EVOLUTIONARY BIOLOGY OF THE PARASITIC ANGIOSPERM *ARCEUTHOBIUM AMERICANUM* (VISCACEAE) AS DETERMINED BY POPULATION GENETIC ANALYSIS AND INFECTIVITY EXPERIMENTS

by

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Department of Botany

A thesis submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy at the University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

In this study, a multidisciplinary approach incorporating population genetic analysis and infectivity experiments was used to explore the evolutionary biology of *Arceuthobium americanum* Nutt. ex Engelm. (Viscaceae). This study represents one of the few comprehensive investigations into the evolutionary, geographical, biological, ecological, and historical factors influencing a parasitic plant.

Arceuthobium americanum infects three principal hosts and has the most extensive geographic range of any N. American dwarf mistletoe. Based on the lack of apparent morphological and phenological differences between populations of *A. americanum*, past researchers have found no evidence for recognizing subspecific taxa. In the present study, molecular analysis using amplified fragment length polymorphism analysis (AFLP) indicated that *A. americanum* is divided into three distinct genetic races, each associated with a different host taxon in regions of allopatry: (1) *Pinus banksiana* in western Canada; (2) *Pinus contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountain ranges in the western U.S.A.; and (3) *Pinus contorta* var. *latifolia* in the western U.S.A. and Canada. These observations suggested that host identity, geographic isolation, and environmental factors have all contributed to race formation in *A. americanum*.

Molecular analysis using AFLP indicated that hosts are divided into only two genetic groups: (1) *P. banksiana* and hybrids; and (2) *P. contorta* var. *latifolia* and var. *murrayana*. This observation suggests that host identity is not the primary factor leading to race formation in *A. americanum*. Findings from infection experiments also question the role played by host identity since there is no indication of host X parasite

interactions. Nonetheless, the role of host cannot be completely ruled out since the genetic races of *A. americanum* can clearly be defined by this parameter.

The lack of fine-scale patterning within *A. americanum* races was attributed to random dispersal of seeds over long distances by animal vectors, as well as to adaptation of parasite populations to non-geographically patterned host genotypes and local environmental conditions. Historical factors such as glaciations and founder events were also found to impact structuring and genetic diversity in *A. americanum* populations. Despite this lack of fine-scale patterning, the existence of three distinct genetic races of *A. americanum* provides insight into the evolutionary potential of this taxon. Given sufficient time, it is possible that these races will become reproductively isolated, and undergo speciation.

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CHAPTER 1: Introduction, Literature Review, and Thesis Goals

1.1 INTRODUCTION

A parasite is "an organism living on or in another organism (host) and obtaining its food from the latter" (Agrios, 1997). In addition, a parasite "completes a whole stage of its life cycle associated with a single host individual in a relationship that is beneficial to the parasite, but not to the host" (Norton and Carpenter, 1998). In recent years, there has been an increased interest in understanding the evolutionary biology of parasites. This is largely due to an increased awareness of their abundance, wide-spread distribution, socioeconomic impact on humans, and their effect on the ecology and evolutionary fate of their hosts.

Speciation via race formation has been implicated as one of the major evolutionary processes affecting parasites (Price, 1980; Thompson, 1994; Norton and Carpenter, 1998). In both parasites and non-parasites, race formation is likely to occur when gene flow between conspecific populations becomes diminished due to factors such as isolation-by-distance (i.e. geographic isolation) and ecological adaptation (i.e. selective divergence). Geographical isolation promotes race formation since allopatric populations are allowed to evolve independently due to the lack of cohesive forces that would normally result from gene flow (Templeton, 1981; Orr, 1995; Orr and Orr, 1996; Via et al., 2000). Divergent selection pressures resulting from adaptation to different environmental conditions facilitate race formation since specific parasite genotypes would be favoured or selected against in different environments (Schluter, 1996, 1998; Via et al., 2000). In parasites, different hosts are an additional factor that can impose

diversifying selection pressures and also drive race formation (Via et al., 2000). Divergent selection pressures from either host or environment can act sympatrically or allopatrically. Race formation resulting from any of these factors has the potential to lead to speciation if reproductive isolation develops. For this reason, observations of gene flow and adaptation can provide insight into the evolutionary forces acting on host-parasite pathosystems.

To date, fungal and animal parasites have been the primary focus of studies examining race formation in parasites. Thus, an important group of parasites -- the parasitic angiosperms -- has been largely neglected. In this thesis, population genetic analysis and infectivity experiments have been used to assess the evolutionary forces acting on a parasitic plant from the genus *Arceuthobium* M. Von Bieb.

The literature review that follows provides background information about parasitic plants in general, and about the genus *Arceuthobium*, in particular. Such information is essential to understanding evolutionary biology since ecological factors and life history traits contribute to the genetic structure of organisms. This section also reviews two commonly used approaches to examining race formation in parasites. These include population genetic analysis and infectivity experiments. Current insights and knowledge gaps from the theoretical and empirical literature are described for parasitic plants, in general, and more specifically, for members of the genus *Arceuthobium*.

1.2 LITERATURE REVIEW

TRAITS OF PARASITIC PLANTS

A parasitic angiosperm is a flowering plant that relies on another plant for part or all of its nutrition (Parker and Riches, 1993). Parasitic plants are connected to their hosts either above ground (aerial parasites) or below ground (root parasites) by an haustorium (a bridge of specialized tissue) which allows for the flow of water and nutrients from host to parasite (Parker and Riches, 1993). Parasitic plants are found in three subclasses and at least 17 families (see Table 1). These plants are estimated to range in number from 3,000 to over 3,700 species, representing 1% of all angiosperm taxa (Kuijt, 1969; Nickrent and Franchina, 1990; Press and Graves, 1995). Parasitism in this polyphyletic group of plants is postulated to have evolved independently numerous times (Kuijt, 1969, Nickrent and Starr, 1994; Nickrent et al., 1998).

Parasitic plants show a wide range of lifestyles (see Table 1). For example, parasitic plants can be divided into either obligate or facultative parasites. Obligate plant parasites are not capable of establishing and/or developing independently of another organism whereas facultative plant parasites have the ability to establish and grow independently, but generally function as a parasite for part of their nutrition (Parker and Riches, 1993). Parasitic plants can also be divided into hemi- or holo- parasites depending on the nutrients that they require from their hosts. Hemiparasites (such as those in the *Loranthaceae* Juss., *Scrophulariaceae* Juss., and *Viscaceae* Batsch.) have their own chlorophyll and photosynthetic ability but obtain water and minerals (as well as some photosynthates) from their hosts (Parker and Riches, 1993). Hemiparasites can be facultative or obligate. Holoparasites (such as those in the *Orobanchaceae* Vent. and

Rafflesiaceae Dumort.), on the other hand, lack chlorophyll and the ability to synthesize organic carbon, and are therefore, strictly obligate (Parker and Riches, 1993).

Parasitic plants show a variety of reproductive strategies ranging from selfing (for some *Orobanchaceae*) to outcrossing through animal or wind pollination (for many *Loranthaceae* and *Viscaceae*). Furthermore, parasitic angiosperms show a range of seed dispersal mechanisms from those that disperse seeds by animal vectors (for many *Loranthaceae* and *Viscaceae*) to those that disperse seeds by wind (for many *Scrophulariaceae* and *Orobanchaceae*).

Parasitic plants are of considerable economic and ecological importance (Kuijt, 1969; Nickrent and Franchina, 1990; Press and Graves, 1995). For example, parasites in the *Cuscutaceae* Bercht. and J. Presl., *Orobanchaceae*, *Scrophulariaceae*, and *Viscaceae* attack commercially important agricultural crops and timber stands, thereby causing significant reductions in yield and subsequent economic loss (Parker and Riches, 1993; Hawksworth and Wiens, 1996). On the other hand, some woody parasites (such as those in the *Santalaceae* R. Br.) are important sources of timber, and hence, have a positive economic benefit. Furthermore, some plant parasites (such as those in the *Viscaceae*) play an important ecological role by increasing diversity in natural communities (Bennetts *et al.*, 1996; Seamans and Gutiérrez, 1995; Parks et al., 1999).

In the past three decades, increased concern about parasitic plants has led to a number of advances in our understanding of the basic biology of these organisms. Press and Graves (1995) identified three factors that provided the impetus for intensified studies of parasitic plants; these included: (1) the publication of a classic and comprehensive text (Kuijt, 1969) on these organisms, entitled The Biology of Parasitic

Flowering Plants; (2) the presence of *Striga asiatica* Kuntze (witchweed) as a threat to maize crops in the United States; and (3) international attention on famines resulting from crop failures in Africa, partially related to parasitic weeds in the 1970's and 1980's.

These factors promoted an intensive collaboration between developing and developed countries to study these problematic parasitic angiosperms. Since 1973, scientists from many nations have been meeting and presenting research every four to six years at international symposia specializing on parasitic plants (Cubero and Moreno, 1996). Although the first three international symposia were poorly attended with less than 60 scientists attending, a steady increase has been seen since that time. At the most recent symposium in 1996 in Cordoba, Spain (the VIth International Congress on Parasitic Weeds), there were 328 attendees and 108 papers presented (Cubero and Moreno, 1996).

The majority of research papers presented at these international symposia have concentrated on understanding the basic biology, physiology, and control measures of parasitic plants. As such, much progress has been made in these fields. Unfortunately, very little emphasis has been placed on understanding the evolutionary biology of these parasites. On average, only 6% of all papers presented at these international symposia have examined the evolution, speciation, and systematics of parasitic plants (Cubero and Moreno, 1996).

TRAITS OF MEMBERS OF THE GENUS *ARCEUTHOBIUM*

Taxonomy

The genus *Arceuthobium* M. Von Bieb. is a group of aerial hemiparasites in the family Viscaceae. This family of mistletoes is well delineated from other mistletoes

based primarily on differences in embryology. Members of the family Viscaceae have *Allium*-type megasporogenesis whereas those in the family Loranthaceae have *Polygonum*-type megasporogenesis (Cronquist, 1981; Mabberly, 1997). The genus *Arceuthobium* is characterized by dioecious plants generally having 3 or 4 - merous dull-coloured flowers, sessile ring-like anthers, bicoloured fruits from which seeds are explosively discharged, and an extensive endophytic system (Hawksworth and Wiens, 1996; Mabberly, 1997).

Based on morphological features such as patterns of secondary branching (e.g. verticillate or flabellate) of shoots and flowers, as well as on molecular data, the genus *Arceuthobium* has been divided into two subgenera, subgenus *Arceuthobium* and subgenus *Vaginata* Hawksw. and Wiens. These subgenera have each in turn been split into sections based on molecular (Nickrent, 1996), morphological, and physiological characters (Hawksworth and Wiens, 1984, 1996). To date, 42 species and four nonautonymic infraspecific taxa have been recognized within these sections. At the species level and below, *Arceuthobium* is considered taxonomically difficult because plants are morphologically reduced and taxa generally resemble each other. Nonetheless, species of *Arceuthobium* can be distinguished using morphological, physiological and phenological characters of the parasite, as well as identity of the host (Hawksworth and Wiens, 1996).

Host Associations

The genus *Arceuthobium* is comprised of a group parasitic plants that infect conifers in the family *Pinaceae* Sprengel ex Rudolphi in both the New World and the Old World, and in the family *Cupressaceae* Gray in the Old World. *Arceuthobium* spp. are

obligate aerial hemiparasites that infect host branches to obtain water, minerals, and photosynthates from their hosts. *Arceuthobium* spp. are known to infect host taxa with varying intensity. This has led to the development of a ranking system for hosts [principal (≈100% infection), secondary (50-90% infection), occasional (5-50% infection), and rare (<5% infection)] that is based upon level of infection of trees within 6 m of an *Arceuthobium* seed source (Hawksworth and Wiens, 1996).

Economic and Ecological Impact

Dwarf mistletoes have been deemed the most damaging pathogens to attack commercially important coniferous timber stands throughout western North America (Hawksworth and Wiens, 1996). Trees attacked with dwarf mistletoes are often deformed by witches' brooms (dense aggregations of host branches initiated at the point of infection by the parasite) (Hawksworth and Wiens, 1996). Conifers attacked by dwarf mistletoes show a reduction in growth (height, diameter and volume), wood quality, and reproductive fitness (reviewed in Hawksworth and Wiens, 1996). Further, infected trees appear to be more susceptible to secondary damage caused by fungi or insects. Thus, in the forest industry and in developed and recreational areas, control of dwarf mistletoes is of great concern. Most often, control is accomplished by silvicultural methods such as clearcutting, pruning of diseased branches, and planting of non-host pine species in heavily infected stands (Hawksworth and Wiens, 1996; Scharpf, 1984). However, such control measures can be extremely time-consuming and costly, making them impractical for most purposes.

Despite their negative economic impact, dwarf mistletoes are ecologically important in forest ecosystems. For example, forest stands infected with dwarf mistletoes

show a higher diversity and abundance of bird, mammal and insect species, due to the food, cover and nesting sites provided by conifers infected with *Arceuthobium* spp. (Bennetts *et al.*, 1996; Seamans and Gutiérrez, 1995; Parks et al., 1999). As well, in the long-term, dwarf mistletoes open up the forest canopy (personal observation), allowing shade-intolerant plant species to re-establish. Ecologists (Bennetts *et al.*, 1996) have proposed that dwarf mistletoe control is unwarranted in stands where timber production is not a primary management goal since these plants have a long association with conifer forests and have a positive influence on forest ecosystem diversity.

Seed Dispersal and Pollen Flow

Seed dispersal in dwarf mistletoes is achieved by a highly effective, hydrostatically controlled explosive discharge mechanism (reviewed in Hawksworth and Wiens, 1996). *Arceuthobium* infected conifer stands can produce hundreds of thousands to millions of seeds per year. However, infection success is low since only 10% of discharged seeds are intercepted by suitable hosts, and of these, only 5% are successful in establishing new infections (Hawksworth and Wiens, 1996). This low rate of natural infection has been largely attributed to seed loss resulting from wash-off by rain water, desiccation, predation and fungal attack (reviewed in Hawksworth and Wiens, 1996). Although seeds can be explosively discharged up to 20 m, early interception of seeds by neighbouring trees restricts the rate of spread of *Arceuthobium* through dense forest stands to 0.3 to 0.6 m per year (Hawksworth and Wiens, 1996). Transport of *Arceuthobium* seeds by animal vectors is thought to be a form of long-distance dispersal in this genus. Support for such a long-distance mechanism (reviewed in Hawksworth and Wiens, 1996) has been provided by documentation of: (1) satellite infection centres that

are geographically isolated from known infected stands; (2) *Arceuthobium* seeds being found on the feathers of birds and on the fur of small mammals in and around infected stands; and (3) rapid dispersal of several *Arceuthobium* taxa into previously glaciated regions of Canada since the retreat of the Cordilleran and Laurentide sheets during the late Wisconsin glaciation.

Members of the genus *Arceuthobium* exhibit floral features that are typical of both wind-pollinated (anemophilous) and insect-pollinated (entomophilous) species (reviewed in Hawksworth and Wiens, 1996). The relative importance of these modes of pollination varies between *Arceuthobium* taxa. Insects in several groups (including ants, flies, wasps and beetles) have been documented carrying pollen of *Arceuthobium* (reviewed in Hawksworth and Wiens, 1996). On the other hand, pollen traps and insect exclusion devices have been used to document the role of wind in dispersing pollen in this genus. The distance over which *Arceuthobium* pollen is transported by wind has not been well studied, with exception of *Arceuthobium americanum* Nutt. ex Engelm. In this species, researchers (Penfield et al., 1976; Gilbert and Punter, 1984; Coppola, 1989) have documented pollen transport by wind at 10 - 500 m from the closest pollen source.

Reproductive Strategy

Since all members of the genus *Arceuthobium* are dioecious, the opportunity for inbreeding is greatly reduced. Using isoenzyme analysis, Nickrent and coworkers (Nickrent and Butler, 1990; Nickrent and Butler, 1991) showed general congruence between observed and expected heterozygosity levels in *A. californicum* Hawksw. and Wiens, *A. campylopodum* Engel., *A. littorum* Hawksw., Wiens and Nickrent, and *A. monticola* Hawksw., Wiens and Nickrent, thereby supporting the conclusion that

outcrossing is the prevalent reproductive strategy in the genus. For this reason, gene flow amongst conspecific populations should be relatively high in *Arceuthobium* spp. However, given the wide geographic and host range for many members of this genus, genetic structuring would not be surprising due to isolation-by-distance and adaptation to different hosts and environmental conditions.

POPULATION GENETIC STRUCTURE

The extent of gene flow within and between conspecific populations (i.e. population structure) plays a major role in determining the evolutionary fate of organisms (Raven, 1980; 1986; Levin, 1993; Travis, 1996). Thus, in parasites, as in other organisms, knowledge of population genetic structure is important since it provides insight into the potential for interpopulation differentiation and speciation (Nadler et al., 1995). Furthermore, an understanding of genetic structure can shed light on factors that have contributed to patterns of diversity. These factors include migration, life history characteristics, geographic range, and selection pressures (Hartl and Clark, 1989; Hamrick and Godt, 1990). Historical events such as founder events, population bottlenecks, recent speciation events and glacial history can also affect population structure (Lewis and Crawford, 1995).

Several researchers (Price, 1980; Tybayrenc and Ayala, 1991; Maynard Smith et al., 1993) have suggested that parasites are likely to be characterized by high population subdivision, geographic differentiation and the existence of cryptic species. This is thought to relate to patchy distributions and their strict dependence on hosts to survive (Price, 1980). Nadler (1995) has disputed this point, arguing that generalizations of parasite population structure are not possible since so many factors are involved. For

example, the life history characters (such as pollen and seed dispersal) of both the parasite and the host play a role in determining the population structure of parasites (Nadler, 1995; Nadler et al., 1995). In addition, influences such as geographic range and stochastic processes such as founder events and population bottlenecks (Slatkin, 1987) can significantly impact their genetic structuring. Empirical data support Nadler's concept (Nadler, 1995) since dependent species have indeed been documented with varying degrees of population differentiation (Bull et al., 1984; Hillburn and Sattler, 1986; Lybeard et al., 1989; Nadler et al., 1990; Mulvey, et al., 1991; Paggi et al., 1991; Vogler et al., 1991; Blouin et al., 1992; Dame et al., 1993; Nascetti et al., 1993; Nadler, et al., 1995; Dybdahl and Lively, 1996; Jobet et al., 2000).

Population Genetic Structure in Parasitic Plants

Population genetic studies on parasitic plants have been limited to a few root and shoot parasites. Shoot parasites such as *Phoradendron* Nutt. (Glazner et al., 1988) and root parasites such as *Orobanche* L. (Gagne et al., 1998; Paran et al., 1997; Verkleij et al., 1989; Verkleij et al., 1991), *Striga* Laur. (Olivier et al., 1996, 1998; Kuiper, et al., 1996), *Pedicularis* L. (Waller et al., 1998; Schmidt and Jensen, 2000) and *Bdallophyton* Eichler (Garcia-Franco et al., 1998) have been shown to have a wide range of genetic structures. This variation can be attributed to differences in geographic ranges and in life history traits such as breeding system and seed dispersal mechanism amongst these taxa. These studies provided some insight into the distribution of genetic diversity amongst populations of parasitic angiosperms. However, most of them failed to examine parasites throughout their geographic or host range. Thus, it is difficult to infer which evolutionary forces are acting on populations of these taxa. For instance, the relative importance of

geographic isolation or divergent selection pressures imposed by host species, local host genotypes, and environment have not been assessed.

Population Genetic Structure in the Genus Arceuthobium

Population genetic studies of shoot parasites have focussed primarily on members of the genus *Arceuthobium*. These studies do not portray a complete picture of evolutionary pressures acting on the genus *Arceuthobium* since researchers generally did not report the level of genetic diversity or extent of population differentiation in the taxa examined (Linhart, 1984; Nickrent et al., 1984; Nickrent and Butler, 1990, 1991; Hawksworth *et al.*, 1992). These studies are, however, of great taxonomic value. For example, isoenzyme analyses have resulted in the detection and naming of a number of new *Arceuthobium* species within the section *Campylopodum* Hawksw. and Wiens (Nickrent and Butler, 1990, 1991; Hawksworth *et al.*, 1992). These cryptic *Arceuthobium* taxa were each found associated with a narrow host range (only one to two principal host taxa). Isoenzyme data also provided evidence for the existence of two subspecies of *A. tsugense* Rosendahl (G.N. Jones), also within the Section *Campylopodum* (Nickrent and Stell, 1990). In this case, however, each subspecies was associated with several different host taxa. An exception to this pattern was the finding of Nickrent and Butler (1990) who used a lack of molecular differentiation to propose that allopatric and sympatric populations of *A. campylopodum* Engelm. and *A. occidentale* Engelm. (parasitic on different principal hosts) should be considered a single biological species (but see Hawksworth and Wiens, 1996). Only two molecular studies (Nickrent *et al.*, 1984; Linhart, 1984), have examined the genetic structure of *Arceuthobium* outside of the section *Campylopodum*. Both of these studies are of limited

scope, since they examined taxa in only a limited portion of their host range and geographic distribution.

PATTERNS OF INFECTIVITY IN PARASITES

Understanding patterns of infectivity in parasites is important for several reasons. Firstly, parasites can have a significant ecological impact on ecosystems (Press and Graves, 1993). Secondly, an understanding of patterns of infectivity can have serious implications for control measures of economically important parasites. Thirdly, adaptation of parasites to hosts may impact their evolution (Price, 1980; Thompson, 1994; Orr and Orr, 1996; Via et al., 2000). For example, the existence of infective parasite races may be indicative of incipient speciation in these organisms.

Adaptation of parasites to their hosts has been attributed to the existence of gene-for-gene coevolution of resistance and infectivity alleles in host and parasite populations (Flor 1956; Frank, 1992, 1993). Since parasites generally have shorter generation times and higher fecundity rates than their hosts, they are expected to exhibit higher evolutionary rates than their hosts (Hamilton et al., 1990; Kaltz and Shykoff, 1998). Based on this premise, models depicting the cycling of resistance and infectivity alleles in host-parasite interactions predict that parasites will generally be more capable of infecting sympatric than allopatric host populations (Gandon et al., 1996; Morand et al., 1996; Kaltz and Shykoff, 1998).

Kaltz and Shykoff (1998) reviewed the literature and indicated that of 38 studies that examined infectivity patterns of dependent species on their hosts, a high number (50%) did show local adaptation of parasites to their hosts. The hosts and parasites in these pathosystems are presumably involved in a tight coevolutionary interaction with

each other. However, 18.4% of studies found that dependent species were actually locally maladapted to their hosts and 31.6% of studies indicated that there was no significant adaptation or maladaptation.

Lack of local adaptation of parasites to hosts can be explained by various factors. For example, models show that temporal aspects such as a time-lag in the response of parasite infectivity alleles to newly evolving host resistance alleles can lead to local maladaptation (Gandon et al., 1996, Morand et al, 1996). Morand and coworkers (1996) suggested that such a time-lag was responsible for local maladaptation in a schistosome-snail pathosystem. Researchers (Gandon et al., 1996; Morand et al, 1996) have also suggested that spatial aspects play an important role in the outcome of host-parasite interactions. For example, differential migration rates by parasites and their hosts, and the extent to which parasites and hosts coevolve, can impact the degree of local adaptation in a particular pathosystem. Models have been used to show that low parasite gene flow will lead to local adaptation of a parasite to its host in situations where the host is not evolving in response to the parasite (Kirkpatrick and Barton, 1997; Gandon and Van Zandt, 1998). However, in situations where a host is evolving in response to its parasite, computer simulations (Gandon et al., 1996) and empirical data (Mukaratirwa et al., 1996; Davies et al., 1999) suggest that high parasite gene flow can increase local adaptation, presumably by introducing novel adaptive traits into parasite populations (Slatkin, 1987; Thompson, 1994). High gene flow by hosts, on the other hand, has been shown by models (Gandon et al., 1996; Morand et al, 1996) and empirical data (Imhoof and Schmid-Hempel, 1998; Delmotte et al., 1999; Kaltz et al., 1999; Oppliger et al., 1999) to decrease adaptation of parasites to local host genotypes. This is thought to result

from the introduction of novel host alleles that can counterbalance or even reverse the advantage that parasites have over their hosts due to localized selective pressures (Thompson, 1994; Lively, 1999). Confounding the above, however, is the role played by parasite virulence. For example, Lively (1999) used a model to show that parasites with low virulence are likely to be maladapted to their hosts, regardless of levels of migration. On the other hand, parasites with high virulence are likely to be locally adapted to their hosts, regardless of migration rates. Clearly, the extent to which parasites adapt to their hosts is a complex phenomenon depending on the interaction of numerous factors.

Infectivity of Parasitic Plants

Very few studies have examined patterns of infectivity of parasitic plants to different host species. Nonetheless, these studies have provided evidence both supporting and refuting the existence of races that are adapted to infecting specific hosts. For example, infection experiments have provided evidence for host specific races of *Phoradendron tomentosum* Engelm. ex A. Gray (Clay et al., 1985) and *Striga hermonthica* Benth. (Mbwaga and Obilana, 1993; Freitag et al., 1996; Olivier et al., 1998). On the other hand, Overton (1994) found no evidence for infective races of *Phrygilanthus sonorae* A. Wats.

Within a host species, geographic patterning to infectivity by parasitic plants is poorly understood. Only one study has explicitly addressed this issue. Mutikainen and coworkers (2000) examined adaptation of a hemiparasitic plant *Rhinanthus serotinus* Oborny to local genotypes of *Agrostis capillaris* L. In this study, there was no evidence for local adaptation of *R. serotinus* to sympatric *A. capillaris* populations. Other experiments examining infectivity of parasitic plants to different host genotypes have not

been properly designed to address local adaptation. These experiments tended to utilize parasitic plants from geographic origin that do not correspond with that of the host (Sillero et al., 1996; Lane et al., 1996).

Infectivity of members of the Genus Arceuthobium

A limited number of studies have been specifically designed to assess adaptation of *Arceuthobium* spp. to specific host taxa or local genotypes within host taxa. Only two studies have examined infectivity of parasitic plants in the genus *Arceuthobium* to different experimental host species. Infectivity of *Arceuthobium douglasii* Engelm. was examined on two principal host taxa and was found to be most infective to the principal host from which it was obtained (Smith, 1974). This implied that host specific races may exist in *A. douglasii.* Smith and Wass (1979) showed a similar pattern for *Arceuthobium tsugense* (Rosendahl) G.N. Jones since it was found to be more infective to the principal host from which it was obtained than to other principal and non-principal hosts. However, these same researchers (Smith and Wass, 1979) showed the opposite pattern for *A. americanum* since it was found to be more infective to *Pinus contorta* Douglas ex Loudon var. *contorta* than to the host from which it was isolated (*P. contorta* var. *latifolia* Engelm.).

What patterns of infectivity might be expected of dwarf mistletoes at the local level within a given host species? Edmunds and Alstad (1978) proposed that dwarf mistletoes would show strong adaptation to local host populations and individuals due to their association with toxin-defended, long-lived hosts. Toxins including various types of terpenes, as well as phenolics such as lignins and tannins, are thought to be involved in plant defense against various parasite species (Agrios, XXXX). Unfortunately, studies

examining infectivity of *Arceuthobium* spp. on different genotypes within a host taxon have not been designed to address questions related to local adaptation since the parasites and experimental hosts used in these studies were of different geographic origin (Smith and Wass, 1979; Scharpf, 1987; Scharpf and Roth, 1992; Scharpf et al., 1992; Smith et al., 1993).

Recent theoretical models suggest that numerous factors should be considered before making any predictions about infectivity of parasites. These include considerations of evolutionary rate of the parasite and its host, the extent of coevolution between parasite and host, migration rates and level of virulence.

In the *Arceuthobium* - conifer pathosystem, both life history characteristics (e.g. generation time) and molecular data (Nickrent and Starr, 1994) suggest that *Arceuthobium* spp., like other parasites, have a higher evolutionary rate than their hosts. However, the extent to which the host and parasite are coevolving in the *Arceuthobium* - conifer pathosystem is uncertain. Several factors may decrease a tight coevolutionary link in this system. Firstly, *Arceuthobium* spp. are commonly found associated with several principal host taxa, often within close proximity of each other. High gene flow between proximate populations may decrease the ability of *Arceuthobium* spp. to coevolve with any one principal host taxon. Secondly, the long-lived tree hosts of dwarf mistletoe are unique in that they are attacked by a wide range of dependent species in their lifetime (Linhart, 1989, 1991). Therefore, the complex patterns of selection driven by these multispecies interactions may decrease the influence played by one particular dependent species (Linhart, 1991). Migration rates in this pathosystem may also differ from that seen in many other pathosystems. In general, parasites are thought to migrate

more than their hosts (Kaltz and Shykoff, 1998). In the *Arceuthobium* - conifer pathosystem, however, hosts likely migrate more than their parasites due to the prevalence of long distance dispersal of gymnosperm pollen by wind (Ledig, 1998). This high outcrossing in the hosts has the potential to decrease adaptation between local host and parasite populations. Finally, the pathological effects (i.e. virulence) of *Arceuthobium* on its hosts may be very important for determining the outcome of host-parasite interactions. Host cone and seed production, as well as seed germination, are reduced in a number of different *Arceuthobium* - conifer interactions (reviewed in Hawksworth and Wiens, 1996). Whether this virulence is sufficient to overcome high migration by hosts is unclear.

1.3 THESIS GOALS

This thesis is divided into three separate studies (Chapters 2, 3, and 4), each utilizing an unique approach to understanding the evolutionary pressures that have shaped populations of *Arceuthobium americanum* Nutt. ex Engelm. Each chapter begins with an introduction to the problem and a statement of specific objectives for the study. Introductions are followed by Materials and Methods, Results, Discussion and Conclusions. The broad statement of goals for each of these chapters is presented below. Chapter 5 presents a general discussion that draws from information provided by the earlier chapters.

THESIS GOAL 1.

The first study (Chapter 2) examines the population genetic structure of *Arceuthobium americanum* using the molecular technique, amplified fragment length polymorphism (AFLP) analysis. The level and distribution of genetic variation in *A. americanum* as characterized by AFLP analysis are examined with the explicit goal of determining the forces that have shaped its popluation structure. The taxonomic and evolutionary implications of findings from this study are also discussed.

THESIS GOAL 2.

The second study (Chapter 3) describes the use of AFLP markers to examine the population genetic structure of three principal hosts of *Arceuthobium americanum* (*Pinus banksiana* Lamb., *Pinus contorta* var. *latifolia* Engelm., and *Pinus contorta* var. *murrayana* (Greville and Balfour) Engelm.). The population genetic structures of the

parasite and its hosts are compared with each other and with geographic distance in order to assess the relative influence of host identity and genotype, as well as isolation-by-distance in shaping the broad- and fine-scale genetic structure of *A. americanum*.

THESIS GOAL 3:

The third and final study (Chapter 4) uses infection experiments to assess adaptation of *Arceuthobium americanum* to different principal host taxa and genotypes. The infection experiment at the Pine Ridge Forest Nursery in Alberta was designed to assess whether genetic races of *A. americanum* are coincident with infective races that have adapted to infecting specific host taxa or genotypes within these taxa. Such information can provide insight into the role that hosts have played in race formation and in shaping the fine-scale genetic structure of *A. americanum*. The infection experiment at the Petawawa Research Forest in Ontario was designed to assess the ability for this parasite to expand onto hosts beyond its current geographic limit. The implications of findings from both of these studies are also discussed in the context of forest management.

Table 1. Documented cases of parasitism in angiosperms using the classification of Cronquist, 1981 (modified from Nickrent and Franchina, 1990; additional information from Press and Graves, 1995; Mabberley, 1997; Punter, 1999 pers. comm.).

Class Magnoliopsida (Dicots)	Number of Genera/Species	Type of Parasite
A. Subclass Magnoliidae		
Order 1. Laurales		
Family 1. Lauraceae	1(20)	Obligate Stem Hemiparasite
B. Subclass Rosidae		
Order 1. Santalales		
Family 1. Olacaceae	22(200)	Facultative Root Hemiparasites
2. Opiliaceae	9(30)	Obligate Root Hemiparasites
3. Santalaceae	30(480)	Facultative Root Hemiparasites
4. Misodendraceae	1(10)	Obligate Shoot Hemiparasites
5. Loranthaceae	74(700)	Obligate Stem Hemiparasites Facult./Obligate Root Hemiparasites
6. Viscaceae	7(350)	Obligate Shoot Hemiparasites
7. Eremolepidaceae	3(11)	Obligate Shoot Hemiparasites
8. Balanophoraceae	18(47)	Obligate Root Holoparasites
Order 2. Rafflesiales		
Family 1. Hydnoraceae	2(10)	Obligate Root Holoparasites
2. Mitrastemonaceae	1(2)	Obligate Root Holoparasites
3. Rafflesiaceae	7(50)	Obligate Root Holoparasites
Order 3. Polygales		
Family 1. Krameriaceae	1(15)	Facultative Root Hemiparasites
C. Subclass Asteridae		
Order 1. Solanales		
Family 1. Cuscutaceae	1(150)	Obligate Stem Holoparasites
Order 2. Lamiales		
Family 1. Lennoaceae	3(5)	Facultative Root Hemiparasites
Order 3. Scrophulariales		
Family 1. Scrophulariaceae	30(1500)	Facult./Obligate Root Hemiparasites Obligate Root Holoparasites
2. Orobanchaceae	17(150)	Obligate Root Holoparasites

CHAPTER 2: AFLP Marker Analysis Reveals Evidence for Three Genetic Races of the Parasitic Angiosperm, *Arceuthobium americanum*

2.1 INTRODUCTION

Speciation via race formation is thought to be one of the major evolutionary processes in parasites (Price, 1980; Thompson, 1994; Norton and Carpenter, 1998). Race formation occurs when the gene flow between conspecific populations is diminished due to factors such as isolation-by-distance (i.e. geographic isolation) (Templeton, 1981; Orr and Orr, 1996; Via et al., 2000) and ecological adaptation to different environmental conditions or host types (Thompson, 1994; Schluter, 1996, 1998; Via et al., 2000).

Understanding the population genetic structure of parasites is important for several reasons (reviewed in Chapter 1). Firstly, this information provides insight into patterns of diversity within and between conspecific populations. Next, genetic structure can be used to understand the evolutionary forces and historical events that have shaped these patterns. Finally, knowledge of population genetic structure provides insight into the potential for interpopulation differentiation, race formation, and speciation (i.e. incipient speciation).

At the present time, the evolutionary forces acting on parasitic plants in the genus *Arceuthobium* are not well understood. Studies on population level genetic diversity in the genus *Arceuthobium* have been restricted to members of the section *Campylopodum*, a clade that is genetically and morphologically distinctive from other *Arceuthobium* spp. (Nickrent and Butler, 1990, 1991; Nickrent and Stell, 1990). Secondly, the *Arceuthobium* taxa examined to date have relatively limited geographic ranges in

comparison with other members of the genus. Thus, the full impact of isolation-by-distance and adaptation across a wide latitudinal and longitudinal range may not be fully understood. Thirdly, only one study (Nickrent and Stell, 1990) has examined a species (*A. tsugense*) currently found in areas that were both glaciated (on-ice) and unglaciated (off-ice) during Pleistocene glaciations. Previous studies on other plants (Hawley and DeHayes, 1994; Lewis and Crawford, 1995; Broyles, 1998; Ford et al., 1998; Wallace and Case, 2000) have shown, however, that Pleistocene glaciations can have a significant impact on the genetic structure of northern species. Finally, past studies of *Arceuthobium* have been taxonomic rather than evolutionary in focus (Nickrent and Butler, 1990, 1991; Nickrent and Stell, 1990). Thus, the evolutionary impact of geographic isolation, host adaptation, and historical events has not been fully addressed for members of this genus.

In order to gain a better understanding of evolutionary forces acting on plants in the genus *Arceuthobium*, the population structure of *Arceuthobium americanum* M. Von Bieb. (subgenus *Arceuthobium*, section *Americana* Nickrent) has been assessed. Despite the fact that this species has the most extensive range of any North American dwarf mistletoe, it has been the subject of surprisingly limited genetic and taxonomic research.

Arceuthobium americanum has the widest distribution of any N. American dwarf mistletoe with a latitudinal distribution of 2,800 km, a longitudinal distribution of 2,400 km, and an elevational range from 200 m to 3,350 m above sea level (Figure 1a). This parasite is capable of naturally infecting 14 different hosts in the family Pinaceae (Hawksworth and Wiens, 1996). The three principal hosts of *A. americanum* are *Pinus banksiana* Lamb. (a pine that is found throughout most of Canada and northeastern U.S.A.), *Pinus contorta* var. *latifolia* Englem. (a pine that is found in western Canada and

the western U.S.A.) and *Pinus contorta* var. *murrayana* (Greville and Balfour) Englem. (a pine that is found in the western U.S.A.) (Figure 1b). In addition to these principal hosts, *A. americanum* is capable of infecting a secondary host (*Pinus ponderosa* var. *scopulorum* Engelm.), four occasional hosts (*Pinus albicaulus* Engelm., *Pinus flexilis* E. James, *Pinus jeffreyi* Greville and Balfour and *Pinus ponderosa* Douglas ex Lawson and C. Lawson var. *ponderosa*), and six rare hosts (*Pinus aristata*, *Picea engelmanii*, *Picea glauca*, *Picea mariana*, *Picea pungens*, and *Pseudotsuga menziesii*).

Based on the lack of apparent morphological, physiological and phenological differences between populations of *A. americanum* on its different hosts, Hawksworth and Wiens (1996) found no evidence for recognizing infraspecific taxa within this species. Additionally, a preliminary isoenzyme study by Linhart (1984) found no evidence for genetic differentiation between *A. americanum* isolated from its principal host, *Pinus contorta* var. *latifolia,* and its secondary host, *Pinus ponderosa* var. *scopulorum,* where the two occur sympatrically. However, this study (Linhart, 1984) was limited in scope since only one isoenzyme locus was examined from *A. americanum* individuals from hosts in a single stand. As well, only one of the three principal hosts to *A. americanum* was examined. Previous taxonomic studies (Nickrent and Butler, 1990, 1991; Nickrent and Stell, 1990) have detected cryptic taxa within several *Arceuthobium* spp. Therefore, such patterns might also be expected in *A. americanum* as a result of adaptation to different principal hosts and variable environmental conditions throughout its range.

This is the first of three studies that examine the evolutionary forces acting on *A. americanum*. In this initial study, the genetic population structure of *A. americanum* has

been examined using a molecular marker, amplified fragment length polymorphism (AFLP) analysis.

The objectives of this study were to:

(1) Assess the pattern of genetic diversity of *A. americanum* within and between conspecific populations over its entire geographic range;

(2) Determine if there is a correlation between genetic distance and geographic distance between conspecific *A. americanum* populations;

(3) Examine for evidence of distinct genetic races of *A. americanum* that specialize on different hosts; and

(4) Elucidate factors responsible for the observed genetic structure in *A. americanum*.

2.2 MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Plant tissue was collected from 51 populations of *Arceuthobium americanum* throughout its geographic range (Table 2; Figure 1a). For each population, twigs infected with dwarf mistletoe shoots were pruned from a single witches' broom on approximately 10 different trees. Prior to DNA extraction, plant tissue was treated in one of two ways; tissue was either: (1) stored fresh at -80°C prior to being dried in a tissue lyophilizer (Dura-stop MP / Dura-Top MP; FTS Systems - Lifescience Division) and stored in Dierite® dessicant (size 8 mesh); or (2) dried in the field by placing plant material directly into bags containing Silica Gel® dessicant (28-200 Mesh, Avg. Pore diameter 22Å, Sigma S-4883) at the time of collection. A herbarium specimen representative of each population was collected and deposited in the University of Manitoba herbarium (WIN) as a permanent record.

DNA EXTRACTION

Arceuthobium americanum DNA was extracted using the DNeasy Plant Mini Kit (Qiagen 69106). Protocols from the kit were followed with a few modifications: (1) only 5 mg of lyophilized tissue was used since DNA became sheared when larger amounts of plant material were loaded onto the columns; (2) to increase yield, DNA was eluted from columns twice with 150 µL of pre-heated AE buffer; and (3) DNA was concentrated by an overnight EtOH precipitation and resuspended in 30 µL of sterile dH_20.

AFLP ANALYSIS

The AFLP procedure was carried out using MseI and EcorR1 restriction enzymes as per Zabeau and Vos (1993) and Vos et al. (1995) with some modifications. Since

silver staining was used to visualize AFLP bands on gels, it was unnecessary to biotinylate or radioactively label the adapters (Ma1.1, Ma1.2, Ea1.1, Ea1.2; see Table 3). Pre-amplification PCR for *Arceuthobium* was performed using a +1/+1 pre-amplification primer combination (M-C and E-A; see Table 3). Two μL of the diluted ligated DNA sample was added to each pre-amp. reaction tube to a total volume of 25 μL. Reaction tubes contained 30 ng pre-amp. primers, 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 1.0 units of Taq polymerase. Denaturation was at 94.0°C for 30 seconds, followed by primer annealing at 56.0°C for 1 minute, and primer extension at 72.0°C for 1 minute. This cycle was repeated 20 times and the pre-amplification PCR product was diluted 1 in 4 with sterile distilled water.

Selective PCR amplification for all 51 populations of *Arceuthobium* was performed using two +3/+3 primer combinations: (1) M-CAC, E-ACG; and (2) M-CCG, E-ACA (see Table 3). A preliminary analysis using five +3/+3 primer combinations for selective PCR amplification was performed across 32 populations of *Arceuthobium*. This preliminary analysis, yielding 178 loci across this population subset, showed the same overall topology (not shown) as that obtained with two primer combinations. Thus, further analyses were restricted to loci obtained from the two primer combinations described above. The selective PCR amplification mix was the same as for the pre-amplification with the exception that 2 μL of diluted preamplification DNA was used and the final volume of the selective amplification reaction was 20 μL. Denaturation was at 94.0°C for 30 seconds, followed by initial primer annealing at 65.0°C for one minute, and primer extension at 72.0°C for 1 minute. With each successive cycle, the primer

annealing temperature was ramped down by 0.7°C until reaching 56.0°C. At this point, 22 cycles were carried out holding the primer annealing temperature constant at 56.0°C.

AFLP products were run on 5% polyacrylamide gels. Following electrophoresis, gels were fixed on the glass plate in 10% acetic acid for 20 minutes. Silverstaining of gels was performed using the Silver Sequence™ DNA Sequencing System kit (Promega) with the following procedural modification to minimize background staining. Following 30 minutes of silver staining, the gel was briefly dipped into an ultrapure water rinse and then transferred through two separate developer solutions (pers. comm., E. Reimer, Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba). The gel was placed into the first developer solution and rocked back and forth until the first hint of bands appeared on the gel. The gel was then quickly transferred into the second developer solution and agitated until the AFLP loci appeared as dark bands. Gels were fixed in acetic acid, air-dried overnight, and scored on a light box for presence or absence of bands across individuals. Both monomorphic and polymorphic loci were included in the analysis. Loci and individuals considered to be ambiguous following scoring by two independent researchers were excluded from the analysis. Any bands that could not be resolved were recorded as missing data. As well, 100ng of pGEM marker (Promega), and representatives from several disparate populations were included on each gel in order to confirm band position. Gels were photocopied and/or scanned for preservation.

DATA ANALYSIS OF *ARCEUTHOBIUM* AFLP

In order to calculate genetic distance and diversity measures, two approaches were used. The first approach was based on Nei's unbiased distance and diversity measures (Nei, 1978). In this case, the frequency of presence/absence bands were first

adjusted using the correction factor (see below) proposed by Lynch and Milligan (1994) for dominant markers:

$$q = x^{1/2}\left(1 - ((1-x)/8Nx)\right)^{-1}$$

$$p = 1 - q$$

where q is the corrected estimate of frequency of the null allele, x is the observed frequency for the null allele, N is the sample size, and p is the corrected estimate of the presence allele. This Lynch-Milligan (L-M) correction factor assumes Hardy-Weinberg equilibrium to correct for problems associated with dominant marker systems (such as AFLP and RAPDs) that underestimate null allele frequencies and overestimate presence allele frequencies due to the masking of null alleles in the heterozygous state. The assumption of Hardy-Weinberg equilibrium is reasonable since species are dioecious (Hawksworth and Wiens, 1996) and genetic studies (Nickrent and Butler, 1990; Nickrent and Butler, 1991) have shown similarity between observed and expected heterozygosity levels of several *Arceuthobium* spp.

Nei's unbiased genetic distances/identities (Nei, 1978), proportion of polymorphic loci, expected heterozygosities (H_{exp}) (Nei, 1978), and an UPGMA (unweighted pair-group method of analysis) phenogram were determined from the L-M corrected allele frequencies among 51 *A. americanum* populations using BIOSYS-2 (Swofford and Selander, 1992). This program was recompiled by Dr. Kermit Ritland (Department of Forest Sciences, University of British Columbia, Vancouver, B.C.) to handle 200 loci and 100 populations. Since H_{obs} could not be observed due to the dominant nature of the marker system (AFLP) used in this study, it is not possible to comment on the fixation index, F, that would provide insight into the mating strategy in effect in these

populations.

Total genetic diversity (H_T), average diversity within (H_S) and among populations (D_{ST}), and the coefficient of genetic differentiation (G_{ST}) were calculated using Nei and Chesser's (1983) procedure (unbiased for sample size) using the output from BIOSYS-2. These measures were examined for *A. americanum* as a whole, and then for six groups of *A. americanum* depending on identity of the host species (see Table 2): (1) *Pinus banksiana*; (2) *Pinus contorta var. latifolia*; (3) hybrid *P. banksiana* X *P. contorta* var. *latifolia*; (4) *Pinus contorta* var. *murrayana*, (5) *Pinus jeffreyi*; and (6) *Pinus ponderosa*. In addition, these measures were examined for two groups of *A. americanum* on *Pinus contorta* var. *latifolia* (those from previously glaciated regions in Canada and those from non-glaciated regions in the U.S.A.) to examine the impact of Pleistocene glaciation on these populations.

Since there is concern about whether the assumption of Hardy-Weinberg equilibrium used in the Lynch-Milligan correction factor is justified when characterizing population structure with dominant markers (Travis et al., 1996; Schmidt and Jensen, 2000) and the accuracy of estimating population allele frequencies with small sample sizes (Lynch and Milligan, 1994), a second approach was also employed for analyzing the data. This involved using an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992). This procedure was performed both with and without group structure to the populations. To perform this analysis, genetic similarity between individuals are first calculated as Euclidean distances from the plus/minus marker data. The AMOVA procedure then calculates variance components and pairwise F_{ST} values for population from the pairwise Euclidean distances between individuals. Pairwise population F_{ST}

values (equivalent to ϕ_{ST} from WINAMOVA, reference) were then used as a measure of genetic distance between populations (Huff et al., 1993). Euclidean distances and AMOVA were calculated using the software program ARLEQUIN (Schneider et al., 2000). An UPGMA from these F_{ST} distance values between populations was then determined using SYNTAX 5.0 (Podani, 1997).

Chord distances (Cavalli-Sforza and Edwards, 1967) and Wagner trees (Farris, 1972) were also examined but are not presented in this paper since they yielded the same general topology as that seen for UPGMA cluster analysis of Nei's unbiased genetic distances. As well, UPGMA cluster analysis of Jaccard (Sneath and Sokal, 1973) and Simple-match (Sokal and Michener, 1958) coefficients were used to examine the genetic distance between all individuals. The resulting dendrograms are not presented here due to the large size of the trees (≈500 individuals) and because they yielded the same general topology as that seen for UPGMA trees of Nei's and Fst genetic distances of populations.

In addition to cluster analyses, genetic structure of Nei's and Fst genetic distances were analyzed using Nonmetric multidimensional scaling (NMDS). NMDS is a non-parametric method suitable for displaying genetic structure of similarity or dissimilarity matrices. This method is particularly effective in depicting genetic relationship among populations when no distinct groupings are present. NMDS was used to examine geographic patterning within the groups (as defined by host species identity). Only groups of *A. americanum* populations from *P. banksiana* or *P. contorta* var. *latifolia* hosts were considered large enough to perform analyses. NMDS ordinations were performed using the program ORDIN of SYNTAX v.5.1 (Podani, 1997)

2.3 RESULTS

The two primers used in this analysis yielded 100 scorable loci ranging in size from 100 to 1,100 bp. Of these loci, 85 were polymorphic and 15 were monomorphic.

GENETIC STRUCTURE OF POPULATIONS AND DETECTION OF RACES

The dendrogram (Figure 2) created by UPGMA cluster analysis of Nei's unbiased genetic distance values (Nei, 1978) (Appendix 1) revealed that *A. americanum* populations were divided into three distinct genetic races, each associated with a different principal host taxon in regions of allopatry: (1) *P. banksiana* in western and central Canada; (2) *P. contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountain ranges; and (3) *P. contorta* var. *latifolia* throughout western U.S.A. and Canada. A couple of exceptions to this pattern were evident in the analysis. Firstly, the six *A. americanum* populations isolated from *P. banksiana* X *P. contorta* var. *latifolia* hybrid hosts in Alberta (AB-17, AB-18, AB-19, AB-20, AB-21 and AB-22) were found in variable positions in the dendrogram. Next, the *A. americanum* population isolated from *P. contorta* var. *latifolia* hosts in the Cypress Hills (AB-26) was associated with *A. americanum* populations isolated from *P. banksiana* hosts. Finally, populations of *A. americanum* that were isolated from both a principal host (*P. contorta* var. *latifolia* or var. *murrayana*) and a secondary (*P. ponderosa* var. *scopulorum*) or an occasional (*P. jeffreyi*) host from the same stand were not well-differentiated from each other (Figure 2). For example, the two *A. americanum* populations isolated from *P. ponderosa* var. *scopulorum* did not cluster together, but did cluster closely with *A. americanum* from their sympatric principal host population.

A dendrogram (Figure 3) based on UPGMA cluster analysis using F_{ST} genetic

distances (Excoffier et al., 1992) derived from AMOVA (Appendix 2) also depicted three groups separated on the basis of host identity. As was previously observed, the *A. americanum* populations isolated from secondary or occasional hosts did not form distinctive races. Overall, differences in the placement of populations between the two dendrograms were minor. Only populations from Cypress Hills (AB-26) and from hybrid hosts (AB-17 to AB-22 inclusive) showed dramatic differences in placement between the two analyses. In the UPGMA based on Fst genetic distance, the population from Cypress Hills was found with other populations from the same (*P. contorta* var. *latifolia*) host. The six *A. americanum* populations isolated from hybrid hosts in Alberta were found in variable but different positions throughout the dendrogram.

The genetic identities within the three *A. americanum* races (0.941 - 0.970) were greater than those observed between races (0.868 - 0.908) (Table 4). These high identities indicated the cohesiveness of these races. *Arceuthobium americanum* populations from hybrid hosts showed higher identities to populations from *P. banksiana* (0.914) and *P. contorta* var. *latifolia* (0.916) than with each other (0.898). As well, *A. americanum* populations from secondary or occasional hosts showed high identity with populations on sympatric principal hosts.

Five unique alleles were found in the *A. americanum* race associated with *P. contorta* var. *murrayana* that were not found in the other two races. Nine unique alleles were found in the *A. americanum* races on *P. banksiana* and *P. contorta* var. *latifolia* that were not found in the race associated with *P. contorta* var. *murrayana.* Finally, six unique alleles were found in the *A. americanum* races associated with *P. contorta* var. *latifolia* and var. *murrayana* that were not found in the race on *P. banksiana.*

GEOGRAPHIC PATTERNING WITHIN RACES

Both dendrograms and NMDS ordinations (Figures 2 - 4) depicted a general lack of geographic patterning among *A. americanum* populations from *P. banksiana* hosts. Populations isolated from different provinces often grouped together. Similarly, a lack of fine-scale patterning was also observed among populations of *A. americanum* isolated from *P. contorta* var. *latifolia* (Figures 2, 3 and 5). However, at the broader level, geographic patterning was present in this latter group of populations since they could be split into two subgroups: (1) northern var. *latifolia* populations from previously glaciated regions in Canada (BC-23, AB-24, BC-25, AB-26, AB-27, BC-28, AB-29, BC-30, BC-31, BC-32, BC-33); and (2) southern var. *latifolia* populations from unglaciated regions in the U.S.A. (ID-34, WY-35, CO-36, OR-38, CO-39, ID-40, UT-41, CO-42, CO-43, WY-44, UT-45, CO-46). Those few populations that did not consistently fall within the expected subgroup using cluster analysis were clarified by ordination analyses. Only one population from Idaho city (ID-37) was not placed with the expected subgroup using either ordinations or cluster analysis. Ordination analysis was also useful for resolving the placement of *A. americanum* from Cypress Hills (AB-26) as part of the *P. contorta* var. *latifolia* group. This population had been placed with populations isolated from *P. banksiana* hosts using UPGMA cluster analysis of Nei's unbiased genetic distances.

POPULATION LEVEL GENETIC VARIABILITY MEASURES

Genetic variability measures ranged considerably across populations (Table 5). The lowest variability measures were observed for *A. americanum* populations isolated from *P. banksiana* (34 - 51 % poly., H_{exp} 0.127 - 0.165) and hybrids (21 - 52 % poly., H_{exp} 0.059 - 0.185). Higher variability measures were observed for *A. americanum*

populations isolated from *P. contorta* var. *latifolia* (45 - 65 % poly., H_{exp} 0.157 - 0.220), *P. contorta* var. *murrayana* (49 - 55 % poly., H_{exp} 0.147 - 0.201), *P. ponderosa* var. *scopulorum* (54 % poly., H_{exp} 0.109 - 0.185), and *P. jeffreyi* (59 % poly., H_{exp} 0.189).

The three populations from hybrid hosts in northern Alberta (AB-18, AB-21 and AB-22) were genetically depauperate, having a significantly lower proportion of polymorphic loci (26.33 ± 4.73% poly.) ($P<0.05$) and genetic variability (H_{exp} 0.085 ± 0.023) ($P=0.02$) than all other populations (49.11 ● 7.92% poly., H_{exp} 0.174 ● 0.024). As well, those populations from northern hybrid hosts were considerably less variable than populations isolated from the more southerly hybrid hosts in central Alberta (AB-17, AB-19, and AB-20) (48.67 ± 3.51% poly., $P=0.007$; H_{exp} 0.181 ± 0.005, $P=0.020$). Populations with the highest variability were found within the *P. contorta* var. *latifolia* host group. Indeed, the populations from Kamloops (BC-30) and Jasper (AB-29) had the highest genetic variability (62.0 ± 4.24% poly., H_{exp} 0.218 ● 0.004, $P<0.0001$) compared with all other populations (49.04 ● 7.88% poly., H_{exp} 0.167 ± 0.032).

GENETIC DIVERSITY AMONG POPULATIONS

Overall Diversity

When all populations of *A. americanum* were considered as a single taxon, Nei and Chesser's (1983) diversity measures indicated that *A. americanum* was quite variable with H_T of 0.238, H_S of 0.170, D_{ST} of 0.068, and G_{ST} of 0.286 (Table 6). This indicates that 71.4% of the genetic variation was found within populations and 28.6% was found between populations. Results of the AMOVA are provided in Table 7. As with Nei's statistics above, the AMOVA indicated that considerably more of the variance was found within the populations (69.70%) than between populations (30.30%) ($p<0.001$) (Table 7).

Group Diversity

When *A. americanum* populations were divided into groups based upon host identity, the following was observed. Nei and Chesser's (1983) genetic diversity statistics (Table 6) indicated that *A. americanum* populations on *P. banksiana* had a low total genetic diversity (H_T 0.198) compared with that observed in *A. americanum* as a whole (H_T 0.238). Those populations from *P. contorta* var. *latifolia* hosts had the highest H_T (0.296). Average genetic diversity values (H_S) within the *A. americanum* groups ranged from 0.133 and 0.152 for hybrid and *P. banksiana* hosts, respectively, to 0.180 and 0.189 for *P. contorta* var. *murrayana* and var. *latifolia* hosts, respectively. Indeed, the average genetic diversity within the *A. americanum* on *P. banksiana* group was significantly lower (H_S 0.152 ● 0.016, $P<0.0001$) than that for *A. americanum* on *P. contorta* var. *latifolia* and var. *murrayana* (H_S 0.187 ± 0.020). Population differentiation values (G_{ST}) ranged from the lowest for *A. americanum* from *P. ponderosa* var. *scopulorum* and *P. contorta* var. *murrayana* hosts (0.044 and 0.095, respectively) to 3.5 - 8X higher from hybrid and *P. contorta* var. *latifolia* hosts (0.351 and 0.361, respectively). When the six host groups were examined using a nested AMOVA, most of the variance (65.92%) remained within the populations, 18.35% was found among the six groups, and 15.74% was found among the populations within these six groups ($P<0.001$) (Table 7).

Arceuthobium americanum populations isolated from *P. contorta* var. *latifolia* hosts from Canada (previously glaciated) were more genetically diverse (H_S 0.198 ● 0.017) than those from the U.S.A. (non-glaciated regions) (H_S 0.180 ± 0.018) ($P<0.0001$). However, populations from the unglaciated regions showed a similar level of differentiation ($G_{ST} = 0.142$) as did those from glaciated regions ($G_{ST} = 0.139$).

2.4 DISCUSSION

FACTORS INFLUENCING OVERALL GENETIC STRUCTURE

Several factors have likely contributed to the overall genetic structuring of *A. americanum*. These factors include host species identity, competitive exclusion by other *Arceuthobium* spp., geographic isolation, climate, and historical factors.

Role of Principal Hosts

Researchers have suggested that pressures imposed by different hosts have played a major role in the evolutionary diversification of *Arceuthobium* (Nickrent and Butler, 1990; Hawksworth and Wiens, 1996). Principal host species identity seems to play a role in delimiting the population structure of *A. americanum* since the three races (*P. banksiana* race, *P. contorta* var. *latifolia* race, and *P. contorta* var. *murrayana* race) can be easily defined by this parameter. These races are clearly delineated from each other as can be seen by: (1) high genetic identities within, compared to between, these groups; (2) the presence of three distinctive clusters in the dendrograms based upon these genetic identity values; and (3) the presence of unique alleles associated with these races. Different host species are often thought to represent unique environments to which a parasite must adapt (Thompson, 1994; Brooks and McLennan, 1996). It seems likely, therefore, that the divergent selection pressures imposed by the three different hosts have favoured certain parasite genotypes. These pressures could have contributed to the formation of three races of *A. americanum* that are genetically distinct from each other.

Role of Non-principal Hosts

Cluster analysis using AFLP data indicated that *A. americanum* has not undergone race formation on *P. jeffreyi* and *P. ponderosa* var. *scopulorum*. The high genetic

identity between *A. americanum* populations found on these non-principal hosts and their sympatric principal hosts indicates that gene flow is high between these populations. Therefore, these non-principal hosts do not seem to influence the population structure of *A. americanum*. This observation may relate to a more recent jump onto these hosts compared to their relatively ancient association with the principal hosts.

The development and spread of *A. americanum* races adapted to non-principal hosts may also be limited by competitive exclusion by other *Arceuthobium* taxa that are already adapted to these hosts. For example, *A. americanum* may be limited from adapting to its secondary host *P. ponderosa* var. *scopulorum* Engelm. due to competition with the major parasite of this host, *A. vaginatum* subsp. *cryptopodum* (Engelm.) Hawksw. and Wiens. Indeed, Hawksworth (1969) showed that infection of *P. ponderosa* var. *scopulorum* hosts by *A. americanum* was 64% in populations where *A. vaginatum* subsp. *cryptopodum* was absent, but only 13% where *A. vaginatum* subsp. *cryptopodum* was present. A similar scenario may also exist for *Pinus jeffreyi* Greville and Balfour where *Arceuthobium campylopodum* Engelm. may competitively exclude *A. americanum* from adapting to this non-principal host.

Role of Geographic Isolation

In many non-parasites, isolation-by-distance leads to race formation due to the lack of cohesive forces that would result from gene flow (Orr, 1995; Orr and Orr, 1996). Given their generally allopatric distribution, geographic isolation may play an important role in the formation the three *A. americanum* races, especially in separating populations on *P. contorta* var. *murrayana* from those on var. *latifolia* (Figure 1a). For example, *A. americanum* populations from var. *murrayana* (CA-47, OR-48, CA-49, OR-50) are found

in the Sierra Nevada and Cascade Mountain ranges. Gene flow by pollen and seed dispersal would be restricted between these populations and those on *P. contorta* var. *latifolia* (CO-36, ID-37, OR-38, OR-39, ID-40, UT-41, CO-42, CO-43, WY-44, UT-45, CO-46) across the Great Basin in the Blue, Salmon River, Uinta, and Rocky Mountains (Figures 1a and 1b). Thus, geographic isolation likely accounts for some of the differentiation between *A. americanum* populations in these regions. The positioning of the *A. americanum* population isolated from *P. contorta* var. *latifolia* in John Day (OR-38) as an outlier to the var. *latifolia* host group does imply, however, that a certain degree of gene flow occurs between *A. americanum* populations found on these two varieties of *P. contorta*. This population is in an intermediate geographic position in the Blue Mountains between the Cascades and Rockies (see Figure 1a).

In Canada, gene flow between *A. americanum* populations on *P. banksiana* and *P. contorta* var. *latifolia* is not restricted by physical barriers such as mountain ranges. In fact, the two host species co-occur and hybridize in a zone in central Alberta (Critchfield and Little, 1966). Outside this hybridization zone, however, the host taxa are geographically separated, presumably due to different environmental preferences (see below). The *A. americanum* races are similarly geographically separated, with contact occurring only in the hybrid zone of Alberta. Thus, geographic isolation could account for some of the differentiation between *A. americanum* races on *P. contorta* var. *latifolia* and *P. banksiana*. Nonetheless, the hybrid zone likely allows for some gene flow across these two *A. americanum* races, as it does for the two hosts.

Although isolation-by-distance appears to play some role, the lack of geographic chaining and the existence of three distinctive clusters in the dendrogram despite the

sampling of *A. americanum* across an almost continuous region (Figure 1a) implies that geographic isolation does not account for all of the differentiation observed amongst the three *A. americanum* races.

Role of Climate

Climatic differences between the regions where the three *A. americanum* races are found may also play an important role in their differentiation. *Arceuthobium americanum* races may each be adapted to growing in the environmental conditions that characterize the regions for each of their principal hosts. *Pinus contorta* and *P. banksiana*, and hence their associated *A. americanum* races, are found in different ecoclimatic zones (Ecoregions Working Group, 1989; Rudolph and Liadly, 1990; Barbour and Christensen, 1993). For example, *P. banksiana* is found in the Boreal Forest which is characterized by extremes in temperature, short growing seasons, relatively dry conditions, and peak precipitation in the summer. *Pinus contorta* on the other hand is found in the Western Mountane Coniferous Forest which is characterized by less extremes in temperature, a longer growing season, wetter conditions, and peak precipitation in the winter. Based on finer scale environmental differences, this ecoclimatic zone is divided into smaller regions with var. *latifolia* being found in the Rocky Mountain Forest region and var. *murrayana* being found in the Pacific Northwest Montane Forest region. Given the broad climatic differences between the zones where these three hosts exist, it is feasible that the *A. americanum* races are responding to divergent selection pressures imposed by different environments rather than to pressures imposed by different hosts.

Role of Historical Events

Despite the strong group structure evident in *A. americanum*, some populations did not fall into the expected clusters predicted by host identity. This phenomenon can likely be explained by historical events. For example, past founder events may be responsible for the variable position of relatively isolated *A. americanum* populations (AB-18, AB-21, and AB-22) from hybrids in northern Alberta. New mutations and genetic drift acting on these initially small and isolated populations could differentiate these populations relative to others (Lewontin, 1965; Slatkin, 1987). On the other hand, populations of *A. americanum* isolated from hybrids in central Alberta (AB-17, AB-19, and AB-20) are not geographically isolated from other populations. However, their intermediate geographic position provides an opportunity for these populations to receive high gene flow from *A. americanum* populations found on either of the two surrounding principal host species (*P. banksiana* and *P. contorta* var. *latifolia*). This may account for the variable and often outlying position of these populations in the UPGMA dendrograms. As well, these parasite populations may be affected by host genotypes in this may impose diversifying selection pressures on parasites that attack them.

Due to the unique Pleistocene history and geographic position of the Cypress Hills in southeastern Alberta and southwestern Saskatchewan (Wheeler and Guries, 1982; Critchfield, 1985), historical migrations and geographic isolation may account for the variable position of *A. americanum* from the Cypress Hills (AB-26) in the two dendrograms. The 1500 m plateau that comprises the Cypress Hills is thought to have served as a refugium for *P. contorta* var. *latifolia* throughout most of the Wisconsin glaciation (Broscoe, 1965; Jungerius, 1966; Wheeler and Guries, 1982, 1987). It is

possible that *A. americanum* from the Cypress Hills has a similar history to its host, having survived the much of the Wisconsin in this refugium. In addition, plant communities in the Cypress Hills are isolated from similar vegetation by 300 - 500 km. This limits gene flow amongst plants in the Cypress Hills and those in other regions. Thus, newly arising mutations and genetic drift due to this geographic isolation have probably played a role in differentiating *A. americanum* from Cypress Hills (AB-26) from other *A. americanum* populations found on *P. contorta* var. *latifolia.*

FACTORS INFLUENCING STRUCTURING WITHIN *A. AMERICANUM* RACES

Animal Dispersal of Seeds

Relatively strong geographic patterning of genetic data would be expected if the primary mechanism for migration of *A. americanum* was explosive discharge of seeds from fruits. Such fine-scale geographic patterning was not observed in this study. This lack of patterning could be attributed to dispersal of seeds through stochastic transport on the feathers of birds or on the fur of small mammals. Aside from the molecular evidence provided by this study, seed dispersal by animal vectors is supported by several lines of evidence. For example, several studies have documented finding *A. americanum* seeds adhered to the feathers of birds and the fur of small mammals in and around infected stands (Hawksworth et al., 1987; Punter and Gilbert, 1989; Nicholls et al, 1984, 1989; Hawksworth and Geils, 1996). Additionally, animal vectors or 'freak' weather events must be responsible for the existence of satellite infection centres of *Arceuthobium* that are geographically isolated from other known infected stands (Hawksworth and Wiens, 1996). Finally, dispersal of *A. americanum* into regions previously covered by the Laurentide ice sheets has occurred at a rate of two orders of magnitude greater than can

be explained by explosive discharge of seed alone (Hawksworth and Wiens, 1996). Taken together, the ecological observations described above and the molecular evidence from this study strongly implicate animal vectors as having an important influence on the fine-scale genetic structure of *A. americanum* populations. Other researchers have suggested that rare events of animal dispersal can indeed have a significant impact on rates of plant migration (Clark et al., 1998) and patterns of genetic structure (Antonovics, 1968; Hamrick, 1987).

Given the importance of long-distance dispersal to the fine-scale structuring of *A. americanum*, why then does this mechanism not impact the cohesiveness of the three genetic races of this parasite? This may be related to several factors. Firstly, many migrating bird species are unlikely to traverse east/west across the Rocky Mountains. Next, even if seeds are dispersed across these regions, it is possible that the introduced seeds from different *A. americanum* races are unable to establish under strong selection pressures imposed by different hosts (but see Chapter 4) and environmental conditions.

Glacial History

The possible impact of Pleistocene glaciation on genetic structuring can be assessed by examining the northern (on-ice) and southern (off-ice) subgroups of *A. americanum* on *P. contorta* var. *latifolia*. It is possible that different origins following glaciation resulted in differentiation between these two subgroups. For example, northern populations may have been derived from a glacial refugium in Canada whereas southern populations likely remained relatively unchanged for thousands of years. Fossil evidence from *A. americanum* supports this idea since pollen from this species has been recorded as far north as 56°30 'N in the Banff-Jasper and Peace River areas in 7,000 to

10,000 year old sediments (White and Mathews, 1986; MacDonald, 1989). These refugial populations may have served as a centre of origin for the spread of *A. americanum* across Canada. On the other hand, populations south of the glacial front would have remained isolated from these northern populations, thereby following a different evolutionary path. The distinction between the north / south subgroups of *A. americanum* on *P. contorta* var. *latifolia* may alternatively reflect isolation-by-distance. However, since *A. americanum* populations are rather continuously distributed across the range of *P. contorta* var. *latifolia*, this seems unlikely. It is also possible that the pattern results from a sampling artifact related to the geographic distribution of sampled populations. Despite efforts to collect *A. americanum* evenly throughout its range, some gaps exist. For instance, *A. americanum* was not collected in northern Montana, Idaho or Washington. If populations were sampled from these regions, it is possible that the break between the northern and southern groups would be weaker.

Other Factors

Numerous other factors may influence the fine-scale patterning within *A. americanum* groups. These factors may include adaptation to local host genotypes or environmental conditions that are themselves not geographically patterned. These influences are examined in more detail in the second paper (Chapter 3) of this thesis by comparing the genetic structure of *A. americanum* with that of its *Pinus* hosts.

FACTORS INFLUENCING DIVERSITY / VARIABILITY IN *A. AMERICANUM*

Genetic Drift

The generally large population sizes and outcrossing nature of *A. americanum* should prevent most populations of this species from suffering loss of genetic diversity

due to random genetic drift or inbreeding. Findings from this study support this hypothesis since most populations are genetically diverse. However, the northern-most populations of *A. americanum* isolated from hybrid hosts (AB-18, AB-21, and AB-22) were genetically depauperate. This lack of diversity can likely be attributed to genetic drift, and provides support for the earlier hypothesis that these populations became established as a result of founder events.

Evolutionary Origin

Levels of genetic diversity within groups of *A. americanum* can provide insight into the evolutionary origin and the migratory history of this parasite. Since *A. americanum* is found throughout the range of *P. contorta* but only in the western-most portion of the range for *P. banksiana* (see Figures 1a and 1b), researchers have hypothesized (Hawksworth and Wiens, 1996) that this parasite originated on *P. contorta* before jumping onto *P. banksiana*. Findings from this study support the idea that *A. americanum* populations evolved on *P. contorta* since they were found to be more genetically diverse than those from *P. banksiana*. Conspecific populations with the highest genetic diversity are generally thought to represent the centre of origin for a taxon (Ruedi et al, 1996; Toumi and Lumaret, 1998).

Effects of Glaciation

The effect of glaciation on population structuring was considered earlier (pages 44 and 45). Here, the impact of glaciation on genetic diversity is discussed. In general, studies show higher genetic diversity in conspecifics or congeners from non-glaciated areas (off-ice) than from glaciated areas (on-ice) (Fowler and Morris, 1977; Copes, 1981; Waller et al., 1987; Lewis and Crawford, 1995; Broyles, 1998). Genetic diversity is

thought to be lost during the stepping-stone migration of individuals from glacial refugia in the south into newly deglaciated northern regions (reviewed in Lewis and Crawford, 1995 and Broyles, 1998). The finding in this study that *A. americanum* populations from *P. contorta* var. *latifolia* are more genetically diverse in glaciated (on-ice) than in unglaciated (off-ice) regions contradicts this expected pattern.

A few other studies (Hawley and DeHayes, 1994; Ford et al., 1998; Wallace and Case, 2000) have reported a similar pattern to that seen in *A. americanum*. These researchers have attributed these anomalous geographic patterns in the distribution of genetic diversity to several factors (reviewed in Wallace and Case, 2000). Firstly, Hawley and DeHayes (1994) proposed that the lower levels of genetic diversity in southern versus northern populations of *Picea rubens* Sarg. resulted from different origins for these two groups of populations. These researchers suggested that two separate glacial refugia, each harbouring different levels of genetic diversity, gave rise to the northern versus southern populations of *Picea rubens*. Ford and coworkers (1998) proposed that either postglacial range contractions in the south or pre-Laurentide patterns of genetic diversity could explain the higher diversity observed in species of *Carex* sect. *Phyllostachys* (J.Carey) L.H. Bailey that have undergone massive migrations following deglaciation compared with those that have persisted in southern glacial refugia. In the third study, Wallace and Case (2000) observed that *Cypripedium parviflorum* L. maintained more diversity in northern populations (previously glaciated) compared to conspecifics from southern populations (unglaciated regions). These researchers suggested the existence of a northern refugium for *C. parviflorum* in a periglacial environment. Alternatively, they suggested that populations in the southern states may

have remained relatively small and isolated throughout glaciation, thereby promoting genetic drift and loss of genetic variation.

Some of these hypotheses could explain the patterns of diversity seen in *A. americanum* from on-ice versus off-ice regions. As was previously discussed, pollen evidence supports the existence of a northern refugium for *A. americanum* in the eastern Rockies (White and Mathews, 1986; MacDonald, 1989). It is possible that this northern refugium acted as a source of genetically diverse individuals that could colonize newly deglaciated land. Alternatively, if *A. americanum* existed south of the glacial front as small populations that were subjected to genetic drift during the time of Pleistocene glaciations, this would explain lower genetic diversity in these populations. While the size of the conifer forests south of the glacial front during the Wisconsin glaciations (Critchfield, 1985) make it unlikely that this parasite existed in small and isolated populations, it is possible that repetitive range contractions during the advance and retreat in the Pleistocene could have reduced the genetic diversity in these off-ice populations.

FACTORS INFLUENCING DIFFERENTIATION AMONGST POPULATIONS

Several researchers (Price, 1980; Tybayrenc and Ayala, 1991; Maynard Smith et al., 1993) have suggested that parasites are likely to be characterized by high population subdivision, geographic differentiation and the existence of cryptic species due to host dependence, adaptation to local host genotypes, and restricted gene flow. In order to ascertain if *A. americanum* is more highly structured than other taxa, a comparison with non-parasitic plants must be made. In their review of >400 plant species, Hamrick and Godt (1990) found that breeding systems accounted for most of the genetic diversity observed amongst populations. Findings from this study show that *A. americanum* has

greater differentiation (G_{ST} 0.286) than other plant species with similar breeding systems (insect-pollinated taxa, G_{ST} 0.197; wind-pollinated taxa, G_{ST} 0.099; taxa with explosive discharge, G_{ST} 0.243; taxa with animal vectors, G_{ST} 0.257). In addition to isozyme data, estimates for population differentiation based on dominant markers are available for a number of plant species. Such a comparison supports the above observation that *A. americanum* populations are highly structured. AMOVA indicated that 30.30% of variation in *A. americanum* was found amongst populations. This value is higher than that estimated for 27 other outcrossing plant species (15.5%) based on AMOVA of the dominant marker RAPDs (Bussell, 1999).

Restricted Gene Flow amongst Races

Much of the genetic differentiation in *A. americanum* as a whole is likely related to limited gene flow across the three races identified in this taxon. Indeed, a closer examination of partitioning of diversity in *A. americanum* using a nested AMOVA reveals that 65.92% of the variance is found within populations, 18.34% is found among the groups, and 15.74% is found among populations within the groups. This indicates that >50% of the among population variation in *A. americanum* can actually be attributed to variation found between the races. Restricted gene flow across *A. americanum* races may be the result of several factors (see above) including geographic isolation or selection against migrants. Interestingly, when genetic differentiation within each of the *A. americanum* races is examined, only *A. americanum* on *P. contorta* var. *latifolia* has a high degree of structuring (G_{ST} 0.361) relative to other outcrossing plants. This may be related to a different origin for northern and southern subgroups (see above) or to adaptation to local host genotypes (see Chapter 3).

Pollen Dispersal

Factors intrinsic to the organism may also play a role in the structuring of *A. americanum* populations. For example, limited pollen dispersal could limit gene flow in this species, thereby increasing population differentiation. Despite being an obligately outcrossing species, *A. americanum* pollen is likely not dispersed over extremely long distances. Pollen in this species is thought to be dispersed through a combination of insect vectors (such as flies and gnats) and wind-dissemination (Penfield et al., 1976; Coppola, 1989; Gilbert and Punter, 1984, 1990, 1991). Maximum pollen dispersal by wind has been reported as 400 - 512 m for *A. americanum* (Penfield et al., 1976; Gilbert and Punter, 1984).

AFLP AS A GENETIC MARKER FOR POPULATION STUDIES

There has been some concern in the literature about the estimation of population genetic parameters from dominant markers such as AFLP or RAPDs (Lynch and Milligan, 1994; Ayres and Ryan, 1999; Fischer et al., 2000). Such estimations require that two assumptions be met: (1) that populations are in Hardy-Weinberg equilibrium; and (2) that null alleles are homologous across populations (Fischer et al., 2000). In this study, it seems probable that the Hardy-Weinberg assumption is met given the dioecious nature of *Arceuthobium*, as well as the high similarity detected between observed and expected heterozygosity in several *Arceuthobium* spp. using codominant isoenzyme markers (Nickrent and Butler, 1990; Nickrent and Butler, 1991). Thus, the application of the L-M correction factor in this study likely compensated for the underestimation of null alleles that is commonly associated with dominant markers. As for the second assumption, it is probable that many of the null alleles observed in this study represent

homologous characters since closely related individuals within a single species were being examined. Furthermore, Fischer and colleagues (2000) have indicated that deviation from either of these assumptions can be countered by high levels of significance to variability measures as determined by AMOVA. In the present study, all variability measures as determined by AMOVA were highly significant ($P<0.001$).

Several researchers (Lynch and Milligan, 1994; Isabel et al., 1999) have also suggested that dominant markers might be biased towards higher values of population differentiation in comparison with codominant markers (Fischer et al., 2000). Thus, it was suggested that data sets be 'pruned' to restrict analysis to markers with relatively high frequencies of null alleles (i.e. the "3/N-criterion"). These researchers also suggested that 100 individuals should be used to represent each population. However, Bartish and coworkers (1999) have shown that using many fewer individuals (ten plants per population) and ignoring the "3/N" criterion yielded comparable results to that obtained by following Lynch and Milligan's (1994) suggestions. Indeed, these researchers suggested that applying the "3/N-criterion" when only a small number of individuals is sampled actually underestimates gene diversity in populations. Examining 100 individuals per population is also highly impractical in broad-spectrum population studies. Bartish and coworkers (1999) have suggested that increasing the number of loci examined can decrease sampling variance and compensate for observing a low number of individuals per population. Use of the AFLP marker system fulfils this recommendation since many loci can be easily scored.

TAXONOMIC IMPLICATIONS

Nickrent and Butler (1990) have argued that the taxonomic classification of a

genus should be internally consistent in terms of the levels of divergence that one accepts for the various component species. Hawksworth and Wiens (1984, 1996) are the primary researchers conducting taxonomic studies for the genus *Arceuthobium*. These researchers (Hawksworth and Wiens, 1996) define species as "population systems that exhibit suites of characteristics that remain constant within prescribed limits of variation from generation to generation on different hosts". Subspecies are defined similarly to species except that "the distinguishing differences are neither as numerous nor of the magnitude that separate species". Races are taxa that associate with different principal hosts but show no consistent morphological or physiological differences.

The implications of the present study on the taxonomy of *A. americanum* are worth considering in this context. *Arceuthobium americanum* is clearly comprised of three groups (denoted as races in this study) that are genetically well-differentiated from each other. The cohesiveness of the three groups suggests that gene flow is limited across these groups, despite the lack of obvious morphological differences.

At the present time, the classification scheme of Hawksworth and Wiens (1984, 1996) is accepted and the three groups of *A. americanum* will be considered as genetic races. However, a future study examining microanatomy and SEM would be useful to determine if micromorphological differences exist between the three races of *A. americanum*. This will aid in determining if a higher taxonomic rank should be applied to these cryptic taxa within *A. americanum*.

EVOLUTIONARY IMPLICATIONS

Understanding gene flow and the population genetic structure of parasites is important to understanding their evolutionary biology since it provides insight into the

potential for interpopulation differentiation, race formation, and speciation (Nadler et al., 1995). Researchers have suggested that pressures imposed by different hosts have played a major role in the evolutionary diversification of *Arceuthobium* (Nickrent and Butler, 1990; Hawksworth and Wiens, 1996). The findings from this study show that *A. americanum* is strongly structured with approximately 30% of its genetic variation distributed among populations. Much of this population differentiation is distributed amongst the three genetic races of *A. americanum*. These races have non-overlapping geographic ranges (with the exception of the hybrid zone in Alberta) and are each associated with a different host taxon. Since gene flow is higher within than between the races, novel alleles may become common in one race, but not another. It is possible that *A. americanum* will undergo speciation via race formation if gene flow continues to be restricted between the three races. Reproductive isolation could occur through genetic changes such as those that control the timing of anthesis.

The concept of speciation via race formation has been criticized by Levin (1993) who argues that there is no evolutionary force that can simultaneously act on all geographically isolated populations of a race to separate them uniformly from a pre-existing race. However, it seems intuitive that limited gene flow amongst races but high gene flow within races due to factors such as selection pressures or geographic isolation could drive races to reproductive isolation and, ultimately, to speciation (Templeton, 1981; Orr, 1995; Orr and Orr, 1996; Via et al., 2000).

2.5 CONCLUSIONS

Molecular analysis of *A. americanum* using AFLP markers has provided insight into the population structure of this species. *Arceuthobium americanum* appears to be divided into three genetic races, each associated with a different host taxon in regions of allopatry. Several factors likely contribute to the overall structuring of populations. Host identity appears to play a role in shaping *A. americanum* into three races as the groups can easily be delineated by this parameter. Limited gene flow due to physical barriers and geographic isolation has likely been important as well since these races generally have allopatric distributions. Additionally, *A. americanum* races may be responding to differences in the environmental conditions that characterize their allopatric distributions. Unfortunately, it was not possible to determine which of these factors was most important in shaping population structure of *A. americanum.* Given sufficient geologic time, complete reproductive isolation could develop and drive these races to speciation.

A relatively recent association with non-principal hosts and competitive exclusion by other *Arceuthobium* spp. has probably inhibited *A. americanum* from adapting to and spreading on non-principal hosts. Historical factors such as founder events may account for the outlying position of *A. americanum* populations from hybrids in northern Alberta. Geographic isolation over a long period of time may explain the unresolved position of the population from Cypress Hills, Alberta. The lack of fine-scale geographic patterning within *A. americanum* races suggests that random dispersal of seeds by animal vectors has been important for the spread of this parasite within infected stands and into new uninfected stands. Factors affecting structuring of *A. americanum* are examined further

in Chapter 3.

Genetic variability and diversity were found to vary across *A. americanum* populations. Genetically depauperate populations of *A. americanum* from northern hybrid hosts may have formed as a result of founder events and the loss of genetic diversity through drift. This observation is also supported by the divergent position of these populations in the dendrograms. The high genetic diversity found in *A. americanum* populations on *P. contorta* supports the hypothesis that this parasite evolved originally as a parasite of this host, and later spread on *P. banksiana.* Surprisingly, northern (on-ice) populations of *A. americanum* on *P. contorta* var. *latifolia* have higher genetic diversity than southern (off-ice) populations on this same host. This pattern was unexpected since conspecifics or congeners from previously glaciated regions generally show low diversity. This implies that *A. americanum* may have survived part of the Wisconsin glaciations in a genetically diverse northern refugium.

Despite the significant molecular divergence between the three races of *A. americanum*, there is no evidence of macromorphological differences. Thus, based on the classification scheme for *Arceuthobium* spp. suggested by Hawksworth and Wiens (1996), these groups should not be assigned the rank of species or subspecies. However, future investigations may result in the detection of micromorphological differences amongst these genetically divergent races and provide support for their description at a higher taxonomic level.

Table 2. *Arceuthobium americanum* populations collected from various host species in Canada and the U.S.A. Vouchers deposited in WIN.

Population	Site and Voucher number
Host: *Pinus banksiana*	
Beauval (SK-1)	Hwy. 165, just east (within 1 km) of Beauval, Saskatchewan; *Jerome 9717 and Chorney.*
Belair (MB-2)	E. & W. side of Hwy. 59; 7.5 km N. of Jct. of Hwys. 12S and 59N; Dwarf mistletoe Forest Disease Control area at Belair Forest, Manitoba; *Jerome 9707 and Chorney.*
Candle Lake (SK-3)	79 km NE of Prince Albert via Hwys. 55 and 120 at Candle Lake Provincial Park campground, Saskatchewan; *Jerome 9713 and Chorney.*
Cowan (MB-4)	E. & W. side of Hwy. 10N at Cowan "Wayside Park", 1-2 km N. of Cowan, Manitoba; *Jerome 9708 and Chorney.*
Devil's Lake (MB-5)	E. side of Hwy 6, 4 km N. of Devil's Lake picnic ground, Manitoba; *Jerome 9703 et al.*
Ft. McMurray (AB-6)	E. side of Hwy. 63, 42.6 km north of Fort McMurray, Alberta (7.6 km north of Syncrude Oil Plant); *Jerome 9720 and Chorney.*
Grand Rapids I (MB-7)	W. side of Hwy. 6; 10 km S. of Grand Rapids, Manitoba; *Jerome 9701 et al.*
Grand Rapids II (MB-8)	E. & W. side of Hwy. 6; 10 km N. of Long Point Road; 36 km S. of Grand Rapids, Manitoba; *Jerome 9702 et al.*
La Loche (SK-9)	Hwy. 155 N, just south (within 1 km) of La Loche, Saskatchewan; *Jerome 9718 and Chorney.*
La Ronge (SK-10)	Jct. of Hwy 165 W and Hwy 2 N, 52 km S of La Ronge, Saskatchewan; *Jerome 9716 and Chorney.*
The Pas (MB-11)	E. & W. side of Hwy. 10 North, 35 km S. of The Pas, Manitoba; *Jerome 9709 and Chorney.*

Prince Albert I (SK-12)	S. side of Hwy. 55, Nisbet provincial forest, Fire Break and Dwarf Mistletoe Management Area, approx. 10 km W. of Prince Albert, Saskatchewan; *Jerome 9714 and Chorney.*
Prince Albert II (SK-13)	Jct. of Hwys. 240 and 263, (along Cookson Rd.), just south of South gate of Prince Albert National Park, Saskatchewan; *Jerome 9715 and Chorney.*
Smeaton (SK-14)	Hwy. 106, 31.3 km N. of Smeaton, Saskatchewan; *Jerome 9712 and Chorney.*
Smoky Lake (AB-15)	E. & W. sides of feeder road, 3 km S. of Hwy. 28, just outside of the Tree Improvement Centre (Alberta Environmental Protection), 15 km E. of Smoky lake, Alberta; *Jerome 9719 and Chorney.*
Tobin Lake (SK-16)	5.3km N. of junction of dirt road and provincial Hwy. 35 at Tobin Lake Provincial Park, Saskatchewan; *Jerome 9711 and Chorney.*

Host: Hybrid *P. banksiana* X *Pinus contorta* var. *latifolia*

Grande Prairie (AB-17)	98 st. (Resources Road), across from The Dunes Golf Course, approx. 3 km. S. of Grande Prairie, Ab; *Jerome 9723 and Chorney.*
High Level (AB-18)	near High Level; no voucher collected.
Slave Lake (AB-19)	E. side of Hwy. 2, just NE of Athabasca River, 46.4 km SE of Slave lake, Alberta; *Jerome 9721 and Chorney.*
Whitecourt (AB-20)	E. & W. side of road, 41 ave., at Sandhills Cross-country ski area, 5.5 km east of Whitecourt, Alberta; *Jerome 9722 and Chorney.*
Whitemud/Peace River (AB-21)	Junction of Whitemud and Peace Rivers, Alberta; no voucher collected.
Wood Buffalo National Park (AB-22)	Peace Point, Wood Buffalo National Park, Alberta; no voucher collected.

Host: Canadian *Pinus contorta* var. *latifolia*

100 Mile House (BC-23)	W. side of Hwy. 97, just at southern limits of 100 Mile House, British Columbia; *Jerome 9729 and Chorney.*
Banff (AB-24)	Tunnel Mountain Road at Tunnel Mountain campground (Village I), Banff National Park, Alberta; *Jerome 9727 and Chorney.*
Castlegar (BC-25)	On Zuckerberg Island, Castlegar, British Columbia; *Jerome 9705 et al.*
Cypress Hills (AB-26)	Near Bull Trail Head at Cypress Hills Interprovincial Park, Alberta; *Jerome 9801 and Chorney.*
DTR (AB-27)	N. & S. side of Hwy. 11, 49.6 km. E of Saskatchewan River Crossing, 1.6 km E. of David Thompson Resort, Alberta; *Jerome 9726 and Chorney.*
Field (BC-28)	N. side of Hwy. 1, at Chancellor Peak campground, 24.1 km W of Field, British Columbia; *Jerome 9728 and Chorney.*
Jasper (AB-29)	Jct. of Hwys. 93A S. and 116 N., just outside of Jasper, Alberta; *Jerome 9724 et al.*
Kamloops (BC-30)	At turnoff to Logan Lake off Hwy. 5; 25 km S of Kamloops, British Columbia; *Jerome 9730 and Chorney.*
Nimpo Lake (BC-31)	308 km W. of Williams Lake, E. of Anahim Lake, 5.5 km W of Nimpo Lake, British Columbia; no voucher collected.
Prince George (BC-32)	500 m E of Jardine Rd. turnoff, N and S sides of Hwy. 16; 65 km W. of Prince George, British Columbia; no voucher collected.
Redstone (BC-33)	N. side of Hwy. 20 W; 150 km W. of Williams Lake; 2 km W. of Redstone, British Columbia; no voucher collected.

Host: U.S.A. *Pinus contorta* var. *latifolia*

Ashton (ID-34)	6.6 miles NE of Ashton on Hwy. 20, Targhee National Forest, Fremont County, Idaho; *Jerome 9910 et al.*

Bondurant (WY-35)	13.3 miles SE of Bondurant on US180, The Rim, Bridger-Teton National Forest, Sublette County, Wyoming; *Jerome 9912 et al.*
Grand Lake (CO-36)	1 mile S. of East gate to Rocky Mtn. National Park on Hwy. 34, Grand Lake, Grand County, Colorado; *Jerome 9915 et al.*
Idaho City (ID-37)	15.6 miles NE. of Idaho city on Hwy. 21, Boise County, Boise National Forest, Idaho; *Jerome 9908 et al.*
John Day (OR-38)	Dixie Pass, Dad's Creek Rd, 0.9 miles E. of its jct. with Hwy. 26, X miles E. of John Day, Grant County, Malheur National Forest, Oregon; *Jerome 9907 et al.*
Kenosha Pass (CO-39)	Kenosha Pass, S. side of Hwy. 285 across from Pike National Forest campground, Pike National Forest, Park County, Colorado; *Jerome 9918 et al.*
Ketchum (ID-40)	23 miles NW. of Ketchum on Hwy. 75, 1.4 miles NW. of Galena Lodge, Sawtooth National Forest, Blaine County, Idaho; *Jerome 9909 et al.*
Manila I (UT-41)	18.5 miles SE of Manila on Rte. 44, Ashley National Forest, Daggett County, Uinta Mtns, Utah; *Jerome 9913 et al.*
Monarch Pass (CO-42)	2 miles SW. of Monarch Pass on US50, San Isabel National Forest, Chaffee County, Colorado; *Jerome 9919 et al.*
Red Feather Lakes I (CO-43)	3 miles W. of Red Feather Lakes on County Road 162, Roosevelt National Forest, Larimer County, Colorado; *Jerome 9916 et al.*
Yellowstone (WY-44)	N and S sides of Hwy. 212, 8.3 miles SW. of NE entrance in Yellowstone National Park, Wyoming (9.3 miles SW of Silver Gate), Gallatin National Forest, Wyoming; *Jerome 9911 et al.*

Host: *P. contorta* var. *murrayana*

Ft. Klamath (OR-47)	4.6 miles N. of Ft. Klamath on Hwy. 62, Klamath County, Oregon; *Jerome 9905 et al.*

Lee Vining I (CA-48)	Feeder road, running S of Hwy. 120, 4.2 miles W. of jct. with 395, Mono County, California; *Jerome 9901 et al.*
Mt. Shasta (CA-49)	Military Pass, on Military Pass Road, Mt. Shasta, Shasta - Trinity National Forest, Siskiyou County, California; *Jerome 9904 et al.*
Sisters (OR-50)	9 miles NW. of Sisters on Hwy. (20)126, Deschutes National Forest, Deschutes County, Oregon; *Jerome 9906 et al.*
Host: *Pinus ponderosa*	
Manila II (UT-45)	18.5 miles SE of Manila on Rte. 44, Ashley National Forest, Daggett County, Uinta Mtns, Utah; *Jerome 9914 et al.*
Red Feather Lakes II (CO-46)	3 miles W. of Red Feather Lakes on County Road 162, Roosevelt National Forest, Larimer County, Colorado; *Jerome 9917 et al.*
Host: *Pinus jeffreyi*	
Lee Vining II (CA-51)	Feeder road, running S of Hwy. 120, 4.2 miles W. of jct. with 395, Mono County, California; *Jerome 9902 et al.*

Table 3. Sequences for primers and adapters used for ligation, pre-amp. PCR and selective amp. PCR for AFLP.

AFLP PRIMER	PRIMER SEQUENCE
Ma 1.1 Adapter	GAC GAT GAG TCC TGA G
Ma 1.2 Adapter	TAC TCA GGA CTC AT
M+C pre-amplification primer	GAT GAG TCC TGA GTA AC
M-CAC selective amplification primer	GAT GAG TCC TGA GTA ACA C
M-CCG selective amplification primer	GAT GAG TCC TGA GTA ACC G
Ea 1.1 Adapter	CTC GTA GAC TGC GTA CC
Ea 1.2 Adapter	AAT TGG TAC GCA GTC
E+A pre-amplification primer	GAC TGC GTA CCA ATT CA
E-ACG selective amplification primer	GAC TGC GTA CCA ATT CAC G
E-ACA selective amplification primer	GAC TGC GTA CCA ATT CAC A

Table 4. Matrix of Nei's unbiased genetic identity coefficients (range) (Nei, 1978) for all pairwise group comparisons of *Arceuthobium americanum*. Genetic identity of *A. americanum* from *Pinus jeffreyi* not reported since based on only one population.

Host	*Pinus banksiana* (n=16)	*Pinus contorta var. latifolia* (n=22)	Hybrids (n=6)	*Pinus contorta var. murrayana* (n=4)	*Pinus ponderosa* (n=2)	*Pinus jeffreyi* (n=1)
Pinus banksiana	0.941 (0.890-0.979)					
Pinus contorta var. latifolia	0.908 (0.844-0.963)	0.954 (0.908-0.988)				
Hybrids	0.914 (0.854-0.957)	0.908 (0.829-0.964)	0.898 (0.844-0.951)			
Pinus contorta var. murrayana	0.868 (0.800-0.915)	0.904 (0.840-0.950)	0.872 (0.847-0.899)	0.970 (0.955-0.981)		
Pinus ponderosa var. scopulorum	0.917 (0.873-0.948)	0.964 (0.932-0.994)	0.916 (0.890-0.942)	0.911 (0.901-0.918)	0.978 (0.978-0.978)	
Pinus jeffreyi	0.861 (0.806-0.903)	0.896 (0.851-0.924)	0.872 (0.844-0.905)	0.971 (0.959-0.992)	0.899 (0.898-0.901)	**** (****-****)

* The average Nei's unbiased genetic identity coefficient (range) across all 51 *A. americanum* populations is 0.918 (0.800-0.988).

Table 5. Genetic variability in 51 populations of *Arceuthobium americanum*. Average number of individuals (N), percentage of unique genotypes (% gene), mean number of alleles per locus ± 1 SE (k), percentage of polymorphic loci at 95% criterion (P), expected heterozygosity ± 1SE (H_{exp}) (unbiased estimate Nei [1978]).

Population	N	k	P	H_{exp}
Host: *Pinus banksiana*				
Beauval (SK-1)	9.9 ± 0.0	1.4 ± 0.0	40.0	0.151 ± 0.021
Belair (MB-2)	9.9 ± 0.0	1.4 ± 0.0	37.0	0.127 ± 0.019
Candle Lake (SK-3)	9.6 ± 0.1	1.4 ± 0.0	38.0	0.141 ± 0.020
Cowan (MB-4)	9.8 ± 0.0	1.4 ± 0.0	43.0	0.150 ± 0.020
Devil's Lake (MB-5)	9.9 ± 0.0	1.4 ± 0.0	38.0	0.143 ± 0.021
Ft. McMurray (AB-6)	10.0 ± 0.0	1.4 ± 0.0	44.0	0.153 ± 0.020
Grand Rapids I (MB-7)	10.0 ± 0.0	1.3 ± 0.1	34.0	0.127 ± 0.020
Grand Rapids II (MB-8)	5.7 ± 0.2	1.3 ± 0.0	34.0	0.143 ± 0.022
La Loche (SK-9)	9.3 ± 0.1	1.4 ± 0.0	42.0	0.156 ± 0.021
La Ronge (SK-10)	9.9 ± 0.0	1.4 ± 0.0	42.0	0.165 ± 0.021
The Pas (MB-11)	9.8 ± 0.0	1.5 ± 0.0	45.0	0.146 ± 0.019
Prince Albert I (SK-12)	9.9 ± 0.0	1.5 ± 0.1	47.0	0.172 ± 0.021
Prince Albert II (SK-13)	9.7 ± 0.1	1.4 ± 0.0	39.0	0.162 ± 0.024
Smeaton (SK-14)	9.4 ± 0.1	1.5 ± 0.1	47.0	0.170 ± 0.021
Smoky Lake (AB-15)	9.6 ± 0.0	1.5 ± 0.1	51.0	0.185 ± 0.021
Tobin Lake (SK-16)	9.9 ± 0.0	1.4 ± 0.0	40.0	0.133 ± 0.019
Host: Hybrids				
Grande Prairie (AB-17)	6.6 ± 0.1	1.5 ± 0.1	49.0	0.183 ± 0.021
High Level (AB-18)	9.6 ± 0.0	1.2 ± 0.0	21.0	0.059 ± 0.014
Slave Lake (AB-19)	10.0 ± 0.0	1.5 ± 0.1	52.0	0.185 ± 0.021
Whitecourt (AB-20)	7.4 ± 0.1	1.5 ± 0.0	45.0	0.175 ± 0.021
Wood Buffalo National Park (AB-21)	9.1 ± 0.0	1.3 ± 0.0	28.0	0.091 ± 0.017
Whitemud/PR (AB-22)	9.9 ± 0.0	1.3 ± 0.0	30.0	0.104± .018
Host: Canadian *Pinus contorta* var. *latifolia*				
100 Mile-House (BC-23)	9.9 ± 0.0	1.6 ± 0.0	58.0	0.203 ± 0.021
Banff (AB-24)	9.2 ± 0.1	1.6 ± 0.0	60.0	0.208 ± 0.021
Castlegar (BC-25)	10.0 ± 0.0	1.5 ± 0.1	47.0	0.157 ± 0.020
Cypress Hills (AB-26)	9.8 ± 0.1	1.5 ± 0.1	54.0	0.181 ± 0.021
DTR (AB-27)	6.0 ± 0.2	1.5 ± 0.1	53.0	0.204 ± 0.022
Field (BC-28)	9.9 ± 0.0	1.6 ± 0.0	57.0	0.197 ± 0.020
Jasper (AB-29)	9.9 ± 0.0	1.6 ± 0.0	59.0	0.215 ± 0.021

Kamloops (BC-30)	9.9 ± 0.0	1.6 ± 0.0	65.0	0.220 ± 0.021
Nimpo Lake (BC-31)	9.9 ± 0.0	1.6 ± 0.0	60.0	0.202 ± 0.020
Prince George (BC-32)	9.5 ± 0.1	1.5 ± 0.1	54.0	0.198 ± 0.022
Redstone (BC-33)	9.4 ± 0.1	1.5 ± 0.1	50.0	0.194 ± 0.022
Host: U.S.A. *Pinus contorta* var. *latifolia*				
Ashton (ID-34)	10.0 ± 0.0	1.5 ± 0.1	54.0	0.170 ± 0.020
Bondurant (WY-35)	10.0 ± 0.0	1.5 ± 0.1	48.0	0.178 ± 0.022
Grand Lake (CO-36)	10.0 ± 0.0	1.5 ± 0.1	48.0	0.166 ± 0.021
Idaho City (ID-37)	10.0 ± 0.0	1.6 ± 0.0	62.0	0.211 ± 0.020
John Day (OR-38)	9.9 ± 0.0	1.6 ± 0.0	56.0	0.202 ± 0.021
Kenosha Pass (CO-39)	9.1 ± 0.0	1.5 ± 0.1	47.0	0.154 ± 0.020
Ketchum (ID-40)	10.0 ± 0.0	1.5 ± 0.0	45.0	0.161 ± 0.020
Manila I (UT-41)	9.6 ± 0.1	1.5 ± 0.1	51.0	0.182 ± 0.021
Monarch Pass (CO-42)	10.0 ± 0.0	1.5 ± 0.1	50.0	0.184 ± 0.022
Red Feathers Lake I (CO-43)	10.0 ± 0.0	1.5 ± 0.1	48.0	0.176 ± 0.021
Yellowstone (WY-44)	10.0 ± 0.0	1.5 ± 0.1	54.0	0.198 ± 0.022
Host: *Pinus ponderosa* var. *scopulorum*				
Manila II (UT-45)	10.0 ± 0.0	1.5 ± 0.1	54.0	0.200 ± 0.022
Red Feathers Lake II (CO-46)	9.9 ± 0.0	1.5 ± 0.1	54.0	0.187 ± 0.021
Host: *Pinus contorta* var. *murrayana*				
Fort Klamath (OR-47)	9.9 ± 0.0	1.5 ± 0.1	49.0	0.147 ± 0.018
Lee Vining I (CA-48)	9.9 ± 0.0	1.5 ± 0.1	53.0	0.176 ± 0.020
Mount Shasta (CA-49)	10.0 ± 0.0	1.7 ± 0.0	66.0	0.201 ± 0.020
Sisters (OR-50)	10.0 ± 0.0	1.5 ± 0.0	55.0	0.194 ± 0.021
Host: *Pinus jeffreyi*				
Lee Vining II (CA-51)	10.0 ± 0.0	1.6 ± 0.0	59.0	0.189 ± 0.020
AVERAGE (n=51)	**9.5**	**1.5**	**48.2**	**0.170**

Table 6. Genetic diversity statistics (Nei and Chesser, 1984) for *Arceuthobium* on the different host species. H_T = total gene diversity, H_S = within-population gene diversity, D_{ST} = among population gene diversity, G_{ST} = coefficient of genetic differentiation.

Host species	H_T	H_S	D_{ST}	G_{ST}
All populations	0.238	0.170	0.068	0.286
Pinus banksiana	0.198	0.152	0.046	0.232
Pinus contorta var. latifolia	0.296	0.189	0.107	0.361
Canadian *Pinus contorta var. latifolia*	0.230	0.198	0.038	0.139
U.S.A. *Pinus contorta var. latifolia*	0.210	0.180	0.030	0.142
Pinus ponderosa	0.203	0.194	0.009	0.044
Pinus contorta var. murrayana	0.199	0.180	0.019	0.095
Pinus jeffreyi	****	0.189	****	****
Hybrids	0.205	0.133	0.072	0.351

Table 7. Analysis of Molecular Variance (AMOVA) for 51 populations of *Arceuthobium americanum*. Populations were divided into six groups based on host species (*P. banksiana*, *P. contorta* var. *latifolia*, *P. banksiana* X *P. contorta* var. *latifolia* hybrids, *P. contorta* var. *murrayana*, *P. jeffreyi*, and *P. ponderosa*). Degrees of freedom (df), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value).

	df	SSD	Variance Components	% Total	P-value
Population Level					
Among Populations	50	2334.160	3.92122	30.30	<0.001
Within Populations	439	3959.211	9.01870	69.70	<0.001
Total	489	6293.37	12.93992	100.00	
Nested Level (Six groups)					
Among Groups	5	999.376	2.50992	18.34	<0.001
Among Populations within Groups	45	1334.784	2.15304	15.74	<0.001
Within Populations	439	3959.211	9.01870	65.92	<0.001
Total	489	6293.371	13.68167	100.00	

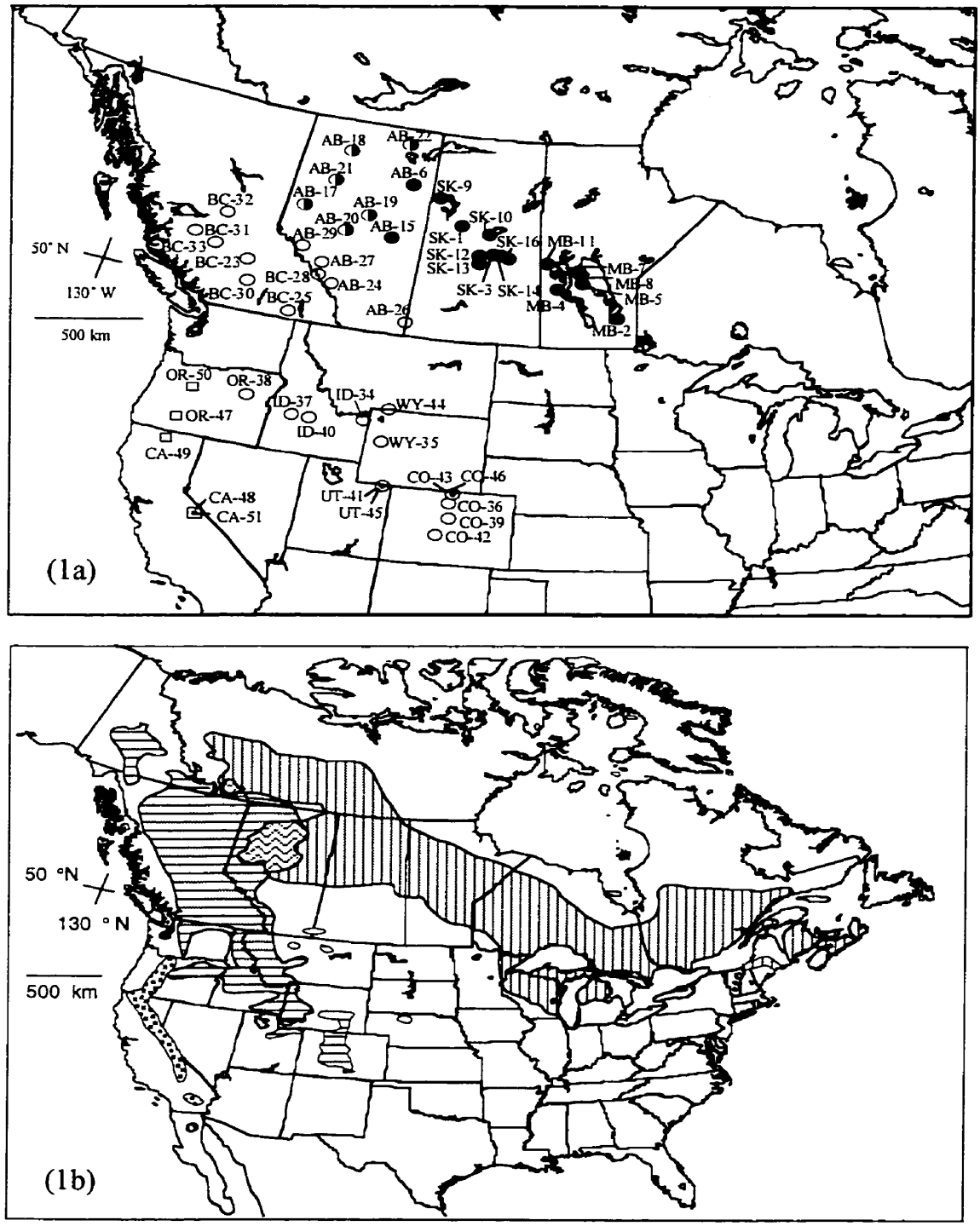

Figure 1. Maps showing: (1a) range of *A. americanum* and collection sites (dots indicate host identity: closed circle - *P. banksiana* host; open circle - *P. contorta* var. *latifolia* host; half open/half closed circle - hybrid host; open square - *P. contorta* var. *murrayana* host; circle with dot in middle - *P. contorta* var. *latifolia* and *P. ponderosa* var. *scopulorum* hosts; square with dot in middle - *P. contorta* var. *murrayana* and *P. jeffreyi* hosts); (1b) range of *P. banksiana* (vertical lines), *P. contorta* var. *latifolia* (horizontal lines), hybrids (zigzag lines), and *P. contorta* var. *murrayana* (dots).

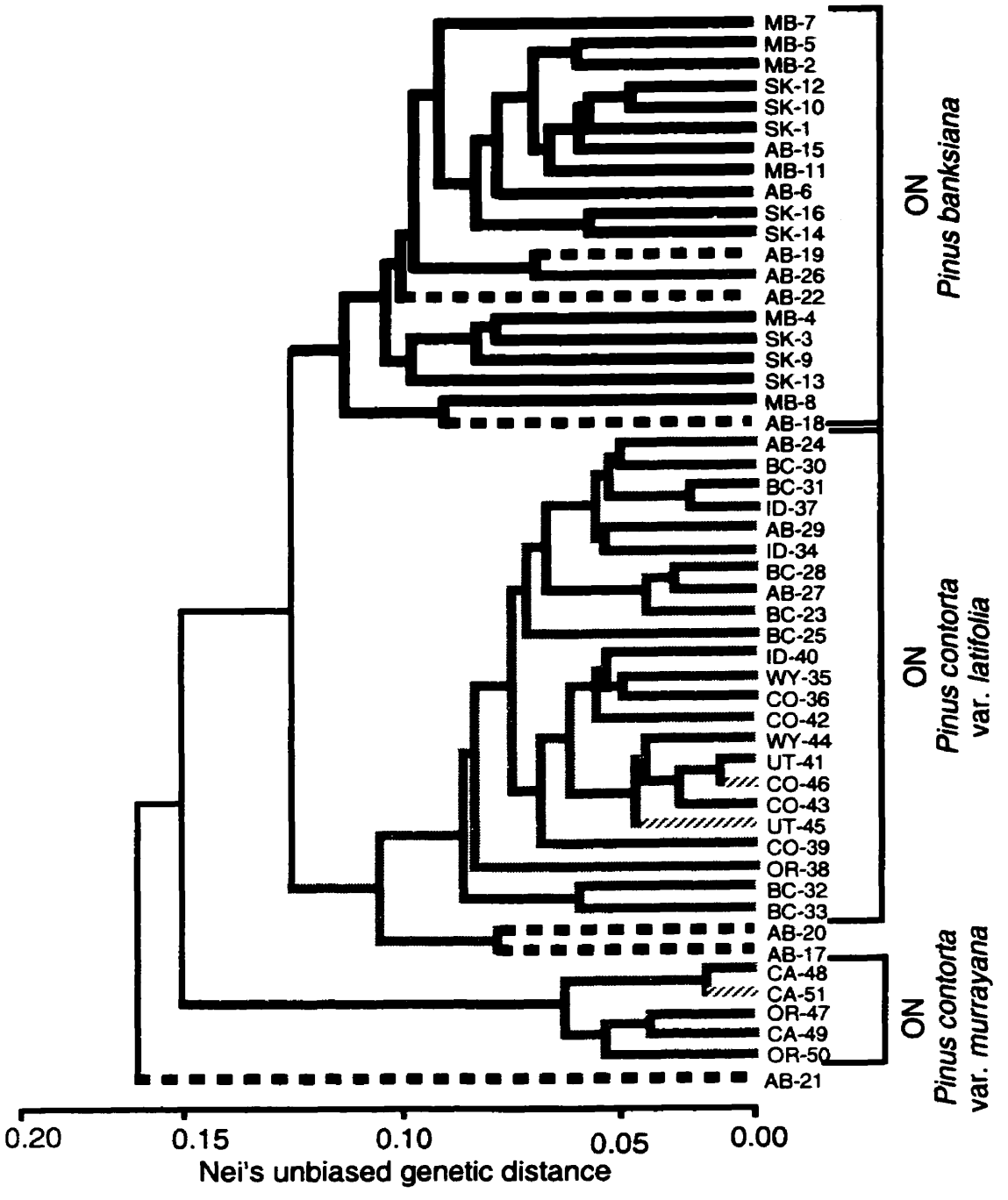

Figure 2. UPGMA dendrogram of 51 populations of *Arceuthobium americanum* based on L-M modified (Lynch and Milligan, 1994) Nei's unbiased genetic distances (Nei, 1978). Line shading indicates host taxon from which the parasite was isolated: black lines - *P. banksiana* hosts, gray lines - *P. contorta* var. *latifolia* and var. *murrayana* hosts, dashed lines - hybrid hosts, slashed lines - non-principal hosts. Cophenetic correlation = 0.802.

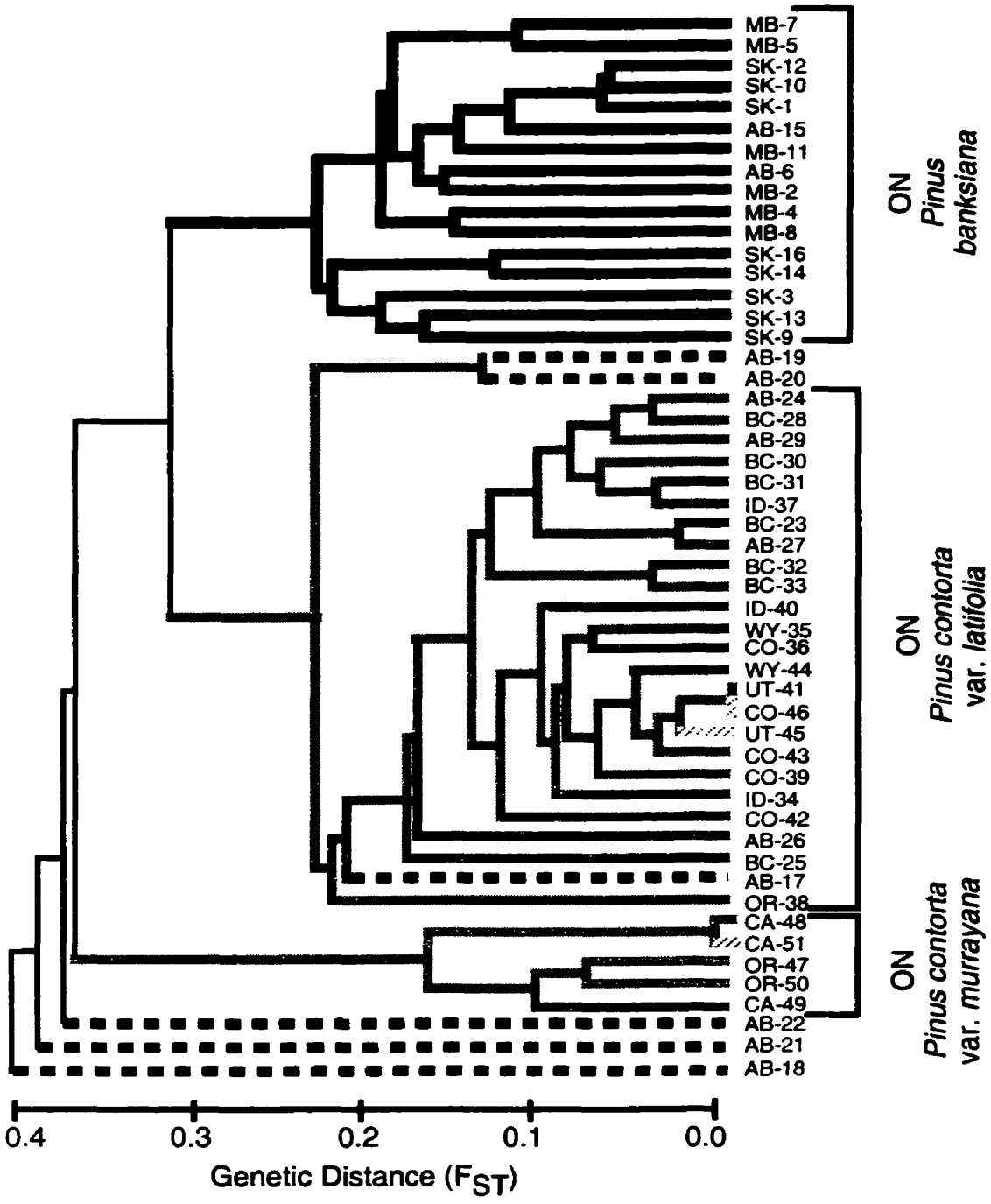

Figure 3. UPGMA dendrogram of 51 populations of *Arceuthobium americanum* based on Fst distance values from AMOVA (Excoffier et al., 1992). Line shading indicates host taxon from which the parasite was isolated: black lines - *Pinus banksiana* hosts, gray lines - *P. contorta* var. *latifolia* and var. *murrayana* hosts, dashed lines - hybrid hosts, slashed lines - non-principal hosts.

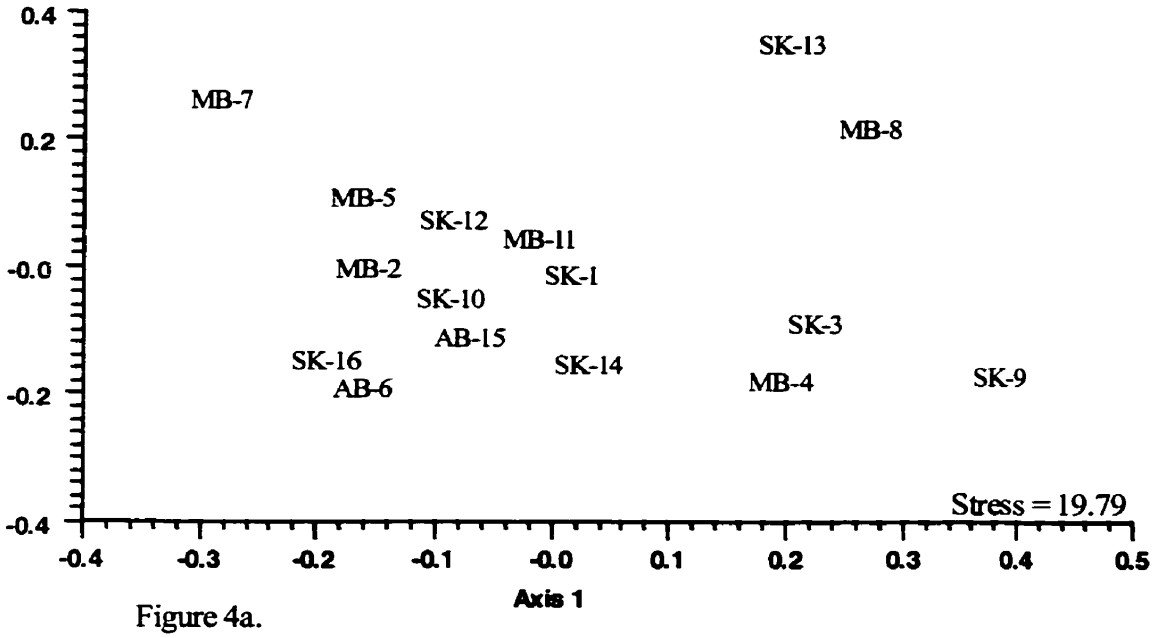

Figure 4a.

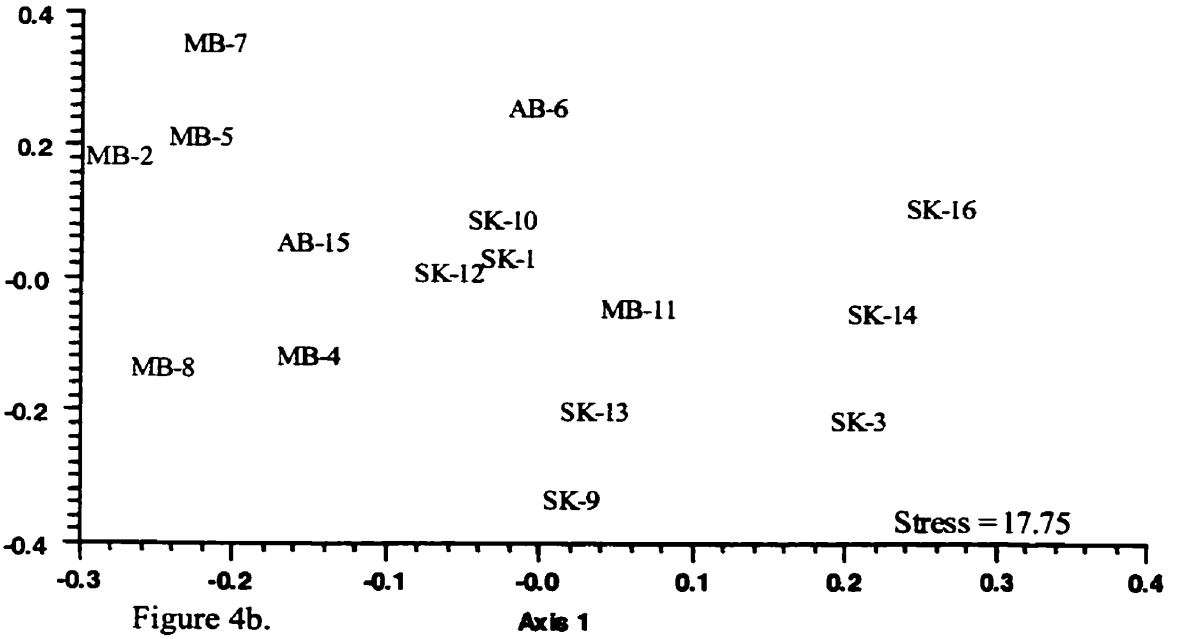

Figure 4b.

Figure 4. NMDS ordinations of pairwise genetic distances between populations of *Arceuthobium americanum* on *Pinus banksiana* using (4a) Nei's unbiased genetic distances (Nei, 1978) or (4b) Fst genetic distances (Excoffier et al., 1992).

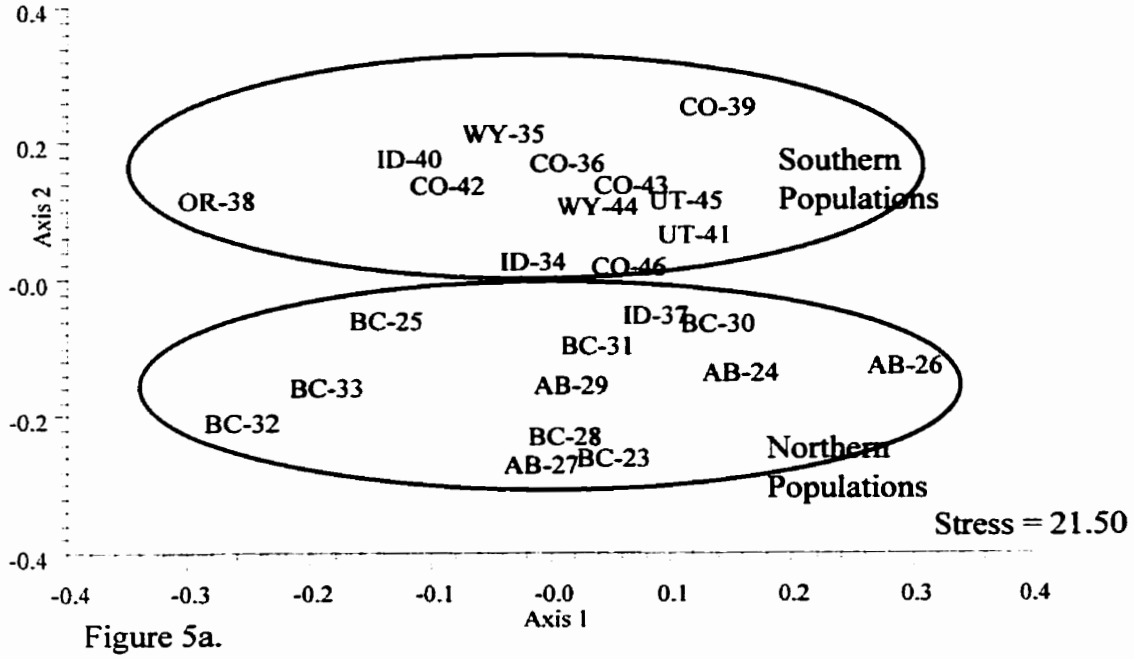

Figure 5a.

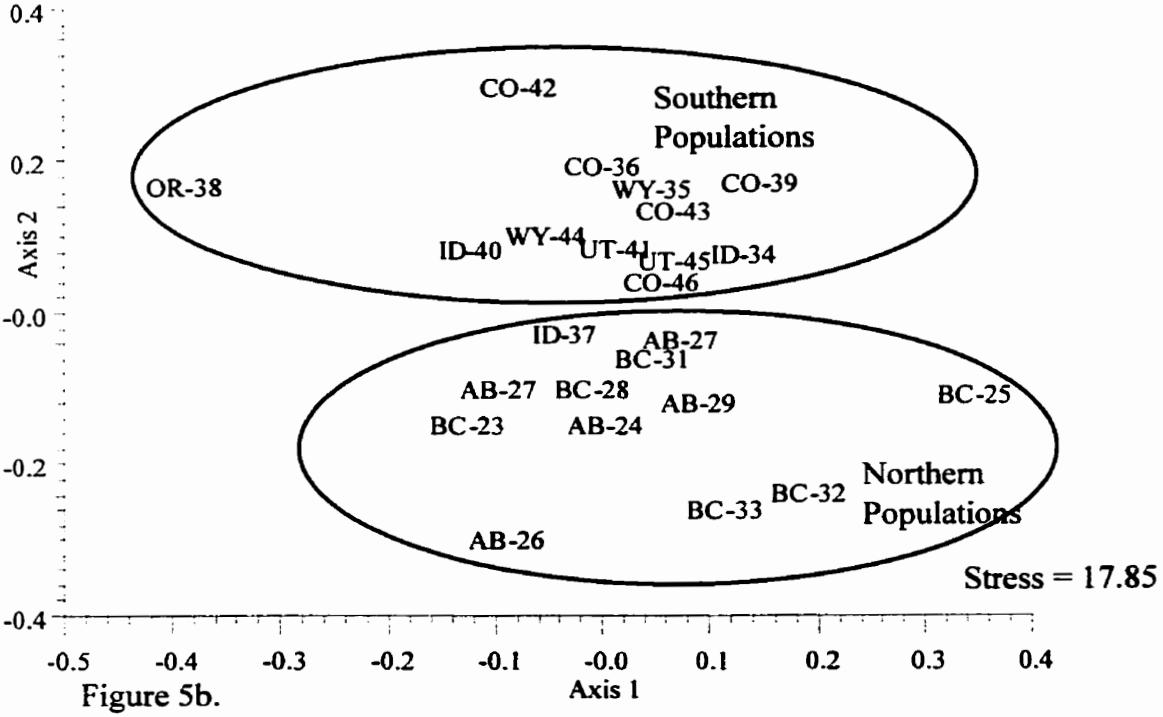

Figure 5b.

Figure 5. NMDS Ordinations of *Arceuthobium americanum* on *Pinus contorta* var. *latifolia* hosts using either: (5a) Nei's unbiased genetic distance (Nei, 1978); or (5b) Fst genetic distance (Excoffier et al., 1992).

CHAPTER 3: Comparative Population Structure and Genetic Diversity of *Arceuthobium americanum* and its *Pinus* spp. Hosts

3.1 INTRODUCTION

Knowledge of population structure is important to understanding evolution since it reflects the extent of gene flow between populations, and hence, the evolutionary potential of an organism (Price, 1980; Thompson, 1994; Nadler, 1995). Genetic population structure is affected by life history characteristics such as mating patterns and dispersal systems, as well as by numerous other influences such as selection pressures, geographic range, stochastic forces, and historical events. Parasites are unique, however, in that their population structure is also influenced by their intimate relationship with their hosts (Mulvey et al., 1991; Nadler, 1995; Nadler et al., 1995).

In host-parasite interactions, the evolutionary outcome is dependent upon the amount of gene flow amongst populations of both the host and the parasite (Dybdahl and Lively, 1996). For example, high gene flow within both host and parasite (Gandon et al., 1996; Morand et al, 1996) could lead to decreased local adaptation. On the other hand, low gene flow by both host and parasite could lead to increased local adaptation (Price, 1980; Kirkpatrick and Barton, 1997; Gandon and Van Zandt, 1998; but see Gandon et al., 1996). This would occur as a parasite adapts to infecting different host species or local genotypes within a given host species. Parasite populations may eventually become differentiated into races as they adapt to the divergent selection pressures driven by hosts (Thompson, 1994). Parasite populations may additionally differentiate into races independent of the role played by interaction with its host. This may occur as a result of

decreased gene flow due to limited migration (i.e. isolation-by-distance) (Price, 1980; Thompson, 1994) or as a result of divergent selection pressures imposed by different environmental conditions (Orr, 1995; Orr and Orr, 1996; Via et al., 2000).

Given the importance of relative gene flow and adaptation, researchers (Thompson, 1994; Dybdahl and Lively, 1996) have suggested that knowledge of population structure of both the parasite and its host is necessary to fully understand evolution of these pathosystems. Adaptation of a parasite to a given host species may be reflected by higher genetic similarity between populations of parasites found within, relative to between, different host species (Price, 1980). Adaptation to local host genotypes may be reflected by similar genetic population structures exhibited by a parasite and its host (Dybdahl and Lively, 1996). The effect of isolation-by-distance would be reflected by congruence between genetic and geographic distances (Dybdahl and Lively, 1996). If environmental parameters are geographically patterned, such an influence can be detected in the same manner as is isolation-by-distance.

In recent years, studies have begun to employ this approach to understanding evolution of parasites (Nadler et al., 1990; Mulvey et al., 1991; Dybdahl and Lively, 1996; Davies et al., 1999; Delmotte et al., 1999; Martinez et al., 1999; Jobet et al., 2000). However, the population structures of parasitic plants and their hosts have never been directly compared. Edmunds and Alstad (1978) predicted that parasitic plants such as dwarf mistletoes would show strong adaptation to local host populations, and even to host individuals, due to their dependence on toxin-defended, long-lived host trees. If strong local adaptation is prevalent, it could have a significant impact on the evolution of these parasitic plants. This hypothesis of local adaptation (Edmunds and Alstad, 1978) has

never been thoroughly examined within a species of *Arceuthobium* to test for genetic races that have resulted from isolation-by-distance or adaptation to local host genotypes and specific host taxa.

This is the second of three studies that examine the evolutionary biology of *Arceuthobium americanum* Nutt. ex Engelm., a parasitic plant that infects three principal hosts (*Pinus banksiana* Lamb., *Pinus contorta* var. *latifolia* Englem., and *Pinus contorta* var. *murrayana* (Greville and Balfour) Engelm.). In the first study (Chapter 2), the population structure of *A. americanum* was examined using AFLP. It was found that *A. americanum* is divided into three genetic races, each associated with a different principal host. Geographic structuring within these races was weak. In this second study, the population genetic structures of *P. banksiana*, *P. contorta* var.*latifolia*, and *P. contorta* var. *murrayana* were determined using AFLP. This made it is possible to assess the overall and fine-scale genetic structure of *A. americanum* in light of knowledge about host genetic structure. To determine the relative influence of isolation-by-distance and host selection pressures on *A. americanum*, the genetic distance matrices of both host and parasite were compared with each other and with geographic distance.

The objectives of this study were to:

(1) Determine the population genetic structures of *P. banksiana, P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana*;

(2) Evaluate the influence of host identity in shaping the overall genetic structure of *A. americanum*; and

(3) Examine the role played by isolation-by-distance and local host genotype in shaping the fine-scale genetic structure of *A. americanum*.

3.2 METHODS

COLLECTIONS

Parasite and host plant tissue were collected from 29 populations: (1) eleven from *Pinus banksiana* - Belair (MB-2), Candle Lake (SK-3), Cowan (MB-4), Ft. McMurray (AB-6), Grand Rapids I (MB-7), La Loche (SK-9), La Ronge (SK-10), The Pas (MB-11), Prince Albert I (SK-12), Smeaton (SK-14), and Smoky Lake (AB-15); (2) five from *P. banksiana* X *Pinus contorta* var. *latifolia* hybrids - High Level (AB-18), Slave Lake (AB-19), Whitecourt (AB-20), Whitemud/Peace River (AB-21), and Wood Buffalo National Park (AB-22); (3) ten from *P. contorta* var. *latifolia* hosts - Banff (AB-24), Castlegar (BC-25), Cypress Hills (AB-26), DTR (AB-27), Jasper (AB-29), John Day (OR-38), Ketchum (ID-40), Manila I (UT-41), Red Feather Lakes I (CO-43), and Yellowstone (WY-44); and (4) three from *Pinus contorta* var. *murrayana* hosts - Ft. Klamath (OR-47), Lee Vining I (CA-48), and Mt. Shasta (CA-49). These populations represent a subset of the 51 *A. americanum* sampled in Chapter 2. Locality information and codes are defined in Chapter 2 (Table 2). For each population, host and parasite individuals were sampled from a single witches' broom from 10 different trees. Prior to DNA extraction, tissue was lyophilized as described in Chapter 2.

DNA EXTRACTIONS

Parasite and host DNA were extracted using the DNeasy Plant Mini Kit (Qiagen 69106). Protocols from the kit were followed with a few modifications. For *Pinus*, the recommended 15-20 mg of lyophilized tissue was used for extraction. However, for *Arceuthobium*, only 5 mg of lyophilized tissue was used since DNA became sheared when larger amounts of plant material were loaded onto the columns. The elution step

was also modified for both *Pinus* and *Arceuthobium* by eluting DNA from columns twice with 150 µL of pre-heated AE buffer to increase final yield. DNA was concentrated by an overnight EtOH precipitation and resuspended in 30 µL of sterile dH_20.

AFLP Analysis

The AFLP procedure was carried out using Mse1 and EcoR1 restriction enzymes as per Zabeau and Vos (1993) and Vos et al. (1995) with modifications as described in Chapter 2. Pre-amplification PCR for *Arceuthobium* was performed using a +1/+1 pre-amplification primer combination (see Table 3, Chapter 2), whereas for *Pinus*, a +1/+2 pre-amplification primer combination, E-A (GAC TGC GTA CCA ATT CA) and M-CC (GAT GAG TCC TGA GTA ACC) was used. Reaction conditions and cycle parameters for *Arceuthobium* are described in Chapter 2. For *Pinus*, reactions were similar to that for *Arceuthobium*. Tubes contained two µL of the diluted ligated DNA sample, 30 ng of each pre-amp. primer, 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 1.3 units of Taq polymerase. Denaturation was at 94.0°C for 30 seconds, followed by primer annealing at 56.0°C for 1 minute, and primer extension at 72.0°C for 1 minute. The cycle was repeated 28 times and the pre-amplification product was diluted 1 in 6 with sterile distilled water.

Selective amplification PCR for *Arceuthobium* was performed using two +3/+3 primer combinations: (1) M-CAC, E-ACG; and (2) M-CCG and E-ACA (see Chapter 2). Due to the large size of the *Pinus* genome, selective amplification PCR for *Pinus spp.* was performed using +3/+4 primer combinations in order to reduce the number of fragments (pers. comm., D. Remington, Forest Biotech. Group, North Carolina State University, Raleigh, NC). A preliminary analysis was performed using five +3/+4

selective PCR primer combinations across 20 *Pinus* spp. populations. This analysis yielded 122 loci and showed the same overall topology (not shown) as that obtained with only two primer combinations. Thus, further analyses were restricted to loci obtained from only two +3/+4 primer combinations: (1) M-CCAG (GAT GAG TCC TGA GTA ACC AG), E-AGG (GAC TGC GTA CCA ATT CAG G); and (2) M-CCGC (GAT GAG TCC TGA GTA ACC GC), E-ACG (GAC TGC GTA CCA ATT CAC G). Reaction conditions and cycles were similar to that for *Arceuthobium* (see Chapter 2). However, for *Pinus*, the selective amplification was carried out in a 25 μL reaction volume and an initial denaturation step at 94.0°C for 3 minutes was added to the cycle parameters. As well, once the annealing temperature had ramped to 56.0°C, 23 cycles were carried out holding this annealing temperature constant.

AFLP products were run on 5% polyacrylamide gels, fixed in 10% glacial acetic acid, and silverstained using the Silver Sequence™ DNA Sequencing System kit (Promega) following the modifications described in Chapter 2. Both monomorphic and polymorphic loci were included in the study. Following scoring by two independent researchers, ambiguous loci or individuals were excluded from the analysis. Bands that could not be resolved were recorded as missing data. To confirm band positions, each gel contained 100ng of pGEM marker (Promega) as well as representatives from several disparate populations. Gels were photocopied and/or scanned for preservation.

DATA ANALYSIS OF *ARCEUTHOBIUM* AND *PINUS* AFLP

To calculate genetic distance and diversity, two approaches were used. The first approach was based on Nei's distance and diversity measures unbiased for sample size (Nei, 1978). In this case, the frequency of presence/absence bands of both *Arceuthobium*

and *Pinus* were first adjusted using the correction factor of Lynch and Milligan (1994) for dominant markers (see Chapter 2). This factor uses an assumption of Hardy-Weinberg equilibrium to correct for problems associated with dominant marker systems (such as AFLP and RAPDs) that underestimate null and overestimate presence allele frequencies due to the masking of null alleles in the heterozygous state. The assumption that populations are in Hardy-Weinberg equilibrium is fair since *Arceuthobium* spp. are dioecious (Hawksworth and Wiens, 1996) and genetic studies (Nickrent and Butler, 1990; Nickrent and Butler, 1991) have shown high similarity between observed and expected heterozygosity levels for members of this genus. This assumption is also valid for the genus *Pinus* since species are known to outcross and transport their pollen over extremely long distances (Nicholls et al., 1978; Ledig, 1998; Campbell et al., 1999). As well, molecular studies have also shown high similarity between observed and expected heterozygosities for *P. banksiana* and *P. contorta* var. *latifolia* (Yeh and Layton, 1979; Dancik and Yeh, 1984; Yeh et al., 1985).

Within-population variability measures including the proportion of polymorphic loci and expected heterozygosities (H_{exp}) were determined from the L-M corrected allele frequencies amongst the 29 populations using BIOSYS-2 (Swofford and Selander, 1992) recompiled by Dr. Kermit Ritland (Department of Forest Sciences, University of British Columbia, Vancouver, BC). Among-population genetic diversity measures including total genetic diversity (H_T), average diversity within (H_S) and among populations (D_{ST}), and the coefficient of genetic differentiation (G_{ST}) were calculated using Nei and Chesser's (1983) procedure (unbiased for sample size) using the output from BIOSYS-2. These measures were examined for the four *Pinus* taxa and the four groups of

Arceuthobium defined on the basis of host identity (*P. banksiana*, *P. contorta var. latifolia*, *P. banksiana* X *P. contorta* var. *latifolia* hybrids, and *P. contorta* var. *murrayana*).

Genetic data were also examined using an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992). Genetic distance was first calculated between individuals as Euclidean distances from the plus/minus marker data. The AMOVA procedure was then used to calculate variance components and pairwise population F_{ST} values from the Euclidean distances. The pairwise population F_{ST} values (equivalent to ϕ_{ST}) were used as measures of genetic distance between populations (Huff et al., 1993). Negative Fst values are considered a sampling artefact and were considered as equivalent to zero (Tansley and Brown, 2000; D. Huff, Pennsylvania State University, pers. comm.). A population level AMOVA was used to examine diversity within and between populations for each of *A. americanum*, *P. banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana*. In addition, a nested AMOVA was performed on the four groups of *A. americanum* to assess the amount of diversity within and between host groups of this parasite. Euclidean distances and AMOVA were calculated using the software program ARLEQUIN (Schneider et al., 2000). An UPGMA dendrogram for both *A. americanum* and *Pinus* spp. was constructed using pairwise population F_{ST} genetic distance values with the program SYNTAX 5.0 (Podani, 1997). UPGMA dendrograms based on Nei's unbiased genetic distances yielded the same overall topology and, thus were not presented in this thesis.

Mantel tests were used to compare host and parasite genetic and geographic distance matrices. Mantel tests (Mantel, 1967; Smouse et al., 1986) are widely used to

compare distance matrices in ecological and population studies to examine for correspondence between different parameters (Winfield et al., 1998; Cooper, 2000). Prior to analysis, parasite and host populations were divided into groups based on identity of the host species. Thus, the effect of local host genotype on parasite genetic structure could be examined independent of the role played by host species identity. For the Mantel tests, only two (*P. contorta* var. *latifolia* and *P. banksiana*) of the potential four host groups were examined. The small number of populations examined (n=3) from *P. contorta* var. *murrayana* precluded the analysis of this species due to bias during matrix randomization. The hybrid host group was not examined since results would be complicated by the nature of these individuals which represent a variety of introgressed forms between *P. banksiana* and *P. contorta* var. *latifolia*.

Two-way Mantel tests (Smouse et al., 1986) were used to compare host and parasite genetic structures to assess the extent to which genotype of the host predicted the genotype of the parasite. If parasites were adapted to local host genotypes, the genetic distance between populations of parasites and hosts should be correlated (Dybdahl and Lively, 1996). Genetic and geographic distance matrices were also compared to assess the role of geography in shaping the fine-scale genetic population structure within a host taxon or parasite race. A correlation between genetic and geographic distances would imply a role for geographic isolation or adaptation to local environmental conditions. Geographic distances (km) were calculated by assuming that dispersal between populations occurred via a straight-line geographic distance "as the crow flies" between all populations. The significance of the standardized correlation (r) for the Mantel tests was determined using matrix randomization. Correlations were considered significant if

the probability of obtaining the observed value of r by chance alone among 1000 reshuffled matrices was small ($P<0.05$). Two-way Mantel tests were performed using the program EVAL of SYNTAX 5.1 (Podani, 1997). In addition, partial correlations between distance matrices were calculated using three-way Mantel tests to examine the effect of either geography or host genetic distance on parasite genetic distance, independent of the influence imposed by the other variable (Smouse et al., 1987). Three way Mantel tests were performed using the Mantel option in the computer program ARLEQUIN (Schneider et al., 2000).

Scatterplots of pairwise distances for pairs of populations were used to portray parasite genetic distance versus host genetic distance, parasite genetic distance versus geographic distance, and host genetic distance versus geographic distance. Scatterplots were constructed using the computer program Data Desk® Version 4.1 © (Vellman, 1993).

3.3 RESULTS

The two primers used in this analysis yielded 79 scorable loci ranging in size from 400 to 1,100 bp. Of these loci, 61 were polymorphic and 18 were monomorphic.

OVERALL GENETIC STRUCTURE OF PARASITE AND HOST POPULATIONS

The dendrogram (Figure 6) constructed by UPGMA cluster analysis using Fst genetic distances (Appendix 3) indicated that the 29 *A. americanum* populations were divided into three distinct genetic races, each associated with different host taxa in regions of allopatry: (1) *P. banksiana* in western and central Canada; (2) *P. contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountain ranges; and (3) *P. contorta* var. *latifolia* throughout western U.S.A. and Canada. *Arceuthobium americanum* populations isolated from the hybrids were found at variable positions throughout the dendrogram (Figure 6), either associated with the *P. banksiana* host cluster, or as outliers to all populations. Genetic identity within groups varied (Table 8) but was relatively high (> 0.940) with the exception of the hybrid group (0.892). However, the genetic identity for *A. americanum* as a whole was relatively low (0.913, range 0.800 - 0.985).

The division of *A. americanum* into three races was recognized in an earlier study based on a larger data set of 51 populations (see Chapter 2). In this second study, however, the pattern observed for *A. americanum* can be interpreted in light of the genetic structure of host populations. Surprisingly, the UPGMA dendrogram (Figure 7) using Fst genetic distances (Appendix 4) of the hosts suggested that *Pinus* spp. were divided into only two distinctive genetic groups: (1) *P. banksiana* and hybrids; and (2) *Pinus contorta* var. *latifolia* and var. *murrayana*. The two varieties of *P. contorta* were not well-differentiated from each other. A closer inspection of the *Pinus* data using

NMDS ordination analysis did reveal (Figure 8), however, that *P. contorta* var. *latifolia* and var. *murrayana* could be weakly distinguished from each other, although the population from var. *latifolia* in John Day (OR-38) was an outlier to both of these groups. Interestingly, this population occupies an intermediate geographic position between the regions where the two host varieties occur. *Pinus* hybrid populations were found associated with the *P. banksiana* group, suggesting a higher contribution from this parent relative to that from *P. contorta* var. *latifolia* parent.

The high genetic identity found within either *P. banksiana* or *P. contorta* relative to the low identity between these species suggests that they are well-differentiated taxa (Table 9). However, the two varieties of *P. contorta* showed a similar level of identity with each other (0.970) as they do with themselves (0.969 - 0.973).

COMPARISON OF PARASITE AND HOST GENETIC / GEOGRAPHIC DISTANCES

Two-way Mantel tests were performed to examine for correspondence between parasite and host genetic and geographic distance matrices (Table 10). For the *P. banksiana* group, correlations were not strong. For example, the correlation between parasite and host genetic distance matrices was moderate (Figure 9a; r=0.326, P=0.009, Table 10). However, the correlation between parasite genetic distance and geographic distance was weak (Figure 9b; r=0.122, P=0.286, Table 10). Finally, the correlation between host genetic distance and geographic distance (Figure 9c; r=0.331, P=0.014, Table 10) was similar to that observed between host and parasite genetic distance. For the *P. contorta* var. *latifolia* group, correlations differed from those seen for the *P. banksiana* group (Table 10). The relationship between host and parasite genetic distance matrices was quite strong (Figure 10a; r=0.586, P=0.007, Table 10). However, when

parasite genetic distances were compared with geographic distances, a weaker relationship was seen (Figure 10b; r=0.185, P=0.154, Table 10). Similarly, a weak correlation was seen between host genetic and geographic distances (Figure 10c; r=0.080, P=0.274, Table 10). Thus, in both cases parasite genetic distance correlated more strongly with host genetic distance than with geographic distance.

Partial correlations (see Table 11) were also performed to examine the effect on parasite genetic structure imposed by either: (a) host genotype (without the confounding influence of geographic isolation), or (b) geographic distance (without the confounding influence of host genotype). When partial correlations were examined for only those populations from *P. banksiana* hosts, 10.4% of the variance in the parasite genetic distance matrix could be attributed to the host whereas 0.0% could be attributed to geography (Table 11). When only those populations isolated from *P. contorta* var. *latifolia* hosts were examined, 33.7% of the variance in the parasite genetic distance matrix could be attributed to host and 2.7% could be attributed geography (Table 11). Thus, in both cases, local host genotype had a greater effect on parasite population structure than did geography. Regardless, only a portion (10.4% for parasites on *P. banksiana* and 36.4% for parasites on *P. contorta* var. *latifolia*) of the total genetic variation in parasite population structure was explained by host and geography combined. This implied that within a given host race of *A. americanum*, there was little evidence for division into further infraspecific taxa as a result of geographic isolation or adaptation to specific host genotypes.

PARASITE GENETIC VARIATION AND DIVERSITY MEASURES

Overall Variation

Population level variability measures for the 29 populations of *A. americanum* examined in this study were previously reported and discussed in Chapter 2 (Table 5). Nei and Chesser's (1983) among-population diversity measures for *A. americanum* were calculated independently for this second study (Table 12) since analyses were restricted to a subset of the initial 51 populations. This allowed for a more direct comparison between host and parasite populations. When all populations were considered as one taxon, *A. americanum* was observed to be genetically diverse (H_T of 0.234; H_S of 0.163) with strong differentiation amongst populations (G_{ST} of 0.303). The population-level AMOVA confirmed this observation since most of the variance was found within (67.07%) rather than between populations (32.93%) ($p<0.001$) (Table 13). These results are similar to that which was seen when all 51 populations of *A. americanum* were included in the analysis (Chapter 2).

Within Group Variation

Diversity measures were also assessed for groups of *A. americanum* defined by host identity. This analysis indicated that total genetic diversity (H_T) measures were similar for each of the host groups of *A. americanum* (0.192 - 0.201) with the exception of the higher diversity seen for the *P. contorta* var. *latifolia* group (0.225). Within group genetic diversity values (H_S) showed a wider range of values (0.123 - 0.188) and were consistently lower than values for H_T. Likewise, values for population differentiation as measured by the coefficient of genetic differentiation (G_{ST} 0.089 - 0.388) ranged widely across groups (Table 12). When the four groups of *A. americanum* were examined using

a nested AMOVA, most of the variance (63.73%) was still found within the populations. Of the remaining variance, 17.80% was found among the four groups, and 18.47% was found among the populations within these groups ($p<0.001$) (Table 13).

Since *A. americanum* on *P. contorta* var. *latifolia* spans regions with drastically different glacial histories, it is interesting to examine diversity in these two groups. Similar to previous findings (Chapter 2), *A. americanum* populations from previously glaciated northern regions were found to be more diverse (H_T 0.225, H_S 0.193) than those from unglaciated southern regions (H_T 0.210, H_S 0.184) (Table 12).

HOST GENETIC VARIATION AND DIVERSITY MEASURES

Population level variability measures ranged considerably (37.7 - 62.3 % poly.; H_{exp} 0.130 - 0.219) across the 29 *Pinus* spp. host populations (Table 14). In contrast with that seen for *A. americanum* populations, the three hybrid pine populations from northern Alberta (AB18, AB22, and AB22) were amongst the most genetically diverse (54.1 - 55.7 % poly.; H_{exp} 0.189 - 0.196) of all populations studied.

Nei and Chesser's (1983) among population diversity measures for individual host species indicated that total genetic diversity (H_T) and within group diversity (H_S) were similar across all pine species (H_T 0.174 - 0.192, H_S 0.166 - 0.172) with the exception of *P. banksiana* X *P. contorta* var. *latifolia* hybrids which had considerably higher levels for these measures (H_T 0.217, H_S 0.193). (Table 15). Interestingly, the parasite races were characterized by even higher total genetic diversity (H_T 0.192 - 0.225) than the host taxa (H_T 0.174 - 0.217) (compare Tables 12 and 15).

In the pines, the small divergence between H_T and H_S values resulted in low values of population differentiation as estimated by G_{ST} (0.046 - 0.130). In comparison,

A. americanum had a G_{ST} of 0.303, indicating that populations of the parasite were 3 - 6 X more strongly structured than its hosts. As with Nei and Chesser's diversity measures reported above, the AMOVA revealed that populations of *Pinus* spp. were much less differentiated (3.77 - 10.55 %) (Table 16) than were populations of its parasite (32.93%) (Table 13).

As with the parasite, *P. contorta* var. *latifolia* spans regions with vastly different glacial histories in the Wisconsin period. When *Pinus contorta* var. *latifolia* populations were split into two subgroups, those from previously glaciated regions had similar levels of total genetic diversity (H_T of 0.183) and within group genetic diversity (H_S 0.169 ± 0.020, P=0.716) as those from unglaciated regions (H_T of 0.196; H_S 0.164 ± 0.020) (Table 15).

3.4 DISCUSSION

FACTORS INFLUENCING OVERALL STRUCTURE OF *A. AMERICANUM*

As was previously observed with 51 *A. americanum* populations (Chapter 2), the 29 populations examined in this study were divided into three genetic races, each infecting a different host taxon in regions of allopatry. This pattern suggested that identity of the host taxon, isolation-by-distance, and environmental parameters have facilitated the formation of three genetic races in *A. americanum.* In this second study, the impact of these factors was further explored by assessing the overall structure of *A. americanum* in light of information about the overall structure of the hosts.

Examination of hosts revealed that only two of the three taxa were genetically distinct. Since *P. banksiana* and *P. contorta* hosts were genetically divergent, they may impose different selection pressures on their respective parasite populations. The role played by host identity does not preclude, however, a role for isolation-by-distance and adaptation to environmental conditions (see Chapter 2). These two latter factors likely play an important role in the diversification of the *A. americanum* races associated with the two *P. contorta* varieties. Since the two host varieties could not be readily discriminated using molecular markers, they are unlikely to impose strong divergent selection pressures on their parasites. Host influence can not be completely ruled out, however, since ordination analysis showed they can be weakly differentiated. Nonetheless, the differences amongst parasite populations in this region are more likely a result of adaptation to different environmental conditions and geographical barriers that limit gene flow (see Chapter 2). Gene flow is likely to be restricted between *A. americanum* populations on *P. contorta var. murrayana* in the Sierra Nevada and

Cascade Mountain ranges and those on *P. contorta* var. *latifolia* in the Blue, Salmon River, Uinta, and Rocky Mountains. These restrictions are more likely to affect the parasite than its host due to intrinsic differences in their mating systems. For example, pine pollen is carried over much longer distances (Ledig, 1998; Campbell et al., 1999) than is *Arceuthobium* pollen (Penfield et al., 1976; Gilbert and Punter, 1984).

FACTORS INFLUENCING PATTERNS WITHIN *A. AMERICANUM* RACES

In Chapter 2, cluster analyses and ordinations indicated weak geographic patterning within *A. americanum* races. The weak correspondence between genetic and geographic distance indicated a negligible role for isolation-by-distance in shaping the fine-scale structure of *A. americanum*. This was used to support the role of random dispersal of *A. americanum* seeds by birds and mammals into new and geographically disparate stands. However, it was also suggested that strong selection pressures that are not geographically patterned might obscure patterns of isolation-by-distance. Such selection pressures could act even if gene flow is not limited by geographic barriers. In this second paper, fine-scale patterning of *A. americanum* was further explored using Mantel tests to assess the importance of adaptation of parasite populations to local host genotypes.

Local Host Genotypes

For *A. americanum* races found on both *P. banksiana* and *P. contorta* var. *latifolia*, a greater proportion of the variation in the parasite genetic distance matrix was accounted for by local host genotype than isolation-by-distance. This observation suggests that populations of *A. americanum* have to some extent become adapted to local host genotypes within a host species. This is perhaps not surprising given that *A.*

americanum is strictly dependent upon its host for survival.

The greater contribution of local host genotype to shaping the genetic structure of *A. americanum* populations isolated from *P. contorta* var. *latifolia* in comparison to those found on *P. banksiana* may be related to its longer association with the former host. Based on fossil pollen evidence and the geographic distribution of *A. americanum* relative to its principal host species, it has been hypothesized that *A. americanum* originated as a parasite of *P. contorta* and later jumped onto *P. banksiana* (Hawksworth and Wiens, 1996). If this is true, the pattern may simply reflect the length of evolutionary time that *A. americanum* has had to adapt to local genotypes of *P. contorta* var. *latifolia*. Indeed, given the wide array of other factors (such as life history traits and historical events) that can influence the structuring of populations, a considerable portion (>30%) of the variation in *A. americanum* on *P. contorta* var. *latifolia* is actually accounted for by local host genotype.

Local Environmental Conditions

Broad scale environmental patterns associated with different ecoclimatic regions have been implicated in this and a previous study (Chapter 2) as facilitating race formation in *A. americanum*. Patterning within *A. americanum* races may also be affected by environmental patterns, but in this case on a much finer-scale. For example, *A. americanum* could be adaptating to local environmental conditions that are not geographically patterned. In a recent study by Cooper (2000), such factors were implicated as playing an important role in shaping the genetic structure in the southern brown bandicoot (a small marsupial). In his study, Cooper (2000) found no correlation between genetic and geographic distance despite a lack of geographic barriers to gene

flow. However, Cooper (2000) did find a strong correlation between genetic distance and both habitat type (swamp or forest) and annual rainfall levels. Cooper (2000) concluded that gene flow amongst populations was being limited due to selection against new migrants imposed by local habitat type and levels of rainfall. In *A. americanum*, habitat type is unlikely to play a role in fine-scale patterning since most stands where this parasite was found are of similar plant composition. However, local environmental conditions such as rainfall, light intensity, and average temperature probably do differ between sites in which *A. americanum* is found. These factors could account for some degree of fine-scale structuring within *A. americanum* races. Unfortunately, measures of local environmental conditions are not available for the sites examined in this study.

Non-Intrinsic Factors

Numerous factors not intrinsic to the organism itself can also affect an organism's population structure. These include factors such as rapid migration, founder events, bottlenecks, recent speciation events, habitat heterogeneity, complex age structure, introgression from other genomes, and Pleistocene and pre-Pleistocene glacial history (see Lewis and Crawford, 1995). Indeed, all of these factors likely contributed (along with random seed dispersal by animal vectors and selection pressures imposed by local genotype and environmental conditions) to the small percentage of variation in genetic structure of *A. americanum* that was attributable to isolation-by-distance.

Comparison with the Literature

Currently, there are no published papers comparing the genetic structure of parasitic angiosperms and their hosts. However, a review of previous studies that have examined genetic population structure of animal parasites and their hosts suggests that no

single factor can explain fine-scale patterning in parasites. For example, Dybdahl and Lively (1996) showed that the partial correlation between genetic distance matrices of a trematode parasite and its snail host was much less than that observed between parasite genetic and geographic distance matrices. Thus, in contrast to that seen for *A. americanum*, geography plays a more important role than host genotype in shaping the fine-scale structure of this organism. Similarly, Martinez and coworkers (1999) found that the genetic population structures of the great spotted cuckoo and its magpie hosts were not correlated. However, the genetic structure of each was postively correlated with geographic distance, indicating the importance of dispersal from native areas. A slightly different conclusion was reached by Mulvey and coworkers (Mulvey et al., 1991) in their study of a liver fluke parasite and its deer host. These researchers found a lack of congruence between parasite and host genetic and geographic distance matrices. This lack of influence by either factor was attributed to the role played by long distance dispersal of the host, and subsequently, its resident parasites (Mulvey et al., 1991). These researchers suggested that this long-distance dispersal may have counteracted factors that would have led to spatial differentiation of these populations.

From these studies, it is evident that generalizations about the role of host and geography in shaping parasite populations are not possible (contra Price, 1980). This likely pertains to the fact that life history traits and non-intrinsic factors vary widely across host-parasite pathosystems (Nadler, 1995).

COMPARISON OF VARIABILITY / DIVERSITY IN *A. AMERICANUM* AND HOSTS

Levels of Genetic Diversity

A comparison of levels of diversity within and between a parasite and its hosts is

important for understanding their interaction. The higher level of total genetic diversity (H_T) observed in the parasite relative to its hosts may be related to several factors. Firstly, the geographic range of the parasite as a whole is larger than that of its individual host taxa. Empirical studies across a large number of plant species indicate that taxa with wide geographic ranges commonly possess higher levels of genetic diversity than do more narrowly distributed taxa (Hamrick and Godt, 1990). Additionally, *A. americanum* must infect different host taxa with varying physiological and genetic characteristics over this range. This may also act to maintain higher diversity in the parasite. Higher levels of genetic diversity are also observed within each of the *A. americanum* races defined on the basis of host identity. In this case, the higher diversity of the parasite may be related to adaptation of parasites to different host genotypes and habitat conditions.

In only one situation were host populations more diverse than parasite populations. Both within population variability values and within group diversity measures indicated that the least diverse parasite populations (i.e. those from hybrid hosts in northern Alberta) were isolated from the most diverse host populations. For the parasite, it was previously speculated (Chapter 2) that founder events and geographic isolation contributed to the low level of genetic diversity in these northern populations. For the hosts, the hybrid nature of these organisms contributes to their higher diversity since they represent a mixture of the genomes of two different species.

Effect of Glaciation on Genetic Diversity

Conspecifics and congeners from from glaciated regions tend to be less genetically diverse than those from unglaciated regions (Fowler and Morris, 1977; Copes, 1981; Waller et al., 1987; Broyles, 1998). This general pattern has been attributed to the

loss of genetic diversity that occurs as a species migrates (often rapidly) from large refugia south of the glacial front into newly deglaciated northern regions (reviewed in Lewis and Crawford, 1995 and Broyles, 1998). In this study, hosts showed an atypical pattern since populations of *P. contorta* var. *latifolia* from unglaciated southern (off-ice) regions had a similar level of genetic diversity as those from previously glaciated northern (on-ice) regions. Thus, past glaciations do not appear to have greatly impacted genetic diversity in the host. The parasite showed an even more dramatic contrast to the typical pattern since *A. americanum* populations from previously glaciated northern (on-ice) regions were actually more diverse than populations from unglaciated southern (off-ice) regions. This pattern was previously seen with all 51 *A. americanum* populations (Chapter 2). The most plausible explanation for these observations is that *A. americanum* and its *P. contorta* var. *latifolia* hosts survived in a genetically diverse glacial refugium along the eastern slope of the Rocky Mountains in Canada during the latter part of the Wisconsin glaciation (Chapter 2). Expansion of the host and its parasite into newly deglaciated territory in the north may have had a bigger impact on the host than it did on the parasite. This could occur if the hosts moved into deglaciated regions at a much quicker rate than did the parasite. This is possible since the obligate parasite would have had to migrate behind the movement of the host.

COMPARISON OF HOST AND PARASITE POPULATION DIFFERENTIATION

Arceuthobium americanum populations were found to be 3 - 6 times more structured than any of the principal host species. This structuring was seen across all 29 *A. americanum* populations, as well as for the *A. americanum* groups defined on the basis of host identity. Strong structuring in *A. americanum* relative to *Pinus* may be attributed

to several factors.

Breeding and Dispersal Mechanisms

Differences in breeding mechanisms between *Arceuthobium* and *Pinus* may account for much of the difference in structuring of the parasite relative to its host. Due to the prevalence of wind-pollination in gymnosperms, these taxa have the highest levels of gene flow recorded for any plant group (Hamrick and Godt, 1990). In the genus *Pinus*, pollen morphology allows it to be transported by wind over extremely long distances (hundreds to thousands of kilometers) (Ledig, 1998; Campbell et al., 1999). In the genus *Arceuthobium*, there is no evidence to suggest that pollen can be carried by the wind over distances comparable to that seen for the *Pinus* hosts (Penfield et al., 1976; Gilbert and Punter, 1984). Interestingly, long-distance dispersal of *A. americanum* seeds by animal vectors seems to increase rather than decrease population differentation. This would result from colonists in neighbouring stands originating from regions with different genotypes.

Selection Pressures

Price (1980) predicted that adaptation and strict dependence of a parasite upon a host would lead to strong structuring of a parasite (but see Nadler, 1995). This and a previous study (Chapter 2) do suggest that differentiation of the parasite can be partially attributed to selection pressures imposed by different host taxa and/or local host genotypes. Differential selection pressures imposed by environmental conditions throughout the range of *A. americanum* may also contribute to the strong partitioning of diversity in this parasite. Such selection pressures could favour specific genotypes of *A. americanum* in certain regions, thereby accounting for strong structuring amongst

populations.

Comparison with the Literature

Other studies that have examined the degree of genetic structuring in parasites relative to their hosts have showed varying results. Similar to that seen for the *A. americanum-Pinus* spp. pathosystem, Delmotte and coworkers (Delmotte et al., 1999) showed that fungal parasites were much more differentiated than their *Silene* L. plant hosts. These researchers attributed this finding to different mating systems since the fungal parasites undergo routine selfing whereas the host plant outcrosses. Martinez and coworkers (1999) observed a similar pattern whereby great spotted cuckoo populations were found to be more strongly structured than their magpie hosts. It was suggested that perhaps the cuckoo parasites impose a selection pressure for their magpie hosts to disperse relatively long distances from their natal sites. Alternatively, these researchers suggested that frequent extinctions and recolonizations of local magpie populations in the presence of these brood parasites could have contributed to this pattern. Some researchers (Dybdahl and Lively, 1996; Davies and coworkers, 1999) also observed the opposite pattern to that seen for the *A. americanum-Pinus* spp. pathosystem. In these studies, the primary hosts (snails) were found to be more strongly structured than their parasites (trematodes). The difference in structuring was attributed to dispersal mechanisms. For example, the primary snail hosts disperse themselves over short distances. However, the parasites are dispersed over longer distances by their subsequent and final hosts (birds, Dybdahl and Lively, 1996; humans, Davies et al., 1999).

As can be seen from the present study and from the literature, the complex nature of forces acting on hosts and parasites will lead to varying degrees of differentiation in

hosts and parasites, depending on the pathosystem in question (contra Price, 1980). This is important since the degree to which a parasite and host are differentiated relative to each other may have an impact on the evolutionary outcome of host-parasite interactions (Dybdahl and Lively, 1996).

AFLP AS A MARKER FOR POPULATION GENETIC STUDIES ON PINES

There is general congruence between genetic diversity measures and differentiation values in pines obtained in this study using dominant AFLP markers and that previously reported based on codominant isoenzyme markers. In the preseent study, values for population differentiation in *P. banksiana*, *P. contorta* var. *latifolia* and var. *murrayana* (G_{ST} 0.046 - 0.130) using AFLP data were similar to those determined for these taxa in several other studies (G_{ST} 0.010 - 0.070) using isoenzymes (reviewed in Ledig, 1998). As well, the average within population genetic diversity (H_S 0.166 - 0.172) based on AFLP falls within the range of values reported for these taxa by several other authors (H_S 0.143 - 0.185) using isoenzymes (reviewed in Ledig, 1998). The small sample size used in this study seems to have had little effect on estimates of diversity and differentiation in *Pinus* spp. Overall, application of Nei and Chesser's (1983) procedure for calculating diversity measures seems appropriate for AFLP data after the L-M correction factor has been applied. Thus, dominant markers such as AFLPs seem reliable for studying population structure in outcrossing plants. To increase robustness, future studies could examine fewer populations but a larger number of individuals per population.

EVOLUTIONARY IMPLICATIONS

Speciation via race formation has been implicated as a major evolutionary force

affecting parasites (Brooks and McLennan, 1993; Thompson, 1994; Norton and Carpenter, 1998). In this and a previous study (Chapter 2), *A. americanum* was found to be divided into three genetic races, each associated with a different host taxon. Given the strong genetic differentiation between these races, it is conceivable that these races will differentiate into distinct species given a sufficient period of time, (Norton and Carpenter, 1998; but see Levin, 1993).

Restricted gene flow amongst parasite populations can also enhance the potential of parasites to track local host genotypes within a host species (Price, 1980). Thus, it is possible for parasites to undergo race formation on local host genotypes within a host taxon. The findings from this study indicate that only a small portion of the parasite genetic structure can be attributed to local host genotypes. Thus, race formation on local host genotypes is unlikely to occur in this taxon. High gene flow between genetically unrelated neighbouring populations due to long-distance seed dispersal may prevent the formation of races adapted to host genotypes within a host taxon.

3.5 CONCLUSIONS

As was observed in a previous study (Chapter 2), *A. americanum* was found to be divided into three genetic races, each associated with a different host taxon in regions of allopatry. In the present study, patterning of parasite populations was further explored in light of knowledge about structuring of *Pinus* host populations. This proved insightful since only two of the three host taxa were found to be genetically distinct. Since *Pinus banksiana* was distinct from *P. contorta*, it was inferred that these hosts may impose differential selection pressures that have facilitated race formation in *A. americanum*. This does not preclude, however, a role for geographic isolation and adaptation to different environmental conditions. On the other hand, the two varieties of *P. contorta* were not genetically distinct from each other. Thus, these host taxa likely did not impose highly divergent selection pressures on their parasite populations. In this case, it appears that isolation-by-distance and adaptation to different environmental conditions have been more important in the divergence of *A. americanum* races associated with these host varieties.

There was a general lack of fine-scale geographic patterning to genetic diversity within the three *A. americanum* races. This suggests that populations are unlikely to become subdivided into additional races. Given the lack of geographic patterning, isolation-by-distance appears to play a negligible role in shaping the fine-scale structure within races. Rather, this pattern was attributed to random dispersal of *A. americanum* seeds over long distances by animal vectors. As well, fine-scale structure may have been limited by adaptation of *A. americanum* populations to non-geographically patterned factors such as local host genotype and environmental variables. Unfortunately,

measurements of local environmental conditions were not available for the sites examined in this study. Thus, the contribution of this factor is undetermined. Local host genotypes do appear to play some role in shaping fine-scale structure of parasite populations though, particularly for *A. americanum* populations on *P. contorta* var. *latifolia*.

Parasite populations were found to have higher genetic diversity and stronger structuring than their hosts. Factors intrinsic to the host may be partially responsible for this observation. For example, weak structuring in *P. banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana* can be attributed to their strongly outcrossing nature. Pine pollen is known to be transported by wind over extremely long distances. On the other hand, *Arceuthobium* spp. pollen is transported over relatively short distances. Furthermore, divergent selection pressures imposed by different hosts and environments may contribute to the high structuring and high genetic diversity seen in *A. americanum*. The wide geographic range of the parasite relative to each of its individual host taxa may also contribute to this high diversity.

Table 8. Matrix of Nei's unbiased genetic identity coefficients (range) (Nei, 1978) for pairwise comparisons among four groups of *Arceuthobium americanum** based on host species identity.

Host Species Identity	*Pinus banksiana* (n=11)	*Pinus contorta var. latifolia* (n=10)	Hybrids (n=5)	*Pinus contorta var. murrayana* (n=3)
Pinus banksiana	0.944 (0.890-0.979)			
Pinus contorta var. latifolia	0.908 (0.844-0.962)	0.952 (0.928-0.985)		
Hybrids	0.915 (0.854-0.957)	0.904 (0.850-0.958)	0.892 (0.844-0.936)	
Pinus contorta var. murrayana	0.868 (0.800-0.915)	0.907 (0.913-0.876)	0.868 (0.847-0.891)	0.973 (0.959-0.981)

* The average genetic identity across all twenty-nine *A. americanum* populations is 0.913 (0.800-0.985).

Table 9. Matrix of Nei's unbiased genetic identity coefficients (range) (Nei, 1978) for pairwise comparisons amongst four *Pinus* spp.

Species	*Pinus banksiana* (n=11)	*Pinus contorta var. latifolia* (n=10)	Hybrids (n=5)	*Pinus contorta var. murrayana* (n=3)
Pinus banksiana	0.976 (0.955-0.995)			
Pinus contorta var. latifolia	0.837 (0.748-0.915)	0.969 (0.931-0.997)		
Hybrids	0.955 (0.918-0.985)	0.897 (0.834-0.967)	0.967 (0.940-0.986)	
Pinus contorta var. murrayana	0.839 (0.797-0.903)	0.970 (0.937-0.993)	0.896 (0.862-0.947)	0.973 (0.966-0.986)

Table 10. Two-way Mantel test results for comparisons between host (*Pinus* spp.) and parasite (*Arceuthobium americanum*) genetic and geographic distance matrices. Number of populations sampled (n), correlation (r), and probability (P).

Host	*Pinus banksiana* (n=11)	*Pinus contorta* var. *latifolia* (n=10)
Parasite vs Host	r=0.326 P=0.009	r=0.586 P=0.007
Parasite versus Geography	r=0.122 P=0.286	r=0.185 P=0.154
Host versus Geography	r=0.331 P=0.014	r=0.080 P=0.274

Table 11. Three-way Mantel test results showing partial correlations (r) between parasite (*Arceuthobium americanum*) and host (*Pinus* spp.) genetic distances, and between parasite (*A. americanum*) genetic and geographic distances to determine the effect of one variable when the other variable is controlled. Number of populations sampled (n), correlation (r), and probability (P).

Host	*Pinus banksiana* (n=11)	*Pinus contorta* var. *latifolia* (n=10)
Parasite versus Host	r=0.307 P=0.026	r=0.584 P=0.008
Parasite versus Geography	r=-0.021 P=0.564	r=0.174 P=0.177
Proportion of genetic variation in parasite attributed to host	10.4%	33.7%
Proportion of genetic variation in parasite attributed to geography	0.0%	2.7%
Proportion of genetic variation in parasite accounted for by both host and geography combined	10.4%	36.4%

Table 12. Genetic diversity statistics (Nei and Chesser, 1983) for a subset of 29 *Arceuthobium americanum* populations on its principal host species. Number of populations sampled (n), total genetic diversity (H_T), within-population genetic diversity (H_S), among-population genetic diversity (D_{ST}), coefficient of genetic differentiation (G_{ST}).

Host Species Identity	H_T	H_S	D_{ST}	G_{ST}
All populations (n=29)	0.234	0.163	0.071	0.303
Pinus banksiana (n=11)	0.198	0.154	0.044	0.222
Pinus contorta var. latifolia (n=10)	0.225	0.188	0.037	0.164
Canadian *Pinus contorta var. latifolia* (n=5)	0.225	0.193	0.032	0.142
U.S.A. *Pinus contorta var. latifolia* (n=5)	0.210	0.184	0.026	0.124
Pinus contorta var. murrayana (n=3)	0.192	0.175	0.017	0.089
Hybrids (n=5)	0.201	0.123	0.078	0.388

Table 13. Analysis of Molecular Variance (AMOVA) for 29 populations of *Arceuthobium americanum*. Populations were analyzed at both the population-level by considering all populations as a single taxon, and at the nested level using four groups based on host species identity (*P. banksiana*, *P. contorta* var. *latifolia*, *P. banksiana* X *P. contorta* var. *latifolia* hybrids, and *P. contorta* var. *murrayana*). Degrees of freedom (df), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value).

	df	SSD	Variance Components	% Total	P-value
Population Level					
Among Populations	28	1367.317	4.17771	32.93	<0.001
Within Populations	251	2135.833	8.50930	67.07	<0.001
Total	279	3503.150	12.68701	100.00	
Nested Level (Four groups)					
Among Groups	3	560.093	2.37773	17.80	<0.001
Among Populations within Groups	25	807.223	2.46593	18.47	<0.001
Within Populations	251	2135.833	8.50930	63.73	<0.001
Total	279	3503.150	13.35296	100.00	

Table 14. Genetic variability measures for 29 populations of *Pinus* spp. Average number of individuals (N), mean number of alleles per locus ± 1 SE (k), percentage of polymorphic loci at 95% criterion (P), expected heterozygosity ± 1SE (H_{exp}) (unbiased estimate Nei [1978]).

Population	N	k	P	H_{exp}
Pinus banksiana				
Belair (MB-2)	9.0 ± 0.0	1.5 ± 0.1	45.9	0.130 ± 0.027
Candle Lake (SK-3)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.201 ± 0.028
Cowan (MB-4)	10.0 ± 0.0	1.6 ± 0.1	57.4	0.219 ± 0.029
Ft. McMurray (AB-6)	10.0 ± 0.0	1.4 ± 0.0	44.3	0.152 ± 0.026
Grand Rapids I (MB-7)	9.9 ± 0.0	1.4 ± 0.1	44.3	0.154 ± 0.027
La Loche (SK-9)	10.0 ± 0.0	1.5 ± 0.1	50.8	0.162 ± 0.025
La Ronge (SK-10)	10.0 ± 0.0	1.5 ± 0.1	54.1	0.188 ± 0.027
Prince Albert I (SK-11)	9.0 ± 0.0	1.5 ± 0.1	52.5	0.186 ± 0.028
Smeaton (SK-14)	10.0 ± 0.0	1.4 ± 0.1	44.3	0.164 ± 0.027
Smoky Lake (AB-15)	10.0 ± 0.0	1.5 ± 0.1	49.2	0.166 ± 0.026
The Pas (MB-11)	8.4 ± 0.1	1.5 ± 0.1	49.2	0.166 ± 0.026
Species Average	9.7	1.5	49.5	0.172
Hybrids				
High Level (AB-18)	9.0 ± 0.0	1.6 ± 0.1	55.7	0.189 ± 0.027
Slave Lake (AB-19)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.189 ± 0.027
Whitecourt (AB-20)	10.0 ± 0.0	1.6 ± 0.1	62.3	0.203 ± 0.027
Wood Buffalo National Park (AB-21)	9.5 ± 0.1	1.5 ± 0.1	54.1	0.190 ± 0.027
Whitemud/PR (AB-22)	9.5 ± 0.1	1.6 ± 0.1	55.7	0.196 ± 0.027
Species Average	9.6	1.6	56.1	0.193
Canadian *Pinus contorta* var. *latifolia*				
Banff (AB-24)	8.0 ± 0.0	1.5 ± 0.1	52.5	0.184 ± 0.27
Castlegar (BC-25)	9.0 ± 0.0	1.5 ± 0.1	49.2	0.168 ± 0.026
Cypress Hills (AB-26)	10.0 ± 0.0	1.5 ± 0.1	45.9	0.149 ± 0.025
David Thompson Resort (AB-27)	10.0 ± 0.0	1.5 ± 0.1	47.5	0.151 ± 0.025
Jasper (AB-29)	10.0 ± 0.0	1.5 ± 0.1	54.1	0.194 ± 0.027
Cdn. Average	9.4	1.5	49.8	0.169

U.S.A. *Pinus contorta* var. *latifolia*				
John Day (OR-38)	9.5 ± 0.1	1.5 ± 0.1	50.8	0.173 ± 0.027
Ketchum (ID-40)	10.0 ± 0.0	1.4 ± 0.1	37.7	0.131 ± 0.025
Manila I (UT-41)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.169 ± 0.025
Red Feathers Lake I (CO-43)	9.5 ± 0.1	1.5 ± 0.1	49.2	0.185 ± 0.027
Yellowstone (WY-44)	10.0 ± 0.0	1.5 ± 0.1	47.5	0.164 ± 0.025
U.S.A. Average	9.8	1.5	47.5	0.164
Species Average	9.6	1.5	48.7	0.167
Pinus contorta* var. *murrayana				
Fort Klamath (OR-47)	10.0 ± 0.0	1.5 ± 0.1	49.2	0.187 ± 0.028
Lee Vining I (CA-48)	9.0 ± 0.0	1.4 ± 0.1	41.0	0.150 ± 0.027
Mount Shasta (CA-49)	9.0 ± 0.0	1.4 ± 0.1	44.3	0.162 ± 0.027
Species Average	9.3	1.4	44.8	0.166

Table 15. Genetic diversity statistics (Nei and Chesser, 1983) for *Pinus* spp. hosts. Number of populations sampled (n), total genetic diversity (H_T), within-population genetic diversity (H_S), among-population genetic diversity (D_{ST}), coefficient of genetic differentiation (G_{ST}).

Species	H_T	H_S	D_{ST}	G_{ST}
Pinus banksiana (n=11)	0.192	0.172	0.020	0.102
Pinus contorta var. latifolia (n=10)	0.192	0.167	0.025	0.130
Canadian *Pinus contorta var. latifolia* (n=5)	0.183	0.169	0.014	0.077
U.S.A. *Pinus contorta var. latifolia* (n=5)	0.196	0.164	0.032	0.163
Pinus contorta var. murrayana (n=4)	0.174	0.166	0.008	0.046
Hybrids (n=5)	0.217	0.193	0.024	0.110

Table 16. Analysis of Molecular Variance (AMOVA) for 29 populations of *Pinus* spp. Populations were divided into three groups based on species identity (*P. banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana*). Degrees of freedom (df), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value).

	df	SSD	Variance Components	% Total	P-value
Pinus banksiana					
Among Populations	10	73.610	0.20888	3.77	$p < 0.001$
Within Populations	96	511.633	5.32951	96.23	$p < 0.001$
Total	106	585.243	5.53840	100.00	
Pinus contorta* var. *latifolia					
Among Populations	9	102.055	0.58621	9.22	$p < 0.001$
Within Populations	85	490.861	5.77484	90.78	$p < 0.001$
Total	94	592.916	6.36105	100.00	
Pinus contorta* var. *murrayana					
Among Populations	2	19.033	0.51500	10.55	$p < 0.001$
Within Populations	27	117.900	4.36667	89.45	$p < 0.001$
Total	29	136.933	4.90311	100.00	

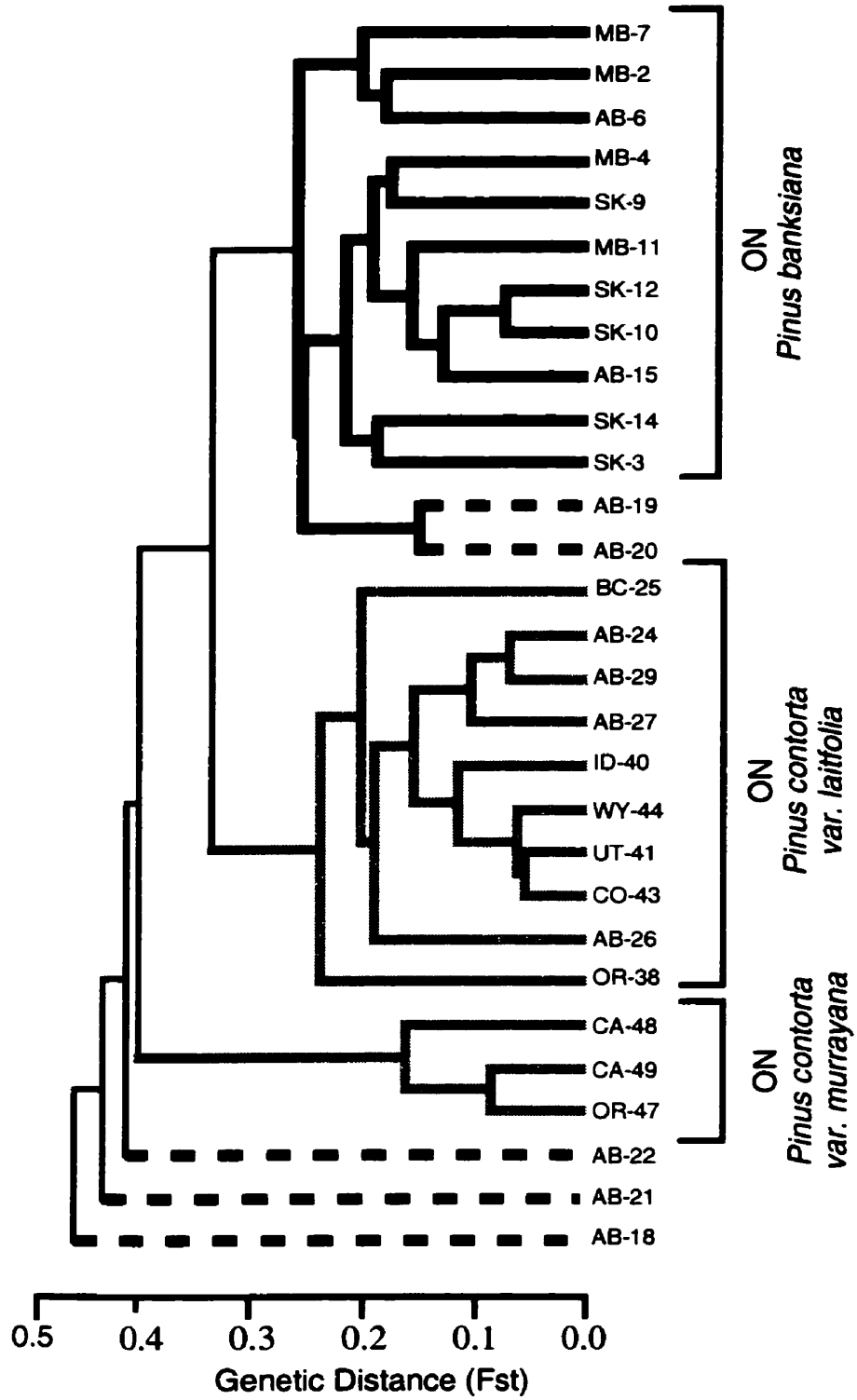

Figure 6. UPGMA dendrogram of 29 *Arceuthobium americanum* populations based on Fst genetic distances (Excoffier et al., 1992). Line colour indicates host identity. Black lines indicate *P. banksiana* host, gray lines indicate *P. contorta* var. *latifolia* and var. *murrayana* hosts, dashed lines indicate hybrid hosts.

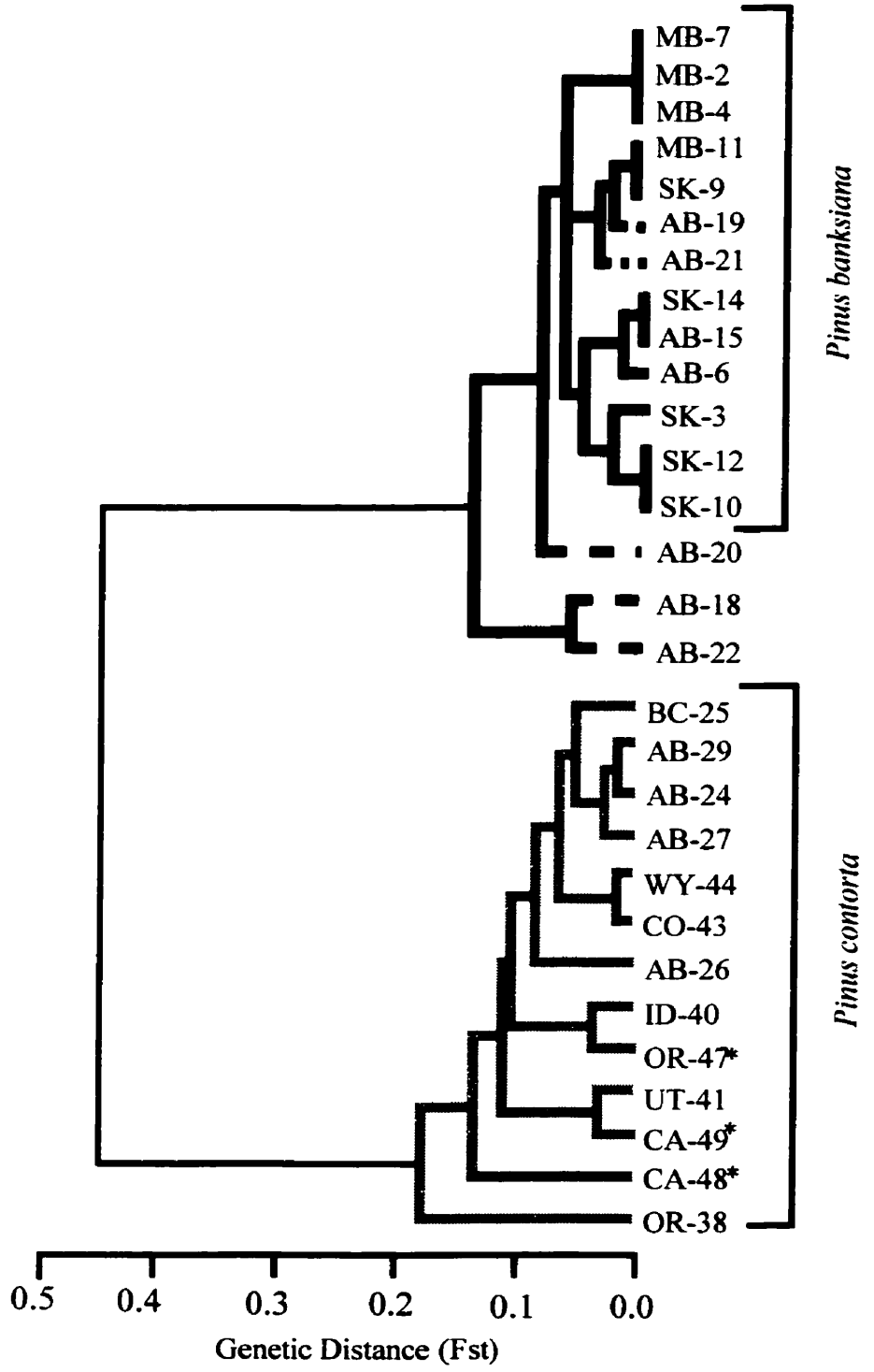

Figure 7. UPGMA dengrogram of 29 *Pinus* spp. populations based on Fst genetic distances (Excoffier et al., 1992). Black lines indicate *P. banksiana*, gray lines indicate *P. contorta* var. *latifolia* and var. *murrayana*, dashed lines indicate hybrids. * Indicates *P. contorta* var. *murrayana*.

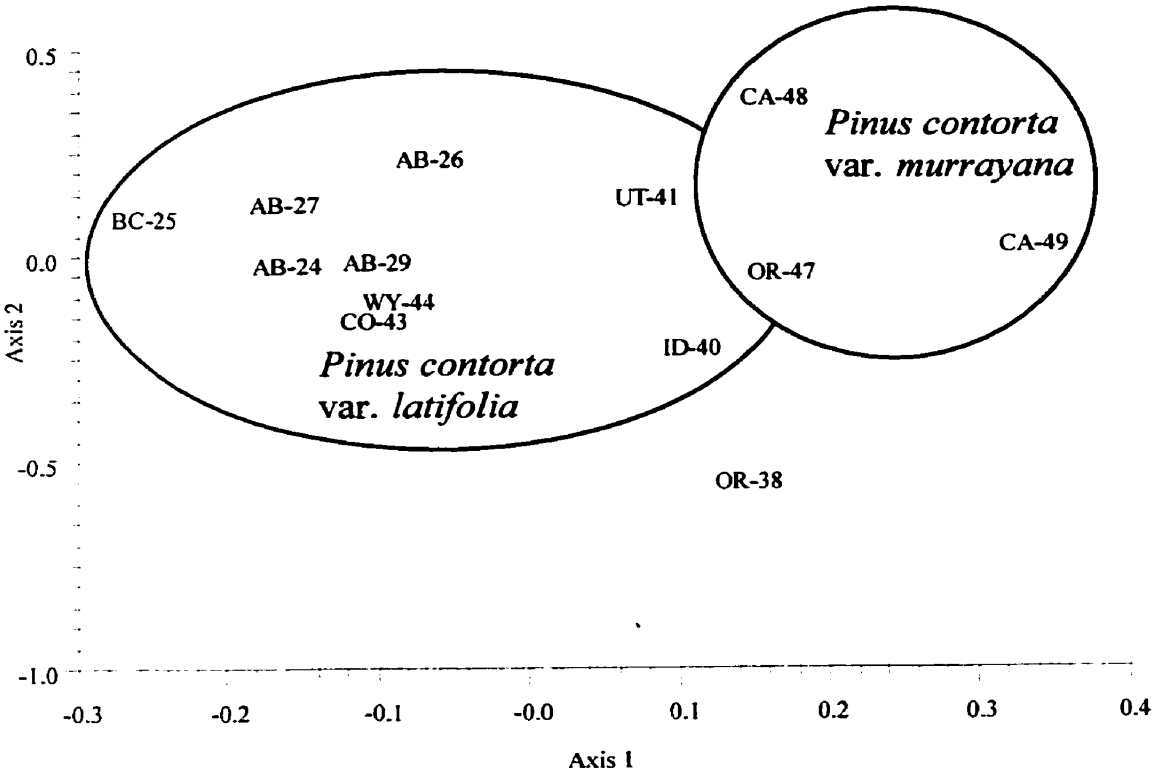

Figure 8. NMDS Ordination of *Pinus contorta* var. *latifolia* and var. *murrayana* using Fst genetic distance values (Excoffier et al., 1992).

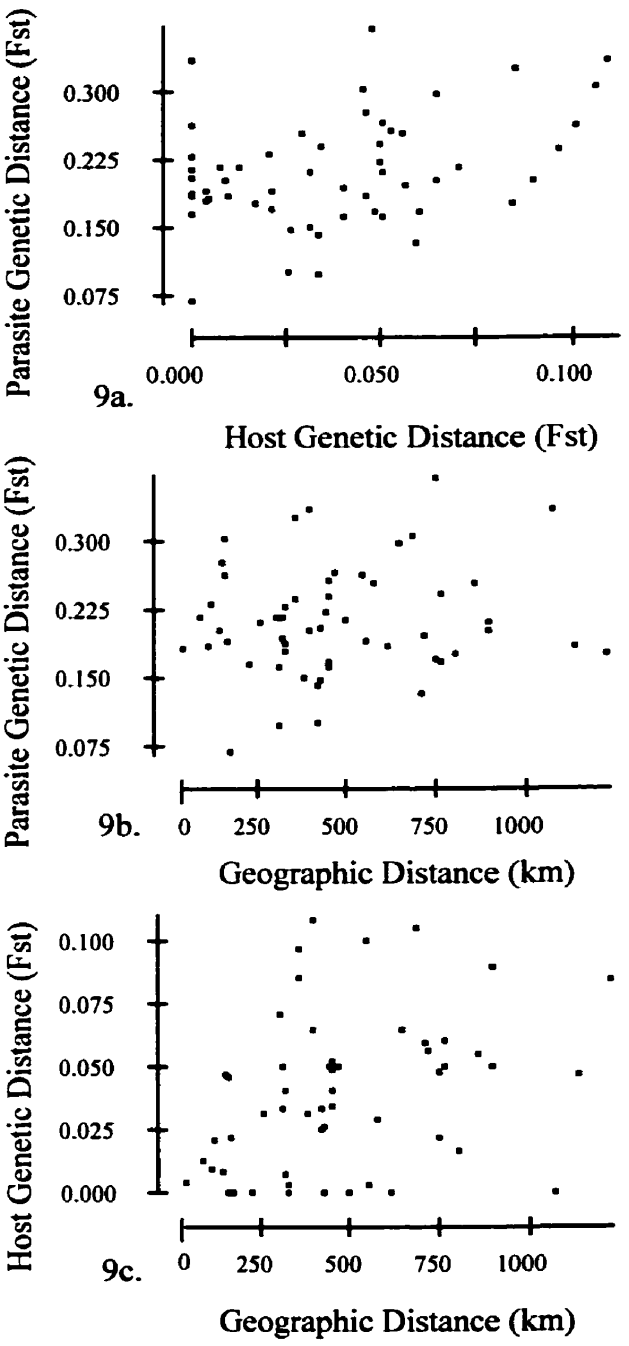

Figure 9. Scatterplots of pairwise distances for 11 populations in the *Arceuthobium americanum* - *Pinus banksiana* pathosystem: (9a) *A. americanum* genetic distance (Fst) versus *P. banksiana* genetic distance (Fst); (9b) *A. americanum* genetic distance (Fst) versus geographic distance (km); (9c) *P. banksiana* genetic distance (Fst) versus geographic distance (km).

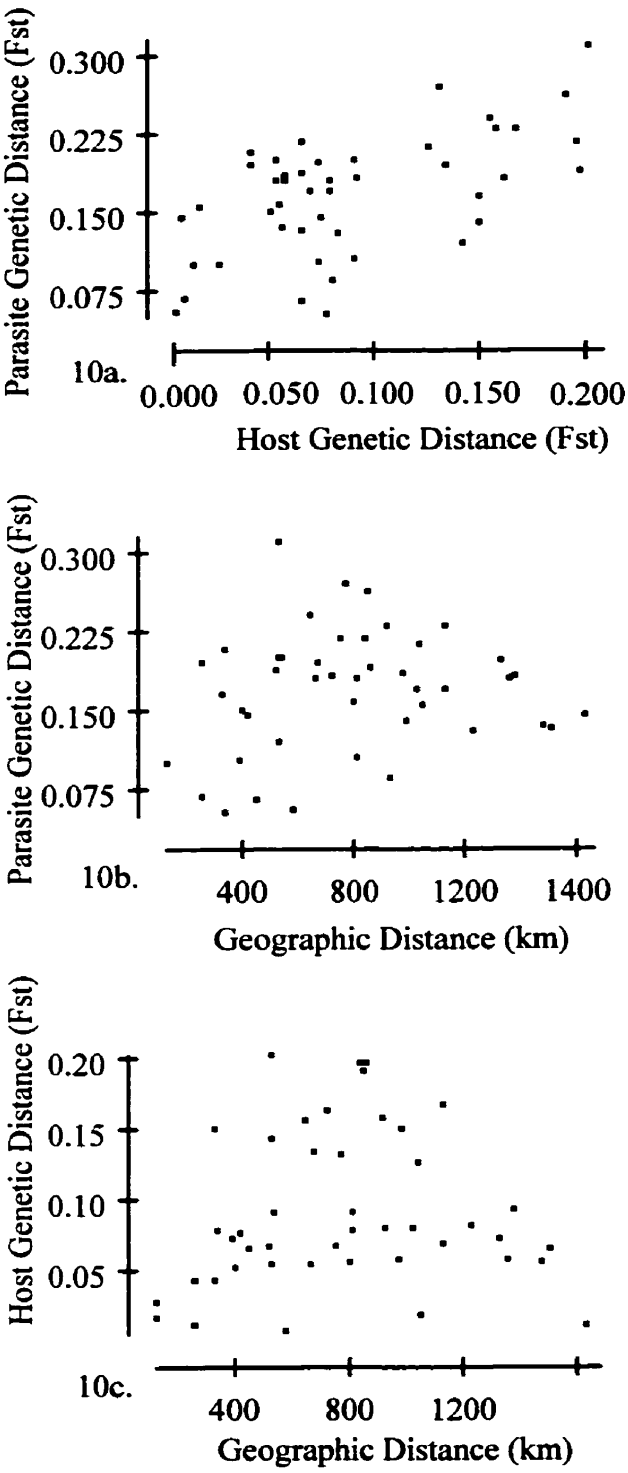

Figure 10. Scatterplots of pairwise distances for 14 populations in the *Arceuthobium americanum* - *Pinus contorta* var. *latifolia* pathosystem: (10a) *A. americanum* genetic distance (Fst) versus *P. contorta* var. *latifolia* genetic distance (Fst); (10b) *A. americanum* genetic distance (Fst) versus geographic distance (km); (10c) *P. contorta* var. *latifolia* genetic distance (Fst) versus geographic distance (km).

CHAPTER 4: Infectivity of the Parasitic Plant, *Arceuthobium americanum* on *Pinus banksiana*, *Pinus contorta*, and their hybrids

4.1 INTRODUCTION

In natural systems, dependent species (sensu Linhart, 1989 - parasites and herbivores) may be adapted to their hosts at different hierarchical levels. For example, genetic races (Bush, 1994; Sembene and Delobel, 1998; Groman and Pellmyr, 2000) and morphologically similar sibling species (Soper et al, 1988; McCarthy, 1990; Hajeck et al, 1991; Nascetti et al, 1993) of herbivores and parasites are often adapted to specific host taxa. Within host taxa, parasites are commonly observed to be adapted to local (sympatric) host genotypes (Parker, 1985; Ballabeni and Ward, 1993; Ebert, 1994; Dybdahl and Lively, 1996; Lively and Jokela, 1996; Morand et al., 1996; Mukaratirwa et al., 1996). At the extreme end of this spectrum, dependent species may even be adapted to specific host individuals within a population (Edmunds and Alstad, 1978; Wainhouse and Howell, 1983). Adaptation of parasites to their hosts has been attributed to the existence of gene-for-gene coevolution of resistance and infectivity alleles in host and parasite populations (Flor 1956; Frank, 1992, 1993). Although local adaptation is common in host-parasite pathosystems, local maladaptation is also frequently observed (reviewed in Kaltz and Shykoff, 1998).

The outcome of host-parasite interactions can be difficult to predict since so many factors can be involved. These include time lags in the response of parasite infectivity alleles to host resistance alleles (Gandon et al., 1996; Morand et al., 1996), the degree of coevolution between a parasite and its hosts (Gandon et al., 1996; Gandon and Van

Zandt, 1998), the migration rates of both host and parasite (Gandon et al., 1996; Kirkpatrick and Barton, 1997; Gandon and Van Zandt, 1998), and virulence of the parasite (Lively, 1999) (reviewed in Chapter 1).

Studies examining patterns of adaptation have primarily focussed on animal and fungal parasites (Morand et al., 1996; Mukaratirwa et al., 1996; Imhoof and Schmidt-Hempl, 1998; Davies et al., 1999; Delmotte et al., 1999; Kaltz et al., 1999; Oppiliger et al., 1999). Only a few studies have determined patterns of infectivity in parasitic plants (Clay et al, 1985; Mbwaga and Obilana, 1993; Overton, 1994; Mutikainenen et al., 2000). Although several researchers have examined infectivity of parasites in the genus *Arceuthobium* (Smith, 1974; Smith and Wass, 1979; Scharpf, 1987; Scharpf and Roth, 1992; Scharpf et al., 1992; Smith et al., 1993; Robinson, 1995), these studies were not specifically designed to assess adaptation to different principal host species or to local genotypes.

Arceuthobium americanum Nutt. ex Engelm. is ideally suited to studying adaptation in the genus *Arceuthobium* since infectivity can be examined at different levels. Firstly, it is possible to test for adaptation of this parasite to different host taxa since *A. americanum* is found on three principal hosts (*Pinus banksiana* Lamb., *Pinus contorta* var. *latifolia* Engelm., and *Pinus contorta* var. *murryana* (Greville and Balfour) Engelm.). Secondly, it is possible to examine for local adaptation to different genotypes within a host taxon since *A. americanum* is found over a wide geographic range. Thirdly, it is possible to study factors that limit the spread of this parasite since *A. americanum* is presently found in only a limited portion of the range of its *P. banksiana* hosts.

This is the final of three studies that examine the evolutionary forces acting on *A. americanum*. Population genetic analysis previously suggested that *A. americanum* is divided into three distinct genetic races, each associated with a different principal host taxon (Chapters 2 and 3). It is possible that these genetic races are paralleled by infective races adapted to specific host taxa. The existence of infective races would support a strong role for host identity in facilitating race formation in *A. americanum*. Findings from a previous study suggest that host identity does play a role in the divergence of the *A. americanum* race associated with *P. banksiana* from the races on *P. contorta* (Chapter 2 and 3). However, host identity does not seem to play a role in the divergence of the two *A. americanum* races associated with the two varieties of *P. contorta* (Chapter 3).

Arceuthobium americanum may also have adapted to local host genotypes within a host taxon. Edmunds and Alstad (1978) predicted that dwarf mistletoes would be adapted to local host populations and individuals due to their dependence on long-lived, toxin-defended host trees. However, high gene flow in the *Pinus* hosts (Chapter 3) may decrease the extent to which *A. americanum* could become locally adapted. Furthermore, findings from Mantel tests suggest that local host genotype may account for a small proportion of the genetic variation within the *A. americanum* races (Chapter 3).

Infection experiments were performed at tree plantations in Alberta and Ontario in order to examine infectivity of *A. americanum* without the confounding influence of environment. The objectives of these experiments were to:

(1) Examine the pattern of infectivity of *A. americanum* within and between host populations / species over its geographic range;

(2) Determine if the genetic races of *A. americanum* are paralleled by

infective races;

(3) Assess if *A. americanum* is more infective to local (sympatric) host genotypes than to disparate genotypes; and

(4) Evaluate the potential for *A. americanum* to spread eastward into new uninfected stands of *P. banksiana*.

This is the first study to examine infectivity of *A. americanum* to both *P. contorta* and *P. banksiana*. Furthermore, it is the only study to examine the ability for *A. americanum* to spread on *P. banksiana* hosts to the east of its natural range.

4.2 METHODOLOGY

COLLECTION OF DWARF MISTLETOE SEEDS

Arceuthobium americanum seeds were collected from three source populations (Table 17, Figure 11): (1) Belair Forest, Manitoba (from *Pinus banksiana*); (2) Pine Ridge Forest Nursery, Alberta (from *Pinus banksiana*); and (3) David Thompson Resort (DTR), Alberta (from *Pinus contorta* var. *latifolia*). In early August 1997, brooms containing fruiting pistillate *A. americanum* plants were covered with fine weave Sofwipe cheesecloth (Grade 40, 24 X 20 threads /sq.inch) to capture seeds as they were explosively discharged from fruits. In early September, cheesecloths containing discharged seeds were removed from the brooms and placed in cold storage at 4°C. Within a couple of days, the sticky *A. americanum* seeds were removed from cheesecloth by hand. *Arceuthobium americanum* seeds collected from numerous trees within a single stand were mixed together to form an inoculum representative of that source population (see Table 17). Seeds were stored in a sterile dry container at 4°C until inoculations were performed (within 10 - 34 days).

SEED VIABILITY

Viability of *A. americanum* seeds from each source population was tested using a 2% solution of 2,3,5 - triphenyl tetrazolium chloride (2,3,5 - TTC). Seeds were presoaked in distilled water at room temperature (24°C) for 24 hours. Next, seeds were either: (1) partially bisected, (2) completely bisected longitudinally, or (3) bisected and the intact embryo removed from seed. Viability was tested by placing either the bisected seeds or the completely dissected embryos in a sterile petri dish containing a 2% aqueous solution of 2,3,5-TTC. Seeds were maintained in the TTC solution at 24°C in the dark

for up to 10 days. Embryos were examined under a dissecting microscope for red coloration indicating that the seeds were respiring and hence, viable.

SEED GERMINATION

Arceuthobium americanum seeds from David Thompson Resort, Alberta and Pine Ridge Forest Nursery, Alberta were tested for germinability. Seeds were surface sterilized on a shaker for 30 minutes in a petri dish containing 3% H_2O_2. Following treatment with H_2O_2, seeds were rinsed three times (for 10 minutes each) in sterilized dH_2O. Finally, seeds were transferred onto 0.8% agar. Petri dishes containing seeds were then placed into an incubator on a 10 hour light: 14 hour dark cycle at 22°C, for up to 3 weeks. Germinating seeds produced radicles as early as 72 hours after incubation.

INOCULATION OF EXPERIMENTAL HOSTS

Experimental Host Preparation

Prior to inoculation, experimental host twigs were marked with a paintstick to indicate where the seed was to be placed. Each twig received three seeds. The paintstick left a permanent mark on inoculated twigs such that inoculation sites could be identified in subsequent years.

In general, twigs were inoculated at a height above the snow line (> 70 cm) since spring snowmelt is thought to result in the loss of seeds from host branches (Robinson, 1995; D. Punter, Dept. of Botany, U. of Manitoba, pers. comm.). Experimental host twigs to be used for inoculations were of similar age (current year, 1 year old, and 2 year old growth).

Arceuthobium Seed Preparation

Twelve to twenty-four hours prior to inoculations, *A. americanum* seeds were soaked in autoclaved dH_20 in sterile petri dishes. The viscin became imbibed with water giving the seeds a mucilaginous appearance and a sticky texture.

Inoculations

Using forceps, imbibed seeds were inoculated onto twigs of experimental hosts by transferring seeds directly from the water in the petri dish on the twig. Seeds were placed at the base of a needle fasicle next to, rather than directly on, the paint marks in order to avoid possible inhibition of germination and host penetration by parasites.

EXPERIMENTAL DESIGN AT PINE RIDGE FOREST NURSERY, ALBERTA

In total, sixty-nine trees (10 - 12 years of age) in a plantation at the Pine Ridge Forest Nursery (PRFN) near Smoky Lake, Alberta were inoculated with over 7,600 *A. americanum* seeds from three source populations (Figures 11 and 12). This plantation contained *P. banksiana, P. contorta* var. *latifolia*, and *P. banksiana* X *P. contorta* var. *latifolia* hybrids from several geographic regions throughout western Canada planted in a randomized block design. Five experimental host populations within this plantation were inoculated. These included *P. banksiana* from S.E. Manitoba, *P. banksiana* from PRFN, Alberta, *P. contorta* var. *latifolia* from Rocky Mountain House, Alberta, *P. contorta* var. *latifolia* from Cypress Hills, Alberta, and *P. banksiana* X *P. contorta* var. *latifolia* hybrids from Whitecourt, Alberta (see Figures 11 and 12). Three of these host populations were chosen to match closely with the three *A. americanum* seed sources: *P. banksiana* from S.E. Manitoba - *A. americanum* from Belair Forest, Manitoba, *P. banksiana* from PRFN, Alberta - *A. americanum* from PRFN, Alberta, and *P. contorta*

var. *latifolia* from Rocky Mountain House, Alberta - *A. americanum* from David Thompson Resort, Alberta (Figure 11). Individual trees to be inoculated with given *A. americanum* seed sources were chosen randomly to avoid microclimatic effects on infection success. Inoculations were performed within 10 - 14 days of seed collection.

EXPERIMENTAL DESIGN AT PETAWAWA RESEARCH FOREST, ONTARIO

Fifteen experimental *P. banksiana* trees (32 years of age) were inoculated with over 1,500 seeds of *A. americanum* at the Petawawa Research Forest near Chalk River, Ontario (Figures 13 and 14). This plantation contained *Pinus banksiana* from several geographic regions throughout Canada and the U.S.A. Fifteen trees, representative of various geographic sources, were divided into three general categories: Eastern, Central, and Western (Figures 13 and 14). Due to an insufficient number of *A. americanum* seeds, the three source populations were pooled together as a single inoculum. Inoculations were performed within 32 - 34 days of seed collection.

ASSESSMENT OF INFECTION SUCCESS IN THE FIELD

In July, 1998 (9 - 10 months post-inoculation), inoculation sites were assessed for germination success. In July and August, 2000 (32 - 35 months post-inoculation), infection success was assessed by examining inoculation sites for successful penetration of host xylem as reflected by branch swelling and/or the emergence of young *Arceuthobium* shoots from the site of initial penetration on the experimental host twigs.

DATA ANALYSES

Lab viability and germination were reported as percentage of total seeds examined. Field germination and infection success was reported as percentage of total seeds inoculated on each tree. Since percentages and proportions form a binomial rather

than normal distribution, all data were arcsin - square root transformed in order to equalize the variances and render the data normal prior to statistical analyses. For proportions greater than 0%, data were transformed using the following equation from Zar (1984):

$p' = \arcsin \sqrt{p}$

where p and p' are the non-transformed and transformed proportions, respectively. Unfortunately, arcsin - square root transformations are not effective at normalizing proportions near the extremes (i.e. 0.0 or 1.0) (Zar, 1984). Thus, in this study, proportions with a value of 0 were transformed as follows:

$p' = \arcsin \sqrt{1/4n}$ where p' is the transformed proportion and n is the number of seeds examined. All data transformations were performed using Microsoft Excel 1998, Version 8.0©.

Two-sample t-tests were used to examine for pairwise differences in viability of seeds from the three *A. americanum* source populations. Similarly, two sample t-tests were used to compare differences in germination between *A. americanum* source populations. Since seeds of *A. americanum* from Belair Forest were unavailable, this comparison was only made for *A. americanum* from Pine Ridge Forest Nursery and from David Thompson Resort, Alberta.

For the infection experiment at the Pine Ridge Forest Nursery in Alberta, infectivity was analyzed at various levels. Firstly, a Factorial Analysis of Variance (FANOVA) was used to examine infectivity across the five host populations and the three parasite populations. Host X parasite interactions could be detected, as well as differences in host susceptibility and parasite infectivity from each of their respective

populations. Data were also examined using FANOVA to test for adaptation / maladaptation of *A. americanum* to given host species. In this case, parasite populations were pooled into two classes: *A. americanum* from *P. banksiana* hosts and *A. americanum* from *P. contorta* var. *latifolia* hosts. Host populations were likewise pooled into three classes: *P. banksiana* hosts, *P. contorta* var. *latifolia* hosts, and hybrid hosts. Finally, in a separate analysis, geographic patterning of infectivity within a given host taxon was examined. For the *A. americanum* - *P. banksiana* pathosystem, FANOVA was used to assess infectivity of *A. americanum* from two locations (S.E. Manitoba and Pine Ridge Forest Nursery, Alberta) on hosts from two locations (Belair Forest, Manitoba and Pine Ridge Forest Nursery, Alberta). For the *A. americanum* - *P. contorta* var. *latifolia* pathosystem, 2 sample t-tests were used to compare infectivity of *A. americanum* from DTR, Alberta on hosts from either Rocky Mtn. House or Cypress Hills, Alberta. For the infection experiment at the Petawawa Research Forest, Ontario, an Analysis of Variance (ANOVA) was used to detect differences in susceptibility of three classes of trees (eastern, western, and central sources) to a pooled *A. americanum* seed source.

All statistical analyses (t-tests, ANOVA, and FANOVA) were performed separately for infectivity as determined by swellings on host twigs versus infectivity as determined by parasite shoot emergence from host twigs. All analyses were performed using the program Data Desk ® Version 4.1 © (Vellman, 1993).

4.3 RESULTS

LABORATORY SEED VIABILITY AND GERMINATION

Mean seed viability and standard deviations around these means ranged widely across the *A. americanum* source populations from DTR (66.68 ± 20.70), Belair Forest (43.68 ± 11.89), and PRFN (33.66 ± 22.57) (see Table 18). Due to the standard deviation around the means, t-tests revealed that seed viability values were not significantly different from each other (P-values from 0.092 to 0.526). Mean germination values are reported in Table 19. A t-test showed no significant difference (P=0.193) between germination for *A. americanum* seeds obtained from PRFN (60.97 ± 5.75) and DTR (67.25 ± 3.50).

FIELD GERMINATION (8 - 9 MONTHS POST-INOCULATION)

In mid-July of 1998, inoculation sites on 15/69 experimental host trees at the Pine Ridge Forest Nursery (PRFN) in Alberta and on 2/15 experimental host trees at the Petawawa Research Forest in Ontario were examined for germination success of *A. americanum* seeds. Unfortunately, *A. americanum* seed loss from inoculation sites on experimental host twigs was high. In addition, most of the seeds that were still on the trees failed to germinate. At the time when field germination was assessed (July 1998), healthy radicles emerging from *A. americanum* seeds were observed for only a small number of the total seeds inoculated on experimental host trees. Field germination values ranged from 1 - 12 % for the 15 trees observed at the PRFN (pers. obs.) and the two trees observed at Petawawa (P. Copis, Petawawa Research Forest, pers. comm.). This was quite low in comparison with germination success of these seeds as assessed under laboratory conditions (55 - 70%, see Table 19).

INFECTION SUCCESS AT PINE RIDGE FOREST NURSERY, ALBERTA

In early August of 2000 (35 months post-inoculation), inoculation sites on experimental host trees at the PRFN were examined for infection success. The overall infection success averaged across all host and parasite sources (Tables 20 and 21) was low in terms of both branch swellings (3.57% ± 3.40) and *A. americanum* shoot emergence (1.79% ● 1.96). Sixteen of the 69 trees (23.19%) inoculated with *A. americanum* lacked signs of infection as depicted by branch swellings (Table 20). Twice that number (46.38%) lacked signs of infection as depicted by emergence of *A. americanum* shoots from inoculation sites. The highest proportion of branch swellings (15.79%) and shoot emergence (7.89%) was observed for *A. americanum* from DTR inoculated on an experimental *P. banksiana* host tree from SE Manitoba. Infection success as depicted by *A. americanum* shoot emergence was consistently lower than that determined by swellings (Tables 20 and 21).

Overall Pattern Across Five Host Sources and Three Parasite Sources

Factorial analyses of variance detected no significant host X parasite (5 X 3) interactions for either branch swellings (P=0.4391) or *A. americanum* shoot emergence (P=0.6170) (Tables 22a and 22b). However, there was a highly significant difference in host susceptibility as depicted by both swellings (P = 0.0001, Table 22a) and shoots (P ≤ 0.0001, Table 22b). The mean susceptibility of *P. banksiana* trees from SE Manitoba was higher than that seen for other experimental host populations (Table 21). Factorial analysis of variance indicated that parasite seed sources showed differential infectivity as depicted by *A. americanum* shoot emergence (P = 0.0122, Table 22b). *Arceuthobium americanum* seeds from DTR had a higher mean infectivity than either PRFN or Belair

(Table 21). However, this pattern of differential *A. americanum* infectivity was not significant for infections as depicted by branch swellings (P = 0.0734, Table 22a).

Host Specific Patterning

Data were also analyzed to test for adaptation / maladaptation of *A. americanum* to given host species. In this case, parasite populations were pooled into two classes: *A. americanum* from *P. banksiana* hosts and *A. americanum* from *P. contorta* var. *latifolia* hosts. Host populations were likewise pooled into three classes: *P. banksiana* hosts, *P. contorta* var. *latifolia* hosts, and hybrid hosts. FANOVA indicated no significant host X parasite interactions for either branch swellings (P = 0.2248) or shoot emergence (P = 0.3309) (Table 23). However, there was a significant difference in susceptibility across host populations as depicted by swellings (P = 0.0059) and shoots (P≤0.0001). *Pinus banksiana* and hybrid hosts appeared to be more susceptible to *A. americanum* than were *P. contorta* var. *latifolia* hosts (Table 24). However, FANOVA showed no significant difference in infectivity between *A. americanum* seed sources from *P. banksiana* hosts in comparison with those from *P. contorta* hosts (swellings, P=0.6074; shoot emergence, P=0.0887).

Geographic Patterning within a Host Taxon

Geographic patterning of infectivity within a given host taxon was also examined. For the *A. americanum* - *P. banksiana* pathosystem, infectivity of *A. americanum* from two locations (Belair, Manitoba, and Pine Ridge Forest Nursery, Alberta) on hosts from two locations (S.E. Manitoba and Pine Ridge Forest Nursery, Alberta) was assessed. In this case, FANOVA showed no significant interaction between host X parasite for either branch swellings (P=0.7115) or *A. americanum* shoot emergence (P=0.6440) (Table 25).

However, there was a significant difference in susceptibility between hosts from S.E. Manitoba and PRFN, Alberta for both swellings (P=0.0197) and for shoots (P=0.0093). *Pinus banksiana* hosts from S.E. Manitoba appear to be more susceptible to infection than *P. banksiana* hosts from PRFN, Alberta (Table 26). FANOVA also indicated a significant difference in the ability for the three *A. americanum* source populations to infect hosts as depicted by both swellings (P=0.0405) and shoot emergence (P=0.0125). *Arceuthobium americanum* from PRFN appears to be more infective than *A. americanum* from SE Manitoba (Table 26).

The same pattern of infectivity was not observed for *A. americanum* from *P. contorta* var. *latifolia* hosts. For the *A. americanum* - *P. contorta* var. *latifolia* pathosystem, infectivity of *A. americanum* from DTR, Alberta onto hosts from either Rocky Mtn. House or Cypress Hills, Alberta was examined. In this case, t-tests showed that there was no significant difference in the ability for *A. americanum* seeds from DTR to infect hosts from Rocky Mtn. House or Cypress Hills as depicted by both swellings (P=0.6914) and shoots (P=0.7779).

INFECTION SUCCESS AT PETAWAWA RESEARCH FOREST, ONTARIO

In mid-June of 2000 (32 months post-inoculation), experimental host trees at the Petawawa Research Forest in Ontario, were examined for infection success at inoculation sites. One of the experimental hosts (that from Big River, SK) died and was excluded from analysis. Infection success on the remaining fourteen experimental hosts at Petawawa (Table 27) was higher than that seen at PRFN in Alberta (Tables 20 and 21). At Petawawa, an average of 16.81% (± 9.24%) of inoculation sites had branch swellings and 5.95% (± 5.16%) had *A. americanum* shoots (Table 27). At PRFN, on the other

hand, an average of 3.57% (± 3.40) of inoculation sites had branch swellings and 1.79% (± 1.96%) had *A. americanum* shoots (Table 21).

At Petawawa, all trees had at least one infection as depicted by swellings at inoculation sites on host twigs (Table 27). As well, 11/14 had at least one infection as depicted by *A. americanum* shoot emergence (Table 27). Furthermore, half of the trees (7/14) had swellings at greater than 20% of their inoculation. The mean regional values for infection success ranged from 14.12% (western sources) to 20.18 % (eastern sources) as depicted by swellings and from 3.36% (western sources) to 8.58% (eastern sources) as depicted by shoot emergence (Table 27). As was seen at the PRFN, standard deviations around mean infectivity were large. An ANOVA showed no evidence for adaptation or maladaptation since means from the three regions were not significantly different (Table 28) for either swellings (P=0.5643) or shoots (P=0.4410). Each of the three regions had trees infected to a variable extent (Table 28, Figure 15). These data indicate that eastern and central *P. banksiana* trees that have not previously been in contact with *A. americanum* are at least as capable of being infected by this parasite as are those from western Canada.

4.4 DISCUSSION

GERMINATION

Estimates of *Arceuthobium americanum* germination success in the field (1 - 12% of the total seeds inoculated) were considerably lower than that observed under laboratory conditions (61 - 67 %). Furthermore, these estimates are lower than those reported for *A. americanum* by Robinson (1995) in one experiment (27.4% germination), but are comparable to that reported in a second experiment (6.5% germination). Robinson (1995) suggested that the low germination observed in the second experiment resulted from a reduction in seed viability due to a winter with below average temperatures. Smith and Wass (1979) reported a wide range of germination values for *A. americanum* across three study sites and several years (n=7, mean 58%, range 2.3% - 78.7%). These researchers also implied that cold winter conditions contributed to the low germination in some instances. In the present study, warm and dry spring conditions in 1998 associated with the 1997/1998 El Niño conditions are more likely to have contributed to low germination than cold winter conditions. Warm temperatures and desiccation have previously been implicated as factors decreasing *Arceuthobium* seed viability, germination, and penetration (Scharpf, 1969). The observed low level of germination may also have been related to high seed loss from experimental host twigs. Unfortunately, this parameter was not specifically quantified in this study. However, in July of 1998 when germination success was assessed in the field, an estimated 50% of seeds were absent from inoculation sites on host twigs (personal observation). This seed loss is considerably higher than that reported for *A. americanum* on *P. contorta* var. *latifolia* (4 - 12%) by Smith and Wass (1979), but lower than that reported for *A.*

campylopodum on *P. jeffreyi* (60 - 95%) by Scharpf and coworkers (1992). It is comparable to that observed by Robinson (1995) who found average seed loss of *A. americanum* on *P. banksiana* over two experiments to be 60%. These researchers (Scharpf et al., 1992; Robinson, 1995) attributed seed loss to overwintering, spring wind and rain wash-off, molding, and insect attack. Similar factors likely affected seed retention in the present study.

PATTERNS OF INFECTIVITY AT PINE RIDGE FOREST NURSERY, ALBERTA

Infection success as depicted by *A. americanum* shoot emergence was consistently lower than that determined by swellings (Tables 22 and 23). This is not surprising since branch swellings generally precede the emergence of *Arceuthobium* shoots during the infection process (Hawksworth and Wiens, 1996).

Infection success as determined by both branch swellings (3.57% ± 3.40) and *A. americanum* shoot emergence (1.79% ± 1.96) from inoculated twigs was extremely low at the PRFN, Alberta. These values were considerably lower than the overall average of 20% reported across seven trials for *A. americanum* by Smith and Wass (1979). However, values were within the range of that reported for three (average 8.3%, range 0.0% - 13.7%) of these seven trials. The values from the present study were also lower than those reported by Robinson (1995) for *A. americanum* (13 - 15%) in one experiment, but comparable to those observed (2 - 4 %) in his second experiment.

Robinson (1995) and Smith and Wass (1979) both related low infection success of *A. americanum* to low seed retention and germination. In the present study, those factors that have the potential to reduce seed retention (overwintering, wind and rain washoff,

molding, and insect attack) and germination (desiccation) may have reduced infection success.

Host X Parasite Interactions

Previous studies (Chapters 2 and 3) examining the population genetic structure of *A. americanum* indicated that this parasite was divided into three genetic races, each associated with a different host taxon: (a) *Pinus banksiana*, (b) *Pinus contorta* var. *latifolia*, and (c) *Pinus contorta* var. *murryana*. One of the goals of this study was to determine if these genetic races of *A. americanum* were paralleled by infective races adapted to the host taxon with which they are associated in nature. Adaptation of *A. americanum* to *P. contorta* var. *murryana* trees could not be assessed since this taxon is not found at the PRFN plantation. The infection experiment did, however, assess adaptation of *A. americanum* to the other two principal hosts, *P. contorta* var. *latifolia* and *P. banksiana*. The results from the infection experiment at the PRFN indicated no host X parasite interactions, suggesting a lack of infective races of *A. americanum* adapted solely to infecting a single principal host taxon. These observations question the role played by host identity in shaping *A. americanum* populations into races.

A second goal of this study was to assess adaptation of *A. americanum* to different genotypes within a given host taxon. Edmunds and Alstad (1979) predicted that local adaptation would be prevalent for dwarf mistletoes since they are associated with toxin-defended long-lived hosts. The results from the infection experiment at the PRFN depicted a lack of host X parasite interaction at the local level within a given host taxon. Thus, *A. americanum* genotypes are neither locally adapted nor maladapted to host genotypes. This is not surprising given that Mantel tests had previously shown only a

weak correlation between *A. americanum* and *P. banksiana* genetic distances, and a moderate correlation between *A. americanum* and *P. contorta* var. *latifolia* genetic distances (Chapter 3).

This observed lack of adaptation of *A. americanum* to their specific host taxa and local genotypes within these host taxa may be related to several factors. As was described in Chapter 1, theoretical models have depicted several circumstances in which local adaptation is unlikely to occur. Firstly, if there is a sufficient time lag in response of parasite infectivity alleles to host resistance alleles, it is possible that parasites will be more infective to allopatric than sympatric hosts. In the present study, a time lag could explain the absence of local adaptation of *A. americanum* to specific host genotypes within a host taxon. Due to this time lag phenomenon, Kaltz and Shykoff (1998) have suggested that numerous sympatric and allopatric combinations should be examined when performing infection experiments in order to obtain a true picture of adaptation. In the present study, three parasite populations and five experimental host populations were observed. Since parasite adaptation was being examined on two levels (e.g. to different host taxa and to different host genotypes within a host taxon), it is possible that this three by five combination was insufficient to detect overall patterns.

A strong coevolutionary relationship has also been described as a prerequisite to local adaptation of parasites to their hosts (Gandon et al., 1996). Such strong coevolution may not be present in the *Arceuthobium* - conifer pathosystem. Firstly, *A. americanum* infects three principal hosts and several non-principal hosts throughout its range (Hawksworth and Wiens, 1996). Diverse selection pressures resulting from such interactions may actually decrease the extent to which *A. americanum* is capable of

adapting to any one host taxon or genotype. Secondly, *P. banksiana* and *P. contorta* are attacked by a wide array of other dependent species including *Cronartium gloeosporioides* Arth. (the causative agent of stalictiform blister rust), *Endocronartium harknessii* (JP Moore) Y. Hiratsuka (the causative agent of western gall rust), *Lophodermella concolor* (Dearn.) Darker (the causative agent of needle cast), and *Synanthedon sequoiae* (Hy.) Edwards (sequoia pitch moth) (Wu et al., 1996). Furthermore, *P. contorta* is host to a number of other *Arceuthobium* taxa including *A. laricis* (Piper) St. John, *A. campylopodum* Engelm., *A. vaginatum* (Willd.) Presl. subsp. *cryptopodum* (Engelm.) Hawksw. and Wiens, *A. cyanocarpum* (A. Nelson ex Rydberg) Coulter and Nelson, and *A. tsugense* (Rosendahl) G.N. Jones. Selection pressures imposed on *Pinus banksiana*, *Pinus contorta*, and *A. americanum* from these multispecies interactions likely decrease the ability for *A. americanum* to coevolve with these hosts.

Migration is also thought to play an important but complex role in host - parasite interactions. Low gene flow by parasites can lead to local adaptation (Kirkpatrick and Burton, 1997; Gandon and Van Zandt, 1998). However, sufficiently high gene flow by hosts can overcome this local adaptation by introducing novel resistance alleles into host populations (Gandon et al., 1996). Population genetic analysis (see Chapter 2 and 3; Ledig, 1998) and pollen records (Ledig, 1998) indicate that gene flow in *Pinus banksiana* and *Pinus contorta* is very high. As a result, conspecific host populations may not present sufficiently different genotypes for *A. americanum* to differentiate upon. High gene flow by hosts has also been used to explain the lack of local adaptation in several other pathosystems including a fungal-plant (Delmotte et al., 1999, Kaltz et al. 1999), a haemogregarine-lizard (Oppliger et al, 1999), a protozoan-bumblebee (Imhoof and

Schmid-Hempel, 1998), and a parasitic plant - plant (Mutikainen et al., 2000).

One other factor that could impact adaptation in this pathosystem is the cost of evolving and maintaining resistance alleles. Resistance to parasites is often thought to come at a fitness cost to the host (Ebert, 1994). As such, hosts in the *A. americanum* - conifer pathosystem are unlikely to evolve resistance alleles to *A. americanum* races that they rarely encounter. Thus, *A. americanum* races could potentially be more capable of infecting host taxa with which they are not commonly associated (i.e. they may be locally maladapted). However, a pattern of maladaptation could be obscured by the transfer of host resistance alleles through genome introgression between *P. banksiana* and *P. contorta* genomes in the hybridization zone in central and northern Alberta. Previous studies have documented the effects of introgression between *P. banksiana* and *P. contorta* on disease resistance and morphological, chemical, and genetic characteristics at considerable distances outside of their hybrid zone (see Wu et al., 1997). Given the complex set of factors impacting the *A. americanum* - conifer pathosystem that have been described here, it is not entirely surprising that host X parasite interactions were not detected.

Susceptible Hosts

Despite the lack of host X parasite interactions at the PRFN, there was a significant difference in host susceptibility regardless of the *A. americanum* seed source. The tendency for *P. banksiana* and hybrid hosts to be more susceptible to infection by *A. americanum* than were *P. contorta* var. *latifolia* may relate to the longer association between *A. americanum* and this latter host. Based on fossil records, current distribution patterns (Hawksworth and Wiens, 1996), and genetic diversity measures (Chapter 2), it

seems likely that *A. americanum* evolved initially as a parasite of *P. contorta* and then jumped on hybrids and *P. banksiana* following the retreat of the Laurentide ice sheets. As a result, *P. contorta* var. *latifolia* may have had sufficient time to evolve a certain level of resistance to *A. americanum* whereas *P. banksiana* has not. *Pinus banksiana* from Manitoba appeared to be particularly susceptible to *A. americanum*. Interestingly, these trees are from the extreme eastern limit of *A. americanum*. Consequently, their association with *A. americanum* is most recent. The high susceptibility in this population may therefore reflect the lack of time for these hosts to evolve resistance to *A. americanum*. On the other hand, hybrid trees from Whitecourt and *P. banksiana* trees from PRFN are further west and would have had a longer period of time to evolve resistance mechanisms to this parasite. Furthermore, introgression of resistance alleles from *P. contorta* populations is more likely in trees from these regions since they are close to the hybridization zone. Introgression between these two genomes has also been implicated in providing resistance to several forest pests in *P. contorta* var. *latifolia* (Wu et al., 1996; Yang et al., 1997).

Experimental design may have contributed to the high susceptibility observed in *P. banksiana* hosts from Manitoba. Although all other experimental hosts used in this study were distributed in a randomized block design throughout the plantation, *P. banksiana* from Manitoba were randomly blocked within the northwest corner of the plantation. It is thus possible that these trees were situated in microclimatic conditions more favourable for infection by *A. americanum*.

Highly Infective A. americanum genotypes

Arceuthobium americanum seeds from David Thompson Resort in Alberta were most infective to all hosts except hybrids. The reason for the higher infectivity of this lot of seeds is unclear. However, it should be noted that this was the only seed source from *P. contorta* var. *latifolia*. Thus, it is possible that the more ancient association of this *A. americanum* race with its host has made it more infective than its counterpart from *P. banksiana*. Other researchers have also reported variable infectivity in different seed sources of *Arceuthobium* spp. (Smith et al., 1993).

Experimental Error

Experimental error must also be considered when interpreting the results of this study. It is possible that any host X parasite interactions that do exist were obscured by the low infection success and high variance that characterized the experiment at the PRFN. In order to confirm the findings of this study, these experiments should be performed again in an attempt to increase seed retention, germination, and overall infection success. This would make the data more robust. Higher levels for some of these parameters have been reported in the literature (Robinson, 1995; Scharpf and Roth, 1992; Scharpf et al., 1992; Smith and Wass, 1979; Smith et al., 1993). However, several studies have also reported comparable or even lower levels for some of these parameters (Smith, 1974; Smith and Wass, 1979; Scharpf et al., 1992; Robinson, 1995). One possible way to greatly increase infection levels in experiments with *Arceuthobium* would be to inoculate experimental hosts in the spring with lab germinated seeds.

Experimental error may also have been introduced by monitoring infection success over a relatively short period of time. Monitoring in the present study (3 years)

was longer than that by Robinson (1995) (one year) who used anatomical sections to confirm establishment of infections. However, the monitoring in the present study was shorter than that carried out by several other researchers (up to 6 years) (Smith, 1974; Smith and Wass, 1979; Scharpf and Roth, 1992; Scharpf et al., 1992) who used macroscopic observations of infection success. These longer-term studies assessed size (length and diameter) of swellings, broom formation, flowering, and fruit production as well as the number of sites with swellings and shoot emergence. It is possible that, in the present study, a greater proportion of inoculation sites may have developed infections given a sufficient period of time. Unfortunately, such long-term observations were not possible during the course of this Ph.D. research.

PATTERNS OF INFECTIVITY AT THE PETAWAWA RESEARCH FOREST

The primary focus of the infection experiment performed at Petawawa, Ontario, was to assess the ability of *A. americanum* to spread east of its current geographic limit. A previous study (Smith and Wass, 1979) showed that *A. americanum* had the ability to infect *P. contorta* var. *contorta* to the west of its current geographic limit. These researchers suggested that natural westward spread of *A. americanum* has been prevented by historical factors such as glaciation, and due to barriers such as alpine and subalpine climates, as well as immune tree species.

The present study is the first to show that *A. americanum* has the ability to spread onto *P. banksiana* far to the east of its current geographic limit. The high infectivity that was observed for *A. americanum* on *P. banksiana* hosts from regions of Canada where the parasite is presently absent (i.e. central sources - 15.61% ● 8.33 swellings; eastern sources - 20.18% ● 8.50 swellings) implies a lack of genetic resistance in these trees. In

fact, these trees were infected to the same extent as were those from western Canada (16.81% ± 9.24) where this parasite is currently found. These findings suggest that the current eastern limit of *A. americanum* at the eastern edge of Manitoba is not related to host resistance factors. Since the experiment was carried out at a plantation in eastern Ontario, it also seems unlikely that environmental factors have limited its spread. Further, since there are no large geographic barriers to create a disjunction in the *P. banksiana* host distribution across eastern Canada, it is unlikely that host availability is a limiting factor. Rather, it seems more plausible that *A. americanum* has simply not had the time to spread into Ontario and eastern Canada since the last glaciation period.

In each of the regions (western, central, and eastern) examined, trees with high and low susceptibility were observed (Figure 13). This pattern depicts a lack of adaptation at the regional level. It does imply, however, that individual trees or populations may have differential susceptibility to *A. americanum*. Unfortunately, inoculated trees were not replicated at the population level so this observation can not be confirmed from these data.

COMPARISON OF INFECTION EXPERIMENTS IN ALBERTA AND ONTARIO

The considerably higher average infectivity of *A. americanum* on hosts at the Petawawa Research Forest in Ontario than those at the PRFN in Alberta may be related to different factors. Firstly, experimental host trees in Petawawa were considerably older (32 years of age) than those at the PRFN (10 - 12 years of age). Thus, it is possible that juvenile resistance to *Arceuthobium* has played some role. Past researchers have proposed such a resistance mechanism to explain observations in natural infections whereby juvenile trees were infected to a lesser degree than older trees in the same stand

(Dowding, 1929; Muir, 1972). However, the ultimate role played by juvenile resistance is unclear. Robinson (1995) examined resistance in trees of varying age classes (3, 5, 7, 12, 17, and 22). This researcher found no difference in susceptibility between the 3, 5, and 7 year olds, but did find a difference between the 12, 17, and 22 year old trees. Older trees were infected to a great extent than were younger trees. Robinson (1995) suggested that the higher infection levels observed in older trees might be explained by the fact that the denser crowns of older trees reduce incoming solar radiation, and consequently reduce seed desiccation.

Environmental factors may also have played a role in different infection levels observed at the PRFN in Alberta compared with those at the Petawawa Research Forest in Ontario. It is possible that milder and wetter conditions at the Petawawa Research Forest (S. D'Eon, Petawawa Research Forest, pers. comm.) compared with those at the PRFN (C. Hansen, PRFN, pers. comm.) allowed for higher establishment of infections at the former site.

FOREST MANAGEMENT IMPLICATIONS

The findings from the infection experiment performed at the PRFN, Alberta, should be interpreted with caution given the low infection success and high variance in the data set. The general lack of adaptation or maladaptation implies that reforesting dwarf mistletoe infected regions with disparate host genotypes or with different principal host taxa without *a priori* knowledge of genetic resistance is unlikely to be effective in decreasing infection levels. Rather, forest managers will need to continue to search for resistant genotypes at the local and host species level. There are, however, several problems with the strategy of replanting dwarf mistletoe infected regions with resistant

trees. Firstly, very few studies have been able to document complete, or even partial, genetic resistance of conifers to dwarf mistletoes. In several instances, cases of reported resistance in natural populations have been dispelled upon closer examination in field trials (reviewed in Hawskworth and Wiens, 1996). However, in some cases, resistance appears to be real (Scharpf, 1987; Scharpf and Roth, 1992; Scharpf et al., 1992). Nonetheless, the overall utility of finding trees with genetic resistance to dwarf mistletoe is unclear. Firstly, given the long generation time of trees, searching for heritable resistance in successive generations will take many years. Furthermore, the stability of the genetic resistance mechanism is unknown. Due to their long generation time, conifers are likely to lag behind *Arceuthobium* in the coevolutionary arms race. Unlike agricultural crops, conifers also require a long time (30+ years) to reach maturity for timber use. Thus, the parasite may evolve new infectivity alleles at a higher rate than a single host generation can maintain resistance in the field and at a higher rate than breeders can develop resistant trees in the lab.

The findings from the infection experiment at the Petawawa Research Forest have significant implications for forest management since they suggest that given sufficient time, there is a great likelihood that *A. americanum* will spread into the forests of eastern Canada. The implications for forest management are substantial since *Arceuthobium* is one of the most damaging forest pathogens to attack commercially important coniferous timber stands in western Canada. Future forest management plans should carefully monitor the spread of *A. americanum* to prevent the further eastward movement of this parasite into managed forest lands.

4.5 CONCLUSIONS

The infection experiments performed in this study provided insight into adaptation of *A. americanum* to its coniferous hosts. Infection levels at the Pine Ridge Forest Nursery in Alberta were extremely low. These low infection levels were attributed to high seed loss and low germination. Unseasonably dry conditions may have resulted in the low germination levels. The absence of host X parasite interactions depicted a lack of adaptation or maladaptation of genetically defined *A. americanum* races to their associated host taxa. This lack of patterning to infectivity of *A. americanum* was also observed at the local level within a host taxon. *Arceuthobium americanum* was neither adapted nor maladapted to hosts in the region from which they were isolated. These observations are in contradiction to Edmunds and Alstad (1978) who predicted that parasites (such as dwarf mistletoes) that exist on toxin-defended, long-lived hosts are likely to be adapted to local host populations and individuals. Indeed, the findings from this study question the extent to which *A. americanum* is coevolving with its hosts, and the role that host identity plays in the formation of the observed genetic races of *A. americanum*.

Infection experiments at the PRFN did reveal, however, that *Pinus banksiana* hosts (in particular those from Manitoba) were more susceptible to *A. americanum* than were *P. contorta* var. *latifolia* hosts. This was attributed to a more ancient association between *A. americanum* and *P. contorta* var. *latifolia* hosts. These hosts would then have had a greater opportunity to evolve resistance mechanisms to this parasite.

Given the overall low infection success in this experiment, the findings should be considered preliminary and interpreted with caution. Future experiments examining

infectivity in *Arceuthobium* could improve robustness of data by increasing the number of combinations examined, as well as by increasing overall infection success in the field.

Infection levels in the experiment at the Petawawa Research Forest in Ontario were higher than those at the PRFN. This was attributed to environmental differences and age of inoculated trees. One of the major findings of the experiment at Petawawa was that trees from regions of Canada currently uninfected with *A. americanum* were easily infected with this parasite. In fact, there was no significant difference in infection success in experimental *P. banksiana* trees from western, central, and eastern Canada infected with *A. americanum* from western Canada. This again suggests that *A. americanum* is neither locally adapted or maladapted to hosts with which it is commonly associated.

Findings from both of these experiments have implications for forest management. The results from the experiment at the PRFN in Alberta suggest that planting of disparate host genotypes and different principal host taxa is unlikely to result in decreased infections by *A. americanum*. The results from the infection experiment at the Petawawa Research Forest indicate that forest managers in Ontario should carefully monitor the spread of *A. americanum* to prevent the further eastward movement of this parasite into managed forest lands.

Table 17. Collection of *A. americanum* seeds from cheesecloth covered brooms from three source populations in 1997; see Figure 11 for location of collection sites.

Seed Source Population	# Brooms	# Trees	Date covered	Date collected
DTR, Alberta *Pinus contorta* var. *latifolia*	175	88	Aug. 7, 1998	Sept. 9 and 10, 1998
BELAIR FOREST, Man. *Pinus banksiana*	175	80	Aug. 15, 16 and 21, 1998	Sept. 10, 1998
PRFN, Alberta *Pinus banksiana*	180	90	Aug. 8 & 9, 1998	Sept. 5, 8 and 9, 1998

Table 18. % Viability (number of seeds examined) as determined by 2% TTC tests for field collected *Arceuthobium americanum* seeds from three source populations.

	Dissected Embryo	Partially Bisected Seed	Completely Bisected Seed	Unweighted MEAN
A. americanum **Source Population**				
DTR, AB *Pinus contorta* var. *latifolia*	50.00 (22)	70.83 (24)	51.61 (31) 94.29 (35)	66.68 ± 20.70
PRFN, AB *Pinus banksiana*	4.55 (23)	33.33 (32)	42.42 (33) 58.33 (36)	34.66 ± 22.57
BELAIR FOREST, MB *Pinus banksiana*	39.29 (38)	57.14 (21)	34.62 (26)	43.68 ± 11.89

Table 19. % Germination success (number of seeds examined) for field collected *Arceuthobium americanum* seeds from various source populations.

Arceuthobium americanum Seed Source	Germination (%)
David Thompson Resort, AB *Pinus contorta* var. *latifolia*	70.59 (51) 69.78 (43) 63.33 (30) 65.31 (49)
Average	67.25 ± 3.50
Pine Ridge Forest Nursery, AB *Pinus banksiana*	54.35 (46) 64.58 (48) 64.00 (50)
Average	60.97 ± 5.75
Belair Forest, MB *Pinus banksiana*	No Seeds Available

Table 20. Infection success for three *Arceuthobium americanum* seed source populations inoculated onto five experimental host populations at the Pine Ridge Forest Nursery in Alberta. Infection success reported as percentage of inoculated seeds successfully infecting hosts as depicted by swellings or by the emergence of *Arceuthobium americanum* shoots from the inoculation site. Three to five replicate trees (R) were inoculated for each population.

		ORIGINAL GEOGRAPHIC SOURCE OF EXPERIMENTAL HOST TREES									
		PRFN, AB *Pinus banksiana*		SE MANITOBA *Pinus banksiana*		WHITECOURT, AB Hybrid		ROCKY MTN HOUSE, AB *Pinus contorta*		Cypress Hills, AB *Pinus contorta*	
	R	Swellings	Shoots	Swellings	Shoots	Swellings	Shoots	Swellings	Shoots	Swellings	Shoots
***Arceuthobium* Seed Source**	1	5.56	1.85	6.06	4.04	6.12	0.00	0.00	0.00	0.58	0.58
	2	1.98	1.98	5.05	4.08	3.88	2.91	0.00	0.00	0.59	0.00
PRFN, AB	3	2.94	2.94	6.02	3.61	3.77	1.89	0.00	0.00	0.57	0.00
Pinus banksiana	4	2.94	0.98	11.61	2.68	6.86	0.98	1.98	0.00	----	----
	5	2.86	2.86	7.92	4.95	2.86	2.86	4.26	0.00	----	----
	1	1.00	0.00	2.50	2.50	3.77	1.89	3.23	0.00	1.89	0.00
BELAIR, MB	2	0.00	0.00	3.03	1.01	10.81	4.50	1.03	0.00	0.00	0.00
Pinus banksiana	3	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.55	0.00
	4	1.00	0.00	8.82	4.92	0.00	0.00	0.00	0.00	----	----
	5	6.42	2.75	6.86	4.90	0.00	0.00	0.00	0.00	----	----
	1	4.55	1.82	8.33	7.84	1.10	1.10	1.00	0.00	1.18	0.00
DTR, AB	2	0.00	0.00	15.79	7.89	4.67	2.80	3.45	2.30	10.18	4.79
Pinus contorta	3	5.10	0.00	10.31	6.19	1.82	0.91	0.00	0.00	0.00	0.00
	4	6.80	5.83	4.00	4.00	2.94	0.98	3.88	2.91	----	----
	5	7.48	2.80	5.56	2.78	0.00	0.00	3.03	0.00	----	----

Table 21. Summary of mean infectivity as depicted by proportion of inoculation sites (± standard deviation) with swellings or *A. americanum* shoot emergence across the three *Arceuthobium americanum* populations on five experimental host populations at the Pine Ridge Forest Nursery, Alberta.

		PRFN, AB *Pinus banksiana*	BELAIR, AB *Pinus banksiana*	DTR, AB *Pinus contorta*	Mean Susceptibility
Host Origin					
PRFN, AB *Pinus banksiana*	Swellings	3.25 ± 1.35	1.88 ± 2.58	4.78 ± 2.93	3.31 ± 2.53
	Shoots	2.12 ± 0.81	0.55 ± 1.23	2.09 ± 2.41	1.59 ± 1.69
S.E. MANITOBA *Pinus banksiana*	Swellings	7.33 ± 2.61	4.24 ± 3.55	8.80 ± 4.61	6.79 ± 3.93
	Shoots	3.87 ± 0.83	1.11 ± 1.53	6.56 ± 3.09	4.03 ± 2.19
WHITECOURT, AB Hybrid	Swellings	4.70 ± 1.70	2.92 ± 4.71	2.11 ± 1.79	3.24 ± 3.05
	Shoots	1.73 ± 1.25	1.28 ± 1.98	1.16 ± 1.02	1.39 ± 1.39
ROCKY MTN HOUSE, AB *Pinus contorta*	Swellings	1.24 ± 1.89	0.85 ± 1.40	2.27 ± 1.68	1.46 ± 1.67
	Shoots	0.00 ± 0.00	0.00 ± 0.00	1.04 ± 1.44	0.35 ± 0.92
CYPRESS HILLS, AB *Pinus contorta*	Swellings	0.58 ± 0.01	2.14 ± 2.29	3.79 ± 5.57	2.17 ± 3.31
	Shoots	0.19 ± 0.33	0.00 ± 0.00	1.60 ± 2.77	0.60 ± 1.58
					OVERALL
Average Infectivity	Swellings	3.37 ± 3.00	2.43 ± 3.13	4.40 ± 4.06	3.57 ± 3.40
	Shoots	1.70 ± 1.62	0.94 ± 1.63	2.39 ± 2.59	1.79 ± 1.96

Table 22. Results for the Factorial Analysis of Variance of infectivity as depicted by proportion (arcsin - square root transformed) of inoculation sites with (22a) swellings and (22b) shoots for three *Arceuthobium americanum* seed sources inoculated on five experimental host populations at the Pine Ridge Forest Nursery, Alberta.

(22a)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	4	578.662	144.665	7.1070	0.0001
Parasite	2	110.472	55.2362	2.7430	0.0734
Host X Parasite	8	162.840	20.3549	1.0108	0.4391
Error	54	1087.41	20.1373		
Total	68	1961.73			

(22b)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	4	488.231	122.058	12.624	≤0.0001
Parasite	2	92.6119	46.3060	4.7893	0.0122
Host X Parasite	8	60.7886	7.59857	0.78590	0.6170
Error	54	522.107	9.66865		
Total	68	1166.54			

Table 23. Results for the Factorial Analysis of Variance of infectivity as depicted by proportion (arcsin - square root transformed) of inoculation sites with (23a) swellings and (23b) shoots for *Arceuthobium americanum* from two host types (*Pinus banksiana* and *Pinus contorta* var. *latifolia*) inoculated onto two experimental host types (*Pinus banksiana* and *Pinus contorta* var. *latifolia*) at the Pine Ridge Forest Nursery, Alberta.

(23a)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	2	269.863	134.932	5.5813	0.0059
Parasite	1	6.44465	6.44465	0.26657	0.6074
Host X Parasite	2	73.9130	36.9565	1.5287	0.2248
Error	63	1523.08	24.1759		
Total	68	1854.66			

(23b)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	2	298.845	149.448	13.049	≤0.0001
Parasite	1	34.2284	34.2284	2.9887	0.0887
Host X Parasite	2	25.7843	12.8921	1.1257	0.3309
Error	63	721.523	11.4527		
Total	68	1137.55			

Table 24. Mean infectivity ± standard deviation of *A. americanum* from either *P. banksiana* (PB) or *P. contorta* var. *latifolia* (PC) inoculated onto *P. banksiana* (PB), *P. contorta* var. *latifolia* (PC), or hybrid hosts as depicted by (24a) swellings or by (24b) *A. americanum* shoot emergence.

(24a)

		EXPERIMENTAL HOST		
		PB	PC	HYBRID
PARASITE	PB	4.16±3.18	2.09±2.27	3.81±3.47
	PC	6.79±4.21	2.84±3.33	2.11±1.79

(24b)

		EXPERIMENTAL HOST		
		PB	PC	HYBRID
PARASITE	PB	2.25±1.72	0.04±0.15	1.50±1.58
	PC	3.92±2.94	1.25±1.86	1.16±1.02

Table 25. Results for the Factorial Analysis of Variance of infectivity as depicted by proportion (arcsin - square root transformed) of inoculation sites with (25a) swellings or (25b) shoots for two sources of *Arceuthobium americanum* (Belair Forest, Manitoba and PRFN, Alberta) on two sources of *Pinus banksiana* (S.E. Manitoba and PRFN, Alberta) at the Pine Ridge Forest Nursery, Alberta.

(25a)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	1	107.054	107.054	6.7146	0.0197
Parasite	1	79.1828	79.1828	4.9664	0.0405
Host X Parasite	1	2.25997	2.25997	0.14175	0.7115
Error	16	255.097	15.9436		
Total	19	443.594			

(25b)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	1	66.0874	66.0874	8.7389	0.0093
Parasite	1	59.7976	59.7976	7.9072	0.0125
Host X Parasite	1	1.6770	1.6770	0.22185	0.6440
Error	16	120.999	7.56244		
Total	19	248.562			

Table 26. Mean infectivity of *Arceuthobium americanum* isolated from *Pinus banksiana* (2 sources, PRFN, Alberta and Belair Forest, Manitoba) onto *P. banksiana* (2 sources, PRFN, Alberta or S.E. Manitoba) as depicted by (26a) swellings and by (26b) shoots.

(26a)

		***Pinus banksiana* HOST**	
		PRFN	SE Manitoba
PARASITE	PRFN	3.26 ± 1.35	7.33 ± 2.61
	Belair	1.88 ± 2.58	4.24 ± 3.55

(26b)

		***Pinus banksiana* HOST**	
		PRFN	SE Manitoba
PARASITE	PRFN	2.12 ± 0.81	3.87 ● 0.83
	Belair	0.55 ● 1.23	2.49 ± 2.04

Table 27. Infection success of a pooled *A. americanum* seed inoculum onto fifteen experimental hosts (3 regions) at the Petawawa Research Forest, Ontario as indicated by % of inoculation sites (± standard deviation) with either branch swellings or *A. americanum* shoot emergence.

Original Geographic Source of Experimental *Pinus banksiana* Hosts		% with Swellings	% with Shoots
EASTERN	Birchtown Brook, NS	29.29	15.15
	Cape Breton Highland, NS	17.65	10.78
	Neils Harbour, NS	21.62	9.91
	Thomson Station, NS	25.25	7.07
	East Bideford, PEI	7.07	0.00
	Average	20.18 ± 8.50	8.58 ● 5.61
CENTRAL	Miller Lake, ON	4.3	1.08
	Gowganda Lake, ON	12.75	0.00
	Cass Lake, MN (USA)	13.33	3.81
	Kenora, ON	24.44	8.89
	Vermillion Bay, ON	23.23	13.13
	Average	15.61 ± 8.33	5.38 ± 5.53
WESTERN	Big River, SK	Entire tree died	
	Cowan, MB	1.11	0.00
	Reindeer Lake, SK	6.67	3.33
	Whitecourt, AB	21.42	2.04
	Fort McMurray, AB	27.27	8.08
	Average	14.12 ± 12.26	3.36 ● 3.43
OVERALL AVERAGE		16.81 ± 9.24	5.95 ± 5.16

Table 28. Results for the Analysis of Variance of infectivity as depicted by proportion (arcsin - square root transformed) of inoculation sites with (28a) swellings or (28b) shoots for a pooled source population of *A. americanum* inoculated onto *Pinus banksiana* hosts from three regions (western, central, and eastern) at the Petawawa Research Forest, Ontario.

(28a)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	2	86.9945	43.4973	0.60298	0.5643
Error	11	793.507	72.1370		
Total	13	880.502			

(28b)

Source	DF	Sum of Squares	Mean Square	F-ratio	Probability
Host	2	90.8141	45.4071	0.88284	0.4410
Error	11	565.765	51.4332		
Total	13	656.579			

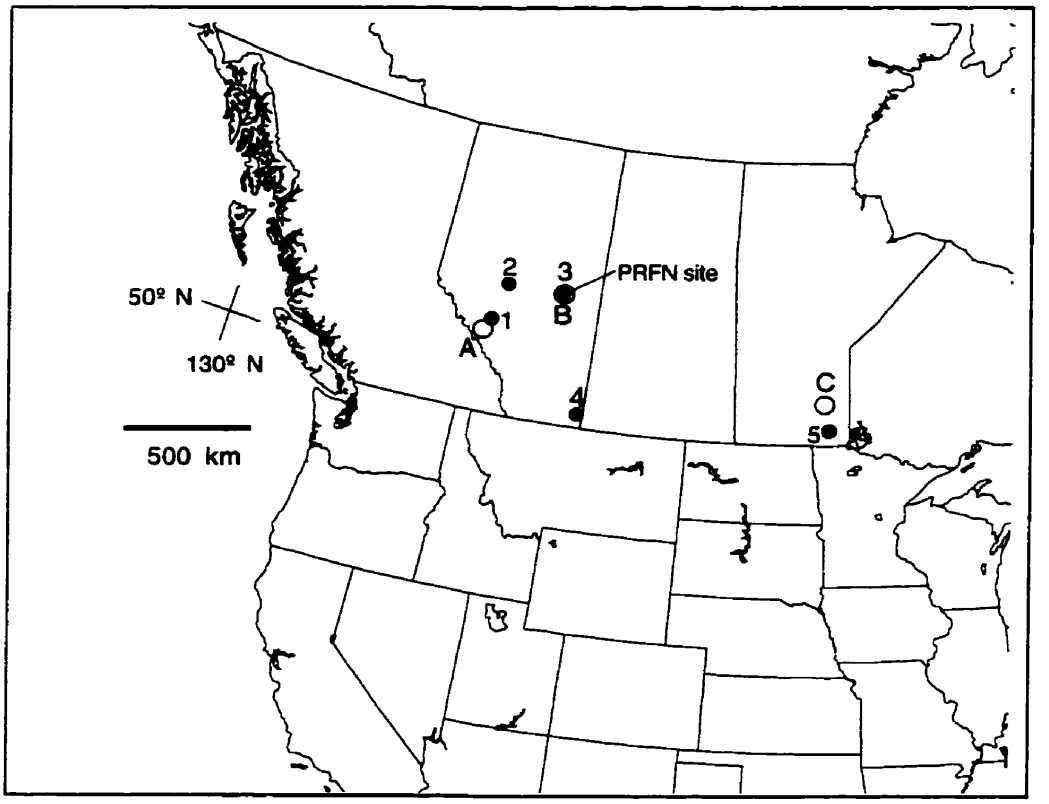

Figure 11. Map showing *A. americanum* seed collection sites (A = DTR, B = PRFN, C = Belair) and *Pinus* spp. host origins (1 = *P. contorta* var. *latifolia* from Rocky Mtn. House, AB, 2 = hybrid from Whitecourt, AB, 3 = *P. banksiana* from PRFN, AB, 4 = *P. contorta* var. *latifolia* from Cypress Hills, AB, 5 = *P. banksiana* from SE Manitoba) for the infection experiment at the Pine Ridge Forest Nursery (PRFN) in Alberta.

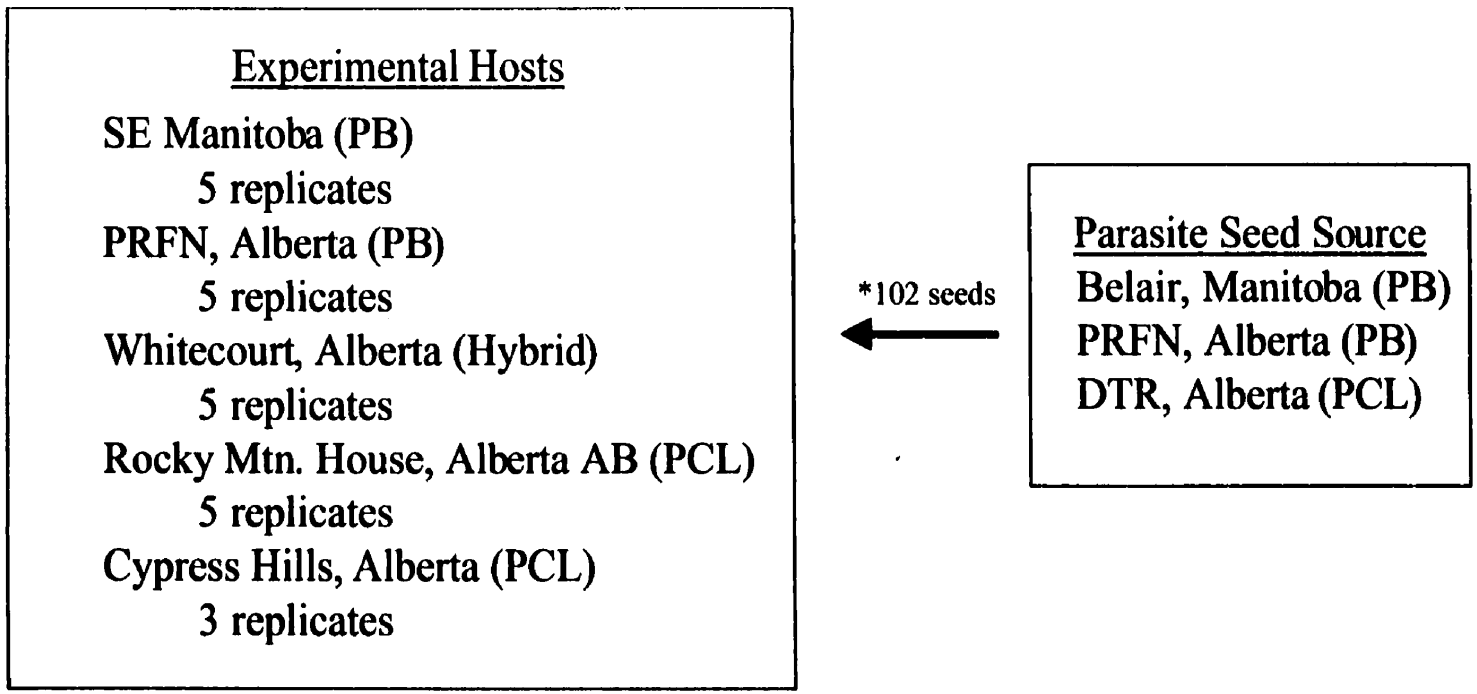

Figure 12. Experimental design of the infection experiment at the provenance stand in the Pine Ridge Forest Nursery, Alberta. Three *Arceuthobium americanum* parasite seed sources (see above; PB = *Pinus banksiana*, PCL = *Pinus contorta* var. *latifolia*) were each inoculated onto five experimental host sources (see above; PB = *Pinus banksiana*, PCL = *Pinus contorta* var. *latifolia*, Hybrid = *P. banksiana* X *P. contorta* var. *latifolia*) in the stand. The total number of seeds inoculated was 7,632. * In general, 102 seeds from each parasite seed source were inoculated onto five replicate trees of each experimental host source (i.e. 510 seeds for each host X parasite combination). However, for the Cypress Hills experimental host source, 168 seeds were inoculated onto three replicate trees (i.e. 504 seeds for each host X parasite combination).

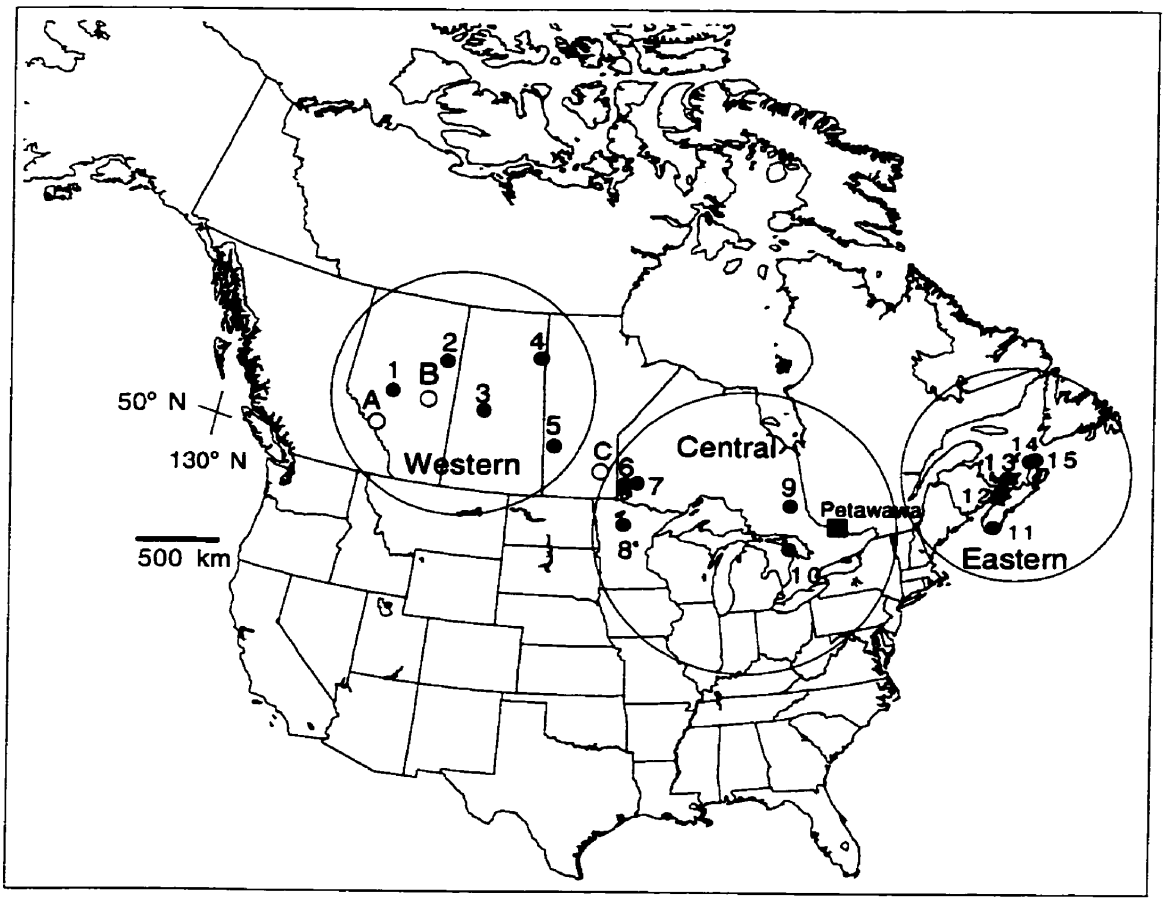

Figure 13. Map showing *Arceuthobium americanum* seed collection sites (A = DTR, B = PRFN, C = Belair) and *Pinus banksiana* host origins (1 = Whitecourt, AB, 2 = Fort McMurray, AB, 3 = Big River, SK, 4 = Reindeer Lake, SK, 5 = Cowan, MB, 6 = Kenora, ON, 7 = Vermillion Bay, ON, 8 = Cass Lake, MN, 9 = Gowganda Lake, ON, 10 = Miller Lake, ON, 11 = Birchtown Brook, NS , 12 = Thomson Station, NS, 13 = East Bideford, PEI, 14 = Cape Breton Highland, NS, 15 = Neils Harbour, NS) for the infection experiment at the Petawawa Research Forest in Ontario.

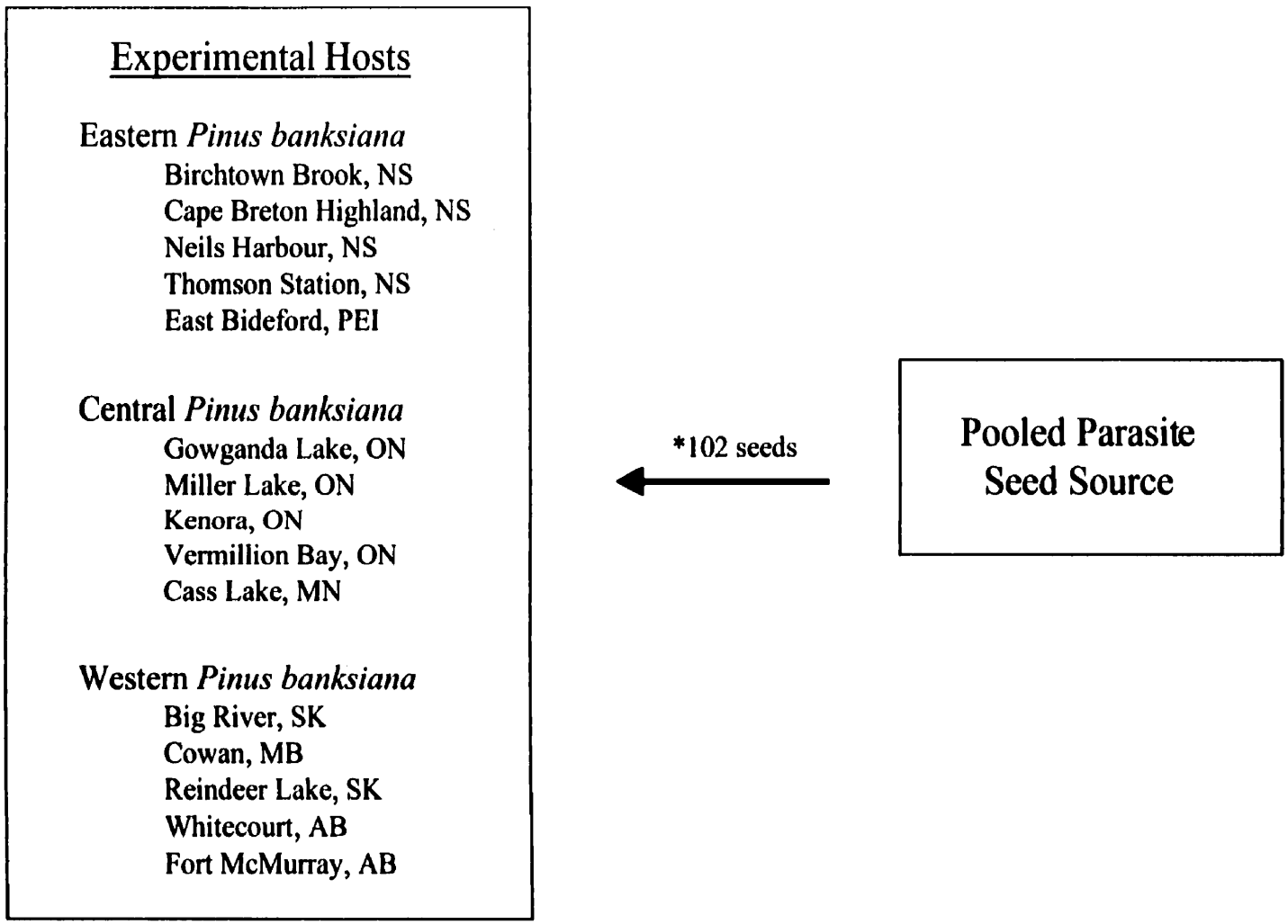

Figure 14. Experimental design of the infection experiment at the provenance stand at the Petawawa Research Station, near Chalk River, Ontario. Three *Arceuthobium americanum* parasite seed sources (from Belair Forest, MB, *Pinus banksiana*, from Pine Ridge Forest Nursery, AB, *Pinus banksiana*, and from David Thompson Resort, AB, *Pinus contorta* var. *latifolia*) were pooled and used to inoculate fifteen trees (see above) from three different regions. *Each of the fifteen trees received 102 *A. americanum* seeds.

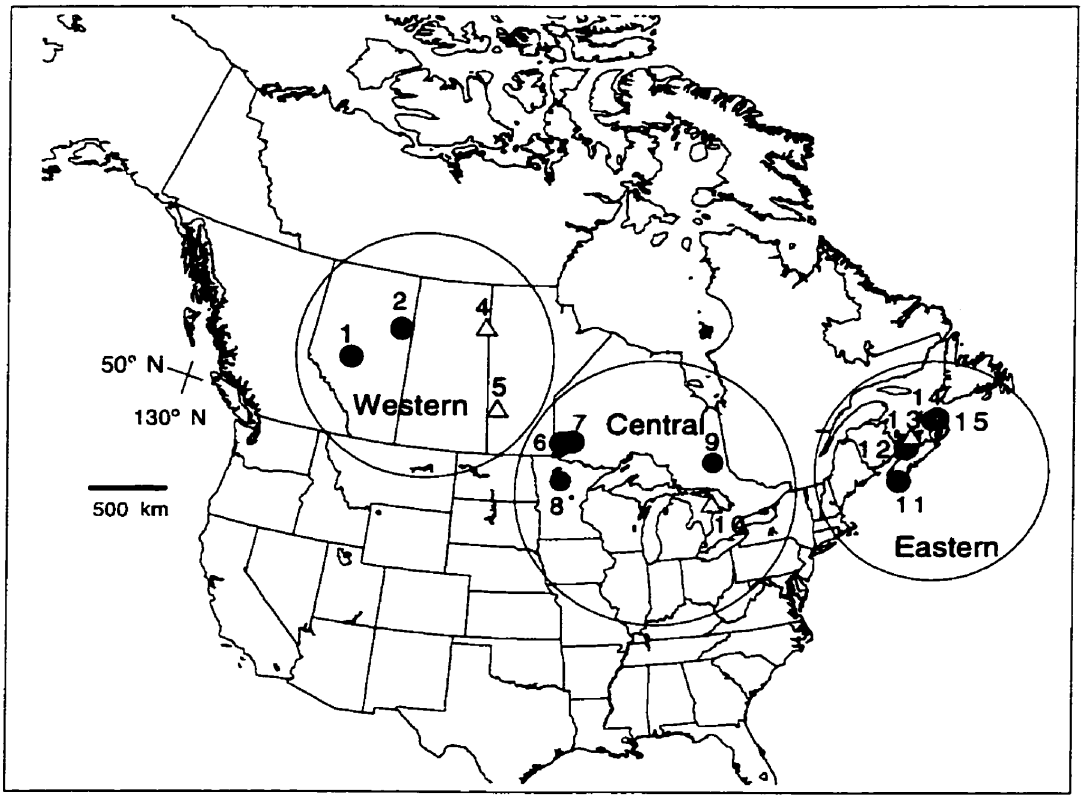

Figure 15. Map showing relative infection success on experimental *Pinus banksiana* hosts (1 = Whitecourt, AB, 2 = Fort McMurray, AB, 3 = Big River, SK, 4 = Reindeer Lake, SK, 5 = Cowan, MB, 6 = Kenora, ON, 7 = Vermillion Bay, ON, 8 = Cass Lake, MN, 9 = Gowganda Lake, ON, 10 = Miller Lake, ON, 11 = Birchtown Brook, NS , 12 = Thomson Station, NS, 13 = East Bideford, PEI, 14 = Cape Breton Highland, NS, 15 = Neils Harbour, NS) at the Petawawa Research Forest in Ontario. Infection levels are reported as proportion of inoculation sites showing branch swellings: dark shaded circles = 20.00 - 30.00% infection, gray shaded circles = 10.00 - 19.99% infection, open triangles = 1.00 - 9.99% infection.

CHAPTER 5: General Discussion

In this study, a multidisciplinary approach incorporating population genetic analysis and infectivity experiments was used to explore the evolutionary biology of *Arceuthobium americanum*. This study is the first comprehensive investigation into the evolutionary, geographical, biological, ecological, and historical factors influencing a parasitic plant.

Population genetic analysis using amplified fragment length polymorphism analysis revealed that *Arceuthobium americanum* is comprised of three distinct genetic races, each associated with different host taxa in regions of allopatry: (1) *P. banksiana* in western and central Canada; (2) *P. contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountain ranges; and (3) *P. contorta* var. *latifolia* throughout western U.S.A. and Canada (Chapter 1). These races are clearly delineated from each other as can be seen by: (1) the presence of three distinct genetic groups in the cluster analyses based on genetic identity values; (2) high genetic identities within compared to between these groups; and (3) the presence of unique alleles associated with these groups. The observation of cryptic taxa within *A. americanum* supports the hypothesis of several researchers (Price, 1980; Tybayrenc and Ayala, 1991; Maynard Smith et al., 1993) who have suggested that parasites are likely to be characterized by high population subdivision, geographic differentiation and the existence of cryptic taxa. It is possible that *A. americanum* will undergo speciation via race formation if gene flow continues to be restricted between the three races and reproductive isolation develops. Speciation via race formation has been implicated as one of the major evolutionary processes acting on parasitic organisms (Price, 1980; Thompson, 1994; Norton and Carpenter, 1998).

Findings from this thesis suggest that race formation in *A. americanum* is facilitated by geographical isolation as well as by divergent selection pressures imposed by different environmental conditions and different hosts (Chapters 2, 3 and 4). Geographical isolation promotes race formation due to the lack of cohesive forces that would normally result from gene flow between conspecific populations (Orr, 1995; Orr and Orr, 1996; Via et al., 2000). Given their allopatric distributions, geographic isolation appears to have played an important role in the formation of three *A. americanum* races (Chapter 2). Gene flow by pollen and seed dispersal is limited by the geographic separation of *A. americanum* populations on *P. contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountains from those on *P. contorta* var. *latifolia* across the Great Basin in the Blue, Salmon River, Uinta, and Rocky Mountains. Gene flow by pollen and seed dispersal is also limited between *A. americanum* populations on *P. banksiana* and those on *P. contorta* var. *latifolia* due to their allopatric distributions. Contact between these taxa occurs only in a hybrid zone in central and northern Alberta. Given these limitations to gene flow, it seems probable that isolation-by-distance has played a major role in formation of the three races of *A. americanum*. However, the strong group structure and the lack of geographic patterning in cluster analyses despite the intensive sampling of *A. americanum* across an almost continuous range suggests that other factors must be involved.

Different host species are thought to represent unique environments to which a parasite must adapt (Thompson, 1994; Brooks and McLennan, 1996). Thus, hosts may impose diversifying selection pressures that favour certain parasite genotypes while selecting against others (Via et al., 2000). Findings from this thesis suggest a role for

host identity in delineating the population structure of *A. americanum* since the three races can be easily defined by this factor (Chapter 2). However, other observations from this thesis question the overall contribution of host identity (Chapters 3 and 4). Firstly, population genetic analysis indicated that hosts were divided into only two distinctive genetic groups: (1) *P. banksiana* and hybrids; and (2) *Pinus contorta* var. *latifolia* and var. *murrayana* (Chapter 3). Since *P. banksiana* and *P. contorta* hosts are genetically divergent they may have imposed different selection pressures on their respective parasite populations. The same may not be true, however, of the two *P. contorta* varieties since these hosts could not be easily discriminated using molecular markers. The findings from infection experiments (Chapter 4) also question the role of host identity in shaping *A. americanum* population structure. Infection experiments indicated a lack of host specific or geographic patterning to infectivity by *A. americanum*. For example, *P. banksiana* hosts did not select against infection by *A. americanum* from *P. contorta* var. *latifolia*. Similarly, *P. contorta* var. *latifolia* hosts did not select against infection by *A. americanum* from *P. banksiana*. Since there was no evidence that host taxa favoured infection by their associated *A. americanum* races, it seems unlikely that this factor has played the primary role in the diversification within *A. americanum*. However, the findings of these experiments should be considered preliminary and interpreted with caution given the low infection success of this experiment.

Divergent selection pressures resulting from adaptation to different environmental conditions is also thought to facilitate race formation since specific genotypes would be favoured or selected against in a given environment (Schluter, 1996, 1998; Via et al., 2000). *Arceuthobium americanum* races may each be adapted to growing in the

environmental conditions (levels or rainfall, temperature, length of growing season, etc...) that characterize the ecoclimatic regions in which their principal hosts grow. Thus, it is possible that *A. americanum* races are responding to divergent selection pressures imposed by abiotic factors rather than to pressures imposed by different host taxa.

The relative importance of geographical isolation, environmental conditions, and host identity was not resolved in this thesis. It seems plausible that these factors interact with each other to facilitate race formation in *A. americanum*. Additional experiments examining infectivity patterns in *A. americanum* could provide greater insight into the relative role played by these factors. For example, replication of infection experiments in each of the ecoclimatic regions where the *A. americanum* races are found could provide insight into the impact of different environments on these races. Additionally, increasing the number of host-parasite combinations examined could shed more light on the role played by host identity and genotype.

Biological traits and ecological associations were also considered to be important in shaping populations of *A. americanum* and its hosts (Chapters 2 and 3). In *A. americanum*, long-distance dispersal of seeds through stochastic transport on the feathers of birds and on the fur of small mammals was thought to contribute to the lack of fine-scale geographic patterning within *A. americanum* races (Chapter 2). Patterning would likely have been much stronger had explosive discharge been solely responsible for dispersing seeds of *A. americanum*. Adaptation to non-geographically patterned host genotypes and local environmental conditions were also implicated in shaping the fine-scale structure within *A. americanum* races. In the hosts, the observed lack of geographic

patterning was attributed to life history characteristics intrinsic to these taxa (Chapter 3). For example, gymnosperms have the highest levels of gene flow recorded for any plant group (Hamrick and Godt, 1990). This is particularly true of members of the genus *Pinus* in which pollen morphology is responsible for allowing pollen grains to be transported by wind over extremely long distances (Ledig, 1998; Campbell et al., 1999).

Historical factors such as founder events and glacial history were also thought to play a role in shaping genetic diversity in populations of both host and parasite (Chapters 2 and 3). For example, it was suggested that founder events were responsible for the origin of *A. americanum* populations on hybrids in northern Alberta. Low genetic diversity in these populations and their divergent position in dendrograms support this hypothesis (Chapter 2). Additionally, the advance and retreat of glaciers during the Wisconsin is thought to have affected population diversity and patterning of both the parasite and its hosts (Chapters 2 and 3). Populations of *P. contorta* var. *latifolia* from unglaciated southern regions were genetically more diverse than those from previously glaciated northern regions (Chapter 3). The pattern observed in *P. contorta* var. *latifolia* is typical of that found in many other taxa (Fowler and Morris, 1977; Copes, 1981; Waller et al., 1987; Broyles, 1998). This pattern has been attributed to the loss of genetic diversity as a species undergoes rapid migration from large refugia south of the glacial front into newly deglaciated northern regions (reviewed in Lewis and Crawford, 1995 and Broyles, 1998). Opposite to the pattern observed in the host, *A. americanum* populations from previously glaciated northern regions were more diverse than populations from unglaciated southern regions (Chapter 2). It was suggested that this observation could be explained by the survival of *A. americanum* in a genetically diverse

glacial refugium along the eastern slopes of the Canadian Rocky Mountains during the late Wisconsin. If this were the case, populations derived from this northern refugium would also have been quite diverse.

In summation, it appears that the genetic structure of the parasitic plant *A. americanum* is affected by many of the factors that affect the structure of non-parasites. These include geographic distribution, life history and ecological traits, selection pressures and historical events. The factor that is unique to *A. americanum* in comparison with non-parasites is the influence played by host identity and genotype. However, findings from this study suggest that although host plays some role, it is not the primary factor shaping populations of *A. americanum*.

In order to determine if the patterns observed for *A. americanum* are applicable to parasitic plants in general, it is recommended that future studies incorporate a multi-disciplinary approach using population genetic analysis and infection experiments to examining parasitic plant taxa with a wide range of biological and ecological attributes. For example, it would be useful to examine parasitic plants with lifestyles ranging from obligate holoparasites (such as those in the *Orobanchaceae* Vent., *Rafflesiaceae* Dumort., and *Balanophoraceae* Rich.) to obligate hemiparasites (such as members of the *Loranthaceae* Juss. and *Viscaceae*) to facultative hemiparasites (such as members of the *Santalaceae* R. Br. and *Lennoaceae* Solms-Laub.). Obligate parasites with a strict dependence on their hosts are likely to behave differently from facultative parasites. Insight could also be gained from studying parasitic plants with different reproductive strategies and dispersal mechanisms. A wide array of reproductive strategies exists in parasitic plants extending from those that self (some *Orobanchaceae*) to those that

outcross through wind and animal pollination (such as the *Loranthaceae*, *Viscaceae* and *Scrophulariaceae*). Seed dispersal mechanisms vary from those that disperse seeds by explosive discharge and animals (many *Loranthaceae* and *Viscaceae*) to those that disperse seeds by wind (such as the *Scrophulariaceae* and *Orobanchaceae*). Both pollination and seed dispersal mechanisms can have strong influences on genetic structure and hence, the evolutionary potential of organisms (Hamrick and Godt, 1990). Finally, future research should examine the impact of host specificity on the evolution of parasitic plants. Host specialization in parasitic plants ranges from taxa that attack a large number of unrelated hosts (as is seen for many root parasites) to those that attack a much narrower host range (as is seen for many shoot parasites). Given this broad spectrum of biological and ecological attributes that characterize these organisms, it is unlikely that a single evolutionary principle will apply to the > 3,000 species of parasitic plants. It is more likely that certain patterns will characterize taxa with similar lifestyles, seed and pollen dispersal mechanisms, and degree of host-specialization.

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Appendix 1. Matrix of pairwise Nei's unbiased genetic distances (Nei, 1978) amongst 51 A. americanum populations.

	MB-7	MB-8	BC-25	MB-5	SK-16	MB-2	MB-4	MB-11	SK-14	SK-1	SK-3	SK-12	SK-13	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-17	BC-25	AB-29	AB-27	AB-24	AB-28	AB-26	BC-30	BC-31	BC-32	BC-33	ID-40	WY-44	UT-41	UT-45	CO-43	CO-46
Grand Rapids I (MB-7)	0.000																																			
Grand Rapids II (MB-8)	0.077	0.000																																		
Castlegar (BC-25)	0.126	0.120	0.000																																	
Devil's Lake (MB-5)	0.037	0.056	0.113	0.000																																
Tobin Lake (SK-16)	0.087	0.076	0.114	0.055	0.000																															
Belair (MB-2)	0.057	0.072	0.118	0.033	0.054	0.000																														
Cowan (MB-4)	0.078	0.060	0.142	0.058	0.065	0.056	0.000																													
The Pas (MB-11)	0.065	0.072	0.094	0.050	0.048	0.054	0.069	0.000																												
Smeaton (SK-14)	0.087	0.087	0.105	0.074	0.030	0.055	0.047	0.044	0.000																											
Beauval (SK-1)	0.062	0.052	0.096	0.042	0.038	0.037	0.051	0.049	0.045	0.000																										
Candle Lake (SK-3)	0.075	0.061	0.106	0.077	0.062	0.062	0.050	0.054	0.040	0.052	0.000																									
Prince Albert I (SK-12)	0.056	0.078	0.114	0.031	0.053	0.036	0.061	0.032	0.051	0.031	0.066	0.000																								
Prince Albert II (SK-13)	0.103	0.070	0.142	0.079	0.077	0.093	0.076	0.068	0.066	0.080	0.061	0.067	0.000																							
La Ronge (SK-10)	0.069	0.076	0.106	0.041	0.050	0.036	0.066	0.035	0.039	0.029	0.058	0.021	0.076	0.000																						
La Loche (SK-9)	0.116	0.105	0.138	0.098	0.095	0.082	0.055	0.073	0.070	0.059	0.052	0.060	0.071	0.065	0.000																					
Smoky Lake (AB-15)	0.060	0.075	0.087	0.058	0.058	0.044	0.068	0.040	0.050	0.030	0.057	0.038	0.083	0.034	0.086	0.000																				
Ft. McMurray (AB-6)	0.061	0.068	0.089	0.054	0.050	0.042	0.068	0.062	0.054	0.051	0.069	0.063	0.096	0.048	0.095	0.037	0.000																			
Slave Lake (AB-19)	0.073	0.096	0.090	0.064	0.074	0.064	0.054	0.063	0.044	0.054	0.068	0.053	0.084	0.054	0.094	0.063	0.058	0.000																		
Whitecourt (AB-20)	0.090	0.126	0.094	0.086	0.100	0.101	0.106	0.087	0.083	0.097	0.117	0.090	0.140	0.084	0.126	0.078	0.065	0.066	0.000																	
Grande Prairie (AB-17)	0.126	0.130	0.074	0.097	0.085	0.102	0.099	0.081	0.061	0.084	0.094	0.097	0.117	0.070	0.105	0.053	0.062	0.084	0.050	0.000																
100 m House (BC-23)	0.097	0.091	0.056	0.101	0.106	0.114	0.086	0.092	0.096	0.091	0.107	0.109	0.132	0.093	0.137	0.064	0.057	0.065	0.072	0.074	0.000															
Jasper (AB-29)	0.116	0.117	0.032	0.113	0.094	0.112	0.130	0.088	0.082	0.075	0.107	0.097	0.137	0.073	0.120	0.065	0.065	0.066	0.083	0.056	0.034	0.000														
DTR (AB-27)	0.092	0.105	0.064	0.103	0.088	0.129	0.117	0.084	0.101	0.106	0.116	0.112	0.142	0.099	0.154	0.067	0.067	0.090	0.066	0.067	0.014	0.032	0.000													
Banff (AB-24)	0.087	0.099	0.053	0.080	0.077	0.087	0.081	0.069	0.058	0.058	0.076	0.081	0.109	0.066	0.108	0.039	0.060	0.057	0.066	0.037	0.039	0.030	0.044	0.000												
Field (BC-28)	0.111	0.111	0.049	0.094	0.092	0.118	0.112	0.090	0.092	0.089	0.111	0.101	0.144	0.087	0.145	0.057	0.071	0.069	0.058	0.055	0.022	0.025	0.014	0.029	0.000											
Cypress Hills (AB-26)	0.087	0.072	0.050	0.074	0.072	0.085	0.065	0.077	0.069	0.051	0.057	0.091	0.103	0.085	0.095	0.064	0.058	0.043	0.091	0.073	0.045	0.051	0.073	0.040	0.064	0.000										
Kamloops (BC-30)	0.097	0.083	0.039	0.084	0.090	0.070	0.103	0.071	0.069	0.061	0.082	0.082	0.103	0.058	0.123	0.038	0.061	0.063	0.079	0.045	0.043	0.030	0.060	0.023	0.046	0.041	0.000									
Nimpo Lake (BC-31)	0.084	0.103	0.027	0.076	0.080	0.079	0.098	0.072	0.071	0.074	0.093	0.081	0.114	0.068	0.128	0.056	0.058	0.053	0.066	0.044	0.032	0.025	0.043	0.028	0.035	0.039	0.018	0.000								
Prince George (BC-32)	0.141	0.135	0.060	0.137	0.137	0.137	0.121	0.123	0.130	0.128	0.113	0.130	0.136	0.133	0.147	0.100	0.101	0.084	0.118	0.094	0.046	0.055	0.073	0.062	0.065	0.055	0.052	0.043	0.000							
Redstone (BC-33)	0.126	0.094	0.047	0.098	0.096	0.105	0.087	0.086	0.091	0.086	0.107	0.091	0.115	0.102	0.119	0.069	0.078	0.071	0.088	0.061	0.049	0.047	0.051	0.059	0.046	0.057	0.046	0.032	0.033	0.000						
Ketchum (ID-40)	0.092	0.079	0.064	0.085	0.094	0.109	0.108	0.106	0.098	0.094	0.107	0.110	0.126	0.104	0.156	0.081	0.052	0.078	0.079	0.084	0.050	0.055	0.056	0.062	0.053	0.058	0.055	0.050	0.066	0.051	0.000					
Yellowstone (WY-44)	0.084	0.075	0.052	0.081	0.094	0.098	0.110	0.084	0.086	0.087	0.104	0.100	0.128	0.090	0.134	0.072	0.061	0.086	0.072	0.083	0.047	0.048	0.042	0.057	0.056	0.059	0.049	0.038	0.077	0.053	0.033	0.000				
Manilla I (UT-41)	0.091	0.089	0.042	0.087	0.090	0.089	0.119	0.082	0.086	0.072	0.095	0.093	0.108	0.087	0.135	0.055	0.070	0.075	0.081	0.065	0.058	0.047	0.062	0.036	0.054	0.046	0.025	0.025	0.063	0.058	0.043	0.023	0.000			
Manilla II (UT-45)	0.086	0.089	0.045	0.087	0.092	0.093	0.118	0.079	0.090	0.089	0.090	0.097	0.110	0.085	0.136	0.056	0.073	0.087	0.073	0.074	0.050	0.049	0.051	0.043	0.057	0.061	0.036	0.038	0.070	0.061	0.042	0.019	0.021	0.000		
RFL I (CO-43)	0.111	0.083	0.041	0.102	0.102	0.113	0.133	0.086	0.106	0.098	0.106	0.113	0.120	0.103	0.140	0.088	0.082	0.096	0.088	0.088	0.061	0.046	0.054	0.057	0.061	0.058	0.043	0.038	0.065	0.050	0.042	0.015	0.016	0.023	0.000	
RFL II (CO-46)	0.086	0.092	0.030	0.082	0.084	0.082	0.104	0.070	0.071	0.067	0.085	0.087	0.107	0.072	0.125	0.053	0.058	0.060	0.072	0.063	0.048	0.027	0.049	0.030	0.040	0.040	0.018	0.015	0.054	0.048	0.044	0.017	0.006	0.022	0.012	0.000

Appendix 1 cont'd...

	MB-7	MB-8	BC-25	MB-5	SK-16	MB-2	MB-4	MB-11	SK-14	SK-1	SK-3	SK-12	SK-13	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-17	BC-25	AB-29	AB-27	AB-24	AB-28	AB-26	BC-30	BC-31	BC-32	BC-33	ID-40	WY-4	UT-41	UT-45	CO-43	CO-46
Kenosha Pass (CO-39)	0.125	0.101	0.049	0.119	0.101	0.116	0.137	0.101	0.092	0.113	0.099	0.119	0.130	0.120	0.144	0.089	0.090	0.115	0.105	0.104	0.080	0.064	0.082	0.056	0.077	0.077	0.059	0.049	0.076	0.069	0.054	0.039	0.044	0.030	0.034	0.040
Bondurant (WY-35)	0.100	0.084	0.062	0.097	0.117	0.117	0.116	0.084	0.116	0.110	0.100	0.108	0.113	0.113	0.131	0.087	0.079	0.093	0.077	0.085	0.057	0.058	0.060	0.061	0.064	0.067	0.052	0.051	0.050	0.048	0.031	0.028	0.039	0.035	0.026	0.036
Grand Lake (CO-36)	0.103	0.076	0.052	0.073	0.105	0.093	0.114	0.085	0.099	0.072	0.098	0.090	0.107	0.090	0.133	0.068	0.075	0.082	0.095	0.084	0.066	0.053	0.071	0.045	0.061	0.063	0.040	0.043	0.061	0.048	0.028	0.032	0.032	0.038	0.030	0.030
Monarch Pass (CO-42)	0.096	0.083	0.047	0.083	0.097	0.112	0.127	0.069	0.099	0.089	0.100	0.097	0.109	0.081	0.139	0.072	0.066	0.095	0.080	0.074	0.048	0.048	0.050	0.055	0.052	0.067	0.046	0.039	0.069	0.060	0.033	0.038	0.039	0.038	0.030	0.035
Idaho City (ID-37)	0.074	0.099	0.048	0.069	0.089	0.086	0.088	0.077	0.075	0.068	0.090	0.087	0.105	0.074	0.119	0.054	0.060	0.051	0.065	0.046	0.037	0.029	0.043	0.024	0.035	0.039	0.031	0.012	0.055	0.047	0.040	0.038	0.027	0.031	0.040	0.017
Ashton (ID-34)	0.090	0.104	0.046	0.094	0.091	0.091	0.124	0.078	0.090	0.075	0.112	0.095	0.135	0.090	0.154	0.060	0.066	0.082	0.100	0.080	0.050	0.028	0.043	0.043	0.043	0.068	0.037	0.027	0.053	0.039	0.036	0.034	0.035	0.037	0.033	0.024
Lee Vining I (CA-48)	0.131	0.152	0.127	0.132	0.155	0.142	0.192	0.135	0.149	0.153	0.172	0.148	0.184	0.140	0.223	0.099	0.099	0.147	0.115	0.106	0.112	0.108	0.133	0.108	0.106	0.132	0.109	0.095	0.174	0.141	0.115	0.103	0.102	0.098	0.114	0.104
Lee Vining II (CA-51)	0.147	0.145	0.112	0.141	0.154	0.153	0.188	0.132	0.141	0.146	0.163	0.147	0.174	0.136	0.216	0.102	0.114	0.141	0.126	0.100	0.107	0.101	0.114	0.095	0.103	0.119	0.094	0.084	0.161	0.135	0.120	0.116	0.102	0.108	0.113	0.104
Sisters (OR-50)	0.138	0.130	0.107	0.131	0.142	0.135	0.183	0.124	0.150	0.133	0.164	0.137	0.154	0.139	0.200	0.099	0.095	0.142	0.135	0.114	0.098	0.084	0.108	0.093	0.101	0.114	0.095	0.076	0.140	0.116	0.101	0.094	0.088	0.096	0.093	0.089
Mt. Shasta (CA-49)	0.129	0.121	0.102	0.122	0.129	0.131	0.177	0.118	0.135	0.134	0.145	0.129	0.154	0.120	0.191	0.089	0.092	0.133	0.123	0.107	0.092	0.081	0.095	0.087	0.082	0.107	0.087	0.080	0.144	0.121	0.096	0.086	0.088	0.089	0.092	0.086
Ft. Klamath (OR-47)	0.134	0.122	0.109	0.136	0.146	0.147	0.181	0.123	0.147	0.143	0.157	0.142	0.164	0.142	0.192	0.108	0.103	0.151	0.132	0.120	0.102	0.095	0.104	0.092	0.107	0.116	0.096	0.095	0.153	0.120	0.100	0.087	0.090	0.087	0.087	0.093
John Day (OR-38)	0.109	0.094	0.074	0.099	0.115	0.126	0.140	0.094	0.124	0.110	0.118	0.121	0.141	0.105	0.170	0.083	0.072	0.099	0.119	0.105	0.050	0.050	0.047	0.069	0.052	0.075	0.063	0.062	0.097	0.083	0.042	0.047	0.063	0.057	0.059	0.053
High Level (AB-18)	0.085	0.062	0.117	0.054	0.084	0.080	0.100	0.090	0.086	0.078	0.100	0.086	0.100	0.068	0.138	0.096	0.073	0.113	0.116	0.122	0.114	0.130	0.122	0.117	0.116	0.127	0.106	0.112	0.188	0.132	0.083	0.104	0.121	0.107	0.120	0.103
Whitemud/PR (AB-21)	0.125	0.128	0.143	0.101	0.128	0.119	0.126	0.118	0.124	0.118	0.137	0.130	0.111	0.108	0.158	0.116	0.088	0.097	0.156	0.114	0.136	0.128	0.162	0.122	0.162	0.104	0.109	0.109	0.168	0.145	0.105	0.119	0.119	0.116	0.134	0.109
WBNP (AB-22)	0.088	0.082	0.111	0.065	0.094	0.054	0.081	0.064	0.071	0.049	0.093	0.057	0.111	0.067	0.113	0.060	0.080	0.066	0.124	0.111	0.096	0.103	0.115	0.072	0.091	0.081	0.071	0.083	0.135	0.103	0.119	0.110	0.097	0.111	0.106	0.121

Appendix 1 cont'd...

	CO-39	WY-35	CO-36	CO-42	ID-37	ID-34	CA-48	CA-51	OR-50	CA-49	OR-47	OR-38	AB-18	AB-21	AB-22
Kenosha Pass (CO-39)	0.000														
Bondurant (WY-35)	0.042	0.000													
Grand Lake (CO-36)	0.034	0.027	0.000												
Monarch Pass (CO-42)	0.049	0.029	0.031	0.000											
Idaho City (ID-37)	0.053	0.050	0.038	0.049	0.000										
Ashton (ID-34)	0.044	0.037	0.031	0.040	0.029	0.000									
Lee Vining I (CA-48)	0.125	0.116	0.136	0.114	0.078	0.104	0.000								
Lee Vining II (CA-51)	0.116	0.120	0.129	0.106	0.079	0.104	0.008	0.000							
Sisters (OR-50)	0.114	0.108	0.108	0.085	0.076	0.092	0.046	0.042	0.000						
Mt. Shasta (CA-49)	0.101	0.099	0.107	0.085	0.073	0.083	0.019	0.024	0.020	0.000					
Ft. Klamath (OR-47)	0.097	0.086	0.108	0.095	0.082	0.091	0.042	0.041	0.033	0.019	0.000				
John Day (OR-38)	0.071	0.055	0.048	0.047	0.049	0.048	0.085	0.092	0.077	0.051	0.082	0.000			
High Level (AB-18)	0.121	0.127	0.092	0.084	0.107	0.113	0.163	0.160	0.155	0.145	0.157	0.102	0.000		
Whitemud/PR (AB-21)	0.141	0.122	0.117	0.111	0.091	0.135	0.165	0.170	0.145	0.148	0.166	0.116	0.136	0.000	
WBNP (AB-22)	0.077	0.118	0.091	0.113	0.085	0.082	0.137	0.127	0.134	0.123	0.131	0.114	0.098	0.170	0.000

Appendix 2. Matrix of pairwise Fst genetic distances (Excoffier et al., 1992) amongst 51 A. americanum populations.

	MB-7	MB-8	BC-25	MB-5	SK-16	MB-2	MB-4	MB-11	SK-14	SK-1	SK-3	SK-12	SK-13	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-17	BC-25	AB-29	AB-27	AB-24	AB-28	AB-26	BC-30	BC-31	BC-32	BC-33	ID-40	WY-44	UT-41	UT-45	CO-43	CO-46
Grand Rapids I (MB-7)	0.000																																			
Grand Rapids II (MB-8)	0.284	0.000																																		
Castlegar (BC-25)	0.472	0.403	0.000																																	
Devil's Lake (MB-5)	0.129	0.205	0.438	0.000																																
Tobin Lake (SK-16)	0.329	0.289	0.407	0.272	0.000																															
Belair (MB-2)	0.190	0.264	0.441	0.162	0.270	0.000																														
Cowan (MB-4)	0.265	0.166	0.438	0.200	0.263	0.228	0																													
The Pas (MB-11)	0.278	0.194	0.339	0.243	0.177	0.259	0.191	0.000																												
Smeaton (SK-14)	0.327	0.283	0.386	0.324	0.141	0.299	0.194	0.165	0.000																											
Beauval (SK-1)	0.243	0.247	0.423	0.200	0.245	0.184	0.181	0.193	0.178	0.000																										
Candle Lake (SK-3)	0.336	0.236	0.419	0.303	0.241	0.307	0.237	0.211	0.184	0.226	0.000																									
Prince Albert I (SK-12)	0.162	0.156	0.372	0.104	0.203	0.134	0.152	0.100	0.186	0.080	0.219	0.000																								
Prince Albert II (SK-13)	0.300	0.160	0.383	0.263	0.241	0.283	0.236	0.168	0.178	0.221	0.180	0.131	0.000																							
La Ronge (SK-10)	0.242	0.196	0.396	0.156	0.194	0.169	0.224	0.163	0.203	0.078	0.233	0.069	0.196	0.000																						
La Loche (SK-9)	0.371	0.284	0.422	0.336	0.296	0.337	0.172	0.186	0.205	0.190	0.204	0.142	0.153	0.217	0.000																					
Smoky Lake (AB-15)	0.255	0.242	0.355	0.243	0.233	0.187	0.177	0.197	0.216	0.142	0.266	0.103	0.216	0.148	0.219	0.000																				
Ft. McMurray (AB-6)	0.203	0.197	0.367	0.223	0.228	0.177	0.211	0.245	0.256	0.198	0.264	0.193	0.269	0.169	0.304	0.181	0.000																			
Slave Lake (AB-19)	0.275	0.280	0.313	0.238	0.262	0.252	0.223	0.233	0.204	0.234	0.281	0.181	0.242	0.214	0.265	0.206	0.211	0.000																		
Whitecourt (AB-20)	0.312	0.272	0.291	0.252	0.311	0.296	0.281	0.245	0.282	0.314	0.344	0.212	0.285	0.233	0.305	0.261	0.254	0.146	0.000																	
Grande Prairie (AB-17)	0.428	0.350	0.270	0.355	0.330	0.374	0.306	0.286	0.252	0.353	0.374	0.289	0.317	0.245	0.328	0.256	0.272	0.262	0.191	0.000																
100 m House (BC-23)	0.377	0.253	0.199	0.345	0.350	0.381	0.287	0.308	0.343	0.379	0.378	0.314	0.337	0.342	0.388	0.295	0.249	0.226	0.212	0.223	0.000															
Jasper (AB-29)	0.396	0.318	0.150	0.383	0.307	0.378	0.353	0.283	0.265	0.323	0.355	0.288	0.320	0.277	0.317	0.290	0.266	0.200	0.200	0.152	0.138	0.000														
DTR (AB-27)	0.403	0.290	0.209	0.366	0.299	0.415	0.317	0.272	0.309	0.400	0.375	0.293	0.314	0.340	0.372	0.285	0.280	0.258	0.188	0.171	0.011	0.100	0.000													
Banff (AB-24)	0.357	0.276	0.195	0.323	0.298	0.323	0.260	0.269	0.241	0.278	0.328	0.266	0.316	0.270	0.293	0.227	0.260	0.204	0.180	0.119	0.144	0.065	0.101	0.000												
Field (BC-28)	0.359	0.271	0.140	0.312	0.298	0.368	0.297	0.279	0.287	0.331	0.340	0.289	0.315	0.291	0.331	0.263	0.273	0.176	0.148	0.162	0.043	0.060	0.037	0.039	0.000											
Cypress Hills (AB-26)	0.327	0.228	0.202	0.284	0.270	0.325	0.226	0.248	0.256	0.280	0.277	0.262	0.266	0.306	0.306	0.263	0.232	0.173	0.222	0.255	0.148	0.181	0.200	0.146	0.166	0.000										
Kamloops (BC-30)	0.333	0.209	0.142	0.296	0.303	0.268	0.304	0.264	0.264	0.306	0.327	0.240	0.245	0.251	0.334	0.215	0.244	0.190	0.152	0.147	0.127	0.095	0.134	0.080	0.099	0.121	0.000									
Nimpo Lake (BC-31)	0.325	0.281	0.091	0.300	0.296	0.308	0.298	0.270	0.288	0.326	0.357	0.258	0.297	0.280	0.348	0.243	0.242	0.182	0.142	0.132	0.089	0.081	0.065	0.097	0.069	0.127	0.040	0.000								
Prince George (BC-32)	0.457	0.357	0.225	0.441	0.416	0.438	0.370	0.373	0.407	0.449	0.430	0.356	0.353	0.420	0.400	0.326	0.363	0.304	0.286	0.270	0.123	0.142	0.161	0.171	0.145	0.199	0.123	0.108	0.000							
Redstone (BC-33)	0.428	0.289	0.170	0.382	0.352	0.381	0.285	0.304	0.327	0.391	0.388	0.288	0.314	0.360	0.356	0.270	0.305	0.258	0.220	0.202	0.117	0.157	0.097	0.161	0.134	0.153	0.109	0.051	0.045	0.000						
Ketchum (ID-40)	0.359	0.271	0.241	0.346	0.346	0.380	0.342	0.329	0.351	0.381	0.376	0.337	0.331	0.342	0.404	0.318	0.254	0.262	0.248	0.245	0.104	0.172	0.140	0.182	0.119	0.184	0.119	0.112	0.203	0.200	0.000					
Yellowstone (WY-44)	0.374	0.248	0.219	0.347	0.320	0.365	0.332	0.292	0.303	0.367	0.364	0.291	0.316	0.318	0.343	0.287	0.284	0.279	0.226	0.221	0.153	0.155	0.087	0.158	0.143	0.189	0.129	0.112	0.214	0.168	0.104	0.000				
Manilla I (UT-41)	0.416	0.338	0.170	0.380	0.374	0.384	0.396	0.334	0.359	0.383	0.402	0.340	0.337	0.356	0.408	0.319	0.339	0.253	0.244	0.254	0.176	0.135	0.180	0.131	0.128	0.185	0.057	0.075	0.185	0.203	0.121	0.063	0.000			
Manilla II (UT-45)	0.359	0.263	0.179	0.323	0.303	0.316	0.320	0.285	0.319	0.354	0.345	0.285	0.285	0.301	0.352	0.245	0.281	0.252	0.228	0.238	0.154	0.166	0.129	0.145	0.145	0.178	0.079	0.093	0.160	0.168	0.097	0.049	0.025	0.000		
RFL I (CO-43)	0.443	0.296	0.199	0.413	0.369	0.427	0.405	0.326	0.372	0.429	0.410	0.362	0.350	0.383	0.411	0.362	0.342	0.320	0.264	0.288	0.185	0.146	0.134	0.184	0.149	0.213	0.106	0.121	0.191	0.171	0.107	0.054	0.051	0.060	0.000	
RFL II (CO-46)	0.360	0.297	0.148	0.341	0.319	0.340	0.342	0.283	0.282	0.339	0.352	0.300	0.316	0.315	0.375	0.282	0.292	0.229	0.213	0.223	0.157	0.102	0.142	0.102	0.101	0.145	0.035	0.067	0.174	0.168	0.097	0.047	-0.026	0.034	0.014	0.000

Appendix 2 cont'd...

	MB-7	MB-8	BC-25	MB-5	SK-16	MB-2	MB-4	MB-11	SK-14	SK-1	SK-3	SK-12	SK-13	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-17	BC-25	AB-29	AB-27	AB-24	AB-28	AB-26	BC-30	BC-31	BC-32	BC-33	ID-40	WY-44	UT-41	UT-45	CO-43	CO-46
Kenosha Pass (CO-39)	0.430	0.323	0.183	0.422	0.348	0.401	0.407	0.324	0.339	0.422	0.383	0.346	0.359	0.392	0.406	0.338	0.351	0.330	0.257	0.293	0.213	0.183	0.163	0.176	0.164	0.242	0.130	0.112	0.221	0.201	0.148	0.072	0.102	0.090	0.060	0.070
Bondurant (WY-35)	0.409	0.299	0.244	0.385	0.375	0.394	0.370	0.294	0.390	0.422	0.390	0.329	0.327	0.377	0.382	0.337	0.328	0.311	0.254	0.282	0.173	0.159	0.161	0.176	0.160	0.197	0.119	0.133	0.137	0.173	0.095	0.119	0.111	0.088	0.082	0.093
Grand Lake (CO-36)	0.408	0.280	0.247	0.360	0.362	0.364	0.376	0.308	0.355	0.365	0.389	0.306	0.327	0.339	0.386	0.286	0.329	0.273	0.276	0.268	0.200	0.160	0.204	0.143	0.149	0.216	0.112	0.122	0.204	0.205	0.106	0.095	0.081	0.103	0.084	0.077
Monarch Pass (CO-42)	0.424	0.338	0.271	0.382	0.386	0.437	0.417	0.321	0.408	0.420	0.416	0.360	0.350	0.358	0.438	0.356	0.340	0.345	0.307	0.305	0.195	0.202	0.190	0.251	0.172	0.274	0.178	0.176	0.261	0.263	0.112	0.147	0.161	0.140	0.113	0.147
Idaho City (ID-37)	0.303	0.280	0.199	0.303	0.313	0.319	0.291	0.277	0.309	0.305	0.354	0.270	0.312	0.298	0.344	0.247	0.250	0.173	0.170	0.153	0.132	0.111	0.096	0.079	0.088	0.130	0.104	0.039	0.151	0.161	0.076	0.126	0.084	0.113	0.137	0.069
Ashton (ID-34)	0.376	0.351	0.192	0.376	0.321	0.353	0.364	0.282	0.332	0.350	0.404	0.316	0.360	0.348	0.406	0.276	0.304	0.283	0.269	0.254	0.182	0.123	0.150	0.134	0.129	0.223	0.133	0.093	0.176	0.187	0.109	0.130	0.096	0.085	0.120	0.054
Lee Vining I (CA-48)	0.480	0.445	0.418	0.464	0.471	0.481	0.478	0.430	0.464	0.510	0.486	0.436	0.442	0.455	0.498	0.430	0.416	0.424	0.361	0.356	0.368	0.374	0.332	0.406	0.355	0.420	0.352	0.335	0.433	0.412	0.355	0.362	0.394	0.370	0.402	0.377
Lee Vining II (CA-51)	0.491	0.420	0.390	0.470	0.462	0.483	0.475	0.414	0.450	0.493	0.468	0.431	0.429	0.452	0.487	0.422	0.427	0.414	0.359	0.345	0.358	0.354	0.322	0.385	0.334	0.400	0.325	0.312	0.414	0.394	0.357	0.363	0.375	0.374	0.380	0.367
Sisters (OR-50)	0.474	0.404	0.404	0.461	0.460	0.455	0.460	0.393	0.464	0.482	0.476	0.415	0.394	0.441	0.461	0.417	0.391	0.410	0.375	0.356	0.336	0.327	0.297	0.368	0.329	0.392	0.319	0.286	0.364	0.348	0.319	0.324	0.358	0.348	0.344	0.356
Mt. Shasta (CA-49)	0.434	0.357	0.355	0.414	0.400	0.430	0.435	0.355	0.419	0.454	0.422	0.373	0.364	0.396	0.431	0.379	0.371	0.374	0.335	0.317	0.307	0.312	0.274	0.346	0.273	0.357	0.282	0.276	0.364	0.351	0.276	0.279	0.328	0.317	0.321	0.319
Ft. Klamath (OR-47)	0.480	0.395	0.411	0.475	0.456	0.482	0.456	0.375	0.455	0.497	0.468	0.423	0.417	0.451	0.472	0.436	0.399	0.432	0.384	0.381	0.333	0.344	0.299	0.370	0.330	0.388	0.331	0.313	0.410	0.387	0.311	0.300	0.360	0.339	0.347	0.346
John Day (OR-38)	0.420	0.314	0.312	0.395	0.356	0.421	0.388	0.326	0.393	0.420	0.401	0.363	0.355	0.379	0.425	0.359	0.329	0.322	0.347	0.308	0.207	0.232	0.191	0.270	0.216	0.264	0.204	0.203	0.295	0.295	0.167	0.197	0.219	0.216	0.232	0.211
High Level (AB-18)	0.429	0.368	0.500	0.328	0.415	0.430	0.430	0.391	0.442	0.413	0.441	0.359	0.392	0.328	0.532	0.439	0.373	0.446	0.469	0.501	0.424	0.473	0.506	0.454	0.402	0.452	0.388	0.412	0.574	0.521	0.383	0.424	0.485	0.405	0.465	0.420
Whitemud/PR (AB-21)	0.459	0.426	0.486	0.409	0.463	0.420	0.407	0.387	0.449	0.427	0.500	0.389	0.402	0.371	0.499	0.393	0.343	0.357	0.451	0.403	0.399	0.413	0.450	0.410	0.429	0.364	0.339	0.339	0.490	0.452	0.370	0.392	0.424	0.370	0.444	0.384
WBNP (AB-22)	0.419	0.396	0.458	0.419	0.398	0.327	0.398	0.277	0.358	0.347	0.438	0.309	0.390	0.353	0.448	0.376	0.379	0.377	0.405	0.444	0.414	0.403	0.463	0.367	0.374	0.390	0.337	0.384	0.508	0.460	0.446	0.439	0.454	0.419	0.446	0.384

Appendix 2 cont'd...

	CO-39	WY-35	CO-30	CO-42	ID-37	ID-34	CA-48	CA-51	OR-50	CA-49	OR-47	OR-38	AB-18	AB-21	AB-22
Kenosha Pass (CO-39)	0.000														
Bondurant (WY-35)	0.130	0.000													
Grand Lake (CO-36)	0.079	0.078	0.000												
Monarch Pass (CO-42)	0.172	0.098	0.116	0.000											
Idaho City (ID-37)	0.154	0.130	0.091	0.179	0.000										
Ashton (ID-34)	0.129	0.084	0.108	0.158	0.075	0.000									
Lee Vining I (CA-48)	0.388	0.398	0.408	0.418	0.317	0.362	0.000								
Lee Vining II (CA-51)	0.361	0.399	0.391	0.408	0.308	0.358	0.006	0.000							
Sisters (OR-50)	0.365	0.358	0.354	0.355	0.281	0.344	0.224	0.198	0.000						
Mt. Shasta (CA-49)	0.321	0.332	0.323	0.325	0.261	0.300	0.100	0.109	0.103	0.000					
Ft. Klamath (OR-47)	0.331	0.342	0.371	0.371	0.288	0.340	0.214	0.208	0.120	0.081	0.000				
John Day (OR-38)	0.244	0.200	0.198	0.223	0.193	0.214	0.313	0.308	0.279	0.215	0.277	0.000			
High Level (AB-18)	0.467	0.503	0.465	0.459	0.420	0.473	0.516	0.504	0.510	0.455	0.509	0.432	0.000		
Whitemud/PR (AB-21)	0.475	0.445	0.424	0.427	0.336	0.420	0.485	0.489	0.449	0.414	0.478	0.385	0.548	0.000	
WBNP (AB-22)	0.441	0.458	0.414	0.496	0.381	0.382	0.477	0.464	0.464	0.418	0.455	0.432	0.499	0.562	0.000

Appendix 3. Matrix of Fst Genetic Distances (Excoffier et al., 1992) between 29 *Arceuthobium americanum* populations.

	MB-7	BC-25	MB-2	MB-4	MB-11	SK-14	SK-3	SK-12	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-29	AB-27	AB-24	AB-26	ID-40	WY-44	UT-41	CO-43	CA-48	CA-49	OR-47	OR-38	AB-18	AB-21	AB-22
Grand Rapids I (MB-7)	0.000																												
Castlegar (BC-25)	0.472	0.000																											
Belair (MB-2)	0.190	0.441	0.000																										
Cowan (MB-4)	0.265	0.438	0.228	0.000																									
The Pas (MB-11)	0.278	0.339	0.259	0.191	0.000																								
Smeaton (SK-14)	0.327	0.386	0.299	0.194	0.165	0.000																							
Candle Lake (SK-3)	0.336	0.419	0.307	0.237	0.211	0.184	0.000																						
Prince Albert I (SK-12)	0.162	0.372	0.134	0.152	0.100	0.186	0.219	0.000																					
La Ronge (SK-10)	0.242	0.396	0.169	0.224	0.163	0.203	0.233	0.069	0.000																				
La Loche (SK-9)	0.371	0.422	0.337	0.172	0.186	0.205	0.204	0.142	0.217	0.000																			
Smoky Lake (AB-15)	0.255	0.355	0.187	0.177	0.197	0.216	0.266	0.103	0.148	0.219	0.000																		
Ft. McMurray (AB-6)	0.203	0.367	0.177	0.211	0.245	0.256	0.264	0.193	0.169	0.304	0.181	0.000																	
Slave Lake (AB-19)	0.275	0.313	0.252	0.223	0.233	0.204	0.281	0.181	0.214	0.265	0.206	0.211	0.000																
Whitecourt (AB-20)	0.312	0.291	0.296	0.281	0.245	0.282	0.344	0.212	0.233	0.305	0.261	0.254	0.146	0.000															
Jasper (AB-29)	0.396	0.150	0.378	0.353	0.283	0.265	0.355	0.288	0.277	0.317	0.290	0.266	0.200	0.200	0.000														
DTR (AB-27)	0.403	0.209	0.415	0.317	0.272	0.309	0.375	0.293	0.340	0.372	0.285	0.280	0.258	0.188	0.100	0.000													
Banff (AB-24)	0.357	0.195	0.323	0.260	0.269	0.241	0.328	0.266	0.270	0.293	0.227	0.260	0.204	0.180	0.065	0.101	0.000												
Cypress Hills (AB-26)	0.327	0.202	0.325	0.226	0.248	0.256	0.277	0.262	0.306	0.306	0.263	0.232	0.173	0.222	0.181	0.200	0.146	0.000											
Ketchum (ID-40)	0.359	0.241	0.380	0.342	0.329	0.351	0.376	0.337	0.342	0.404	0.318	0.254	0.262	0.248	0.172	0.140	0.182	0.184	0.000										
Yellowstone (WY-44)	0.374	0.219	0.365	0.332	0.292	0.303	0.364	0.291	0.318	0.343	0.287	0.284	0.279	0.226	0.155	0.087	0.158	0.189	0.104	0.000									
Manilla I (UT-41)	0.416	0.170	0.384	0.396	0.334	0.359	0.402	0.340	0.356	0.408	0.319	0.339	0.253	0.244	0.135	0.180	0.131	0.185	0.121	0.063	0.000								
RFL I (CO-43)	0.443	0.199	0.427	0.405	0.326	0.372	0.410	0.362	0.383	0.411	0.362	0.342	0.320	0.264	0.146	0.134	0.184	0.213	0.107	0.054	0.051	0.000							
Lee Vining I (CA-48)	0.480	0.418	0.481	0.478	0.430	0.464	0.486	0.436	0.455	0.498	0.430	0.416	0.424	0.361	0.374	0.332	0.406	0.420	0.355	0.362	0.394	0.402	0.000						
Mt. Shasta (CA-49)	0.434	0.355	0.430	0.435	0.355	0.419	0.422	0.373	0.396	0.532	0.379	0.371	0.374	0.335	0.312	0.274	0.346	0.357	0.276	0.279	0.328	0.321	0.100	0.000					
Ft. Klamath (OR-47)	0.480	0.411	0.482	0.456	0.375	0.455	0.468	0.423	0.451	0.472	0.436	0.399	0.432	0.384	0.344	0.299	0.370	0.388	0.311	0.300	0.360	0.347	0.214	0.081	0.000				
John Day (OR-38)	0.420	0.312	0.421	0.388	0.326	0.393	0.401	0.363	0.379	0.425	0.359	0.329	0.322	0.347	0.232	0.191	0.270	0.264	0.167	0.197	0.219	0.232	0.313	0.215	0.277	0.000			
High Level (AB-18)	0.429	0.500	0.430	0.430	0.391	0.442	0.441	0.359	0.328	0.532	0.439	0.373	0.446	0.469	0.473	0.506	0.454	0.452	0.383	0.424	0.485	0.465	0.516	0.455	0.509	0.432	0.000		
Whitemud/PR (AB-21)	0.459	0.486	0.420	0.407	0.387	0.449	0.500	0.380	0.371	0.499	0.393	0.343	0.357	0.451	0.413	0.450	0.410	0.364	0.370	0.392	0.424	0.444	0.485	0.414	0.478	0.385	0.548	0.000	
WBNP (AB-22)	0.419	0.458	0.327	0.398	0.277	0.358	0.438	0.309	0.353	0.448	0.376	0.379	0.377	0.405	0.403	0.463	0.367	0.390	0.446	0.439	0.454	0.446	0.477	0.418	0.455	0.432	0.499	0.562	0.000

Appendix 4. Matrix of Fst Genetic Distances (Excoffier et al., 1992) between 29 *Pinus* spp. populations.

	MB-7	BC-25	MB-2	MB-4	MB-11	SK-14	SK-3	SK-12	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-29	AB-27	AB-24	AB-26	ID-40	WY-44	UT-41	CO-43	CA-48	CA-49	OR-47	OR-38	AB-18	AB-21	AB-22
Grand Rapids I (MB-7)	0.000																												
Castlegar (BC-25)	0.499	0.000																											
Belair (MB-2)	-0.041	0.483	0.000																										
Cowan (MB-4)	-0.014	0.410	-0.015	0.000																									
The Pas (MB-11)	0.047	0.442	0.053	0.022	0.000																								
Smeaton (SK-14)	0.086	0.452	0.065	0.041	0.000	0.000																							
Candle Lake (SK-3)	0.109	0.432	0.106	0.097	0.032	0.005	0.000																						
Prince Albert I (SK-12)	0.041	0.452	0.060	0.032	0.034	0.010	0.013	0.000																					
La Ronge (SK-10)	0.035	0.460	0.061	0.050	0.051	0.009	0.021	-0.006	0.000																				
La Loche (SK-9)	0.048	0.471	-0.007	0.022	-0.013	0.000	0.065	0.034	0.071	0.000																			
Smoky Lake (AB-15)	0.056	0.462	0.047	0.017	0.057	-0.006	0.051	0.026	0.027	0.008	0.000																		
Ft. McMurray (AB-6)	0.090	0.518	0.085	0.051	0.050	0.030	0.101	0.004	0.049	0.046	0.004	0.000																	
Slave Lake (AB-19)	0.093	0.389	0.081	0.049	0.009	0.029	0.051	0.074	0.063	0.020	0.026	0.073	0.000																
Whitecourt (AB-20)	0.125	0.310	0.132	0.044	0.072	0.049	0.098	0.043	0.070	0.092	0.061	0.070	0.029	0.000															
Jasper (AB-29)	0.456	0.052	0.442	0.355	0.411	0.414	0.404	0.409	0.425	0.420	0.406	0.467	0.347	0.262	0.000														
DTR (AB-27)	0.558	0.041	0.548	0.459	0.511	0.508	0.491	0.500	0.521	0.521	0.506	0.561	0.454	0.355	0.015	0.000													
Banff (AB-24)	0.499	0.042	0.485	0.407	0.435	0.436	0.420	0.434	0.470	0.448	0.431	0.504	0.352	0.276	0.011	0.027	0.000												
Cypress Hills (AB-26)	0.522	0.092	0.505	0.421	0.469	0.468	0.458	0.468	0.478	0.480	0.460	0.522	0.396	0.305	0.055	0.054	0.076	0.000											
Ketchum (ID-40)	0.590	0.156	0.573	0.497	0.532	0.539	0.520	0.544	0.551	0.529	0.533	0.598	0.454	0.394	0.080	0.151	0.079	0.163	0.000										
Yellowstone (WY-44)	0.497	0.067	0.465	0.388	0.441	0.436	0.424	0.441	0.460	0.435	0.441	0.501	0.377	0.285	0.018	0.081	0.056	0.067	0.074	0.000									
Manilla I (UT-41)	0.507	0.070	0.500	0.409	0.467	0.471	0.446	0.450	0.459	0.480	0.461	0.529	0.396	0.304	0.057	0.059	0.083	0.058	0.143	0.066	0.000								
RFL I (CO-43)	0.532	0.074	0.504	0.504	0.467	0.461	0.454	0.473	0.485	0.468	0.463	0.538	0.417	0.327	0.010	0.066	0.093	0.127	0.091	0.007	0.078	0.000							
Lee Vining I (CA-48)	0.567	0.127	0.547	0.465	0.512	0.497	0.486	0.514	0.505	0.521	0.499	0.579	0.441	0.360	0.126	0.176	0.140	0.105	0.119	0.119	0.093	0.131	0.000						
Mt. Shasta (CA-49)	0.518	0.143	0.511	0.441	0.483	0.497	0.471	0.478	0.495	0.494	0.486	0.555	0.440	0.375	0.150	0.111	0.145	0.133	0.188	0.139	0.026	0.173	0.153	0.000					
Ft. Klamath (OR-47)	0.528	0.133	0.520	0.436	0.484	0.494	0.469	0.480	0.496	0.487	0.486	0.544	0.423	0.343	0.072	0.084	0.081	0.095	0.031	0.061	0.044	0.071	0.122	0.049	0.000				
John Day (OR-38)	0.514	0.202	0.494	0.429	0.438	0.462	0.456	0.472	0.496	0.445	0.478	0.544	0.405	0.330	0.159	0.198	0.132	0.192	0.150	0.135	0.197	0.168	0.232	0.161	0.112	0.000			
High Level (AB-18)	0.147	0.213	0.157	0.098	0.107	0.105	0.093	0.132	0.090	0.129	0.478	0.208	0.078	0.050	0.212	0.286	0.222	0.240	0.334	0.208	0.209	0.262	0.283	0.235	0.265	0.259	0.000		
Whitemud/PR (AB-21)	0.137	0.369	0.120	0.092	0.031	0.077	0.120	0.115	0.121	0.027	0.106	0.169	0.035	0.071	0.325	0.425	0.336	0.376	0.412	0.346	0.385	0.378	0.407	0.390	0.378	0.340	0.049	0.000	
WBNP (AB-22)	0.174	0.366	0.203	0.154	0.117	0.137	0.136	0.132	0.135	0.155	0.177	0.262	0.138	0.106	0.322	0.426	0.358	0.404	0.461	0.371	0.382	0.377	0.437	0.391	0.406	0.381	0.040	0.051	0.000

Appendix 5. Matrix of Geographic Distances (km) between 29 populations of both *Arceuthobium americanum* and *Pinus* spp.

	MB-7	BC-25	MB-2	MB-4	MB-11	SK-14	SK-3	SK-12	SK-10	SK-9	AB-15	AB-6	AB-19	AB-20	AB-29	AB-27	AB-24	AB-26	ID-40	WY-44	UT-41	CO-43	CA-48	CA-49	OR-47	OR-38	AB-18	AB-21	AB-22
Grand Rapids I (MB-7)	0.0																												
Castlegar (BC-25)	1349.7	0.0																											
Belair (MB-2)	335.7	1517.8	0.0																										
Cowan (MB-4)	151.1	1237.0	329.3	0.0																									
The Pas (MB-11)	142.2	1222.6	453.3	157.5	0.0																								
Smeaton (SK-14)	362.5	1028.0	651.5	324.1	220.4	0.0																							
Candle Lake (SK-3)	400.5	996.7	687.8	359.5	258.3	38.0	0.0																						
Prince Albert I (SK-12)	453.6	919.8	715.2	386.8	314.5	108.9	83.6	0.0																					
La Ronge (SK-10)	458.3	1020.6	769.4	447.9	320.3	134.1	113.0	171.4	0.0																				
La Loche (SK-9)	754.5	969.4	1074.4	754.6	622.2	435.8	405.8	423.0	306.7	0.0																			
Smoky Lake (AB-15)	866.6	649.5	1136.5	808.9	724.4	504.1	473.9	422.2	434.8	320.5	0.0																		
Ft. McMurray (AB-6)	903.2	953.1	1222.8	901.7	770.8	580.6	548.8	556.9	454.1	148.8	335.1	0.0																	
Slave Lake (AB-19)	999.1	687.9	1285.3	956.0	857.3	639.6	602.0	571.0	547.4	333.4	168.3	270.5	0.0																
Whitecourt (AB-20)	1084.0	553.4	1350.3	1024.0	941.9	721.5	683.6	638.1	647.6	470.1	217.5	413.5	142.9	0.0															
Jasper (AB-29)	1261.5	396.3	1504.1	1185.4	1120.9	902.3	865.1	808.4	844.4	686.9	410.3	625.1	358.3	216.8	0.0														
DTR (AB-27)	1168.3	331.0	1395.5	1082.4	1029.8	815.2	779.1	715.4	773.0	661.0	353.2	627.8	359.1	222.3	131.6	0.0													
Banff (AB-24)	1135.8	254.3	1337.7	1036.5	1002.2	796.9	763.3	691.1	776.3	711.5	395.5	706.9	449.2	325.2	255.9	130.8	0.0												
Cypress Hills (AB-26)	853.6	546.3	979.1	722.7	745.9	599.2	580.4	497.4	653.3	327.8	523.8	841.6	675.2	619.2	664.9	533.6	425.6	0.0											
Ketchum (ID-40)	1527.4	645.5	1557.3	1381.5	1140.9	1317.0	1300.0	1217.4	1372.7	1149.1	1148.6	1483.3	1249.4	1139.8	1030.0	993.8	815.2	727.7	0.0										
Yellowstone (WY-44)	1204.8	753.1	1194.2	1054.6	1138.4	1055.4	1047.1	970.0	1138.7	1286.7	1031.5	1356.6	1170.0	1096.0	1058.5	935.4	805.9	526.8	382.2	0.0									
Manilla I (UT-41)	1572.2	1132.1	1484.7	1421.9	1529.8	1479.7	1476.2	1403.5	1574.4	1738.9	1485.5	1811.0	1621.3	1540.5	1481.0	1365.2	1236.4	980.6	534.4	454.5	0.0								
RFL I (CO-43)	1449.9	1335.3	1297.0	1306.6	1438.1	1437.2	1442.5	1381.5	1550.7	1764.9	1557.3	1860.2	1710.7	1655.0	1637.8	1511.7	1383.0	1046.2	818.6	584.5	337.7	0.0							
Lee Vining I (CA-48)	2279.7	1270.7	2275.5	2131.4	2199.4	2078.9	2061.5	1978.7	2131.8	2185.2	1872.3	2200.6	1947.0	1819.9	1662.2	1599.0	1501.6	1488.3	761.9	1084.3	882.0	1207.9	0.0						
Mt. Shasta (CA-49)	2144.1	937.0	2210.2	2005.4	2041.0	1883.6	1859.3	1776.4	1909.1	1901.8	1581.3	1890.1	1623.5	1485.8	1300.9	1264.6	1191.4	1299.4	667.2	1049.3	1050.7	1385.6	470.4	0.0					
Ft. Klamath (OR-47)	2042.5	804.7	2124.8	1906.9	1934.9	1770.6	1744.9	1662.6	1790.3	1772.4	1451.9	1756.8	1489.2	1350.5	1163.2	1130.1	1061.0	1193.2	611.5	990.3	1051.7	1379.2	588.1	139.7	0.0				
John Day (OR-38)	1702.0	535.1	1783.4	1565.4	1634.9	1438.6	1450.5	1331.6	1466.4	1475.5	1156.7	1477.0	1217.5	1086.8	926.3	865.0	774.2	854.9	322.6	674.8	840.8	1139.4	736.0	445.1	342.2	0.0			
High Level (AB-18)	1230.2	1005.0	1549.8	1227.4	1098.2	904.7	871.5	870.6	780.6	476.1	542.4	327.3	386.8	472.4	614.4	682.1	796.4	1050.1	1611.6	1555.5	2005.2	2096.9	2275.0	1912.2	1773.6	1539.0	0.0		
Whitemud/PR (AB-21)	1215.2	815.8	1517.4	1188.8	1076.2	866.0	829.7	810.7	757.8	478.9	424.2	345.7	255.9	299.9	423.7	497.2	616.4	907.7	1430.3	1395.4	1840.0	1950.3	2085.1	1721.4	1582.9	1349.1	191.0	0.0	
WBNP (AB-22)	1057.2	1141.7	1388.5	1080.6	936.7	771.9	744.9	767.2	638.7	344.4	559.9	232.5	457.6	588.5	778.5	810.8	903.1	1063.0	1701.4	1587.6	2042.1	2092.3	2404.6	2073.1	1937.0	1674.0	254.9	394.6	0.0