INTRASPECIFIC DIVERSITY IN TREMBLING ASPEN

(*POPULUS TREMULOIDES*, SALICACEAE) IN

WATERTON LAKES NATIONAL PARK, ALBERTA:

A BIOGEOGRAPHIC PERSPECTIVE

by

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ABSTRACT

Trembling aspen (*Populus tremuloides* Michx), a clonal angiosperm, is the most geographically widespread tree in North America. It is widely thought that most extant populations in the western interior of Canada and the United States became established shortly after glacial retreat, but sexual recruitment effectively ceased soon thereafter owing to inimical climatic conditions. Six populations of *P. tremuloides* were studied in the prairie and montane environments of Waterton Lakes National Park, Alberta. Vegetative tissues were analyzed for electrophoretically-detectable variation in 13 enzymes encoded by 16 loci, 14 of which were polymorphic. Six populations maintained high levels of inter- and intra-population diversity ($\bar{P} = 0.891$; $\bar{H} = 0.319$; $\bar{A} = 2.4$). The mean fixation index, F, was -0.102 indicating some deviation from Hardy-Weinberg expectations. Genetic differentiation ($F_{ST} = 3.0$) was apparent in this ecologically diverse, but geographically small spatial setting. Some of this structure was attributed to the effects of selection. The pattern of allele frequencies was analyzed in relation to a surrogate measure of fitness, mean annual increment of ramets, which revealed the presence of a significant, albeit weak, positive correlation between growth and heterozygosity. This was interpreted as evidence of balancing selection acting to maintain diversity. A chemical analysis of dormant twigs for crude protein, macro- and micro-chemical elements, and fiber demonstrated that there were considerable differences between clones within populations and between sites. The relative intensity of browsing by mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*) also varied substantially between clones and between sites. Correlation analysis of browsing intensity with chemical composition demonstrated that herbivory by ungulates was not linked to protein, elemental composition, or fiber. Moreover, it is unlikely that selective feeding is cost effective given the nature of chemical differences between clones.

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It is postulated that while herbivores may affect genetic diversity, the precise effects are random with respect to the assayed chemical components. In conclusion, it is suggested that the maintenance of diversity in the absence of modern-day recruitment, and resistance to geographic differentiation in a spatially heterogeneous environment is largely due to clonality, specifically, the species' phalanx growth habit and concomitant physiological integration between ramets, the combination of which spreads the risk of death and buffers the effects of selection over time and space.

DEDICATION

To my son, Jeff, who sacrificed the most throughout my years of graduate study

ACKNOWLEDGMENTS

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"The more diversified the descendants from any one species become in structure, constitution, and habits, by so much they will be better enabled to seize on many and widely diversified places in the polity of nature, and so be enabled to increase in numbers."

Charles Darwin (1859)

PROLOGUE

A Biogeographical Perspective on the Genetic Structure of Natural Plant Populations

Biogeography, as a specialization within geography, is concerned with the integration and synthesis of phenomena as they pertain to the distribution of organisms. It relies heavily on ecology, population biology, systematics, evolutionary biology, natural history and the geosciences, anatomy, cytogenetics, and physiology (Brown and Gibson 1983). Within the context of plant and animal distributions these disciplines are woven together to pose that distinctively geographical question: "Why are spatial distributions structured the way they are?".

Contemporary biogeographers, in the tradition of the 19th and early 20th century biogeographers such as von Humboldt, deCandolle, Wallace, Warming and Raunkiaer, have worked within a paradigm that has emphasized large-scale spatio-temporal processes, concerning themselves, for example, with distributions of major taxa, vegetation, or plant "formations". They invariably assumed that conspecific populations of organisms were comparatively uniform in life history traits, species interactions, and habitat requirements. Recently, O'Neill et al. (1986) criticized biogeographers for typically "smoothing over" small-scale variation and paying little attention to short-term dynamics in their search "to find constancy beyond the perceptual scale of a single human investigator." Notwithstanding, it can be argued that this broad-brush approach was sufficient, and

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perhaps necessary, at ecosystem or larger organizational levels. However, similar conceptualization was often also invoked for smaller systems, for the need to consider community, population, and individual variation was generally perceived as being of little consequence, or as a hindrance to the development of mathematical descriptions. For example, the predilection of biogeographers (and ecologists) to perceive the species as a homogeneous and static ecological unit underlies the tangled history of community organization. The notion of spatial constancy can be seen in Clements' (1916, 1936) ideas of plant communities evolving as superorganisms over time culminating in a common, stable endpoint, the climax association. The concept of the biologically-homogeneous superorganism also permeated the phytosociological ideas of Braun-Blanquet (1966) and is central to the concept of group selection.

A final example of how delimiting geographical boundaries is often confounded by holistic thinking can be found in the analysis of species' distributions. Description of a species' ecological characteristics or delineation of its geographic distribution is largely based on the taxonomist's unit (Harper 1982). Unfortunately, these criteria are founded on stable and conservative characters, yet as pointed out by Harper (1982), individuals which comprise such distributions often have significantly different ecologies that are, in part , genetically driven. For example *Betula pubescens* occupies a latitudinal gradient in Finland, but when populations from the north and south are reciprocally transplanted, there are manifest differences in defoliation by herbivores (*Oporinia* sp.) (Haukioja 1980). A similar trend occurs along an elevational gradient. Indeed, some geographical distributions comprise general-purpose genotypes (*sensu* Baker 1965) which engender highly plastic phenotypes that respond similarly to a given set of environmental conditions. However, many other distributions are the sum of local or regional specialists (Levins 1968; Harper 1982).

For effective description, Harper (1982) emphasized the need for examining species' distributions through detailed studies of the autecology and genetics of species. Unfortunately, the marriage of population genetics to theory in biogeography and ecology has been slow in gaining acceptance. Yet, the contribution of genetics to biogeography and ecology is clear in R.J. Berry's (1989) recent Presidential Address to the British Ecological Society, entitled "Ecology: where genes and geography meet", in which he stated that "Ecologists tend to operate on as simplistic a notion of genetics as biologists did of species in Linnaeus's time: that individuals or populations can be treated as if they were genetically identical with any other individual or population of the same species" and that "we cannot understand why is what where . . . unless we concentrate on the interactions between genome and environment in time and space".

Evolutionary theory, by way of the "neo-Darwinian" or "modern" synthesis, has broad implications for ecological biogeography because the amount and distribution of genetic variation within and between populations affects a species' ecological amplitude, and hence, geographic distribution. This is evident from the findings of many ecogenetic investigations, beginning with the work of Turesson (1922), Gregor (1930, 1938) and the Carnegie group of Clausen, Keck, and Hiesey (Clausen et al. 1940, 1948; Clausen and Hiesey 1958). They demonstrated that for some species, the range of individual tolerance and developmental plasticity was insufficient to accommodate the environmental range and phenotypic response necessary for survival. Moreover, they clearly showed that under such conditions the distribution of species often comprises a number of genetic races each adapted to a particular environment. More recently, Bradshaw and his collaborators, who examined adaptation of plants to soils with high heavy metal contents (e.g., Jain and Bradshaw 1966; McNeilly 1968; Antonovics and Bradshaw 1970; Wu et al. 1975), showed that the distribution of genotypes may follow a clinal pattern at a microhabitat scale.

Their work spawned numerous other studies which also demonstrated the existence of locally-adapted populations (e.g., Hamrick and Allard 1975; Snaydon and Davies 1976; Turkington and Harper 1979; Waser and Price 1985). From an ecogenetical perspective, these and other studies have prompted rejection of the classic hypothesis of uniformity (Grant 1971), in favor of the balance hypothesis of great genetic variability maintained by various kinds of selection (Stebbins 1950; Lewontin 1974).

Over the past two decades, numerous studies have described the levels and distribution of genetic variation in natural plant populations (see reviews Heslop-Harrison 1964; Langlet 1971; Hamrick 1982), much of which has been recently analyzed in relation to various life history traits (Loveless and Hamrick 1984; Hamrick and Godt 1989). Most of this information concerns short-lived plants; comparatively little is known about woody perennials. A second poorly-studied feature of plant life histories is clonality, despite the commonness of this trait. Over half of the most cosmopolitan angiosperms show some asexual reproduction (Silander 1985). One angiosperm species which is particularly intriguing is trembling aspen (*Populus tremuloides* Michx.), which is a clonal woody perennial. Although it is the most geographically widespread tree in North America (Jones 1985), this species has a tremendous propensity for vegetative reproduction and development of large clones (Barnes 1966; Kemperman and Barnes 1976). Only three studies have addressed questions related to the genetic diversity and spatial structures of this species (Mitton and Grant 1980; Cheliak and Dancik 1982; Hyun et al. 1987). None of these investigations have undertaken extensive analyses of genetic diversity and population structure in relation to adaptive traits of the species.

This study, therefore, focuses on the interplay of ecological and evolutionary forces controlling the local distribution of *P. tremuloides*. The study is divided into three main sections. In the first section, I address two questions: 1) What are the levels of genetic

variation (as indicated by enzyme electrophoresis) in populations of *P. tremuloides* in one part of its range and, 2) How is this genetic variation partitioned within and among populations? I then evaluate the amount and distribution of genetic variation with respect to life history traits and environmental influences. The second section focuses on the ecological significance of the electrophoretically-assayed genetic variation. Here, I examine the statistical and biochemical associations between heterozygosity and growth, an important measure of fitness, to ascertain the importance of balancing selection as a force in maintaining genetic diversity. The final section deals with the way in which the interaction of species at the plant-animal interface may affect genetic diversity. *P. tremuloides* is an important food resource for several members of the Cervidae (e.g., deer, moose, and elk). In this section, I evaluate clonal variation in the chemical composition of *P. tremuloides*, assess these results in terms of ungulate nutritional requirements, determine if selective foraging takes place, and consider the implications of herbivory on genetic diversity in populations of *P. tremuloides*. The dissertation concludes with a synthesis of the findings of the study, focusing on the ecology and evolution of *P. tremuloides* in local populations with comments on the evolutionary implications at macrogeographic scales.

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CHAPTER 1

Genetic Diversity and Geographic Structure of *Populus tremuloides* in a Heterogeneous Landscape

1.1 INTRODUCTION

Species characterized by extensive biogeographic ranges are often grouped into genetically distinct subpopulations that are largely isolated from one another. The substructuring of a species' range into eco-genetically discrete populations may arise because of three fundamental evolutionary forces: limited dispersal of pollen and propagules, genetic drift, and variable selection (Clausen et al. 1940, 1948; Ehrlich and Raven 1969; Bradshaw 1972; Levin and Kerster 1974; Slatkin 1985, 1987). These populations, sometimes classified as ecotypes, may exhibit morphological or physiological characteristics that are particularly suited for growth within a local geographic region (Turesson 1922; Clausen et al. 1940, 1948; Clausen and Hiesey 1958; Hamrick and Allard 1972; Chapin and Chapin 1981; Turkington and Harper 1979). The evolution of locally-adapted populations in the presence of strong selection pressures is, however, contingent upon the presence of preexisting genetic variants or variation continually arising by mutation.

There is some evidence for macrogeographic genetic differentiation in *Populus tremuloides* along a latitudinal gradient based on laboratory and common garden experiments (Vaartaja 1960; Pauley 1963; Pauley et al. 1963; Brissette and Barnes 1984). On a regional scale, and using electrophoretic evidence, Hyun et al. (1987) found that on average 6.8% of the total gene diversity in *P. tremuloides* from southwestern Ontario could

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be attributed to population differentiation among eight "geographic regions". In the western interior of Canada and the United States, modern-day sexual recruitment in *P. tremuloides* is widely believed to be a rare event because of the exacting seedbed requirements for germination and early seedling development (Pearson 1914; Baker 1925; Moss 1938; Cottam 1954; Barnes 1966; Kemperman and Barnes 1976; Cheliak and Dancik 1982; McDonough 1985). Mitton and Grant (1980) hypothesized that the effects of selection on populations of *P. tremuloides* might be manifest more in the absence of recruitment because gene flow can often prevent selection (and drift) from establishing locally differentiated populations (see review in Slatkin 1985, 1987). To test this hypothesis, they analyzed *P. tremuloides* population structure along an elevational gradient over 500 km^2 in Colorado using electrophoresis. Little differentiation amongst three polymorphic enzymes was found in relation to elevation. Still lacking is information on the population structure of *P. tremuloides* on a small geographic scale. The principal advantage of working at such a scale (from the plants' perspective) is that the confounding influence of limited dispersal and genetic drift are significantly reduced. The amount of genetic variation and the degree of population differentiation that exists within a population from a locally heterogeneous landscape may also reflect the adaptive ability of constituent populations as well as the modes and mechanisms of evolutionary change, even in the face of gene flow. Such a study may also reveal populations that have become differentiated in ways that are similar to divergence over large geographic areas.

In this portion of the study, genetic diversity and population differentiation in *P. tremuloides* is surveyed in a diverse montane and prairie environment in Waterton Lakes National Park (WLNP), Alberta. This area is particularly well suited to such a study because: 1) the stands are fairly open and could therefore be mapped and subdivided spatially into discrete groups along geographic and ecological gradients; 2) the physical

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environment is heterogeneous which is indicated by the altitudinal range (2940-1310 m) and is reflected in the diverse flora [approximately 870 vascular plant species from 313 genera and 78 families in 292 km^2 (Kuijt 1982)]; 3) the environment is relatively undisturbed by human activities; and 4) geographic distance is unlikely to have been a major impediment to historical gene exchange, thus obviating, to some extent, the effects of limited gene flow and associated genetic drift. The specific hypotheses tested herein are that *P. tremuloides* populations in this heterogeneous environment that have experienced considerable environmental change since colonization should be comprised of a suite of genetically-similar individuals, and spatially-variable selection pressures should generate spatially-discrete populations.

1.1.1 The species

Trembling aspen is one of three members of the family Salicaceae, section Leuce, subsection Tripidae, which also includes the North American bigtooth aspen (*P. grandidentata* Michx.) and the Eurasian aspen (*P. tremula* Michx.). The latter is the only deciduous tree in the world to have a wider range than *P. tremuloides* (Jones 1985). *Populus tremuloides* is dioecious, wind pollinated, and has a diploid number of chromosomes ($2n$ =38), although triploidy has been reported (Every and Wiens 1971; Einspahr and Winton 1976). *Populus tremuloides* reaches reproductive maturity by 10 to 20 years of age. Seed production peaks at about 50 years with light to heavy seed crops at 3-5 year intervals (McDonough 1985). The species is also characterized by a propensity for tremendous vegetative reproduction (Barnes 1966; Kemperman and Barnes 1976). *P. tremuloides* is generally considered to be a fire opportunist, but notwithstanding its affinity for burnt-over sites, populations may, in some instances, form stable vegetation

assemblages [see Mueggler (1985) for a review of various aspen communities in the western United States].

1.1.2 Working definition of a population

The geographic unit of interest in this study is that of a "population". Intuitively, there seems little difficulty with the term "population"; however, defining or operationalizing it is more problematic. Geneticists typically focus on a "Mendelian" population, also known as a panmictic unit, deme, gamodeme, or local group (Stebbins 1950; Crawford 1984), i.e., a community of interbreeding organisms. Even populations of sedentary organisms are not necessarily discrete units, nor have they fixed boundaries, so delineation is often arbitrary (Solbrig and Solbrig 1979; Dewey and Heywood 1988). Moreover, gene flow may be restricted between members of a population as a result of geographic separation, geographically varying selection, and asynchronous flowering (Hamrick 1983). Therefore, a population may not function as a single reproductive unit, but rather as a system of subpopulations which are more or less reproductively isolated from each other (Ehrlich and Raven 1969). Information on populations circumscribed by gene exchange is rare. For the purposes of this study, each local assemblage of stands of *P. tremuloides* is treated as a population. Operationally, these units fit Solbrig and Solbrig's definition of a population (1979:4) as "inhabitants of a given geographical locality."

1.2 STUDY AREA

The study area is located in Waterton Lakes National Park (WLNP), Alberta, Canada, adjacent to the Canada/U.S. International Boundary and Glacier National Park, Montana (Figure 1). It encompasses 292 km^2 and comprises two major geographic units: the Rocky Mountains and the Foothills. The Foothills region is found in the eastern section of the Park. Here, soft bedrock and overlying glacial and glacial-fluvial deposits result in a rolling topography where relative relief ranges from 10 - 30 m. The mountainous region of the Park, which is part of the Lewis Range and one of the Front Ranges of the Rocky Mountains, rises abruptly from the Foothills. Intense deformation of the sedimentary rocks in WLNP began about 100 million years ago and continued sporadically for at least the next 50 million years (Harrison 1976). Precambrian rocks were thrust up and over the younger Cretaceous formations along the Lewis Thrust; rocks of Paleozoic and Mesozoic age have since been eroded away. The area was most recently glaciated by Cordilleran ice of late Wisconsin age. Deglaciation was probably complete by 11,000 B.P. (Harrison 1976).

The winters in WLNP are normally cold, with varying amounts of precipitation, occurring mostly as snowfall. Mild Pacific airmasses have a moderating effect on winter conditions. This effect is even more pronounced when these airmasses are further warmed under downslope (i.e., chinook) wind conditions. Chinook frequency in WLNP is the highest in the province. Summers are short and cool with only 97 frost-free days on the low elevation, southern boundary of the Park (Longley 1967). Summer precipitation peaks in late May and early June. Precipitation generally increases with altitude and an east-to-west horizontal gradient. Apart from solar irradiance, the dominant climatic factor, in terms of its effect on vegetation and soil moisture conditions, is the prevailing southwesterly wind. These winds maintain an average velocity of 6.7 m s^{-1} but with occasional
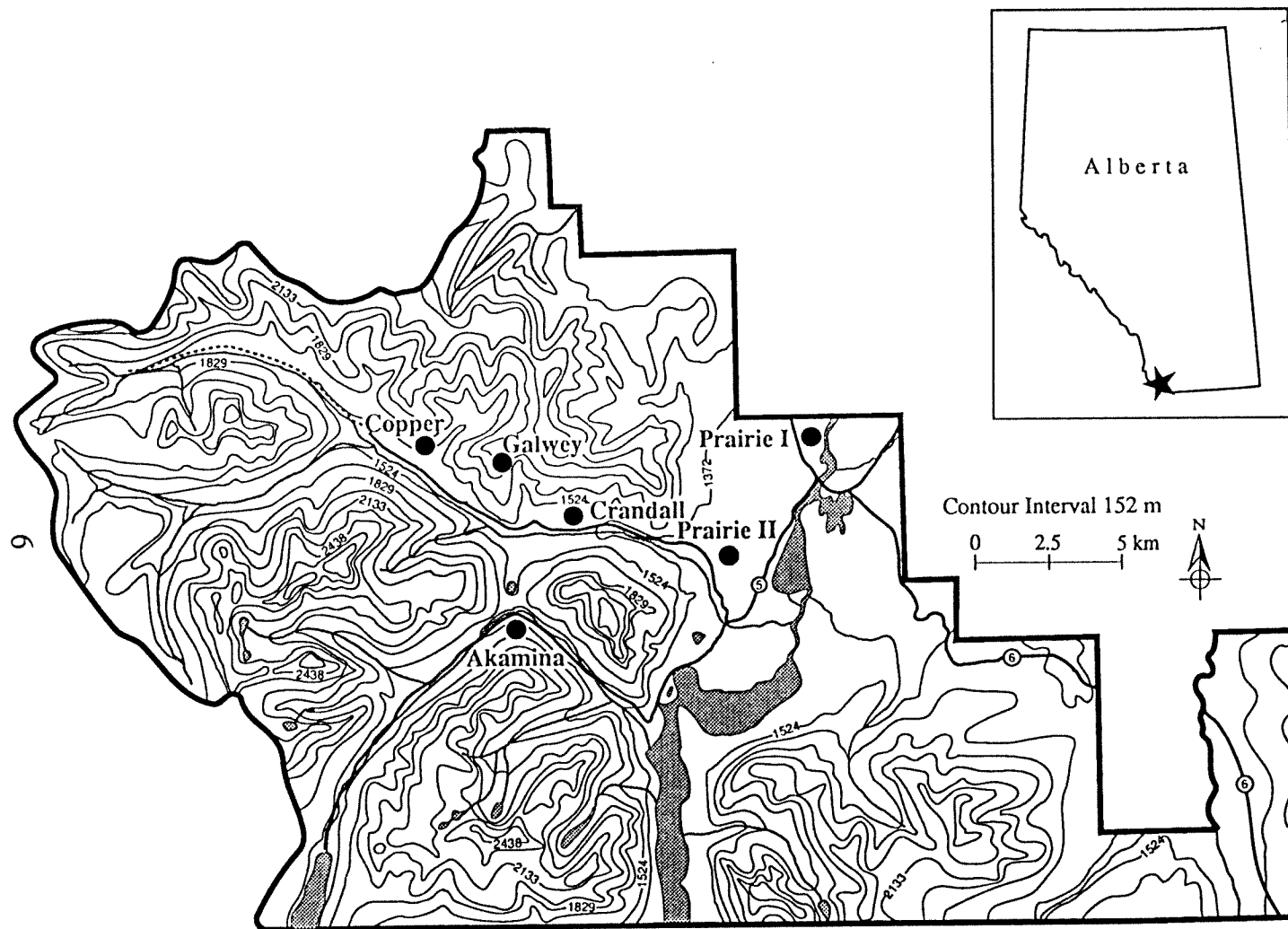
Figure 1. Location of the six *Populus tremuloides* populations, Waterton Lakes National Park, Alberta
Source: National Topographic System MCR 211

gale-force strength of 44.4 m s $^{-1}$ (Poliquin 1973). Owing to the varied physiography of the region there are a variety of local climatic conditions which, in turn, have a marked effect on the vegetation within WLNP (Finklin 1986).

The Foothills region is characterized by gray luvisolic and chernozemic soils (Coen and Holland 1973). The parent materials are slightly alkaline in reaction and are calcareous. The former tend to be low in organic matter and exchangeable bases. The chernozems, however, are typically high in organic matter with moderately high amounts of exchangeable bases. Brunisols occur at moderately high elevations (Coen and Holland 1973). They tend to be fairly acidic, low in organic matter, and often shallow in depth. Podzols tend to be found in the higher and moister western regions of WLNP. These soils are strongly acidic, have moderately high amounts of organic matter, and are well drained (Coen and Holland 1973). Finally, regosols can be found on the relatively recent or unstable land surfaces. They are poorly differentiated, well drained, slightly acidic to neutral, and variable in the amounts of organic matter and coarse fragments (Coen and Holland 1973).

Watt (1984) united the vegetation descriptions of Lopoukhine (1970), Kuchar (1973), and MacKenzie (1973) within the framework of an ecological scheme. The dry prairie region, or Fescue Grassland, covers approximately 6.7% of the total park area. The dominant vegetation is the Festuca/Danthonia assemblage consisting of *Festuca scabrella* and *Danthonia parryi*. These two species dominate on low elevation glacial till. The Fescue Grassland on the dry south-facing slopes is dominated by *F. idahoensis*, *Koeleria cristata*, and *Selaginella densa*. *F. scabrella* and *D. intermedia* can be found in the moister depressions. Flat, low elevation, and moist disturbed areas are primarily occupied by the introduced species *F. pratensis*, *Phleum pratense*, and *Bromus inermis*. Dominant shrubs

in this assemblage are *Amelanchier alnifolia* and *Symphorocarpos albus*. *P. tremuloides*, while present, is not widespread in this assemblage and is confined to moist depressions.

The Aspen Parkland assemblage covers approximately 6.1% of the park area. *Populus tremuloides* dominates this region, and can be considered as transitional between the prairie grasslands and the coniferous forest habitat types. It spans an elevational range from 1280 to 1435 metres (Watt 1984). Strong and Leggat (1981 in Watt 1984) suggested that increased moisture during July and August separates this assemblage from the Festuca/Danthonia assemblage. Watt (1984) divided the aspen forest into two types: low elevation and upland. Typically, the associated vegetation in the lowland sites consists of *Equisetum arvense*, *Heracleum lanatum*, and *Urtica lyalli* . Upland sites tend to be more "shrubby", characterized by *A. alnifolia* and *S. albus*, as well as *Berberis repens*. Kuijt (1982) mentioned that *P. tremuloides* understory typically includes species such as *Geranium viscosissimum* and *G. richardsonii*, *Clematis columbiana*, *Fragaria virginiana*, *Lathyrus ochroleucus*, *Silene menziesii*, *Thalictrum venulosum*, *Viola canadensis*, and *Smilacina stellata*. In the protected, or more moist regions, *P. tremuloides* is generally succeeded by the longer-lived and also fire-dependent *Pinus contorta*.

The moisture regime of the Montane ecoregion is intermediate between the coniferous forest and the grassland. This is a savanna-like habitat distinguished from other ecoregions by the presence of *Pseudotsuga menziesii* and *P. flexilis*. Its upper elevational range in Waterton has been reported to be 1675 m (Lopoukhine 1970). There is no mention by Watt (1984) of *P. tremuloides'* place in this assemblage. However, personal observations indicate that while *P. tremuloides* is not abundant, it can be found in "pockets" of habitat where later successional species have yet to dominate, or where habitat conditions are otherwise unsuitable for late successional species.

The Subalpine ecoregion occurs at altitudes above the Montane and Aspen Parkland habitat types but below the Alpine ecoregion. At the lower elevations of this vegetation type, *P. contorta* is the dominant tree. Above this is the more representative spruce-fir forest consisting of stands of *Picea engelmannii* and *Abies lasiocarpa*. Near the upper boundary *Pinus albicaulis* and *Larix lyalli* may be found. This complex habitat type accounts for approximately 35% of the total park area. Here, *P. tremuloides* is sparse, clone size is small and tree architecture has krummholtz characteristics except in protected or moist depressions.

The Alpine ecoregion occurs in areas above the "treeline". Snow cover has been suggested to be the most important factor in affecting plant distribution (Watt 1984). *Salix nivalis* and *Betula* spp. dominate the lower zones of this assemblage while *Cassiope* spp. and *Carex* spp. dominate the mid-elevational portions. Only lichens can be found at the highest elevations.

1.3 METHODS AND MATERIALS

1.3.1 Description of populations

This study is centered around six populations which were delineated on the basis of topography, soil type, altitude, climate, and geographic distance (Figure 1). The populations were separated by an average of 6.7 km (range, 1.0 - 13 km). Putative clones within a population were separated by at least 30-50 m of terrain lacking ramets of *P. tremuloides*, or by some impenetrable barrier (e.g., rock outcropping). An average of 26 clones were sampled per population (range, 22 to 37 clones) for a total of 156 clones. Although it might be desirable to have a larger sample size per population, sampling of larger geographic areas may confound the results owing to inter-habitat variation. The exception was the Akamina population where, because of succession, *P. tremuloides* is sparse. The geographic distance over which this population was sampled was greater than that of the other populations; however, the habitat within which clones were sampled was relatively homogeneous. Clones occurring at each sampling site comprise each population. Populations were selected from environments which were perceived to be relatively homogeneous, at least on a landscape scale as opposed to a fine scale. The latter is virtually impossible because of the propensity of *P. tremuloides* to form large clones in this area. Two populations (Prairie I and II) lie in what is known as the Badlands, a low elevation area underlain by glacial deposits (mostly till). Prairie I has lithic orthic brown chernozemic soils that have developed on shallow glacio-fluvial outwash over bedrock. Moisture storage is poor and potential evapotranspiration high. Mountains on either side of the Pass Creek valley rise abruptly 800 m on the west side of the Badlands and Lower Waterton valley plain. The Prairie II population lies at the junction of this valley within an area of glacio-fluvial deposits, outwash plains, and esker complexes. Prairie II has orthic dark

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brown and black chernozemic soils. They are medium textured with many gravel and cobble-sized fragments. Water permeability, however, is restricted because the till material of Cordilleran origin is very dense and compacted. Evapotranspiration losses and low water permeability result in extremely arid conditions. For both these populations, precipitation is low, varying between 75-99 mm y $^{-1}$ (Poliquin 1973). The dominant vegetation of both these populations is a Festuca-Danthonia assemblage (Kuchar 1973).

The Galwey and Crandall populations lie 4 km up the Pass Creek valley, a narrow U-shaped valley approximately 500 m wide and 7 km long. The Copper Creek population is found a further 3 km northwest. The Galwey population lies at the upper limits of *P. tremuloides* and close to the timberline (1810 m). Galwey lies on bedrock locally covered in debris or drift. Soils are regosols or lithic orthic regosols. The latter are characterized by rock outcrops interspersed with coarse textured soils having variable amounts of coarse fragments. The soils are shallow, having formed on unconsolidated materials such as resistant dolomites, medium sandstones, and fissile red shales. The dominant grasses are *Festuca scabrella, F. idahoensis, Agropyron spicatum* and *Danthonia parryi.* Shrubs include *Arctostaphylos uva-ursi, Juniperus communis* and *J. horizontalis. Pinus flexilis* is found on the drier sites, while *P. contorta* is found in the nearby mesic sites.

Lying below the Galwey population on the same landform is the Crandall site which has orthic dark brown chernozems that have formed on gravelly and cobbley alluvial fan deposits with south-facing slopes. The vegetation here is also grassland, dominated by more drought-tolerant species including *Festuca idahoensis, Koeleria cristata,* and *Selaginella densa. F. scabrella* and *Danthonia intermedia* may be found in moister depressions. The shrubs *Amelanchier alnifolia* and *Symphorocarpus albus* are also common in this population. The elevation of this population is about 1360 m. The

precipitation in the areas occupied by the Galwey and Crandall populations ranges between 75-100 mm y $^{-1}$.

To the northwest lies the Copper Creek population situated on bedrock. Soils are largely luvisolic. *P. tremuloides* is fairly abundant near the valley bottom. *P. contorta* is otherwise dominant. Elevation averages 1400 m and precipitation is moderate ranging from 100-124 mm y $^{-1}$.

The Akamina population is located in the narrow and confined Cameron Creek valley. This population is geographically most isolated from the others by a series of mountains with summits ranging from about 2400 m to 3000 m, as well as dense coniferous forest. The soils are orthic humo-ferric podzols and orthic regosols. The podzols are medium to coarse textured, porous, and contain abundant coarse fragments. Organic matter is high, as is moisture availability. The regosols are also coarse textured and contain coarse fragments. The dominant vegetation is *Picea engelmannii* and *Abies lasiocarpa* forest. Some *P. contorta* is also found in this population. Recently disturbed sites (e.g., avalanche chutes) have dense growth of *Salix* spp. Elevation ranges from 1370 to 1430 m. Precipitation is high ranging from 125-150 mm y $^{-1}$.

1.3.2   Tissue collection and electrophoresis

Dormant vegetative buds were collected from a single ramet chosen to represent each clone and stored in vials under refrigeration until use. Electrophoretic procedures generally follow Cheliak and Pitel (1984). At the Petawawa National Forestry Institute, crude enzyme extracts were prepared by grinding 10-50 mg of dormant bud tissue (scales removed) and treated in cold extracting buffer: 2% Tergitol 15-S-9, 2% Polyethylene glycol (20M), 8% polyvinylpyrrolidone, 50 mM Ascorbic acid, 0.4 mM beta-nicotinamide adenine dinucleotide, 0.1% Bovine serum albumin, 0.2 mM Pyridoxal 5'-phosphate, 0.3

M Sucrose, 12 mM Cysteine-HCl, 0.66 ml beta-mercaptoethanol which was dissolved in 0.05M borate buffer (pH 7.1) (Cheliak and Pitel 1984). The mixture was homogenized with a teflon-head, motorized grinder in a cold room (4°C). The homogenate was soaked onto filter-paper wicks (Whatman No. 3) before being loaded onto horizontal starch gels (1:1 Electrostarch, Connaught Starch). Enzymes were resolved on 12.5% starch gels using two buffer systems: (i) gel buffer ("B") consisting of 121.1 M Tris-192.1 M citric acid, pH 8.1, with electrode buffer ("B") consisting of Lithium hydroxide, 61.8 M boric acid adjusted to pH 8.1; and ii) gel buffer ("H") consisting of 0.05 M DL-Histidine-HCL titrated to 7.0 with HCL, and 1.40 mM EDTA with electrode buffer ("H") consisting of 125 M Tris titrated to 7.0 with 1.0 M Citric acid. Enzymes were designated by capital letters (e.g., AAT); isozyme loci were numbered if more than one locus was present (e.g., AAT-1). Loci were numbered sequentially from the most anodal to the most cathodal. The highest frequency allele of a locus was designated 100 and other alleles of that locus were identified by their mobilities relative to it. The enzyme systems, plus their Enzyme Commission (EC) reference numbers are shown in Table 1.1. No controlled pollinations were made to confirm the genetic control of the allozyme variants observed. Rather, genetic interpretations of the enzyme phenotypes were inferred from the known functional form of the enzyme.

1.3.3 Quantitative analyses

Genotypic diversity of *P. tremuloides* populations was examined by calculating the number of unique 14-locus genotypes divided by the sample size. Genetic variation was initially assessed using three descriptive statistics: i) proportion of polymorphic loci (P), which is the ratio of the number of loci exhibiting electrophoretic variability to the total number of polymorphic and monomorphic loci resolved; ii) average number of alleles per

Table 1.1. Allozymes scored in populations of *Populus tremuloides* from Waterton Lakes National Park, Alberta.

Enzyme System	EC number	Variation[1]
Asparate aminotransferase	2.6.1.1	
AAT-1		P
AAT-2		P
Alcohol dehydrogenase	1.1.1.1	
ADH-1		M
ADH-2		P
Aldolase	4.1.2.13	
ALD		P
Esterase	3.1.1.1	
EST		P
Glycerate-2-dehydrogenase	1.1.1.29	
G2DH		P
Glucose-6-phosphate dehydrogenase	1.1.1.49	
G6PD		M
Glutamate dehydrogenase	1.4.1.3	
GDH		M
Leucine aminopeptidase	3.4.11.1	
LAP-1		M
LAP-2		P
Phosphoglucomatase	2.7.5.1	
PGM		P
Peroxidase	1.11.1.7	
PER		P
Phospoglucose isomerase[2]	5.3.1.9	
PGI		-
6-Phosphogluconate dehydrogenase	1.1.1.44	
6-PGD-1		P
6-PGD-2		P
Shikimic acid dehydrogenase	1.1.1.25	
SDH		P
Malate dehydrogenase	1.1.1.37	
MDH		P
Menadione reductase[2]	1.6.99.2	
MR		-

[1] P and M refer, respectively, to polymorphic and monomorphic loci
[2] Refers to enzyme systems that were assayed but inconsistent

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locus (over all polymorphic and monomorphic enzyme systems); and iii) expected and observed proportion of loci heterozygous per individual. Expected heterozygosity is calculated as

$$H_e = 1 - \sum_{i=1}^{k} x_i^2 \qquad [1.1]$$

where x_i is the frequency of the i th allele, summed over k alleles, and averaged over all loci to yield H_e, the average proportion of heterozygous loci per individual. It provides an unambiguous measure of gene diversity as it measures gene frequencies (Nei and Roychoudhury 1974). Observed (H_O) and expected (H_E) proportion of loci heterozygous within a population were compared using chi-square tests to ascertain whether populations deviated from Hardy-Weinberg equilibrium conditions. The formula used was

$$\chi^2 = 2N \sum_x (\sigma^2{}_{kx}/\bar{p}_{kx}) \qquad [1.2]$$

with $(r-1)(m-1)$ degrees of freedom where m is the number of alleles, $\bar{p}_{kx}$ is the weighted mean allele frequency, and N is the the total sample size. The quantity H_T expresses the level of heterozygosity given the pooling of populations and random mating.

Having ascertained the amount of genetic diversity within the total population, the question was how this diversity was distributed and whether it was differentiated into an organized pattern or distributed at random. First, chi-square analysis was used to test the null hypothesis that the observed allele frequencies in the different populations are a random sample drawn from a single population (Workman and Niswander 1970). A significant value indicates that the amount of differentiation between populations was greater than that expected by chance alone. Wright's F statistics (1943, 1951, 1965, 1969) were used to elucidate how the detected variation was structured genetically. Wright originally

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formulated the statistics in terms of correlations between uniting gametes at different hierarchical levels of a population. Wright showed that intrapopulational deficiency of heterozygotes could be measured by the fixation index, F, and that the most likely cause of positive values is population structuring which can be due to a variety of factors. F compares observed and expected heterozygote frequencies (Jain and Workman 1967; Kirby 1975) where

$$F = 1 - H_O / H_E \qquad [1.3]$$

The value of F ranges from -1.0 to + 1.0. Positive values indicate a deficit of heterozygotes and negative values an excess. The quantity F_{IT} measures the average reduction in individual heterozygosity relative to the total sample (i.e., all populations). This reduction can be partitioned into two components: reduction due to inbreeding within populations (F_{IS}), and another contribution due to differentiation between populations (F_{ST}). When the populations are panmictic, F_{IS} is 0 and the overall reduction in heterozygosity is equal to the amount due to subdivision alone. Similarly, if there is no differentiation between populations $F_{ST} = 0$ and the only reduction in heterozygosity will be due to population level phenomena. The relationship between the three F statistics can be summarized as

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST}) \qquad [1.4]$$

The F statistics were calculated according to the methods of Weir and Cockerham (1984) using the computer programs of Black and Krafsur (1985). This method explicitly accounts for sample size and variation in sample size among populations and uses jackknifing to estimate F statistic variances (i.e., by recalculating statistics after loci or populations are sequentially removed). F_{IS} was calculated by taking the weighted average

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of $F = 1 - H / 2pqN$, where H is the observed number of heterozygotes and $2pqN$ represents the expected number, across all populations; weighting reflects the allele frequencies in each population (Kirby 1975; Nei 1977). F_{ST} was calculated as $\sigma_p^2 / \bar{p} (1 - \bar{p})$ where $\bar{p}$ and σ_p^2 are the mean and variance, respectively, of allele frequencies among populations.

From the allozyme data, Nei's (1972) standard genetic distance, D, which gauges the genetic similarity and evolutionary relationships between pairs of populations (X and Y), was calculated as

$$D = -ln \, (J_{xy}/\sqrt{J_x \, J_y}) \qquad [1.5]$$

where J_x, J_y, J_{xy}, are the arithmetic means across loci of $\Sigma \, x_i^{\,2}$, and $\Sigma \, x_i$, and Σy_i are the frequencies of the $i^{\,th}$ allele in populations X and Y. D is a measure of the accumulated number of detectable gene substitutions per locus. If the rate of gene substitutions per unit time is constant, D will be linearly related to time since divergence of two populations. D varies from 0.0 to 1.0. A related measure is Nei's (1972) identity measure, I, which in this case is a multilocus genetic statistic calculated from single-locus I values. It is a measure of the mean similarity of all pairs of populations within a species and is calculated as

$$I = J_{xy} \, /\sqrt{J_x \, J_y} \qquad [1.6]$$

The identity measure ranges from $I = 0.0$ for populations that have no alleles in common to $I = 1.0$ for populations with identical allele frequencies.

Two measures were used to measure clone diversity. The first was simply G/N where G is the number of distinct clones and N is the number of individuals sampled (Pleasants and Wendel 1989). The second was Simpson's index of diversity ($d = 1 - \Sigma \, p_i^{\,2}$) adjusted

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for finite sample size ($d = 1 - \Sigma \{[n_i(n_i - 1)] / [N(N-1)]\}$ (Pielou 1969). This latter index measures the probability that two clones (genotypes) selected at random from a population of N individuals will be genotypically identical.

1.4 RESULTS

1.4.1 Genetic and genotypic diversity

Of the 15 different enzyme systems assayed, 13 were consistently scorable (Table 1.1). The latter yielded 16 loci; LAP-1 and ADH-1 were found to be invariant in the total population. A total of 38 alleles were detected in the 14 polymorphic loci (Table 1.2). Averaged over populations, 89.1% of the loci were polymorphic (99% criterion). Most of the polymorphic loci had two or three alleles. The greatest variability was observed at the AAT-1 locus where five alleles were detected. Allelic frequencies at the 14 polymorphic loci and estimates of intrapopulation genetic variability (percentage of polymorphic loci, average number of alleles per locus and observed heterozygosity) for the six populations are presented in Table 1.3. The mean number of alleles per population was 2.4 (range 2.2-2.5). The proportion of polymorphic loci per population ranged from 75 (Galwey and Prairie II) to 87.5% (Prairie I). The unweighted mean polymorphism across all populations was 81.3%.

Expected heterozygosities ranged from 0.246 (Copper Creek) to 0.346 (Akamina) and averaged 0.290 over all populations. Observed heterozygosities were greater than Hardy-Weinberg expectations and ranged from 0.280 (Copper Creek) to 0.349 (Prairie II) with a mean of 0.319 (Table 1.3). Deviations from Hardy-Weinberg expectations were measured for each locus by the inbreeding coefficient or fixation index, F (Table 1.4). Nineteen of 78 (24.5%) tests had statistically significant departures from Hardy-Weinberg equilibrium which is considerably higher than the 5% expected. Five of the fourteen loci did not deviate significantly from their expected genotype frequencies in any of the six populations. In total, significant deficiences of heterozygotes were found in 10 instances and excesses in 9. Deviations from random mating on a per population basis ranged from one locus at the

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Table 1.2. Allele frequencies for polymorphic loci in six subpopulations of *Populus tremuloides* [1].

Locus (allele)	Prairie I	Prairie II	Akamina	Galwey	Crandall	Copper
G6PD						
1	0.203	0.220	0.340	0.304	0.409	0.250
2	0.797	0.780	0.660	0.696	0.591	0.750
ADH-2						
1	0.730	0.700	0.680	0.783	0.591	0.750
2	0.257	0.260	0.320	0.217	0.386	0.229
3	0.014	0.040	------	------	0.023	0.021
G2DH						
1	0.784	0.780	0.700	0.848	0.591	0.771
2	0.216	0.220	0.300	0.152	0.409	0.229
PGM						
1	0.851	0.900	0.880	0.935	0.818	0.896
2	0.095	0.100	0.100	0.043	0.136	------
3	0.054	------	0.020	------	0.045	0.083
4	------	------	------	0.022	1.000	0.021
LAP-2						
1	0.986	1.000	0.940	0.978	0.977	0.958
2	0.014	____	0.060	0.022	0.023	0.042
ALD						
1	0.730	0.780	0.640	0.826	0.682	0.750
2	0.270	0.220	0.360	0.174	0.318	0.250
PER						
1	0.730	0.920	0.520	0.870	0.977	0.917
2	0.270	0.080	0.480	0.130	0.023	0.083
6-PGD-1						
1	0.216	0.300	0.160	0.391	0.182	0.125
2	0.784	0.700	0.840	0.609	0.818	0.875
6-PGD-2						
1	0.149 -	-----	------	------	0.068	0.021
2	0.851	1.000	1.000	1.000	0.932	0.979
AAT-1						
1	0.694	0.913	0.800	0.875	0.750	0.854
2	0.139	0.043	0.120	0.025	0.068	0.021
3	0.083	------	------	0.050	--------	------
4	0.083	0.022	0.040	0.025	0.136	0.083
5	------	0.022	0.040	0.025	0.045	0.042
AAT-2						
1	0.946	0.840	0.940	0.717	0.955	0.958
2	0.014	0.160	0.020	0.239	--------	0.042
3	0.027	------	------	------	--------	--------
4	0.014	------	0.040	0.043	0.045	--------
EST						
1	0.568	0.520	0.320	0.478	0.341	0.563
2	0.324	0.440	0.360	0.478	0.341	0.354
3	0.108	0.040	0.320	0.043	0.318	0.083
SDH						
1	0.338	0.380	0.380	0.239	0.273	0.188
2	0.662	0.620	0.620	0.761	0.727	0.813
MDH						
1	0.959	0.940	0.860	1.000	1.000	0.958
2	0.041	0.020	------	------	------	------
3	------	0.040	0.140	------	------	0.042

[1] Dashes indicate alleles not detected in specific populations

Table 1.3. Genetic variability in *Populus tremuloides* in Waterton Lakes National Park, Alberta.

Population	% of loci polymorphic	Average no of alleles per locus	Heterozygosity	
			Observed	Expected
Prairie I	87.5	2.5	0.302	0.315 (0.042)
Prairie II	75.0	2.4	0.349	0.258 (0.047)
Akamina	81.3	2.2	0.306	0.346 (0.046)
Galwey	75.0	2.4	0.340	0.267 (0.047)
Crandall	81.3	2.4	0.334	0.306 (0.056)
Copper	87.5	2.5	0.280	0.246 (0.040)
Mean	81.3	2.4	0.319	0.290

Values in parentheses are ±SE

Table 1.4. Pooled within-population fixation indices (*F*) estimated for six populations of *Populus tremuloides*.

Locus	Prairie I	Prairie II	Akamina	Galwey	Crandall	Copper
AAT-1	0.312***	0.202	0.299*	0.314	0.449**	0.522***
AAT-2	-0.039	-0.049	-0.107	-0.122	1.000***	-0.043
ADH-2	0.057	-0.361	-0.103	-0.278	-0.270	-0.300
ALD	0.041	-0.282	0.132	-0.211	-0.048	-0.333
EST	-0.156	-0.647***	0.519***	-0.769***	0.386***	-0.285
G2PG	0.203	-0.282	0.333	-0.179	-0.316	-0.297
G6PD	0.080	-0.282	0.020	-0.437*	-0.692***	-0.111
LAP-2	-0.014	------	-0.064	-0.022	-0.023	-0.043
PER	-0.178	-0.087	0.038	-0.150	-0.023	-0.191
6PGD-1	-0.276	-0.429	-0.190	-0.278	-0.222	-0.143
6PGD-2	0.253	------	------	------	-0.073	-0.021
PGM	-0.129	-0.111	0.628***	0.649***	-0.173	-0.096
MDH	-0.042	0.049	-0.502**	------	------	1.000***
SDH	-0.027	-0.613**	-0.613**	-0.314	-0.375	-0.231
Mean	0.006	-0.203	0.065	-0.150	-0.029	-0.041

Dashed line indicates that a single allele was fixed in the population; hence *F* is undefined.

[1] The value of *F* ranges is from -1.0 to +1.0; positive values indicate a deficit of heterozygotes and negative values an excess.

* $P \leq 0.05$

**$P \leq 0.01$

***$P \leq 0.001$

Prairie I population to five at the Akamina population. No loci deviated significantly from Hardy-Weinberg expectations for all populations, although EST showed significant departures in four of the populations and approached statistical significance ($P = 0.09$) in the Copper Creek population. As for other instances, where two or more individual loci departed signficantly from Hardy-Weinberg expectations across populations, AAT-1 displayed a consistent deficit of heterozygotes. Conversely, SDH and PGM had higher levels of heterozygosity than predicted. The highest levels of heterozygosity were noted in the Prairie II population. When heterozygosity is considered across loci, but within populations, the Prairie II and Copper Creek populations had both an excess and deficit of heterozygotes, respectively (Table 1.4). All other loci deviated in different directions within each population. Deviations from Hardy-Weinberg expectations were also discovered in four of six populations at EST though no consistent directional trend was found. Excess heterozygosity at G6PD locus was found for the Crandall and Akamina populations.

When the sample size was accounted for by the method of Weir and Cockerham (1984), the within-population fixation index weighted over 14 loci was -0.102 indicating a 10.2% excess of heterozygotes relative to Hardy-Weinberg expectations. Indices on a per population basis are presented in Table 1.5. F_{IS} values were highly variable ranging from -0.331 at SDH to 0.359 at MDH; if the populations were panmictic $F_{IS}=0$. Negative values are evidence of a deficiency of heterozygotes within population subdivisions while positive values indicate an excess of heterozygotes. The F_{IT} values, also highly variable, are consistent in sign with F_{IS} values; F_{IT} values range from -0.310 at SDH to 0.382 at MDH. Again, negative values imply no subdivision is evident, while positive correlations indicate the existence of population subunits. The average heterozygote excess relative to Hardy-Weinberg expectations is -0.070. This suggests that heterogeneity within

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Table 1.5. Estimates of F_{IS}, F_{IT}, F_{ST} for 14 polymorphic loci in *Populus tremuloides*

Locus	F_{IS}	F_{IT}	F_{ST}
EST	-0.101	-0.062	0.035
SDH	-0.331	-0.310	0.015
MDH	0.359	0.382	0.036
G6PD	-0.208	-0.189	0.016
ADH-2	-0.169	-0.166	0.002
G2DH	-0.039	-0.019	0.019
PGM	0.074	0.075	0.001
LAP-2	-0.022	-0.024	0.003
ALD	-0.067	-0.063	0.003
PER	0.059	0.210	0.160
6-PGD-1	-0.258	-0.221	0.029
6-PGD-2	0.182	0.230	0.059
AAT-1	0.354	0.358	0.007
AAT-2	0.059	0.117	0.062
Mean	-0.102	-0.070	0.030

populations with respect to heterozygote excess is greater than heterogeneity among populations where the populations are considered as a single panmictic unit.

1.4.2 Population structure

Patterns of allele frequency distribution indicated a moderate amount of genetic heterogeneity among populations (Tables 1.5 and 1.6). Chi-square tests for heterogeneity of allele frequencies among populations were significant ($P \leq 0.02$) at seven loci (AAT-1, AAT-2, EST, MDH, PER, 6PGD-1, 6PGD-2) (Table 1.6). This suggests that a genetically-based geographic structure is present in the Waterton populations. Since the primary component of F_{IS} was F_{IT}, most diversity lies within populations. Values of F_{ST} range from 0.003 at LAP to 0.160 at PER and average 0.030 over all loci from which I infer that approximately 97% of the detected genetic variation in *P. tremuloides* from WLNP resides within populations. Most PER heterogeneity arose in the Akamina population where a slight heterozygote excess was noted. A deficiency of heterozygotes was noted in all other populations at this loci. Other loci which exceeded the mean F_{ST} value include AAT-2, 6PGD-2, EST, and MDH (Table 1.5).

Table 1.7 presents pairwise comparisons of genetic identities and genetic distances for all combinations of the six populations. No populations were genetically identical. However, they tended to be uniformly quite similar averaging 0.987; pairwise genetic values ranged from 0.973 for Galwey and Akamina to 0.995 for Prairie I and Prairie II. Genetic distances between populations were low ($D = 0.013$), as was the correlation between geographic and genetic distance ($r^2 = 0.026$). While the statistical significance of this coefficient cannot be tested since there is no method of determining the degrees of freedom for such pairwise comparisons of two matrices of intercorrelated populations

Table 1.6. Chi-square analyses of heterogeneity of allele frequencies among loci.

Locus	No. of alleles	Chi-square	DF	P
EST	3	37.30	10	0.001
SDH	2	7.32	5	0.20
MDH	3	29.15	10	0.001
G6PD	2	7.98	5	0.16
ADH-2	3	8.39	10	0.59
G2DH	2	9.76	5	0.08
PGM	4	19.87	15	0.18
LAP-2	2	4.27	5	0.51
ALD	2	5.49	5	0.36
PER	2	47.13	5	0.001
6-PGD-1	2	13.26	5	0.02
6-PGD-2	2	24.90	5	0.001
AAT-1	5	35.69	20	0.02
AAT-2	4	45.66	15	0.001

Table 1.7. Genetic identities (upper triangle) and genetic distances (lower triangle) for all pairwise comparisons of six subpopulations of *Populus tremuloides*.

	Prairie I	Prairie II	Akamina	Galwey	Copper	Crandall
Prairie I	-------	0.995	0.986	0.986	0.996	0.984
Prairie II	0.005	-------	0.978	0.997	0.998	0.988
Akamina	0.014	0.023	-------	0.973	0.978	0.979
Galwey	0.014	0.003	0.027	-------	0.992	0.976
Copper	0.004	0.002	0.022	0.008	-------	0.992
Crandall	0.016	0.013	0.022	0.024	0.008	-------

(Jorde 1980), the low correlation coefficient strongly suggest genetic and geographic distances are uncorrelated.

1.4.3   Clonal diversity

All populations were polyclonal. Of the 156 putative clones, 144 clones had uniquely defined multilocus electrophoretic genotypes thereby generating an average G/N value of 0.92 with a range from 0.86 to 1.0. Therefore, within a population, the chance of discovering a new clone was 92%. Twelve instances of clonal duplication were found; eight of these were in one population (range 2-5 ; $\bar{x} = 2.8$). Clonal duplication was confined to a single population in eight of the 12 instances. No clones were duplicated in all populations; one clonal type was found in four populations. No clonal duplication was found within the Akamina and Galwey populations, though the former shared one clonal genotype with two other populations. The estimates of genotype diversity, as measured by Simpson's index for finite sample (D) ranged from 0.98 to 1.00 with an average of 0.99.

1.5 DISCUSSION

1.5.1 Genetic Diversity

Populations of *P. tremuloides* in Waterton Lakes National Park exhibit significant levels of genetic diversity as revealed by enzyme electrophoresis. The percentage of electrophoretically-detectable polymorphic loci per population (81.3%) and the mean number of alleles per locus (2.4) are considerably greater than the average for plants in general (50.0%; 1.53) (Hamrick and Godt 1989). These results are, however, comparable with those reported in other studies of genetic variation in *P. tremuloides*. Cheliak and Dancik (1982) reported values of 92%; 2.3 and Hyun et al. (1987) 79%; 2.7, respectively, for polymorphism and mean number of alleles per locus. The expected level of heterozygosity in this study (30.5%) is intermediate between the values of 42% and 23.5% in the *P. tremuloides* studies of Cheliak and Dancik (1982) and Hyun et al. (1987), respectively. Studies of heterozygosity in other tree and shrub species indicate that these values are not substantially greater than those reported elsewhere for obligate outcrossers. Heterozygosity in *Camellia japonica* was observed to be 23% (Wendel and Parks 1985). Bousquet et al. (1987, 1988) reported estimates of heterozygosity for *Alnus crispa* and *A. rugosa* to average 13.5% and 16.5%, respectively. Heterozygosity in *Robinia pseudoacacia* averaged 29% (Surles et al. 1989). Similarly, the levels of heterozygosity in ecologically related conifers ranges from 20-30% (Yeh and Layton 1979; Yeh and O'Malley 1980; Conkle 1981; Wheeler and Guries 1982; Alden and Loopstra 1987) with a mean of 20.7% based on 20 species (Hamrick et al. 1981).

It is germane to also make comparison to the levels of genetic diversity in populations of clonal plants similarly characterized by an ability to reproduce vegetatively. Table 1.8, adapted from Pleasants and Wendel (1989), presents such an overview. The level of

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Table 1.8. Comparison of genetic and clonal diversity in natural plant populations[1]

| Species | Genetic diversity | | | | Genotypic diversity | | | | | | |
| | Within species | | | Among pops | Within pops. | | | | Pops/G | Local | Wide spread |
	$\%P$[2]	A[3]	H_T[4]	G_{ST}[5]	Pops/N[6]	G[7]	G/N[8]	D[9]	G[10]	(%)[11]	(%)[12]
Erythronium albidum[a]	38	3.0	0.45	0.01	7/70	63	0.90	0.96	1.0	100	0
Erythronium propullans[a]	24	1.8	0.31	0.33	7/93	21	0.23	0.66	1.5	86	5
Puccinellia x phryganodes[b]	100	----	0.42	0.33	3/30	21	0.70	0.81	1.0	95	0
Agrostis stolonifera[c]	----	----	-----	----	6/178	42	0.24	0.69	1.1	98	0
Spartina patens[d]	48	----	0.43	0.12	4/34	101	0.29	0.93	1.4	62	0
Typha (4 spp.)[e]	0	----	0	----	----	1	----	0.00	----	0	100
Alnus incana[f]	40	3.0	0.57	0.07	4/409	5	0.01	0.69	4.0	0	100
Puccinellia maritima[g]	----	----	----	----	4/328	86	0.26	0.80	1.0	99	0
Cyperus esculentus[h]	33	2.0	0.24	----	10/200	9	0.05	0.44	2.1	66	0
Cyperus rotundus[h]	10	2.0	0.05	----	4/80	2	0.03	----	3.0	0	50
Sarracenia purpurea[i]	50	1.7	0.21	----	11/458	----	----	----	----	----	----
Populus tremuloides[j]	92	2.3	0.42	----	7/222	222	1.00	1.00	1.0	100	0
Populus tremuloides[k]	89	2.4	0.31	0.30	6/156	144	0.81	0.99	1.0	100	0

[1] Adapted from Pleasants and Wendel (1989)

[2] % Polymorphic loci, [3] number of alleles per locus; [4] genetic diversity; [5] proportion of genetic diversity among populations; [6] number of sampled individuals; [7] number of unique clones; [8] genotype diversity; [9] genotype diversity; [10] number of populations per genotype; [11] found in only 1 population; [12] found in 75% of populations.

[a] Pleasants and Wendel (1989); [b] From Pleasants and Wendel (1989); [c] Wu et al. 1975; [d] Silander 1984; [e] Sharitz et al. 1980; [f] Huenneke 1985; [g] Gray et al. 1979; [h] Horak et al. 1987; [i] Schwaegerle and Schaal 1979; [j] Cheliak and Dancik 1982; [k] this study

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polymorphism in this study tends to be considerably higher than that documented for most of these species, which is, in part, a reflection of each species breeding system. Populations of *P. tremuloides*, *Alnus incana*, *Spartina patens* and *Erythronium albidum* are founded by sexual propagules, and accordingly, have high levels of genetic diversity. Lower levels of genetic diversity are associated with species such as *Agrostis stolonifera* and *Cyperus* sp., which, while capable of producing sexual reproduction, tend to "reproduce" by vegetative means under most conditions. Last, species such as *Puccinellia* x *phryganodes* are not known to reproduce by sexual means; rather, all reproduction has been attributed to vegetative expansion (stolons) (Jeffries and Gottleib 1983). Overall, however, genetic diversity remains relatively high despite the absence of recombination. With regard to heterozygosity, the levels I found are somewhat lower than that found in several clonal species listed in Table 1.8, but are in accord with the H_T values of 0.311 and 0.293 for sexual species and wind-mediated outcrossing species, respectively (Hamrick and Godt 1989).

There was a slight excess of heterozygotes over that expected under random mating, a result consistent with the findings of Cheliak and Dancik (1982) but in partial disagreement with those of Hyun et al. (1987) who found a consistent deficiency of heterozygotes. Hyun et al. reported fixation indices, F, to range from 0.295 to 0.568 and average 0.462. They suggested that the disparity between their results and those of Cheliak and Dancik (1982) may be owing to differences in loci and tissue used in the electrophoretic analysis. The differences may also have arisen because Hyun et al. (1987) pooled stands from the same forest cover type into "populations" and, as they point out, if indeed these pooled stands are genetically distinct, a deficiency of heterozygotes will arise because of the Wahlund effect (Nei 1977). Small heterozygote deficiencies are common to outcrossing plants for this reason as well as consanguineous matings and partial selfing.

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In all populations, the genotypic frequencies deviated significantly from Hardy-Weinberg equilibrium. Both deficiencies and excesses of heterozygotes were observed. Heterozygote deficiences may arise due to nonrandom mating, drift, and selection. Heterozygote deficiencies were not widespread at other loci in the four populations where significant deviations from Hardy-Weinberg expectations occurred. Therefore, nonrandom mating, gene flow and drift are probably not involved. One particularly interesting pattern to emerge was at the AAT-1 loci where high levels of homozygosity (20-50%) were noted in all populations, suggesting that selection might be operating against heterozygotes. If inbreeding were taking place, then the direction of deviations would be similar for all loci. All populations, save for Akamina, had negative F values, a characteristic that might reflect its relative isolation or habitat conditions. Overall, the absence of any strong singular pattern suggests that several forces are probably acting in concert differentially affecting different loci in different populations.

1.5.2 Clonal diversity

In general, the influence of gene flow in maintaining clonal diversity is not considered to be of major importance in most clonal populations as they are composed of a common clonal structure (i.e., where one or a few genotypes numerically dominate the population) [see review in Ellstrand and Roose (1987)]. The findings in this study run counter to this trend in that *P. tremuloides* has a high degree of within-population genotypic diversity. Cheliak and Dancik (1982) similarly found high levels of clonal diversity; in fact, all 222 clones that they analyzed were electrophoretically unique. Additional comparison to other clonal populations with vegetative reproduction using data compiled by Ellstrand and Roose (1987) and Pleasants and Wendel (1989) is made in Table 1.8. It is best to make comparisons between studies by evaluating values for *D*. Other measures such as the total

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number of genotypes and G/N tend to be particularly affected by the intensity of sampling effort, the geographic proximity of sampled individuals and locations, and the number of characters (including loci) used to distinguish between clones (Ellstrand and Roose 1987; Pleasants and Wendel 1989). In this table it can be seen that the only other sexual/vegetatively-reproducing species that are characterized by equally high values of clonal diversity are *Erythronium albidum* and *Spartina patens*.

Two explanations may account for those cases where genotype duplication occurred within a population. First, a single genotype may have established at more than one location, or second, I may have unwittingly sampled a single clone more than once, despite the procedure for selecting putative clones whereby each was separated by at least 30-50 m of terrain or some impassable barrier (e.g., rock outcropping). Presumably, clones expand and contract in relation to changing environmental conditions. In four of the six instances where I observed common multilocus genotypes in this population, the clones were in close proximity to one another; thus each could have fragmented from a single larger clone. In the Prairie I population, I found the preserved remains of a *P. tremuloides* root system below what is now a grassland community. The nearest clone was about 25 m away. This root system may have been part of the adjacent clone or other nearby clones, or, alternatively, that of a now extinct clone.

With regard to among-population diversity, there were four cases where a single genotype was found at two to four sites, each separated by several kilometres. It is inconceivable that a single clone could have once been so large as to span this distance. The largest reported *P. tremuloides* clone consisted of some 47,000 "mature" stems covering 43 ha in Utah; however this clone was south of the glacial maxima and may date to the Pleistocene (Kemperman and Barnes 1976). In southern Manitoba, Steneker (1973) found the area of most clones to fall in the 0.006 to 0.08 ha range with the largest clone

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being 1.5 ha. In northern Manitoba, Wall (1969) found considerable variation in clone size but none larger than 0.2 ha. Therefore, it is concluded that the clonal replicates found in different populations represent separate individuals. Additionally, as indicated above, these genotypes may differ at other loci, and as such, they would not be members of a common clone. Interestingly, Galwey was the only population in which all clones were unique. Unlike the other populations, the terrain here is rugged and the soils patchy, which greatly limits clone expansion. Further, there is reason to believe (see section 1.5.5.2) that the clones at this site might be of more recent age, and accordingly, one can expect that the suite of recombinants would be different in structure from those of previous colonization events, thus reducing the probability of finding similar multilocus genotypes.

1.5.3 Origin and maintenance of genetic and genotypic diversity

Genetic diversity in populations of species with an outcrossing breeding system like *P. tremuloides* initially arises through the process of recombination following mutation, and is augmented by long-distance dispersal of both pollen and seeds. Several life-history traits of *P. tremuloides* promote substantial gene flow, including the outcrossing mode of reproduction, high fecundity, wind-mediated pollen and seed dispersal, and long generation times (Loveless and Hamrick 1984). These life history features may result in: 1) large effective population sizes, and 2) high allelic diversity in temporally and spatially variable environments.

Despite the fact that *P. tremuloides* frequently produces large seed crops, sexual recruitment is thought to be rare in western North America (Pearson 1914; Baker 1925; Moss 1938; Cottam 1954; Barnes 1966; Maini 1968; Einspahr and Winton 1976; Cheliak and Dancik 1982; McDonough 1985). The apparent rarity of modern-day sexual recruitment is suggested to result from a brief period of seed viability combined with

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exacting seedbed requirements for germination and early seedling development. Moss (1938) found that mature *P. tremuloides* seeds were viable for only 2-4 weeks. Seeds protected from precipitation, but not humidity or temperature fluctuations, declined in viability from 40-60% after four weeks, and 75-100% after eight weeks (McDonough 1979). Optimum conditions for successful germination and survival include a well-drained seedbed, moderate temperatures and freedom from competition (McDonough 1985). Germination and emergence are also reduced in the face of competition by inhibitors in litter [e.g., coumarin at concentrations as low as 100 ppm (McDonough 1979)] and low water potentials [e.g., -0.4 to -0.5 MPa (McDonough 1971, 1975)]. Once germinated, the root hairs often have difficulty penetrating the soil surface where they must perform an essential water-absorbing function until significant root growth occurs (Moss 1938; Day 1944). Furthermore, anchorage tends to be weak, the root and hypocotyl grow slowly, and the hypocotyl quickly etiolates under poor light conditions (McDonough 1985). Even upon seedling establishment, small soil water deficits quickly bring on wilting, dessication and possibly death (Moss 1938; Einspahr and Winton 1976). In Michigan, Barnes (1966) set up a series of plots in moist and sheltered "microsites" containing 18-450 newly germinated seedlings; all seedlings were dead within two years. The only reports of apparently well-established seedling populations in the dry western portions of *P. tremuloides'* range are those of Dixon (1935), Faust (1936), Ellison (1943), Larson (1944) and Barnes (1966). Establishment of seedlings in all of these cases was because of extremely favorable moisture conditions (spring banks and drawn-down reservoirs), an absence of competition and favorable substrates (e.g., burned sites), or a combination of these conditions. There was no indication of recent seedling establishment in Waterton except in disturbed roadside habitats.

Various types of selection could account for the presence of genotypically diverse populations. Diversifying selection might have taken place not only between the populations occupying the different environments in the study area, but also operate within populations. Genotypic diversity may also reflect a latent response to historical selection regimens characterized by fluctuating selection pressures (e.g., pathogenicity, herbivores, climatic change, competition, and disturbance) (Burdon 1980; Silander 1985; Jelinski and Hutchinson 1988). Such temporal dynamism may thus generate niche differences among coexisting clones (Harberd 1961; Solbrig and Simpson 1974; Williams 1975; Burdon 1980; Ellstrand and Levin 1982). Others (e.g., Hebert and Crease 1980; Loaring and Hebert 1981) have also suggested in clonal species that maintenance of diversity in the face of fitness differentials could be aided by differing reproductive strategies that occasionally allow for higher fitness of the otherwise maladapted clones. Given the long-term variability in the physical environment, however, competitive dominance amongst the potentially immortal clones may well be reversible (Sebens and Thorne 1985), and even maladapted genotypes may persevere for long periods of, for example, unfavorable climate. Perseverance under such conditions is probably due in large part to the species' propensity for vegetative reproduction which allows the clones to "spread risk" (Cook 1979 and see section 1.5.5.2).

Balancing selection may be implicated in maintaining genetic diversity in clonal populations though its role is poorly known (Silander 1985). It has been suggested that heterozygotes may have greater physiological versatility and hence permit a more flexible response to the environment, though this is a matter of considerable debate (see reviews in Mitton and Grant 1984; Zouros and Foltz 1987). Generally, if heterozygotes have a fitness advantage this tends to generate negative F values although this is not a sufficient condition for heterozygote advantage (Lewontin and Cockerham 1959). F_{IS} in this study

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was -0.102 indicating a 10.2% excess of heterozygotes relative to Hardy-Weinberg expectations. The role of heterozygote superiority is the focus of the following chapter. There, it is demonstrated that heterozygosity is positively correlated with growth.

In Chapter 3, I show that differential herbivory takes place, and postulate that such an effect, especially during the early stages of clone development, may also affect genetic diversity. Diversity could be enhanced when ungulates browse, thus protracting the early successional stage of growth (DeByle 1980), and limiting lateral expansion. In turn, this reduces the probability of competitive domination of patch space and resource by a particular clone. The net result in this instance is an increase in genotypic diversity (Jelinski and Hutchinson 1988). On the other hand, diversity could be reduced if the herbivore choose disproportionately more of rarer genotypes from the mixture available (Cahn and Harper 1976).

High levels of genetic diversity in *P. tremuloides* may also be maintained simply because many of the allozymes assayed in this study are selectively neutral, i.e., equal in fitness and therefore not subject to the pressures of selection. Variation may also arise due to mutations that are selectively equivalent in functional efficiency (Lewontin 1974; Kimura 1983). Neutral theory also predicts that, in the absence of sexual recruitment, each locus should become heterozygous for a unique mutation. These mutations inevitably accumulate as a consequence of Muller's ratchet (Muller 1964). Normally, recombination cleans the germ line of harmful mutations by the purging of defective gametes. However, the process of purging the germ line of deleterious mutations does not operate to the same extent on plants that produce multiple meristems. Damaged meristems do not contribute to the gene pool but there should be sufficient number of undamaged meristems, some of which may carry mutations, from which reproductive organs are differentiated and still make a sizeable contribution to the gene pool (Stebbins 1988).

Whitham and Slobodchikoff (1981) argued that for large-sized, long-lived clones with annually regenerated meristematic tissue, favorable mutations may be incorporated into the gene pool and hence facilitate adaptive evolution. Recently, King and Schaal (1990) analyzed restriction site variation in DNA that encodes rRNA in asexual lineages of *Taraxacum officinale*. When clonal offspring were compared to their parents, King and Schaal found that somatic events altered rDNA variation in the siblings. Mutation which arose early during some development was in fact incorporated into the germ line. On the basis of these findings, they proposed that asexual species can generate genotypic variation which may then be subject to selection. Whitham and Slobodchikoff (1981) suggested that even if beneficial mutations are not incorporated into the germ line, they could still be perpetuated by vegetative reproduction. If, however, mutation which occurs at a metabolically critical heterozygous locus consistently results in death, natural selection may prevent heterozygotes from becoming fixed at some loci. This may explain the absence of a totally heterozygous situation with respect to this study. Nothwithstanding, given a mutation rate of say 10^{-5} - 10^{-6} mutations per locus per generation (Lewontin 1974), over time it is clear that an old clone may be endowed with a great number of mutations (Slobodchikoff and Whitham 1981). It follows that populations may consist of genetically diverse clones which may contribute to the high levels of diversity found in this study. Unfortunately, information on the degree to which mutations are important in maintaining and augmenting genetic diversity in natural plant populations is not known (Schaal 1988).

1.5.4 Population structure and ecological determinants

In general, population structure can be expected to arise where gene flow is restricted and natural selection is operative (Slatkin 1985, 1987). These conditions were assumed to be characteristic of *P. tremuloides* in WLNP. However, *P. tremuloides* exhibited small to

moderate amounts of genetic differentiation in this ecologically diverse arena. F_{ST} was 3.0%, a finding which is in accordance with the mean genetic identity value comparing all six populations, I = 0.987. Unfortunately, for comparative purposes, there are no studies of genetic differentiation in woody angiosperms on a geographically small scale similar to that examined in this study.

Comparison with conifers and other gymnosperms, which share some life-history traits found in the above angiosperms, reveals value F_{ST} values that range from 1% in closely spaced populations of *Pinus contorta* (Knowles 1984) and *Picea mariana* (Boyle and Morgenstern 1987) to 12% for *P. ponderosa* (O'Malley et al. 1979). Mitton et al. (1980) also found clinal differentiation along an elevational gradient at one locus in three populations of *P. ponderosa*. For a population of *Pseudotsuga menziesii* located in two mountainous areas in southwest Oregon, Moran and Adams (1989) reported that only 1.8% of the total genetic diversity in stands resides within the populations. Alden and Loopstra (1987) surveyed allozyme variation in four *Picea glauca* populations along a 650 m altitudinal gradient in Alaska and found that 97% of the genetic diversity occurred among trees within stands, 1% among north-south locations, and 2% among populations. Table 1.9 lists the extent of microgeographic differentiation for several outcrossing plant species. It can be seen that the distribution of genetic diversity among *P. tremuloides* populations in WLNP is comparable with ecologically similar *Picea glauca*, *Pinus ponderosa* and *P. longaeva*. It is important to recognize that relatively few gymnosperms are capable of vegetative reproduction and none form extensive, long-lived clones with virtually no sexual recruitment into contemporary populations. Comparison of genetic differences among populations of clonal plants that are capable of vegetative reproduction is made in Table 1.8. The values reported herein are most similar to that for *Erythronium albidum*. Its

Table 1.9. Studies of microgeographic differentiation in outbreeding plants

	Sub-populations	H_T[1]	G_{ST}[2] %	Reference
Lolium multiflorum	4	0.26	1	Mittton et al. (1978) in Levin (1986)
Lolium multiflorum	4	0.31	1	Mitton et al. (1978) in Levin (1986)
Silene maritima	6	0.32	4	Baker et al. (1974) in Levin (1986)
Spartina patens	3	0.43	12	Silander (1984)
Liatris cylindracea	66	0.09	7	Schaal (1975)
Pinus ponderosa	8	0.33	3	Mitton et al. (1980)
Pinus ponderosa[3]	6	0.30	4	Linhart et al. (1981)
Pseudotsuga menziesii	4	0.19	7	El-Kassaby and Sziklai (1982)
Pseudotsuga menziesii	2	0.17	2	Moran and Adams (1989)
Pinus longaeva	11	0.48	4	Hiebert and Hamrick (1983)
Picea glauca	4	0.28	3	Alden and Loopstra (1987)
Populus tremuloides	6	0.31	3	This study

[1] H_T = total allelic diversity

[2] G_{ST} = % of allelic diversity among populations to total allelic diversity (computationally the same a F_{ST}.)

[3] A single population with six groups of trees.

probable derivative, *Erythronium propullans*, is thought to have comparatively limited gene flow (Pleasants and Wendel 1989).

More information is available for comparison of population structure in angiosperms at larger spatial scales. In a near province-wide study of genetic variation in *P. tremuloides* in Ontario, Hyun et al. (1987) found that, on average, 6.8% of the total gene diversity could be attributed to population differentiation among eight "geographic regions". Weber andStettler (1981) examined isozyme variation in *P. trichocarpa* in the Pacific Northwest and reported that 6.3% of total genic variation was between populations. Conversely, Farmer et al. (1988) presented data from a study of isozyme variation in *P. balsamifera* along a 1200 km long transect in northwestern Ontario which yielded differentiation estimates of only 1.4%. Bousquet et al. (1987) found that approximately 5% of the total genetic diversity resided among 22 populations of *Alnus crispa* in central Quebec. In a Japan-wide study of population structure in *Camellia japonica* , Wendel and Parks (1985) noted that 1.2% of total variation could be ascribed to interpopulation differentiation. It is important to reiterate that the geographic scale at which these studies were conducted is several orders of magnitude larger than in this study. Increasing geographic distance correlates with increasing genetic distance as shown by a stepping-stone process and the infinite alleles model (Kimura and Weiss 1964; Nei 1972).

Since F_{ST} values varied almost 100-fold (0.160-0.001), this sharp difference in estimates for gene flow again provides evidence for selection acting on individual loci. Moreover, gene flow and drift should affect all loci and all alleles equally (Lewontin and Krakauer 1973) and no structuring in *P. tremuloides* can occur due to selfing. The greatest divergence among populations occurred at PER (F_{ST}=0.160), and given the magnitude of difference between this locus and others, it strongly argues for some type of selection. Interestingly, Mitton and Grant (1980) and Hyun et al. (1987) similarly noted marked

heterogeneity for PER. An excess of PER heterozygotes occurred at all sites except the moist Akamina site. Mitton et al. (1977, 1980) also found that allelic frequencies at this locus in populations of *Pinus ponderosa* were coincident with habitat differences with respect to temperature and moisture regimens in the Colorado Front Range. They suggested heterozygotes are associated with xeric sites and that these differences might be attributable to kinetic variation between PER genotypes. PER has been shown to be a growth-related isoenzyme in hybrid poplars (Wray and Gordon 1975).

It is clear that the populations are limited in the degree to which they are differentiated. In the following sections, the potential role of gene flow in homogenizing the populations and the buffering effects of clonality on halting or delaying environmental selection are discussed. Recognition need also be given to the fact that it is presently impossible to unequivocally specify a direct relationship between enzyme systems and polygenic variation (Lewontin 1974). This problem represents a major hiatus in the analysis of the adaptive significance of allozyme variation. Differentiation of populations could be related, for example, to edaphic conditions. Numerous studies have documented differentiation in response to soil properties such as heavy metal content, nutrient availability and aridity (Jain and Bradshaw 1966; Antonovics and Bradshaw 1970; Snaydon 1970; Heywood and Levin 1985; Nevo et al. 1986). For example, the Prairie I and II populations are windswept and soils on the Galwey site are limited in terms of pedogenic development, presumably low in organic matter and nutrients, extremely porous, and hence, prone to rapid drying. Since germination, seedling establishment and clonal growth are affected by moisture availability, aridity may operate directly on some loci in *P. tremuloides* at these three locations. There may also be ecogenetic differences in the populations based on a elevational gradient. Peck and Wallner (1982) documented variable heat resistance in *P. tremuloides* leaves along a 490 m elevational gradient in Colorado (this compares to the 470

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m gradient in this study). Trees were transplanted to a common elevation where it was found that heat tolerance in leaves was greatest in trees growing at the lowest site. Similarly, the Galwey site is the coldest, so there could also be ecogenetic adaptation to cooler soils and concommitant effects on root respiration similar to that found in populations of *Abies lasiocarpa* and *Picea engelmannii* distributed along an elevational gradient (Sowell and Spomer 1986). Clearly the problem is disentangling the relative role of a multiplicity of environmental factors and their interactions with the above or closely linked loci. Reciprocal-transplant or common-garden experiments would be valuable in this regard. Notwithstanding these problems, the following chapter demonstrates a relationship between protein heterozygosity and allozymes.

1.5.5 Factors constraining differentiation

Despite the manifest differences in the physical environment, which presumably have the potential to affect the selective environment, I propose that there are two evolutionary and ecological forces which have considerable potential to retard or delay the structuring of populations in WLNP, specifically: 1) features of the mating system and the environment which affect gene flow, and 2) *P. tremuloides* phalanx clonal growth habit.

1.5.5.1 Gene flow

In general, a very small amount of gene flow can reduce differentiation, especially if there is little or no selection (Wright 1946, 1951; Levin and Kerster 1974), though the precise way in which populations are affected depends on the action of several life-history traits and features of the physical environment, including mode of reproduction, efficacy of pollen and seed dispersal, vectors of dispersal, floral phenology, seed-bank lifespan, physical barriers to dispersal, population density and spatial arrangement (Loveless and

Hamrick 1984). The amount of pollen movement is influenced by pollen size and pollen density, physical barriers, spatial separation, and phenology (Levin and Kerster 1974). *Populus tremuloides* ' pollen is extremely small (Moore and Webb 1978), thus lending itself to long-distance transport and hence, large genetic neighborhoods (Levin 1988). Levin (1988) calculated that the size of the paternity pool for *P. deltoides* exceeds 1000 km^2 and that females may draw pollen from over 30,000 males. However, the size of this pool is not an invariant property; rather, it is subject to change in response to meteorological and ecological conditions. Like pollen, *P. tremuloides* seeds are also very small (1-2 x 10^6 kg^{-1}) (McDonough 1985) and are adapted for wind dispersal of 500 m, or several km in high winds (Stoekler 1960 in McDonough 1985). With increased age, the lower shoots on *P. tremuloides* stems become abscised and the canopy consists primarily of short shoots (Pollard 1970; Kozlowski and Clausen 1966). Consequently, seed release is from the upper portions of the stand, a feature that promotes long-distance wind dispersal of both pollen and seeds (Levin and Kerster 1974). Wind speed tends to be particularly influential on dispersal distance (Augsberger and Franson 1987). In the Waterton region, winds are generally high. The diurnal peak in mid-summer afternoons averages 3.9-4.44 m s^{-1}. In summer, however, winds of at least 11.1-13.9 m s^{-1} with stronger gusts have been documented (Finklin 1986). While the number of migrants exchanged per generation (Nm) could be estimated from the F_{ST} values (Wright 1931), the interpretation of these values is made difficult because of potentially long and overlapping generations characteristic of species capable of vegetative reproduction.

In WLNP, the greatest physical barriers to gene flow among the complex of populations are the mountains and the coniferous forest that separates the Akamina population from the other populations. Given retardation of gene flow, the genetic distance between it and other populations should increase (Slatkin 1985, 1987). Yet, if one

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variation (as indicated by enzyme electrophoresis) in populations of *P. tremuloides* in one part of its range and, 2) How is this genetic variation partitioned within and among populations? I then evaluate the amount and distribution of genetic variation with respect to life history traits and environmental influences. The second section focuses on the ecological significance of the electrophoretically-assayed genetic variation. Here, I examine the statistical and biochemical associations between heterozygosity and growth, an important measure of fitness, to ascertain the importance of balancing selection as a force in maintaining genetic diversity. The final section deals with the way in which the interaction of species at the plant-animal interface may affect genetic diversity. *P. tremuloides* is an important food resource for several members of the Cervidae (e.g., deer, moose, and elk). In this section, I evaluate clonal variation in the chemical composition of *P. tremuloides*, assess these results in terms of ungulate nutritional requirements, determine if selective foraging takes place, and consider the implications of herbivory on genetic diversity in populations of *P. tremuloides*. The dissertation concludes with a synthesis of the findings of the study, focusing on the ecology and evolution of *P. tremuloides* in local populations with comments on the evolutionary implications at macrogeographic scales.

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examines the genetic distance values, the Akamina population is quite similar to the remaining populations. Pollen and seeds could conceivably travel over the intervening mountains and coniferous forest, or through a col between Blakiston and Cameron valleys.

Assortive mating owing to phenological differences imposed by elevation is likely to promote divergence unless unusual weather conditions occur which promote synchronicity in phenology (Loveless and Hamrick 1984). Although floral phenology was not monitored, leafing phenology can be used as a surrogate measure because it generally follows the pattern of the former (Greene 1971). Leaf flushing among clones was evaluated on May 8-9 and May 21-22, 1986 following the scoring system of Brissette and Barnes (1984). Seven stages were recognized: (1) dormant winter condition, buds at rest; (2) buds swelling; (3) leaves breaking bud, bud scales still tight; (4) leaves expanded beyond bud scales but still clustered; (5) leaves separating yet still folded, bud scales falling away; (6) leaves unfolded but still succulent; and (7) summer condition, leaves mature and hardened off.

When leaf flushing was first scored on May 8-9, 1986 both Prairie I and II populations were phenologically more advanced than the remaining populations. Of the clones in the Prairie I population, 57% had leaves breaking through the bud scales (stage 3), while Prairie II lagged somewhat behind at 39%. The trees at the cooler Galwey, and shaded Akamina and Crandall sites had virtually no leaves at the bud-breaking stage. Thirteen days later 100% and 83% of the clones of the Prairie I and Prairie II sites, respectively, had reached stage 3. Meanwhile, the Galwey and Crandall sites were still in the earliest flushing stages. Leaf phenology was most retarded at the Galwey site where 93% of the clones were only beginning to flush (stage 2). Seventy-five percent of the clones at the Copper Creek site, which lies below Galwey, were at least at stage 3. Interestingly, at the time of the second survey, all clones at the Akamina site had leaves breaking buds (stage

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3). In sum, the low elevation populations were, phenologically speaking, about 2 weeks more advanced than the clones in the cooler Akamina, and the high elevation Galwey sites were at least two weeks behind that of the Prairie populations. This is coincident with Hopkins (1918) bioclimatic law which states that the date of any phenological event is about four days later per 120 m increase in altitude during spring and summer. Accordingly, the magnitude of difference in elevation between the highest and lowest sites is 470 m, or about 16 "phenological days". Female flowers remain receptive for about 2 weeks or so (Greene 1971). It is concluded that differentiation is not likely to occur owing to differences in flowering phenology because of the combination of the window for mating, within-site clonal variation in flowering, and strong upslope winds. Last, while hermaphroditism in *P. tremuloides* has been reported (Erlanson and Hermann 1927; Santamour 1956; Barnes 1966), the condition is not believed to be widespread (Lester 1963; Mitton and Grant 1980).

1.5.5.2 Clone stability

Despite the potential for the evolution of locally adapted genotypes, as indicated by the amount of gene diversity and strong selection pressures in this heterogeneous environment, the long generation times characteristic of *P. tremuloides* clones can be expected to delay, or even arrest, the slow decay of genetic variance and inhibit population subdivision (Loveless and Hamrick 1984). Unfortunately, there is no reliable way to age *P. tremuloides* clones. It has been suggested, however, that most of the *P. tremuloides* clones found in the Great Basin are at least 8,000 years old (Cottam 1954; Barnes 1975; Kemperman and Barnes 1976; Cheliak and Dancik 1982). Moreover, Barnes (1975) suggested that some clones south of glacial maxima (e.g., Utah and Colorado) might date

back to the Pliocene. That clones can achieve large size and great age has been documented for several other species (see review in Cook 1985; Sebens and Thorne 1985).

The age of some clones in WLNP is probably closely linked to the paleoclimatic history of the region. Christensen and Hills (1971) suggested that the Holocene climate of the Waterton Lakes region can be classified into 3 climatic divisions beginning with the warming trend that caused the disappearance of the Cordilleran ice sheet by 11,000 years B.P. The earliest of these, the Anathermal, was a cool and moist period (11,500-7,000 years B.P.) immediately following Wisconsin glaciation. The post-glacial pollen record (Bujak 1974) shows that *P. tremuloides* was initially abundant until succeeded by *Picea glauca* forests that persisted for the duration of the Anathermal. This period was followed by the Altithermal (8,000-5,000 years B.P.) which was characterized by warm dry conditions which gave way to a mixed-forest cover of *Pinus* spp., *Populus* spp., *Betula spp.*, and *Alnus* spp. along with extensive grasslands. This climatic event was succeeded by the Medithermal interval which was characterized by cool, moist conditions and periodic neo-glacial events including two cool periods around 3,500 and 900 years B.P. (Harrison 1976). Apparently, the forest "closed" during the Medithermal and treeline shifted downward. The dominant vegetation was *Abies* spp., *Picea* spp., *Betula* spp., and *Alnus* spp. Grasslands were reduced in aerial extent. Noteworthy is the fact that *P. tremuloides* pollen is prone to rapid degradation because of its delicate exine (Axelrod and Ting 1960; Sangster and Dale 1961), and thus its relative abundance may be underestimated. Nevertheless, based on palynological evidence, known climatic change, and early-successional tendencies, it is clear that the abundance of *Populus* spp. has fluctuated widely.

During this period of climatic change, clones would have variously gone through periods of expansion, contraction and, for some, extinction. The oldest clones are likely

those in the low lying Prairie populations. The high elevation populations (e.g., Galwey and Akamina) are at the aspen treeline and the cool climate of neoglacial events would presumably result in treeline depression, thus these clones are likely of more recent origin. The Akamina population resides in the Cameron Valley where glacial retreat was slower (Harrison 1976); therefore, vegetation reestablishment would have been delayed. Moreover, whereas populations of *P. tremuloides* form stable vegetation assemblages in the more xeric sites (e.g., the Prairie, Galwey, Crandall and to a certain extent, Copper populations), arboreal competition by conifers in the Akamina population poses considerable threat to *P. tremuloides*. While roots may persist beneath the coniferous canopy, and thus perpetuate the clone, even in the absence of above-ground growth, there is no information on the length of time they can remain nutritionally unsupported (Schier et al. 1985). Notwithstanding, indefinite survival in such a condition is unlikely; thus clones in this valley could conceivably be of more recent age.

If clones are as old as widely thought, such antiquity may well be a result of the species' phalanx clonal growth habit and physiological integration among ramets. The phalanx growth form is one in which a genet consists of dense, closely-packed ramets with distinct abrupt boundaries at the leading edge of the clone (Lovett Doust 1981). The phalanx growth form contrasts with the other extreme growth form, the "guerilla" strategy, where ramets are patchily distributed in relation to resources and maximize interspecific contacts (e.g., *Viola blanda*: Cook 1985). Lateral expansion is slow (Lovett Doust 1981), but resource exploitation is efficient (Harper 1985). Despite the inherent ability of species characterized by the phalanx clonal growth tactic to deny space to other genets, Mitton and Grant (1980) mapped the distribution of ramets from two clones and found some interdigitation.

Physiological integration among the dense network of ramets within large clones is also suggested to contribute to the resilience and persistence of old clones. Resource sharing among member ramets of a clone has been shown to buffer the impacts of unfavorable microsites for other species (e.g., Salzman and Parker 1985; Pitelka and Ashmun 1985; Lau and Young 1988; Tissue and Nobel 1988). There is some evidence that root connections can transmit water and solutes from ramet to ramet in *P. tremuloides* (DeByle 1964; Gifford 1966; Tew et al. 1969), although it is not known whether carbohydrates can be similarly translocated. Given the large area a single clone can occupy, it would seem highly probable that, because of the phalanx growth strategy, some members of the clone would be positioned in microsites of suboptimal or poor conditions or, what Hartnett (1987) called "patch specific selective influences". Physiological integration would be especially advantageous for those ramets at the leading edge of the clone faced with competition from neighbors. These ramets would be able to draw upon metabolic reserves of interior ramets, and so buffer the impacts of competitively dominant neighbors. For example, it has been suggested that the stomata of *P. tremuloides* in the eastern U.S. do not close effectively under water stress and hence are subject to desiccation (Jones et al. 1985). As such, where water may be limiting, physiological connections among ramets may buffer this effect by sharing of water and other solutes. Clonal integration would be especially advantageous in the xeric Prairie and high-elevation Galwey populations. Although degeneration of root connections to the parent ramet eventually occurs, the independent ramet may become "reintegrated" by the production of its own daughter ramets, although photoassimilate transfer appears to be directed from older ramets to younger ramets in nongrameneous species (Pitelka and Ashmun 1985). In sum, the clone is an integrated unit that buffers negative impacts and spreads the risk of death (Cook 1979, 1985; Tissue and

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Nobel 1988). Patch specific influences and suboptimal environments which pose serious consequences for aclonal species may not be detrimental to *P. tremuloides*.

Clone persistence may also be related to the presence of favorable somatic mutations in that mutation may provide a vehicle for clonal adaptation to contemporary environmental conditions (Whitham and Slobodchikoff 1981, Schaal 1988, King and Schaal 1990). Whitham and Slobodchikoff (1981) were among the first to speculate that heritable mutations arising in long-lived, large-sized clones capable of complete regeneration of meristematic tissue (e.g., buds) alter gene frequency and hence may facilitate adaptive evolution over time. Further, mutations arising in, and confined to, meristematic tissue such as root suckers, could potentially quickly spread via ramet (and other modular units) duplication (Whitham and Slobodchikoff 1981). Harmful mutations may be purged from the population by natural pruning of the modular unit carrying the defective genetic material (Whitham and Slobodchikoff 1981). Therefore, even in the absence of sexual reproduction, somatic mutation may provide a source of heritable variation that permits the clone to adapt to changing local conditions. Unfortunately, little specific information is available on the role of mutation in augmenting suvival of clones from natural plant populations.

Given the ability of *P. tremuloides* clones to reproduce vegetatively and share resources, and perhaps evolve as a result of mutation, the obvious question is "why is there not domination of the physical environment by a few large clones as William's (1975) Strawberry-Coral model predicts?" Kemperman and Barnes (1976) suggested that small clone size is a function of inter- and intraspecific competition and time. Similarly, frequent or severe disturbance may also prevent monopolization of patch space (Sebens and Thorne 1985; Jelinski and Hutchinson 1988). Finally, and perhaps most importantly to this study, WLNP is a heterogeneous and fractured environment; hence, there is a limited number of

"safe sites" (Harper 1977) and these are restricted in size. Over time, individual clones could occupy an entire patch, but their areal extent is limited by the microgeographic variation in habitat. This would explain some of the apparent random pattern found in this study. Such patterns often emerge where densities are low (Elliot 1977).

1.6   SUMMARY AND CONCLUSIONS

Allozyme analysis of six populations of *Populus tremuloides* from a locally-heterogeneous environment reveals that both inter and intra-population diversity is high. There also appears to be important subdivision in this relatively small geographic, but relatively diverse ecological landscape. It is postulated that diversity is possibly maintained by various forms of selection, somatic mutation, occasional gene flow (despite the widespread belief that such events are extremely rare) and finally, the species' phalanx growth habit and concomitant physiological integration which spreads the risk of death and buffers the effects of selection over time and space. Waser (1987:255) recently suggested that we may lack the summary statistics or theory to account for evolutionary forces that may foster indistinguishable patterns, or as Waser stated, "the available theory is not sufficiently well-developed to handle many biologically realistic conditions." Such may be the case for *P. tremuloides* given the confounding influence of a complicated population history, near immortality of established clones, and the propensity to form large clones. Because of these factors, it may be impossible to precisely determine the mechanisms to account for the distribution of *P. tremuloides*. There is the related problem of defining a plant's "viewpoint". There may be a lack of congruence, between *P. tremuloides* ' "view" of the environment (*sensu* Turkington and Harper 1979), and those environmental factors which were perceived, and hypothesized, to be transitionally sharp enough to result in local-scale differentiation. Finally, it is also important to recognize that, while differentiation is not more pronounced at the small number of loci assayed in this study, this is certainly not *ipso facto* proof that spatial structure does not exist at other loci, nor more importantly, does it mean that fitness differences do not exist among clones. This latter point is examined in the following chapter by comparing genetic loci and the growth

rates of ramets as indicators of performance and local adaptation among *P. tremuloides* clones.

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CHAPTER 2

Environmental Heterogeneity, Protein Heterozygosity,
and Growth of *Populus tremuloides*

2.1 INTRODUCTION

In the preceding chapter, it was shown that the level of genetic diversity within

populations of trembling aspen (*Populus tremuloides*) in Waterton Lakes National Park

was high, and that the pattern of genetic variation in space was largely random. I proposed

that such a distribution may result from long-range pollen and seed dispersal, ramet

multiplication (i.e., clonal growth), and physiological integration of ramets. These factors

increase neighborhood size, restrict the establishment of new propagules, and prolong the

life of genotypes which once had a better fit with the environment and are now maladapted.

Therefore, taken together, these phenomena might have led to the homogenization of the

populations.

The absence of striking geographic patterns in allelic isozymes, but the presence of

considerable enzyme polymorphism among individual *P. tremuloides* clones, raises the

question: What is the biological significance of this genetic diversity? Although genetic

variation is generally ubiquitous in almost all taxa of natural plant populations, the

production and maintenance of genetic polymorphism is not well understood (Chakraborty

1987), and the role of polymorphism in controlling traits such as competitive ability,

physiological performance, and response to disturbance is meagre (Hamrick et al. 1979;

Turkington and Harper 1979; Antonovics 1984; Goldberg 1988; Jelinski and Hutchinson

1988). Nevertheless, over the past decade or so, there have been attempts to relate patterns

of heterozygosity to phenotypic traits such as growth rate (Zouros et al. 1980; Knowles and Grant 1981; Mitton et al. 1981; Singh and Zouros 1978; Koehn and Shumway 1982; Ledig et al. 1983; Mitton and Grant 1984; Govindaraju and Dancik 1986; Strauss 1986; Koehn et al. 1988; Zouros et al. 1988). The majority of these studies revealed positive correlations between heterozygosity and growth rate (see reviews in Mitton and Grant 1984; Watt 1985*a*; Zouros and Foltz 1987).

Lerner (1954) first hypothesized a causal relationship between the degree of heterozygosity and growth rate, and other measures of performance and developmental homeostasis (i.e., stability and consistency) based on observations primarily made on cultivated plants and domesticated animals. He suggested that heterozygotes may impart a buffering action against environmental stress more strongly than homozygous enzyme genotypes. Since Lerner's initial formulation, two basic mechanisms have been forwarded about the relation between heterozygosity and observed increased performance (i.e., fitness). First, the enzyme variants themselves, or closely linked loci, are directly involved in the expression or determination of a trait (e.g., growth). In this scenario heterozygosity *per se* is advantageous. This is called the "overdominance" hypothesis. The second explanation for association between heterozygosity and increased fitness is the "dominance hypothesis". This hypothesis holds that allozymes at polymorphic loci are selectively neutral and the observed level of heterozygosity is merely representative of the overall levels of genetic variation in the genome. Therefore, in predominantly outcrossing populations, variations in growth may be due to rare, deleterious, or recessive alleles that express themselves under conditions of inbreeding.

It has also been proposed that individual loci may contribute to a heterotic condition (i.e., increased vigour or reproduction) if kinetic differences exist between allozymes at polymorphic loci. Koehn and Shumway (1982) first suggested that this phenomenon may

arise because heterozygotes are metabolically more efficient than homozygotes. It is known that gene products (enzyme variants) can have specific properties that mold metabolic pathways critical to maintenance and growth. Since heterozygotes tend to have intermediate properties, there should be less catalytic variation in a biochemical pathway. It follows that a reduction in the biosynthetic costs to facilitate regulation (Koehn et al. 1988) ultimately frees more energy for other energy-dependent traits such as growth or reproduction. Because of the resulting increase in fitness, it is widely thought, therefore, that the combined effect of multiple and (or) single locus heterozygosity may be a potent force in maintaining genetic polymorphisms (Lerner 1954; Mitton and Grant 1984; Livshits and Kobyliansky 1985; Zink et al. 1985). Indeed, Mitton and Grant (1984:495) suggested that "an individual organism's level of heterozygosity is a major organizing principle in natural populations of both plants and animals."

Given the high level of genetic diversity in *P. tremuloides* in WLNP, and the fact that fitness differences can arise owing to multiple and single-locus heterozygosity, the study of heterozygosity in relation to growth may provide valuable insight into the impact of genetic polymorphisms within populations of this species. In this paper, I hypothesize that there may be differences in components of Darwinian fitness in *P. tremuloides* of WLNP owing to the different biochemical properties in the diverse set of 14 polymorphic genes that were revealed in Chapter 1. In general, the direct measurement of "fitness" is an extremely difficult task and is virtually impossible for long-lived clonal species such as *P. tremuloides*. The simple and intuitively-appealing model of instantaneous growth rate would be an ideal metric since this approach permits tracking of selection at various stages of a ramet's life. Such a model , however, requires precise information on birth, death, and migration rates, population parameters that I have no way to assay or estimate with a reasonable degree of confidence. Other measures of vegetative growth such as stem

volume, number of ramets, area of the clone, or total plant biomass could also be used as a measure of performance (Grant and Mitton 1979), but are difficult to measure accurately and cannot be estimated over a number of decades. The surrogate measure of fitness I employed was mean annual incremental growth (radial) which can be easily measured and provides a historical perspective on the ramet's response to variable environmental conditions. This measure has been widely used (e.g., Grant and Mitton 1979; Knowles and Mitton 1980; Mitton and Grant 1980; Knowles and Grant 1981; Linhart and Mitton 1985). By employing direct study of clonal performance, I have attempted to determine if there is a correlation between individual heterozygosity at 14 polymorphic loci and the adaptive expression of a phenotypic trait, growth rate, as a first approximation of fitness. While this is a very small sample of the total genome, it does provide a reasonable estimate of the overall level of heterozygosity (Mitton and Pierce 1980; Chakraborty 1981; Turelli and Ginzburg 1983). The results will also be interpreted within a framework of evolutionary processes that may explain associations between electrophoretically measured biochemical variation and growth, as well as the evolutionary consequences of allelic isozyme variation as manifest through growth.

The only study of the effect of heterozygosity on *P. tremuloides* growth is that of Mitton and Grant (1980). They examined the relationship between three protein polymorphisms and the annual width increments in relation to elevation and sex of 106 aspen clones in the Colorado Front Range. Their main conclusions were that natural selection affects allelic frequencies between sexes and that heterozygosity was positively correlated with growth rate. Unfortunately, their analysis was somewhat limited in that they were only able to resolve three polymorphic loci. Typically, the number of polymorphic enzymes that have been examined in relation to growth varies between five and six (Koehn et al. 1988). It is generally accepted that even this is too few loci to assess

the potential for fitness differences (Mitton and Pierce 1980; Zouros and Foltz 1987; Koehn 1987; Koehn et al. 1988).

The effects of genetic variation on growth are complicated by sex-related variation in dioecious plants such as *P. tremuloides*. Female plants generally have lower growth rates (Lloyd and Webb 1977) as a result of greater allocation to sexual reproductive structures. Grant and Mitton (1979) found female ramets of *P. tremuloides* had higher mean annual incremental growth than male ramets at all elevations. However, in a follow-up study, when the effects of age, heterozygosity, and elevation were controlled for, there were no sex-related differences in the mean annual incremental growth (Mitton and Grant 1980), and Einspahr (1960) and Barnes (1966, 1969) found no significant sex-related differences in age, volume, or mean diameter at breast height (dbh). In a 25-year longitudinal study of Barnes' (1966) *P. tremuloides* clones, Sakai and Burris (1985) were unable to find statistical differences in mean annual incremental growth between male and female clones. Similarly, in a study of clonal growth in male and female bigtooth aspen (*Populus grandidentata*), Sakai and Sharik (1988) also reported no sex-related differences in basal area density or mean dbh. Therefore, it is concluded that sex has little or no effect on vegetative growth of *P. tremuloides*.

2.2 METHODS AND MATERIALS

2.2.1 Growth

Tree cores were extracted from each of the 156 trembling aspen clones used for analyses in the previous chapter. The methodology of Mitton and Grant (1984) was used to reduce the probability of sampling multiple clones; i.e., the five largest stems in a plot with a 10 m diameter were selected for core samples (Mitton and Grant 1980). Cores were extracted from the trees at breast height (1.3 m). Just prior to lab analysis, the cores were soaked in tap water for two hours. A razor was then used to remove a thin upper section, thus providing a clean cross-sectional surface for subsequent measurement. Tree ring widths were measured (to the nearest 0.01 mm) using the Tree Ring Increment Measuring (T.R.I.M.) system (Fayle and MacIver 1986)

Each core was placed on a Sony Linear Magnascale electronic ruler, which included a Sony linear recoder and a digital display unit. A fluorescent light source was placed beneath those cores that were difficult to "read" using a standard overhead light source. Individual ring measurements were taken through a binocular microscope at 10X magnification with one ocular fitted with a cross-hair to mark each ring. Each measurement recorded was transferred into an Apple IIe microcomputer for processing using T.R.I.M. software. The mean annual width increment and standard deviation was calculated for each ramet.

2.2.2 Site Characteristics

While genetic factors are the basis of physiological and morphological variation, the interactions between the genotype and environment ($G \times E$) are also important, as well as covariance components. As indicated above, assessment of environmental controls is

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difficult owing to the fact that during the lifetime of a ramet, developmental stage, climatic conditions, and competitive interactions are all subject to change. Therefore, I employed proximate variables that should provide a measure of the structure of the environment which was known, or believed to have, an effect on growth, or is correlated with a variable that does. Emphasis was placed on variables related to abiotic moisture stress as water was deemed to be the greatest limiting factor (Fischer and Turner 1978; Fralish and Loucks 1975). Additionally, each of the selected variables was quickly and precisely measurable with non-destructive sampling procedures. These variables included elevation, slope, slope position, aspect, and exposure. The elevation of each clone was estimated with a survey altimeter. Slope and aspect were measured using Brunton compass. Exposure of clones to wind and wind-related influences was estimated by the method of Fralish and Loucks (1975) (Appendix I). Slope position was coded following Bowersox and Ward (1972) (Appendix II). Finally, growth rates tend to be greater in younger trees than older trees (Fritts 1976). Thus, the effects of age were controlled for by analysis of covariance (see section 2.4) after examination for statistical significance.

2.2.3 Genetic variation

The individual clones were examined electrophoretically for 16 enzyme loci encoded by 13 enzyme systems. Of these loci, 14 were found to be polymorphic. The details of the electrophoresis and subsequent results were presented in Chapter 1. For the purposes of this paper, heterozygosity was simply assessed as the number of loci heterozygous.

2.2.4 Statistical analyses

Population differences in mean annual incremental growth were compared with one-way analysis of variance (ANOVA). The association between age, environmental variables

and growth rate was tested with stepwise multiple regression to yield a parsimonious set of covariates that would minimize the degrees of freedom in subsequent analyses. Aspect was transformed following the equation of Beers et al. (1966) to facilitate conversion of this variable to a quantitative measure such that

$$A' = \text{cosine } (A_{max} - A) + 1 \tag{1}$$

where A' = transformed aspect code

A_{max} = aspect which is to be assigned the highest numerical value on the transform scale.

A = direction of prevailing slope measured in azimuth degrees clockwise from the north.

A_{max} was set at 45 degrees which, according to Beers et al. (1966), is near the optimum aspect for most forest tree species.

The variables were examined for normality, linearity and homoscedasticity of residuals. The dependent variable, mean annual increment, was log transformed to reduce heteroscedasticity. A square root transformation was used on the highly-skewed variable, slope. The relationship between heterozygosity and growth was then examined using the reduced suite of variables as covariates in an analysis of covariance (ANCOVA), which allows for statistical control over those variables that produce potentially confounding effects. Since previous studies documented an inverse relationship between the degree of heterozygosity and growth (see Mitton and Grant 1984; Zouros and Foltz 1987), a one-tailed test of significance was employed.

2.3 RESULTS

Ten of the 156 clones were eliminated from the data set because of difficulty in extracting complete age profiles. The average mean annual increment in ring width was 1.35 mm (SD = 0.355). There were significant differences between populations in the mean annual growth rate (ANOVA, P < 0.01). However, there were no statistical differences when the effects of slope position, exposure, and age were controlled for. Growth was greatest in the two Prairie populations followed by the population in the more moist Akamina location. Growth was least in the high elevation Galwey site which was about two-thirds that of the nearby low-lying Prairie populations (Table 2.1).

When mean annual growth rate was regressed against slope position, exposure, age, elevation, slope, and aspect, 56 % of the variance in growth rate was explained (P < 0.001). The stepwise procedure isolated growth as being inversely correlated with slope position, elevation (P < 0.001), and age (P < 0.05), and positively correlated with exposure (P < 0.01) ($r = 0.74$) (Table 2.2). I also examined the relationship between mean annual incremental growth and the independent variables on a bivariate basis. As expected, the results are in accordance with the multiple regression analysis (Figure 2.1). These four variables were then used as covariates in an analysis of covariance to test for a correlation between growth rate and the degree of heterozygosity. Using this test, the results in Table 2.3 show growth is positively correlated with the degree of heterozygosity after adjustment for the effect of the covariates ($r = 0.66$, $P = 0.032$). This analysis is based on all data points ($N = 146$), as opposed to heterozygosity means ($N=9$). By comparison, a less conservative (Zouros et al. 1988) method of examining the relationship between heterozygosity and growth involves the regression of average growth against the degree of heterozygosity, which again yields a positive relationship ($r = 0.86$, $P<0.05$); Kendall's

Table 2.1. Sample means for growth rates, and heterozygosity among populations of *Populus tremuloides* in Waterton Lakes National Park, Alberta.

Subpopulation	Annual width increment (mm)		Heterozygosity[1]	
	Mean	St. dev.	Mean	Range
Prairie I	1.54	0.41	4.1	0-8
Prairie II	1.52	0.25	3.1	0-8
Akamina	1.32	0.26	2.8	0-8
Galwey	1.03	0.23	3.3	2-8
Crandall	1.26	0.31	3.1	1-8
Copper	1.28	0.28	2.7	0-8

[1]Observed number of heterozygous loci per individual clone among 14 loci

Table 2.2. Multiple regression of mean growth rate in *Populus tremuloides* from Waterton Lakes National Park, Alberta

	Correlation Coefficients	Probability
Multiple r	0.746	0.001
Independent variables		
Slope position	- 0.654	0.001
Elevation	- 0.481	0.001
Exposure	0.461	0.003
Age	- 0.050	0.038
Aspect	- 0.015	0.846
Slope	- 0.710	0.125

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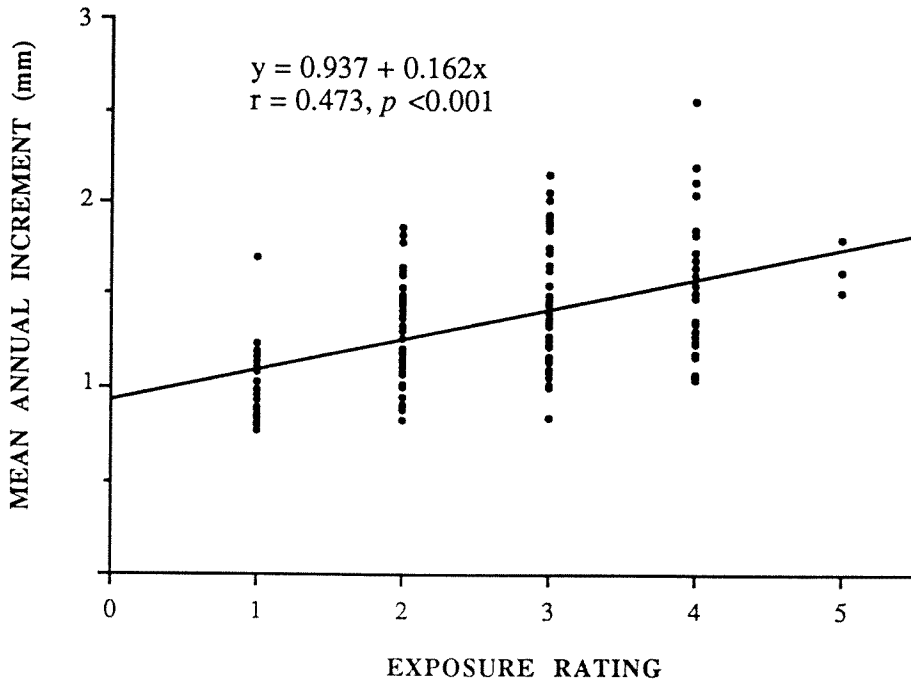
Fig. 2.1a. Scattergram showing the relationship between mean annual incremental growth (mm) and exposure rating. The exposure rating is developed after Fralish and Loucks (1975) (Appendix I).
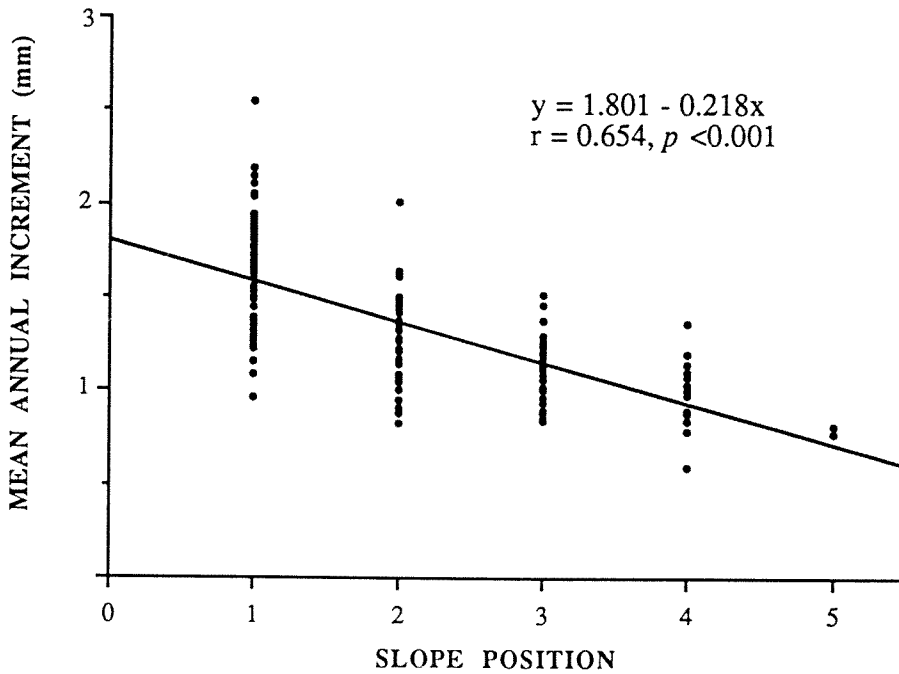
Fig. 2.1b. Scattergram showing the relationship between mean annual incremental growth (mm) and slope position rating. The slope position rating is developed after Bowersox and Ward (1972) (Appendix II).

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$$y = 2.534 - 9.570^{-4}x$$
$$r = 0.407, p < 0.001$$
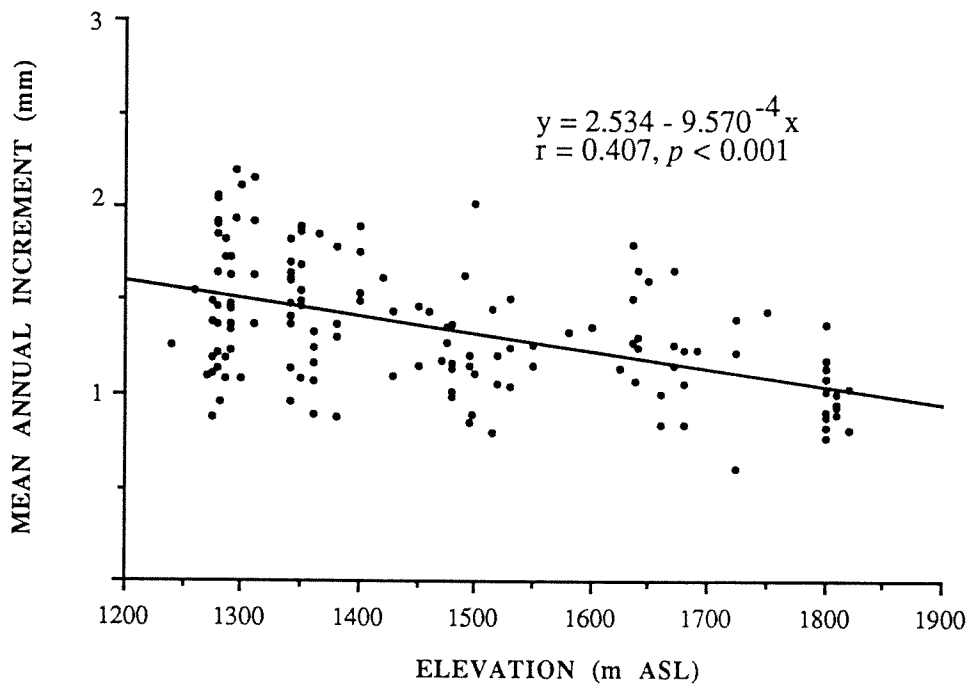
Fig. 2.1c. Scattergram showing the relationship between mean annual incremental growth (mm) and elevation (metres above sea level).

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Table 2.3. Analysis of covariance of growth with degree of heterozygosity controlling for the effects of elevation, slope position, exposure, and age.

Source of variance	df	Sum of Squares	F
Covariates			
Elevation	1	1.06	22.92*
Slope position	1	2.17	47.01*
Exposure	1	0.81	17.48*
Age	1	0.84	18.12*
Main Effects			
Heterozygosity	8	1.55	13.80*

*$P<0.001$

rank correlation (τ) was 0.733 (one-tailed P <0.05) (Figure 2.2).

When examined on a single locus basis, again controlling for the effect of slope position, elevation, age, and exposure, growth and heterozygosity were positive for nine loci, negative for four loci, and equal for both homozygotes and heterozygotes at one locus; no negative effects were significant (Table 2.4). There were significant differences at the G6PD, ADH-2, and ALD loci ($P < 0.001$) and G2DH ($0.10 > P > 0.05$); heterozygotes had higher growth rates than did homozygotes. The unadjusted and adjusted means for these loci are presented in Table 2.5. When the various genotypes at each locus are analyzed by analysis of covariance, the basic pattern which emerges is one in which the heterozygotes are more fit than the common homozygotes which, in turn, are more fit than the rare homozygotes.
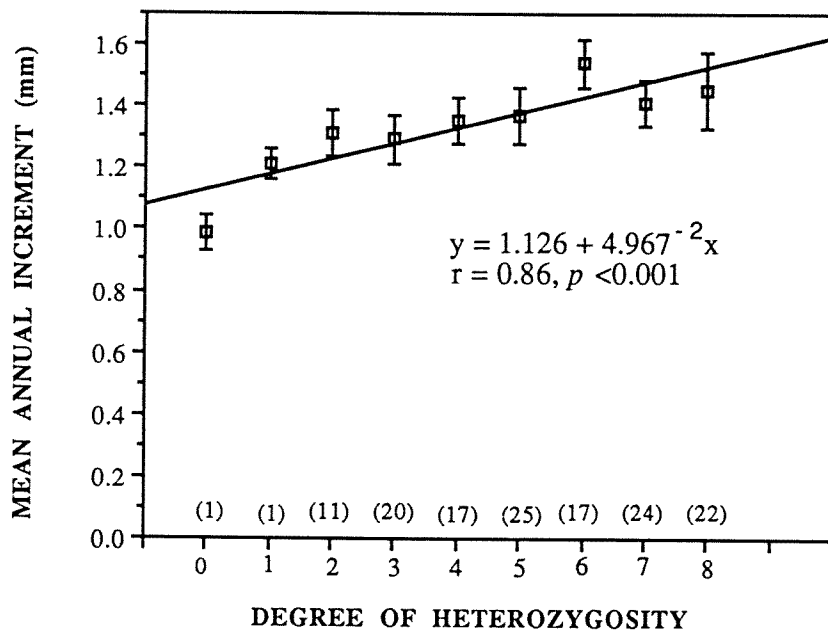
Fig. 2.2. Relationship between mean annual incremental growth (mm) and degree of heterozygosity after adjustment for slope position, exposure, elevation, and age. Vertical bars are ± 1SE around the mean. The numbers in parentheses are the observed number of clones in each heterozygosity class.

Table 2.4. Comparison of single locus statistics in mean annual increment in growth rate between heterozygotes and homozygotes.

| Locus[2] | Ho | He | Mean growth rate (mm)[1] | | | | |
			Homozygotes	N	Heterozygotes	N	F
EST	0.638	0.590	1.31	51	1.37	95	2.22
SDH	0.560	0.414	1.35	64	1.41	82	0.21
MDH	0.057	0.090	1.35	136	1.33	10	0.11
G6PD	0.482	0.397	1.30	75	1.40	71	7.65[3]
ADH	0.511	0.424	1.30	74	1.40	72	7.15[3]
G2DH	0.383	0.367	1.32	92	1.40	54	3.63[4]
PGM	0.192	0.209	1.35	118	1.36	28	0.03
LAP	0.052	0.052	1.34	136	1.44	10	0.19
ALD	0.433	0.396	1.31	88	1.39	58	5.33[3]
PER	0.255	0.269	1.36	111	1.32	35	0.38
6-PGDH-1	0.412	0.330	1.37	82	1.32	64	1.98
6-PGDH-2	0.078	0.093	1.35	135	1.32	12	0.63
AAT-1	0.235	0.346	1.35	115	1.37	31	0.66
AAT-2	0.184	0.191	1.35	118	1.35	28	0.95

[1] Comparisons of annual growth increment are based on analysis of covariance with slope position, exposure, elevation, and age as covariates. Adjusted values represent adjustments for covariates.

[2] Enzymes are: EST (Esterase), SDH (Shikimic acid dehydrogenase), MDH (Malate dehydrogenase), G6PD (Glucose-6-phosphate dehydrogenase), ADH (Alcohol dehydrogenase), G2DH (Glycerate-2-dehydrogenase), PGM (Phosphoglucomutase), LAP (Leucine-amino peptidase), ALD (Aldolase), PER (Peroxidase), 6-PGDH (Glucose-6-phosphate dehydrogenase), AAT (Aspartate aminotransferase).

[3] $P < 0.001$

[4] $0.10 > P > 0.05$

Table 2.5. Analysis of covariance of mean annual increment variabilities among G6P, ADH-2, ALD, and G2PG genotypes in *Populus tremuloides*.

Genotype	N	Unadjusted Mean	Adjusted Mean[1]	F - ratio[2]	P [3]
G6PD					
11	6	1.14	1.18		
12	71	1.36	1.39	3.06	0.025
22	69	1.36	1.32		
ADH-2					
11	67	1.29	1.31		
12	67	1.41	1.39	2.32	0.040
13	5	1.52	1.48		
22	7	1.28	1.25		
G2DH					
11	82	1.30	1.32		
12	55	1.44	1.41	2.75	0.034
22	9	1.28	1.27		
ALD					
11	77	1.30	1.32		
12	60	1.43	1.41	3.65	0.015
22	9	1.23	1.23		

[1] Based on analysis of covariance controlling for the effects of slope position, exposure rating, elevation and age.

[2] F-statistics apply to adjusted data.

[3] One-tailed.

2.4 DISCUSSION

2.4.1 General pattern of variation in growth in relation to habitat

There were differences in the mean annual incremental growth rate between populations of *Populus tremuloides* in Waterton Lakes National Park. Variation in growth was found to be positively correlated with increasing protection from wind and inversely associated with elevation, slope, and age. More specifically, change in mean annual incremental growth appears to be due in part to inter-site variation in water availability and potential evapotranspiration, as estimated by slope and exposure. The effects of abiotic stress, especially water availability, in the growth of trembling aspen in WLNP is perhaps best discussed with reference to the Galwey population.

Mean annual increment at the high elevation Galwey site was 1.03 mm y^{-1}, compared to maximum growth of 1.54 mm y^{-1} (Table 2.1) at the low elevation Prairie I and II sites. The Galwey population is situated on a south-facing slope which is characterized by a comparatively short growing season, and dry, poorly developed soils. The soils on this ephemeral habitat are Regosols or Lithic Orthic Regosols having variable amounts of coarse fragments and the underlying substrate is unconsolidated material. These soils have poor water retention capabilities. The existence of frequent strong winds, as evidenced by the krummholz trees situated on exposed reaches at this site, would tend to rapidly deplete soil moisture. Furthermore, such winds would increase transpirational losses that could curtail growth (Woodward 1987). Although vertical sinker roots are part of the root system, they would have a difficult time penetrating the rocky soils to perform critical water-absorbing functions. The majority of the roots in this species occur in the comparatively dry upper 0.60 m of the soil (Day 1944; DeByle 1964; Barnes 1966) .

It is well known that where water is limiting, transpirational costs increase because of the difficulty in acquiring sufficient quantities to maintain guard cell turgor (Parkhurst and Loucks 1972; Woodward 1987). Maintenance of this condition is particularly problematic for aspen as leaves continue to lose water at very low leaf-water potentials (e.g., -6 MPa) (Tobiessen and Kana 1974). Similar results were found in *P. trichocarpa* by Schulte et al. 1987) though they also noted that the effects of preconditioning has a pronounced effect on whether drought-stressed stomata can close in response to water stress. Notwithstanding, if leaf-water potential falls below some critical value, leaf abscission may take place (Schulte et al. 1987; Woodward 1987) or metabolic damage may occur (Kramer and Kozlowski 1979). Thus, *P. tremuloides'* lack of control of water loss on droughty or exposed sites (such as Galwey) may result in considerable inhibition of growth. Furthermore, inadequate water for transpiration can directly affect leaf extension and canopy development, factors which also can affect growth (Woodward 1987). Accordingly, save for the Prairie sites, the Akamina population exhibited the highest growth rate (Table 2.1), despite its comparatively short growing season, because this site was the most moist of all those sampled. By comparison, Fralish and Loucks (1975) similarly found that four variables, all related to water availability, affected growth: soil texture, available water-holding capacity, water-table depth and stand exposure. Other studies have have found similar controls on growth of *P. tremuloides* (Graham et al. 1963; Einspahr and Benson 1967; Steneker 1976) and other plants in arid environments (Fischer and Turner 1978).

Lower growth rates at the Galwey site are likely due to low water availability and the shorter growing season. The pattern of leaf flushing at this site was at least two weeks behind that of the Prairie populations. Here, growth cessation could be expected to be earlier since trees are genetically adapted to the temperature regimen of their native habitat

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(Brissette and Barnes 1984). Since average lapse rates range from 1°C to 2°C per 300 m (Hopkins 1938; Price 1981), temperatures at this high elevation site should accordingly be about 4-8° C cooler than the low elevation Prairie sites. These values only suffice as a first approximation to the growing temperature regimen; site specific lapse rates vary with changing climatic conditions (Price 1981). At this elevation there is also a greater probability of late-season killing frosts, which if they occur before the plant is able to recover mineral nutrients, could result in reduced growth the following year. Frost resistance is a genetic character and thus these genotypes may be frost-resistant ecotypes; however, there is no evidence for this in trembling aspen (Egeberg 1963). Finally, the presumably low nutrient status of this high elevation location could further reduce growth, and this effect would be compounded by the fact that under such situations leaf senescence is typically early (Kramer and Kozlowski 1979).

Mitton and Grant (1980) similarly found a decrease in growth of trembling aspen with elevation, though its relative influence was not as pronounced as in this study. Unfortunately, no descriptive or summary statistics were provided with their results. Greene (1971) also reported a substantial decrease in the mean annual growth of aspen in Colorado which was correlated with elevation. She found that at 2000 m the mean annual radial increment of stems in six clones was 3.73 mm, which decreased to 1.13 mm at 3400 m. Although not examined in relation to elevational gradients, Morgan (1969) extracted cores from seven aspen trees in Colorado that ranged in age from 48-59 years and found growth rates of 3.7-4.8 mm y^{-1}. The annual increment in growth reported by Sakai and Burris (1985) for aspen at two sites in Michigan were 1.90 and 0.95 mm y^{-1}.

Typically, growth in trees decreases with time (Kramer and Kozlowski 1979), yet the age of the putative oldest ramets for each clone was poorly correlated with incremental growth. However, Lieffers and Campbell (1984) were unable to find a statistically

significant decrease in growth of trembling aspen in relation to age. While Mitton and Grant (1980) found growth rate to decrease with age, they also found a positive, albeit small, partial correlation ($r = 0.221, P < 0.001$) between ring width and stem diameter. They suggested that incongruence between these results might arise if some trees grow faster than others, yet have similar overall longevities.

2.4.2 Ecological implications of genetic variation of multiple loci

When the effects of the potentially extraneous environmental variables were held constant, no differences among populations were found to exist. This result is in agreement with the findings of Chapter 1 wherein populations are not spatially discrete with respect to allozymes. There was, however, a statistically significant ($P < 0.01$) positive relationship between growth and the degree of heterozygosity after controlling for the effects of the environmental variables. Assuming that growth is a fitness character, this finding suggests that fitness differences do exist between clones. The most complete evidence for a positive association between heterozygosity and growth comes from the studies of molluscs (see review in Zouros and Foltz 1987), but several investigators have found similar results in natural plant populations. In the only other study examining the effect of heterozygosity on growth rate in trembling aspen, Mitton and Grant (1980) also found an increase in growth in relation to allelic isozyme heterozygosity. As for other plant species, Schaal and Levin (1976) found a positive relationship between individual heterozygosity in *Liatris cylindracea* and fecundity, longevity and speed of development. Linhart and Mitton (1985) documented a reduction in the variability in growth rate and female cone production in ponderosa pine (*Pinus ponderosa*) in relation to increased levels of heterozygosity. Strauss (1986) also found some evidence that growth in knobcone pine (*P. attenuata*) was positively correlated with heterozygosity. Stutz and Mitton (1988) found

that natural selection favored uridine diphosphoglucose pyrophosphorylase (UDP) heterozygotes in mature stands of Engelmann spruce (*Picea engelmannii*) occupying both dry and wet sites, but acted against PGM heterozygotes on a wet site.

The proposition of a positive relationship between heterozygosity and growth rate is not universal. Heterozygosity was not found to be positively correlated with growth in ponderosa pine (*P. ponderosa*) and lodgepole pine (*P. contorta*) (Knowles and Grant 1981; Mitton et al. 1981). Ledig et al. (1983) suggested that the lack of positive association in these studies may be due to the low number of loci studied ($n=3$ and $n=4$), for it is unlikely that so few loci characterize the average heterozygosity for the entire genome (Mitton and Pierce 1980). Ledig et al. (1983) failed to find an association between heterozygosity and growth rate in a juvenile cohort of pitch pine (*P. rigida*) but such a relationship was present in older stands. Mitton and Grant (1984) suggested that a negative or non-significant relationship between heterozygosity and growth might arise if there is differential apportionment of energy between adults and juveniles; juveniles shunt most surplus energy to growth, while adults utilize some of this for reproduction. They also pointed out that characters differ in their degree of canalization whereby some traits are buffered against genetic (and environmental) variability. Last, a lack of association may also arise if those loci analyzed are not associated either directly or indirectly with complex polygenic traits such as growth.

If the degree of heterozygosity is indeed associated with growth, what mechanistic explanation might exist for such an association? As was pointed out in the introduction, there are two main hypotheses to account for the positive correlation between heterozygosity and fitness-related traits. The first explanation forms the basis of the overdominance hypothesis. From a physiological point of view, plant growth, the conversion of energy for use in the synthesis of new protoplasm, involves complex

interactions between numerous biochemical pathways. In a broad sense, growth depends on the plant's ability to acquire, store, and release energy as required. The process of photosynthesis governs the acquistion and storage of energy. The rate of growth, however, is largely influenced by enzymes that catalyze energetic conversions at ordinary temperatures. This is accomplished by momentarily binding substrate molecules on the surface of the enzyme molecules, thereby increasing the probability of a reaction occurring (Lehninger 1975). Electrophoretically-measured biochemical variation has been found to have measurable physiological consequences, some of which affect phenotypic expressions. Even though electrophoresis taps into only a small segment of the genome, some loci may be involved in specific metabolic pathways affecting growth. Those enzymes involved in protein catabolism, preglycolysis and glycolysis are thought to be particularly important in the multilocus heterozygosity-growth relationship (Koehn et al. 1988). It has been suggested that phenotypic variability in relation to enzyme polymorphism is owing to the fact that heterozygotes may enjoy an energetic advantage and have greater physiological versatility because they code for two (or more) versions of the same enzyme which may differ in catalytic optima (Koehn 1969; Johnson 1979; DiMichelle and Powers 1982; Koehn and Shumway 1982; Watt 1985b). Thus, for example, each of the two (or more) forms of an enzyme may exhibit different optima for temperature, pH, hydration or other factors compared to the single form characteristic of the homozygote. This physiological plasticity may result in individuals (i.e., genotypes) being less affected by fluctuating or stressful environmental conditions such as drought or frost (Mitton and Grant 1984; Mitton and Koehn 1985).

The second explanation for association between heterozygosity and increased fitness is the "dominance hypothesis". This hypothesis holds that allozymes at polymorphic loci are selectively neutral and the observed level of heterozygosity is merely representative of the

overall levels of genetic variation in the genome. Moreover, low levels of heterozygosity are believed to be inversely correlated with homozygosity for deleterious alleles in the genome as a whole. Therefore, in predominantly outcrossing populations, variations in growth may be due to rare, deleterious, or recessive alleles that express themselves under conditions of inbreeding. There is, however, no evidence to suggest that *P. tremuloides* in WLNP suffer the effects of inbreeding. From the results in the preceding chapter, populations appear to intercross, and there were no serious deficiencies of heterozygotes relative to Hardy-Weinberg expectations. This evidence thus favors the first hypothesis; however, unequivocally invoking one explanation over the other requires determination of the loci that directly govern the expression of a phenotypic trait (Chakraborty 1987). Unfortunately, this latter problem represents a major hiatus in our understanding of the biological significance of genetic variation.

2.4.3 Ecological implications of genetic variation of single loci

In this study, mean annual incremental growth rate was found to be significantly correlated with heterozygosity at four individual loci including G6PD (glucose-6-phosphate dehydrogenase), ADH (alchohol dehydrogenase), and ALD (aldolase) ($P<0.001$) and G2DH (glycerate-2-dehydrogenase) ($0.10 > P > 0.05$). Probability theory predicts less than one significant correlation by chance. There were no statisticstically significant correlations between homozygotes and growth rate.

Allozymes at a single locus are not functionally equivalent. Polymorphism in even a single gene can have significant effects on physiological performance (Koehn 1987). As a result, some investigators have narrowed their focus from a multi-locus perspective to the impact of single-locus variation on fitness, albeit it is widely recognized that these genes may well be linked to others (see reviews in Koehn et al. 1983; Watt 1985b). If

heterozygosity itself is in fact directly linked to increased fitness, it has been argued that this may be due to catalytic functions of single loci (Mitton and Grant 1984; Koehn 1987). However, to relate biochemical diversity of alleles at a locus to physiological variability requires an understanding of the biochemical function of an enzyme as well as its role in metabolism (Koehn 1987). Again, there is a dearth of information on the precise physiological consequences of genetic variation, particularly as it pertains to plants (Rainey et al. 1987; Zouros and Foltz 1987; Koehn 1987). For example, only recently, Rainey et al. (1987) suggested that respiratory efficiency in perennial ryegrass (*Lolium perenne*) may be related to, or correlated with, 6PGD and PGM.

Part of the problem in ascribing variation in growth to specific enzyme loci relates to the fact that: 1) the metabolic function of all polymorphic enzymes is not known (Koehn 1987), and 2) a large number of loci may control a phenotypic expression (Lewontin 1974); many of these are likely neutral (Kimura 1968, 1983). Notwithstanding these arguments, some researchers (e.g., Mitton and Grant 1980, 1984; Kasule and Cook 1988) have commented that while it may seem somewhat remarkable that a few enzyme loci, often picked at random, can be used to predict fitness-related characters, enzyme loci have a high probability of directly or indirectly influencing general fitness. Rather than relying on indirect correlation, Koehn et al. (1983, 1988) argued that if one can determine the precise physiologic phenotypic effects of genes associated with known metabolic function, it is hard to accept the proposition that the known physiologic phenotypes are produced by genes of unknown function (i.e., linked deleterious alleles).

Although the effects of heterozygosity at single loci in relation to energy status are presently unknown for most genes (Koehn et al. 1988), three of the four enzymes found to be correlated with growth in this study occupy positions of regulatory potential; the fourth variable loci, G2DH, is a minor enzyme (Figure 2.3). G6PD is a major enzyme whose

GLYCOLYSIS

PENTOSE PHOSPHATE PATHWAY

Glucose

G6PD

Glucose-6-phosphate $\longrightarrow$ 6-phosphogluconate

Fructose-6-phosphate   Ribulose-5-phosphate

Fructose-1,6-biphosphate

ALD

Dihydoxyacetone-P   Glyceraldehyde-3-phosphate

FERMENTATION

Pyruvate $\longrightarrow$ Acetaldehyde $\longrightarrow$ Ethanol

ADH

KREBS
CYLCLE
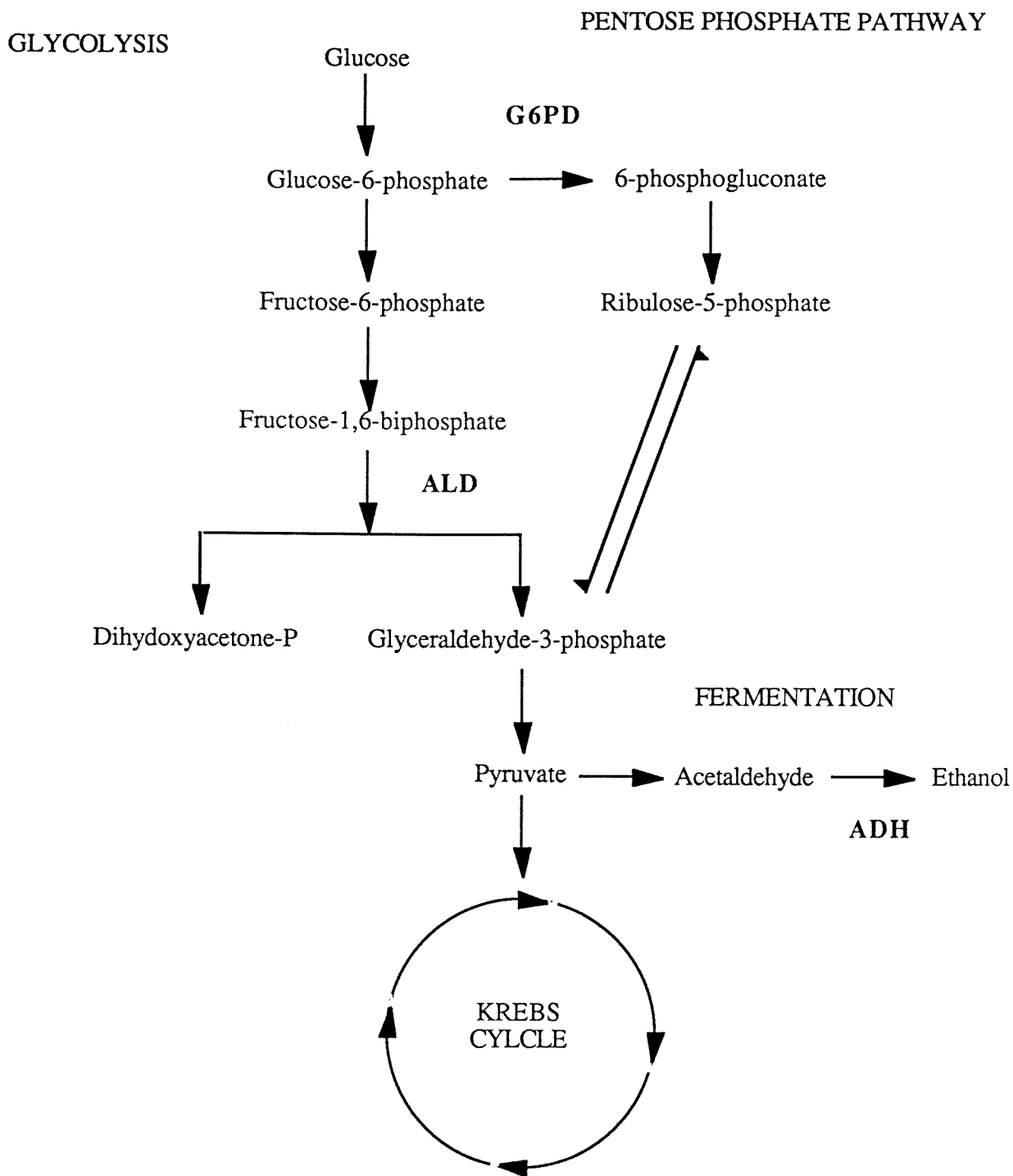
Figure 2.5. An outline of the principal steps in the glycolytic, pentose shunt, and anaerobic pathways showing the positions of G6PD, ALD, and ADH. Adapted from Lehninger (1975)

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function is central in the pentose shunt. The pentose shunt is an alternate pathway for the catabolism of glucose. Although less important than glycolysis in terms of the percent glucose oxidized, this process is important in generating reduced NADPH (nicotinamide adenine dinucleotide phosphate), which is a high-energy compound that serves in redox balance and reduction of carbon in the process of photosynthesis. The first reaction in the pentose shunt involves the enzymatic dehydrogenation of G6P by G6PD to form 6-phosphogluconate. Cavener and Clegg (1981) demonstrated that two homozygous alleles at this locus in *D. melanogaster* had functional differences in the activity of the pentose shunt which translated to differences in the balance of carbon flow. Although the effects of heterozygotes at this locus are known to be approximately intermediate between the homozygotes in terms of activity, the precise consequences of heterozygosity on metabolic rate and energy status are unknown for this and nearly all enzyme systems (Koehn et al. 1988). Nevertheless, while the effect of heterozygotes at this locus is unknown, G6PD is linked to 6PGD (Watt 1985*b*), the second enzyme in the pentose shunt. Rainey et al. (1987) found that individuals of *Lolium perenne* that were heterozygous for both 6PGD and PGM were better able to withstand heat stress than homozygous individuals. Conceivably, there may be some some such role for G6PD.

While direct evidence of the role of ADH in relation to growth is unknown, it is known that it too occupies a position with regulatory potential (Figure 2.3). ADH catalyzes removal of hydrogen atoms from ethanol which yields acetaldehyde. In the presence of high induced levels of ADH this is converted to ethanol. Recently, it has been shown (Heinstra et al. 1983) that ADH also catalyzes the conversion of acetaldehyde into useful metabolic acetate. The role of ADH is best understood as it pertains to *D. melanogaster* (reviewed in Van Delden 1982, 1984). Oakeshott et al. (1982) found that the geographic patterns of ADH alleles in natural populations of *D. melonogaster* are strongly correlated

with environmental gradients, specifically latitude, topography, and rainfall. The role of ADH in plants is poorly known, however. Selection at this locus has been shown in maize (Marshall et al. 1973). It is also known that high levels of this enzyme can produce toxic accumulations of ethanol during prolonged periods of flooding (Johnson 1979). Habitat conditions in WLNP do not lend themselves to the production of toxic levels of acetaldehyde or ethanol via flooding. Hence, it is somewhat surprising that growth is related to ADH. It may be possible that the presence of ADH signals the ability to withstand short-term soil anoxia during spring thaw. Although the soils are "droughty" for most of the growing season, tolerance of anoxia might give significant advantage to tolerant genotypes. Heywood and Levin (1985) analyzed 15 allozymes in *G. pulchella* in relation to 20 quantitative soil variables and found that ADH and PGM served to discriminate between calcareous and noncalcareous soil types. There was no genotypic analysis however, nor were the investigators able to state whether selection took place at these loci or whether there was genetic linkage. Under soil conditions somewhat similar to WLNP, Farris and Schaal (1983) conducted a drought-stress experiment in sheep sorrel (*Rumex acetosella*) and found that drought-tolerant survivors had fewer ADH bands than non-survivors; complex banding patterns prevented more definitive genotypic designations. No mechanism was proposed for the precise role of such a banding pattern. It seems apparent, however, that this locus is closely related to water relations either directly because of the functional differences between ADH allozymes or by regulatory variants closely linked to this locus.

Aldolase (also known as fructose diphosphate aldolase) (ALD) is involved in the second of two priming reactions of glycolysis (Figure 2.3). Specifically, aldolase catalyzes the reaction of fructose, 1, 6-diphosphate to yield two triose phosphates, glyceraldehyde 3-phosphate (an aldose), and dihdroxyacetone phosphate (a ketose). The former is the all

important product of the first phase of glycolysis. Although this locus has been analyzed in relation to fitness in various organisms, the role of heterozygotes or alternate homozygous alleles is poorly known. Strauss (1986) found heteroygote inferiority at this locus in *P. attenuata* when examined in relation to growth; heterozygotes were also low in frequency. The relationship between growth rate and ALD in *Pinus rigida* (Bush et al. 1987), when analyzed with Smouse's adaptive distance model (Smouse 1986), resulted in mixed findings. There was a positive correlation between heterozygosity and growth in two of eight populations, a negative correlation in three, and a further three populations had zero correlations.

Glycertate-2-dehydrogenase (G2DH), as indicated at the onset of this section, is a minor enzyme with no specific metabolic function. Notwithstanding, Zouros and Foltz (1987) argued that even if no known relationship exists between an enyzme variant and some physiological phenotype, studies of any polymorphic loci in relation to fitness characters are of value. Their contention was based on a proposition taken from population ecology whereby selection can be examined through the use of life table analyses (e.g., schedules of fecundity or viability). Irrespective of a genetic variant's precise physiologic effect, its ultimate fate is manifest through either or both reproduction and viability. Therefore, a polymorphic loci may affect some physiologic function but it may not affect fecundity or viability. In this case it can be regarded as a neutral character since it has no effect on fitness. Given this situation, any genetic variant can be studied in relation to fitness characters.

The critical question remains as to whether heterozygosity *per se* is responsible for the heterotic effects or whether it is the consequence of the less frequent occurrence of deleterious recessive alleles as homozygotes. Smouse (1986) proposed a method for inferring the relationship between fitness and heterozygote superiority at a particular locus.

Essentially, if overdominance is the underlying cause of increased fitness, then heterosis should be stronger among the more polymorphic loci. Therefore, assuming that the locus of interest is overdominant, and that the allelic frequencies are approximately in equilibrium, heterozygotes should be more fit than common homozygotes which, in turn, should be more fit than rare homozygotes. In this study, using growth as a surrogate for fitness, Smouse's model can be used to predict whether selection is acting directly on the aforementioned loci whose polymorphism was found to be related to growth. Based on these results, if faster growth is indeed a component of fitness, then heterozygotes at the G6PD, ADH, ALD, and G2DH loci in *P. tremuloides* in WLNP are more fit and selection is acting directly on these loci. It is still unknown, however, just how the different variants exert their influence on *P. tremuloides*' physiology.

2.4.4 Evolutionary implications of heterozygosity and growth

Faster growth rates, whether they accrue from the action of multiple genes or from the way in which a single enzyme variant may alter the effectiveness of a biochemical pathway, may confer several adaptive advantages. Genotypes which grow faster are likely to reach sexual maturity more quickly, and therefore, contribute earlier, and hence, in greater abundance to the gene pool than slower growing genotypes. Furthermore, if colonization is episodic following disturbance, those genotypes which are characterized by faster growth are more likely to outcompete slow growing genotypes through crown competition for light. As thinning occurs, less fit genotypes may be eliminated. For example, Ledig et al. (1983) found that heterozygote superiority was greatest in dense stands of *P. rigida* where competition for resources could be expected to be great. Similarly, since *P. tremuloides* is generally considered an early successional species, the effects of competition from later successional species (e.g., conifers) are likely to be protracted or reduced for those

individuals capable of attaining canopy dominance. Furthermore, *P. tremuloides* experience mast years during which radial and terminal growth are reduced. During this time, resources are channeled into sexual reproduction at the expense of incremental growth. Thus, rapid incremental growth in the intervening years might reduce the incidence of suppression by competitors. A similar resource tradeoff apparently takes place between vegetative reproduction and mean annual ring width (Sakai and Burris 1985). Last, rapid growth, especially in the first few years, would also enable faster growing genotypes to have a greater percentage of above-ground biomass out of reach of vertebrate herbivores.

2.4.5 Other possible influences on mean annual incremental growth

Clearly, much of the growth within clones and between populations is probably related to several genetic and environmental factors (such as competition, nutrient availability, history of browsing, or the effects of pathogens) which were not measured in this study. For example, those allozymes assayed may not be directly or closely linked to the energetic or physiological pathway responsible for growth. The particular enzymes examined in this study were selected for their ability to be analyzed electrophoretically and not for assessment of their potential role in physiological processes *per se*. As well, their physiological role, and ultimately, selective role, may not be manifest under present environmental conditions (Koehn et al. 1983; Hartl et al. 1985; Burton and Place 1986). This is an important consideration, particularly in the case of *P. tremuloides* for it is widely believed that clones in the Great Plains region are of great age (see Chapter 1). Hence, "mapping" genotype profiles in relation to growth under present environmental conditions may lead to poor predictability for both multi- and single-locus relationships if the environments for which genotypes were selected have undergone considerable change.

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Also, as indicated in the previous chapter, physiological integration among member ramets of a clone may buffer the effects of selection thereby "masking" the potential effects of heterozygosity as might be manifest in species with physiologically independent stems. Finally, and perhaps most importantly, the role of the assayed electrophoretic loci may be of more significance in the early stages of clonal development (e.g., germination and seedling development). In general, plant mortality is high at this time (Harper 1977) but evidence of selection has been erased. The impact of competition for resources, say from grasses, during the first few years of life is not easy to interpret a number of decades later. Once again, to disentangle environmentally-generated fitness variation from genetically-determined variation (and $G \times E$ interactions), there is a need for long-term studies of clone performance using a series of reciprocal transplant or common garden experiments.

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2.5 SUMMARY AND CONCLUSIONS

While pronounced population differentiation in allelic isozymes was not readily evident in the analyses of the preceding chapter, there was considerable enzyme polymorphism among individual *P. tremuloides* clones in WLNP. To gain insight into the effects of genetic polymorphism, I examined the relationship between environmental heterogeneity, allozyme heterozygosity and growth in 146 clones. Mean annual incremental growth was determined for five ramets per clone; clonal genotypes were scored at 14 polymorphic enzyme loci. A significant relationship was found to exist between multi-locus heterozygosity and mean annual incremental growth rate after controlling for the effects of elevation, age, slope position and exposure. Furthermore, there was evidence of a single-locus relationship between heterozygosity and fitness at the ADH, ALD, G2DH, and G6PD loci. Heterozygotes appeared more fit and it is suggested that natural selection may be acting directly at the these loci. Bradshaw (1972) pointed out that where populations are perennial, selection in a particular niche will be cumulative over time. Thus only slight differences in the fitness of genotypes such as that observed in this study need be present to exclude less well adapted genotypes from a particular niche or patch. On the basis of these results, it is therefore concluded that clones of *P. tremuloides* in WLNP are characterized by environmentally- and genetically-related fitness differences.

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CHAPTER 3

Clonal Variability in the Chemical Composition of *Populus tremuloides* and the Implications for Herbivore and Plant Populations

3.1 INTRODUCTION

Vertebrate herbivores have long been known to be selective in their choice of plant species on which to graze or browse (Swift 1948; Radwan 1972; Sinclair 1975; Lindroth 1979; Radwan and Crouch 1974; Klein 1977; Belovsky and Jordan 1978). Selection by herbivores for individuals or varieties within plant species is less well-documented. Selectivity could have significant effects on the genetic diversity of natural plant populations if the genotypes eaten are not selected in proportion to their availability. For example, diversity could be enhanced if a herbivore chooses disproportionately more of a common genotype from a mixture available. Leopold (1933) and Squillace and Silen (1962) were among the first to note marked differences in palatability within a species. They recorded variations in palatability of genotypes of *Pinus ponderosa* grown on a common site. More recently, others (Radwan 1972; Dimock et al. 1976; Silen and Dimock 1978; Silen et al. 1986) examined differences between Douglas fir (*Pseudotsuga menziesii*) genotypes in relation to browsing by hares (*Lepus americanus*) and black-tailed deer (*Odocoileus hemionus columbianus*) and found genotypes that sustained lower levels of browsing had depressed dry-matter and cellulose digestibilities, and high levels of secondary chemical compounds. These studies also demonstrated that the traits linked to the palatability of Douglas fir genotypes are genetically transmittable and additive (i.e., genes that interact only by enhancement of a trait). Domestic sheep grazing on white clover (*Trifolium*

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repens) respond to a proximate cue, leaf markings, though the ultimate basis for this selectivity is not known (Charles 1964; Cahn and Harper 1976). Dickman (1978) surveyed 28 hybrid poplar clones for damage by eastern cottontail rabbits (*Sylvilagus floridanus*) and found significantly higher rates of herbivory on two *P. nigra* clones. While Dickman did not examine, nor speculate upon, the underlying cause for this pattern, *P. nigra* is high in flavonoids (Wollenweber 1974).

Populus tremuloides is a major food item for many temperate region vertebrate herbivores such as moose (*Alces alces*), elk (*Cervus elaphus*), and deer (*Odocoileus* spp.) (Kufeld 1973; Peek 1974; Bryant and Kuropat 1980; Olmsted 1980). The phytochemical composition of *P. tremuloides* has been widely assessed (e.g., Kubota et al. 1970; Tew 1970; McColl 1980; Collins and Urness 1983; Alban 1985; Canon et al. 1987; Schwartz et al. 1988). However, intraclonal variability in plant chemistry or browse use has not been assessed save for the study of McNamara (1980). McNamara examined the protein and mineral content of twigs of three clones and suggested that elk showed preference for the genotype possessing highest protein content. The conclusions of her study are limited by the small sample size and the absence of data on trace elements and structural constituents. Notwithstanding, McNamara's results are consistent with the findings of others who have found considerable clonal polymorphism for a number of allozyme (genetic) and quantitative characters (Schier et al. 1985; Jones and DeByle 1985; Cheliak and Dancik 1982; this study). Clones of *P. tremuloides* also have a tremendous propensity for vegetative reproduction and, as such, can attain great size. The largest reported clone consisted of some 47000 mature stems covering 43 ha in size (Kemperman and Barnes 1976). This potential for attaining such large size means that deer could conceivably browse exclusively on one or a few clones. The nutritional consequences thus could be

profound and varied if there exists significant interclonal differences in the chemical composition of the browse.

The objectives of this study were to: (1) examine the nutritive value of *P. tremuloides* dormant, current-year twigs relative to the dietary requirements of large herbivores; (2) evaluate the pattern of *in situ* browse use as a first approximation of selectivity; (3) compare browse use to interclonal variability in chemical composition; and 4) evaluate variability in browse use and chemical composition in terms of short-term ecological implications and long-term evolutionary consequences for *P. tremuloides*. Specifically, I tested two hypotheses: 1) that clonal differences exist in the chemical composition of *P. tremuloides* twigs, and 2) that nutritionally-superior clones should be browsed to a greater extent than nutritionally-inferior clones. The analysis of aspen as a forage resource was restricted to protein and mineral elements as these are known to be major limiting nutrients in the diets of free-ranging ungulates (Sinclair 1975; Price 1978; Lindroth 1979; Belovsky 1981; Robbins 1983), and fiber constituents. Cell wall constituents were evaluated because: 1) they interfere with the extraction of nutrients contained in the cell (e.g., protein, sugars, starches); 2) they are difficult to digest (Van Soest 1982); and 3) they affect voluntary intake (Spalinger et al. 1986; Baker and Hobbs 1987). Secondary chemicals, although well-known both as deterrents and for their effect on biological functions in browsing ruminants (Bryant and Kuropat 1980; Cooper and Owen-Smith 1985; Palo 1985; Palo et al. 1985; Robbins et al. 1987*a*, 1987*b*), were not included in the assay for reasons outlined below.

Phenolic glycosides are the only well-known class of secondary compounds known to occur in the Salicaceae (Palo 1984). A review of the literature, however, suggests that soluble phenolics do not inhibit digestion of cell walls and cell contents in dormant *P. tremuloides* twigs. Sinclair and Smith (1984) reported that *P. tremuloides* twigs had a low

ratio of protein-complexing phenols to total phenols. Noting that only a small fraction of total phenolic extract may be biologically active, Tahvanainen et al. (1985) analyzed various phenolic fractions in mature and juvenile twigs in five *Salix* species. The plant material was offered to mountain hares (*Lepus timudus*) in field diet experiments with highly palatable *Populus tremula* (a European species of aspen closely related to *P. tremuloides*) serving as reference material. They found that the concentration of total phenolics and leucoanthocyanins was lowest in *P. tremula*; concentration of phenolic glycosides ranked second lowest. Hares also showed a distinct preference for *P. tremula* and *Salix caprea* twigs. Masslich et al. (1988) did not find any tannins (a polyphenol) or terpenes in dormant *P. tremuloides* twigs. Robbins et al. (1987*a*) showed that, in general, dormant stems of deciduous tree species (including *P. grandidentata*) had very low levels of precipitating tannins, and protein availability was similar to that for grass, legumes, and pelleted rations. Robbins (*personal communication*) suggested that *P. tremuloides* would be similarly characterized. Jogia et al. (1989) recently reported on the presence of 2,4,6-trihydroxydihydrochalcone in *P. balsamifera* juvenile twigs but not in mature twigs of *P. balsamifera* or any twigs of *P. tremuloides*. In sum, there is little evidence to suggest that these well-known allelochemicals affect browsing on dormant *P. tremuloides* twigs by ruminants, albeit there may be some hitherto unknown secondary compounds involved.

3.2 METHODS

3.2.1 Selection of clones and assessment of browse use

Eight clones genetically unique at 14 polymorphic loci were randomly selected from each of three different environments, each environment corresponding to a population described in Chapter 1, namely Prairie, Galwey and Akamina (see chapter 1). Based on both electrophoretic and phenotypic characters [e.g., leafing patterns, and bark texture and color (Barnes 1969]. The clones were of approximate equal size (0.01 ha) and, therefore, assumed to have equal proportions of available browse.

Browse surveys were conducted about one month prior to leaf-out on clones from two environments, the Prairie and Galwey sites. Clones from the Akamina study site were not surveyed for browse use because of very low ungulate densities, but twigs from this area were still collected for chemical analysis. Browse use was enumerated at 40-50 ramets on the outer edge of each clone. Browsed and unbrowsed twigs were counted to yield percent utilization (Stickney 1966; Jensen and Scotter 1977). Twigs were defined as the plant material distal to the stem ≥25 mm in length and situated between ground level and 2 m.

Within a few days of the browse survey, twigs were collected for chemical analysis from 5 unbrowsed ramets at each of the 24 clones. Only unbrowsed twigs were sampled since there is evidence that indicates browsing can alter the chemical content of vegetation (Bryant 1981; Danell and Huss-Danell 1985). Samples were placed in an ice chest and later frozen until lab analysis. Because juvenile ramets have different chemical compositions than older trees (Bryant 1981), sampled ramets were ≥ 8 years of age. Similarly, only the current annual growth was sampled to control for heterogeneity among test material. Last, to control for micro-site influences (e.g., canopy cover), the samples were taken from ramets situated at the border of each clone and receiving full exposure to sunlight.

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3.2.2 Laboratory analysis

Prior to analysis, twigs were oven-dried (70°C) to constant dry weight and then ground in a Wiley mill (#20 sieve). Samples were analyzed for neutral detergent fiber, acid detergent fiber (ADF), and acid lignin according to the methods of Goering and Van Soest (1970) but without sodium sulfite and decahydronapthalene (Mould and Robbins 1981). All determinations were corrected to 100% dry matter. The detergent analysis was carried out at Agriculture Canada's Agassiz Agricultural Research Station. Elemental analysis was carried out for N, P, K, Ca, Mg, Na, Mn, Fe, Cu and Zn. Chemical concentrations were determined following the wet oxidation procedure of Parkinson and Allen (1975) on 1.0 g samples. N determinations were made on a Technicon Auto Analyzer II and other mineral elements on a Thermo-Jarell Ash Autocomp 81 Inductively-Coupled Argon Plasma spectrophotometer. These analyses were carried out at the University of British Columbia, Plant and Soil Testing Laboratory. Crude protein was calculated as N x 6.25. Due to a comparatively poor understanding of ungulate nutritional requirements, National Research Council (NRC) (1976) standards for domestic animals are sometimes used by wildlife ecologists to identify potential nutritional limitations of a food resource (e.g., Risenhoover 1989), and will be used accordingly here (Table 3.1).

Dry matter digestion (DMD) was estimated using the summative equations Robbins et al. (1987*b*).

$$DMD = (0.9231e^{-0.0451X})(NDF) + [(-16.03 + 1.02\ NDS) - 2.8\ (P) \quad [3.1]$$

where: X = lignin content of the ADF (%)

NDF = neutral detergent fiber (%)

NDS = neutral detergent solubles (%)

P = reduction in protein digestion (%) (Robbins et al. 1987*a*)

Table 3.1. Nutrient requirements of beef cattle[1].

	Growing and finishing steers and heifers	Dry pregnant cows	Lactating cows	Mature bulls
	Percent			
Crude protein	8.5 - 9.5	5.9 - 8.8	9.2 -10.9	8.5 -10.2
P	0.18 - 0.70	0.18	0.18 - 0.39	0.18 - 0.39
K	0.6 - 0.18	0.6 - 0.18	0.6 - 0.8	0.6 - 0.8
Ca	0.18 - 1.04	0.18	0.18 - 0.44	0.18 - 0.44
Mg	0.04 - .10	0.04 - 1.10	0.18	0.18
Na	0.06	0.06	0.06	0.06
	Parts per million			
Cu	4.0	4.0	4.0	4.0
Mn	1.0 - 10.0	20.0	1.0 - 10.0	1.0 - 10.0
Fe	10.0	10.0	10.0	10.0
Zn	20.0 - 30.0	20.0 - 30.0	20.0 - 30.0	20.0 - 30.0

[1] source: NRC (1976)

Digestible protein (DP) was predicted as

$$DP = -3.87 + 0.9283X - 11.82Y \qquad [3.2]$$

where   X = crude protein content, in % dry matter

Y = bovine serum albumin (BSA) precipitation, mg mg^{-1} forage dry matter

BSA was estimated as 0.10 mg mg^{-1} forage dry matter following Robbins et al. (1987*b*).

Inter-clonal and inter-site variations in chemical composition were separately examined with one-way analysis of variance (ANOVA). Where necessary, data were logarithmically transformed to normalize the distributions. Browse use was compared to crude protein and phosphorus using simple correlation analysis. This analysis was not extended to the other elements since there is little evidence to suggest that, except for Na, they are critical in diet selection by ungulates. They are nevertheless important dietary components; hence they were included in the assessment of aspen twigs as a food resource.

3.3 RESULTS

3.3.1 Browse use

All clones in the low elevation Prairie site had some twigs browsed, but none more than 15% (Table 3.2). On average, only 8.8% (SD=2.96) of the surveyed twigs were browsed. Most of this browsing is attributed to 700 or so elk that reside in the area (Parks Canada 1984). Greater use of aspen browse was found at the high elevation (Galwey) site. Here all clones were browsed to some extent at rates, on average, more than twice the low elevation rates ($\bar{x}$=21%; SD=5.81); two were subjected to levels of 28% (Table 3.2). This is attributed to mule deer, for only a small band of bull elk occasionally frequent this site (R. Watt, Park Warden, *pers. communication*).

3.3.2 Elemental composition

There were clonal differences ($P \leq 0.05$) in crude protein and the macroelements (Table 3.3). Mean crude protein concentration ranged from 4.56 to 7.44% and averaged 6.25%. Phosphorus varied from 0.13 to 0.21% and averaged 0.17% across clones. Some clones exhibited a considerable amount of intraclonal variability (e.g., clones 6, 95, 124). There was no relationship ($P>0.05$) between P concentration and browse use at either site. Among the major elements, Ca exhibited the greatest amount of interclonal variability ranging from 0.52 to 2.09% with a mean Ca concentration of 0.94% (Table 3.3). The Ca:P ratios averaged 5.62:1 and interclonally varied from 3.02:1 to 12.92:1. Interclonal variation in K was small with clonal means ranging from 0.58 to 0.84%, with an overall mean of 0.69%. Magnesium ranged from 0.13 to 0.22%; average concentration was 0.18%. One clone (clone 6) had particularly high within-clone variation.

The trace elements also varied significantly ($P \leq 0.05$) between clones (Table 3.4); but in general, interclonal variation was greater in the micronutrients than the macronutrients.

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Table 3.2. Percent browse use for each of 16 clones in the Prairie and Crandall/Galwey sites over the winter of 1986.

Site	Clone	% twigs browsed
Prairie	3	9
	6	15
	10	8
	11	9
	15	7
	53	5
	74	10
	95	7
	mean	8.8
Galwey/Crandall	59	15
	111	28
	112	26
	117	28
	121	21
	124	19
	126	13
	129	18
	mean	21

Table 3.3. Macronutrient contents in dormant current-year twigs from 24 clones of *Populus tremuloides*, Waterton Lakes National Park, Alberta. The mean and standard errors (in parentheses) are based on 5 replicates per per clone. Measured as percent dry matter.

Clone	Crude protein	P	K	Mg	Ca	Ca:P
Prairie						
3	7.18 (0.15)	0.16 (0.01)	0.84 (0.03)	0.16 (0.00)	2.09 (0.17)	12.92
6	6.57 (0.13)	0.13 (0.02)	0.64 (0.08)	0.19 (0.03)	1.19 (0.17)	8.98
10	6.45 (0.23)	0.19 (0.01)	0.62 (0.02)	0.22 (0.01)	0.91 (0.06)	4.68
11	5.05 (0.19)	0.13 (0.01)	0.67 (0.04)	0.14 (0.00)	1.06 (0.07)	8.46
15	6.71 (0.12)	0.19 (0.00)	0.80 (0.04)	0.20 (0.01)	1.70 (0.09)	9.03
53	4.56 (0.13)	0.16 (0.01)	0.81 (0.03)	0.15 (0.00)	0.65 (0.02)	4.17
74	6.65 (0.09)	0.19 (0.01)	0.70 (0.03)	0.13 (0.01)	1.02 (0.05)	5.45
95	6.54 (0.44)	0.17 (0.01)	0.70 (0.02)	0.17 (0.01)	0.99 (0.11)	5.72
Akamina						
23	6.67 (0.16)	0.21 (0.01)	0.66 (0.04)	0.16 (0.01)	0.89 (0.08)	4.33
31	5.42 (0.13)	0.17 (0.01)	0.65 (0.02)	0.21 (0.01)	1.00 (0.07)	6.00
32	5.62 (0.12)	0.19 (0.01)	0.75 (0.03)	0.20 (0.01)	0.80 (0.01)	4.20
35	5.51 (0.22)	0.17 (0.01)	0.63 (0.01)	0.22 (0.01)	0.69 (0.03)	4.04
36	6.80 (0.17)	0.20 (0.01)	0.61 (0.01)	0.19 (0.00)	0.68 (0.03)	3.40
42	5.25 (0.15)	0.14 (0.00)	0.58 (0.01)	0.20 (0.01)	0.70 (0.07)	5.14
43	5.65 (0.12)	0.19 (0.00)	0.72 (0.02)	0.22 (0.01)	1.26 (0.05)	6.66
44	5.35 (0.15)	0.15 (0.01)	0.72 (0.03)	0.21 (0.00)	1.03 (0.08)	7.10
Galwey						
59	6.54 (0.38)	0.19 (0.01)	0.65 (0.00)	0.15 (0.01)	0.87 (0.10)	4.70
111	7.44 (0.22)	0.19 (0.00)	0.63 (0.02)	0.16 (0.01)	0.66 (0.06)	3.48
112	6.68 (0.05)	0.15 (0.00)	0.71 (0.03)	0.20 (0.00)	0.75 (0.01)	4.90
117	7.39 (0.31)	0.17 (0.00)	0.65 (0.02)	0.21 (0.01)	0.86 (0.03)	5.22
121	6.90 (0.40)	0.18 (0.02)	0.70 (0.03)	0.17 (0.00)	0.76 (0.03)	4.23
124	6.72 (0.34)	0.15 (0.02)	0.65 (0.02)	0.16 (0.01)	0.66 (0.11)	4.68
126	6.39 (0.14)	0.18 (0.01)	0.65 (0.03)	0.16 (0.00)	0.52 (0.04)	3.02
129	6.43 (0.12)	0.19 (0.01)	0.69 (0.03)	0.18 (0.01)	0.80 (0.04)	4.33
Grand mean	6.25	0.17	0.69	0.18	0.94	5.62
F	12.84***	6.69***	5.35***	10.08***	17.27***	

*** $P < 0.001$

Table 3.4. Micronutrient contents in dormant current-year twigs from 24 clones of *Populus tremuloides*, Waterton Lakes National Park, Alberta. The mean and standard errors (in parentheses) are based on 5 replicates per clone. Measured as ppm[1].

Clone	Mn		Fe		Zn	
Prairie						
3	23.58	(2.25)	75.64	(9.79)	58.47	(4.98)
6	28.71	(3.86)	106.91	(39.48)	83.22	(11.83)
10	19.05	(0.80)	51.36	(4.66)	110.93	(3.94)
11	25.85	(0.38)	124.34	(41.57)	98.77	(3.27)
15	17.37	(0.76)	92.59	(13.00)	78.96	(4.41)
53	20.82	(0.56)	49.83	(8.46)	87.34	(4.68)
74	31.31	(0.84)	35.15	(1.86)	72.59	(5.72)
95	58.30	(4.96)	64.80	(5.72)	123.33	(7.88)
Akamina						
23	19.21	(1.52)	72.40	(7.30)	110.17	(4.82)
31	45.79	(10.02)	38.76	(2.17)	97.35	(11.27)
32	22.43	(0.81)	57.49	(4.86)	63.24	(2.67)
35	15.42	(1.88)	53.11	(8.05)	115.76	(8.76)
36	21.61	(1.61)	83.88	(33.04)	46.42	(2.80)
42	21.52	(2.95)	51.93	(9.18)	70.70	(2.14)
43	34.23	(0.33)	42.77	(1.40)	77.80	(3.18)
44	25.81	(1.60)	47.19	(5.34)	54.94	(2.43)
Galwey						
59	35.57	(1.72)	68.19	(7.42)	92.68	(6.84)
111	16.53	(0.94)	58.10	(27.20)	72.71	(2.87)
112	19.04	(1.90)	48.70	(3.07)	82.79	(6.61)
117	29.76	(1.41)	150.36	(79.02)	83.01	(2.15)
121	25.54	(1.05)	55.07	(8.58)	104.19	(6.92)
124	23.73	(2.53)	41.66	(7.36)	76.89	(7.61)
126	17.85	(3.46)	150.46	(65.70)	127.02	(4.79)
129	32.61	(3.77)	63.08	(15.76)	106.01	(5.85)
Grand mean	26.32		70.16		87.30	
F	9.32***		3.24***		11.72***	

[1]see text re: Na and Cu concentrations
*** $P < 0.001$,

The most pronounced between-clone differences occurred for Mn and Zn (Table 3.4). Mean Mn concentration among clones was 26.32 ppm and varied from 15.42 to 58.30 ppm. Zinc averaged 87.3 ppm and varied from 46.42 to 127.02 ppm. Interclonal Fe concentrations varied widely from 35.15 to 150.46 ppm and averaged 70.16 ppm. Intraclonal variability was also high. Sodium concentrations were too low in solution (<51 ppm) for accurate spectrophotometric determination. Copper concentrations were also beneath the instrument's detection limits (<10.2 ppm), save for 2 clones, 23 and 117, in which concentrations averaged 10.5 and 12.2 ppm, respectively.

3.3.3 Structural constituents and dry matter digestibility

Overall, relatively small but significant differences were found for the structural constituents (Table 3.5). NDF ranged from 39.48 to 52.81% and averaged 45.76%. ADF averaged 32.56% and ranged from 28.32 to 37.37%. Among clones, cellulose contents averaged 21.27% and ranged from 17.43 to 25.73%. Lignin exhibited the least interclonal variability of the structural elements. Intraclonally, values ranged from 9.45 to 13.09% and averaged 12.29%. Estimates of dry matter digestibility derived from the summative equation of Robbins et al. (1987*a,b*) averaged 51.98%, with an interclonal range of 45.89 to 56.16%. Significant differences ($P < 0.05$) were found between clones for all fiber constituents.

3.3.4 Environmental differences in chemical composition

When the results of the chemical assay were partitioned and examined by environment, differences ($P \leq 0.05$) were found for crude protein, K, Ca, and Mg as well as lignin and ash/cutin (Table 3.6). Clones in the Prairie environment had significantly ($P \leq 0.01$) higher concentrations of K, Ca, and ash/cutin than the other two environments (Tables 3.6 and

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Table 3.5. Fibre composition in dormant current-year twigs from 24 clones of *P. tremuloides*. The mean and standard errors (in parentheses) are based on five replicates per clone. Measured as percent dry matter.

Clone	NDF	ADF	Cellulose	Ratio Lignin	Ash	Ratio Lignin: Cellulose	Ratio Lignin: ADF	Dry Matter Digestibility
Prairie								
3	43.83 (0.95)	31.30 (0.23)	18.75 (0.30)	12.56 (0.22)	6.41 (0.42)	.671	.401	48.82 (0.575)
6	42.82 (0.83)	30.75 (0.95)	19.84 (0.68)	10.91 (0.41)	4.49 (0.07)	.551	.355	51.91 (0.616)
10	45.72 (1.06)	32.02 (0.97)	18.93 (0.65)	13.09 (0.57)	3.37 (0.17)	.694	.409	48.35 (1.165)
11	44.60 (0.92)	33.39 (0.52)	22.01 (0.51)	11.38 (0.27)	3.79 (0.28)	.518	.341	53.54 (0.510)
15	40.65 (0.77)	29.54 (0.36)	17.43 (0.53)	12.11 (0.19)	5.39 (0.05)	.698	.410	50.60 (0.439)
53	50.83 (1.17)	37.37 (0.94)	25.77 (0.75)	11.60 (0.29)	3.17 (0.12)	.451	.311	53.73 (0.507)
74	43.30 (1.22)	30.18 (0.80)	20.53 (1.19)	9.65 (0.46)	4.04 (0.14)	.481	.322	54.67 (0.473)
95	47.03 (2.05)	34.28 (1.46)	21.99 (1.15)	12.29 (0.36)	3.74 (0.40)	.562	.359	46.45 (0.598)
Akamina								
23	50.65 (0.86)	35.77 (0.83)	23.77 (0.69)	12.00 (0.29)	3.40 (0.15)	.506	.336	52.36 (1.262)
31	45.24 (1.03)	32.79 (0.73)	21.94 (0.68)	10.85 (0.14)	3.52 (0.11)	.496	.331	54.18 (0.833)
32	43.16 (0.77)	30.20 (0.33)	19.75 (0.48)	10.45 (0.25)	3.32 (0.08)	.531	.346	50.03 (1.692)
35	47.20 (1.03)	33.65 (1.29)	22.42 (1.10)	11.23 (0.29)	2.98 (0.14)	.504	.335	56.16 (0.913)
36	39.48 (0.73)	28.32 (0.69)	17.93 (0.55)	10.39 (0.22)	3.26 (0.07)	.581	.367	52.58 (0.824)
42	49.34 (1.49)	35.50 (0.98)	23.76 (0.74)	11.75 (0.29)	3.07 (0.19)	.495	.331	50.91 (0.754)
43	43.37 (0.67)	30.62 (0.61)	21.17 (0.54)	9.45 (0.40)	4.89 (0.14)	.448	.309	49.19 (1.955)
44	47.55 (0.48)	34.81 (0.57)	23.69 (0.47)	11.12 (0.42)	4.27 (0.28)	.470	.319	53.98 (0.563)
Galwey								
59	48.21 (1.13)	35.24 (1.35)	23.93 (1.11)	11.30 (0.33)	3.83 (0.26)	.475	.322	50.29 (1.320)
111	45.00 (0.46)	31.37 (0.41)	20.47 (0.54)	10.90 (0.23)	3.20 (0.15)	.535	.348	49.48 (0.614)
112	47.30 (2.21)	33.43 (1.51)	20.58 (1.04)	12.85 (0.63)	3.39 (0.17)	.626	.385	45.89 (2.259)
117	45.46 (1.59)	30.88 (1.17)	20.96 (0.77)	9.92 (0.65)	3.65 (0.10)	.474	.321	52.99 (0.437)
121	45.46 (1.63)	31.06 (1.24)	20.09 (1.41)	10.97 (0.22)	3.24 (0.16)	.558	.356	48.92 (1.213)
124	52.81 (0.80)	36.48 (0.33)	24.85 (0.40)	11.63 (0.14)	2.77 (0.17)	.469	.319	47.23 (1.658)
126	47.44 (1.37)	33.17 (0.86)	20.95 (0.54)	12.22 (0.46)	2.67 (0.07)	.583	.368	47.58 (0.832)
129	41.69 (0.21)	29.29 (0.81)	19.04 (0.80)	10.25 (0.26)	3.34 (0.15)	.543	.351	53.65 (0.471)
Grand mean	45.76	32.56	21.27	11.29	3.69	.538	.348	53.00
F	7.97***	7.94***	7.87***	7.29***	15.41***			6.26***

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 3.6. Chemical concentrations in dormant current-year twigs from 24 clones of *Populus tremuloides* in three environments within Waterton Lakes National Park, Alberta. Mean ± SE (in parentheses) are given. Based on composite samples from 8 clones per environment and 5 ramets per clone.

Chemical constituent	Prairie	Galwey	Akamina	F
		Percent		
NDF	45.00(0.583)*a*	46.52(0.663)*a*	45.75(0.625)*a*	1.51
ADF	32.47(0.485)*a*	32.50(0.482)*a*	32.71(0.484)*a*	0.71
Cellulose	20.90(0.490)*a*	21.12(0.385)*a*	21.80(0.385)*a*	1.24
Lignin	11.58(0.196)*a*	11.38(0.204)*ab*	10.91(0.155)*b*	3.41*
Ash/Cutin	4.26(0.172)*b*	3.26(0.084)*a*	3.59(0.111)*a*	16.59***
DMD[1]	51.07(0.416)	49.22(0.634)	52.80(0.545)	10.10***
Crude protein	6.25(0.025)*a*	6.88(0.019)*b*	5.75(0.017)*a*	17.09***
P	0.16(0.005)*a*	0.17(0.004)*a*	0.17(0.004)*a*	1.09
K	0.72(0.017)*b*	0.66(0.008)*a*	0.68(0.011)*a*	7.18**
Ca	1.16(0.076)*a*	0.73(0.028)*b*	0.73(0.038)*b*	16.40***
Mg	0.17(0.006)*ab*	0.17(0.004)*a*	0.21(0.004)*b*	18.26***
		Parts per million		
Mn	28.6(2.08)*a*	25.1(1.35)*a*	26.8(2.29)*a*	0.68
Fe	72.6(8.27)*a*	84.7(14.60)*a*	54.5(5.04)*a*	2.58
Zn	89.3(3.79)*a*	92.7(3.66)*a*	79.3(4.50)*a*	2.93

NOTE: All weights are dry weights. Means with common letter in each row do not differ significantly ($P > 0.05$; Scheffe's Test) between environments.

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

[1] Dry matter digestibility for black-tailed and white-tailed deer as estimated by the method of Robbins et al. (1987*b*)

3.7). Twigs from the Prairie clones were more lignified ($P \leq 0.05$) than the Akamina clones (Table 3.6 and 3.7). Clones in the high elevation environment (Galwey) had, on average, more crude protein ($P < 0.001$) than the Prairie and Akamina sites (Table 3.6). Magnesium was significantly higher ($P < 0.001$) in the Akamina site relative to the other two sites. There were no differences ($P > 0.05$) in the concentrations of P or the trace elements between environments (Table 3.7). Apparent dry matter digestibility was significantly ($P < 0.001$) higher in the Akamina site relative to the Galwey site (Table 3.6).

3.3.5 Differences in chemical composition within clones within environments

There was considerable variation between environment (Table 3.7). The greatest amount of within-environment variability was found at the Prairie site where there were significant interclonal differences for all chemical constituents ($P < 0.001$). Differences, albeit not as pronounced, were also found for most constituents in the Akamina environment. The Galwey site exhibited the least amount of within-site clonal variability (Table 3.6). Here, there were no differences for crude protein or K and where statistical differences did arise, the relative concentrations tended not to vary to the extent found in the other two environments.

3.3.6 Correlative patterns between browse use and plant chemistry

There was no relationship (simple linear regression and Kendall's rank correlation; $P>0.05$) between percent browse use and crude protein, phosphorus, cell-wall constituents, or DMD for the Prairie site. Regression of crude protein against browse use was not undertaken for the Galwey site because of greater within-clone to between-clone

Table 3.7. Analysis of variance for differences in chemical concentrations in dormant current-year twigs from 8 clones of *Populus tremuloides* in each of three environments. Based on composite samples from 5 ramets per clone and 8 clones per environment.

Chemical constituent	Prairie	Galwey	Akamina
NDF	10.07***	4.64***	15.65***
ADF	10.30***	4.66***	11.66***
Cellulose	13.58***	3.74**	9.62***
Lignin	8.44***	6.37***	7.32***
Ash/Cutin	21.12***	4.08***	14.66***
Dry matter digestibility[1]	6.10***	3.11***	7.49***
Crude protein	19.50***	2.04	13.60***
P	8.51***	2.77*	11.99***
K	4.88***	1.49	8.62***
Ca	18.64***	5.08**	12.39***
Mg	7.07***	13.49***	5.54***
Mn	21.15***	14.35***	5.34**
Fe	4.45***	2.80*	1.81
Zn	10.42***	11.29***	14.56***

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

[1] Dry matter digestibility for black-tailed and white-tailed deer as estimated by the method of Robbins et al. (1987*b*)

variability. There was no relationship ($P>0.05$) between browsing and the other dietary constituents (phosphorus, cell-wall constituents, or DMD) at the Galwey site.

3.4 DISCUSSION

3.4.1 Browse use and chemical composition

Considerable variabililty in browse use was found between the Prairie and Galwey study sites. The low rate of browse use at the low-elevation Prairie site, which is utilized predominantly by elk, is consonant with the classification of elk as intermediate feeders tending to graze rather than browse (Hobbs et al. 1983; Kasworm et al. 1984). In nearby west-central Alberta, Morgantini and Hudson (1985, 1989) found that grasses dominated (>90%) the winter diet of elk. They also found that grass species tended to be high in digestible energy but comparatively low in protein (ca. 3.5%) during winter (Morgantini and Hudson 1985). In Colorado, by contrast, elk shifted their diet to woody browse as winter advanced in response to lowering nitrogen content of grasses (Hobbs et al. 1981). Morgantini and Hudson (1985) suggested that the disparity between their results and those of Hobbs et al. (1981) might be owing to comparatively greater digestibility of grasses used by elk in west-central Alberta. Waterton and Banff have similar grassland communities (Kuchar 1973) which may therefore account for the low rate of browse use by elk in WLNP. More detailed diet assessment requires knowledge of composition and quality of the total diet.

For wintering elk, it is thought that diets must contain 5-7% crude protein to meet maintenance requirements (Mould and Robbins 1981; Hobbs et al. 1981). Only two clones fell outside this range, one each below and above the 5 and 7% values. The average ($\bar{x} =$ 6.25%) is higher than that cited above for grasses. That elk do not appear to browse to a greater extent on comparatively high-protein *P. tremuloides* may signal that an alternative source of protein is available, or perhaps they rely on the synthesis of amino acids and protein by urea recycling (Robbins 1983), though this cannot substitute for maintenance requirements. Additionally, elk may compensate for protein deficiencies encountered in

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winter by maximizing, to some degree, protein intake in summer (Morgantini and Hudson 1989).

Increased use of aspen browse at the Galwey site, used almost exclusively by mule deer, is consistent with their food habits in that mule deer tend to browse more than elk (Hobbs et al. 1983; Kasworm et al. 1984). Kasworm et al. (1984) found that *Populus* spp. constituted 9.8% of the winter diet of mule deer in nearby Montana. The crude protein requirements for wintering adult mule deer are believed to range between 6% and 9% (the level at which efficient rumen bacterial digestion takes place (Van Soest 1982; Robbins 1983). All clones at this site satisfied the lower limit of this requirement though none approached the upper limit. Since much of the mule deer winter diet falls below the 6% threshold (Short et al. 1966; Hobbs et al. 1983), aspen twigs may constitute an important source of protein.

It is unlikely that current browse use is directly affected by historical patterns of utilization. In all probability, the use of aspen would fluctuate over time, and as such, it is conceivable that ungulates could have overbrowsed the nutritionally-superior clones and now have no alternative but to browse the nutritionally-inferior clones. However, the populations of elk and mule deer have remained stable for several decades (Parks Canada 1984), and thus an equilibrium in browse availability should have been reached. Elk populations have fluctuated from an estimated high of 1500 in 1947 to 325 in 1949, but populations have stabilized around 600-700 animals since 1963. The size of the mule deer population over the last few decades is poorly known. Populations were thought to number 1000-2000 animals in the 1940's (Banfield 1947 in Parks Canada 1984). Their numbers declined and have remained around 350 since the mid 1950's. This argument assumes that year-to-year variability is not extreme. Relative to domestic crop species, nutrient concentrations of wild plants are not particularly sensitive to annual variations in

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nutrient availability and local environmental conditions (Chapin 1980). Any change in the pattern of browse use would then be in response to factors such as alternative forage availability and snow cover. So, while ungulates may increase browse use during periods of increased snow cover, it is thought that these conditions would be highly transient because these sites are wind swept and subject to frequent chinooks.

Minimum reported P requirements for mule deer vary between 0.20 and 0.35% (French et al. 1956; Ullrey et al. 1975; Verme and Ullrey 1972; Short 1981); only one clone of the total sample had a P content > 0.20%. P requirements for elk are not known but are likely similar to those for deer. Similarly, by NRC (1976) standards, 12 of the 24 clones barely met the minimum P requirement for beef cattle, while the balance did not (Table 3.1). However, for a winter forage the values are probably sufficient given that the animals can likely call upon P reserves accumulated during summer (Hanley and McKendrick 1985).

According to Robbins (1983), white-tailed deer (*O. virginianus*) require 0.40-0.64% Ca, in which case the mean concentration of all but one clone exceeds Ca requirements. Most clones and ramets satisfied minimum Ca concentrations for cattle also. Since the Ca content of grasses is often below animal nutritional requirements (Van Soest 1982), aspen may provide an important supplementary source. There were, however, departures from the ideal 1:1 or 2:1 Ca:P ratio that is best for proper absorption and metabolism of Ca. Other studies of Ca and P in aspen have also noted departures from the optimal ratio. McNamara (1980) reported an average Ca:P ratio of 6.2:1 in three clones respectively, while Masslich et al. (1988) found Ca:P ratios in twigs of mature aspen to average 11.6:1. In general, however, Ca excesses have comparatively little effect on P absorption, as opposed to excesses of P on Ca absorption (Robbins 1983); thus, the average (5.6:1) noted in this study is unlikely to have any significant deleterious effects. Further, some calcium

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may be tied up as insoluble calcium oxalate, thus lowering the effective Ca:P ratio (Harbers et al. 1980). That high ratios can be accommodated by ruminants is suggested by their extensive use of alfalfa (Robbins 1983). Notwithstanding this, ratios greater than 7:1-10:1 are generally accepted as being sufficiently high to reduce availability of P. Thus the high Ca:P ratios noted for four clones, particularly clones 3 and 15, could have potentially serious consequences on P metabolism if twigs from such clones were to constitute a large part of the diet.

Magnesium and K requirements for deer and elk are poorly known. By NRC standards (1976) there was sufficient Mg in all clones for growing and finishing heifers and steers, and dry pregnant cows. Half (12) of the clones just met the minimum requirements for bulls. There were no clones with excess Mg. Twigs from most clones satisfied NRC (1976) requirements for K. Two clones (3 and 53) slightly exceeded the upper limits of the nutritional requirements for beef cattle, and one clone (42) was just under the lower boundary. Neither K nor Mg deficiencies are common in free-ranging wildife (Robbins 1983).

Grimshaw and Allen (1987) pointed out that because microelements in plant tissues are generally present in low concentrations, extraneous influences in both the field and lab often make accurate estimates rather problematic. Notwithstanding, the trace elements, especially Mn, appeared to exceed minimum nutritional requirements. Manganese toxicity at the levels recorded in this study is unlikely since much higher levels have been found in dairy cattle with no known serious adverse effects (Miller et al. 1972). Similarly, the concentrations of Zn and Fe are not believed to be detrimental. While Cu was not precisely determined, it is no more than 10.2 ppm, except for two clones. It is concluded, therefore, that there is little potential for copper toxicity. Copper deficiencies are possible however. Kubota et al. (1970) reported that Cu concentrations in *P. tremuloides* twigs average 6.5

ppm. While beef cattle require about 4 ppm (Table 3.1), elk seem to have unusually high requirements (Freudenberger et al. 1987; Gogan et al. 1989). If other elements are balanced, 10-15 ppm is probably sufficient for wintering elk (R.J. Hudson, *personal communication*). Since grasses average 5 ppm (NRC 1977 in Gogan et al. 1989), and the values reported herein might fall well below the minimum requirement, there clearly exists the potential for a copper deficiency. Sodium was not precisely determined but even the maximum estimates (0.005%) fall well below basic requirements (Table 3.1). Sodium deficiencies are common in wildlife foods and animals must either conserve ingested sodium or locate supplementary sources such as mineral licks (Robbins 1983). Overall, the importance of trace elements in wildlife nutrition is poorly understood (Robbins 1983).

3.4.2 Structural constituents

Neutral detergent fiber (NDF) consists mainly of the total cell wall fraction which contains hemicellulose, cellulose, lignin and cutin. The most digestible twigs are those with the lowest NDF content (i.e., cell solubles = 1-NDF) (e.g., clones 15 and 36). Increasing NDF is positively related to increased particle retention times (Fig. 3.6; Spalinger et al. 1986) but the relationship is asymptotic and the values (i.e., interclonal range) reported herein are thus unlikely to have a substantial affect on rumination time. Twigs from clones in WLNP had lower NDF fractions ($\bar{x}$ = 46%) than those reported by Schwartz et al. (1988) in Alaska who found that NDF fractions in winter aspen twigs averaged 54.9%. Mean NDF for most clones in this study was less than the average (53%) for four species of browse used by elk in Colorado over two years (Hobbs et al. 1981). However, some caution need be exercised in making such interspecific comparisons because NDF digestibility depends on the lignin content of NDF (Robbins et al. 1987b). Notwithstanding, Baker and Hobbs (1987) showed that elk are superior to mule deer in

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their ability to digest NDF. This reflects the former's preference for graminoids with highly digestible cell walls (Hobbs et al. 1983).

Cellulose is the predominant carbohydrate in higher plants. Cellulose digestion is partially dependent upon the degree of lignification. As such, lignin:cellulose ratios are sometimes calculated as another measure of the amount and type of fiber (Van Soest 1982). Low ratios are desired. For example, some clones (e.g., clones 53 and 112) had a relatively high cellulose component (Table 3.6). Accordingly, clone 53 has a ratio of 0.45:1, whereas clone 112 was 0.63:1; thus clone 53 is superior with respect to cellulose digestibility. Overall, ratios ranged from 0.448:1 to 0.698:1 and averaged 0.538:1.

Cellulose digestion is maximized when it is retained in the rumen for sufficient time to complete digestion. Compared to elk, mule deer retain a given particle for a shorter time period (Baker and Hobbs 1987). Accordingly, mule deer tend to select diets with a high proportion of cell solubles, but sometimes with low NDF digestibility (Hobbs et al. 1983). Elk, on the other hand, have longer particulate retention times and are more efficient at digesting cell walls (Baker and Hobbs 1982). Therefore, if all other forage elements were equal, clones high in cellulose (e.g., clone 53) would be, nutritionally speaking, more appropriate for elk than mule deer while clones such as clone 36 are more suitable for mule deer, because they are characterized by higher concentrations of cell solubles.

Acid detergent fiber (ADF) is a preparative residue for the determination of cellulose, Maillard products (heat-damaged proteins), lignin, and biogenic silica (Van Soest 1982). ADF in itself has no theoretical basis for food selectivity; rather, some investigators (e.g., Klein 1977; Cooper and Owen-Smith 1985; Schwartz et al. 1988) use it as a predictor of digestibility because it reflects fiber content. Both the mean concentration and range of concentrations found in this study ($\bar{x}$ = 32.6%; range 28.3-37.4%) are below the average

values of 41.2% and 40.1% that were measured by Klein (1977) and Schwartz et al. (1988), respectively.

As an alternative method of estimating fiber content, Van Soest (1982) compared the ratio of lignin to ADF. Lignin consists of polyphenols that are highly polymerized thus providing structural rigidity for the plant. Lignin is virtually indigestible because it is resistant to normal enzymatic and acid hydrolysis and is most commonly associated with the reduced digestibility of fiber (Van Soest 1982). Thus high lignin:ADF ratios reflect increased lignification. Lignin also interferes with cellulose, and especially hemicellulose digestibility (Smith et al.1971, 1972; Robbins and Moen 1975; Jung 1989). In this study, lignin:ADF ratios varied from 0.309:1 to 0.410:1 ($\bar{x}$ = 0.348:1). The data of Klein (1977) yielded a high ratio, 0.636:1, while that of Schwartz et al. (1988) yielded a low ratio, 0.262:1.

Increased lignification results in forage particles becoming more resistant to comminution and increases particulate retention time (Spalinger et al. 1986; Baker and Hobbs 1987). The values found in this study are about 2-3 times higher than the mean lignin concentration reported for grasses [$\bar{x}$ = 4.4-5.1% (Hobbs et al. 1981)]. However, the range of values (9.5-13.1%) is well above the 5% threshold (Fig. 4 in Spalinger et al. 1986), the point at which increased lignification does not result in significantly higher retention times. Mule deer can partially compensate for increased forage retention time by increasing gut fill which allows for constant intake of digestible energy, despite lower digestive efficiency (Baker and Hobbs 1987). This is in contrast to large-bodied foragers (e.g., elk) that tend to fill to capacity (Short et al. 1965; Hofmann 1989).

3.4.3 Dry matter digestibility

Arguably, the most important factor in the nutritional value of winter forage is dry matter digestibility. *In vitro* digestion trials were not part of the experimental protocol in this study because of insufficient material on the sampled ramets. Traditionally, most *in vitro* analyses use domestic ruminants as a model for simulating forage digestion. However, cell wall digestion varies between grazing and browsing ruminants which complicates studies of diet selection and evaluation (Cooper and Owen-Smith 1985; Palo 1985; Palo et al. 1985). Ruminants accustomed to high tanniferous diets are less affected by tannins than predominant grazers (Robbins et al. 1987*b* and references therein). For this reason, Robbins et al. (1987*b*) advocate the use of summative equations, and accordingly, this procedure was adopted by this study.

Overall digestibility was high among clones. Inter-clonal differences were found and these could variously impact on the energy balance of foraging ungulates if sizable differentials occurred in the relative use of these clones. However, most clones meet the 50% minimum requirement for maintenance (Amman et al. 1973). That browse represents an important source of energy is clear from Hobbs' (1989) model of energy balance for mule deer which predicted that during a severe winter each percentage point decrease in the digestibility of shrubs (in the model the levels of shrub digestibility ranged from 25-35%) decreases doe mortality by 10%. Empirical field data validated this prediction (Hobbs 1989). When the values found in this study are compared to composite grass samples on elk range in west-central Alberta that average 67-68% (Morgantini and Hudson 1985), it can be seen why elk browsing of *P. tremuloides* is low. This is in contrast to the digestibility of grasses in Colorado which averaged 44-45%, and where there was concomitant increased use of shrubs (Hobbs et al. 1981). The variation between these two studies might reflect the species' composition for each site, respectively, that were used to

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generate the pooled estimates. Schwartz et al. (1988) found *in vitro* digestibility of aspen terminal shoots to be 42% (SD = 1.1%). Bergstom and Danell (1987) determined *in vitro* digestibility for 4, 5 and 6 mm diameter-long shoots from *P. tremula* to average 41% irrespective of twig diameter. Similarly, Bergstom and Danell (1987) reported that *in vitro* digestibility of elongated 4-6 mm shoots of *P. tremula* averaged 41%. Miquelle and Van Ballenberghe (1989) reported a value of 66.5%, though this was based on one sample only. Renecker and Hudson (1988) used the nylon bag technique to estimate digestibility and found that winter *P. tremuloides* twigs averaged 44.9%. *P. tremuloides* has higher digestibilities than most other browse species (Klein 1977; Hobbs et al. 1981; Morgantini and Hudson 1985; Palo 1985; Risenhoover 1989).

3.4.4   Variation between and within sites

Chemical content varied little with respect to environment. Relative to domestic crop species, nutrient concentrations of wild plants are not particularly sensitive to annual variations in nutrient availability and local environment conditions (Chapin 1980). The only major exception where there could be biological implications is the Prairie site. Here, Ca concentration was about 64% higher than the other two sites which may reflect the high concentrations of exchangeable Ca cations typically found on chernozemic soils. Where other environmental differences exist, they are not considered to be of significant biological importance.

The greater variability in chemical composition within sites relative to between sites is also consistent with the results of the genotypic analysis (Chapter 1). Genetic diversity was much higher within sites than between sites and may be a significant control for the observed pattern of chemical variation. The vast majority (144) of the 156 clones electrophoretically analyzed at 14 polymorphic loci revealed little clonal duplication. The

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genetic uniqueness among clones is also consistent with the results of the chemical assay in that most clones differ from one another irrespective of environment.

The reason for intraclonal variation may be due to slight differences in local site conditions such as water availability or nutrient conditions. It has also been suggested that leaves and branches, for example, may vary spatially and temporally in terms of defensive compounds, nutritional quality and vulnerability to disease because of somatic mutation (Whitham and Slobodchikoff 1981). The accumulation of a mosaic of mutations may be particularly advantageous in defending against herbivores and pathogens because predators and parasites would have a difficult time predicting vulnerable tissues and hence in evolving specialized adaptations (Whitham and Slobodchikoff 1981; Whitham et al. 1984).

3.4.5 Possible impact of herbivory on the ecology and evolution of *P. tremuloides*

Clones at the Galwey site are probably the most sensitive to the effects of browsing for two reasons. First, browsing in this environment was 2-5 times higher than that found in the Prairie environment and second, growth in this short growing season site is slower than that found in the Prairie site. In the previous chapter, it was shown that average growth of clones at this site was only 67% of that attained by the Prairie populations. As for the Akamina site, Both deer and *P. tremuloides* are sparse in the successionally-advanced region of the Park. Nevertheless, twigs from clones in these regions are be nutritionally comparable to clones in the other sites. Though Mg concentrations were higher here, the mean difference (0.04%) would be of negligible biological importance.

Vertical growth of twigs and branches allows the tree to escape from the high-risk browsing zone. When twigs are browsed, however, the branch dies back to the preceding

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annual growth node. Not only does browsing thus curtail growth, but continued and intense herbivory depletes carbohydrate reserves and results in stand deterioration (Sampson 1919; DeByle 1980; Olmsted 1980; Weinstein 1980; and see review in DeByle 1985). Unfortunately, the exact levels at which this occurs is poorly known. Olmsted (1980) estimated (on the basis of limited data) that at least 70% of current annual growth has to remain unbrowsed for stand perpetuation. Studies of simulated browsing on other woody plants also indicate that production increases until browsing is greater than 25-40% of current year's growth (Lay 1965; Willard and McKell 1978; Oldemeyer 1981 in Danell and Bergstrom 1989; Danell and Bergstrom 1989), though Danell and Bergstrom (1989) found that one species of birch (*Betula pendula*) maintained browse production even when >75% of twigs were removed. If, however, we assume a 25-40% threshold for *P. tremuloides*, the levels of browsing noted at the Galwey site may result in clone deterioration. On the other hand, more moderate levels of browsing (< 20%) may actually stimulate production.

Browsing may also control the architecture of ramets and clones. Browsing can break apical dominancy which then stimulates the production of adventitious shoots (Schier 1973, 1978). The extent of sucker production depends on the levels of carbohydrates (Tew 1970; Schier and Johnston 1971), hormonal growth promoters (see review Schier et al. 1985), and genetic factors (Schier 1974, 1981; Schier and Campbell 1980). Low or moderate levels of browsing may actually enhance vegetative reproduction as well as promote clonal expansion (Jelinski and Hutchinson 1988). Apical dominance is so strong in some clones, however, that vegetative regeneration is sparse or nonexistent (Schier 1975). Clones thus characterized are at an ecological and evolutionary disadvantage if browsing is severe. Paradoxically, heavily browsed trees often assume a shrub-like architecture. These effects have been noted in *P. tremuloides* browsed by elk in Colorado (Olmsted 1980), as well as

cottonwoods (*Populus fremontii*) (McGinley and Whitham 1985) and pinyon pine (*Pinus edulis*) (Whitham and Mopper 1985). Adoption of this shrub-like growth form renders a greater proportion of biomass available to the browsers, and may initiate a positive feedback between browse availability and intensity until the ramet's resources are exhausted. Ramets that revert back to the juvenile growth form also run the increased risk of being over-topped by both intra- and interspecific competitors. Last, reversion to a shrub-like form renders the plant more susceptible to breakage by snow (*personal observation*).

No clones stood out as being particularly high in crude protein, or phosphorus, two major and often limiting elements in herbivore nutrition (Sinclair 1975; Lindroth 1979; Belovsky 1981; Hanley and McKendrick 1983; Hobbs 1989). The absence of chemical responsiveness may indicate that vertebrate herbivores have little affect on the genetic architecture of *P. tremuloides*. Inherently rapid vertical growth (Jones and Schier 1985) permits escape from the high risk browsing zone. Also, the propensity to spread risk through ramet duplication (Cook 1979, 1983) and physiological connection (DeByle 1964; Tew et al. 1969) may permit browsed ramets to draw upon the resources of unbrowsed ramets. However, vegetative growth takes place at the expense of increased vertical growth (Sakai and Burris 1985). As such, while the risk of genet death may decrease, individual shoot mortality may increase because more biomass is within reach of browsing animals. Since it is several years before significant root development and vegetative growth take place (Zahner and DeByle 1965), the probability of death due to browsing during the early stages of clone development is likely high. In general, plant seedlings are most vulnerable to a range of selective pressures and random events during the early stages of development (Harper and White 1974; Harper 1977), and the specific hazards posed by herbivores are probably most extreme during a genet's first few years of life when a large proportion of

biomass is within reach of browsers (Klein 1977). If highly nutritious genotypes are produced, browsers may identify and eliminate them during the early stages of clone development.

3.5 SUMMARY AND CONCLUSIONS

The data were consistent with the hypothesis that clonal differences exist in the chemical composition of *P. tremuloides* twigs. That genetics plays an important role in affecting plant chemistry, albeit one not directly tested, is suggested given the general pattern of low intra clonal variation in chemical composition within each environment but high variability between clones within each environment. Clark (1983) reviewed the literature on genotype-plant chemistry variations and concluded that considerable genotypic differences exist in the uptake, translocation, accumulation, and use of mineral elements. The hypothesis that nutrionally-superior clones are browsed to a greater extent than inferior clones, however, was rejected. It may be that the differences between clones, albeit statistically significant, are not physiologically detectable. Other factors such as distance to thermal cover, microgeographic variation in slope, and disturbance were not controlled for but may be critical in browse selection. To control for variation in residency time and twig availability, cafeteria-type food trials (e.g., Bergstrom and Danell 1987), where twigs of all genotypes are equally available and offered in the absence of alternative foods, might be useful in this regard. However, even if differences are detectable, I suspect that the time spent searching for the nutritional best twig would not be cost effective in terms of nutritional gain. Notwithstanding, there were clones that would be preferable as sources of browse and deer or elk may benefit by fortuitously browsing on these clones. Vertebrate herbivores are postulated to have a profound influence on the plant population's genetic architecture during the initial stages of development. Once *P. tremuloides* clones are established, physiological connections permit spreading of risk and hence reduce the probability of death due to browsing.

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EPILOGUE

Ecological Genetics and the Biogeography of *Populus tremuloides*

Summary

There is widespread belief among both ecologists and population geneticists that population recruitment of *P. tremuloides* in the western interior has effectively ceased. Support for this contention comes from studies that show the conditions necessary for germination and seedling establishment of *P. tremuloides* are both extremely demanding and exceedingly rare. Since successful sexual recruitment is a rare event for this species, I predicted that populations in a heterogeneous environment that have experienced considerable temporal environmental change since initial colonization should be comprised of a suite of genetically restricted individuals and spatially-variable selection pressures should generate spatially discrete populations. I found, contrary to the first hypothesis, that there exists a large pool of genetic and genotypic diversity, both within and between populations. Several explanations were proposed to account for the existence of such a high degree of polymorphism including neutrality of the allozymes, insufficient time for evolutionary equilibrium, various types of selection, somatic mutation, and differential herbivory. As well, it was suggested that the basic premise upon which this prediction was founded, the absence of sexual reproduction, might be relaxed on occasion, in which case gene flow would then be a contributing factor. It is virtually impossible to unravel the relative contribution of each of these factors. I was, however, able to partially test for balancing selection due to heterozygote superiority by examination of allele frequencies in relation to a surrogate measure of fitness, growth rate. Increased growth was correlated with increased heterozygosity and I concluded that heterozygotes have some selective

advanatage. Further, there was positive correlation between growth and four individual loci, three of which occupy positions of regulatory potential.

I also considered the potential effect of vertebrate browsing as a disruptive selective force. More specifically, I hypothesized that polymorphism in chemical composition might lead to variation in browsing rates. While substantial differences were found in browsing rates, the results of extensive chemical analysis indicated that there is little evidence to suggest that mule deer or elk would be selective in their choice of which clones to browse. Thus, it was concluded that herbivore impacts on the genetic structure of the populations are random with respect to chemical composition.

Consistent with the second hypothesis there was significant differentiation, albeit a small amount, between *P. tremuloides* populations in these diverse montane and pre-montane habitats. It is unlikely that local populations are inbred because the species is self-incompatable and exhibits long distance, wind-mediated, dispersal of pollen and seeds. Thus it was concluded from the analysis of population structure that some type of selection has taken place. It was recognized that it is impossible to determine the precise contribution of a particular evolutionary force to a particular pattern of variation among natural populations solely from the pattern. Unfortunately, failure in growing plantlets for reciprocal and common garden experiments precluded more direct assessment of selection and the presence of locally adapted populations.

Despite some evidence for selection, it became apparent that this evolutionary force is not as effective as it might be in aclonal populations because *P. tremuloides'* clonal growth habit both maintains genetic diversity and renders populations comparatively immune to structuring. From a spatial point of view, iterative and physiologically-integrated modular growth, as manifest through the phalanx growth strategy, allows clones to adjust to fine-scale environmental variability through sharing of water (and perhaps ions and organic

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materials), and hence, to occupy otherwise unfavorable or suboptimal habitat and achieve considerable size. Similarly, during periods of moderate climatic challenge, say drought, perhaps only radial growth or vegetative reproduction is affected, and not ramet survivorship. When faced with severe or protracted environmental challenge, some ramets may die and the clone contracts, but if the number of ramets is high, and mortality selective, the integrity of the genet is preserved. Upon arrival of more favorable conditions these genotypes can recover through vegetative reproduction and resume normal rates of sexual reproduction. The net effect is a diverse array of persistent old clones.

While plasticity *per se* could not be examined, the growth rates in a wide variety of multilocus genotypes suggests that the populations are composed of uniformly robust clones. Thus, it appears that the species is both genetically variable and plastic, at least in terms of this important metric character. It also follows that if clones are as ancient as widely thought, and as resilient as argued in this study, then the clones that have survived over the past several thousand years have done so by virtue of being able to tolerate fluctuating environmental conditions or by accumulating adaptive somatic mutations.

Evolutionary Consequences

Although sexual reproduction is thought to be rare, there is little evidence to suggest that the species is at any particular disadvantage in terms of decay of genetic variation. the source of this diversity remains to be investigated but somatic mutation may be important (Whitham and Slobodchikoff 1981). Regardless, the species does not appear to be in the sort of evolutionary blind alley as envisaged for species in which sexual reproduction has ceased (Darlington 1939; Stebbins 1950; De Wet and Stalker 1974). On the contrary, this study has shown that there exists a large storehouse of cryptic genetic material that may be

adaptively advantageous and can be used by recombinants when environmental conditions are conducive to seed germination and establishment.

Although decadal periods of climatic variation likely affect reproductive and establishment rates, most forest tree species are capable, within limits, of making phenotypic adjustments to longer-term (e.g., centennial) climatic variation by altering physiological and morphological characters. Hostile conditions which last longer than the life span of most tree species thus will result in major displacements of the species range. *Populus tremuloides*, however, can persist through such periods of longer-term climatic change by vegetative propagation, and consequently, suffer little or no appreciable change in areal range. Even when conditions change to favor competitors, *P. tremuloides'* propensity to form large, dense stands of physiologically-integrated ramets can lead to both spatial and temporal domination of the landscape.

These considerations have important implications for explaining the distributional limits of *P. tremuloides* on regional and transcontinental scales. The large pool of genetic material, outcrossing and wind dispersal of pollen and seeds generates a vast array of recombinants and also ensures extensive sampling of habitats beyond the species' extant range. Among the large number of multilocus genotypes there likely exist novel genotypes, preadapted to new habitats. Meanwhile, asexual reproduction permits extensive colonization and monopolization of the parental environment, a feature maximized by the phalanx growth strategy. When habitat conditions become more challenging, physiological integration among clones buffers these effects and spreads the risk of genet death and produces, concomitantly, little or no range retreat. Given new ecological opportunity, sexual reproduction again commences and once more challenges the existing range limits. Successive cycles of invasion, colonization and stabilization thus gradually facilitate range extension. Further, heterozygosity might be implicated in fine-scale adaptation and

correlate with temperature gradients along the northern range limits of this species, where conditions are presumably stressful (see also Chapters 2 and 3). It was interesting to note in this regard that the two coldest sites, Akamina and Galwey were characterized by populations with particularly high amounts of heterozygosity.

Several other authors have recently suggested that vegetative reproduction has important genetic and evolutionary consequences (Ellstrand and Roose 1987; Gliddon et al. 1987; Stevens and van Damme 1988). Even John Harper, one of the most influential population biologists of the century, who vigorously argued that ramet production is not a form of reproduction (Harper 1977), recently stated that "the concept of reproductive value has to be reexamined for all modular organisms" (Harper 1985:30). He pointed out that reproduction may increase exponentially throughout the lifetime of the genet and that genet death usually comes through environmental challenge, not the programmed senescence that is typical of aclonal species.

Future Research Directions

There are a number of advantages to studying questions of evolutionary significance to biogeography using clonal species. First, there is no reshuffling of the genome, save that due to mutation; this makes for a less confounding set of conditions for studying the significance of genetic variation. The effects of selection might also be more easily detectable when manifest over centuries or millennia rather than over a few generations. Second, ramets from clones provide replicates which are useful for field and laboratory experimentation. Third, in the absence of recombination, individuals can simply be expressed as a function of the number of clones present. Last, since clones may carry a genetic memory of past environmental conditions, their date of establishment might also provide a window into the particular environmental regimen under which recruitment took

place. Conversely, the disadvantages of working with clones, as shown in this study, are that it is difficult to age them and physiological integration can buffer the effects of selection. Thus, in some ways, they are poor models for examining plant-habitat relationships.

Our current understanding of the factors affecting the distribution of *P. tremuloides* is far from complete, but the results of this study do provide a base on which future investigation can be built. Several important questions need particular attention. First, because there is a plethora of selection pressures in a heterogeneous environment such as WLNP, it is impossible to establish from allozyme surveys alone which traits are being selected for, a point emphasized throughout the study and which calls for examination. Similarly, information on the relative plasticity of individual genotypes and their ability to adapt to a range of environmental conditions is also required.

Second, the adaptive significance of clonal integration in heterogeneous environments requires study. Specifically, the degree to which clonal integration and resource sharing among ramets moderates the effects of environmental heterogeneity in space and time requires examination. While there is some evidence that xylem integration permits transfer of water between ramets, it is still unknown whether phloem transfer of carbon (or other resources) exists. Further, analyses of clone architecture are required to determine the degree of physiological independence of ramets within a clone. Because ramets are capable of physiological independence, a clone can be considered as a "colony" of genetically identical genotypes. The probability of genet death is thus even further diminished since the integrity of the clone is not compromised by the death of one or a few members of the colony. There are no such demographic studies for *P tremuloides*. It would also be interesting to determine if genotypes can control the branching pattern of ramets in marginal habitats, for it makes little sense to pack modules into a resource-poor environment. In

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such environments, resources for maintenance and growth are scarce yet must be spread among the competing modules (Harper 1985). There is a tradeoff in that if modules (such as suckers) are too loosely packed, there is a cost for supporting these tissues (Harper 1985). There is also the risk of competitors invading these spaces and perhaps outcompeting neighboring ramets. In extreme cases, the phalanx could be broken and ramets lose integration with neighbors. Yet, by strategic placement of modules in a highly fractured environment such as WLNP, the clones may be able to bridge the resource-poor environments and invade favorable sites.

Third, a knowledge of clone age and genet demography would be particularly useful for separating the effects of age on population structure (i.e., for determining if sufficient time and generations have passed in which an equilibrium condition could come about). To date, the frequency of successful seedling establishment is largely based on anecdotal information (Ellstrand and Roose 1987). In Chapter 2 it was pointed out that the ages of clones of *P. tremuloides* are generally considered to be 8000-10000 years old (Barnes 1975; Kemperman and Barnes 1976; Cheliak and Dancik 1982), an estimate deduced from knowledge of glacial retreat. Marked climatic fluctuations occurred earlier in the Holocene. While I have argued that clones represent formidable competitive entities, arboreal competition from conifers would take its toll. Evidence for this can be seen in the Akamina Valley of WLNP where such competition severely limits the size and density of clones. Pollen diagrams of the vegetation history of the western interior of Canada (reviewed by MacDonald and Ritchie 1986) indicate fairly extensive establishment by *Populus* spp. between 3500 and 7000 B.P., a period which roughly corresponds with the warm conditions of the Hypsithermal 4000-8000 years B.P. It is somewhere in this range that I would place the age of most extant clones; since 3500 B.P. vegetation in the western interior has been relatively stable (MacDonald and Ritchie 1986).

Fourth, additional study of the role of somatic mutation in generating genetic variability is required, as is assessment of the cost in terms of genetic load, for even a small mutation rate in these putatively long-lived clones could lead to large accumulations. Valuable insight into the rates of mutation might be made using recent advances in technology for assessing mitochondrial DNA (mtDNA). Unlike nuclear DNA, which is shuffled during recombination, changes in mtDNA are due only to mutation.

Finally, and from a more general point of view, the precise role of protein variability is still largely unresolved after 20 years of debate between the neutralist and selectionist schools of thought. Few studies have probed the nature of differences in the biochemical properties of specific protein polymorphisms and physiological and morphological variation particularly as it pertains to plants. More studies similar to that of Rainey et al. (1987) who examined the relationship between respiration and enzyme genotypes would contribute greatly to our understanding of the physiologic consequenes of genomic organization. Once these questions are better answered, it will be easier to test ecological models and assess the significance of factors such as competition and ecotypic adaptation.

Toward a Unified Biogeography

In this thesis, I have attempted to place a distinctively geographical question against a background that biogeographers are wont to dismiss, the role of evolutionary forces in affecting species' distributions. I maintain that for a comprehensive understanding of the geography of species, irrespective of scale, biogeographers (particularly those housed in geography departments) must be prepared to fuse the disciplines of ecology and genetics. For as Harper (1982) stated, "Problems of the distribution and abundance of species may need to be seen as essentially problems of genetics!". The widespread post-Darwinian desire to find pattern in nature has often resulted in gross oversimplification. Current

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thinking among biogeographers predominantly bypasses the role of the genotype, assumes a steady state, and promotes the notion that species' distributions are controlled primarily by abiotic elements. This study has attempted to show the importance of integrating ecological evolutionary theory to problems of biogeographic distributions. It has stressed the importance of genetic variation at the individual and population level and the need to consider that present-day plant distributions may have established under different conditions than exist today. Further, it suggested that evolutionary, physiological and ecological processes play major roles in influencing a distributional pattern on a local scale, and that this has ramifications for understanding larger-scale patterns. Biogeographers and ecologists alike need to integrate levels of biological organization if they are to develop a unified quantitative theory of plant geographic distribution. To accomplish this, as paradoxical as this may sound since geographers pride themselves on being synthesists, geographers must free themselves from the perils of intellectual isolation and specialization and recognize that the analysis of organic pattern must take place beyond the traditional confines of the subdisciplines that they consider within the purview of geography. Pantin (1968) said that biologists should "follow the analysis of their problems into every other kind of science". The eminent geographer, Carl Sauer (1956), clearly acknowledged the import of biology to biogeographers when he stated that "The field of biogeography requires more knowledge of biology than can be demanded of most of us. It is, however, so important to us and so inadequately cultivated. . . We need more workers who like and are able to live on frontiers, such as those of biology." Knowledge of the way in which genetics and ecology fuse to affect species' distributions is coincident with Sauer's notions and essential to a comprehensive understanding of plant geography.

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Appendix I. Classification, description, and rating of wind exposure[1].

Rating value	Classification	Description
1	Exposed	Open on all sides; ridge tops
2	Moderately exposed	Protected on one or two sides with south and west exposure or east and west exposure
3	Intermediate	Protected on three sides with west or south exposure; on two sides with either west or south exposure; level or gentle slopes
4	Moderately sheltered	Protect on two or three sides; not exposure to west or south sides
5	Sheltered	Protected on all sides by adjacent forest or landform

[1] Adapted from Fralish and Loucks (1975)
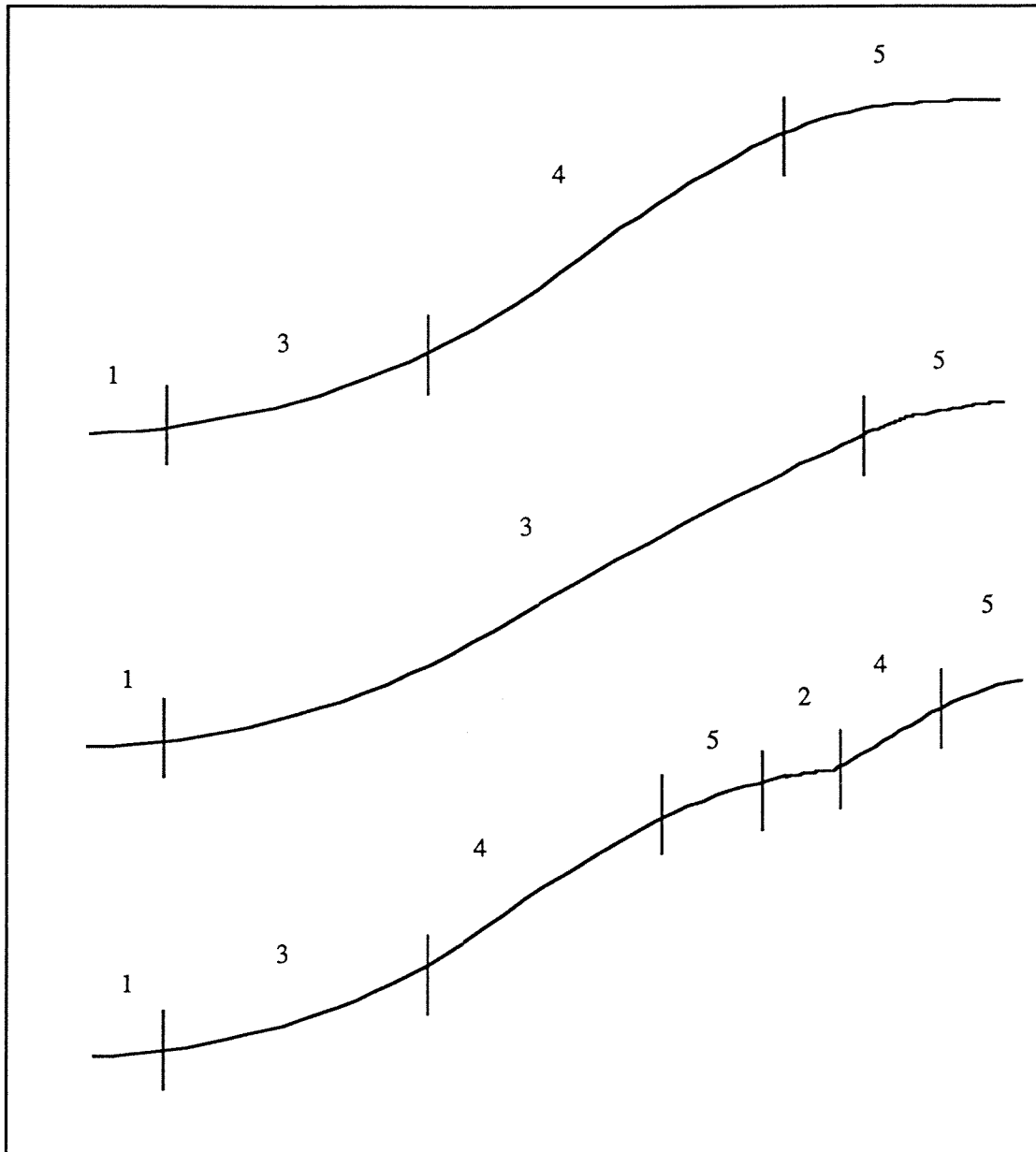
Appendix II. Slope position coding scheme for classification of topography in Waterton
Lakes National Park, Alberta. Adapted from Bowersox and Ward (1972).

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