan–Baikal–Mongolian Mts	0.8645 ± 0.0541	0.0490 ± 0.0245
14b–Alai–Pamir–Tien Shan	0.9526 ± 0.0304	0.0540 ± 0.0276
15a–West Himalaya–Karakoram–AfgPak Mts	0.9637 ± 0.0205	0.0253 ± 0.0130
15b–East Himalaya	0.9827 ± 0.0183	0.0315 ± 0.0162
16–Tibet–Chinese Mts	0.9089 ± 0.0328	0.0398 ± 0.0201
average	**0.9163**	**0.0314**

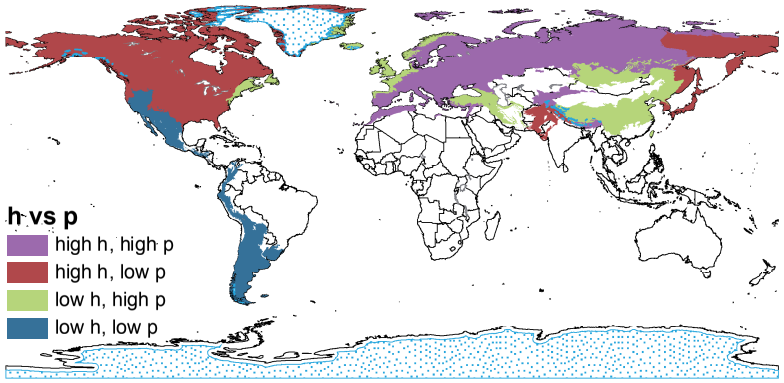
Table 5.1: Genetic diversity statistics for population structure with 21 groups. Measures were calculated in Arlequin 3.01 (Excoffier et al. 2005). Where h is gene diversity and p is nucleotide diversity. The average was used to define which populations have high or low values which is further used for phylogeographic inferences (see Figure 5.5c).



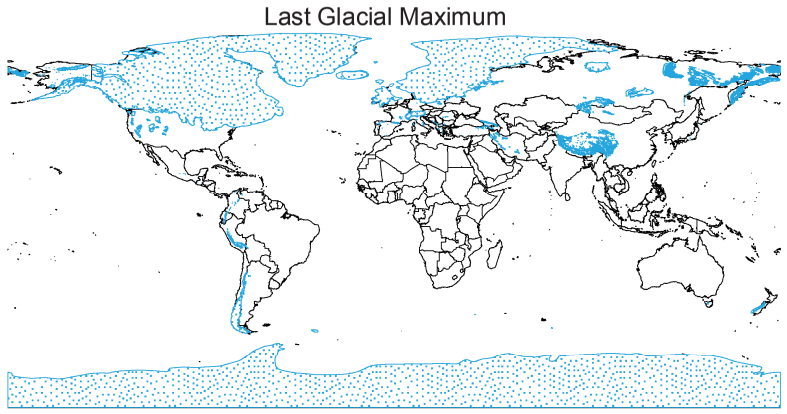
(a) Gene diversity (h).



(b) Nucleotide diversity (p).



(c) Comparing high and low h and p for phylogeographical inferences.



(d) Last glacial maximum taken from Ehlers and Gibbard (2004) shown here for pattern recognition with *Draba*'s phylogeographic history.

Figure 5.5: Global maps comparing diversity statistics from which exact values for each region are listed in Table 5.1 and geography. All maps were created in ESRI ArcView 9.

(Figure 5.6). The overall mean of pairwise differences for the entire dataset is 0.018. Even though *Draba* has very low values indicating the sequences are very similar, proving that genetic diversity is relatively low for all levels of the dataset, a structure can be seen from this relatively young radiating species. At the base of the UPGMA tree is group F–Turkey–Caucasus–Iran, a result almost too good to be true. It also seems to explain the same migration route from there westward through the G–European and Mediterranean region and eastward into the H–Central Asian Mountains as the correlation between h and p from above. The H–Central Asian Mountain lineage evolves into the youngest lineages of the North and South American Cordilleras (groups A and B) and the arctic (groups C, D, and E). What is interesting is the separation of the D–Amphi–Atlantic group from the G–European and Mediterranean group indicating the dominance of arctic lineages that originated from the Central Asian Mountains rather than from the European continent.

In conjunction with the results from the pairwise differences between groups as shown in Figure 5.6, AMOVA also indicates there is greater differentiation within the 8 groups of populations (82.4%) rather than among them (10.7%) (Table 5.3). This leads us to the conclusion that the populations are highly diverse and at the same time not that much different from each other. This could be due to their relative isolation in some cases, but on a short time scale as to have not had time to diverge enough to develop a large number of unique mutations. It also could be a result of populations that have been highly effected by migration and have had reoccurring opportunities to create new population structures, but with lineages that may have been in contact multiple times. This would result in populations that eventally appear more identical to eachother given their shared history. It also could be our pure lack of samples of true populations. However, we have shown that the populations are significant, that they show a genetic and geographical structure, and the ability of the data allowing the inference of probable migration routes.

Phylogeographic Inference	**high** p	**high** h	**low** p	**low** h
prolonged or severe demographic bottleneck (or a selective sweep) in recent times			x	x
stable pop with large long–term N_e or samples from historically separated pops	x	x		
rapid pop growth from an ancestral pop with small N_e (if enough time passed for variation, but too short for large differences)		x	x	
transient bottleneck in large ancestral pop, or samples from small, geographically subdivided pops	x			x

Table 5.2: Phylogeographic inferences comparing haplotype diversity h and nucleotide diversity p, from Avise 2000, pgs 59–60. Where N_e is effective population size.

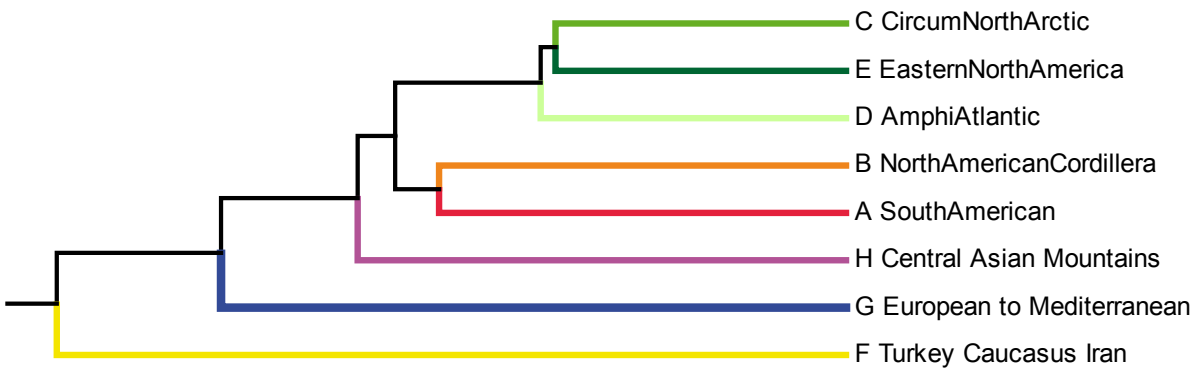


Figure 5.6: UPGMA tree of pairwise differences between eight geographic groups. Pairwise difference matrix was calculated in Arlequin, tree was calculated and drawn in MEGA4. This figure uses the same color scheme as in the networks, and especially shows the same hypothetical migration route as discussed in the haplotype sharing analysis in the TCS network.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among groups	7	781.312	1.18638 Va	10.7
Among populations within groups	13	364.729	0.75783 Vb	6.86
Within populations	541	4925.36	9.10419 Vc	82.4
Total	**561**	**6071.41**	**11.0484**	

Table 5.3: AMOVA of the populations subdivided into 8 groups was calculated in Arlequin.

5.3.5 Dating the Core *Draba* radiations

A brief analysis of the age of Core *Draba* gave an age of approximately 2.3 million years. A UPGMA tree of all the unique coded haplotypes was constructed and linearized by calibrating the tree, assuming a constant molecular clock with a mutation rate of 8.24×10^{-9} (Richardson et al. 2001) (done in MEGA4) (shown in Figure 5.7). The lineage through time (LTT) plot shows a gradual increase in lineages with a few points of increased radiation events. By placing important geological events on the time scale of this estimation, we can make a hypothesis about times which may be correlated with an expansion in lineages. Points in the tree where there is an increase in number of branches can be correlated with the lineage through time plot below the tree. It appears that there was an establishment of *Draba* lineages during the Pre–Pastonian glacial stage. Then some time after the interglaical period of the Pastonian, during the Beestonian/Pre–Illinoian stages saw an increase in *Draba* lineages. The next radiation was in the early part of the Ionian stage. The final radiation coincides with the end of the Illionian glacial period.

5.4 Discussion

It can be proposed that older lineages with lower haplotype diversity and higher sequence diversity that have experienced a transient bottleneck from a large ancestral population or geographically subdivided population can describe the Amphi–Atlantic region (9), the mountain ranges of the Altai, Sayan, Baikal and Mongolian regions (14a), the Tibetan Plateau and mountains of central and southwestern China (16), and the mountainous regions of eastern and northern Turkey Caucasus, and Iranian highlands (11). In correlation with the basal position of Caucasus and surrounding areas, as well as the inclusion of some Central Asian lineages at the base of all three Cores, indicates the center of origin of *Draba* is somewhere between these areas (see Figure 5.8a). This is in correlation with the results from the phylogeny. Interestingly, is the similarity in genetic structure to Amphi–Atlantic group. This could be due to the fact it could have been a major corridor of subdivided populations throughout the islands and ice–free refugia proposed to occur there during the last glaciation cycles (Brochmann et al. 2003, 2004). It must be noted that the Beringian Land Bridge accessions do not have a similar phylogeographic history as those from the Amphi–Atlantic group, this can be explained by the complete difference in ecosystems during the emergences of each land bridges. The North American Cordillera lineages represent the lower reaches of the lineages which are possibly relics from the LGM. Their common sharing with the arctic and Asian haplotypes indicates this. North and South American Cordilleran lineages appear to consist of both old and new haplotypes, with the new haplotypes having less divergence between sequences. We can therefore infer that the first migration of *Draba* to the southward was much earlier than the most recent one. The sharp difference in phylogeographic history between the Southern North American Cordillera and the Central North American Cordillera indicate this is possibly the suture zone initially proposed by Koch and Al-Shehbaz (2002) (see Figure 5.8b). As the lineages which were pushed down by the expanding glaciers into the Central Rocky Mountains collided with the more established and older lineages of both the Central and Southern North American Cordillera, it created the area of high species diversity. It also could be the direct reason why the Central Rocky Mountains and neighboring areas have such a wide variety of basal chromosome numbers, unusual ploidy levels, and increased asexual reproduction via apomixis (see chapter 4).

So when exactly could have this suture zone been created, and how old is this center of species diversity in comparison to the age of the genus? The high amount of singletons in all regions indicate there has been strong pressures for differentiation, and the evaluation of the sequence divergence both indicate these events are very recent. In the estimation of the Core *Draba*, we have concluded there have been a number major radiation events. The first occurred during the divergence of *Draba* from its closest lineages (*D. verna* and *D. hederifolia* possibly as early as 11 mya in the Miocene (not shown in Figure 5.7). These basal *Draba* lineages may have gone extinct for there are no relatives seen in the current Core species. Except for the two haplotypes mentioned in the phylogenetic section of this chapter, *D. jorullensis* and *D. crypthantha*. These two accessions may hold the ancestral chloroplast genome of the most common ancestor between the outgroup lineages, which are also mostly from the Southern North American Cordillera and South American Cordillera (see chapter 4) and Core *Draba* lineages. Which puts these ancient and mostly extinct lineages of *Draba* to be highly correlated with the known 'Ring of Fire' where the modern lineages of *Draba* still strongly inhabit. The creation of the Isthmus of Panama during the end of Pliocene, which initiated the cooling of Earth (beginning the Pleistocene) could be considered the turning point in Core *Draba* lineage evolution.

However, focusing on the Core *Draba* species, they must have had a relatively stable population during the Pliocene, and began a slow expansion about 2.3 mya during the beginning of the Pleictocene in the Galasian epoch, when lineages began to increase and create the Cores which we see today (refer to Figure 5.7). During the Galasian (2.6 – 1.8 mya), which is officially accepted by the International Union of Geological Sciences (IUGS) to be the beginning of the Pleistocene rather than the end of the Pliocene (Gibbard et al. 2009), therefore marking it as the beginning of the cooling of Earth, *Draba* gradually speciated in preferably colder habitats than its direct ancestors. Within the Galasian, numerous global glaciation cycles occurred (Gibbard et al. 2005). The first sharp increase in lineages occurred approximately 1.6 mya, while in the Calabrian epoch. The next is in the beginning portion of the Ionian epoch. The last sharp increase is at the end of the Ionian, beginning of the Tarantian, which is marked by the end of the Illionian glacial period approximately 125,000 years ago. At this time, an interglacial period of approximately 20,000 years dominated until the beginning of the last glacial period (Weichselian/Devensian/Valdaian/Wisconsinan). A final possible series of events that could have effected the most recent populations of *Draba* is the emergence of the 1st and 2nd Beringian Land Bridges fig:DrabaMigrationc. It seems that there has been little lineage radiation since the beginning of the present interglacial cycle, the Flandrian (since 12,000 years ago). However, as we mentioned above, the lineages pushed down from the North American Cordillera into the Southern Cordillera may have created this very recent center of species diversity and suture zone at LGM, which is a completely different time frame and location from the origin of the Core itself at 2.3 mya.

It is possible to say that during the interglacial period following the Illionian glacial, *Draba* lineages increased, but it could also be said that *Draba* has seemingly had a steady increase in lineages throughout these cycles. If *Draba* only radiated during interglacials and was stable during glacials, such a pattern could be seen from the data. This assists us in the conclusion that *Draba* can easily adapt to changing habitats in the face of such large global climate changes, possibly due to its ability to undergo polyploidization and hybridization. *Draba* seems to be unaffected by vicarience events such as glaciation growth and retreats, as the lineages seemed to steadily increase throughout this time of great environmental variability. It must be noted that these are estimates made without fossil evidence and therefore it is fully possible that the time frame is not accurate. However, in the following section we discuss the possibility of *Draba* being closely linked with the fauna and glacial boundaries of the Pleistocene.

5.4.1 Modes of Migration

With regards to seed dispersal, again no studies have been made to as the actual method for dispersion. However, having a seed head that can break off easily after maturity, like most Brassicaceae, and with dehiscent siliques (and silicles), it can be assumed that the seeds and/or siliques themselves are possibly distributed by birds and wind. Birds that inhabit rocky outcrops find similar shelter in the same locations as *Draba*. Specifically D. grandis, which is distributed in Alaska, British Columbia and The Russian Far East (Kuril and Ramanov islands) is found almost exclusively in sea bird rockeries and rocky bluffs above salt-water beaches. Also, several Amphi-Atlantic or Amphi-Beringian taxa show similar patterns of distribution and localization. I personally have found numerous herbarium specimens with feathers entangled in the plant, often with leaves having ciliate or tomentus trichomes, seemingly buried into the rosette. There is no reason to dismiss the possibility of small seeds or siliques attaching themselves to the feathers of their nesting partners and being carried from one migration point to the next. This hypothesis

could possibly be supported by the presence of chloroplast haplotypes that are found in far–reaching areas even when the species themselves do not have a similar wide range. It is also possible that the seeds, after ingestion, were carried from one location to the next, given the seed coat is highly sclarified (personal observation). Further work needs to be done to correlate specific bird species which nest in preferred *Draba* habitats to specific *Draba* species in order to make such claims.

Second, is the evidence of *Draba* on the Mammoth Steppe (as described by Guthrie 1990, 2001) in the ancient flora of North American Beringia associated with woolly mammoths (Zazula et al. 2002) and a packrat (*Neotoma* spp.) (Zazula et al. 2005) and directly from preserved colon contents of a woolly mammoth from northeastern Siberia (what I have referred to as Asian Beringia) (van Geel et al. 2008). Even though it is impossible to prove that mammoth herds directly assisted in migration of *Draba*, it can, however, be assumed they were linked by their direct overlap in habitat, and that the tiny seeds of Draba species are distributed by these mammals though the mud on their feet or on their thick, shedding fur as they cover substantial distances. It is possible that the mammoth and other mammal populations, such as bison and bears (Barnes et al. 2002; Shapiro et al. 2004) which migrated, assisted in the gradual speciation of *Draba* throughout the Mammoth Steppe region. However, the migration of mammoths appears to be relatively unimpressive. In isotope studies of mammoth tooth enamel, it was concluded that the high plains mammoth of North America (from Colorado to Texas) were shown to have a migration of no more than 600km and most likely only 200km (Hoppe 2004). Also, Siberian mammoths were stated to have only migrated 650km from the comparison with modern–day elephants (Olivier 1982). This little amount of migration could not very well contribute greatly to the dispersal of plant species. However, given enough time, for example the expansion and retreating of glaciers (even if this is less than 100 years), and the behavior of males moving between populations of females and their young, the mammoth's range would too have changed. With the consideration of this, we can see how a close association with the animal and its plant habitat can very well contribute to the dispersal of lineages. Similarly, intercontinental or intracontinental long-distance bird flights can easily account for the North-South American migrations or the Arctic–Altai disjunctions.

5.4.2 Do areas of high species and genetic diversity overlap?

We can conclude there is almost no direct correlation between these two centers of diversity for *Draba* and can be seen in Figure 5.8c. As described in of this thesis, the three major centers of species richness/diversity are the North American central Cordillera (namely the Greater Rocky Mountain range with 56 species), the Tibetan Plateau and mountains of southwestern China (52 species), the Sierra Nevadas of Mexico and southern U.S.A., Great Basin, and neighboring mountains (49 species), the Northern Andes of Ecuador (48 species), and the Himalaya, Karakoram, and Afghan and Pakistan mountains (45 species). We have shown here that the center of genetic diversity is mostly found in the region between Eastern Turkey, Caucasus, and Iran, and the lineages of this region hold the most basal position in all analysis. The other regions with relatively high genetic diversity are the mountain ranges of the Alai, Pamir, Tien Shan, Altai, Sayan, Baikal, and Mongolia, the Mediterranean, Amphi–Atlantic, the Tibetan Plateau and southwestern Chinese mountains, European, Asian Arctic and Eastern Himalaya. The only similarities between these two lits of regions is Tibetan Plateau and southwestern Chinese mountains and eastern part of the Himalaya. Most striking is the difference between the Ecuadorian Andes between the species richness and genetic diversity. It is one of the highest regions of species richness and endemics (48 species)

mostly which occur only within that portion of the Andes, and at the same time has the absolutely lowest value for both nucleotide diversity (p) and haplotype diversity (h). However, if the origin of the species is found to be in the Tibetan Plateau and southwestern Chinese mountains or Eastern Himalaya, then one can say that species richness, genetic diversity, and origin of species are all in the same location. However, since the region with the highest of these measures is desired then the center of species richness is in the Greater Rocky Mountains, the center of genetic diversity is surrounding the Caucasus region, and the center or origin is somewhere between the Caucasus and the Central Asian mountains. With this said and the data presented both in the phylogeny and phylogeography, we can state that the center origin partially overlaps with the center for genetic diversity, but NOT with the center for species diversity. It was also shown in the age estimation of the Core *Draba* species that the development highest center for species diversity in the Central Rocky Mountains is a very recent event compared to the origin of the genus itself. The prevailing thought of identifying the origin of a genus simply by its center for species diversity during the 20th century cannot be supported in this case. This study on *Draba* shows that in order to identify the origin of a genus or species, one must analyze the genetics of the organism.

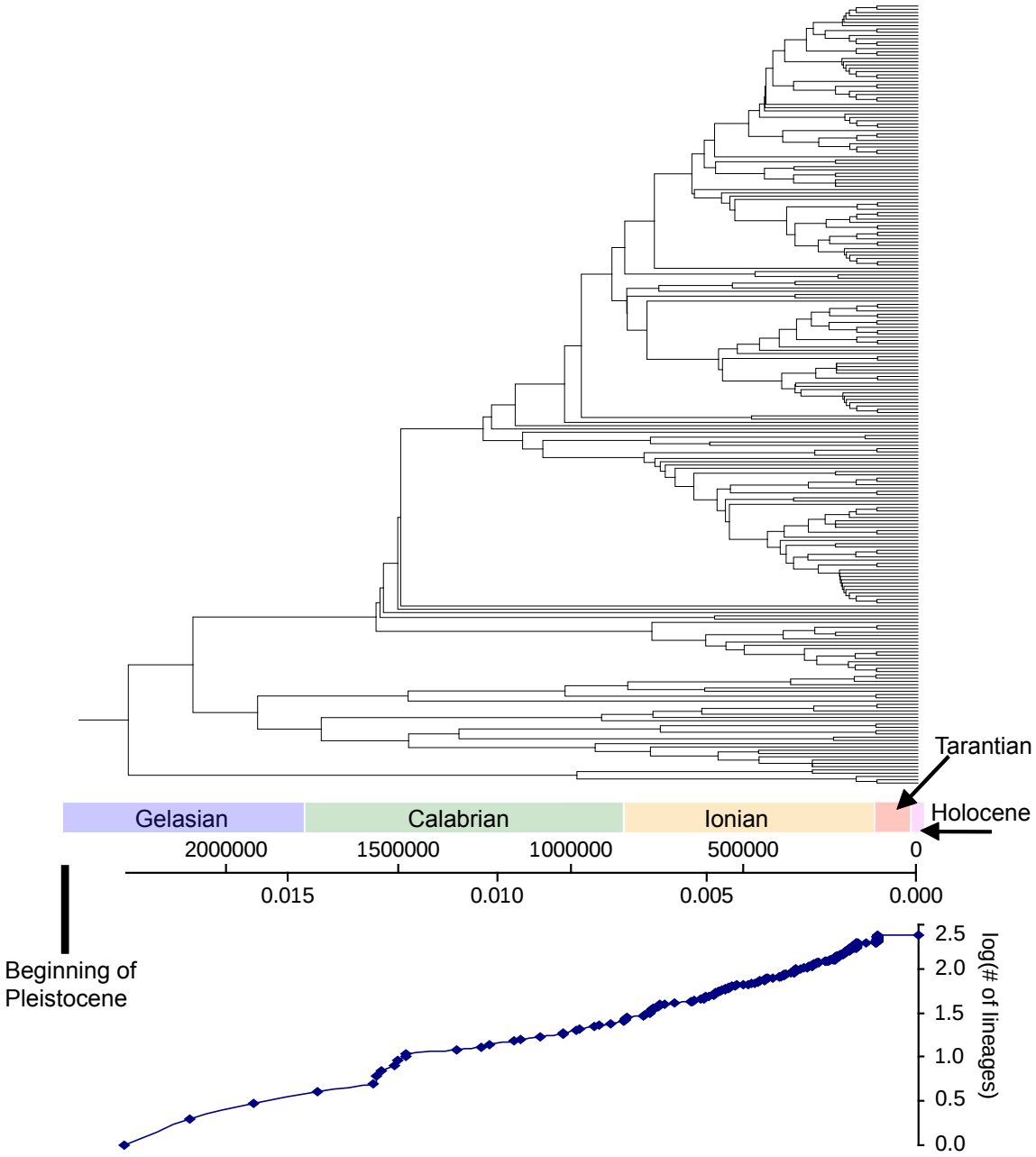
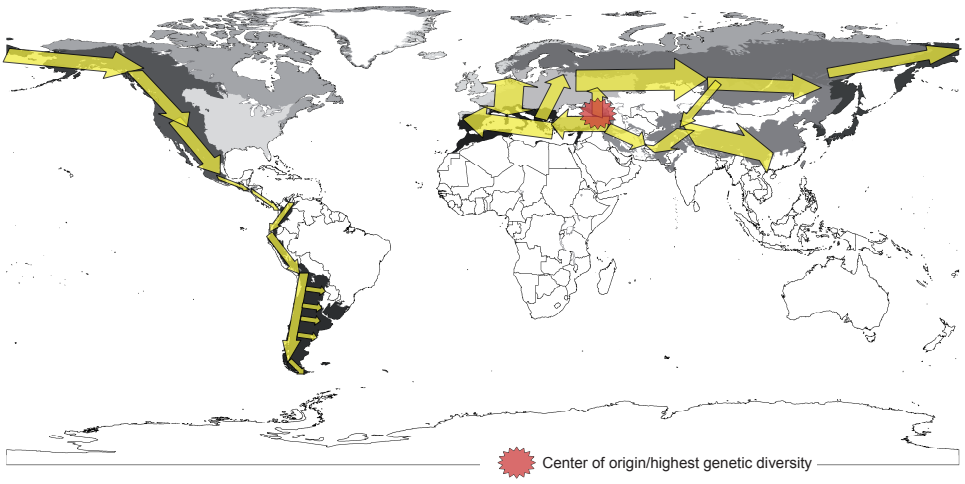
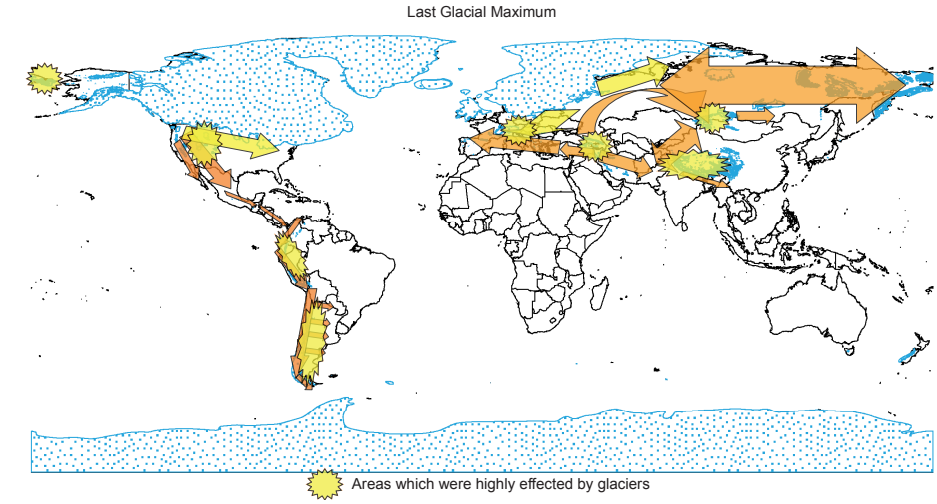


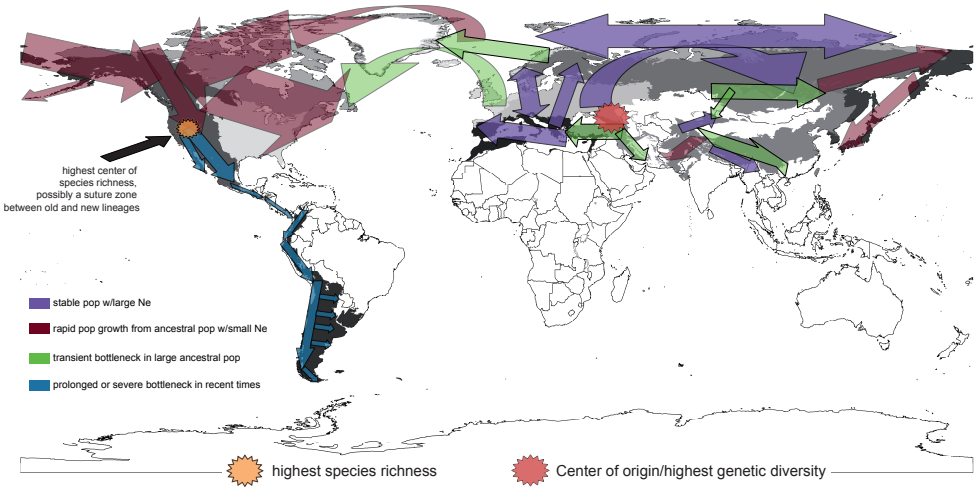
Figure 5.7: A linearized UPGMA tree constructed from unique coded haplotypes in MEGA4 is shown here. An estimated rate of evolution used was 8.24×10^{-9} (Richardson et al. 2001) assuming a constant molecular clock mutation rate. A lineage through time plot was constructed from this UPGMA tree in Genie 3.0 and is shown on the same time scale below the tree. Hypotheses about specific geological events that may have contributed to *Draba* lineage radiation shown. Correlation between the increased number of lineages and these events can be seen. Further work is being done to improve the calibration of the mutation rate so as to better determine the exact time frame. Regardless of the possible inaccuracies, it can be shown that Core Draba have been gradually increasing in lineages throughout the Pleistocene with a few instances of increased radiation.



(a) Early distribution of older lineages.



(b) Proposed lineages present during the Last Glacial Maximum between 20,000 and 8,000 years ago.



(c) Most recent migration events after the Last Glacial Maximum.

Figure 5.8: The compilation of the data herein leads to the final conclusion of these migration routes for Core *Draba* lineages across the globe in the past 2.7 million years.

Chapter 6

Conclusions

In retrospect, *Draba* as a model for speciation processes has been an absolutely spectacular group to study. The number of species occurring throughout the world, the patterns of high species richness in polyploids, the phylogenetic conclusions of the genus as a whole, and the phylogeographic pattern of radiation, all give us the unique opportunity to study speciation at the genus level on a world-wide basis. *Draba* encompasses many types of distribution patterns, from widespread in the arctic, to endemic on isolated mountains. At the same time there are distinct groups of *Draba* species with overlapping distribution ranges, resulting in groups that could be studied as a unit for such complex facets as the effective population size variation within a genus. In the face of this extensive compilation of information, however complete the *Draba* richness accounts appear to be, there are still mountain peaks that most likely hold undiscovered *Draba* species. There is also an incomplete assessment of the rarity or endangered status of *Draba* on a world-wide scale. Also, could *Draba* be an indicator species for healthy alpine and/or arctic ecosystems to understand conservation efforts or the effect of global warming on plants found at the margins (see Crawford 2008)? Collecting the needed data on *Draba* will take many years and a lot of money. However, regardless of all of these shortcomings, we are well on the way to understanding the distribution, speciation, and migration of this unique global genus.

In this report we have fully illustrated the trends of polyploidy occurring most often in the higher alpine and arctic regions of the world. A bold statement has been made here about the possibility that speciation and subsequent polyploidization are accelerated by forced migration and recolonization induced by glaciation cycles. With *Draba* it can be seen that speciation and polyploidization rates are accelerated compared to other herbaceous plants. *Draba* is a genus that survives at the margins of high alpine and arctic ecosystems. The higher the altitude and latitude, the higher the ploidy level in *Draba*. In addition, the presence of alternative base chromosome numbers and apomictic species (Windham 2000, 2003) is isolated in the Central Rocky Mountains of North America, and have been identified as the center of species diversity and a suture zone between arctic/northern Cordilleran with southern Cordilleran species. Further work needs to be done on other areas of proposed suture zones (for example in the Central Asian Mountains and between the arctic/alpine zones) in collecting chromosome numbers of other endemic and narrowly distributed species. However, even with only 40% of the species with chromosome counts, one can still see these trends in *Draba*'s speciation and polyploidzation processes, in conjunction with the genetic data presented herein. However, the ultimate questions to as exactly what processes cause genome

duplication are still not determined. Only after extensive population studies, greenhouse experiments, physiological experiments and genome-wide analysis will these questions be answered.

Prior to the study of a complex genus such as *Draba*, it was vital to understand what constitutes a *Draba* and which species are so far apart that they can't be compared in relation to the process of polyploidization. After the studies herein, we have determined that true *Draba* species are almost all higher alpine and arctic or subarctic perennials or short-lived perennials, excluding most of the low-altitude annuals species, especially the eastern and some southwestern North American *Tomostima* and *Abdra* species. It remains to determine the placement of *Draba verna* and *D. hederifolia* within or outside of *Draba*. After this study, they are apparently at the base of the Core *Draba*. However, the addition of the remaining *Draba* annuals not included herein could reflect light on this conundrum, as well as assist us in better dating the split between these groups. It was important to compare this phylogenetic study, which was based both on nuclear and chloroplast data, to the only 'current' morphological classification system for *Draba*. Our studies showed without a doubt that most of the previous morphological groups and subdivisions of the genus (e.g., Schulz 1927; Tolmachev 1939) are artificial and do not warrant their sectional organization from an evolutionary standpoint. Similarity did lie within the three groups briefly stated in a smaller study by Beilstein and Windham (2003), but the scale of this study greatly improved upon that rather meek conclusion. Regardless of the few outlying species in the present phylogeny dataset not having a geographic pattern, it is most appropriate to accept a classification of *Draba* species that is outlined by general regional similarities, i.e. Cores I, II, and III. However, in order to actually resolve the interior branches of the Cores within the phylogenetic trees resulting from these data, it will be necessary to combine morphological features that are currently believed to show real evolutionary relationships, such as trichome types, flower color, and presence vs. absence of cauline leaves (Al-Shehbaz, personal communication), as well as chromosome numbers (e.g., Windham 2000, 2004; Warwick and Al-Shehbaz 2006) that show considerable promise in testing relationships. Only after the addition of this morphological matrix to a sequence matrix could we really begin to resolve the closer relationships between *Draba* species.

It is an understatement to say that the historical migration of *Draba* has been complex. However, one can definitely say that there have been multiple and recent (i.e. latest glaciation cycles) radiation events responsible for the patterns seen herein. Despite the surprisingly older date of extinct *Draba* lineages, all results show that these events effecting the Core *Draba* lineages may have been so recent that they have been influenced by the animals of the mammoth steppe and possible bird migrations throughout the Pleistocene. The currently observed properties of *Draba* lineages could be relics of a continuous population or very large population sizes, that may have been severed during the glaciation cycles and at the same time often had contact with one another regardless of the isolation on mountain ledges or edges of glaciers. The relatively low nucleotide diversity (i.e., sequences very similar to one another) and at the same time very high genetic diversity (i.e., many different haplotypes) allow us to conclude that what is currently seen today is the result of the large effect of population sizes that once dominated *Draba*'s distribution. However, this is still speculation, considering the inability to accurately date the radiation events seen here. Ideally one could integrate the data in this study with ancient DNA from *Draba* seeds found in permafrost or preserved mammoth dung found within the sediment of lakes or the floor of the shallow seas where mammoth bones are often found (i.e., North Sea, English Channel, Bering Strait). One could then determine a exact rate of mutation for the newest *Draba* species that are well within the radiations seen from the chloroplast sequences. This could then help us estimate the radiation events

possibly to even the nearest 1,000th year, ultimately leading to detect exact migration events of certain lineages. Overall, *Draba* is truly a great example to study recent migration of species that resulted in isolation and subsequent radiation and speciation. That speciation is evident in the distinct morphological characters, the stability of the ploidy levels whether high or low, the large amount of haplotype diversity in the maternally inherited chloroplast genome, and at the same time the large amount of haplotype sharing over geographic barriers normally considered to hinder gene flow in rock-loving, extreme-living perennials. However, with the short genetic distances between the mutations, it can be said that after this series of migration and radiation events, Core *Draba* lineages has been undergoing speciation only for a relatively short period of time.

Appendix A

Floras and Checklists Used

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Appendix B

Supplementary Materials

Additional information provided on the included data DVD.

- Species Matrix
- Accession List
- Herbarium Photos
- Alignments
  - ITS phylogeny
  - trnLF phylogeny
  - Combined phylogeny
  - phylogeographic
  - network only
- Heuristic Tree ITS
- Heuristic Tree trnLF
- Heuristic Tree Combined
- Bayes Tree ITS
- Bayes Tree trnLF
- Bayes Tree Combined
- AWTY Plot ITS
- AWTY Plot trnLF
- AWTY Plot combined
- Bayes Consensus Network with Names

- Neighbor-Net-Network with Names
- Full TCS Network

Zusammenfaßung

Die vorliegende Doktorarbeit befasst sich mit Phylogeografie und Phylogenie von *Draba*, der größten Gattung innerhalb der Brassicaceae. In der Gattung *Draba* sind über 370 Arten bekannt; sie besitzen eine weltweite Verbreitung in extremen Ökosystemen einschließlich Hochgebirgen und Gebieten hoher geografischer Breite (Verbreitungslücken umfassen Australien, tiefer Lagen Südasiens und nahezu ganz Afrika bis auf das Atlasgebirge). Draba ist eine ausgezeichnete Modellpflanze für Studien von großflächigen Wanderungsbewegungen von Arten in alpinen/arktischen Habitaten, die sowohl rezent als auch in der Vergangenheit relativ kontinuierlich in den Hauptgebirgsregionen und -polarregionen zu finden sind beziehungsweise waren. Daten hinsichtlich des Artenreichtums wurden gesammelt, und Verbreitungsmuster der Arten wurden verglichen. Im Vorliegenden stelle ich den ersten Überblick der geografischen, weltweiten Verbreitung der gesamten Gattung anhand einer Matrix, die Art-Vorhandensein/-Nichtvorhanden zusammenfasst, sowie Karten, die mittels ArcView 9 generiert wurden, vor. Plastiden- und Kernsequenzen von 580 Individuen wurden, ausgehend von in Herbarien gesammeltem Blattmaterial, analysiert. Die Erstellung von Phylogenien und Genverwandtschaftsbeziehungen basierte auf molekulare Methoden der Evolutionsforschung. Im Rahmen dieser Doktorarbeit wurde die erste, erfolgreiche, gattungsweite Phylogenie von Draba erstellt und drei Hauptgruppen von Draba beschrieben, die mit geografischen Regionen übereinstimmen. Außerdem identifizierte ich Arten, die historisch betrachtet *Draba* zugeordnet wurden, aber nicht Teil der Hauptgruppe sind, und ich schlage vor, die Namen dieser Taxa zu ändern. Ich habe erforscht, unter welchen Umständen sich eine Art entwickelt, indem ich die Auswirkungen von Umweltfaktoren auf die Wanderungsbewegungen der Linien im Verlauf der Zeit diskutiere. Ich untersuchte, warum die Gattung Draba artenreicher als andere zu sein scheint und schlussfolgerte, dass ihr bevorzugtes Habitat in Hochgebirgen und hoher geografischer Breite ihre Fähigkeit zur Anpassung, und damit ihre Artbildungs- und Polyploidisierungsrate beeinflusst hat. Ihre Artbildungsraten wurden berechnet, und genetische Mutationsraten wurden in Bezug zur Zeit gesetzt, wodurch ich in der Lage war, das Alter der zentralen Draba-Arten mit 2,3 Millionen Jahren zu bestimmen; die ältesten Arten spalteten sich vor 11-18 Millionen Jahren von ihrem Vorfahren ab. Ihre Wanderungsrouten folgten zahlreichen weltweiten Mustern, können im Besonderen jedoch mit den Phasen der Vereisung während des Pleistozäns korreliert werden. Draba hat sich angepasst, an den Rändern dieser Ökosysteme zu überleben, indem sie Reproduktionsstrategien wie asexuelle Samenentwicklung (Apomixis), Polyploidisierung, Selbstbefruchtung und Hybridisierung entwickelt hat. Zudem besteht hinsichtlich des Ursprungs einer Gattung die weitgehend etablierte Hypothese, dass der Ort der höchsten Diversität, sowohl an Artenzahl in einem Gebiet als auch an entsprechender genetischer Diversität, gleichzeitig sein Ursprungsgebiet ist. Im Fall von Draba zog ich den Schluss, dass das Gebiet der höchsten genetischen Diversität mit der Ursprungsregion übereinstimmt, welche zwischen Kaukasus und den zentralasiatischen Gebirgen liegt, jedoch nicht mit dem Gebiet der höchsten Arten-/Alphadiversität oder dem höchsten Artenreichtum entspricht, wie sie in den zentralen Rocky Mountains gefunden wurde. Dies steht im Gegensatz zu der akzeptierten Theorie, dass Diversitätszentren anhand von Artenzahlen

identifiziert werden, während wir hier zeigen, dass die genetische Diversität ebenfalls eine Schlüsselrolle für die Arterhaltung spielt, was für Artenschutzstrategien wichtig sein kann.

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Figure B.1: A tribute to all my plant friends, enjoy and thanks Doonesbury, by Garry Trudeau.