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Changes in Genetic Diversity of Whitebark Pine (*Pinus albicaulis* Engelm.) Associated with Inbreeding and White Pine Blister Rust Infection

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Abstract

We investigated the association of inbreeding and infection by the introduced disease white pine blister rust (caused by the fungus *Cronartium ribicola* J. C. Fisch) with genetic diversity of whitebark pine (*Pinus albicaulis* Engelm.) by genetically comparing cohorts of different ages in natural stands. Isozyme analysis of bud tissue was used to estimate expected and observed heterozygosity (H_e and H_o), and Wright's fixation index (F_{is}) for three age cohorts (seedling, young, and mature), sampled from 14 sites in British Columbia, Oregon, Idaho, and Montana. Comparison of genetic diversity parameters among cohorts within a site was used to assess the extent and persistence of inbreeding with age, while comparisons of parameters among sites within a cohort were used to assess the impact of the disease on genetic diversity. Significant evidence of inbreeding ($F_{is} > 0$) was found in all age cohorts. When sites were stratified by level of blister rust infection, differences in F_{is} and H_o among cohorts were only significant when level of infection was low. A significant negative association was found between level of blister rust infection and H_o in the mature cohort. This suggests that when differential selection due to blister rust is weak, more heterozygous individuals may be favored; however, more

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homozygous individuals may have higher fitness under higher blister rust levels

Key words: whitebark pine, *Pinus albicaulis*, white pine blister rust, genetic diversity, isozymes, inbreeding, cohort analysis, heterozygosity.

Introduction

Exotic diseases can have a devastating impact on populations that have not coevolved with pathogens and have little or no natural resistance, potentially leading to rapid declines in effective population size and loss of genetic variation. They pose unusual challenges to conservation because of their potential to drive rapid changes in host abundance and genetic composition (ALTIZER et al., 2003). Species in many genera of forest trees have been severely impacted by introduced diseases (e.g. *Pinus*, *Chamaecyparis*, *Quercus*, *Castanea*, *Ulmus*, *Fagus*, *Juglans*, *Cornus*) (BINGHAM et al., 1971; LEDIG, 1991; DAUGHTREY ans HIBBEN, 1994; MALONEY et al., 2005; LOO, 2009), but data on the effects of these diseases on genetic structure and diversity is sparse. It is well known that disease can have a significant impact on both individual species and on the structural composition of forests (BURDON et al., 2006); however, empirical demonstrations of the effect of diseases on host demographics are difficult to conduct (ALEXANDER et al., 1996). In addition, studies of the effects of diseases on genetic diversity are difficult because baseline, pre-epidemic genetic data are impossible to collect after a disease has swept through (MCDONALD et al., 1998).

Conifer populations often show increasing levels of heterozygosity with age, as embryos typically have a deficiency of heterozygotes, yet populations of mature trees usually have an excess (PLESSAS and STRAUSS, 1986; POLITOV et al., 1992; LEDIG et al., 2000). The excess homozygosity in seeds is most likely due to inbreeding (LEDIG et al., 2000), including self-pollination and consanguineous matings, and selection against inbreds is the most likely reason for the reduction in F_{is} with age (KRUTOVSKII et al., 1995). For example, a study of Bosnian pine (*Pinus leucodermis* Ant.), which has reported selfing rates in the same range as whitebark pine (18–28%), found that inbred individuals were not efficiently selected against during seed maturation and germination, but were eliminated by age five (MORGANTE et al., 1993). A heterozygote deficiency may be the result of genetic substructuring within stands producing a Wahlund effect, but in most cases, inbreeding appears to be the primary cause and this declines with age due to selection over time against more homozygous inbred individuals (PLESSAS and STRAUSS, 1986).

Whitebark pine (*Pinus albicaulis* Engelm.) experiences inbreeding from selfing, and to a lesser extent, consanguineous mating (KRAKOWSKI et al., 2003; BOWER and AITKEN, 2007). In addition, the inbreeding coefficient (F_{is}) of seedlings is higher than that of mature trees, indicating selection against inbred individuals over time (BOWER and AITKEN, 2007). A previous study reported that F_{is} decreased and observed heterozygosity (H_o) increased from west to east in whitebark pine in British Columbia (KRAKOWSKI et al., 2003). Stand-level infection by the introduced disease white pine blister rust (caused by the fungus *Cronartium ribicola* J. C. Fisch.) in British Columbia also increases from west to east (ZEGLEN, 2002), although infection varies widely among stands even within a mountain range (CAMPBELL and ANTOS, 2000). Trends in genetic diversity observed in British Columbia populations could be the result of either postglacial colonization patterns or selection by the disease against more homozygous, inbred individuals (KRAKOWSKI et al., 2003). However, in that study, any potential effects of inbreeding are confounded with the level of disease, due to the field sampling design.

The goals of this study were to examine the individual effects of inbreeding and blister rust infection on genetic diversity of whitebark pine. The effects of inbreeding were assessed by comparing F_{is} and H_o among three age cohorts within populations, and the effects of blister rust were assessed by comparing these genetic diversity values for a given cohort among populations distributed across a wide geographic area.

Materials and Methods

Sample Materials

Thirty individuals from each of three age cohorts were sampled from 14 sites in British Columbia, Idaho, Montana, and Oregon in 2002 and 2003 (*Table 1* and *Figure 1*). The seedling cohort was represented by three-year-old trees grown from seed collected from natural populations at these sites and grown in a common garden experiment in Vancouver, British Columbia. The young and mature cohorts were sampled in the field from the same natural stands where seeds were previously collected for the common garden experiment. The young cohort comprised trees estimated to be less than 30 years old, while the mature cohort included larger, older trees of reproductive age. To assess any potential genetic differences between putatively resistant (clean) and susceptible (infected) trees, the health status of each individual was recorded. Whitebark pine often grows in multistemmed clusters, and without genetic analysis, it is impossible to determine if these comprise one or more genotypes (TOMBACK and SCHUSTER, 1994). Therefore, the stems in each cluster were conservatively regarded as comprising a single genotype and only a single stem was sampled from each cluster. If any stems in a sampled cluster were infected, that sample was considered to be from an infected individual. Buds were sampled from all individuals in both the field and the common garden experiment, and placed separately into separate plastic vials for transportation and subsequent storage at –80°C.

A disease infection survey was performed on each site to estimate the level of white pine blister rust infection. The survey protocol generally followed SMITH and HOFFMAN (2000). Strip transects were used to estimate stand infection levels by categorizing 50 trees on each site as 1) alive and clean: no evidence of blister rust; 2) infected and alive: alive with cankers, flagged or dead branches, or dead stems in multiple stemmed clumps with some living stems; 3) infected and dead: dead with clear evidence of blister rust cankers; or 4) dead unknown: dead

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but unable to determine cause of death. Four to six transects were assessed on most sites, except one site with very low whitebark pine density, where only one transect was completed which encompassed most of the trees on this site (Lunch Peak). Infection percentage was calculated as: # of infected trees (both alive and dead)/ (total # of trees assessed – # dead from unknown causes). The mean infection percentage over all transects on a site was used to estimate the level of blister rust infection. For comparative purposes, sites were classified by infection level as low (<50% infection, $n=5$), moderate (50–75% infection, $n=5$), or high (>75% infection, $n=4$).

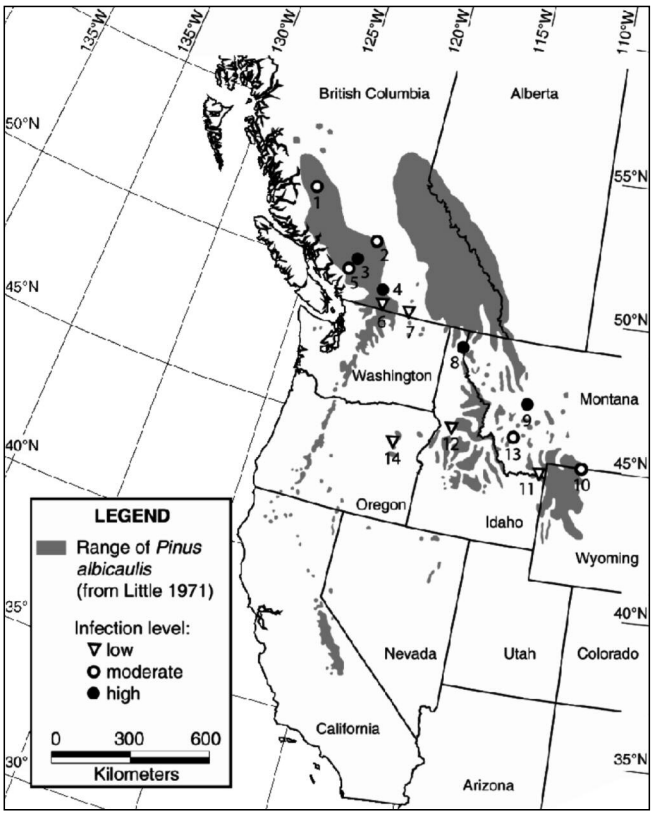

Figure 1. – Map of the range of whitebark pine and locations and level of blister rust infect of 14 sample locations. Infection level: low = <50%, moderate = 50–75%, high = >75%.

Isozyme Analysis

Whole buds were ground in a buffer slightly modified from MITTON et al. (1977) and the supernatant absorbed onto 3 x 15 mm Whatman filter paper wicks. Samples were analyzed using starch gel electrophoresis on gels of 10.5% starch and 7.5% sucrose (w/v). Two buffer and electrode systems were used to analyze 10 loci as follows: 1) lithium borate/tris-citrate at a pH of 8.3 (SELANDER et al., 1971) was used for *PGI* (E.C. 5.3.1.9), *SKDH 1, 2* (E.C. 1.1.1.25), *PGM* (E.C. 2.7.5.1), and *TPI* (E.C. 5.3.1.1); 2) histadine-EDTA (CHELIAK and PITEL, 1984) was used for *IDH* (E.C. 1.1.1.42), *MDH* 1, 2 (E.C. 1.1.1.37), *GDH* (E.C. 1.4.1.3) and *ALD* (E.C. 4.1.2.13). Gels were electrophoresed with wicks in place at half voltage for 30 minutes, then wicks were removed and the lithium borate gels run at 300 V and the histadine-EDTA gels run at 225 V. Electrophoresis continued until a marker dye reached to within 1 cm of the edge of the gel. Gels were sliced and stained using recipes as detailed in CHELIAK and PITEL (1984), then fixed in a mixture of 1:5:5 glacial acetic acid:distilled water: methanol.

Data Analysis

The Fstat program (GOUDET, 1995) was used to calculate expected (H_e) and observed (H_o) heterozygosities and Wright's fixation index (F_{is}) (WRIGHT, 1951) for each cohort on each site. WEIR and COCKERHAM's (1984) Θ (F_{st}) and f (F_{is}) were estimated with Fstat for each cohort, and for sites grouped by infection level within each cohort. Fstat calculates F-statistics per locus and globally over all loci for a site or age cohort (GOUDET, 1995).

SAS Version 8 (SAS INSTITUTE, 1999) was used for all statistical analysis. Means and standard errors were calculated for genetic parameters, and the differences among cohorts tested using PROC GLM for one-way analysis of variance (ANOVA) with a Duncan's multiple range test. The ANOVA was also conducted separately on groups of sites stratified by level of infection. Simple regression with PROC REG was used to determine if there was a significant association between level of blis-

Table 1. – Study site names, geographic locations, and white pine blister rust infection.

No.[a]	Site Name	State/ Prov.	Lat. (°N.)	Long. (°W.)	Elev. (m)	Infection %	# mature clean	# mature infected
1	Heckman Pass	BC	52.5	125.8	1525	57.0	2	28
2	Jesamond	BC	51.3	121.8	1850	55.4	12	18
3	D'Arcy	BC	50.5	122.6	1800	81.0	6	24
4	Thynne Mt.	BC	49.7	120.9	1785	82.5	11	19
5	Blackcomb	BC	50.1	122.9	1900	71.0	20	10
6	Manning Park	BC	49.1	120.7	2000	40.0	13	17
7	Mt. Baldy	BC	49.2	119.3	2150	44.3	11	19
8	Lunch Peak	ID	48.4	116.2	1850	81.6	5	25
9	Granite Butte	MT	46.9	112.5	2340	82.2	3	27
10	Hellroaring	MT	45.0	109.4	3000	70.0	8	22
11	Sawtel Peak	ID	44.5	111.4	2400	47.5	16	14
12	Gospel Hill	ID	45.6	115.9	2100	45.5	8	22
13	Quartz Hill	ID	45.7	112.9	2650	62.7	12	18
14	Vinegar Hill	OR	44.7	118.6	2340	30.1	26	4

[a] Refers to number on *figure 1.*

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ter rust infection and site latitude and longitude or genetic parameters.

Paired t-tests were performed on data from the mature cohort to determine if clean and infected trees paired by site differed in H_o and F_{is}. Sites varied in the number of clean and infected trees in this cohort (*Table 1*). To eliminate potential confounding due to variation in sample size between clean and infected trees on each site, F_{is} was calculated as 1- (H_o for the group of clean or infected trees/H_e calculated for all trees on the site). The t-test was limited to sites that had a minimum of four individuals in each group.

Results

Significant inbreeding ($F_{is} > 0$ at $\alpha = 0.05$) was detected in all cohorts when analyzed across all sites, even when sites were stratified by level of rust infection (*Figure 2*). Across all sites, differences in mean F_{is} values were marginally significant among cohorts ($p = 0.056$) but in the multiple range test, only means of the seedling and young cohorts were significantly different at $\alpha = 0.05$. By locus, the difference among cohorts was only significant for SKD 1 ($p = 0.014$), with only the seedling and young cohort differing. Differences in H_o among sites were not significant when analyzed over all loci, but differed significantly for PGM and SKD 1 when loci were analyzed individually. In both cases, the seedling cohort differed from both the young and mature cohorts, When sites were stratified by the level of rust infection, the mean of the seedling cohort was significant different from the young and mature cohorts for both F_{is} ($p = 0.025$) and H_o ($p = 0.039$) when the rust level was low. However, no differences were detected among cohorts when the rust level was moderate or high (*Figures 2* and *3*).

When populations were analyzed by mountain range, average H_e and H_o were slightly higher in the Coast Range (populations 1–7 in *Figure 1*) ($H_e = 0.222$ and $H_o = 0.188$) than in the Rocky Mountains (populations 8–14 in *Figure 1*) ($H_e = 0.210$ and $H_o = 0.177$). F_{is} was higher in the Rocky Mountains than in the Coast Range (0.156 vs. 0.144); however, no differences were significant in T-tests of group means. No significant associations were found between stand level blister rust infection and latitude ($R^2 = 0.081$, $p = 0.324$) or longitude ($R^2 = 0.003$, $p = 0.843$). Regressions of the genetic parameters on latitude were only significant for H_e in the young cohort ($R^2 = 0.334$, $p = 0.031$), and all regressions on longitude were not significant ($p > 0.05$). Regressions of the genetic parameters for each of the cohorts with blister rust infection levels showed a significant association only for H_o in the mature cohort ($p = 0.018$). Plots of F_{is} and H_o versus infection percentage indicated that the association between F_{is} and infection increased with cohort age while the association of H_o with infection decreases (*Figures 4a–f*). *Figure 4d* shows one outlier site with high infection but low H_o. This point represents Lunch Peak (site #8 *Figure 1*, *Table 1*). This site had a very low density of whitebark pine, with large distances between trees. Only a single infection survey transect could be performed on this site because of the small population size. It is likely that the low density and wide spacing among whitebark pine trees at this site resulted in a higher rate of selfing. This is reflected in the high F_{is} (*Figure 4a* – filled circle and *table S1*-supplimentary data) and low H_o values (*Figure 4d* – filled circle and *table S2* – supplementary data) in the seedling cohort. Cook's distance can be used to measure the influence of a data point on a regression (WEISBERG, 1985), and the Cook's distance value for H_o from this site was an order of magnitude higher than most of the other sites, indicating that this outlier had a large influence on the regression. When this data point was removed from the analysis, the regression of H_o on infection percentage for the seedling cohort was also significant ($p = 0.033$), but with a positive slope (*Figure 4d*).

Comparison of clean and infected trees within a site showed that for nine of the fourteen sites, H_o was higher on average in the infected trees than in clean trees. However, paired T-tests for both F_{is} and H_o showed that the differences between the group means were not sta-

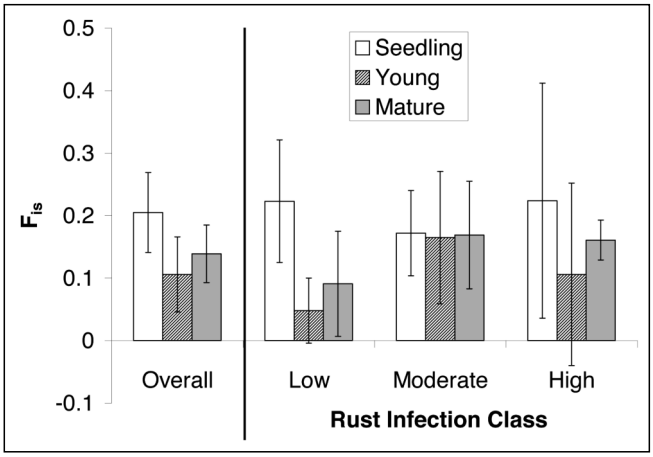

Figure 2. – Mean fixation index (F_{is}) by cohort for low, moderate, and high rust infection level sites (error bars are 95% confidence intervals determined by bootstrapping over loci 1000 times in Fstat).

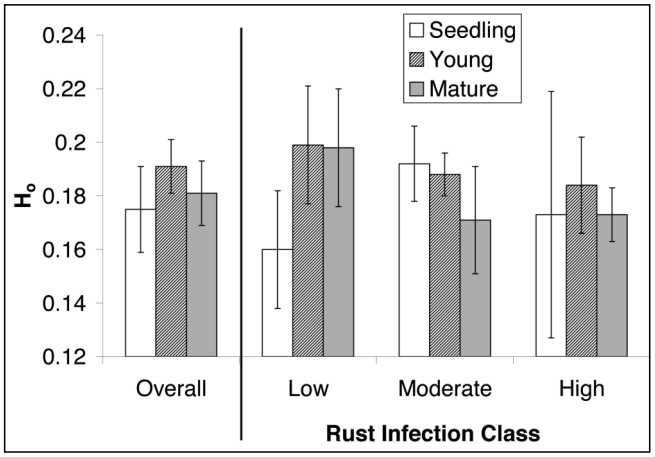

Figure 3. – Mean observed heterozygosity (H_o) by cohort for low, moderate, and high rust infection level sites (error bars are 95% confidence intervals determined by bootstrapping over loci 1000 times in Fstat).

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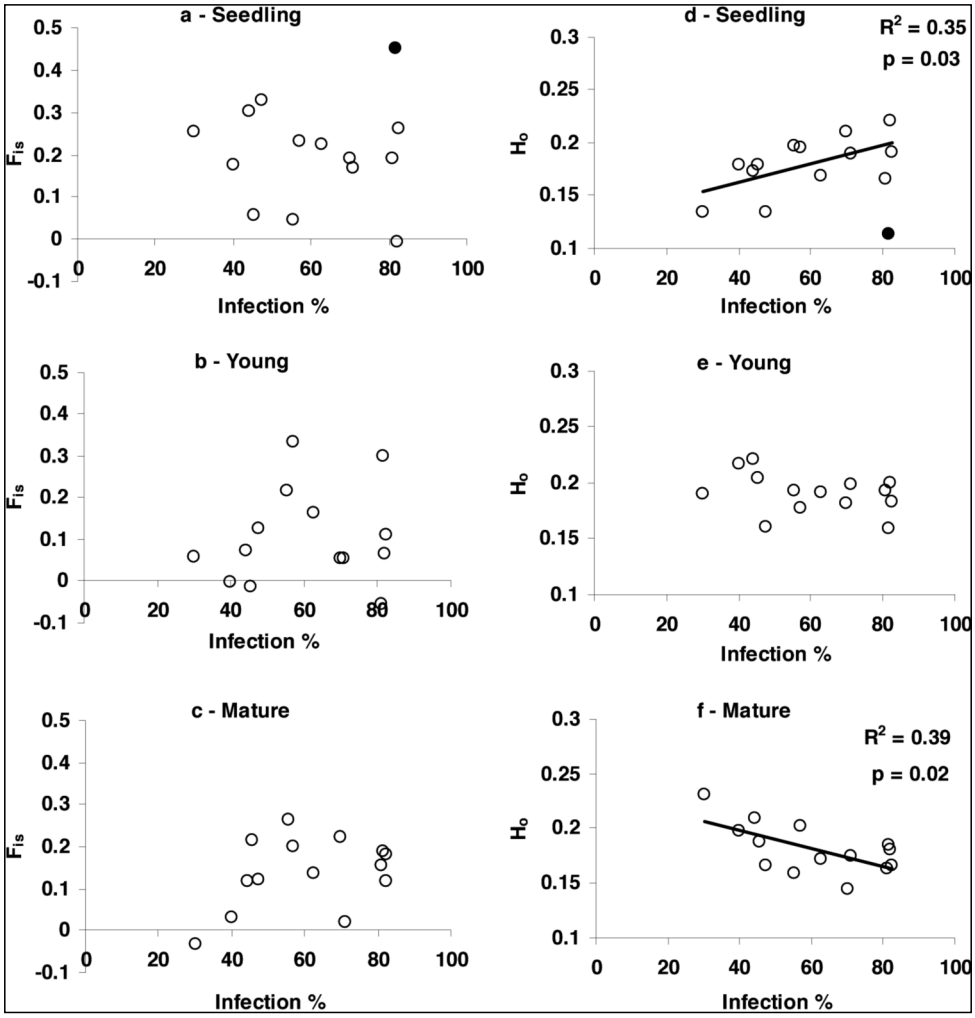

Figure 4. – Scatterplot of (a-c) fixation index (F_{is}) and (d-f) observed heterozygosity (H_o) of whitebark pine versus infection percent for 14 sites.

Table 2. – Fixation Indices (F_{is}) at three life stages for several conifers.

Species	F_{embryo}	$F_{seedling}$ (age)	F_{mature} (age)	Reference
Pinus albicaulis		0.203 (3)	0.141	This study
"	0.093		-0.034[a]	Bower and Aitken 2007
P. sibirica	~0.06		-0.025	Politov et al. 1992
P. koraiensis	~0.04		-0.046	Politov et al. 1992
P. cembra	0.012		-0.174	Krutovskii et al. 1995
P. pumila	0.208		0.009	Politov et al. 2006
P. sylvestris	0.143	0.266 (10)	-0.123 (40)	Starova et al. 1990
"	0.12	0.006 (3)		Muona et al. 1987
P. radiata	0.081	0.038 (3-6)	-0.119 (17-20)	Plessas & Strauss 1986
P. leucodermis	0.126	-0.101 (5)	-0.095	Morgante et al. 1993
"	0.142	-0.121 (5)	-0.079	"
P. ponderosa	0.171	-0.022		Farris & Mitton 1984
P. monticola	0.011		-0.105 (10-34)	El-Kassaby et al. 1987
P. banksiana	0.010		-0.064	Cheliak et al. 1985
P. washoensis		0.11 (2)	-0.037	Mitton et al. 1997
Pseudotsuga menziesii	0.050		-0.027 (>25)	Shaw & Allard 1982
Picea engelmanii	0.081		-0.034 (<80)	Shea 1987
Abies lasiocarpa	0.074		-0.102 (<80)	Shea 1987
A. bracteata	0.388		0.049	Ledig et al. 2006
Sequoiadendron giganteum	0.043		-0.021	Fins & Libby 1982

[a] Determined from inferred maternal genotype.

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Table S1. – F_{is} values for 10 individual isozyme loci and over all loci from 14 sites.

A: Seedling cohort.

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.15	*	0.15	0.56	0.14	-0.06	*	*	*	0.23	0.23
Jesamond	-0.06	-0.04	-0.13	0.21	0.32	-0.06	*	0	*	-0.07	0.05
D'arcy	-0.02	-0.02	0.49	0.16	-0.01	0.00	*	0	*	0.21	0.19
Thynne Mtn.	-0.07	0.36	0.12	0.22	0.19	-0.05	*	-0.02	*	-0.16	0.26
Blackcomb	-0.02	1.0	0.40	-0.04	0.06	0.10	*	*	*	-0.09	0.17
Manning Park	-0.04	0.85	0.16	0.10	0.20	-0.06	*	-0.06	*	-0.06	0.18
Mt. Baldy	-0.01	1.0	0.06	0.30	0.61	0.28	*	*	*	0.37	0.30
Lunch Peak	*	0.85	0.33	0.71	0.57	*	*	-0.06	*	-0.14	0.45
Granite Butte	-0.06	-0.02	0.15	-0.17	-0.28	-0.02	*	0.66	*	0.20	-0.01
Hellroaring	0.01	0.66	0.25	0.07	0.32	*	*	-0.04	1.0	0.13	0.19
Sawtell Peak	0.06	-0.01	0.13	0.48	0.60	*	*	0	*	0.00	0.33
Gospel Hill	-0.18	-0.02	-0.17	0.31	0.16	*	*	*	*	-0.02	0.06
Quartz Hill	-0.08	0.37	-0.12	0.40	0.39	*	*	-0.02	*	0.52	0.23
Vinegar Hill	-0.06	0.66	0.18	0.65	-0.02	*	*	-0.09	*	0.00	0.25

* Monomorphic

B: Young cohort.

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.29	0.0	0.66	0.22	0.65	-0.14	*	*	*	0.16	0.33
Jesamond	0	0.54	0.01	0.19	0.32	-0.12	*	*	*	0.38	0.22
D'arcy	-0.06	*	-0.14	-0.09	-0.01	-0.02	1.0	-0.02	*	0.02	-0.05
Thynne Mtn.	-0.06	-0.02	0.15	-0.13	0.38	-0.04	*	0	*	0.17	0.11
Blackcomb	0.30	-0.01	0.24	-0.25	0	-0.07	*	*	*	0.15	0.06
Manning Park	-0.01	0.37	0.04	0	0.01	-0.06	*	*	*	-0.22	0
Mt. Baldy	-0.03	*	-0.17	0.34	0.05	-0.16	*	*	*	0.24	0.07
Lunch Peak	-0.04	0	0.39	-0.10	0.38	-0.06	*	0.79	*	0.47	0.30
Granite Butte	0.24	0	0.10	0.05	0.04	0.	*	0	*	-0.12	0.07
Hellroaring	0.25	0	0.09	0.08	-0.09	*	*	0	*	-0.06	0.05
Sawtell Peak	0.25	*	0.33	-0.07	0.05	*	*	*	*	0.0	0.13
Gospel Hill	-0.21	*	-0.19	0.14	0.01	*	1.0	*	*	-0.08	-0.02
Quartz Hill	0.05	1.0	-0.01	0.21	-0.11	*	*	1.0	0	0.27	0.16
Vinegar Hill	0.66	*	0.13	0.01	-0.12	0	*	-0.04	*	0.18	0.06

* Monomorphic

C: Mature cohort

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.30	*	0.07	0.18	0.58	0.26	*	*	*	-0.18	0.20
Jesamond	0.36	*	0.02	0.50	0.67	-0.10	*	*	*	0.04	0.26
D'arcy	*	-0.04	0.11	0.10	0.39	0.0	*	-0.02	*	0.02	0.15
Thynne Mtn.	-0.02	*	0.33	-0.01	0.12	-0.04	*	*	*	0.12	0.12
Blackcomb	-0.02	0	0.15	-0.21	0.01	-0.09	*	*	*	0.13	0.02
Manning Park	-0.06	-0.02	-0.23	0.16	0.16	-0.01	*	0	*	0.11	0.03
Mt. Baldy	-0.03	1.0	0.13	0.22	0.05	-0.07	*	0	*	0.13	0.12
Lunch Peak	0.21	0.29	-0.08	0.10	0.46	-0.02	1.0	*	*	0.04	0.19
Granite Butte	0.03	0.0	0.34	0.27	-0.09	-0.02	*	0	*	0.53	0.18
Hellroaring	0.17	-0.02	0.21	0.44	0.15	*	*	0	*	-0.06	0.22
Sawtell Peak	0.02	0	0.28	0.03	0.14	*	*	0	*	0	0.12
Gospel Hill	0.76	0.66	0.07	0.09	-0.13	1.0	*	1.0	*	-0.13	0.22
Quartz Hill	-0.08	0	0.23	0.02	0.14	*	*	1.0	*	0.13	0.14
Vinegar Hill	-0.21	*	0.09	-0.06	0.15	0	*	*	*	-0.29	-0.03

* Monomorphic

tistically significant ($p = 0.297$ and 0.247 for F_{is} and H_o, respectively).

Genetic differentiation among populations varied among cohorts ($F_{st} = 0.075$, 0.025, and 0.040 for the seedling, young, and mature cohorts, respectively), but all values are within the range previously reported for whitebark pine (YANDELL, 1992; JORGENSEN and HAMRICK, 1997; STUART-SMITH, 1998; RICHARDSON et al., 2002; KRAKOWSKI et al., 2003).

Discussion

We observed a significant decrease in F_{is} between the seedling and young cohort when the level of rust infection was low, but no differences among cohorts when the level of rust was moderate or high. In our study, inbreeding and blister rust affect the genetic diversity of whitebark pine in different ways. Inbreeding usually leads to lower genetic diversity within individuals and a heterozygote deficiency in seeds and seedlings, but this

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effect decreases over time as trees age and density-dependent selection occurs (*Table 2*). This pattern can be seen when the level of rust infection is low (*Figure 2*). However, when selection pressure due to blister rust is higher, heterozygosity levels are nearly the same across cohorts, and the heterozygote deficiency typical of seedlings was maintained in both young and mature trees in the more infected populations studied. Selection against heterozygotes by the rust may be a reason why F_{is} values stay high in the young and mature cohorts on sites with higher infection levels, and why H_o decreases with level of rust infection in the mature cohort. There are other reports of differentiation of enzyme loci associated with environmental variation which suggest that genotypes for specific enzymes may result in physiological differences that are acted on by natural selection

Table S2. – H_o values for 10 individual isozyme loci and over all loci from 14 sites.

A: Seedling cohort.

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.35	**	0.30	0.21	0.43	0.20	*	*	*	0.47	0.20
Jesamond	0.13	0.10	0.47	0.31	0.24	0.13	*	0.05	*	0.53	0.20
D'arcy	0.07	0.07	0.24	0.41	0.48	0.03	*	0.05	*	0.31	0.17
Thynne Mtn.	0.19	0.09	0.39	0.39	0.34	0.12	0.44	0.06	*	0.30	0.19
Blackcomb	0.07	0.00	0.30	0.36	0.46	0.24	*	*	*	0.47	0.19
Manning Park	0.10	0.04	0.41	0.35	0.41	0.14	*	0.20	*	0.15	0.18
Mt. Baldy	0.33	0	0.48	0.35	0.20	0.13	0	0	0	0.23	0.17
Lunch Peak	*	0.03	0.33	0.13	0.22	*	*	0.13	*	0.28	0.11
Granite Butte	0.18	0.07	0.43	0.54	0.60	0.07	*	0.03	*	0.29	0.22
Hellroaring	0.57	0.03	0.38	0.44	0.34	*	*	0.10	0	0.23	0.21
Sawtell Peak	0.27	0.07	0.45	0.27	0.22	*	*	0.03	*	0.03	0.13
Gospel Hill	0.33	0.07	0.55	0.36	0.41	*	*	*	*	0.07	0.18
Quartz Hill	0.19	0.10	0.62	0.31	0.28	*	*	0.07	*	0.11	0.17
Vinegar Hill	0.15	0.05	0.40	0.17	0.35	*	*	0.20	*	0.03	0.13

B: Young cohort.

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.21	0.03	0.17	0.39	0.17	0.33	*	*	*	0.46	0.18
Jesamond	0.03	0.10	0.52	0.41	0.33	0.23	*	*	*	0.30	0.19
D'arcy	0.13	*	0.43	0.47	0.33	0.07	*	0.07	*	0.43	0.19
Thynne Mtn.	0.13	0.07	0.35	0.57	0.30	0.10	*	0.03	*	0.29	0.18
Blackcomb	0.20	0.07	0.37	0.54	0.45	0.17	*	*	*	0.20	0.20
Manning Park	0.27	0.10	0.37	0.41	0.47	0.13	*	*	*	0.43	0.22
Mt. Baldy	0.33	*	0.46	0.33	0.47	0.30	*	*	*	0.31	0.22
Lunch Peak	0.11	0.03	0.24	0.50	0.30	0.13	0	0.03	*	0.24	0.16
Granite Butte	0.23	0.03	0.45	0.48	0.50	0.03	*	0.03	*	0.23	0.20
Hellroaring	0.23	0.03	0.39	0.46	0.53	*	*	0.03	*	0.13	0.18
Sawtell Peak	0.20	*	0.37	0.54	0.47	*	*	*	*	0.04	0.16
Gospel Hill	0.37	*	0.60	0.43	0.47	*	0	*	*	0.17	0.20
Quartz Hill	0.30	0	0.50	0.40	0.41	*	*	0	0.03	0.27	0.19
Vinegar Hill	0.03	*	0.41	0.50	0.57	0.03	*	0.10	*	0.27	0.19

* Monomorphic

C: Mature cohort

Site	PGI 1	MDH 1	MDH 2	SKD 1	SKD 2	IDH	GDH	PGM 2	ALD	TPI	Overall
Heckman Pass	0.20	*	0.40	0.35	0.21	0.33	*	*	*	0.53	0.20
Jesamond	0.10	*	0.43	0.23	0.14	0.21	*	*	*	0.47	0.16
D'arcy	*	0.10	0.37	0.41	0.31	0.03	*	0.07	*	0.33	0.16
Thynne Mtn.	0.07	*	0.28	0.50	0.32	0.10	*	*	*	0.39	0.17
Blackcomb	0.06	0.03	0.35	0.37	0.39	0.20	*	*	*	0.35	0.18
Manning Park	0.13	0.10	0.57	0.38	0.33	0.07	*	0.03	*	0.37	0.20
Mt. Baldy	0.33	0	0.41	0.37	0.47	0.17	*	0.03	*	0.31	0.21
Lunch Peak	0.17	0.13	0.39	0.44	0.28	0.07	0	*	*	0.37	0.18
Granite Butte	0.33	0.03	0.30	0.37	0.53	0.07	*	0.03	*	0.13	0.18
Hellroaring	0.20	0.07	0.39	0.29	0.33	*	*	0.03	*	0.13	0.14
Sawtell Peak	0.30	0.03	0.38	0.48	0.40	*	*	0.03	*	0.03	0.17
Gospel Hill	0.10	0.03	0.46	0.43	0.57	0	*	0	*	0.29	0.19
Quartz Hill	0.17	0.03	0.32	0.50	0.40	*	*	0	*	0.30	0.17
Vinegar Hill	0.37	*	0.47	0.54	0.43	0.03	*	*	*	0.47	0.23

* Monomorphic

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(MOPPER et al., 1991; MITTON, 1995). An alternate explanation may be that where selection or local mating system has produced highly heterozygous stands, rust infection levels do not reach moderate or high levels. This could be due to local environmental conditions in which more heterozygous individuals have higher fitness. However, while they were not statistically significant, our results showed that on individual sites, infected trees often have higher heterozygosity than clean trees, which argues against this hypothesis.

As the level of rust infection increases, overall H_o of the mature trees within the stands we assessed decreased, yet the seed cohort produced from these stands had higher H_o than stands with lower rust infection. One potential explanation for this is that if rust is killing stems or even just the cone-bearing branches of individuals within clusters, this could result in a reduction in matings among related individuals on sites with higher rust infection. This would lead to higher heterozygosity in the seed than in the mature individuals that are reproducing. A decrease in density leading to a loss of spatial genetic structure (e.g. due to harvesting) often has been reported to result in increases in inbreeding (MARQUARDT et al., 2007), and wind-pollinated species would be expected to show a positive relationship between population density and outcrossing rate (FARRIS and MITTON, 1984). However, empirical tests of this relationship are inconsistent (DYER and SORK, 2001), and studies of the effect of different silvicultural treatments on genetic diversity showed an increase in heterozygosity in naturally regenerated seedlings of *Pinus strobus* after harvesting compared to old-growth populations (MARQUARDT et al., 2007). The authors suggested that logging decreased the spatial genetic structure, reducing spatially caused biparental inbreeding for the seedlings produced post-disturbance. The relationship we observed in H_o in the seedling cohort may also reflect a decrease in selfing or biparental inbreeding in stands with higher rust infection, however, this cannot be tested with our data. This hypothesis is speculative, but warrants further investigation. A study to investigate genetic diversity in mature parent trees with their offspring that includes both a measure of local tree density and level of blister rust infection may be able to test this hypothesis.

Previous studies exploring the effects of disease epidemics on genetic diversity have yielded varying results in both forest trees and agricultural crops (MCDONALD et al., 1998; OUBORG et al., 2000; KIM et al., 2003). In wild plant-pathogen populations, hosts should evolve towards increased resistance (GILBERT, 2002). However, it is not always possible to determine if changes in genetic diversity are correlated with increases in resistance (MCDONALD et al., 1998) and depending on the pathosystem, genetic control of resistance varies. For example, in a study of American beech (*Fagus grandifolia* Ehrh.), trees susceptible to beech bark disease had higher H_o values than resistant trees and showed an excess of heterozygotes, while resistant trees showed a deficiency of heterozygotes, suggesting recessive control of resistance (HOUSTON and HOUSTON, 2000). Conversely, a heterozygote excess detected in populations of American chestnut (*Castanea dentata*) that have persisted through the chestnut blight epidemic through sprouting suggests that selection has favored more heterozygous individuals (STILWELL et al., 2003).

Introduced pathogens can result in high selection pressure when levels of natural resistance are low, and evolution towards increased resistance could result in decreased levels of diversity due to a genetic bottleneck or selective sweep. In a comparison of populations of western white pine (*Pinus monticola*), a closely related species in the *Pinus* subsection Strobus, KIM et al. (2003) found that a natural population under low rust pressure had higher polymorphism (P) and heterozygosity (H_e), and more private alleles than a population with high rust pressure. Assuming that levels of genetic diversity in the two populations were similar prior to invasion by blister rust, this suggests that selection by rust resulted in reduced diversity. Inferences regarding the level of resistance in the natural whitebark pine populations sampled in this study are not possible; however, if there is stronger differential selection on high rust sites, this may lead to higher levels of overall resistance in the remaining population, albeit at the risk of dramatic reductions in population size and genetic diversity, and even local extirpations if resistance genes are recessive.

Both dominant and recessive resistance genes have been identified in a variety of crop plants (TOYODA et al., 2002; CHU et al., 2004). To date, only one dominant vertical resistance mechanism to blister rust (hypersensitivity) has been identified in the white pines (KINLOCH and DUPPER, 2002), while several horizontal mechanisms have been identified with dominant, recessive, and additive modes of inheritance hypothesized for a number of pine-rust pathosystems (see references in KINLOCH, 1982 and KINLOCH et al., 2008). Evidence of recessive gene segregation was found in early inoculation trials of western white pine (BINGHAM et al., 1960; BINGHAM, 1966) and recessive inheritance of genes conferring high resistance or immunity in Idaho western white pine were hypothesized by MCDONALD and HOFF (1970) and HOFF and MCDONALD (1971). Disease resistance genes are often dominant (BURDON, 2001) although about 10% of resistance genes in crops are recessive (BURDON and THOMPSON, 1995). In a large, truly panmictic population, recessive genes would rarely be expressed and thus would be of little selective value (BURDON, 2001), but forest trees typically have mixed mating systems with some selfing and bi-parental inbreeding (LEDIG, 1998). In wild populations, this mixed mating will facilitate the expression of recessive phenotypes produced by such alleles in some individuals. Although this will likely be associated with some inbreeding depression (WILLIAMS and SAVOLAINEN, 1996), the cost could be small compared with the selective advantage of disease resistance (BURDON, 2001).

The relationship between H_o and level of blister rust infection found in our study suggests, but does not confirm, that more homozygous individuals have a higher fitness when challenged by blister rust possibly due to recessive genes for resistance being expressed under

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inbreeding. Our results suggest that, contrary to our expectations, more heterozygous individuals may be more likely than more homozygous whitebark pine genotypes to suffer mortality due to the introduced pathogen white pine blister rust. Whitebark pine may have some type of resistance to white pine blister rust that is recessively controlled. However, this must be confirmed by identification of specific resistance mechanisms and determination of their genetic control in common garden experiments with controlled exposure to spores of this introduced disease.

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Genetic diversity of *Anadenanthera colubrina* Vell. (Brenan) var *cebil*, a tree species from the South American subtropical forest as revealed by cpSSR markers

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Abstract

Anadenanthera colubrina var *cebil* is a tree species native to the Upper Parana Atlantic Forest where human activities have severely impacted causing deep fragmentation. Microsatellites are not available in this species. Therefore, the first objective of this study was to generate chloroplast simple sequence repeats (cpSSR) by cross-species transfer. Understanding the evolutionary dynamics of subdivided populations is an important matter. In this way, a first approach to the characterization of the haplotypic diversity within and between populations as well as the genetic structure of native Argentinean populations were the main goals of this study.

Twenty four individuals from two populations of the Misiones province were studied and four cpSSR *loci* were tested. Two of them exhibited polymorphic patterns leading to the identification of 11 cpDNA haplotypes with high mean genetic diversity ($GD = 0.73$). The minimum spanning network defined three clear groups which can be assigned to at least three subpopulations. AMOVA indicated that the total variance showed the highest percentage of variation (48%) within subpopulations with a fixation index (F_{ST}) statistically significant ($F_{ST} = 0.520$; $p < 0.05$). Brown's two *loci* component analysis indicated that substructure population is present. Jost's differentiation global index (D_{est}) was 0.049 while D_{est} pairwise comparison reflected a certain level of genetic structure.

The high diversity level detected in the adult trees of *A. colubrina* var *cebil* from the populations under study could be due to recent human influence. In this way, the reduction in population size caused a reduction in the number of trees leading to surviving trees showing the historical diversity of the populations analyzed.

Key words: Upper Parana Atlantic Forest, Curupay, intraspecific genetic diversity, cpSSR cross-species transfer.

Introduction

The study of the genetic diversity distribution, population structure and evolutionary features of a species native range is of particular relevance when evaluating their long-term variability and fitness (GÓMEZ et al., 2002).

Anadenanthera colubrina Vell. Brenan var *cebil*, (Fabaceae, Mimosoideae) known locally as curupay, is a tree species native to the South American subtropical forest. Curupay trees can be found in the Upper Parana Atlantic Forest, which extends from Brazil into the northern part of Argentina, where human activities have had a severe impact leading to deep fragmentation. These trees can reach up to 35 m in height, with flowers arranged in hermaphrodite inflorescences and long legume fruits with narrow and flattened seeds (JUSTINIANO and FREDERICKSEN, 1998; CIALDELLA, 2000). Their floral traits show that pollination is performed by bees and seed dissemination occurs over short distances by anemochory – autochory after pod dehiscence (JUSTI-

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