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COEVOLUTIONARY GENETICS OF *PINUS CONTORTA - PINUS BANKSIANA* COMPLEX AND *ENDOCRONARTIUM HARKNESSII*

BY

TERRANCE ZHIHONG YE

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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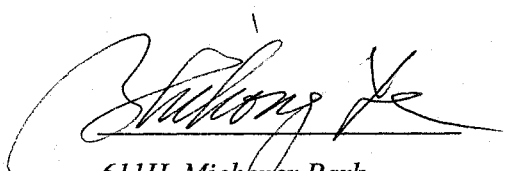

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **COEVOLUTIONARY GENETICS OF *PINUS CONTORTA* - *PINUS BANKSIANA* COMPLEX AND *ENDOCRONARTIUM HARKNESSII*** submitted by **TERRANCE ZHIHONG YE** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in Forest Biology and Management.

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ABSTRACT

This thesis examined the coevolutionary genetics of the lodgepole pine *Pinus contorta*) - jack pine (*P. banksiana*) complex in west central Alberta and western gall rust (WGR) fungus (*Endocronartium harknessii*). It consisted of three studies. First, a greenhouse inoculation study of 23 lodgepole pine, 9 jack pine, and 8 putative hybrid populations using two WGR sources, one each from lodgepole pine and jack pine hosts, was carried out to investigate if hybrids differ from the parental species in WGR susceptibility and if there is host-pathogen interaction. Lodgepole pine and hybrids were significantly more susceptible to WGR infection than jack pine. Both host species were more susceptible to their own rust spore sources, causing significant spore source × host group interactions.

In the second study, the random amplified polymorphic DNA (RAPD) variability was assessed to elucidate single-locus and multilocus structure of hosts using the same populations as in the greenhouse study, and to determine if the level of gene exchange in the hybrid zone is related to WGR susceptibility. Averaged estimates of RAPD diversity were 0.143 for lodgepole pine, 0.156 for jack pine, and 0.152 for hybrids. Based on RAPD variation, lodgepole pine could be separated from jack pine, but hybrids had a closer genetic affinity with lodgepole pine. Significant multilocus associations were found in 29 of 40 populations. Such associations were due largely to Wahlund effect that accounted for 40.7% of multilocus heterozygosity in hybrids, but only 18.6% in lodgepole

pine and 16.0% in jack pine.

The level of introgression, as measured by genetic admixtures of parental species, was significantly correlated with WGR susceptibility. Thus, WGR prevalence in this hybrid area would be closely linked to the introgression.

The third study was designed to develop a new complementary model of host - pathogen interaction that allowed for arbitrary levels of dominance in a diploid host. Computer simulations based on this model allowed for examining effects of overdominance, incomplete dominance and underdominance on equilibrium frequencies of resistance gene in a host population and on stability of equilibria.

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Introduction and Literature Review

1 Introduction to Lodgepole and jack Pine

Lodgepole pine (*P. contorta* Dougl. ex Loud.) and jack pine (P. *banksiana* Lamb.) are widely distributed and economically important pine species in Canada (Yeatman 1967; Critchfield 1985). They are closely related species with extensive hybridization between them where their ranges overlap (Moss, 1949; Critchfield 1985). Lodgepole pine is a primary species for intensive silviculture and tree improvement in western Canada because of its desirable silvicultural traits, including fast-growing, quality wood, relatively short rotation, and the ability to grow under diverse site conditions. It is the number one species both in harvesting and planting in British Columbia (B.C. Ministry of Forests 1992) and the second most important commercial forest species after white spruce (*Picea glauca*) in Alberta (Dhir and Barnhardt 1993). Jack pine is an important source of pulpwood, lumber, and round timber (Benzie 1977; Cayford and Bickerstaff 1968; Cayford et al. 1967), growing farther north than any other American pine and is the most widely distributed pine species in Canada. It is a pioneer species in succession and invades areas where mineral soil has been exposed by major disturbances such as fires.

1.1 Geographic distribution of species

Lodgepole pine is widespread in western North America. Its distribution, centered in British Columbia, spans about 33° of latitude from Yukon down to Southern California, 35° of longitude from the Pacific coast to the center of Colorado and 3900 m of elevation (Wheeler and Critchfield 1985). It has generally been recognized to be comprised of three subspecies, ssp. *contorta*, ssp. *latifolia*, ssp. *murrayana* and one edaphic ecotype, ssp. *bolanderi* (Wheeler and Critchfield 1985). Subspecies *latifolia* is the most abundant, ranging along the Rocky Mountain region, from the Yukon to Colorado and eastern Oregon. This subspecies is commonly known as lodgepole pine.

The major portion of the jack pine range is in Canada where its northern boundary extends eastward from the Mackenzie River in the Northwest Territories across the country to the Cape Breton Island, NS. The range then extends southwest through Maine, New Hampshire, northern New York, central Quebec and northern Ontario, Michigan, extreme northwest Indiana, northeast Illinois, then northwest through Wisconsin, Minnesota, Manitoba, Saskatchewan, central Alberta, to the extreme northeast British Columbia. Within its range, jack pine is widely but not continuously distributed. In Canada, it is most abundant in Ontario, and in the United States, the largest acreages are in Minnesota, Wisconsin, and Michigan. The only significant artificial extensions of the jack pine range have been on strip-mined areas in the central and northeastern States and on the sand hills of Nebraska (Rudolph and Laidly 1990).

1.2 Biogeography of species

Lodgepole pine and jack pine probably evolved from a common progenitor and became differentiated following cooling of the climate and crustal uplift in western North America in the late Tertiary (Yeatman 1967). The divergence and speciation of jack pine and lodgepole pine probably resulted from allopatric processes due to the range disruption as a consequence of continental glaciation (Dancik and Yeh 1983; Critchfield 1985).

Hypotheses about the origins and migration routes of lodgepole pine following glaciation are of significant interest. Studies of fossil pollen showed that lodgepole pine was probably present in mountain valleys in the western United States by 12200 BP (MacDonald and Cwynar 1985). It migrated northwards along the eastern slop of the Rocky Mountains, reaching central eastern British Columbia by 8000 BP. Lodgepole pine reached the Yukon border region by 5000 BP and extended to its present northern limits in the Yukon in the last thousand years (MacDonald and Cwynar 1985, 1991). There is no pollen evidence of a refugial population existing in the unglaciated portions of the Yukon during the last glacial maximum and the lodgepole pines in Canada probably originated from glacial refugia in the Rocky Mountains (MacDonald and Cwynar 1985). However, the fossil record conflicts with the evidence from extant lodgepole pine in the Yukon (Critchfield 1985) and genetic evidence reported by Wheeler and Guries (1982). The genetic distinctness of northern populations supports the inference that pine was present in the Yukon during the last glacial period. Wheeler and

Critchfield (1985) indicated that if lodgepole pine had persisted throughout the Wisconsin period, it probably did so in very small isolated patches that would have been hard to capture through pollen core sampling. Lodgepole pine surviving in northern refugia probably recolonized most of the Yukon and extended south in British Columbia where it was met by pine moving north, probably within a few hundred kilometers of the border (Wheeler and Critchfield 1982).

Jack pine probably migrated into the northern interior from the southeast and entered from near the Great Lakes at about 10,000 BP (MacDonald et al. 1998). The subsequent northwestward spread of jack pine has been reconstructed from a series of pollen records from western Canada (MacDonald 1987; McLeod 1991). The pollen evidence suggests a progressive northward movement similar to that of lodgepole pine. By 8500 BP, jack pine was in central Saskatchewan and Alberta. It reached northern Alberta by 8000 BP and the central Northwest Territories by 4500 BP. It probably did not reach its extreme northern limits in the Mackenzie River valley until the last 2000 years (MacDonald et al. 1998).

1.3 Natural hybridization between lodgepole and jack pine

1.3.1 Pinus contorta – Pinus banksiana complex (PCBC)

Putative natural hybrids between lodgepole pine and jack pine were first reported by A. C. Holman after observations in northern Alberta (Austin 1929), and documented by Critchfield and others (e.g., Critchfield 1980, 1984, 1985; Wheeler and Guries 1987). Across much of central and northern Alberta and the

southwest corner of the Northwest Territories, lodgepole pine and jack pine occur sympatrically and hybridize, giving rise to populations of individuals intermediate for a number of morphological and biochemical traits (Wheeler and Guries 1987). Stands in the Alberta overlap region range from nearly pure lodgepole pine through hybrid swarms to nearly pure jack pine (Moss 1949; Mirov 1956; Schoenike 1962; Wheeler 1981; Pollack and Dancik 1985), suggesting varying levels of introgression and adaptation to wide ecological amplitudes across the parental species' ranges. Differences in cone morphology provide the strongest diagnostic features for distinguishing the hybrids in the field (Rudolph and Laidly 1990), which has been confirmed through comparing with artificial hybrids from the crosses between the two species (Zavarin et al. 1969). The mature cone of lodgepole pine is spreading or reflexed, ovoid or conical in shape, the scales conspicuously umbonate, each scale armed with a minute recurved prickle. The mature cone of jack pine is erect (directed toward the apex of the shoot), strong incurved or slightly spreading, the scales variously thickened and unarmed. The hybrids are somewhat morphological intermediate (Moss 1949). Natural stands in this hybrid zone and its neighboring areas are referred to as *Pinus contorta - banksiana* complex (PCBC).

1.3.2 Pattern and extent of introgressive hybridization

The extent of introgressive hybridization is determined by many factors such as differing ecological preferences (edaphic preference in particular), internal reproductive barriers (only 31% of lodgepole-jack pines crossability) and differing

flowering times (Critchfield 1985). In the overlap region, edaphic preference is an important determinant of distributions of the parental species and their hybrids, with jack pine being the more xerophytic, lodgepole pine the more mesophytic and tolerant of clay soils and bogs, and the hybrids occupying a wide range of intermediate sites (Yeatman 1967). Outside the overlap region, some investigations concur with earlier observations that introgression from jack pine has taken place, but there is little agreement concerning the amount or geographic distribution of jack pine influence (Critchfield 1985). Introgression at considerable distances from the overlap region has been reported for cone orientation (Critchfield 1957), cortex resin composition (Forrest 1980, 1981), leaf oil terpenes (von Rudloff and Nyland 1979; von Rudloff and Lapp 1987), isozymes (Wheeler 1981; Wheeler and Guries 1987; Dancik and Yeh 1983), and disease and insect resistance (Wu et al. 1996).

Hybridization between lodgepole pine and jack pine results in relatively unfit individuals in the earliest generations. Thus, Critchfield (1984) has related the narrowness of the zone of present-day hybridization to the fact that crosses between these species result in "... a high incidence of reproductive breakdown ..." and hybrids that demonstrate "... a moderately high percentage of aborted pollen." Crosses to form the F1 generation are unsuccessful 69% of the time relative to control cross (Critchfield 1980). In addition, F1 individuals possess 0-50% aborted pollen and F2 and F3 individuals demonstrate 0-42% pollen abortion (Critchfield 1980). Although the strength of the barrier that

impedes the formation of initial hybrid generation is consistent with the relative narrowness of contemporary hybrid zones, it is not predictive of the distribution of genetic and morphological variation seen in these two species. For example, introgression from jack pine into lodgepole pine is limited near the hybrid zone. There are, however, populations of lodgepole pine separated from the present-day hybrid zone by long distances that contain introgressed genes from jack pine (Critchfield 1984). Critchfield (1985) hypothesized that these latter populations have resulted from ancient contact and hybridization, and introgression from jack pine into lodgepole pine is "... the result of genetic contacts long before the last glacial period."

2 Introgressive Hybridization in Hybrid Zones

2.1 General concepts of introgression

Introgression is the incorporation (through hybridization and back crossing) of an allele from one species into the gene pool of a second species (Anderson and Hubricht 1938; Harrison 1990). Although traditionally defined in the context of movement of "foreign" alleles along a defined geographic transect, the term introgression can also be applied within a single mixed/hybrid population, when limited hybridization results in leakage of alleles between sympatric "species" but not fusion of these parental types. Botanists have distinguished these two situations by describing localized (sympatric) and dispersaled (allopatric)

introgression (Heiser 1973; Rieseberg and Wendel 1993; Arnold and Bennett 1993).

Although some researchers suggested that introgression may be of minor evolutionary significant, having only localized and transient effects (Randolph et al. 1967; Wagner 1970; Heiser 1973; Hardin 1975), many studies have demonstrated that introgression is of evolutionary significance because it extends a species' gene pool, releases novel gene combinations to be sifted by natural selection and serves as a means of gene dispersal (Wiegand 1935; Anderson 1949; Anderson and Stebbins 1954; Stebbins 1959; Baker 1951; Lewontin and Birch 1966; Watt 1972; Potts and Reid 1988).

2.2 Methodology

2.2.1 Identification of hybrids using molecular markers

Studies of the ecological and evolutionary consequences of hybridization and introgression have long been hampered by difficulty in identifying the hybrids. Accurate identification of hybrids is crucial to correct interpretations of hybridization phenomena or the interaction of hybrids with their biotic or abiotic environment. For example, documentation of introgressive race formation, or measurements of pest abundance on hybrid and parental genotypes, relies on accurate methods of hybrid identification. Although the classification of hybrids remains problematic, considerable progress has been made over the past 60 years. The first major contributions were by Edgar Anderson, who devised a number of

morphometric approaches for detecting and describing hybrids, such as the hybrid index and the scatter diagram (Anderson 1949). Although these methods were useful for describing morphological variation in hybrid populations, it soon became clear that the character correlations and morphological intermediacy detected by these approaches could result from evolutionary phenomena other than hybridization (Dobzhansky 1941, Baker 1947, Barber and Jackson 1957, Heiser 1973). Dobzhansky (1941) recognized that intermediacy could arise from convergent morphological evolution. He also noted that remnants of the ancestral population from which two species differentiated might also exhibit intermediacy, i.e., an early and explicit recognition of symplesiomorphy (shared ancestral characters). Barber and Jackson (1957) recognized that differentiation within a series of populations in continuous contact (primary intergradation) could be difficult to distinguish from zones of hybridization involving secondary contact between previously isolated species (secondary intergradation). As a result, they questioned the assumption that steep clines always result from the merger of previously differentiated populations. Other authors were skeptical of the use of hybrid indices and other biometric tools in the absence of information regarding the genetic basis of the characters being assessed (e.g., Baker 1947, Gottlieb 1972, Lamb and Avise 1987, Rieseberg et al. 1988). For example, morphological analysis of genetically characterized treefrog hybrids revealed that >40% of individuals with a known hybrid ancestry would have been misclassified as "pure" parental species based on morphology (Lamb and Avise 1987). Likewise, a review

of 46 studies reporting morphological character expression in plant hybrids (Rieseberg and Ellstrand 1993) revealed that only 45% of morphological characters displayed "intermediate" expression in first generation hybrids. The remaining characters were either the same as one parent or the other (45%) or were extreme relative to either parent (10%). By contrast, Floate et al. (1994) reported surprisingly strong correlations between morphology and genotype of hybrid cottonwoods (*Populus spp.*), suggesting that in some instances morphology can be a reliable indicator of hybrid ancestry.

Despite the uncertainties associated with morphological classification of hybrids, many studies continue to rely on this approach, including studies of parasite resistance (e.g., Boecklen and Spellenberg 1990, Siemens et al. 1994, Whitham et al. 1994). Clearly, hybrid morphology as a predictor of genotype will continue to be valuable for future study in introgressive hybridization.

The development of molecular markers has proved to be sensitive tool for the detection of hybrids and for the dissection of hybrid genomes (e.g., Levin 1975; Keim et al. 1989; Harrison 1990; Arnold 1992; Rieseberg et al. 1996; Howard et al. 1997). Advantages of molecular markers include (1) the large number of independent markers available for analysis, (2) simple genetic control, (3) low levels of non-heritable variation, (4) apparent selective neutrality, and (5) the ability to distinguish between maternal and paternal parents (e.g., Palmer et al. 1983, Powell 1983). Several authors also note the advantages of employing linked molecular markers to distinguish between morphological patterns resulting from

hybridization and those resulting from primary intergradation, convergence, or symplesiomorphy (e.g., Avise and Saunders 1984, Doebley 1989, Rieseberg et al. 1990).

One of the difficulties with this approach is that hybrid and parental genotypic classes often differ minimally (Nason and Ellstrand 1993; Floate et al. 1994; Boecklen and Howard 1997). Thus, multilocus genotypes and haplotypes are now being used to increase the power of discriminating among hybrid categories (e.g., Ellstrand et al. 1987; Paige et al. 1991; Nason et al. 1992; Paige and Capman 1993).

2.2.2 Measures of introgression

A major biological problem in studying hybrid zone and introgression is to recognize the present form and manner of the disposition of the infiltrants and thus their possible evolutionary influences (Namkoog 1966). For instance, the introgressants may form a completely intergrading series from one species to the other or may be developing into a coherent population. Alternatively, the introgressants may be affecting a uni- or bi-directional gene flow or tending toward oblivion. The analytical problem is that of finding a good scale to measure the proportions in which the parental species are represented in a sampling of introgressants. Statistical methods developed to deal with gene migration and estimation of gene flow within and among animal species has been widely used in human populations (Roberts and Hiorns 1965; MacLean and Workman 1973a, 1973b; Elston 1971; Korey 1978; Wijsman and Cavalli-Sforza 1984). In plants,

however, only a few studies have attempted to quantify the extent of introgression among species. Wheeler and Guries (1987) used the least-squares procedure developed by Roberts and Hiorns (1965) and subsequently modified by Elston (1971) to quantify introgression between lodgepole and jack pines using allozyme data. The estimated index values (*M*), based on species differences in gene frequencies at 11 loci, provided consistently similar and reasonable estimates of introgression given what was known about the populations studies. Nason et al. (1992) used a maximum likelihood (ML) method to examine hybridization and introgression in mixed populations of black oaks (*Quercus kelloggii* and *Q. wislizenli* var. *frutescens*), manzanitas (*Arctostaphylos patula* and *A. viscida*), and irises (*Iris fulva* and *I. hexagona*) based on codominant molecular markers (allozyme and RFLP). Under one assumption, that only first and second generation products of hybridization are present in a population, the ML method provided good estimates of the true parental species, hybrid, and first generation backcross frequencies.

As hybrid swarms evolve from slightly introgressed to strongly introgressed, the loss of one parental type and a shifting of the population mode towards the range between the F_1s and the other parental type would be expected (Grant 1981). Documenting introgression in natural populations from morphological and historical inferences remains difficult largely because other factors that could explain the variational patterns, including intraspecific variation, convergent evolution, retention of ancestral characters, and phenotypic plasticity

(Bloom 1976; Heiser 1973; Reiseberg et al. 1988). To detect introgression, species specific diagnostic or nearly diagnostic genetic markers must be present in the donor species.

2.3 Hybrid zone theories and patterns of introgression

Hybrid zones occur where genetically distinct groups of individuals meet and mate, resulting in offspring of mixed ancestry (Barton and Hewitt 1989; Harrison 1990, 1993). Three hybrid zone models are often used to explain various patterns of introgressive hybridization (Moore 1977; Arnold 1992, 1997): Tension Zone, Mosaic and Bounded Hybrid Superiority. The Tension Zone model has selection against hybrids in the hybrid zone and gene flow from the parental populations. Tension zones are in fact clines maintained by a balance between dispersal and selection against hybrids. Because they are not maintained by a response to local environmental conditions, they can move from place to place (Barton and Hewitt 1985). The Mosaic model has a mosaic of different selective habitats, with gene flow among them, and the patches of different habitats are equally abundant in the centre of the zone. The Bounded Hybrid Superiority model has a central zone where hybrids are more fit and outer margins where parental species are more fit. The Tension Zone model is, by far, the most popular due to the series of elegant models of hybrid zones and their successful applications to grasshoppers and frogs by Barton and his coworkers in 1980s (e.g., Barton and Hewitt 1989). In the bulk of the literature, a hybrid zone is synonymous with a cline (Barton and Hewitt 1985). However, in many cases, plant hybrid zones fail

to fit the classical pattern of clinal variation with a monotonic shift in allele frequencies along a transect. Mosaic variation may be the rule, rather than the exception, for plant hybrid zones (and may be common in animal systems as well, e.g., see Harrison 1986, 1990). Around the hybrid zone of lodgepole and jack pines, gene exchange between the two species is evident but parental contributions to the hybrids appear to be geographically mosaic (i.e., there is little relation between level of introgression and the distance from the nearest stands of parental species). Critchfield (1985, Table 1) summarized 'irregular' geographic distributions of jack pine influences in lodgepole populations for cone orientation, terpene patterns and isozyme markers in regions around the PCBC hybrid zone. Such irregularity and the lack of the agreement between different kinds of data probably arise from the wide but uneven dispersal of individual jack pine genes through lodgepole populations. However, knowledge on patterns and levels of introgression and population differentiation is still lacking.

Hybrid zones can be formed in two ways: primary intergradation and secondary contact. In primary intergradation subsets of a larger population diverge in their genetic makeup, behavior, morphology, etc. because of environmental influences. Therefore, these subpopulations have different selective pressures acting on them. This, theoretically, should lead to differentiation of these subpopulations into genetically distinct groups. Secondary contact is a result of two populations that were once separated coming back together again. This is probably the easiest scenario to envision and as a result is the most widely

accepted method of hybrid zone formation. For outcrossing species, when hybrid zones are the result of secondary contact, gametic disequilibria will be high initially and may gradually erode over time. However, dispersal of parental types into the zone, selection against hybrid or recombinant genotypes, and positive assortative mating will slow or prevent the decay of genetic disequilibria. Even neutral markers will be affected because their behavior will be determined by linkage relationships to loci under selection and/or loci that cause positive assortative mating (Barton and Hewitt 1981, 1989). If hybrid zone represents cases of primary intergradation or parapatric differentiation, selection may "create" nonrandom associations, and disequilibria values will increase over time. In fact, the two scenarios are not mutually exclusive, because selection might act within secondary zones to favor particular allelic combinations that were not present initially (Harrison and Bogdanowicz 1997). In discussing the biogeography of jack pine and lodgepole pine, Critchfield (1980) hypothesized that post-Pleistocene influence of jack pine to lodgepole pine would be restricted mostly to the regions of overlap (secondary contact) whereas, outside these regions, widespread but non-uniform jack pine influence would be the result of earlier contacts between the species during Pleistocene interglacials (primary intergradation). This hypothesis, however, still needs to be tested.

Observations of patterns of introgression across hybrid zones provide insights into the importance of intrinsic barriers to gene exchange and ultimately enable us to infer the genetic architecture of these barriers. The nature of the

genotypic array found within a hybrid zone population depends both on the sample of genotypes that initially established that population and on the evolutionary forces that have operated subsequently (Harrison and Bogdanowicz 1997). Because the genetic variation found within a hybrid zone is greatest when all classes of hybrids are present, any factor that eliminates one or more classes should diminish the genetic variation. For example, the greatest genetic variation should be found where F1 hybrids are fertile, interbreed, and backcross with both parents (i.e., bidirectional introgression). This results in all combinations and permutations of the two species' genomes and produces a continuum of intermediates between both species. *Eucalyptus risdonii* × *E. amygdalina* hybrid zones (Potts and Reid 1985, Potts 1986) represent this pattern. Another pattern of hybridization exhibited in hybrid zones of *Populus fremontii* × *P. angustifolia* results in unidirectional introgression where fertile F1 hybrids backcross with only one of the parent species (Keim et al. 1989). With this hybridization pattern, the continuum of hybrid types is one sided, creating a morphological and genetic gap with an associated decline in hybrid zone genetic diversity.

3 Introduction to Western Gall Rust

Western gall rust (WGR), caused by *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J. P. Moore), is an important fungal disease of hard pines in North America (Allen et al. 1990). During the last several decades, high incidences of WGR in lodgepole pine and jack pine have been frequently reported from Nova Scotia to the Yukon, especially in young

plantations (Powell and Hiratsuka 1973; van der Kamp and Spence 1987; Bella and Navratil 1988; Yanchuk et al. 1988; Wu et al. 1996). WGR is one of the serious diseases of lodgepole pine in British Columbia (van der Kamp and Spence 1987) and Alberta (Hiratsuka and Maruyama 1985), and of jack pine in Ontario (Juzwik and Chong 1990) and Manitoba (Klein et al. 1991).

3.1 Life cycle of western gall rust fungus

Endocronartium harknessii was first described by Moore in 1876 as *Peridermium harknessii* on *Pinus radiata* (Hopkin 1986). Since that time, many studies towards understanding the true life cycle of the rust fungus have been conducted. Fromme (1916) suggested that the rust exhibits pine to pine infection and is therefore an autoecious rust fungus. In the next a couple of years, however, some researchers reported that the rust fungus infected *Castilleja miniata* Dougl. as the alternative host (Weir and Hubert 1917; Meinecke 1920, 1929). These observations were subsequently doubted. Wagener (1964) suggested that the surface contamination of *Castilleja miniata* by *Peridermium stalactiforme* might contribute to the apparent heteroecism observed by Meinecke (1929). Inoculation tests and cytological studies later indicated that the fungus was purely autoecious (Zalasky and Riley 1963; Wagener 1964; Ouellette 1965; Hiratsuka et al. 1966). During the life cycle of this rust fungus only one spore stage named "aeciospore" is observed (Epstein and Buurlage 1988).

Hiratsuka et al. (1966) studied cytology of the rust fungus by staining nuclei of subsets of spores that were incubated for given time intervals. He

observed that during aeciospore maturation and germination young, immature aeciospores had two nuclei, mature aeciospores were uninucleate, and in the germ tubes there were two, three or four nuclei. This was considered as evidence of meiosis, although no actual phases of meiotic division were reported. Based on nuclear cycle, Hiratsuka (1969) placed *P. harknessii* along with *P. pini* into a new genus, *Endocronartium*, which was characterized by still questionable meiosis within a germ tube; fusion of two haploid nuclei, followed by nuclear division into four daughter cells. Uninucleate branches of germ tubes would be functionally basidiospores, in which basidiomycetes generally establish the haploid life form. Based on the studies of Hiratsuka (1991) and Hiratsuka et al. (1966), the life cycle of *Endocronartium harknessii* can be described as followings. Aeciospores of the fungus are produced on the galls and infect the green tissue of young shoots directly. Immature spores usually have two nuclei, while the mature spores just before germination have one nucleus due to the nuclear fusion. Upon germination the spores produce germ tubes and subsequent meiotic nuclear division results in 2 to 4 uninucleates in the germ tube. Germ tubes usually divide into three, four, or five cells separated by septa and the growth is determinate. Each segment of a septa germ tube usually has one nucleus. The germ tubes often have side branches that are functional and are involved in the host penetration. Dikaryotization of the monokaryotic and haploid hyphae then take place at the base of sorus and the dikaryotic cells divide to produce the spores that annually sporulate on the gall surface.

However, Hiratsuka's theory was strongly criticized by Laundon (1976), who saw no evidence for meiosis in Hiratsuka's paper (1968). Several other studies have suggested that the life cycle of *Endocronartium harknessii* is asexual. Epstein and Buurlage (1988) observed only nuclear divisions in the germ tubes of mainly binucleate aeciospores and later population studies by Vogler et al. (1991, 1997) showed that this rust fungus comprised two distinct zymodemes (multilocus electrophoretic phenotypes), which also differed by the nuclear number of the spore. Predominantly binucleate type I was characterized by single marker at each isozyme locus, while uninucleate type II had additional markers. Based on photometric measurements, uninucleate zymodeme had the same amount of DNA in one nucleus as the binucleate had in two nuclei. Also the number of nuclei in aeciospore germlings increased arithmetically over time. These data suggested that aeciospore nuclei, in both types I and II, divide mitotically, as is consistent with an asexual life cycle. It was interpreted that the uninucleate type was the result of karyogamy of two binucleate types or that the binucleate type was the result of haploidization of the uninucleate type (Vogler et al. 1997).

Thus, the central issue is whether the spore stage infecting pines is a true aeciopore (avegetative product of mitosis) or a functional teliospore that yields meiotic products but only resembles an aeciospore morphologically (Vogler et al. 1997). Within all above studies, no phases of cell divisions were documented. Neither was any mechanism for the haploidization of the fungus suggested, although it is known that *Peridermium* hyphae are uninucleate within pine tissue

(Hiratsuka 1969). It can be considered that neither meiotic nor mitotic events within germ tubes of *Endocronartium harknessii* are adequately reported. Even if cytotological observations based on nuclear number were correlated, the actual phases of meiosis should still be documented microscopically, or evidence of meiosis should be obtained with analysis of genetic markers in controlled matings.

Overall, the interpretations of nuclear status of *Endocronartium harknessii* aeciospores or aecial germ tubes by Hiratsuka (1969, 1991), Epstein and Buurlage (1988) and Vogler et al. (1997) remain largely controversial.

3.2 Disease cycle, damage, and control of western gall rust

WGR is characterized by the formation of woody swellings (galls) on branches and stems. Although the galls are generally globose, they may be asymmetrical and are sometimes deeply fissured. In spring and early summer (May-July, depending on climate), masses of orange-yellow spores are produced by and released from galls on diseased trees (Peterson 1973; Hiratsuka 1987). The airborne spores infect new young shoot tissues of hard pines directly. After germination, the germ tubes of the spore germlings grow either perpendicular or parallel to the epidermal ridges of the hypocotyles (Hopkin et al. 1988). Once the monokaryotic and haploid hyphae reach the cambium, they stimulate repeated division of the cambial cells, causing the production of excess xylem and ray parenchyma (Allen et al. 1990; Hiratsuka and Powell 1976) and resulting in the gall formations 1-2 year after infection. Galls continue to increase in diameter as the host tree grows, and typically reach sizes of 5-10 cm in diameter (although

larger galls sometimes develop on main stems). Galls become inactive with the death of the branch or stem, or are killed by hyperparasitic fungi. But the woody swellings remain on the tree. Damage is not significant on mature trees where most infections occur on branches (Gross 1983). Branch galls do not result in serious growth losses. However, infections on young trees often result in main stem galls that can cause stem malformations and predispose the tree to breakage in high winds or under heavy snow loads (Ziller 1974).

Unlike other pine stem rust pathogens, it is capable of infecting directly from pine to pine without the need for alternate hosts. Therefore, efforts to control the disease must focus on the pine host (Allen et al. 1990). Despite possible control of WGR infection by the use of fungicides (Huber 1980) and removal of infected trees (Peterson and Walla 1986), genetic improvement for WGR resistance is perhaps the most economic and effective way of controlling the disease (Yang et al. 1997).

3.3 Genetic variation of fungus

Understanding the variability in WGR fungus is prerequisite for effective management of the pathogen. Early studies on the fungus mainly focused on the taxonomic classification and the biology (Anderson and French 1965; Hiratsuka et al.1966; Hiratsuka and Maruyama 1968; Hiratsuka 1969). However, genetic variation in the pathogen populations has been explored recently.

Isozyme data revealed that selective pressure for host specificity in WGR fungus seems to be weak. Tuskan et al. (1991) examined the isozyme variability of

201 WGR fungal isolates collected from 13 geographic locations throughout North Dakota and northwest Minnesota. The study involved three host species, *Pinus ponderosa*, *P. banksiana* and *P. sylvestris*. Five of the 13 putative loci were found polymorphic and their frequencies were heterogeneous among the locations. Small isozyme difference among isolates from different pine species was found and might suggest that selective pressure for host specificity in WGR fungus in sampled populations was minimal. This lack of host specificity in WGR fungus was also supported by the study of isozyme variability among 341 isolates collected from 13 Pinus species at 39 Northern American locations (Vogler et al. 1991).

Recently, molecular techniques have enabled the study of geographic variability in WGR fungus at the DNA level. However, most studies have used localized samples. Analysis of restriction fragment length polymorphisms in 25 single-gall aeciospores from lodgepole pine host at five locations in British Columbia revealed variability within and among locations at the ribosomal DNA region (Sun et al. 1995). A study of the random amplified polymorphic DNA (RAPD) in the western gall rust fungal isolates from lodgepole pine host at 12 locations across British Columbia also showed variability within and among the locations (Sun et al. 1995). In Ontario, the western gall rust fungal isolates sampled from jack pine hosts exhibited variability at 16 of 24 RAPDs, but the geographic variability among the isolates was not apparent, probably due to limited sample size (Hubbes and Lin 1995).

A RAPD survey on the broader geographic distribution of WGR fungus by Li et al. (2001) consisted of isolates taken from lodgepole pine host at four locations in BC and Alberta and from jack pine host at nine locations in Alberta, Saskatchewan, Manitoba, and Ontario. The RAPD pattern of WGR isolates from lodgepole pine was uniform. However, isolates from jack pine differed significantly among locations, with an east-west trend of decreasing similarity in RAPD. The authors suggested that the migration of refugium after glaciation and the selection along macrogeographic gradients might account for this east-west trend. The large differentiation between WGR fungal isolates sampled in lodgepole pine and jack pine hosts might suggest that selective pressure for host specificity in sampled populations was strong (Li et al. 2001).

3.4 Geographic variation on resistance to western gall rust in pines

An important prerequisite for studying pine-rust coevolution and genetic improvement of WGR resistance is knowledge on the extent and patterns of geographic and genetic variability in lodgepole pine and jack pine within their natural ranges.

The evaluation of WGR resistance in lodgepole pine and jack pine was usually carried out by a field investigation of different provenances and/or different families exposed to natural sources of the rust. In the early 1970s, a series of lodgepole pine provenance trials were established by the British Columbia Forest Service in several geographic locations in B.C. and Yukon. Field observations in these provenance trials showed that lodgepole pine demonstrated

remarkable variation in the resistance to WGR. In the provenance trial of 53 lodgepole pine provenances located at Red Rock at age 5 from planting, Martinsson (1980) observed that high elevation and high latitude provenances were particularly susceptible to the WGR infection. Yanchuk et al. (1988) reported a 10-year-old provenance-family test near Red Rock B.C. with 214 open-pollinated families from 24 provenances. The variation in resistance to the WGR existed at provenance, population and the individual tree levels, with the coastal provenances showing the highest infection. Wu et al. (1996) investigated the incidence of the WGR disease in a 21-year old provenance-family test plantation at Red Rock, B.C. The plantation contained 778 wind-pollinated families from 53 provenances from western Canada. The results indicated that the resistant provenances concentrated mainly in the northeast part of the lodgepole pine's natural distribution such as the Peace River region and along the low elevation sites of the wet belt of southern interior B.C. The most susceptible provenance (Lime Lookout, BC) had an infection rate of 100% while the most resistant provenance (Inonoaklin, BC) had only 17% of WGR infection incidence.

Variation of resistance in lodgepole pine to the WGR disease was also revealed in a greenhouse screening by artificial inoculation. Yang et al. (1997) inoculated 291 open-pollinated families originating from west central Alberta with a mixture of WGR spores collected from Hinton, Alberta. They observed an east-west trend of WGR resistance, with the western and the high-elevation families being more susceptible. The geographic trends of the WGR susceptibility in

lodgepole pine detected by Yang et al. (1997) supported the field observations in that lodgepole pine provenances and families from the low elevation and the contact regions with jack pine were more resistant to WGR.

Jack pine is also highly variable in responses to WGR infection (Klein et al. 1991). Growth and disease (*Cronartium quercuum* and *Endocronartium harknessii*) susceptibility of jack pine provenances are related to environmental gradients in latitude and in length and temperature of growing season associated with seed origin (Yeatman 1974, Rudolph and Yeatman 1982, Burnes et al. 1989).

4 Host-Pathogen Coevolution and Mathematical Models

4.1 Host-pathogen interaction and coevolution

The coexistence of host plants and pathogens in nature indicates that they evolve together. Changes in the virulence of pathogens must be continually balanced by changes in the host resistance and vice versa so that a dynamic equilibrium of resistance and virulence is maintained if both host and parasite are to survive. Many hypotheses have been proposed to describe the genetics of host-pathogen systems. The concept of gene-for-gene coevolution is a model of paramount importance to research on the evolution of resistance against pathogens in crops and on the evolution between species in natural plant populations (Thompson and Burdon 1992).

In the 1940's, working with flax and the obligate flax rust pathogen, Flor hypothesized a genetic mechanism by which plants were resistant to pathogens

(Flor 1942, 1955, 1971). This hypothesis, termed the gene-for-gene concept, is now generally accepted to mean that "for each gene determining resistance in the host there is a corresponding gene for avirulence in the parasite with which it specifically interacts". The gene-for-gene concept has become a powerful tool in evolutionary biology because it suggests very specific ways in which pairs of species interact (Thompson and Burdon 1992). It also served as the paradigm in genetic breeding programs for the past 50 years (Buell 1999).

Except for those elegant genetic studies that confirmed the existence of single genes in the host and pathogen, few studies supported the model until 1984. With the advent of molecular biology, Staskawicz et al. (1984) were able to isolate the first pathogen avirulence gene. Using a molecular approach, an avirulence gene from *Pseudomonas syringae* pv. *glycinea* race 6 that confers race-cultivar specific resistance on soybeans (*Glycine max* (L.) Merrill) was cloned. To date, over 30 avirulence genes have been isolated from bacterial pathogens alone (see review of Leach and White 1996). In contrast to the rapid isolation of avirulence genes upon the advent of molecular biology, the first plant disease resistance gene that conforms to the gene-for-gene hypothesis was not cloned until 1993. Using a positional cloning approach, the tomato (*Lycopersicon Esculentum* Mill.) *Pto* gene that confers resistance to the bacterial speck pathogen was isolated (Martin et al. 1993). Up to the year 1998, numerous disease resistance genes that conform to the gene-for-gene hypothesis have been cloned, bringing the total number to 25 (Buell 1999).

The gene-for-gene concept has been documented for many host-pathogen systems, especially in cultivated crops. While the dynamics of natural host-pathogen systems is complex, the gene-for-gene theory also works in natural populations (Thompson and Burdon 1992; Frank 1993). There are generally two approaches for studying the genetics of host-pathogen interaction in natural populations: (1) examine the role of genetic variation through comparisons of host family lines grown in natural situations; (2) examine the interaction of hosts and pathogens and genetic analysis of resistance (Thompson and Burdon 1992). For example, the simultaneous study in hosts and pathogens in a number of populations of *Linum marginale* and *Melampsora lini* growing in the same geographic region have provided a new perspective in understanding how plants and pathogens continue to coevolve in natural systems (Burdon and Jarosz 1991, 1992; Jarosz and Burdon 1991). In comparison to crop pathosystem, natural forest pathosystems may possess complex population structures that reflect great genetic diversity in both hosts and pathogens as a result of their adaptation to each other and to their highly heterogeneous environments. Many individual host and pathogen populations constitute metapopulations at which gene-for-gene coevolution exists and genetic variations in resistance and virulence genes are maintained. Further complication in the natural pathosystems arises from gene exchange between populations that disrupt the local dynamic balance between hosts and pathogens, and therefore bring endemic disease levels to epidemic outbreaks (Harlan 1976; Frank 1993).

4.2 Mathematical models and computer simulations

The basic model for studying the coevolution of pathogen and host populations should include both genetics and epidemiology in an explicit way. Such an approach is fraught with technical complications and a proliferation of parameters making it difficult to extract a clear message (May and Anderson 1983). All existing models can be classified into three classes: (1) Class 1 - incorporates the population genetics of both pathogen and host populations in an explicit way but ignores the frequency-dependent effects on fitness. (2) Class 2 - focuses on genetics of the host populations, with the genetic dynamics of the pathogens subsumed in frequency-dependent fitnesses of the host genotypes. (3) Class 3 - seeks a relatively accurate account of the density-dependence and epidemiology of the interaction between hosts and different strains of pathogens, without retaining the explicit genetics (May and Anderson 1983).

There is a large and still-growing body of literature in which the fitnesses of various host genotypes under exposure to various pathogen genotypes are specified, and then study the ensuing dynamical behavior of host and pathogen gene frequencies. Much of this work is related, metaphorically or in detail, to crop breeding (Mode 1958, 1961; Person, 1966; Yu 1972; Leonard 1977; Lewis 1981; Levin and Udovic 1977; Fleming 1980; van der Plank 1975; Day 1974). The usual models are based on the "gene-for-gene" assumption, in which a one locus / two allele system in a diploid host interacts with a diallelic locus in a haploid pathogen.

Theoretical models are often complex such that their algebraic description and manipulation are possible in only some extreme cases. This has resulted in a major gap between the experimentalists dealing with the facts of the real world and the theoreticians dealing with the obscure complexities of the artificial models. Computer simulations can partially bridge this gap (Fraser and Burnell 1970). Many theoretical analyses and computer simulations (e.g., Leonard 1977; Frank 1993) have been carried out to assess the effects of selection and genetic and demographic processes on changes in the gene frequencies in hosts and pathogens. However these studies are limited to the dynamics in a single host species. Thus, theoretical and/or computer simulation to include interspecific gene flow (introgression) and its impact on the change in gene frequencies in host and pathogen population is needed to validate the hypotheses developed from the empirical studies (Whitham 1989; Floate and Whitham 1993).

5 Host-Pathogen Interaction in Hybrid Zones

Studies of differential responses of parental species and their hybrids to infection with pathogen under controlled or natural conditions have been considered to be a fruitful approach to assessing the adaptive effects of hybridization and introgression (see Strauss 1994 for review). It has been clearly established that the interactions between pathogens and their plant hosts can be modified in hybrid zones (Moulia 1999).

5.1 Pathogen distribution in plant hybrid zones

The impact of hybridization on the distribution of pathogens in hybrid zones has been documented in many plant models (Strauss 1994). Fritz et al. (1994) proposed four alternative hypotheses to synthesize the diversity of results on the studies of pathogen of hybrid plant to predict the distribution of pathogen in hybrid populations. The assessment of the level of pathogen could vary according to the models. Briefly, they are: (1) The Additive hypothesis - hybrids will not differ from the mean of the resistances of the two parents. Thus F1 hybrids are intermediate between the resistances of the parental species, suggesting that resistances of hybrids are due to the additive inheritance of resistance traits from both patents. Gange (1995) found that growth rates of aphid (*Pterocallis alni*) were intermediate in F1 hybrids between two alder (*Alnus spp.*) species. Similarly, studies found intermediate abundances or growth rates of parasites on hybrid plants (Manley and Fowler 1969; Hall and Townsend 1987; Soetens et al. 1991; Aguilar and Boecklen 1992; Eisenbach 1996; Orians et al. 1997). (2) The Dominance hypothesis – resistance in hybrid differed significantly from the mean resistance of parents, but it did not differ significantly from that of either parents. Resistance in hybrid could resemble the more resistant or the more susceptible parent. Dominance has been frequently discovered in field studies and towards the susceptible parent (Fritz et al. 1996; McClure 1985; Siemens et al. 1994; Hjalten 1997; Mattson et al. 1996). Dominance towards the resistant parent has been shown in a few studies (Paige and Capman 1993; Graham et al. 1995; Fritz et al.

1996). (3) The Hybrid Susceptibility hypothesis - higher density of pathogen and/or higher rate of pathogen infection on hybrids compared to the parental species. This hypothesis predicts that a larger than expected fraction of the pathogen population would reside on hybrid individuals relative to the parental species. Whitham et al (1989) found that backcross hybrids between *Populus angustifolia* and *P. fremontii* were more susceptible to the gall-forming aphid, *Pemphigus betae*, than was either parental species. Several other studies also found similar results (Drake 1981; Fritz et al. 1994, 1996; Whitham et al 1994; Christensen et al. 1995; Mattson et al. 1996; Orians et al. 1997). (4) The Hybrid Resistance hypothesis – hybrids would be more resistant than either parent, resulting in lower density of the pathogen. However, increased resistance of hybrids relative to both parents has infrequently been demonstrated. Boecklen and Spellenberg (1990) found support for this hypothesis in an oak hybrid zone.

These hypotheses were first stated for F1 hybrids, but the authors suggested that they could be applied to more complex backcrosses and recombinant hybrid populations (Fritz et al. 1994, 1996; Whitham et al. 1994; Christensen et al. 1995).

Considering the range of organisms in these studies, it is not surprising that patterns of host susceptibility and pathogen occurrence in hybrid zones varied greatly from case to case. In general, the susceptibility of hybrids would likely depend on the complex interplay of genetic, ecological and life-history attributes of both host and pathogen. Thus, the interaction could be affected by the genetic

basis of resistance in each parent (single or multigenic; Fritz et al. 1994), the phylogenetic distance between the parental species, the taxonomic identity (e.g., insect or fungus) and the degree of speciation of pathogen (Whitham et al. 1994) and the ecological characteristics of areas occupied by the hybrid swarms (Paige and Capman 1993). Furthermore, intercrossing between F_1s and the backcross of hybrids with either parent might produce a complex diversity of genotypes within a hybrid swarm (Paige and Capman 1993; Fritz et al. 1994; Strauss 1994). Different categories of hybrids are usually indistinguishable even though their susceptibilities to pathogens may be very different. For example, F1 might be highly resistant while backcrossing hybrids might be susceptible (Paige et al. 1990). Hence, valid comparisons between different hybrid systems need to consider the type of hybrids.

To support any of these hypotheses may be implicit of a probable underlying mechanism of inheritance of resistance such that testing these hypotheses is a useful first step for investigating the genetics of resistance (Fritz et al. 1994). However, whatever mechanisms are involved, changes in the distribution of pathogen can have evolutionary consequences. Some proposed that hybridization could limit or extend the host spectrum of a pathogen. In the first case, susceptible hybrids would act as "sinks" for pathogens. Their level of susceptibility would prevent the pathogens from adapting to the resistant parental species (Whitham 1989). In the second case, hybrids that were genotypically

intermediate would act as "bridges", favoring the adaptation of pathogens to new closely related host taxa (Floate and Whitham 1993).

5.2 Effects of introgression on plant resistance

When plant hybrid zones function as sinks for pathogens, the susceptible hybrids could serve as a refugium to buffer the selection pressure on the evolution of virulence in pathogens that overcomes resistance in parental species (Whitham 1989). The breakdown of co-adapted gene complex due to interspecific hybridization could also render hybrids more susceptible (Whitham 1989). Floate and Whitham (1993) extended this theory to account for interspecific introgression as a co-evolutionary mechanism in host-pathogen interaction, i.e., introgressive hybridization through backcrossing would form a series of genetic gradients that functioned as a hybrid bridge and facilitated the shifting of the host. It is easy to predict that host shifting will be affected by the pattern of plant hybridization as it determines the degree to which hybrid genotypes span the genetic gap between plant species. For example, in the progression from F1 sterility to unidirectional introgression and to bidirectional introgression, the genetic gaps between parental species diminish to form a continuum of intermediates.

The literature suggests that interspecific introgression might have played a significant role in the evolution of host-pathogen relationship in tree species. Resistance of loblolly pine (*Pinus taeda* L.) to fusiform rust (*Cronatium fusiforme*) has been attributed to the influence of introgression of shortleaf pine (*Pinus echinata* Mill., Hare and Switzer 1969; Florence and Hicks 1980). Manley and

Fowler (1969) reported a positive correlation in resistance to spruce budworm (*Choristoneura fumiferania*) and increasing genetic introgression of red spruce (*Picea rubens* Sarg.) into black spruce (*Picea mariana* (Mill.) BSP). Some studies suggested that putative hybrids between Sitka (*Picea sitchensis* (Bong.) Carr.) and white spruce (*Picea glauca* (Moench) Voss) are highly resistant to spruce leader weevil (*Pissodes strobi* Peck) (Mitchell et al. 1990; Ying 1991). However, hybrids have also been found to be more susceptible to insect and disease infestation than their parental species (Floate and Whitham 1993; Lefevre et al. 1994; Strauss 1994). Van der Kamp (1988, 1989) conducted studies involving inoculation of seedlings of lodgepole pine and Scots pine (*Pinus sylvestris*) with geographically separate spore sources of WGR fungus from lodgepole pine and Scots pine. These studies have shown differential intra- and inter-specific responses to WGR. Furthermore, significant interactions between spore sources and pine populations were apparent in some of these studies. In assessing the geographic variation in susceptibility of lodgepole pine to WGR and other pathogens, Wu et al. (1996) found a positive correlation ($r = 0.60$) between the level of WGR resistance in lodgepole pine populations and their distance from the western limit of the natural range of jack pine. This might suggest that introgression of jack pine played an important role in the evolution of WGR resistance in lodgepole pine such that long-term breeding for WGR resistance in lodgepole pine require the exploitation of lodgepole-jack pines interspecific gene pool.

6 Research Objectives and Structure of the Thesis

The objective of my thesis research was to understand coevolution of lodgepole and jack pines with WGR fungus in the PCBC hybrid zone. This was accomplished through three different studies. First, I examined if different host populations would respond differentially to different WGR spore sources by studying the response of host populations to two genetically distinct WGR sources, one from lodgepole pine hosts and another from jack pine hosts. This was a greenhouse inoculation study of 40 populations sampled from 23 lodgepole pine, 9 jack pine, and 8 putative hybrid populations from the PCBC complex. Second, I tested hypotheses concerning biogeographic and multilocus structures of the PCBC complex and determined whether the level of introgression is related to WGR resistance. This was accomplished by studying a set of neutral DNA markers using the same 40 populations. Third, I refined current theory on the coevolutionary dynamics of natural pathosystems with special reference to the interaction of pathogens with hybrid host populations. This was accomplished by incorporating partial dominance and overdominance into Leonard's (1977) model with complete dominance and by examining the stability of equilibrium resistance and virulence polymorphisms via computer simulation.

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Chapter 2

Susceptibility to *Endocronartium harknessii* in *Pinus contorta – Pinus banksiana* Complex: Host - Pathogen Interactions

1 Introduction

Lodgepole pine (*P. contorta* Dougl. ex Loud.) and jack pine (*Pinus banksiana* Lamb.) are the most northern and most widely distributed of the North American pines (Yeatman 1967; Critchfield 1985). Unique life history characteristics such as large reserve supply of seeds in serotinous cones, low shade tolerance and rapid juvenile growth have made these species aggressive pioneers after major disturbances such as fire (Critchfield 1985; Yang et al. 1996). With their wide natural ranges, jack pine from the east and lodgepole pine from the west meet and hybridize in central and northwestern Alberta (Moss 1949). Natural stands in this hybrid zone and its neighboring areas are referred to as *Pinus contorta – Pinus banksiana* complex (PCBC). Edaphic preference is an important determinant of distributions of the parental species and their hybrids, with jack pine being the more xerophytic, lodgepole pine the more mesophytic and tolerant of clay soils and bogs, and the hybrids occupying a wide range of intermediate sites (Yeatman 1967). Numerous morphological, biochemical and molecular studies (e.g., Moss 1949; Mirov 1956; Zavarin et al. 1969; Pollack and Dancik 1985; Wheeler and Guries 1987) have shown that stands in the PCBC hybrid zone

range from nearly pure jack pine through hybrid swarms to nearly pure lodgepole pine, suggesting varying levels of introgression and adaptation to wide ecological amplitudes across the parental species' ranges.

Because these hybrid swarms harbor a great amount of genetic diversity, they host a potentially unique array of ecological and evolutionary interactions. An important interaction is the interaction between these long-lived conifer hosts and pathogenic fungi that have coevolved in complementary genetic systems, regulating host and pathogen populations in dynamic balance with one another. While the dynamics of natural host-pathogen systems is complex, it is believed that the gene-for-gene theory established by Flor (1956) for agricultural crops and their pathogens also works in natural populations (Thompson and Burdon 1992; Frank 1993). In comparison to crop pathosystems, natural forest pathosystems may possess complex population structures that reflect great genetic diversity in both hosts and pathogens as a result of their adaptation to one another and to highly heterogeneous environments. Further complication in the natural pathosystems arises from the fact that gene exchange between populations or taxa across the hybrid zones may disrupt the local dynamic balance between hosts and pathogens, thereby bringing endemic disease levels to epidemic outbreaks (Harlan 1976; Frank 1993).

Western gall rust fungus (WGR), *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka, is an autoecious, gall forming stem rust of hard pines (*Pinus* spp.) with a widespread occurrence in North America. It is one of the most serious

diseases of lodgepole pine in British Columbia (Van der Kamp and Spence 1987) and Alberta (Hiratsuka and Maruyama 1985), and of jack pine in Ontario (Juzwik and Chong 1990) and Manitoba (Klein et al. 1991). WGR infection in natural pine populations varies temporally (Peterson 1971) and spatially (Van der Kamp 1988*a*). As an important first step towards understanding the complex population structures in pine–WGR pathosystems, experiments have been designed to determine if pine–rust interactions are important by inoculating pine hosts with WGR isolates (e.g., Van der Kamp 1988*b*, 1989; Blenis et al. 1993). While these studies have shown that the host specificity in WGR is detectable particularly when WGR isolates are collected from a wide geographic range, they have focused on WGR resistance in pure stands from one or two pine species. Recent assessments of geographic variation in susceptibility of lodgepole pine to WGR suggest that introgression from jack pine might have played an important role in the evolution of rust resistance in lodgepole pine (Wu et al. 1996; Yang et al. 1997).

In this study, we investigated the interaction of lodgepole pine, jack pine, and their natural hybrids across the PCBC hybrid zone with WGR by inoculating 40 PCBC populations with two geographically diverse WGR spore sources in a greenhouse. Our specific objectives were (*i*) to determine if the hybrids would differ from the parental species in their susceptibility to WGR infection and (*ii*) to examine if different host populations would respond differentially to different WGR spore sources.

2 Materials and Methods

2.1 Study populations and seedling growth

Cones were collected from a total of 23 lodgepole pine, 9 jack pine, and 8 putative hybrid populations covering the hybridization region in Alberta and British Columbia (Figure 2-1). A population assigned to a species or hybrid at the time of collection based on overall tree and stand appearance was subsequently confirmed using the most diagnostic cone and seed traits as suggested by Wheeler and Guries (1987). Each population was represented by a bulk collection consisting of 10 or more cones per tree from at least 15 trees per stand. Of 23 lodgepole pine populations sampled, populations 1–16 were from the main natural range of the species; populations 17–20 from the areas representing known outliers of lodgepole pine in Alberta (Moss 1949; Smithers 1961), and populations 21–23 were three B.C. sources near the hybrid zone. Seeds of all cones from each population were extracted, bulked, and stored by population at –4°C with the moisture content being kept between 5 and 10%. Seed germination rate at the time of seed collection averaged 89.7% across populations.

A random sample of about 70 seeds from each of the 40 seedlots was taken for sowing to ensure 50 seeds per population. Prior to sowing, seeds were stratified to ensure more uniform germination rates. In May 1996, stratified seeds were sown in Format 600 (20 × 700 mL) Styroblock^{TM} containers (Beaver Plastics Ltd., Edmonton, Alta.) with peat moss adjusted to pH 5.5 by adding hydrated lime. The seedlings were placed in a greenhouse with an 18-h photoperiod and a

constant temperature of 20°C. They were fertilized every second week with Plant Prod® 20:20:20 ($N - P_2O_5 - K_2O$) fertilizer (Plant Products Co. Ltd., Brampton, Ont).

2.2 Spore sources and inoculation

Mature WGR galls at ages 2–5 years were collected from two heavily infected stands, one from lodgepole pine near Hinton, Alberta (53°12'N; 117°32'W) (WGR_{PL}) and the other from jack pine in Dragline Lake, Manitoba (51°35'N; 100°40'W) (WGR_{PJ}). These two spore sources were chosen because they showed distinct random amplified polymorphic DNA (RAPD) profiles based on a survey of geographic variation among WGR isolates across Canada (Li 1998). At least 15 spore lots from each location were collected, each spore lot containing five or more galls sampled from different trees. After transportation to the laboratory, the galls were air dried on open bench space overnight. The spores were passed through a 50-μm sieve, placed into 30-mL vials with a few silica gel crystals or a capsule and stored in a freezer (–10 to -15°C). The day before each inoculation, a sample of stored spores were placed on 0.2% water agar for 24 h, after which the percent germination of 100 spores were determined.

When 7 weeks old, seedlings were inoculated with WGR_{PL} and WGR_{PJ} spores. A total of 2000 seedlings (40 populations × 2 spore sources × 25 replications) were used for inoculation. A split-plot design with 25 blocks (or replications) was employed. Seedlings in each block were separated into two groups. Each group consisted of 40 populations (23 lodgepole pine, 9 jack pine,

and 8 putative hybrids) each represented by a single seedling. Group I was inoculated with WGR_{PL} and group II was inoculated with WGR_{PJ}.

The inoculation was carried out at the growth chambers, first for the 50 containers marked for inoculation with WGR_{PL} and then for the other 50 containers with WGR_{PJ}. While several conventional inoculation methods including spore–water suspension (Blenis and Hiratsuka 1986), spore–talc mixture (Blenis and Pinnell 1988), spore–oil suspension (Burnes et al. 1988), and brush method (Klein et al. 1991) have been used in the past, we chose the torn needle method of Myrholm and Hiratsuka (1993) because it minimized the possibility of "escaping" infection and allowed for the production of galls on young seedlings. It was implemented as follows. After the seedlings were first misted with distilled water, a single primary needle of a seedling was removed with downward pull and dry spores were then applied directly on and around the small scar left by the needle. Spores were applied with a small artists' paint brush. After inoculation, the seedlings were lightly atomized with distilled water and covered with a plastic sheet to maintain high humidity for spore germination and infection and kept at 15°C without artificial lighting. The seedlings were uncovered from the plastic sheet and returned to the normal growing conditions 48 h after inoculation.

2.3 Rust assessment and data analysis

Red stain symptoms on seedlings were observed within 4–6 weeks after inoculation. This early observation was a preliminary indicator of the successful infection. In other studies using different inoculation methods (e.g., Burnes et al.

1988; Allen et al. 1990; Kojwang and Van der Kamp 1992), weak correlations were observed between early symptoms and pine resistance. However, because of the rapid rust development using the torn needle method, a reliable assessment of the inoculated seedlings could be conducted as early as 3 months after inoculation (Y. Hiratsuka, unpublished). Assessments of WGR infection were carried out when the seedlings were 6 months and 12 months old. The WGR assessments were based on a rating system (0–6): 0, no symptoms; 1, visible discoloration or a definite indication of infection, such as acute bending of stem; 2, a definite canker but no swelling; 3, some swelling with rough bark and open necrotic canker; 4, partial gall, often with rough bark and necrotic canker; 5, complete gall formation; and 6, multiple galls. This was a modification of an earlier rating system (0-5) proposed by Klein et al. (1991) to account for the occurrence of multiple galls on the infected seedlings. Tree height was also measured for each seedling at both assessment times.

Analysis of variance (ANOVA) was used to determine significant differences among spore sources (WGR_{PL} and WGR_{PJ}), host groups (lodgepole, jack pines and hybrids), spore sources × host groups, populations and spore sources × population interaction using the following linear model:

$$Y_{ijkl} = \mu + B_i + R_j + BR_{ij} + G_k + RG_{jk} + P_{l(k)} + RP_{jl(k)} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the observation of the seedling in the lth population within kth host group and jth spore source in the ith replication, μ is the overall mean, B_i is the effect of ith replication, R_j is the effect of the jth rust spore source, BR_{ij} is the

replication × spore source interaction, G_k *is* the effect of the *k*th host group, RG_{jk} *is* the spore source × host group interaction, $P_{l(k)}$ is the effect of the *l*th population within *k*th host group, $RP_{jl(k)}$ *is* the spore source × population interaction and ε_{ijkl} *is* the experimental error. All effects except for μ, R_j, G_k, and RG_{jk} were considered random. Because of this mixture of random and fixed effects in the model and because of imbalance in the data caused by slow germination and mortality, SAS GLM Type III sums of squares (SAS Institute Inc. 1990) was used for the analysis. Several data transformations such as square root transformation used did not seem to mitigate the departure from normality of the residual of rust scores. Since ANOVAs based on both untransformed and transformed data provided similar results in terms of (1) plot of predicted values and residuals, (2) magnitude of coefficient of variation, and (3) significance levels of *F* tests, we only present the results from ANOVA based on the raw data.

The relative importance of random effects such as population and rust source × host population interaction was determined using the estimates of variance components. Thus, population differentiation was simply the proportion of total variation accounted for by the among-population variation

$$\rho_{ST} = \sigma_P^2 / (\sigma_P^2 + \sigma_{R\times P}^2 + \sigma_e^2)$$

where σ_P^2, $\sigma_{R\times P}^2$ and σ_e^2 are the variance components for among-population effect, rust source × host population interaction, and error, respectively. Similarly, the intraclass correlation

$$\rho_{HP} = \sigma_P^2 / (\sigma_P^2 + \sigma_{R\times P}^2)$$

was used to assess if the rust source × host population interaction was important. This quantity is similar to the measure of crossover genotype-environment interaction (e.g., Yang et al. 1998). A perfect correlation ($\rho_{HP} = 1$) is expected when there is no host-pathogen interaction (i.e., $\sigma_{R\times P}^2 = 0$). The standard errors for estimated ρ_{ST} and ρ_{HP} were calculated using Kempthorne (1969).

3 Results

Due to low germination rate and poor seedling establishment in some populations (e.g. three BC populations), only 1733 PCBC seedlings were available for the 6-month and 12-month assessments of WGR severity. The 6-month-old seedlings with scores 0, 1, 2, 3, 4, 5, and 6 were distributed with frequencies of 16.5, 9.0, 12.2, 9.6, 21.0, 31.7, and 0%, respectively (Table 2-1). None of the 6-month-old seedlings got a score of 6 (the occurrence of multiple galls). The 12-month assessment showed an increase of WGR severity, with the respective frequencies of scores 0–6 being 13.3, 7.6, 9.9, 7.6, 18.1, 34.9, and 8.7%. Of 286 seedlings that were scored 0 in the 6-month assessment, 231 remained disease free and 55 became infected with varying levels of severity. Lodgepole pine and jack pine showed different distributional patterns of WGR scores (Figure 2-2). Lodgepole pine had much high frequencies of scores 5-6 while jack pine had high frequencies of scores 0-2. WGR inoculum from lodgepole pine (WGR_{PL}) caused higher levels of infection than WGR from jack pine (WGR_{PJ}) did. For example,

43.9% of 6-month-old seedlings (n = 864) infected with WGR_{PL} had a score of 5 but only 19.6% of 6-month-old seedlings (n = 869) infected with WGR_{PJ} had the same score.

The 40 populations of lodgepole pine, jack pine, and their hybrids varied considerably in their responses to WGR infection (Table 2-2). For example, the 6-month assessment of WGR infection with WGR_{PL} showed that average severities ranged from 1.16 for a jack pine population (population 37) to 4.61 for a hybrid population (population 27). The disease progression between the two assessments was evident. The maximum increase of the disease was over 48% for a jack pine population (population 34) infected with WGR_{PL}. It should be noted that two of the three B.C. lodgepole populations (populations 21 and 23) had seeds with slow germination and poor post-sowing establishment. Thus only five or fewer seedlings from these two populations were available for inoculation. The remaining 38 populations had at least 16 seedlings each during the two assessments.

The analysis of variance (ANOVA) of the 6-month and 12-month WGR assessments showed significant differences among two rust sources, three host groups (lodgepole pine, jack pine, and their hybrids) and rust source – pine host group interactions (Table 2-3). Lodgepole pine and hybrids were significantly more susceptible to WGR than jack pine regardless of rust sources (Table 2-4). The overall WGR score of the 6-month-old lodgepole, hybrid, and jack pine seedlings were 3.37, 3.11, and 2.28, respectively, whereas the overall WGR scores

of those 12-month-old seedlings were 3.59, 3.60, and 2.59, respectively. Overall, spores collected from lodgepole pine (WGR_{PL}) were more virulent to the pine hosts than those from jack pine (WGR_{PJ}) with respective WGR scores of 3.40 for WGR_{PL} and 2.70 for WGR_{PJ} at the 6-month assessment, and 3.56 for WGR_{PL} and 3.13 for WGR_{PJ} at the 12-month assessment. However, lodgepole pine was significantly more susceptible to WGR_{PL} than WGR_{PJ}. In contrast, jack pine was more susceptible to WGR_{PJ} than WGR_{PL} even though such difference did not reach statistical significance at 12-month assessment. Such pine-rust interaction could be visualized by the pattern of "crossover" lines in Figure 2-3.

There were significant differences in height growth among the three host groups with the overall growth ranking of lodgepole pine (13.1 cm) < hybrids (14.8 cm) < jack pine (15.9 cm) when the seedlings were 6 months old, and lodgepole pine (17.1 cm) < hybrids (19.5 cm) < jack pine (20.6 cm) when the seedlings were 12 months old (Table 2-5). The seedlings infected with WGR_{PL} grew slower than those with WGR_{PJ}. When all host groups were considered, there was about a 2 cm decrease of height growth for the seedlings infected with WGR_{PL} at both 6-month and 12-month assessments.

Differences among blocks (replications) were not significant for WGR severity and height growth at both assessments (Table 2-3). Strictly speaking, the replications used here are not the "true" replications because the pine seedlings from the same population are genetically heterogeneous. In other words, part of the replication variation would be due to the genetic variation among the pine

seedlings from the same population. However, the insignificant replication differences would suggest that the seedlings from the same population used as the replications have similar genetic background.

The ANOVA of WGR score and height growth also showed significant variation among populations within the three host groups and their interactions with rust sources (Table 2-3). Estimates of variance components for among-population effect (σ_P^2), rust source × host population interaction ($\sigma_{R\times P}^2$) and error (σ_e^2) for WGR infection were presented in Table 2-6. For comparison, the estimated variance components when the 40 populations are not grouped were also presented (see the right-hand side of Table 2-6). The among-population variation accounted for 7.5% and 5.4% of total variation for 6-month and 12-month WGR assessments, respectively, when the 40 host populations were grouped into three classes (lodgepole pines, jack pines, and their hybrids), but accounted for 10.3 and 8.0% of total variation, respectively, when they were not grouped. These estimates were all significantly greater than zero, as the differences of the estimates were more than 2 standard errors. There were significant host–pathogen interactions for 6-month and 12-month WGR assessments, as the differences of the estimated ρ_{HP} from the expected value of one were more than 2 standard errors. The estimated values of ρ_{HP} were similar for grouped and ungrouped host populations.

4 Discussion

Our results point out that lodgepole pine and hybrids are more susceptible to WGR than jack pine regardless of whether WGR inoculum is collected from lodgepole pine or jack pine hosts. Lodgepole pine and its artificial hybrids with jack pine were also more susceptible to sweetfern rust and to eastern gall rust than local jack pine in test plantations in Minnesota, Wisconsin, Michigan (Anderson and Anderson 1965), and Ontario (Yeatman 1974). Van der Kamp (1989) examined responses of lodgepole pine and Scots pine (*Pinus sylvestris*) to infection with WGR isolates from both host species in B.C. and found that lodgepole pine was much more susceptible to WGR than Scots pine.

To explain varying hybrid responses to pest infestation, Strauss (1994) presented four genetically based alternative hypotheses to the null hypothesis of no differences between the two species: (*i*) hybrid intermediacy (additive hypothesis); (*ii*) hybrid < both parents (hybrid resistance hypothesis); (*iii*) hybrid > both parents (hybrid susceptibility hypothesis or hybrids-as-sinks hypothesis); and (*iv*) hybrid = one parent (dominance hypothesis). Hypotheses (*ii*) and (*iii*) are just different forms of overdominance. Our results appear to be consistent with hypothesis (*iv*), i.e., the dominant gene effect may be important in producing the observed responses of lodgepole – jack pine hybrids to WGR infection, though it remains to determine the genetic basis of the phenotypic similarity between lodgepole pine and hybrids. However, such explanation may be overly simplistic for the two reasons. First, the above models refer primarily to hybrids in the F_1 generation.

Since the PCBC natural hybrids have certainly resulted from many generations of hybridization and backcrossing, they contain varying proportions of parental genes (Table 3 of Wheeler and Guries 1987). Second, the use of group averages ignores the wide range of WGR susceptibility across hybrid populations that overlap with those of parental populations (cf. Table 2-2). Perhaps, a more realistic model would be one that allows the presence of both additive and dominant genes controlling WGR resistance. A similar suggestion was made by Kojwang and Van der Kemp (1991).

The two pine species were more susceptible to the WGR source from their own species; responses of hybrids to both WGR_{PL} and WGR_{PJ} were similar to those of lodgepole pine. This host-specific virulence corroborates the host-specific differentiation as revealed by random amplified polymorphic DNA (RAPD) markers (Li 1998). In the RAPD survey of geographic variation among isolates collected from 13 locations with 4 locations for lodgepole pine and 9 for jack pine across Canada, Li (1998) identified 10 RAPD markers that were unique to the isolates of lodgepole or jack pine hosts. The isolates from lodgepole pine at different locations showed remarkable homogeneity of RAPD profiles (Li 1998) and isozyme profiles (Vogler et al. 1990). In contrast, RAPD variation was detected among isolates collected from jack pine (Li 1998). While it remains unclear whether such RAPD variation is associated with pathogenicity, more WGR sources particularly from jack pine are certainly needed in future inoculation studies.

The results from ANOVA (Table 2-3) and subsequent analyses (Tables 2-4 and 2-6, Figure 2-3) showed strong pine host × WGR source interactions. This is hardly surprising given the host-specific virulence of the two WGR sources as shown above. In an experiment of inoculating 50 lodgepole and Scots pine populations with two bulk WGR spore lots, one from lodgepole pine and the other from Scots pine, Van der Kamp (1989) found no host specificity in the rust (i.e., no pine host × WGR source interactions). However, the two spore sources, while collected from the two host species, were from in the same area, Vancouver, B.C. (Van der Kamp 1989). Thus, they might be the same WGR race adapted to both host species or no new WGR races particularly adapted to non-native Scots pine would have developed (Van der Kamp 1989). In contrast, the two spore sources used in this study, one from lodgepole pine in Hinton, Alta. and the other from jack pine in Dragline Lake, Man., were almost 17° longitude apart. Thus, our spore sources are perhaps different WGR races adapted to different host species and site conditions. It was shown that susceptibility of lodgepole pine to WGR depended strongly on spore sources when WGR spores were collected over a wide geographic range (Van der Kamp 1988*b*; Yanchuk et al. 1988), but such dependence was less evident when WGR spores were from a restricted geographic range (Blenis et al. 1993).

Since WGR has a wide host range, including most native hard pines and exotic hard pine species (Hiratsuka and Powell 1976), its populations have exhibited a great deal of genetic variability (Tuskan et al. 1990; Vogler et al. 1990;

Li 1998). In addition, the pine host – WGR pathogen coevolutionary dynamics suggests that the more resistant jack pine would have exerted a greater selection pressure on WGR than lodgepole pine, resulting in a greater diversity among the WGR populations collected from jack pine. This is certainly consistent with the observation that there is a great amount of RAPD variation among isolates from jack pine in contrast to monomorphic RAPD patterns for isolates from lodgepole pine (Li 1998). In the PCBC hybrid zone, however, this coevolutionary dynamics is more complicated because continued gene exchanges among lodgepole pine, jack pine, and their hybrids may have produced recombinant pine genotypes with differing levels of WGR susceptibility, thereby disrupting the pine–rust balance in various parts of the hybrid zone and bringing WGR from endemic to epidemic levels locally. This may in part explain why some hybrid populations (populations 29, 39, and 40) have very high WGR scores (cf. Table 2-2).

Jack pine grew significantly faster than lodgepole pine with the hybrids intermediate at both 6-month and 12-month assessments (Table 2-5). Similar results were found in 5–10 years and 15–20 years of testing artificial hybrids and their lodgepole and jack pine parents on an Idaho site (Lotan 1967; Rehfeldt and Lotan 1970). While it remains to be determined whether the height growth is truly associated with degree of affinity to one or the other parent, the intermediacy of the hybrid performance suggests a polygenic inheritance with predominant additive gene action.

The torn needle method used in our inoculation experiment permitted a

rapid and uniform assessment of susceptibility of the PCBC pine seedlings to WGR infection (Myrholm and Hiratsuka 1993; Yang et al. 1997). It also minimized the possibility of "escaping" infection and allowed for the production of galls on young seedlings. A dependable WGR assessment could have been made as early as 3 months after inoculation (Y. Hiratsuka, unpublished). Thus our 6-month and 12-month assessments probably reflect true levels of WGR susceptibility in young PCBC seedlings. However, two potential complications may arise from the fact that the torn-needle method is not the natural way in which trees become infected. First, it likely bypasses the first stage of tree resistance mechanisms of preventing germinated spores from penetrating the epidermis. However, our observation indicated no penetration into the wounds, as needle scars were covered by exuded resins. The penetration occurred through the exposure of thin and vulnerable epidermis above the needle scar to the WGR inoculum. Second, the level of WGR infection with the torn needle method might be inflated due to the fact that some of the host reactions (e.g., bending and canker formation) resulted from the wounding rather than WGR infection. Given that about 34 and 37% of the seedlings rated as 1 (stem bending) and 2 (canker formation), respectively, at the 6-month assessment had higher WGR scores (up to 6) at the 12-month assessment (Table 2-1), the host reactions would probably be due to the true WGR infection. Nevertheless, there is a need to consolidate the WGR rating system by including wounded but un-inoculated controls with the torn needle method.

From a standpoint of breeding for WGR resistance in these conifers, it is desirable to investigate the concordance of the early WGR assessment based on the greenhouse inoculation studies with the field performance at later stages of tree growth. However, apart from some difficulties with field or nursery assessments, such as inability to control inoculum sources, natural infections in "wave" years (Peterson 1971) and site effect, the concordance between juvenile and mature WGR resistance may be far from perfect because different defense mechanisms to rust infection might have been evolved between juvenile and mature trees (Czabator 1971) and between juvenile tissues formed from a germinated seed and adult-plant tissues formed after bud break. In particular, pioneer species such as lodgepole and jack pines concentrate on rapid height growth during the seedling and juvenile stages, but as the trees reach reproductive maturity and more energy is invested in reproduction and defense, with a corresponding decrease in the energy invested in growth (Loehle 1988). Thus, both greenhouse and field assessments are needed to understand the ontogenetic effects on WGR resistance. Furthermore, the WGR rating system used in our greenhouse assessments may also need to be modified to account for the ontogenetic changes when used to assess older trees.

In conclusion, our greenhouse inoculation study is the first systematic assessment of WGR resistance in pine populations across the PCBC hybrid zone in central and northwestern Alberta. Lodgepole pine and the hybrids were more susceptible to WGR than jack pine regardless of the spore sources. The two

geographically diverse spore sources, one from lodgepole pine and the other from jack pine, were more adapted to their own host species. Significant spore source × pine host interactions indicate host specificity in the rust. However, such host specificity is far from stabilized (equilibrium) because of continued gene exchanges among the two parental species and hybrids.

5 Summary

Lodgepole (*Pinus contorta* Dougl. ex Lound.) and jack (*Pinus banksiana* Lamb.) pines occur sympatrically and hybridize in central and northwestern Alberta, providing opportunities for studying unique ecological and evolutionary interactions. A greenhouse inoculation experiment was conducted to investigate interactions between 40 populations of lodgepole and jack pines and their putative hybrids across this hybrid zone and two sources of the western gall rust fungus, *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka, one from lodgepole pine and the other from jack pine. Rust susceptibility and height were assessed when the seedlings were 6 months and 12 months old. Lodgepole pine and the hybrids were significantly more susceptible to the rust infection than jack pine. Jack pine grew significantly faster than the hybrids and lodgepole pine. In addition, the seedlings infected with spores from lodgepole pine grew significantly slower than those with spores from jack pine. While the overall rust scores indicated that spores from lodgepole pine were more virulent to the hosts than those from jack pine, both host species were more susceptible to their own rust sources, causing significant spore source × host group interactions. However, such host specificity

in the western gall rust is far from stabilized (equilibrium) because of continued gene exchanges among the two parental species and their hybrids.

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Table 2-1. Number of PCBC seedlings with WGR scores (0-6) at 6-month (WGR1) and 12-month (WGR2) assessments

WGR1	WGR2							
	0	1	2	3	4	5	6	Total
0	231	28	11	9	5	2	0	286
1		103	27	9	12	2	3	156
2			133	34	37	7	0	211
3				80	61	23	3	167
4					198	149	17	364
5						421	128	549
Total	231	131	171	132	313	604	151	1733

Table 2-2. Mean severity (± standard error) of two western gall rust sources, one from lodgepole pine (WGR_{PL}) and the other from Jack pine (WGR_{PJ}), on 6-month-old and 12-month-old lodgepole pines, jack pines and their hybrids.

Pop	Location	Latitude (°N)	Longitude (°W)	Altitude (m)	WGR_{PJ}		WGR_{PL}	
					6 months	12 months	6 months	12 months
				Lodgepole Pine (LP)				
1	Blairmore, AB	49°35'	114° 35'	1584	3.14 ± 0.41	3.23 ± 0.43	3.78 ± 0.41	4.30 ± 0.41
2	Fallen Timber, AB	51°29'	115° 11'	1650	2.75 ± 0.39	3.21 ± 0.40	4.29 ± 0.26	4.76 ± 0.15
3	Prairie Creek, AB	52°16'	115° 13'	1175	3.00 ± 0.36	3.40 ± 0.39	3.35 ± 0.42	3.83 ± 0.42
4	Colt Creek, AB	52°38'	116° 05'	1520	2.59 ± 0.39	3.09 ± 0.42	4.41 ± 0.26	4.77 ± 0.26
5	Hinton, AB	53°12'	117° 32'	1360	3.52 ± 0.31	4.22 ± 0.34	4.23 ± 0.35	4.59 ± 0.34
6	Edson River, AB	53°44'	116° 35'	1080	2.74 ± 0.38	3.13 ± 0.38	3.91 ± 0.32	4.27 ± 0.32
7	Saddle Hills, AB	55°44'	119° 40'	825	3.00 ± 0.40	3.50 ± 0.41	4.45 ± 0.21	4.68 ± 0.22
8	Grande Prairie, AB	54°38'	119° 07'	1100	2.71 ± 0.37	3.17 ± 0.43	3.77 ± 0.37	4.09 ± 0.38
9	Grande Prairie, AB	54°39'	119° 06'	1065	2.92 ± 0.39	3.28 ± 0.38	4.43 ± 0.23	4.70 ± 0.25
10	Grande Prairie, AB	54°32'	117° 49'	825	1.36 ± 0.33	1.82 ± 0.40	4.22 ± 0.31	4.43 ± 0.28

11	Virginia Hills, AB	54°28'	115° 52'	1127	2.50 ± 0.44	2.86 ± 0.46	3.95 ± 0.27	4.43 ± 0.28
12	Swan Hills, AB	54°44'	115°18'	1064	2.04 ± 0.39	2.26 ± 0.40	3.86 ± 0.32	4.24 ± 0.36
13	Swan Hills, AB	54°42'	115°30'	1130	2.29 ± 0.39	2.90 ± 0.39	3.06 ± 0.46	3.35 ± 0.48
14	Judy Creek, AB	54°26'	115°35'	1097	3.18 ± 0.33	3.77 ± 0.31	4.09 ± 0.25	4.55 ± 0.29
15	Highwood, AB	50°40'	115°05'	1860	4.35 ± 0.30	4.52 ± 0.31	4.50 ± 0.13	5.00 ± 0.13
16	Diamond Hills, AB	52°37'	115°04'	976	1.73 ± 0.39	2.00 ± 0.43	3.75 ± 0.36	4.33 ± 0.36
17	Cypress Hills, AB	49°30'	110°15'	1160	2.15 ± 0.36	2.65 ± 0.41	3.00 ± 0.45	3.38 ± 0.43
18	Pelican Mountains, AB	55°38'	113°27'	915	2.38 ± 0.40	2.79 ± 0.43	3.70 ± 0.34	4.04 ± 0.34
19	Cameron Hills, AB	59°42'	117°59'	730	3.64 ± 0.33	4.27 ± 0.33	4.29 ± 0.28	4.71 ± 0.30
20	Watt Mountain, AB	58°42'	117°23'	590	3.00 ± 0.32	3.57 ± 0.35	3.75 ± 0.35	4.17 ± 0.35
21	Tetsa River, BC	58°40'	124°10'	760	_a	_	3.33 ± 1.67	3.33 ± 1.67
22	Jack Fish Creek, BC	58°32'	122°42'	455	3.88 ± 0.34	4.13 ± 0.39	4.13 ± 0.46	4.33 ± 0.49
23	Stone Mountain, BC	58°39'	124°46'	1179	_	_	4.20 ± 0.58	4.40 ± 0.68
	Mean				2.80	3.24	3.95	4.06

Putative Hybrid (HB)

24	Blue Ridge, AB	54°06'	115°32'	978	1.88 ± 0.40	2.08 ± 0.45	2.60 ± 0.37	3.00 ± 0.41
25	Chickadee Creek, AB	54°13'	115°54'	829	2.79 ± 0.34	3.58 ± 0.31	3.46 ± 0.35	4.04 ± 0.37
26	Virginia Hills, AB	54°16'	116°13'	978	2.32 ± 0.39	2.60 ± 0.40	3.12 ± 0.38	3.84 ± 0.39
27	Clear Hills South, AB	56°36'	119°42'	960	2.70 ± 0.33	3.43 ± 0.35	4.61 ± 0.20	4.91 ± 0.23
28	Wabasca Road, AB	55°32'	114°51'	670	2.04 ± 0.44	2.78 ± 0.47	1.33 ± 0.27	1.88 ± 0.30
29	Hotchkiss Road, AB	57°14'	118°16'	792	3.83 ± 0.21	4.22 ± 0.20	4.08 ± 0.29	4.58 ± 0.32
39	Chinchaga, AB	57°13'	118°16'	780	3.48 ± 0.32	3.76 ± 0.36	4.05 ± 0.33	4.38 ± 0.34
40	Hawk Hills, AB	57°38'	117°27'	732	3.68 ± 0.34	4.09 ± 0.33	4.22 ± 0.33	4.74 ± 0.33
	Mean				2.81	3.29	3.41	3.97
				Jack Pine (JP)				
30	Calling Lake, AB	55°25'	113°23'	640	1.58 ± 0.35	2.17 ± 0.40	2.38 ± 0.29	2.92 ± 0.29
31	Wandering River, AB	55°32'	112°18'	762	2.62 ± 0.39	3.05 ± 0.44	3.68 ± 0.40	3.91 ± 0.42
32	Tower Road, AB	54°55'	111°27'	701	2.08 ± 0.37	2.40 ± 0.39	2.17 ± 0.32	2.58 ± 0.37
33	La Corey, AB	54°25'	110°40'	579	2.58 ± 0.33	3.21 ± 0.36	1.92 ± 0.33	2.32 ± 0.38
34	Stony Mountain, AB	56°16'	111°36'	610	2.24 ± 0.36	3.00 ± 0.40	1.52 ± 0.25	2.26 ± 0.32

35	Smoky Lake, AB	54°04'	112°12'	610	1.80 ± 0.35	2.20 ± 0.40	1.60 ± 0.28	2.04 ± 0.33
36	Otter Creek, AB	56°42'	116°16'	700	3.29 ± 0.43	3.67 ± 0.43	3.86 ± 0.32	4.36 ± 0.31
37	Tall Creek, AB	57°57'	115°47'	340	2.72 ± 0.36	3.20 ± 0.37	1.16 ± 0.24	1.44 ± 0.27
38	Hay River, AB	59°04'	117°42'	332	2.74 ± 0.34	3.57 ± 0.34	1.65 ± 0.34	2.04 ± 0.40
	Mean				2.38	2.80	2.18	2.37
	Overall Mean				2.70	3.13	3.40	3.56

Note: [a] All tree seedlings were dead

Table 2-3. Analysis of variance for 6- and 12-month assessments of WGR score (0 - 6) and height growth (cm).

Source of variation	df	Mean squares				Expected mean squares
		WGR score		Height		
		6-month	12-month	6-month	12-month	
Blocks (B)	24	3.0	3.0	59.5	40.9	$\sigma_e^2 + k_{33}\sigma_{B\times R}^2 + k_{41}\sigma_B^2$
Rust sources (R)	1	85.3 ***	32.0 *	979.0 ***	730.1 ***	$\sigma_e^2 + k_{13}\sigma_{R\times P}^2 + k_{32}\sigma_{B\times R}^2 + Q_R$
B*R	24	4.1 *	5.4	36.8	57.6	$\sigma_e^2 + k_{31}\sigma_{B\times R}^2$
Groups (G)	2	131.9 ***	115.3 ***	883.3 ***	823.1 **	$\sigma_e^2 + k_{12}\sigma_{R\times P}^2 + k_{22}\sigma_P^2 + Q_G$
R*G	2	47.3 ***	29.1 *	78.5	11.5	$\sigma_e^2 + k_{12}\sigma_{R\times P}^2 + Q_{R\times G}$
Populations in G (P)	37	15.5 **	14.3 *	101.3 ***	127.2 ***	$\sigma_e^2 + k_{11}\sigma_{R\times P}^2 + k_{21}\sigma_P^2$
R*P	37	5.7 ***	6.9 **	25.8	30.8	$\sigma_e^2 + k_{11}\sigma_{R\times P}^2$
Error	1605	2.7	4.0	32.0	39.0	σ_e^2

Note: *, **, *** Significant at the 5%, 1 % and 0. 1% level, respectively.
For 6-month assessments: $k_{11} = 21.418$, $k_{12} = 19.229$, $k_{13} = 19.068$, $k_{21} = 42.836$, $k_{22} = 38.458$, $k_{31} = 34.389$, $k_{32} = 25.17$, $k_{33} = 34.389$, $k_{41} = 68.779$. For 12-month assessments: $k_{11} = 15.253$, $k_{12} = 13.946$, $k_{13} = 13.492$, $k_{21} = 30.506$, $k_{22} = 27.892$, $k_{31} = 24.293$, $k_{32} = 17.81$, $k_{33} = 24.293$, $k_{41} = 48.585$.

Table 2-4. Average disease scores of lodgepole pines, jack pines, and their hybrids to infection of two western gall rust (WGR) sources (WGR_{PL} and WGR_{PJ}).

Host	6 Months			12 Months		
	WGR_{PL}	WGR_{PJ}	Overall	WGR_{PL}	WGR_{PJ}	Overall
Lodgepole	3.95 a	2.80 a	3.37 a	4.06 a	3.24 a	3.65 a
Hybrid	3.41 b	2.81 a	3.11 b	3.97 a	3.29 a	3.63 a
Jack	2.18 c	2.38 b	2.28 c	2.37 b	2.80 b	2.59 b
All Hosts	3.40	2.70	3.05	3.56	3.13	3.43
All Hosts (Weighted)	3.44	2.70	3.06	3.66	3.15	3.36

Note: (1) Differences between WGR_{PL} and WGR_{PJ} at both 6-month and 12-month assessments are significant at $P < 0.05$ for each of host groups except for jack pine. Values within columns followed by the same letter are not significantly different from each other at $P = 0.05$, according to Tukey's multiple comparisons procedure. (2) All Host (Weighted) are the overall means calculated by using number of populations in each host group as weights.

Table 2-5. Height growth (cm) of lodgepole pines, jack pines, and their hybrids with infection of two western gall rust (WGR) sources (WGR_{PL} and WGR_{PJ}).

Host	6 Months			12 Months		
	WGR_{PL}	WGR_{PJ}	Overall	WGR_{PL}	WGR_{PJ}	Overall
Lodgepole	11.3 b	14.4 b	12.9 c	15.5 b	18.3 b	16.9 c
Hybrid	14.3 a	15.3 b	14.8 b	18.7 a	20.2 a	19.5 b
Jack	15.1 a	16.7 a	15.9 a	20.0 a	21.2 a	20.6 a
All Hosts	13.2	15.1	14.2	17.5	19.5	18.5
All Hosts (Weighted)	12.8	15.1	14.1	17.2	19.0	18.4

Note: (1) Differences between WGR_{PL} and WGR_{PJ} at both 6-month and 12-month assessments are significant at $P < 0.05$ for each of host groups except for jack pine. Values within columns followed by the same letter are not significantly different from each other at $P = 0.05$, according to Tukey's multiple comparisons procedure. (2) All Host (Weighted) are the overall means calculated by using number of populations in each host group as weights.

Table 2-6. Estimates of variance components, population differentiation (ρ_{ST}) and host-pathogen interaction (ρ_{HP}) for 6-month and 12-month assessments of western gall rust infection with the 40 populations being grouped and being ungrouped.

	Grouped		Ungrouped	
	6-month	12-month	6-month	12-month
σ_P^2	0.23	0.24	0.34	0.38
$\sigma_{R\times P}^2$	0.14	0.19	0.29	0.31
σ_e^2	2.68	4.01	2.68	4.01
ρ_{ST}	0.075 ± 0.019	0.054 ± 0.018	0.103 ± 0.026	0.080 ± 0.023
ρ_{HP}	0.620 ± 0.111	0.561 ± 0.152	0.537 ± 0.101	0.548 ± 0.121

Note: $\rho_{ST} = \sigma_P^2/(\sigma_P^2 + \sigma_{R\times P}^2 + \sigma_e^2)$ and $\rho_{HP} = \sigma_P^2/(\sigma_P^2 + \sigma_{R\times P}^2)$, where $\sigma_P^2, \sigma_{R\times P}^2$ and σ_e^2 are variance components for pine populations, spore source × pine population interaction and error, respectively.

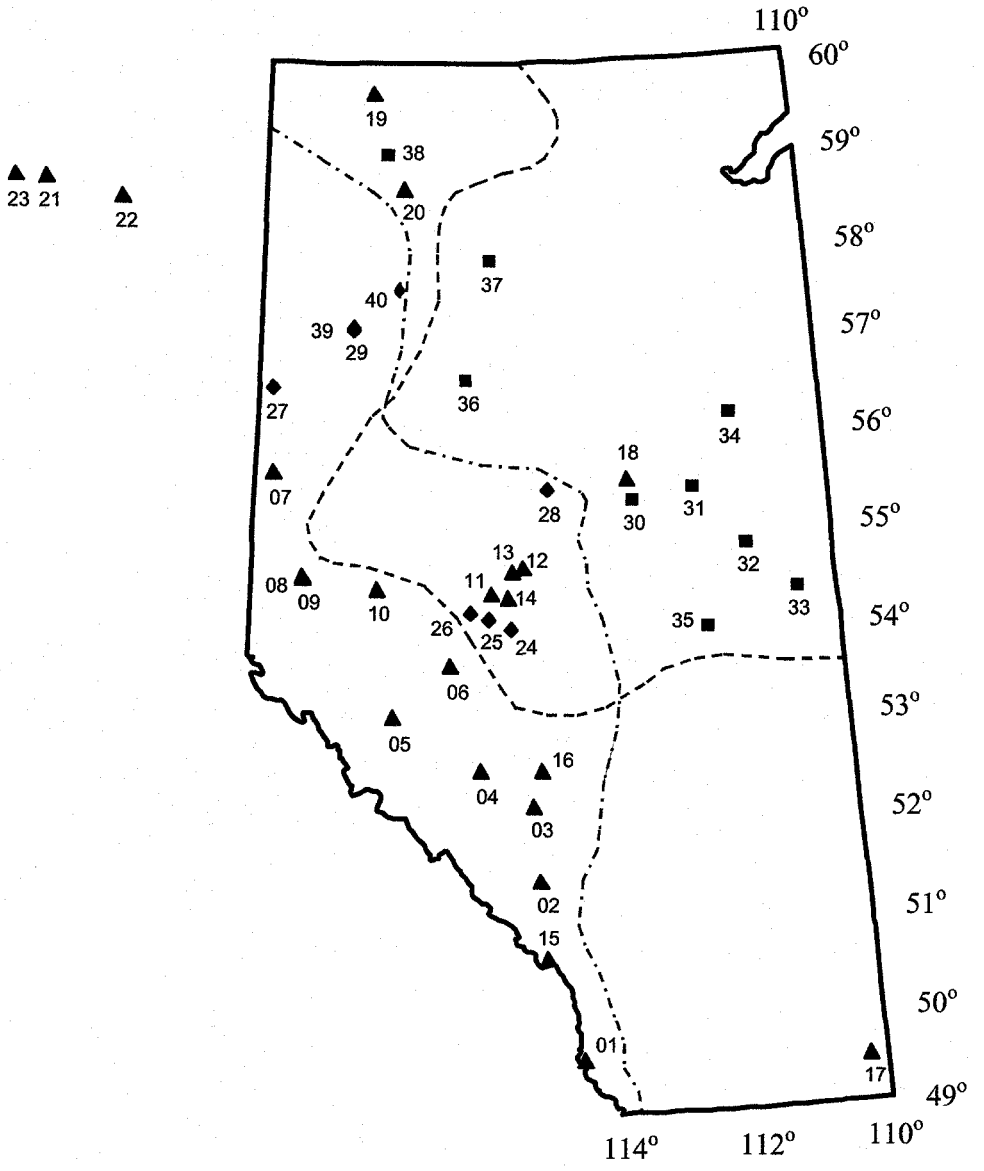

Figure 2-1. Geographic locations of 40 populations assigned to lodgepole pine (▲), jack pine (■) and hybrids (◆) based on their cone and seed morphology. The hybrid zone (the overlap and neighboring areas) is located in west central Alberta. The symbols for hybrid populations 29 and 39 are almost completely overlapped because the two populations are in their close proximity (< 2 km). Note: -·- is the eastern limit of lodgepole pine, and - - - is the western & southern limits of jack pine (from Moss (1949)).

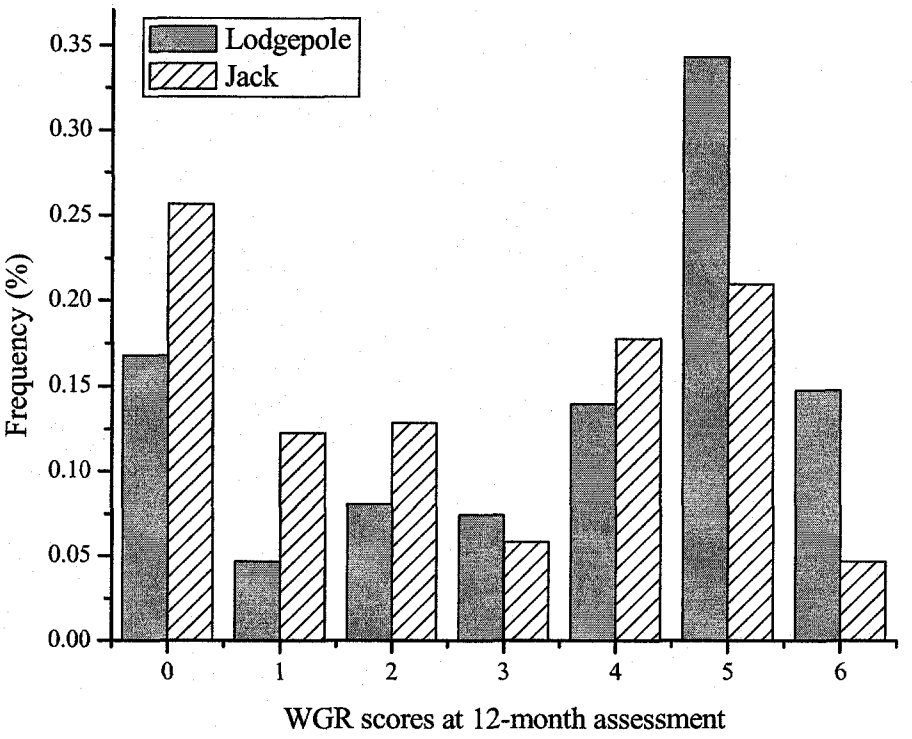

Figure 2-2. Distribution of seedlings for 12-month assessments of WGR scores (0-6).

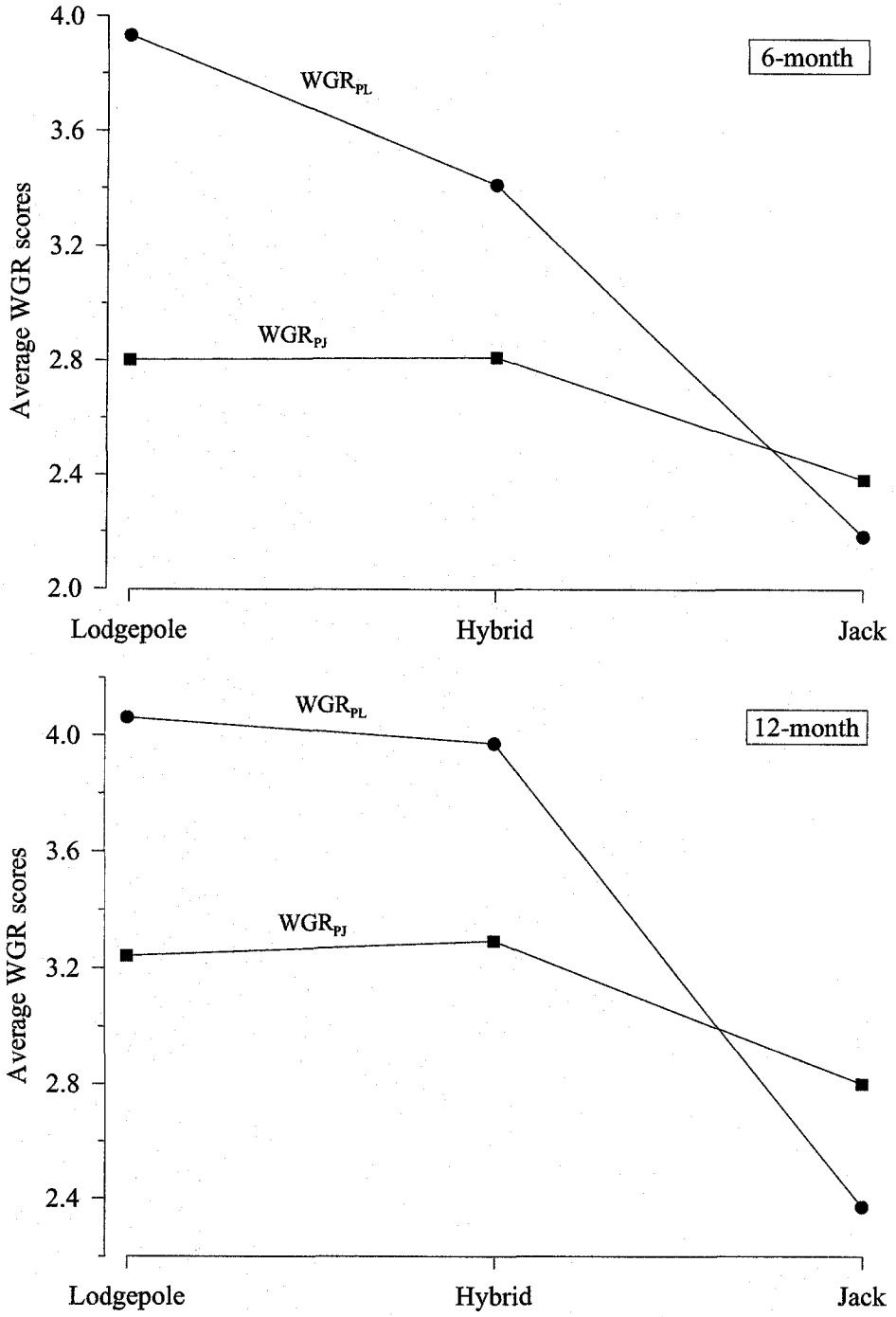

Figure 2-3. Average WGR scores of lodgepole pine, jack pine, and their hybrids to infection of two western gall rust sources (WGR_{PL} and WGR_{PJ}).

Chapter 3

Population Structure in *Pinus contorta* - *Pinus banksiana* Complex and Its Relation to *Endocronartium harknessii* Resistance

Part I Single-Locus Population Structure

1 Introduction

Natural hybridization between species with different adaptive norms is of evolutionary significance. It extends a species' gene pool and hence ecological range by introgression (Anderson 1949; Stebbins 1959), releases novel gene recombinations to be sifted by natural selection (Lewontin and Birch 1966; Arnold 1997) and serves as a dispersal mechanism (Potts and Reid 1988). One of the major issues in studying interspecific hybridization and introgression has been the origin of hybrid zones. Two alternative scenarios could explain the patterns of variation in hybrid zones and infer their origins (Mayr 1963; Harrison 1990). The first scenario proposed that a hybrid zone occurs within a series of populations in continuous contact of the two species (*primary intergradation*) in response to local environmental gradients or patchy environments. The other scenario implies that a hybrid zone is the result of *secondary contact* between species that are geographically isolated. When a hybrid zone evolved from the secondary contact,

it would be characterized by clines (Barton and Hewitt 1985) or patterns of "isolation by distance" (Wright 1943). Such patterns would not be expected in the primary intergradation scenario. However, patterns arising from primary and secondary contacts are often indistinguishable unless a zone of intergradation within a few hundred generations of secondary contact was observed (Endler 1977).

Lodgepole pine (*P. contorta* Dougl. ex Loud.) and jack pine (*P. banksiana* Lamb.) are the most northern and most widely distributed of the North American pines (Critchfield 1980; Rudolph and Yeatman 1982). With their wide natural ranges, lodgepole from the west and jack pine from the east meet and hybridize in central and northern Alberta and the southwest corner of the Northwest Territories (Moss 1949; Yeatman 1967; Scotter 1974; Critchfield 1985). Natural stands in these overlap regions and their neighborhoods are referred to as the *Pinus contorta* - *Pinus banksiana* complex (PCBC). Studies of morphology (Moss 1949; Pollack and Dancik 1985; Yang et al. 1999), terpene (Mirov 1956; Zavarin et al. 1969; Pollack and Dancik 1985), isozyme (Wheeler and Guries 1987) and chloroplast DNA markers (Wagner et al. 1991) have shown that stands in the PCBC hybrid zones would range from nearly pure jack pine through hybrid swarms to nearly pure lodgepole pine. While these studies have indicated varying levels of introgression and adaptation of the PCBC populations to the wide ecological amplitudes across the parental species' ranges, there is little agreement concerning the geographic distribution of the introgression among parental species and

hybrids. In discussing the biogeography of jack pine and lodgepole pine, Critchfield (1980, p.27-28) hypothesized that post-Pleistocene influence of jack pine to lodgepole pine would be restricted mostly to the regions of overlap (secondary contact) whereas, outside these regions, widespread but non-uniform jack pine influence would be the result of earlier contacts between the species during Pleistocene interglacials (primary intergradation). Critchfield's hypothesis would predict a weakening pattern of "introgression by distance" in the area that is larger than the hybrid zone. However, this prediction is yet to be tested.

In the present study, Critchfield's hypothesis was examined based on a survey of random amplified polymorphic DNA (RAPD) diversity in 40 populations sampled from a lodgepole pine-jack pine complex in west central Alberta and its neighboring areas. As diallelic markers, RAPDs have been shown to mostly inherit in a biparental dominant Mendelian fashion (e.g., Carlson et al. 1991; Rieseberg et al. 1993; Li and Yeh 2001). They are well suited for population genetic studies due to the availability of a large number of markers and to the random distribution of these markers over an organism's genome (e.g., Chalmers et al. 1992; Yeh et al. 1995; Fazekas and Yeh 2001). RAPDs are particularly attractive to conifer population geneticists because the problem of dominance can be avoided through the use of haploid megagametophytes, available in all conifer species. The objectives were (i) to assess RAPD diversity in lodgepole pine, jack pine and their hybrids, and (ii) to determine if there were patterns of "introgression by distance" as predicted under the secondary contact scenario in the PCBC

complex.

2 Materials and Methods

2.1 PCBC populations

Forty PCBC populations including 23 lodgepole pine, 9 jack pine and 8 putative hybrid populations in Alberta and British Columbia (BC) were sampled (see Chapter 2 for more detailed description of populations' geography and distribution). A population was assigned to a species or hybrid at the time of collection based on overall tree and stand appearance. This classification was subsequently corroborated using the most diagnostic cone and seed traits as suggested by Wheeler and Guries (1987). Each population was represented by a bulk collection of ten or more cones per tree from at least 15 trees per stand. The procedures for cone collection, seed extraction, and seed storage are described elsewhere in Chapter 2.

2.2 DNA extraction and amplification

A random sample of 30 seeds from each of the 40 seedlots was taken for DNA extraction. Prior to DNA extraction, the seeds were rehydrated overnight in distilled water. Seed coats were removed and the haploid female megagametophytes were separated from the embryos. Total DNA was extracted from each megagametophyte using a phenol/chloroform method modified from Lee and Taylor (1990). DNA concentration was quantified on a 0.7% agarose gel by comparison with a lambda DNA standard. Working samples were diluted to 0.2

- 0.4 ng/µl. PCR amplification of DNA was carried out in a GeneAmp 9600 Thermo-cycler (Perkin-Elmer). Amplification reactions contained: 10 mM reaction buffer (Perkin-Elmer), 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 2.5 mM MgCl2, 0.2 µM primer, 0.6 units of Taq DNA polymerase, 2 - 4 ng sample DNA, in a total volume of 20 µl. The reaction temperature regime was as follows: 92 °C for 2 min., 40 cycles of: 92 °C for 30s, 36 °C for 30s, 72 °C for 1 min., and then 72 °C for 8 min. Amplification products were loaded into a 1.4% agarose gel along with a lambda ladder and electrophoresis was performed at 70V for 5 h. Gels were stained with ethidium bromide and photographed under UV illumination.

Despite reports on the sensitivity of RAPD expression to experimental conditions (Smith et al. 1994; Harris 1995), I obtained clear, reproducible RAPD profiles through controlling a consistent amount of DNA per amplification (2-4 ng) and using high quality Taq polymerase. Primer screening also played a key role in obtaining reproducible RAPD profiles. For screening diagnostic primers, I used the seeds of three pure lodgepole pine and three pure jack pine populations sampled from allopatric regions in Saskatchewan (jack pine) and BC (lodgepole pine). These two groups of pure populations were referred as "Saskatchewan type" and "BC type" populations. Two hundred 10-nucleotide random primers purchased from the University of British Columbia (UBC) Biotechnology Laboratory were initially screened to identify ten UBC primers (Table 3-1) that consistently amplified 39 sharp and reproducible RAPDs over several independent

runs. These ten primers were then used for identification of species-specific RAPDs in the six pure lodgepole and jack pine populations and for subsequent assays of the 40 PCBC populations.

The inheritance of RAPDs in lodgepole pine was previously interpreted from segregation patterns of 60 to 90 megagametophytes of seeds from each of three maternal trees (Li and Yeh 2001). Seventy-two percent of RAPDs used here were covered by Li and Yeh's study. Chi-square analysis indicated that 53 of 803 (7%) RAPDs distorted significantly from a 1:1 Mendelian ratio. Due to their random nature, RAPDs are often assumed to generate markers from across the genome. Research on RAPD genomic maps by Li and Yeh (2001) indicated that these RAPDs generated from the same primers chosen in this study were either located on different linkage groups or far apart on the same linkage groups across the genome.

2.3 Data collection and analysis

Photographs taken from ethidium bromide stained agarose gel were used to score the RAPDs. Each RAPD was named by its primer number plus a hyphenated alphabet (*e.g.*, 061-A) to indicate its relative distance from the origin. Thus, RAPD 061-A was closer to the origin than RAPD 061-B. The distance from the origin corresponded to the size of a fragment (in bp) generated by the primer in question. A total of 39 RAPDs which were generated by the 10 UBC primers was scored: 036 A-B, 061 A-B, 162 A-E, 251 A-D, 256 A-E, 337 A-D, 428 A-C, 590 A-F, 635 A-E, 681 A-C. For each of the 39 RAPDs, each megagametophyte was scored for

the presence (1) or absence (0) of a RAPD frequency. Thus, there was a 30 × 39 matrix of ones and zeros for each of the 40 PCBC populations. Occasionally, the RAPDs of a few (usually 2-3) megagametophytes in a population were too faint to be scored and they were treated as missing values.

Frequencies of RAPDs were estimated in each population. Population genetic parameters including diversity (h), population differentiation (G_{ST}) for one-level and two-level hierarchies, and unbiased genetic distance (D) were estimated using POPGENE 1.32 (Yeh et al. 1999) based on Nei's (1973; 1978) algorithms. The Ewens-Watterson test for neutrality (Manley 1985) indicated that all 39 RAPDs were selectively neutral. The estimates of gene flow among the populations under Wright's (1943) island model were calculated from the G_{ST} estimates (Slatkin and Barton 1989). A dendrogram of all 40 PCBC populations was constructed from the matrix of Ds using the unweighted pair-group method with arithmetic average (UPGMA) as described in Nei (1987). The standard errors of all connecting branches were computed using the method of Ritland (1989).

Differences in frequencies of RAPDs between two "type" populations were tested using z-test. Only the RAPDs which were statistically different between two "type" populations and their ranges of frequencies were not overlapped were considered to be diagnostic markers.

Heterogeneity of RAPD frequencies among populations in each of the three groups (lodgepole pine, jack pine and hybrids) was analyzed using χ^2 test of a $2 \times r$ contingency table (r is 23 for lodgepole pine, 9 for jack pine, and 8 for

hybrid) (Workman and Niswander 1970). Two contrasts, the hybrids versus the average of lodgepole and jack pines, and lodgepole pine versus jack pine, based on the RAPD frequencies were constructed and tested using the CATMOD procedure of SAS (SAS for Windows 8.10; SAS Institute 1990).

To test Critchfield's hypothesis concerning the patterns of introgression within and outside the PCBC hybrid zone, we estimated Slatkin's (1993) index of 'genetic similarity' ($\hat{M}$) from the relationship under Wright's (1943) island model: $F_{ST} = 1/(4M + 1)$, where F_{ST} was a measure of genetic divergence between a pair of sampled populations. Note that Wright's F_{ST} is identical to the parametric value of Nei's G_{ST} when there are only two alleles at a locus as in our RAPD data. Geographic distance in kilometers (d) between the same pair of populations was also computed. Two analyses were carried out: (i) all 40 populations and (ii) 33 populations within or in the vicinity of the sympatric region [i.e., populations 17-23 (four outliers and three BC lodgepole pine populations) were excluded]. Thus, there were 780 (40 × 39/2) pairs of $\hat{M}$ and d values for analysis (i) and 528 (33 × 32/2) pairs for analysis (ii). Significance in the introgression by distance relationship can be tested statistically using a Mantel test (Mantel 1967). This test assesses whether $\log_{10}(\hat{M})$ is correlated with $\log_{10}(d)$. A null distribution is generated by randomizing rows and columns of one matrix while holding the other constant. Because entire rows (populations) are treated as a single unit, the Mantel test is more appropriate than alternatives which assume that each population pair is independent (Manly 1994).

A large, negative correlation coefficient between $\log_{10}(\hat{M})$ and $\log_{10}(d)$ would indicate a strong pattern of "introgression by distance". This holds reasonably well under a variety of population structure models (Slatkin 1993). There are two commonly used estimators of F_{ST}, $\hat{\theta}$ (Weir and Cockerham 1984) and $\hat{G}_{ST}$ (Nei 1973). While $\hat{G}_{ST}$ is more biased than $\hat{\theta}$ (Yang 1998), its use for calculating $\hat{M}$ avoids the problem associated with negative estimates of gene flow. Thus, $\hat{G}_{ST}$ was used to calculate the $\hat{M}$ value in our study.

3 Results

3.1 RAPD frequency and genetic variability

Thirty-nine RAPDs were scored on 30 megagametophytes of seeds from each of the six pure populations sampled in the allopatric regions. None of the 39 RAPDs was specific to either lodgepole or jack pines. However, frequencies of eight RAPDs (061-A, 256-A, 337-A, 337-D, 428-A, 590-A, 635-B and 635-D) were consistently homogeneous among the three populations sampled for each species, but markedly different between the two species. The frequencies of the eight RAPDs 061-A, 256-A, 337-A, 337-D, 428-A, 590-A, 635-B and 635-D calculated using all 90 megagametophytes from the three pure lodgepole populations were 0.258, 0.090, 0.455, 0.307, 0.114, 0.045, 0.101 and 0.966. The corresponding frequencies from the three pure jack pine populations were 0.818, 0.716, 0.078, 0.889, 0.876, 0.456, 0.578 and 0.422. The difference between the

species was significant for each of the eight RAPDs. Thus, these eight RAPDs were considered to be diagnostic markers for describing the differences among the 40 PCBC populations.

Frequencies of RAPDs for populations of lodgepole pine and jack pine were similar to those from the "type" populations (Table 3-2). Despite significant heterogeneity of frequencies across populations within each species at all the diagnostic RAPDs except for 061-A, the frequency ranges between the species were nearly non-overlapping. While the frequency ranges for hybrids were generally larger, there was a tendency of hybrids towards lodgepole pine. The contrasts of lodgepole versus jack pines were highly significant ($P < 0.001$) at all eight diagnostic RAPDs (Table 3-2). However, the expectation of hybrid intermediacy held only for two RAPDs (428-A and 635-B) with the remaining six RAPDs showing significant departures from the expectation.

Of the 39 RAPDs including the eight diagnostic markers, 14 (036-B, 061-B, 162-A, 162-B, 162-D, 251-C, 256-A, 337-A, 227-D, 428-A, 590-A, 590-B, 635-A and 635-B) were dimorphic in each of the 40 PCBC populations. Thirteen RAPDs (251-B, 251-D, 251-E, 256-B, 256-C, 256-D, 256-E, 428-C, 590-C, 590-D, 590-E, 590-F and 635-C) were monomorphic across all 40 populations. An additional RAPD, 162-E, was dimorphic only in population 34 at frequencies of 0.793 and 0.207. The remaining 11 RAPDs were fixed in varying numbers of populations: 036-A (5), 061-A (2), 162-C (9), 337-B (6), 337-C (6), 428-B (11), 635-D (14), 635-E (29), 681-A (13), 681-B (4) and 681-C (7).

A RAPD was considered polymorphic when both band present and null occurred irrespective of their frequencies. A RAPD was labeled rare when its frequency was less than 0.05. On average, jack pine populations exhibited higher levels of polymorphism and diversity than lodgepole and hybrid populations. However, RAPD diversity differed among populations within groups with the ranges of 0.085 - 0.190 for lodgepole pine, 0.144 - 0.165 for jack pine and 0.114 - 0.183 for hybrids, respectively. In all PCBC populations, the diversity estimates varied greatly among RAPDs as indicated by large standard deviations. Hybrid populations had fewer rare RAPDs (an average of 1.6 rare RAPDs per population) than lodgepole pines (an average of 3.0 rare RAPDs per population) and jack pines (an average of 2.3 rare RAPDs per population) (Table 3-3).

3.2 Population structure and genetic affinity among PCBC populations

The estimate of G_{ST} for all 40 PCBC populations was 0.247 for a one-level hierarchy, suggesting that about a quarter of the total RAPD diversity was due to difference among the populations. The rate of gene flow derived from the G_{ST} value under Wright's (1943) island model was about 1.5 migrants per generation per population. By expanding to two levels of hierarchy, we found $G'_{SC} = 0.1617$ (population differentiation within same species group) and $G'_{ST} = 0.1017$ (diversity among species groups. The estimates of G_{ST} for the three groups were appreciably lower, with population differentiation for the hybrids (G_{ST} = 0.168) and lodgepole pines (G_{ST} = 0.162) being greater than jack pines (G_{ST} = 0.155). The

corresponding estimates of gene flow were 2.5 for hybrids, 2.6 for lodgepole pines and 2.7 for jack pines.

Genetic distances estimated for the 780 pairs of the 40 PCBC populations ranged from 0.004 between populations 1 and 3 to 0.203 between populations 6 and 32 [the full set of (40 × 39)/2 genetic distances is available upon request]. On average, population 23 exhibited the lowest genetic distance (0.041) whereas population 32 had the largest genetic distance (0.138) from the other populations. The genetic distances among populations within the three groups averaged 0.034 for lodgepole pines, 0.039 for jack pines, 0.043 for the hybrids (Table 3-4). The among-group genetic distances were 0.103 for lodgepole-jack pines, 0.050 for lodgepole pine - hybrids and 0.077 for jack pine - hybrids. The UPGMA cluster analysis based on genetic distances uncovered two groups (Figure 3-1). The two groups were considered to be significantly different because the shaded bars measuring sizes of standard errors of the two main branches were less than half their branch lengths. The first group consisted of all jack pine populations and population 28, a hybrid population based on morphological classification. The second group included all lodgepole and the remaining seven hybrid populations. Populations 31 and 36 were originally designated as jack pines based on cone and seed morphology, but they were members of group II based on the UPGMA cluster analysis of RAPD variability. No further divisions within group II could be identified that separated lodgepole pine from the hybrids. In fact, the standard

error bars of connecting branches within either group were generally greater than half their branch lengths (the results not presented).

3.3 Introgression by distance

All 40 PCBC populations were included in analysis *i*. There were a total of 780 pairwise values of genetic similarity ($\hat{M}$) along with their geographic distances (*d*). The geographic distances ranged from 1.8 km between populations 29 and 39 to 1384.1 km between populations 17 and 23. The estimates of genetic similarity ($\hat{M}$) ranged from 0.37 between populations 6 and 32 to 18.26 between populations 1 and 3. Estimated correlation coefficient of log($\hat{M}$) on log(*d*) for all 780 population pairs was negative and small ($r = -0.1373$), but it was significantly less than zero ($P \leq 0.0240$). In analysis *ii*, four outlier lodgepole pine populations, populations 17–20 (Chapter 2), and three distant BC populations (21–23) were excluded. The exclusion of these seven populations resulted in a total of 528 pairs of $\hat{M}$ and *d* values. The estimated correlation coefficient was almost doubled (–0.2589) and this estimate was highly significant ($P \leq 0.0002$). The two scatter plots of pairwise $\hat{M}$ values against geographic distances in a logarithmic scale for analyses *i* and *ii* were produced, but only the plot for analysis *ii* is presented (Figure 3-2). The dashed line ($\hat{M} = 1$) in Figure 3-2 represents Wright's (1943) criterion to indicate the relative strengths of gene flow and random drift. Wright (1943) suggested that random drift would result in substantial local differentiation if $\hat{M} < 1$, but not if $\hat{M} > 1$. One hundred and thirty-two of the 528 $\hat{M}$ values

shown in Figure 3-2 were <1. In comparison, 173 of the 780 $\hat{M}$ values in analysis *i* were found to be <1. These small values ($\hat{M} < 1$) occurred in a wide range of geographic distances, from 24.4 km between populations 18 and 30 to 1166.7 km between populations 17 and 38.

4 Discussion

The moderate to high levels of RAPD diversity (based on all loci) observed in the PCBC populations (Table 3-3) are similar to those found in a RAPD survey of 15 *P. contorta* ssp. *latifolia* populations (Fazekas and Yeh 2001), and are in the middle to high ends of the ranges of other estimates reported from allozyme electrophoretic surveys for lodgepole pine (Wheeler and Guries 1982, $h = 0.116$; Dancik and Yeh 1983, $h = 0.184$; Yang and Yeh 1995, $h = 0.194$ for ssp. *latifolia*), jack pine (Dancik and Yeh 1983, $h = 0.115$; Ross and Hawkins 1986, $h = 0.192$; Gauthier et al. 1992, $h = 0.158$) and hybrids (Wheeler and Guries 1987, $h = 0.146$ with a range of 0.127 - 0.155). On average, the diversity estimated from this RAPD data would be expected to be at the lower end of the range because the mean number of alleles per RAPD locus (A = 1.38 - 1.62) was less than the number of alleles per allozyme locus (e.g., Dancik and Yeh 1983, A = 2.00 - 3.00; Wheeler and Guries 1987, A = 1.62 - 2.24). The maximum possible diversity is 0.50 (1/2) for two equally frequent alleles at a locus, 0.67 (2/3) for three equal frequent alleles at a locus, ..., and $(n\text{-}1)/n$ for n equal frequent alleles at a locus. However, it is well known (e.g., Brown and Weir 1983) that the value of gene

diversity depends on both allele "richness" (allele number) and "evenness" (allele frequency). In other words, the diversity for the two evenly distributed alleles would be higher than that for a multi-allelic case where one predominant allele is accompanied by a series of rare alleles (< 0.05). Furthermore, in this RAPD assay, null alleles across all PCBC populations could not be detected so that the overall monomorphism was probably underestimated.

The estimates of population differentiation (G_{ST}) for lodgepole pine and jack pine showed that more than 15% of the total genetic variation is among populations and the remainder resides within populations for each species. These estimates are among the highest obtained for *Pinus* species as cited by Ledig (1998). Three of his examples showed G_{ST} > 20% (G_{ST} = 32.0% for *P. cembra*, G_{ST} = 22.2% for *P. ayacahuite* and G_{ST} = 22.0% for *P. muricata*), but most studies cited including those for lodgepole and jack pines showed that the G_{ST} values were usually less than 10% and often less than 5%, confirming the conclusion drawn from a larger survey of conifer species by Hamrick et al. (1992). Nevertheless, none of these studies included populations in the sympatric hybrid regions. The high levels of population differentiation would be expected in the PCBC hybrid regions because gene exchanges among the PCBC populations might be limited by topographic, ecological, phenological or genetic barriers (Critchfield 1985).

The use of genetic distances between the 40 PCBC populations enabled the separation of most jack pines from lodgepole and hybrid populations (Figure 3-1). However, no further subdivisions could be identified to separate lodgepole pine

from the hybrid populations. In contrast, Wheeler (1981, p. 65-70) found in his allozyme survey that all four hybrid populations grouped with the jack pine populations whereas all lodgepole pine populations were clustered into a single group. While the present study and that of Wheeler (1981) could distinguish lodgepole from jack pines, neither could classify hybrids into a distinct group. The inability to differentiate hybrids from lodgepole pine would correspond to significant departures from the usual expectation of hybrid intermediacy observed in six out of the eight diagnostic RAPDs (Table 3-2). In fact, a close inspection of mean RAPD frequencies at these eight markers (Figure 3-3) indicates that hybrids are genetically more similar to lodgepole pine than to jack pine. Interestingly, previous greenhouse evaluation of the same PCBC populations for resistance to western gall rust (WGR) [*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka] also suggests that hybrids were more closely related to lodgepole pine in WGR resistance than to jack pine (Chapter 2).

What could account for the difference in the tendency of hybrids towards a different parental species observed in this study and Wheeler (1981)? It is quite obvious that different populations have been sampled by the two studies. While this study focused on sampling populations within or in the vicinity of the PCBC hybrid zone, Wheeler (1981) sampled a much wider range, particularly for lodgepole pine. The 15 lodgepole pine populations in his study included six populations from the west side of the Rocky Mountains in south BC and one U.S. population from Washington. These lodgepole pine populations would probably

be among the least related to the hybrid populations within or near the PCBC hybrid zone. On the other hand, even when the populations sampled by the two studies were in close proximity to each other, they could be quite different. For example, populations 18 and 30 were separated only by a distance of 24.4 km, but were assigned to the different species based on cone and seed morphology. As expected, these two populations had very limited gene exchange ($\hat{M} = 0.9$). The use of different markers, RAPDs by this study versus allozymes by Wheeler (1981), might also have contributed to the difference in sorting out hybrids though a valid comparison could be made only through a study of the same populations by the two sets of markers.

Results from this study suggest a mild trend of "introgression by distance" in the PCBC hybrid zone. With all 40 populations included, the trend was weak (the correlation coefficient was -0.1373). By excluding four outlier and three BC populations, the area covered by the remaining 33 populations was confined to the sympatric region and its surrounding areas and the trend of "introgression by distance" was substantially strengthened (the correlation coefficient was almost doubled) (Figure 3-2). While the exclusion of the seven populations led to a reduced number of population pairs (from 780 to 528), it was still a large number for determining the correlation. The increased strength of the trend of "introgression by distance" for the populations within or in the vicinity of the PCBC hybrid zone is consistent with the prediction under Critchfield's hypothesis that introgressive hybridization in this hybrid zone might have been through the

post-Pleistocene secondary contact, but the uneven, mosaic distribution of gene exchange outside of the zone would be the result of earlier contacts during Pleistocene interglacials.

An east-west cline was evident at 13 RAPDs (036-A, 036-B, 061-A, 162-B, 256-A, 337-D, 428-A, 590-A, 635-A, 635-B, 635-D, 681-B); frequencies of these markers were significantly correlated with longitude (results not presented). Such cline would be expected under the secondary contact scenario. Hybridization between lodgepole and jack pines followed by repeated backcrossing would probably have produced populations of various intermediate genotypes that lead to the genetic gradient as revealed at the 13 RAPDs. Under the primary intergradation scenario, the gradient would be less evident because of population responses to local, patchy environments.

There is an array of genetic, ecological and demographic factors influencing the observed pattern of introgression. In general, the pattern would be more evident in species with limited dispersal capabilities, small effective population sizes or in populations that are at or near the migration-drift equilibrium (Wright 1943; Slatkin 1993). It should be noted that the extent of gene flow may be overestimated in non-equilibrium populations particularly in those recently formed populations in which gene exchange has reduced considerably after initial colonization period (Pogson et al. 2001). In studying the patterns of gene flow in populations belonging to three subspecies of lodgepole pine, ssp. *contorta*, ssp. *latifolia*, and ssp. *murrayana*, Yang and Yeh (1995) found a weak

pattern of "isolation by distance" for *contorta*, but no pattern for *latifolia* and *murrayana*. This lack of strong pattern of "isolation by distance" in lodgepole pine was attributed to extensive seed and pollen dispersal (Critchfield 1980) as well as large effective population sizes (N_e = 2577 - 8419) estimated for the 66 lodgepole pine populations (Yang and Yeh 1995). Thus, the effect of migration likely overpowers that of random drift in lodgepole pine. The weak pattern observed in subspecies *contorta* could be due to its approximately linear distribution along the Pacific Coast (Yang and Yeh 1995). Such linear geographic structure is required to form the pattern of "isolation by distance" (Wright 1943). Judging from similar levels of population differentiation estimated for jack pine (Dancik and Yeh 1983; Ross and Hawkins 1986; Gauthier et al. 1992), there would have been little pattern of "isolation by distance" in this pine species as well.

Selections induced by topographical, ecological, phenological and genetic barriers would also affect the pattern of "introgression by distance" in the PCBC hybrid zone. The following factors would likely limit success of seed and pollen dispersal or pollination among the PCBC populations, thereby limiting gene exchanges and producing unfit hybrids. First, edaphic preference is known to be an important determinant of geographic distributions of PCBC populations, with jack pine being the more xerophytic, lodgepole pine the more mesophytic and tolerant of clay soils and bogs, and the hybrids occupying a wide range of intermediate sites (Yeatman 1967; Rudolph and Yeatman 1982). Second, lodgepole and jack pines could be easily crossed by hand, but the crosses could

produce about a third as many germinable seeds as intraspecific crosses (Critchfield 1980). Third, jack pine could flower 2-3 weeks earlier than lodgepole pine (Critchfield 1985). Despite possible production of relatively unfit hybrids under these selection pressures, frequent habitat disturbances such as widespread forest fire and abundant serotinous cones in both lodgepole and jack pines have provided favorable conditions for species mixing and hybrid survival (Wheeler and Guries 1987). Thus, the mild pattern of gene exchanges observed in the PCBC hybrid zone is likely a result of interactions among migration, random drift and selection induced by different barriers and habitat disturbances.

In conclusion, it is evident from this RAPD survey and previous studies (e.g., Moss 1949; Mirov 1956; Zavarin et al. 1969; Pollack and Dancik 1985; Wheeler and Guries 1987; Yang et al. 1999) that lodgepole and jack pines should remain as distinct species. However, the identification and classification of the PCBC hybrids would continue to be difficult because of (i) discordant assignments of hybrids and (ii) inconsistent tendency of hybrids to the parental species as shown in many morphological, biochemical and molecular studies. These and other difficulties in accurate classification of hybrids need to be overcome for confident interpretation of hybridization events and the interaction of hybrids with their biotic and abiotic environments. Mild but significant pattern of "introgression by distance" observed in this study of the PCBC hybrid zone signals that sufficient introgressive hybridization has occurred to allow for the infiltration of genes of one species into another through repeated backcrossing of hybrids to one or both

parental species after initial interspecific hybridization. Varying types of intermediate hybrids arising from the introgression would be potentially novel genotypes for evolution in these pines. Research is needed to further investigate whether the observed trend of introgression is related to the patterns of variation for the traits of adaptive importance in this hybrid zone.

5 Summary

Population structure of a lodgepole pine (*Pinus contorta* Dougl.) and jack pine (*P. banksiana* Lamb.) complex in west central Alberta and neighboring areas was studied by assessing random amplified polymorphic DNA (RAPD) variability in 23 lodgepole pine, nine jack pine and eight putative hybrid populations. Of 200 random primers screened, ten that amplified 39 sharp and reproducible RAPDs were chosen for the study. None of the 39 RAPDs was unique to the parental species. RAPD diversity ranged from 0.085 to 0.190 among populations and averaging 0.143 for lodgepole pine, 0.156 for jack pine, 0.152 for hybrids and 0.148 for all 40 populations. The estimated population differentiation based on G_{ST} was 0.168 for hybrids, 0.162 for lodgepole pine, 0.155 for jack pine and 0.247 for across all 40 populations. Cluster analysis of genetic distances generally separated jack pine from lodgepole pine and hybrids, but no division could be identified that further separated lodgepole pine from hybrids. Observed weak to mild trend of "introgression by distance" in the complex and its neighborhoods was consistent with the view that introgressive hybridization between lodgepole and jack pines

within and outside the hybrid zone might have been through secondary contact and primary intergradation, respectively.

Table 3-1. Sequences of the 10 University British Columbia (UBC) Biotechnology Laboratory 10-mer primers used for assessing genetic diversity in 40 PCBC populations. The number of RAPD fragments scored for each primer is presented in the last column.

UBC Primer	Sequence	Number of Scored Fragments
36	5'-CCCCCCTTAG-3'	2
61	5'-TTCCCCGACC-3'	2
162	5'-AACTTACCGC-3'	5
251	5'-CTTGACGGGG-3'	4
256	5'-TGCACTCGAA-3'	5
337	5'-TCCCGAACCG-3'	4
428	5'-GGCTGCGGTA-3'	3
590	5'-CCGGCATGTT-3'	6
635	5'-CTCAGCTCAG-3'	5
681	5'-CCCCCGGACT-3'	3
Total		39

Table 3-2. Frequencies of the 8 most diagnostic RAPDs in the 40 PCBC populations and 2 pure "type" populations.

Population	061-A	256-A	337-A	337-D	428-A	590-A	635-B	635-D
				Lodgepole pine (LP)				
1	0.200	0.250	0.690	0.276	0.033	0.267	0.103	1.000
2	0.321	0.250	0.379	0.241	0.000	0.207	0.148	0.889
3	0.300	0.207	0.633	0.167	0.033	0.133	0.133	0.933
4	0.233	0.138	0.767	0.333	0.000	0.033	0.167	1.000
5	0.172	0.000	0.467	0.267	0.033	0.033	0.000	1.000
6	0.233	0.035	0.321	0.071	0.000	0.167	0.000	1.000
7	0.379	0.071	0.696	0.348	0.067	0.103	0.000	1.000
8	0.367	0.033	0.533	0.300	0.100	0.069	0.067	0.933
9	0.267	0.214	0.500	0.200	0.185	0.069	0.133	1.000
10	0.214	0.037	0.233	0.233	0.033	0.172	0.200	0.967
11	0.407	0.133	0.600	0.167	0.167	0.567	0.000	0.000
12	0.345	0.000	0.267	0.300	0.103	0.100	0.167	0.900
13	0.241	0.267	0.414	0.345	0.167	0.233	0.267	0.767

14	0.300	0.667	0.433	0.367	0.000	0.800	0.167	1.000
15	0.393	0.207	0.900	0.400	0.100	0.276	0.400	0.967
16	0.267	0.138	0.333	0.133	0.103	0.200	0.000	1.000
17	0.071	0.067	0.467	0.167	0.000	0.300	0.033	1.000
18	0.333	0.138	0.690	0.207	0.138	0.100	0.167	1.000
19	0.464	0.167	0.333	0.467	0.167	0.000	0.207	1.000
20	0.333	0.200	0.333	0.333	0.167	0.867	0.067	0.900
21	0.267	0.233	0.533	0.367	0.200	0.067	0.035	0.931
22	0.333	0.214	0.552	0.241	0.133	0.033	0.100	0.833
23	0.310	0.071	0.500	0.233	0.100	0.467	0.067	0.967
Mean	0.293	0.162	0.503	0.268	0.088	0.229	0.114	0.912
			Putative hybrid (HB)					
24	0.690	0.400	0.233	0.567	0.500	0.267	0.367	0.833
25	0.267	0.300	0.767	0.300	0.267	0.600	0.276	0.897
26	0.241	0.222	0.467	0.133	0.067	0.552	0.207	1.000
27	0.379	0.103	0.621	0.276	0.069	0.724	0.138	1.000
28	0.962	0.600	0.067	0.733	0.467	0.533	0.267	0.333

29	0.241	0.107	0.467	0.400	0.133	0.300	0.067	1.000
39	0.167	0.133	0.267	0.267	0.133	0.067	0.036	0.964
40	0.393	0.233	0.667	0.167	0.100	0.133	0.200	0.933
Mean	0.418	0.262	0.445	0.355	0.217	0.397	0.195	0.870
			Jack pine (JP)					
30	1.000	0.933	0.100	0.800	0.267	0.867	0.400	0.500
31	0.929	0.241	0.300	0.267	0.333	0.500	0.276	0.828
32	0.867	0.833	0.000	0.867	0.767	0.667	0.500	0.233
33	0.862	0.867	0.035	0.793	0.700	0.833	0.567	0.200
34	1.000	0.889	0.033	0.800	0.500	0.933	0.517	0.690
35	0.821	0.821	0.000	0.828	0.533	0.655	0.393	0.286
36	0.815	0.733	0.333	0.267	0.133	0.633	0.138	0.724
37	0.929	0.889	0.000	0.833	0.533	0.724	0.400	0.267
38	0.897	0.833	0.000	0.690	0.500	0.733	0.233	0.267
Mean	0.902	0.782	0.089	0.683	0.474	0.727	0.380	0.444
			Pure lodgepole pine (BC "type")					
Mean	0.259	0.090	0.456	0.307	0.114	0.045	0.102	0.967

	Pure jack pine (Saskatchewan "type")							
Mean	0.816	0.721	0.078	0.889	0.876	0.456	0.578	0.422
	Chi Square (χ^2) tests for heterogeneity of RAPD frequencies across populations							
χ^2 (LP)	22.6	92.4***	75.5***	29.8	38.2*	207.1***	64.2***	351.2***
χ^2 (HB)	59.0***	30.8***	49.0***	38.2***	38.1***	49.1***	16.1*	92.2***
χ^2 (JP)	12.3	60.2***	51.9***	63.9***	39.0***	21.5**	20.0*	57.1***
χ^2 (Overall)	363.0***	465.2***	285.3***	260.9***	299.8***	429.0***	177.2***	598.7***
	Chi Square (χ^2) tests for two contrasts among the three pine groups							
χ^2 [HB vs. (LP+JP)]	35.4***	23.3***	28.8***	9.1**	0.1	4.0*	0.6	14.9***
χ^2 (LP vs. JP)	188.2***	253.5***	104.2***	125.9***	151.0***	177.6***	79.9***	198.2***

Note:. *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Table 3-3. Estimates of RAPD variability for the 40 PCBC populations

Population	Mean Number of alleles per RAPD locus	Polymorphic RAPDs		Rare RAPDs		RAPD Diversity	
		No.	%	No.	%	Estimate	Std. Dev.
			Lodgepole pine (LP)				
1	1.54	21	53.8	4	10.3	0.121	0.156
2	1.44	17	43.6	4	10.3	0.107	0.147
3	1.51	20	51.3	3	7.7	0.137	0.164
4	1.51	20	51.3	3	7.7	0.149	0.170
5	1.51	20	51.3	8	20.5	0.107	0.145
6	1.38	15	38.5	9	23.1	0.085	0.140
7	1.49	19	48.7	2	5.1	0.139	0.173
8	1.62	24	61.5	1	2.6	0.181	0.182
9	1.56	22	56.4	0	0.0	0.164	0.170
10	1.59	23	59.0	4	10.3	0.149	0.156
11	1.41	16	41.0	5	12.8	0.115	0.165
12	1.62	24	61.5	1	2.6	0.177	0.163
13	1.62	24	61.5	0	0.0	0.178	0.172
14	1.44	17	43.6	5	12.8	0.105	0.161
15	1.49	19	48.7	2	5.1	0.146	0.189

16	1.56	22	56.4	1	2.6	0.175	0.170
17	1.46	18	46.2	5	12.8	0.119	0.166
18	1.54	21	53.8	1	2.6	0.145	0.164
19	1.59	23	59.0	1	2.6	0.190	0.190
20	1.46	18	46.2	4	10.3	0.113	0.150
21	1.62	24	61.5	1	2.6	0.177	0.169
22	1.56	22	56.4	2	5.1	0.171	0.185
23	1.54	21	53.8	3	7.7	0.128	0.166
LP mean	1.52	20.4	52.4	3.0	7.7	0.143	0.166
			Putative hybrids (HB)				
24	1.59	23	59.0	2	5.1	0.182	0.192
25	1.56	22	56.4	1	2.6	0.150	0.168
26	1.54	21	53.8	0	0.0	0.145	0.164
27	1.51	20	51.3	2	5.1	0.114	0.146
28	1.62	24	61.5	2	5.1	0.183	0.187
29	1.49	19	48.7	2	5.1	0.130	0.165
39	1.62	24	61.5	2	5.1	0.176	0.180
40	1.54	21	53.8	2	5.1	0.134	0.153
HB mean	1.56	21.8	55.8	1.6	4.2	0.152	0.169
			Jack pine (JP)				
30	1.54	21	53.8	2	5.1	0.144	0.174

31	1.62	24	61.5	1	2.6	0.165	0.169
32	1.54	21	53.8	2	5.1	0.156	0.174
33	1.54	21	53.8	4	10.3	0.158	0.182
34	1.62	24	61.5	2	5.1	0.155	0.178
35	1.59	23	59.0	1	2.6	0.157	0.167
36	1.56	22	56.4	1	2.6	0.165	0.171
37	1.54	21	53.8	4	10.3	0.150	0.175
38	1.51	20	51.3	4	10.3	0.153	0.172
JP mean	1.56	21.9	56.1	2.3	6.0	0.156	0.173
Overall mean	1.54	21.0	57.5	2.6	6.6	0.148	0.168

Table 3-4. Averages of Nei's genetic distances within and between the three groups of PCBC populations. The ranges of the genetic distances are given in the parenthesis.

Group	Lodgepole pine	Hybrids	Jack pine
Lodgepole pine	0.034 (0.004 - 0.083)		
Hybrids	0.050 (0.008 - 0.129)	0.043 (0.006 - 0.097)	
Jack pine	0.103 (0.020 - 0.203)	0.077 (0.020 - 0.182)	0.039 (0.011 - 0.107)

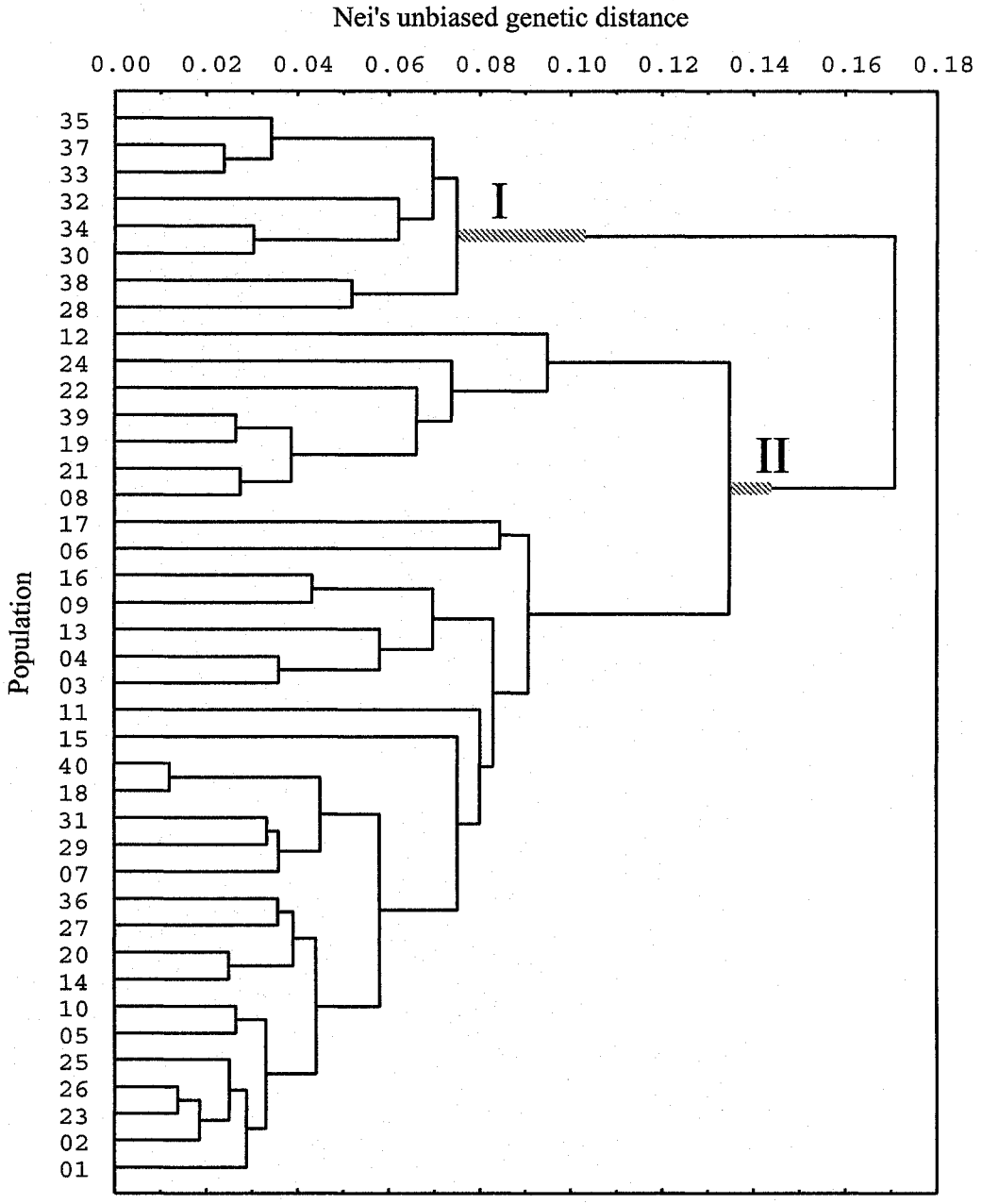

Figure 3-1. Dendrogram of 40 PCBC populations derived from Nei's genetic distances. Cluster analysis was performed using UPGMA method. The standard errors of two branches for Groups I and II were indicated by the shaded bars. The two groups were considered to be significantly different because the shaded bars on the two branches were less than half their branch lengths.

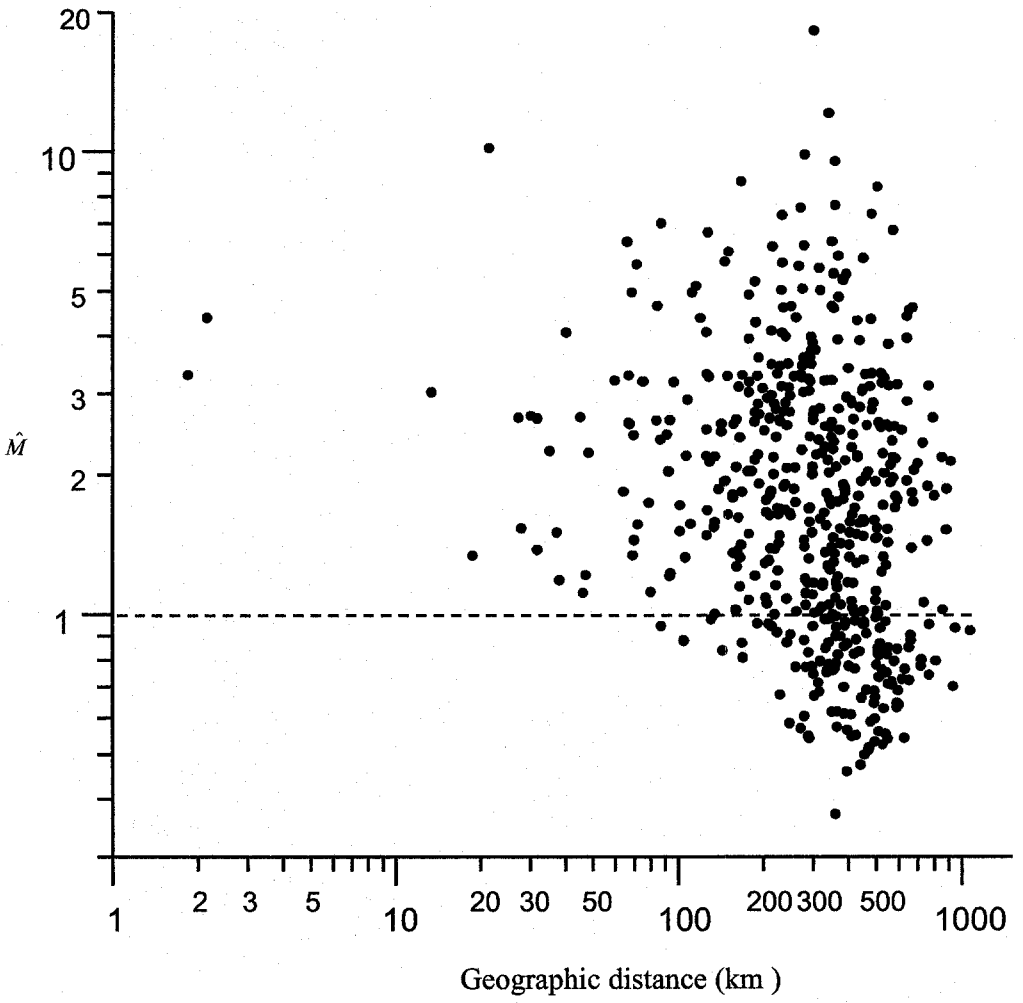

Figure 3-2. Scatter plot of the estimated genetic similarity ($\hat{M}$) against geographic distance (km) in a log-log scale for 528 pairs of 33 populations in a lodgepole-jack pine hybrid zone in Alberta. The estimated correlation coefficient (-0.2589) between Log_{10} ($\hat{M}$) and Log_{10} (geographic distance) was significantly less than zero, judging from Mantel test ($P \leq 0.0002$).

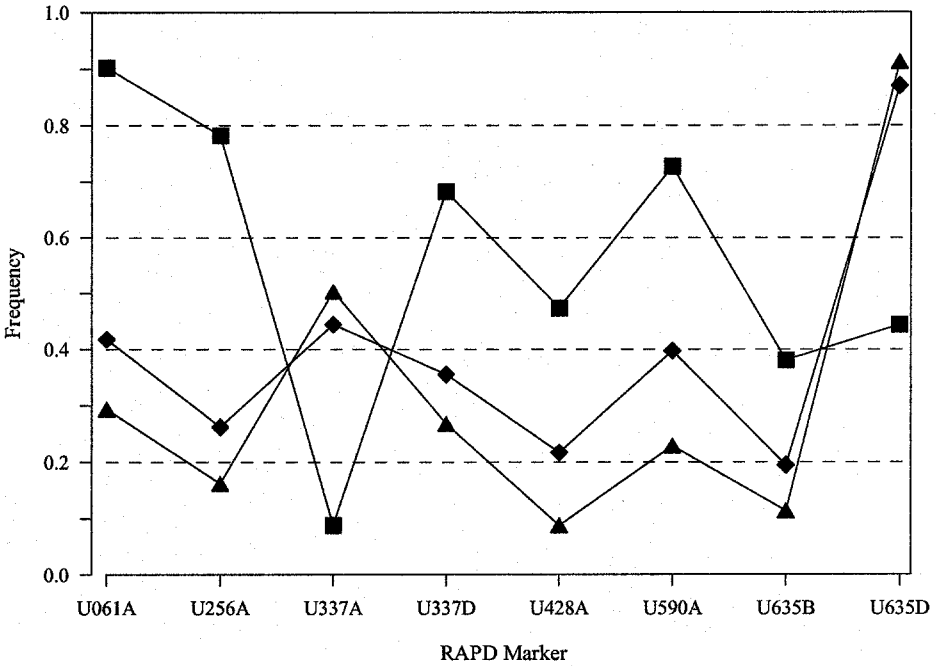

Figure 3-3. RAPD frequencies of eight diagnostic markers averaged across lodgepole pine (▲), jack pine (■) and hybrid (◆) populations.

Part II Multilocus Population Structure

1 Introduction

Genetic structure of coniferous forest trees has been studied extensively since the 1970's (Mitton 1983; Hamrick et al. 1992). Conifers with wide geographic range are generally characterized by having over 90% of genetic variability residing within populations, but with little population differentiation at individual allozyme loci (Hamrick et al. 1981; Yeh et al. 1994). If loci essentially behave as independent units, evolutionary processes can be effectively studied on a single locus basis and results averaged over loci. However, there are substantial reasons to suspect that not all evolutionary processes involve loci acting independently and that in many species multiple-locus associations are important. In such cases, it is inappropriate to use descriptive statistics of single-locus measures to summarize effects over loci. Epistatic selection, chance from population subdivision and founder effect, genetic hitch-hiking, mixing of two or more distinct gene pools, nonrandom mating, and mutation can produce linkage disequilibria among loci and further impede the rate at which such disequilibria decay (Hedrick et al. 1978; Barton and Clark 1990; Clegg et al. 1972; Brown et al. 1977; Smouse and Neel 1977; Muona and Szmidt 1985; Waller and Knight 1989; Laurie-Ahlberg and Weir 1979). Many studies conducted have revealed the prevalence of gametic disequilibria in conifers (e.g., Mouna and Szmidt 1985;

Roberds and Brotschol 1985; Yeh and Morgan 1987; Yang and Yeh 1993; Yeh et al. 1994; Fazekas and Yeh, 2001).

In hybrid zones where two or more distinct gene pools meet and hybridize, alleles at different loci derived from the same population tend to cluster together in the same individual (Barton and Hewitt 1989, Barton and Gale 1993). Therefore, association between loci, or linkage disequilibria would expect to be high in hybrid zones (Szymura and Barton 1991; Bert and Harrison 1988). Such associations should be halved in every generation and so must continually be replenished. Physical linkage between alleles that define the parental taxa, the influx of parental combinations of genes into the center of the hybrid zone, pre-mating and post-mating barriers, and selection against heterozygotes are often cited as causes to create and maintain multilocus associations (Barton and Gale 1993, Barton 1983, Moore and Price 1993). Study on multilocus structure is therefore essential in explaining the formation, maintenance, and properties of hybrid zones.

The two most widely distributed and economically important pine species in Canada, lodgepole pine (*Pinus contorta*) from the west and jack pine (*P. banksiana* Lamb.) from the east, meet and hybridize in west central Alberta (Moss 1949; Yeatman 1967; Critchfield 1985). Natural stands in the overlap and its neighboring areas are referred to as *Pinus contorta – Pinus banksiana* complex (PCBC). In the Part I of this chapter the population structure at single loci has been investigated for the parental and putative hybrid populations sampled from

PCBC area. Results showed that these populations had moderate to high variability and were characterized by larger population subdivision on single-locus level. However, allelic associations at different loci may arise through a variety of causes such as epistatic selection or hitch-hiking effects (Hedrick 1985), Wahlund effects (Nei and Li 1973; Feldman and Christiansen 1975), and genetic drift (Ohta 1982a, b). Although significant linkage disequilibria have been reported by several authors using samples from lodgepole pine in North America (Yang and Yeh 1993; Fazekas and Yeh 2001; Epperson and Allard 1987), little is known about the multilocus structure in the PCBC hybrid zone yet.

In this study, we conducted a multilocus survey of genetic structure among 40 populations, including 23 lodgepole pine, 9 jack pine and 8 putative hybrid populations, in the PCBC hybrid zone using RAPDs. The objectives were to investigate and compare the differences in multilocus structures between parental species and putative hybrids in the PCBC hybrid zone. The results could also provide insight into the processes of selection and dispersal in hybrid populations, which is essential for predictions concerning the evolutionary fate of hybrid zones.

2 Materials and Methods

2.1 Population samples, DNA extraction and amplification, Data collection

Forty PCBC populations (23 lodgepole pine, 9 jack pine and 8 putative hybrid populations) in Alberta and British Columbia (BC) were sampled. The location of each population and the procedures for cone collection and extraction,

and seed storage were described in Chapter 2. A random sample of 30 seeds from each of the 40 seedlots was taken for DNA extraction and amplification. The detailed procedures for DNA extraction and amplification and data collection were also described in Part I. A total of 39 RAPDs generated by the 10 UBC primers were scored and used in this study. Eight of them were considered to be diagnostic markers for describing the differences among the 40 PCBC populations (Part I).

2.2 Data analysis

With 39 RAPDs, we looked for a set of summary statistics that would adequately define the "average" multilocus association in each of the 40 PCBC populations. The coefficient of linkage disequilibrium between the *i*th allele at locus *j* and the *k*th allele at locus *l* is defined as $D_{ik,jl} = g_{ik,jl} - p_{ji}p_{lk}$, where $g_{ik,jl}$ is the frequency of the two-locus haploid genotype (gamete) and $p_{ji}p_{lk}$ is the product of the frequencies of the corresponding alleles. Several studies (Brown et al. 1980; Souza et al. 1992; Haubold et al. 1998) have described the average levels of linkage disequilibrium in haploid populations using various moments from the distribution of the number of heterozygous loci (K) in two randomly chosen gametes. The most useful is the variance of K (Brown et al. 1980),

$$\sigma_K^2 = \sum_j h_j - \sum_j h_j^2 + 2\sum_j \sum_{l>j} \sum_i \sum_k (2p_{ji}p_{lk}D_{ik,jl} + D_{ik,jl}^2)$$

where $h_j = 1 - \sum_i p_{ji}^2$ is Nei's (1973) gene diversity. If there is no disequilibrium among loci (i.e., all $D_{ik,jl} = 0$), the expected variance of the pairwise differences

reduced to

$$\sigma_K^2(0) = \sum_j h_j - \sum_j h_j^2 .$$

For a sample of n gametes, we calculated the sample variance of K (s_K^2) from the empirical distribution of the number of heterozygous loci based on the $n(n-1)/2$ pairs of gametes. s_K^2 is an estimate of σ_K^2 and thus a high value of s_K^2 is an indication of strong multilocus association. Following Brown et al. (1980), we tested the null hypothesis $H_0 : \sigma_K^2 = \sigma_K^2(0)$ by constructing an upper 95% confidence limit (L) for s_K^2. The average level of linkage disequilibrium in a population would be considered significant if $s_K^2 > L$.

The total and average variances in the number of heterozygous loci found in random pairs of gametes in each of three population groups (lodgepole pine, jack pine, and putative hybrid) were then partitioned into different components (Brown and Feldman 1981). The components of total variance are mean gene diversity (*MH*), variance of gene diversity (*VH*), single-locus Wahlund effect (*WH*), mean disequilibrium (*MD*), two-locus Wahlund effect (*WC*), and interaction between *MD* and *WC* (*AI*). The average variance can be split into five components: *MH*, *MD*, *AI*, variance of disequilibrium (*VD*), and covariance of interaction (*CI*). Thus, the combinations of these components could be used to characterize the multilocus associations. For example, high *MD*, positive *AI*, low *VD*, and low *CI* are usually associated with systematic, repeatable selection; founder effects might

be invoked when *VD* and *CI* are high; and population subdivision might be invoked when *WC* is high but *AI* is low (Brown et al. 1981).

To distinguish between the different evolutionary forces that may generate disequilibria, we employed Ohta's (1982a, b) method of subdividing linkage disequilibria into within- and between-populations components, which is similar to Nei's (1973) subdivision of the total genetic diversity into the same components. A series of five different components was calculated and compared (Ohta 1982a): D_{IS}^2 and $D_{IS}^{'2}$ are within-subpopulations components, D_{ST}^2 and $D_{ST}^{'2}$ are among-populations components and D_{IT}^2 is the total population component of disequilibria. Analytical calculations have shown that when migration is limited among subpopulations, e.g. when disequilibria are created mainly by genetic drift, then D_{IS}^2 is less than D_{ST}^2 and $D_{IS}^{'2}$ is greater than $D_{ST}^{'2}$ (Ohta 1982b). On the other hand, if systematic effects such as natural selection contribute to maintain allelic associations in the different populations, then D_{IS}^2 is greater than D_{ST}^2 and $D_{IS}^{'2}$ is less than $D_{ST}^{'2}$. The comparison of the values of the different components allows therefore an inference about stochastic and systematic causes of genetic disequilibria.

3 Results

Of the 40 variances (s_K^2) observed 29 exceeded their upper 95% confidence limits, implying the significance of cumulative two-locus gametic

disequilibria in the populations from PCBC hybrid zone (Table 3-5). The mean values of s_K^2 were higher in putative hybrid populations (6.3134) than in parental populations (6.1393 for lodgepole pine and 6.2033 for jack pine). Multilocus associations in 7 of 8 putative hybrid populations were statistically significant, which was higher than that in both lodgepole pine (74%) and jack pine (56%) populations.

The components of variance (Table 3-6) indicated that 27.6% and 21.1% of the total variance were due to the two-locus effects in the groups of parental species (lodgepole and jack pines, respectively). In contrast, the variance component of the two-locus effects in the group of putative hybrids was 38.9%. This suggests that two-locus association is more important in putative hybrid populations than in parental populations. The most prominent component was mean gene diversity (*MH*), which accounted for 50-67% of the variance. Putative hybrids had the intermediate single locus effects including mean gene diversity (*MH*), variance of diversity (*VH*), and Wahlund effect (*WH*). However, the difference among three groups for all the single-locus effects was relatively small.

Of the two-locus effects, however, the Wahlund effect (*WC*) and the interaction between mean disequilibrium and Wahlund effect (*AI*) were much higher in putative hybrid populations than that in both parental populations (Table 3-6). The relatively high mean disequilibrium (*MD*) components in all three groups of species indicate that the average population exhibited substantial multilocus structure in this hybrid zone. Among all two-locus components, the

variance of disequilibrium (*VD*) was the most important component in parental populations and the second important component in putative hybrid populations.

The variance of the 741 two-locus disequilibria was divided into a between- and a within-population components. The apportionment of the variation attributable to these components was similar among different two-locus combinations. Results (Table 3-7) showed that D'^2_{ST} (0.0002 and 0.0005 for lodgepole and jack pines, respectively) is much smaller than D'^2_{IS} (0.0493 and 0.0527 for lodgepole and jack pines, respectively), which implies that the maintenance of high level of disequilibria could largely be attributed to stochastic forces, i.e. random drift in small founding populations.

4 Discussion

Our analysis showed that the multilocus allele combinations observed in natural populations from PCBC hybrid zone are not random samples. Nonrandom associations of alleles were apparent in both parental populations and putative hybrid populations.

For both parental species, the variances of disequilibrium (*VD*) are the most important contributor to the two-locus effect, which accounts for 46.6% (lodgepole pine) and 46.0% (jack pine) (Table 3-6). The high values of *VD* mean that gametic disequilibrium is not systematic and the most common gametic types are dissimilar in different populations. It indicates that the multilocus associations present in the parental species in PCBC area are largely explained by founder

effects (Brown and Feldman 1981). This result is also supported by using Ohta's method of partitioning the total variance of disequilibrium (Table 3-7).

Several possible causes could be accounted for the high level of founder effect in PCBC area. First of all, populations in PCBC area are at the edges of the distribution of species and fragmented due to their demographical histories and ecological preferences. Limited gene exchange among populations leads to relatively small effective population size and large population subdivision. Populations are therefore more exposed to genetic drift. Linkage disequilibria due to large founder effects have been reported in many other studies (e.g. Fazekas and Yeh 2001; Kremer and Zanetto 1997; Yeh et al. 1994; Muona and Szmidt 1985). Secondly, population bottlenecks caused by glaciation or repeated fire events could be another reason. Gametic disequilibria generated by severe bottlenecks would remain in the population for a long time even after an increase in population size (Avery and Hill 1979). Biogeography of lodgepole pine and jack pine based on the fossil record suggested that both species have undergone population bottlenecks during the last glacial period (Chritchfield 1980; MacDonald et al. 1998). Moreover, both lodgepole pine and jack pine forests are fire-originated (Chritchfield 1980; Rudolph and Yeatman 1982) and have evolved as components of fire-prone ecosystems (Rowe and Scotter 1973). In North America boreal forest have a natural fire regime of large-scale, high-intensity fires that occur at long intervals in periods of severe drought (Heinselman 1981). Storage of most or the entire annual seed crop in serotinous cones in both pine species augments the

potential for rapid increases in population size in the event of fire (Chritchfield 1980). Yang and Yeh (1993) found an extensive multilocus association of alleles in lodgepole pine thought to be due to a severe population bottleneck (reduction of the effective population size) formerly experienced by the populations as a consequence of fire.

Like parental populations, founder effect is still an important contributor to two-locus disequilibria in the hybrid populations, which accounted for 30.8% of two-locus variation. Wahlund effect (*VC*), however, was the most important component among all two-locus effects in hybrid populations. As shown in Table 3-6, the two-locus Wahlund effect (*WC*) in hybrid populations is much stronger than that in the parental populations. *WC* accounts for 40.7% of two-locus variation in hybrid, while only accounts for 18.6% (lodgepole pine) and 16.0% (jack pine) in parental species. *WC* measures the pooling of genes from different populations that have different multilocus associations (Brown and Feldman 1981). The large value of *WC* indicates that allelic combinations exist in different frequencies in different populations.

Previous study (see Chapter 3, Part I) has suggested that the introgressive hybridization between lodgepole pine and jack pine within the hybrid zone may have been through one or more secondary contacts (the coming together of previously isolated and genetically distinct populations), which is largely consistent with the postglacial histories of lodgepole and jack pines (Rudolph and Yeatman 1982; Wheeler and Guries 1982; Critchfield 1985). Harrison and

Bogdanowicz (1997) summarized that when hybrid zones are the results of secondary contact; gametic disequilibria will be high initially and may gradually erode over time. This is especially true for the predominantly outcrossing species with large population size (Strauss et al. 1992). However, dispersal of the parental type into the zone, selection against hybrid or recombinant genotypes, and positive assortative mating will slow or prevent the disappearance of disequilibria (Harrison and Bogdanowicz 1997). In hybrid populations, high disequilibrium could occur and be maintained only if there were sufficient migration into the populations and if mating and survivale of hybrids were not random. Apparently, migration of parental genotypes into hybrid populations has played the most important role in maintaining the disequilibrium in hybrid populations in this study by the fact that two-locus Wahlund effect was 2.4 times higher than that in parental species. That most disequilibria are significant suggests that these parental genotypes are not freely mixing in hybrid populations. Biologically, the nonrandom mating and survival of hybrids could also be expected since natural hybridization in the PCBC area is restricted by differing ecological preferences, internal reproductive barriers, and difference in flowing time (Critchfield 1985). It has been reported that hybridization between lodgepole pine and jack pine results in relatively unfit individuals in the earliest generations (Critchfield 1985). Many lodgepole × jack pine hybrids produced larger quantities of aborted pollen grains, less than half the size of normal grains (Saylor and Smith 1966; Righter and Stockwell 1949). One or more natural hybrids in Alberta produced about 50%

aborted pollen (Moss 1949) and trees with up to 14, 27, and 28% aborted pollen were present in three stands in and near PCBC hybrid zone (Pollack 1980). Critchfield (1985) reported that jack pine flowers 2-3 weeks earlier than lodgepole pine in a plantation. Due to the nonrandom mating and survival, alleles derived from parental populations or species tend to cluster together in the same individual (Barton and Gale 1993), which create and maintain strong Wahlund effect and thereby retain high level of linkage disequilibrium.

High *MD*, positive *AI*, low *VD*, and low *CI* are usually cited as evidences of systematic and repeatable selection (Brown and Feldman 1981). Our results, however, showed that *VD* was higher than *MD* and *WC* is the most prominent two-locus effects in hybrid populations (Table 3-6). This indicates that effects of migration and random drift are more important than systematic selection effects in maintaining the multilocus association found in the hybrid populations. Ohta's analysis also confirmed the less importance of selection: among-population component (D'^2_{ST}) is much smaller than within-population component (D'^2_{IS}) in hybrid populations (Table 3-7).

Selection models in hybrid zones can be classified into (1) exogenous selection (fitness defined in relation to the environment) and (2) endogenous selection (fitness defined by within-genome interactions such as heterozygote disadvantage or epistasis, and being independent of environment) (Moore and Price 1993). In reality, natural hybrid zones may well include both kinds of selection. Nevertheless, the relative predominance of either type has been the

subject of considerable debate in the hybrid zone literature. Reviews of studies of hybrid zones (Barton and Hewitt 1985) concluded that most are maintained by selection against hybrids independent of the environment, i.e., they are tension zones (key 1968). However, there is also substantial evidence of an important role for exogenous selection in hybrid zones (Arnold 1997; Harrison 1990). In PCBC area, Critchfield (1985) observed that hybrids generated relatively unfit individuals in the earliest generations. This might be the indication that systematic endogenous selection against heterozygotes was invoked as a general explanation for the occurrence of linkage disequilibrium in hybrid populations in the early stages. With the increase of generations, however, hybrids repeatedly backcrossed with one or both parental species and hybrid populations actually consisted of successive backcrosses which may have better fitness. Endogenous selection thus tends to be weak and non-systematic exogenous selection due to microenvironments might become more important in maintaining the multilocus associations.

Theoretically, high levels of two-locus disequilibrium could be estimated if the loci involved are linked. Although detailed linkage maps for lodgepole pine and jack pine are still lacking, of the 39 neutral RAPDs scored in this study 28 were shown on the gene maps of lodgepole pine (Li, 1998) and either not physically linked or far apart. In addition, it could be expected that jack pine might have similar linkage groups due to the genetic similarity between these two related species. Therefore, the gametic disequilibria in the samples of this study were

mostly unique and developed between independent loci.

The population differentiation in terms of G_{ST} has been reported to be higher in PCBC than that estimated in other studies (See Part I). Our multilocus analysis further confirmed this finding. The interaction between *MD* and *WC* (*AI*) is positive but small, accounting for only 1% to 3% (Table 3-5). A positive value of *AI* indicates that the correlation of alleles between populations is repetitive of the pattern within populations (Brown and Feldman 1981). High *WC* with low *AI* is the characteristics of population subdivision defined by Brown and Feldman (1981). The high levels of overall population differentiation would be expected in the PCBC hybrid regions because gene exchanges among the PCBC populations might be limited by topographic, ecological, phenological or genetic barriers (Critchfield 1985).

5 Summary

Multilocus population structure was investigated from 40 populations (23 lodgepole pine, 9 jack pine, and 8 putative hybrids) in lodgepole pine – jack pine complex using random amplified polymorphic DNA (RAPD) markers. Significant two-locus gametic disequilibrium was found in 29 of 40 populations studied. Putative hybrids have stronger multilocus association than parental populations. Wahlund effect accounts for 40.7% of two-locus variation in hybrids, while only accounting for 18.6% (lodgepole pine) and 16.0% (jack pine) in parental species. Wahlund effect and founder effect are the two prominent forces in hybrid populations for maintaining the linkage disequilibria from decay with time.

Founder effect plays the most important role in the existence of linkage disequilibria in parental populations in PCBC hybrid zone, which accounted for 46% of two-locus variation. Contribution of epistatic natural selection to the multilocus structure might also be involved in hybrid populations, but smaller than effects of migration and random drift. Study of multilocus population structure confirmed the existence of large population differentiation and gene diversity which were found in the previous single-locus analysis.

Table 3-5. Estimates of multilocus associations in 40 populations from PCBC area (s_K^2 and $\sigma_K^2(0)$ are the observed and expected variances of the number of heterozygous loci between two randomly chosen gametes, respectively. L is the upper 95% confidence limit for s_K^2.

Populations	s_K^2	$\sigma_K^2(0)$	L	Significance
		Lodgepole Pine (LP)		
1	5.0262	3.1680	4.9029	*
2	4.9426	2.8875	4.5281	*
3	6.3079	3.6060	5.4340	*
4	6.8679	3.9006	5.9094	*
5	5.2738	2.9828	4.5918	*
6	2.5123	2.1113	3.3217	
7	4.9444	3.2755	5.3828	
8	7.3867	4.5010	6.9109	*
9	6.3832	4.0527	6.3636	*
10	7.9053	3.4974	5.4530	*
11	6.1221	2.8072	4.4043	*
12	7.3920	4.5907	7.1080	*
13	7.7608	4.3696	6.9119	*
14	3.8460	2.6603	4.0522	
15	6.4147	3.5056	5.4911	*

16	5.7468	4.3818	6.6847	
17	3.9020	2.9028	4.4259	
18	6.2845	3.7778	5.7656	*
19	7.4626	4.6050	7.0687	*
20	4.5851	3.0566	4.5852	
21	8.5618	4.6205	7.0976	*
22	7.6885	4.3183	6.6737	*
23	7.8857	3.2944	5.0999	*
LP mean	*6.1393*	*3.6032*	*5.5725*	
	Putative Hybrid (HB)			
24	6.4496	4.2114	6.4148	*
25	6.0479	3.8086	5.8129	*
26	5.6281	3.5070	5.5546	*
27	5.6050	3.1590	4.8616	*
28	8.7563	4.2584	6.7357	*
29	5.6571	3.2432	4.9508	*
39	7.3102	4.4570	6.8452	*
40	5.0532	3.5915	5.4530	
HB mean	*6.3134*	*3.7795*	*5.8286*	
	Jack Pine (JP)			
30	5.4095	3.6283	5.5386	

31	8.1473	4.1235	6.4255	*
32	7.2523	3.7418	5.7112	*
33	5.6814	3.8668	5.8909	
34	5.1735	3.6912	5.7097	
35	7.0393	3.8617	6.0628	*
36	6.4530	4.1364	6.3918	*
37	4.6372	3.3524	5.7098	
38	6.0364	3.7157	5.7453	*
JP mean	*6.2033*	*3.7909*	*5.9095*	

Note: * Significant at 5% level

Table 3-6. Components of variance of multilocus association in hybrid zone

	Lodgepole	Jack	Putative hybrid
Single locus effect			
Mean gene diversity (MH)	3.6110	3.8075	3.7733
Variance of diversity (VH)	0.4311	0.2927	0.4025
Wahlund effect (WH)	0.5280	0.3787	0.4683
Total	4.5701	4.4789	4.6441
Two-locus effect			
Mean disequilibrium (MD)	1.0749	0.6430	1.0055
Wahlund effect (WC)	0.5765	0.4680	1.7072
Interaction between MD and WC (AI)	0.0902	0.0869	0.2404
Variance of disequilibrium (VD)	1.4460	1.3414	1.2938
Covariance of interaction (CI)	-0.0826	0.3773	-0.0501
Total variance (MH + VH + WH + MD + WC + AI)	6.3115	5.6768	7.5973
Average variance (MH + MD + AI + VD + CI)	6.1393	6.2561	6.2630

Table 3-7. Ohta's variance components of linkage disequilibrium (averaged over all pairs of loci)

	Within subpopulation components		Between subpopulation components		Total population component
	D^2_{IS}	D'^2_{IS}	D^2_{ST}	D'^2_{ST}	D^2_{IT}
Lodgepole pine	0.0011	0.0493	0.0485	0.0002	0.0496
Jack pine	0.0011	0.0527	0.0518	0.0005	0.0532
Putative hybrid	0.0011	0.0575	0.0571	0.0008	0.0583

Part III Pattern of Introgression and Resistance to WGR

1 Introduction

Western gall rust (WGR) caused by *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka is a widespread and damaging rust of lodgepole pine (*P. contorta* Dougl. ex Loud.) and jack pine (*P. banksiana* Lamb.) as well as of several other hard pines in North America. Ziller (1974) and Van der Kamp and Spence (1987) described WGR as one of the serious diseases of lodgepole pine in British Columbia. Bella (1985), Hiratsuka and Maruyama (1985), and Powell and Hiratsuka (1973) reported serious damage by WGR in plantations and in natural stands of lodgepole pine in Alberta. Lodgepole pine and jack pine differ in their resistance to WGR. Our previous greenhouse inoculation study (Chapter 2) suggested that both pine species were more susceptible to their own rust sources, causing significant rust source × host group interactions.

Natural hybridization between the lodgepole pine and jack pine has been documented by Critchfield and others (e.g., Critchfield 1980, 1984, 1985; Wheeler and Guries 1987). These two closely related pine species occur sympatrically and hybridize across much of central and northern Alberta and the south-west corner of the Northwest Territories of Canada. Numerous morphological, biochemical and molecular studies (Moss 1949; Mirov 1956; Zavarin et al. 1969; Pollack and Dancik 1985; Wheeler and Guries 1987) have shown that stands in this *Pinus contorta* - *Pinus banksiana* complex (PCBC) ranged from nearly pure jack pine

through hybrid swarms to nearly pure lodgepole pine, suggesting varying levels of introgression and adaptation to the wide ecological amplitudes across the parental species' ranges.

Plant host-pathogen interactions in natural populations are subject to the effects of various genetic, ecological, epidemiologic, and historical factors (May and Anderson 1983). The literature suggests that interspecific introgression might have played a significant role in the evolution of host-pathogen interaction on tree species. For examples, Whitham (1989) showed that clinal variation in resistance to aphid (*Pemphigus betae*) in cottonwood (*Populus* spp.) is genetically based and related to the level of hybridization. Resistance of loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii*) to fusiform rust (*Cronartium fusiforme*) has been attributed to the influence of shortleaf pine (*Pinus echinata* Mill.) (Hare and Switzer 1969; Florence and Hicks 1980; Dorman and Keith 1976). A positive correlation of resistance to spruce budworm (*Choristoneura fumiferania* (Clements)) with increasing genetic introgression of red spruce (*Picea rubens* Sarg) into black spruce (*Picea mariana* (Mill.) BSP) was reported by Manley and Fowler (1969). Introgression can result in a transfer of new genes or gene complexes across the usual species boundaries through repeated hybridization and backcrossing (Stebbins 1959; Heiser 1973; Levin 1975). Host-pathogen dynamics could be altered due to introgression of resistance genes or gene complexes followed by selections.

Little is known about the evolutionary significance of genetic introgression in relation to pathogen resistance in lodgepole and jack pines (Critchfield 1980; Rudolph and Yeatman 1982). Wu et al. (1996) found a strong relationship between resistance of lodgepole pine to pest infection and provenance distance to the western limit of jack pine, suggesting that the observed geographic clines might represent a genetic gradient of introgression from jack pine. Yang et al. (1997) observed an east-west trend for WGR resistance for lodgepole pine in two lodgepole pine breeding regions in Alberta. However, neither jack pine nor hybrid populations were sampled in these studies. In addition, morphological markers are limited in determining the extent and pattern of introgression due to their unknown genetic basis (Rieseberg and Ellstrand 1993). These markers may also be functionally or developmentally correlated, thus reducing the total information content of each trait.

The development of molecular genetic markers has greatly facilitated studies of hybridization and introgression. Unlike morphological characters, molecular markers are independent and have simple modes of inheritance and expression. Also, the number of potentially available molecular markers far exceeds the number of morphological characters. Moreover, many molecular markers have apparent selective neutrality. Thus, using molecular markers is much more likely to properly detect patterns and levels of introgression (Rieseberg and Ellstrand 1993). In the study of introgression between lodgepole and jack pines using isozyme markers and morphological traits, Wheeler (1981) found that a

relatively precise numerical estimate of gene flow can be attained by using gene frequency data, although certain sets of morphological traits (corn and seed traits) may provide good estimates of gene flow.

In this study, the evolutionary effect of introgression on pine-rust interaction in PCBC hybrid zone was investigated by combining the results from a greenhouse inoculation study with the genetic information revealed by RAPD markers. The specific objectives were (1) to document the extent and geographic pattern of introgression; and (2) to exam the effect of introgression on resistance to WGR.

2 Materials and Methods

2.1 Study populations and reference populations

Cones were collected from a total of 23 lodgepole pine, 9 jack pine, and 8 putative hybrid populations in Alberta and British Columbia (Table 3-8). The detailed procedures in cone collection, seed extraction and germination were provided elsewhere (Chapter 2). Parental populations and putative hybrids (intermediate taxa) were pre-classified based on overall tree and stand appearance and diagnostic cone/seed traits as suggested by Wheeler and Curies (1987). In addition, 3 pure jack pine and 3 pure lodgepole pine populations sampled from allopatric regions in Saskatchewan (jack pine) and British Columbia (lodgepole pine) were used as reference populations for screening primers and estimating genetic admixtures of each study population.

2.2 Spore sources, inoculation, and greenhouse assessments

Inoculation on seedlings using two different western gall rust (WGR) sources, one from lodgepole pine in Alberta (WGR_{PL}) and the other from jack pine in Manitoba (WGR_{PJ}), was conducted in a greenhouse under controlled environmental conditions. These two spore sources were chosen because they showed distinct random amplified polymorphic DNA (RAPD) profiles based on a survey of geographic variation among WGR isolates across Canada (Li 1998). WGR severity scores and height growth were measured when the seedlings were 6 and 12 months old. See Chapter 2 for detail information.

2.3 DNA extraction and amplification

DNA was extracted from a random sample of 30 megagametophytes from each of 46 populations (40 study populations from PCBC and 6 "type" populations from BC and Saskatchewan), and amplified using the methods described in Part I. A total of 39 RAPDs generated by 10 UBC primers was scored. Eight RAPDs (061-A, 256-A, 337-A, 337-D, 428-A, 590-A, 635-B, and 635-D), with consistently homogeneous allele frequencies among the 3 "type" populations sampled for each species and markedly different allele frequencies between the two species, were regarded as diagnostic markers.

2.4 Data analysis

To quantify and compare the nature of the differences between host groups by using morphological data (height growth and WGR severity at 6- and 12-month

assessments) and molecular data (frequencies of 39 RAPDs), canonical discriminant analysis was conducted for each data set using SAS Proc CANDISC (SAS Institute Inc. 1990).

The relative contributions from each parental species to the investigated populations were estimated using a least-squares procedure developed by Roberts and Hiorns (1965) and Elston (1971) and used by Wheeler and Guries (1987) in his study of introgression. The calculated index of gene migration or genetic admixture (m) is considered an estimate of the proportion of genes in a population derived from the reference taxa. Suppose there are $p = 2$ reference taxa (lodgepole and jack pines) and for each we have frequencies of the same $k = 8$ diagnostic RAPDs, Let $\mathbf{X}^{(0)} = \{\mathbf{x}_{ij}^{(0)}\}$ be a $k \times p$ matrix, $\mathbf{x}_{ij}^{(0)}$ being the estimate of the ith allele frequency in the jth reference taxon. Let the $k \times 1$ vector $\mathbf{y}^{(0)}$ have as its elements the corresponding allele frequencies in the putative hybrid population. $\mathbf{X}$ is a $k \times (p-1)$ matrix whose jth column is $\mathbf{x}_j^{(0)} - \mathbf{x}_p^{(0)}$, and $\mathbf{y} = \mathbf{y}^{(0)} - \mathbf{x}_p^{(0)}$. Thus the least-squares estimate is given by

$$\mathbf{m} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y}$$

and

$$m_p = 1 - \sum_{j=1}^{p-1} m_j$$

provided $\mathbf{X}'\mathbf{X}$ is non-singular, where m_j ($j = 1, \ldots, p$) are the genetic admixtures of the hybrid population expressed as the proportion of genes derived from the jth parental species. Since all estimated m_j are positive in this study, therefore we stop here. Otherwise, $\mathbf{m}$ should be recomputed with the smallest m_j set equal to 0. This

process should be repeated so long as there remains an element in **m** that is negative (Elston 1971). All calculations were made as the proportion of genes derived from jack pine.

An empirical distribution of m_j was observed by bootstrapping (resample 1000 time). Estimates of 95%, 99%, and 99.9% confidence intervals were constructed. For testing the null hypothesis H_0: $m_j = 0$ and/or H_0: $(1\text{-}m_j) = 0$, The confidence limits that were both less or higher than zero would imply a rejection of H_0.

The geographic patterns of morphological traits (height growth and WGR severity) may be affected by the geographic variation in hosts and rusts. Therefore, partial correlations between geographic variables (latitude, longitude, and altitude) and morphological traits were calculated and tested by controlling host groups and rust sources. Partial correlations were also estimated for investigating the effect of introgression on WGR infection by controlling longitude, latitude, and height growth, and on height growth by controlling longitude, latitude, and WGR infection. SAS Proc CORR was used for the analysis (SAS Institute Inc. 1990).

3 Results

3.1 Pattern of genetic admixture

Canonical discriminant analysis based on the allele frequencies of 39 RAPDs revealed highly significant differences among populations within the hybrid zone. The first canonical variable ($P < 0.001$) explained approximately

98% of the variation among populations. Coefficients for the first 11 RAPDs variables were large; the remaining 28 RAPDs accounted for only limited amounts of additional variance ($< 28\%$). The grouping pattern (Figure 3-5) closely resembled the results from the UPGMA cluster analysis based on genetic distances using 8 diagnostic RAPDs (See Part I, Figure 3-1). There was good separation between the parental species. Most morphologically determined "pure" jack pine populations formed a distinct cluster that was primarily separated along the first canonical variable. Geographically neighboring populations (such as 11, 13, 14 and 24) in the central of the hybrid zone were separated largely by the second canonical variable. "Pure" lodgepole pine and putative hybrid populations exhibited a considerable overlap.

Estimates of genetic admixture, which were expressed as the proportion of genes derived from jack pine, were largely in agreement with the expectations based on the field observations, with a few exceptions (Table 3-8). Most "pure" lodgepole pine populations had low values of genetic admixture (mean: 6.9%, range: 0.0 - 29.8%). In contrast, the primary genetic entity in seven "pure" jack pine populations (30, 32, 33, 34, 35, 37, 38) was of jack pine origin (mean: 96.4%, range: 90.8 - 100.0%). In "pure" jack pine populations 31 and 36, however, more than 50% of admixtures from lodgepole pine were detected. Similar to lodgepole pine, most putative hybrid populations had relatively small proportion of genes from jack pine, such as populations 25 (21.0%), 26 (7.2%), 27 (10.5%), 29 (5.7%), 39 (0.1%) and 40 (4.6%) (Table 3-8). Putative hybrid population 28 was an

exception which had 81.8% of jack pine genes. Seven other populations (11, 14, 20, 24, 25, 31, 36) had significant proportion (> 20%) of genes from both parental species and spread across "pure" lodgepole pine, "pure" jack pine, and putative hybrid groups.

Correlation analysis revealed a significant geographic pattern of genetic admixture across the PCBC hybrid zone. Significant negative correlation was observed between genetic admixture and longitude ($r = -0.343, P = 0.003$).

Figure 3-4 showed a geographic clinal trend in genetic admixture from east to west. However, there were some patches in the east areas showing the opposite trend. For example, "pure" lodgepole pine population 18 ($m = 0.0$) was located among "pure" jack pine and hybrid populations close to the east part of the zone.

3.2 Geographic pattern of western gall rust severity

Significant relationships between longitude and WGR severity at 6-month-old ($r = 0.405, P < 0.001$) and 12-month-old ($r = 0.423, P < 0.001$) were found, suggesting a significant east-west trend with western populations being more susceptible to WGR infection (Table 3-9). For example, the mean WGR score for the populations from east of 117°W was 3.00 while the mean WGR score for the populations from west of 117°W was 3.81. Seventeen out of the 20 most resistant populations were located on the east of 117°W.

Canonical discriminant analysis showed that there were significant differences in WGR severity (at both 6 and 12 months old) among populations. The first canonical variable ($P < 0.001$) accounted for more than 99% of variation.

The grouping pattern (Figure 3-6) was similar to that based on frequencies of 39 RAPDs (Figure 3-5). For example, populations from group I in UPGMA cluster analysis based on genetic distances (Part I, Figure 3-1) still clustered together when they were classified solely based on their resistance to WGR.

3.3 Relationship between genetic admixture and morphological traits

Partial correlation analyses indicated the existence of significant relationship between genetic admixture and WGR severity. The level of infection by the WGR from lodgepole pine (WGR_{PL}) at both 6-month-old and 12-month-old assessments were negatively correlated with genetic admixture ($r = -0.769, P < 0.001$ and $r = -0.614, P < 0.001$, respectively) (Table 3-10, Figure 3-7). However, the correlation between genetic admixture and severity of the WGR from jack pine (WGR_{PJ}) was much weaker and statistically not significant ($r < -0.139, P > 0.10$) (Table 3-10, Figure 3-8). Marginally significant correlation ($r = 0.233, P = 0.048$) was found between genetic admixture and height growth at 12-month assessment (Table 3-10).

4 Discussion

The Quaternary histories of pines have been used to explain the current distribution of genetic diversity in the species (Wheeler and Guries 1982; Critchfield 1985; Cwynar and MacDonald 1987). The postglacial histories of lodgepole and jack pines have been reconstructed from a serial of pollen records by MacDonald and Cwynar and others (MacDonald et al. 1998). Their studies

suggested that lodgepole pine probably migrated northwards along the eastern slope of the Rocky Mountains from southwestern corner of the northern interior, reaching B.C. by 8000 BP. In the meanwhile, jack pine migrated from near the Great Lakes, spread northwestward, and reached northern Alberta (see Fig. 6.3 in MacDonald et al. 1998). By using RAPD markers, our results showed that the geographic pattern of population genetic admixture (Figure 3-4) is largely in concordance with the pattern of postglacial spread of both pine species detected by pollen data in this area. This might be the indication that the observed variational pattern in genetic composition among populations was largely due to interspecific gene flow, or introgression rather than adaptation to the gradient of environment.

Whether or not introgression leads to adaptive evolution is a fundamental question and needs to be addressed (Arnold 1997). The strong correlation between genetic admixture and WGR severity (Table 3-10) and the similar geographic patterns in terms of RAPD markers and WGR severity (Fig. 3-5, 3-6) led us to assume that WGR prevalence in the populations in PCBC area is closely linked to the introgression of resistance genes or gene complexes from parental species. WGR susceptibility exhibited an increasing gradient from jack pine, through the intermediate hybrid swarms, to lodgepole pine. Similar results of hybrid intermediacy have been found for resistance to rusts on pines (Wu et al. 1996; Florence and Hicks 1980) and resistance to spruce budworm defoliation in spruce (Manley and Fowler 1969).

Our findings illustrate the great importance of the concept of "hybrids-as-filters". Barton and Bengtsson (1986) hypothesized that hybrid populations act as evolutionary filters or barriers which allow beneficial genes through but prevent introgression of negative genes. Most hybrid zones are thought to be maintained by a balance between the dispersal of parental genes into the zone and selection (Haldane 1948; Barton 2001). Both endogenous and exogenous selection can result in barriers to the movement of genes across a hybrid zone (Hewitt 1988). Endogenous selection may be due to the breakdown of coadapted gene complexes (Grant and Grant 1994), whereas exogenous selection may result from a wide variety of environmental gradients. To across such a barrier, an allele must be recombined into a favorable, or at least viable, genetic background (Anderson 1949; Stebbins 1959; Barton 1979) so that it would not be lost by drift. Strong resistance to pathogens can definitely be a favorable adaptive trait. Rapid juvenile growth also has a definite fitness advantage, especially for pioneer tree species such as lodgepole and jack pines. Rapid growth alone is a good defense against environmental stresses.

Although mean WGR susceptibility was significantly correlated with the genetic composition of populations ($r = -0.322 \sim -0.416, P < 0.001$), infection caused by WGR from different host species exhibited different patterns of response to introgression. For example, strong negative correlation between WGR_{PL} infection and genetic admixture (m) was found ($r = -0.614 \sim -0.769, P < 0.001$) in this study. However, correlation between

WGR_{PJ} infection and genetic admixture was quite low and did not reach a statistically significant level ($r = -0.014 \sim -0.139, P > 0.930$). This finding suggested that introgression might have played an important role in the pine-rust interaction as well. Millar and Kinloch (1991) summarized that rusts apparently followed their hosts during glacial and interglacial periods and would thus evolve with the hosts. Rusts might be able to adapt to their hosts in relatively short time due to their short generations. Two spore sources used here are likely different WGR populations adapted to different host species and site conditions. WGR_{PL} came from and was adapted to lodgepole pine in PCBC area. New resistance genes or gene complexes from jack pine infiltrated into lodgepole pine populations through introgression, and increased their resistance to WGR_{PL}. Thus, the more resistance genes from jack pine, the lower WGR_{PL} severity the population, which caused the significant correlation between WGR_{PL} severity and genetic admixture. In contrast, WGR_{PJ} came from jack pine in Manitoba, which is far away from PCBC area. It has poor adaptation to the populations in PCBC. In addition, evidence showed that rust collections of jack pine origin might have experienced more selection pressure than that from lodgepole pine origin (Li 1998). We could be expected to observe more differentiated patterns when more WGR sources from jack pine are included.

When a hybrid zone is formed through secondary contacts, a shift in the frequencies of characters would be expected to occur along a cline (Rieseberg and Ellstrand 1993). In the PCBC hybrid zone, the proportions of genetic admixture

showed significant geographic patterns along the longitude and the altitude. The transition, however, was not uniform (Figure 3-4). Despite having an overall shift in genetic admixture, the clinal variation in the hybrid zone revealed occasional reversals of the admixture along the transition. Thus, as suggested by Rieseberg and Ellstrand (1993), this variation can be regarded as a mosaic pattern on a two-dimensional microgeographic scale. Several possible reasons could explain this complex pattern of interspecific gene flow in the PCBC hybrid zone. For example, gene flow by seed is generally much more restricted than gene flow by pollen. Hence, long-distance gene flow by pollen could "leap-frog" species-specific genes to regions beyond those of direct physical contact between the parental types (dePamphilis and Wyatt 1989; Potts and Reid 1988). If one or more hybrids are established in suitable habitat islands, interspecific gene flow can proceed by repeated backcrossing. Alternatively, a mosaic structure in a hybrid swarm could be the result of the local spread of stabilized introgressants (Rieseberg and Brunsfeld 1992). In either case, the eventual distribution of specific genes is a function of gene flow, selection, and drift. Moreover, jack pine is more xerophytic and lodgepole pine is more mesophytic and tolerant of clay soils and bogs. The hybrids occupy a wide range of intermediate sites (Yeatman 1967; Rudolph and Yeatman 1982). Different edaphic preferences among parental hosts and their hybrids may be the another important factor contributing to the mosaic pattern.

Documenting introgression in natural populations from morphological and historical data remains difficult largely because many factors could well explain

the observed variational patterns, including intraspecific variation, convergent evolution, retention of ancestral characters, and phenotypic plasticity (Bloom 1976; Heiser 1973; Reiseberg et al. 1988). Hence, diagnostic or even species-specific genetic markers need be present in the donor species to accurately detect introgression.

5 Summary

The patterns and level of introgression, suggested by the genetic admixtures of parental species, have been investigated by using diagnostic random amplified polymorphic DNA (RAPD) markers in 23 lodgepole pine, 9 jack pine and 8 putative hybrid populations. We studied the effect of introgression on response to infection of seedlings by western gall rust (WGR) in a greenhouse. Significant variation in the level of introgression was found among populations. A general east-west trend was observed, with western populations having less introgressed genes from jack pine sources than eastern populations. Strong correlation and similar geographic pattern between introgression and WGR severity implies that WGR prevalence in this area is closely linked to the introgression of resistance genes or gene complexes from parental species. Populations which have a higher proportion of genes from jack pine tend to be more resistant to WGR in seedlings. A strong negative correlation between WGR_{PL} severity and genetic admixture was found ($r = -0.614 \sim -0.769$). However, the relationship between WGR_{PJ} severity and genetic admixture was

statistically insignificant ($r = -0.014 \sim -0.139$). This suggested that introgression might have played an important role in pine-rust interaction.

Table 3-8. Estimates of introgression based on genetic admixture for 40 populations of lodgepole pine, jack pine, and putative hybrids. Genetic admixtures (*m*) were calculated as the proportion of genes derived from jack pine.

Population	Latitude (°N)	Longitude (°W)	Altitude (M)	Genetic admixture	
				Jack pine (*m*)	Lodgepole pine (*1-m*)
Lodgepole pine (LP)					
1	49°35'	114°35'	1584	0.0000	1.0000 ***
2	51°29'	115°11'	1650	0.0686	0.9314 ***
3	52°16'	115°13'	1175	0.0000	1.0000 ***
4	52°38'	116°05'	1520	0.0000	1.0000 ***
5	53°12'	117°32'	1360	0.0000	1.0000 ***
6	53°44'	116°35'	1080	0.0000	1.0000 ***
7	55°44'	119°40'	825	0.0000	1.0000 ***
8	54°38'	119°07'	1100	0.0000	1.0000 ***
9	54°39'	119°06'	1065	0.0287	0.9713 ***

10	54°32'	117°49'	825	0.0018		0.9982	***
11	54°28'	115°52'	1127	0.2979	**	0.7021	**
12	54°44'	115°18'	1064	0.0525		0.9475	***
13	54°42'	115°30'	1130	0.1834	*	0.8166	***
14	54°26'	115°35'	1097	0.2758	**	0.7242	**
15	50°40'	115°05'	1860	0.1228		0.8772	***
16	52°37'	115°04'	976	0.0000		1.0000	***
17	49°30'	110°15'	1160	0.0000		1.0000	***
18	55°38'	113°27'	915	0.0000		1.0000	***
19	59°42'	117°59'	730	0.1440		0.8560	***
20	58°42'	117°23'	590	0.2328	**	0.7672	**
21	58°40'	124°10'	760	0.0701		0.9299	***
22	58°32'	122°42'	455	0.0562		0.9438	***
23	58°39'	124°46'	1179	0.0446		0.9554	***

Putative hybrid (HB)

24	54°06'	115°32'	978	0.5138	**	0.4862	**
25	54°13'	115°54'	829	0.2097	**	0.7903	**
26	54°16'	116°13'	978	0.0722		0.9278	***
27	56°36'	119°42'	960	0.1047		0.8953	***
28	55°32'	114°51'	670	0.8180	***	0.1820	*
29	57°14'	118°16'	792	0.0570		0.9430	***
39	57°13'	118°16'	780	0.0016		0.9984	***
40	57°38'	117°27'	732	0.0462		0.9538	***
			Jack pine (JP)				
30	55°25'	113°23'	640	0.9082	***	0.0918	
31	55°32'	112°18'	762	0.4171	**	0.5829	***
32	54°55'	111°27'	701	1.0000	***	0.0000	
33	54°25'	110°40'	579	1.0000	***	0.0000	
34	56°16'	111°36'	610	0.9704	***	0.0296	
35	54°04'	112°12'	610	0.9521	***	0.0479	

36	56°42'	116°16'	700	0.4709	*	0.5291	**
37	57°57'	115°47'	340	1.0000	***	0.0000	
38	59°04'	117°42'	332	0.9148	***	0.0852	

Note: *, **, *** Significant at 5%, 1%, and 0.1% level, respectively.

Table 3-9. Partial correlations between geographic variables (latitude, longitude, and altitude) and WGR severity at 6-month (WGR6) and 12-month assessments (WGR12) of 40 populations by controlling host groups and WGR sources.

	Latitude	Longitude	Altitude	WGR6
Longitude	0.6905***			
Altitude	-0.7821***	-0.4233***		
WGR6	0.0260	0.4050***	0.1198	
WGR12	0.0282	0.4230***	0.1305	0.8964***

Note: ***, significant at 0.1% level, respectively.

Table 3-10. Partial correlations between genetic admixture and WGR severity (mean severity of WGR, severity of WGR_{PL}, and severity of WGR_{PJ}) and height growth at 6-month and 12-month assessments of 40 populations.

	WGR Severity[1]						Height[2]	
	6-month			12-month			6-month	12-month
	WGR	WGR_{PL}	WGR_{PJ}	WGR	WGR_{PL}	WGR_{PJ}		
Genetic admixture	-0.4159***	-0.7693***	-0.1390	-0.3215***	-0.6136***	-0.0141	0.2127	0.2328*

Note: *, ***, significant at 5% and 0.01% level, respectively.

[1] Partial correlations were calculated by controlling effects of longitude, latitude, and height growth.

[2] Partial correlations were calculated by controlling effects of longitude, latitude, and WGR severity.

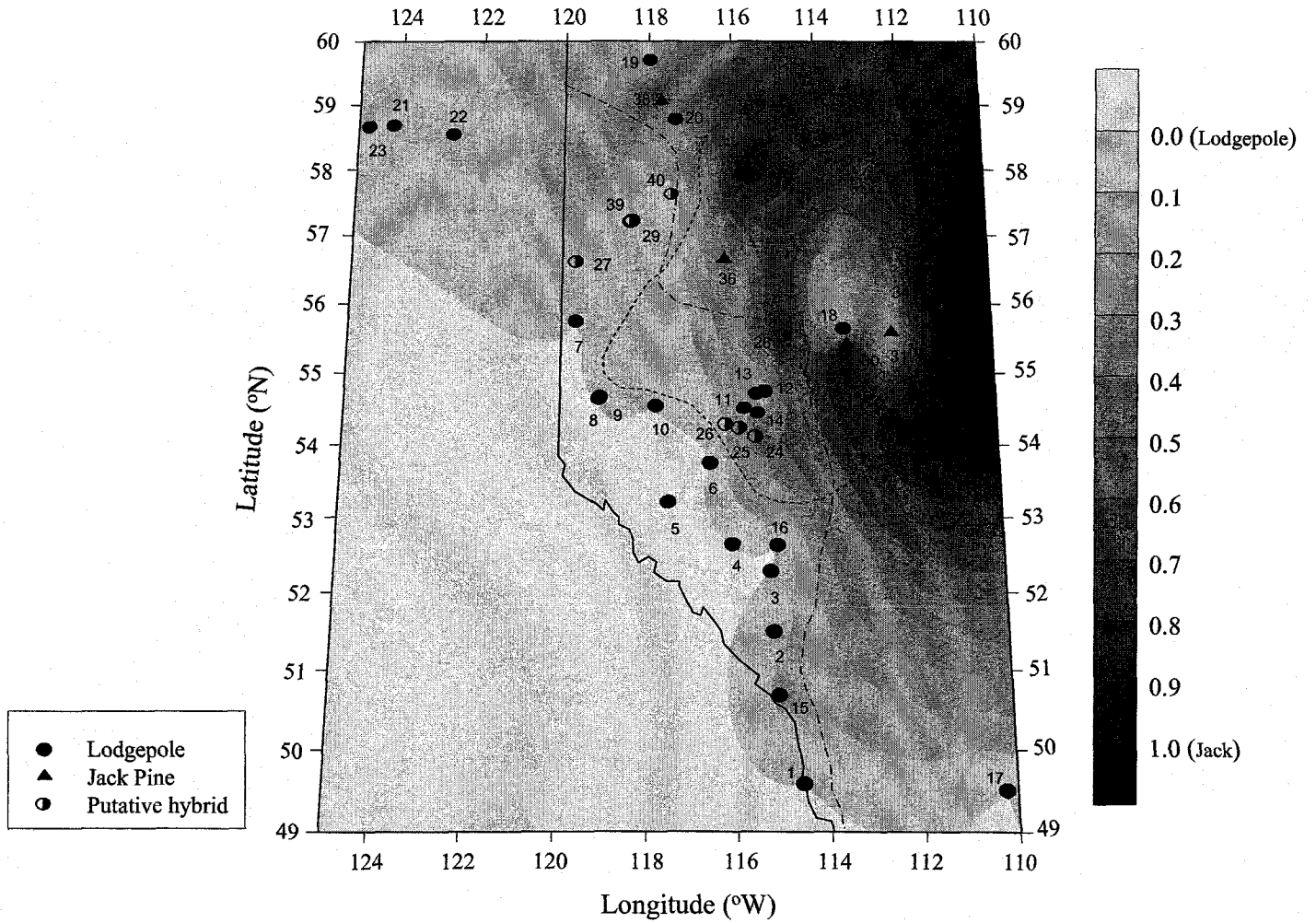

Figure 3-4. Geographical distribution of genetic admixture (*m*, proportion of genes derived from jack pine). Note: — · — Eastern limit of lodgepole pine; – – – Western & southern limits of jack pine (Fig.1 from Moss (1949)).

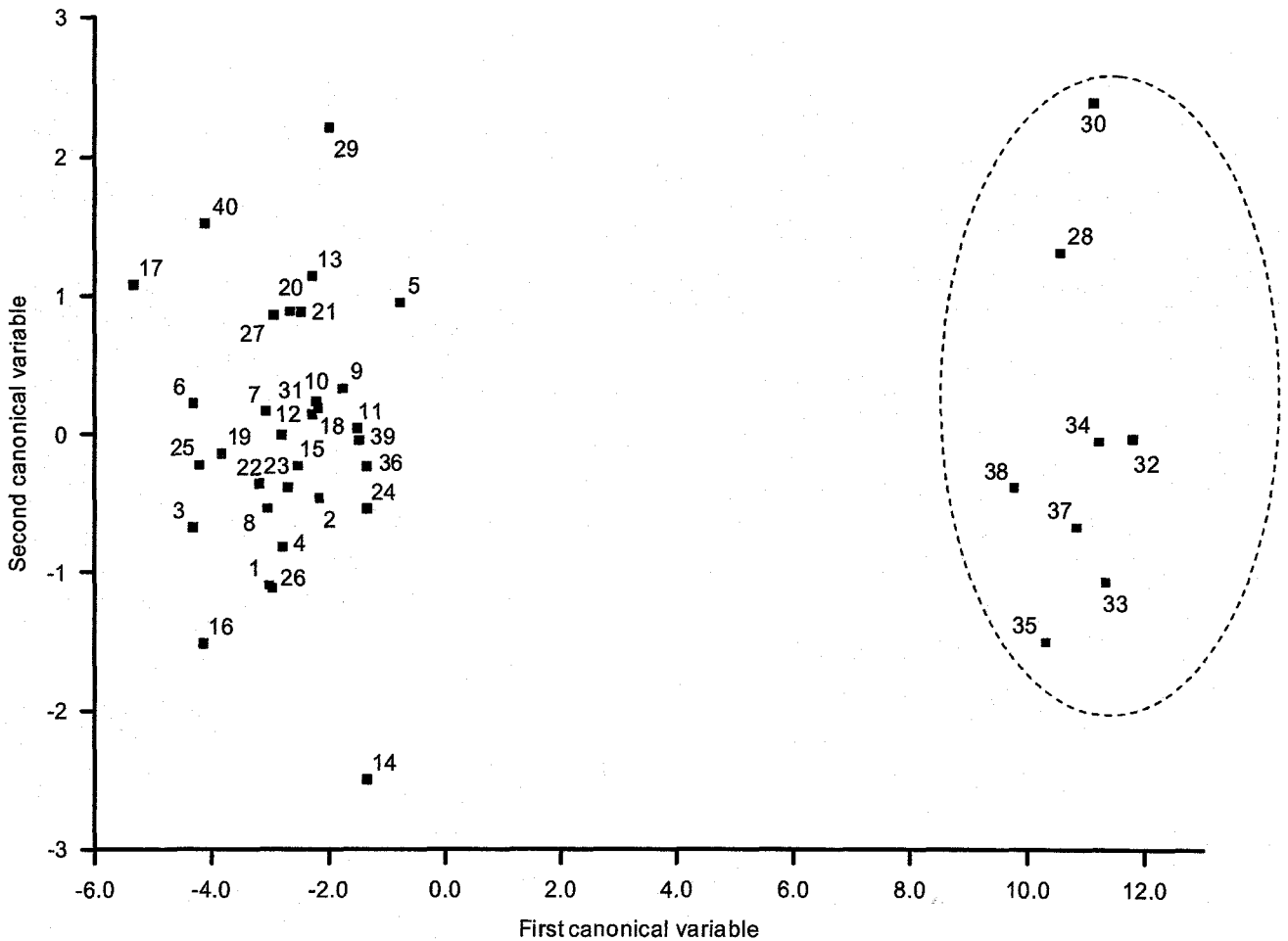

Figure 3-5. Plot of first two canonical variables resulting from a canonical discriminant analysis based on the frequencies of 39 RAPDs. Populations within the dash circle are from group I in UPGMA cluster analysis of genetic distances.

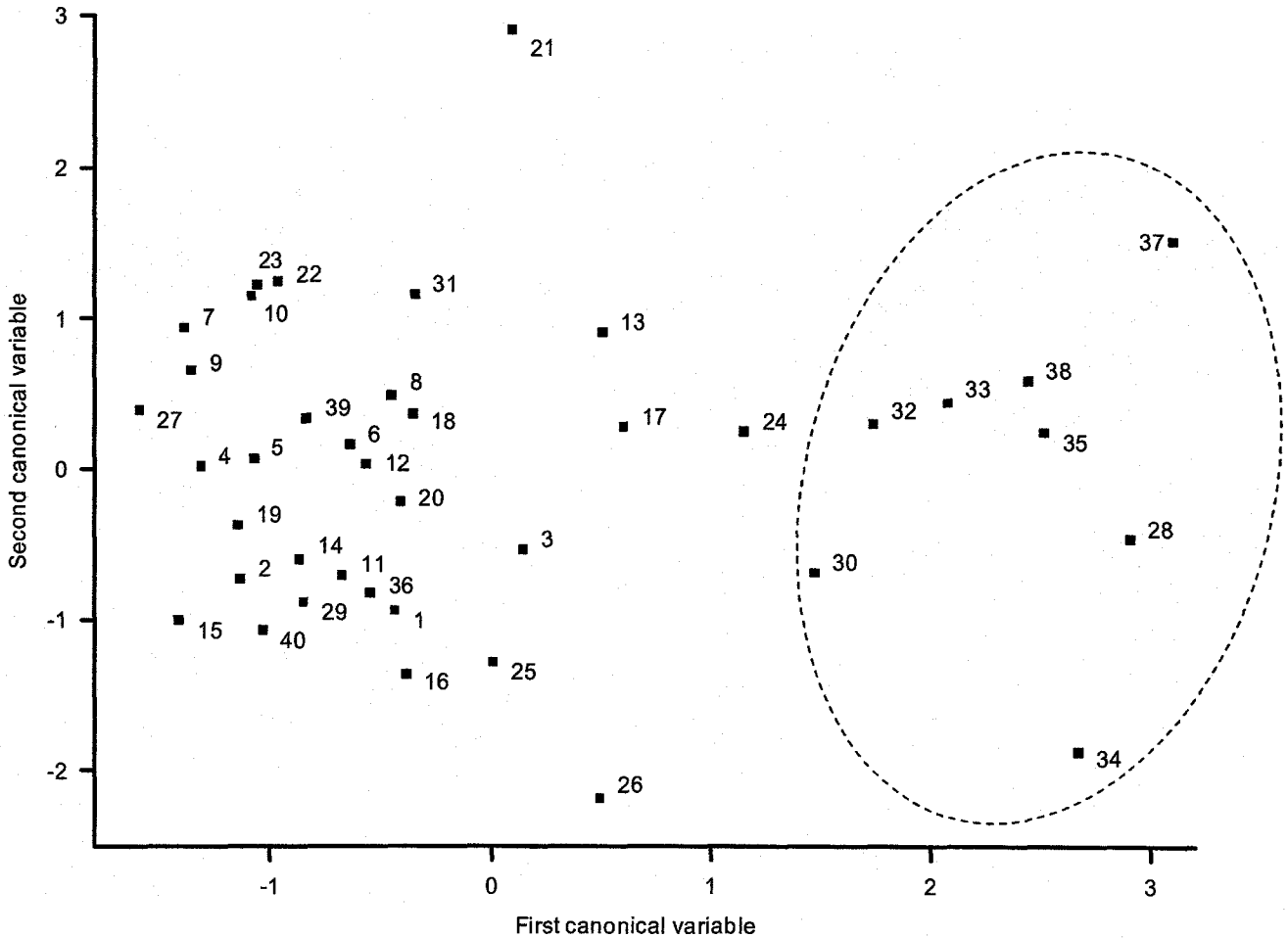

Figure 3-6. Plot of first two canonical variables resulting from a canonical discriminant analysis based on severity of WGR from lodgepole pine at 6 and 12 months old. Populations within the dash circle are from group I in UPGMA cluster analysis of genetic distances.

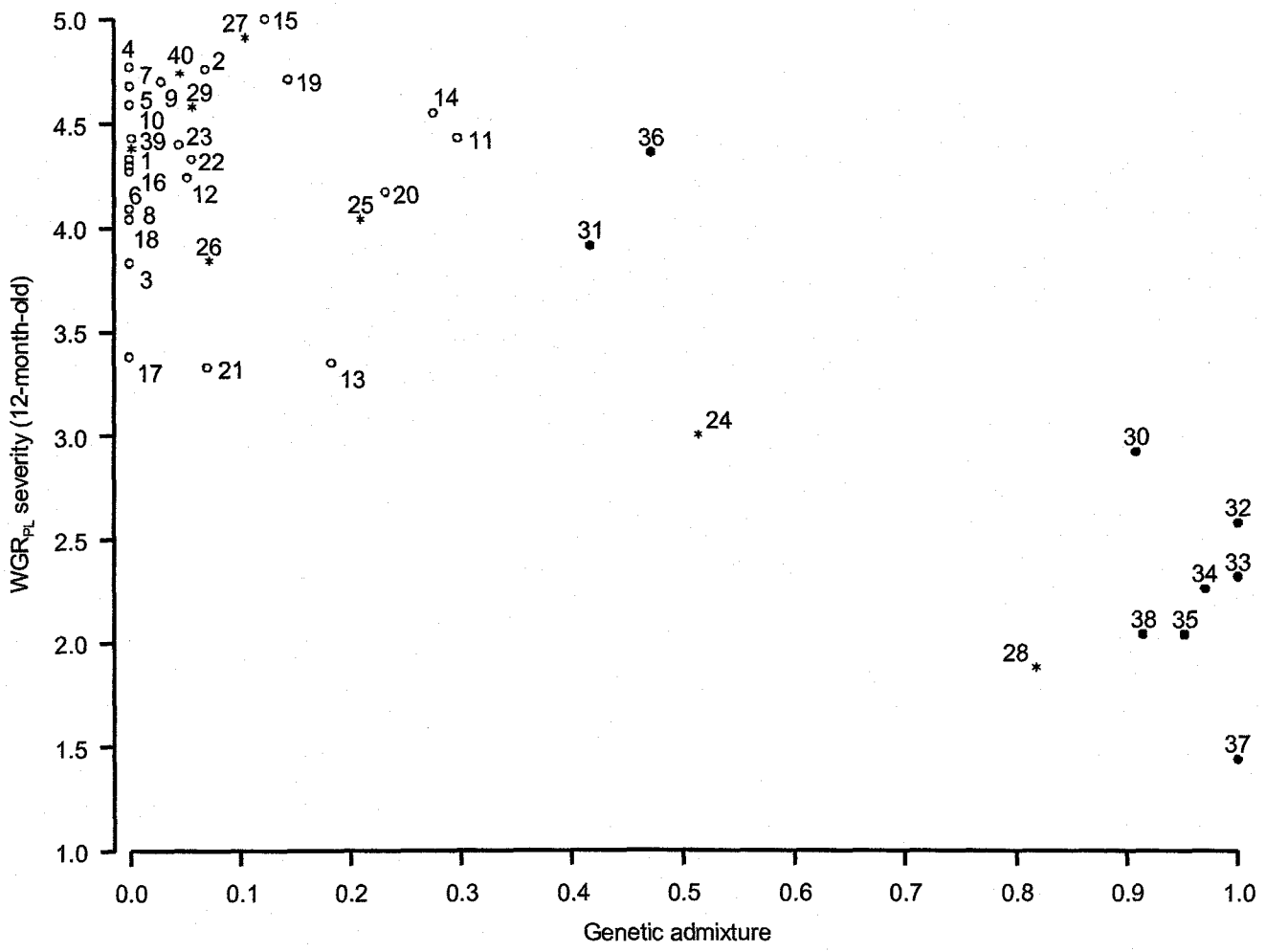

Figure 3-7. Relationships between genetic admixture and severity of WGR from lodgepole pine at 12-month assessment of 40 populations of lodgepole pine (○), jack pine (●) and putative hybrids (✱).

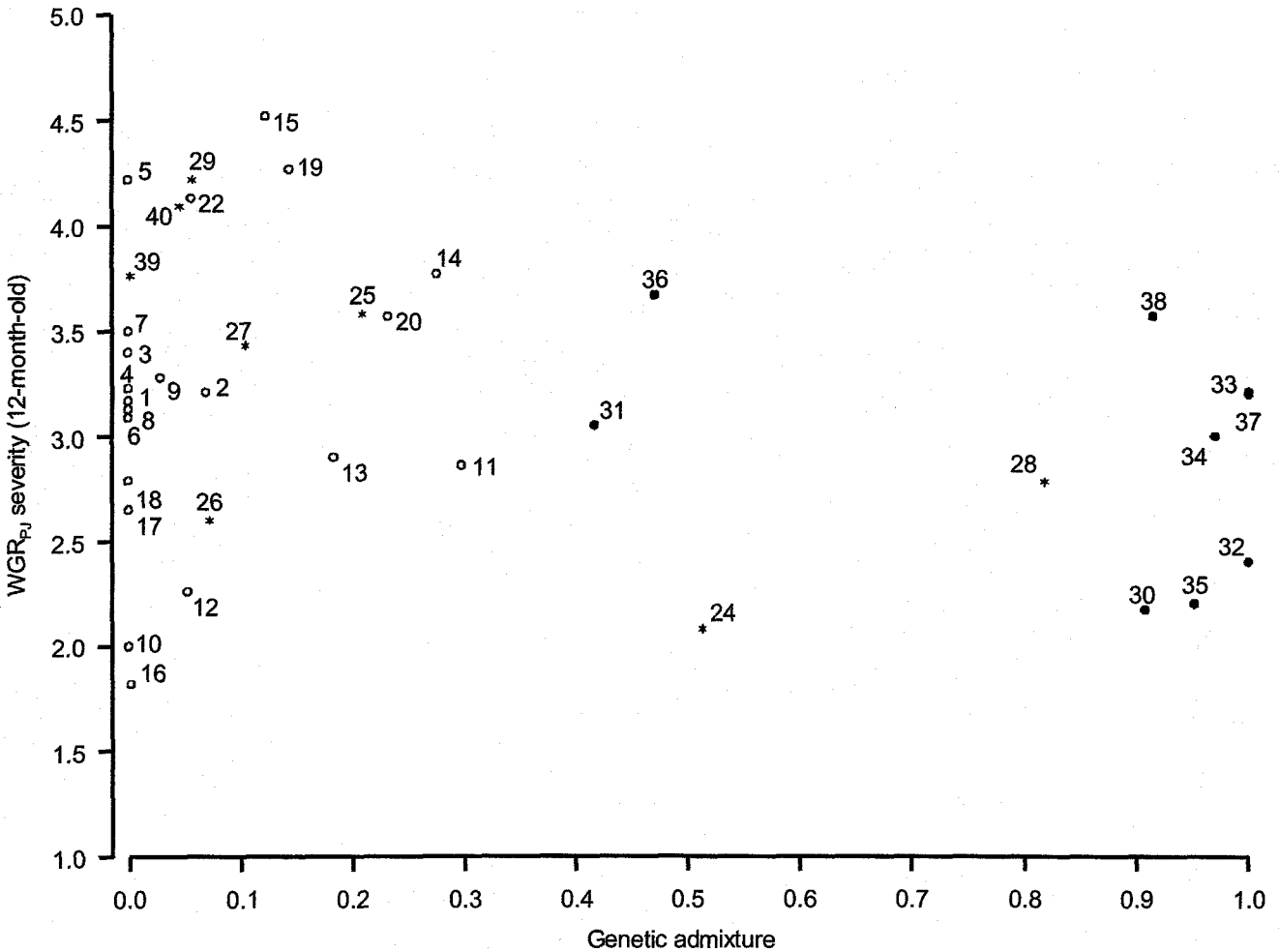

Figure 3-8. Relationships between genetic admixture and severity of WGR from jack pine at 12-month assessment of 40 populations of lodgepole pine (○), jack pine (●) and putative hybrids (✱).

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Chapter 4

Coevolution in Natural Pathosystems: Effects of Dominance on Host-Pathogen Interactions

1 Introduction

Interspecific hybridization and introgression combine different species' genomes in hybrids and generate greater genetic variation than that found in the individual species. Because hybrid swarms harbor a great amount of genetic variability, they host unique arrays of ecological and evolutionary interactions. One such interaction is between host plants and their pathogens that have coevolved in complementary genetic systems. The dynamics of natural host-pathogen systems is necessarily complex, but the gene-for-gene relationship proposed for agricultural crops and their pathogens (Flor 1956) works in natural populations as well (Thompson and Burdon 1992; Frank 1993). Natural pathosystems may maintain high levels of polymorphism in both hosts and pathogens as a result of their adaptation to one another and in response to heterogeneous environments. Furthermore, gene exchange between host populations or taxa across their hybrid zones can disrupt the local dynamic balance between hosts and pathogens, thereby bringing endemic disease levels to epidemic outbreaks (Harlan 1976; Frank 1993).

Population genetic analysis is essential for characterizing the properties of host-pathogen systems and for predicting magnitudes and trends of their coevolution. Several genetic models have been proposed to study the gene-for-

gene relationships or complementary gene interactions between host and pathogen (Mode 1958; Jayakar 1970; Leonard 1977; Lewis 1981; Levin 1983; Frank 1993). Of these, Leonard's (1977) single-locus model of selection in a natural host-pathogen system that conforms to the gene-for-gene relationship in identifying the conditions for maintaining resistance and virulence polymorphisms has received considerable attention. While Leonard's model could predict the equilibrium frequencies of resistance and virulence genes, there was debate on the stability of equilibria attained from the model (e.g., Sedcole 1978; Fleming 1980). Recently, Leonard (1994) carried out extensive computer simulations to investigate the stability of equilibria attained from his model and revealed the unstable limit cycles in which convergence to the equilibrium frequencies failed when initial allele frequencies were outside a critical cycle. This new analysis has since been extended to include the effects of mutation and migration (Kirby and Burdon 1997) and human perturbations such as replacement of resistant genotypes to the host population every generation (Sun and Yang 1998, 1999).

Despite these new analyses, one important feature that has been ignored is the fitness differences between heterozygote and homozygote for the dominant allele because all these analyses of Leonard's model assume the complete dominance of resistance in host populations. This assumption is in general well fit into cultivated crops pathosystems where hosts are usually pure lines (homozygotes) only. However, it becomes implausible when we apply the model into pathogen - hybrid plant pathosystems where extensive interspecific hybridization occurs. The genomes of two different species are combined during

the formation of F_1s. Subsequent hybridization leads to recombination of genes and changes in allele frequency in the formation of F_2s, backcrosses, or more complex hybrids. Therefore, hybrids possess different gene combinations from the parental species and exhibit different levels of dominance for resistance. In explaining varying hybrid responses to pest infestation, Strauss (1994) identified four hypotheses in resistance between the two species: (*i*) hybrid intermediacy (additive hypothesis).; (*ii*) hybrid > both parents (hybrid resistance hypothesis); (*iii*) hybrid < both parents (hybrid susceptibility hypothesis or hybrids-as-sinks hypothesis); and (*iv*) hybrid = one parent (dominance hypothesis). Many evidences for each of the above four patterns can be found in forest trees, such as hybrid intermediacy in resistance to fusiform rust in shortleaf pine – loblolly pine introgression (Hare and Switzer 1969; Florence and Hicks 1980); hybrid susceptibility in resistance to the gall-forming aphid in backcrossing hybrids of cottonwoods (Whitham 1989); dominance towards susceptible parent in resistance to gall-forming adelgids in hybrid spruce (Mattson et al. 1996); and hybrid resistance in resistance to gall-forming wasps in an oak hybrid zone (Boecklen and Larson 1994). These four hypotheses signal the pivotal role that the dominance effect could play in host resistance: no dominance in hypothesis (*i*), overdominance in hypothesis (*ii*), underdominance in hypothesis (*iii*) and complete dominance in hypothesis (*iv*). It is, therefore, essential to include the effect of dominance in the genetic models for pathogen-hybrid plant pathosystems.

In this paper, we develop a new complementary model of gene interaction between host and pathogen by extending Leonard's (1977, 1994) model to allow for arbitrary levels of dominance in a diploid host genotype. Our objective is to investigate how dominance could affect equilibrium frequencies and the stability of equilibria. The potential use of the model to pine-rust pathosystems is also discussed.

2 Theory

2.1 Leonard's model

Before developing our model, we present a brief overview of Leonard's (1977, 1994) model. In essence, Leonard' model is a single-locus population genetic model for a gene-for-gene interaction between a diploid plant host and its haploid pathogen, both with discrete generation intervals. There are two alleles at the host locus, one for resistance (R) and one for susceptibility (r), and similarly there are two alleles at the pathogen locus, one for virulence (V) and one for avirulence (v). The allele for resistance in the host is assumed to be dominant, but dominance is ignored in the haploid pathogen. Thus, there are two host phenotypes, resistant (RR or Rr) and susceptible (rr), and two pathogen phenotypes, virulent (V) and avirulent (v). The gene-for-gene interaction stipulates that the resistant host is resistant to the avirulent pathogen but susceptible to the virulent pathogen, whereas the susceptible host is susceptible to both pathogen phenotypes. Thus, the relative fitnesses of host or pathogen phenotypes differ,

depending on the counterpart's phenotypes. This model further assumes that there are fitness costs associated with unnecessary resistance or virulence. This will ensure that the coevolutionary process does not reach an unstable state in which all hosts are resistant and all pathogens are virulent.

The fitnesses of pathogen and host phenotypes for Leonard's model can be obtained from Table 4-1 by letting $h = 1$ (complete dominance) so that the fitnesses for genotypes *Rr* and *RR* are the same. Five parameters with unambiguous biological means were introduced in defining the fitnesses of host and pathogen genotypes and simulating host-pathogen interactions. In the model, fitness values for pathogen genotypes are designated relative to a fitness of 1 for the avirulent pathogen genotype on the susceptible host genotype. (1) *Effectiveness of the resistance in the host* (t): On the resistant host, the fitness of the avirulent genotype is reduced by a factor, t, which represents the effectiveness of that allele for resistance. Leonard summarized that the value of t may be in the range of 0.95 to 1.0 in several cultivated crops (Leonard 1977). (2) *Cost of virulence in the parasite* (k): Evidences showed that selection acts against unnecessary alleles for virulence in gene-for-gene relationships involving a number of pathogens (Grant and Archer 1983; Leonard 1977; Vanderplank 1968). Thus, the model assumes that there is some cost to the pathogen in situations in which the virulence is unnecessary. The fitness of the virulent genotype on susceptible host is designated as $1-k$. Experiments conducted both in greenhouse and in field have yielded values of k in the range of 0.1 to 0.4 (Leonard 1977) and

0.04 to 0.06 (Grant and Archer 1983) respectively. (3) *Advantage of virulent pathogen on hosts with corresponding gene for resistance* (*a*): This parameter was added to account for the possible special advantage that the pathogen may receive when its virulence allele matches a specific resistance allele in the host. When a = 0, there is a cost of virulence even when it is necessary to parasitize the resistant host, which means that the presence of the virulence allele causes a reduction in the intrinsic rate of reproduction by the parasite. This can be thought as hard selection (Wallace 1975). On the other hand, when $a = k$, virulence has a fitness cost only when it is unnecessary, which means that the fitness of virulent pathogen is reduced in competition with the avirulent pathogen on a susceptible host. This can be thought as soft selection (Wallace 1975). Some data for Phytophthora infestans indicates that the value of a may be as great as 0.8 (Denward 1967; Leonard 1977). (4) *Severity of disease* (*s*): Most of pathogens cannot kill their host plants or destroy their reproductive capacity with a single infection. For rusts fungi, several hundred or even thousands of infections per plant may be required to drastically reduce the plant's seed production (Leonard 1985). Therefore, the parameter s is introduced and expressed as the amount of damage caused by the avirulent genotype on the susceptible host genotype. The value of s could vary greatly from geographical region to geographic region and even from year to year within the same region. (5) *Cost of resistance in the host* (*c*): This parameter expresses the cost of resistance when it is unnecessary (i.e., the pathogen is not present or cause no damage) or when it is ineffective (as when only the virulent

genotype of the pathogen is present). Judging from the fact that the incorporation of several major genes resistance into crop cultivars has not noticeably reduced yield, the value of c for most resistance genes should be very low, perhaps in the range of 0.01 to 0.05 or even less (Leonard 1977).

Frequencies of alleles for virulence and avirulence in the pathogen population at the ith generation are n_i and m_i with $n_i + m_i = 1$. Frequencies of alleles for resistance and susceptibility in the host population at the ith generation are p_i and q_i with $p_i + q_i = 1$. Leonard's model assumes that the host population undergoes random mating so that Hardy-Weinberg equilibrium is achieved in each generation. Thus, frequencies of three genotypes, *RR*, *Rr* and *rr*, at the ith generation are p_i^2, $2p_iq_i$ and q_i^2, and the frequency of host phenotype for the dominant resistance allele (*RR* or *Rr*) is $1-q_i^2$. During the ith generation, selection changes the frequency of virulence allele in the pathogen population from n_i to n_{i+1},

$$n_{i+1} = g(p_i, n_i) = \frac{n_i[1-k+(1-q_i^2)a]}{1-(1-q_i^2)t+n_i[(1-q_i^2)(a+t)-k]} \qquad (1)$$

The selection induced by the pathogen infection on reproduction of resistant and susceptible host phenotypes at the ith generation determines the frequency of resistance allele in the host population at the (i+1)th generation,

$$p_{i+1} = f(p_i, n_{i+1}) = \frac{p_i[1-c-s(1-t)+n_{i+1}s(k-a-t)]}{1-s+n_{i+1}ks+(1-q_i^2)[ts-c-n_{i+1}s(a+t)]} \qquad (2)$$

It is evident that genetic feedback between host and pathogen populations is delayed rather than immediate. In other words, the composition of pathogen population may change in response to selection during the current pathogen generation, but the impact of the changed pathogen population on the relative fitness of the host population is not shown until the next host generation.

When the equilibrium is reached between selection pressures exerted by host resistance and pathogen virulence [i.e., there is no change in allele frequencies for resistance in host ($p_{i+1} = p_i = p_e$) and for virulence in pathogen ($n_{i+1} = n_i = n_e$)], the equilibrium frequencies for the resistance allele in host and for the virulence allele in pathogen are functions of fitness parameters as defined in Table 4-1,

$$p_e = 1 - q_e = 1 - \sqrt{1 - \frac{k}{a+t}} \tag{3}$$

and

$$n_e = \frac{ts - c}{s(a+t)} \tag{4}$$

Mathematical analysis of the model showed that the equilibrium point was not locally stable. Extensive computer simulations by Leonard (1994) revealed the presence of an unstable limit cycle for each combination of parameter values, i.e. allele frequency starting inside the limit cycle spiraled inward toward an internal equilibrium point; those starting outside the limit cycle spiraled outward toward fixation or extinction. Leonard (1994) concluded that the internal equilibrium

point was stable in a biological sense, although it might not meet mathematical criteria for the stability.

2.2 Extension of Leonard's model

To extend Leonard's model and allow for an arbitrary degree of dominance in the diploid host population, we introduce a new parameter (h) called the heterozygous effect (Gillespie 1998) to measure the fitness of the heterozygote relative to the selective difference between the two homozygotes (Table 4-1). If resistance is completely dominant ($h=1$), the fitness for the heterozygote (*Rr*) is the same as that for the resistant homozygote (*RR*) (i.e., Leonard's original model as described in the previous section). If susceptibility is completely dominant (h = 0), the fitness for the heterozygote (*Rr*) is the same as that for the susceptible homozygote (*rr*). Incomplete dominance ($0 < h < 1$) indicates that the fitness for heterozygote (*Rr*) lies between those for the two homozygotes (*RR* and *rr*). In particular, with h = 0.5, there is no dominance and the fitness for heterozygote is the average of those for the two homozygotes. Finally, heterozygote inferiority (underdominance) or superiority (overdominance) needs to be described with respect to the type of pathogens. In the presence of avirulent pathogens, underdominance of resistance is indicated by $h < 0$ because heterozygote *Rr* is more susceptible to the pathogen than recessive homozygote *rr*, whereas overdominance is indicated by $h > 1$ because heterozygote *Rr* is more resistant to the pathogen than dominant homozygote *RR*. In the presence of virulent pathogens, however, the reverse is true provided that there is advantage of virulent

pathogen on resistant hosts (i.e., $a > 0$): underdominance and overdominance of resistance are indicated by $h > 1$ and $h < 0$, respectively. Thus, to avoid any confusion in using h as a measure of dominance, we will hereafter refer $h < 0$ as underdominance of resistance and $h > 1$ as overdominance of resistance.

When two pathogen phenotypes, *V* and *v*, attack three genotypes (*RR*, *Rr* and *rr*) in the host population whose frequencies are p_i^2, $2p_iq_i$ and q_i^2 at the *i*th generation, their relative fitnesses change according to the composition of host population and the parameters given in Table 4-1,

$$\begin{aligned} W_v &= q_i^2 + 2p_iq_i(1-ht) + p_i^2(1-t) \\ &= 1 - p_i^2 t - 2p_iq_iht \end{aligned}$$

and

$$\begin{aligned} W_V &= q_i^2(1-k) + 2p_iq_i(1-k+ha) + p_i^2(1-k+a) \\ &= 1 - k + p_i^2 a + 2p_iq_iha \end{aligned}$$

The frequency of *V* after one generation of selection is,

$$\begin{aligned} n_{i+1} &= \frac{n_i W_V}{n_i W_V + m_i W_v} \\ &= \frac{n_i(1-k+p_i^2 a + 2p_iq_iha)}{n_i(1-k+p_i^2 a + 2p_iq_iha) + m_i(1-p_i^2 t - 2p_iq_ih)} \end{aligned} \tag{5}$$

which reduces to equation (1) if $h = 1$ (Leonard's model). The change in frequency of *V* due to selection is,

$$\Delta n = n_{i+1} - n_i = \frac{m_i n_i (W_V - W_v)}{W_v + n_i(W_V - W_v)} \tag{6}$$

where $W_V - W_v = (1-2h)p_i^2 + 2hp_i - k/(a+t)$. Obviously, the change in frequency of V causes the change in fitnesses of host genotypes and the frequency of resistance allele (R). The fitnesses of the three host genotypes are,

$$\begin{aligned}
W_{RR} &= m_{i+1}[1-c-s(1-t)] + n_{i+1}[1-c-s(1-k+a)] \\
&= 1-c-s(1-m_{i+1}t-n_{i+1}k+n_{i+1}a) \\
W_{Rr} &= m_{i+1}[1-ch-s(1-ht)] + n_{i+1}[1-ch-s(1-k+ha)] \\
&= 1-ch-s(1-m_{i+1}th-n_{i+1}k+n_{i+1}ah) \\
W_{rr} &= m_{i+1}(1-s) + n_{i+1}[1-s(1-k)] \\
&= 1-s+n_{i+1}ks
\end{aligned}$$

The frequency of R after one generation of selection is,

$$\begin{aligned}
p_{i+1} &= \frac{p_i^2 W_{RR} + p_i q_i W_{Rr}}{p_i^2 W_{RR} + 2p_i q_i W_{Rr} + q_i^2 W_{rr}} \\
&= \frac{p_i[1-c\theta_i - s(1-t\theta_i) + n_{i+1}s(k-a\theta_i - t\theta_i)]}{1-s+n_{i+1}ks + (1-q_i)(1-q_i+2hq_i)[ts-c-n_{i+1}s(a+t)]}
\end{aligned} \tag{7}$$

where $\theta_i = h + (1-h)p_i$. This frequency reduces to equation (2) if $h = 1$ (Leonard's model). The change in frequency of R due to selection is,

$$\begin{aligned}
\Delta p = p_{i+1} - p_i &= \frac{p_i q_i [p_i(W_{RR} - W_{Rr}) + q_i(W_{Rr} - W_{rr})]}{p_i^2 W_{RR} + 2p_i q_i W_{Rr} + q_i^2 W_{rr}} \\
&= \frac{p_i q_i^2 [ts-c-n_{i+1}s(t+a)](2h-1)}{1-s+n_{i+1}ks + (1-q_i)(1-q_i+2hq_i)[ts-c-n_{i+1}s(a+t)]}
\end{aligned} \tag{8}$$

When the equilibrium is reached between selection pressures from the resistant hosts and virulent pathogens, there is no change in the frequencies of resistance and virulence genes over generations, $n_i = n_{i+1} = n_e$ and $p_i = p_{i+1} = p_e$. The

equilibrium frequency of resistance allele (p_e) can be obtained by solving the following equation (setting $\Delta n = 0$),

$$(1-2h)p_e^2 + 2hp_e - \frac{k}{a+t} = 0 \qquad (9)$$

In the special case where there is no dominance (i.e., h = 0.5), equation (9) reduces to $p_e - k/(a + t) = 0$ and thus the equilibrium frequency is,

$$p_e = \frac{k}{a+t} \qquad (10a)$$

In all other cases, the equilibrium frequency is affected by the dominance as measured by the heterozygous effect (h),

$$p_e = \frac{h - \sqrt{h^2 - \frac{(2h-1)k}{a+t}}}{2h-1} \qquad (10b)$$

In the case of complete dominance for resistance (h = 1; Leonard's model), equation (10b) reduces to equation (3). Similarly, the equilibrium frequency of virulence allele (n_e) is obtained by setting $\Delta p = 0$ in equation (8). In other words, we need to solve for n_e in the following equation given that the equilibrium frequency of resistance allele is non-boundary ($0 < p_e < 1$),

$$ts - c - n_e s(t+a) = z$$

where constant z is zero if there is dominance ($h \neq 0.5$), but z may be non-zero if there is no dominance ($h = 0.5$). Thus,

$$n_e = \frac{ts - c - z}{s(a+t)} \qquad (11)$$

which is the same as equation (4) when $h \neq 0.5$. In general, dominance does not affect the equilibrium frequency of the virulent allele in a haploid pathogen population as expected. In addition to the internal equilibrium points (p_e, n_e,), there are four trivial equilibrium points, (0, 0), (0, 1), (1, 0) and (1, 1).

3 Numerical Analysis and Results

3.1 Effect of dominance on equilibrium frequency

It is evident from (10b) and (11) that only the equilibrium frequency for resistance (p_e) in the diploid host population is affected by dominance whereas that for virulence (n_e) in the haploid pathogen population is not. However, p_e is also affected by the fitness-related factors (a, k and t) as given in Table 4-1 (also cf. Leonard 1977). Thus, we now examine the effect of dominance along with a series of values of the fitness-related parameters.

For our numerical analysis, h takes values ranging from -1 to 2 to cover underdominance of resistance ($-1 \leq h < 0$), complete dominance of susceptibility ($h = 0$), incomplete dominance ($0 < h < 1$) including no dominance ($h = 0.5$), complete dominance of resistance ($h = 1$) and overdominance of resistance ($1 < h \leq 2$). Leonard (1977, 1994) described in detail the possible ranges for a, k and t. Here, we use the following ranges for our numerical evaluation: $0 \leq a \leq 0.5$, $0 \leq k$

≤ 0.5 and $0.5 \leq t \leq 1$. While our ranges for a and k are consistent with Leonard's, our range for t is much greater. Leonard (1977) determined his t values based on the disease data from annual crop plants where 'pure' lines with major resistance genes were grown to overcome the attack by prevalent virulent pathogens and thus his t values were equal to or very close to 1 as expected. In natural pathosystems on which our study is focused, however, t may be appreciably less than 1 since genes with partial resistance may be prevalent in natural populations but rare in cultivated crops because detection and introgression of these genes with small effects are difficult for most of crop improvement programs. To calculate the equilibrium frequency for virulence allele (n_e), the range of the two other fitness-related parameters (c and s) needs to be determined. We calculate n_e using c values in the range of 0.0 ~ 0.1 and s values in the range of 0.2 ~ 0.8. These values are within the ranges proposed by Leonard's (1977).

The numerical results show an interesting pattern on the equilibrium frequencies for resistance gene (p_e). Across the range of $0 \leq h \leq 1$, p_e can take values from 0 to 1. However, the value for p_e with underdominance of resistance is proportional to the degree of underdominance, but the value for p_e with overdominance of resistance is inversely proportional to the degree of overdominance. Figure 4-1 shows plots of p_e against h ($-1 \leq h \leq 2$) for three cases of a, k and t: (i) $a = 0.0$, $k = 0.0$ and $t = 0.5$; (ii) $a = 0.5$, $k = 0.5$ and $t = 0.5$; (iii) $a = 0.0$, $k = 0.5$ and $t = 0.5$. Cases (i) and (iii) display the constrained minimum and maximum p_e values for underdominance ($h < 0$) and overdominance ($h > 1$),

respectively. It is evident from equation (10b) that p_e in case (i) and other cases where $k = 0$ is determined by

$$p_e = \frac{h - |h|}{2h - 1} = \begin{cases} 2h/(2h-1), & if \ \ h < 0 \\ 0, & if \ \ h \geq 0 \end{cases} \qquad (12a)$$

where $|h|$ is the absolute value of h. Note that if $k = 0$, $p_e > 0$ only if $h < 0$, which signifies underdominance of resistance. This would make the fitness of the avirulent pathogen type greater on the *Rr* host than on either the susceptible host or the homozygous resistant host. Similarly, p_e in case (i) and other cases where $a = 0$ and $k = t$ is determined by

$$p_e = \frac{h - |h-1|}{2h - 1} = \begin{cases} 1/(2h-1), & if \ \ h > 1 \\ 1, & if \ \ h \leq 1 \end{cases} \qquad (12b)$$

Cases (ii) is an example with non-boundary p_e values.

It is clear from Figure 4-1 that when there is underdominance of resistance ($h < 0$), the internal equilibrium points (p_e, n_e) exist even when there is no cost of unnecessary virulence ($k = 0$). This is in contrast to Leonard's (1994) conclusion that there could be no internal equilibrium point when $k = 0$. Leonard's conclusion was based on the assumption of complete dominance of resistance ($h = 1$). Thus, when $k = 0$ the whole population becomes susceptible to the pathogen (i.e., $p_e = 0$). Similarly, when there is overdominance ($h > 1$), the internal equilibrium points (p_e, n_e) exist even when there is a cost of necessary virulence ($a = 0$) at the balance between the cost of unnecessary virulence and effectiveness of resistance (i.e., $k = t$). While such balance is needed to obtain equation (12b), it must be viewed as an

extreme case where the fitnesses of avirulent and virulent pathogens on the resistant (*RR*) plant are identical. In general, the condition of $k < t$ is required for a unequivocal definition of resistance. With $a = 0$ and $k = t$, Leonard's model ($h = 1$) would lead to the fixation of resistance gene (i.e., $p_e = 1$) at equilibrium.

3.2 Stability of equilibria

To examine the effect of dominance on the stability of internal equilibrium points (p_e, n_e), we identify unstable limit cycles for all combinations of fitness parameters and dominance effect (h) that generate the internal equilibrium points. Our results are in close agreements with the Leonard's three observations (1994): (i) allele frequencies starting on an unstable limit cycle would continue in its closed trajectory; (ii) allele frequencies outside the unstable limit cycle would spiral outward toward fixation or loss of virulence and resistance genes; (iii) allele frequencies inside the unstable limit cycle would spiral inward toward the internal equilibrium point. As an example, Figure 4-2(A-D) show plots of the frequencies of resistance and virulence genes (p_i, n_i) using equations (5) and (7) for four levels of dominance (h = 1.2, 1.0, 0.5 and -0.2). The fitness parameters are $a = k = 0.2$, s = 0.8, $t = 1.0$ and $c = 0.03$. Initial frequencies are $n_0 = 0.3$ for virulence and $p_0 = 0.2$ for resistance. The frequencies (p_i, n_i) for 30,000 generations are plotted in a phase plane. In all four plots, the frequencies of genes spiral inward toward their internal equilibrium point but never reach that point even after 200,000 generations (results not presented). It is also evident from these plots that the rate at which the frequencies of resistance and virulent genes are approaching to the

internal equilibrium point (p_e, n_e) increases with decreasing h values. Thus, the level of dominance greatly affects the path and pace of host-pathogen coevolution.

Simulations are carried out to determine the position of the limit cycle for each of combinations of parameter values over the following ranges: s = 0.2, 0.5, or 0.8; t = 0.5, 0.8 or 1.0; c = 0.00, 0.01, 0.02, 0.03, 0.05, 0.07, 0.10, 0.15 and 0.30; k = 0, 0.1, 0.2, 0.3, 0.4, or 0.5; a = 0 or k; and h = -0.9, -0.75, -0.50, -0.25, 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75 or 1.90. We test all combinations of parameter values that produce an internal equilibrium point. In addition, the two sets of parameter values that give rise to the lower and upper boundaries for internal equilibrium points in Figure 4-1 are included. The initial frequency of the resistance allele, p_0, in the test simulations is fixed to its equilibrium frequency. The choice of the initial frequency of virulence, n_0, requires a trial-and-error approach. When the initial run produces an inward spiral, then the value of n_0 is increased in each consecutive run until the allele frequencies would spiral outward. The size of unstable limit cycle is measured by the value of $-\log(n)$, where n is the frequency of virulence at its lowest point on the limit cycle. As Leonard (1994) pointed out, the stability of equilibrium or frequency polymorphism in a pathosystem increases with the size of limit cycle and thus with high values of $-\log(n)$. There is no stable polymorphism when $-\log(n)$ is zero and stability increases with increasing value of $-\log(n)$.

Figure 4-3 shows the plots of $-\log(n)$ against the h values for three sets of parameters used to produce Figure 4-1 and with s = 0.5 and c = 0.03. For all three

sets of parameters, there is little stability with $0 \leq h \leq 1$ and when the values of $-\log(n)$ are close to or equal to zero. However, the nonzero values of $-\log(n)$ occur at $1 < h \leq 2$ for the cases of $a = 0.0$, $k = t = 0.5$, $s = 0.5$ and $c = 0.03$; and $a = k = t = 0.5$, $s = 0.5$ and $c = 0.03$, and at $-1 \leq h < 0$ for the cases of $a = k = 0.0$, $t = 0.5$, $s = 0.5$ and $c = 0.03$; and $a = k = t = 0.5$, $s = 0.5$ and $c = 0.03$. There are also many cases where the stability of polymorphism is increased at $0 \leq h \leq 1$ (results not presented). Thus, the occurrence of stable polymorphism is strongly dependent on the level of dominance.

4 Discussion

This study has extended Leonard's (1977, 1994) model of host-pathogen interactions in natural pathosystems to assess the effects of overdominance, incomplete dominance and underdominance on the equilibrium frequencies of resistance allele in a diploid host population and on the stability of equilibria. Our model reduces to Leonard's original model when complete dominance of resistance is assumed. Two new features of our model emerge from our numerical analyses. First, when there is overdominance or underdominance of resistance ($h > 1$ or $h < 0$), the internal equilibrium points exist even when $k = 0$ or when there is a cost of necessary virulence ($a = 0$) at the balance between cost of unnecessary virulence and effectiveness of resistance (i.e., $k = t$). In contrast, with $0 \leq h \leq 1$, the resistant allele is either lost ($p_e = 0$) or fixed ($p_e = 1$) and there is no internal equilibrium point in these two cases (cf. Figure 4-1). This is the conclusion from

the analysis of Leonard's model for the special case of complete dominance (Leonard 1977, 1994). Second, it is evident from Figure 4-3 that the occurrence of a stable polymorphism is strongly dependent on the level of dominance (h). Even in the case where there is no cost of unnecessary virulence ($k = 0$), some level of stability is possible with underdominance ($h < 0$), confirming a well-known result from population genetic analysis of balancing selection (Gillespie 1998). In contrast, Leonard (1977, 1994) concluded from the analysis of his model of complete dominance that no stable polymorphism would be possible when $k = 0$. These two new features from our model suggest that care must be exercised in using Leonard's model particularly when there is evidence of overdominance or underdominance.

It is evident from equation (12a) and Figure 4-1 that if $k = 0$, $p_e > 0$ only if $h < 0$, which signifies underdominance of resistance. In this case, the fitness of the avirulent pathogen type would be greater on the *Rr* host than on either homozygote host, implying that the heterozygous host is actually more susceptible to the avirulent pathogen than the homozygous susceptible host. To the best of our knowledge, this 'heterozygote susceptibility' phenomenon has not yet been documented in the plant pathology literature. However, similar phenomena have been observed in many plant-herbivore interaction systems (Strauss 1994; Whitham et al. 1999). For example, Whitham et al. (1999) found that cottonwood hybrids between *Populus angustifolia* and *P. fremontii* attracted more gall-forming aphids (*Pemphigus betae*) than did either resistant or susceptible parental species.

Unfortunately, because most of these studies are conducted on unknown genotypes of hybrid plants in natural habitats, the genetic and environmental causes of the observed patterns remain to be investigated.

The assumption of complete dominance in Leonard's model of gene-for-gene coevolution is reasonable in an agricultural pathosystem where a major dominant allele for resistance to a disease is usually found. In natural pathosystems, however, several modes of gene action for resistance may exist including complete dominance overdominance and underdominance. For example, there are numerous responses of natural hybrids to attacks by pests, including hybrid susceptibility (underdominance of resistance), hybrid dominance (complete dominance) and hybrid intermediacy (no dominance) (e.g., Strauss 1994; Fritz 1999). Our model allows for an arbitrary level of dominance and thus may be particularly useful for studying the coevolutionary dynamics between hybrid populations and their pathogens. In the past, the effect of dominance on the equilibrium of resistance and its stability has been examined using simpler models (see Bergelson et al. 2001 for review). For example, Levin (1983) considered a symmetric fitness model in which the host genotypes, *RR*, *Rr* and *rr*, have respective fitnesses of α, β and γ in the presence of avirulent pathogens, but have respective fitnesses of γ, β and α in the presence of virulent pathogens. Even with this simplified model, a linearization approximation is required to find the necessary and sufficient condition for stable polymorphism in the host, which is $\beta < (\alpha + \gamma)/2$. The condition

$$\beta^2 < \left(\frac{\alpha+\gamma}{2}\right)^2 - 2\left(\frac{\alpha-\gamma}{2}\right)^2$$

is required to maintain stable polymorphisms in both the host and pathogen. Thus, Levin's (1983) model concurs with our model that stable polymorphisms are possible with overdominance or underdominance (heterozygote non-intermediacy) in the host.

The effects of mutation and population subdivision studied by Kirby and Burdon (1997) on Leonard's model can also be incorporated into our model. When the mutation rates in forward or backward direction are μ_p for pathogen and μ_h for host, the recursion equations for frequencies of resistant and virulent genes in (5) and (7) become,

$$n_{i+1} = g(p_i, n_i) + \mu_p[1 - 2g(p_i, n_i)]$$

$$p_{i+1} = f(p_i, n_{i+1}) + \mu_h[1 - 2f(p_i, n_{i+1})]$$

To illustrate the joint effects of dominance and mutation, we calculate the frequencies of resistance and virulence for 30,000 generations using the fitness values of Kirby and Burdon (1997): $a = k = 0.05$, $t = 1$, $c = 0.03$, $s = 0.8$. The initial point of $n_0 = 0.00001$ and $p_0 = 0.2$ is chosen because it is well outside the limit cycle for all h values examined. Figure 4-4(A-D) show the results of 30,000 generations when there is no mutation ($\mu_h = \mu_p = 0.0$) for $h = 1.0$ and when the mutation rate is low ($\mu_h = \mu_p = 10^{-10}$) for three levels of dominance ($h = 1.2$, 1.0, and –0.2) As was found by Kirby and Burdon (1997), in the absence of mutation the virulence allele is fixed rapidly (in the 8-12th generation) regardless of the

dominance effect. The addition of a tiny amount of mutation allows for converging cycles and dominance affects the sizes of the limit cycles in the same way as found in Figure 4-2(A-D).

Effect of population subdivision can be considered for different models of population structure (Wright 1969), from the simplest island model to the complex stepping stone model. Kirby and Burdon (1997) used the island model to study the effect of population subdivision on the behaviors of Leonard's model. Under the island model, a large population is split into geographic subpopulations and a proportion (m) of migrants is exchanged among them every generation. With subpopulations of finite size (N), the effect of random drift will balance the effect of migration at equilibrium. The net result at equilibrium is the increased homozygosity and decreased heterozygosity in the whole population. Likely, population subdivision would diminish the effect of dominance in our model.

5 Practical implications

The new complementary model was designed to extend Leonard's original model to study host-pathogen interaction in natural hybrid zones. The population structure and pattern of inheritance are more complicated in natural than in agricultural crops. Therefore, several basic questions need to be discussed when applying the model to the real world.

Question 1: Is there any evidence showed that the major gene models might be applicable to natural forest pathosystems, specifically for pine-rust pathosystems?

Little was known about the genetic control of resistance genes in forest trees in the past. Recently, however, studies on the genetic basis of the natural pathosystems using molecular techniques such as genomic mapping indicated that disease resistance in forest pathosystems is not exclusively polygenic. Major gene resistance occurred in many forest pathosystems as well, especially in pine-rust systems. Kinloch (1982) summarized the mechanisms and inheritance of resistance in pines to pine stem rusts caused by *Cronartium* spp. and *Endocronartium harknessii*. Only 3 of 14 known pine-rust pathosystems were hypothesized as polygenic inheritance. One example of major gene resistance in a forest tree is the sugar pine (*Pinus lambertiana*) gene for resistance to nonendemic white pine blister rust pathogen. This resistance gene was recognized many years ago by Kinloch et al. (1970) and recently mapped by Devey et al. (1995). Previous breeding efforts to improve the level of fusiform-rust resistance in loblolly pine (*Pinus taeda*) have assumed a polygenic basis for resistance and followed quantitative breeding models (Zobel and Talbert 1984). However, Walkinshaw (1991) proposed on the basis of infection percentages and reciprocal specificities with single gall isolates of the fungus that host resistance to fusiform rust in loblolly pine followed a gene-for-gene model. Other examples of major gene resistance include: loblolly pine – fusiform rust (Wilcox et al. 1996), white pine – blister rust (Kinloch et al. 1999; Kinloch and Dupper 2002), sugar pine – white pine blister rust (Kinloch and Comstock 1981), and slash pine – fusiform rust

(Stelzer et al. 1999). These studies suggested that the major gene models are quite common in pine-rust pathosystems.

Question 2: In this model, what are the basic assumptions which might be in contradiction to the lodgepole/jack pine-western gall rust pathosystem?

First of all, this is a genetic model which is based on gene-for-gene (GFG) relationship. Although GFG concept was proposed for agricultural crops and their pathogens (Flor 1956), it works in natural populations as well (Thompson and Burdon 1992; Frank 1993). For example, Kinloch and Walkinshaw (1991) proposed on the basis of infection percentages and reciprocal specificities with single gall isolates of the fungus that host resistance to fusiform rust in slash pine followed a GFG model. In studying the white pine – blister rust pathosystem, Kinloch and Dupper (2002) reported classic GFG interactions in this pathosystem. Although the detailed information about the genetic base of lodgepole/jack pine – WGR pathosystem is still lacking, GFG relationship might exist as well.

For purpose of simplicity, this model assumes the pathogen is haploid. This assumption was made based on the following reasons. First, there is disagreement over whether the nuclei fuse prior to spore germination and whether subsequent nuclear divisions are meiotic or mitotic. Hiratsuka et al. (1966) proposed that *Endocronartium harknessii* was an endocyclic rust, undergoing nuclear fusion as the spore matured, followed by meiosis at germination, and concluded that the spore was more correctly an "aecidoid teliospore". Others (Christenson 1968; Epstein and Buurlage 1988), making similar observations,

concluded that neither fusion nor meiosis occurred in the rust collections they sampled. In studying isozyme structure of this fungus in the western United States, Vogler et al. (1997) mentioned that their isozyme data support the latter interpretation. They found that this rust fungus comprised two distinct zymodemes (multilocus electrophoretic phenotypes), which also differed by the nuclear number of the spore. Isolates showed the phenotype of one zymodeme or the other but never a recombinant type. Second, by sampling 166 individual galls from 11 sites throughout North Dakota and northwestern Minnesota, Tuskan and Walla (1989) found that spore samples of *Endocronartium harknessii* appear to be homozygous, possibly homokaryotic. That is, if the aeciospores are dikaryotic, as reported by Epstein and Buurlage (1988), and only single bands are detected for each polymorphic locus, then the nuclei of the rust fungus must be homozygous.

The model assumes that host and pathogen have the same generation length, which definitely differs from the reality. In pine-rust pathosystems, one host generation actually contains several dozens of pathogen generations. Luckily, this problem could be partially solved by adjusting the parameter s (severity of disease) in the model since multiple attack of pathogen within one host generation increases the severity of disease and the delayed response of host could only be seen in the next host generation.

Question 3: What needs to be done in the future studies?

In most GFG models of natural populations, both plants and pathogens reproduce naturally and the objective is to understand the dynamics of allele

frequencies and the path of coevolution. The application of models depends largely on whether the model parameters are correctly used. Unfortunately, information about the values of model parameters in pine-rust pathosystems is still lacking and needs to be studied in the future.

Ideally, the basic model for studying the coevolution of pathogen and host populations should include both genetics and epidemiology in an explicit way. Such an approach is fraught with technical complications and makes it difficult to extract a clear message. Perhaps for these reasons, most of existing studies tend to make one or another kind of simplification (May and Anderson 1983). Therefore, it is necessary to include some information about demographic structure and population dynamics into the model. Besides, the new version of the model could be expected to incorporating at least two loci and resulting in three or more genotypes within a population, produce cycles that are even more complex and chaotic, and sometimes qualitatively different, from one-locus models. This is especially important when the model is used in pathogen-hybrid plant pathosystems where two or more species are involved.

6 Summary

We developed a new complementary model of gene interaction between host and pathogen by allowing for arbitrary levels of dominance in the diploid hosts. This model enables us to assess the effects of overdominance, incomplete dominance and underdominance on the equilibrium frequencies of resistance allele in a diploid host population and on the stability of equilibria. Our model reduces to

the established gene-for-gene model when complete dominance of resistance is assumed. Computer simulations show that this model has two novel features. First, when there is overdominance or underdominance of resistance, the internal equilibrium points exist even when there is no cost of unnecessary virulence or when there is a cost of necessary virulence at the balance between cost of unnecessary virulence and effectiveness of resistance. Second, the occurrence of stable resistance and virulence polymorphism is strongly dependent on the level of dominance. These two features suggest the need for due diligence when using the former gene-for-gene model, especially when there is evidence of overdominance or underdominance. In particular, our model is suitable for studying the coevolutionary dynamics between hybrid populations and their pathogens in natural pathosystems. The potential problems in the application of the model to natural pine-rust pathosystems have also been addressed.

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Table 4-1. Relative fitnesses of haploid pathogen and diploid host genotypes in a complementary gene interaction model. Three host genotypes respond differently to attacks by either avirulent or virulent pathogens and their responses are measured by the respective fitness values. The genotypic frequencies are those for the *i*th generation [1].

Genotype		*rr*	*Rr*	*RR*
	Frequency	q_i^2	$2p_iq_i$	p_i^2
		Fitnesses of two pathogen genotypes on three host genotypes		
v (avirulent)	m_i	1	$1-ht$	$1-t$
V (virulent)	n_i	$1-k$	$1-k+ha$	$1-k+a$
		Fitnesses of three host genotypes on two pathogen genotypes [2]		
v (avirulent)	m_i	$1-s$	$1-ch-s(1-ht)$	$1-c-s(1-t)$
V (virulent)	n_i	$1-s(1-k)$	$1-ch-s(1-k+ha)$	$1-c-s(1-k+a)$

Note: (1) h = heterozygous effect in the diploid host; k = cost of virulence in the parasite; t = effectiveness of the resistance in the host; a = advantage of virulent pathogen on hosts with corresponding gene for resistance; s = severity of disease; c = cost of resistance in the host.

(2) Assume that the effects of the two pathogen genotypes are additive and the distribution of pathogen genotypes is random so that the fitness of each host genotypes will be determined by the frequency of each pathogen genotype multiplied by the damage that it does to that host.

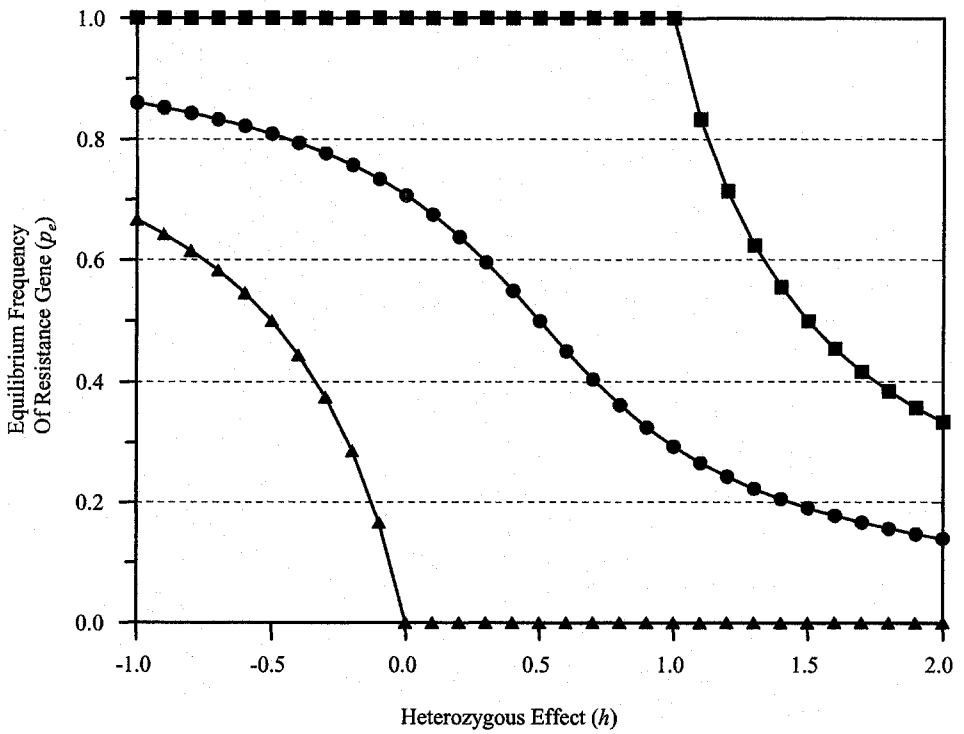

Figure 4-1. Equilibrium frequencies of resistance gene (p) over the range of heterozygous effects ($-1 \leq h \leq 2$) for three sets of fitness parameters: (▲) $a = k = 0.0$, $t = 0.5$, $s = 0.5$ and $c = 0.03$; (•) $a = k = t = 0.5$, $s = 0.5$ and $c = 0.03$; and (■) $a = 0.0$, $k = t = 0.5$, $s = 0.5$ and $c = 0.03$, where k is cost of virulence, t is effectiveness of the resistance, a is advantage of virulent pathogen on hosts with corresponding gene for resistance, s is severity of disease and c is cost of resistance in the host. The parameter h takes values ranging from -1 to 2 to cover underdominance ($-1 \leq h < 0$), complete recessiveness for resistance ($h = 0$), incomplete dominance ($0 < h < 1$) including no dominance ($h = 0.5$), complete dominance for resistance ($h = 1$) and overdominance ($1 < h \leq 2$).

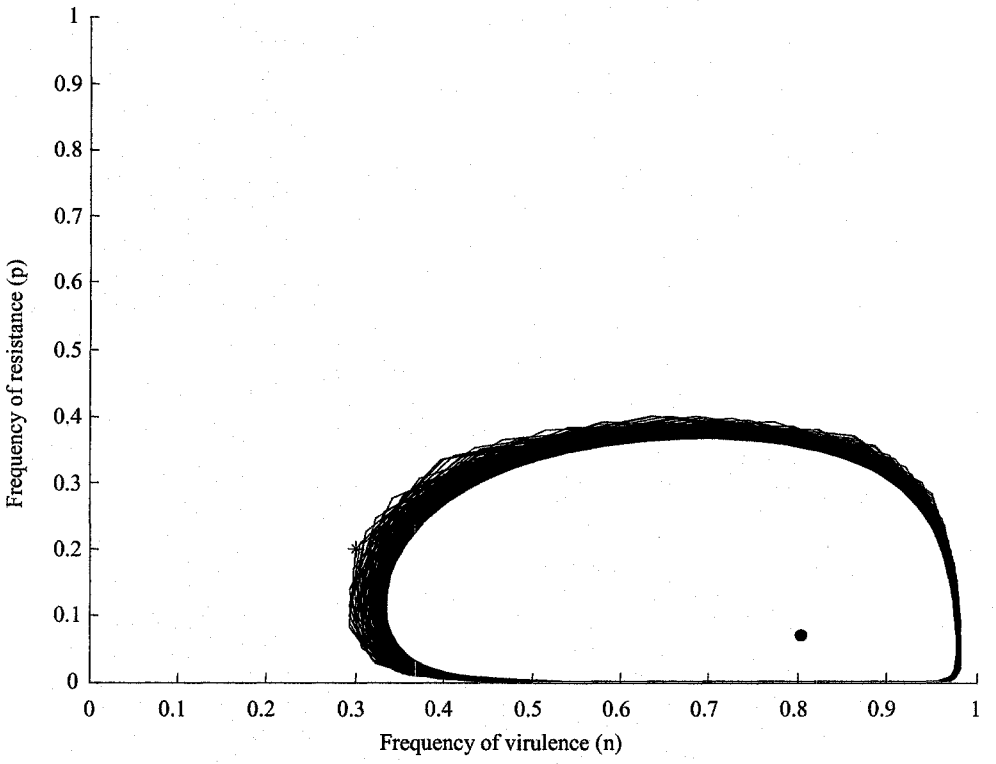

Figure 4-2A. Phase planes from simulations of a host-parasite coevolution model with underdominance effect: $h = 1.2$. In the plot, p is the frequency of gene for resistance in the diploid host and n is the frequency of gene for virulence in the haploid pathogen. Fitness parameters used are $a = k = 0.2$, $s = 0.8$, $t = 1.0$ and $c = 0.03$. Simulations start with the same initial frequencies ($n_0 = 0.3$, $p_0 = 0.2$) at the 0^{th} generation and continue until the 30000^{th} generation. The dots indicate the internal equilibrium points.

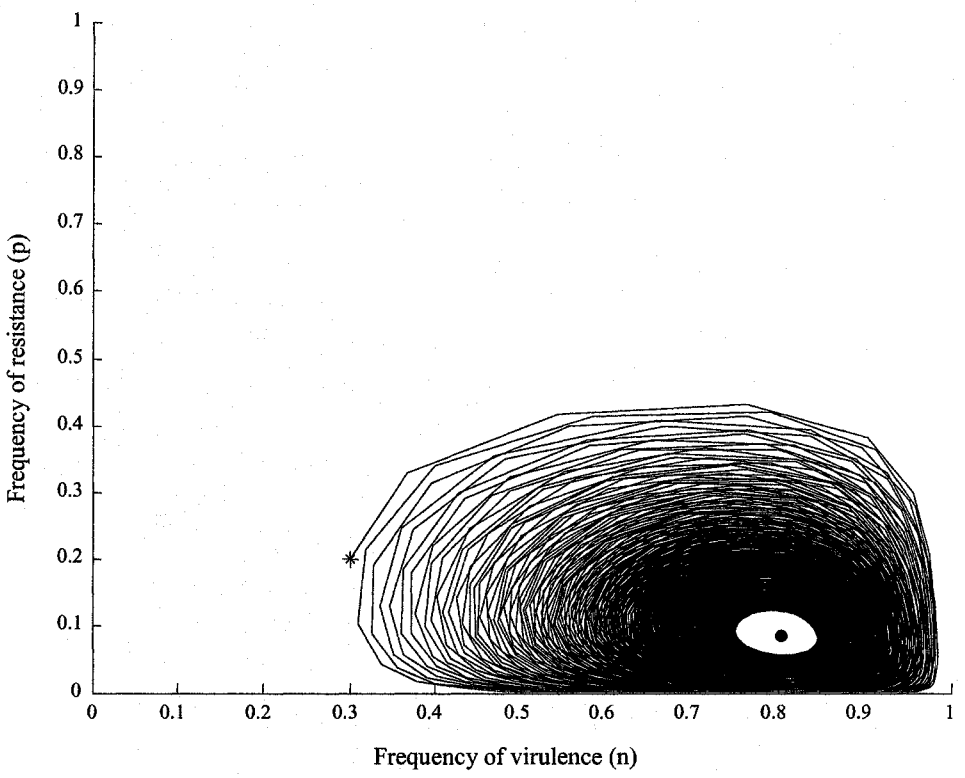

Figure 4-2B. Phase planes from simulations of a host-parasite coevolution model with complete dominance effect: h =1.0. In the plot, p is the frequency of gene for resistance in the diploid host and n is the frequency of gene for virulence in the haploid pathogen. Fitness parameters used are $a = k = 0.2$, $s = 0.8$, $t = 1.0$ and $c = 0.03$. Simulations start with the same initial frequencies ($n_0 = 0.3$, $p_0 = 0.2$) at the 0^{th} generation and continue until the 30000^{th} generation. The dots indicate the internal equilibrium points.

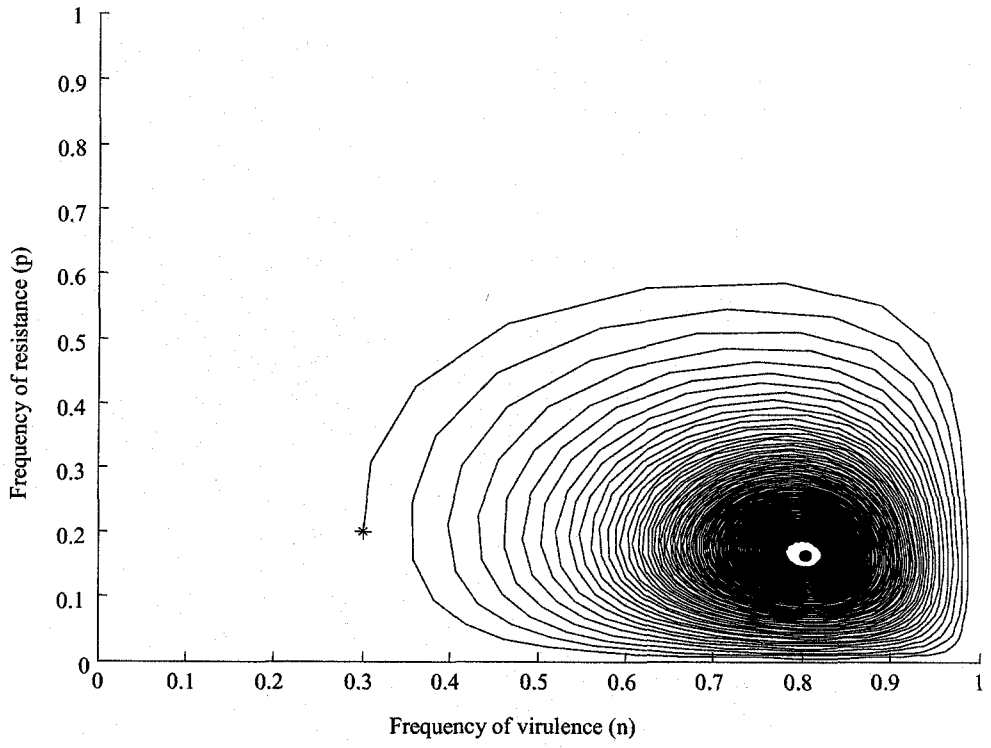

Figure 4-2C. Phase planes from simulations of a host-parasite coevolution model without dominance effect: $h = 0.5$. In the plot, p is the frequency of gene for resistance in the diploid host and n is the frequency of gene for virulence in the haploid pathogen. Fitness parameters used are $a = k = 0.2$, $s = 0.8$, $t = 1.0$ and $c = 0.03$. Simulations start with the same initial frequencies ($n_0 = 0.3$, $p_0 = 0.2$) at the 0^{th} generation and continue until the 30000^{th} generation. The dots indicate the internal equilibrium points.

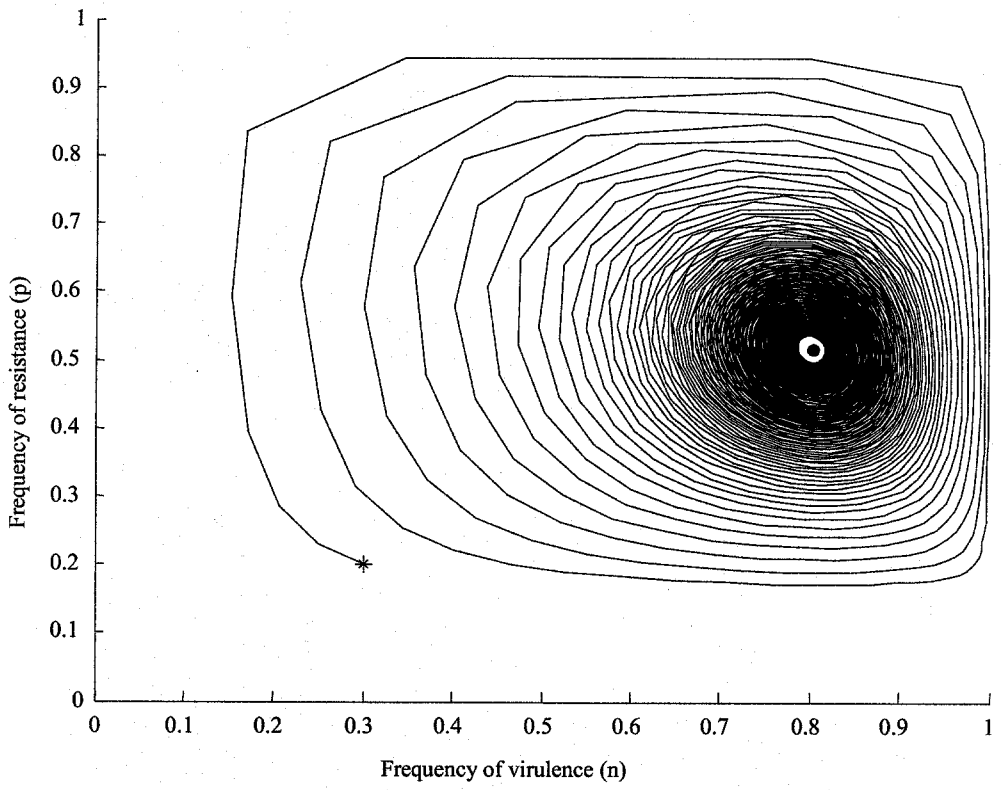

Figure 4-2D. Phase planes from simulations of a host-parasite coevolution model with overdominance effect: h =-0.2. In the plot, p is the frequency of gene for resistance in the diploid host and n is the frequency of gene for virulence in the haploid pathogen. Fitness parameters used are $a = k = 0.2$, $s = 0.8$, $t = 1.0$ and $c = 0.03$. Simulations start with the same initial frequencies ($n_0 = 0.3$, $p_0 = 0.2$) at the 0^{th} generation and continue until the 30000^{th} generation. The dots indicate the internal equilibrium points.

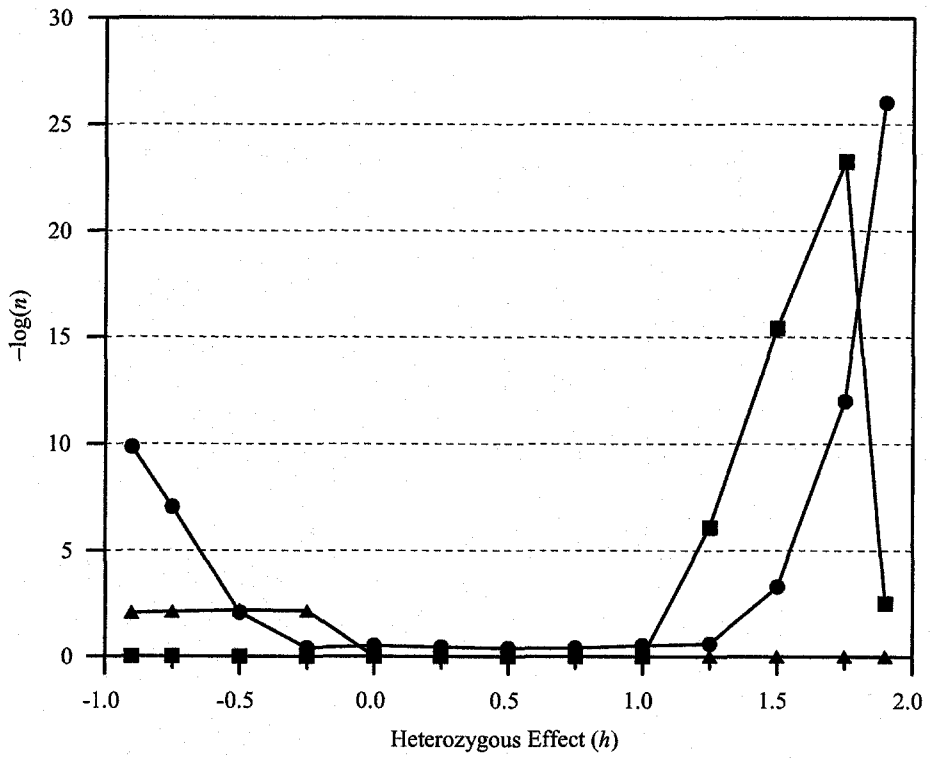

Figure 4-3. Effect of dominance (h) on the stability of polymorphisms as measured by the position of the unstable limit cycle [$-\log(n)$] in a host-parasite coevolution model, where n is the frequency of gene for virulence at its lowest point on the limit cycle. Three sets of fitness parameters are used: (▲) $a = k = 0.0$, $t = 0.5$, $s = 0.5$ and $c = 0.03$; (●) $a = k = t = 0.5$, $s = 0.5$ and $c = 0.03$; and (■) $a = 0.0$, $k = t = 0.5$, $s = 0.5$ and $c = 0.03$, where k is cost of virulence, t is effectiveness of the resistance, a is advantage of virulent pathogen on hosts with corresponding gene for resistance, s is severity of disease and c is cost of resistance in the host. The parameter h takes values ranging from -1 to 2 to cover underdominance ($-1 \leq h < 0$), complete recessiveness for resistance ($h = 0$), incomplete dominance ($0 < h < 1$) including no dominance ($h = 0.5$), complete dominance for resistance ($h = 1$) and overdominance ($1 < h \leq 2$).

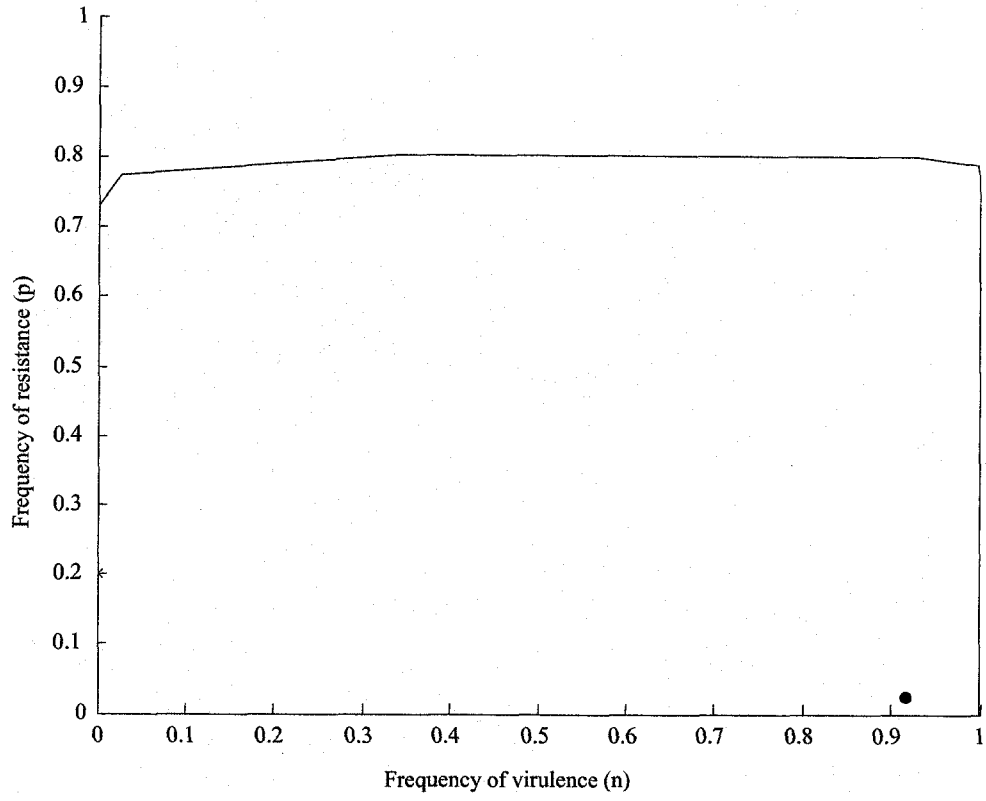

Figure 4-4A. Phase planes from simulations to examine the joint effects of mutation (μ_h and μ_p) and dominance (h) using fitness values: $a = k = 0.05$, $t = 1$, $c = 0.03$ and $s = 0.8$. The initial point are $n_0 = 0.00001$ and $p_0 = 0.2$. Results of 30,000 generations are shown in the case of no mutation ($\mu_h = \mu_p = 0.0$) for $h = 1.0$. The dots indicate the internal equilibrium points.

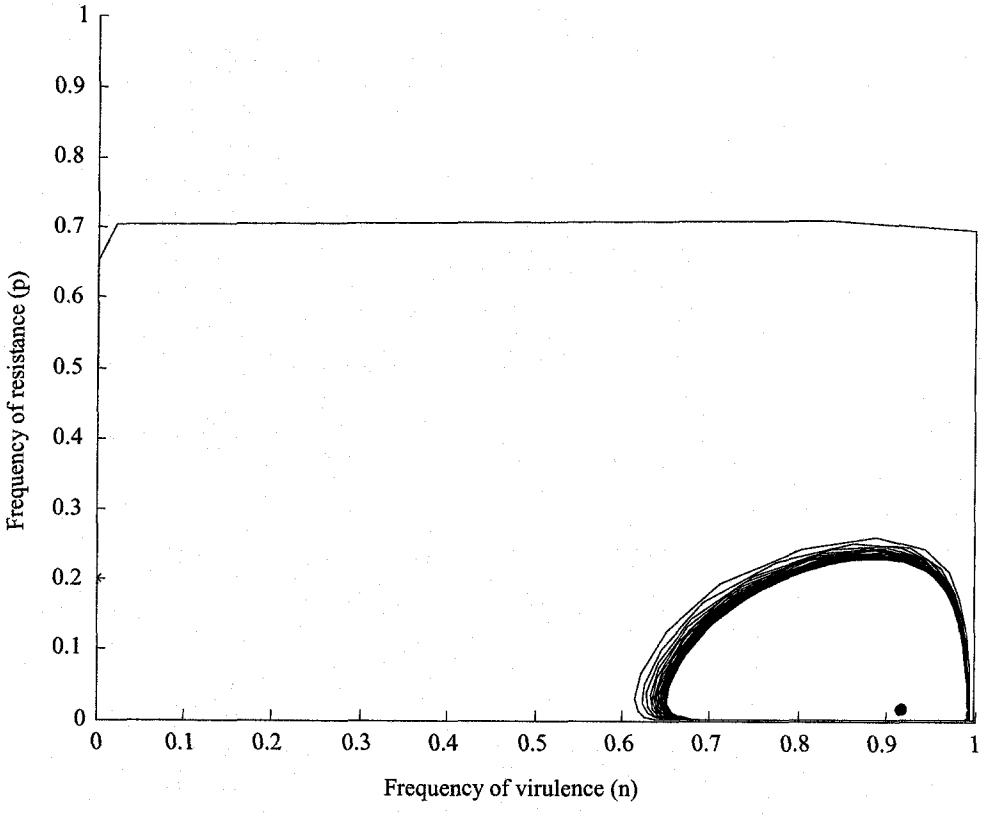

Figure 4-4B. Phase planes from simulations to examine the joint effects of mutation (μ_h and μ_p) and dominance (h) using fitness values: $a = k = 0.05$, $t = 1$, $c = 0.03$ and $s = 0.8$. The initial point are $n_0 = 0.00001$ and $p_0 = 0.2$. Results of 30,000 generations are shown in the case of low mutation rate ($\mu_h = \mu_p = 10^{-10}$) for $h = 1.2$. The dots indicate the internal equilibrium points.

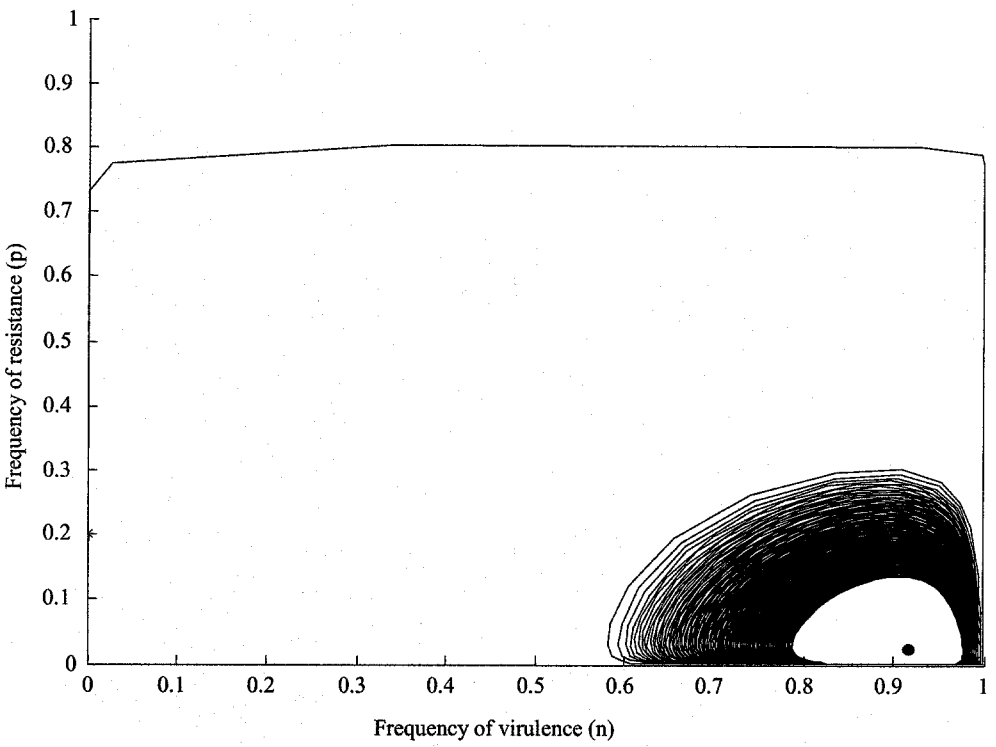

Figure 4-4C. Phase planes from simulations to examine the joint effects of mutation (μ_h and μ_p) and dominance (h) using fitness values: $a = k = 0.05$, $t = 1$, $c = 0.03$ and $s = 0.8$. The initial point are $n_0 = 0.00001$ and $p_0 = 0.2$. Results of 30,000 generations are shown in the case of low mutation rate ($\mu_h = \mu_p = 10^{-10}$) for $h = 1.0$. The dots indicate the internal equilibrium points.

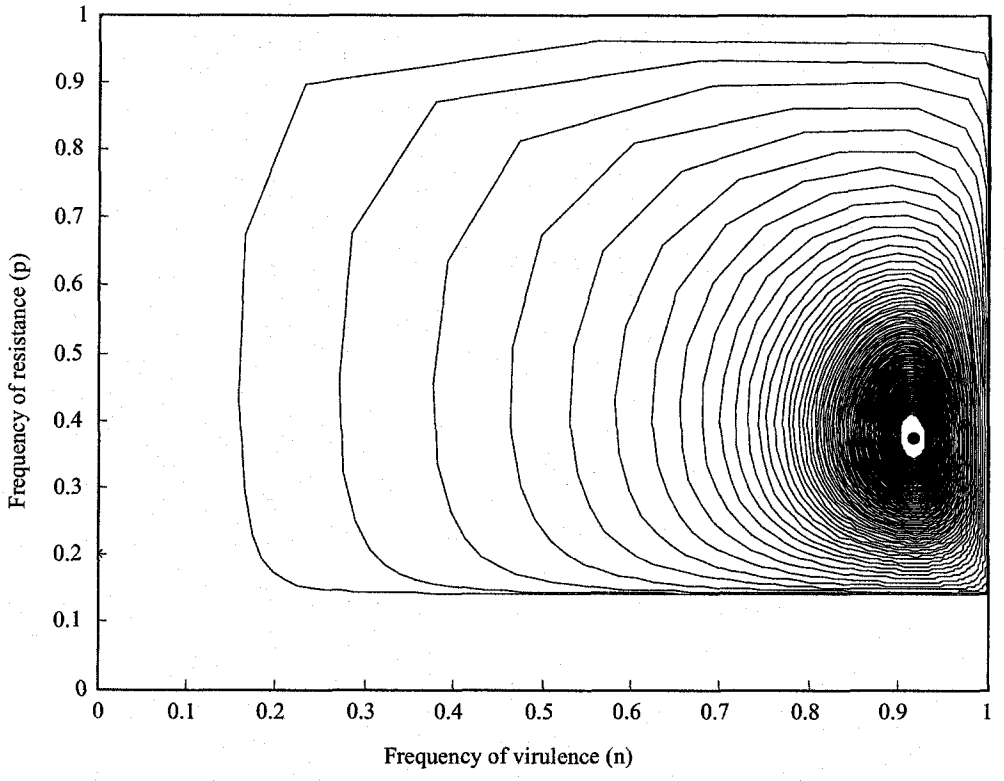

Figure 4-4D. Phase planes from simulations to examine the joint effects of mutation (μ_h and μ_p) and dominance (h) using fitness values: $a = k = 0.05$, $t = 1$, $c = 0.03$ and $s = 0.8$. The initial point are $n_0 = 0.00001$ and $p_0 = 0.2$. Results of 30,000 generations are shown in the case of low mutation rate ($\mu_h = \mu_p = 10^{-10}$) for $h = -0.2$. The dots indicate the internal equilibrium points.

Chapter 5

General Discussion and Implications

1 General Discussion, Conclusions, and Implications

Natural populations rarely exist in isolation. They interact with con-specific populations, closely related species, and biotic and abiotic factors. The coevolution between two species is affected by an array of genetic, life history and ecological characteristics as well as the forces of mutation, random genetic drift due to chance fluctuation, migration and natural selection. Accordingly, host specificity of pathogens and the genetic structure of host and parasite populations are important factors underlying the history of host-pathogen coevolution. To the best of my knowledge, the research presented here represents the first rigorous documentation of the dynamics of host-pathogen interaction and the important factors underlying their coevolutionary history.

There were three components in this research. First, a systematic greenhouse inoculation study of 23 lodgepole pine, 9 jack pine, and 8 putative hybrid populations using two genetically distinct WGR sources, one each from lodgepole pine and jack pine hosts was conducted. It addressed a central question in the study of host-pathogen coevolution by explaining host specificity difference underlying the dynamics of the pine-rust system. While the overall rust scores indicated that spores from lodgepole pine were more virulent to the hosts than those from jack pine, both host species were more susceptible to their own rust

sources, causing significant spore source × host group interactions. However, the dynamics of host specificity in the pine-rust coevolution was far from being in equilibrium because of the continued gene exchanges among the two parental species and their hybrids. This might in part explain why hybrid populations 29, 39, and 40 exhibited very high WGR scores.

The movement of lodgepole and jack pines into west-central Alberta after glacial retreat has created a broad range of metapopulation structures with different genetic constitutions. Rusts apparently followed and coevolved with their hosts during glacial and interglacial periods (Millar and Kinloch 1991). The PCBC is, therefore, expected to have considerably more complex pine-rust dynamics than that in single-species systems.

My finding of a strong host-specificity in the pine-rust system implies that the effective control of WGR fungus will largely depend on an understanding of genetic relationships among WGR and the pine hosts. To date, however, the process guiding the coevolution of WGR fungi with their pines remains poorly understood. It is probably complex (Millar and Kinloch 1991). Thus, there is the need to discern the relative roles of historical factors, ecological factors and contemporary factors, such as gene flow, genetic drift, mating system, and selection in the present-day patterns of geographic structure.

The second component in my research dissected the genetic structure of hosts using the same greenhouse populations. I specifically selected RAPD markers of known map positions to cover the genome with little physical linkage

between pairs of markers in order to provide a representative estimate of diversity. In addition, it is often assumed that due to their random nature, RAPDs are particularly useful for studying migration pattern and gene flow.

On average, jack pines exhibited higher levels of polymorphism and diversity than lodgepole and hybrid populations. Population differentiations for the hybrids (G_{ST} = 0.168) and lodgepole pines (G_{ST} = 0.162) were higher than jack pines (G_{ST} = 0.155). The corresponding estimates of gene flow were 2.5 for hybrids, 2.6 for lodgepole pines and 2.7 for jack pines. The apparent host specificity of the WGR and the greater differentiation among the lodgepole pine and the hybrid populations might have contributed to their higher WGR scores than that observed in the jack pine populations. This is because rusts apparently followed their hosts during glacial and interglacial periods (Millar and Kinloch 1991) and would thus evolve with the hosts. Although we were not be able to test the effect of genetic differentiation of WGR sources on the magnitude of pine-rust interaction since only two WGR sources were tested, we would expect that this effect is also strong.

Populations from *Pinus contorta – Pinus banksiana* complex maintained significant multilocus structure, with putative hybrids exhibiting stronger multilocus association than the parental populations. Introgressive hybridization between lodgepole pine and jack pine within the hybrid zone might have been through the coming together of previously isolated and genetically distinct populations, consistent with the postglacial histories of lodgepole and jack pines

(Rudolph and Yeatman 1982; Wheeler and Guries 1982; Critchfield 1985). Migration of parental genotypes into the hybrid populations played an important role in maintaining the apparent disequilibrium in hybrid populations. This indicates that effects of migration and random drift were more important than systematic selection effects in maintaining the multilocus association found in the hybrid populations.

The genetic gradient in the hybrid swarms caused by migration between species was parallel to the traits of adaptive importance in PCBC area. The genetic admixture of each population (ranging from 0.02 to 1.0) was significantly correlated with WGR severity in the greenhouse ($r = -0.32 \sim -0.46$) after controlling the effects of longitude and latitude. The greater the genetic contribution from jack pine, the more resistant to rust the population.

The third component of my research was to develop a new complementary model of gene interaction between host and pathogen by allowing for arbitrary levels of dominance in the diploid hosts. The model is particular suitable for studying the coevolutionary dynamics between hybrid populations and their pathogens in natural pathosystems. I showed that when there was overdominance or underdominance of resistance, the internal equilibrium points existed even when there was no cost of unnecessary virulence or when there was a cost of necessary virulence at the balance between cost of unnecessary virulence and effectiveness of resistance. In addition, the occurrence of stable resistance and virulence polymorphism was strongly dependent on the level of dominance.

This model could be used as one of the base models for studying pine-rust coevolution in the PCBC area. Lodgepole and jack pines proved to be closely related species and likely share a common gene pool including resistance genes. Therefore, it is reasonable to assume that their differences in resistance to rust were caused by different resistant genotypes. The hybrid swarms could be represented by using different levels of dominance. However, the application of this model to the real world should be cautious. Proper use of the parameters is of key important. The estimates of most of the parameters found in the literature were from annual crops, which are likely to differ from woody plants.

2 Future Studies Recommended

This study provided valuable knowledge about the pine-rust dynamics and the important factors underlying their coevolutionary history in the PCBC hybrid zone. Here are some suggestions for future studies.

Our study clearly indicated that there is strong pine-rust interaction between three parental groups (lodgepole pine, jack pine, and hybrids) and two genetically different rust sources. The sampling scheme of WGR fungi was designed based on the study of RAPD variation among geographic isolates (Li et al. 2001) and some economical considerations. Rigorous documentation of pine-rust interaction and host specificity in PCBC hybrid zone could be done by selectively choosing several host populations which represent different levels of introgression based on this study, and collecting WGR fungal isolates from each of those populations.

Although strong host specificity in this pine-rust system has been found in the greenhouse study, further observations on the changes of the resistance need to be conducted for several years for the out-planted individuals. This information is important for documenting the pine-rust interaction since little is known about the juvenile-mature correlation of the interaction yet.

It has been found that there is significant relationship between frequencies of diagnostic RAPD markers and resistance to WGR in this study. Further identification and mapping of QTLs for resistance to WGR using set of samples originating from the PCBC area can be useful for further exploration of the nature of pine-rust interaction and marker-assisted selection.

The development of a two-locus gene-for-gene coevolution model could be initialized by extending the single-locus model developed in this study. This extension would allow the model to simulate the situations where there are different resistance genes from hosts and different virulence genes from pathogens.

Different combinations of parameters representing various situations for the coevolution models need to be investigated and simulated for looking at the fates of pine-rust coevolution in PCBC.

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