Variation in reproductive characteristics of lodgepole pine (*Pinus contorta* var. *latifolia*) in British Columbia

by

Anne Berland
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Supervisory Committee

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Dr. Patrick von Aderkas, Department of Biology
Co-Supervisor

Dr. Bradley Anholt, Department of Biology
Co-Supervisor

Dr. Gerry Allen, Department of Biology
Departmental Member

Abstract

Supervisory Committee
Dr. Patrick von Aderkas, Department of Biology
Co-Supervisor
Dr. Bradley Anholt, Department of Biology
Co-Supervisor
Dr. Gerry Allen, Department of Biology
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Lodgepole pine (*Pinus contorta* var. *latifolia*) is the most wide-ranging pine in North America. Populations in British Columbia vary widely in phenotypic and genotypic characteristics. The effect of climate on variation in reproductive characteristics has never been examined, yet is vital to the production of seed necessary for reforestation. This study aims to determine the relationship between the climate in B.C and variation in female cone and seed characteristics.

The study makes use of the Illingworth provenance trial, sixty common garden plots that are distributed throughout British Columbia. Female cones from six provenances were collected at 21 sites during the summer of 2012. The number of scales was counted and maximum length was measured for each cone. Seed was extracted and counted. Variables were pooled for each tree. The climate at each site was described using data from ClimateWNA. Principal components analysis was used to reduce the highly correlated data set to the first two principal components (PC1 and PC2), which together described 76.7% of the variation in the data. PC1 was most closely aligned with variables related to temperature, the number of frost-free days, and degree-days above 5°C or below 0°C. PC2 was most closely aligned with precipitation and moisture variables.

The reproductive variables were moderately positively correlated with one another. Analysis of variance indicated that average cone length and the average number of seeds per cone were significantly affected by both site and provenance, however the average number of seeds per cone was not. Average values of each reproductive trait for each site were modelled against the first two principal components using multiple analysis of variance and univariate linear modelling. The best-fit model for the average number of scales per cone included PC1 and PC2, however the model only described 4.9% of the variation in the data. The best-fit model for the number of seeds included only PC1, and the model only explained 4.1% of the variation in the data. The model for average cone length had the strongest results, with a model that included PC2 and explained 18.7% of the data.

The results of the study indicate that climate is not the most important factor in predicting reproductive characteristics such as cone length, and the number of scales and seed per cone. The significant effect of moisture on average cone length was the strongest relationship identified in the study. The reproductive traits were best described by their stability across the climates of the test sites. High genetic variation in lodgepole pine populations may be contributing to the stability of reproductive traits. Lodgepole pine female cone and seed traits were stable for mature trees over a wide range of provenances and climate regions.

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Chapter 1: Introduction

Lodgepole pine (*Pinus contorta* Douglas ex Loudon) is widespread in western North America. In British Columbia, it is one of the primary species used in intensive reforestation and tree improvement programs as part of sustainable forestry practices (Wu et al., 1996; Wu et al., 2005; Wallis et al., 2011). The recent mountain pine beetle (*Dendroctonus ponderosae*) epidemic has caused widespread tree mortality. Salvage harvesting, and increased seed demand for reforestation have been the main forestry responses to this problem (Anonymous, 2009).

Seed orchards were established in the province to provide high quality, genetically selected seed for reforestation (Owens et al., 2005). Unfortunately, low seed yields have plagued the programs, and the orchards have been unable to meet the demand (Owens et al., 2005; Owens 2006). This problem is most evident in the Okanagan where the majority of seed orchards are located. Orchards located at more northern latitudes in the province are more productive in terms of seed output, while producing fewer cones per tree (Owens et al., 2005).

The difference in seed production between regions in B.C. impacts lodgepole pine forestry in a negative way and raises questions about the genetic and phenotypic variation that exists within the species. If intensive reforestation efforts in the province are going to continue, the problem of producing adequate volumes of high quality seed must be addressed.

Description of *Pinus contorta*

Pinus contorta is distributed over 33° of latitude from the Mackenzie basin in the Yukon Territory, to the Rocky Mountains in southern Colorado in the east, and to northern Baja peninsula in California to the west. It is the most wide-ranging pine in North America, and one of the most variable (Critchfield, 1980; MacDonald & Cwynar, 1985; Rehfeldt et al., 1999; Wu and Ying, 2001; Owens, 2006). The species occupies a wide elevation range – from sea level to over 3400 m. In B.C., it is naturally found in twelve of the fourteen biogeoclimatic (BEC) zones that make up the province, and is a major component species in six of these zones (Wu et al., 2005). The species is adapted to many different environments, which include coastal, montaine, subalpine, and boreal forests (Rehfeldt et al., 1999). In addition, *Pinus contorta* is a pioneer species, has low shade tolerance and is able to grow in very diverse, and sometimes quite extreme, conditions (Ledgard, 2001; Wu et al., 2005; Owens, 2006).

There are four geographical varieties or subspecies of *Pinus contorta*. They are distinct based on morphological and ecological traits. *Pinus contorta* var. *contorta*, common name shore pine, occurs on the Pacific coast. *Pinus contorta* var. *murrayana* is found in the southern Cascade and Sierra Nevada mountains and *Pinus contorta* spp. *bolanderi* is found in the Mendocino White Plains of California. *Pinus contorta* var. *latifolia* is distributed throughout the interior, and is commonly referred to as lodgepole pine (Critchfield, 1957; Owens, 2006). Variety *latifolia* is not only the most widely distributed of the four, but it is also the most commercially valuable (Wu and Ying, 2001). Its wide and dense distribution is attributed to serotinous cones, which provide a seed bank when released by fire (Critchfield, 1980). As it is the focus of this research, the name lodgepole pine will be used to refer specifically to *Pinus contorta* var. *latifolia* for the remainder of this document.

Characteristics such as fast growth, high quality wood and short rotation time make lodgepole pine a desirable forest species (Wu et al., 1996). Lodgepole pine is the most widely distributed, harvested and planted of the forest tree species in B.C. Due to its use in the pulp and paper industry, lodgepole pine is also the most commercially valuable (MacDonald & Cwynar, 1985; Owens, 2006; Wallis et al., 2011).

Since the 1990s, populations of lodgepole pine in B.C. have been devastated by an infestation of mountain pine beetle. The beetle epidemic is the largest ever recorded in the province and has affected 170,000 km^2 of lodgepole pine forest in the province (Anonymous, 2009, Maness et al., 2013). The wide-ranging extent of lodgepole pine forest that has either been directly affected or threatened by mountain pine beetle has led to salvage harvesting. Between 2004 and 2009, 44% of the provincial annual allowable cut was lodgepole pine. To combat the losses, planting has been very intensive. In 2007, lodgepole pine made up one half of all seedlings used in reforestation in B.C. (Anonymous, 2009).

Provincial policy in B.C. dictates that high quality seed is required to meet the reforestation demands. In practice, the majority of lodgepole pine seed for reforestation comes from wild stands, or from wild populations identified as having superior performance. The rest is produced from recently expanded seed orchards throughout the province. Select seed orchard genotypes have resulted in high yield. Increases of 6% to 22% can be expected for stem volume (Anonymous, 2012). Seed orchards are generally located in the southern portion of a species range. The warmer climates of these areas relative to the northern portion of the range helps to promote earlier and more abundant flowering in young trees, and to avoid cold damage (Schmidtling, 1987). For forestry species grown in orchards in the Northern Okanagan, including interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*), western larch (*Larix occidentalis*), and interior spruce (*Picea* cross), this strategy has been a success. The exception is lodgepole pine (Gertzen, 1999). For a variety of reasons, seed orchard cannot meet the demand for seed (Anonymous, 2009).

The lodgepole pine orchards in the Okanagan Valley have not met expectations for seed output (Owens, 2006). By comparison, the trees at seed orchards near Prince George have low numbers of cones per tree, but each cone will yield a comparatively large number of filled viable seed. The trees at orchards in the Okanagan, however, have greater number of cones per tree, but relatively few filled viable seeds and many empty seeds. It is clear that the northern region is much more productive (Owens et al., 2005). This disparity is true for ramets of clones that were planted at both seed orchard locations (Owens, 2006). The difference in seed production among the same genotypes at two locations raises the question of whether these trees display a phenotypically plastic response to unfavourable climate conditions.

Lodgepole Pine Cone Development

The reproductive cycle of pine species is longer than that of many other conifers (Owens and Blake, 1985). The full cycle from bud initiation to seed and cone maturity lasts twenty-six months. The first stage begins in the early summer when pollen cones are initiated. In the late months of this first summer, buds are initiated that may become either lateral buds or seed cones. Buds begin to differentiate in the fall, but do not complete development until the following spring (Owens et al., 1981). Hormones, day length and temperature affect the timing of female strobili initiation (Critchfield, 1980). Before the first dormancy, seed cones typically have 30 to 35 scales, and primordia for two-thirds of the final scales. Pollen cones are dormant for the winter months, until the following spring when pollen forms. Seed cones, after breaking dormancy, develop bracts quickly, with two ovuliferous scale primordia initiating soon after on scales in the distal portion of the cone. Pollination of the female cones occurs in May or June when pollen grains germinate in the ovules. Around the same time, meiosis occurs and megagametophytes develop in the ovule. After a few weeks this female development ceases. The conelets will lie dormant until the following spring. The next April, growth continues, and male gametes and eggs are formed by June, when fertilization occurs. The development of the embryo, seed and cone are finished by the end of the summer (Owens et al., 1981).

Climate has been found to adversely effect embryo development in the northern portion of the species range (Critchfield, 1980; Owens, 2006). Unlike the coastal varieties of *Pinus contorta*, var. *latifolia* cones are serotinous and remain unopened on the tree for years (Owens, 2006). Species with long reproductive cycles, such as lodgepole pine, inherently have more opportunities for things to go wrong, complicating the task of identifying the causes of low seed yield in these species (Owens and Blake, 1985).

Phenotypic and genotypic variation

The phenotype of an organism is made up of characteristics, such as form and function that are the result of interactions between an organism's genotype and both the internal and external environments (Sultan, 2000). The range of possible phenotypic outcomes is constrained, however, both by the genetic makeup of an organism, and by the conditions in which an organism develops (DeWitt et al. 1998).

The traits that make up a particular phenotype are controlled at the most basic level by the genotype of an organism. Traits may be qualitative, meaning that they are either present, absent, or represented by discreet phenotypes, or they may be quantitative and occur along a continuum, such as height growth (Ehrenfeld, 2003). Quantitative traits are often under the control of multiple genes (Via and Lande, 1985).

For a long time, variation in the expression of phenotypes was deemed to be environmental noise that clouded the true genetic expression of an organism, which is the reason for controlled experimental conditions. However, over the past few decades this view has changed. It is accepted that the environment can influence the development of organisms, and therefore, their phenotypes. Environmentally induced variation in the particular characteristics of an organism, commonly known as phenotypic plasticity, is now understood and accepted as a natural form of diversity that influences development, function and adaptation (Sultan, 2000). Plasticity can provide species with a range of possible phenotypes that may be better suited to novel environments, or changing conditions (Sánchez-Gómez et al., 2011). This view of phenotypes led to the idea of a ‘norm of reaction’, which is made up of the full range of environmentally determined possible forms that the phenotype could take (Sultan, 2000). The individual responds to environmental cues, but the ability to respond is controlled by developmental systems (Schlichting, 1986; Callaway et al., 2003). Plastic responses can be adaptive, maladaptive or neutral in regards to an organism’s fitness (Ghalambor et al. 2007). Genotype by environment interactions occur when there is variation in a species or variety’s genetic ability to respond. Phenotypic plasticity can be expressed in the development, physiology or life history of an organism, and, once induced, responses may become either fixed or dynamic (Sultan, 2000; Callaway et al., 2003).

Epigenetics

A relatively new branch of biology, epigenetics, is focussed on another aspect of gene expression and phenotypic variation. Epigenetics examines variation in gene expression that is not due to changes in a DNA sequence (Grant-Downton and Dickinson, 2005). The presence of epigenetic variants contributes to the overall phenotypic variation in a population (Hirsh et al., 2013). These mechanisms of gene regulation are heritable, and potentially reversible (Hirsh et al., 2013; Nicotra et al., 2010). Mechanisms include DNA methylation, histone modification, and transposable element activation (Grant-Downton and Dickinson, 2005; Nicotra et al., 2010). In addition to plasticity, epigenetic changes offer sessile plants yet another method of adapting to the changing external environment (Hirsh et al., 2013).

Phenotypic plasticity in nature

Developmental, physiological and behavioural plasticity can be seen in marine and freshwater invertebrates, land and aquatic plants, amphibians, fish, reptiles, and mammals (Sultan, 2000; Sultan, 2003).

Growth rate, shell thickness and shell shape in gastropods are traits that can be strongly influenced by genotype by environment interactions. The New Zealand freshwater snail *Potamopyrgus antipodarum* exhibits clinal variation across water depth for several life-history traits. The most apparent difference is that snails occupying deeper habitats grow larger and reproduce later (Negovetic and Jokela, 2001). Negovetic and

Jokela (2001) were able to attribute the variation between habitats to phenotypic plasticity for juvenile and adult size, the proportion of brooding females, brood size, number of surviving offspring, and juvenile survival. After transplantation between habitats, the snails were able to attain the specific phenotype of the new environment, indicating strong environmentally induced variation (Negovetic and Jokela, 2001).

Phenotypic plasticity is a cause of variation in the sexual ornamentation of female collared flycatchers (*Ficedula albicollis*). Sexual selection based on female ornaments can be used as a method to assess parental quality (Johnstone et al., 1996). In collared flycatchers, female wing patch size varies between years: i.e. wing patches are smaller on yearling females than older females. After studying wing patch sizes over six years, Hegyi et al. (2008) found that the negative effects of original patch size on changes in patch size was stronger for younger than older females. The North Atlantic Oscillation (NAO) index had a positive effect on changes in patch size. Previous years' clutch size had a negative effect on patch size, but only in years with high NAO indices, indicating favourable weather conditions. Overall, the poor weather conditions in certain years have stronger effects than reproductive effort. Within the same individual, changes in sexual ornamentation were strongly related to the number of mates obtained. They concluded that phenotypic plasticity was age-dependent, and changes in patch size indicated trade-offs between current and future ornamentation and fitness (Hegyi et al., 2008).

In small mammals, organ size and ability can fluctuate with aerobic performance and metabolic rate (Hammond et al., 2001). Changes to environmental conditions, such as temperature, oxygen concentration and altitude were found to affect organ mass in deer mice (*Peromyscus maniculatus sonoriensis*). Organ responses included a positive correlation between small intestine mass and energy intake, a negative correlation between heart mass and average ambient temperature, a positive correlation between altitude and lung mass, and negative correlations between altitude and both small intestine and kidney mass. Abiotic variables have effects on the organs of small mammals at altitude; such changes are likely the result of whole-body plastic responses (Hammond et al., 2001).

Both growth responses and developmental plasticity can be quite dramatic in plants. Consequently, they have been the subjects of the majority of plasticity research (Sultan, 2000). Variation in a species can appear as differences in size, growth rates, biomass allocation, reproduction, or chemical properties (Callaway et al., 2003). Experiments with plants are somewhat simpler than with other organisms as they can be more easily cloned and grown in a myriad of environmental conditions (Sultan, 2000). Additionally, the sessile nature of plants makes plastic responses to the environment even more important to their fitness (Schlichting, 1986). For plants, environmental triggers can vary depending on abiotic factors, the presence, absence and identity of neighbours, disturbance, parasitism, or herbivory (Callaway et al., 2003).

The most well understood plastic response in plants is to light, in particular, red:far-red light ratio. A reduction in this ratio is the cue that stimulates elongation. Plants in dense environments experience shading from neighbours; in response plants may grow taller. This response can be triggered under experimental conditions, and has been used to compare the fitness of short and bushy phenotypes to that of tall phenotypes, which have better access to light (Sultan, 2000).

Plant responses to nutrients and water are also well known (Baquedano et al., 2008). Biomass allocation can be plastic. Limited water or nutrient resources are environmental cues that trigger increased allocation of biomass to root tissue, whereas under low light plants may produce leaves with the greatest leaf specific areas possible, both by increasing size and decreasing thickness (Sultan, 2003). Phenotypic plasticity is a significant way in which plants can adapt to or be influenced by their environments (Sultan, 1995).

Epigenetic changes in *Diplacus* species have been found to be the cause for changes in petal colour, morphology and scent. In southern California, the inland species *Diplacus australis* has yellow flowers and insect pollinators. A sibling species, *Diplacus puniceus* is found on the coast. It has red flowers and bird pollinators. In the region between the coastal and inland plant, an intermediate orange form occurs, which was thought to be a hybrid of the two species. However, a phenotypic transition over time from yellow flowers pollinated by insects to red flowers pollinated by birds has been identified on numerous individual plants in this region. This epigenetic change allows individual plants to change their flower colour and floral morphology, with the result that the pollination syndrome is altered. The plant attracts a new pollinator (Hirsh et al., 2013).

Phenotypic variation in conifers

Phenotypic plasticity can be important for long-lived species as a method of adaptation that allows for the continued persistence of the species through rapid environmental change (Grulke, 2010). Climatic adaptation is viewed as the most important factor in the evolution of temperate and boreal conifer species (Skrøppa and Johnsen, 2000). Testing for plasticity in forest trees requires large areas for planting, and is time consuming (Pigliucci, 2005). However, recently plasticity in growth and development has been described for long-persisting species, such as conifers.

For boreal and temperate conifer species, both the cessation of growth and the concomitant development of cold acclimation are important for survival, and are induced by short days and low temperatures. In Norway spruce (*Picea abies*) variation in geography and climate is linked to variation in these traits. The species varies along altitudinal and latitudinal gradients (Johnsen and Skrøppa, 2000).

Stone pine (*Pinus pinea*) is a Mediterranean species that, in spite of its low genetic variability, is able to exist and thrive in a wide array of diverse environments (Sánchez-Gómez et al., 2011). In addition to responding to changes in light (Awada et al. 2003), the species varies across clones in terms of cone yield, and across populations for growth and germination. There is also strong inter-clonal variation in performance under water deficit for the trees (Sánchez-Gómez et al., 2011).

Maritime pine (*Pinus pinaster*) is a Mediterranean species with a fragmented distribution that is planted worldwide (Alía et al., 1997; Garzón et al., 2011). The species shows genetic differentiation and local adaptation between populations (Garzón et al., 2011). Significant genotype by environment interactions have been identified in this species for survival (Alía et al., 1997; Garzón et al., 2011), wood volume and polycyclism (Alía et al., 1997).

Scots pine (*Pinus sylvestris*) has a broad distribution (Garzón et al., 2011). In Finland, populations show genotype by environment effects for germination of seed. The viability of seed from northern areas is much lower than that from areas in the south. Temperature, frost, photoperiod, precipitation and varying mating patterns influenced the amount of mature seed produced (Harju, 1996). Phenotypic plasticity has been positively linked with survival of the species in the Mediterranean (Garzón et al., 2011).

Turkish pine (*Pinus brutia*) is widely distributed in the eastern Mediterranean. Growth morphology varies over this range depending on the environment. Genetic variation is high within and between populations. The populations on the island of Crete are genetically differentiated from other populations on mainland Greece. Morphological and anatomical variation in traits is related to both environmental conditions and natural selection along altitudinal gradients (Dangasuk and Panetsos, 2004).

Phenotypic variation in lodgepole pine

Lodgepole pine populations vary widely in growth, pest tolerance, cold hardiness and phenology. Variation generally occurs along environmental clines that follow latitude in B.C. and the Yukon, and elevation south of the border in Utah and Idaho (Wu and Ying, 2001). The species is made up of multiple populations that are adapted to the climatic conditions of the local environment (Rehfeldt et al., 1999; Wu and Ying, 2001). Local climate can strongly influence height growth and survival of individual trees (Wu et al., 2005). The differences between populations can be generalized to the scale of BEC zones in the province, and the various climates that the zones delineate (Wu et al., 2005).

There are statistically significant differences in genetic variation, growth characteristics and survival between lodgepole pine populations in B.C. (Xie and Ying, 1995). However, while statistically significant, the genetic variation between populations has been found to be less than that contained within populations (Wheeler and Guries, 1982). Individuals are highly adaptable. Populations have broad fundamental niches.

Climatic optima for height and survival can vary widely between populations (Rehfeldt et al., 1999).

Variation across the distribution of lodgepole can be seen in the incidence of diseases and insects. There are differences between populations for resistance to attacks by a variety of pests and diseases, including western gall rust (*Endocronartium harknessii*; Yanchuk et al., 1988; Wu et al., 1996; Wu et al., 2005), stalactiform blister rust (*Cronartium coleosporiodides*; Yanchuk et al., 1988, Wu et al., 1996), needle cast (*Lophodermella concolor*; Wu et al., 1996; Wu et al., 2005), sequoia pitch moth (*Synanthedon sequoia*; Wu et al., 1996), and terminal weevil (*Pissodes terminalis*; Wu et al., 2005). Differences between populations in these traits can, in some cases, be linked to geographic predictors, such as latitude, longitude and elevation (Wu et al., 1996), or to differences between BEC zones (Wu et al., 2005).

History of forestry research

The wide variation in the quality and growth potential of forest trees had already drawn attention in France as early as the mid-eighteenth century, due to the naval and commercial importance of forestry. The French botanist Duhamel du Monceau carried out the first comparative study using Scots pine between 1745 and 1755. In the 1820s André de Vilmorin continued this research, proposing clinal variation and differences between populations. Forest species used in large-scale forestry were generally widely distributed, and local variation in growth was identified mostly as a concern over an apparent declining supply of quality timber. The effects of seed origin and growing region climatic conditions on wood characteristics were early acknowledgements of phenotypic variation. These ideas led to the introduction of provenance testing (Mátyás, 1996). The term provenance refers to the origin of a population sample. Provenance trials are the forestry equivalent of common garden experiments. They involve systematic collections of populations from various geographic regions across a species' range. These provenances are then planted at test sites, which may cover a geographic gradient (Mátyás, 1996; Ying and Yanchuk, 2006). The lifespan and space requirement of trees makes these experiments both time-consuming and expensive. However, due to the economic importance of the forest industry they have become standard practice in forestry since the beginning of the twentieth century (Mátyás, 1996).

In the late eighteenth and early nineteenth centuries, French botanists initiated the first provenance tests by planting seed from various sources in common gardens in order to conduct growth comparisons. Throughout the nineteenth and early twentieth centuries, provenance trials were established and maintained at large scales across Russia and Europe. The effects of climatic and ecological conditions on the broad intraspecific variability in forest trees and the ability to use this information to improve cultivated forests were recognized around the turn of the twentieth century (Mátyás, 1996).

The aim of provenance trials is to measure the responses of the provenances as they develop under the local conditions at the test site (Mátyás, 1996, Ying and Yanchuk, 2006). Together, these responses can be taken as geographic patterns of adaptive variation (Wu and Ying, 2004). This information can be used to identify provenances with the most desirable characteristics. Growth characteristics, such as crown and stem form, wood quality, and volume, and survival are common traits of interest in forestry research. Other traits include reproductive developmental traits (e.g. number of cones, or age of first cone), cold hardiness, and resistance to insects or disease (Mátyás, 1996;

Nelson and Johnsen, 2008). More complex phenomena such as genetic variation can be examined using provenance methods as well (Mátyás, 1996). Provenance responses can be correlated to climate data or relevant proxies, such as latitude, longitude and elevation, with the goal of uncovering causal relationships (Ying and Yanchuk, 2006).

The Illingworth Provenance Trial

British Columbia has one of the most complete provenance studies in the world. From 1968 to 1974, a provenance study commonly known as the "Illingworth provenance trial" was established by the British Columbia Forest Service (Illingworth, 1978; Rehfeldt et al., 1999; Wu and Ying, 2001). *Pinus contorta s*eed from 142 provenances (Figure 1) were collected and established at more than sixty test sites in B.C. and the Yukon Territory (Figure 2) with a common experimental design (Rehfeldt et al., 1999; Wu et al., 2005). The seed sources cover the range of lodgepole pine's widespread geographic distribution, and include all subspecies and varieties of *Pinus contorta*. Due to its importance to the forest industry in B.C., the trial largely focussed on *Pinus contorta* var. *latifolia* (Rehfeldt et al., 1999). This long-term program offers a rare opportunity to analyze the responses of mature lodgepole pine trees to climate in the interior region of B.C. (Wu et al., 2005).

Figure 1. Illingworth trial provenance locations

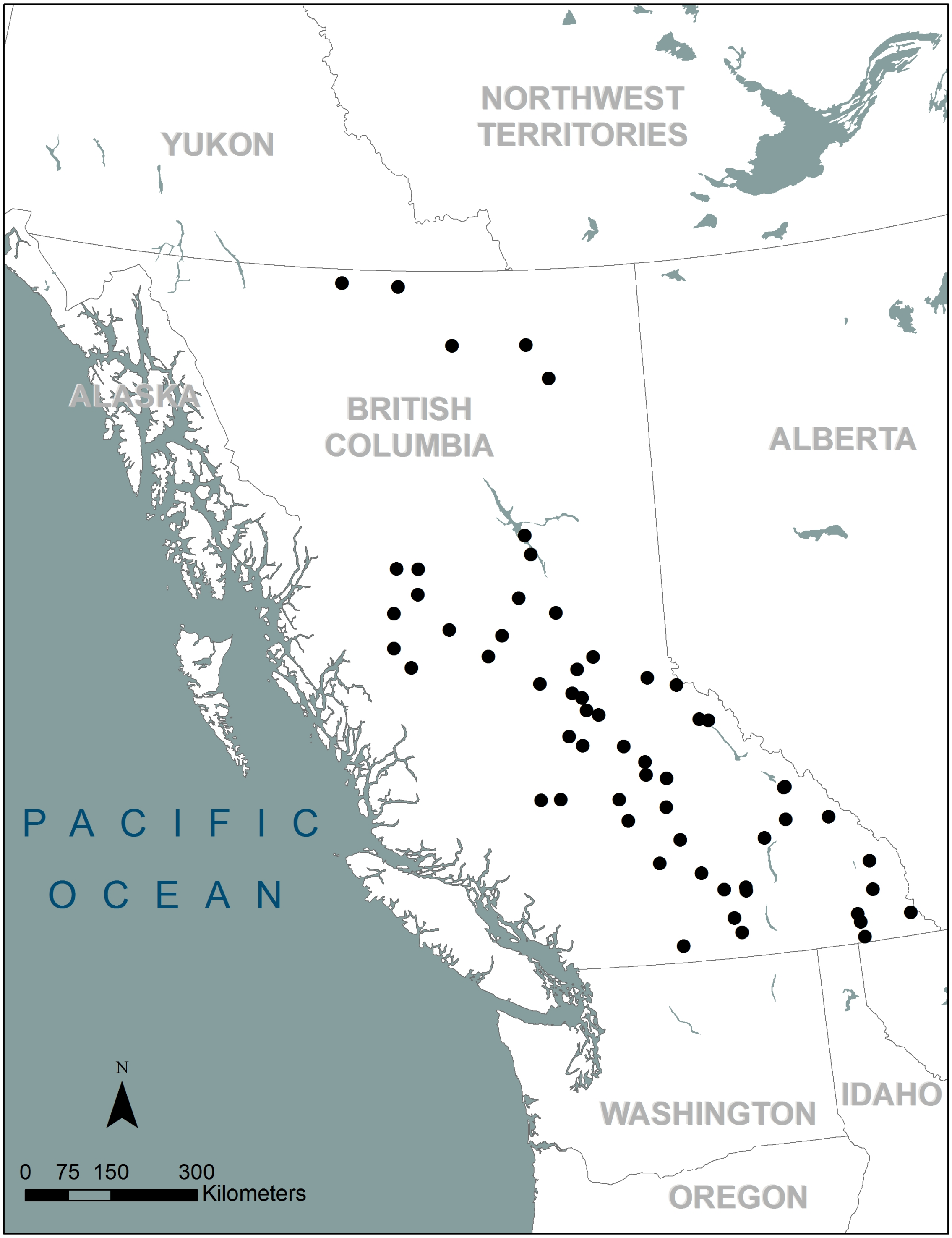

Figure 2. Illingworth trial test site locations.

The sixty study sites in B.C. were selected to cover a range of latitudes, precipitation regimes, soil types, and elevations. The sites span 11° of latitude, 15° of longitude and 1450 metres of elevation. They cover eight BEC zones; lodgepole pine is the major component species in all but the Spruce-Willow-Birch zone. Fifty of the sixty sites are in the following four BEC zones: Interior Cedar Hemlock, Sub-Boreal Spruce, Mountain Spruce, and Engelmann Spruce-Subalpine Fir. With few exceptions, lodgepole pine is a typical species at all sites (Illingworth, 1978; Wu et al., 2005).

The Illingworth trial was designed with the explicit purpose of controlling for genetic composition, stand age, and competition (Wu et al., 2005). A subset of sixty of the 142 source populations of *Pinus contorta* was planted at each site (Rehfeldt et al., 1999; Wu et al., 2005). Each population was represented at an average of thirty-three sites. Each study site was planted with all populations indigenous to the region, most of the populations from surrounding regions and several populations from the extreme ends of the species' range (Rehfeldt et al., 1999). Ten standard provenances were planted at all sixty sites, seven of which are *Pinus contorta* var. *latifolia* (Rehfeldt et al., 1999, Wu and Ying, 2001).

At each site, two completely randomized blocks were planted with provenances represented by nine seedlings each. Trees were spaced 2.5 metres apart in three by three tree square plots (Illingworth, 1978; Rehfeldt et al., 1999; Wu et al., 2005). Competing trees and shrubs were periodically removed. Inter-tree competition was minimal (Rehfeldt et al., 1999).

The Illingworth provenance trial has been used to answer many research questions regarding lodgepole pine. This information has contributed significantly to the development of lodgepole pine forestry guidelines. Results of these studies indicate that there is large variation across provenances and study sites (Yanchuk et al., 1988; Xie and Ying, 1995; Wu et al., 1996; Rehfeldt et al., 1999; Wu and Ying, 2001; Wu et al., 2005).

Seed Transfer and Tree Improvement

The patterns of growth in forest trees that led to provenance experiments throughout the world also led to the establishment of seed transfer guidelines. Reforestation is an integral part of forest industrial activity. The importance of using the best-adapted seed sources in reforestation efforts has long been recognized (Zobel and Talbert, 1984). Seed transfer guidelines provide criteria for choosing sources to plant at specific locations. In effect, this restricts the movement of tree seeds. Seed can only be planted in areas where the trees are deemed fully able to meet their genetic potential based on environmental limitations. Seed is matched to a particular environment, which, in lieu of often difficult-to-measure variables, can be broadly defined using latitude, longitude and elevation. Adherence to seed transfer guidelines is helpful in avoiding cold injury, drought, and attack by insects and disease (Ying and Yanchuk, 2006).

Seed transfer guidelines are dictated by transfer and response functions, models that are used to analyze the fundamental niche of a seed source and to predict the climate that will optimize growth and performance (Thomson et al., 2008). Transfer functions measure the productivity of multiple provenances at a test site and relate that to the climatic distance over which the each source population was transferred. Response functions measure individual provenances across multiple sites, relating the different climatic regimes to trait variation. Differences in productivity across provenances are often pooled and used to fit curves that approximate the impacts on productivity (O’Neill et al. 2008). Response and transfer functions provide information on both local adaptation and the effects of transfer (Wang et al., 2010). Climate variables that have been found to be biologically relevant and are therefore commonly included in transfer function modeling include mean annual temperature, mean warmest month temperature, mean coldest month temperature, mean annual precipitation, summer precipitation, annual heat to moisture index and summer heat to moisture index (Wang et al., 2006).

Tree improvement programs are primarily concerned with increasing the quality and quantity of forest products and decreasing the relative effort, resulting over the long term in the production of high quality, adapted, high yield trees. The ideal products of tree improvement programs are adapted trees that will grow well in a wide range of environments. While the end result is an improved or more desirable phenotype, the research focus is, by necessity, on the genetic variation available in species, populations and even individuals. Genetic variation relating to adaptability may be quite large between geographic regions (Zobel and Talbert, 1984). Genotype by environment interactions can hamper improvement programs, because a family, or population, of trees may perform in an unpredictable manner under different abiotic conditions. For this reason, the most generally desired phenotypes are those that are most stable within a particular group of environments (Alía et al., 1997; Burdon, 1977; Zobel and Talbert, 1984).

Once the best-adapted source populations are identified for a particular region, acquiring adequate seed for reforestation can be a difficult task. To meet the demand, seed orchards are created from desirable genotypes. The physical location of seed orchards can have a tremendous impact. An orchard site should have the appropriate soil texture and fertility, airflow, water supply, and a geographic location that conforms to seed transfer guidelines. Further consideration should be given to the accessibility of the site to workers, and the susceptibility of the site to insects and disease (Zobel and Talbert, 1984).

Cone and seed literature review

Tree improvement projects have primarily focussed on tree attributes that relate to wood characteristics (Zobel and Talbert, 1984). As seed production is vital for reforestation, some research has also studied variability in seed yield (Sarvas, 1970; Schmidtling, 1987). However, research on lodgepole pine cone and seed development has lagged behind wood quality focussed tree improvement projects.

Seed production represents a significant resource investment for plants. In years of high reproductive output, resources are given to reproduction at the cost of lower vegetative growth rates (Despland and Houle, 1997). The amount of viable seed produced by a conifer tree in a given year depends on several factors. First, the conditions during early development will dictate not only how many cones a tree produces, but also how many scales are initiated on each strobilus, and how many scales are fertile. Generally, only the central and distal scales are fertile, while proximal scales are entirely vegetative. Second, conifer pollen is wind dispersed; as a consequence a risk of self-fertilization (geitonogamy) exists. Third, seed losses can occur due to disease or seed-eating insects (Caron and Powell, 1989).

Relationships exist between cone morphology and seed yield for many conifer species. For black spruce (*Picea mariana*), Caron and Powell (1989) found that cone length, width, and the number of scales were each correlated with total seeds and filled seeds per cone. Seed yield was affected by cone size, pollen availability and the presence of cone or seed consumers (Caron and Powell, 1989). In jack pine (*Pinus banksiana*), Despland and Houle (1997) determined that the number of fertile scales, and the viability and germination rates of seed were correlated with the number of cones. In addition, seed mass, germination velocity and the proportion of filled and viable seeds were all correlated (Despland and Houle, 1997).

Climatic conditions during cone development are important. The temperature during the seed maturation season had a positive effect on seed mass and germination rates for jack pine (Despland and Houle, 1997). Sirois (2000) examined four black spruce populations at varying latitudes and found a decreasing trend from south to north in the proportion of filled seeds and germination ability.

The influence of temperature on tree reproduction is still unclear. Results published by Webber et al. (2005) on hybrid white and Engelmann spruce (*Picea glauca* x *Picea engelmannii*) showed that while elevated temperatures decreased the time to pollen shed, it also reduced pollen yields, in vitro viability and seed yields. However, heat-treating seed cones after pollination until early embryo development resulted in increased seed yields and seed masses, and reduced seed germination time (Webber et al., 2005).

Wheeler and Guries (1982) surveyed morphological traits from cones and seeds collected at 28 sites from throughout the natural range of lodgepole pine. They found that the populations were significantly different for many seed and cone traits, including cone width, weight, curvature, and angle of attachment, and seed wing length and width, seed weight, and umbo and prickle development. Cone length, seed coat and wing colour did not significantly vary between provenances. Subspecies *latifolia* displayed less population differentiation than the other subspecies (Wheeler and Guries, 1982).

Pilot study findings

Two pilot projects have examined lodgepole pine cones from various seed orchards in B.C. to test for potential differences in cone morphology and seed yield. These projects have provided evidence that variation in lodgepole pine trees due to different environmental conditions may be contributing to the low seed yields in Okanagan seed orchards.

MacDonald (2009) assessed variation in cone morphology among three seed orchards, Red Rock near Prince George, and Kettle River and Vernon in the Okanagan Valley. Five cones were collected from ramets of three different genotypes that were planted at all three orchards. For each cone, maximum width and length, mass, scale number, fertile scale number and seed number were recorded (MacDonald, 2009).

The results indicated significant genotype by environment interactions for the number of seeds, scales and fertile scales. There were no significant site effects for cone width or mass. The Red Rock seed orchard had significantly more seed than the more southern orchards, with an average of 34.73 seeds per cone, compared to 23.8 or 7.8 at Kettle River and Vernon, respectively. Red Rock had significantly more fertile scales than the other two orchards, however Vernon had significantly more total scales than Red Rock or Kettle River. Only Kettle River and Vernon, the two Okanagan orchards, were significantly different in terms of cone length, but neither had cone lengths that were significantly different from Red Rock. Cone mass was positively correlated with the number of seeds, and with the ratio of seeds to fertile scales. In addition, there were positive correlations between seeds and fertile scales, scales and length, length and number of seeds, and length and ratio of seeds to fertile scales. Overall, there was high phenotypic variation in the lodgepole pine genotypes sampled, and phenotypic plasticity was considered to be the source of the variation in the number of seeds, scales and fertile scales. However, none of the cone characteristics measured in the study could be concretely linked to changes in seed yield between orchards (MacDonald, 2009).

Smith (2010) examined cone development over the final four months of maturation to test whether differences in cone morphology and seed yield were due to variation in cone development over this period. The study included cones from three Okanagan seed orchards, specifically Eagle Rock, Vernon and Kalamalka. Three to five cones were collected biweekly from two trees of five genotypes at each orchard from April to July. None of the genotypes were sampled at more than one orchard. Average cone mass was recorded for each tree and sampling date. A selection of five cones from the last sampling date was used to determine the average number of scales per cone for each genotype. Cones were collected from two different genotypes at three points in the final summer of cone development. The number of fertile ovules per cone was determined by dissecting three cones from each genotype (Smith, 2010).

There were significant differences in cone development among the three orchards. The rate of cone growth over the final months of cone development was the highest in Vernon and the lowest in Eagle Rock. The final measurements of cone mass were significantly different between the orchards, even as the growth rate between consecutive sampling dates throughout the growing season were not significantly different. Scale number and mass were correlated for all orchards, however there were no significant differences in scale number between orchards. The number of fertile scales was positively correlated with cone mass as well. The final conclusions of the study were that there were differences between the genotypes examined, and that phenotypic plasticity might be the explanation for the differences in cone size between orchards, even within the small geographic region that the orchards represented. These differences in cones then dictated differences in seed yield (Smith, 2010).

The results of these pilot projects suggest that there are environmental effects on cone development and seed yield in lodgepole pine. Cone morphology differs between seed orchards, i.e. by site, and while results do not directly show a causal relationship, the differences do appear to be connected to seed yield.

Project Aims

The Illingworth provenance trial provided a unique opportunity to expand these pilot projects across a substantial portion of the geographic distribution of lodgepole pine in B.C. The large number of study sites and provenances available increased the power of the analysis, broadened the range over which any results may be applied, and related the observed variation to climatic factors. The underperformance of currently established seed orchards would benefit from a seed response function based explicitly on seed yield.

This study investigated the influence of climate and provenance on lodgepole pine cone and seed traits. The main objective was to model the variation in lodgepole pine reproductive characteristics in order to describe the reproductive variation of lodgepole pine populations in B.C. In addition, my hope was that information from this study could be used to determine optimal climatic conditions for seed orchards to provide adequate seed for reforestation sustainably for the foreseeable future. My hypotheses were that the climatic regimes at test sites would have an effect on reproductive traits in lodgepole pine, and that these effects would vary between provenances.

Chapter 2: Analysis

Study Design

This study made use of the trees that are part of a lodgepole pine provenance test, the Illingworth trial, (Experimental Plot 657.06), established and maintained by the BC Ministry of Forests and Range (Illingworth, 1978).

The provenances included in this analysis were a subset of the ten common provenances that were planted at all sites. The six selected provenances were all of the subspecies *latifolia* and originate from within B.C (Figure 3). The selected provenances were distributed across over 10° of latitude within B.C. The provenance origins covered an elevation range of 884 meters, a mean annual temperature (MAT) range of 7.3°C, a mean annual precipitation (MAP) range of 404 mm, and a growing degree-day (GDD) range of 385 (Table 1).

Data available for the present day survival and tree condition at the Illingworth trial sites were used as a guide for site selection. Sites with fewer than four living trees for each of the selected provenances were eliminated from consideration for sampling. Climate data for the remaining sites were downloaded from ClimateWNA (Wang et al., 2012). Eighteen sites were selected to represent a wide range of climates and geographic locations within B.C. Backup sites with similar climate parameters were also identified in order to provide alternative options in cases of inadequate cone load or high mortality of trees. Climate variables used for selection include MAT and MAP for the 1961-1990 time period.

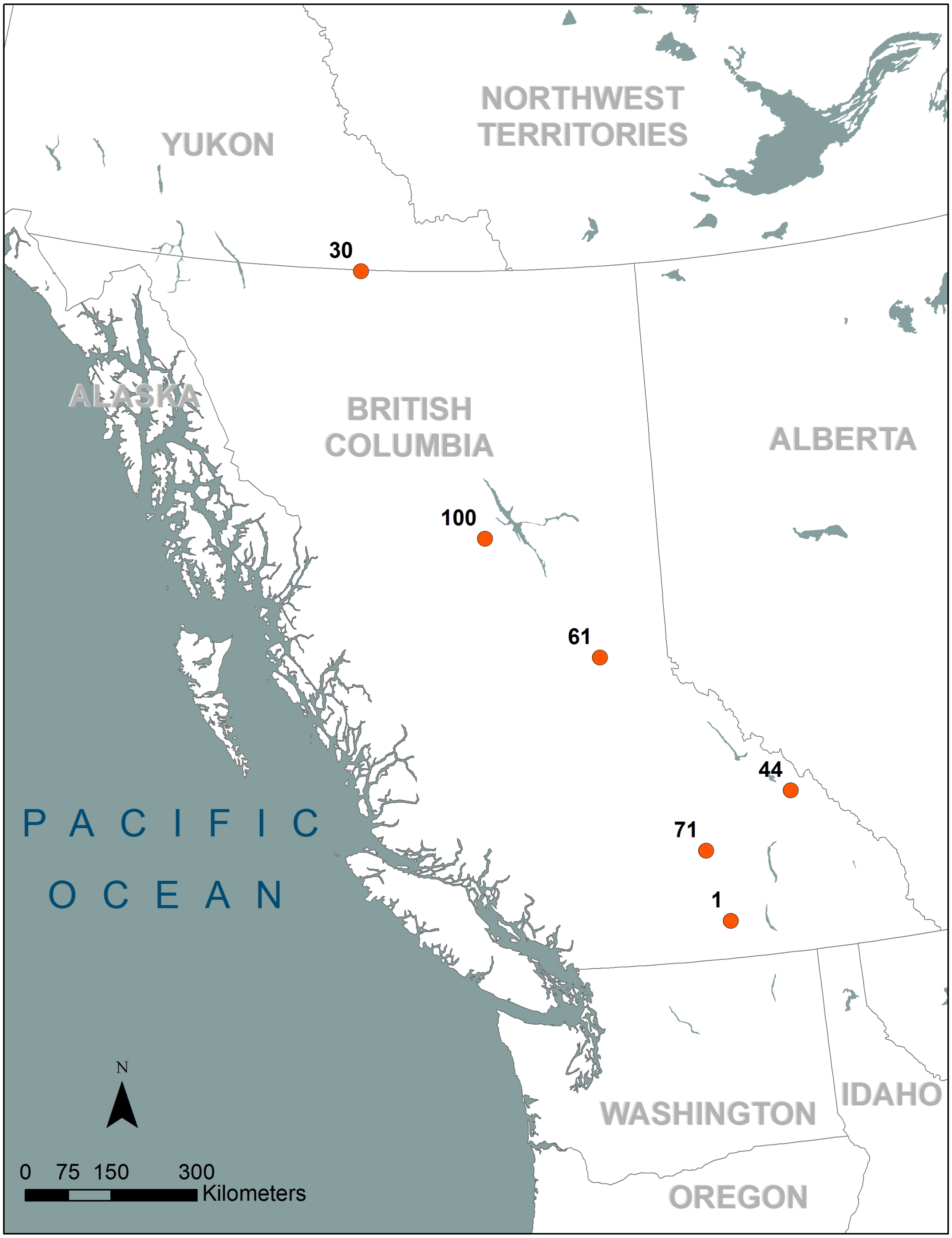

Figure 3. Locations of origin for the six provenances included in the study, with provenance numbers shown.

Table 1. Geographic locations of sampled provenance origins. The 30-year averages for mean annual temperature (MAT), mean annual precipitation (MAP), and growing degree-days (GDD) are listed.

Provenance Name	Provenance Number	Latitude	Longitude	Elevation (m)	MAT (°C)	MAP (mm)	GDD
Trapping Creek	1	49.58°N	-119.02°W	1006	4.6	547	1325
Lower Post	30	59.98°N	-128.55°W	640	-2.7	449	945
Marl Creek	44	51.52°N	-117.18°W	945	3.2	640	1207
Purden Lake	61	53.87°N	-121.80°W	838	2.7	750	1077
Flyhills	71	50.72°N	-119.45°W	1524	2.6	853	975
Nina Creek	100	55.8°N	-124.82°W	762	1.3	522	940

Fieldwork

Fieldwork occurred in June and July of 2012. Twenty-one sites in total were sampled (Figure 4 and Table 2). Due to the poor condition of trees, or the low number of cones for certain provenances, the following four backup sites were included in the study: Roddy Creek, Niquidet Lake, Elk Creek and Kloakut Lake. The 1961-1990 long term normal MAP and MAT were used to ensure that a range of climate regimes were included in the study (Figure 5).

At each site, trees from each provenance were assessed for cone load. Trees identified as bearing adequate numbers of cones for sampling were identified, with emphasis placed on the availability of closed cones. From these trees, four were randomly selected. Cones were removed from each tree using a pole pruner that could be extended to a maximum of 34 feet, however the height from which cones were collected varied. Cones were assessed for condition and approximate age. Closed cones were preferred, but were not available in quantity from every tree, and therefore some open cones were sampled. Samples were kept in paper bags and were labelled according to the site, provenance and tree number.

Figure 4. Locations of sample sites, with site codes shown.

Table 2. Geographic locations of sampling sites. The provenances collected at each site are listed according to provenance number.

Site Name	Site Code	Latitude	Longitude	Elevation (m)	Provenances Sampled
70 Mile	70MI	51.28°N	-121.33°W	1070	1, 30, 44, 61, 71, 100
Bateman Creek	BATE	53.88°N	-121.98°W	730	1, 30, 44, 61, 71, 100
Beaverfoot	BEAV	51.03°N	-116.33°W	1370	1, 30, 44, 61, 71, 100
Cuisson Lake	CUIS	52.5°N	-122.38°W	850	1, 30, 44, 61, 71, 100
Decker Lake	DECK	54.37°N	-125.82°W	930	1, 30, 44, 61, 71, 100
Elk Creek	ELKC	50.25°N	-115.48°W	1190	1, 30, 44, 61, 71, 100
Freeman	FREE	49.08°N	-115.85°W	1520	1, 30, 44, 61, 71, 100
Kloakut Lake	KLOK	51.67°N	-123.5°W	1520	1, 30, 44, 61, 71, 100
Lassie Lake	LASI	49.62°N	-118.92°W	1370	1, 30, 61, 71, 100
Lussier River	LUSS	49.8°N	-115.5°W	1650	1, 30, 44, 61, 71, 100
Muncho Lake	M451	58.8°N	-125.72°W	1100	1, 30, 44, 61, 71, 100
McLatchie Creek	MCLA	49.35°N	-114.68°W	1550	1, 44, 61, 71, 100
Mons Lake	MONS	51.67°N	-123°W	1280	1, 30, 44, 61, 71, 100
Nilkitkwa	NILK	55.32°N	-126.67°W	760	1, 30, 44, 61, 71, 100
Niquidet Lake	NIQU	52.45°N	-121.32°W	950	44, 71
Ootsa Lake	OTSA	53.77°N	-126.83°W	1040	1, 30, 44, 61, 71, 100
Pettigrew Creek	PETI	49.25°N	-120.2°W	1520	1, 30, 44, 61, 71, 100
Roddy Creek	RODY	50.08°N	-118.57°W	1460	71, 104
Sue Blackwater	SUEB	51.07°N	-117.4°W	1550	1, 30, 44, 61, 71, 100
Sue Fire	SUEF	51.57°N	-117.35°W	1190	1, 30, 44, 61, 71, 100
Wigwam	WIGW	50.82°N	-117.98°W	790	1, 30, 44, 61, 71, 100

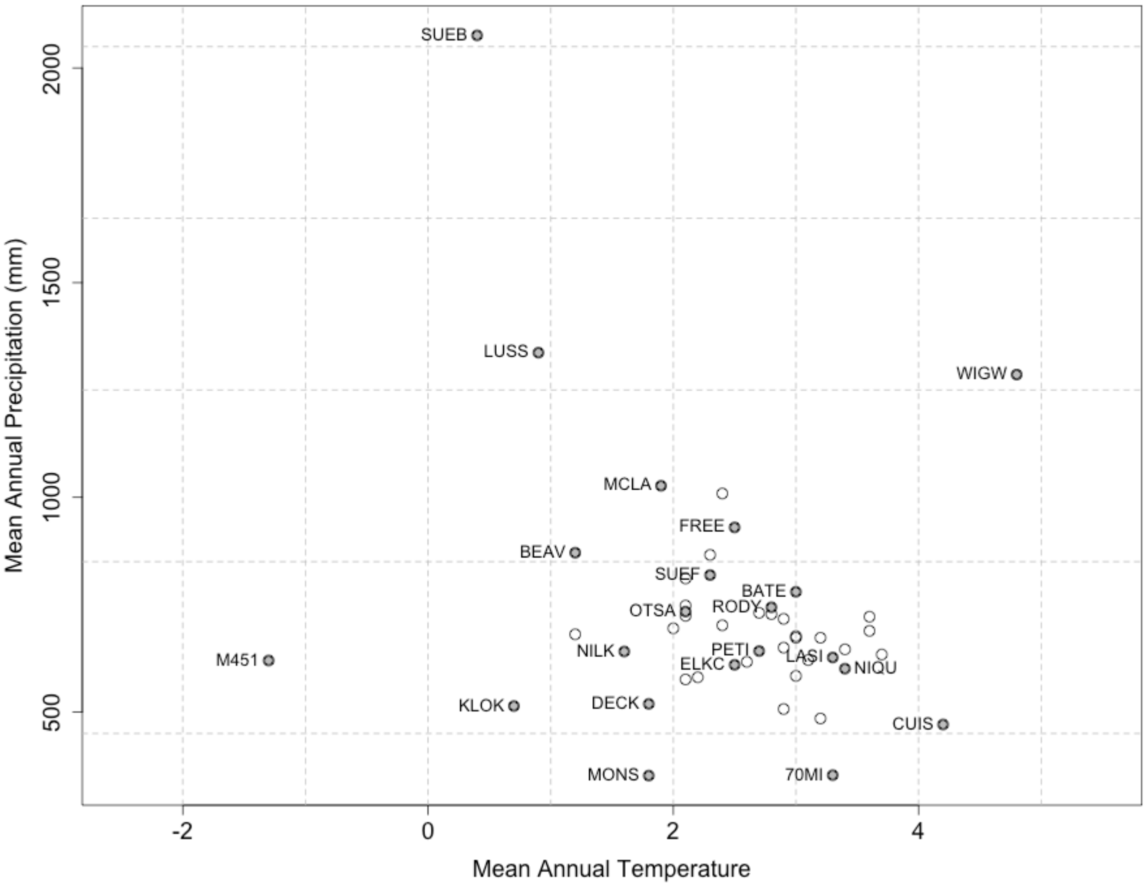

Figure 5. Mean annual temperature and precipitation for potential Illingworth trial sites. Sampled sites are shown in gray and labelled with site codes. Unvisited sites are represented by open circles.

Methodology

Cone Processing

A maximum of five cones per tree, randomly selected from those sampled, were used in the analysis. For each cone, the number of scales was counted, and the open or closed condition of each cone was noted. Cone length was measured to the nearest 0.01 mm with digital calipers. To induce the serotinous cones that remained closed to open, cones were placed in a warming oven at 65°C for two days. Any cones that remained closed after the initial warming were retreated at 72°C. After opening, seed was extracted and counted. Cones that were already opened did not have seed extracted, and these cones were excluded from the analysis of seed variation.

All analysis for this study was carried out in R version 3.0.1 (R Core Team, 2013). An average value for cone length, and for the number of scales and seed per cone was calculated for each tree. The effects of site (SITE) and provenance (PROV) on the three reproductive traits were investigated using analysis of variance (ANOVA). After the ANOVA were performed for each reproductive trait, the residuals were tested using the Shapiro-Wilk test to ensure that they met the assumption of normality, and transformations were made where the assumption was not met.

For modeling purposes, an average value for each provenance at each site was calculated for cone length (AVG_LENGTH), and the number of scales (AVG_SCALES) and seed (AVG_SEEDS) per cone using the individual un-transformed tree averages.

Climate Data

While an emphasis was placed on collecting younger cones, the precise ages of cones were unknown, and therefore the specific years in which the cones developed are also unknown. Climate data made available by ClimateWNA for each site were averaged over the years 2001-2009 in order to describe the climatic properties at the sites for the time period in which the cones developed (Wang et al., 2012). This range of years was chosen to best approximate the period of time over which all sampled cones developed. Annual, seasonal, and monthly variables were included in the analysis (Table 3).

The internal structure of the climate data was investigated using correlation analysis. Due to the highly correlated nature of the climate data, principal component analysis (PCA) was undertaken in order to attain climate variables that would represent the climate space while not being correlated. Prior to this, several variables, which had zero values for all sites, were removed from the dataset, leaving 167 variables for the PCA. Upon calculation of the principal components (PCs) of the climate data, two different methods of selection criteria were employed, the Kaiser-Guttman criterion and the Broken Stick Model. The results of these methods were used to select the first two PCs to represent the variation in the climate data. The loadings for the selected PCs were examined to determine the most closely aligned climate variables. Scores were calculated for each site within the climatic space represented by the PC model.

Table 3. List of climate variables accessed from ClimateWNA and included in the analysis.

Annual Variables	
MAT	Mean annual temperature (°C)
MWMT	Mean warmest month temperature (°C)
MCMT	Mean coldest month temperature (°C)
TD	Continentality – the temperature difference between MWMT and MCMT (°C)
MAP	Mean annual precipitation (mm)
MSP	Mean summer (May to Sept.) precipitation (mm)
AH:M	Annual heat:moisture index (MAT+10)/(MAP/1000))
SH:M	Summer heat:moisture index ((MWMT)/(MSP/1000))
DD_0	Degree-days below 0°C, chilling degree-days
DD5	Degree-days above 5°C, growing degree-days
NFFD	Number of frost-free days
FFP	Frost-free period
bFFP	Julian date on which FFP begins
eFFP	Julian date on which FFP ends
PAS	Precipitation as snow (mm) between August in previous year and July in the current year
EMT	Extreme minimum temperature over a centered 30 year period
EXT	Extreme maximum temperature over a centered 30 year period
Eref	Hargreaves reference evaporation
CMD	Hargreaves climatic moisture deficit
Seasonal variables	
Tave_wt	Winter (December (previous year) – February) mean temperature (°C)
Tave_sp	Spring (March – May) mean temperature (°C)
Tave_sm	Summer (June – August) mean temperature (°C)
Tave_at	Autumn (September – November) mean temperature (°C)
Tmax_wt	Winter mean maximum temperature (°C)
Tmax_sp	Spring mean maximum temperature (°C)
Tmax_sm	Summer mean maximum temperature (°C)
Tmax_at	Autumn mean maximum temperature (°C)
Tmin_wt	Winter mean minimum temperature (°C)
Tmin_sp	Spring mean minimum temperature (°C)
Tmin_sm	Summer mean minimum temperature (°C)
Tmin_at	Autumn mean minimum temperature (°C)
PPT_wt	Winter precipitation (mm)
PPT_sp	Spring precipitation (mm)
PPT_sm	Summer precipitation (mm)
PPT_at	Autumn precipitation (mm)

Table 3. Continued.

Monthly variables	
Tave01 – Tave12	January – December mean temperatures (°C)
Tmax1 – Tmax12	January – December maximum mean temperatures (°C)
Tmin01 – Tmin12	January – December minimum mean temperatures (°C)
PPT01 – PPT12	January – December precipitation (mm)
DD_0_01 – DD_0_12	January – December degree-days below 0°C
DD5_01 – DD5_12	January – December degree-days above 5°C
NFFD01 – NFFD12	January – December number of frost-free days
PAS01 – PAS12	January – December precipitation as snow
Eref01 – Eref12	January – December Hargreaves reference evaporation
CMD01 – CMD12	January – December Hargreaves climatic moisture deficit

Modelling

To fully explore the questions posed in this study, a number of different modelling approaches were undertaken. First, the relationships between climatic PCs and reproductive response variables were visualized in scatterplots. The approximate shapes of the relationships between the variables were examined. Previous research has identified many lodgepole pine traits as showing quadratic shapes in variation over climate (Wang et al., 2006). For this reason, and due to the parabolic shape of several scatterplots, squared versions of the predictor variables were included in the model selection process. The overall effect of climate on the measured reproductive traits was examined using multivariate analysis of variance (MANOVA). The best model fit was obtained by using a method of backwards elimination starting from a complete model, including all interaction terms of the following formula (1):

$$(\text{AVG\_SCALES}, \text{AVG\_LENGTH}, \text{SQRT\_SEEDS}) \sim \text{PC1} + \text{PC1}^2 + \text{PC2} + \text{PC2}^2 + \text{PROV} + \text{PC1:PC2} + \text{PC1:PROV} + \text{PC2:PROV} + \text{PC1:PC2:PROV} \tag{1}$$

The normality of the multivariate model was tested using the Shapiro-Wilk Multivariate Normality Test. The residuals for each reproductive variable were also tested for normality using the Shapiro-Wilk test. Variables for which the residuals did not meet the assumption of normality were transformed. Both multivariate and univariate ANOVA tables were examined to determine the effects of the resulting model. Univariate linear models were run for each response variable using the resulting model in order to obtain more information about the predictor effects.

Individual linear models were also calculated for each reproductive trait using a procedure of backwards elimination starting from a complete model using Formula 1, listed above. This was used to identify any significant predictor effects on single reproductive traits. Again, normality was tested in a post-hoc fashion, using the residual values resulting from of each model.

Results

Field Observations

Cones were collected from 419 lodgepole pine trees. The distribution of trees sampled across provenances is provided in Table 4. With few exceptions, at least 5 cones were collected from each tree. Due to the large number of cones sampled, it was difficult to make field predictions for trends in the reproductive traits. Freeman Creek and 70 Mile were two sites that stood out as having particularly larger, or smaller cones, respectively. The sites appeared to have a strong effect on the condition of trees in general, with some sites having particularly hardy and healthy looking trees overall, while at other sites trees were uniformly suffering. The presence of mountain pine beetle was a hindrance to sampling at sites in the central interior, such as Cuisson Lake, Decker Lake, and Niquidet Lake. The high mortality at these, and more moderate levels of mortality at others, reduced the number of trees available to sample.

Table 4. Summary of the number of trees and cones sampled for each provenance.

Provenance Number	Number of trees	Number of cones
1	71	347
30	65	322
44	69	338
61	68	333
71	70	339
100	76	376
Total	419	2055

Initial Cone Results

In total, traits were measured for 2055 cones. Across provenances, the average number of scales and seeds per cone did not vary substantially, whereas cone length appeared to vary according to provenance (Figure 6). Provenance 30 in particular appeared to have longer cones than the other provenances. The pattern across sites was less clear (Figure 7). There were obvious differences in mean trait values among some sites, and the variance among sites was not consistent, particularly for the average number of seeds per cone. Individual sites, such as 70 Mile for shorter cone lengths, and Beaverfoot Creek for large variation in the number of scales per cone, stood out as performing differently amongst the sites.

The correlation coefficients between the three reproductive traits were the following: average number of scales per cone: average cone length, R = 0.43; average number of scales per cone: average number of seeds per cone, R = 0.30; average cone length: average number of seeds per cone, R = 0.39. Somewhat unexpectedly the correlation between length and seeds per cone was slightly larger than the correlation between scales and seeds.

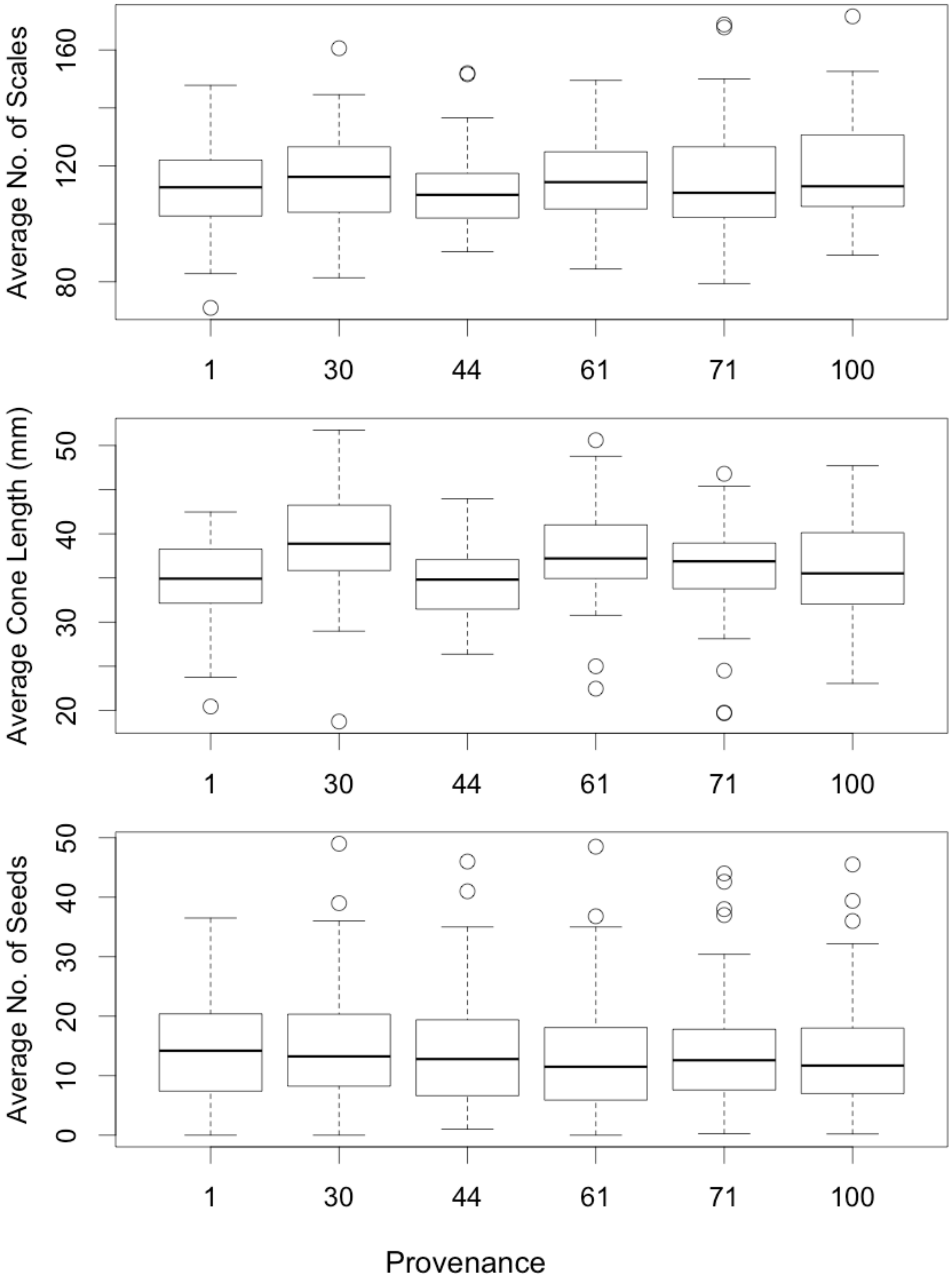

Figure 6. Boxplots of the average number of scales per cone, average cone length, and the average number of seeds per cone for the six provenances included in the study.

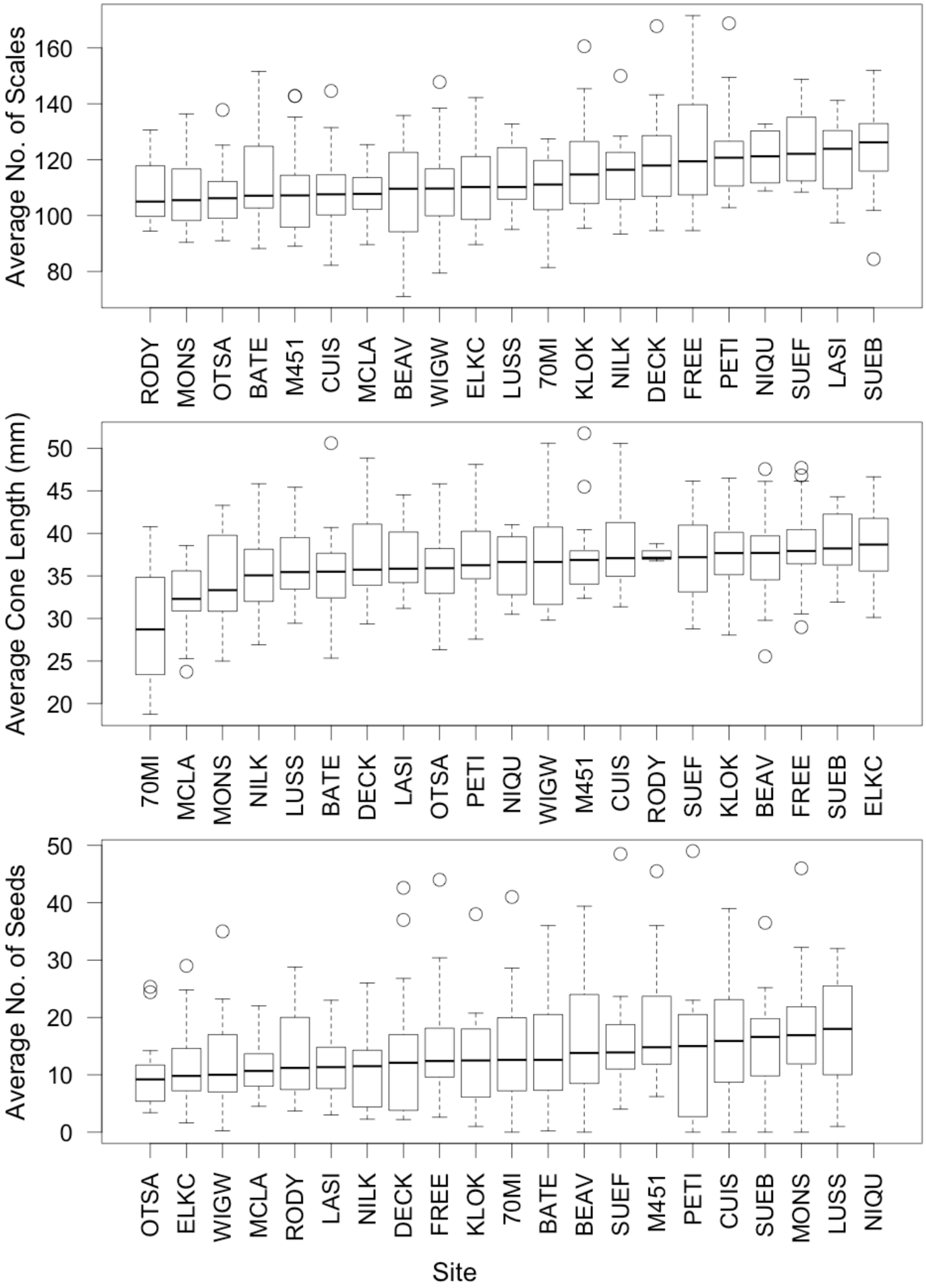

Figure 7. Boxplots of the average number of scales per cone, average cone length, and the average number of seeds per cone for the 21 sites included in the study, with sites ordered by increasing median values.

The residuals of the ANOVA for the average number of scales per cone, and average cone length met the assumption of normality, however the residuals for the average number of seeds per cone did not (average number of scales per cone: W = 0.995, P-value = 0.183; average cone length: W = 0.998, P-value = 0.891; average number of seeds per cone: W = 0.978, P-value = 0.000). The average number of seeds per cone was transformed using a square root, meeting the normality assumption (W = 0.992, P-value = 0.0802).

There were significant effects of site, provenance and the interaction of site and provenance for both the average number of scales per cone and average cone length (Table 5). There were no significant effects on the average number of seeds per cone, however, the interaction effect of site and provenance was close to being significant.

For the average number of scales per cone and the average cone length, these results show that not only did these traits vary significantly according to site and provenance, but that this variation was not necessarily predictable across provenance and site. Tukey Honest Significant Differences was used to identify which provenances, sites, and interactions were identified as being significantly different (Tables 6 and 7).

Table 5. Analysis of variance results for the effects of site and provenance on the average number of scales per cone, average cone length and the average number of seeds per cone.

	Df	SS	MSS	F-value	P-value
Average no. of scales per cone					
SITE	20	15100	757	4.11	0.000
PROV	5	2170	434	2.36	0.041
SITE:PROV	89	29400	3330	1.79	0.000
Residuals	303	55900	184		
Average length					
SITE	20	2250	112	5.83	0.000
PROV	5	975	195	10.1	0.000
SITE:PROV	89	2790	31.4	1.63	0.001
Residuals	303	5840	19.3		
Average no. of seeds per cone					
SITE	19	27.6	1.45	0.874	0.616
PROV	5	2.8	0.560	0.337	0.890
SITE:PROV	80	178	2.22	1.34	0.051
Residuals	225	374	1.66		

Table 6. List of significant differences from the Tukey Honest Significant difference test for the average number of scales per cone.

	Difference	P-value
Provenances		
100-44	7.38	0.0152
Sites		
FREE-BEAV	15.0	0.0375
FREE-MONS	14.7	0.0474
FREE-OTSAFREE	15.8	0.0281
PETI-OTSA	15.2	0.0419
SUEB-BEAV	15.5	0.0178
SUEB-MCLA	16.3	0.0315
SUEB-MONS	15.2	0.0231
SUEB-OTSA	16.3	0.0134
SUEF-70MI	15.0	0.0309
SUEF-BEAV	16.3	0.0103
SUEF-CUIS	15.3	0.0319
SUEF-MCLA	17.0	0.0194
SUEF-MONS	16.0	0.0135
SUEF-OTSA	17.1	0.00781
Site:Provenance Interactions		
FREE:100-70MI:1	44.3	0.027
FREE:100-70MI:100	46.2	0.012
FREE:100-70MI:71	51.2	0.001
FREE:100-BEAV:1	53.8	0.000
FREE:100-BEAV:44	50.1	0.002
FREE:100-BEAV:71	44.1	0.030
FREE:100-CUIS:61	48.0	0.006
FREE:100-CUIS:71	61.3	0.002
FREE:100-ELKC:1	50.9	0.001
FREE:100-ELKC:30	50.0	0.002
FREE:100-ELKC:44	43.0	0.047
FREE:100-LUSS:1	45.4	0.018
FREE:100-M451:100	43.7	0.035
FREE:100-M451:61	49.5	0.003
FREE:100-MCLA:100	43.9	0.033
FREE:100-MCLA:71	47.6	0.030
FREE:100-MONS:100	48.9	0.004
FREE:100-MONS:44	45.0	0.021
FREE:100-MONS:61	48.4	0.005
FREE:100-NILK:44	53.1	0.003
FREE:100-OTSA:100	45.3	0.019
FREE:100-OTSA:30	49.4	0.016
FREE:100-OTSA:44	50.0	0.002
FREE:100-WIGW:44	51.1	0.001
FREE:100-WIGW:71	54.5	0.000

Table 7. List of significant differences from the Tukey Honest Significant difference test for average cone length.

	Difference	P-value
Provenances		
30-1	4.08	0.000
30-100	2.87	0.002
30-44	4.16	0.000
30-71	2.96	0.002
61-1	3.07	0.001
61-44	3.14	0.001
Sites		
BATE-70MI	6.41	0.000
BEAV-70MI	8.26	0.000
CUIS-70MI	9.67	0.000
DECK-70MI	8.08	0.000
ELKC-70MI	9.09	0.000
FREE-70MI	9.62	0.000
KLOK-70MI	8.15	0.000
LASI-70MI	8.21	0.000
LUSS-70MI	7.06	0.000
M451-70MI	8.28	0.000
MCLA-CUIS	-6.38	0.002
MCLA-ELKC	-5.80	0.007
MCLA-FREE	-6.32	0.003
MONS-70MI	5.61	0.002
NILK-70MI	6.08	0.001
OTSA-70MI	6.50	0.000
PETI-70MI	8.17	0.000
SUEB-70MI	9.67	0.000
SUEB-MCLA	6.38	0.001
SUEF-70MI	7.98	0.000
WIGW-70MI	7.79	0.000
Site:Provenance Interactions		
70MI:100-BEAV:61	-14.9	0.012
70MI:100-CUIS:30	-20.0	0.000
70MI:100-DECK:30	-16.4	0.001
70MI:100-ELKC:30	-17.7	0.000
70MI:100-ELKC:61	-15.7	0.004
70MI:100-FREE:71	-14.1	0.035
70MI:100-LUSS:30	-13.9	0.048
70MI:100-M451:30	-17.5	0.000
70MI:100-PETI:30	-14.7	0.017
70MI:100-PETI:61	-15.3	0.033
70MI:100-SUEB:30	-16.0	0.003
70MI:100-SUEB:44	-14.9	0.014
70MI:100-SUEF:61	-14.2	0.033
70MI:100-WIGW:61	-21.6	0.000

Table 7. Continued.

	Difference	P-value
Site:Provenance Interactions		
70MI:71-BEAV:61	-16.7	0.001
70MI:71-CUIS:30	-21.8	0.000
70MI:71-CUIS:61	-15.0	0.012
70MI:71-DECK:1	-14.5	0.021
70MI:71-DECK:30	-18.2	0.000
70MI:71-ELKC:30	-19.5	0.000
70MI:71-ELKC:61	-17.5	0.000
70MI:71-LUSS:30	-15.7	0.005
70MI:71-M451:30	-19.3	0.000
70MI:71-MONS:1	-15.3	0.008
70MI:71-NILK:61	-16.7	0.006
70MI:71-PETI:30	-16.5	0.001
70MI:71-PETI:61	-17.1	0.004
70MI:71-SUEB:30	-17.8	0.000
70MI:71-SUEB:44	-16.7	0.001
70MI:71-SUEF:61	-16.0	0.003
70MI:71-WIGW:61	-23.4	0.000
BEAV:100-70MI:100	14.2	0.031
BEAV:100-70MI:71	16.0	0.003
BEAV:61-70MI:1	14.0	0.043
CUIS:100-70MI:71	14.2	0.031
CUIS:30-70MI:1	19.0	0.000
CUIS:30-70MI:30	14.4	0.026
CUIS:30-NILK:1	14.9	0.014
DECK:30-70MI:1	15.4	0.006
ELKC:30-70MI:1	16.7	0.001
ELKC:61-70MI:1	14.7	0.017
FREE:100-70MI:1	16.8	0.001
FREE:100-70MI:100	17.8	0.000
FREE:100-70MI:71	19.6	0.000
FREE:71-70MI:71	15.9	0.003
KLOK:100-70MI:71	15.3	0.008
KLOK:71-70MI:71	14.0	0.041
LASI:100-70MI:71	13.9	0.045
M451:30-70MI:1	16.5	0.001
MCLA:100-CUIS:30	-17.7	0.000
MCLA:100-DECK:30	-14.2	0.032
MCLA:100-ELKC:30	-15.5	0.006
MCLA:100-FREE:100	-15.5	0.005
MCLA:100-M451:30	-15.3	0.008
MCLA:100-WIGW:61	-19.3	0.000
MONS:100-CUIS:30	-14.8	0.015
MONS:100-WIGW:61	-16.5	0.008
OTSA:44-CUIS:30	-14.1	0.038
SUEB:100-70MI:100	14.4	0.024
SUEB:100-70MI:71	16.2	0.002

Table 7. Continued.

	Difference	P-value
Site:Provenance Interactions		
SUEF:100-70MI:100	14.2	0.033
SUEF:100-70MI:71	16.0	0.003
SUEF:44-CUIS:30	-14.0	0.040
SUEF:71-70MI:71	15.4	0.007
WIGW:61-70MI:1	20.6	0.000
WIGW:61-70MI:30	16.0	0.015
WIGW:61-70MI:61	17.2	0.003
WIGW:61-KLOK:44	15.0	0.047
WIGW:61-MCLA:1	15.1	0.043
WIGW:61-MONS:44	15.3	0.033
WIGW:61-NILK:1	16.5	0.008
WIGW:61-NILK:44	16.1	0.046
WIGW:61-OTSA:44	15.7	0.021
WIGW:61-SUEF:44	15.7	0.022

For the average number of scales, there were several significantly different sites that stand out. Sue Blackwater and Sue Fire, located in close proximity in the northern Kootenays had more scales per cone than several other sites. However, Beaverfoot, also located in this region had fewer scales per cone than other sites. Ootsa Lake, located near Smithers, B.C., is another notable site with fewer average scales per cone.

Provenance 100 had a significantly greater average number of scales per cone than provenance 44. Provenance 100 is from a more northerly location than provenance 44, however it is not the most northern provenance. The significant interaction between site and provenance was due to the large average number of scales per cone for provenance 100 at site Freeman Creek. Freeman Creek, located in the southern Kootenays represented the largest transfer distance for provenance 100.

Significant differences across sites for average cone length were most commonly related to site 70 Mile. This significant effect was not a surprise as the cones from this site were noticeably smaller than those at other sites. Not only were the cones significantly smaller than others, but also anecdotally, the trees at this site in the central interior were also quite small and in poor condition. In addition to 70 Mile, the average cone length at site McLatchie Creek was also significantly smaller than at other sites.

Differences between provenances for average cone length can be traced to provenances 30 and 61. Provenances 30 and 61 are from northern B.C., with 30 being the most northern provenance. Cones from provenance 30 are significantly longer than all of the other provenances (1, 44, 71, 100), with the exception of provenance 61. Cones from provenance 61 are only significantly longer than two of the southern provenances, 1 and 44. There are a large number of site and provenance combinations that are significantly different than the site or provenance effect would dictate alone for average cone length. Once again, site 70 Mile stands out as being a significantly different site for cone length.

Initial Climate Results

As expected, the climate variables were highly correlated with one another. These correlations fell into predictable groupings, most notably with the temperature and precipitation variables being correlated accordingly. The PCA of the 167 climate variables resulted in 20 PCs (Table 8). The Kaiser-Guttman criterion indicated that the first four principal components were adequate for explaining the variation in the data. However, using the Broken Stick Model only the first two were needed to represent the climatic variation. After examining the cumulative variation across the eigenvalues, it was decided that the first two PCs, which represent 76.7% of the variation in the data, would be interpreted and incorporated in further analysis.

Table 8. Eigenvalues resulting from the principal components analysis of the climate variables, and their contribution to the correlations.

	Eigenvalue	Proportion Explained	Cumulative Proportion
PC1	98.2	0.588	0.588
PC2	29.8	0.178	0.767
PC3	12.2	0.073	0.839
PC4	9.00	0.054	0.893
PC5	5.45	0.033	0.926
PC6	3.49	0.021	0.947
PC7	2.48	0.015	0.962
PC8	1.72	0.010	0.972
PC9	1.57	0.009	0.981
PC10	0.827	0.005	0.986
PC11	0.537	0.003	0.990
PC12	0.481	0.003	0.992
PC13	0.367	0.002	0.995
PC14	0.216	0.001	0.996
PC15	0.183	0.001	0.997
PC16	0.160	0.001	0.998
PC17	0.148	0.001	0.999
PC18	0.0982	0.001	0.999
PC19	0.0544	0.000	1.00
PC20	0.0438	0.000	1.00

The first PC represented 58.8% of the variation in the data. PC1 was negatively correlated with annual, seasonal, and monthly temperature variables, degree-days above 5°C, frost-free days and Hargreaves reference evaporation (Table 9). PC1 was positively correlated with degree-days below 0°C, and precipitation variables. Lower values of PC1 indicated higher temperatures and evaporation, and a greater number of degree-days above 5°C and frost-free days. Higher values of PC1 indicated a greater number of degree-days below 0°C, and higher precipitation. PC2, which represented 17.8% of the variation in the data, was most strongly correlated with variables relating to precipitation and moisture. Annual, seasonal and monthly precipitation, and precipitation as snow loaded negatively with PC2. Annual and summer heat:moisture indices, and annual, seasonal and monthly Hargreaves climatic moisture deficit index loaded positively. Lower values of PC2 indicate higher precipitation. Higher values of PC2 indicate higher moisture. Both precipitation and temperature variables were correlated with PC1, making it a complex variable to interpret. PC2, which was most strongly aligned with precipitation and moisture variables, was more straightforward. The PC1 and PC2 scores for each site were extracted for use in modelling (Figure 8). Combined, these two PCs appeared to adequately represent the variation in the temperature averages, extremes, precipitation and moisture regimes, and the growing season for the range of study sites.

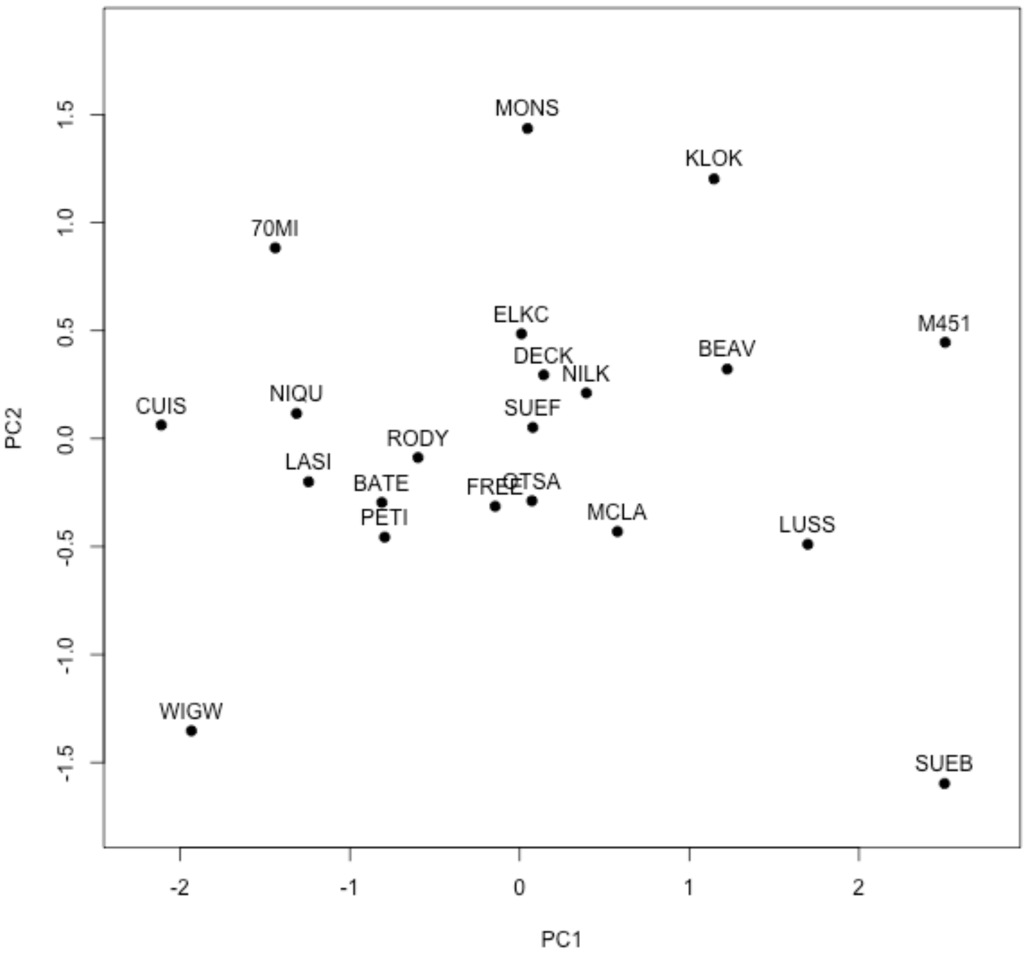

Figure 8. Scatterplot of PC1 and PC2 scores for sampling sites.

Table 9. Loadings of climate variables on the first two principal components.

Variable	PC1	PC2	Variable	PC1	PC2
Tmax01	-0.495	0.106	PPT09	0.293	-0.455
Tmax02	-0.461	0.204	PPT10	0.261	-0.472
Tmax03	-0.526	0.057	PPT11	0.226	-0.489
Tmax04	-0.501	0.079	PPT12	0.201	-0.495
Tmax05	-0.509	0.110	DD_0_01	0.526	0.149
Tmax06	-0.476	0.152	DD_0_02	0.533	-0.002
Tmax07	-0.445	-0.027	DD_0_03	0.527	0.065
Tmax08	-0.508	-0.007	DD_0_04	0.552	-0.021
Tmax09	-0.523	0.007	DD_0_05	0.555	-0.096
Tmax10	-0.491	0.208	DD_0_06	0.464	-0.147
Tmax11	-0.486	0.101	DD_0_09	0.529	0.075
Tmax12	-0.500	0.097	DD_0_10	0.522	-0.075
Tmin01	-0.449	-0.296	DD_0_11	0.520	0.096
Tmin02	-0.492	-0.189	DD_0_12	0.515	0.132
Tmin03	-0.503	-0.216	DD5_01	-0.517	-0.131
Tmin04	-0.541	-0.182	DD5_02	-0.534	-0.010
Tmin05	-0.519	-0.232	DD5_03	-0.537	-0.121
Tmin06	-0.395	-0.241	DD5_04	-0.518	-0.083
Tmin07	-0.378	-0.366	DD5_05	-0.524	-0.060
Tmin08	-0.391	-0.350	DD5_06	-0.486	0.000
Tmin09	-0.377	-0.337	DD5_07	-0.477	-0.158
Tmin10	-0.424	-0.223	DD5_08	-0.546	-0.147
Tmin11	-0.481	-0.256	DD5_09	-0.548	-0.153
Tmin12	-0.432	-0.265	DD5_10	-0.561	-0.030
Tave01	-0.528	-0.149	DD5_11	-0.540	-0.128
Tave02	-0.535	0.004	DD5_12	-0.484	-0.119
Tave03	-0.541	-0.077	NFFD01	-0.438	-0.224
Tave04	-0.549	-0.030	NFFD02	-0.428	-0.166
Tave05	-0.547	-0.021	NFFD03	-0.493	-0.251
Tave06	-0.486	0.005	NFFD04	-0.521	-0.207
Tave07	-0.478	-0.160	NFFD05	-0.523	-0.225
Tave08	-0.546	-0.148	NFFD06	-0.361	-0.243
Tave09	-0.551	-0.140	NFFD07	-0.306	-0.338
Tave10	-0.550	0.020	NFFD08	-0.351	-0.317
Tave11	-0.530	-0.102	NFFD09	-0.373	-0.339
Tave12	-0.515	-0.130	NFFD10	-0.418	-0.237
PPT01	0.187	-0.479	NFFD11	-0.490	-0.273
PPT02	0.277	-0.463	NFFD12	-0.407	-0.229
PPT03	0.227	-0.486	PAS01	0.273	-0.435
PPT04	0.199	-0.465	PAS02	0.315	-0.437
PPT05	0.252	-0.417	PAS03	0.327	-0.417
PPT06	0.389	-0.361	PAS04	0.370	-0.328
PPT07	0.295	-0.190	PAS05	0.495	-0.230
PPT08	0.327	-0.272	PAS09	0.472	-0.282

Table 9. Continued.

Variable	PC1	PC2	Variable	PC1	PC2
PAS10	0.419	-0.301	DD_0_sm	0.441	-0.154
PAS11	0.325	-0.424	DD_0_at	0.542	0.053
PAS12	0.264	-0.456	DD5_wt	-0.538	-0.102
Eref01	-0.218	0.012	DD5_sp	-0.528	-0.071
Eref03	-0.501	-0.153	DD5_sm	-0.536	-0.109
Eref04	-0.538	0.039	DD5_at	-0.564	-0.126
Eref05	-0.499	0.131	NFFD_wt	-0.446	-0.216
Eref06	-0.487	0.193	NFFD_sp	-0.525	-0.231
Eref07	-0.382	0.004	NFFD_sm	-0.361	-0.294
Eref08	-0.421	-0.001	NFFD_at	-0.432	-0.296
Eref09	-0.396	-0.010	PAS_wt	0.288	-0.449
Eref10	-0.454	0.172	PAS_sp	0.377	-0.370
Eref11	-0.399	-0.121	PAS_at	0.380	-0.387
CMD03	-0.297	0.148	Eref_wt	-0.218	0.012
CMD04	-0.313	0.336	Eref_sp	-0.561	0.027
CMD05	-0.362	0.366	Eref_sm	-0.451	0.059
CMD06	-0.443	0.317	Eref_at	-0.467	0.054
CMD07	-0.370	0.122	CMD_sp	-0.358	0.360
CMD08	-0.385	0.211	CMD_sm	-0.444	0.238
CMD09	-0.306	0.338	CMD_at	-0.287	0.351
CMD10	-0.201	0.353	MAT	-0.580	-0.082
CMD11	-0.149	0.166	MWMT	-0.487	-0.173
Tmax_wt	-0.497	0.148	MCMT	-0.534	-0.098
Tmax_sp	-0.535	0.084	TD	0.217	-0.055
Tmax_sm	-0.511	0.039	MAP	0.276	-0.485
Tmax_at	-0.525	0.106	MSP	0.358	-0.395
Tmin_wt	-0.476	-0.255	AHM	-0.350	0.401
Tmin_sp	-0.542	-0.219	SHM	-0.424	0.299
Tmin_sm	-0.406	-0.332	DD_0	0.560	0.074
Tmin_at	-0.463	-0.282	DD5	-0.554	-0.106
Tave_wt	-0.548	-0.085	NFFD	-0.483	-0.278
Tave_sp	-0.570	-0.049	bFFP	0.478	0.298
Tave_sm	-0.536	-0.106	eFFP	-0.348	-0.350
Tave_at	-0.567	-0.080	FFP	-0.442	-0.335
PPT_wt	0.228	-0.486	PAS	0.338	-0.422
PPT_sp	0.231	-0.480	EMT	-0.457	-0.272
PPT_sm	0.374	-0.306	EXT	-0.513	0.009
PPT_at	0.261	-0.486	Eref	-0.525	0.049
DD_0_wt	0.547	0.101	CMD	-0.439	0.325
DD_0_sp	0.553	0.037			

Modelling

The relationships between the climate and reproductive variables contained a lot of scatter (Figure 9). There was high variation within each variable. The average number of scales per cone did appear to show a slight negative parabolic relationship with PC1.

The initial model fit resulted in residual values that were normally distributed for the average number of scales per cone, and average cone length, but not for the average number of seeds per cone (AVG_SCALES: W = 0.988, P-value = 0.485; AVG_LENGTH: W = 0.992, P-value = 0.794; AVG_SEEDS: W = 0.966, P-value = 0.008). A square root transformation of the average number of seeds per cone resulted in normally distributed residuals (SQRT_SEEDS: W = 0.989, p-value = 0.559).

The model resulting from the MANOVA analysis was the following:

$$(\mathrm{AVG\_SCALES}, \mathrm{AVG\_LENGTH}, \mathrm{SQRT\_SEEDS}) \sim \mathrm{PC1}+\mathrm{PC2}+\mathrm{PROV}+\mathrm{PC1}:\mathrm{PC2} \quad (2)$$

The results of the MANOVA indicate that there were significant effects of provenance and the interaction of PC1:PC2 (Table 10). PC2 was close to being significant. The quadratic terms did not significantly contribute to describing the variation in the model. The significant interaction between PC1 and PC2 indicated that the climatic regimes of B.C. were influencing the reproductive traits of lodgepole pine in complex ways. The response variables met the assumption of multivariate normality for the MANOVA (W = 0.984, P-value = 0.250).

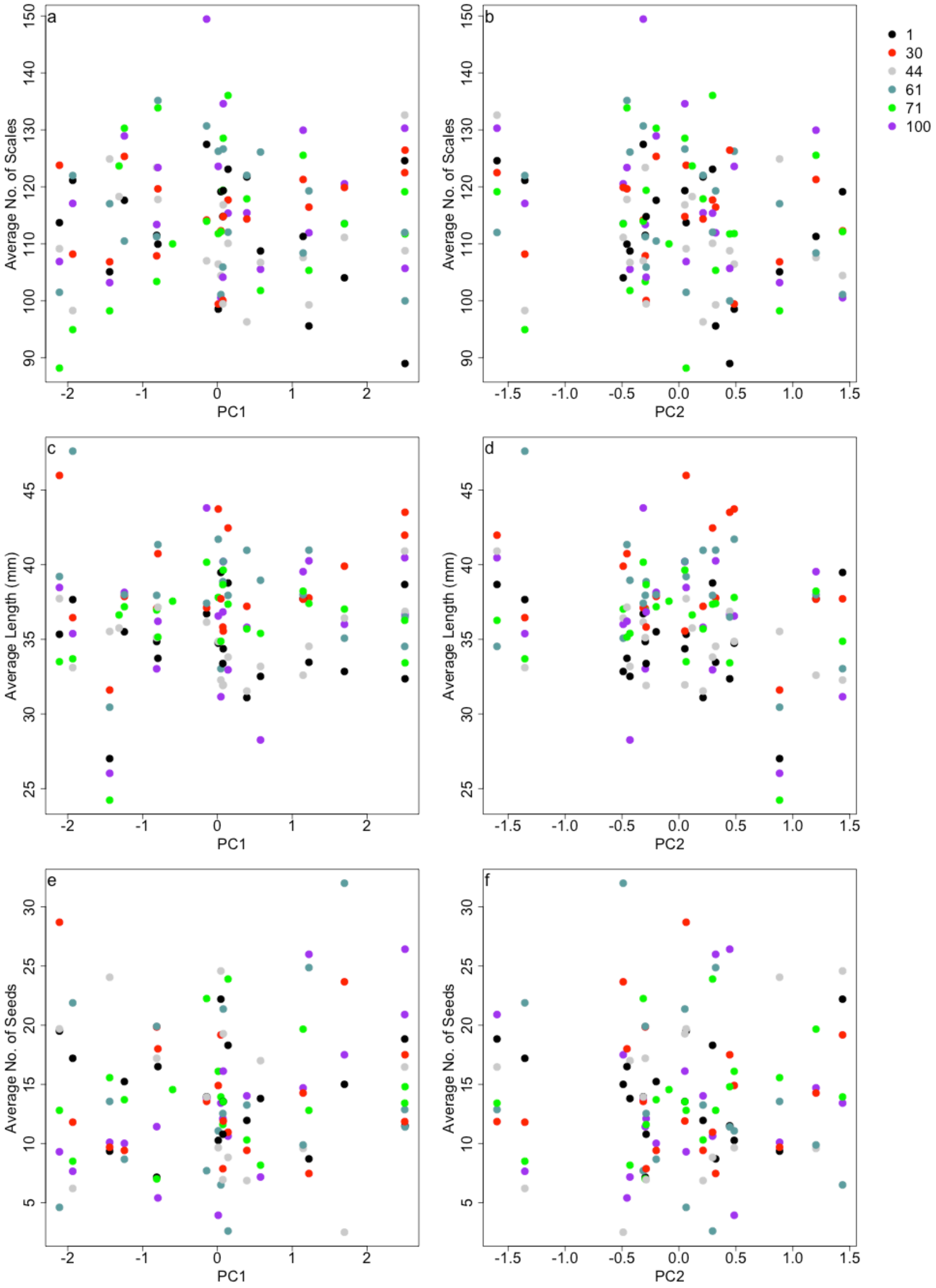

Figure 9. Scatterplots of PC1 (a-c) and PC2 (d-f) versus the average number of scales per cone, average cone length and the average number of seeds per cone, coloured according to provenance number

Table 10. ANOVA summary table of MANOVA analysis of reproductive traits according to the model: (AVG_SCALES, AVG_LENGTH, SQRT_SEEDS) ~ PC1 + PC2 + PROV + PC1:PC2

	Df	Pillai	Approx F	DF	den DF	P-value
PC1	1	0.0259	0.834	3	94	0.479
PC2	1	0.0765	2.60	3	94	0.057
PROV	5	0.316	2.26	15	288	0.005
PC1:PC2	1	0.0952	3.30	3	94	0.024
Residuals	96					

When the univariate responses of the reproductive traits were examined, further details emerged (Table 11). None of the terms in the model had significant effects on the average number of scales or seeds per cone. The significant terms in the model were all related to describing the variation in average cone length. Average cone length was significantly affected by provenance, matching the trend observed in the boxplots of average cone length, and by PC2. The significant interaction between PC1 and PC2 that was noted in the multivariate ANOVA was not significant for any of the individual traits.

Table 11. Univariate model results for the average number of scales per cone, average cone length and the average number of scales according to the model: (AVG_SCALES, AVG_LENGTH, SQRT_SEEDS) ~ PC1 + PC2 + PROV + PC1:PC2

	Df	**SS**	**MSS**	**F-value**	**P-value**
AVG_SCALES					
PC1	1	126	126	1.10	0.298
PC2	1	287	287	2.48	0.118
PROV	5	582	116	1.01	0.417
PC1:PC2	1	159	159	1.38	0.244
Residuals	96	11000	116		
AVG_LENGTH					
PC1	1	18.9	18.9	1.60	0.208
PC2	1	70.7	70.7	6.01	0.016
PROV	5	292	58.4	4.96	0.000
PC1:PC2	1	31.7	31.7	2.69	0.104
Residuals	96	1130	11.8		
SQRT_SEEDS					
PC1	1	1.15	1.15	1.76	0.188
PC2	1	0.089	0.0894	0.137	0.713
PROV	5	0.755	0.151	0.231	0.948
PC1:PC2	1	0.289	0.289	0.443	0.508
Residuals	96	62.8	0.654		

Linear models for individual reproductive traits using Formula 2 revealed that the reproductive traits were not necessarily modelled as closely as hoped (Table 12 and Figure 10). There were no significant terms in the model, and the model did not significantly explain the variation in the average number of scales per cone ($F_{8, 106} = 1.38$, P-value = 0.212, Adjusted $R^2 = 0.0262$). The residuals for the average number of scales met the assumption of normality using the Shapiro-Wilk test (W = 0.991, P-value = 0.690). Neither the model, nor any of the terms in the model were significant for explaining the variation in the average number of seeds per cone ($F_{8, 96} = 0.437$, P-value = 0.896, Adjusted $R^2 = -0.0453$). A square root of the average number of seeds per cone was taken in order to meet the assumption of normality (AVG_SEEDS: W = 0.966, P-value = 0.008; SQRT_SEEDS: W = 0.989, P-value = 0.559). The model did, however, significantly explain 21.0% of the variation in average cone length ($F_{8, 106} = 4.78$, P-value = 0.000, Adjusted $R^2 = 0.210$). Average cone length met the assumption of normality (W = 0.992, P-value = 0.757). This analysis also indicated that provenances 30 and 61, both originating from the northern part of the province, were influencing the significant effect on average cone length. The interaction between PC1 and PC2 was close to being significant for cone length. It appeared that the variation in the data was strongly influenced by cone length, leading to results that were only significant for this particular variable.

Table 12. Summary of linear models for the average number of scales per cone, average cone length, and the average number of seeds per cone according to the formula Y = PC1 + PC2 + PROV + PC1:PC2

	Estimate	Std. Error	t value	P-value
AVG_SCALES				
(Intercept)	112	2.47	45.5	0.000
PC1	-0.0201	0.829	-0.0240	0.981
PC2	-2.22	1.43	-1.56	0.122
PROV30	2.69	3.54	0.758	0.450
PROV44	-1.98	3.50	-0.566	0.573
PROV61	3.42	3.50	0.980	0.330
PROV71	1.81	3.41	0.531	0.597
PROV100	5.45	3.50	1.56	0.122
PC1:PC2	-1.10	0.904	-1.21	0.227
AVG_LENGTH				
(Intercept)	34.7	0.760	45.6	0.000
PC1	0.465	0.255	1.83	0.071
PC2	-1.33	0.438	-3.03	0.003
PROV30	4.19	1.09	3.84	0.000
PROV44	0.0950	1.07	0.0880	0.930
PROV61	3.60	1.07	3.35	0.001
PROV71	1.34	1.05	1.28	0.203
PROV100	1.34	1.07	1.25	0.214
PC1:PC2	0.520	0.278	1.87	0.064
SQRT_SEEDS				
(Intercept)	3.71	0.191	19.5	0.000
PC1	0.0656	0.0661	0.992	0.324
PC2	0.0580	0.109	0.531	0.597
PROV30	0.0161	0.270	0.059	0.953
PROV44	-0.170	0.283	-0.601	0.549
PROV61	-0.152	0.274	-0.557	0.579
PROV71	-0.0145	0.270	-0.054	0.957
PROV100	-0.181	0.266	-0.682	0.497
PC1:PC2	-0.0463	0.0695	-0.665	0.508

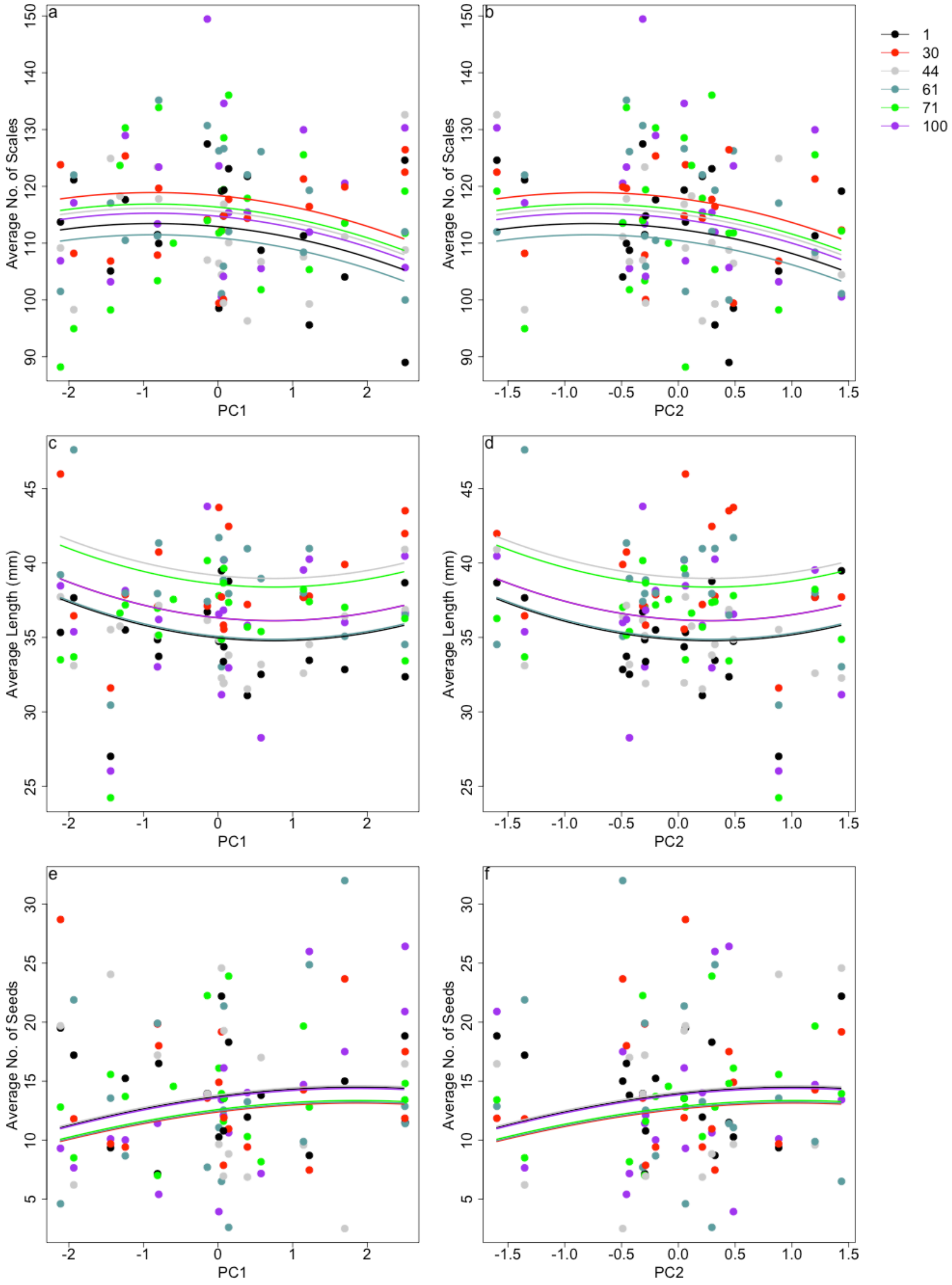

Figure 10. Scatterplots of PC1 and PC2 versus (a-b) average number of scales per cone, (c-d) average cone length, and (e-f) the average number of seeds per cone with curve shown for the model Y = PC1 + PC2 + PROV + PC1:PC2, coloured according to provenance number.

The shapes of the curves provide some information about the relationships with the reproductive variables. The model predicted a negative parabolic relationship between PC1 and PC2 and the average number of scales per cone, although the amount of variation explained was not significant. Similar to the average number of scales, the model also predicted a non-significant negative parabolic relationship between the climate variables and the average number of seeds. The relationship between PC1 and PC2 and average cone length was positively parabolic, indicating that larger cone lengths will occur at the more extreme ends of the climate spectrum. However, there was a large amount of scatter around the modelled curves. For all but the average number of scales, a quadratic model was not necessarily the best fit for the reproductive variables.

When the reproductive traits were modelled individually, the best-fit models varied for the three reproductive variables. The following formulas were determined to best describe the variation in the data:

$$\text{AVG\_SCALES} \sim \text{PC1} + \text{PC1}^2 + \text{PC2} + \text{PROV} \tag{3}$$

$$\text{AVG\_LENGTH} \sim \text{PC2} + \text{PROV} \tag{4}$$

$$\text{AVG\_SEEDS} \sim \text{PC1} + \text{PROV} + \text{PC1:PROV} \tag{5}$$

The residuals of all three models were found to meet the assumption of normality (AVG_SCALES: W = 0.991, P-value = 0.642; AVG_LENGTH: W = 0.980, P-value = 0.079; AVG_SEEDS: W = 0.980, P-value = 0.116). The models once again did not provide a large amount of predictive value for the data. For the average number of scales per cone, the terms PC1^2, and PC2 were significant, while the model was not quite significant, and only explained 4.9% of the variation in the data ($F_{8,\,106} = 1.74$, P-value = 0.098, Adjusted $R^2 = 0.0490$, Table 13). Similarly, the model was not significant for the average number of seeds per cone, and only the interaction between PC1 and PROV was significant ($F_{11, 93}$ = 1.41, P-value = 0.181, Adjusted R^2 = 0.0416). The model of average cone length was significant and explained 18.7% of the variation in the data ($F_{8, 106}$ = 5.38, P-value = 0.000, Adjusted R^2 = 0. 187). Significant terms in the model included PC2 and PROV.

The models determined through univariate analysis, though not necessarily highly predictive, were informative about the nature of the variation in the data (Figure 11). The model for the average number of scales per cone was negative and parabolic. The curves had maxima at approximately PC1= -1, and PC2 = -0.5. While there were no sites for this value for PC2, the sites Lassie Lake and Bateman Creek were approximately located at the maximum point of PC1. The position of the maxima indicated that moderate temperatures were ideal for cones to develop larger numbers of scales. The model for average cone length was linear with a negative slope relative to PC2. The provenances followed the same pattern with no interaction effects. The model predicted that wetter sites would have longer cones than drier sites. The model for the average number of seeds per cone was linear. Provenances 1, 44, 71 and 100 all had small positive slopes, while provenance 30 had a steep positive slope, and provenance 61 had a negative slope. As this model was not significant overall, and the only significant term was the interaction between PC1 and PROV, it was difficult to gain insight from the model results.

Table 13. ANOVA results for the linear models AVG_SCALES ~ PC1 + PC1^2 + PC2 + PROV, AVG_LENGTH ~ PC2 + PROV, and AVG_SEEDS ~ PC1 + PROV + PC1:PROV.

	Estimate	Std. Error	t value	P-value
AVG_SCALES				
(Intercept)	114	2.61	43.8	0.000
PC1	0.830	0.806	1.03	0.306
PC1_2	-1.11	0.552	-2.01	0.047
PC2	-3.82	1.47	-2.60	0.011
PROV30	2.81	3.50	0.803	0.424
PROV44	-1.92	3.46	-0.554	0.581
PROV61	3.42	3.45	0.991	0.324
PROV71	1.84	3.37	0.545	0.587
PROV100	5.45	3.45	1.58	0.118
AVG_LENGTH				
(Intercept)	34.7	0.771	45.1	0.000
PC2	-1.11	0.428	-2.58	0.000
PROV30	4.18	1.10	3.78	0.000
PROV44	0.0786	1.09	0.0720	0.943
PROV61	3.60	1.09	3.31	0.000
PROV71	1.29	1.06	1.22	0.227
PROV100	1.34	1.09	1.23	0.220
SQRT_SEEDS				
(Intercept)	3.72	0.183	20.3	0.000
PC1	-0.0317	0.140	-0.226	0.822
PROV30	0.0210	0.259	0.0810	0.935
PROV44	-0.164	0.271	-0.604	0.548
PROV61	-0.165	0.263	-0.629	0.531
PROV71	-0.0174	0.258	-0.067	0.947
PROV100	-0.223	0.255	-0.875	0.384
PC1:PROV30	-0.0112	0.197	-0.0570	0.955
PC1:PROV44	-0.193	0.217	-0.891	0.375
PC1:PROV61	0.194	0.198	0.982	0.329
PC1:PROV71	0.119	0.201	0.589	0.557
PC1:PROV100	0.476	0.196	2.42	0.017

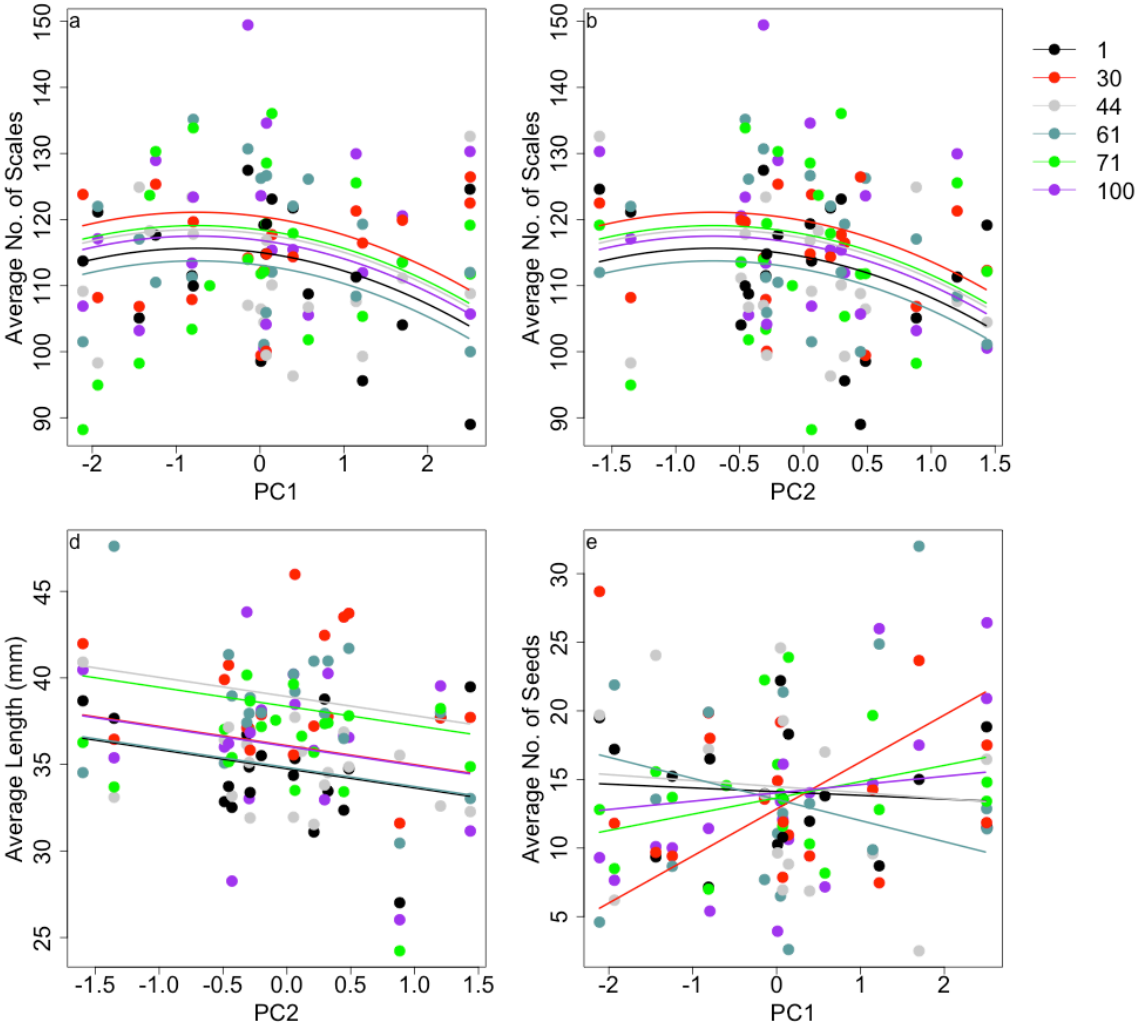

Figure 11. Scatterplots of (a-b) PC1 and PC2 and average number of scales per cone, (c) PC2 and average cone length, and (d) PC1 and the average number of seeds per cone with curves shown for the model according to formulas 3, 4, and 5, coloured according to provenance number.

Chapter 3: Discussion

Interpretation of results

We hypothesized that the variation in climate across the interior of B.C. would significantly relate to variation in reproductive traits in lodgepole pine. The results of the study indicate that climate is not the most important factor in predicting reproductive characteristics such as cone length, and the number of scales and seed per cone. The low explanatory values of the models resulting from this analysis leave most of the variation in reproductive traits still unexplained. Climate information and differences between provenances were not adequate in explaining the variation in the reproductive variables.

The measured reproductive traits remained relatively stable across climate regimes, and between populations. This stability is somewhat surprising, as other lodgepole pine traits such as growth and survival vary widely across the climate regimes represented by the Illingworth trial (see Rehfeldt et al., 1999; Wu et al., 2005; Wang et al., 2006; O'Neill et al., 2008). Height in particular can be modeled well using the mean annual temperature of study sites (Wang et al., 2006). The difference between the strong variation in growth traits, and the relative stability of reproductive traits is the most substantial finding of this study.

Our results support the findings by Caron and Powell (1989) for positive correlations between cone traits and seed. This is somewhat expected, as a greater number of scales per cone will increase the potentiality for a greater number of seeds. However, even more important appears to be the overall cone length, in this study taken as an indicator of cone size. Cone length was slightly more closely correlated with the number of seeds. After having extracted thousands of seeds, I confirmed lab observations that larger cones have a greater number of seeds. Scales on longer cones may have the potential to be longer and larger themselves. While this is untested, cones with larger scales may have more fertile scales. Scale size, and its impact on the potential for fertile ovules, both unaddressed in this study, may be the missing link between cones and seed yield.

Negative parabolic relationships have been identified as being biologically relevant for lodgepole pine characteristics such as height and survival (Wang et al., 2006). A negative parabolic relationship indicates that a response variable will achieve a maximum at certain optimal values in the predictor variable, while extreme values of the predictor will lead to minimum values, or even zero. This relationship was weakly identified as being significant for the average number of scales per cone, but explained a very small portion of the total variation. There was no significant variation in the number of scales per cone between provenances, when modelled with climate. The six provenances appeared to produce relatively uniform numbers of scales per cone across a climate gradient.

Average cone length was, however, found to vary between provenances. Two of the three northern provenances 30, Lower Post, and 61, Purden Lake, were identified as having average cone lengths that were significantly longer than the other provenances. Northern provenances were, by design, transferred across the longest distance in a southerly direction. With the exception of provenance 100, the southern transfer direction may have released the trees in these provenances from the environmental pressures of their native northern origins, and allowing them to produce larger cones in less severe environments.

In addition to differences between provenances, average cone length was significantly affected by the principal component mostly closely related to moisture. Considering this result in combination with the moderate positive correlation between cone length and number of seed suggests that, while not identified as having significant effects on seed number, higher moisture levels could theoretically lead to more seeds per cone. Wetter sites were predicted to have longer cones. Longer cones may have larger scales leading to more seed per cone.

The variation in the number of seeds per cone, while one of the main foci of the study, remained stable across the study sites. There were several sites, provenances and site:provenance interactions that were significantly related to different average numbers of scales per cone and average cone lengths. However, none of these effects were strong enough to impact the average number of seeds per cone, and these effects explained little of the variation in average seed number. Sites such as 70 Mile, with very short cones, and Freeman Creek, with large numbers of scales per cone, did not stand out as having significantly different numbers of seed. There were no sites that stood out as significantly different. The strongest piece of evidence regarding variation in seed numbers relates to a significant interaction effect between the first principal component and provenance, with provenance 100, Nina Creek, however, the variation explained by the model is small. The average number of seeds per cone remained relatively stable regardless of provenance or climate.

Stability in tree characteristics across environments is a major goal in tree improvement programs. The occurrence of genotype (or population) by environment-type effects complicates tree improvement programs, as the relative rankings of populations or varieties can change when planted in different environments. These changes in performance can be expensive when these effects result in lower yields or high tree mortality (Burdon, 1977). Our finding of stability in numbers of seed per cone indicate that population by environmental effects have little impact in this regard.

A study of the geographic variation of cone and seed traits in white pine (*Pinus strobus*) found that the provenance of samples had a significant effect on the variation in reproductive traits, but was not the major source of variation. Rather, between and within tree variation far exceeded variation between populations (Beaulieu and Simon, 1995). Out study did not test the variation within tree, but our results did support the finding that provenance has a significant but not major impact on reproductive variation. Provenance explained only a small portion of the variation in the measured traits. The same study also found that the number of scales per cone varied considerably more than cone length between populations. The source population was found to significantly affect the number of seeds per cone (Beaulieu and Simon, 1995). These effects were not replicated for the lodgepole pine provenances included in our study. However, much like lodgepole provenances 30, 61, and 100, the northernmost population white pine population included in the study was also the most morphologically different (Beaulieu and Simon, 1995).

One area of lodgepole pine reproduction that has been the focus of a concentrated research effort is the effect of the seed predators, such as red squirrels (*Tamiasciurus hudsonicus*) and red crossbills (*Loxia curvirostra*), on lodgepole pine seeds and cones. Benkman (1999) argued that abiotic factors were unlikely to have as strong an impact on cone and seed traits as seed predation. Instead, the pattern of presence and absence of these two seed predators were found to better describe the differences in reproductive traits between populations. Seed and cone trait differences between populations were attributed to the evolution of predator defenses, such as larger and wider cones, fewer seeds per cone, and thicker scales, particularly thicker basal scales as a defense against red squirrels. Unlike our study, Benkman's (1999) found that cone length did not vary between the sample populations. Additionally, scale and seed number were not included in the analysis (Benkman, 1999).

Our study did not include information about seed predation, or the subsequent evolutionary responses to predation, that may have resulted in selective pressures or defense responses at the provenance source locations. Therefore, it is not clear whether predation could have contributed to the significant effect of provenance on cone length. In addition, Benkman's (1999) study did not examine trees planted outside of their natural population range. Therefore, the population level cone and seed responses to different environmental conditions in combination with predation pressure are unknown.

Coniferous tree species maintain large amounts of genetic variation within populations. This variation may allow reproductive traits to remain stable across geographic gradients. Several aspects of lodgepole pine biology contribute to maintaining genetic variability. Wind-dispersed pollen can travel long distances. Lodgepole pine has high outcrossing rates, and a general intolerance of selfing. Like pollen, coniferous seed can also undergo long distance dispersal (Wheeler and Guries, 1982). Lodgepole pine and jack pine have the smallest seeds of any pines, making their seed the most readily dispersible. It has been argued that lodgepole pine trees may be able to establish at distances exceeding 10 kilometers from their parents (Critchfield, 1980). These factors all combine to decrease the genetic variation among populations. In addition, the ability to respond plastically to environmental changes has equipped lodgepole pine with the capacity to occupy many different environments (Rehfeldt et al., 1999; Wu and Ying, 2001). Plasticity may contribute to lessening the selective pressures of the environment on reproductive characteristics of lodgepole pine.

Lodgepole pine trees reproduce regularly. The species does not display mast years for cone production (Critchfield, 1980; Smith and Balda, 1979). Serotinous cones allow trees to build up a canopy seed bank over time. Seeds are released when the resin on cones melt and cones are opened due to the heat from forest fires (Critchfield, 1980). Mitton et al. (1981) examined the relationships between heterozygosity and growth variability. High heterozygosity in lodgepole pine was found to correlate with lower variation in annual ring width growth rates, whereas in ponderosa pine (*Pinus ponderosa*) high heterozygosity correlated with high variation in annual ring width. In contrast to the highly regular annual reproduction of lodgepole pine, annual cone production in ponderosa pine is highly variable. The observed regular annual ring growth in lodgepole pine may be related to low annual variability in cone production, as resource partitioning between biological functions could remain relative stable over time. One theory is that high heterozygosity leads to the full expression of a species' reproductive frequency, causing the observed differences between ponderosa and lodgepole pines (Mitton et al., 1981). It is possible that the same processes that are regulating annual ring width and annual cone production may also regulate cone morphology and seed yield.

The Okanagan Seed Orchard Problem

One of the aims of this project was to help to explain the problem of low seed yields at Northern Okanagan seed orchards. While our results show stability of seed numbers across the measured climates, instability is the current status of the lodgepole pine seed production industry in the Northern Okanagan. The relationships between cone length and moisture and cone length and seed number may shed some light on the problem. The Okanagan is a very hot dry region, particularly for B.C. The low moisture in this region may be part of the problem in regards to low seed yields.

The Okanagan problem, however, remains unresolved. It is nearly impossible to relate the results of this analysis to the current situation at the Okanagan seed orchards. Strong relationships between the reproductive traits and the climate variables would allow us to speculate on the optimal conditions for seed production, even at the extreme ends of the climate spectrum. However, the models have very little predictive value. In addition, none of the Illingworth trial sites are directly comparable to the climatic conditions at the seed orchards. There are no Illingworth sites representative of the hot, dry, low elevation conditions at the seed orchards, therefore none of the results from individual sites can be used to infer the causes of the seed yield problem.

Study Limitations

The main goal of the study was to determine the relationships between climate and reproduction characteristics in lodgepole pine.

The Illingworth trial offers an even aged experimental design that is one of the most comprehensive provenance trials in the world. However, initial site selection covered a narrow range of mean annual temperatures. More recent provenance research has made an attempt to represent extreme high and low temperature within trial design in order to identify relationships with tree characteristics across the climate spectrum. These extremes help to determine limits in biological traits.

The lack of extreme climate representation in the initial trial design, combined with 40 years of natural forest processes have lead to a limited present day availability of sites with an adequate number of living trees. The mountain pine beetle epidemic has contributed to this problem by increasing tree mortality at many sites. These factors reduced the number of trees available to sample at the sites, and limited the number of sites that remain from which to sample.

While newer cones were the focus of sampling, cones of unknown and various ages were collected. Nine years of climate data was averaged in order to represent an estimated range of development years for all cones. Cone development in lodgepole pine occurs over the course of three consecutive years, therefore some of the climate data came from years before cone initiation, or after development was completed.

One insight gained from the study is that provenance trials are not necessarily best suited for reproductive research in lodgepole pine. Unlike growth and survival traits, which are, by nature, cumulative over time, reproduction occurs over a much shorter cycle. Research in this area, therefore, necessitates known time periods over which development has occurred. As lodgepole pine produces serotinous cones that are retained in the canopy, indefinitely in some cases, it is virtually impossible to obtain a sample of cones for which the years of development for each cone is accurately known.

Seed orchards offer the most easily accessible collections of trees from which to collect cones of known ages. Seed orchards also offer the opportunity to sample from ramets of different genotypes that may be planted in several locations. At the same time, seed orchards have a limited geographic distribution. Sampling at seed orchards would restrict the climate regimes available for analysis and the overall sample size for the study. The information gained from a study based on seed orchard samples would be relevant only for specific locations.

Perspectives

There are a number of different approaches that could be taken to look at the wide-scale climate effects on seed production in lodgepole pine. A broader range of climatic extremes would allow us to more realistically describe the full extent of the natural variation in lodgepole pine populations. Ideally, samples would be taken at the limits of the range of lodgepole pine. This would require sampling outside of B.C., such as in California and Yukon Territory. The Illingworth trial as it stands presently does not adequately address the extreme environments that lodgepole pine can occupy.

A finely tuned examination of climatic impacts on reproductive traits would require that the years in which cones developed be accurately known, allowing the most accurate climate data to be used in analysis. Although the number of seeds per cone did not vary significantly between sites, regardless of the climatic impacts, it may be valuable to analyze cones with respect to the specific time period in which they developed.

Other cone or seed traits may have yielded more information about the effects of climate on reproductive variation. In particular, some measure of scale size, how this varied with climate, and its relation to seed number may be worth investigating. Scale thickness, which has been shown to vary with regard to the presence or absence patterns of seed predators, could be measured (Benkman, 1999). The number of fertile scales per cone would also help to determine the link between scale traits and seed.

The high genetic variation in lodgepole pine populations in B.C. may be the cause of the stable reproductive trait measurements. Testing for heterozygosity in lodgepole pine, and comparing this information with annual growth, annual cone production, cone morphology, and seed yield may indicate if increasing heterozygosity of alleles leads to increased stability of reproductive characteristics, as was previously found for annual ring growth. The seed orchards outside of the Okanagan, such as those in the Prince George region, could be potential sites for this type of analysis. The orchards would provide the ability to sample many genotypes, and information from several years of collections could be combined for a long-term study.

Conclusions

Our hypothesis for this study was that climate would have a significant effect on reproductive characteristics in lodgepole pine. I identified the strongest relationship between climate and the reproductive variables to be between average cone length and moisture, with significant variation also identified between provenances. However, this relationship did not explain a majority of the variation in cone length. I found that the reproductive traits remained relatively stable across the climate gradients represented by the study sites. The root cause of this stability is unknown. High genetic variation in lodgepole pine populations, due to long distance pollen and seed dispersal, high out-crossing rates, and an intolerance of selfing, may be contributing to the stability of reproductive traits. Lodgepole pine female cone and seed traits are stable in 40-year old trees over a wide range of provenances and climate regions.

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