SYSTEMATICS AND GLACIAL POPULATION HISTORY OF THE ALTERNIFOLIUM GROUP OF THE FLOWERING PLANT GENUS CHRYSOSPLENIUM (SAXIFRAGACEAE)

BY

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ABSTRACT:

The flowering plant genus *Chrysosplenium* comprises approximately 57 species of herbaceous perennials. These species are mainly distributed in the Northern Hemisphere where they occur in moist habitats. Though the center of diversity, and presumed location of origin, for the genus is east temperate Asia, more recently radiating taxa have invaded the arctic of North America and Europe. There are six species of *Chrysosplenium* in North America and four of them (i.e., *C. iowense, C. tetrandrum, C. wrightii,* and *C. rosendahlii*) belong to the section *Alternifolia*. Termed the Alternifolium group, this collection of species presents an excellent opportunity to study the evolution of variation in arctic and alpine environments. Similar to many arctic taxa, these species display very little morphologic or genetic variation, but they exhibit diversity in chromosome number, breeding system, geographic distribution, and ecology. Though the Alternifolium group has been the subject of numerous taxonomic studies, no thorough investigation of its evolutionary history has been conducted. This study used a combination of genetic and phenotypic data (e.g., DNA sequence, Inter-Simple Sequence Repeat, morphology) to determine the patterns of variation present within the Alternifolium group and then used these patterns to infer historical processes that might have contributed to them. Through the course of the study, however, it also became necessary to investigate the applicability of genetic estimates derived from different molecular markers and statistical methods. Appropriate comparisons among genetic estimates are critical to accurately interpret results and generate new predictions.

CHAPTER ONE:

INTRODUCTION

From the time of its inception, the field of evolutionary biology has ascribed special importance to the study of so-called "natural laboratories", particularly as they are manifest in the environments of oceanic islands (Darwin, 1859; Wallace, 1881; MacArthur & Wilson, 1967). This focus is predicated on the existence of a suite of common insular characteristics (e.g., discreteness and isolation, small size, ecological diversity, and dynamic geologic history), which simultaneously render a biological system unique while also capable of providing insight into the formulation of complex and broadly applied evolutionary theory (MacArthur & Wilson, 1967; Emerson, 2002). It is largely this capability, conjoined with an undoubted affinity of biologists for the exotic and generally equatorial, that explains why so many of the venerable works of evolutionary biology concern the diversification of island lineages (e.g., finches, Darwin, 1859; Anolis lizards, Losos et al., 1998; Hawaiian silverswords, Baldwin et al., 1991). However, islands or island-like features are not restricted to the world's oceans, but exist even within the continental expanse in the form of caves, gallery forests, tide pools, and arctic and alpine tundra (MacArthur & Wilson, 1967). The Arctic, in particular, presents an intriguing opportunity for evolutionary research, as the region exhibits many of the attributes that make oceanic islands amenable to such investigations, while maintaining a markedly distinct taxonomic, climatic, and geological character (Yurtsev, 1994). Certainly, the history

of the arctic biota is a global history amplified, as its patterns of variation bear, with unmatched fidelity, the marks of upheaval inflicted by a geological and climatic revolution that even the founders of evolutionary thought considered to be of rare importance (Darwin, 1859; Wallace, 1881). Today, the arctic biota comprises a patchwork of individual lineages, each having evolved through the forceful coaction of historical, stochastic (e.g., genetic drift), and deterministic (e.g., natural selection) processes (Weider & Hobæk, 2000). It is in regard to the interplay of these processes that the role of the arctic as a "natural laboratory" is most powerfully applied, as it offers a prospective understanding of the individual and collective influence of evolutionary mechanisms operating therein (U.S. Polar Research Board, 1998; Weider & Hobæk, 2000). However, despite all prospects, the arctic biota remains relatively unknown with regard to the nature of both evolutionary pattern and process (Murray, 1987; Steltzer et al., 2008). The following studies represent an effort to delineate patterns of genetic and morphologic variation within a group of closely related arctic and boreal plant species, as well as determine the behavior of molecular and analytical methods that might best be applied in the pursuit of such delineation. Perhaps, with the future proliferation of similar studies, biologists might more extensively exploit the impressive research potential of the arctic biota.

The Arctic and its flora

The Arctic

Any discussion of evolution in the arctic flora must begin with a definition of that region, especially considering with what difficulty a precise and universally accepted circumscription has faced in the past. The Arctic is a region vast in both size (~2 300 000 sq. mi.) and complexity, however, delimitation of its boundaries is often made in the most simplistic terms (Polunin, 1951; Downes, 1965). The common biological definition of the Arctic, which is by no means wholly uninformative, assigns to it all the treeless area beyond the climatic timberline (Billings & Mooney, 1968; Murray, 1987). Though this definition is operational and has been employed throughout most studies of arctic plants, more detailed descriptions have been proposed (e.g., Polunin, 1951; Elvebakk et al., 1999). The more contemporary of these definitions (i.e., Elvebakk et al., 1999) equates the Arctic to the Arctic Bioclimatic Zone, which is characterized by both arctic tundra vegetation and an arctic climate. In contrast, Polunin's (1951) description

> *...I have come to accept as truly arctic only certain areas of land, fresh water, and adjacent sea. These are in general those that lie north of whichever of the following is situated farthest north in each sector of the northern hemisphere: (1) a line 80 km. (50 miles) north of the northern limit of coniferous forest or at least more or less continuous taiga, i.e. terrain with sparsely scattered trees; (2) north of the present-day northern limit of at least microphanerophytic growth (i.e., of trees 2-8 m. in height but excluding straggling bushes in unusually favourable situations), the northern extremities of tongues or outliers separated by not more than fifteen degrees of longitude*

being united across; or (3) north of the northern Nordenskiöld line, which is determined by the formula V =9 – 0.1K, where V is the mean of the warmest month and K is the mean of the coldest month, both in degrees Centigrade.

places less emphasis on plant community composition. Both authors, however, provide a more nuanced view of arctic boundaries than is traditionally available, offering an important basis for the consideration of ecological variation within the Arctic.

A popular depiction of the Arctic is that of an area that is uniformly cold and desertic (Polunin, 1951). While true that the Arctic is primarily a peripheral environment with very little biologically usable heat and low levels of precipitation (additional attributes include: short growing season, strong wind, long photoperiod, low light intensity, and low nitrogen supply), such generalizations cannot fully or accurately represent this ecologically complex region (Billings & Mooney, 1968; Savile, 1972; Murray, 1987). In fact, efforts to reflect even large-scale differences in arctic ecology have produced up to five subdivisions of this biome (Elvebakk et al., 1999). Though these subdivisions are primarily defined by plant community composition, their differentiation is ultimately the result of variation in physiographic conditions, which influence species' distributions (Hansell et al., 1998). This variation is spatially and temporally structured with factors such as periglacial (e.g. formation of ice mounds and frost blisters) and thermokarst (e.g., formation of thaw lakes and sinkholes) processes effecting changes in local and regional topography,

temperature, moisture, etc. (Murray, 1987; Trenhaile, 2004). Recognition of arctic habitat diversity is a critical component for understanding adaptive evolution and phytogeography in arctic plant species.

The arctic flora

An arctic plant species is typically and simply defined as one that has the main part of its range in the Arctic (Polunin, 1951). However, what distinguishes these species from all but their alpine relatives is the ability to metabolize, grow, and reproduce at low temperatures (Billings & Mooney, 1968). Few vascular plants have this capability, and its rarity is signified by the relatively small size (~1500 species) of the Arctic Flora (Murray, 1995). In contrast, the much smaller Cape Floristic Region (~88 000 sq. km.) is home to over 9000 species (Cowling & Heijnis, 2001; Goldblatt & Manning, 2002). Modest as it is, consideration of the Arctic as a floristic region, by some (Yurtsev, 1994) emphasizes the unique taxonomic, ecological, and genetic qualities of the flora.

As previously alluded to, the arctic flora displays an unusual taxonomic structure that has been artfully described by one author (Savile, 1972) as "a depauperate miscellany". Indeed, the arctic flora consists of relatively few endemic genera and in most cases, a small number of arctic species per genus or family are observed (Yurtsev, 1994). Though there are a number of endemic arctic species (> 10% of all

arctic species), many more can be described as having arctic-alpine or largely boreal distributions (Bliss, 1971). Consideration of the taxonomic distribution of arctic species within prominent arctic genera, such as *Carex* or *Saxifraga*, suggests that the flora was formed by repeated invasions from multiple geographic origins, rather than any *in situ* radiation of taxa (Savile, 1972).

The perceived lack of diversity in the arctic flora is, perhaps, its most well known feature (Willig et al., 2003; Grundt et al., 2006). This condition has long been attributed to aspects of arctic plant biology and environmental history (Steltzer et al., 2008). Specifically, substantial range reductions, experienced during periods of Pleistocene glaciation, are thought to have reduced variation in arctic species, while self-pollination and clonality, modes of reproduction believed common in the arctic, combine with severe selection regimes to maintain variation at low levels (Billings & Mooney, 1968; Murray, 1987; Hewitt 1996; Pamilo & Savolainen, 1999; Abbott et al. 2000). The issue with this entire perception is that is it is based on a number of questionable assumptions regarding the arctic flora and its history. Of these assumptions, the following refutations or critiques may be offered: it is well established that glacial action was also capable of increasing levels of intra- and inter-specific variation (Abbott et al., 2000; Alsos et al., 2005; Marr et al., 2008); the prominence of inbreeding and asexuality in arctic plants has never been broadly or intensively tested and is beginning to prove less frequent than thought (Murray, 1987; Gabrielsen & Brochmann, 1998; Steltzer et al., 2008); and, the arctic is a complex

environment for which it would be ill-informed to envision a uniform direction and strength of selection (Murray 1987; Hansell et al., 1998). However, beyond the problems with its theoretical underpinnings, the view that the arctic flora is wholly depauperate has been repeatedly contradicted by empirical study. This includes demonstrations of high polyploid frequency (Packer, 1969; Brochmann et al. 2004), high genetic diversity (Bauert, 1996; Gabrielsen & Brochmann, 1998; Abbott & Brochmann, 2003), and prolific cryptic species formation in arctic taxa (Grundt et al., 2006). The continued discovery of novel variation within and among arctic plant species not only revises our view of this flora but also offers biologists intriguing new insights into its evolutionary history.

The history of the Arctic and evolution of the arctic flora

> *If glacial epochs in temperate lands and mild climates near the poles have, as now believed by men of eminence, occurred several times over the past history of the earth, the effects of such great and repeated changes, both on the migration, modification, and extinction of species, must have been of overwhelming importance—of more importance, perhaps, than even the geological changes of sea and land.* (Wallace, 1881)

> *The glacial periods and their climatic consequences have apparently played the most prominent part in the development of present arctic and boreal biota, and unless these features are studied in parallel to the variation and present area of different*

> *species the problems of their origin and evolution will remain unsolved.* (Hultén, 1937).

The importance of the climatic and geologic history of the Arctic cannot be understated with regard to the evolution of the arctic flora. For, it is surely a biota that was forged in the freezing crucible of a rapidly changing planet. The history of the modern Arctic begins in the middle Miocene (~15 mya); prior to this, northern polar-regions supported a vast, continuous boreal forest that extended across the Bering Land Bridge to cover both North America and Asia (Bliss, 1971; Savile, 1972; Murray, 1995). However, at that time a global cooling trend, which continued into the Pleistocene, was re-established and thermophilic taxa began to recede from high latitude environments (Tiffney & Manchester, 2001). Contemporaneous orogenic processes, which gave rise to the North American Cordillera, the Alps, and other mountain ranges, provided routes of migration for alpine plant species to move into the newly transformed arctic environments (Savile, 1972; Murray, 1995). These alpine migrants, along with a few cold-tolerant boreal species, formed the precursors of the arctic flora and established the first tundra-dominated plant communities (Savile, 1972). Though, the circumpolar tundra belt did not exist until the late Pliocene (~3 mya; Bliss, 1971; Murray, 1995).

The Pleistocene (1.8 – 0.01 mya) was a formative event in the history of global plant diversity. Significant changes in climate throughout this period resulted in the large-scale redistribution of species and strongly influenced evolutionary process

(Hewitt 1996). Climate change was experienced most severely at the polar-regions, where species redistribution was additionally compelled by the movements of massive continental ice sheets (Pielou, 1991). Four major glacial stages mark the Pleistocene, each followed by a warmer inter-glacial period. The most recent of these glacial stages, referred to in North America as the Wisconsin, reached its maximum approximately 18 000 years ago (Pielou, 1991). At that time, ice covered much of what are now Canada and the northern portion of the United States, and plant populations were forced to persist in ice-free areas, mostly located beyond the glacial margins (Dahl, 1946; Abbott & Brochmann, 2003). These ice-free areas are more commonly known as glacial refugia and can be classified into two types: open and closed (Lindroth, 1969). The open refugium was located south of the continental ice sheets and included the large 'southern' North American refugium described by Darwin (1859). This area harbored huge numbers of species and served as a major source for the recolonization of previously glaciated landscapes. Closed refugia were located within the margins of the continental glaciers and were either completely or partially surrounded by ice (Lindroth, 1969). These included the areas referred to as 'Nunataks' (Blytt, 1876), or mountain top refugia, and coastal refugia (Hultén, 1937; Dahl, 1946; Heusser, 1960). Perhaps the most important example of a closed refugium is Beringia (Hultén, 1937), the region that includes modern day Alaska, much of the Yukon Territory, and northeastern Asia. Beringia is known to have remained ice-free throughout the Pleistocene and to have harbored a great number of arctic and boreal plant species (Colinvaux, 1967; Hopkins, 1967; Abbott &

Brochmann, 2003). The exchange of arctic species between Asia and North America through this region was an important factor in the development of the arctic flora (Murray 1987). Additional examples of North American closed refugia include southwestern Kodiak Island (Karlstrom, 1969), the Driftless Area (Baker et al., 1980; Pusateri et al., 1993), and 'Nunatak' refugia in the southern Canadian Rockies (Packer & Vitt, 1974; Loehr et al., 2005; Marr et al., 2008). In the past it was often difficult or impossible to identify the specific refugia that harbored a given species (Abbott & Brochmann, 2003). Today, hypotheses concerning the location of refugia and their putative roles in post-glacial recolonization are tested using patterns of genetic variation. These studies serve to reinforce the understanding of the Pleistocene as a period fundamental to the evolution of arctic plants.

Taxon

Saxifragales

Saxifragales is a distinctive angiosperm order, consisting of approximately 2470 species (Jian et al., 2008). It is confidently placed within the Eudicot clade, though its relationship to other Eudicot lineages is largely unresolved (Soltis et al., 2005). The latest circumscription of the order (The Angiosperm Phylogeny Group II, 2003) ascribes to it 12 families (Altingiaceae, Cercidiphyllaceae, Crassulaceae, Daphniphyllaceae, Grossulariaceae, Haloragaceae, Hamamelidaceae, Iteaceae,

Paeoniaceae, Peridiscaceae, Pterostemonaceae, and Saxifragaceae), and though this composition has been described as surprising, it is well supported by molecular phylogenetic analyses (Chase et al., 1993; Soltis et al., 1997a, 1998, 2000; Soltis & Soltis, 1997; Hoot et al., 1999). Despite the success at broad circumscription and extensive genetic sampling efforts, relationships within the Saxifragales remain difficult to ascertain (Jian et al., 2008). This condition is primarily attributed to the ancient (100-120 mya) but rapid radiation of the order, which may also account for its impressive level of morphological diversity (Magallón et al., 1999; Soltis et al., 2005; Jian et al., 2008). Only two groups, commonly resolved as sister taxa, are consistently supported by molecular phylogenetic analyses; the Saxifragaceae alliance (i.e. Saxifragaceae sensu stricto, Grossulariaceae, Iteaceae, and *Pterostemon*) and the Crassulaceae + Haloragaceae alliance (Fishbein & Soltis, 2004; Soltis et al., 2005; Jian et al., 2008). Phylogenetic analyses conducted within the last year using nearly 51 000 bp of DNA sequence data failed to improve resolution within the Saxifragales (Jian et al., 2008).

Saxifragaceae sensu stricto includes approximately 30 genera of herbaceous perennials (Soltis et al., 2001). The narrow circumscription of the family, supported by phylogenetic analyses (Chase et al., 1993; Morgan & Soltis, 1993; Soltis & Soltis, 1997) and phenotypic characters (e.g., iridioid chemistry, embryology, and serology), follows the taxonomic treatments of Takhtajan (1987) and Thorne (1992). The largest genera include *Saxifraga* (300 spp.), *Chrysosplenium* (~57 spp.), and

Heuchera (~50 spp.; Judd et al., 2002). This is primarily a northern hemisphere family; centers of diversity include western North America as well as alpine regions of Europe and Asia (Soltis et al., 2001). The recent and rapid radiation of the family appears to have resulted in a high degree of morphological similarity among the genera, which, in turn, has led to difficulty in resolving evolutionary relationships (Judd et al., 2002). The nature of these origins has also been implicated in the widespread hybridization known from some Saxifragaceous genera, which may also complicate phylogenetic investigation (Soltis et al., 2001). Analyses of DNA sequence data (cpDNA: *matK*, *rbcL*, *trnL-trnF*, and *psbA-trnH*; rDNA: ITS and 26S) have helped greatly in understanding many of the relationships within the family, especially those at the deeper-level (Soltis et al., 2001).

The genus *Chrysosplenium* L. comprises ~ 57 herbaceous perennial species, which are native to moist habitats (Hara, 1957). The genus is a member of the well-supported Heucheroid clade (Soltis et al., 2001) and has typically been placed as sister to *Peltoboykinia* (Johnson & Soltis, 1994, 1995; Soltis et al., 1993, 1996, 2001). *Chrysosplenium* is distinguished from other Saxifragaceae by their tetramerous, apetalous flowers (pentamery is the inferred ancestral state for the family; Ronse Decraene et al., 1998), flavonoid chemistry (Collins et al., 1975; Bohm et al., 1977; Bohm & Wilkins, 1978), and DNA sequence characters (Nakazawa et al., 1997; Soltis et al., 2001). In addition, members of *Chrysosplenium* utilize a rare (also found in *Mitella* as well as other genera outside of Saxifragaceae) seed dispersal mechanism

that is effected when raindrops strike the dehisced fruit ('splash cup') containing the seed and ejects the seeds up to a meter from the parent plant (Savile, 1953; Nakanishi, 2002). The genus is broadly distributed throughout arctic, alpine, and boreal environments of the northern hemisphere; two Chilean endemics represent the only species to occur in the Southern Hemisphere (Hara, 1957). Hara (1957) suggested that the region of origin for the genus was South America, but evidence from molecular phylogenetic analyses (Soltis et al., 2001), as well as consideration of rust parasite evolution (Savile, 1975) point to eastern temperate Asia. The genus has been subdivided into two sections (i.e., *Alternifolia* and *Oppositifolia*; Franchet, 1890) based on phyllotaxis. Monophyly of the sections is supported by DNA sequence data (Nakazawa et al., 1997; Soltis et al., 2001) and flavonoid chemistry (Bohm & Collins, 1979). The first large-scale molecular phylogenetic investigation of the genus was conducted by Nakazawa et al. (1997) and used the cpDNA regions *matK* and *rbcL* to determine relationships among the 16 Japanese species. That study supported the monophyly of the genus as its sections. More recently, Soltis et al. (2001) employed cpDNA *matK* sequence data for an expanded data set comprising 29 members of the genus. That study resolved a number of deeper-level relationships and provided a framework for testing a number of theories regarding character evolution and biogeography. Despite these positive attributes, the utility of the Soltis et al. (2001) phylogeny is limited, especially with regard to species-level relationships, by incomplete taxon sampling and low resolution. This is particularly true of the North American, alternate-leaved species, forming what will hereafter be referred to as the

Alternifolium group, for which their relationships among each other as well as to the rest of the genus were left largely unresolved.

The Alternifolium group comprises four species (*C. wrightii* Franch. & Savigny, *C. iowense* Rydb., *C. tetrandrum* (Lund ex Malmgr.) Th. Fr., and *C. rosendahlii* Packer) that occur in arctic, alpine, and boreal environments in North America, as well as in Europe and Asia (Packer, 1963). With the exception of *C. wrightii*, the group exhibits very little morphological diversity and members are similar in appearance to the Old World species, *C. alternifolium*. This condition has resulted in some taxonomic controversy, each member of the Alternifolium group having been included in *C. alternifolium* by some authors at the ranks of subspecies and variety, resulting in great fluctuations in the range of the latter (Rose, 1897; Gray, 1950; Packer, 1963). Despite the close morphological similarity, the group displays considerable variation in chromosome number (*C. iowense*, 2n = c. 120; *C. tetrandrum*, 2n = 24; *C. wrightii*, 2n = 24; *C. rosendahlii*, 2n = 96) and geographic distribution (Packer, 1963). There also appears to be some variation in breeding system; *C. iowense* exhibits a mixed mating system and *C. tetrandrum* is an obligate selfer (Warming, 1909; Packer, 1963; Weber, 1979). Though their North American ranges vary greatly in size and location, each has clearly been influenced by Pleistocene glaciation, as evidenced by a number of distributional disjunctions (Packer, 1963). The most striking of these disjunctions are exhibited by *C. tetrandrum* and *C. iowense*. A few populations of *Chrysosplenium tetrandrum* occur

in the Rocky Mountains of Colorado, Montana, and Idaho, isolated from the species' main circumpolar distribution (Packer, 1963; Weber, 2003). *Chrysosplenium iowense* is primarily distributed in the southern Canadian boreal forest, however, isolated populations persist in the Driftless Area of northeastern Iowa and southeastern Minnesota, where they are strongly associated with ice caves (Weber, 1979). Though this group presents great potential for comparative investigations into ice age influences on patterns of intra-specific genetic variation, only one previous molecular study has been conducted on any species of the Alternifolium group and that was quite limited in both its scope and results (Schwartz, 1985).

Molecular and analytical methods

Heritable variation is an integral component of Darwinian evolution and assessing levels of this variation is, quite naturally, critical to evolutionary study (Darwin, 1859; Wright, 1931). Despite Darwin's early recognition of their importance, such assessments only became possible, almost half a century after the publication of *The Origin of Species* (Darwin, 1859), with the rediscovery of Mendelian genetics (Wright, 1931). For the next 60 years, genetic variation was estimated via analyses of phenotypic trait differences in populations. However, this method was not readily informative for genetic investigation, as accurate results required the following conditions:

> *(1) Phenotypic differences caused by allelic substitution at single loci must be detectable in single individuals. (2) Allelic substitutions at one locus must be distinguishable from substitutions at other loci. (3) A substantial portion of (ideally, all) allelic substitutions must be distinguishable from each other. (4) Loci studied must be an unbiased sample of the genome with respect to physiological effects and degree of variation.* (Hubby & Lewontin, 1966).

The situation was greatly improved with the advent of enzyme electrophoresis and the birth of molecular genetics (Hunter & Markert, 1957; Harris, 1966; Hubby & Lewontin, 1966; Lewontin & Hubby, 1966; Stebbins, 1989). By visualizing protein variation, for the first time researchers were able to assess directly allele frequencies at a given locus. The continued development of molecular approaches has produced various DNA-based methods, each with certain advantages and disadvantages. Today, a major division of these methods concerns the information content of the data (i.e., dominant versus codominant) that each type of approach produces. The differences require important analytical considerations and at present it is not well understood how analogous estimates of gene-statistics derived from each of these method types might compare (Nybom & Bartish, 2000). This is problematic, as it is often desirable to apply knowledge gained in one study to the interpretation of results in another. Without a proper comparative context, vast reserves of information regarding genetic patterns and evolutionary process may become useless.

Codominant markers

The use of codominant molecular markers (i.e., marker that allows discrimination of heterozygotes) began with the development of enzyme electrophoresis in the 1960s (Lewontin and Hubby, 1966; Hubby & Lewontin, 1966). Since that time, allozyme variation has been studied in a number of plant species and the method is still applied, in some circumstances to great effect (e.g., Crawford et al., 2005; Crawford et al., 2006; Grundmann et al., 2007). The nature of their appeal is that enzyme studies are relatively inexpensive and allow a direct estimation of allele frequencies in populations (Clegg, 1989). The latter point is critical, as a direct observation allows more accurate calculation of genetic statistics (e.g., F_{ST}, F_{IS}, G_{ST}, H_S, H_T; Wright, 1943; Nei, 1973) and thus, a more informed consideration of evolutionary process. Recently, a DNA-based codominant marker, known as a microsatellite, has become popular (Powell et al., 1996; Varshney et al., 2005). This marker is able to overcome some of the traditional shortfalls of allozymes, including lack of variation and sampling bias, which were largely attributable to the inherent methodological requirements of allozyme loci (i.e., coding regions; Clegg, 1989; Hamrick & Godt, 1989; Wendel & Weeden, 1989). Codominant markers remain the preferred choice for most analyses of genetic variation due to the availability of analytical options. As microsatellites become easier to develop, they are beginning to displace the subject of the next section, the dominant marker.

Dominant markers

Arbitrarily amplified DNA (AAD) methods (e.g., AFLP, inter-simple sequence repeat, randomly amplified polymorphic DNA), to which they are sometimes referred, are hyper-variable PCR-based methods that produce dominant data (i.e., type of data that does not allow discrimination of heterozygote from dominant homozygote; Wolfe & Liston, 1998). These data are produced in the form of band presences or absences, which represent the presence or absence of a single 'dominant allele' at an anonymously amplified 'locus' (Nybom & Bartish, 2000). Because these methods are generally incapable of determining 'allele' number at each 'locus', one cannot determine whether a band phenotype represents a heterozygote or a dominant homozygote (Nybom & Bartish, 2000). Advantages of these methods include: the capability to produce large data sets, low expense for development and use, and significant levels of variation (Huang & Sun, 2000; Archibald et al., 2006). However, because allele frequencies cannot be directly determined from dominant data, analytical approaches have proven challenging (Meudt & Clarke, 2007). In recent years, probabilistic statistical approaches have been developed to infer allele frequencies from these data sets based on band frequencies (Zhivotovsky, 1999; Holsinger et al., 2002; Vekemans, 2002). From these inferences it becomes possible to calculate analogues of the traditional genetic statistics used for codominant markers (Holsinger et al., 2002). Given their ability to overcome some of the disadvantages of dominant data, these methods have become widely used. However, we do not yet understand how statistical estimates derived from different marker

and/or analytical types differ. Once such an understanding can be reached, we can reduce the limitations on the application of knowledge, utilizing the full collection of the past.

CHAPTER TWO:

PHYLOGENY OF *CHRYSOSPLENIUM* (SAXIFRAGACEAE) BASED ON NUCLEAR AND CHLOROPLAST DNA SEQUENCE DATA

Abstract

Chrysosplenium is a genus of approximately 57 perennial herbaceous species. These species are distributed primarily in arctic, alpine, and boreal environments throughout the Northern Hemisphere. Members of the genus are readily distinguished from other Saxifragaceae by their tetramerous, apetalous flowers, however morphological and genetic variation within the genus is low and many species relationships remain unresolved. The alternate-leaved species that occur in North America (*C. iowense*, *C. tetrandrum*, *C. wrightii*, and *C. rosendahlii*) represent one group within *Chrysosplenium* that is relatively unknown phylogenetically, though it exhibits striking patterns of variation in chromosome number and biogeographic distribution. Parsimony and likelihood analyses of a combined data set of chloroplast and nuclear DNA sequences provide the first intensive investigation of relationships within the group and place it, with strong support, as sister to *C. japonicum*. Most relationships within the group were poorly supported or unresolved. A multivariate statistical approach to analyzing data from five morphological characters is able to distinguish clearly among the three species for which taxonomic identification can be difficult.

Introduction

The genus *Chrysosplenium* L. (Saxifragaceae) comprises approximately 57 species of herbaceous perennials that are native to moist habitats (Hara, 1957). These species are distinguished from other Saxifragaceae by their tetramerous, apetalous flowers (pentamery is considered ancestral in the Saxifragaceae; Ronse Decraene et al., 1998) and their flavonoid chemistry (Bohm & Collins, 1979). Representatives of the genus are found in arctic, alpine, and boreal environments throughout the Northern Hemisphere. Only two species, *C. valdivicum* Hook. and *C. macranthum* Hook., occur in the Southern Hemisphere, and these are native to extreme southern Chile (Hara, 1957). Hara (1957) suggested that the Chilean range represented the geographic origin for the genus, however, species diversity, patterns of rust-parasite evolution, and phylogeny-based biogeographic analyses strongly support east temperate Asia in this role (Savile, 1975; Soltis et al., 2001).

Aside from leaf arrangement, which was used by Franchet (1890) to subdivide the genus into two sections (*Alternifolia* and *Oppositifolia*), the high level of morphological similarity within *Chrysosplenium* has made determining relationships among taxa difficult. Nakazawa et al. (1997) were the first to employ a DNA sequencing approach to a phylogenetic study within *Chrysosplenium*. Using the chloroplast genes *rbcL* and *matK*, they were able to confirm the monophyly of *Chrysosplenium* as well as begin to determine relationships among Japanese members

of the genus. That study was followed by a genus-wide phylogeny produced by Soltis et al. (2001). Again using *matK*, the study included 29 species, sampled from across the generic distribution. Soltis et al. (2001) also showed strong support for the monophyly of *Chrysosplenium* as well as for section *Oppositifolia*. Though the Soltis et al. (2001) phylogenetic analysis was successful in resolving deeper-level relationships within the genus and testing theories of character evolution and biogeography, its utility is limited by incomplete taxon sampling and low resolution. This is especially the case for the North American alternate-leave species of the genus, which form what I will refer to as the Alternifolium group.

The Alternifolium group consists of four species (i.e., *C. wrightii* Franch. & Savigny, *C. iowense* Rydb., *C. tetrandrum* (Lund ex Malmgr.) Th. Fr., and *C. rosendahlii* Packer) that occur in arctic, alpine, and boreal environments in North America as well as in Europe and Asia (Packer, 1963). Aside from *C. wrightii*, the species are morphologically very similar to each other as well as to *C. alternifolium*, an Old World species (Packer, 1963). The lack of distinguishing morphological characters, despite impressive variation in chromosome number, has caused multiple revisions to the taxonomic status of each of these species, particularly with respect to *C. alternifolium* (Gray, 1950; Packer, 1963). Stamen number is the primary morphological character for differentiating among the Alternifolium group species, and while seed size and flower shape measurements provide some information, variation in these traits results in overlapping value ranges (Packer, 1963). Soltis et

al. (2001) only sampled two species, *C. iowense* and *C. tetrandrum*, from the Alternifolium group, for which a sister-species relationship was only very weakly supported. Considering the relative attention that has been paid to this group of species in the taxonomic literature (e.g., Rose, 1897; Simmons, 1906; 1913; Hara, 1957; Hultén, 1960; Packer, 1963) it would be of interest to apply a molecular phylogenetic approach to test hypotheses of relationships within the Alternifolium group, as well as between it and the rest of the genus. We used DNA sequence data from four gene regions (one rDNA, three cpDNA) to address the following questions: **(1)** does the addition of the nuclear ribosomal region increase support for tip groups within the genus; **(2)** what is the position of the Alternifolium group within *Chrysosplenium*; and **(3)** what are the species-level relationships within the Alternifolium group. In addition, we use a multivariate statistical approach (i.e., Principal Components Analysis) of quantitative taxonomic characters to determine if a combined analysis of these data might better differentiate *C. iowense*, *C. tetrandrum*, and *C. rosendahlii*.

Materials and methods

Phylogenetic taxon sampling

We sampled 34 individual accessions (Table 2.1) representing 21 ingroup taxa and 3 outgroup taxa. With a main goal of understanding relationships among members of

the Alternifolium group, sampling was concentrated on those species (i.e., *C. iowense*, *C. tetrandrum*, *C. wrightii*, and *C. rosendahlii*) and each is represented by three or four individuals. Plant material was obtained from natural populations or herbarium specimens. The non-Alternifolium group samples (except *Mitella* spp.) are the same as those used in Nakazawa et al. (1997) and Soltis et al. (2001). Sequences for the *matK* gene were obtained from GenBank (AB003044-AB003060).

DNA extraction and sequencing

Total DNA was extracted either using DNeasy Plant Mini Kits (Qiagen, Valenci, CA) or a CTAB protocol (see Nakazawa et al., 1997; Soltis et al., 2001). Two chloroplast loci (*trnL-F* spacer and *rpL16*) and nuclear ribosomal ITS (including 5.8s) were PCR amplified. PCR primers used for the *trnL-F* spacer were "C" and "F" (Taberlet et al., 1991), for *rpL16* they were F71 and REx2 (Shaw et al., 2005), and were NNC-18S10 and C26A for ITS (Wen & Zimmer, 1996). PCR reactions included 1X Biomix (Midwest Scientific, St. Louis, Missouri) and 0.64 μM forward and reverse primer. In ITS amplifications, 0.5% dimethylsulfoxide was included to reduce secondary structure. PCR amplifications were carried out under the following conditions: 2 min at 95°C; 30 cycles of 45 s at 95°C, 45 s at 48° C, and 4 min at 72°C; and a final extension of 10 min at 72°C. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea). The internal primers, ITS-1 and ITS-4 (White et al., 1990), were used for sequencing of ITS.

Phylogenetic analyses

DNA sequence alignment was accomplished by eye using Se-Al version 1.0 (Rambaut, 1996); insertion/deletion events were subsequently scored using the complex gap coding option in the program SEQSTATE (Müller, 2005). Parsimony analyses were conducted in PAUP* (Swofford, 1998) with all characters equally weighted. Analyses were first performed on individual combined DNA sequence and gap character data sets. Comparison of the resulting topologies revealed no instances of well-supported topological differences. All data were combined into a single data matrix for subsequent analyses. Initial searches were conducted using 1000 replicates of RANDOM taxon addition and NNI branch swapping. Each set of shortest trees from these initial searches was used for subsequent analyses employing TBR branch swapping. Relative support for the recovered clades was assessed using jackknife analyses with 1,000 replicates, 37% deletion, TBR branch swapping and the "emulate Jac" command.

MODELTEST 3.6 (Posada & Crandall, 1998) was used to determine the appropriate model for the DNA sequence data set. Maximum liklihood (ML) analyses were performed using GARLI 0.942 (available at http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html) employing the model

determined by MODELTEST. To determine node support, we did 100 bootstrap replicates in GARLI.

Morphometric sampling and analyses

Population sampling was conducted across the western North American ranges of *C. tetrandrum*, *C. iowense*, and *C. rosendahlii*, but primarily in regions where species ranges occur in close proximity or are overlapping (e.g., northern Alaska, western Alberta). The total number of sampled individuals included in the morphometric study was 294 (*C. tetrandrum* = 243, *C. iowense* = 31, and *C. rosendahlii* = 20). Six quantitative traits were measured on each sampled individual: seed length, seed width, sepal length, sepal width, hypanthium length, and hypanthium width. The presence or absence of leaf and sepal maculation was also assessed as a binary character. Floral measurements were taken from one of the central flowers in the inflorescence. Length and width measurements of each sepal and hypanthium measured were combined to produce ratio characters, which were used to perform a Principal components analyses (PCA; PC-ORD; McCune & Mefford, 1999) . Individuals with missing measurement values were excluded from those analyses.

Results

The combined ITS-cpDNA data set included 3656 nucleotide characters and 134 insertion/deletion characters. Of the 3790 total characters, 500 were parsimony informative. Parsimony analyses recovered six minimum length trees of 1594 steps (CI = 0.6953, RI = 0.8318; Fig. 2.1).

Our analyses support the monophyly of *Chrysosplenium* (100% jackknife; 100% likelihood bootstrap) as well as that of section *Alternifolia* (94% jackknife; 96% likelihood bootstrap). In both analyses, a clade including two opposite-leaved species (i.e., *C. pseudofauriei* H. Lev. and *C. grayanum* Maxim.) is placed as sister to the alternate-leaved clade, making section *Oppositifolia* paraphyletic. Relationships among the rest of the opposite leaved species were generally well supported. Among the alternate-leaved species, a strongly supported (92% jackknife; 80% likelihood bootstrap) clade was recovered including all accessions of the Alternifolium group as well as individuals of *C. alternifolium*. With the exception of one strongly supported group (99% jackknife; 93% likelihood bootstrap) that includes all accessions of *C. iowense* and one weakly (63% likelihood bootstrap) supported group including *C. wrightii* and *C. tetandrum*, most relationships within this clade are unresolved or poorly supported. The only difference between the likelihood and parsimony topologies involved the placement of the Chilean species *C. valdivicum*. This taxon was placed as sister to the *album-rhabdospermum-pilosum* clade in the likelihood analysis and alternatively, sister to the nesting clade of *album-rhabdospermum-*

pilosum. However, neither of these placements is well supported (51% jackknife and bootstrap).

Morphology

Relationships among sampled individuals in the PCA are shown in Fig. 2.2. The results of the analysis show three clearly differentiated clusters of individuals corresponding to pre-identified species groups. The first two axes account for 70.513% of the variance in the data set. The eigenvector output shows that differentiation along the first axis is largely determined by seed length and seed width, while differentiation in the second axis is determined by sepal shape and maculation presence/absence.

Discussion

Phylogenetic relationships

The results of our phylogenetic analyses are largely congruent with previous efforts by Nakazawa et al. (1997) and Soltis et al. (2001) but do differ rather conspicuously with regard to the monophyly of section *Oppositifolia*. This difference may be a result of taxon sampling or the use of the nuclear ribosomal gene ITS. One benefit to using multiple data partitions in phylogenetic analysis is the ability to identify inter-

specific gene flow events. Though there is no direct biological evidence to suggest that our topology is the result of chloroplast capture or hybridization, these processes are known to be common in some genera of the Saxifragaceae (Soltis et al., 2001).

The likelihood and parsimony topologies were mostly congruent, and both analyses showed higher levels of relative support for tip groups than those reported in the Soltis et al. (2001) phylogeny. Both analyses supported a monophyletic section *Alternifolia*, though this taxon was not broadly sampled in these analyses. The Alternifolium group was recovered as paraphyletic in the parsimony analyses with regard to *C. alternifolium*. This is not surprising given the morphological similarity of these elements (Packer, 1963). The strong support for a *C. iowense* clade, which is sister to the rest of the Alternifolium group and *C. alternifolium*, provides evidence for its status as a distinct species (Hara, 1957; Packer, 1963). A sister group relationship between *C. tetrandrum* and *C. wrightii* is intriguing as these are the only diploid species in the clade and they share overlapping ranges (Packer, 1963). *Chrysosplenium wrightii* is the most morphologically distinct species of the Alternifolium group, and it grows at elevations above 1200 m. in Alaska and Siberia. *Chrysosplenium tetrandrum* is circumpolar and occurs along wet stream margins and bogs. Further phylogenetic studies to demonstrate more conclusively the relationship between these two species would provide an evolutionary context for studies of adaptive evolution in the arctic.

Morphology

With the exception of *C. wrightii*, members of the Alternifolium group display little morphological diversity and species identification often relies on accurate assessments of stamen number. Additional taxonomic characters have been proposed (e.g., hypanthium shape, seed length; Packer, 1963); however, because of variation in trait values, these do not provide diagnostic tests of identity. Our morphometric analysis used five taxonomic characters (hypanthium shape, sepal shape, seed length, seed width, and presence/absence of maculation) of limited utility. The results of this analysis show that these three species can be effectively differentiated from one another using a combination of quantitative and qualitative traits.

The lack of morphological and genetic differentiation among the North American alternate leaved species may reflect a recent radiation of the genus into the arctic. Nonetheless, the variation that is exhibited in this group (e.g., ecological, cytological) warrants continued study with the goal of providing a phylogenetic basis for studies of arctic and boreal diversification.

Table 2.1 Species of *Chrysosplenium* included in the combined phylogenetic analyses.

Species	Locality	Collector and Collection Number
C. alternifolium	Siberia, Russia	Petrovsky
C. alternifolium	Siberia, Russia	Solstad (04/1026)
C. japonicum	Miyagi, Japan	Wakabayashi & Nakazawa (943113)
C. carnosum	Dubhkund, Nepal	Wakabayashi et al. (9710339)
C. pseudofauriei	Tokushima, Japan	Wakabayashi (93045)
C. grayanum	Tochigi, Japan	Mishima (943099)
C. delavayi	Hoang Lien Son, Vietnam	Wakabayashi & Nakazawa (943129)
C. ramosum	Iwate, Japan	Wakabayashi & Nakazawa (943181)
C. valdivicum	Antillanca, Chile	Ono & Nakazawa (942100)
C. kamschaticum	Iwate, Japan	Wakabayashi & Nakazawa (943117)
C. echinus	Tochigi, Japan	Setogucchi (943091)
C. macrostemon	Kanagawa, Japan	Nakazawa (942009)
C. nagasei	Gifu, Japan	Wakabayashi & Nakazawa (943024)
C. kiotense	Gifu, Japan	Wakabayashi & Nakazawa (943059)
C. fauriae	Gifu, Japan	Wakabayashi & Nakazawa (943059)
C. album	Hiroshima, Japan	Wakabayashi (89034)
C. rhabdospermum	Nagasaki, Japan	Nakazawa (942014)
C. pilosum	Gifu, Japan	Wakabayashi (93039)
C. iowense	Iowa, USA	Levsen (NL061904-85)
C. iowense	Alberta, Canada	Levsen (NL062405-244)
C. iowense	Manitoba, Canada	Levsen (NL062506-450)
C. rosendahlii	Banks Island, Canada	Foote
C. rosendahlii	Banks Island, Canada	Foote
C. rosendahlii	Alaska, USA	Levsen (NL080306-814)
C. wrightii	Alaska, USA	Jansen (02-224)
C. wrightii	Alaska, USA	Larsen (02-1686)
C. wrightii	Alaska, USA	Larsen (10-0822)
C. wrightii	Kodiak Is., Alaska, USA	Parker
C. tetrandrum	Colorado, USA	Levsen (NL080704-132)
C. tetrandrum	Washington, USA	Levsen (NL062105-149)
C. tetrandrum	Alberta, USA	Levsen (NL070305-353)
P. tellimoides	Tokyo, Japan	Wakabayashi & Nakazawa (943165)
M. japonica	Japan	Wakabayashi
M. yoshinagae	Japan	Wakabayashi

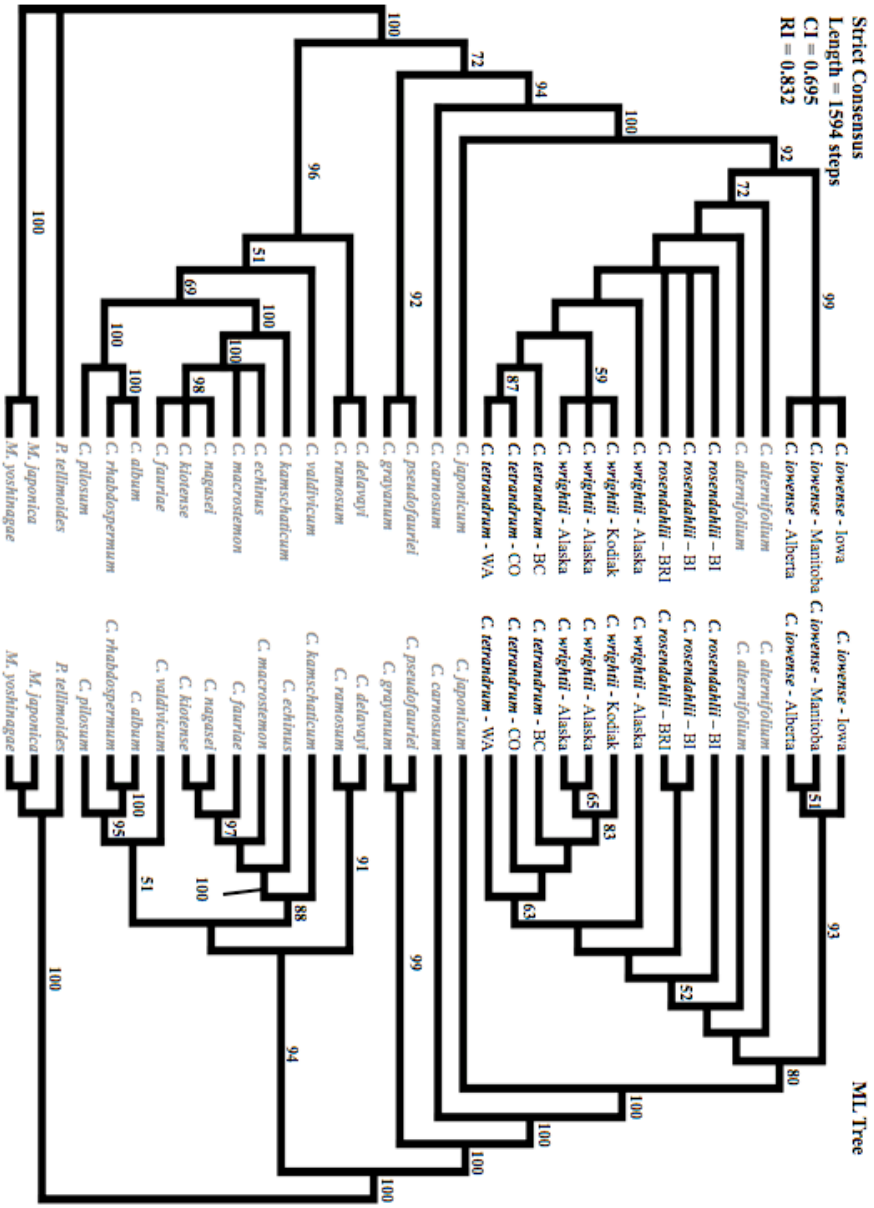

Figure 2.1 A strict consensus of 6 most parsimonious trees on the left and a Maximum Likelihood tree on the right. Numbers above branches on the strict consensus tree are jackknife support values ≥ 50% and numbers above branches on ML tree are bootstrap values above ≥ 50%. Taxa in black font are members of the Alternifolium group.

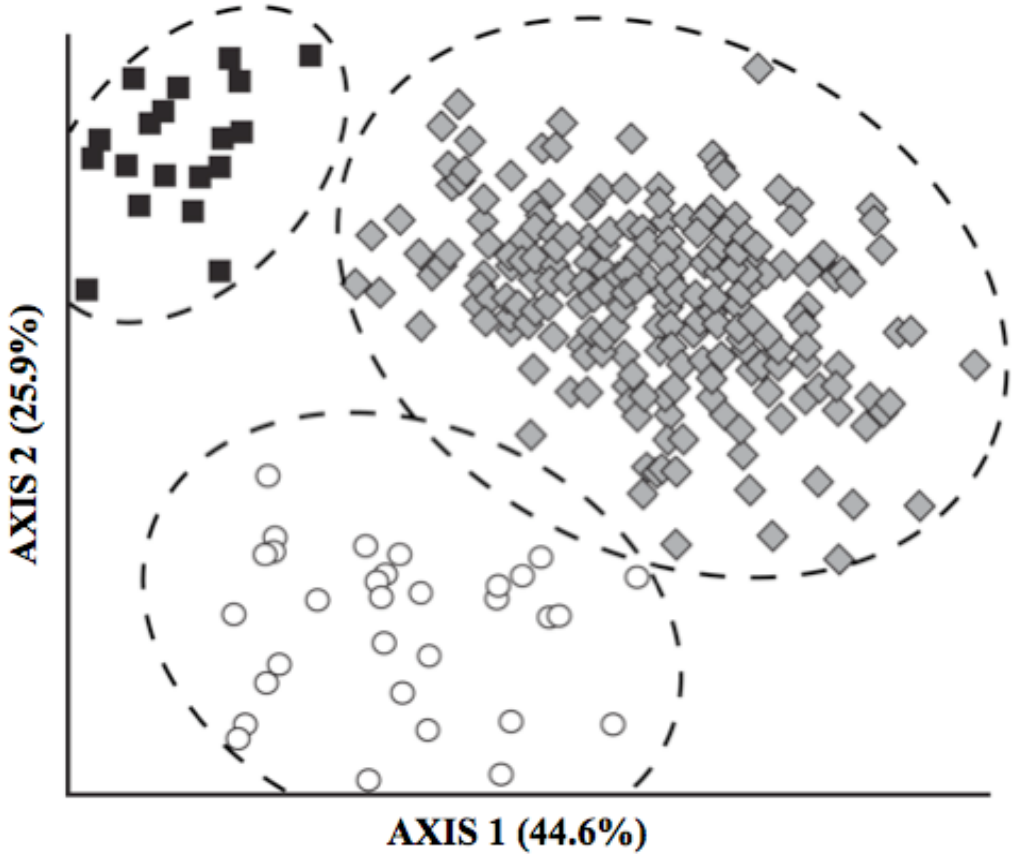

Figure 2.2 Results from a Principal Components Analysis. The two axes shown are those accounting for greatest variance. The gray diamond represents *C. tetrandrum* individuals, the open circles are *C. iowense*, and the dark squares are *C. rosendahlii*.

CHAPTER THREE:

DETERMINING PATTERNS OF GENETIC DIVERSITY AND POST-GLACIAL RECOLONIZATION OF WESTERN CANADA IN THE IOWA GOLDEN SAXIFRAGE, *CHRYSOSPLENIUM IOWENSE* (SAXIFRAGACEAE), USING INTER-SIMPLE SEQUENCE REPEATS (ISSR)

Abstract

Chrysosplenium iowense Rydb. (Saxifragaceae) is a southern Canadian boreal forest species with a small number of disjunct populations occurring in the Driftless Area of northeastern Iowa and southeastern Minnesota. This disjunction is attributed to the actions of glacial movement and climate change during the Pleistocene. Populations within each of these distributions may have been isolated for 115 000 years or more and though levels of genetic divergence between these regions may be significant, there is no morphological or cytological variation associated with this geographic break. We employed inter simple sequence repeat (ISSR) markers to determine patterns of genetic diversity within 12 populations (6 Canadian; 6 Iowan) of *C. iowense* and elucidate the routes of post-glacial recolonization for the species. Despite finding relatively high levels of genetic divergence ($\theta^{II} = 0.383$, $\theta^{II} = 0.299$) between Driftless Area and Canadian populations, there is no conclusive evidence of a speciation event within *C, iowense*. Analyses show moderate levels of genetic diversity within the species ($H_T = 0.188$), the majority of which is partitioned among individuals within populations (68.18%), which were similar across the northern (H_T

= 0.234, H_T = 0.28) and southern (H_T = 0.189) ranges. Finally, the patterns of genetic diversity within *C. iowense* suggest that the Canadian range was established by migrants originating in now extinct refugial populations that existed outside the Driftless Area.

Introduction

Throughout the Quaternary Period, global climate changes along with cycles of glacial advance and retreat repeatedly altered the physical distribution of plant species (Abbott et al., 2000). For many high latitude plant taxa, the recency and magnitude of these redistributions have had important effects on their current genetic structure (Hewitt, 1996; Hewitt, 2004). In some cases, range fragmentation and subsequent isolation of populations introduced high levels of genetic drift, producing strong genetic differentiation without, necessarily, a correlated change in phenotype (Lande, 1980; Hewitt, 2001; Petit et al., 2003). At its most pronounced, this process may have led to the prolific formation of cryptic species in the arctic and sub-arctic (Grundt et al., 2006). However, few groups of arctic or sub-arctic plants have been investigated to corroborate this finding. We have applied a hyper-variable molecular genetic approach to determine whether a Pleistocene-age range disjunction in the boreal species *Chrysosplenium iowense* Rydb. (Saxifragaceae) has resulted in a level of genetic divergence between isolated ranges that is indicative of a speciation event.

Chrysosplenium iowense is a perennial herb native to the boreal forests of North America (Packer, 1963). Though it is primarily distributed in southwest and central Canada, the species is also found in small isolated populations throughout the Driftless Area of Iowa and Minnesota (Packer, 1963; Weber, 1979). This "northern" range disjunction is shared by other boreal and arctic plant species (e.g., *Mertensia paniculata*, *Ribes hudsonianum*, *Carex media*) and is likely the product of climatic and glacial dynamics operating in the late Pleistocene (Pusateri et al., 1993). Though no identified morphological or cytological differences distinguish populations from the two ranges of *C. iowense* (Packer, 1963), prolonged isolation may be presumed to have resulted in strong genetic differentiation.

The Driftless Area of the midwestern United States comprises adjacent regions of Iowa, Minnesota, Wisconsin, and Illinois (Hartley, 1966). The name refers to the region's lack of Wisconsin age (115-15 kya) glacial drift, strong evidence that it remained ice-free during the most recent glaciation (Hartley, 1966). In Iowa and Minnesota, the limits of the Driftless Area are coincident with those of the Paleozoic Plateau, a regionally unique physiographic feature characterized by a rugged, bedrock controlled landscape highly dissected by deeply entrenched streams (Hartley, 1966). Differential weathering of bedrock has resulted in the widespread development of karst features (e.g., caves, sinkholes) throughout the Driftless Area, providing a diversity of microhabitats for numerous exotic plant species (Hedges, 1972; Pusateri et al., 1993). Prominent among these features, north-facing algific talus slopes are

perforated with “ice caves” and “cold air vents” that maintain the summer temperature of nearby soil at close to 15°C (Hedges, 1972; Weber, 1979). The temperature requirements for successful sexual reproduction in *C. iowense* mean that Driftless Area populations of this species occur only within close proximity of these slopes (Weber, 1979). Because of this physiologically imposed range restriction, Driftless Area *C. iowense* constitutes only about 15 small populations and is thus, listed as threatened in Iowa and endangered in Minnesota (Weber, 1979; The PLANTS Database, 2007). The limited southern range of *C. iowense* is in contrast with its more extensive northern range (Fig. 3.1), in which populations are patchily distributed among wet stream margins and bog habitats of the Canadian boreal forest (Packer, 1963).

Aside from simply geographic distance, additional causes of restricted gene flow among populations of *C. iowense* may be aspects of the species’ reproductive biology. Though *C. iowense* exhibits a mixed mating system, the putative insect pollinators are collembolans with presumably small (e.g., < 10cm/day; Weber, 1979; Bengtsson et al., 1994) dispersal distances. Seeds are dispersed via a splash cup mechanism (Savile, 1953; Nakanishi, 2002) and the maximum, experimentally determined dispersal distance is 45cm (Weber, 1979). Despite potentially larger secondary dispersal distances achieved through water transport in streams and rivers, the majority of gene flow in *C. iowense* appears to operate on a relatively small spatial scale.

To date, there has been only limited investigation into the genetic structure of *C. iowense*. Using eight isozyme loci, Schwartz (1985) was unable to show any variation within or among five Driftless Area populations. This result is probably less a reflection of a complete lack of genetic variation within the species and more a demonstration of the need for more variable molecular markers to detect existing polymorphism (Clegg, 1989; Coates & Byrne, 2005). No genetic study has been conducted that has included Canadian populations and thus, there is no information as to the relationships among populations of the two ranges or relative levels of genetic diversity within each range. The lack of information regarding these points is significant when we consider the goal of conserving populations of *C. iowense* in Iowa and Minnesota.

To determine patterns of genetic diversity within *C. iowense*, across the species' distribution, we employed analyses of hypervariable, PCR-based inter-simple sequence repeat (ISSR) markers (Huang & Sun, 2000). We use these data to address the following questions: **(1.)** what is the level of genetic divergence between Canadian and Driftless Area populations of *C. iowense*; **(2.)** do patterns of genetic diversity differ between the different ranges of this species; **(3.)** what is the route(s) of post-glacial recolonization for Canadian populations of *C. iowense*?

Materials and methods

Population sampling

Twelve populations of *C. iowense* were sampled for this study, including six from the Driftless Area of northeastern Iowa, four from central Alberta, and two from western Manitoba (Fig. 3.1; Table 3.1). Sample sizes varied between 10 and 20 individuals per population, depending on total population size. From each sampled individual, leaf material was removed and stored in silica gel. To avoid obtaining multiple samples from a single genet, which for *C. iowense* form discernible clumps typically < 1 m in diameter (Weber, 1979), a minimum spacing requirement of 0.5 m between sampled clumps was used.

DNA extraction and ISSR survey

Eight individuals per population (96 total) were randomly selected for ISSR genotyping. DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Valencia, CA). Methods for DNA amplification, as well as PCR product electrophoresis, visualization and sizing of products, and scoring are discussed in detail by Archibald et al. (2006). All PCR reactions were a total of 25μL, including 0.5μL DNA, 50μM dye-labeled primer (D4, WellRED, Proligo, St. Louis, MO), 25mM $MgCl_2$, and 2x Bullseye (0.05 units/μL Bullseye *Taq* polymerase, 150mM Tris-HCl pH 8.5, 40mM $(NH_4)_2SO_4$, 1.5mM $MgCl_2$, 0.2% Tween 20, 0.4mM dNTP's, and stabilizer; MIDSCI,

St. Louis, MO). The four primers (Table 3.2) chosen for this study demonstrated utility during screening. Each PCR run involved 5 min at 94°C; 40 cycles of 45 s at 94°C, 45 s at 49-60°C, and 90 s at 72°C; 10 min at 72°C.

ISSR electrophoresis and analysis was performed on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA), using the Fragment Analysis Module. Fluorescently labeled primers allowed detection by the automated sequencer and fragment sizes were estimated using a custom 1000 bp size standard (MapMarker 1000, BioVentures, Murfeesboro, TN); bands of a given size were considered to represent a single locus. Fragment analysis was conducted using the manufacturer's software (Beckman Coulter, Fullerton, CA). Analysis parameters were set to default for minimum acceptable peak height and relative height. Each reaction was analyzed in two separate runs and only those bands that appeared in both were included in the final data set.

Data analysis

Numbers of polymorphic loci and private bands for populations and regional groups were determined with the program FAMD v1.108 (Schlüter & Harris, 2006). HICKORY v1.1 (Holsinger & Lewis, 2003) analyses produced estimates of expected heterozygosity, H_S (average panmictic heterozygosity) and H_T (total panmictic heterozygosity), analogous to those described by Nei (1973). The Bayesian

hierarchical model employed by HICKORY is useful for the analysis of dominant data, which do not allow a direct determination of allele frequency or heterozygosity. Statistical differences among heterozygosity estimates are determined by comparing Bayesian credible intervals. Significance is assigned when neither 95% credible interval includes the other estimate's mean value.

A distance matrix, derived from the Dice similarity coefficient, was computed in FAMD. Dice's (1945) coefficient is appropriate for dominant genetic data because it excludes "shared-absence" characters, which are less likely to be homologous than "shared-presences" (Archibald et al., 2006). Dice is calculated as

$$S_{ij} = \frac{2n_{11}}{2n_{11} + n_{01} + n_{10}}$$

where n_{11} is the number of shared bands between individuals and n_{01}/n_{10} are the number of mismatches between individuals. The distance transformation is expressed as D = 1 – S.

Analyses of molecular variance (AMOVA) were performed, using ARLEQUIN v3.1 (Excoffier, 2005), to determine the apportionment of genetic variation within *C. iowense*. These include one three-level AMOVA and three two-level AMOVAs, one

for each geographic region. The significance of observed variance components was determined with 5000 permutations.

A Mantel test was performed in ALLELES IN SPACE v1.0 (Miller, 2005) to determine the level of association between genetic and geographic distance in the data set. The analysis used a Dice distance matrix of individuals and geographic distances, which were calculated from latitude/longitude coordinates of sample locations and then log transformed. Significance was assessed by 5000 permutations.

To determine the genetic affinities of the 96 individuals involved in this study, a principal coordinates analysis (PCoA), based on the Dice distance matrix, was performed in FAMD. Levels of genetic differentiation among populations and regional groups were determined by calculating θ^{II}, a Bayesian analogue of Wright's (1951) F_{ST} (Holsinger, 2002). Unlike the AMOVA-based measures of differentiation produced in ARLEQUIN, which are derived from a matrix of squared Euclidean distances among haplotypes generated from 'multi-locus' band phenotypes (Excoffier, 2005), θ^{II} is estimated from the variance in inferred allele frequencies at each sampled locus (Holsinger & Lewis, 2003). The Bayesian hierarchical model implemented in HICKORY v1.1 (Holsinger & Lewis, 2003) permits θ^{II} to be estimated from dominant ISSR data without the need to invoke assumptions regarding the level of inbreeding in populations (Holsinger, 2002). The HICKORY analyses were conducted under the *f*-free model, following the authors' (Holsinger & Lewis, 2003)

suggestion for dominant marker data sets. MCMC sampling parameters were set to default values: burn-in (50,000), sampling (250,000), and thinning (50). Two runs were performed for each analysis to ensure convergence of the MCMC sampling algorithm. Statistical comparisons of θ^{II} estimates were performed using the posterior comparisons option in HICKORY. This method approximates the posterior distribution of $\theta^{II}_{A} - \theta^{II}_{B}$ (*i.e.*, the difference between estimates of θ^{II} derived from data sets A and B) from a sample of $\theta^{II}_{Ai} - \theta^{II}_{Bi}$ (*i.e.*, the difference between paired random samples from the posterior distribution of θ^{II} for each data set; Holsinger & Lewis, 2003; Holsinger & Wallace, 2004). If the 95% credible interval of this distribution includes zero, then the estimates cannot be considered statistically different (Holsinger & Lewis, 2003).

Results

Four ISSR primers produced 1195 scorable loci of which 1165 (97.5%) were polymorphic across all samples. The distribution of polymorphic loci (pl) and private bands (pb) for individual populations and groups is summarized in Table 3.1. When the data set is partitioned based on geography, the Driftless Area has the highest number of both polymorphic loci (936) and private bands (179). The Canadian regions have comparably smaller counts: Alberta, 857 pl and 84 pb, and Manitoba, 553 pl and 33 pb. The number of polymorphic loci and private bands for individual populations range from 411 to 595 (mean 501 ± 61.5) and 2 to 18 (mean 8.33 ± 4.75),

respectively. There is no statistical evidence to suggest significant differences among these values.

The program HICKORY produced estimates of expected heterozygosity, among these the species-wide estimate for the total expected heterozygosity, H_T = 0.188 (Table 3.3). Additional analyses revealed significant differences among heterozygosity estimates for geographic regions. Comparisons of Bayesian 95% credible intervals showed statistically higher values of heterozygosity in Manitoba (H_S = 0.276; H_T = 0.28) as compared to Alberta (H_S = 0.219; H_T = 0.234) and the Driftless Area (H_S = 0.18; H_T = 0.189), which were different from each other.

A three-level AMOVA (Table 3.4), with all 12 populations grouped into three geographic regions, found the majority (68.18%) of genetic variation partitioned among individuals within populations. Differences among regions accounted for 26.89% of the total variation, while differences among populations within regions accounted for 4.93%. Three two-level AMOVAs were performed to determine the apportionment of genetic variation within each geographic region. In the Driftless Area, 92.68% of the genetic variation was partitioned among individuals within populations and 7.32% was found among populations. These percentages were comparable to the results found for the Alberta and Manitoba regions.

The Mantel test, performed in ALLELES IN SPACE, was significant ($r = 0.55$, $p < 0.0001$) for a correlation between Dice and geographic distance in the data set. A PCoA (Fig. 3.2), based on Dice distances among all 96 individuals, produced three discrete clusters of individuals, which were grouped based on geographic affinities. The three primary axes explained 30%, 7.15%, and 5.85% of the total genetic variance.

Bayesian estimates of genetic differentiation within and among each of the three geographic regions are summarized in Table 3.5. Posterior comparisons of θ^{II} show statistical differences among values. The greatest level of inter-regional genetic differentiation ($\theta^{II} = 0.383$) occurs between the Driftless Area and Manitoba, while measures of differentiation between Alberta and Manitoba ($\theta^{II} = 0.259$) and the Driftless Area and Alberta ($\theta^{II} = 0.299$) are statistically indistinguishable. Within region θ^{II} estimates suggest low levels of differentiation. Values for the Driftless Area ($\theta^{II} = 0.063$) and Alberta ($\theta^{II} = 0.086$) are not statistically different, while the estimate for Manitoba ($\theta^{II} = 0.028$) is smaller.

Discussion

Automated ISSRs present a number of benefits to the study of closely related lineages, which include increasing the number of scorable loci per primer, while also improving the accuracy of locus size estimation and homology assessment (see

Archibald et al., 2006). These benefits serve to produce larger, more accurate datasets than manual ISSR or enzyme studies, with a greater ability to resolve relationships among groups, populations, or even individuals (Crawford & Mort, 2004). In this investigation, an automated ISSR approach produced many polymorphic loci, discounting Schwartz's (1985) earlier inferences about *C. iowense* biology, which were based on an invariant enzyme dataset.

Patterns of genetic diversity

A cessation or reduction in gene flow among population subdivisions is expected to increase differentiation at selectively neutral loci through the enhanced action of genetic drift (Epperson, 2003). Under most circumstances (see Ibrahim, 1996), the prolonged isolation of subdivisions will produce relatively high levels of genetic structure, while decreasing population-level gene diversity (Epperson, 2003). This pattern of genetic variation is predicted for many high latitude plant taxa, which experienced numerous instances of range fragmentation and/or restriction throughout the Pleistocene and whose current populations are mutually isolated by factors of distance, breeding system, etc. (Hewitt 1996; Hewitt, 2001; Petit et al., 2003). For some, the extent of genetic differentiation, absent concomitant phenotypic change, has led to the formation of new cryptic species, a process that may be particularly common in the arctic and greatly enhance our view of biodiversity in that region (Grundt et al., 2006). One goal of this study is to determine the levels of divergence

among populations of *C. iowense* and evaluate whether those levels are suggestive of a speciation event.

Chrysosplenium iowense, a high latitude species with a significant distributional disjunction and a limited dispersal capability, exhibits a pattern of differentiation expected among geographically disparate ranges. Namely, that genetic distance among individuals is strongly associated with the geographic distance between them (see results of Mantel Test), suggestive of a scenario of "isolation by distance" (IBD; Wright, 1943; Epperson, 2003). A PCoA (Fig. 3.2) grouped individuals into three units, which correspond to geographic regions (i.e., Driftless Area, Alberta, Manitoba). According to the results of the PCoA and θ^{II} estimation (Table 3.5), the greatest level of genetic divergence among these units occurred between the Driftless Area and the Canadian regions. When we compare the θ^{II} values among these regions, only the estimate of divergence between the Driftless Area and Manitoba (θ^{II} = 0.383) exceeds the average Φ_{ST} value (0.35 ± 0.18) for intra-specific ISSR studies reported by Nybom (2004). However, both the Driftless Area/Manitoba and Driftless Area/Alberta (θ^{II} = 0.299) divergence estimates show significantly higher values than that resulting from a comparison between *C. iowense* and its sister species, *C. tetrandrum* (θ^{II} = 0.238; 95% credible interval, 0.201 – 0.281). In their study of cryptic speciation in arctic *Draba*, Grundt et al. (2006) describe a correlation between genetic distance and the accumulation of hybrid sterility factors, which leads to speciation. While our results show relatively high levels of genetic divergence

among the ranges of *C. iowense*, they are not high enough to clearly indicate a speciation event and lack the conclusive evidence of reproduction isolation that could be provided by a biosystematic study such as was applied to *Draba*.

In addition to the development of genetic structure, aspects of life history, breeding system, and spatial distribution can affect the levels of genetic diversity within a species or population (Hamrick & Godt, 1989; Nybom & Bartish, 2000). Considering the physical (e.g., size, location) and ecological (e.g., available pollinators, length of growing season, forest community, etc.) differences between the two ranges of *C. iowense*, we compared their respective levels of gene diversity to determine any dissimilarity. The results show that the patterns of genetic diversity do differ across the species, but not substantially. The largest differences were observed for levels of panmictic heterozygosity (H_S and H_T; Table 3.3), which were highest for the Manitoba region and lowest for the Driftless Area. Due, likely to complex glacial population histories, it is difficult to unambiguously place the Driftless Area and Manitoba regional groups of *C. iowense* in categories of geographic range (e.g., widespread or narrow distribution) based on heterozygosity values. However, levels of heterozygosity for the Alberta region are consistent with other ISSR-based values for widespread species (Sica et al., 2005; Al et al., 2007). A three-level AMOVA (Table 3.4) found that the majority (68.18%) of genetic variability in *C. iowense* is accounted for by differences among individuals within populations. This result, which was confirmed by two-level AMOVAs conducted within each geographic

region, is generally reflective of outcrossing species (Chung, 2004) and confirms earlier reports of this breeding system for *C. iowense* (Weber, 1979). Though the Driftless Area had the largest number of private bands, when combined, the Canadian regions slightly exceed this value (Table 3.1). This comparably similar level of genetic variation may be attributable to *C. iowense*'s status in the Driftless Area as a putative glacial relict (Schwartz, 1985; Abbott et al., 2000; Petit et al., 2003).

Historical biogeography

The current distribution of *C. iowense* is most readily attributable to environmental forces operating during the last glaciation (115-15 kya), specifically the advance and retreat of continental glaciers (Hewitt, 1996; Abbott et al., 2000). As the glaciers scoured much of northern North America, most plant and animal species migrated south and persisted in a large southern refugium (Darwin, 1859; Abbott & Brochmann, 2003). Boreal and arctic plants like *C. iowense* would have persisted relatively close to the glacial front in large swaths of tundra and boreal forest (Pusateri, 1993; Petit et al., 2001). When the glaciers receded, these species migrated north and recolonized previously ice-covered habitat (Hewitt, 2001). However, due to a lack of palynological and fossil evidence, we are often unsure of the origin of these colonists or the routes of their recolonization (Abbott et al., 2000). In these cases, it is necessary to test historical biogeographic scenarios using analyses of patterns of genetic diversity (Abbott et al., 2000). Three potential scenarios of post-

glacial recolonization for *C. iowense* include: **(1.)** a long distance dispersal event either from the Driftless Area to Canada or vice versa; **(2.)** a gradual northern migration of populations from the Driftless Area back to Canada; **(3.)** the establishment of Canadian populations by colonists from an alternate source.

Reid's paradox states that for many trees and herbs, the observed mean dispersal distance of a species cannot explain the extent of its current distribution (Reid, 1899). This principle certainly applies to *C. iowense* and may be solved by invoking the occasion of a rare long distance dispersal event. However, these events often leave the signature of a genetic bottleneck, including a loss of genetic diversity, the extent of which depends on the length and severity (i.e., reduction in population) of the bottleneck (Hewitt, 1996; Ibrahim et al., 1996). This is not the pattern observed for *C. iowense*. Instead, levels of genetic diversity are moderate for all regions and even comparatively high in Manitoba.

The second scenario describes a gradual "stepping-stone" dispersal of populations from the Driftless Area to the area of the current Canadian distribution. Under this scenario, effective population sizes would remain moderately large and the ancestors of current Driftless Area and Canadian populations would have been exchanging genes until quite recently (9 – 10kya; Pusateri, 1993). Again, this pattern is not entirely supported by the genetic evidence. The level of divergence and respective amounts of accumulated private bands between these distributions is suggestive of a longer period of isolation than that allowed under this scenario.

While the southern refugium was the primary region for glacial survival of species, there is evidence of the existence of other, smaller refuges within the glacial margins (Stehlik, 2003). Nunataks (Blytt, 1876), as these refugia are known, are proposed to have existed within the Canadian Rocky Mountains, near the current distribution of Alberta populations of *C. iowense* and may have served as a center of recolonization for the species (Packer & Vitt, 1974). Current Canadian populations may also have been established by migrants from relict populations existing in the southern refugium, isolated from those that established the Drifltess Area distribution. Though this hypothesis is extremely difficult to demonstrate with confidence, given the current nonexistence of these alternative populations, our genetic data is most supportive of this scenario. The level of divergence between the two distributions, as well as the relative levels of genetic diversity within each suggests that Canadian populations were established by migrants, separate from the ancestors of current Driftless Area populations.

Conservation

In both Iowa and Minnesota, *C. iowense* is restricted to the Driftless Area where it occurs in approximately 15 populations and is legally protected from extirpation (Weber, 1979; The PLANTS Database, 2007). The species is not known from any other locality within the United States. An extensive study of the species' biology of Driftless Area *C. iowense* was produced by Weber (1979) and reported that the

southern distribution of this species was not limited by "its functioning biology", but rather the small amount of available habitat. From this we may conclude that the restricted condition of its Driftless Area range has probably existed for thousands of years, since the retreat of the Wisconsin glaciers. However, the rarity of this species in Iowa and Minnesota combined with its environmental sensitivity and the prospect of imminent climate change should warrant concern for its long-term viability. This case is strengthened by the potential that prolonged isolation has rendered this southern element a new cryptic species, truly endemic to the Driftless Area. Our study is the first informative genetic survey of *C. iowense* and provides some of the first (see Chung, 2004) estimates of patterns of genetic diversity for northern species occurring in the Driftless Area. Though the observed levels of genetic divergence between the Driftless Area and Canadian populations cannot substantively determine whether the relict populations represent a novel species, they do indicate that Driftless Area *C. iowense* is a unique genetic element within the species. Clearly, further study of morphology, cytology, biosystematics, and genetics are required to understand the taxonomic status and future viability of *C. iowense*.

Table 3.1 The numbers of private bands and polymorphic loci found for regions and populations of *C. iowense*. Values in parentheses indicate percentage of polymorphic loci.

Region	Population	*n*	Polymorphic Loci	Private Bands
Driftless Area		**48**	**936 (78)**	**179**
	A	8	411 (34)	3
	B	8	478 (40)	10
	C	8	563 (47)	14
	D	8	449 (38)	10
	E	8	523 (44)	2
	F	8	570 (48)	10
Canada		**48**	**982 (82)**	**229**
Alberta		**32**	**857 (72)**	**84**
	M	8	595 (50)	18
	N	8	467 (39)	4
	O	8	555 (46)	5
	S	8	519 (43)	11
Manitoba		**16**	**553 (46)**	**33**
	BA	8	421 (35)	6
	BB	8	461 (39)	7

Table 3.2 Four dye-labeled ISSR primers used to genotype *C. iowense*.

Sequence (5'-3')	T_m (celsius)	Loci
CT_9TD	59	304
GT_7YG	49	235
GAG_5RC	57	288
CTC_7RC	58	368

Table 3.3 Estimates of average panmictic heterozygosity (H_S) and total pooled heterozygosity (H_T) for regional groups of *C. iowense*. Values in parentheses show the lower and upper bounds of a 95% Bayesian credible interval. Asterisks indicate values that are statistically different from others within the same column.

Region	*n*	H_S	H_T
Driftless Area	48	0.18 (0.16, 0.204) *	0.189 (0.167, 0.215)
Alberta	32	0.219 (0.204, 0.235) *	0.234 (0.217, 0.251) *
Manitoba	16	0.276 (0.262, 0.29) *	0.28 (0.266, 0.294) *
C. iowense	96	N/A	0.188 (0.169, 0.209)

Table 3.4 Results of a three-level AMOVA performed on ISSR data from *C. iowense*.

Source of Variation	**d.f.**	Variance Components	Percent of Variation
Among Regions	2	2468.74	26.89
Among Populations Within Regions	9	1333.906	4.93
Among Individuals Within Populations	84	7885.5	68.18

Table 3.5 Pairwise θ^{II} comparisons among geographic regions of *C. iowense*. Values in parentheses show the lower and upper bounds of a 95% Bayesian credible interval. Asterisks indicate a value that is statistically different.

	Driftless Area	Alberta	Manitoba
Driftless Area	0.063 (0.054, 0.073)		
Alberta	0.299 (0.269, 0.333)	0.086 (0.07, 0.105)	
Manitoba	0.383 (0.355, 0.412) *	0.259 (0.228, 0.293)	0.028 (0.012, 0.05) *

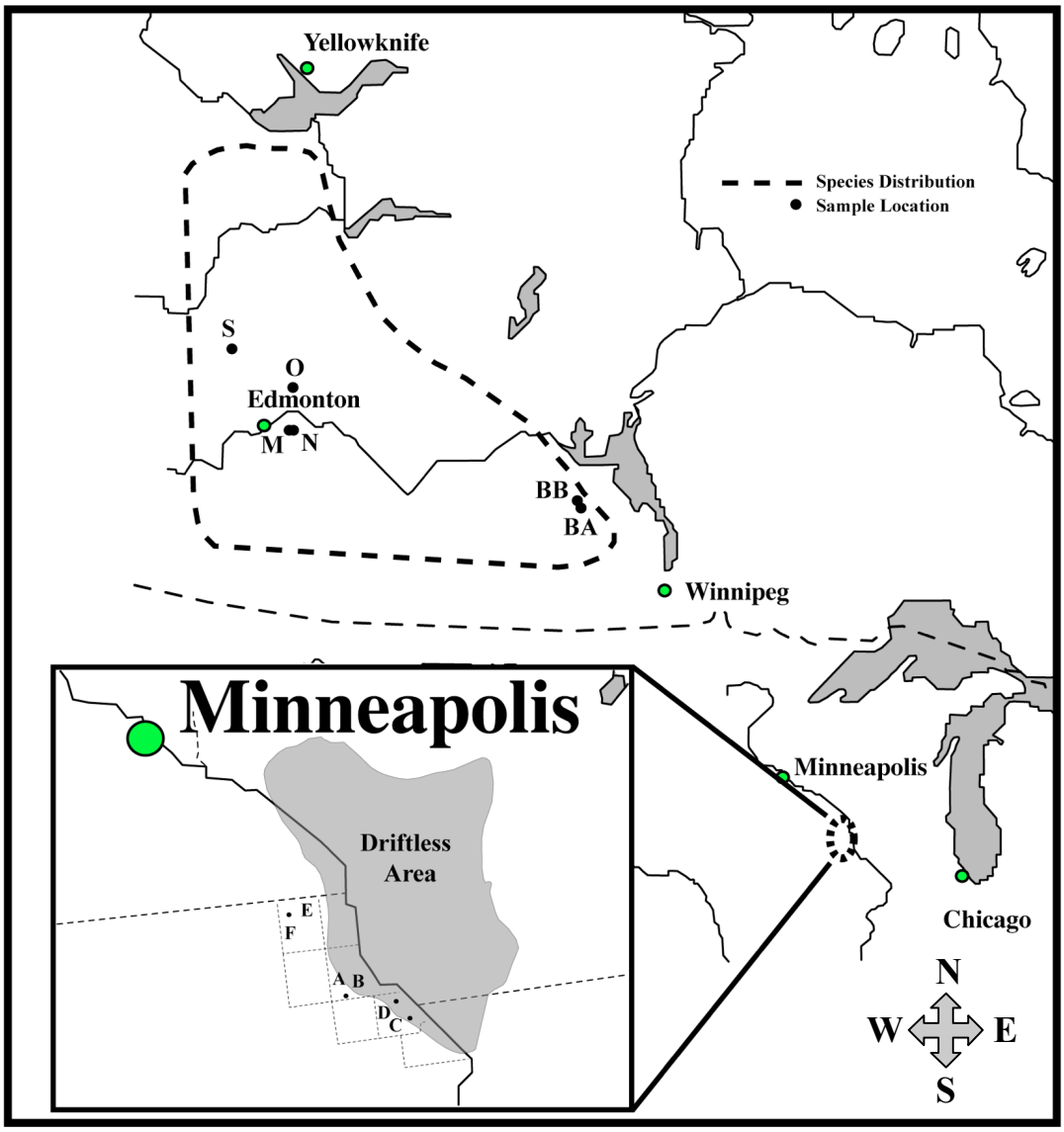

Figure 3.1 Map shows the species distribution of *C. iowense*, as well as sampling locations for the 12 populations analyzed in this study. The location of the Driftless Area is indicated in gray in the inset map.

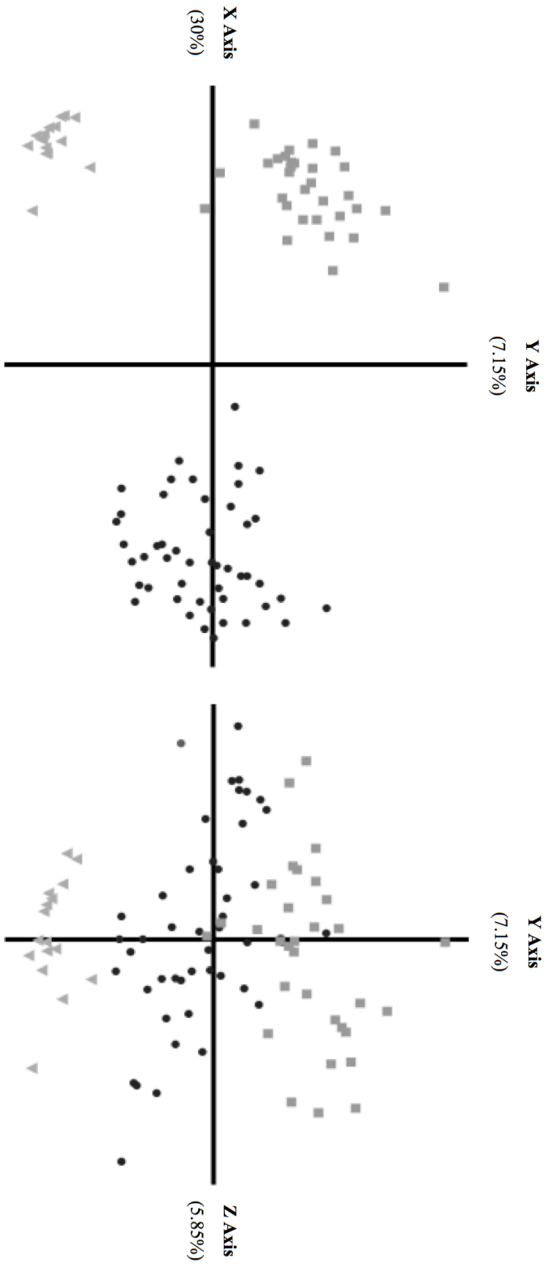

Figure 3.2 Results of principal coordinates analysis (PCoA). The first three axes are represented in two 2-dimensional graphs. Individuals are coded by shape and color according to geographic origin (light gray triangle = Manitoba, dark gray square = Alberta, black circle = Driftless Area).

CHAPTER FOUR:
THE GENETIC STRUCTURE AND GLACIAL HISTORY OF *CHRYSOSPLENIUM TETRANDRUM*, DETERMINED WITH INTER-SIMPLE SEQUENCE REPEATS

Abstract

The herbaceous perennial *Chrysosplenium tetrandrum* (Lund ex Malmgr.) Th. Fr. (Saxifragaceae) is a self-pollinating, circumpolar species with a broad latitudinal distribution and significant North American range disjunction. The southernmost populations are isolated in the Colorado and Montana Rocky Mountains, distantly separated from elements of the main range, which is largely coincident with known glacial refugia (e.g., Beringia and Canadian Arctic Archipelago) as well as recently deglaciated areas. We employed analyses of inter-simple sequence repeats (ISSRs) and morphology to determine patterns of variation within the western North American range of *C. tetrandrum* and test hypotheses of glacial population history and reproductive strategy. We found very low levels ($\theta^{II} = 0.085$) of range-wide genetic differentiation and genetic diversity ($H_S = 0.077$, $H_T = 0.084$), which are consistent with findings in other arctic species and attributed to recent population establishment and clonality. Low levels of genetic differentiation within the Alaska range ($\theta^{II} = 0.072$), which might signify a recent bottleneck or range expansion into the region. Both the Colorado and Alaska regions showed strong differentiation between themselves and other geographic groups, suggesting long-term isolation. The findings generally demonstrate a complex glacial history for the species.

Introduction

Factors that influence patterns of genetic variation within plant species are myriad. They include contemporary as well as historical attributes of an organisms' biology (e.g., life history, physiology) and environment (e.g., physiography), which serve to shape networks of gene flow within and among populations and determine the local effects of genetic drift and natural selection (Stebbins, 1950; Silvertown, 2001). To date, studies have revealed the genetic structure of hundreds of plant species, while extensive reviews have detailed the specific roles of physical and biological attributes in determining that structure (e.g., Hamrick et al., 1979; Loveless & Hamrick, 1984; Hamrick & Godt 1989; Nybom & Bartish, 2000; Nybom, 2004). Despite the wealth of genetic data available for many plant species, there still exist taxa for which, the patterns of genetic variation and their causes remain largely unknown. Notable among these groups is the arctic flora, which is thought to be lacking in biodiversity at all levels but has been largely unexamined (Murray, 1987; Steltzer et al., 2008).

Throughout the late Tertiary and Quaternary periods, polar and sub-polar regions of the world experienced substantial alterations to their physical environment. Cycles of global climate change together with repeated periods of glacial advance and retreat redistributed species across the landscape and established harsh new ecological regimes at high latitude (Ewing & Donn, 1956; Billings & Mooney, 1968; Pielou, 1991). These events are believed to have had significant effects on the genetic

structure of arctic and boreal plant species (Hewitt, 1996; Hewitt, 2004). The actions of glacial expansion, particularly regarding the extirpation of populations and the reduction of species' ranges, are credited with establishing a general state of low intra-specific genetic diversity within the arctic flora (Pamilo & Savolainen, 1999). The maintenance of this condition is largely attributed to the assumed frequency of self-pollination in many arctic species, but may also stem from the influence of powerful selective forces operating in stressful polar environments (Billings and Mooney, 1968; Bliss, 1971). It is important to note, however, that these hypothesized patterns of variation have not been widely confirmed (Murray, 1987; Steltzer et al., 2008). To date, only a few arctic species (e.g., *Saxifraga oppositifolia* L. and *Dryas integrifolia* Vahl.) have been the subject of intensive genetic study and, for these, levels of genetic variation are often higher than traditional assumptions suggest (Tremblay & Schoen, 1999; Abbott et al., 2000). Such findings have emphasized additional aspects of arctic plant biology and glacial history, including the persistence of populations in glacial refugia and higher-than-expected levels of outcrossing, which contribute to the generation or maintenance of intra-specific genetic variation (Hewitt, 1996; Gabrielsen & Brochmann, 1998; Hewitt, 2000; Abbott & Brochmann, 2003). Discordance between expected and observed results serves to underscore the difficulty in establishing generalized theory to explain or predict patterns of variation in a complex flora. An improved understanding of the evolution of the arctic flora will require a consideration of this complexity, certainly as it relates to species' glacial history, ecology, breeding system, and phylogenetic affinity. In the present

study, we investigate patterns of variation within the western North American range of the arctic species *Chrysosplenium tetrandrum* (Lund ex Malmgr.) Th. Fr. (Saxifragaceae) to elucidate further, arctic plant diversity.

Chrysosplenium tetrandrum is a widespread, circumpolar species with a complex glacial and pre-glacial history (Fig. 4.1; Packer, 1963). The species range reaches as far north as Murchisonfjord, North-East Land, Spitsbergen Archipelago (80° 3' N; Aiken et al., 1999) and is largely continuous throughout the arctic, but at its southern extent, high elevation populations in Colorado remain strongly disjunct from the central distribution (Packer, 1963). Weber (2003) suggested that these disjunct elements may be late Tertiary-age relicts of a once prominent Oroboreal flora. These populations, he contended, survived the Pleistocene glaciations *in situ*, persisting in isolated Nunatak (i.e., high mountain) refugia (Blytt, 1876; Weber, 2003). Additional ice-free regions existed in North America during the Pleistocene glaciations, including the area to the south of the major ice-sheets (Darwin, 1859), Beringia (i.e., Alaska, Yukon Territory, and parts of Siberia; Hultén, 1937), coastal areas of western British Columbia and southeastern Alaska (Heusser, 1960), and the Canadian Arctic Archipelago (Hultén, 1937). The current distribution of *C. tetrandrum* (Fig. 4.1) includes portions of each of these regions and it is possible that all of them played a role in the glacial survival of the species. The long-term isolation and range reduction associated with glaciation and refugial survival would have had significant effects on patterns of genetic variation within species such as *C. tetrandrum*.

While glacial history has an important effect on levels of intra-specific genetic diversity, so too do contemporary patterns of gene flow within species. However, at present, these patterns are completely unknown within *C. tetrandrum*. The mode of reproduction and breeding system of the species, which represent important determinants in these patterns, are not confirmed, but preliminary evidence suggests that *C. tetrandrum* is obligately self-pollinating and capable of vegetative reproduction via stoloniferous growth (Warming, 1909; Packer, 1963; Weber, 1979). These characteristics, combined with a short dispersal distance (< 0.5 m; Weber, 1979; Nakanishi, 2002), implies that intra-specific gene flow in *C. tetrandrum* is quite localized and significantly limited.

As with many arctic plants species, the morphology of *C. tetrandrum* is highly reduced and largely uniform across its range (Savile, 1972; Billings, 1987). Though knowledge of the cause of taxonomically significant morphological variation in *C. tetrandrum* is desirable, the relationship between genetic and morphological variation in the species is unclear. Phenotypic plasticity is viewed as an important contributor to phenotypic variation in arctic species and may account for the relatively high degree of variation observed in traits of *C. tetrandrum* (Savile, 1972; Stenström et al., 2002).

Instances of inter-specific gene flow can also have important consequences for the evolution of a species. Such exchanges can produce novel genetic variation and ultimately result in rapid speciation (Rieseberg, 1997; Rieseberg & Willis, 2007). In the arctic, hybridization is thought to be quite common and consequently, important to the evolution of the flora (Brochmann et al., 2004). In the case of *C. tetrandrum*, the species is sympatric with a closely related species at two separate points in its North American range (arctic coast, *C. rosendahlii* Packer; Alberta, *C. iowense* Rydb.; Table 4.1). Within the sympatric zones, species have been found growing in close proximity and exhibiting approximately identical phenology (Packer, 1963). Despite this, there exists no evidence of hybridization between these taxa in either region (Packer, 1963). A lack of gene flow may be attributable to biological barriers, such as self-pollination and differences in chromosome number among species (i.e., *C. tetrandrum* 2n = 24, *C. iowense* 2n = c. 120, *C. rosendahlii* 2n = 96; Packer, 1963). While hybridization between *C. tetrandrum* and either *C. iowense* or *C. rosendahlli* appears unlikely, the prospect has not been thoroughly investigated. Both the frequency of hybridization in other members of Saxifragaceae (e.g., *Heuchera* L., *Mitella* L.; Soltis et al., 2001) and the moderate levels of outcrossing described in *C. iowense* suggest the possibility of even limited inter-specific gene flow.

The goal of this study was to determine patterns of genetic diversity within the western North American range of *C. tetrandrum*. To achieve this, we employed inter-simple sequence repeat (ISSR) markers (Huang & Sun, 2000) and used these data to

address the following questions: **(1)** what do contemporary levels of genetic diversity indicate about patterns of gene flow within western North American *C. tetrandrum*; **(2)** what is the level of genetic divergence between the Colorado and Montana distribution of *C. tetrandrum* and its main Canadian and Alaskan distribution; **(3**) are patterns of morphological variation correlated with those of genetic variation; and **(4)** is there evidence of inter-specific gene flow between *C. tetrandrum* and *C. iowense* and/or *C. rosendahlii*?

Materials and methods

Population sampling

Forty populations of *C. tetrandrum* were sampled from across the species' western North American range (Fig. 4.1; Table 4.1). These include two Alaskan populations from which *C. rosendahlii* individuals were also collected and one Albertan population from which *C. iowense* individuals were also collected (mixed populations were not included in species-wide analyses). Whole flowering shoots were collected from between 3 and 20 individuals per population, depending on total population size. To avoid obtaining multiple samples from a single genet, a minimum spacing requirement of 1 m between sampled clumps was used. The shoots were pressed and stored in coin envelopes. Leaf material was removed from each shoot and dried in

silica gel for DNA extraction. For ten random individuals, duplicate DNA collections were made to determine the reproducibility of the ISSR analyses (Bonin et al., 2004).

DNA extraction and ISSR survey

Between 3 and 16 individuals per population (402 total) were randomly selected (when possible) for ISSR genotyping. DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Valencia, CA). Methods for DNA amplification, as well as PCR product electrophoresis, visualization and sizing of products, and scoring are discussed in detail by Archibald et al. (2006). All PCR reactions were a total of 25μL, including 0.5μL DNA, 50μM dye-labeled primer (D4, WellRED, Proligo, St. Louis, MO), 25mM $MgCl_2$, and 2x Bullseye (0.05 units/μL Bullseye *Taq* polymerase, 150mM Tris-HCl pH 8.5, 40mM $(NH_4)_2SO_4$, 1.5mM $MgCl_2$, 0.2% Tween 20, 0.4mM dNTP's, and stabilizer; MIDSCI, St. Louis, MO). The four primers (Table 4.2) chosen for this study demonstrated utility during screening. Each PCR run involved 5 min at 94°C; 40 cycles of 45 s at 94°C, 45 s at 49-60°C, and 90 s at 72°C; 10 min at 72°C. ISSR electrophoresis and analysis was performed on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA), using the Fragment Analysis Module. Fluorescently labeled primers allowed detection by the automated sequencer and fragment sizes were estimated using a custom 1000 bp size standard (MapMarker 1000, BioVentures, Murfeesboro, TN); bands of a given size were considered to represent a single 'locus'. Fragment analysis was conducted using the manufacturer's

software (Beckman Coulter, Fullerton, CA). Analysis parameters were set to default for minimum acceptable peak height and relative height.

Morphological measurement

Six quantitative traits were measured on each sampled individual: seed length, seed width, sepal length, sepal width, hypanthium length, and hypanthium width. The presence or absence of leaf and sepal maculation was also assessed as a binary character. Floral measurements were taken from one of the central flowers in the inflorescence. Length and width measurements of each sepal and hypanthium measured were combined to produce ratio characters, which were used for morphometric analyses. Individuals with missing measurement values were excluded from those analyses.

Data analysis

The ISSR band profiles for duplicate samples were compared, and those loci that exhibited a high frequency ($\geq 40\%$) of mismatch across duplicate comparisons were removed from the dataset. ISSR reproducibility was assessed, using the edited dataset, as one minus the average frequency of mismatch for all duplicate comparisons (Bonin et al., 2004).

We calculated the percentage of polymorphic loci (FAMD v1.108; Schlüter and Harris 2006) and the number of private bands (i.e., a band that is present only in a specific group and occurs in ≥ 4 individuals within that group) for each population and regional group. To determine levels of genetic diversity within and among populations, estimates of Nei's (1973) average panmictic heterozygosity (H_S) and total panmictic heterozygosity (H_T) were calculated using a Bayesian hierarchical model (HICKORY v1.1; Holsinger & Lewis, 2003). Morphological diversity within populations was measured by calculating average pair-wise morphometric (i.e., squared Euclidean distance) distances (APD) among individuals (PC-ORD; McCune & Mefford, 1999). Levels of statistical significance among estimates of H_S and H_T were determined by comparing 95% Bayesian credible intervals. Significance is assigned when neither 95% credible interval includes the other estimate's mean value. Significant differences among APD estimates were determined with a permutation analysis of variance (ANOVA; PERMDISP2; Anderson, 2004). A nonparametric permutation approach was necessary given the non-independence of pair-wise distance values.

Distance matrices were calculated for both the ISSR (FAMD v1.108; Schlüter & Harris, 2006) and morphological (PC-ORD; McCune & Mefford, 1999) datasets. The ISSR distance matrix was computed using the Dice similarity coefficient (Dice, 1945), which is desirable for use with dominant genetic data like ISSRs because it excludes 'shared absence' characters (Archibald et al., 2006). Dice is calculated as

$$S_{ij} = \frac{2n_{11}}{2n_{11} + n_{01} + n_{10}}$$

where n_{11} is the number of shared bands between individuals and n_{01}/n_{10} are the number of mismatches between individuals. The distance transformation is expressed as D = 1 – S. The morphological distance matrix is based on the squared Euclidean distance measure, which was calculated from five morphological traits (i.e., seed length, seed width, sepal length/width, hypanthium length/width, and presence/absence maculation). A matrix of geographic distances (kilometers) among sampled populations was also calculated (GEOGRAPHIC DISTANCE MATRIX GENERATOR v1.2.2; Ersts, 2008).

Three Mantel tests were conducted to identify correlations between distance matrices. The first test, ISSR distance versus geographic distance, was computed using the software program ALLELES IN SPACE v1.0 (Miller, 2005), while the other two, morphological distance versus geographic distance and morphological distance versus ISSR distance, were computed using the program zt (Bonnet & Van de Peer, 2002). In each test, statistical significance was assessed by 10 000 permutations.

To analyze relationships among individuals, we performed a principal coordinates analysis (PCoA; FAMD v1.108; Schlüter & Harris, 2006) using the Dice distance matrix.

Levels of genetic differentiation among populations and regional groups were estimated by calculating θ^{II} (HICKORY v1.1; Holsinger & Lewis, 2003), a Bayesian analogue of Wright's (1951) F_{ST}. The Bayesian approach is popular for producing estimates of genetic differentiation from dominant data sets. This is because the method permits θ^{II} to be estimated from the variance in inferred allele frequencies, which cannot be directly observed from dominant data, without the need to invoke assumptions regarding the level of inbreeding in populations (Holsinger et al. 2002). HICKORY v1.1 (Holsinger & Lewis, 2003) analyses were conducted under the *f*-free model, following the authors' (Holsinger & Lewis, 2003) suggestion for dominant marker data sets. MCMC sampling parameters were set to default values: burn-in (50 000), sampling (250 000), and thinning (50). Two runs were performed for each analysis to ensure convergence of the MCMC sampling algorithm. Statistical comparisons of θ^{II} estimates were performed using the posterior comparisons option in HICKORY. This method approximates the posterior distribution of $\theta^{II}_{A} - \theta^{II}_{B}$ (*i.e.*, the difference between estimates of θ^{II} derived from data sets A and B) from a sample of $\theta^{II}_{Ai} - \theta^{II}_{Bi}$ (*i.e.*, the difference between paired random samples from the posterior distribution of θ^{II} for each data set; Holsinger & Lewis, 2003; Holsinger & Wallace, 2004). If the 95% credible interval of this distribution includes zero, then the estimates cannot be considered statistically different (Holsinger & Lewis, 2003).

A locus-by-locus analysis of molecular variance (AMOVA) was performed (ARLEQUIN v3.1; Excoffier et al., 2005) to determine the apportionment of genetic

variation within *C. tetrandrum*. Unlike the standard AMOVA computed in ARLEQUIN, the locus-by-locus AMOVA treats each marker in a multi-locus genotype as unlinked (Excoffier et al., 2005). Significance of observed variance components was determined with 5000 permutations.

Analysis of mixed populations

To investigate the possibility of inter-specific gene flow between *C. tetrandrum* and either *C. iowense* or *C. rosendahlii*, we constructed new, smaller genetic and morphological data sets for each case. For *C. tetrandrum* - *C. iowense*, the data sets consisted of five populations, including: two of *C. tetrandrum* (i.e., BD and CM), two Alberta populations of *C. iowense* and one mixed population (i.e., BC; Table 4.1). For *C. tetrandrum* – *C. rosendahlii*, the data sets consisted of six populations, including: two of *C. tetrandrum* (i.e., BX and CE), two Northern Alaskan populations of *C. rosendahlii*, and two mixed populations (i.e., BZ and CC; Table 4.1). Principal components analyses (PCA) were performed (PC-ORD; McCune & Mefford, 1999) on the two morphological data sets to identify intermediate phenotypes. Locus-by-locus AMOVAs (ARLEQUIN v3.1; Excoffier et al., 2005) were performed on each of the genetic data sets to identify loci that exhibit high (≥ 0.5) levels of differentiation, ϕ_{ST}, between species. These, highly differentiated loci, composed new genetic data sets that were analyzed in NEWHYBRIDS v1.1 (Anderson, 2003), which implements a Bayesian approach to testing hypotheses of hybrid ancestry. The program computes

the posterior probability that individuals belong to different hybrid categories: pure *C. tetrandrum*, pure *C. iowense* or *C. rosendahlii*, F_1, F_2, backcross with *C. tetrandrum*, and backcross with *C. iowense* or *C. rosendahlii*. Two replicate runs of each analysis were performed to ensure consistency among results.

Results

Genetic and morphological variation

ISSR analyses produced 701 loci, of which, 659 (94%) were polymorphic across all samples. Repeatability of ISSR markers was 95%. Table 4.1 summarizes the distribution of private bands (PB) and polymorphic loci (PL) among populations and regions. The percentage of polymorphic bands varied among populations (range, 10 - 50%; mean, 24% ± 11) and regions (range, 29 – 76%; mean, 55% ± 18). The most polymorphic region was Alaska/Yukon (76%) followed closely by Washington (70%). Private bands (PB) were found in only two populations, population H (five bands) and population U (one band), and three regions, Washington (eight), British Columbia (three), and Alaska/Yukon (nine).

Range-wide estimates of average panmictic heterozygosity and total panmictic heterozygosity were 0.077 (± 0.004) and 0.084 (± 0.005), respectively. Heterozygosity estimates were statistically invariant across populations, but did differ

among regions, with values for Alaska/Yukon (H_S = 0.085; H_T = 0.092) significantly lower than those of other groups (Table 4.1). The average pair-wise distance (APD) measure, which indicates levels of morphological variation within groups, was found to be statistically invariant among populations and regions (Table 4.1).

Three Mantel tests produced the following estimates of association between sets of distance matrices: ISSR versus geographic (r = 0.015, P = 0.238), morphological versus geographic (r = -0.104, P = 0.086), and ISSR versus morphological (r = -0.013, P = 0.433). In all three tests, we found no significant correlation between matrices.

The first three plotted axes of the PCoA (Fig. 4.2) accounted for 18.43% of the total variation at the molecular level. The analysis did not indicate clear genetic structure within *C. tetrandrum*. This finding is consistent with the range-wide Bayesian estimate of genetic differentiation (θ^{II} = 0.085 ± 0.004). Pair-wise estimates of genetic differentiation within and among regions are summarized in Table 4.3. The highest intra-regional θ^{II} values were found in Alberta (0.173) and Colorado (0.214). Inter-regional F_{ST} values were highest in comparisons made to Alaska/Yukon, Colorado, and Montana.

A locus-by-locus AMOVA (Table 4.4) reported that the greatest percentage of genetic variation (84.59%) in the data set was accounted for by differences among

individuals within populations. In contrast, very little variation was accounted for by differences among populations (11.9%) or regions (3.52%).

Inter-specific gene flow

Principal components analyses

In the case of each species-to-species comparison, a PCA (Fig. 4.3) of morphological data clearly grouped all individuals into one of two species groups and did not indicate any individuals with intermediate phenotypes that might suggest mixed ancestry.

Genetic analyses

To identify genetic evidence of gene flow between *C. tetrandrum* and *C. iowense*, a reduced ISSR data set was constructed and consisted of 33 loci, which were found to be highly differentiated ($\phi_{ST} \geq 0.5$) between species. Genetic analyses placed 38 out of 50 individuals into pure species categories with a posterior probability greater than 0.8. One individual, belonging to the mixed population and indicated in Fig. 4.3 by an arrow, was placed with high posterior probability (0.933) in the *C. tetrandrum* backcross category.

The data set for the *C. tetrandrum*-*C. rosendahlii* comparison consisted of six highly differentiated loci. Nineteen of seventy-two individuals were placed within a pure species category with a posterior probability above 0.8 and no individuals were placed within a hybrid category with a probability higher than 0.3.

Discussion

Genetic diversity and breeding system in C. tetrandrum

Reproductive strategy is one of the most important factors to influence patterns of genetic diversity within plant species (Loveless & Hamrick, 1984; Nybom & Bartish, 2000). Components of reproductive strategy (e.g., mode of reproduction and breeding system) dictate a fundamental level of gene flow within and among populations, which can then be modified by additional aspects of a species' biology and ecology (Hamrick & Godt, 1989). Asexual modes of reproduction eliminate chromosomal recombination and gene flow among individuals, though multiclonal populations are often as genetically diverse as their sexual counterparts (Ellstrand & Roose, 1987; Hamrick & Godt, 1989; Coates & Byrne, 2005). Alternatively, sexual reproduction allows for recombination and gene flow, though the rates of these processes are determined by breeding system. Predominant outcrossing systems, characterized by gene flow among unrelated individuals, are associated with high levels of intra-populational diversity. Predominant self-pollinating systems result in

reduced rates of recombination and lower levels of diversity within populations (Jain, 1976; Hamrick & Loveless, 1984).

Chrysosplenium tetrandrum has been described (Warming,1909) as a purely selfing species. The results of this study are mixed in their support for this assertion. Analyses of ISSR markers yielded very low range-wide estimates of expected heterozygosity (H_S = 0.077, H_T = 0.084) and similarly low values for individual populations and regions (Table 1). These results are consistent with an autogamous *C. tetrandrum*. Alternatively, low range-wide estimates of genetic differentiation (θ^{II} = 0.085) contradict this hypothesis. Predominant selfing species lack gene flow among individuals and populations, leading to high levels of genetic differentiation, which were not observed in this study (Hamrick & Godt, 1989). We consider two factors to be central to reconciling this contradiction; the first is the importance of asexual reproduction suggested for *C. tetrandrum* and the second is the recent (8 -10 kya) establishment of many populations in the western range of this species as a result of glacial dynamics. Balloux et al. (2003) demonstrated that, by effectively fixing heterozygotes, asexual reproduction in diploid species increases gene diversity at individual loci and decreases population differentiation. The authors also suggested that with predominant asexual reproduction there is an expected decrease in genotypic diversity (e.g., H_S, H_T), as we see with our results (Balloux et al., 2003). However, despite its explanatory ability, we cannot conclusively identify asexuality as a main cause of the observed genetic patterns given that evidence of its effect (e.g.,

heterozygote excess at single loci) is not available to investigations using dominant molecular markers (Balloux et al., 2003). Short evolutionary history can also explain low levels of species-wide differentiation at high latitudes (Odasz & Savolainen, 1996; Max et al., 1999; Pamilo & Savolainen, 1999). For many high latitude populations, the period since glacial retreat may have been too short for many population processes to have reached equilibrium (Varvio et al., 1986; Pamilo & Savolainen, 1999). With the presumed frequency of clonality in arctic plants, both of these processes may be operating interactively to reduce levels of genetic differentiation within some high latitude species.

Genetic structure

As previously mentioned, the overall measure of genetic differentiation was low (θ^{II} = 0.085), particularly when compared to the ϕ_{ST} value (0.35 ± 0.18) reported by Nybom (2004) as an average for intra-specific ISSR studies. However, our result is comparable to the average species-wide F_{ST} estimate from other high latitude species (0.036; Hamrick & Godt, 1989). The θ^{II} estimates are consistent with the results of an AMOVA (Table 4.4) and a PCoA (Fig. 4.3), which report that most of the variation in the data set is accounted for by differences among individuals within populations. There is no statistical support (see results of ISSR v geographic distance Mantel test) for the hypothesis that levels of genetic differentiation were the result of isolation-by-distance (Wright, 1943). Again, we attribute this pattern largely to

recent population establishment and, perhaps also, the fixation of ancestral allele frequencies by of asexual reproduction.

Glacial refugia and post-glacial migration

The Pleistocene history of glaciation in northwestern North America is complex (Heusser, 1960). Over a period of two million years this region experienced four major glacial stages (i.e., Nebraskan, Kansan, Illinoian, Wisconsin), the most recent of which, Wisconsin, reached its maximum extent about 18 kya (Trenhaile, 2004). Throughout each of these stages large ice-sheets covered much of northern North America (Pielou, 1991). The arctic, boreal, and north temperate plant species that occur in high latitude environments today are descendants of ancestors that survived these glaciations in ice-free areas, known as glacial refugia (Pielou, 1991). Two of the largest and most important refugia in western North America were the Southern refugium (Darwin, 1859), which consisted of the entire region south of the Laurentide and Cordilleran glaciers, and Beringia (Hultén, 1937), which includes large tracts of what is today Alaska and the Yukon Territory. Additional, smaller refugia existed along the coasts of British Columbia and southern Alaska, as well as possibly in the southern Canadian Rocky Mountains (Heusser, 1960; Loehr et al., 2005). The use of molecular data to determine the role of these North American refugia has become commonplace (e.g., Tremblay & Schoen, 1999; Eidesen et al., 2007; Marr et al., 2008) and this accumulating information provides the basis for further formulation of

genetic predictions given specific historical scenarios. In the following, we discuss the putative roles (i.e., glacial refuge or area of recolonization) of sampled geographic regions as determined by patterns of genetic variation.

Alaska/Yukon

Alaska and the Yukon Territory form the western portion of the Beringia refugium (Hultén, 1937). Paleontological and molecular evidence (Hopkins, 1967; Abbott et al., 2000; Abbott & Brochmann, 2003) confirm both the existence of arctic plant species in the area throughout the Wisconsin glaciation (115-15 kya) and the region's role as a center for post-glacial recolonization. Thus, we expected Alaska/Yukon populations of *C. tetrandrum* to exhibit comparatively high levels of genetic diversity (Table 4.1) and differentiation among populations (Table 4.3), reflecting long-term persistence and large population sizes (Hewitt, 2004). The results, however, show very low levels of genetic diversity (Table 4.1) and differentiation within the region (Table 4.3), patterns that are more consistent with scenarios of rapid range expansion or recent bottleneck events, or both (Ibrahim et al., 1995; Hewitt, 1996). Given then, a scenario of recent range expansion, it is difficult to determine if contemporary Alaska/Yukon populations are derived from Beringian ancestors or non-Beringian migrants that moved into Alaska following glacial retreat. If the former is correct, the high levels of genetic differentiation between this region and others, evidenced by θ^{II} estimates and private band numbers, strongly suggest that those resident Beringian

populations did not serve as important sources of post-glacial recolonization for this species.

Washington

Sampled Washington populations occur at the northern edge of the southern refugium and perhaps also served as a source of post-glacial recolonization (Heusser, 1960; Soltis et al., 1996). While genetic diversity estimates (Table 4.1) provide inconclusive evidence of a refugial history for this region, low levels of genetic differentiation between these populations and the Canadian ranges suggest recent connections or the presence of recurrent gene flow (Cruzan & Templeton, 2000).

British Columbia and Alberta

Most of British Columbia and Alberta were completely glaciated as late as 10 kya (Heusser, 1960; Trenhaile, 2004) and the contemporary *C. tetrandrum* range in these regions is expected to have been established during a period of post-glacial recolonization. Generally, low levels of genetic diversity and differentiation would characterize a scenario of recent range expansion, however, if populations in these areas are derived from separate refugia they may exhibit comparatively high levels of differentiation (Hewitt, 1996; Marr et al., 2008). In fact, the results are consistent with both explanations. British Columbia populations maintain low levels of genetic

diversity and differentiation, while Albertan populations show much higher levels of intra-regional divergence. The relatively low levels of differentiation between Alberta and both British Columbia and Washington suggest that either of the latter regions served as one source of migrants during the recolonization process. The other putative refugial source is not known, though it may be the unglaciated portion of the southern Canadian Rockies that occurred between the Laurentide and Cordilleran glaciers (Packer & Vitt, 1974; Loehr et al., 2005).

Colorado and Montana

According to Weber (2003), Colorado and possibly Montana populations of *C. tetrandrum* may be oroboreal relicts from the late Tertiary (2-3 mya). This suggests a considerable period of isolation for these populations and would require high levels of differentiation from the main distribution (Hewitt, 1996). While the levels of divergence are higher than most in the data set, they do not demonstrate conclusively a long period of isolation, certainly no more in some cases than that experienced by the Alaskan populations. The high levels of differentiation and genetic diversity are consistent with long-term persistence in this region (Hewitt, 2004; Abbott et al., 2000).

Morphological variation

In predominant self-pollinating species, recombination rates are decreased and the persistence of linkage disequilibrium is increased (Jain, 1976; Flint-Garcia et al., 2003). Thus, compared to predominant outcrossing species, self-pollinators are expected to exhibit stronger associations between neutral genetic and morphological variation (Price et al., 1984). However, we find that ISSR variation is not correlated with morphological variation in *C. tetrandrum*. This non-association may be attributed to the independent segregation of genetic and quantitative trait loci, perhaps due to some sexual reproduction in populations, or the highly plastic nature of measured morphological traits.

Mixed populations

The North American range of *C. tetrandrum* becomes sympatric with those of its sister species, *C. iowense* (in western Alberta) and *C. rosendahlii* (on arctic coast). Packer (1963) posited that habitat differences between these species were not substantial and that, while they had not yet been found, there was no reason that mixed populations could not occur. In this study, we identified mixed populations between *C. tetrandrum* and *C. iowense* and *C. tetrandrum* and *C. rosendahlii*. However, despite the spatial proximity of these conspecific individuals, there exist some compelling barriers to inter-specific gene flow. These barriers include different chromosome numbers for each species and predominantly selfing mode of reproduction (Warming, 1909; Packer, 1963). We found no evidence of

morphological intermediates in any of the mixed populations that might suggest hybrid ancestry. Likewise, genetic analyses were largely unable to exhibit evidence of gene flow. One individual in the *C. tetrandrum*-*C. iowense* mixed population was identified with high confidence as a backcross. This result is not compelling evidence of widespread gene flow, but should promote further investigation of these mixed populations, especially with regard to chromosome numbers.

Table 4.1 Population sampling information of *Chrysosplenium tetrandrum* including population sample sizes for morphological and genetic analyses, numbers of private bands (PB), percentage of polymorphic loci (PL), average pair-wise morphometric distance (APD), average panmictic heterozygosity (H_S), and total panmictic heterozygosity (H_T). Ninety-five percent Bayesian credible intervals are indicated in parentheses following heterozygosity values. Significance of heterozygosity estimates are indicated by an asterisk.

Region	Population	ISSR *N*	Morphology *N*	APD	PL (%)	PB	H_S	H_T
Washington		**54**	**15**	**0.27**	**70**	**8**	**0.133 (0.118, 0.151)**	**0.139 (0.123, 0.159)**
	H	16	0		50	5	0.111	
	J	15	15	0.27	41	0	0.084	
	K	15	0		46	0	0.086	
	L	8	0		23	0	0.08	
Alberta		**40**	**11**	**0.26**	**54**	**0**	**0.133 (0.118, 0.15)**	**0.151 (0.132, 0.173)**
	P	8	0		15	0	0.063	
	Q	8	0		16	0	0.066	
	R	16	3	0.3	36	0	0.079	
	BC	*16*	*14*					
	BD	8	8	0.28	35	0	0.103	
British Columbia		**68**	**17**	**0.32**	**63**	**3**	**0.105 (0.095, 0.116)**	**0.114 (0.102, 0.127)**
	T	5	0		12	0	0.068	
	U	15	0		4	1	0.085	
	BE	10	4	0.17	2	0	0.063	
	BG	5	4	0.21	14	0	0.073	
	BH	7	3	0.31	31	0	0.102	
	CF	10	0		18	0	0.063	
	CM	10	6	0.27	23	0	0.069	
	CN	6	0		13	0	0.069	
Montana		**8**	**0**		**29**	**0**	**0.105 (0.096, 0.115)**	
	Z	8	0		29	0	0.105	
Colorado		**23**	**9**	**0.29**	**38**	**0**	**0.159 (0.108, 0.179)**	**0.179 (0.14, 0.205)**
	G	7	2		24	0	0.085	
	CP	16	7	0.29	22	0	0.059	
Alaska/Yukon		**171**	**155**	**0.32**	**76**	**9**	***0.085 (0.077, 0.095)**	***0.092 (0.083, 0.103)**
	BI	14	3	0.24	28	0	0.072	
	BJ	8	9	0.28	29	0	0.085	
	BK	7	6	0.31	28	0	0.091	
	BL	3	0		10	0	0.075	
	BM	8	2		32	0	0.091	
	BN	8	9	0.34	31	0	0.091	
	BO	16	16	0.31	37	0	0.078	
	BQ	5	0		12	0	0.069	
	BR	8	5	0.36	15	0	0.063	
	BS	16	10	0.26	22	0	0.061	
	BT	14	11	0.3	26	0	0.062	
	BU	15	19	0.3	29	0	0.073	
	BV	12	13	0.26	27	0	0.07	
	BW	11	26	0.33	22	0	0.065	
	BX	12	13	0.31	21	0	0.063	
	BY	4	4	0.34	14	0	0.077	
	EZ	*12*	*12*					
	CC	*10*	*9*					
	CD	0	3	0.39				
	CE	10	6	0.32	18	0	0.064	

Table 4.2 Four dye-labeled ISSR primers used to genotype *Chrysosplenium tetrandrum*.

Sequence (5'-3')	Tm (celsius)	Loci
CT9TD	59	304
GT7YG	49	235
GAG5RC	57	288
CTC7RC	58	368

Figure 4.3 Symmetrical matrix of pairwise θ^{II} comparisons among geographic regions of the western North American range of *Chrysosplenium tetrandrum*. Values in boxes are within region comparisons. Significant differences among within-region comparisons are indicated by superscript letters A and B and differences among between region comparisons within columns are indicated by superscript letters W, X, Y, and Z.

	Alaska/Yukon	British Columbia	Alberta	Washington	Montana	Colorado
Alaska/Yukon	0.072 ± 0.005^{A}	0.221 ± 0.045^{Z}	0.123 ± 0.038^{Y}	0.225 ± 0.035^{Y}	0.353 ± 0.024^{Y}	0.329 ± 0.03^{Y}
British Columbia	0.221 ± 0.045^{W}	0.088 ± 0.007^{A}	0.015 ± 0.004^{W}	0.05 ± 0.008^{W}	0.189 ± 0.017^{W}	0.12 ± 0.009^{W}
Alberta	0.123 ± 0.038^{W}	0.015 ± 0.004^{W}	0.173 ± 0.017^{B}	0.034 ± 0.006^{W}	0.167 ± 0.021^{W}	0.121 ± 0.012^{W}
Washington	0.225 ± 0.035^{W}	0.05 ± 0.008^{X}	0.034 ± 0.006^{X}	0.058 ± 0.006^{A}	0.163 ± 0.017^{W}	0.113 ± 0.009^{W}
Montana	0.353 ± 0.024^{Y}	0.189 ± 0.017^{Z}	0.167 ± 0.021^{Y}	0.163 ± 0.017^{Y}	N/A	0.254 ± 0.018^{X}
Colorado	0.329 ± 0.03^{Y}	0.12 ± 0.009^{Y}	0.121 ± 0.012^{Y}	0.113 ± 0.009^{X}	0.254 ± 0.018^{X}	0.214 ± 0.019^{B}

Table 4.4 Results of a locus-by-locus AMOVA performed on populations of the western North American range of *Chrysosplenium tetrandrum*.

Source of Variation	Variance Components	Percent of Variation
Among regions	1.205	3.517
Among populations within regions	4.077	11.9
Among individuals within populations	28.98	84.585

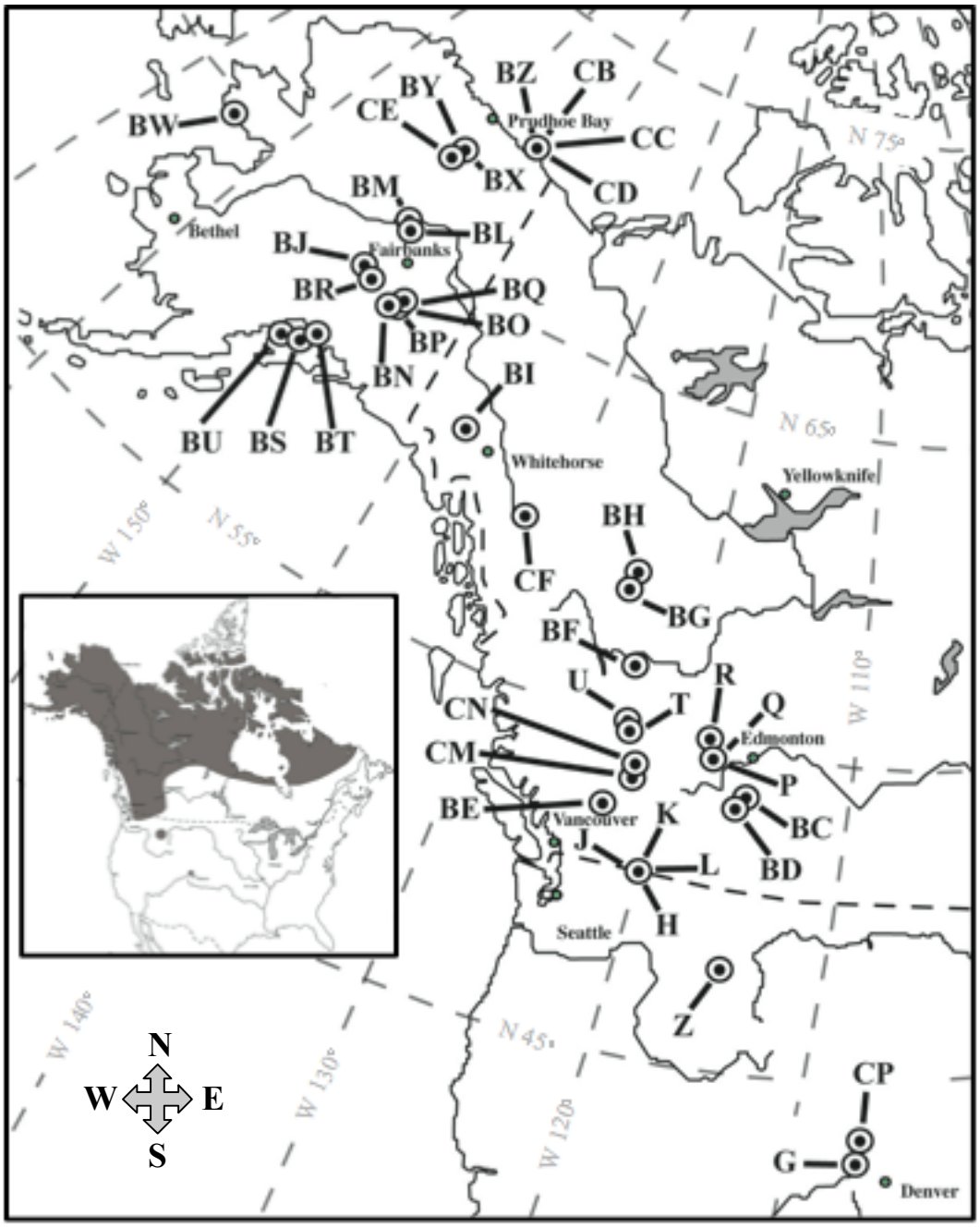

Figure 4.1 Map shows the species distribution of *Chrysosplenium tetrandrum*, as well as sampling locations for 40 populations analyzed in this study.

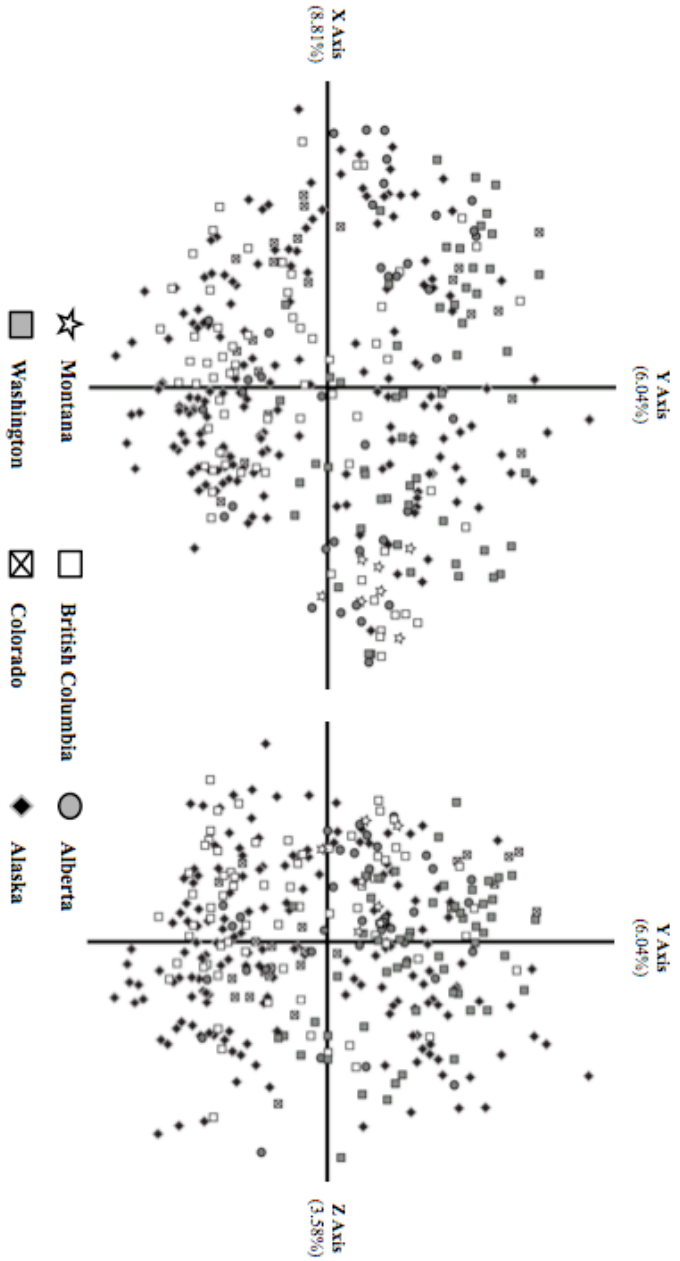

Figure 4.2 Principal coordinates analysis (PCoA) of ISSR profiles. The first three axes are represented in two 2-dimensional graphs. *Chrysosplenium tetrandrum* individuals are coded by shape and color according to geographic origin.

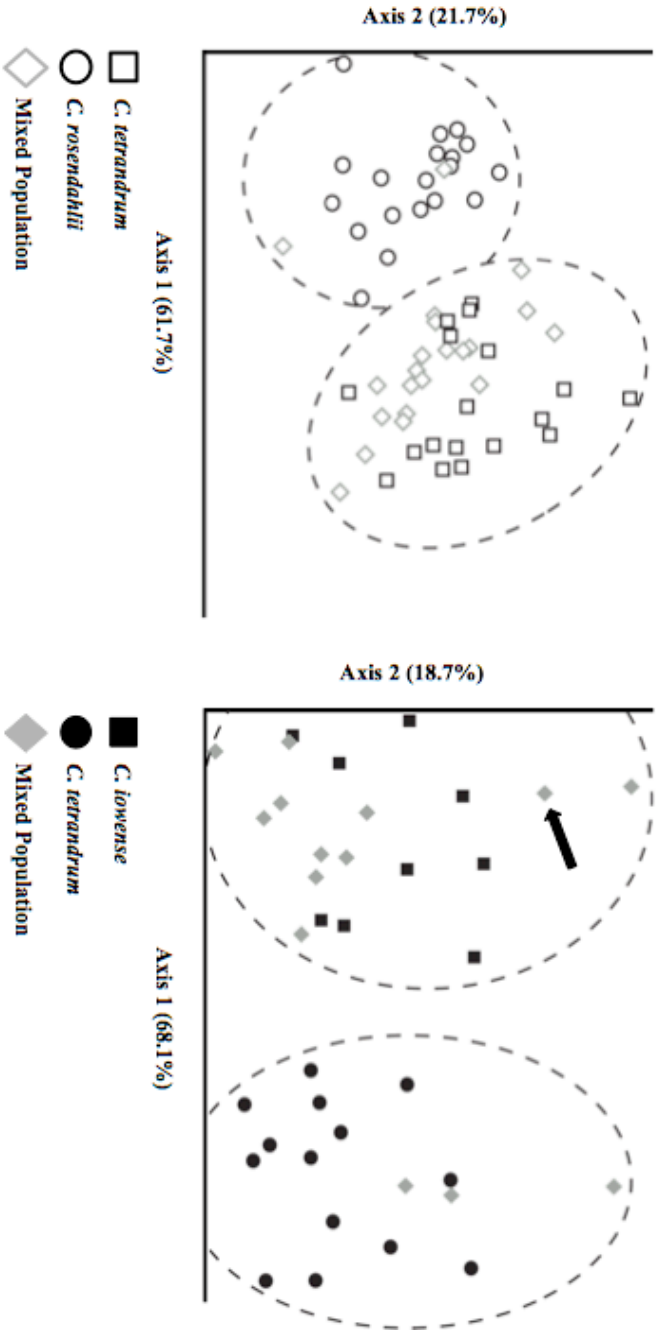

Figure 4.3 Principal components analyses (PCA) of morphological data. The result on the left is from analysis between *Chrysosplenium tetrandrum* and *C. rosendahlii* and the result on the right is from a *C. tetrandrum* and *C. iowense* comparison. The arrow indicates the individual, which was recovered with high probability as a *C. tetrandrum* backcross.

CHAPTER FIVE:

NEI'S TO BAYES': COMPARING COMPUTATIONAL METHODS AND GENETIC MARKERS FOR ESTIMATING PATTERNS OF GENETIC VARIATION IN *TOLPIS* (ASTERACEAE)

Abstract

Accurate determination of patterns of genetic variation provides a powerful inferential tool for studies of evolution and conservation. For more than 30 years, enzyme electrophoresis was the preferred method for elucidating these patterns. As a result, evolutionary geneticists have acquired considerable understanding of the relationship between patterns of allozyme variation and aspects of evolutionary process. Myriad molecular markers and statistical analyses have since emerged, enabling improved estimates of patterns of genetic diversity. With these advances, there is a need to evaluate results obtained with different markers and analytical methods. We present a comparative study of gene statistic estimates (F_{ST}, G_{ST}, F_{IS}, H_S, and H_T) calculated from an ISSR and an allozyme data set derived from the same populations using both standard and Bayesian statistical approaches. Significant differences were found between estimates, owing to the effects of marker and analysis type. Most notably, F_{ST} estimates for codominant data differ between Bayesian and standard approaches. Levels of statistical significance are greatly affected by methodology and, in some cases are not associated with similar levels of

biological significance. Our results suggest that caution should be used in equating or comparing results obtained using different markers and/or methods of analysis.

Introduction

The patterns of genetic variation within and among populations are of interest to diverse fields in plant biology including population genetics, systematics, and conservation. For the past four decades, following the demonstration of the utility of enzyme electrophoresis (Harris, 1966; Hubby & Lewontin, 1966; Lewontin & Hubby, 1966), there has been an ever-increasing use of various types of molecular markers to assess genetic variation. The basic rationale for molecular markers replacing earlier approaches such as quantitative characters as a means of assessing genetic variation is the more direct equation between genotype and phenotype obtained with molecular methods (Lewontin & Hubby, 1966; Schulman, 2007). However, as the science has progressed, considerations of improved efficiency and sensitivity have promoted the development of new molecular markers, many of which present significant analytical challenges to accurately assessing genetic variation (Sunnucks, 2000). Although ongoing developments in statistical analyses offer the potential to overcome these challenges, there is still a general inability to knowledgeably compare analogous estimates derived from different marker classes and/or statistical methods (Bonin et al., 2007).

For more than four decades, allozyme markers have been an invaluable tool for studies of evolutionary genetics, providing plant biologists with a straightforward, low cost means of estimating levels of intra-specific genetic variation (Cruzan, 1998). Allozymes produce codominant data, which permit direct observation of allele frequencies at allozyme loci and can be used rather simply to calculate various gene statistics (Hubby & Lewontin, 1966; Lewontin & Hubby, 1966; Hamrick, 1989; Weeden & Wendel, 1989). In addition, because of the highly conserved nature of allozyme loci in flowering plants (Gottlieb, 1982) homologous loci can be compared between closely related species. Practical advantages of allozymes include the relative procedural simplicity and low cost of the method (Clegg, 1989). Because of the large database that has accrued for allozymes, their estimated patterns of variation can be compared among plants with different ecological and life history traits (e.g., Hamrick & Godt, 1989). One of the major criticisms of allozyme data concerns the level of genome sampling; allozyme variation can only be determined for protein-coding genes (many of the assays are for enzymes of glycolysis and the citric acid cycle) of which, in plants, there is a rather small (~ 40) potential pool of useful candidates (Clegg, 1989; Wendel & Weeden, 1989). This number is further reduced for within-species studies where often only about 50% of the loci are polymorphic (Hamrick & Godt, 1989). In addition, variation at each of these loci may only be detected if it affects the electrophoretic mobility of the enzyme with the standard conditions employed (Lewontin & Hubby, 1966; Clegg, 1989). As much as 20% of the base substitutions may go undetected (Coates & Byrne, 2005). Allozyme

variation is often absent in groups of recently radiated taxa for which allozymes frequently provide limited and/or imprecise estimates of population genetic structure (e.g., Schwartz, 1985; Crawford et al., 1987).

In contrast to allozymes, arbitrarily amplified DNA (AAD) methods (e.g., AFLP, inter-simple sequence repeat, and random amplified polymorphic DNA) are able to produce large data sets, especially when loci are visualized using polyacrylamide gels. These loci presumably represent neutral, rapidly evolving regions from across the genome (Clegg, 1989; Huang & Sun, 2000; Krauss, 2000; Archibald et al., 2006). Very small amounts of plant material are needed for these markers, making them ideal for use with rare species. Utilizing PCR, AAD methods amplify a specific region of DNA or 'allele', which is visualized on an electrophoretic gel as a band presence or absence. Because band presence can indicate either the dominant homozygote or heterozygote, genotype and allele frequencies cannot be directly determined and estimation of gene statistics can be problematic (Meudt & Clarke, 2007). This represents a significant disadvantage compared to allozymes. However, the increased level of variation often seen with AAD markers and the increased ease of obtaining the required amount of plant material has led to these markers being preferred over allozymes in many studies; particularly in cases where genetic variation within and among populations and/or species is low (Crawford et al., 1994; Crawford et al., 2001).

Although codominant markers like allozymes are preferred for most genetic studies, especially those that calculate statistics requiring knowledge of allele frequencies, recently developed analytical techniques have allowed researchers to take greater advantage of the benefits of dominant markers (Krauss, 2000; Bonin et al., 2007). The traditional approach to estimating levels of population structure with allele frequency data has typically involved the use of F-statistics (i.e., F_{IT}, F_{IS}, F_{ST}). These were originally defined by Wright (1943; 1951) and based on correlations between uniting gametes at different hierarchical levels, total population (T) and population subdivision (S). Nei (1973; 1977), seeking to expand the use of Wright's F-statistics beyond a single locus two allele system, redefined them as functions of partitioned gene diversity and calculated levels of inbreeding (F_{IS} and F_{IT}) and genetic differentiation (G_{ST}) using measures of observed (H_O) and expected (H_S and H_T) heterozygosity. Nei's (1973) coefficient of gene differentiation, G_{ST}, is a multi-locus, multi-allele equivalent of Wright's F_{ST}.

Though F-statistics have been used extensively with codominant data, the methodological requirement of allele frequency estimates has made their application to dominant data problematic. Bayesian statistical analysis is an approach that is increasingly applied to evolutionary genetic studies because it ostensibly offers investigators the ability to overcome some of the analytical shortfalls of dominant data sets (Zhivotovsky, 1999; Holsinger et al., 2002). One of the more prominent implementations of Bayesian statistics to the analysis of dominant data is the method

described by Holsinger et al. (2002). The method is based on a Bayesian hierarchical model and directly estimates an F_{ST} analogue (θ^B) from dominant or codominant data, while incorporating the effect of uncertainty in inbreeding (F_{IS}) on this estimate (Holsinger et al., 2002). Holsinger et al. (2002) has demonstrated that this method produces reliable estimates of F_{ST} (although see Bonin et al., 2007) and allele frequencies without the assumption of a known inbreeding coefficient, which is required by other approaches (Lynch & Milligan, 1994; Zhivotovsky, 1999). By using the Bayesian estimate of mean allele frequency to calculate expected panmictic heterozygosities (H_S and H_T), the method is also able to produce an estimate of Nei's G_{ST}, known as $G_{ST}{}^B$. Under this framework, θ^B corresponds to a random-effects model of population sampling, which produces estimates from all potentially sampled populations and presumably reduces sampling error (Weir, 1996, p. 162; Holsinger, 1999). Alternatively, the $G_{ST}{}^B$ estimate corresponds to a fixed-effects model and is derived from all actually sampled populations (Weir, 1996, p. 162; Holsinger, 1999). Unlike standard implementations of the random-effects model (i.e., Weir and Cockerham, 1984), the Bayesian method is able to use it without a specified model of population divergence (Holsinger, 1999).

Despite their respective limitations, both allozyme and dominant data offer viable, low cost alternatives to more resource intensive methods (e.g., microsatellites; Schulman, 2007; Agarwal et al., 2008). At present, the greatly increased use of dominant markers over allozymes and the aforementioned large database available for

allozymes provides the potential to compare results from the two types of markers and relate them to many traits of plant ecology and life history. However, the relationships among analogous gene statistics derived from different marker classes and statistical methods are not well known. A better understanding of the comparability of gene statistic estimates derived from different methods will allow a more complete synthesis of the knowledge accumulated throughout more than 40 years of molecular genetic research. The opportunity for researchers to employ the full body of this knowledge will benefit a broad range of studies across the fields of plant genetics, systematics, and conservation, placing in a larger biological context the results of newer methods of data production and analysis.

For insight into the relationships among gene statistic estimates, comparisons of these estimates should be conducted across a broad range of biological conditions (i.e., patterns of genetic variation) and include empirical data for both codominant and dominant markers from the same populations. The purpose of this study was to respond to this need for comparative data among different commonly used markers and methods of analysis. We present a comparison of analogous estimates of F_{ST}, G_{ST}, F_{IS}, and expected heterozygosity derived from dominant (inter-simple sequence repeat, ISSR) and codominant (allozyme) data sets for the angiosperm genus *Tolpis* (Asteraceae) using the Holsinger et al. (2002) Bayesian method, as well as standard approaches to codominant and dominant data. *Tolpis* represents a recent radiation for which both dominant and codominant data have been used to assess genetic

relationships among taxa, as well as other aspects of species biology (Crawford et al., 2006; Archibald et al., 2006). By comparing specific estimates (Table 5.1), our goal was to determine patterns of relationships among analogous gene statistics and provide additional information as to the operation of Bayesian analysis when applied to individual empirical studies.

Materials and methods

Data

Tolpis (Asteraceae) is a Macaronesian and Mediterranean angiosperm genus, of which the majority of species represent a recent radiation within the Canary Island archipelago (Park et al., 2001; Crawford et al., 2006). From this Canarian radiation, we sampled 15 populations across five species (Table 5.2), constituting the bulk of the so-called *T. laciniata*-*T. lagopoda* complex (Archibald et al., 2006; Crawford et al., 2006). This complex comprises a high level of morphological and ecological variation, though all species share a perennial habit and are highly self-incompatible (Crawford et al., 2008). *Tolpis laciniata* and *T. lagopoda*, in particular, form large outcrossing populations (Crawford et al., 2006).

Individuals from each of the 15 populations sampled were genotyped at 10 polymorphic allozyme loci (GPI-2, PGM-1, TPI-1, TPI-2, PGD-1, PGD-2, GDH,

AAT-2, AAT-3, MDH-3) and at 1510 polymorphic ISSR loci. Protocols for enzyme electrophoresis and ISSR amplification and scoring are described in Crawford et al. (2006) and Archibald et al. (2006), respectively. Population sample sizes varied between marker classes. The allozyme data set averaged 17 individuals per population (range, 6-29), while the ISSR data set averaged almost eight individuals per population (range, 3-14). All 10 allozyme loci had three or more alleles (seven maximum) at a locus.

Analyses

Allozyme data were analyzed using both the Bayesian approach implemented in the program HICKORY v1.1 (Holsinger & Lewis, 2003) and the standard methods described by Nei and Chesser (1983), which were manually calculated. HICKORY estimates the Bayesian analogue of Weir and Cockerham's (1984) F_{ST} and Wright's F_{IS} (1951), designated in the program as θ^{II} and f, respectively (Holsinger, 1999; Holsinger et al., 2002; Holsinger & Lewis 2003). It also provides estimates of Nei's (1973) average expected panmictic heterozygosity (H_S), total expected panmictic heterozygosity (H_T), and coefficient of gene differentiation (G_{ST}), denoted by G_{ST}^{B}. Bayesian analyses of the codominant allozyme data set in HICKORY were performed with default parameter settings (burn-in = 50 000, sampling = 250 000, thinning = 50) under the full model analysis, which provides estimates of both θ^{II} and f. Nei and Chesser's (1983) methods produce unbiased estimates of G_{ST}, F_{IS}, H_S, and H_T. The

G_{ST} statistic is a multi-locus, multi-allele estimate of Wright's F_{ST} (Nei, 1973) and is often (as in the cases of Hall et al., 1994*;* Yeh et al., 1997) designated as the latter for ease of discussion. For this reason, we too refer to this estimate as F_{ST}. However, considering its statistical affinity we compared it to both θ^{II} and $G_{ST}{}^{B}$.

The ISSR data set was analyzed using HICKORY, under both the *f*-free and full model options and with ARLEQUIN v3.1 (Excoffier et al., 2005). Under HICKORY's full model, estimates of *f* influence those of θ^{II} and vice versa (Holsinger et al., 2002). Because estimates of *f* have been shown to be unreliable when calculated from dominant data sets, especially with small population sample sizes, Holsinger and Lewis (2003) suggest that under these conditions the *f*-free model, which removes the constraints of *f* on θ^{II} estimation, may be more appropriate. Both of these analyses were performed under the default parameter settings described above. Two replicate runs of each HICKORY analysis, for both marker classes, were produced to ensure convergence of the Markov chain Monte Carlo (MCMC) sampler.

In ARLEQUIN, a locus-by-locus analysis of molecular variance (AMOVA) produced locus-specific estimates of ϕ_{ST}, which is an F_{ST} analogue based on pair-wise squared Euclidean distances (Excoffier et al., 1992; Excoffier et al., 2005). The average of these individual values was calculated to produce the multi-locus estimate reported in the results. The AMOVA is a commonly used method for producing estimates of genetic differentiation from dominant data.

Statistical comparisons among estimates were conducted using 95% Bayesian credible intervals and confidence intervals for individual estimates. In HICKORY, the production of a sample log file during analysis allowed a statistical comparison of θ^{II} and f estimates (described in Holsinger & Wallace, 2004) using the posterior comparison option. Pairwise comparisons involving non-Bayesian estimates required the calculation of a 95% confidence interval (Zar, 1999), which could be compared to a similar Bayesian credible interval. Estimates were considered statistically different if each confidence/credible interval did not overlap the other estimate's mean value.

The following comparisons (summarized in Table 5.1) were considered for statistical analysis and discussion: **i.** comparison between Bayesian and standard analyses of an allozyme data set; **ii.** comparison between models of Bayesian analysis applied to an ISSR data set; **iii.** comparison between Bayesian and AMOVA estimates applied to an ISSR data set; **iv.** comparison between ISSR and allozyme estimates produced by Bayesian analyses.

Sample size differences

Population sample size is an important factor in gene statistic estimation and differential sampling of a given population or set of subpopulations may yield disparate estimates of genetic structure (Bonin et al., 2007). In this study, differences

in population sample sizes between marker classes could potentially account for differences in estimates of population parameters between those classes. To determine if, and in what way, the differences in sample size between the ISSR and allozyme data sets have influenced estimates of genetic differentiation in this study, the following analyses were conducted: **1.)** populations with ISSR sample sizes of three individuals (i.e., 17 and 1869; Table 5.2) were removed from the allozyme and ISSR data sets and F_{ST} and/or θ^{II} were recalculated for both; **2.)** population sample sizes in the allozyme data set were reduced, randomly, to equal the corresponding sample sizes in the ISSR data set, then F_{ST} and θ^{II} estimates were recalculated.

Data simulation and analysis

Two data simulation studies were conducted to address aspects of Bayesian gene statistic estimation that surfaced in the results of our analyses of the empirical data sets. Simulation study 1 was designed to investigate divergent allozyme F_{ST} estimates for standard and Bayesian methods, while simulation study 2 addressed the effects of sample size on accurate *f* estimation with dominant data.

Simulation study 1

The program EASYPOP v1.7 (Balloux, 2001), a forward-time simulator employing an individual based model of evolution, was used to produce 110 codominant data sets.

Evolution was simulated at 15 multiallelic loci (i.e., with five alleles each) for four populations of 100 diploid individuals each and 20 individuals per population were randomly subsampled for analysis. There was no migration, mutation, or selfing, and mating was random. Simulation durations (i.e., number of generations) were varied to produce different final F_{ST} values. Each of the data sets was analyzed using a standard approach in the program POPGENE v1.32 (Yeh and Boyle, 1997; Yeh et al., 1997) and the Bayesian full model from HICKORY. The differences between the F_{ST}/G_{ST} estimates produced by these methods were plotted against the simulated F_{ST}.

Simulation study 2

Using the program EASYPOP v1.7 (Balloux, 2001), we simulated five data sets at each of five F_{ST} value ranges (0.01-0.1, 0.275-0.325, 0.4-0.45, 0.55-0.6, 0.8-0.85) for four different sample sizes (20, 50, 100, and 200 individuals). Data sets consisted of 10 populations of 500 individuals each. Each population was randomly subsampled to produce the sample sizes listed above. Individuals' genotypes comprised 100 biallelic loci, which were converted to dominant data. Simulated F_{IS} values ranged between 0.13 and 0.15. Evolution input parameters for simulations included: no migration, no mutation, and a 0.25 selfing value. Simulated data sets were analyzed in HICKORY under the full model.

Results

F_{ST} and G_{ST}

Our estimates of F_{ST} differed according to analytical method (Fig. 5.1). The standard method (**A_S**; see Table 5.1 for designation of methods**)** estimate for allozymes (0.388 ± 0.22) exceeded all three of the Bayesian θ^{II} values, including that produced (0.172 ± 0.017) for the same allozyme data set (**A_B**). Different model analyses of ISSR data in HICKORY produced significantly different results for θ^{II} (**I_B**, 0.165 ± 0.003; **I_{Bf},** 0.147 ± 0.007), though neither could be distinguished statistically from the **A_B** estimate. The AMOVA-based ϕ_{ST} estimate (**I_S**, 0.137 ± 0.151) was statistically different from the **I_B** estimate, but not from that of **I_{Bf}**.

Only one of the listed methods (**I_{Bf}**) produced statistically overlapping estimates of G_{ST} and F_{ST}, and comparisons among G_{ST} estimates gave slightly different results than those with F_{ST} (Fig. 5.1). Most notable among these results, the G_{ST}^{B} estimate for **A_B** (0.258 ± 0.018) was not found to be different from the **A_S** estimate reported above. The **I_{Bf}** (0.138 ± 0.008) and **I_B** (0.157 ± 0.003) methods produced significantly different estimates of G_{ST}^{B} and each differed from the **A_B** estimate.

The effect of sample size differences between data sets was investigated by altering, either through the removal of whole populations or individuals within populations, the sample design of the full data analysis and recalculating F_{ST} and θ^{II}

values. The removal of populations 17 and 1869, each with an ISSR sample size of three, produced estimates for $\mathbf{A_S}$, $\mathbf{A_B}$, and $\mathbf{I_{Bf}}$ that were not significantly different from the corresponding values in the full data analysis. Reducing population sample sizes in the allozyme data set did not significantly change the $\mathbf{A_S}$ F_{ST} estimate (0.439), but did lower the $\mathbf{A_B}$ θ^{II} estimate (0.129) as compared to the full data analysis.

In simulation study 1, we found a strong positive linear relationship (Fig. 5.2) between the difference in F_{ST} estimates and the simulated F_{ST}. We observe the same positive relationship for Bayesian $G_{ST}{}^{B}$ estimates (Fig. 5.3), though the increase in the difference between estimates over the span of F_{ST} values is of a much smaller magnitude.

F_{IS}

Mean estimates of inbreeding based on allozymes ($\mathbf{A_S}$, 0.317 ± 0.339; $\mathbf{A_B}$, 0.28 ± 0.037) are not statistically distinguishable (Fig. 5.4). The ISSR-based Bayesian estimate ($\mathbf{I_B}$, 0.999 ± 0.0009) produced by the full model analysis is extremely high and is not corroborated by any other biological data.

From simulation study 2, we report the average difference between *f* and the simulated F_{IS} for each F_{ST} value and sample size (Fig. 5.5). We found that increasing sample size up to 200 individuals per population had little effect on the accuracy of *f*

estimation. While changing F_{ST} values did affect the relationship of simulated and estimated F_{IS} values, it, likewise, did not result in a change in the accuracy of the Bayesian *f* estimate.

Expected heterozygosity

Average expected panmictic heterozygosity (H_S) estimates differ between model runs (**I_B**, 0.173 ± 0.0008; **I_{Bf}**, 0.147 ± 0.007) conducted on ISSR data (Fig. 5.6). Both of these values also differ from the **A_B** estimate (0.228 ± 0.007). The large degree of error surrounding the **A_S** estimate (0.194 ± 0.175) does not allow it to be distinguished from the other estimates, despite, in some cases, large differences in mean value. This is also the issue when considering individual population H_{iS} estimates (Fig. 5.7), where the **A_S** estimate is almost never statistically different from the **A_B** value, except in the case of populations 6 and 1883. Comparisons made among HICKORY-based heterozygosity estimates for individual populations show consistently that there are differences between model runs and data sets. The Bayesian total pooled expected heterozygosity (H_T) estimates differed between models and data sets (**I_B**, 0.2047 ± 0.001; **I_{Bf}**, 0.1707 ± 0.009; **A_B**, 0.307 ± 0.008; Fig. 5.8). While the **A_S** estimate of H_T (0.316 ± 0.229) is most similar to that of the **A_B** method, it cannot be distinguished from any of the three Bayesian H_T estimates.

Discussion

The increasing use of recently developed molecular genetic markers and their concomitant statistical analyses necessitates an improved understanding of the comparability of genetic estimates across various methodological approaches. Such an understanding is especially important given the wealth of genetic and life history information that is restricted to interpretations of allozyme data sets, now that allozymes are being increasingly supplanted by AAD and other markers. However, despite a number of comparative reviews of molecular markers (e.g., Nybom & Bartish, 2000; Nybom, 2004; Coates & Byrne, 2005), there are few (e.g., Virk et al., 2000) that conduct exhaustive comparisons of alternative marker data sets produced from identical populations and under similar analytical conditions. Even more rare are studies in which both different markers and different methods of analysis have been compared (Holsinger & Wallace, 2004). In order to contribute to the overall understanding of relationships among analogous gene statistic estimates, we conducted a narrowly focused, comparative study of a set of gene statistics calculated in three different analytical environments from empirically derived dominant and codominant data. The study shows that estimates differ between analytical method and marker type, though not always in the manner suggested by the literature.

Consider first the confidence with which estimates of population differentiation obtained by different markers and analyses can be compared. The apportionment of genetic diversity within and among populations is one of the most important measures

for biologists because it is broadly informative. For example, it is often employed in conservation strategies aimed at preserving maximum genetic diversity in a species, specifically as an aid to identifying genetically distinct (e.g., highly differentiated) elements within a species. Estimates of genetic differentiation among populations based on allozymes are available for a multitude of taxa, and the level and pattern of differentiation are often associated with a variety of life history attributes, especially breeding system (Hamrick & Godt, 1989). However, the lack of variation at allozyme loci, particularly in rare species, may preclude calculations of genetic differentiation. In these cases it would be advantageous to determine whether estimates of differentiation from AADs are comparable to estimates from allozymes for species with similar life history and ecological attributes.

F_{ST} and G_{ST}

The F_{ST} statistic estimates the level of population differentiation based on the degree of fixation of alleles among populations, and is subject to the effects of selection, drift, and mutation. The influence of these processes is an important consideration in comparing statistical estimates produced by different molecular markers. The expected differences in the detectable rate of mutation and level of selective control operating at allozyme and AAD loci may contribute to divergence in F_{ST} estimates between these marker classes. In fact, under a broad range of conditions, mutation rate appears to be the prime factor in determining the degree of genetic differentiation

among populations, with higher mutation rates corresponding to lower F_{ST} values (Hedrick, 1999; Fu et al., 2003; Holsinger & Wallace, 2004). Despite the presumably higher mutation rates in AAD markers than in allozyme markers, we did not find that the ISSR data set produced a comparably lower θ^{II} estimate than the allozymes (Fig. 5.1). Although not consistent with the expected result, this finding does not appear to be uncommon in genetic studies employing both allozymes and AAD markers and may reflect the influence of other evolutionary processes (e.g., natural selection or genetic drift; Zeng et al., 2003; Volis et al., 2005).

However, when we consider estimates of $G_{ST}{}^{B}$, we find that the amount of genetic differentiation in the ISSR data set is significantly lower than it is in the allozyme data set (Fig. 1). A similar relationship is revealed when comparing the $\mathbf{A_S}$ estimate to the $\mathbf{I_{Bf}}$ or $\mathbf{I_B}$ θ^{II} estimates, and both of these findings reflect the expected pattern of genetic differentiation under the assumption that a differential mutation rate between marker types is a strong determining force. In addition, the non-significant difference between allozyme and ISSR θ^{II} estimates, which contradicts the expected result, seems to be more a product of the Bayesian method employed, than an indication of biological reality. This explanation is supported by the results of our simulated data study, which demonstrate that as "true" F_{ST} values become larger in a codominant data set HICKORY will underestimate levels of genetic differentiation to an increasingly greater degree (Figs. 5.2 and 5.3). We are unsure of the reason for the underestimation by θ^{II}, but suggest that it may be caused by the random-effects

sampling model implemented in HICKORY. Random-effects estimates of θ^{II} are calculated from Bayesian estimates of allele frequency in all potentially sampled populations (Holsinger, 1999). In a standard analysis of variance approach, the random-effect model is expected to produce smaller test statistics relative to the fixed-effect model by increasing mean square values (Weir, 1996, p. 162). This expectation is supported by the findings of simulated data study 1, which generally produced larger estimates of G_{ST}^{B} (i.e., fixed-effect estimate) when compared to θ^{II} (Figs. 5.2 and 5.3).

Statistically significant differences observed between full and f-free model ISSR-based estimates of θ^{II} and G_{ST}^{B} may be due to the confounding effects of erroneous f estimates under the full model (Holsinger & Lewis, 2003). However, despite statistical significance the respective mean estimates of these models do not differ substantially. In fact, the statistically significant finding is almost definitely due to the large number of ISSR loci used in these analyses, illustrating the occasional discordance between statistical and biological significance (Hedrick, 1999).

Our manipulation of population and individual sampling produced only slight, and mostly nonsignificant, changes in F_{ST} estimates for both allozymes and ISSRs, and in no circumstance did it change relationships among estimates. Given this, we conclude that differences in sample size between data sets seem to have played little

role in producing the divergent estimates of F_{ST} in allozymes and ISSRs that were observed.

While our results for the *Tolpis* data sets cannot be viewed as generally applicable guidelines for estimating population differentiation, several comments are warranted. The most significant finding is the difference between Bayesian and standard estimates of F_{ST} for codominant markers. While the underlying statistical affinities of these procedures do differ, it appears more likely that the influence of some component(s) of the HICKORY implementation (e.g., the random-effects sampling design) is primarily responsible for the significant differentiation between these estimates. Alternatively, the close approximation of the Bayesian G_{ST}^{B} estimate to the standard F_{ST} (Fig. 5.3), both of which are based on the unbiased G_{ST} statistic of Nei and Chesser's (1983), suggests that these are much more comparable values. Thus we caution against comparing allozyme-based F_{ST} values estimated from a standard method to those from the Bayesian procedure implemented in HICKORY. With regard to estimates of population differentiation (θ^{II} and G_{ST}^{B}) from ISSR markers, significantly different, but very similar values were obtained with the full and f-free models. Given the possible confounding effects of erroneous f estimates under the full model (Holsinger & Lewis, 2003), in view of our results, we recommend using the f-free model. Or, if both models are used and substantially different results are obtained, more confidence should be placed in results from the f-free model. Comparison of population differentiation estimates between codominant

and dominant markers is not straightforward because of the aforementioned issues with both the methods and markers. However, given that our simulation study suggests that, with codominant data, $G_{ST}{}^{B}$ is a better approximation of Nei and Chesser's (1983) unbiased F_{ST} than is θ^{II}, it may be more meaningful to use this statistic for comparison with dominant data as well. Finally, considering our results as well as those of a more extensive review conducted by Bonin et al. (2007), we find that the AMOVA-based ϕ_{ST} estimate, a commonly used approach for dominant data, is generally quite similar to the θ^{II} value estimated under the f-free model of HICKORY. Nevertheless, we believe it is prudent, given the limited investigation into the comparability of these statistics and the ease of formatting for their respective analyses, for one to calculate both estimates for dominant data sets and compare only like statistics.

F_{IS}

Our results for inbreeding are quite clear in demonstrating that the co-dominant allozyme markers, whether using standard or Bayesian analyses, are to be preferred over AAD markers. These results are not surprising as Holsinger and Lewis (2003) cautioned that dominant data might not be appropriate for estimating f, at least under the tested Bayesian framework. Though Holsinger and Lewis (2003) did suggest that a large sample size might overcome these issues, our own simulations show no evidence of this. Even at sample sizes of 200 individuals per population, we found

significant differences between estimated f and the expected (i.e., simulated) value (Fig. 5.5). This result suggests that estimating f in HICKORY with dominant data is largely uninformative.

Expected heterozygosity

Although the large confidence intervals for heterozygosity estimates of H_S and H_T using standard analyses of allozymes result in no statistical difference between them and all other analyses, a few comments can be proffered about the results. The Bayesian estimates for allozymes are substantially higher than those for ISSR markers. Nybom and Bartish (2000) and Coates and Byrne (2005) reviewed estimates of diversity from allozyme and AAD (mostly RAPD) markers. Because data in those reviews are from standard analyses of both types of markers and there is variation in the method used to produce their estimates, such as whether to include monomorphic loci (excluded in our study), they are of limited value for our purposes. However, with these caveats in mind, higher diversity estimates with AAD markers appear to be more common than those with allozymes. By contrast, our results suggest higher estimates for allozymes. Neither published data nor results of the present study provide compelling reasons for predicting *a priori* the relative levels of diversity estimates provided by the two markers. Additional studies are needed before a more definitive assessment can be made.

Biological vs. statistical significance

Earlier in the discussion we alluded to the difference between statistical and biological significance (Hedrick, 1999), and several additional comments are in order because the significance issue is important to the interpretation of our analyses. In particular, the relatively small number of loci sampled in the standard allozyme approach resulted in a large degree of error in estimation and made it difficult to find this estimate significantly different from those of other methods, though, in comparison, its mean value was often quite divergent. In contrast, significant differences in estimation between the two Bayesian models (full and f-free) with ISSRs were common, despite very similar mean estimates. These findings can be attributed to two effects, that of the large number of ISSR loci analyzed and the statistical sampling procedure of the Bayesian method, which also greatly decreased error for the analysis of the 10-locus allozyme data set. Certainly, the differences presented between θ^{II} and F_{ST}, given the large error surrounding the mean, may be interpreted as more substantial than those between the two Bayesian models, especially considering that the former influences interpretations of method comparisons much more meaningfully than the latter.

Table 5.1 The five combinations of molecular marker and statistical method considered in this study are shown along with their respective abbreviations. The far right column lists the four methodological comparisons described in this study of gene statistic estimates from the genus *Tolpis*.

Marker	Analysis	Abbreviation	Comparisons
Allozyme	standard	A_S	A_S x A_B
Allozyme	Bayesian full model	A_B	A_B x (I_{Bf}/I_B)
ISSR	Bayesian *f*-free model	I_{Bf}	I_{Bf} x I_B
ISSR	Bayesian full model	I_B	(I_{Bf}/I_B) x I_S
ISSR	AMOVA	I_S	

Table 5.2 Population sampling information detailing *Tolpis* taxonomic affinities, sample size for the respective markers, and geographic origin within the Canarian Archipelago.

Species	Populations	*n* (allozyme)	*n* (ISSR)	Island
T. sp. nov. 1	4	18	7	La Palma
T. webbii	6	6	14	Tenerife
	11	15	8	Tenerife
T. laciniata	14	14	6	La Palma
	17	9	3	La Palma
	1860	14	7	El Hierro
	1869	17	3	El Hierro
	1883	8	8	La Palma
	1893	10	8	La Palma
	1918	16	10	La Gomera
T. lagopoda	1941	20	8	Tenerife
	1949	27	8	Tenerife
T. sp. nov. 3	1975	16	8	Tenerife
	1987	14	8	Tenerife
T. proustii	1863/10	29	9	El Hierro

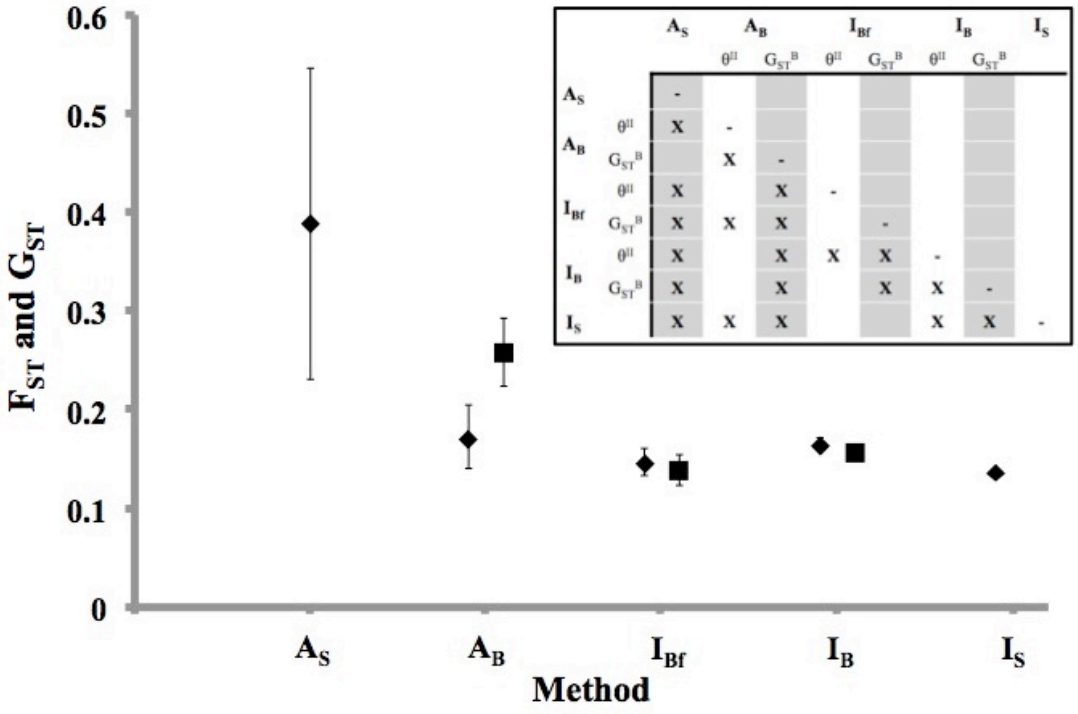

Figure 5.1 Mean values for F_{ST} among *Tolpis* populations, including θ^{II} and ϕ_{ST}, (diamond) and G_{ST}^B (square) estimates across five analysis categories (**A_S**, **A_B**, **I_{Bf}**, **I_B**, and **I_S**). Bars indicate 95% credible/confidence interval. The table in the upper right shows comparisons among statistically different (indicated with an **X**) estimates of genetic differentiation produced by the different methods.

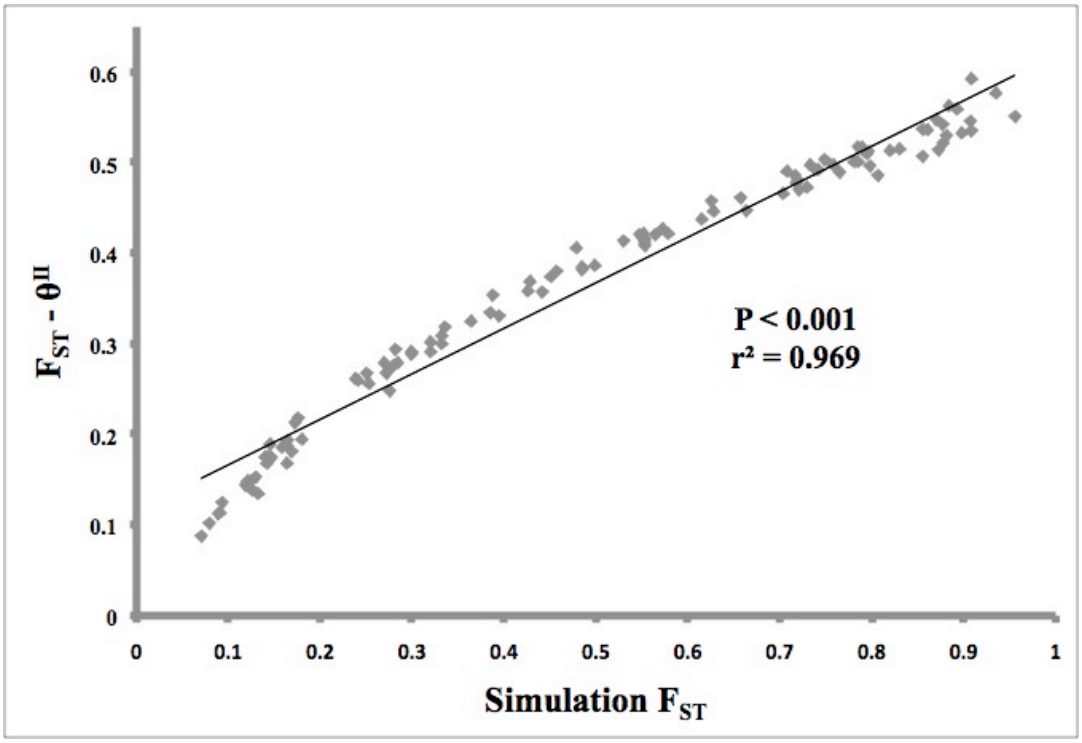

Figure 5.2 Correlation between the difference in standard and Bayesian estimates of F_{ST} and the simulated F_{ST}.

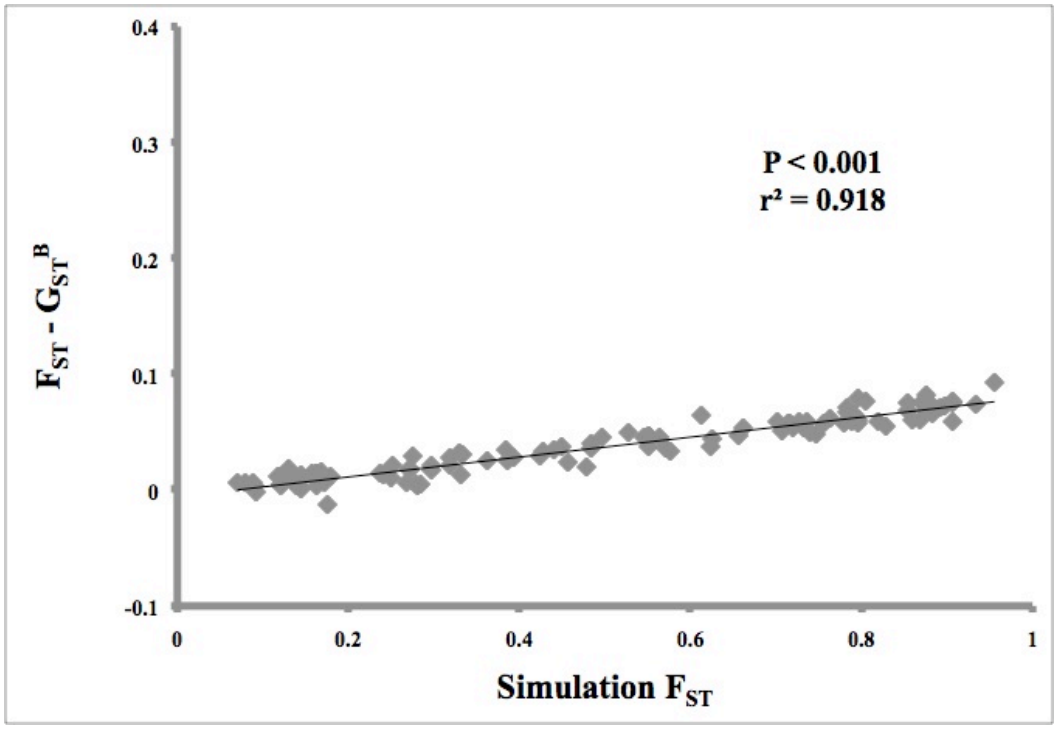

Figure 5.3 Correlation between the difference in standard F_{ST} and Bayesian G_{ST} estimates and the simulated F_{ST}.

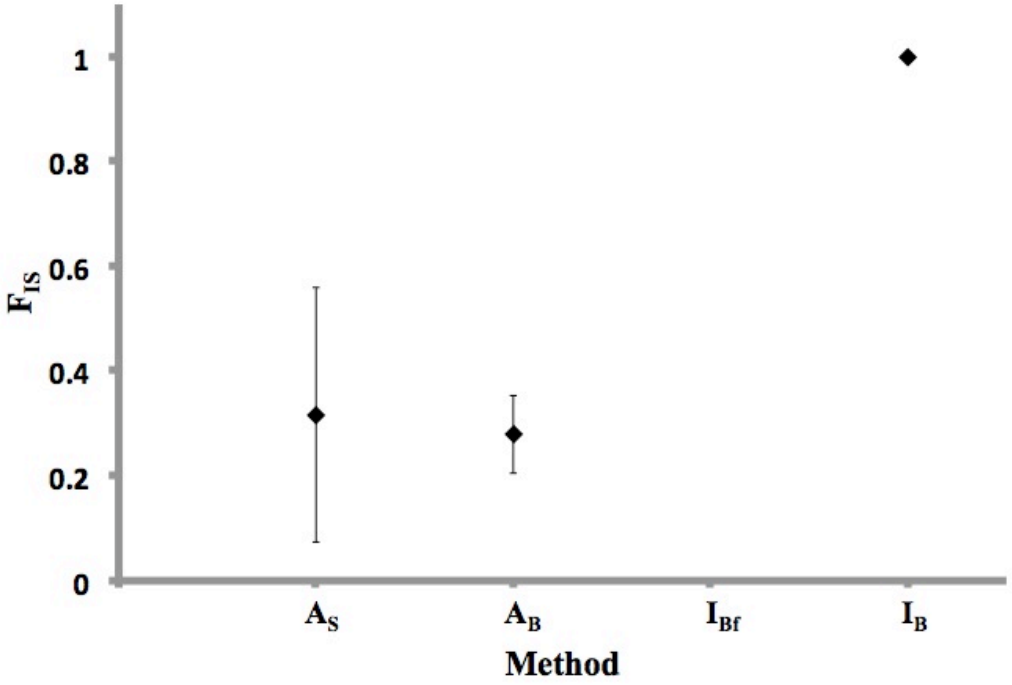

Figure 5.4 Mean values for F_{IS} and f estimates for *Tolpis* populations across three analysis categories (no estimate produced in f-free Bayesian model; $\mathbf{A_S}$, $\mathbf{A_B}$, and $\mathbf{I_B}$). Bars indicate 95% credible/confidence interval.

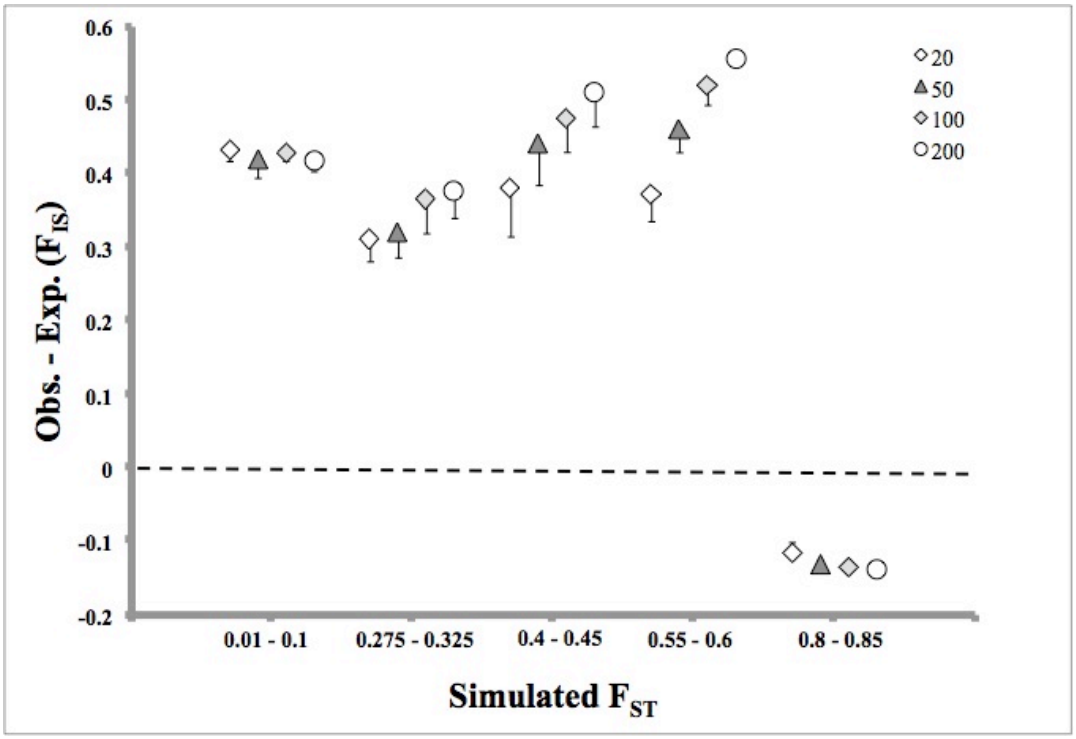

Figure 5.5 Average difference between f estimation and simulated F_{IS} for data sets at four sample sizes (20, 50, 100, and 200 individuals per population). Bars show the negative or positive aspect of the 95% confidence interval. Intervals that do not intersect the zero line (broken) indicate statistically significant differences between the estimated and simulated F_{IS}.

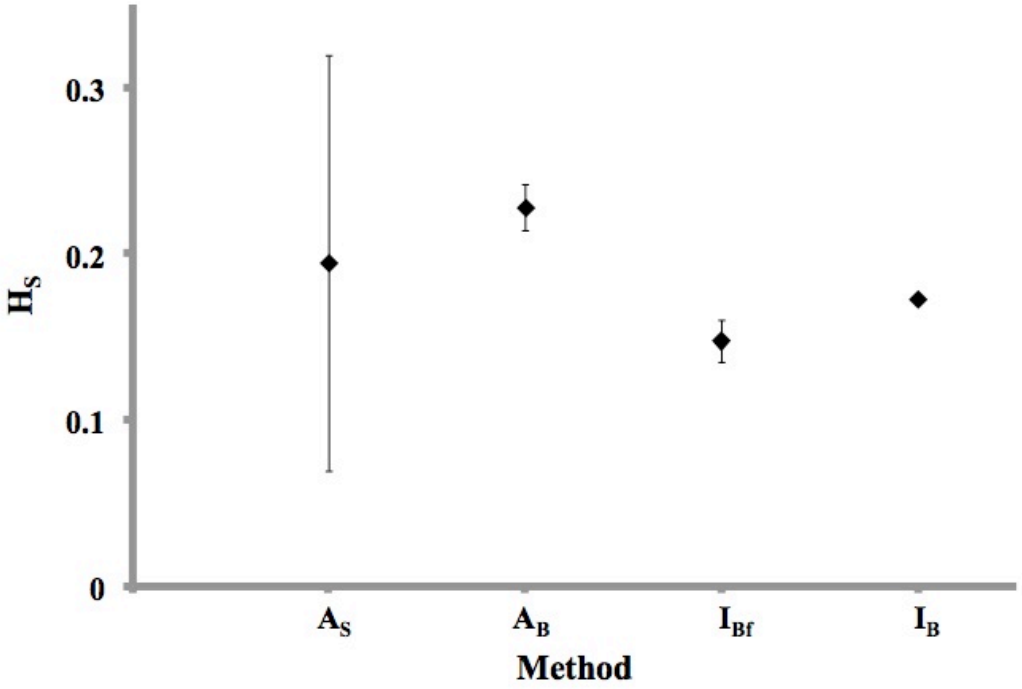

Figure 5.6 Mean values for H_S estimates for *Tolpis* populations across four analysis categories ($\mathbf{A_S}$, $\mathbf{A_B}$, $\mathbf{I_{Bf}}$, and $\mathbf{I_B}$). Bars indicate 95% credible/confidence interval.

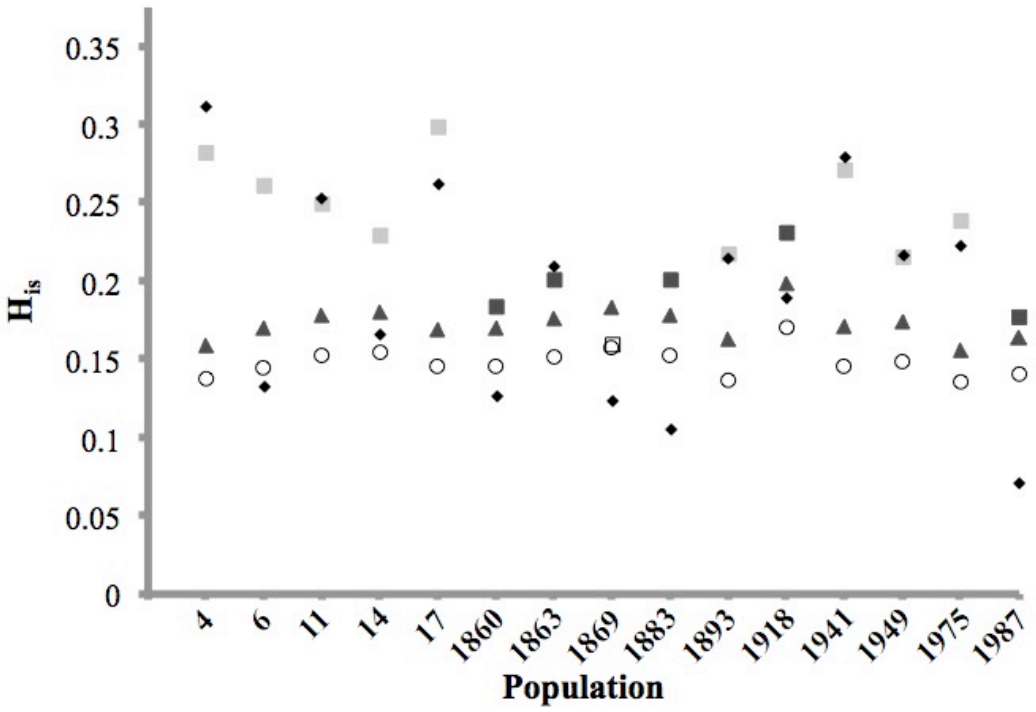

Figure 5.7 Mean values for within population expected panmictic heterozygosity (H_{IS}) estimates for *Tolpis* populations across four analysis categories ($\mathbf{A_S}$ = diamond; $\mathbf{A_B}$ = square; $\mathbf{I_{Bf}}$ = circle; $\mathbf{I_B}$ = triangle). Shared colors indicate a lack of significance among estimates. Standard allozyme ($\mathbf{A_S}$) estimates did not differ statistically from any of the other three estimates in any population other than 6 and 1883.

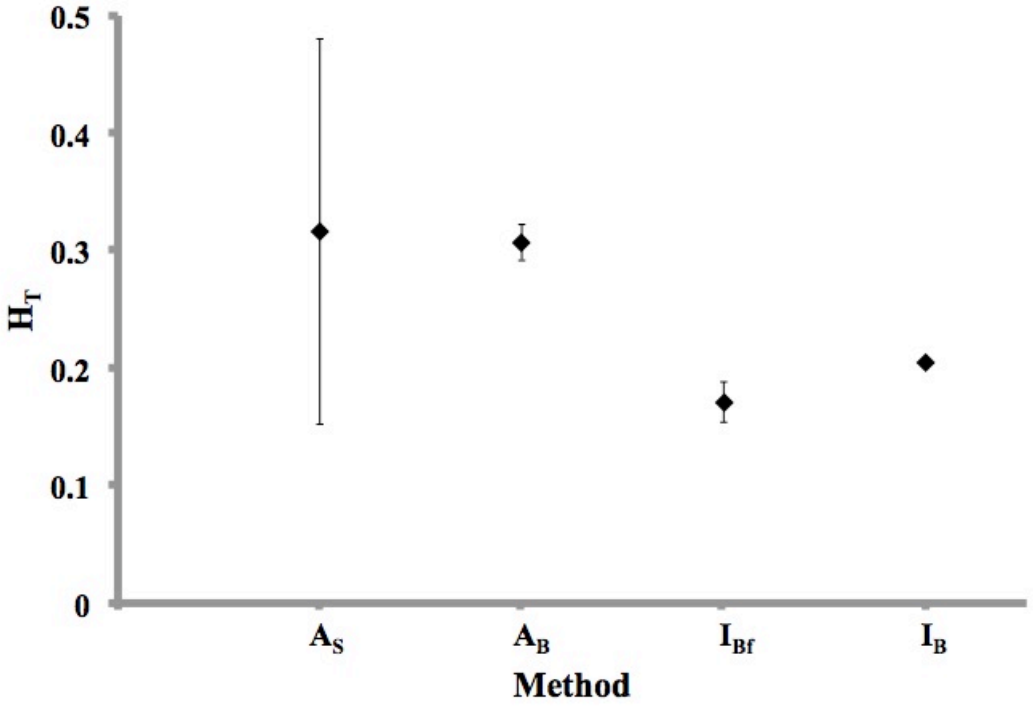

Figure 5.8 Mean values for H_T estimates for *Tolpis* populations across four analysis categories ($\mathbf{A_S}$, $\mathbf{A_B}$, $\mathbf{I_{Bf}}$, and $\mathbf{I_B}$). Bars indicate 95% credible/confidence interval.

LIST OF REFERENCES:

Abbott, R., & Brochmann, C. (2003). History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology , 12*, 299-313.

Abbott, R., & Comes, H. (2004). Evolution in the Arctic: a phylogeographic analysis of the circumarctic plant, *Saxifraga oppositifolia* (Purple saxifrage). *New Phytologist , 161*, 211-224.

Abbott, R., Chapman, H., Crawford, R., & Forbes, D. (1995). Molecular diversity and derivations of populations of *Silene acaulis* and *Saxifraga oppositifolia* from the high Arctic and more southerly latitudes. *Molecular Ecology , 4*, 199-207.

Abbott, R., Smith, L., Milne, R., Crawford, R., Wolff, K., & Balfour, J. (2000). Molecular analysis of plant migration and refugia in the arctic. *Science , 289*, 1343-1346.

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports , 27*, 617-631.

Al, C.-X., Zhang, L.-S., Wei, H.-R., Jin, S.-N., Yuan, K.-J., & Liu, Q.-Z. (2007). Study on the genetic diversity of natural chestnut of Shandong by ISSR. *Chinese Journal of Biotechnology , 23*, 628-633.

Alsos, I., Engelskjon, T., Gielly, L., Taberlet, P., & Brochmann, C. (2005). Impact of ice ages on circumpolar molecular diversity: insights from an ecological key species. . *Molecular Ecology , 14*, 2739-2753.

Anderson, E., & Thompson, E. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics , 160*, 1217-1229.

Anderson, L., Hu, F., Nelson, D., Petit, R., & Paige, K. (2006). Ice-age endurance: DNA evidence of a white spruce refugium in Alaska. *Proceedings of the National Academy of Sciences of the United States of America , 103*, 12447-12450.

Anderson, M. (2004). *PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests.* New Zealand: Department of Statistics, University of Aukland.

Archibald, J., Crawford, D., Santos-Guerra, A., & Mort, M. (2006). The utility of automated analysis of inter-simple sequence repeat (ISSR) loci for resolving relationships in the Canary Island species of *Tolpis* (Asteraceae). *American Journal of Botany , 93*, 1154-1162.

Baker, R., Van Zant, K., & Dulian, J. (1980). Three late glacial pollen and plant macrofossil assemblages from Iowa. *Palynology , 4*, 197-203.

Baldwin, B., Kyhos, D., Dvorak, J., & Carr, G. (1991). Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae).

Proceedings of the National Academy of Sciences of the United States of America , 88, 1840-1843.

Balloux, F. (2001). EASYPOP (Version 1.7): A computer program for population genetics simulation. *The Journal of Heredity , 92*, 301-302.

Balloux, F., & Lugon-Moulin, N. (2002). The estimation of population differentiation with microsatellite markers. *Molecular Ecology , 11*, 155-165.

Balloux, F., Lehmann, L., & de Meeus, T. (2003). The population genetics of clonal and partially clonal diploids. *Genetics , 164*, 1635-1644.

Bauert, M., Kaelin, M., Baltisberger, M., & Edwards, P. (1998). No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps uisng RAPD markers. *Molecular Ecology , 7*, 1519-1527.

Bell, K., & Bliss, L. (1980). Plant reproduction in a high arctic environment. *Arctic and Alpine Research , 12*, 1-10.

Bengtsson, G., Hedlund, K., & Rundgren, S. (1994). Food- and density-dependent dispersal: evidence from a soil Collembolan. *The Journal of Animal Ecology , 63*, 513-520.

Berg, L., & Lascoux, M. (2000). Neutral genetic differentiation in an island model with cyclical parthenogenesis. *Journal of Evolutionary Biology , 13*, 488-494.

Billings, W. (1987). Constraints to plant growth, reproduction, and establishment in Arctic environments. *Arctic and Alpine Research , 19*, 357-365.

Billings, W., & Mooney, H. (1968). The ecology of arctic and alpine plants. *Biological Reviews of the Cambridge Philosophical Society , 43*, 481-529.

Bliss, L. (1971). Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics , 2*, 405-438.

Bliss, L., Courtin, G., Pattie, D. R., & Whitfield, D. W. (1973). Arctic tundra ecosystems. *Annual Review of Ecology and Systematics , 4*, 359-399.

Blytt, A. (1876). *Essay on the immigration of the Norwegian Flora during alternating rainy and dry periods.* Christiana: Cammermeyer.

Bocher, T. (1951). Distributions of plants in the circumpolar area in relation to ecological and historical factors. *The Journal of Ecology , 39*, 376-395.

Bohm, B., & Wilkins, C. (1978). Chemosystematic studies in the Saxifragaceae s.l., 8. The flavanoids of *Elmmera racemosa* (Watson) Rydberg. *Brittonia , 30*, 327-333.

Bohm, B., & Collins, F. (1979). Flavonoids of some species of *Chrysosplenium*. *Biochemical Systematics and Ecology , 7*, 195-201.

Bohm, B., Collins, F., & Bose, R. (1977). Flavonoids of *Chrysosplenium tetrandrum*. *Phytochemistry , 16*, 1205-1209.

Bonin, A., Bellemain, E., Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology , 13*, 3261-3273.

Bonin, A., Ehrich, D., & Manel, S. (2007). Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology , 16*, 3737-3758.

Bonnet, E., & Van de Peer, Y. (2002). zt - a software tool for simple and partial Mantel tests. *Journal of Statistical Software , 7*, 1-12.

Brochmann, C., Brysting, A., Alsos, I., Borgen, L., Grundt, H., Scheen, A., et al. (2004). Polyploidy in arctic plants. *Biological Journal of the Linnean Society , 82*, 521-536.

Brubaker, L., Anderson, P., Edwards, M., & Lozhkin, A. (2005). Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. *Journal of Biogeography , 32*, 833-848.

Brunsfeld, S., & Sullivan, J. (2005). A multi-compartmented glacial refugium in the northern Rocky Mountains: evidence from the phylogeography of *Cardamine constancei* (Brassicaceae). *Conservation Genetics , 6*, 895-904.

Chase, M., Soltis, D., Olmstead, R., Morgan, D., Les, D., Mishler, B., et al. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden , 80*, 528-580.

Chung, M., Gelembiuk, G., & Givnish, T. (2004). Population genetics and phylogeography of endangered *Oxytropis campestris* var. *chartacea*. *Molecular Ecology , 13*, 3657-3673.

Clegg, M. (1989). Molecular diversity in plant populations. In A. Brown, M. Clegg, A. Kahler, & B. Weir, *Plant populations genetics: breeding and genetic resources.* Sunderland, MA: Sinauer.

Coates, D., & Byrne, M. (2005). Genetic variation in plant populations: assessing cause and pattern. In R. Henry, *Plant diversity and evolution: genotypic and phenotypic variation in higher plants.* Cambridge, MA: CABI Publishing.

Colinvaux, P. (1967). Quaternary vegetational history of arctic Alaska. . In D. Hopkins, *The Bering Land Bridge.* Stanford: The Stanford University Press.

Comes, H., & Kadereit, W. (1998). The effect of Quaternary climatic changes on plant distributions and evolution. *Trends in Plant Sciences , 3*, 432-438.

Cowling, R., & Heijnis, C. (2001). The identification of broad habitat units as biodiversity entities for systematic conservation planning in the Cape Floristic Region. *South African Journal of Botany , 67*, 15-38.

Crandall, K., & Templeton, A. (1993). Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics , 134*, 959-969.

Crawford, D., & Mort, M. (2004). Single-locus molecular markers for inferring relationships at lower taxonomic levels: observations and comments. *Taxon , 53*, 631-635.

Crawford, D., Archibald, J., Santos-Guerra, A., & Mort, M. (2006). Allozyme diversity within and divergence among species of *Tolpis* (Asteraceae-Lactuceae) in the Canary Islands: systematic, evolutionary, and biogeographical implications. *American Journal of Botany , 93*, 656-664.

Crawford, D., Archibald, J., Stoermer, D., Mort, M., Kelly, J., & Santos-Guerra, A. (2008). A test of Baker's law: breeding systems and the radiation of *Tolpis* (Asteraceae) in the Canary Islands. *International Journal of Plant Sciences , 169*, 782-791.

Crawford, D., Stuessy, T., Cosner, M., Haines, D., Wiens, D., & Penailillo, P. (1994). *Lactoris fernandeziana* (Lactoridaceae) on the Juan Fernández Islands: allozyme uniformity and field observations. *Conservation Biology , 8*, 277-280.

Crawford, D., Stuessy, TF, & Silva, M. (1987). Allozyme divergence and the evolution of Dendroseris (Compositae: Lactuceae) on the Juan Fernández Islands. *Systematic Botany , 12*, 435-443.

Crawford, D., Tago-Nakazawa, M., Stuessy, T., Anderson, G., Bernardello, G., Ruiz, E., et al. (2001). Intersimple sequence repeat (ISSR) variation in Lactoris

fernandeziana (Lactoridaceae), a rare endemic of the Juan Fernández Archipelago, Chile. . *Plant Species Biology , 16*, 185-192.

Cruzan, M. (1998). Genetic markers in plant evolutionary ecology. *Ecology , 79*, 400-412.

Cruzan, M., & Templeton, A. (2000). Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. *Trends in Ecology and Evolution , 15*, 491-496.

Dahl, E. (1946). On different types of unglaciated areas during the Ice Ages and their significance to phytogeography. *New Phytologist , 45*, 225-242.

Darwin, C. (1859). *The origin of species.* London: John Murray.

Dice, L. (1945). Measures of the amount of ecological association between species. *Journal of Ecology , 26*, 297-302.

Downes, J. (1965). Adaptations of insects in the arctic. *Annual Review of Entomology , 10*, 257-274.

Eidesen, P., Carlsen, T., Molau, U., & Brochmann, C. (2007). Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. *Journal of Biogeography , 34*, 1559-1574.

Ellstrand, N., & Elam, D. (n.d.). Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* .

Elvebakk, A., Elven, R., & Razzhivin, V. (1999). Delimitation, zonal and sectorial subdivision of the Arctic for the Panarctic Flora Project. In I. Nordal, & V. Razzhivin, *The species concept in the High Arctic-A Panarctic Flora Initiative.* Olso: The Norwegian Academy of Science and Letters.

Emerson, B. (2002). Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology , 11* (6), 951-966.

Epperson, B. (2003). *Geographical genetics.* Princeton, NJ: Princeton University Press.

Ersts, P. (2008). *Geographic Distance Matrix Generator (version 1.2.2).* (C. f. American Museum of Natural History, Producer) Retrieved 2008, from http://biodiversityinformatics.amnh.org/open_source/gdmg

Ewens, W. (1982). On the concept of the effective population size. *Theoretical Population Biology , 21*, 373-378.

Excoffier, L., Laval, L., & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online , 1*, 47-50.

Excoffier, L., Smouse, P., & Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes:application to human mitochondrial DNA restriction data. *Genetics , 131*, 479-491.

Gray, A. (1950). *Gray's Manual of Botany.* Revised by Fernald, M. New York: van Nostrand Co.

Fernald, M. (1925). Persistence of plants in unglaciated areas of boreal America. *Memoirs of the American Academy of Arts and Sciences , 15*, 238-342.

Fishbein, M., & Soltis, D. (2004). Further resolution of the rapid radiation of Saxifragales (Angiosperms, eudicots) supported by mixed-model Bayesian analysis. *Systematic Botany , 29*, 883-891.

Fishbein, M., Hibsch-Jetter, C., Soltis, D., & Hufford, L. (2001). Phylogeny of Saxifragales (angiosperms: eudicots): analysis of rapid, ancient radiation. *Systematic Biology , 50*, 817-847.

Franchet, A. Monographie du genere Chrysosplenium Tournfort. *Nouvelles Archives du Muséum d'histoire naturelle publiées par les professeurs-administrateurs de cet établissement. Troisiéme Série., (Paris) iii. 2*, 87-114.

Fu, R., Gelfand, A., & Holsinger, K. (2003). Exact moment calculations for genetic models with migration, mutations, and drift. *Theoretical Population Biology , 63*, 231-243.

Gabrielsen, T., & Brochmann, C. (1998). Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. *Molecular Ecology , 7*, 1701-1708.

Goldblatt, P., & Manning, J. (2002). Plant diversity of the Cape Region of southern Africa. *Annals of the Missouri Botanical Garden , 89*, 281-302.

Gottlieb, L. (1982). Conservation and duplication of isozymes in plants. *Science , 216*, 373-380.

Grundmann, M., Ansell, S., Russel, S., Koch, M., & Vogel, J. (2007). Genetic structure of the widespread and common Mediteranean bryophyte *Pleurochaete squarrosa* (Brid.) Lindb. (Pottiaceae) - evidence from nuclear and plastidic DNA sequence variation and allozymes. *Molecular Ecology , 16*, 709-722.

Grundt, H., Kjølner, S., Borgen, L., Reiseberg, L., & Brochmann, L. (2006). High biological species diversity in the arctic flora. *Proceedings of the National Academy of the United States of America , 103*, 972-975.

Hall, P., Orrell, L., & Bawa, K. (1994). Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). *American Journal of Botany , 81*, 1104-1111.

Hamrick, J. (1989). Isozymes and the analysis of genetic structure in plant populations. In D. Soltis, & P. Soltis, *Isozymes in Plant Biology.* Portland, Oregon: Dioscorides Press.

Hamrick, J., & Godt, M. (1989). Allozyme diversity in plant species. In A. Brown, M. Clegg, A. Kahler, & B. Weir, *Plant population genetics: breeding and genetic resources.* Sunderland, MA: Sinauer.

Hansell, R., Malcolm, J., Welch, H., Jefferies, R., & Scott, P. (1998). Atmospheric change and biodiversity in the Arctic. *Environmental Monitoring and Assessment , 49*, 303-325.

Hara, H. (1957). Synopsis of the genus *Chrysosplenium* L. *Journal of the Faculty of Science University of Tokyo Section III Botany , 7*, 1-90.

Harris, H. (1966). Enzyme polymorphisms in man. *Proceedings of the Royal Society of London Series B, Biological Sciences , 164*, 298-310.

Hartley, T. (1966). The flora of the "Driftless Area". *The University of Iowa studies in natural history , 21*, 87-127.

Hedges, T. (1972). Expanded joints and other periglacial phenomena along the Niagara Escarpment. . *Biuletyn Peryglacjalny , 21*, 87-127.

Hedrick, P. (1999). Perspective: highly variable loci and their implications in evolution and conservation. *Evolution , 53*, 313-318.

Heusser, C. (1960). *Late-Pleistocene environments of North Pacific North America.* New York: American Geographical Society.

Hewitt, G. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society , 359*, 183-195.

Hewitt, G. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society , 58*, 247-276.

Hewitt, G. (2001). Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology , 10*, 537-549.

Holsinger, K. (1999). Analysis of genetic diversity in geographically structured populations: a Bayesian perspective. *Hereditas , 130*, 245-255.

Holsinger, K., & Lewis, P. (2003). Retrieved from Hickory v0.8: http://darwin.eeb.uconn.edu/hickory/hickory.html

Holsinger, K., & Wallace, L. (2004). Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). *Molecular Ecology , 13*, 887-894.

Holsinger, K., Lewis, P., & Dey, D. (2002). A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology , 13*, 887-894.

Hoot, S., Magallon, S., & Crane, P. (1999). Phylogeny of basal eudicots based on three molecular data sets: *atpB*, *rbcL*, and 18S nuclear ribosomal DNA sequences. *Annals of the Missouri Botanical Garden , 86*, 1-32.

Hopkins, D. (1967). *The Bering Land Bridge.* Stanford, CA: Stanford University Press.

Huang, J., & Sun, M. (2000). Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics , 100*, 1050-1060.

Hubby, J., & Lewontin, R. (1966). A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics , 54*, 577-594.

Hulten, E. (1937). *Outline of the history of Arctic and Boreal Biota during the Quaternary period.* New York: Lehre J Cramer.

Hunter, R., & Markert, C. (1957). Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science , 125*, 1294-1295.

Ibrahim, K., Nicholas, R., & Hewitt, G. (1996). Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity , 77*, 282-291.

Jain, S. (1976). The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics , 7*, 469-495.

Jian, S., Soltis, P., Gitzendanner, M., Moore, M., Li, R., Hendry, T., et al. (2008). Resolving an ancient, rapdi radiation in Saxifragales. *Systematic Biology , 57*, 38-57.

Johnson, L., & Soltis, D. (1994). matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany , 19*, 143-156.

Johnson, L., & Soltis, D. (1994). matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany , 19*, 143-156.

Judd, W., Campbell, C., Kellogg, E., Stevens, P., & Donoghue, M. *Plant Systematics: a phylogenetic approach.* Sunderland, MA: Sinauer .

Karlstrom, T., & Ball, G. (1969). *The Kodiak Island Refugium: its geology, flora, fauna and history*. Toronto: The Ryerson Press.

Krauss, S. (2000). Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology , 9*, 1241-1245.

Lande, R. (1980). Genetic variation and phenotypic evolution during allopatric speciation. *The American Naturalist , 116*, 463-479.

Leberg, P. (1992). Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution , 46*, 477-494.

Lewontin, R., & Hubby, J. (1966). A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics , 54*, 595-609.

Lindroth, C. (1969). The biological importance of Pleistocene refugia. In T. Karlstrom, & G. Ball, *The Kodiak Island Refugium: its geology, flora, fauna and history.* Toronto: The Ryerson Press.

Linhart, Y., & Grant, M. (1996). Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics , 27*, 237-277.

Loehr, J., Worley, K., Grapputo, A., Carey, J., Veitch, A., & Coltman, D. (2005). Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *Journal of Evolutionary Biology , 19*, 419-430.

Losos, J., Jackman, T., Larson, A., de Queiroz, K., & Rodriguez-Schettino, L. (1998). Contingency and determinism in replicated adaptive radiations of island lizards. *Science , 279*, 2115-2118.

Loveless, M., & Hamrick, J. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics , 15*, 65-95.

Lynch, M., & Milligan, B. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology , 3*, 91-99.

MacArthur RH, W. E. (1967). *The theory of island bigeography* . Princeton, NJ: Princeton University Press.

Magallon, S., Crane, P., & Herendeen, P. (1999). Phylogenetic pattern, diversity and diversification of eudicots. *Annals of the Missouri Botanical Garden , 86*, 297-372.

Marr, K., Allen, G., & Hebda, R. (2008). Refugia in the Cordilleran ice sheet of western North America: chloroplast DNA diversity in the Arctic-alpine plant *Oxyria digyna*. *Journal of Biogeography , 35*, 1323-1334.

Max, K., Mouchaty, S., & Schwaegerle, K. (1999). Allozyme and morphological variation in two subspecies of *Dryas octopetala* (Rosaceae) in Alaska. *American Journal of Botany , 86*, 1637-1644.

McCune, B., & Mefford, M. (1999). *PC-ORD. Multivariate analysis of ecological data.* Gleneden Beach, Oregon: MjM Software Design.

Meudt, H., & Clarke, A. (2007). Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science , 12*, 106-117.

Meusel, H., Jaeger, E., & Weinert, E. (1965). *Vergleichende chorologie der zentraleuropaeischen flora.* Jena: Bd 9. Fischer.

Miller, M. (2005). Alleles In Space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity , 96*, 722-724.

Morgan, D., & Soltis, D. (1993). Phylogenetic relationships among members of Saxifragaceae s.l. based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden , 80*, 631-660.

Müller, K. (2005). SeqState: primer design and sequence statistics for phylogenetic DNA datasets. Application note. *Applied Bioinformatics , 4*, 65-69.

Murray, D. (1987). Breeding systems in the vascular flora of Arctic North America. In K. Urbanska, *Differentiation Patterns in Higher Plants.* New York: Academic Press.

Murray, D. (1995). Causes of arctic plant diversity: origin and evolution. In F. Chapin III, & C. Korner, *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences.* Heidelberg: Springer.

Nakanishi, H. (2002). Splash seed dispersal by raindrops. *Ecological Research , 17*, 663-671.

Nakazawa, M., Wakabayashi, M., Ono, M., & Murata, J. (1997). Molecular phylogenetic analysis of *Chrysosplenium* (Saxifragaceae) in Japan. *Journal of Plant Research , 110*, 265-274.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America , 70*, 3321-3323.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America , 70*, 3321-3323.

Nei, M. (1977). F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics , 41*, 225-233.

Nei, M., & Chesser, R. (1983). Estimation of fixation indices and gene diversities. *Annals of Human Genetics , 47*, 253-259.

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology , 13*, 1143-1155.

Nybom, H., & Bartish, I. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics , 3*, 93-114.

Odasz, A., & Savolainen, O. (1996). Genetic variation in populations of the Arctic perennial *Pedicularis dasyantha* (Scrophulariaceae) on Svalbard, Norway. *American Journal of Botany , 83*, 1379-1385.

Packer, J. (1963). The taxonomy of some North American species of *Chrysosplenium* L., Section Alternifolia Franchet. *Canadian Journal of Botany , 41*, 85-103.

Packer, J., & Vitt, D. (1974). Mountain Park: a plant refugium in the Canadian Rocky Mountains. *Canadian Journal of Botany , 52*, 1393-1409.

Pamilo, P., & Savolainen, O. (1999). Post-glacial colonization, drift, local selection and conservation value of populations: a northern perspective. *Hereditas , 130*, 229-238.

Park, S.-J., Korompal, E., Francisco-Ortega, J., Santos-Guerra, A., & Jansen, R. (2001). Phylogenetic relationships of *Tolpis* (Asteraceae: Lactuceae) based on *ndhF* sequence data. *Plant Systematics and Evolution , 226*, 23-33.

Petit, R., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., et al. (2003). Glacial refugia: hotspots but not melting pots of genetic diversity. *Science , 300*, 1563-1565.

Petit, R., Bialozyt, R., Brewer, S., Cheddadi, R., & Comps, B. (2001). From spatial patterns of genetic diversity to postglacial migration processes in forest trees. In J. Silvertown, & J. Antonovics, *Integrating ecology and evolution in a spatial context:*

14th special symposium of the British Ecological Society. Cambridge: Cambridge University Press.

Pielou, E. (1991). *After the ice age: the return of life to glaciated North America.* Chicago: The University of Chicago Press.

Polunin, N. (1951). The real Arctic: suggestions for its delimitation, subdivision, and characterization. *The Journal of Ecology , 39*, 308-315.

Posada, D., & Crandall, K. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics , 14*, 817-818.

Powell, W., MMachray, G., & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science , 1*, 215-222.

Pusateri, W., Roosa, D., & Farrar, D. (1993). Habitat and distribution of plants special to Iowa's Driftless Area. *Journal of Iowa Academy of Science , 100*, 29-53.

Rambaut, A. (1996). *Se-Al, sequence alignment program v1. d1.* Oxford: University of Oxford.

Reid, C. (1899). *The Origin of the British Flora.* London: Dulau.

Rieseberg, L., & Willis, J. (2007). Plant speciation. *Science , 317*, 910-914.

Ronse Decraene, L., Roels, P., Smets, E., & Backlund, A. (1998). The floral development and floral anatomy of *Chrysosplenium alternifolium*, an unusual member of the Saxifragaceae. *Journal of Plant Research , 111*, 573-580.

Rose, J. (1897). Preliminary revision of the North American species of *Chrysosplenium*. *Botanical Gazette , 23*, 274-277.

Savile, D. (1972). Arctic adaptations in plants. *Monograph No. 6* , 1-81.

Savile, D. (1975). Evolution and biogeography of Saxifragaceae with guidance from their rust parasites. *Annals of the Missouri Botanical Garden , 62*, 354-361.

Savile, D. (1953). Splash-cup dispersal mechanism in *Chrysosplenium* and *Mitella*. *Science , 117*, 250-251.

Shaw, J., Lickey, E., Beck, J., Farmer, S., Liu, W., Miller, J., et al. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany , 92*, 142-166.

Schlüter, P., & Harris, S. (2006). Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes , 6*, 569-572.

Schulman, A. (2007). Molecular markers to assess genetic diversity. *Euphytica , 158*, 313-321.

Schwartz, O. (1985). Lack of protein polymorphism in the endemic relict *Chrysosplenium iowense* (Saxifragaceae). *Canadian Journal of Botany , 63*, 2031-2034.

Sica, M., Gamba, G., Montieri, S., Gaudio, L., & Aceto, S. (2005). ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. *BMC Genetics , 6*.

Silvertown, J. (2001). Plants stand still, but their genes dont't: non-trivial consequences of the obvious. In J. Silvertown, & J. Antonovics, *Integrating Ecology and Evolution in a Spatial Context.* (pp. 3-20). London: Blackwell Science.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science , 236*, 787-792.

Soltis, D., & Soltis, P. (1997). Phylogenetic relationships among Saxifragaceae sensu lato: a comparison of topologies based on 18S and *rbcL* sequences. *American Journal of Botany , 84*, 504-522.

Soltis, D., Gitzendanner, M., Strenge, D., & Soltis, P. (1997). Chloroplast DNA intraspecific phylogeography of plants for the Pacific Northwest. *Plant Systematics and Evolution , 206*, 353-373.

Soltis, D., Hibsch-Jetter, C., Soltis, P., Chase, M., & Farris, J. (1997a). Molecular phylogenetic relationships among angiosperms: an overview based on *rbcL* and 18S rDNA sequences. In K. Watsuki, & P. Raven, *Evolution and diversification of land plants.* Tokyo: Springer.

Soltis, D., Kuzoff, R., Conti, E., Gornall, R., & Ferguson, K. (1996). *matK* and *rbcL* gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. *American Journal of Botany , 83*, 371-382.

Soltis, D., Kuzoff, R., Mort, M., Zanis, M., Fishbein, M., Hufford, L., et al. (2001). Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. *Annals of the Missouri Botanical Garden , 88*, 669-693.

Soltis, D., Soltis, P., Chase, M., Mort, M., Albach, D., Zanis, M., et al. (2000). Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL* and *atpB* sequences. *Botanical Journal of the Linnean Society , 133*, 381-461.

Soltis, D., Soltis, P., Endress, P., & Chase, M. (2005). *Phylogeny and evolution of angiosperms.* Sunderland, MA: Sinauer Associates.

Soltis, D., Soltis, P., Mort, M., Chase, M., Savolainen, V., Hoot, S., et al. (1998). Inferring complex phylogenies using parsimony: an empirical approach using three large DNA data sets for angiosperms. *Systematic Biology , 47*, 32-42.

Soltis, D., Tago-Nakazawa, M., Xiang, Q.-Y., Kawano, S., Murata, J., Wakabayashi, M., et al. (2001). Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on matK sequence data. *American Journal of Botany , 88*, 883-893.

Stebbins, G. (1950). *Variation and Evolution in Plants.* New York: Columbia University PRess.

Stehlik, I. (2003). Resistance or emigration? Response of alpine plants to the ice ages. *Taxon , 52*, 499-510.

Steltzer, H., Hufbauer, R., Welker, J., Casalis, M., Sullivan, P., & Chimner, R. (2008). Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: Implications for a terrestrial feedback to climate change in the High Arctic. *Journal of Geophysical Research , 113*, G03S10.

Stenstrom, A., Jonsdottir, I., & Augner, M. (2002). Genetic and environmental effects on morphology in clonal sedges in the Eurasian arctic. *American Journal of Botany , 89*, 1410-1421.

Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology and Evolution , 15*, 199-203.

Swofford, D. (1998). *PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0.* Sunderland, MA: Sinauer .

Takhtajan, A. (1987). *System of Magnoliophyta.* Leningrad: Academy of Sciences.

The Angiosperm Phylogeny Group. (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society , 141*, 399-436.

Thorne, R. (1992). An updated phylogenetic classification of the flowering plants. *Aliso , 13*, 365-389.

Tiffney, B., & Manchester, S. (2001). The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Science , 162*, S3-S17.

Tremblay, N., & Schoen, D. (1999). Molecular phylogeography of *Dryas integrifolia*: glacial refugia and past recolonization. *Molecular Ecology , 8*, 1187-1198.

Trenhaile, A. (2004). *Geomorphology: a Canadian perspective.* (Second Edition ed.). New York: Oxford University Press.

USDA, NRCS. (2007). Retrieved August 2007, 13, from The PLANTS Database: http://plants.usda.gov

US Polar Research Board. (1998). *Future Directions for the National Science Foundation's Arctic Natural Sciences Program.* Commission on Geosciences, Environment, and Resources . National Research Council.

Varshney, R., Graner, A., & Sorrells, M. (2005). Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology , 23*, 48-55.

Varvio, S.-L., Chakraborty, R., & Nei, M. (1986). Genetic variation in subdivided populations and conservation genetics. *Heredity , 57*, 189-198.

Vekemans, X. (2002). *AFLP-SURV version 1.0*. (Laboratoire de Genetique et Ecologie Vegetale, Universite Libre de Bruxelles) Retrieved from hyyp://ulb.be/sciences/lagev/aflp-surv.html

Virk, P., Zhu, J., Newbury, H., Bryan, G., Jackson, M., & Ford-Lloyd, B. (2000). Effectiveness of different classes of molecular markers for classifying and revealing variation in rice (*Oryza sativa*) germplasm. *Euphytica , 112*, 275-284.

Volis, S., Yakubov, B., Shulgina, I., Ward, D., & Mendlinger, S. (2005). Distinguishing adaptive from nonadaptive genetic differentiation: comparison of QST and FST at two spatial scales. *Heredity , 95*, 466-475.

Walker, D., Raynolds, M., Daniels, F., Einarsson, E., Elvebakk, A., Gould, W., et al. (2005). The circumpolar Arctic vegetation map. *Journal of Vegetation Science , 16*, 267-282.

Wallace, A. (1881). *Island life.* New York: Harper & Bros.

Warming, E. (1909). Saxifragaceae. Morphology and biology. *Meddelelser om Gronland , 7*, 169-236.

Watterson, G. (1984). Allele frequencies after a bottleneck. *Theoretical population Biology , 26*, 387-407.

Weber, R. (1979). Species biology of *Chrysosplenium iowense*. *Masters Thesis* . University of Northern Iowa.

Weber, W. (2003). The Middle Asian Element in the Southern Rocky Mountain Flora of the western United States: a critical biogeographical review. *Journal of Biogeography , 30*, 649-685.

Weeden, N., & Wendel, J. (1989). Genetics of plant isozymes. In D. Soltis, & P. Soltis, *Isozymes in Plant Biology.* Portland, Oregon: Dioscorides Press.

Weider, L., & Hobaek, A. (2000). Phylogeography and arctic biodiversity: a review. *Annales Zoologici Fennici , 37*, 217-231.

Weir, B. (1996). *Genetic Data Analysis II.* Sunderland, MA: Sinauer Associates.

Weir, B., & Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution , 38*, 1358-1370.

Wendel, J., & Weeden, N. (1989). Visualization and interpretation of plant isozymes. In D. Solits, & P. Soltis, *Isozymes in Plant Biology.* Portland, Oregon: Dioscorides Press.

White, T., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In D. Gelfand, J. Sminsky, & T. White, *PCR protocols: a guide to methods and applications.* San Diego: Academic Press.

Whitlock, M., & Barton, N. (1997). The effective size of a subdivided population. *Genetics , 146*, 2427-2441.

Widmer, A., & Lexer, C. (2001). Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends in Ecology and Evolution , 18*, 267-268.

Willig, M., Kaufman, D., & Stevens, R. (2003). Latitudinal gradients of biodiversity: pattern process, scale, and synthesis. *Annual Review of Ecology, Evolution and Systematics , 34*, 273-309.

Wolfe, A., & Liston, A. (1998). Contributions of PCR-based methods to plant systematics and evolutionary biology. In D. Soltis, P. Soltis, & J. Doyle, *Plant Molecular Systematics II.* Boston: Kluwer Academic Publishers.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics , 16*, 97-159.

Wright, S. (1943). Isolation by distance. *Genetics , 28*, 114-138.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics , 15*, 323-354.

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution , 19*, 395-420.

Yeh, F., & Boyle, T. (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany , 129*, 157.

Yeh, F., Yang, R., & Boyle, T. (1997). POPGENE version 1.32.

Yurtsev, B. (1994). Floristic division of the Arctic. *Journal of Vegetation Science , 5*, 765-776.

Zar, J. (1999). *Biological Statistics.* Upper Saddle River, NJ: Prentice Hall.

Zeng, J., Zou, Y., Bai, J., & Zheng, H. (2003). RAPD analysis of genetic variation in natural populations of *Betula alnoides* from Guanxi, China. *Euphytica , 134*, 33-41.

Zhivotovsky, L. (1999). Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology , 8*, 907-913.

APPENDIX: SPECIMEN LIST

***Taxon*, Herbarium, Accession #, *Collection* #, Location**
* represented in morphological data sets
† represented in molecular data sets

Chrysosplenium alternifolium, UTRECHT, U 0248432, *Iltis 15*, Russia

Chrysosplenium alternifolium, UTRECHT, U 0248388, Russia

Chrysosplenium alternifolium, UTRECHT, U 0248398, Scotland

Chrysosplenium alternifolium, UTRECHT, U 0248431, *Mennega 145*, Greece

Chrysosplenium alternifolium, UTRECHT, U 0248423, *Mennega 85*, Belgium

Chrysosplenium alternifolium, UTRECHT, U 0248397, *Burg H*, Belgium

Chrysosplenium alternifolium, UTRECHT, U 0248430, *Hekking 3545*, Belgium

Chrysosplenium alternifolium, UTRECHT, U 0248390, Belgium

Chrysosplenium alternifolium, UTRECHT, U 0248399, *185*, Yugoslavia

Chrysosplenium alternifolium, UTRECHT, U 0248412, *68-1959*, Yugoslavia

Chrysosplenium alternifolium, UTRECHT, U 0248387, *Lampinen 11494*, Finland

Chrysosplenium alternifolium, UTRECHT, U 0248405, Moldavia

Chrysosplenium alternifolium, UTRECHT, U 0248429, *Rentrop 70-1082*, Norway

Chrysosplenium alternifolium, UTRECHT, U 0248416, *101*, Luxemburg

Chrysosplenium alternifolium, UTRECHT, U 0248419, *43*, France

Chrysosplenium alternifolium, UTRECHT, U 0248420, *185*, Italy

Chrysosplenium alternifolium, UTRECHT, U 0248409, Bulgaria

Chrysosplenium alternifolium, UTRECHT, U 0248410, Czech Republic

Chrysosplenium alternifolium, UTRECHT, U 0248425, *Groet 27*, Duitsland

Chrysosplenium alternifolium, UTRECHT, U 0248402, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248401, *Florschuetz*, Duitsland

Chrysosplenium alternifolium, UTRECHT, U 0248415, *Willems 414*, Germany

Chrysosplenium alternifolium, UTRECHT, U 0248392, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248391, *Kooper*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248389, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248386, Germany

Chrysosplenium alternifolium, UTRECHT, U 0248433, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248385, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248417, *168*, Germany

Chrysosplenium alternifolium, UTRECHT, U 0248427, *67-586*, Austria

Chrysosplenium alternifolium, UTRECHT, U 0248428, *67-619*, Austria

Chrysosplenium alternifolium, UTRECHT, U 0248426, *Kramer 1130*, Austria

Chrysosplenium alternifolium, UTRECHT, U 0248396, *Nigg*., Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248404, *Kramer 4133*, Austria

Chrysosplenium alternifolium, UTRECHT, U 0248403, *65-1383*, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248400, *Kramer 1330*, Austria

Chrysosplenium alternifolium, UTRECHT, U 0248435, *8084*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248422, *Kramer 7313*, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248418, *289*, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248421, *65-1352*, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248411, *Kramer 3*, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248394, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248393, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248395, *Swart*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248408, Switzerland

Chrysosplenium alternifolium, UTRECHT, U 0248407, *Palkowa*, Poland

Chrysosplenium alternifolium, UTRECHT, U 0248424, *Frey*, Poland

Chrysosplenium alternifolium, UTRECHT, U 0248406, *Frey*, Poland

Chrysosplenium alternifolium, UTRECHT, U 0248444, *Groet 26*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248443, *Willemsen*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248442, *Jonker*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248441, *de Viries*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248440, *Arnolds 1279*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248439, *Jonker*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248438, *Rubers*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248445, *Oudemans 403*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248446, *van Steenis*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248413, *van Royen 623*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248448, *Dijkstra 3119*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248437, *Wttewaal*, Netherlands

Chrysosplenium alternifolium, UTRECHT, U 0248436, *Mennega 8124*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248434, *71-114*, Western Europe

Chrysosplenium alternifolium, UTRECHT, U 0248447, *Gadella*, Netherlands

Chrysosplenium alternifolium, LEIDEN, 951,239-305, *Jacobs*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1437, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1438, *Baenitz*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1431, *Meissner*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1432, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1422, Western Europe

Chrysosplenium alternifolium, LEIDEN, 910,130-351, Suringar, Switzerland

Chrysosplenium alternifolium, LEIDEN, 910,130-369, Germany

Chrysosplenium alternifolium, LEIDEN, 910,130-356, Suringar, Switzerland

Chrysosplenium alternifolium, LEIDEN, 984,166-325, Western Europe

Chrysosplenium alternifolium, LEIDEN, 933, 54 72, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1364, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1436, Western Europe

Chrysosplenium alternifolium, LEIDEN, 972,050-271, *Mennema 1747*, Duitsland

Chrysosplenium alternifolium, LEIDEN, 972,050-270, *Mennema 1748*, Duitsland

Chrysosplenium alternifolium, LEIDEN, 908,234-1363, Western Europe

Chrysosplenium alternifolium, LEIDEN, 984. 7 924, *Renaud-nooy*, Duitsland

Chrysosplenium alternifolium, LEIDEN, 5955, *Rh lep*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-597, Western Europe

Chrysosplenium alternifolium, LEIDEN, 937 19 223, Germany

Chrysosplenium alternifolium, LEIDEN, 10017, *Groeplep*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 936,184 194, Western Europe

Chrysosplenium alternifolium, LEIDEN, 944,280-122, *Bayer*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 987,251-785, *11281*, Germany

Chrysosplenium alternifolium, LEIDEN, 936,160-122, *Weber*, Germany

Chrysosplenium alternifolium, LEIDEN, 949,180-150, Western Europe

Chrysosplenium alternifolium, LEIDEN, 951,255-965, *de Wit 4533*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 951,256-320, Western Europe

Chrysosplenium alternifolium, LEIDEN, 466107, *Timmermans*, Germany

Chrysosplenium alternifolium, LEIDEN, 908,234-1440, *Hartsen*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 957. 72 922, Germany

Chrysosplenium alternifolium, LEIDEN, 954,354-119, *Summerhayes 2344*, England

Chrysosplenium alternifolium, LEIDEN, 956,245-480, *Sandwith*, England

Chrysosplenium alternifolium, LEIDEN, 972,090-400, *Sprague*, England

Chrysosplenium alternifolium, LEIDEN, 297008, *Sandwith*, England

Chrysosplenium alternifolium, LEIDEN, 297007, *Sandwith*, England

Chrysosplenium alternifolium, LEIDEN, 511408, *Anoniem*, Poland

Chrysosplenium alternifolium, LEIDEN, 224005, *Frey*, Poland

Chrysosplenium alternifolium, LEIDEN, 937,217-305, *Pawlowska*, Poland

Chrysosplenium alternifolium, LEIDEN, 979,280-530, *Gugnacka*, Poland

Chrysosplenium alternifolium, LEIDEN, 979,284-528, *Nikolov*, Bulgaria

Chrysosplenium alternifolium, LEIDEN, 908,234-1428, *Pristan*, Germany

Chrysosplenium alternifolium, LEIDEN, 983,209-720, Russia

Chrysosplenium alternifolium, LEIDEN, 224027, *Madalski*, Russia

Chrysosplenium alternifolium, LEIDEN, 979,290-079, *Dadakova*, Czech Republic

Chrysosplenium alternifolium, LEIDEN, 191202, *Olaru*, Romania

Chrysosplenium alternifolium, LEIDEN, 909,234-1429, Western Europe

Chrysosplenium alternifolium, LEIDEN, 984. 86 645, , France

Chrysosplenium alternifolium, LEIDEN, 957. 72 217, *Pucktler*, Germany

Chrysosplenium alternifolium, LEIDEN, 159, *Nooteboom*, France

Chrysosplenium alternifolium, LEIDEN, 908,234-1435, France

Chrysosplenium alternifolium, LEIDEN, 964,41-611, *Jansoncius*, France

Chrysosplenium alternifolium, LEIDEN, 964,41-612, *Louis*, France

Chrysosplenium alternifolium, LEIDEN, 908,235-311, France

Chrysosplenium alternifolium, LEIDEN, 908,275-689, *Gerard*, France

Chrysosplenium alternifolium, LEIDEN, 908,280-494, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,280-60, France

Chrysosplenium alternifolium, LEIDEN, 908,238-60, France

Chrysosplenium alternifolium, LEIDEN, 908,234-1433, Western Europe

Chrysosplenium alternifolium, LEIDEN, 953,5-919, *Koster 3820*, France

Chrysosplenium alternifolium, LEIDEN, 50401, *Verbiest 835*, Luxemburg

Chrysosplenium alternifolium, LEIDEN, 965,350-348, Luxemburg

Chrysosplenium alternifolium, LEIDEN, 92891, *Balgooy 1162*, Luxemburg

Chrysosplenium alternifolium, LEIDEN, 959,115-536, Luxemburg

Chrysosplenium alternifolium, LEIDEN, 953,5-933, *Koster 3819*, Luxemburg

Chrysosplenium alternifolium, LEIDEN, 951,98-646, *Hiusman*, Belgium

Chrysosplenium alternifolium, LEIDEN, 979,280-700, *Van der Veken 9611*, Belgium

Chrysosplenium alternifolium, LEIDEN, 12327, Belgium

Chrysosplenium alternifolium, LEIDEN, 466108, *Timmermans*, Switzerland

Chrysosplenium alternifolium, LEIDEN, *Wieffering 6302*, Switzerland

Chrysosplenium alternifolium, LEIDEN, *Wieffering 6208*, Switzerland

Chrysosplenium alternifolium, LEIDEN, *Wieffering 6126*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 15873, *Mennema 553*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 211015, *Van der Land*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 952,304-501, *Gattiker-Horgen*, Switzerland

Chrysosplenium alternifolium, LEIDEN, *Groep I00I7*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 953,5-932, *Koster 3822*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 953,346-532, *van Royen 1723*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 954,183-968, Western Europe

Chrysosplenium alternifolium, LEIDEN, 952,100-420, *Becking*, Switzerland

Chrysosplenium alternifolium, LEIDEN, 959,288-250, Switzerland

Chrysosplenium alternifolium, LEIDEN, 959,169-063, *Barkman 1952*, Switzerland

Chrysosplenium alternifolium, LEIDEN, Zw.69, Switzerland

Chrysosplenium alternifolium, LEIDEN, 947,143-826, Switzerland

Chrysosplenium alternifolium, LEIDEN, 953,5-924, *Koster 3821*, Switzerland

Chrysosplenium alternifolium, LEIDEN, Austria

Chrysosplenium alternifolium, LEIDEN, Austria

Chrysosplenium alternifolium, LEIDEN, *Groep 10097*, Western Europe

Chrysosplenium alternifolium, LEIDEN, *H.N. 5608*, Austria

Chrysosplenium alternifolium, LEIDEN, 953,132-251, *Barkman 3562*, Austria

Chrysosplenium alternifolium, LEIDEN, 936,184-42, Austria

Chrysosplenium alternifolium, LEIDEN, 466106, Timmermans, Austria

Chrysosplenium alternifolium, LEIDEN, 958,137-778, *Stam*, Austria

Chrysosplenium alternifolium, LEIDEN, 958,137-771, *Stam*, Austria

Chrysosplenium alternifolium, LEIDEN, 955,141-228, *Stam*, Austria

Chrysosplenium alternifolium, LEIDEN, 908,234-1424, Austria

Chrysosplenium alternifolium, LEIDEN, 959,115-582, *D.D.,* Austria

Chrysosplenium alternifolium, LEIDEN, 950,124-70, *Alm 1048*, Sweden

Chrysosplenium alternifolium, LEIDEN, 959,351-934, Sweden

Chrysosplenium alternifolium, LEIDEN, 948,146-243, Sweden

Chrysosplenium alternifolium, LEIDEN, 18, Sweden

Chrysosplenium alternifolium, LEIDEN, 512845, *Huttunen*, Finland

Chrysosplenium alternifolium, LEIDEN, 224007, *Jokela*, Finland

Chrysosplenium alternifolium, LEIDEN, 224014, *Larjomaa*, Finland

Chrysosplenium alternifolium, LEIDEN, 908, 235-408

Chrysosplenium alternifolium, LEIDEN, 908,234-1426, Western Europe

Chrysosplenium alternifolium, LEIDEN, 204243, Denmark

Chrysosplenium alternifolium, LEIDEN, 956,062-710, Italy

Chrysosplenium alternifolium, LEIDEN, 908,234-1427, Italy

Chrysosplenium alternifolium, LEIDEN, 150291, *Schabel*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1368, Western Europe

Chrysosplenium alternifolium, LEIDEN, 959,115-580, *D.D.*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 926,236-74, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1425, Western Europe

Chrysosplenium alternifolium, LEIDEN, 104183, Western Europe

Chrysosplenium alternifolium, LEIDEN, *Groep 19616*, Western Europe

Chrysosplenium alternifolium, LEIDEN, 947,143-888, Western Europe

Chrysosplenium alternifolium, LEIDEN, 947,143-889, Western Europe

Chrysosplenium alternifolium, LEIDEN, 947,143-890, Western Europe

Chrysosplenium alternifolium, LEIDEN, 908,234-1430, Western Europe

Chrysosplenium alternifolium, LEIDEN, 948,115-7, *van Steenis*, Western Europe

Chrysosplenium alternifolium, ALA, V079760, *Elias 7715*, Russia

Chrysosplenium alternifolium, ALA, V94624, Murray 634, Russia

Chrysosplenium alternifolium, ALA, V90907, *Petrovsky 77-19P*, Russia

Chrysosplenium alternifolium, ALA, V115127, Russia

Chrysosplenium alternifolium, ALA, V083300, *Petrovsky*, Russia

Chrysosplenium alternifolium, ALA, V90906, *Petrovsy 77-104P*, Russia †

Chrysosplenium alternifolium ssp. Arctomontanum, ALA, V88610, *Petrovsky*, Russia

Chrysosplenium alternifolium ssp. sibiricum, ALA, V154523, *Solstad 04/1026*, Russia †

Chrysosplenium alternifolium ssp. Sibiricum, ALA, V154470, *Solstad 04/0343*, Russia

Chrysosplenium alternifolium ssp. Sibiricum, ALA, V129008, *Kharkevick*, Russia

Chrysosplenium iowense, KANU, *Levsen NL061704-29*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-06*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-59*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-13*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061904-83*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-19*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-07*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-76*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL061704-24*, Iowa *†

Chrysosplenium iowense, KANU, *Levsen NL062506-457*, Canada *†

Chrysosplenium iowense, KANU, *Levsen NL062506-471*, Canada *†

Chrysosplenium iowense, KANU, *Levsen NL063005-347*, Canada *†

Chrysosplenium iowense, KANU, *Levsen NL062906-489*, Canada *†

Chrysosplenium iowense, LEIDEN, 168590, *Thorne 12450*, Iowa

Chrysosplenium oppositifolium, UTRECHT, U 0248414, *van Groyen 623*, Netherlands

Chrysosplenium oppositifolium, LEIDEN, 959,228-759, Switzerland

Chrysosplenium rosendahlii, ALA, V129262, *Eriksen*, Canada

Chrysosplenium rosendahlii, ALA, V95055, *Edlund 12619*, Canada

Chrysosplenium rosendahlii, ALA, V105136, *Murray 3182*, Alaska

Chrysosplenium rosendahlii, ALA, V105214, *Murray 4588*, Alaska

Chrysosplenium rosendahlii, ALA, 75102, *Murray 4525*, Alaska

Chrysosplenium rosendahlii, ALA, V105111, *Murray 3511*, Alaska

Chrysosplenium rosendahlii, KANU, *Foote NL0705-379*, Canada *†

Chrysosplenium rosendahlii, KANU, *Foote NL0705-383*, Canada *

Chrysosplenium rosendahlii, KANU, *Foote NL0705-386*, Canada *

Chrysosplenium rosendahlii, KANU, *Levsen NL080306-824* Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062105-167*, Washington *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062105-180*, Washington *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062205-216*, Washington *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062205-198*, Washington *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062205-232*, Washington *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062405-245*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062405-258*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062705-276*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062805-296*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062805-314*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062905-331*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070305-396*, Montana *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070305-407*, Montana *†

Chrysosplenium tetrandrum, KANU, *Levsen NL062906-499*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070106-512*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070406-514*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070606-519*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070606-530*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL070906-544*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071506-568*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071506-578*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071606-590*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071306-557*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071606-616*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071606-617*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071606-630*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071606-622*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071906-647*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL071906-662*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL072106-684*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL072106-711*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL072706-739*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080106-757*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080106-761*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080306-781*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080106-757*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080306-785*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080306-805*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080306-825*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080306-844*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080606-856*, Alaska *†

Chrysosplenium tetrandrum, KANU, *Levsen NL080806-885*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL072707-910*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL072707-916*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL081807-927*, Canada *†

Chrysosplenium tetrandrum, KANU, *Levsen NL081807-928*, Canada *†

Chrysosplenium tetrandrum, LEIDEN, 961,40-242, Canada

Chrysosplenium tetrandrum, LEIDEN, 951,241-225, *Calder 3379*, Canada

Chrysosplenium tetrandrum, LEIDEN, 529463, *Collet 1537*, Alaska

Chrysosplenium tetrandrum, LEIDEN, 525477, *Collet 1591*, Alaska

Chrysosplenium tetrandrum, LEIDEN, Sweden

Chrysosplenium tetrandrum, LEIDEN, 912,250-38, *Montell*, Finland

Chrysosplenium tetrandrum, LEIDEN, 921,6-150, *Montell*, Finland

Chrysosplenium tetrandrum, LEIDEN, 921,6-152, *Mentell*, Finland

Chrysosplenium tetrandrum, LEIDEN, 908,234-1421

Chrysosplenium tetrandrum, LEIDEN, 122649,30, Svalbard

Chrysosplenium tetrandrum, LEIDEN, 957,72-218, *Schaefer*, Svalbard

Chrysosplenium tetrandrum, ALA, 32284, *Bos*, Alaska

Chrysosplenium tetrandrum, ALA, V151589, *Cook 02-418B*, Alaska

Chrysosplenium tetrandrum, ALA, V141230, *Roland 4721*, Alaska

Chrysosplenium tetrandrum, ALA, V96919, *Zabel 43*, Alaska

Chrysosplenium tetrandrum, ALA, V139801, *Parker 12055*, Alaska

Chrysosplenium tetrandrum, ALA, 96524, *Hutson*, Alaska

Chrysosplenium tetrandrum, ALA, 76651, *Wright 85*, Alaska

Chrysosplenium tetrandrum, ALA, 9501, *Clein 76*, Alaska

Chrysosplenium tetrandrum, ALA, 96510, *Hutson*, Alaska

Chrysosplenium tetrandrum, ALA, 48721, *Roberson 658*, Alaska

Chrysosplenium tetrandrum, ALA, 17486, *694*, Alaska

Chrysosplenium tetrandrum, ALA, 37509, *Welsh 7360,* Canada

Chrysosplenium tetrandrum, ALA, V134129, *Parker 10342*, Alaska

Chrysosplenium tetrandrum, ALA, V135000, *Parker 11126*, Alaska

Chrysosplenium tetrandrum, ALA, V112297, *Murray 10928*, Alaska

Chrysosplenium tetrandrum, ALA, 50445, *Laursen*, Alaska

Chrysosplenium tetrandrum, ALA, V112852, *Caswell 92-226*, Alaska

Chrysosplenium tetrandrum, ALA, V122064, *Parker 6277*, Alaska

Chrysosplenium tetrandrum, ALA, V111382, *70-505*, Canada

Chrysosplenium tetrandrum, ALA, V129316, *Simpson*, Alaska

Chrysosplenium tetrandrum, ALA, V129315, *Simpson*, Alaska

Chrysosplenium tetrandrum, ALA, V119515, *Parker 5580*, Alaska

Chrysosplenium tetrandrum, ALA, V122031, *Parker 6625*, Alaska

Chrysosplenium tetrandrum, ALA, V122208, *Parker 6584*, Alaska

Chrysosplenium tetrandrum, ALA, V117976, *Parker 5259*, Alaska

Chrysosplenium tetrandrum, ALA, V120369, *Duffy 95-249*, Alaska

Chrysosplenium tetrandrum, ALA, 12960, *Viereck*, Alaska

Chrysosplenium tetrandrum, ALA, 93624, *Thompson 1378*, Alaska

Chrysosplenium tetrandrum, ALA, V132473, *Elven*, Canada

Chrysosplenium tetrandrum, ALA, V131183, *Duffy 98-297*, Alaska

Chrysosplenium tetrandrum, ALA, V81119, *Parker 1542*, Alaska

Chrysosplenium tetrandrum, ALA, V124249, *Caswell 96357*, Alaska

Chrysosplenium tetrandrum, ALA, 78811, *Murray 798*, Canada

Chrysosplenium tetrandrum, ALA, 78812, *Murray 1266*, Canada

Chrysosplenium tetrandrum, ALA, 16220, *Raup 9241*, Canada

Chrysosplenium tetrandrum, ALA, 41163, *Porsild 6*, Canada

Chrysosplenium tetrandrum, ALA, 41173, *Porsild*, Canada

Chrysosplenium tetrandrum, ALA, V96461, *Keller 1347*, Alaska

Chrysosplenium tetrandrum, ALA, V084383, *Marvin 651*, Alaska

Chrysosplenium tetrandrum, ALA, 77553, *Correll 45710*, Alaska

Chrysosplenium tetrandrum, ALA, 76342, *Murray 6542*, Alaska

Chrysosplenium tetrandrum, ALA, 93627, *Stone 21*, Alaska

Chrysosplenium tetrandrum, ALA, V75742, *Meyers 80-139*, Alaska

Chrysosplenium tetrandrum, ALA, 86172, *Batten 75-19*, Alaska

Chrysosplenium tetrandrum, ALA, 2158, *Rynning 981*, Alaska

Chrysosplenium tetrandrum, ALA, 69018, *Komarkova 273*, Alaska

Chrysosplenium tetrandrum, ALA, V101053, *Durst 161*, Alaska

Chrysosplenium tetrandrum, ALA, V071748, *Helmstetter 80-27*, Alaska

Chrysosplenium tetrandrum, ALA, V126176, *Talbot 304*, Alaska

Chrysosplenium tetrandrum, ALA, 6475, *Palmer*, Alaska

Chrysosplenium tetrandrum, ALA, 936, Alaska

Chrysosplenium tetrandrum, ALA, V101138, *Durst 145*, Alaska

Chrysosplenium tetrandrum, ALA, 92824, *Siplivinsky 392*, Alaska

Chrysosplenium tetrandrum, ALA, V96982, *Rutledge*, Alaska

Chrysosplenium tetrandrum, ALA, 53136, *Batten 469*, Alaska

Chrysosplenium tetrandrum, ALA, V96585, *Wright 85*, Alaska

Chrysosplenium tetrandrum, ALA, 91842, *Mason 227*, Alaska

Chrysosplenium tetrandrum, ALA, V100275, *Lewis*, Alaska

Chrysosplenium tetrandrum, ALA, V70674, *Kelso 232*, Alaska

Chrysosplenium tetrandrum, ALA, V69826, *Khokhryakov 6926*, Alaska

Chrysosplenium tetrandrum, ALA, V105958, *Grant 90-1300*, Alaska

Chrysosplenium tetrandrum, ALA, V118694, *Duffy 124*, Alaska

Chrysosplenium tetrandrum, ALA, V104777, *DeLapp 598*, Alaska

Chrysosplenium tetrandrum, ALA, V104802, *DeLapp 664*, Alaska

Chrysosplenium tetrandrum, ALA, V104518, *Sattler 47*, Alaska

Chrysosplenium tetrandrum, ALA, V108339, *Caswell*, Alaska

Chrysosplenium tetrandrum, ALA, V110501, *Parker 2866*, Alaska

Chrysosplenium tetrandrum, ALA, 38483, *Roberson 412*, Alaska

Chrysosplenium tetrandrum, ALA, 4101, *Shetler 678*, Alaska

Chrysosplenium tetrandrum, ALA, 10868, *Smith 2079*, Alaska

Chrysosplenium tetrandrum, ALA, 934, *Clark*, Alaska

Chrysosplenium tetrandrum, ALA, V139532, *Parker 13146*, Alaska

Chrysosplenium tetrandrum, ALA, 20546, *Raup 10045*, Alaska

Chrysosplenium tetrandrum, ALA, V143086, *Jansen 02-355*, Alaska

Chrysosplenium tetrandrum, ALA, V142096, *Batten 02-483*, Alaska

Chrysosplenium tetrandrum, ALA, V143704, *Sturdy 10-49*, Alaska

Chrysosplenium tetrandrum, ALA, V143879, *Parker 14031*, Alaska

Chrysosplenium tetrandrum, ALA, V144206, *Parker 14367*, Alaska

Chrysosplenium tetrandrum, ALA, V146809, *Larsen 02-1802*, Alaska

Chrysosplenium tetrandrum, ALA, V146810, *Larsen 02-2005*, Alaska

Chrysosplenium tetrandrum, ALA, V146811, *Larsen 02-2592*, Alaska

Chrysosplenium tetrandrum, ALA, V141229, *Roland 4732*, Alaska

Chrysosplenium tetrandrum, ALA, V78021, *Murray 3537*, Alaska

Chrysosplenium tetrandrum, ALA, V70437, *Ebersole 393*, Alaska

Chrysosplenium tetrandrum, ALA, 93629, *Thomas*, Alaska

Chrysosplenium tetrandrum, ALA, 86283, *Batten 11*, Alaska

Chrysosplenium tetrandrum, ALA, 93625, *Spetzman*, Alaska

Chrysosplenium tetrandrum, ALA, V70767, *Hultén*, Alaska

Chrysosplenium tetrandrum, ALA, V69193, *Khokhryakov 6574*, Alaska

Chrysosplenium tetrandrum, ALA, 91703, *Mason 41*, Alaska

Chrysosplenium tetrandrum, ALA, 68099, *Young 4359*, Alaska

Chrysosplenium tetrandrum, ALA, V128063, *Parker 8767*, Alaska

Chrysosplenium tetrandrum, ALA, 41672, *Porsild*, Canada

Chrysosplenium tetrandrum, ALA, 41637, *Porsild 17044*, Canada

Chrysosplenium tetrandrum, ALA, V122777, *Talbot 355*, Alaska

Chrysosplenium tetrandrum, ALA, V124776, *Parker 7823*, Alaska

Chrysosplenium tetrandrum, ALA, V127951, *Parker 8650*, Alaska

Chrysosplenium tetrandrum, ALA, V081920, *Coghill*, Alaska

Chrysosplenium tetrandrum, ALA, V78914, *Kelso 84193*, Alaska

Chrysosplenium tetrandrum, ALA, V084738, *Foote 3892*, Alaska

Chrysosplenium tetrandrum, ALA, V96648, *Sherburne 11*, Alaska

Chrysosplenium tetrandrum, ALA, V97454, *Kildaw*, Alaska

Chrysosplenium tetrandrum, ALA, V99714, *Walker 84-213*, Alaska

Chrysosplenium tetrandrum, ALA, 37722, *Etaender 26*, Alaska

Chrysosplenium tetrandrum, ALA, V113521, *Murray 11185*, Alaska

Chrysosplenium tetrandrum, ALA, V150033, *Parker 15599*, Alaska

Chrysosplenium tetrandrum, ALA, V151314, *Roland 5842A*, Alaska

Chrysosplenium tetrandrum, ALA, 47939, *Keeley 1925*, Alaska

Chrysosplenium tetrandrum, ALA, 35001, *Staender 60*, Alaska

Chrysosplenium tetrandrum, ALA, 52959, *Batten 425*, Alaska

Chrysosplenium tetrandrum, ALA, 9329, *Johnson*, Alaska

Chrysosplenium tetrandrum, ALA, 22502, *Johnson*, Alaska

Chrysosplenium tetrandrum, ALA, V151585, *Cook 02-334*, Alaska

Chrysosplenium tetrandrum, ALA, V151584, *Cook 02-074*, Alaska

Chrysosplenium tetrandrum, ALA, V151586, *Cook 02-418A*, Alaska

Chrysosplenium tetrandrum, ALA, V151587, *Cook 02-647*, Alaska

Chrysosplenium tetrandrum, ALA, V151588, *Cook 02-705*, Alaska

Chrysosplenium tetrandrum, ALA, V138786, *Larsen 01-0076A*, Alaska

Chrysosplenium tetrandrum, ALA, V138787, *Cook 01-0349*, Alaska

Chrysosplenium tetrandrum, ALA, V138788, *Larsen 01-0712*, Alaska

Chrysosplenium tetrandrum, ALA, V138789, *Larsen 01-0867*, Alaska

Chrysosplenium tetrandrum, ALA, V084516, *Marvin 1770*, Alaska

Chrysosplenium tetrandrum, ALA, 23801, *Frohne 49-115*, Alaska

Chrysosplenium tetrandrum, ALA, 30639, *Trent*, Alaska

Chrysosplenium tetrandrum, ALA, 23767, *Frohne 54-152*, Alaska

Chrysosplenium tetrandrum, ALA, V134783, *Parker 10836*, Alaska

Chrysosplenium tetrandrum, ALA, V133669, *Roland 3707*, Alaska

Chrysosplenium tetrandrum, ALA, V083590, *Marvin 1491*, Alaska

Chrysosplenium tetrandrum, ALA, V084027, *Ware 8480*, Alaska

Chrysosplenium tetrandrum, ALA, V137716, *Parker 11837*, Alaska

Chrysosplenium tetrandrum, ALA, V153951, *Calhoun 113*, Alaska

Chrysosplenium tetrandrum, ALA, V144929, *Parker 14999*, Alaska

Chrysosplenium tetrandrum, ALA, 4590, *Argus 702*, Alaska

Chrysosplenium tetrandrum, ALA, 63070, *Raceine 84*, Alaska

Chrysosplenium tetrandrum, ALA, 48568, *Roberson 252*, Alaska

Chrysosplenium tetrandrum, ALA, 72015, *Drury 2058*, Alaska

Chrysosplenium tetrandrum, ALA, 5609, *Palmer*, Alaska

Chrysosplenium tetrandrum, ALA, 75242, *Metzner 72*, Alaska

Chrysosplenium tetrandrum, ALA, 72036, *Drury 1601*, Alaska

Chrysosplenium tetrandrum, ALA, 3494, *Viereck*, Alaska

Chrysosplenium tetrandrum, ALA, 10095, *Anderson 7532*, Alaska

Chrysosplenium tetrandrum, ALA, 10094, *Anderson 7217*, Alaska

Chrysosplenium tetrandrum, ALA, 55878, *Murray 3984*, Alaska

Chrysosplenium tetrandrum, ALA, V072276, *Anderson 0061*, Alaska

Chrysosplenium tetrandrum, ALA, 10872, *Smith 1824*, Alaska

Chrysosplenium tetrandrum, ALA, V072151, *Parker 111*, Alaska

Chrysosplenium tetrandrum, ALA, 37721, *Staender 26-B*, Alaska

Chrysosplenium tetrandrum, ALA, V79494, *Keller 1173*, Alaska

Chrysosplenium tetrandrum, ALA, V79568, *Keller 1248*, Alaska

Chrysosplenium tetrandrum, ALA, V82625, *Batten 85-471*, Alaska

Chrysosplenium tetrandrum, ALA, 54039, *Packer 72-117*, Alaska

Chrysosplenium tetrandrum, ALA, 52991, *Batten 431*, Alaska

Chrysosplenium tetrandrum, ALA, 93628, *Lindsay*, Alaska

Chrysosplenium tetrandrum, ALA, 55876, *Murray 4368*, Alaska

Chrysosplenium tetrandrum, ALA, V138337, *Parker 13984*, Alaska

Chrysosplenium tetrandrum, ALA, 86665, *Helmstetter 94-79*, Alaska

Chrysosplenium tetrandrum, ALA, V123466, *Parker 7469*, Alaska

Chrysosplenium tetrandrum, ALA, V73597, *Boise 6-61*, Alaska

Chrysosplenium tetrandrum, ALA, 82445, *Viereck 5819*, Alaska

Chrysosplenium tetrandrum, ALA, 83953, *Boise 75-170*, Alaska

Chrysosplenium tetrandrum, ALA, 2104, *Miller 305*, Alaska

Chrysosplenium tetrandrum, ALA, 17700, *Johnson*, Alaska

Chrysosplenium tetrandrum, ALA, 79349, *Murray 6028*, Alaska

Chrysosplenium tetrandrum, ALA, 23806, *Frohne 49-140*, Alaska

Chrysosplenium tetrandrum, ALA, V96726, *Grant 88-2*, Alaska

Chrysosplenium tetrandrum, ALA, 4622, *Argus 732*, Alaska

Chrysosplenium tetrandrum, ALA, V100021, *Lewis*, Alaska

Chrysosplenium tetrandrum, ALA, 93626, *Wiggins 12580-A*, Alaska

Chrysosplenium tetrandrum, ALA, V109310, *Talbot 99-X-3*, Alaska

Chrysosplenium tetrandrum, ALA, V79589, *Keller 1269*, Alaska

Chrysosplenium tetrandrum, ALA, V118591, *Foote 4649*, Alaska

Chrysosplenium tetrandrum, ALA, V123486, *Parker 7489*, Alaska

Chrysosplenium tetrandrum, ALA, V123822, *Morane 151*, Alaska

Chrysosplenium tetrandrum, ALA, V125262, *Sigafoos 5943*, Alaska

Chrysosplenium tetrandrum, ALA, V76529, *Parker 505*, Alaska

Chrysosplenium tetrandrum, ALA, 87854, *Rice 206*, Alaska

Chrysosplenium tetrandrum, ALA, 59187, *Viereck 7283*, Alaska

Chrysosplenium tetrandrum, ALA, 48643, *Roberson 333*, Alaska

Chrysosplenium tetrandrum, ALA, 87711, *Ward 72*, Alaska

Chrysosplenium tetrandrum, ALA, 933, *Nelson 3518*, Alaska

Chrysosplenium tetrandrum, ALA, V111549, *Parker 3457*, Alaska

Chrysosplenium tetrandrum, ALA, 72030, *Drury 2990*, Alaska

Chrysosplenium tetrandrum, ALA, 3683, *Cessel*, Alaska

Chrysosplenium tetrandrum, ALA, 935, *Nelson 4066*, Alaska

Chrysosplenium tetrandrum, ALA, 67041,*Wiliams 3443*, Alaska

Chrysosplenium tetrandrum, ALA, 4269, *Shetler 869-AF*, Alaska

Chrysosplenium tetrandrum, ALA, 4221, *Shetler 886-AF*, Alaska

Chrysosplenium tetrandrum, ALA, 32773, *Pegau 176*, Alaska

Chrysosplenium tetrandrum, ALA, V150358, *Parker 15848*, Alaska

Chrysosplenium tetrandrum, ALA, V149323, *Roland 5669*, Alaska

Chrysosplenium tetrandrum, ALA, 91978, *McNulty 46*, Alaska

Chrysosplenium tetrandrum, ALA, 92926, *Siplivinsky 125*, Alaska

Chrysosplenium tetrandrum, ALA, 8819, *Clemson*, Alaska

Chrysosplenium tetrandrum, ALA, 40010, *Welsh 7977*, Alaska

Chrysosplenium tetrandrum, ALA, V113852, *Parker 3956*, Alaska

Chrysosplenium tetrandrum, ALA, 51607, *Robuck 1321*, Alaska

Chrysosplenium tetrandrum, ALA, V85821, *Parker 1914*, Alaska

Chrysosplenium tetrandrum, ALA, V98691, *Dick*, Alaska

Chrysosplenium tetrandrum, ALA, V69677, Russia

Chrysosplenium tetrandrum, ALA, V86626, Russia

Chrysosplenium tetrandrum, ALA, V115451, *Parker 4453*, Russia

Chrysosplenium tetrandrum, ALA, V119879, *Parker 5957*, Russia

Chrysosplenium tetrandrum, ALA, V129016, *Kharkevick*, Russia

Chrysosplenium tetrandrum, ALA, V154533, *Solstad 04/1180*, Russia

Chrysosplenium tetrandrum, ALA, 23155, Finland

Chrysosplenium tetrandrum, ALA, V144657, *Elven*, Svalbard

Chrysosplenium tetrandrum, ALA, 17028, *Dahl*, Svalbard

Chrysosplenium tetrandrum, ALA, 8044, Norway

Chrysosplenium wrightii, ALA, 83922, *Weiler*, Alaska

Chrysosplenium wrightii, ALA, V73720, *Murray 6242*, Alaska

Chrysosplenium wrightii, ALA, V75788, *Wetzel 15*, Alaska

Chrysosplenium wrightii, ALA, V131184, *Duffy 98-327*, Alaska

Chrysosplenium wrightii, ALA, V116526, *Barker 15*, Alaska

Chrysosplenium wrightii, ALA, V75233, *Friedman 80-71*, Alaska

Chrysosplenium wrightii, ALA, V79373, *Parker 1159*, Canada

Chrysosplenium wrightii, ALA, 78827, *Murray 1807*, Canada

Chrysosplenium wrightii, ALA, 78955, *Murray 1370*, Canada

Chrysosplenium wrightii, ALA, 78954, *Murray 512*, Canada

Chrysosplenium wrightii, ALA, 55879, *Murray 3882*, Alaska

Chrysosplenium wrightii, ALA, V133670, *Roland 4000*, Alaska

Chrysosplenium wrightii, ALA, 932, *Nelson 4120*, Alaska

Chrysosplenium wrightii, ALA, 5848, *Palmer 643*, Alaska

Chrysosplenium wrightii, ALA, 9334, *Johnson*, Alaska

Chrysosplenium wrightii, ALA, 10093, *Mexia 2143*, Alaska

Chrysosplenium wrightii, ALA, V138790, *Larsen 10-0822*, Alaska †

Chrysosplenium wrightii, ALA, V138791, *Larsen 01-1165*, Alaska

Chrysosplenium wrightii, ALA, V78913, *Kelso 84-75*, Alaska

Chrysosplenium wrightii, ALA, V125081, *Parker 8059*, Alaska

Chrysosplenium wrightii, ALA, V126177, *Talbot 368*, Alaska

Chrysosplenium wrightii, ALA, V124171, *Phillips 96356*, Alaska

Chrysosplenium wrightii, ALA, V146813, *Larsen 02-1686*, Alaska †

Chrysosplenium wrightii, ALA, 84923, *Murray 6877*, Alaska

Chrysosplenium wrightii, ALA, V146812, *Larsen 02-1585*, Alaska

Chrysosplenium wrightii, ALA, V146335, *Lipkin 205*, Alaska

Chrysosplenium wrightii, ALA, V115969, *Kildaw*, Alaska

Chrysosplenium wrightii, ALA, 82262, *Viereck 6053*, Alaska

Chrysosplenium wrightii, ALA, 24768, *Harbo 26*, Alaska

Chrysosplenium wrightii, ALA, 25271, *Schene*, Alaska

Chrysosplenium wrightii, ALA, 83921, *Weiler 5*, Alaska

Chrysosplenium wrightii, ALA, V081545, *Parker 1255*, Alaska

Chrysosplenium wrightii, ALA, V140022, *Parker 12379*, Alaska

Chrysosplenium wrightii, ALA, 86174, *Batten 75-443A*, Alaska

Chrysosplenium wrightii, ALA, 84921, *Murray 6874*, Alaska

Chrysosplenium wrightii, ALA, 79960, *Murray 6245*, Alaska

Chrysosplenium wrightii, ALA, 93630, *Shetler 3342*, Alaska

Chrysosplenium wrightii, ALA, 12969, *Viereck*, Alaska

Chrysosplenium wrightii, ALA, V140111, *Parker 12438*, Alaska

Chrysosplenium wrightii, ALA, V128126, *Parker 8833*, Alaska

Chrysosplenium wrightii, ALA, V127481, *Roland 3332*, Alaska

Chrysosplenium wrightii, ALA, V120105, *Caswell 331-95*, Alaska

Chrysosplenium wrightii, ALA, V122884, *Parker 7601*, Alaska

Chrysosplenium wrightii, ALA, V123255, *Parker 7254*, Alaska

Chrysosplenium wrightii, ALA, V123725, *Moran 84A*, Alaska

Chrysosplenium wrightii, ALA, V123782, *Moran 50*, Alaska

Chrysosplenium wrightii, ALA, V143357, *Jansen 02-224*, Alaska †

Chrysosplenium wrightii, ALA, 68653, *Viereck 5165*, Alaska

Chrysosplenium wrightii, ALA, 78828, *Murray 2029*, Alaska

Chrysosplenium wrightii, ALA, 82261, *Viereck 5621*, Alaska

Chrysosplenium wrightii, ALA, 84142, *Winters 229*, Alaska

Chrysosplenium wrightii, ALA, V119902, *Parker 6007*, Russia

Chrysosplenium wrightii, ALA, 78484, *Murray 3026*, Canada

Chrysosplenium wrightii, ALA, V97953, *Bosworth*, Alaska

Chrysosplenium wrightii, ALA, V86346, *Murie*, Alaska

Chrysosplenium wrightii, ALA, V103991, *Murie*, Alaska

Chrysosplenium wrightii, ALA, V98483, *Kildaw*, Alaska

Chrysosplenium wrightii, ALA, 47723, *Reich 777*, Alaska

Chrysosplenium wrightii, ALA, 47722, *Reich 784*, Alaska

Chrysosplenium wrightii, ALA, 24807, *Harbo 3*, Alaska

Chrysosplenium wrightii, ALA, 28774, *Belson*, Alaska

Chrysosplenium wrightii, ALA, V072192, *Parker 216*, Alaska

Chrysosplenium wrightii, ALA, 86173, *Batten 75-8*, Alaska

Chrysosplenium wrightii, ALA, 25752, *Hamilton 2*, Alaska

Chrysosplenium wrightii, ALA, 52117, *Batten 45*, Alaska

Chrysosplenium wrightii, ALA, 21748, *Flock 32*, Alaska

Chrysosplenium wrightii, ALA, 52137, *Batten 23*, Alaska

Chrysosplenium wrightii, ALA, 52133, *Batten 27*, Alaska

Chrysosplenium wrightii, ALA, 55877, *Murray 4029*, Alaska

Chrysosplenium wrightii, ALA, 51914, *Batten 862*, Alaska

Chrysosplenium wrightii, ALA, V109835, *Caswell*, Alaska

Chrysosplenium wrightii, ALA, 76494, *Densmore 267*, Alaska

Chrysosplenium wrightii, ALA, 78829, *Murray 2246*, Alaska

Chrysosplenium wrightii, ALA, V127275, *Batten 96-112*, Alaska

Chrysosplenium wrightii, ALA, 67301, *Halliday A497/75*, Alaska

Chrysosplenium wrightii, ALA, V148764, *Lipkin 34B*, Alaska

Chrysosplenium wrightii, ALA, V141251, *Roland 4331*, Alaska

Chrysosplenium wrightii, ALA, V151164, *Cook 4824*, Alaska

Chrysosplenium wrightii, ALA, V153170, *Loomis 2452*, Alaska

Chrysosplenium wrightii, ALA, V77057, *Murray 7337*, Alaska

Chrysosplenium wrightii, ALA, V139627, *Parker 13291*, Alaska

Chrysosplenium wrightii, ALA, 92381, *Mason 76-94*, Alaska

Chrysosplenium wrightii, ALA, V70122, *Levkovsky*, Russia

Chrysosplenium wrightii, ALA, 24814, *Harbo 32*, Alaska

Chrysosplenium wrightii, ALA, 78464, *Murray 2195*, Alaska

Chrysosplenium wrightii, ALA, V70673, *Kelso 231*, Alaska

Chrysosplenium wrightii, ALA, V76362, *Parker 625*, Alaska

Chrysosplenium wrightii, ALA, V76731, *Parker 670*, Alaska

Chrysosplenium wrightii, ALA, V73747, *Strickland*, Alaska

Chrysosplenium wrightii, ALA, 6317, *Palmer*, Alaska

Chrysosplenium wrightii, ALA, 48028, *McCartney*, Alaska

Chrysosplenium wrightii, ALA, V76735, *Parker 872*, Alaska

Chrysosplenium wrightii, ALA, V90908, *Andreev 77-49P*, Russia

Chrysosplenium wrightii, ALA, V133953, *Kharkevick*, Russia

Chrysosplenium wrightii, ALA, V119944, *Parker 6076*, Russia