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THE UNIVERSITY OF ALBERTA

Mating System and Genetic Structure of *Thuja orientalis* Linn.

by

Chang Yi Xie

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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(SIGNED) Xiechangyi.....

PERMANENT ADDRESS:

Beijing Forestry University

Beijing.....

China.....

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Mating System and Genetic Structure of *Thuja orientalis* Linn. submitted by Chang Yi Xie in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Supervisor

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External Examiner

Date Mar. 6, 1989

Abstract

Isozyme inheritance and linkage, mating system, and genetic structure of *Thuja orientalis* were studied using isozyme electrophoresis.

Mendelian inheritance was inferred for 26 isozymes of 19 enzyme systems by analysing segregation ratios, comparing the phenotypes of the megagametophytes and the corresponding embryos, and by using similarities among the isozymes of other conifers. A relatively large number of segregation distortions (38 out of a total of 179 tests) were detected. Natural selection seemed to be the most important cause of the observed distortions. Linkages were suggested for four out of 47 tested locus pairs, but none of them were tight.

Mating system parameters in four natural populations were estimated, and the estimated level of selfing is generally higher than those of most other conifers. The multilocus estimates of outcrossing rate among populations varied from 0.68 to 0.81, with an average of 0.75. Significant heterogeneity of the outcrossing estimate was detected both among trees within populations and among populations. It was suggested that a certain amount of detected selfing was due to consanguineous matings rather than self-fertilization.

Estimates of Wright's fixation index showed an overall deficiency of heterozygotes as measured by mating system equilibrium in each filial population. However, there were excesses of heterozygotes in three of the four maternal

populations even compared to those expected under Hardy-Weinberg equilibrium. Although an overall heterozygote deficiency was observed in Shandong Changqin, it is only about one fifth of that observed in the corresponding filial population. These results suggest that a large amount of heterozygotes are removed at the later stages of the life cycle (after seed germination).

Genetic structure was studied using eighteen populations. Compared with most other conifer species, *Thuja orientalis* exhibited relatively lower within-population variation and higher among-population differentiation. Small and isolated populations scattered over a large geographical range with diverse environmental conditions are the most probable cause of the observation. The variation pattern revealed in this study is generally consistent with that derived from provenance tests, and the three climatic factors, annual mean temperature, annual mean relative humidity and annual precipitation, have been found to have significant influences on both patterns. It was evident that natural selection is an important force in maintaining the observed genetic variation.

The results obtained in this study have implications in many aspects of tree improvment practice, such as gene conservation, seed production, progeny test, and the estimation of genetic parameters.

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I would like to express my sincere appreciation and gratitude to my supervisor Dr. Bruce Dancik and the other members of my graduate committee for their assistance, persistence and encouragement during this study. I would also like to express my thanks to professor S. H. Shen and Mr. X. M. Wu for providing seeds and related information. I wish to acknowledge the financial support provided by the Chinese government and the Natural Sciences and Enginering Research Council of Canada. Finally, I wish to thank my wife Fangmin for all her help and support during this study.

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1. General Introduction

1.1 Taxonomy and Ecology of *Thuja orientalis* Linn.

Thuja orientalis is a member of the genus *Thuja* and the family *Cupressaceae*. Six species have been recognized in this genus, two in China (*T. orientalis* Linn. and *T. suthchuenensis* F.), two in North America (*T. plicata* Donn. and *T. occidentalis* L.), and two in eastern Asia (*T. standishii* (G.) C. and *T. koraiensis* N.) (Chun, 1921; Dallimore and Jackson , 1931; Hosie, 1979; Hough, 1947; Li, 1972; Steward, 1958). *Thuja suthchuenensis* is a small tree with a limited distribution in the eastern part of Sichuan province (Chun, 1921; Dallimore and Jackson, 1931) and is not considered to be an important forest tree species in China. In contrast, *Thuja orientalis* is a large tree with a wide distribution and plays a very important role in the Chinese forestry.

An adult tree of *Thuja orientalis* can attain a height of 20 m and a diameter of 1 m (Figure 1.1). This species can be distinguished from the others by its vertically flattened branches and branchlets, thick cone-scales and unwinged seeds (Figure 1.2) (Dallimore and Jackson, 1931).

Thuja orientalis is not only extensively distributed in China but also in Japan and Korea (Ohwi, 1965). It has also been introduced to and widely cultivated in North America (Li, 1972). In China, this species has a very long history of cultivation (many thousand years) and therefore has been

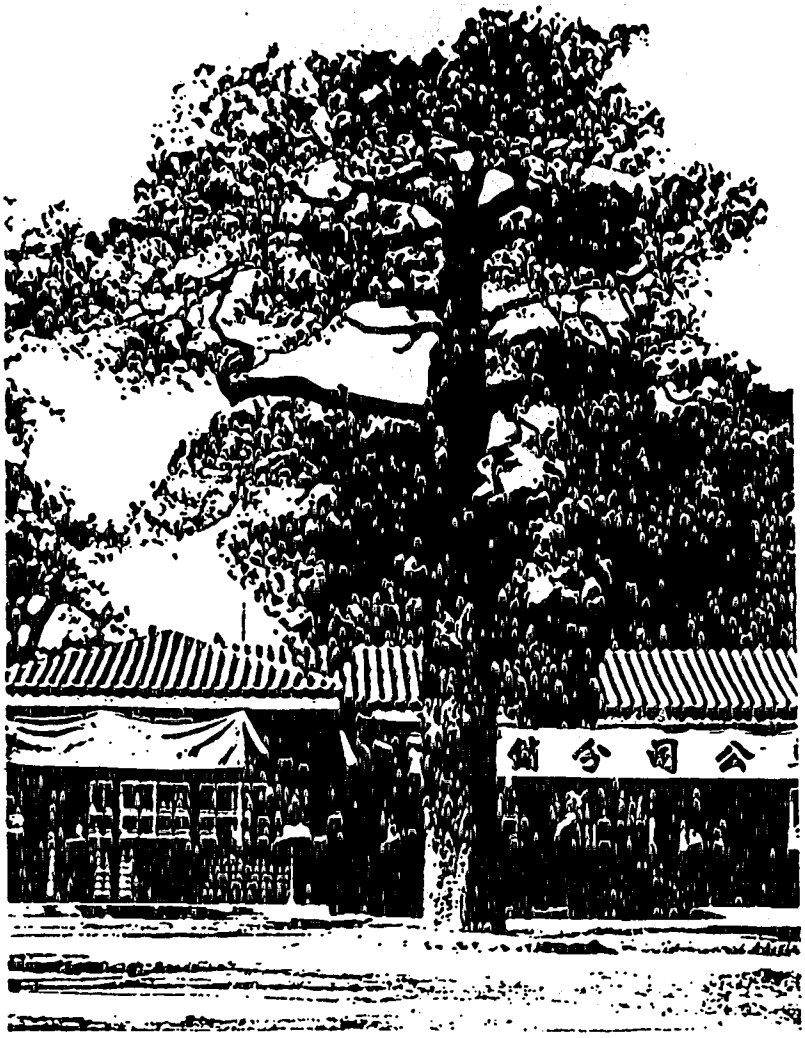

Figure 1.1 Old Specimen of *Thuja orientalis* Linn. in a Park in Beijing, China

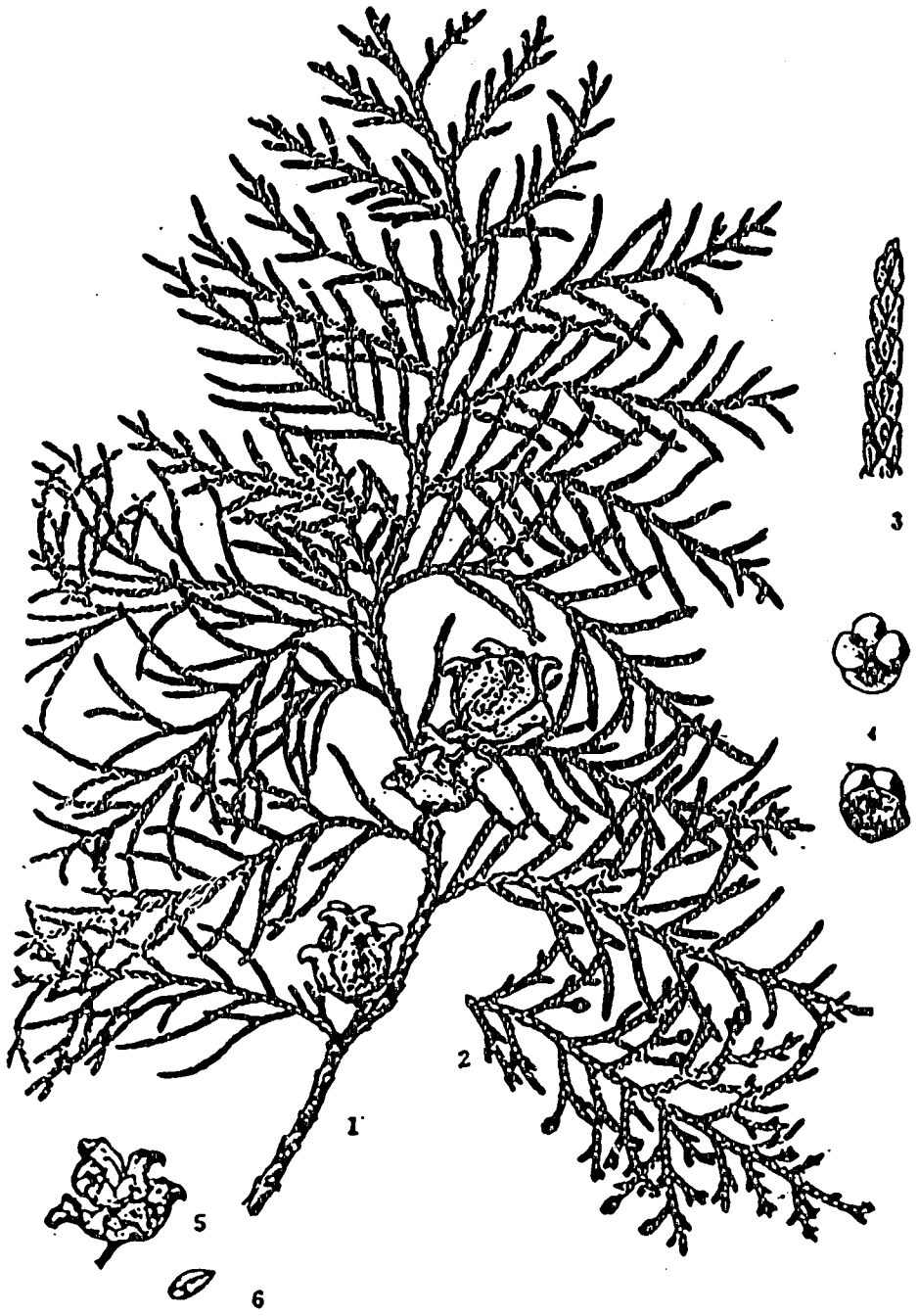

Figure 1.2 Young Branch of *Thuja orientalis* Linn.

1, Fruiting branch. 2, Flowering branch. 3, Scaly leaf. 4, Staminate cone. 5, Cone. 6, Seed.

planted almost all over the country. The natural range of the species covers a vast expanse in China, spanning about 20 degrees of latitude and 40 degrees of longitude, within which about 20 provinces, municipalities and autonomous regions are covered (Figure 1.3). However, the distribution is not continuous but scattered, and the majority of the forests in the range are second growth. The vertical (or elevational) distribution increases from northeast to southwest and the elevation varies from under 150m to over 3300m. The northern boundary of distribution is relatively well established, but the southern boundary has not been clearly defined yet. The central range of the species is between the Yellow River valley and the Huai River valley. Since the natural distribution of this species in many regions is not completely known, the information provided in Figure 1.3 is tentative, and supplements and revisions are necessary.

The value of *Thuja orientalis* is mostly attributable to its great adaptability. It plays a very important role in afforestation in China, especially in dry barrens where many other species cannot be afforested successfully. It can grow well in both dry-cold and wet-warm conditions, with a range of rainfall from 300mm to 1600mm per year and annual average temperature from 8°C to 16°C. It also can grow well on soils with different kinds of bedrock, such as limestone, granite and purple shale and varying soil acidity and alkalinity. Since the early 1950s, *Thuja orientalis* has been used to

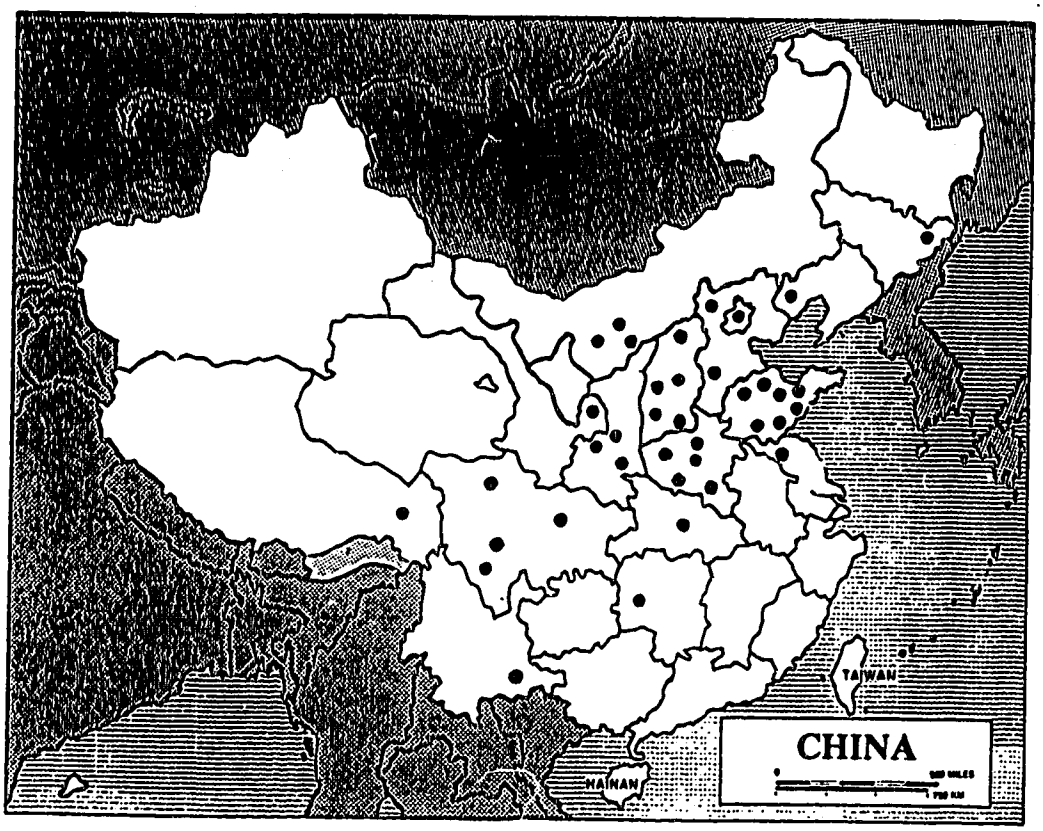

Figure 1.3 Natural Distribution of *Thuja orientalis* **Linn. Dots in the figure indicate the locations where natural distribution of** *Thuja orientalis* **Linn. has been reported.**

afforest a large number of barren hills, and a lot of closed stands have been established.

Besides its value in afforestation, the wood of *Thuja orientalis* can be used for many purpose (e.g. furniture and construction), and its seed, root, leaf and bark all have medicinal uses.

1.2 Objectives of the Present Study

Because of its value, there has been increasing interest in managing *Thuja orientalis* in China. However, some fundamental information, such as the mating system and genetic structure of the species, is not currently available. In order to maximize the effectiveness and efficiency of genetic improvement and gene conservation efforts, studies on the mating system and genetic structure should be carried out before initiating any genetic improvement and gene conservation practice.

Genetic variation is the raw material for the genetic improvement of forest trees. Information on the relative magnitude of genetic variation within a species would help us to justify the cost of its improvement and determine the proper strategy to be taken in this species. For instance, if a large amount of genetic variation exists in a species, greater gain per unit of investment would be expected through a selective breeding program, while the lack of genetic variation may require the use of some other techniques such as interspecific breeding for its

improvement. Knowledge of subpopulations containing large amounts of genetic variation within a target species can be used for defining areas to be set aside for *in situ* gene conservation and selecting trees to be used to increase the gene pool of previously selected breeding populations. Since tree improvement and *ex situ* gene conservation programs usually start with sampling trees from natural populations, information on the pattern of genetic variation of the target species would be very useful in determining sampling strategies (Marshall and Brown, 1975).

Knowledge of the mating system of a species is important in the selection of parent trees and populations in a tree improvement program. Significant inbreeding depression in the form of decreased survival and growth has been observed in many coniferous tree species (Franklin, 1970; Geburek, 1986; Sorensen and Miles, 1982; Wilcox, 1983). Therefore, collection of wind-pollinated seed from parent trees or populations with high selfing rates should be avoided. In addition, since the presence of inbreeding violates the assumptions made in the analysis of wind-pollinated progeny tests, leading to overestimation of additive genetic variance and genetic gain (Namkoong, 1966; Squillace, 1974), adjustments are needed and could be made based on knowledge of the mating system (Cheliak, 1983).

Besides the practical utility, mating system and genetic structure studies of a species are also of theoretical significance. A major concern in population

genetics is to characterize the genetic composition of natural populations and evaluate the influence of evolutionary forces such as mutation, selection, migration and genetic drift. The mating system is an important determinant of genetic structure and evolutionary potential of a population (Allard, 1975; Brown *et al.*, 1975; Clegg, 1980; Jain, 1976; Kahler *et al.*, 1975; Lande and Schemske, 1985). Outcrossing provides the opportunity for higher levels of genetic recombination, and thus results in higher levels of heterozygosity (Brown, 1979) and creates potentially adaptive new genotypes, while inbreeding increases homozygosity and may results in severe performance depression. Since outbreeding populations generally have a greater potential for gene flow, populations within an outbreeding species are expected to be more homogeneous, whereas populations within an inbreeding species are expected to be more differentiated.

For both practical utility and theoretical importance, characterizing the genetic structure and mating system of *Thuja orientalis* has been the major goal of the present study.

Traditionally, patterns of genetic variation in forest trees have been investigated by measuring quantitative traits of various seed sources that are growing in "common gardens". This kind of investigation can provide useful information concerning broad scale genetic variation patterns and seed transfer. However, such studies have the

following drawbacks: 1) Due to the complex inheritance of such traits and their sensitivity to environmental influences, patterns of variation revealed in common garden studies often vary with the trait measured and the environment used for testing (Campbell and Sorensen, 1978). 2) Since the number of genes governing quantitative traits is usually unknown, it is difficult to quantify the degree of genetic variation involved in the observed patterns and to make comparison between populations and species. 3) Due to the expense and size constraints involved in common garden studies of forest trees, seed sources are normally represented by only a few parent trees and, therefore, little information about the levels of genetic variation within populations can be achieved from these studies (Adams, 1981).

Because of those drawbacks of common garden studies, researchers have tried to detect genetic variation directly at the DNA level so that the genetic variation within individual trees, populations or species can be quantifiable, comparable and entirely free of environmental effects. However, the study of DNA polymorphism is still in its infancy, particularly its application in the study of forest trees. Time, expense and technical difficulty are other constraints which limit the usefulness of DNA sequencing techniques in investigating genetic variation involving a large number of trees and populations at the present.

Earlier studies of mating systems of forest trees were largely based on morphological characters (e.g. seedling albinism). There are at least two shortcomings associated with those studies: 1) Such morphological characters (or genetic markers) may not always be available, and 2) Genetic crosses are required to identify the marker carriers.

Isozyme electrophoresis is a powerful method for studying genetic structure and mating systems. By employing this technique, a large number of loci can be examined quickly and relatively inexpensively. Variation revealed by isozyme electrophoresis is relatively close to the DNA level, relatively free of environmental effects and directly quantifiable and comparable. It has been noted that estimates of genetic variation within species from isozyme studies are generally consistent with those from provenance/progeny tests (Copes, 1981; Flower and Morris, 1977; Wheeler and Guries, 1982; Yeh and El-kassaby, 1980). Mating system studies can be greatly simplified by the use of isozyme electrophoresis, for virtually any tree bearing seeds can be employed. Since allozymes are generally codominant, homozygous and heterozygous genotypes can be distinguished from each other without the necessity of making genetic crosses. Meanwhile, the technique is easy to learn, relatively cheap and rapid. In this study, isozyme electrophoresis was employed.

To use isozyme electrophoresis effectively for genetic structure and mating system studies, knowledge of the

inheritance of electrophoretically detectable allozyme polymorphisms and their linkage relationships is required.

Non-Mendelian segregation or segregation distortion at heterozygous isozyme loci would affect the estimates of allele frequencies in the parental generation from bulked seed samples, if the distortion cannot be balanced off by some forces (such as gametic selection) until assay. For individual tree collections, if the individual tree genotypes are inferred by examining a few megagametophytes, the probability that a heterozygote is misclassified as a homozygote increases as the degree of segregation distortion increases. If the direction of distortion is not random among trees, estimates of allele frequencies based on the inferred individual genotypes would also be affected. Since the measures of genetic structure such as the expected heterozygosity, Nei's standard genetic distance and the coefficient of gene differentiation are all functions of gene frequencies, they could be affected, too.

The influence of segregation distortion on the estimates of mixed mating system parameters can be in both directions when the distortion is strictly a phenomenon of the female gametophyte (Cheliak *et al.*, 1984). If the distortion is toward alleles of high frequency in the population, a positive assortative mating system will result and this would cause an overestimate of common allele frequency and an underestimate of outcrossing rate. Similarly, if the distortion is toward low-frequency alleles

in the population, outcrossing rate would be overestimated.

Study of segregation distortion is also an important aspect of population genetics. Meiotic drive as an evolutionary force has significant influence on the genetic structure and evolution of natural populations. It can drastically alter allele frequencies in natural populations, and certain deleterious genes can inexorably increase in frequency, or even become fixed, in spite of their selective disadvantage (Sandler and Novitski, 1957).

Knowledge of linkage relationships permits assessment of the distribution of available loci in a genome and therefore the reliability of estimates of genetic variation in natural populations based on those loci. Independent segregation of isozyme loci is one of the assumptions made in our multilocus estimation procedure of mating system parameters. Thus, the loci employed in mating system studies should not be closely linked. Furthermore, studies on linkage can provide evidence useful in elucidating conifer evolution. Many studies have revealed that gene arrangement is highly conservative in the family *Pinaceae* (Conkle, 1981) and thus indicate that evolution within this family has proceeded primarily through gene substitution rather than through large structural rearrangement within and between gene blocks. Similar results have been obtained from cytological studies (Sax, 1960; Saylor, 1972).

In summary, the specific objectives of the present study are:

1. To analyze the inheritance and linkage relationships of isozymes in *Thuja orientalis*.

2. To estimate the mating system parameters in natural populations of *Thuja orientalis*.

3. To characterize the genetic structure of populations of *Thuja orientalis*.

Corresponding to the above specific objectives, the results of this study have been organized into three chapters. An additional chapter has been added to discuss the practical implications of the results achieved from the study in gene conservation and tree improvement practice.

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2. Inheritance and Linkage of Isozymes

2.1 Introduction

Knowledge of inheritance of the electrophoretically detectable isozyme polymorphisms and their linkage relationships are prerequisites if allozymes are to be used as gene markers for population genetic studies. Since the haploid endosperm (female gametophyte or magagametophyte) tissue of mature seed of conifers allows individual genotypes to be determined probabilistically without the necessity of performing crosses and direct observation of Mendelian segregation at heterozygous allozyme loci (Guries and Ledig, 1978), inheritance and linkage studies of isozymes in conifers are simplified.

There have been many reports of inheritance and linkage studies of allozyme polymorphisms in coniferous species since the electrophoretic technique was first used in forest genetics (Table 2.1). In this chapter, the inheritance and linkage relationships of isozymes in *Thuja orientalis* are described and the following information supplied:

1) Variation patterns of 19 enzyme systems.
2) Segregation distortion at 14 polymorphic loci.
3) Linkage relationships among those loci.

Table 2.1 Inheritance and Linkage Studies in Some Conifers

Species	No.of Enzymes	No.of Loci	Type of study	Reference
Calocedrus decurrens (Torr.)	21	27	I,L	Harry, 1986
Camellia japonica L.	8	12	I,L	Wendel & Parks, 1982
Larix decidua Mill.	2	3	I	Mejnartowicz & Bergmann, 1975
Picea abies (L.)	1	1	I	Bartel, 1971
	2	2	I	Bergmann, 1973
	2,3	2,6	I,L	Bergmann, 1974
	1	1	I	Lundkvist, 1974, 1975
	1	2	I	Lundkvist, 1977
	4	6	L	Lundkvist, 1979
	13	17	I,L	Muona *et al.*, 1987
Picea glauca (Moench) Voss	6	6	I	Cheliak & Pitel, 1984a
	2	14	I	Feret, 1971
	16	26	I,L	King & Dancik, 1983
Picea mariana (Mill.) B.S.P.	12	18	I,L	Yeh *et al.*, 1982
Picea sitchensis (Bong.) Carr.	4	14	I,L	Simonsen & Wellendorf, 1975
Pinus albicaulis Engelm.	12,9	14,11	I,L	Furnier *et al.*, 1986
Pinus attenuata Lemm.	26	58	I,L	Strauss & Conkle, 1986
	2	3	I,L	Conkle, 1971
Pinus banksiana Lamb.	8,4	12,5	I,L	Cheliak *et al.*, 1984
	7	10	I	Tobolski, 1979
Pinus densiflora Sieb. & Zucc.	1	4	I,L	Bab, 1976
Pinus monticola Dongl.	9	14	I	El-Kassaby *et al.*, 1987
Pinus nigra Arnold.	1	2	I	Nikolic & Bergmann, 1974

Pinus palustris Mill.	1	11	I	Snyder & Hamaker, 1978
Pinus ponderosa Laws.	1	1	I	Linhart, 1981
	12	23	I,L	O'Malley *et al.*, 1979
Pinus rigida Mill.	8	14	I	Guries & Ledig, 1978
	7	11	L	Guries *et al.*, 1978
	11	18	L	O'Malley *et al.*, 1986
Pinus strobus L.	10	17	I,L	Eckert *et al.*, 1981
Pinus sylvestris L.	1	2	I	Rudin, 1975,1977
	6	12	L	Rudin & Ekberg, 1978
	1	7	I	Szmidt, 1979
	2	2	I	Yazdani & Rudin, 1982
Pinus taeda L.	10	17,12	I,L	Adams & Joly, 1980a,b
	1	11	I	Snyder & Hamaker, 1978
Pinus virginiana Mill.	1	2	I	Feret & Witter, 1977
	1	2	I	Witter & Feret, 1978
Pseudotsuga menziesii (Mirb.) Franco	2	2	I	Copes, 1979
	18,7	27,19	I,L	El-Kassaby, *et al.*, 1982

I=inheritance study;
L=linkage study.

2.2 Material and Methods

2.2.1 Sampling Strategy

A total of 89 dominant or codominant trees were sampled from four natural populations in the central range of the species (Table 2.2 and Figure 2.1). To reduce the possibility of sampling closely related individuals (i.e. full- or half-siblings), at least 30 meters spacing between sample trees was maintained. More than 200 cones were collected from the south-central portion of the crown of each individual tree and seeds from each tree were bagged seperately and storted at 4°c.

Eight seeds from each tree were used for initial genotyping. The probability of detecting a heterozygote is $P=1-(0.5)^7$ or 0.99 under the assumption of equal distribution and survival of alternate gametes in a heterozygous individual. An additional 30 to 112 seeds of each of 70 trees were examined. Those 70 trees were chosen because they were heterozygous at one or more loci.

2.2.2 Electrophoretic Methods

Seeds were germinated in petri dishes at room temperature until the radicle had emerged at least 2mm (it took about 7 days). They were then either assayed immediately or kept in the refrigerator for later use. After the seed coat was removed, individual megagametophytes and embryos were ground separately in auto-analyzing cups(0.5ml)

Table 2.2 Location and Sample Size for the Four Natural Populations of *Thuja orientalis* Linn. Sampled in the Present Study

Population No.	Population Name	Latitude (°N)	Longitude (°E)	Elevation (Meter)	No. of Trees
1	Shandong Changqin (SCQ)	36.60	116.73	300	34
2	Shanxi Changzhi (SCZ)	36.32	118.17	250[1]	20
3	Shanxi Houxian (SH)	36.57	111.92	1000	16
4	Beijing Miyun (BM)	40.38	116.83	650	$9^{2}+10^{3}$

1: estimated from a map;

2: 1982 collection;

3: 1983 collection.

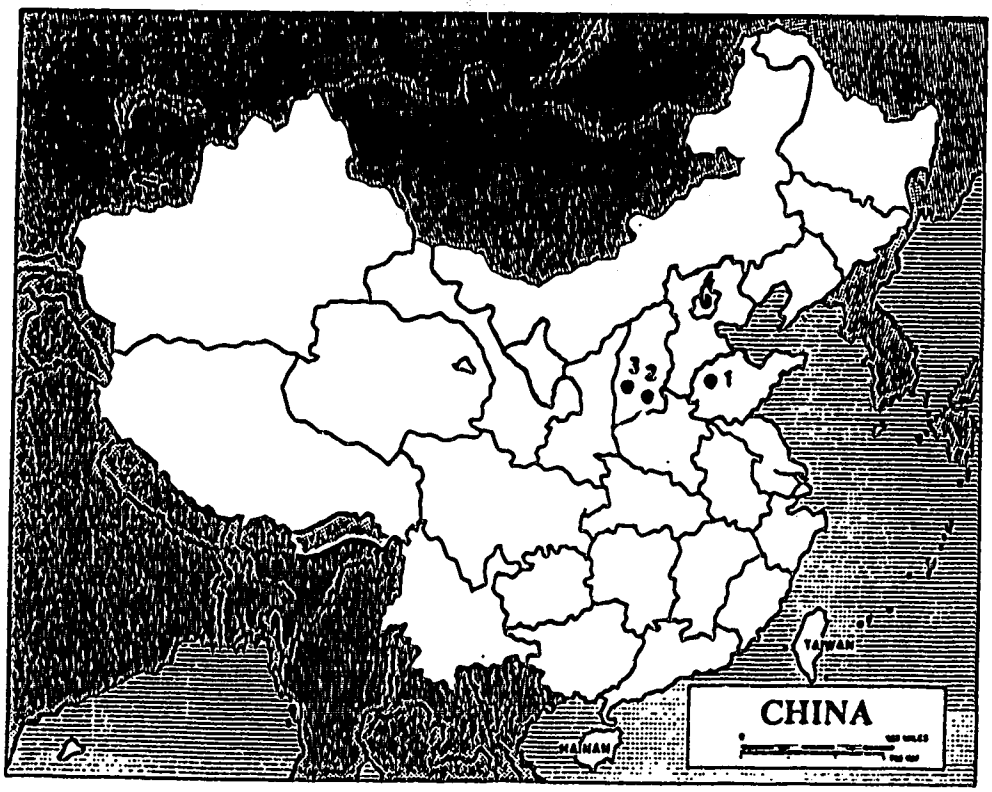


Figure 2.1 Geographical Locations of the 4 Natural Populations of *Thuja orientalis* Linn. Sampled in This Study

with two drops of extraction buffer (Yeh and O'Malley, 1980). The homogenate was absorbed onto 13x1 mm filter paper (Whatman No. 3) wicks, and these wicks then inserted into a slice (the origin) in the gel, 2 cm from cathodal end. Each megagametophyte sample was adjacent to the corresponding embryo sample. Starch gels (12.5% (WT/VOL)) were prepared from Electrostarch (Electrostarch Co., Madison WI, LOT 307). The components of the gel and electrode buffers are detailed in Table 2.3. Electrophoresis was performed under refrigeration at about 4°c. The gels were run at half voltage for 10 to 15 minutes, the wicks were removed , an ice pack was applied to the upper surface of the gel, and total voltage was applied. Electrophoresis was timed until the red tracking dye had migrated about 5 cm beyond the origin. Following electrophoresis, gels were cut into 1 mm (buffer system TC, SPB and RW) or 2 mm (buffer system POL) thick horizontal slices and stained for different enzyme systems.

Enzymes were designated by capital letters (e.g. AAT); isozyme loci were italicized and numbered when more than one isozyme was identified (e.g. *Aat1*). Isozymes were numbered in order of their mobilities, with 1 being assigned to the fastest locus. The most common allele of a locus was designated 100 and the others were identified by their mobility relative to it.

Table 2.3 Gel and Electrode Buffers and Running Voltages

1. Tris-Citrate buffer system (TC)[1]:

   Gel buffer: (63 g tris + 33.04 g citric acid)/4 L. PH 7.0 with 5.6 M HCl or NaOH
   Electrode buffer: the same as gel buffer
   Total running voltage: 200 V.

2. Modified NCS dehydrogenase buffer (SPB)[2]:

   Gel buffer: (41.92 g L-Histidine + 1.6 g EDTA) / 4 L. PH 7.0 with 1.0 M tris
   Electrode buffer: 60.54 tris / 4 L. PH 7.0 with 1.0 M citric acid (anhydrous)
   Total running voltage: 200 V.

3. Ridgeway buffer system (RW)[3]:

   Gel buffer: (72.6 g tris + 19.2 citric acid (anhydrode) + 200 ml RW electrod buffer) /2 L. PH 8.5 .
   Electrode buffer: (10.07 g LIOH + 74.16 g boric acid)/ 4 L. PH 8.1.
   Total running voltage: 300 V.

4. Poulik buffer system (POL)[4]:

   Gel buffer: 9.68 g tris / 1 L. PH 8.65 with 1.0 M citric acid (anhydrous).
   Electrode buffer: (76.64 g boric acid + 10.08 g NaOH)/ 4 L. PH 8.1.
   Total running voltage: 300 V.

1=Siciliano and Shaw, 1976;

2=Modified from Namkoong *et al.*, 1979;

3=Ridgway *et al.*, 1970;

4=Schaal and Anderson, 1974.

2.2.3 Statistical Methods

2.2.3.1 Test of Segregation and Its Heterogeneity

A G-test was used to detect segregation distortion in megagametophytes (Sokal and Rohlf, 1981). The G statistic in this case is defined as:

$$G=2(f_1\ln(f_1/f_{1e})+f_2\ln(f_2/f_{2e})),$$

where

f_1, f_2=observed number of alleles 1 and 2,

f_{1e}, f_{2e}=expected number of alleles 1 and 2,

$f_{1e}=f_{2e}=n/2$, and

n=total number of megagametophytes used.

G has an approximately χ^2 distribution with 1 degree of freedom.

The heterogeneity of segregation over individual trees was tested by calculating the G_h-statistic, which is given by (Sokal and Rohlf, 1981):

$$G_h=(\sum_{i=1}^{N} G_i)-G_p,$$

where

G_i=value of G-statistic for the i^{th} tree,

G_p = value of G-statistic for the pooled trees.

G_h has an approximately χ^2 distribution with N-1 degrees of freedom, where N is the number of trees surveyed. A similar G_h-statistic was calculated to test the heterogeneity of segregation among populations and between sampling years.

2.2.3.2 Test of Linkage and Its Heterogeneity among Trees

To detect deviations from expected joint segregation values for individual and pooled trees with pairwise comparison, The following G-statistic was calculated:

$$G=2\left(\left((a+d)\ln\frac{a+d}{n(P_aP_b+Q_aQ_b)}\right)+\left((b+c)\ln\frac{b+c}{n(P_aQ_b+Q_aP_b)}\right)\right),$$

where

$P_a=(a+b)/n$, $Q_a=1-P_a$,

$P_b=(a+c)/n$, $Q_b=1-P_b$, and

$n=(a+b+c+d)$,

in which, a, b, c and d are the number of gametes observed in category A_1B_1, A_1B_2, A_2B_1 and A_2B_2.

A presumption for this test of linkage is that no more than one of the segregating loci shows a significant deviation from the expected 1:1 ratio. In the present study, a linkage test was not performed whenever segregation was unbalanced at both loci involved. The same G_h-test given in last section was employed to examine the heterogeneity of the joint segregation among trees.

2.2.3.3 Estimation of Recombination Frequency

Although many estimators are available for the estimation of recombination frequency (Nordheim *et al.*, 1983), the maximum likelihood estimator proposed by Bailey (1961) is most frequently used in forest genetics (Rudin and Ekberg, 1978; El-Kassaby *et al.*, 1982; King and Dancik, 1983; Strauss and Conkle, 1986). This estimator and its standard error are given by:

Recombination frequency (R)=r/n,

Standard error (SE)=$((R(1-R))/n)^{0.5}$,

where r=min(a+d,b+c) is the number of recombinant types observed.

This estimator is easy to understand, easy to compute, and has a relatively low mean squared error (Nordheim *et al.*, 1983). It was employed in the present study.

2.3 Results

2.3.1 Inheritance

A total of 42 enzymes was surveyed, but 23 of them were omitted from this and further studies because of their faint, blurred or inconsistent phenotypes in both megagametophytes and embryos. Two of the remaining 19 enzyme systems were monomorphic with a single band (Table 2.4). A total of 24 polymorphic loci was recorded, and a detailed description of the detected allozyme variants and results of segregation analysis for each of them follow (some rare alleles observed in bulked samples of megagametophytes are also presented).

2.3.1.1 Asparate Aminotransferase (EC 2.6.1.1)

Three zones of activity were observed on gels scored for asparate aminotransferase and coded as three loci (Figure 2.3). No tree was found to be heterozygous at *Aat1* and *Aat2*, but three single-banded alleles for each locus were observed in the bulked samples. *Aat3* had three alleles,

Table 2.4 Enzymes Surveyed in *Thuja orientalis* Linn.

Enzyme	Buff.	S.P.*	Locus	Alleles 1	2	3	4
Asparate aminotransferase	PUL	I	*Aat1*	104	100	84	
(EC 2.6.1.1)			*Aat2*	119	100	97	
			Aat3	106	100	94	
Aconitase (EC 4.2.1.3)	SPB	I	*ACO*	115	100	96	
Alcohol dehydrogenase	RW	II	*Adh1*	113	100	95	
(EC 1.1.1.1)			*Adh3*	107	100	94	
Acid phosphatase	SPB	I	*Aph3*	133	100		
(EC 3.1.3.2)			*Aph4*	126	100		
Diaphorase (EC 1.6.4.3)	RW	I	*Dia*	100	94		
Fluorescent-esterase	RW	I	*Feat1*	103	100	96	91
(EC 3.1.1.1)			*Feat2*	108	100		
α-Galactosidase	TC	IV	*αgal*	113	100	88	
(EC 3.2.1.22)							
Glutamate dehydrogenase	PUL	I	*Gdh*	109	100		
(EC 1.4.1.3)							
Hexoseaminidase	TC	V	*Ha*	143	100	71	
(EC 3.2.1.30)							
Isocitrate dehydrogenase	TC	I	*Idh1*	123	100	81	
(EC 1.1.1.42)			*Idh2*	117	100	93	
Leucine-amino peptidase	RW	I	*Lap*	100			
(EC 3.4.11.1)							
Malic enzyme (EC 1.1.1.40)	RW	I	*Me*	114	100	92	

Marmose-6-phosphate isomerase (EC 5.3.1.18)	SPB	IV	*Mpi*	117	100		
Menadione reductase (EC 1.6.99.2)	SPB	III	*Mr*	111	100	94	
Phosphoglucose isomerase (EC 5.3.1.9)	SPB	I	*Pgi2*	141	100	59	
Phospholucomutase (EC 2.7.5.1)	TC	I	*Pgm1*	112	108	100	88
Shikimate dehydrogenase (EC 1.1.1.25)	TC	VI	*Skdh1*	107	100	90	
Superoxide dismutase (EC 1.15.1.1)	RW	I	*Sod*	100			
6-phosphogluconate	SPB	I	*6pg1*	113	100	94	
dehydrogenase(EC 1.1.1.44)			*6pg2*	115	100		

*: S.P.=staining procedure:

I=Yeh and O'Malley, 1980;

II=Shaw and Prasad, 1970;

III=Conkle et al., 1982;

IV=O'Malley et al., 1980;

V=Cheliak and Pitel, 1984b;

VI=Tanksley and Rick, 1980 (modified).

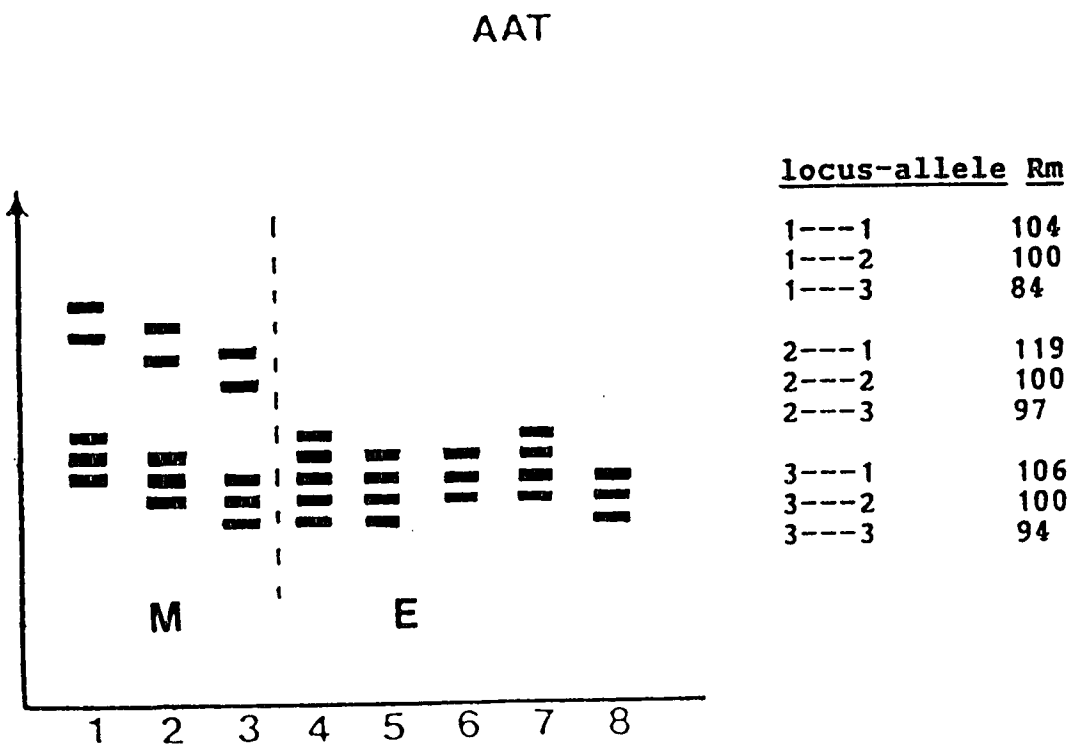


Figure 2.3 Diagram of asparate aminotransferase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring *Aat3* in each lane: 4=13, 5=23, 6=22, 7=12 and 8=33.

each with a triple-banded phenotype. Alleles with a double- or triple-banded phenotype at a cathodal locus of AAT also have been reported in many other conifers (Adams and Joly, 1980a; Eckert *et al.*, 1981; Furnier, *et al.*, 1986; Guries and Ledig, 1978; King and Dancik, 1983; Rudin and Ekberg, 1978). Since heterozygous embryos showed either four or five bands at *Aat3*, the subunit structure of this enzyme could not be determined by the phenotypes of embryos at this locus. However, AAT has been inferred to be a dimer in some conifers (Adams and Joly, 1980a; Cheliak and Pitel, 1984; El-Kassaby *et al.*, 1982; Guries and Ledig, 1978; O'Malley *et al.*, 1979; Rudin, 1975; Wendel and Parks, 1982).

Twenty-seven trees were involved in the segregation test, and significant distortion was detected for four of them. These four trees came from Beijing Miyun; three were sampled in 1982 and one was sampled in 1983. Segregation was significantly heterogeneous among trees in both collections ($G_h(3)=9.52$ and $G_h(1)=12.86$, respectively), but not between the two sampling years ($G_h(1)=0.92$). The deviation from a 1:1 ratio was in the direction of an excess of allele 3 to allele 1 . No segregation distortion was detected for any of the three allelic combinations in any other populations and segregation in those populations was homogeneous over individuals. Significant heterogeneity of segregation among populations was detected between alleles 1 and 3 ($G_h(3)=15.70$). This heterogeneity was due to an overpresence of allele 3 to allele 1 in Beijing Miyun.

2.3.1.2 Aconitase (EC 4.2.1.3)

One zone of activity with three variants was observed on gels stained for aconitase (Figure 2.4). Similar observations have been recorded in *Picea abies* (Muona *et al.*, 1987), *Pinus albicaulis* (Furnier *et al.*, 1986) and *Pinus jeffreyi* (Conkle, 1979). ACO was reported as a dimer in *Picea abies* but a monomer in *Populus tremuloides* (Hyun *et al.*, 1987) and humans (1975; Zouros, 1976). The subunit structure of ACO could not be studied in *Thuja orientalis* with this material because of the blurred staining of the enzyme in embryos. Poor and inconsistent activity of ACO in embryos has been observed in *Pinus rigida* (Adams and Joly, 1980a; Guries and Ledig, 1978) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982). The haploid phenotypes of ACO were single-banded in this species although double-banded patterns have been observed in *Calocedrus decurrens*, another member of the same family (Harry,1986).

Three individuals with genotype 12 were available for segregation analysis. Significant segregation distortion was detected for the two trees sampled in 1982 from Beijing Miyun and the pooled data. The distortion was due to an excess of the common allele (allele 2) over one of the rare alleles (allele 1). In contrast to these two trees, the tree from Shanxi Houxian showed an exact 1:1 segregation ratio.

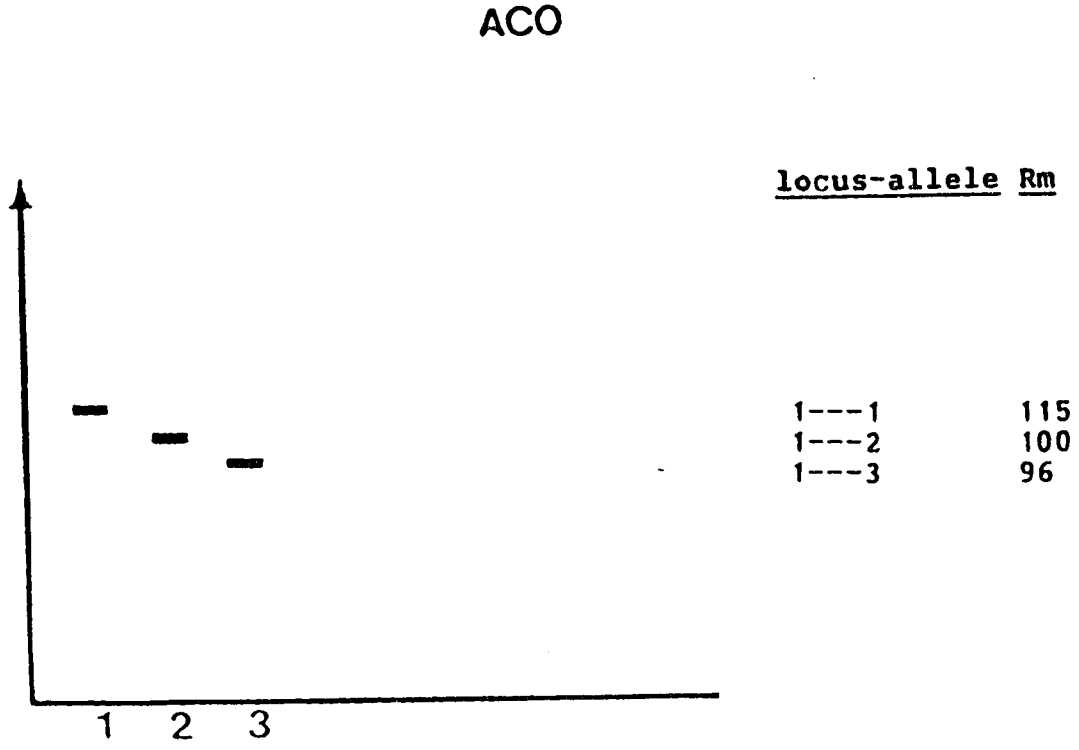


Figure 2.4 Diagram of aconitase gels indicating haploid megagametophytes in lanes 1 to 3.

2.3.1.3 Alcohol dehydrogenase (EC 1.1.1.1)

Alcohol dehydrogenase patterns consisted of three zones of activity (Figure 2.5). The middle zone was very faint and even absent on most gels, so that only zone 1 (*Adh1*) and zone 3 (*Adh3*) were scored. Although three alleles were observed at *Adh1* and *Adh3*, allele 1 and allele 3 were rare at both loci. ADH has been interpreted as a dimer in *Camellia japonica* (Wendle and Parks, 1982). However, the subunit structure of this enzyme could not be studied in *Thuja orientalis*, because it did not resolve clearly in embryos.

Since no tree was heterozygous for *Adh1* or *Adh3*, no segregation distortion analysis was carried out.

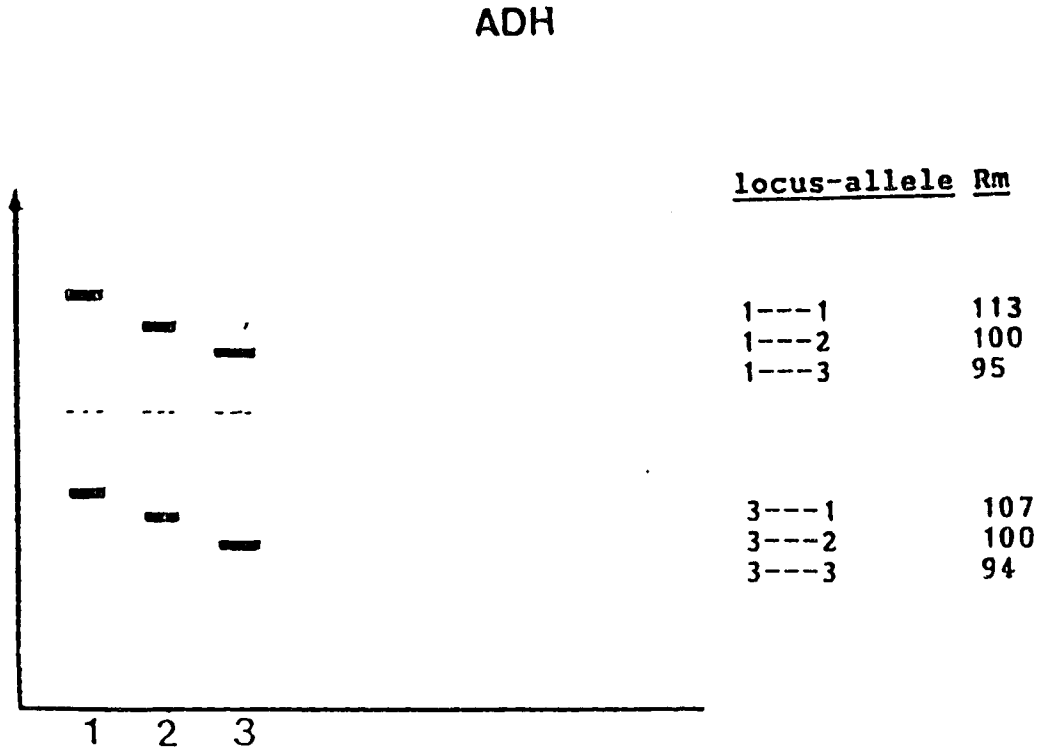


Figure 2.5 Diagram of Alcohol dehydrogenase gels indicating the haploid megagametophytes in lanes 1 to 3.

2.3.1.4 Acid phosphatase (EC 3.1.3.2)

Four zones of activity were observed on gels stained for acid phosphatase, but only the two most cathodal zones were clear enough to be scored (Figure 2.6). Two alleles at each locus appeared and each showed a single-banded pattern. Allele 1 at both loci was observed only in the bulked samples; thus, no data were available for segregation analysis. The heterozygous embryo phenotypes at both loci were too blurred to be identified, so that the subunit structure of APH could not be studied. Poor resulation of APH has also been reported in diploid tissue of *Pinus taeda* (Adams and Joly, 1980a) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982). Dimeric structure of APH has been recorded in *Picea abies* (Lundkvist, 1975) and *Pinus banksiana* (Cheliak *et al.*, 1984).

The banding patterns of APH in *Thuja orientalis* was similar to those reported for Douglas-fir (El-Kassaby *et al.*, 1982), and Mendelian inheritance of APH loci was confirmed in that species.

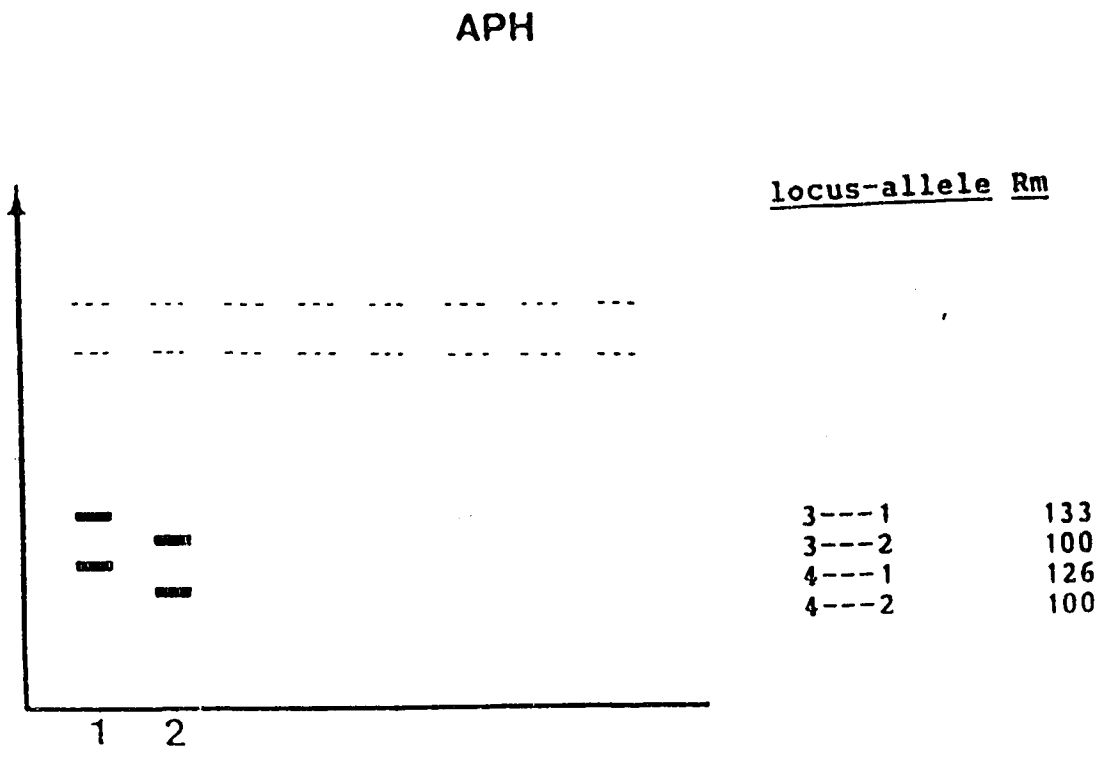


Figure 2.6 Diagram of acid phosphatase gels indicating the haploid megagametophytes in lanes 1 and 2.

2.3.1.5 Diaphorase (EC 1.6.4.3)

Diaphorase had one zone of activity in *Thuja orientalis* (Figure 2.7). Even though the activity of DIA was not very strong, it was sufficient to allow scoring in haploid tissue. Two alleles with single-banded phenotypes were observed at *Dia*, but allele 1 was rare and no heterozygous tree was avaialable for a segregation ratio test. Two alleles with single-banded phenotypes at Dia were also observed in *Camellia japonica*, and those two alleles were found to segregate according to a 1:1 ratio (Wendel and Parks, 1982). The phenotype of heterozygotes of *Camellia japonica* suggested that DIA was a functionally monomeric enzyme in that species. However, it was not possible to determine the subunit structure in this material because of the poor resolution of embryo enzymes.

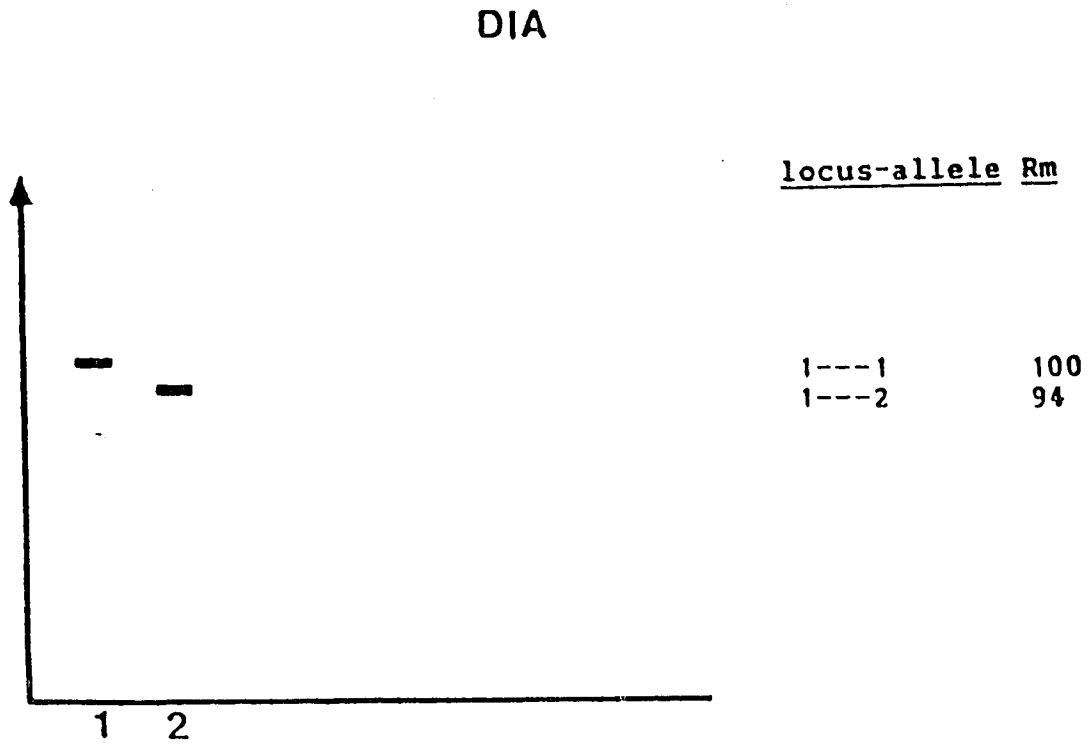


Figure 2.7 Diagram of diaphorase gels indicating the haploid megagametophytes in lanes 1 and 2.

2.3.1.6 Fluorescent esterase (EC 3.1.1.1)

Two zones of activity appeared on fluorescent esterase gels (Figure 2.8). Four single-banded alleles at *Fest1* and two at *Fest2* were observed. The heterozygous embryo phenotypes at F-EST loci consisted of two bands with mobilities identical to parental alleles. Comparison of phenotypes from megagametophytes and embryos suggested that F-EST is a monomer in *Thuja orientalis* , as has been reported in *Calocedrus decurrens* (Harry, 1986). However, dimeric structure has been identified for F-EST in other coniferous species (Mitton *et al.*, 1979; Muona *et al.*, 1987; Wendel and Parks, 1982; Yazdani and Rudin, 1982).

At *Fest1*, Segregation analysis was carried out only for two of the six possible allelic combinations. Segregation between alleles 2 and 3 fit the expectation and no significant heterogeneity was observed between the two heterozygous trees surveyed, while segregation between alleles 2 and 4 in one of the two studied trees significantly deviated from the expected ratio and was heterogeneous between those two trees. The tree that showed significant segregation distortion was from Shanxi Changzhi, and the observed distortion was due to an excess of the common allele (allele 2). No data were available for segregation analysis at *Fest2*, since allele 1 at this locus was rare.

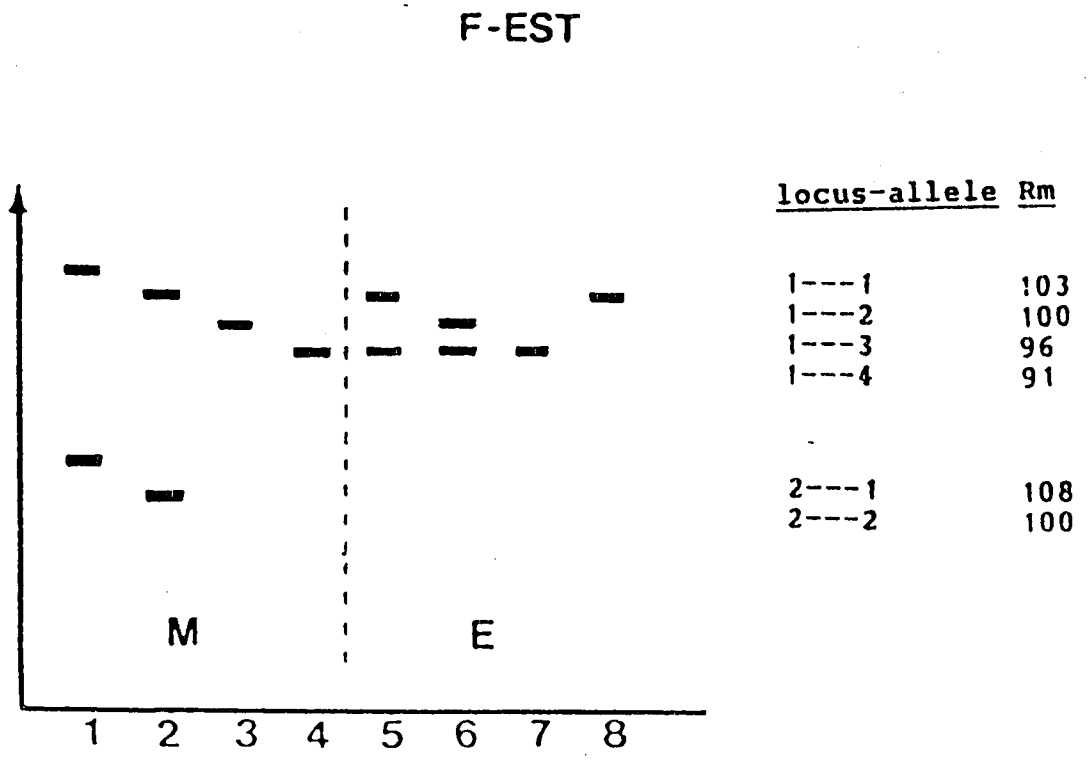
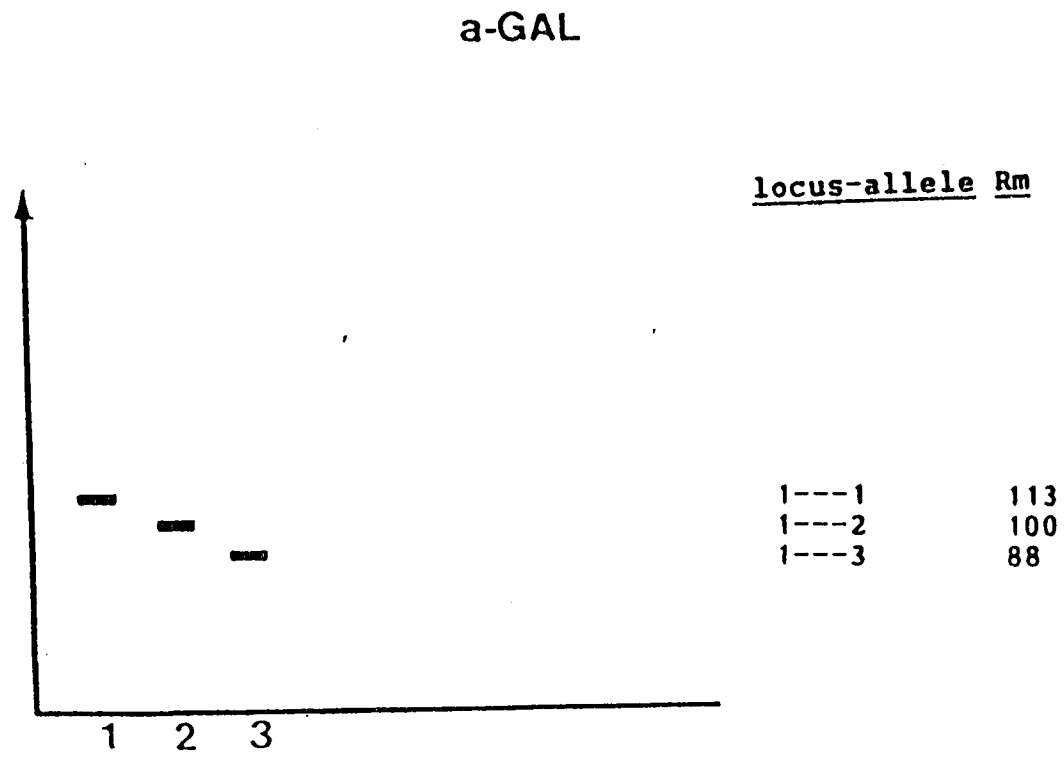


Figure 2.8 Diagram of fluorescent esterase gels indicating the haploid megagametophytes in lanes 1 to 4 and diploid embryo phenotypes in lanes 5 to 8. Scoring of *Fest1* in each lane: 5=24, 6=34, 7=44, 8=22.

2.3.1.7 α-Galactosidase (EC 3.2.1.22)

One zone of activity on α-galactosidase gels was observed and three single-banded allozyme variants appeared (Figure 2.9). Heterozygous embryo phenotypes were so blurred that the subunit structure of this enzyme could not be determined. αGAL rarely has been studied in forest trees and no information on its inheritance is available.

One tree was heterozygous at *αgal* and segregation was not significantly different from the expected Mendelian ratio.



Flgure 2.9 Diagram of α-galactosidase gels indicating the haploid megagametophytes in lanes 1 to 3.

2.3.1.8 Glutamate dehydrogenase (EC 1.4.1.3)

Glutamate dehydrogenase showed one zone of activity in *Thuja orientalis* (Figure 2.10). Although it did not stain strongly, it was clear enough to be scored. Two single-banded allozymes were observed at *Gdh* in this species. The observation that GDH is controlled by a single locus has been reported in many other conifers (Adams and Joly, 1980a; Cheliak and Pitel, 1984a; Conkle, 1979, Eckert *et al.*, 1981; El-Kassaby *et al.*, 1982, 1987; Furnier *et al.*, 1986; King and Dancik, 1983; Mitton *et al.*, 1979; Muona *et al.*, 1987; Neal and Adams, 1981; Strauss and Conkle, 1986; Wendel and Parks, 1982). The phenotype of heterozygous embryos was a single broad band approximately midway between the band positions of the parental alleles. The same observation has also been recorded in *Picea glauca* (Cheliak and Pitel, 1984a), *Pinus ponderosa* (Mitton *et al.*, 1979) and *Pinus taeda* (Adams and Joly, 1980a). It has been hypothesized that GDH may be multimeric, requiring the combination of many polypeptide chains to form a functional enzyme (Pryor, 1974).

Four trees were involved in the segregation test at *Gdh*. One tree from Shanxi Houxian showed a significant deviation from the expected segregation ratio, and such deviation was a result of an excess of the common allele (allele 2). However, segregation among the three trees sampled from this population was homogeneous, and the pooled data did not show significant segregation distortion.

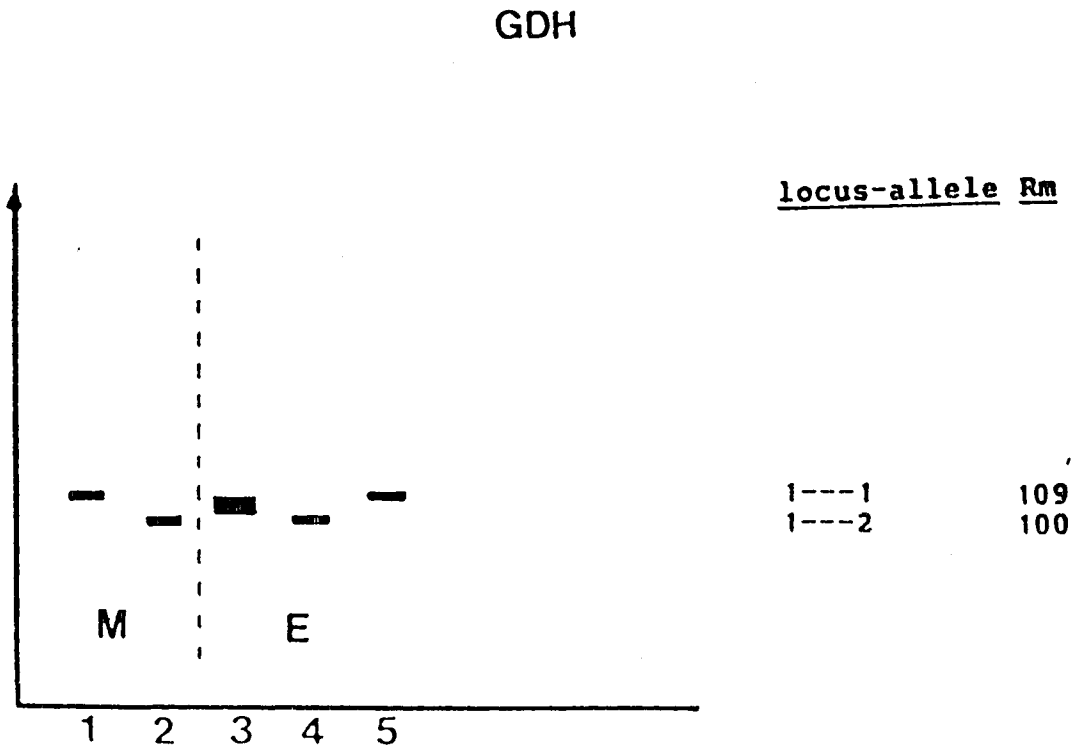


Figure 2.10 Diagram of glutamate dehydrogenase gels indicating the haploid megagametophytes in lanes 1 and 2, and diploid embryo phenotypes in lanes 3 to 5. Scoring in each lane: 3=12, 4=22, 5=11.

2.3.1.9 Hexoseaminidase (EC 3.2.1.30)

One zone of activity with three single-banded allozyme variants was observed on the gels stained for hexoseaminidase (Figure 2.11) . The subunit structure of HA could not be studied with this material because the bands of heterozygous embryos were too blurred. Inheritance of HA in *Pseudotsuga meziesii* was similar (El-Kassaby *et al.*, 1982).

Five trees sampled in 1983 from Beijing Miyun were used for the test of segregation distortion at *Ha*. Of the three possible allelic combinations, a significant excess of allele 2 in comparison to allele 1 was observed for one of the two trees. Segregation between these two trees was homogeneous and the pooled data fit the expected 1:1 ratio. One tree was available to test the segregation distortion between alleles 1 and 3, and two trees for that between alleles 2 and 3. No significant distortion was detected for these two allelic combinations.

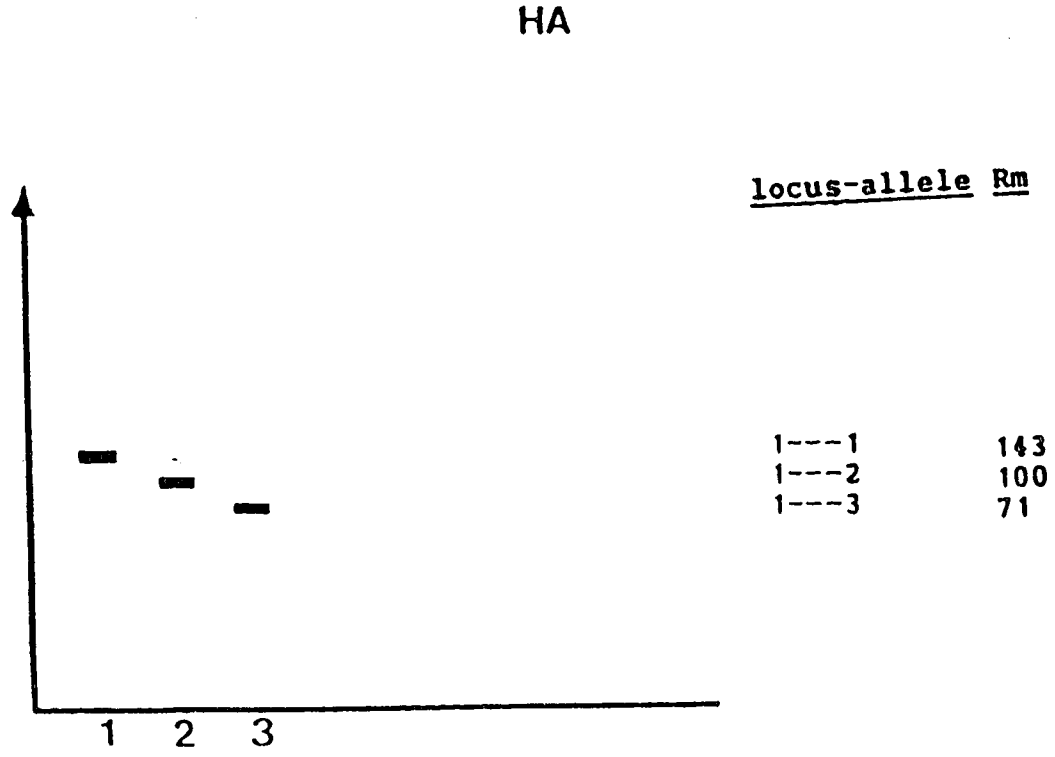


Figure 2.11 Diagram of hexoseaminidase gels indicating the haploid megagametophytes in lanes 1 to 3.

2.3.1.10 Isocitrate Dehydrogenase (EC 1.1.1.42)

Two zones of activity appeared on isocitrate dehydrogenase gels (Figure 2.12). The anodal zone was strongly stained, while the cathodal zone was stained lightly but could be easily scored. Three single-banded alleles were present at each locus, and heterozygous embryos showed triple-banded patterns at both loci with the intermediate band darker. This observation suggests that IDH is functionally dimeric in *Thuja orientalis*, as has been reported in other conifers (El-Kassaby *et al.*, 1982; Guries and Ledig, 1978; Hyun *et al.*, 1986; Muona *et al.*, 1987; O'Malley *et al.*, 1979). While single-banded allozyme variants at IDH have been recorded for most of the studied tree species, double-banded patterns have also been reported (Conkle *et al.*, 1982).

Segregation distortion was detected for two of the five trees heterozygous for *Idh1*, and the distortion was due to an overpresence of allele 2 compared to allele 3. Significant deficiency of allele 1 compared to allele 2 at *Idh2* was detected for one of the three trees studied. Segregation among trees was significantly heterogeneous for the above allelic combinations. Two trees, one heterozygous for alleles 1 and 2 at *Idh1* and another heterozygous for alleles 2 and 3 at *Idh2*, were studied but neither showed significant segregation distortion.

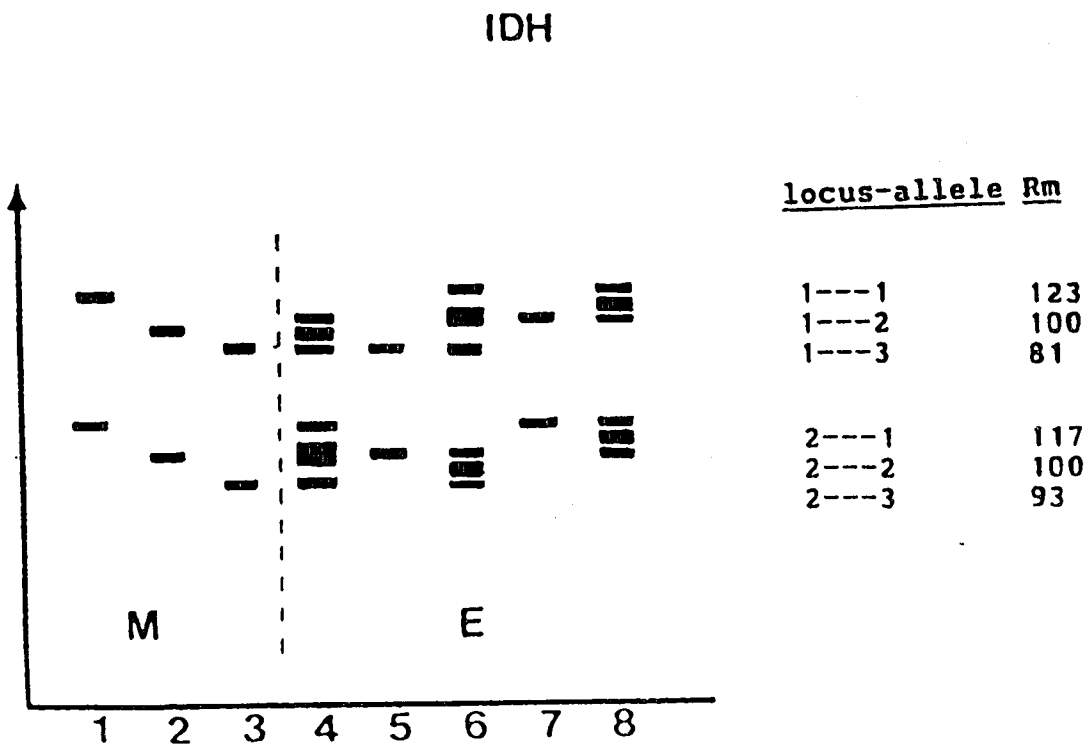


Figure 2.12 Diagram of isocitrate dehydrogenase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring in each lane: *IDH1*: 4=23, 5=33, 6=13, 7=22, 8=12 *IDH2*: 4=13, 5=22, 6=23, 7=11, 8=12.

2.3.1.11 Leucine aminopeptidase (EC 3.4.11.1)

One monomorphic zone of activity on LAP gels was observed in *Thuja orientalis*, which is unusual. Two or three polymorphic loci have been reported in most studied tree species (Adams and Joly, 1980a; Conkle, 1971, 1979; Eckert *et al.*, 1981; Guries and Ledig, 1978; Harry, 1986; King and Dancik, 1983; Mejnartowice and Bergmann, 1975; Muona *et al.*, 1987; Rudin, 1977;), and this enzyme has been inferred to be monomeric in *Calocedrus decurrens* (Harry, 1986), *Picea abies* (Lundkvist and Rudin, 1977), *Pinus attenuata* (Conkle, 1971) and *Pinus sylvestris* (Rudin, 1977).

2.3.1.12 Malic enzyme (EC 1.1.1.40)

One zone of activity appeared on ME gels, and three single-banded alleles were observed (Figure 2.13). Alleles 1 and 3 were observed only in the bulked samples, so that no data were available for segregation analysis. The subunit structure of this enzyme could not be confirmed in *Thuja orientalis* due to the unclear phenotypes of heterozygous embryos. Little work has been done on the inheritance of ME in forest trees, and the results presented here are similar to those found in *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982). It has been reported that ME displayed a tetrameric structure in human tissues (Harries and Hopkinson, 1976)

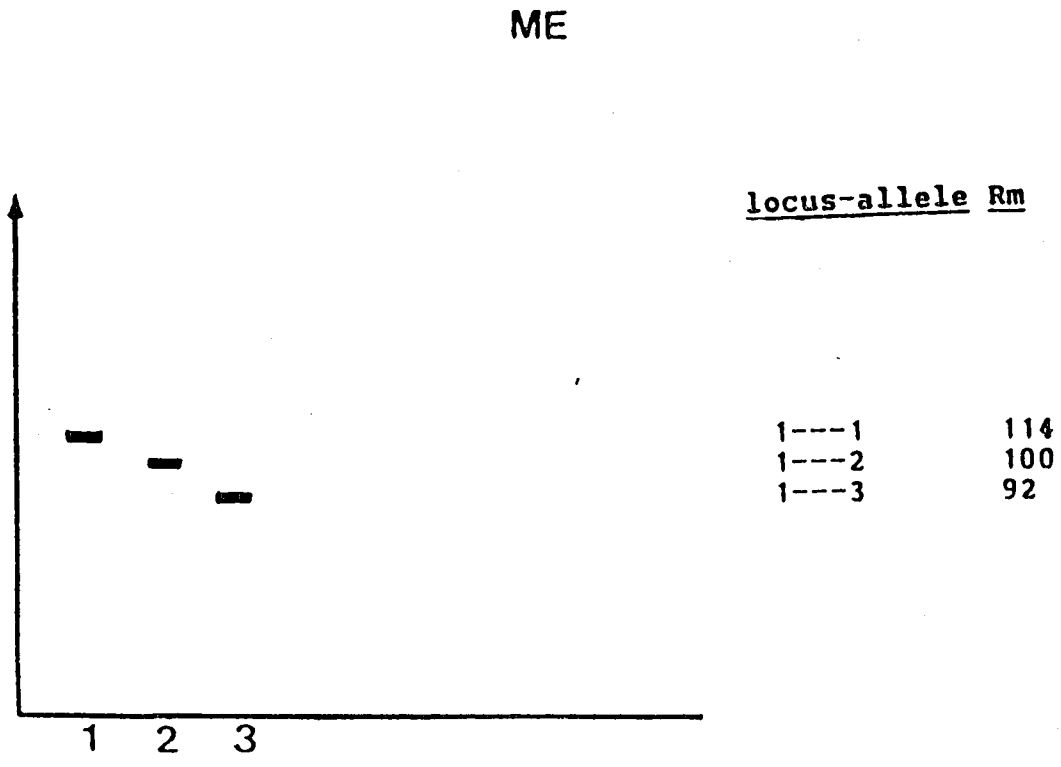


Figure 2.13 Diagram of malic enzyme gels indicating the haploid megagametophytes in lanes 1 to 3.

2.3.1.13 Mannose phosphate isomerase (EC 5.3.1.18)

MPI was stained faintly and inconsistently in *Thuja orientalis* , similar to the situation reported for *Pinus attenuata* (Strauss and Conkle, 1986). However, one zone of activity with two single-banded allozyme variants could be identified (Figure 2.14). The heterozygous embryo phenotypes consist of two bands that were identical to the parental alleles, suggesting that MPI is a monomer in *Thuja orientalis*.

2.3.1.14 Menadione reductase (EC 1.6.99.2)

Menadione reductase showed one zone of activity with three single-banded alleles (Figure 2.15). Heterozygous embryo phenotypes were triple-banded, suggesting MR is functionally dimeric in *Thuja orientalis*. This enzyme has not been commonly studied in forest trees. One zone of activity with two allozymes in *Pinus albicaulis* (Furnier *et al.*, 1986), three zones in *Pinus attenuata* (Strauss and Conkle, 1986) and two zones in *Pinus muricata* D. Don(Millar, 1985) has been recorded, but the subunit structure of MR has not been reported in those species.

Forty-four heterozygous trees were involved in segregation analysis of this locus. No segregation distortion was detected in Shandong Changqin or in the collection of 1982 from Beijing Miyun. Although two out of six trees from Shanxi Houxian showed a significant excess of allele 3 over allele 2, segregation among trees was hemogeneous and the pooled data fit the expectation well.

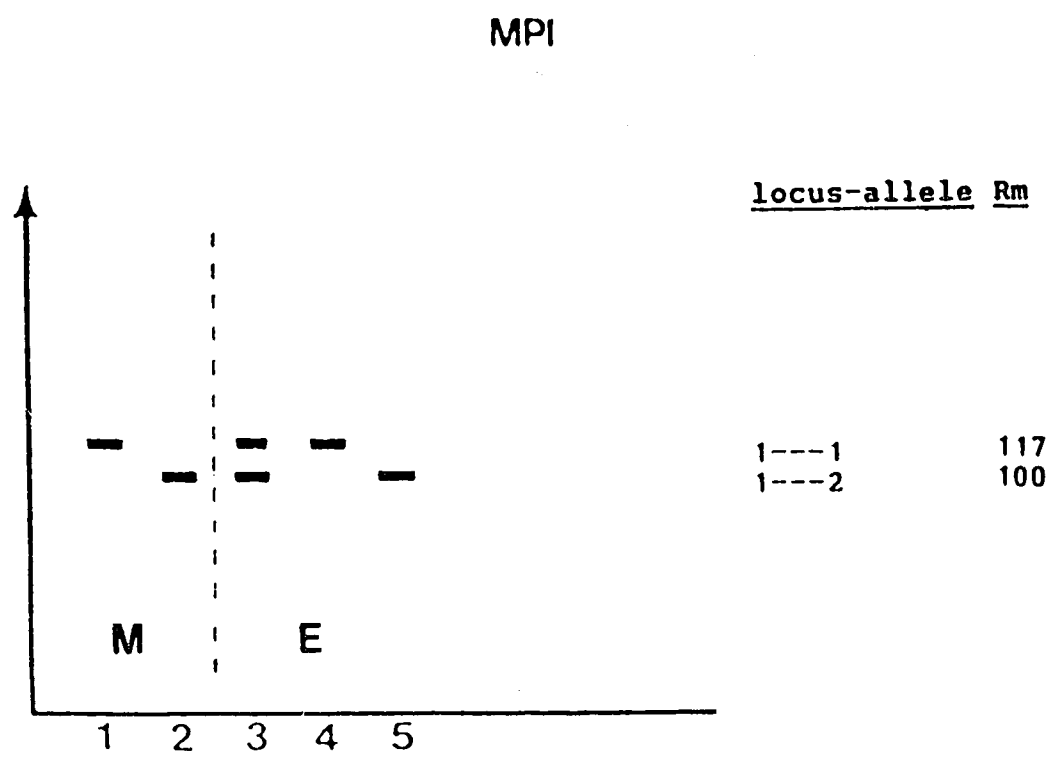


Figure 2.14 Diagram of mannose phosphate isomerase gels indicating the haploid megagametophytes in lanes 1 and 2 and diploid embryo phenotypes in lanes 3 to 5. Scoring in each lane: 3=12, 4=11, 5=22.

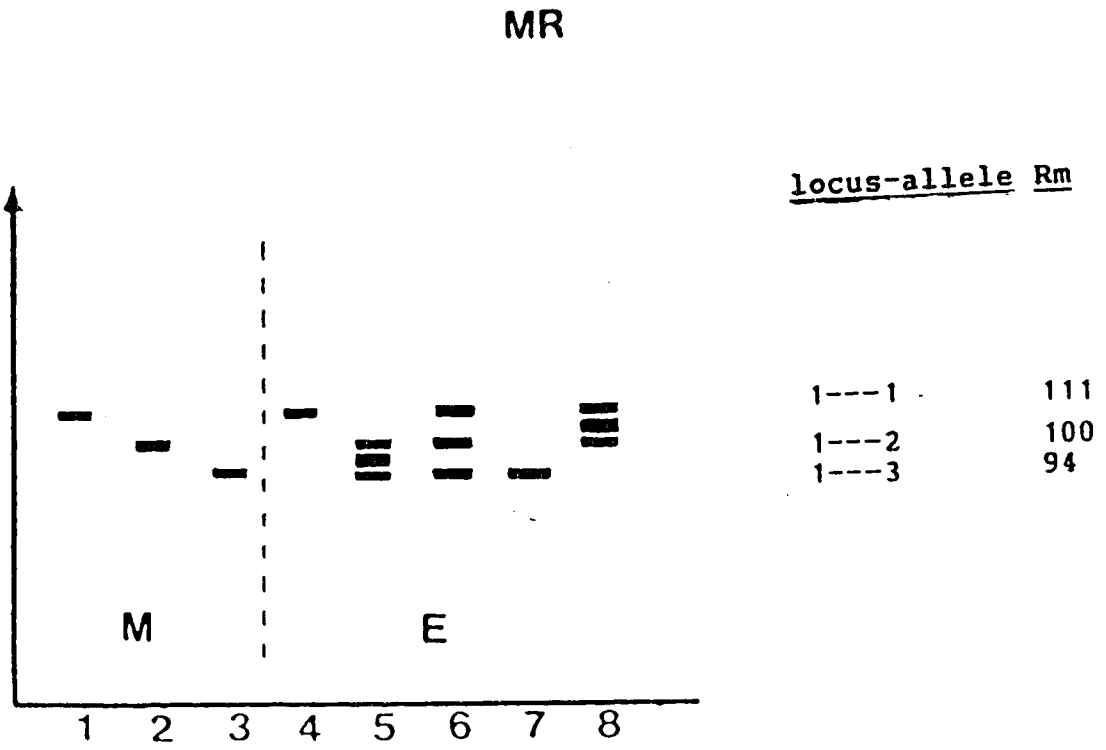


Figure 2.15 Diagram of menadione reductase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring in each lane: 4=11, 5=23, 6=13, 7=33, 8=12.

Three of the nine trees sampled from Shanxi Changzhi displayed a significant deviation of segregation from the Mendelian ratio. An overpresence of allele 2 to allele 3 was observed for two of them, while a deficiency of allele 1 to allele 3 was found for the remaining trees. Segregation of both allelic combinations (i.e. 23 and 13) was significantly heterogeneous in this population ($G_h(2)=8.80$ and $G_h(5)=25.66$, respectively). Only one of the eight trees sampled from Beijing Miyun in 1983 showed significant segregation distortion, and such distortion was due to an excess of the common allele (allele 2) to one of the rare alleles (allele 3). Segregation between these two alleles was significantly heterogeneous in the collection of 1983 ($G_h(7)=14.98$), but not in that of 1982 ($G_h(3)=4.30$). Segregation between the two collections was homogeneous and the pooled data fit the expected segregation ratio. No significant heterogeneity of segregation among populations was detected for any of the possible allelic combinations, and the pooled data fit the expected 1:1 ratio well.

2.3.1.15 Phosphoglucose isomerase (EC 5.3.1.9)

Two zones of activity were observed on gels stained for PGI in *Thuja orientalis* (Figure 2.16). This observation agrees with the findings common for other conifers (Adams and Joly, 1980a; Conkle, 1979; El-Kassaby *et al.*, 1982, 1987; Furnier *et al.*, 1986; Harry, 1986; Hyun *et al.*, 1987; King and Dancik, 1983; Muona *et al.*, 1987; O'Malley *et al.*, 1979; Strauss and Conkle, 1986; Wendel and Parks, 1982). The

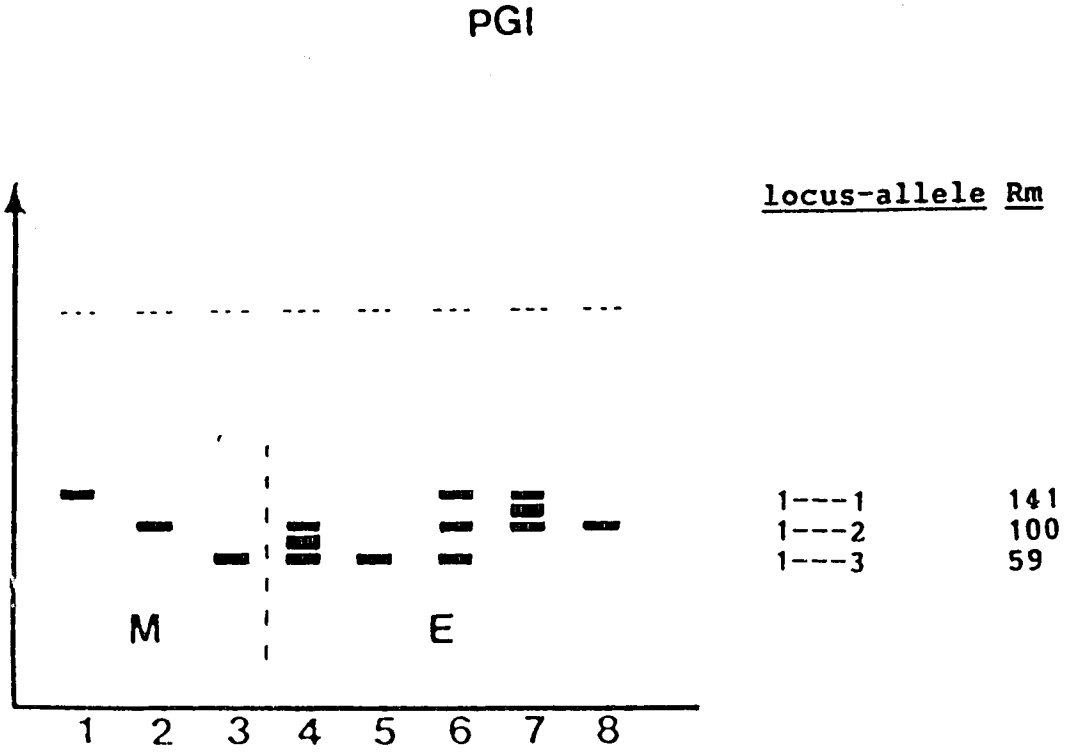


Figure 2.16 Diagram of phosphoglucose isomerase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring in each lane: 4=23, 5=33, 6=13, 7=12, 8=22.

anodal zone (*Pgi1*) was blurred and inconsistent; therefore, it was excluded from this and further studies. Three single-banded alleles were present at *Pgi2*, and heterozygous embryo phenotypes were triple-banded. Mobilities of the fast and slow bands were identical to those of parental alleles. Our finding strongly suggests that PGI is functionally dimeric in *Thuja orientalis*. Dimeric subunit structure of PGI has also been reported in *Calocedrus decurrens* (Harry, 1986), *Camellia japonica* (Wendel and Parks, 1982), *Picea glauca* (Cheliak and Pitel, 1984a), *Picea Sitchensis* (Simonsen and Wellendorf, 1975), *Pinus rigida* (Guries and Ledig, 1978), *Pinus taeda* (Adams and Joly, 1980a), *Populus tremuloides* (Hyun *et al.*, 1987) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982).

A total of thirty-three trees was employed for the test of segregation distortion at *Pgi2*. None of the eight trees from Shonxi Houxian showed unbalanced segregation, while both trees sampled in 1982 from Beijing Miyun displayed significant distortion (one showed an overpresence of allele 1 to allele 2, while another displayed the opposite direction of segregation distortion). Although a significant excess of allele 1 to allele 2 was detected for two out of eleven trees from Shandong Changqin, the segregation was homogeneous among trees and not distorted for the pooled data. In Shanxi Changzhi, segregation is homogeneous among the seven individuals tested and the pooled data fit the expectation well. Significant segregation distortion was

detected for one of the five trees sampled in 1983 from Beijing Miyun, and the distortion was due to an excess of allele 2 to allele 1.

2.3.1.16 Phophoglucomutase (EC 2.7.5.1)

Phosphoglucomutase had two zones of activity in *Thuja orientalis* (Figure 2.17). The faster zone was strongly stained, with four allozyme variants observed, while the slower zone was faint and blurred and therefore excluded from this and further studies. Homozygous embryo phenotypes were single-banded, while heterozygous embryo phenotypes were double-banded, which suggests that PGM is a monomer in this species. Two zones of activity for PGM have been reported in many other conifers (Adams and Joly, 1980a; El-Kassaby *et al.*, 1982, 1987; Furnier *et al.*, 1986; Hyun *et al.*, 1987; Muona *et al.*, 1987; Neal and Adams, 1981; Rajora, 1986; Strauss and Conkle, 1986; Weber and Stettler, 1981; Yeh and El-Kassaby, 1980). One PGM zone has been found in *Calocedrus decurrens* (Harry, 1986), *Picea glauca* (King and Dancik, 1983) and *Populus tremuloides* (Cheliak and Dancik, 1982). Three zones have been recorded in *Camellia japonica* (Wendel and Parks, 1982), *Pinus muricata* (Miller, 1985), *Populus deltoides* and *P. maximowiczii* (Rajora, 1986). PGM is monomeric in all studied tree species, and the alleles are single-banded except in *Camellia japonica*, where alleles at *Pgm1* are double-banded (Wendel and Parks, 1982).

It has been reported that one of the PGM zones showed variation synonymous to that observed for IDH in *Pinus*

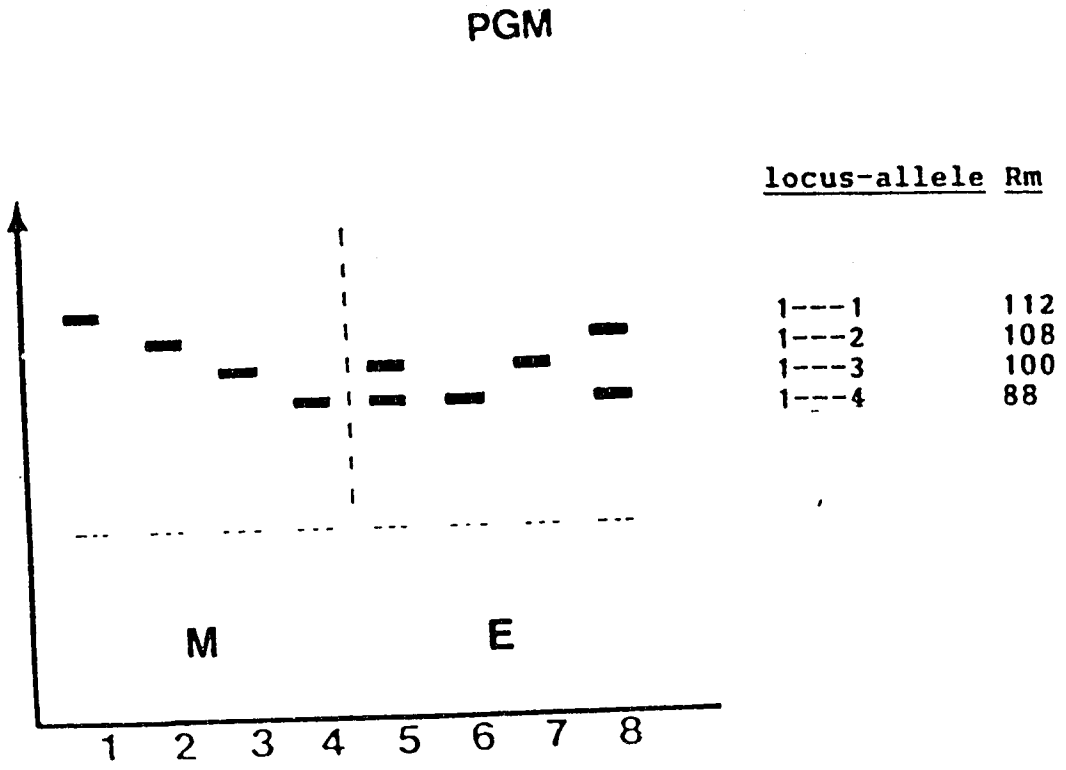


Figure 2.17 Diagram of phosphoglucomutase gels indicating the haploid megagametophytes in lanes 1 to 4 and diploid embryo phenotypes in lanes 5 to 8. Scoring in each lane: 5=34, 6=44, 7=33, 8=24.

attentuata (Strauss and Conkle, 1986) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982). In *Thuja orientalis*, variation at *Pgm1* was not synonymous to that of either *Idh1* or *Idh2*.

A total of 9 trees were available for segregation analysis at *Pgm1*. Segregation distortion was detected for 5 out of 6 trees from Beijing Miyun; two were sampled in 1982 and the other three were collected in 1983. The distortion was due to a deficiency of allele 2 relative to allele 3. Segregation over trees was heterogeneous within both collections in this population and between the two sampling years. No segregation distortion was detected for the other three trees with genotype 34.

2.3.1.17 Shikimate Dehydrogenase (1.1.1.25)

Resolution of shikimate dehydrogenase was poor, especially in some individuals. However, three zones of activity on SKDH gels could be clearly observed (Figure 2.18). Three single-banded allozyme variants were detected at the most anodal zone (*Skdh1*), while the other two zones were too diffused to enable scoring of allelic variation. Heterozygous embryo phenotypes were double-banded and mobilities of these two bands were identical to those of parental alleles, suggesting that this enzyme is a monomer in *Thuja orientalis*. Three zones of activity for SKDH have also been reported in *Pinus muricata* (Miller, 1985). Two zones were observed in *Pinus attenuata* (Strauss and Conkle, 1986) and one zone in *Picea abies* (Muona *et al.*, 1987).

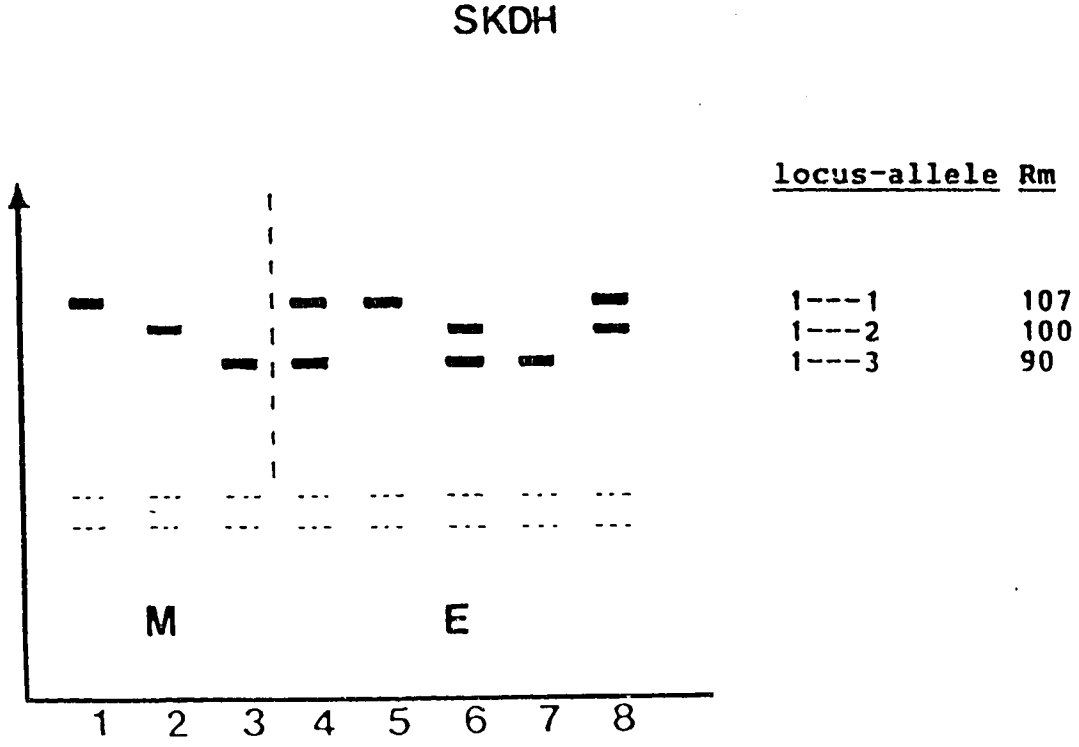


Figure 2.18 Diagram of shikimate dehydrogenase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring in each lane: 4=13, 5=11, 6=23, 7=33, 8=12.

However, subunit structure of SKDH has not been studied in those species. Linhart *et al.* (1981) reported that SKDH was a monomer in needles of *Pinus ponderosa*.

Fifteen trees were available for segregation analysis at *Skdh1*, and distortion was detected for two of them. Those two trees were sampled in 1982 from Beijing Miyun and had the 23 genotype. The direction of distortion of the two trees was opposite, and segregation was significantly heterogeneous between them. Segregation distortion was not detected for any allelic combinations in other collections. Segregation among populations and between sampling years was homogeneous, and the pooled data fit the expected segregation ratio.

2.3.1.18 Superoxide dismutase (EC 1.15.1.1)

Superoxide dismutase showed one monomorphic zone of activity in *Thuja orientalis*, as has been reported in *Picea glauca* (King and Dancik, 1983) and *Pinus monticola* (El-Kassaby *et al.*, 1987). However, polymorphic SOD has been observed in many other tree species (Cheliak and Pitel, 1985; Conkle, 1979; El-Kassaby *et al.*, 1982; Harry, 1986; Wendel and Parks, 1982; Yeh, 1988; Yeh and O'Malley, 1980). SOD has been found to be dimeric in *Calocedrus decurrens* (Harry, 1986) and *Pseudotsuga menziesii* (El-Kassaby *et al.*, 1982).

2.3.1.19 6-Phosphogluconic dehydrogenase (EC 1.1.1.44)

Two zones of activity were observed on the gels stained for 6PG. Three and two alleles were detected at *6pg1* and *6pg2*, respectively, and those alleles showed single-banded patterns (Figure 2.19). Heterozygous embryos displayed triple-banded patterns, suggesting 6PG is functionally dimeric in *Thuja orientalis*. Dimeric subunit structure of 6PG has also been reported in many other tree species (Adams and Joly, 1980a; El-Kassaby *et al.*, 1982; Harry, 1986; Hyun, *et al.*, 1986; Wendel and Parks, 1982). One to five loci coding for 6PG have been recorded (Adams and Joly, 1980; El-Kassaby *et al.*, 1982; King and Dancik, 1983; Rajora, 1986).

Thirty-three trees were available for the test of segregation distortion at *6pg2*. No tree showed unbalanced segregation in Shandong Changqin and in the collection of

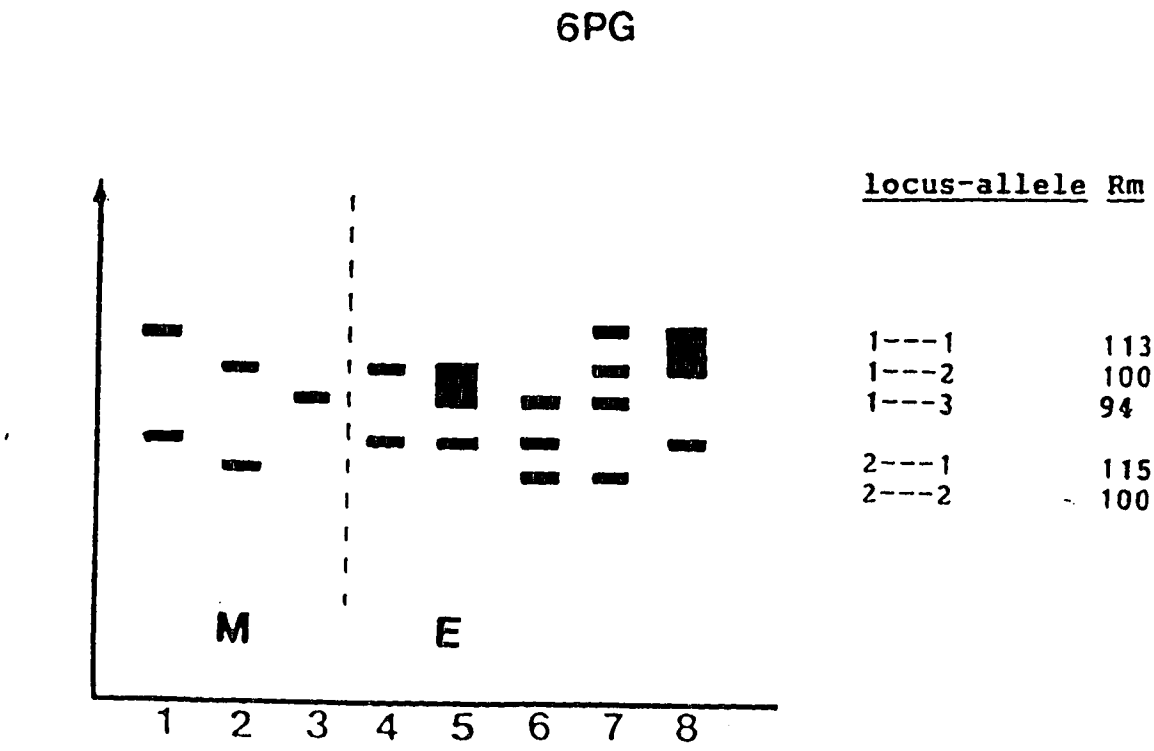


Figure 2.19 Diagram of 6-phosphogluconic dehydrogenase gels indicating the haploid megagametophytes in lanes 1 to 3 and diploid embryo phenotypes in lanes 4 to 8. Scoring in each lane: *6PG1*: 4=22, 5=23, 6=33, 7=13, 8=12 *6PG2*: 4=11, 5=11, 6=12, 7=22, 8=11.

1983 from Beijing Miyun. A significant overpresence of allele 2 to allele 1 was detected for one of the seven trees sampled from Shanxi Changzhi, and three out of eight trees from Shanxi Houxian. Segregation distortion was detected for three of the four trees sampled in 1982 from Beijing Miyun; one showed an overpresence of allele 1 to allele 2, while the remaining two showed distortion in the opposite direction. Segregation between sampling years in Beijing Miyun was significantly hetrogeneous ($G_h(1)=4.34$). Segregation was homogeneous among trees in Shandong Changqin and the 1983 collection from Beijing Miyun. However, it was heterogeneous in the other collections. After the tree showing severe segregation distortion was removed, segregation was homogeneous among the remaining trees in both Shanxi Changzhi and Shanxi Houxian, and the pooled data in both populations fit the expectation. Segregation between sampling years in Beijing Miyun and among populations was significantly heterogeneous. The heterogeneity of segregation among populations was largely contributed by Beijing Miyun.

One tree was heterozygous for alleles 1 and 3 at *6pg1* and the segregation ratio did not deviate significantly from Mendelian expectation.

2.3.2 Linkage Relationships

Fifty-eight trees were available to test for linkage among 47 of 91 possible locus pairs with the 14 polymorphic

loci studied. The maximum number of trees employed for each locus pair was 18 and the total number of tests (not including the tests for pooled data) was 179. Of those 179 tests, 13 were significant (Table 2.5). The results can be grouped into three classes:

1) Locus pairs without significant linkage for both individual trees and pooled trees. Thirty-seven locus pairs, accounting for about 79% of the total, were included in this class (Table 2.6).

2) Locus pairs with significant linkage that could not be accepted. Six locus pairs, accounting for about 13% of the total, were grouped into this class. Four to seventeen trees were used to test the linkage of the six locus pairs, but only one tree displayed significant deviation from the expected joint segregation ratio for each of them. The joint segregation among trees was homogeneous for all locus pairs and no significant linkage was detected for the pooled data (Table 2.7). Therefore, the detected linkages for those locus pairs could be attributed to chance alone.

3) Locus pairs with significant linkage that could be accepted. Four locus pairs were involved in this class, accounting for the remaining 8% of the total studied locus pairs. In this class, each locus pair showed significant linkage for one or more trees and/or pooled data (Table 2.8). Those detected linkages could not be explained by chance alone and, therefore, true linkages were suggested.

Table 2.5 Summary of Linkage Analysis in *Thuja orientalis* Linn.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.*Pgm1*		8	1	1	1	1	-	3	3	-	-	2	1	-
2.*Mr*	0		18	9	2	1	1	18	17	-	2	2	3	1
3.*Aat3*	0	0		4	-	-	-	9	12	-	1	1	1	-
4.*Skdh1*	0	1	0		-	-	-	8	7	-	2	1	-	-
5.*Aco*	0	0	-	-		-	-	2	2	-	-	1	-	-
6.*Gdh*	0	0	-	-	-		-	1	2	-	-	-	-	-
7.*6pg1*	-	0	-	-	-	-		-	-	1	-	-	-	-
8.*6pg2*	1	2	1	1	0	0	-		11	1	3	-	2	1
9.*Pgi*	3	1	1	0	0	0	-	0		2	2	4	1	-
10.*Ha*	-	-	-	-	-	-	0	1	0		-	1	-	1
11.*Fest1*	-	0	0	0	-	-	-	0	0	-		-	-	-
12.*Idh1*	0	0	0	0	0	-	-	-	1	0	-		-	-
13.*Idh2*	0	0	0	-	-	-	-	0	0	-	-	-		-
14.*αgal*	-	0	-	-	-	-	-	0	-	0	-	-	-	

The numbers above the diagonal represent the number of trees studied within the same locus pairs. The numbers below the diagonal represent the number of trees showing significant association between the two involved loci.

Table 2.6 Locus Pairs Without Significant Linkage

Pgm1:Mr	*6pg1:Ha*	*Idh1:Aco*	*αgal:Ha*
Pgm1:Idh2	*Mr:Aat3*	*Idh1:Skdh1*	*Aco:Pgi2*
Pgm1:Skdh1	*Mr:Idh1*	*Idh1:Ha*	*Aco:Pgm1*
Pgm1:Gdh	*Mr:6pg1*	*Idh2:Aat3*	*Aat3:Idh1*
Pgm1:Idh1	*Mr:Gdh*	*Pgi2:Idh2*	*Aat3:Pgm1*
6pg2:Pgi2	*Mr:Idh2*	*Pgi2:Gdh*	*Aat3:Ha*
6pg2:Aco	*Mr:Aco*	*Pgi2:Skdh1*	*Aat3:Skdh1*
6pg2:Gdh	*Mr:αgal*	*Pgi2:Ha*	*Aat3:Fest1*
6pg2:Fest1	*Mr:Fest1*	*Pgi2:Fest1*	*Fest1:Skdh1*
6pg2:αgal			

Table 2.7 Test of Linkage for the Locus Pairs in Class 2 Using Pooled Data

Locus Pair	No. of Trees	No. of Seeds	Gametic Frequencies A_1B_1	A_1B_2	A_2B_1	A_2B_2	G_h	GI	GII	G
Mr:Pgi2	17	874	216	213	212	233	20.857	0.292	0.370	0.640
Mr:Skdh1	9	562	159	141	130	132	8.800	2.571	0.455	0.637
Pgi2:Idh1	4	218	73	36	64	45	6.434	0.000	14.548*	1.646
6pg2:Aat3	11	703	124	186	186	207	10.550	9.822*	9.822*	-
6pg2:Pgm1	4	245	46	74	43	82	4.510	0.102	18.558*	0.379
6pg2:Skdh1	9	566	113	121	185	147	8.218	17.052*	1.591	2.947

Table 2.8 Locus Pairs with Significant Linkage that Could Be Accepted

Locus Pair	Gametic Frequencies A_1B_1	A_1B_2	A_2B_1	A_2B_2	GI	GII	G	R	SE	NL/NS#
Pgi2:Aat3	106	253 (pooled data)	202	216	0.001	12.216*	6.339*	0.456	0.019	1/13
Pgi2:Pgm1	10	27	0	23	3.291	29.110*	3.953*	0.450	0.064	3/3
	24	5	8	23	0.067	0.267	20.610*	0.217	0.053	
	23	9	11	28	0.671	0.127	13.745*	0.282	0.053	
6pg2:Ha	18	11	9	22	0.067	0.601	6.663*	0.333	0.061	1/1
6pg2:Mr	2	29	21	8	0.067	3.297	28.492*	0.167	0.048	2/18
	17	15	6	19	0.862	2.136	4.737*	0.368	0.064	

#: number of trees showed significant linkage/number of trees studied

Although linkages were detected for four locus pairs, these linkages were relatively weak. The six loci involved in the linkage pairs likely belong to two linkage blocks, i.e., *Mr-6pg2-Ha* and *Aat3-Pg2-Pgm1*, although I am not sure whether those two linkage blocks are located on the same or different chromosomes.

2.4 Discussion

2.4.1 Inheritance

A total of 188 segregation tests was performed for the 14 isozyme loci, and significant deviations from the expected 1:1 ratio were detected for 38 of them. Of those 38 deviations, 23 (about 61%) were contributed by Beijing Miyun (Table 2.9). Although such a large number of deviations were observed, Mendelian inheritance could be inferred from segregation analysis for most of the isozymes in each population, except the 1982 collection from Beijing Miyun. In Shandong Changqin, only two out of 67 tests were significant, and Mendelian inheritance could be inferred for all the loci tested in this population. Since none of the isozymes showed consistent segregation distortion in all the populations, and since segregation was not assayed right at meiosis but a substantially later time, thus providing opportunity for gametic selection, I do not doubt the mode of Mendelian inheritance for these isozymes and would attribute most of the observed deviations to natural selection. The interpretation of Mendelian inheritance for these isozymes can be strengthened by comparing the phenotypes of the megagametophytes and the corresponding embryos and by using similarities among the isozymes of other conifers.

Segregation data were not available for *Lap* and *Sod* (both were monomorphic) and for *Aat1*, *Aat2*, *Adh1*, *Adh3*,

Table 2.9 Summary of Segregation Analysis for the 14 Polymorphic Loci

Loci	SCQ		SCZ		SH		BM83		BM82	
	NT	ND	NT	ND	NT	ND	NT	ND	NT	ND
Aat3	15	0(*)	3	0(*)	2	0(*)	3	1	4	3
Aco	-		-		1	0	-		2	2
αgal	1	0	-		-		-		-	
Fest1	3	0(*)	1	1	-		-		-	
Gdh	-		-		3	1(*)	-		1	0
Ha	-		-		-		5	1(*)	-	
Idh1	-		2	0	1	1	2	1	-	
Idh2	2	0(*)	1	0	-		1	1	-	
Mr	17	0(*)	9	3	6	2(*)	8	1	4	0(*)
Pgi2	11	2(*)	7	1(*)	8	0(*)	5	1	2	2
Pgm1	1	0	-		2	0(*)	3	3	3	2
Skdh1	6	0(*)	3	0(*)	2	0(*)	2	0(*)	2	2
6pg1	-		-		-		1	0	-	
6pg2	11	0(*)	7	1	9	3	2	0(*)	4	3
Total	67	2	33	6	34	7	32	9	22	14

*: Segregation was homogeneous among trees, and pooled data fit 1:1 segregation ratio

NT=number of trees tested

ND=number of distortions detected.

Aph3, *Aph4*, *Fest1*, *Me* and *Mpi* (low levels of polymorphism). However, the observation that embryo phenotypes were consistent with a single-locus interpretation and/or the fact that inheritance of those isozymes has been documented in other conifers makes it resonable to infer that the isozymes described above are also inherited in a simple Mendelian fashion.

Segregation distortion has been found to be relatively common in studies of conifer isozymes using viable seed (for references see Strauss and Conkle (1986)). Such phenomena could be caused by many factors, including meiotic drive and natural selection. However, the detected significant heterogeneity of segregation among trees within populations, among populations and between sampling years does not suggest meiotic drive as an important cause of the observed distortion. Alternatively, natural selection seems to be of great importance. Since segregation was assayed using mature seeds, there is ample opportunity for prezygotic and postzygotic selection. Megaspores with certain genotypes might suffer developmental difficulties and thus infrequently mature into female gametophytes. Even if megaspores with different genotypes can develop to mature female gametophytes with equal frequencies and can be equally fertilized, differential survival of female gametophytes with different genotypes may occur due to the difference in the fitness of the embryos. It has been demonstrated that conifers typically have large numbers of

embryonic-lethals (Fowler, 1965; Sorensen, 1969). If a marker gene is linked to a recessive embryonic-lethal, selection against the recessive, homozygous embryos caused by inbreeding would result in a deficiency of the female gametophyte carring that marker gene.

It should be pointed out that the seeds used in the present study had been stored for more than two years before use. Embryos with different genotypes might have been influenced by different selection pressure during this storage period. Thus, segregation ratios might have exhibited further deviation from expectation. Comparing the results of segregation analysis for the two collections from Beijing Miyun has shown that segregation distortion is much more frequent in the 1982 collection than that of 1983 (Table 2.7); the former has been stored one year longer than the latter. This observation seems to support the above speculation.

The observed segregation distortions could also result from mislablling of sampled materials. For instance, if seeds from two trees were labled as if from a single tree, and if one tree is homozygous and another is heterozygous at a particular locus, segregation distortion will be generated even if the heterozygote displays an exact 1:1 segregation ratio.

No matter what is the true mechanism leading to the observed segregation distortion, the net effect, observed segregation distortions, would affect the estimates of mixed

mating system parameters (Cheliak, *et al.*, 1984). Therefore, those loci showing systematic segregation distortion should be excluded from mating system studies.

Heterogeneity of segregation among trees within populations was frequently detected. Such heterogeneity may indicate that the outcrossing pollen pools are heterogeneous or that the genetic backgrounds of those trees are different with respect to linked recessive lethals or semi-lathals upon selfing (Cheliak, *et al.*, 1984).

2.4.2 Linkage

It has been found that linkage among allozyme loci is highly conserved in the *Pinaceae*, and such findings corroborate karyotypic studies (Saylor and Smith, 1966; Runquist, 1968; Sax and Sax, 1933). In *Cupressaceae*, such studies rarely have been carried out and I believe that linkage among allozyme loci has been reported in only one species, *Calocedrus decurrens* (Harry, 1986). In that study, tight linkage between *Got2* and *Pgi2* was detected (R=0.060). Harry believed that the *Got2:Pgi2* linkage found in *Calocedrus decurrens* represents the same linkage group as that described in *Pinaceae* that has been maintained since before the divergence of the two families.

In the present study, no data were available to test the linkage between *Got2* and *Pgi2* in *Thuja orientalis*, so I could not confirm if *Got2:Pgi2* is a conserved linkage group in *Cupressaceae*. However, I found that those locus pairs

showing independent segregation in *Calocedrus decurrens* were also independent in *Thuja orientalis* (e.g. *Aco:Fest*, *Aco:Skdh1*, *Fest:Pgi2*, *Pgi2:Skdh1*). Therefore, linkage among allozyme loci in *Cupressaceae* might also be highly conserved. Of course, to be more certain, more locus pairs should be compared between the two species and among more species in this family.

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3. Mating System

3.1 Introduction

The mating system is the pattern by which gametes are united to transmit genes from generation to generation (Stern and Roche, 1974), and is regarded as an important determinant of the genetic structure and evolutionary potential of a population (Allard, 1975; Brown *et al.*, 1975; Clegg, 1980; Jain, 1976; Kahler *et al.*, 1975; Lande and Schemske, 1985). Studies on the mating system of natural populations of conifers have been greatly stimulated by the use of isozyme markers. During the past decade, mating system studies have been reported for a large number of coniferous species (some of them are given in Table 3.1), and it has been commonly observed that conifers are predominantly outcrossers with outcrossing rates usually higher than 90 percent. It has also been noted that estimates of outcrossing rate vary significantly from population to population (Furnier and Adams, 1986), year to year (Cheliak *et al.*, 1985), tree to tree (Neal and Adams, 1985; Shaw and Allard, 1982), and from different positions of a tree's crown (Shaw and Allard, 1982). However, most of the results reported were obtained from species in the *Pinaceae*. Information on the mating systems of conifers in any other family is rare. In this chapter, the results of mating system studies in four natural populations of *Thuja orientalis* , a member of the *Cupressaceae*, are reported.

Table 3.1 Mating System Studies in Natural Populations of Some Conifers

Species	No. of Pops.	No. of Loci	Outcrossing Rates	Reference
Abies lasiocarpa	2	7	0.89(t_m)[1]	Shea,1987
Picea engelmannii	2	6	0.93(t_m)	Shea,1987
Picea glauca	1	4	0.90(t_m)	King *et al.*,1984
Picea mariana	6	6	0.92(t_m)	Boyle & Morgenstern,1985
Pinus banksiana	1	4	0.88(t_m)	Cheliak *et al.*,1985
Pinus contorta	2	7	0.99(t_m)	Epperson & Allard, 1984
Pinus jeffreyi	5	11	0.94(t_m)	Furnier & Adams, 1986
Pinus monticola	1	6	0.98(t_m)	El-Kassaby *et al.*, 1987
Pinus ponderosa	1	6	0.96(t_s)[2]	Mitton *et al.*,1981
Pseudotsuga menziesii	1	4	0.90(t_m)	El-Kassaby *et al.*,1981
	2	10,9	0.98(t_m)	Neal & Adams,1985
	8	11	0.90(t_m)	Shaw & Allard,1982
	1	10	0.89(t_m)	Yeh & Morgan,1987

1. t_m=multilocus estimate;

2. t_s=single-locus estimate.

3.2 Material and Methods

Four populations were investigated in the present study (Table 3.2 and Figure 3.1). The sampling strategies, tissue preparation and electrophoretic conditions were described in chapter two. A total of seven polymorphic loci (*Fest1*, *Idh1*, *Idh2*, *Mr*, *Pgi2*, *Skdh1* and *6pg2*) were chosen for this study based on the criteria of: 1) the enzyme banding patterns were clear and consistent in both megagametophytes and embryos; 2) segregation was not systematically distorted; 3) the loci were not tightly linked; and 4) they had relatively high polymorphisms.

Both single-locus and multilocus mating system parameters, i.e. outcrossing rate (t) and parental gamete frequencies in the outcrossing pollen pool (p's), were estimated using the expectation-maximization (E-M) algorithm procedures. These procedures (see Appendix) were formulated based on the mixed mating model and therefore have the following assumptions:

1. There are only two types of mating, i.e. random outcrossing and self-fertilization. The relative proportion of these two types of mating in a population is t:s (s=1-t).

2. Both the parental gamete frequencies in outcrossing pollen pool (p's) and the outcrossing rate (t) are uniform over all the maternal trees sampled.

Table 3.2 Location and Sample Size for the Four Natural Populations of *Thuja orientalis* Linn. Sampled in the Present Study

No.	Population Name	Latitude (°N)	Longitude (°E)	Elevation (Meter)	No. of Trees	No. of Seeds
1	Shandong Changqin (SCQ)	36.60	116.73	300	31	1708
2	Shanxi Changzhi (SCZ)	36.32	118.17	250*	20	1006
3	Shanxi Houxian (SH)	36.57	111.92	1000	16	684
4	Beijing Miyun (BM)	40.38	116.83	650	9	435

*: estimated from a map.

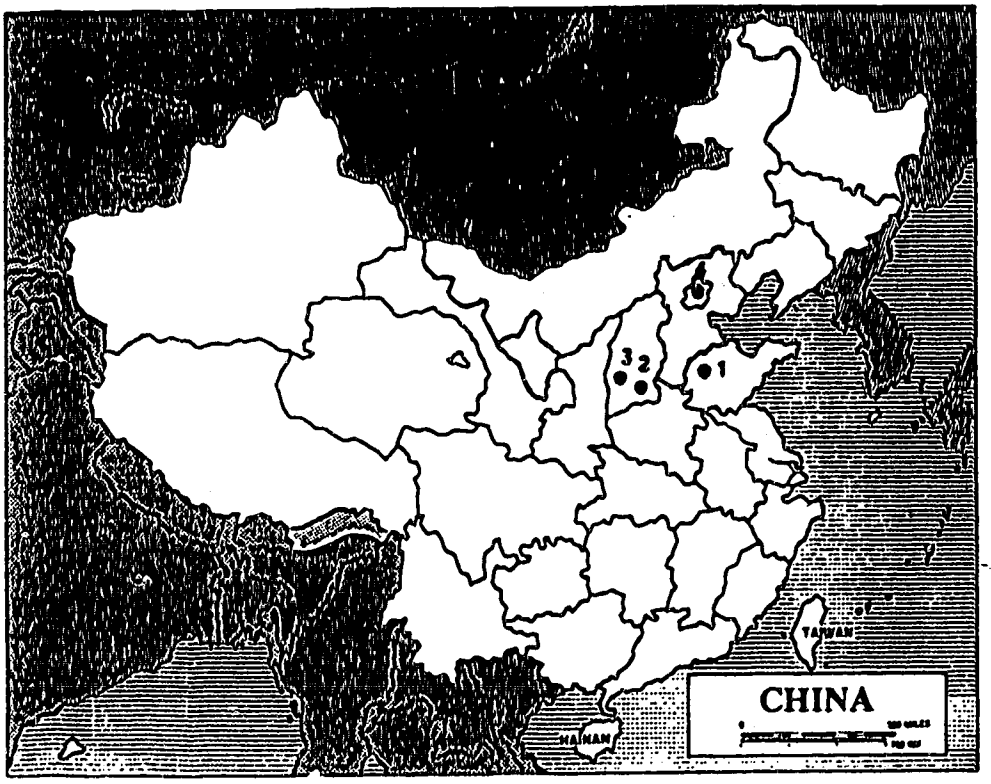

Figure 3.1 Geographical Locations of the Four Natural Populations of *Thuja orientalis* Linn. Sampled in This Study

3. All embryos, regardless of mating event, have equal fitness between fertilization and the assay of progeny genotypes.

4. Segregation of the alleles in heterozygous maternal trees fits a Mendelian 1:1 ratio.

5. For the multilocus procedure, the loci employed segregate independently.

Based on the results reported in chapter two, the last two assumptions do not seem to have been violated severely for the loci used in this study. However, each of the first three assumptions is likely not satisfied. Some degree of assortative matings or mating between nearby related individuals could occur in most natural populations. The parental gamete frequencies in the outcrossing pollen pool could be heterogeneous over maternal trees due to microhabitat selection causing similar genotypes to be clustered within populations and/or the tendency of seeds to fall and grow near their maternal parent causing relatives to be clustered (Shaw *et al.*, 1981). Genetic differences among individual trees in their selfing ability may result in significant heterogeneity of outcrossing rate among maternal trees sampled. Finally, different forms of selection could be present between fertilization and the assay of progeny genotypes.

Inferences about inbreeding rather than selfing in populations can be drawn by comparing single-locus and multilocus population estimates. Single-locus procedures

would underestimate the outcrossing rate when mating between relatives occurs, while multilocus procedures are more robust to such violation and more efficiently discriminate between selfed and outcrossed matings, especially if a large number of polymorphic loci are assayed (Shaw *et al.*, 1981).

Heterogeneity of the multilocus individual tree estimates of outcrossing rate within populations was tested by calculating the following likelihood-ratio test statistic:

$$G_h=-2\left(\sum_{i}^{N}\sum_{j}^{k_i}\mathrm{Log}(L'(G_{ij}))-\sum_{i}^{N}\sum_{j}^{k_i}\mathrm{Log}(L(G_{ij}))\right),$$

where

N=number of maternal trees sampled,

k_i=number of parental gamete types for the i^{th} maternal tree,

$L'(G_{ij})$=likelihood of the j^{th} parental gamete type of the i^{th} maternal tree when $t_{m1}=t_{m2}=\ldots=t_{mN}=t_m$, and

$L(G_{ij})$=likelihood of the j^{th} parental gamete type of the i^{th} maternal tree when $t_{m1}\neq t_{m2}\neq\ldots\neq t_{mN}$.

G_h is asymptotically distributed as χ^2 with N-1 degrees of freedom. Since G_h is calculated under the pre-assumption of homogeneity of outcrossing pollen frequencies among maternal trees, the heterogeneity detected by the G-test could be a result of the heterogeneity of outcrossing rates,

outcrossing pollen frequencies, or both.

A similar G-test was employed to examine the heterogeneity of single-locus outcrossing rate estimates over loci and multilocus estimates among populations. However, homogeneity of outcrossing pollen frequencies over loci and populations was not presumed.

To determine whether outcrossing in each population is complete, the null hypothesis of t=1 was tested by calculating the G-statistic:

$$G=-2\left(\sum_{j}^{k} \mathrm{Log}(L'(G_j))-\sum_{j}^{k} \mathrm{Log}(L(G_j))\right),$$

where

k=number of parental gamete types observed in the population,

$L'(G_i)$=likelihood of the i^{th} parental gamete type when t=1, and

$L(G_i)$=likelihood of the i^{th} parental gamete type when $t=t_m$.

Here, G is asymptotically distributed as χ^2 with 1 degree of freedom.

In order to measure the deviation of observed heterozygosity from that expected under Hardy-Weinberg equilibrium, Wright's fixation index (Wright, 1965) was calculated for both filial and maternal populations by

$$F=1-(H_o/H_e),$$

where

$H_o = 1 - \sum_i P_{ii}$ is the observed heterozygosity,

$H_e = (2N/(2N-1))(1 - \sum_i P_i^2)$ is the unbiased estimate of expected heterozygosity for a small number of individuals (Nei, 1978),

P_i = estimated frequency of the i^{th} allele,

P_{ii} = observed frequency of the i^{th} homozygous class, and

N = number of embryos or maternal trees sampled from the population.

Observed genotype frequencies were tested against those expected under Hardy-Weinberg equilibrium by a goodness of fit G-test, which is equivalent to a test of the null hypothesis of F=0. When deviation from Hardy-Weinberg equilibrium was detected, an expected equilibrium inbreeding coefficient, F_e, and the expected genotype frequencies under the mating system equilibrium were calculated by

$$F_e = (1-t_m)/(1+t_m) \text{ (Fyfe and Bailey, 1951)},$$

and

$$P_{ii} = p_i F_e + p_i^2 (1-F_e),$$

$$H_{ij} = 2 p_i p_j (1-F_e).$$

The same G-test was employed to compare the observed genotype frequencies with the expected ones under mating

system equilibrium.

Finally, the estimated common allele frequencies in the outcrossing pollen pool (male) were compared with those in the maternal population (female) by means of an independence G-test based on percentage (Sokal and Rohlf, 1981).

3.3 Results

Estimates of common allele frequencies for maternal parents (the ovule pool) and in the outcrossing pollen pools in the four populations are given in Table 3.3. Significant differences in allele frequencies between the two gamete pools were detected in seven out of twenty cases, and six of them were due to an excess of the common alleles in the outcrossing pollen pool. Of those seven cases, four were in Beijing Miyun, three were in Shanxi Changzhi, and one was in Shanxi Houxian.

Single-locus population estimates of outcrossing rate (t_s) fluctuated widely among loci within each population, varied over a 2.8-fold range, and were significantly heterogeneous (Table 3.4). The unweighted means of single-locus estimates of outcrossing rate was 0.70.

In all populations, the multilocus population estimates (t_m) were higher than the unweighted means of single-locus estimates (t_s) by an average of 5 percent, but all were significantly lower than 1.0 (Table 3.5). t_m values were significantly heterogeneous among populations. The overall mean multilocus outcrossing estimate was 0.75.

Table 3.3 Common Allele Frequencies (%) in Maternal Populations (F) and the Outcrossing Pollen Pools (M)

Locus	Population															
	Shandong Changqin				Shanxi Changzhi				Shanxi Houxian				Beijing Miyun			
	F	M	G	P	F	M	G	P	F	M	G	P	F	M	G	P
6pg2	80.6	77.0	0.39	ns[1]	68.4	86.7	9.87	**[2]	75.0	76.7	0.10	ns	72.2	50.0	10.50	**
Pgi2	67.7	77.6	2.47	ns	68.4	59.7	1.66	ns	67.9	64.1	0.34	ns	50.0	66.7	5.78	*
Mr	61.3	62.5	0.04	ns	50.0	75.8	14.50	***	67.9	74.4	1.04	ns	55.6	64.1	1.52	ns
Idh1		---[3]			92.1	92.5	0.02	ns		---			83.3	97.5	12.90	***
Idh2	96.8	94.0	0.92	ns		---			96.4	92.5	1.49	ns		---		
Fest1	95.2	96.8	0.34	ns	94.7	98.2	1.89	ns		---				---		
Skdh1		---				---			39.3	53.9	4.31	*	50.0	65.1	4.70	*

1. ns: P>0.05;

2. significance level: *: P<0.05; **: P<0.01; ***: P<0.001;

3. ---: locus was not used for estimating the mating system parameters in the particular population;

G= independence G-test based on percentage.

Table 3.4 Single-locus Population Estimates of Outcrossing Rate (t_s)

Locus	Population SCQ	SCZ	SH	BM
6pg2	0.74	0.78	0.74	0.36
Pgi2	0.56	0.39	0.83	0.63
Mr	0.92	0.75	0.84	0.82
Idh1	-	0.32	-	0.82
Idh2	0.94	-	0.75	-
Fest1	0.81	0.91	-	-
Skdh1	-	-	0.61	0.52
Unweighted Mean	0.79	0.63	0.75	0.63
G_h (df=4)	134.60	113.18	21.56	23.42
P	<0.001	<0.001	<0.001	<0.001

G_h=likelihood-ratio test of heterogeneity.

Table 3.5 Multilocus Population Estimates of Outcrossing Rate (t_m)

Populations	Number of Loci	t_m	Likelihood-ratio Test of Hypothesis: $t_m=1$ G(df=1)	P
SCQ	5	0.81	296.11	<0.001
SCZ	5	0.68	369.20	<0.001
SH	5	0.81	103.71	<0.001
BM	5	0.69	82.03	<0.001
Unweighted Mean of Outcrossing Rate				0.75
Likelihood-ratio Test of Heterogeneity				
G_h (df=3)				32.41
P				<0.001

Individual-tree multilocus estimates of outcrossing rate (t_{mi}) were significantly heterogeneous in each population and ranged from 0.45 to 1.00 in Shandong Changqin, 0.15 to 1.00 in Shanxi Changzhi, 0.47 to 1.00 in Shanxi Houxian, and from 0.38 to 1.00 in Beijing Miyun (Table 3.6).

A deficiency of the observed heterozygosity compared to that expected under Hardy-Weinberg equilibrium was observed in 19 out of 20 cases in the filial populations (Table 3.7). Of those 19 cases, 16 were significant at the 5 percent level or higher. The extent of heterozygote deficiency for individual loci ranged from 2.2% (*Mr* in Shandong Changqin) to 53% (*6pg2* in Beijing Miyun). The overall deficiency in each of the four filial populations was 13.8%, 23.5%, 27.9% and 33.0% for Shandong Changqin, Shanxi Changzhi, Shanxi Houxian and Beijing Miyun, respectively. Compared with that expected under mating system equilibrium, there also was a significant deficiency of observed heterozygosity in 12 cases in the filial populations. Although less extensive, an overall deficiency of heterozygosity to that expected under mating system equilibrium was also observed in each population.

In contrast to the filial populations, an excess of heterozygosity as measured by Hardy-Weinberg equilibrium was found in most cases (16 out of 20) in the maternal populations (Table 3.8). The magnitude of excess varied from 1.3% (*Mr* in Shanxi Changzhi) to 70.0% (*Mr* in Beijing Miyun).

Table 3.6 Multilocus Individual-Tree Estimates of Outcrossing Rate (t_{mi})

Population	Maternal Tree	No. of Loci	No. of Progeny	t_{mi}
Shandong,Changqin				
	SCQ-1	5	60	0.81
	SCQ-2	5	40	1.00
	SCQ-3	5	58	0.97
	SCQ-4	5	60	1.00
	SCQ-5	5	60	1.00
	SCQ-6	5	60	0.88
	SCQ-7	5	60	0.76
	SCQ-8	5	20	0.94
	SCQ-9	5	60	0.99
	SCQ-10	5	60	0.84
	SCQ-11	5	40	0.94
	SCQ-13	5	85	0.63
	SCQ-14	5	60	0.87
	SCQ-15	5	60	0.94
	SCQ-16	5	60	1.00
	SCQ-17	5	60	1.00
	SCQ-18	5	47	0.66
	SCQ-19	5	60	0.83
	SCQ-20	5	60	0.89
	SCQ-21	5	60	0.70
	SCQ-22	5	59	0.45
	SCQ-24	5	40	0.87
	SCQ-25	5	60	0.74
	SCQ-26	5	60	0.78
	SCQ-27	5	60	0.51
	SCQ-28	5	60	0.76
	SCQ-29	5	60	0.90
	SCQ-30	5	53	1.00
	SCQ-32	5	60	0.90
	SCQ-33	5	35	1.00
	SCQ-34	5	31	1.00
Unweighted Mean				0.86
G_h (df=30)				156.32
P				<0.001
Shanxi Changzhi				
	SCZ-1	5	60	0.99
	SCZ-3	5	60	0.61
	SCZ-4	5	70	0.47
	SCZ-5	5	60	0.94
	SCZ-6	5	60	0.53
	SCZ-7	5	51	0.87
	SCZ-8	5	60	1.00
	SCZ-9	5	50	0.62
	SCZ-10	5	60	0.23
	SCZ-11	5	60	0.50

Table 3.6 (continued)

Population	Maternal Tree	No. of Loci	No. of Progeny	t_{mi}
	SCZ-12	5	40	0.15
	SCZ-13	5	55	0.73
	SCZ-14	5	40	0.69
	SCZ-15	5	25	0.45
	SCZ-16	5	60	0.98
	SCZ-17	5	48	1.00
	SCZ-18	5	60	0.81
	SCZ-19	5	27	0.42
	SCZ-20	5	60	0.77
Unweighted Mean				0.67
G_h (df=18)				178.47
P				<0.001
Shanxi Huoxian				
	SH-2	5	60	0.60
	SH-3	5	52	0.47
	SH-4	5	53	0.97
	SH-5	5	53	0.70
	SH-6	5	21	0.83
	SH-8	5	39	0.59
	SH-9	5	59	0.69
	SH-11	5	39	1.00
	SH-12	5	51	0.81
	SH-15	5	51	0.82
	SH-16	5	33	0.61
	SH-18	5	60	1.00
	SH-19	5	53	0.92
	SH-20	5	60	0.89
Unweighted Mean				0.78
G_h (df=13)				156.32
P				<0.001
Beijing Miyun				
	BM-2	5	52	0.72
	BM-9	5	59	0.61
	BM-11	5	34	1.00
	BM-12	5	32	0.61
	BM-15	5	55	0.87
	BM-17	5	50	0.95
	BM-18	5	52	0.38
	BM-19	5	56	0.44
	BM-20	5	45	0.69
Unweighted Mean				0.70
G_h (df=8)				30.03
P				<0.001

G_h=Likelihood-ratio test of heterogeneity.

Table 3.7 Estimates of Observed (H_o) and Expected (H_1[a] and H_2[b]) Heterozygosities, Wright's Fixation Index (F), and Expected Inbreeding Coefficient (F_e) for the Filial Populations.

Locus		Population SCQ	SCZ	SH	BM
6pg2	H_o	0.282	0.271	0.294	0.210
	H_1	0.325	0.327	0.368	0.447
	H_2	0.290	0.265	0.329	0.365
	F	0.132	0.171	0.201	0.530
	P	***[c](ns)[d]	***(ns)	***(*)	***(***)
Pgi2	H_o	0.315	0.342	0.360	0.428
	H_1	0.465	0.475	0.442	0.611
	H_2	0.416	0.383	0.434	0.499
	F	0.323	0.280	0.186	0.300
	P	***(***)	***(***)	***(*)	***(***)
Mr	H_o	0.541	0.459	0.387	0.398
	H_1	0.553	0.598	0.439	0.478
	H_2	0.494	0.485	0.394	0.391
	F	0.022	0.232	0.118	0.163
	P	ns(***)	***(*)	***(***)	**(ns)
Idh1	H_o	-	0.097	-	0.127
	H_1	-	0.132	-	0.139
	H_2	-	0.106	-	0.113
	F	-	0.265	-	0.086
	P	-	***(**)	-	ns(ns)
Idh2	H_o	0.097	-	0.076	-
	H_1	0.095	-	0.082	-
	H_2	0.085	-	0.073	-
	F	-0.021	-	0.073	-
	P	ns(**)	-	ns(ns)	-
Fest1	H_o	0.077	0.034	-	-
	H_1	0.083	0.041	-	-
	H_2	0.074	0.033	-	-
	F	0.072	0.171	-	-
	P	***(ns)	***(ns)	-	-
Skdh1	H_o	-	-	0.305	0.343
	H_1	-	-	0.637	0.572
	H_2	-	-	0.571	0.468
	F	-	-	0.521	0.400
	P	-	-	***(***)	***(***)

Table 3.7 (continued)

Locus	Population SCQ	SCZ	SH	BM
Unweighted Means:				
H_o	0.262	0.241	0.284	0.301
H_1	0.304	0.315	0.394	0.449
H_2	0.272	0.254	0.360	0.367
F	0.138	0.235	0.279	0.330
F_e	0.105	0.190	0.105	0.183

a. Expected heterozygosity under Hardy-Weinberg equilibrium;

b. Expected Heterozygosity under mating system equilibrium;

c. Rejection of the null hypothesis of F=0 at 0.1% level;

d. The null hypothesis that $F=F_e$ can not be rejected ($P>0.05$).

Table 3.8 Estimates of Observed (H_o) and Expected (H_e[a]) Heterozygosities, Wright's Fixation Index (F), and Expected Inbreeding Coefficient (F_e) for the Maternal Populations.

Locus		Population			
		SCQ	SCZ	SH	BM
	H_o	0.323	0.526	0.500	0.556
6pg2	H_e	0.317	0.444	0.389	0.425
	F	-0.019	-0.185	-0.285	-0.308
	P	ns(ns)[b]	ns(ns)	ns(ns)	ns(ns)
	H_o	0.387	0.526	0.571	0.889
Pgi2	H_e	0.492	0.494	0.500	0.646
	F	0.213	-0.065	-0.142	-0.376
	P	**(*)[c]	ns(ns)	ns(ns)	ns(ns)
	H_o	0.548	0.632	0.571	0.889
Mr	H_e	0.556	0.624	0.473	0.523
	F	0.014	-0.013	-0.207	-0.700
	P	ns(ns)	ns(ns)	ns(ns)	**(**)
	H_o	-	0.158	-	0.333
Idh1	H_e	-	0.152	-	0.294
	F	-	-0.039	-	-0.133
	P	-	ns(ns)	-	ns(ns)

	H_o	0.065	-	0.071	-
Idh2	H_e	0.063	-	0.072	-
	F	-0.032	-	0.014	-
	P	ns(ns)	-	ns(ns)	-
	H_o	0.097	0.105	-	-
Fest1	H_e	0.094	0.102	-	-
	F	-0.032	-0.029	-	-
	P	ns(ns)	ns(ns)	-	-
	H_o	-	-	0.429	1.000
Skdh1	H_e	-	-	0.685	0.660
	F	-	-	0.374	-0.515
	P	-	-	ns(ns)	***(***)
Unweighted Means:					
	H_o	0.296	0.389	0.428	0.733
	H_e	0.304	0.363	0.424	0.510
	F	0.029	-0.066	-0.049	-0.406
	F_e	0.105	0.190	0.105	0.183

a. Expected Heterozygosity under Hardy-Weinberg equilibrium;

b. Both null hypotheses of F=0 and F=F_e can not be rejected;

c. Rejection of the null hypothesis of F=0 at 1% and of F=F_e at 5% level.

In cases where heterozygote deficiency was observed, such deficiency is much less extensive compared with that observed in the corresponding filial populations. Three populations, Shanxi Changzhi, Shanxi Houxian and Beijing Miyun, showed an overall excess of heterozygosity of 6.6%, 4.9% and 40.6%, respectively. Although an overall heterozygosity deficiency of 2.9% was observed in Shandong Changqin, it is only about one fifth of that observed in the corresponding filial population.

3.4 Discussion

The estimates of outcrossing rate derived from the present study are lower than those reported for most other conifers (Table 3.1), although similarly low levels of outcrossing have recently been recorded (Knowles *et al.*, 1987; Sproule, 1988). Many factors such as selfing ability of the species, population substructuring, year-specific factors and the sampling strategies used in this study could have contributed to the observed low levels of outcrossing in this species.

In most naturally outbreeding plants, such as conifers, reduction of seed set and germination rate after selfing have been commonly observed, and the degree of such reduction has been found to vary greatly among species (Bingham and Squillace, 1955; Charlesworth and Charlesworth, 1987; Franklin, 1970; Sorensen, 1969; Wright, 1976). In a species with high selfing ability, there may be a significantly lower loss of selfed progeny before the time at which the genotypes are scored (i.e. as viable embryos), thus resulting in a relatively low estimate of outcrossing rate. Unfortunately, the significance of this factor to the observed low outcrossing rates could not be evaluated, since information on the selfing fertility of *Thuja orientalis* is not available.

Population substructuring or clustering of similar genotypes often occurs within natural populations due to microhabitat selection and/or the tendency of seeds to fall

and grow near their maternal parent, thus forming family structures (Shaw *et al.*, 1981). Regardless of the mechanism, clustering of similar genotypes in natural populations will result in heterogeneity of the pollen cloud over maternal trees. Since trees are more likely to receive pollen from nearby pollen sources than randomly (Muller, 1977), trees with similar genotypes are more likely to mate. Mating system estimation procedures formulated based on mixed selfing and random outcrossing models could not distinguish consanquineous matings as distinct from self-fertilization. Thus, clustering of similar genotypes or population substructuring could bias the estimates of outcrossing rate downwards; and the more substructured the populations, the lower will be the estimated rate of outcrossing (Ennos and Clegg, 1982). Extensive family structures seem to exist in the natural populations of *Thuja orientalis*. The effective seeding range of *Thuja occidentalis* L., a close relative of *Thuja orientalis*, was estimated to be from about 45 to 60 meters (Nelson, 1951; Watson, 1936). *Thuja orientalis* is shorter than *T. occidentalis*, and the seed is wingless; therefore, the effective seeding range of this species is expected to be much shorter. In order to test the heterogeneity of the pollen pool over maternal trees (or the presence of population substructures), the number of homozygous and heterozygous embryos borne by each homozygous mother was entered in a 2 x m contingency table (Brown *et al.*, 1975), where m was the number of maternal trees of one

homozygous genotype in the sample, and examined by a likelihood-ratio test of heterogeneity (Sokal and Rohlf, 1981). Significant heterogeneity of the frequencies of genetically detectable outcrosses (heterozygous embryos) was detected in 7 out of 16 cases tested (Table 3.9). Although such heterogeneity could also result from variation in the probability of outcrossing among trees, the fact that the level of heterogeneity differed greatly among loci suggests that the pollen pools are likely not homogeneous. This is because variation in the probability of outcrossing would affect all loci simultaneously (Brown *et al.*, 1975). Evidence for the presence of population substructures within the sampled populations also could be made from the observation of 1) the average single-locus estimates of outcrossing are lower than the corresponding multilocus estimates in all the populations, and 2) the estimates of Wright's fixation index are higher than the corresponding equilibrium inbreeding coefficients (F_e's) in all the filial populations.

Temporal variation of outcrossing estimates has been reported in forest trees (Moran and Brown, 1980; Cheliak, *et al.*, 1985). Yearly fluctuation of some environmental factors resulting in differential production and survival of selfed progeny in different years could be one of the possible explanations for this phenomenon. The amount of alien pollen reaching the female strobili could be reduced by lack of

Table 3.9 Results of Pollen Pool Heterogeneity Test

Locus	Population SCQ	SCZ	SH	BM
6pg2	26.56(17)	13.10(6)*	16.89(6)**	4.01(3)
Pgi2	52.74(13)***	78.13(5)***	18.01(5)**	-
Mr	14.99(8)	7.00(5)	1.61(4)	-
Idh1	-	86.77(13)***	-	3.13(5)
Idh2	74.06(29)***	-	21.92(15)	-
Fest1	12.57(26)	23.53(15)	-	-
Skdh1	-	-	-	-

*,**,***: Rejection of the null hypothesis of homogeneity of pollen pool among maternal trees at the 5%, 1% and 0.1% level.

wind and abundant rain during the flowering period (Sarvas, 1962), thus increasing the proportion of selfed seed. Higher percentages of filled seed in warmer conditions during and for some time after pollination were observed in *Thuja plicata*, which may suggest a higher rate of survival of selfed embryos in warmer environments (Owens and Molder, 1980). Since weather records for those locations where the populations were sampled are not available, I could not be certain whether unusual weather during the sampling year (1983) may have contributed to the low outcrossing estimates derived.

A systematic bias of outcrossing rate estimates may result from the sampling strategies used in the present study. In this study, all the seed cones were sampled from the south-central or lower portion of the crown, where the male flowers have been found to be more abundant (Sarvas, 1962) and the levels of outcrossing have been shown to be lower in many coniferous species (Fowler, 1965; Franklin, 1971; Sarvas, 1962; Shaw and Allard,1982). In *Thuja orientalis*, a similar pattern of male strobili distribution in the crown has been observed (Shi, 1982) and, therefore, a lower proportion of outcrossed seeds from this portion of the crown would be expected.

Significant variation of single-locus estimates of outcrossing rate over loci was observed not only in *Thuja orientalis* but also in other conifers, such as *Picea glauca* (King *et al.*, 1984), *Pinus contorta* (Epperson and Allard,

1984), *Pinus jeffreyi* (Furnier and Adams, 1986), *Pinus ponderosa* (Mitten *et al.*, 1981), *Pinus radiata* (Moran *et al.*, 1980), and *Pseudotsuga menziesii* (Shaw and Allard, 1982; Yeh and Morgan, 1987). Theoretically, actual outcrossing rates should be the same for all loci, since the single-locus estimates of mating system parameters for each locus were derived from the same set of embryos, and the alleles at all loci were transmitted in the same gametes. The observed variability could be due to violations for some loci of one or more of the assumptions made in formulating the estimation procedures. Differential survival of inbred progeny due to recessive deleterious genes that are closely linked to the isozyme loci could be a possible contributor to the observed heterogeneity of single-locus estimates of outcrossing rate. In addition, differences among loci in the spatial arrangement of genotypes within natural populations likely exist, and such differences would also result in heterogeneous estimates of outcrossing rates among loci within populations.

Since the probability of identifying outcrosses through direct observation of the phenotypes of progeny that carry non-maternal alleles increases as more and more loci are examined, and since the sensitivity to failures of the assumptions made to the mixed mating model decreases as more and more outcrosses are detected by direct observation (Shaw *et al.*, 1981), multilocus estimates of outcrossing rate are considered more accurate and generally favored over

single-locus estimates.

As all multilocus population estimates were significantly less than 1.00, outcrossing in these four populations was not complete, or in other words, there were significant amounts of selfing in these populations. However, the fact that all the means of t_s were lower than the t_m values suggests that a certain amount of selfing detected in each population is due to consanguineous matings rather than self-fertilization.

Heterogeneity of outcrossing rates over maternal trees within populations should be considered normal, since different individual trees in a population may differ in their selfing ability and other factors that may influence outcrossing rate. Although variation in self-fertility among individual trees has not been studied in *Thuja orientalis*, it has been well documented in other conifers. Several studies indicate that self-fertility is highly variable among individual trees in Douglas-fir, with the relative self-fertilities ranging from 0 to 94 percent (Orr-Ewing, 1954; Sorensen, 1969; Piesch and Stettler, 1971). Different self-fertilities among trees have also been reported in western white pine (Bingham and Squilance, 1955). In a species where there is polyzygotic polyembryony, selection among embryos of unequal vigor during early embryo development may occur (Sorensen, 1982). Differences among trees in the number of embryonic recessive lethals and deleterious alleles may generate tree-to-tree variation in

outcrossing rate. In this study of *Thuja orientalis*, polyembryony was observed. It has been demonstrated that both polyzygotic and cleavage polyembryony are present in *Thuja orientalis* (Singh and Oberoi, 1962). Therefore, it can be regarded as a possible contributor to the observed heterogeneity of outcrossing rate among individual trees in this species. Variation in pollen yield among trees within natural populations has been observed in many conifers (Sarvas, 1962; Shea, 1987), and such variation could be caused both by genetic and environmental factors. In *Thuja orientalis*, young trees that are predominantly male or female have been reported (Shi, 1982), and predominantly unisexual adult individuals also have been commonly observed (Shen, S.H., personal communication). Microhabitat could affect pollen yield and the ratio of male and female flowers. Cajander (1917) indicated that "the formation of female flowers is most profuse in pines grown in full light, whereas those grown in the shade have only a small amount or none at all. Male flowers on the other hand, are often formed in trees growing in considerable shade." (translated from Finish by Sarvas, 1962). Soil fertility and moisture are also well-known factors influencing flowering. The frequency of self-produced pollen seems to be higher in the pollen cloud of trees with a larger pollen yield, and therefore more selfed seeds are likely produced. The observation that trees with higher levels of male-cone production, thus larger pollen yields, consistently have

lower outcrossing rates in both *Picea engemannii* and *Abies lasiocarpa* (Shea, 1987) supports this speculation. Similar relationships between pollen yield and selfing rate have also been reported in *Picea glauca* (Denti and Schoen, 1988). While investigating the factors that may generate the tree-to-tree variation of observed outcrossing rate, we need to note that the detected heterogeneity of outcrossing rates among maternal trees may not be totally due to variation of the actual outcrossing rates. Heterogeneity of outcrossing pollen frequencies over maternal trees may have played an important role in the observed variation.

Interpopulation variation of outcrossing rate is a common observation in plant species (for review see Clegg (1980) and Hamrick (1982)). Such variation could be attributable to the genetic differences and environmental variation among the populations. Since the four populations sampled in this study are located in different regions, their genetic composition, population structure, stand age, stand density, and climatic and edaphic conditions could be different, and those differences could result in significant heterogeneity of population outcrossing eatimates. In addition, the sampling strategies may partially account for the observed variation. A higher level of outcrossing is expected on the windward side of the crown, since there is a higher concentration of alien pollen compared to that on the leeward side (Sarvas, 1962). Our seed cones were sampled from the south part of the crown in all four populations. If

wind directions were different among populations, heterogeneity of outcrossing rate among populations would be generated even if overall levels of outcrossing within the four populations are the same.

Since there is a significant amount of inbreeding in each population, the observation of heterozygote deficiency in the filial populations (as measured by Hardy-Weinberg equilibrium) is not surprising. More attention needs to be paid to the deficiency after a correction for the mating system. A variety of factors can be responsible for such a deficiency (Brown, 1979); of those, the Wahlund effect, positive assortative mating and restricted neighbourhood size have been most commonly cited. If a population is subdivided into many subpopulations with variation in gene frequencies among them, an excess of homozygosity (or a deficiency of heterozygosity) will result when those panmictic subpopulations are considered as a single large panmictic unit. Positive assortative mating may occur in natural populations. For instance, trees with similar phenological timing of flowering are more likely to mate. Since individuals with similar phenotypes will usually be somewhat similar in their genotypes, the effect of positive assortative mating is similar to that of inbreeding, i.e. increasing the frequency of homozygotes in a population (Crow and Felsenstein, 1968). It has been discussed earlier that due to limited disperal distance of seed, family structures likely exist in the sampled populations. Mating

between relatives will certainly increase the homozygosity of the populations. Those factors that increase homozygosity in a population would also lower the population estimate of outcrossing rate and, therefore, increase the value of the expected equilibrium inbreeding coefficient (F_e). However, since our expected equilibrium inbreeding coefficients were calculated based on the multilocus outcrossing estimates that would be less affected by those factors, F_e values were less inflated than those of F's.

Excesses of heterozygotes after a correction for the mating system in the filial populations (such as *Mr* in Shandong Changqin) could also result from many causes (Brown, 1979). Heterotic selection is a very general phenomenon and is regarded as an important factor (Clegg, 1980). Such selection could be either for the marker locus itself or for the chromosome segment containing the marker locus. Negative assortative mating is another important factor resulting in heterozygote excess over the expectation under mating system equilibrium. Differences in male and female phenology on the same individual have been observed in many monoecious species. For instance, in *Pinus ponderosa* (Roeser, 1941; Wang, 1977), *P. palustris* (Snyder *et al.*, 1977) and *Quercus* (three spp.; Sharp and Sprague, 1967), opening of male flowers commonly precedes opening of female flowers. The converse occurs in *Pinus contorta* (Critchfield, 1980) and *Betula alleghaniensis* (Dancik and Barnes, 1972). More interestingly, male flowers of *Cupania guatemalensis*

are usually borne before and after female flowers on particular individuals (Bawa, 1977). Differences in relative timing of male and female flower maturation on the same tree and similarity between genotypically different trees thus could result in negative genotypic assortative mating. Gametic selection, such as by retardation of pollen germination and pollen tube growth of the pollen with a genotype similar to that of the female gamete is another possible cause of negative assortative mating.

Although differences in allele frequency between the male and female gamete pools could result in excesses of heterozygosity after a correction for the mating system (Brown, 1979; Robertson, 1965; Workman, 1969), it is not considered as an important factor in monoecious plant populations, since each individual is expected to contribute equally to both the male and female gamete pools (Cheliak, *et al.*, 1985). In fact, those loci that displayed significant differences in allele frequency between the two gamete pools did not show significant heterozygote excesses, whereas significant heterozygote excesses were detected at the loci where the allele frequencies were essentially equal in the two gamete pools (Table 3.3 and Table 3.7). Differences in allele frequency in the two gamete pools has been observed not only in this study but also in previous studies (El-Kassaby, *et al.*, 1981; Mitton *et al.*, 1981; Moran and Brown, 1980; Moran *et al.*, 1980; Ritland and El-Kassaby, 1985; Yeh, *et al.*, 1983). Such differences may

indicate that the maternal trees sampled are not representative of the local population, and/or the effective pollen pool is not a random sample of the adult trees.

Viable seeds could be produced by self-fertilization or other forms of inbreeding, and inbreeding depression could be expressed at various later stages of the life cycle (after seed germination). Therefore, inbreds would be continuously removed from a population, leaving an adult population composed largely of outcrossed individuals. This seems to be the situation in the studied populations of *Thuja orientalis*, since the adult populations all appeared to be considerably less inbred than the filial populations and even showed significant excesses of heterozygotes compared to those expected under Hardy-Weinberg equilibrium.

3.5 Conclusion

The present study indicates that *Thuja orientalis*, like other conifers, is a predominant outcrosser, and the rate of outcrossing varies significantly among trees within populations and among populations. This study also indicates that the level of effective selfing is generally higher than those of most other conifers and a certain part of this is likely due to consanguineous matings rather than self-fertilization. Many factors such as possibly high selfing ability of the species, extensive family structures within the populations, and the sampling strategies employed may have contributed to the observed high level of effective

selfing, although their relative contribution could not be evaluated due to the lack of related information.

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3.7 Appendix

3.7.1 Single-locus estimation procedure

Consider a population that is polymorphic for many codominant alleles at a locus (A_1, A_2, ...A_n). Let t_s denote the proportion of ovules which are fertilized by random outcrossing, and $1-t_s$ the proportion of ovules which are self-fertilized. Let p_i denote the frequency of the allele A_i in the outcrossing pollen pool. Then, the probability of observing the i^{th} single-locus pollen genotype in the pollen pool can be given by

$$P(G_{mi})=(0.5)^j(1-t_s)+t_sp_i,$$
$$P(G_{oi})=t_sp_i,$$

where

j=0 for homozygous maternal tree, and 1 for heterozygous maternal tree,

G_{mi}=the i^{th} single-locus pollen genotype which could be produced by the maternal tree, and

G_{oi}=the i^{th} single-locus pollen genotype which can not be produced by the maternal tree.

Let the observed number of single-locus pollen genotype G_{mi} and G_{oi} be N_{mi} and N_{oi}, the expected number of selfing and outcrossing pollens (N_{se} and N_{oe}) can be expressed as

$$N_{se}=\sum_{i}^{N}\frac{(0.5)^{j}(1-t_s)}{(0.5)^{j}(1-t_s)+t_s p_i}N_{mi},$$

$$N_{oe}=\sum_{i}^{N}\left(\frac{t_s p_i}{(0.5)^{j}(1-t_s)+t_s p_i}N_{mi}+N_{oi}\right),$$

where N is the total number of single-locus pollen genotypes observed in the population. Thus, the maximum-likelihood estimates of t_{se} and p_{ie} can be presented as

$$t_{se}=\frac{N_{oe}}{N_{oe}+N_{se}},$$

$$p_{ie}=\frac{n_{oie}}{N_{oe}},$$

where

$$n_{oie}=\frac{t_s p_i}{(0.5)^{j}(1-t_s)+t_s p_i}N_{mi}+N_{oi}$$

is the expected number of pollen i in the outcrossing pollen pool.

The estimates of outcrossing rate and pollen frequencies in the outcrossing pollen pool are obtained by iterating the above estimation procedure until successive estimates of the parameters converge to a specified

criterion. Intially, arbitary values of t_s and p_i's ($\sum p_i=1$) are provided to start the iterative procedure.

3.7.2 Multilocus estimation procedure

Extension of the above single-locus procedure to multilocus case is straightforward. The maximum likelihood multilocus estimates of t_{me} and p_{mie} are given below

$$t_{me}=\frac{N_{oe}}{N_{oe}+N_{se}},$$

$$p_{mie}=\frac{n_{oie}}{N_{oe}},$$

where

$$N_{se}=\sum_{i=1}^{N}\sum_{j=0}^{m}\frac{(0.5)^j(1-t_m)}{(0.5)^j(1-t_m)+t_m p_{mi}}N_m(i,j),$$

$$N_{oe}=\sum_{i=1}^{N}\left(\sum_{j=0}^{m}\frac{t_m p_{mi}}{(0.5)^j(1-t_m)+t_m p_{mi}}N_m(i,j)+N_o(i)\right),$$

$$n_{oie}=\sum_{j=0}^{m}\frac{t_m p_{mi}}{(0.5)^j(1-t_m)+t_m p_{mi}}N_m(i,j)+N_o(i),$$

$N_m(ij)$=observed number of the i^{th} multilocus pollen genotype which could be produced

by the maternal tree which is assumed to have j heterozygous loci,

$N_o(i)$=observed number of the i^{th} multilocus pollen genotype which can not be produced by the maternal tree,

p_{mi}=frequency of the i^{th} multilocus pollen genotype in the outcrossing pollen pool.

m=number of loci used, and

N=total number of multilocus pollen genotypes observed in the population.

4. Genetic Structure

4.1 Introduction

One of the most active areas in population genetics studies using isozyme polymorphisms is the characterization of genetic structure of the species of interest. During the past two decades, a large number of diverse species have been investigated; conifers on average have been found to be considerably more variable than other plants (Hamrick, 1979). Studies have further indicated that most of the isozyme variation in conifers is maintained within populations and differentiation among populations is usually not pronounced (Dancik and Yeh, 1983; Guries and Ledig, 1981; Wheeler and Guries, 1982; Yeh and Layton, 1979; Yeh and O'Malley, 1980). Some biotic characteristics such as occurrence over broad environmental spectra, large population sizes, long generation times, high fecundities, predominantly outcrossing mating systems, and the potential for long distance gene flow via pollen and seed dispersal are considered to be responsible for this general picture of genetic structure of conifer trees (Hamrick *et al.*, 1981)

Thuja orientalis is a coniferous tree species with all the biotic characteristics mentioned above; thus its genetic structure would be expected to be similar to those of other conifers. However, because of its discontinuous distribution, relatively small population sizes and low outcrossing rate, as well as the great differences in

environmental conditions among populations, relatively lower genetic variability within populations and greater genetic differentiation among populations might be expected. In this chapter, the genetic structure of *Thuja orientalis* is characterized and the possible forces responsible for such structure are discussed.

4.2 Materials and Methods

Sampling strategies, tissue preparation, electrophoretic conditions and enzymes assayed were described in chapter two.

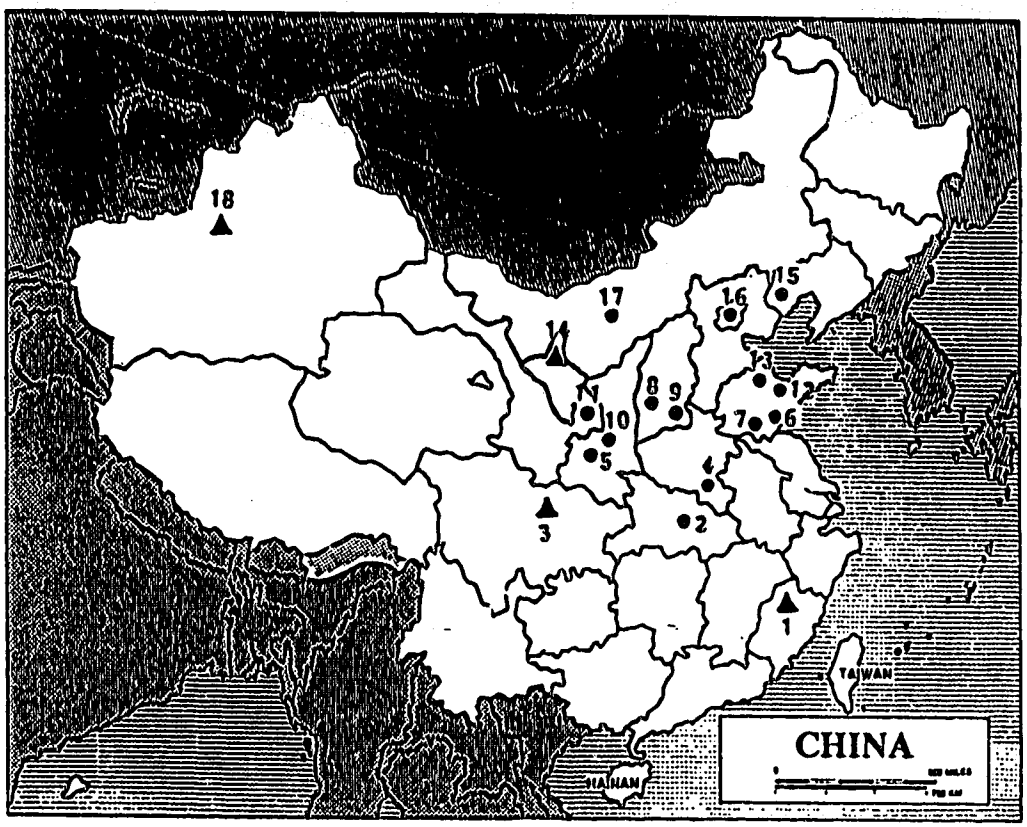
Eighteen populations including four plantations located throughout the species range were sampled (Table 4.1 and Figure 4.1). Seeds from at least 20 trees in each population were collected and gene frequencies at each of the 28 loci were estimated from a bulked sample of about 120 megagametophytes .

4.2.1 Genetic Variation Within Populations

To quantify the levels of genetic variation within populations, four commonly used measures, i.e., average number of alleles observed per locus (n_a), effective number of alleles per locus (n_e), percentage of polymorphic loci (P) and expected average heterozygosity (H), were used. Apparently, n_a is a measure of allelic richness , while n_e and H are measures of allelic evenness since both the number

No.	Population	Latitude (°N)	Longitude (°E)	Elevation (M)
1	Fujiang Nanping	26.65	118.17	120
2	Hubei Jingmen	31.23	112.20	223
3	Sichuan Mianyang	31.47	104.80	472
4	Henan Queshan	32.80	114.03	380
5	Shannxi Chunhua	34.80	108.52	910
6	Shandong Changshan	34.90	118.10	150
7	Shandong Zaozhuang	34.92	117.50	200*
8	Shanxi Jieshan	35.58	110.95	650
9	Shanxi Jincheng	35.58	113.12	1040
10	Shannxi Huangling	35.58	109.27	990
11	Gansu Huexian	36.12	108.68	1200
12	Shandong Boshan	36.32	117.68	50*
13	Shandong Licheng	36.68	117.47	50
14	Ningxia Yinchuan	38.48	106.22	1110
15	Liaoling Chaoyan	40.28	119.35	650
16	Beijing Miyun	40.38	116.83	650
17	Neimong Wulashan	40.73	108.65	1021
18	Xinjiang Yili	43.95	81.33	660

*: estimated from a map.



●: Natural Populations ▲: Plantations

Figure 4.1 Geographical Locations of the 18 Populations of *Thuja orientalis* Linn. Sampled in This Study

and the frequencies of the alleles are taken into account.

Average number of alleles observed per locus (n_a) is equal to the total number of alleles observed divided by the number of loci examined. n_a is of particular value in choosing strategies for gene conservation, since decisions as to which populations to conserve *in situ* or to sample for *ex situ* conservation should be based primarily on the total number of different alleles (gene complexes) in the populations (Marshall and Brown, 1975). However, as a general measure of genetic variability, n_a has a serious drawback: it is highly dependent on sample size. Therefore, comparison of this quantity between different samples is not very meaningful unless sample size is more or less equal.

Effective number of alleles per locus (n_e) is the number of equally frequent alleles that would be required to produce the same homozygosity as in an actual population (Hartl, 1981). It is given by:

$$n_e = 1/((\sum_{i}^{r}\sum_{j} P_{ij}^{2})/r)$$ (Crow and Kimura, 1970),

where

P_{ij}=estimated frequency of the j^{th} allele at the i^{th} locus, and

r=number of loci examined.

A locus was defined as polymorphic if the frequency of the most common allele at that locus was equal to or less than 0.99. This is a common but arbitrary definition, and

there is no reason why the distinction between polymorphic and monomorphic loci should not be made at some other value. Except for its arbitrariness, the statistic P is also imprecise. For each locus, it only establishes whether or not that locus is polymorphic but can not quantify the polymorphism at the locus. Another problem with P is that when the number of loci examined is small, it is subject to a large sampling error.

The expected average heterozygosity is defined as the minimum number of codon difference per locus between two randomly chosen genomes (Nei, 1975). Thus,

$$H=\sum_i h_i/r,$$

where

$h_i=1-\sum_j p_{ij}^2$=the expected heterozygosity of the i^{th} locus.

4.2.2 Genetic Differentiation Among Populations

4.2.2.1 Heterogeneity of Gene Frequencies Among Populations

Heterogeneity of gene frequencies among populations for each locus was tested by calculating the following chi-square statistic:

$$\chi^2=N\sum_i^k \sigma_i^2/p_i \qquad \text{(Workman and Niswander, 1970)},$$

where

$N=\sum_{j}^{m} N_j$=total number of megagametophytes examined for all the populations,

N_j=number of megagametophytes examined for the j^{th} population,

$\sigma_i^2=\sum_{j}^{m}(N_j/N)(P_{ij}^2-P_i^2)$=the weighted variance of the i^{th} allele frequency across the populations,

P_{ij}=allele frequency of the i^{th} allele in the j^{th} population,

p_i=the weighted mean frequency of the i^{th} allele across the populations,

K=number of alleles at the locus, and

m=number of populations.

The degree of freedoms for the chi-square statistic is $(m-1)(k-1)$.

4.2.2.2 Genetic Distance between Populations

The genetic difference between populations expressed as a function of gene frequencies is defined as genetic distance, which measures the accumulated number of detectable gene substitutions per locus (Nei, 1975). Different measures of genetic distance have been proposed, but the "standard genetic distance" given by Nei (1973) is most widely used. Nei's standard genetic distance measure for n loci is given by

$$D_n=-\ln(J_{xy}/(J_xJ_y)^{0.5}),$$

where

J_x=arithm. mean of $j_x=\sum_k (x_k^i)^2$ over all loci in population X,

J_y=arithm. mean of $j_y=\sum_k (y_k^i)^2$ over all loci in population Y,

J_{xy}=arithm. mean of $j_{xy}=\sum_k (x_x^i y_y^i)$ over all loci in population X and Y,

x_k^i=frequency of the k^{th} allele at the i^{th} locus in population X, and

y_x^i=frequency of the k^{th} allele at the i^{th} locus in population Y.

D_n ranges from zero, for populations with identical allelic frequencies, to infinity, for populations that do not share any alleles.

4.2.2.3 Extent of Genetic Differentiation among Populations

The extent of genetic differentiation among all the sampled populations can be quantified by Nei's gene diversity statistics. The total gene diversity, H_T, a measure of total genetic variation in the entire sample of populations, is defined by

$$H_T=1-\sum_i p_i^2,$$

where p_i is the weighted mean frequency of the i^{th} allele in the entire populations. H_T can be further partitioned as

$$H_T=H_S+D_{ST},$$

in which

H_S and D_{ST} are average gene diversities within and among populations. The proportion of total gene diversity due to differences among populations, or the extent of genetic differentiation among populations is

$$G_{ST}=D_{ST}/H_T \ .$$

Quantitatively speaking, the range of 0.05 to 0.15 for G_{ST} may be considered to indicate moderate differentiation; 0.15 to 0.25 to indicate great differentiation; and above 0.25 to indicate very great differentiation (Hartl, 1980).

4.2.2.4 Relative Contribution of Individual Populations to Total Genetic Differentiation

Nei's coefficient of gene differentiation, G_{ST}, can only quantify the total amount of genetic differentiation among populations. In order to evaluate the relative contribution of each single population to total differentiation, the genetic differentiation measures defined by Gregorius and Roberds (1986) were also employed in this study. Based on their definition, the amount of genetic differentiation for the j^{th} population (D_j) is the genetic distance between this population and its complement and is expressed as

$$D_j=\tfrac{1}{2}\sum_i |(p_i(j)-p_i)/1-C_j)|,$$

where

$p_i(j)$=frequency of the i^{th} allele in the j^{th} population,

$p_i=\sum_j^m p_i(j)C_j$=weighted frequency of the i^{th} allele in the entire populations, and

C_j=the relative size of the j^{th} population, $\sum_j^m C_j=1$.

When the genetic composition in the j^{th} population is equivalent to that of the entire populations, $D_j=0$.

The total amount of genetic differentiation among populations, D_T , is defined as the weighted mean of D_j's; thus,

$$D_T=\sum_j^m C_j D_j.$$

Since information on population size is not available, equal population size was assumed and equal weight to all D_j's was given in this study, i.e. $C_1=C_2=\ldots=C_m$.

For n loci examined, the corresponding genetic differentiation for each population and among all populations are given by

$$D_j'=(1/n)\sum_1^n D_{j1}, \text{ and}$$

$$D_T'=(1/n)\sum_1^n D_{T1}.$$

4.2.2.5 Genetic Relationships among Populations

The genetic relationships among populations was illustrated by two methods. First, phenetic clustering based

on Nei's standard genetic distance estimates was performed using the UPGMA (unweighted pair group mean analysis) procedure (Sneath and Sokal, 1973). Second, a discriminant analysis was carried out using megagametophyte genotype scores. The megagametophyte genotype scores were transformed from the original allelic data using Merkle *et al.*'s (1988) scoring procedure . Based on that procedure, a megagametophyte genotype score for a given locus with k alleles consists of k-1 positions coded with 0's and 1's. For the first k-1 positions, a 1 in the i-th position indicates the i-th allele is present, a 0 indicates that it is absent. If all the k-1 positions are filled with 0, it means the k-th allele is present. Thus, the megagametophyte genotype score for a given locus is a vector of ones and zeros. Since in some cases, the original allelic data of *αgal*, *Ha* and *Skdh* were not obtained from the same set of megagametophytes as those of the other loci , these three loci were excluded from the discriminant analysis.

4.2.3 Geographic Patterns of Genetic Variation

Product-moment correlation analysis (Sokal and Rohlf, 1981) was employed to detect the linear relationships between:

(1), average heterozygosity and three major geographic factors, i.e. latitude, longitude, and elevation; and

(2), the commom allele frequency at each locus and the three geographic factors.

Since the detected geographic patterns of genetic variation may result from the environmental gradients in which gradients of natural selection are present, the same product-moment correlation analysis was carried out to relate the average heterozygosity and common allele frequencies to three major climatic factors (i.e., annual mean temperature, annual mean relative humidity and annual precipitation). The significance of all the above correlations was examined by a t-test (Sokal and Rohlf, 1981).

4.3 Results

4.3.1 Genetic Variation Within Populations

With the exception of *Lap* and *Sod*, all other 24 loci surveyed were polymorphic in at least one of the 18 populations. The number of alleles observed at each of the polymorphic loci ranges from two (*Aat2*, *Fest2*, *Gdh*, *6pg2*, *Aph3*, and *Aph4*) to four (*Fest1* and *Pgm1*). Three generalizations could be drawn from the estimated allele frequences and heterozygosities at the 24 polymorphic loci for the 18 populations (Table 4.2). First, there is a large amount of inter-locus variation in heterozygosity. The 24 polymorphic loci could be classified into three groups based on their average heterozygosities over populations. Nine loci (*Aat2*, *Aco*, *Adh1*, *Adh3*, *Fest2*, *Dia*, *Gdh*, *Idh1*, *Mpi*) have very low heterozygosities (lower than 0.05); eight loci (*Aat1*, *Fest1*, *Idh2*, *Me*, *Pgm1*, *6pg1*, *Aph3*, *Aph4*) have moderate levels of heterozygosity (from 0.05 to 0.15); and the remaining seven loci (*Aat3*, *αgal*, *Ha*, *Mr*, *Pgi2*, *Skdh1* and *6pg2*) have high levels of heterozygosity (higher than 0.15). This indicates that different loci do not contribute equally to overall mean heterozygosity. Second, the levels of heterozygosity at a locus vary widely among populations. Some loci in the first group have very high levels of heterozygosity in one or two populations (For example, *Aat1* in population 10 (h=0.32), *Fest1* in population 13 (h=0.37), *Pgm1* in populations 10 and 16 (h=0.35 and 0.37), *6pg1* in

Table 4.2 Allele Frequencies and Heterozygosities at 26 Loci in the Populations of *Thuja orientalis* Linn.

Locus	Natural Population															Plantation				
	2	4	5	6	7	8	9	10	11	12	13	15	16	17	Mean	1	3	14	18	Mean
Aat1																				
1	.00	.00	.00	.00	.00	.01	.01	.15	.00	.00	.00	.00	.00	.01	.01	.00	.00	.00	.00	.00
2	.97	1.0	1.0	1.0	1.0	.94	.92	.81	.99	.93	1.0	.96	.98	.99	.96	.95	.98	.97	1.0	.97
3	.03	.00	.00	.00	.00	.05	.07	.04	.01	.07	.00	.04	.02	.00	.03	.05	.02	.03	.00	.03
h	.06	.00	.00	.00	.00	.11	.15	.32	.02	.13	.00	.08	.04	.02	.07	.10	.04	.06	.00	.05
Aat2																				
1	.00	.00	.00	.00	.00	.02	.03	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	1.0	1.0	1.0	1.0	1.0	.98	.97	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
h	.00	.00	.00	.00	.00	.04	.06	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00	.00	.00
Aat3																				
1	.50	.04	.16	.65	.03	.11	.05	.14	.22	.15	.10	.04	.26	.08	.19	.36	.59	.07	.43	.36
2	.00	.19	.11	.04	.59	.00	.16	.14	.08	.13	.01	.03	.23	.13	.10	.00	.00	.02	.01	.01
3	.50	.77	.73	.31	.38	.89	.79	.72	.70	.72	.89	.93	.51	.79	.71	.64	.41	.91	.56	.63
h	.50	.37	.43	.48	.51	.19	.35	.44	.46	.44	.20	.13	.62	.35	.39	.46	.48	.17	.50	.40
Aco																				
1	.00	.00	.00	.00	.00	.04	.03	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.01	.02	.01
2	1.0	1.0	1.0	1.0	1.0	.96	.96	1.0	1.0	1.0	1.0	.98	1.0	1.0	.99	.99	1.0	.99	.98	.99
3	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00	.00	.02	.00	.00	.00	.01	.00	.00	.00	.00
h	.00	.00	.00	.00	.00	.08	.08	.00	.00	.00	.00	.04	.00	.00	.01	.02	.00	.02	.04	.02
Adh1																				
1	.00	.00	.02	.00	.00	.00	.05	.00	.01	.00	.00	.00	.00	.01	.01	.00	.00	.00	.01	.00
2	1.0	1.0	.96	.98	1.0	.99	.94	1.0	.99	1.0	1.0	1.0	1.0	.93	.98	1.0	1.0	.97	.99	.99
3	.00	.00	.02	.02	.00	.01	.01	.00	.00	.00	.00	.00	.00	.06	.01	.00	.00	.03	.00	.01
h	.00	.00	.08	.04	.00	.02	.11	.00	.02	.00	.00	.00	.00	.13	.03	.00	.00	.06	.02	.02
Adh3																				
1	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00	.00	.02	.00	.01
2	1.0	1.0	.99	1.0	1.0	1.0	.95	1.0	.96	1.0	1.0	1.0	.98	.99	.99	1.0	1.0	.98	1.0	.99
3	.00	.00	.01	.00	.00	.00	.05	.00	.04	.00	.00	.00	.01	.01	.01	.00	.00	.00	.00	.00
h	.00	.00	.02	.00	.00	.00	.10	.00	.08	.00	.00	.00	.04	.02	.02	.00	.00	.04	.00	.01
Fest1																				
1	.00	.00	.01	.00	.00	.01	.00	.00	.02	.00	.00	.01	.00	.04	.01	.00	.00	.00	.00	.00
2	.98	.95	.97	1.0	.94	.95	.98	.97	.96	.92	.76	.99	1.0	.96	.95	1.0	1.0	.97	.95	.98
3	.00	.00	.02	.00	.00	.04	.02	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00	.00	.00

Table 4.2 (continued)

Locus	Natural Population															Plantation				
	2	4	5	6	7	8	9	10	11	12	13	15	16	17	Mean	1	3	14	18	Mean
4	.02	.05	.00	.00	.06	.00	.00	.03	.02	.08	.24	.00	.00	.00	.03	.00	.00	.03	.05	.02
h	.04	.10	.06	.00	.12	.10	.04	.06	.08	.15	.37	.02	.00	.08	.09	.00	.00	.06	.10	.04
Fest2																				
1	.00	.03	.00	.00	.00	.02	.00	.00	.00	.07	.00	.00	.00	.00	.01	.00	.07	.01	.02	.03
2	1.0	.97	1.0	1.0	1.0	.98	1.0	1.0	1.0	.93	1.0	1.0	1.0	1.0	.99	1.0	.93	.99	.98	.97
h	.00	.06	.00	.00	.00	.04	.00	.00	.00	.13	.00	.00	.00	.00	.02	.00	.13	.02	.04	.05
Dia																				
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.01	.00	.00	.00	.00	.00	.00	.00
h	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.02	.00	.00	0.0	0.0	0.0	0.0	0.0
αgal																				
1	.87	.16	.32	.05	.17	.06	.12	.23	.41	.18	.02	.76	.01	.16	.26	.03	.02	.14	.07	.07
2	.09	.47	.48	.86	.78	.76	.66	.47	.47	.60	.88	.09	.84	.59	.56	.86	.86	.61	.84	.79
3	.04	.37	.20	.09	.06	.18	.22	.30	.12	.22	.10	.15	.15	.25	.18	.11	.12	.25	.09	.14
h	.23	.62	.62	.25	.36	.39	.50	.65	.60	.56	.22	.39	.27	.56	.44	.25	.25	.55	.29	.33
Gdh																				
1	1.0	1.0	.99	.99	1.0	.97	1.0	.97	.99	1.0	1.0	.99	.97	1.0	.99	1.0	1.0	.97	.98	.99
2	.00	.00	.01	.01	.00	.03	.00	.03	.01	.00	.00	.01	.03	.00	.01	.00	.00	.03	.02	.01
h	.00	.00	.02	.02	.00	.06	.00	.06	.02	.00	.00	.02	.06	.00	.02	.00	.00	.06	.04	.03
Ha																				
1	.06	.13	.21	.03	.04	.05	.09	.23	.26	.14	.26	.04	.12	.33	.15	.13	.03	.25	.33	.19
2	.12	.14	.51	.23	.12	.38	.68	.45	.12	.42	.70	.59	.44	.32	.38	.11	.12	.26	.38	.22
3	.82	.73	.28	.74	.84	.57	.23	.32	.62	.44	.04	.37	.44	.35	.48	.76	.85	.49	.29	.60
h	.31	.43	.62	.40	.28	.53	.48	.64	.53	.61	.44	.51	.60	.67	.50	.39	.26	.63	.67	.49
Idh1																				
1	.00	.00	.00	.00	.00	.00	.00	.00	.03	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	1.0	1.0	.98	1.0	1.0	1.0	1.0	.99	.95	.98	1.0	1.0	.93	1.0	.99	.95	1.0	1.0	1.0	.99
3	.00	.00	.02	.00	.00	.00	.00	.01	.02	.02	.00	.00	.07	.00	.01	.05	.00	.00	.00	.01
h	.00	.00	.04	.00	.00	.00	.00	.02	.10	.04	.00	.00	.13	.00	.02	.10	.00	.00	.00	.02
Idh2																				
1	.04	.16	.03	.07	.07	.04	.03	.10	.12	.04	.00	.01	.04	.00	.05	.01	.00	.09	.02	.00
2	.96	.84	.97	.93	.93	.94	.97	.90	.88	.96	1.0	.99	.94	1.0	.94	.99	1.0	.91	.98	1.0
3	.00	.00	.00	.00	.00	.02	.00	.00	.00	.00	.00	.00	.02	.00	.00	.00	.00	.00	.00	.00

Table 4.2 (continued)

Locus	Natural Population															Plantation				
	2	4	5	6	7	8	9	10	11	12	13	15	16	17	Mean	1	3	14	18	Mean
h	.08	.27	.06	.13	.12	.11	.06	.18	.21	.08	.00	.02	.11	.00	.10	.02	.00	.17	.04	.06
Lap																				
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
h	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Me																				
1	.02	.00	.04	.00	.00	.05	.06	.00	.06	.00	.00	.02	.01	.01	.02	.00	.00	.00	.02	.01
2	.98	.97	.92	1.0	.98	.92	.90	1.0	.93	.94	.84	.94	.99	.99	.95	.95	1.0	.94	.91	.95
3	.00	.03	.04	.00	.02	.03	.04	.00	.01	.06	.16	.04	.00	.00	.03	.05	.00	.06	.07	.05
h	.04	.06	.15	.00	.03	.15	.19	.00	.13	.11	.27	.11	.02	.02	.09	.10	.00	.11	.17	.09
Mpi																				
1	.00	.00	.00	.00	.00	.00	.00	.00	.03	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.97	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
h	.00	.00	.00	.00	.00	.00	.00	.00	.06	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Mr																				
1	.15	.11	.08	.04	.08	.10	.09	.01	.12	.13	.15	.01	.00	.17	.09	.00	.04	.18	.00	.06
2	.85	.83	.84	.95	.88	.82	.64	.88	.74	.72	.47	.65	.59	.71	.75	.88	.94	.51	1.0	.85
3	.00	.06	.08	.01	.04	.08	.27	.11	.14	.15	.38	.34	.41	.12	.15	.12	.02	.21	.00	.10
h	.26	.30	.27	.10	.21	.31	.51	.22	.42	.44	.61	.46	.48	.45	.36	.21	.11	.59	.00	.23
Pgi2																				
1	.00	.02	.33	.00	.00	.13	.25	.19	.14	.19	.07	.33	.25	.15	.15	.00	.00	.37	.17	.14
2	1.0	.91	.53	.99	.98	.85	.64	.74	.70	.80	.93	.61	.63	.68	.78	.99	1.0	.57	.81	.85
3	.00	.07	.15	.01	.02	.02	.11	.07	.17	.01	.00	.06	.12	.17	.07	.01	.00	.06	.02	.02
h	.00	.17	.59	.02	.03	.26	.51	.41	.46	.32	.13	.52	.53	.49	.32	.02	.00	.53	.32	.22
Pgm1																				
1	.00	.00	.00	.00	.00	.00	.00	.00	.02	.00	.00	.01	.00	.00	.00	.00	.00	.00	.00	.00
2	.00	.02	.02	.00	.00	.03	.08	.20	.03	.02	.00	.08	.24	.08	.06	.00	.00	.02	.00	.01
3	1.0	.98	.98	1.0	1.0	.97	.91	.78	.91	.98	1.0	.91	.76	.88	.93	1.0	1.0	.96	.90	.97
4	.00	.00	.00	.00	.00	.00	.01	.02	.04	.00	.00	.00	.00	.04	.01	.00	.00	.02	.10	.03
h	.00	.04	.04	.00	.00	.06	.17	.35	.17	.04	.00	.17	.37	.22	.12	.00	.00	.08	.18	.07
Skdh1																				
1	.11	.15	.06	.24	.11	.14	.34	.18	.18	.25	.00	.27	.16	.20	.17	.62	.10	.42	.17	.33
2	.53	.51	.83	.73	.78	.64	.50	.75	.42	.55	.75	.41	.56	.75	.62	.25	.61	.36	.53	.44
3	.36	.34	.11	.03	.11	.22	.16	.07	.40	.20	.25	.32	.28	.05	.21	.13	.29	.22	.30	.24

Table 4.2 (continued)

Locus	Natural Population															Plantation				
	2	4	5	6	7	8	9	10	11	12	13	15	16	17	Mean	1	3	14	18	Mean
h	.58	.61	.29	.41	.37	.52	.61	.40	.63	.59	.38	.66	.58	.40	.50	.55	.54	.65	.60	.58
Sod																				
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
h	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
6pg1																				
1	.00	.00	.00	.00	.02	.00	.00	.00	.01	.02	.00	.30	.07	.00	.03	.00	.00	.02	.02	.01
2	1.0	1.0	.88	1.0	.98	.97	.92	.98	.83	.98	1.0	.70	.78	1.0	.93	1.0	1.0	.98	.97	.99
3	.00	.00	.12	.00	.00	.03	.08	.02	.16	.00	.00	.00	.15	.00	.04	.00	.00	.00	.01	.00
h	.00	.00	.21	.00	.03	.06	.15	.04	.29	.04	.00	.42	.36	.00	.12	.00	.00	.04	.06	.03
6pg2																				
1	.33	.32	.23	.02	.10	.11	.19	.43	.15	.09	.22	.10	.20	.21	.20	.19	.28	.19	.12	.20
2	.67	.68	.77	.98	.90	.89	.81	.57	.85	.91	.78	.90	.80	.79	.80	.81	.72	.81	.88	.80
h	.44	.44	.35	.04	.18	.19	.31	.49	.26	.17	.34	.18	.32	.33	.29	.31	.46	.31	.21	.32
Aph3																				
1	.00	.04	.06	.51	.02	.03	.15	.29	.03	.04	.45	.03	.02	.07	.13	.03	.00	.08	.08	.05
2	1.0	.96	.94	.49	.98	.97	.85	.71	.97	.96	.55	.97	.98	.93	.87	.97	1.0	.92	.92	.95
h	.00	.08	.11	.50	.03	.06	.26	.41	.06	.08	.50	.06	.04	.13	.17	.06	.00	.15	.15	.09
Aph4																				
1	.05	.05	.05	.00	.05	.03	.01	.01	.03	.03	.00	.06	.06	.06	.04	.03	.00	.02	.00	.01
2	.95	.95	.95	1.0	.95	.97	.99	.99	.97	.97	1.0	.94	.94	.94	.96	.97	1.0	.98	1.0	.99
h	.10	.10	.10	.00	.10	.06	.02	.02	.06	.06	.00	.11	.11	.11	.07	.06	.00	.04	.00	.03

population 15 and 16 (h=0.42 and 0.36), and *Aph3* in population 6, 10 and 13 (h=0.50, 0.41 and 0.50)). In contrast, some loci in the third group have extremly low heterozygosities in some populations (For instance, *Mr* in population 18 (h=0.00), *Pgi2* in population 1, 2, 3, 4, 6, 7 (h=0.02, 0.00, 0.00, 0.02,and 0.03) , and *6pg2* in population 6 (h=0.04)). Third, the allele frequencies are essentially uniform over populations at most of the polymorphic loci, while great change occurs at some other highly polymorphic loci (e.g., *Aat3*, *Ha*, *Skdh*).

Among the 18 populations, population 16 exhibited the highest level of heterozygosity, while population 3 showed the lowest (Table 4.3). The overall means for the four measures of genetic variability are 1.83, 1.16, 57% and 0.133 for n_a, n_e, P, and H, respectively.

Looking at the 14 natural populations and 4 plantations separately, we can see that the average genetic variability of plantations is only slightly lower than that of natural populations in terms of all the four measures employed (Table 4.2 and Table 4.3).

4.3.2 Genetic Differentiation Among Populations

A significant amount of genetic differentiation among populations of *Thuja orientalis* could be inferred from the detected significant heterogeneity in allele frequencies at 23 out of 24 polymorphic loci among the fourteen natural

Table 4.3 Average Genetic Variability over 28 Loci for the 18 Populations of *Thuja orientalis* Linn.

Population	No. of Loci	n_a	n_e	P(%)	H
Natural Populations					
2	26	1.53	1.11	38	0.100
4	26	1.81	1.16	54	0.139
5	26	2.00	1.18	65	0.156
6	26	1.62	1.10	38	0.093
7	26	1.69	1.10	50	0.092
8	26	2.12	1.15	73	0.128
9	26	2.15	1.22	69	0.179
10	26	1.88	1.22	54	0.179
11	26	2.23	1.22	73	0.177
12	26	1.92	1.18	65	0.154
13	26	1.53	1.15	35	0.132
15	26	2.08	1.18	65	0.150
16	26	2.00	1.22	65	0.180
17	26	1.88	1.18	50	0.153
Unweighted Mean		1.89	1.17	57	0.144
Standard error		0.228	0.044	13.1	0.031
Plantations					
1	26	1.65	1.11	42	0.101
3	26	1.46	1.10	35	0.088
14	26	2.08	1.18	77	0.166
18	26	1.88	1.15	69	0.131
Unweighted Mean		1.77	1.14	56	0.122
Standard Error		0.270	0.037	20.4	0.035

populations studied (Table 4.4). Such inter-population genetic differentiation could account for more than 14% of the total genetic variation in the entire sample of natural populations as indicated by a mean G_{ST} value of 0.1405 (Table 4.5). However, individual populations contributed differently to the total amount of genetic differentiation. Based on the estimates of Gregorius and Roberds' differentiation measure, population 2 is most strongly differentiated from its complement (D_2=0.0980) and, therefore, contributed most to overall differentiation (Table 4.6 and Figure 4.2). On the other hand, population 12 showed the lowest degree of differentiation (D_{12}=0.0354), indicating the genetic structure of this population is nearly representative for the species in China. Similar conclusions could be drawn from the estimates of Nei's standard genetic distance (Table 4.7). Population 2 has the largest average genetic distance (D_{n2}=0.042), while population 12 has the smallest (D_{n12}=0.016). Taking the four artificial populations into account, it was found that population 3 displayed strong differentiation, ranking fourth among the 18 populations, while the other three populations were weakly differentiated (Table 4.8 and Figure 4.3).

Levels of genetic differentiation among populations vary considerably from locus to locus, as indicated by the large ranges of χ^2 values (Table 4.4), G_{ST} estimates (Table 4.5), and the estimates of Gregorius and Roberds' genetic

Table 4.4 Heterogeneity of Allele Frequencies at the 24 Polymorphic Loci among the 14 Natural Populations of *Thuja orientalis* Linn.

Locus	χ^2	df	P
Aat1	220.61	26	***
Aat2	34.87	13	***
Aat3	471.55	26	***
Aco	55.79	26	***
Adh1	91.83	26	***
Adh3	60.01	26	***
Fest1	256.88	39	***
Fest2	74.37	13	***
Dia	14.91	13	ns
αgal	635.68	26	***
Gdh	22.83	13	*
Ha	403.07	26	***
Idh1	96.08	26	***
Idh2	93.65	26	***
Lap	-	-	-
Me	128.38	26	***
Mpi	44.20	13	***
Mr	258.46	26	***
Pgi2	263.91	26	***
Pgm1	213.26	39	***
Skdh1	188.42	26	***
Sod	-	-	-
6pg1	479.86	26	***
6pg2	117.67	13	***
Aph3	375.01	13	***
Aph4	22.95	13	*

*: $P<0.05$;

***: $P<0.001$;

ns: $P>0.05$.

Table 4.5 Gene Diversity and Degree of Differentiation at 26 Loci among the 14 Populations Natural Populations of *Thuja orientalis* Linn.

Locus	H_T	H_S	D_{ST}	G_{ST}
Aat1	0.0757	0.0659	0.0098	0.1295
Aat2	0.0080	0.0066	0.0014	0.1750
Aat3	0.4536	0.3904	0.0636	0.1393
Aco	0.0145	0.0143	0.0002	0.0138
Adh1	0.0316	0.0286	0.0030	0.0949
Adh3	0.0198	0.0177	0.0021	0.1060
Fest1	0.0906	0.0856	0.0050	0.0552
Fest2	0.0218	0.0160	0.0058	0.2661
Dia	0.0020	0.0014	0.0006	0.3000
αgal	0.5875	0.4433	0.1442	0.2454
Gdh	0.0178	0.0175	0.0003	0.0169
Ha	0.6115	0.5030	0.1085	0.1774
Idh1	0.0238	0.0232	0.0006	0.0252
Idh2	0.1060	0.1024	0.0036	0.0340
Lap	0.0000	0.0000	0.0000	0.0000
Me	0.1016	0.0911	0.0105	0.1033
Mpi	0.0045	0.0041	0.0004	0.0889
Mr	0.3990	0.3593	0.0397	0.0995
Pgi2	0.3655	0.3172	0.0483	0.1321
Pgm1	0.1281	0.1166	0.0115	0.0898
Skdh1	0.5451	0.5009	0.0442	0.0811
Sod	0.0000	0.0000	0.0000	0.0000
6pg1	0.1326	0.1147	0.0179	0.1350
6pg2	0.3164	0.2888	0.0276	0.0872
Aph3	0.2262	0.1646	0.0616	0.2723
Aph4	0.0660	0.0659	0.0001	0.0015
Unweighted Mean	0.1673	0.1438	0.0235	0.1405

Table 4.6 Genic Differentiation (Djx100) among the 14 Natural Populations of *Thuja orientalis* Linn.

Locus	Population 2	4	5	6	7	8	9	10	11	12	13	15	16	17	Mean
Aat1	1.29	3.64	3.64	2.46	2.46	2.64	2.32	14.54	1.96	4.64	2.46	1.64	1.64	2.64	3.43
Aat2	0.36	0.36	0.36	0.36	0.36	1.64	2.64	0.36	0.36	0.36	0.36	0.36	0.36	0.36	1.69
Aat3	31.93	14.07	4.21	28.04	30.46	20.21	7.96	2.46	4.53	3.21	10.11	24.21	17.78	10.21	14.96
Aco	0.71	0.71	0.71	0.64	0.64	3.43	2.86	0.64	0.64	0.71	0.64	1.29	0.71	0.71	1.07
Adh1	1.50	1.50	2.50	0.57	1.07	0.64	4.43	1.07	0.43	1.50	1.07	1.50	1.50	5.50	1.77
Adh3	0.93	0.93	0.14	0.50	0.50	0.93	2.07	0.50	1.57	0.93	0.50	0.93	1.07	0.14	0.83
Fest1	2.00	0.71	3.57	2.71	0.93	2.00	1.71	1.21	1.07	2.21	9.93	2.36	3.00	2.36	2.56
Fest2	0.86	2.14	0.86	0.86	0.86	1.14	0.86	0.86	0.86	6.14	0.86	0.86	0.86	0.86	1.35
Dia	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.93	0.07	0.13
αgal	61.86	19.57	9.43	24.36	14.36	19.14	10.86	6.29	13.14	7.14	26.86	50.86	26.57	9.14	21.40
Gdh	0.93	0.93	0.07	0.07	0.93	2.07	0.93	2.07	0.07	0.93	0.93	0.07	2.07	0.93	0.93
Ha	33.50	24.50	20.50	12.71	17.71	9.71	17.93	8.29	18.57	4.64	22.29	21.64	6.64	18.79	16.96
Idh1	1.21	1.21	1.00	0.71	0.71	1.21	0.71	0.21	3.29	1.00	0.71	1.21	6.00	1.21	1.46
Idh2	1.64	10.64	2.64	1.50	1.50	1.71	2.50	4.50	6.50	1.64	5.50	4.64	1.71	5.64	3.73
Me	3.32	2.32	2.68	3.79	1.29	2.75	4.21	4.43	2.71	3.04	6.57	1.04	4.32	4.32	3.34
Mpi	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	2.79	0.21	0.21	0.21	0.21	0.21	0.39
Mr	15.50	9.50	9.50	12.18	6.68	7.50	5.82	10.18	2.32	4.07	17.32	18.50	25.50	8.07	10.90
Pgi2	21.54	12.54	25.96	17.54	17.04	6.54	12.46	4.46	5.04	5.96	11.04	18.46	15.46	10.46	13.18
Pgm1	6.32	4.32	4.71	2.96	2.96	3.32	1.25	7.25	2.25	4.32	2.96	2.68	17.89	3.89	4.79
Skdh1	14.93	12.93	21.14	9.04	11.11	3.07	14.39	7.04	10.39	7.93	12.11	22.86	6.93	11.07	11.85
6pg1	7.00	7.00	8.00	5.00	3.00	4.00	2.00	4.00	6.00	5.00	5.00	27.00	15.00	7.00	7.50
6pg2	13.71	12.71	3.71	17.29	9.29	8.29	0.29	23.71	4.29	10.29	2.71	9.29	0.71	1.71	8.43
Aph3	12.43	8.43	6.43	38.57	10.43	9.43	2.57	16.57	9.43	8.43	32.57	9.43	10.43	5.43	12.90
Aph4	1.50	1.50	1.50	3.50	1.50	0.50	2.50	2.50	0.50	0.50	3.50	2.50	2.50	2.50	1.93
Mean	9.80	6.35	5.57	7.74	5.67	4.67	4.31	5.85	4.11	3.54	7.47	9.22	7.07	4.72	6.14

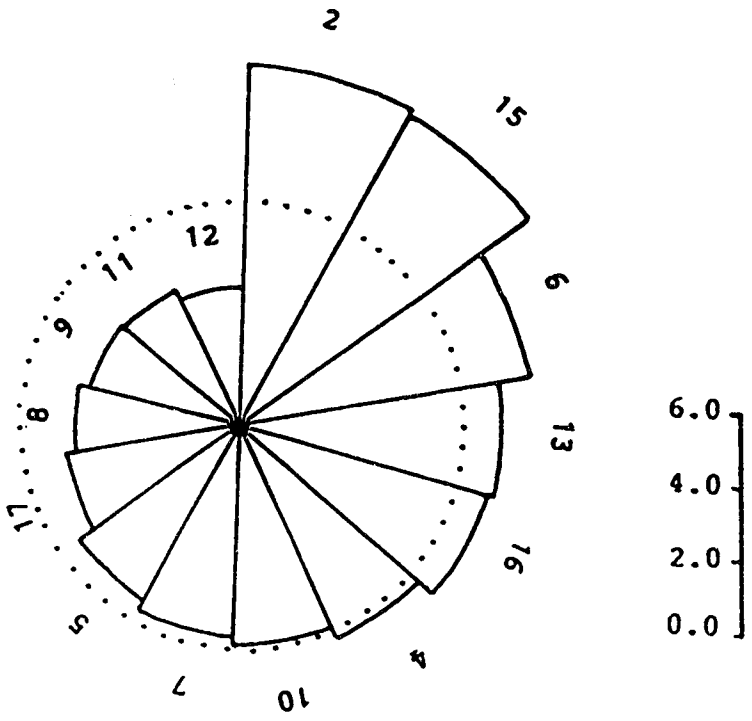


Figure 4.2 Differentiation Snail for the Gene Pool of the 14 Natural Populations of *Thuja orientalis* Linn. Indicating the Relative Contribution of Each Population to the Total Genetic Differentiation. In the snail the dotted circle has a radius equal to the total level of genetic differentiation. The solid sectors represent the contribution of individual populations to the total differentiation of the collection of populations. The radii of the solid sectors equal the amounts of differentiation of the individual populations.

Table 4.7 Pair-Wise Geographic Distance (above the Diagonal) and Genetic Distance (below the Diagonal) Estimates between Populations of *Thuja orientalis* Linn.

Pop.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1		761	1506	804	1261	957	958	1239	1131	1261	1413	1131	1131	1631	1609	1522	1740	3567
2	.040		804	326	500	739	717	587	565	522	696	870	804	913	1326	1087	1087	2827
3	.013	.030		1044	522	1435	1348	913	1022	696	674	1479	1392	783	1827	1544	1174	2196
4	.019	.025	.018		609	435	369	435	348	500	696	543	500	891	1022	826	957	2849
5	.039	.038	.034	.021		957	848	391	522	174	696	757	891	435	1305	1022	739	2305
6	.025	.049	.017	.037	.043		87	630	478	630	935	174	195	1087	652	565	9787	3023
7	.025	.039	.019	.017	.031	.027		522	369	696	848	195	152	1000	674	543	891	2936
8	.016	.039	.017	.010	.013	.029	.019		152	239	326	609	522	478	913	609	522	2436
9	.029	.055	.039	.024	.011	.043	.036	.012		348	478	478	391	630	826	565	609	2588
10	.035	.041	.034	.018	.011	.036	.034	.018	.015		174	829	739	391	1152	848	609	2349
11	.020	.020	.022	.010	.016	.038	.024	.013	.021	.024		935	848	217	1196	892	522	2175
12	.017	.033	.021	.011	.010	.030	.019	.004	.007	.015	.010		87	1044	478	391	891	2936
13	.054	.081	.055	.045	.031	.048	.056	.028	.020	.029	.047	.026		957	522	369	804	2871
14	.020	.046	.035	.016	.018	.047	.037	.012	.010	.022	.012	.008	.035		1218	913	391	1957
15	.055	.036	.066	.037	.024	.078	.060	.034	.025	.036	.023	.024	.057	.026		304	913	2917
16	.026	.054	.025	.025	.018	.038	.027	.016	.012	.023	.019	.013	.033	.018	.039		630	2675
17	.026	.041	.028	.014	.006	.037	.024	.009	.010	.010	.014	.006	.026	.010	.031	.015		2196
18	.018	.042	.015	.022	.016	.023	.027	.011	.018	.021	.017	.010	.030	.021	.045	.017	.014	
Mean	.028	.042	.028	.022	.022	.038	.031	.018	.023	.025	.021	.016	.041	.023	.041	.025	.018	.022

Table 4.8 Genic Differentiation (Djx100) among the 18 Populations of *Thuja orientalis* Linn.

Locus	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Mean
Aat1	1.31	1.00	1.39	3.39	2.19	2.19	3.39	1.31	4.61	14.81	2.39	4.61	3.39	0.69	1.61	1.39	2.39	2.19	3.01
Aat2	0.28	0.28	0.28	0.28	0.28	0.28	0.28	1.72	2.72	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.50
Aat3	12.14	27.89	36.89	18.11	3.36	24.64	48.61	10.75	17.11	5.86	2.50	7.11	21.50	11.75	25.50	16.50	14.11	15.14	17.75
Aco	0.42	0.78	0.78	0.78	0.69	0.69	0.78	3.31	3.22	0.69	0.78	0.78	0.78	0.31	1.22	0.78	0.78	1.31	1.05
Adh1	0.92	1.33	1.33	1.33	2.08	0.64	1.33	0.42	4.67	0.92	0.78	1.33	1.33	0.42	1.33	1.33	5.67	0.36	1.53
Adh3	0.50	0.83	0.83	0.83	0.17	0.50	0.83	0.50	4.33	0.50	3.33	0.83	0.83	1.50	0.83	1.17	0.33	0.50	1.06
Fest1	2.33	1.56	2.56	0.89	0.83	2.33	1.29	0.67	3.72	0.83	1.06	2.39	10.39	0.83	2.06	2.56	2.06	0.67	2.17
Fest2	1.22	1.22	5.78	1.78	1.22	1.22	1.22	0.78	1.22	1.22	1.22	5.78	1.22	0.22	1.22	1.22	1.22	0.78	1.71
Dia	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.96	0.06	0.06	0.11
agal	21.86	66.00	23.72	20.08	12.64	19.86	15.72	14.36	9.00	8.64	20.00	5.28	25.72	4.14	55.00	21.70	8.28	17.86	20.50
Gdh	1.00	1.00	1.00	1.00	0.00	0.00	1.00	2.00	1.00	2.00	0.00	1.00	1.00	2.00	0.00	2.00	1.00	1.00	1.00
Ha	12.47	30.99	34.00	22.00	11.53	11.47	33.00	7.14	34.11	9.53	21.89	8.11	47.00	8.81	25.11	10.11	17.83	11.03	19.79
Idh1	1.97	1.22	1.22	1.22	0.47	0.69	1.22	0.69	1.22	0.19	3.78	0.94	1.22	0.69	1.22	5.94	1.22	0.69	1.43
Idh2	3.94	1.06	5.06	11.17	1.94	2.06	2.17	0.89	2.06	5.06	7.17	1.06	5.06	4.06	4.06	1.78	5.06	2.94	3.70
Me	1.06	3.31	5.25	2.25	2.44	3.56	1.75	2.94	4.75	4.61	4.14	2.69	12.69	1.39	0.75	4.25	4.25	1.94	3.56
Mpi	2.83	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.32
Mr	9.31	14.28	10.44	8.28	4.14	10.81	10.44	3.14	13.56	8.81	3.81	5.56	30.56	15.69	19.72	26.72	8.83	15.31	12.52
Pgi2	16.78	20.25	20.25	12.31	22.72	16.78	18.25	3.28	15.75	5.22	11.08	4.92	13.25	22.72	18.75	16.75	11.75	1.94	14.04
Pgm1	2.36	5.36	5.36	3.36	1.36	2.36	5.36	0.86	3.44	7.81	3.19	3.36	5.36	1.36	3.64	18.81	4.81	2.36	4.47
Skdh1	37.14	14.39	10.56	12.39	19.86	9.31	20.17	6.36	13.44	9.86	18.39	4.44	20.56	21.64	16.83	6.39	12.17	4.19	14.34
6pg1	4.14	5.72	5.72	5.72	4.42	4.14	3.72	2.64	4.83	3.14	12.83	3.72	5.72	2.14	27.44	16.28	5.72	1.64	6.65
6pg2	0.33	13.67	8.67	12.67	3.67	17.33	9.33	8.33	0.33	23.67	4.33	10.33	2.67	0.33	9.33	0.67	1.67	7.33	7.51
Aph3	7.72	10.72	10.72	6.72	4.72	40.28	8.72	7.72	4.28	18.28	7.72	6.72	34.28	2.72	7.72	8.72	3.72	2.72	10.79
Aph4	0.03	1.97	3.03	1.97	1.97	3.03	1.97	0.03	2.03	2.03	0.03	0.03	3.03	1.03	3.47	2.97	2.97	3.03	1.92
Mean	5.77	9.38	8.38	6.63	4.29	7.26	7.95	3.38	6.32	5.59	6.47	3.40	10.33	4.38	9.47	7.06	4.84	3.98	6.38

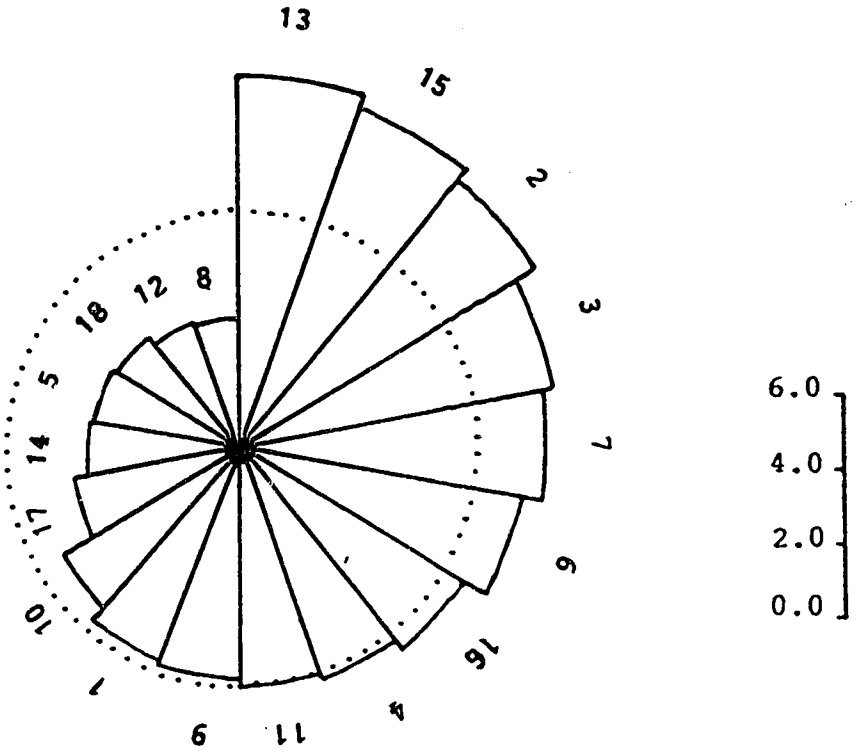


Figure 4.3 Differentiation Snail for the Gene Pool of 18 Populations of *Thuja orientalis* Linn. Indicating the Relative Contribution of Each Population to the Total Genetic Differentiation. In the snail the dotted circle has a radius equal to the total level of genetic differentiation. The solid sectors represent the contribution of individual populations to the total differentiation of the collection of populations. The radii of the solid sectors equal the amounts of differentiation of the individual populations.

differentiation measure (Table 4.6). Since G_{ST} is highly dependent on gene diversity in the total population (H_T), a large G_{ST} may result from a small H_T even if the absolute genetic differentiation is small (A typical example is the large G_{ST} at *Dia*). Therefore, the ranking of individual loci in the extent of genetic differentiation may be better presented using the estimates of Gregorius and Roberds' measure. As illustrated in Figure 4.4, populations on the average exhibited the strongest differentiation at *αgal*, while almost no differentiation was shown at *Dia*.

As can be seen in Table 4.6, ranking of population differentiation changes considerably from locus to locus. For instance, population 2 differentiated most strongly at *αgal* but most weakly at *Aat1*.

Discriminant analysis generated two highly significant discriminant functions (P=0.0000) that accounted for more than 43% of the total variation in the megagametophyte genotype scores of the 20 polymorphic loci (Table 4.9). Based on the coefficients of the two functions we can see that the first function was dominated by *Aph3*, *Aat3*, *Pgi2* and *6gi1* while the second function had larger loadings at *Aat3*, *Aph3*, *Aat1*, *Fest2* and *Pgi2*. Plotting the mean scores of the 18 populations for the first two discriminant functions we may find that populations 6, 13, 15, 10 and 17 are not only highly separated from the rest of populations but also from each other (Figure 4.5). No simple geographic

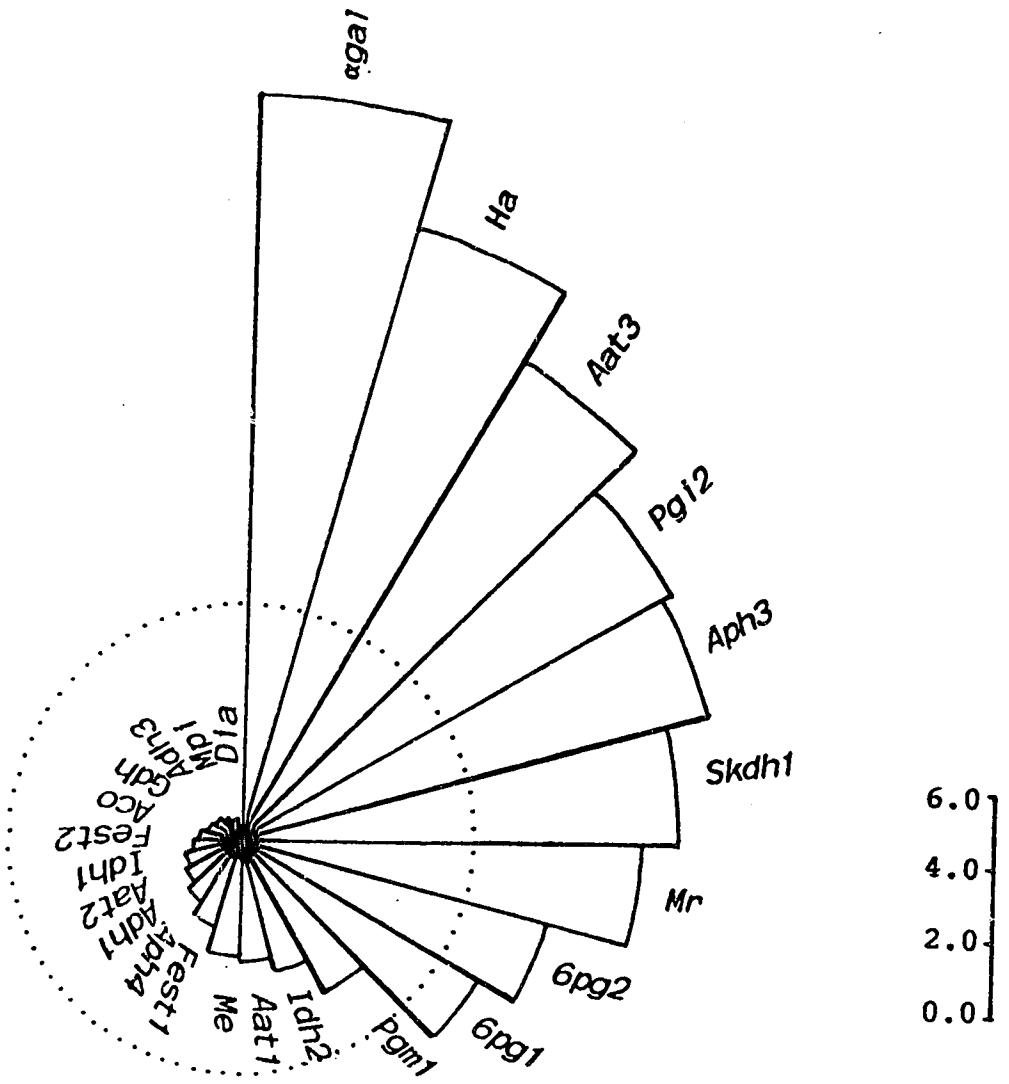


Figure 4.4 Differentiation Snail for the Gene Pool of the 14 Natural Populations of *Thuja orientalis* Linn. Indicating the Relative Contribution of Each locus to the Total Genetic Differentiation. In the snail the dotted circle has a radius equal to the total level of genetic differentiation. The solid sectors represent the contribution of individual loci to the total differentiation of the collection of populations. The radii of the solid sectors equal the amounts of differentiation at the individual loci.

Table 4.9 Coefficients for the First Two Canonical Discriminant Functions

Locus	Function 1	Function 2
Aat1	+0.10519	+0.30881
Aat2	-0.03071	-0.00532
Aat3	+0.54435	-0.61679
Aco	-0.07637	+0.00769
Adh1	-0.05220	+0.03706
Adh3	-0.04122	+0.02408
Fest1	-0.15608	-0.03014
Fest2	+0.02649	-0.21791
Gdh	+0.01037	-0.09419
Idh1	-0.09460	+0.04048
Idh2	-0.00567	+0.06666
Me	-0.08208	-0.03667
Mpi	-0.03551	-0.03205
Mr	-0.00886	+0.02624
Pgi2	-0.28516	+0.20744
Pgm1	-0.11558	+0.10746
6pg1	-0.25622	+0.10234
6pg2	+0.02452	-0.00701
Aph3	+0.69532	+0.61679
Aph4	-0.06808	-0.04841
Eigenvalue	0.4114	0.2477
Percent of variance	27.00	16.26
Canonical correlation	0.5399	0.4456
Significance level	0.0000	0.0000

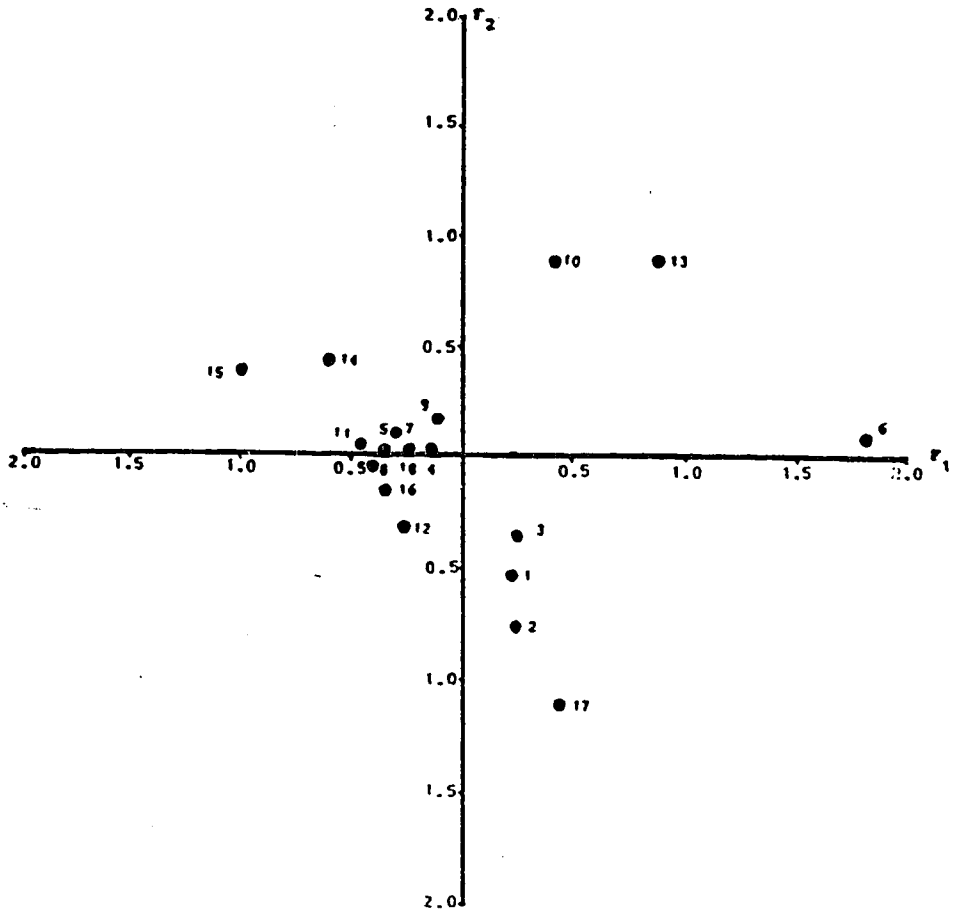


Figure 4.5. Plot of the Mean Scores of the 18 Populations on the First Two Discriminant Functions (F_1, F_2)

interpretation of those two discriminant functions could be made, but geographically nearby populations do not seem to be genetically more similar than distant populations. In fact, those geographically nearby populations were highly separated (e.g., populations 6 and 7, 13 and 12, 10 and 5 etc.).

Cluster analysis based on Nei's standard genetic distance estimates gave similar results (Figure 4.6). Two major differences are: 1) population 7 was not only highly separated from its neighbor, population 6, as shown by discriminant analysis, but also separated from the others; and 2) population 2 was more strongly differentiated from the other populations than what we have seen in Figure 4.5. These differences are not surprising since the methods of those two analyses are quite different and since the three most polymorphic loci, *αgal*, *Ha* and *Skdh*, were excluded from the discriminant analysis.

4.3.3 Geographic Patterns of Genetic Variation

While no significant linear relationship between average heterozygosity and either latitude or longitude was detected, a significant positive correlation between average heterozygosity and elevation was revealed ($r=+0.782$, $P<0.001$). Such a correlation is in good agreement with the observation that the common allele frequencies at most of the polymorphic loci tend to decrease as elevation increases

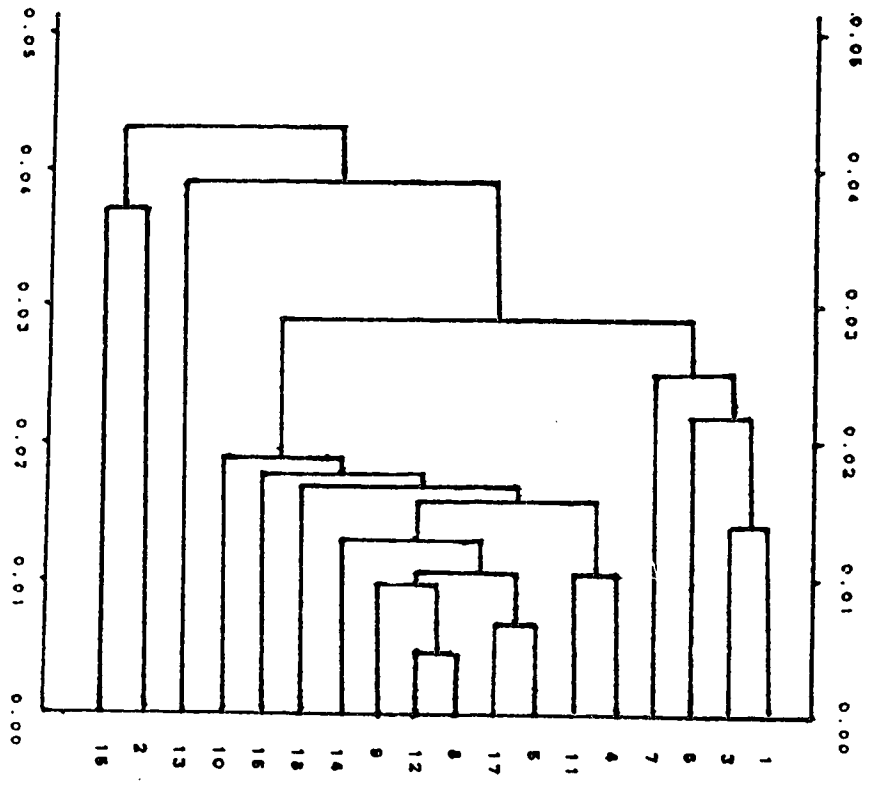


Figure 4.6 Population Clustering Based on Nei's Standard Genetic Distance Using the UPGMA Method

(Table 4.10). The results of correlation analysis between the common allele frequencies and the three major climatic factors indicate that in most of the cases, common alleles are favored in warm and wet environments (Table 4.11). Therefore, the observed positive correlation between average heterozygosity and elevation likely resulted from the decrease of annual mean temperature, annual mean relative humidity and annual precipitation as elevation increases. This hypothesis is strongly supported by the significant negative correlations between elevation and the three climatic factors (r_1=-0.7120, $P<0.01$; r_2=-0.5152, $P<0.05$; r_3=-0.6329, $P<0.01$). In fact, average heterozygosity is negatively correlated with those climatic factors (r_1=-0.6854, $P<0.01$; r_2=-0.6677, $P<0.01$; r_3=-0.5102, $P<0.05$).

Table 4.10 Linear Correlation between the Most Common Allele at the 24 Polymorphic Loci and Geographic Factors

Locus	Latitude	Longitude	Elevation
Aat1	+0.224	-0.092	-0.377
Aat2	+0.076	-0.069	-0.306
Aat3	+0.295	+0.142	+0.410
Aco	-0.090	+0.142	-0.294
Adh1	-0.190	+0.112	-0.624**
Adh3	-0.119	-0.067	-0.569*
Fest1	-0.179	-0.036	+0.286
Fest2	+0.066	+0.368	+0.092
Dia	-0.242	-0.183	-0.034
αgal	-0.068	-0.181	-0.195
Gdh	+0.350	+0.247	-0.446
Ha	-0.509*	+0.149	-0.432
Idh1	-0.191	-0.271	+0.103
Idh2	-0.210	-0.098	-0.109
Me	-0.181	+0.096	+0.057
Mpi	+0.012	+0.345	-0.440
Mr	-0.223	-0.435	-0.191
Pgi2	-0.424	+0.080	-0.838***
Pgm1	-0.382	+0.106	-0.543*
Skdh1	+0.188	-0.051	+0.103
6pg1	-0.326	-0.247	-0.250
6pg2	+0.397	+0.484	-0.130
Aph3	-0.139	-0.225	+0.283
Aph4	+0.158	-0.339	-0.164

*: $P<0.05$;

**: $P<0.01$;

***: $P<0.001$.

table 4.11 Linear Correlation between the Most Common Allele at the 24 Polymorphic Loci and Climatic Factors

Locus	AMT[1]	AMRH[2]	AP[3]
Aat1	+0.116	+0.016	-0.033
Aat2	+0.034	+0.014	+0.354
Aat3	-0.394	-0.593*	-0.410
Aco	+0.165	+0.157	+0.237
Adh1	+0.512*	+0.427	+0.458
Adh3	+0.315	+0.267	+0.253
Fest1	-0.276	+0.268	+0.165
Fest2	-0.365	-0.462	-0.069
Dia	+0.093	+0.198	+0.017
αgal	+0.232	+0.188	+0.108
Gdh	+0.469	+0.458**	+0.654**
Ha	+0.657**	+0.694**	+0.593*
Idh1	-0.184	-0.104	-0.403
Idh2	+0.016	-0.151	-0.022
Me	+0.076	+0.221	+0.203
Mpi	-0.044	-0.381	-0.128
Mr	+0.287	+0.662**	+0.318
Pgi2	+0.819***	+0.674**	+0.658**
Pgm1	+0.591*	+0.505*	+0.388
Skdh1	-0.251	-0.209	-0.324
6pg1	+0.425	+0.467	+0.233
6pg2	-0.180	-0.241	-0.197
Aph3	+0.280	+0.096	+0.005
Aph4	+0.199	+0.341	+0.029

*: P<0.05;

**: P<0.01;

***: P<0.001;

1, AMT=annual mean temperature;

2, AMRH=annual mean relative humidity;

3, AP=annual precipitation.

4.4 Discussion

4.4.1 Genetic Variation Within Populations

The levels of genetic variation within populations of *Thuja orientalis* detected in the present study are within the range of values reported for other conifers. However, these estimates of n_a (1.89), P (57%) and H (0.144) are somewhat lower than the average values (2.29, 67.7%, 2.07) reported for over 20 conifers (Hamrick *et al.*, 1981). Unusually low levels of isozyme variation have been reported in a few conifers, such as *Pinus resinosa* (Folwer and Morris, 1977), *Pinus torreyana* (Ledig and Conkle, 1983) and *Thuja plicata* (Copes, 1981; Yeh, 1988). An extremely restricted geographic range was considered to be responsible for the observed low variability in *Pinus torreyana*, while a bottleneck in population size was speculated to explain the corresponding observation in *Pinus resinosa* and *Thuja plicata*. In *Thuja orientalis*, the observed level of isozyme variation is only slightly lower than the average of other conifers. Such a difference may possibly result from the species' discontinuous distribution, its relatively small population sizes and low outcrossing rates, as well as intensive family structures within populations, or merely result from sampling errors, the enzyme systems surveyed, and the experimental conditions used.

Considerable variation in the amount of genetic polymorphism among isozyme loci has been observed in most

coniferous species and other organisms studied by isozyme techniques. Such variation may be related to differences in metabolic functions, tertiary structures and the subunit weight of the enzymes coded by those loci. Johnson (1974) divided enzymes into three classes based on their metabolic functions: variable-substrate enzymes, regulatory enzymes, and nonregulatory enzymes. Variable-substrate enzymes are expected to have high levels of genetic variability, since those enzymes act on a variety of substrates that may vary greatly in concentrations, and many of those substrates originate outside the organism and thus reflect variation in the environment (Kojima *et al.*, 1970). Johnson (1974) further indicated that enzymes regulating the flow of metabolites in a given metabolic pathway would be the most important in determining the fitness of individuals and therefore would also show high levels of genetic variability. In addition, the level of isozyme variation has been found to be negatively correlated with the number and positively correlated with the weight of the subunits of the enzyme (Koehn and Eanes, 1978; Zouros, 1976). Those correlations are likely due to the decline of the mutation rate as the subunit number increases and the molecular weight decreases (Kimura, 1983; Koehn and Eanes, 1978).

Although the above hypotheses and observations could not be confirmed from this study due to the lack of information on metabolic functions, tertiary structures and the subunit molecular weight for many of the enzymes

surveyed, great differences in the level of genetic variability among loci coding for different enzymes were observed, and such differences suggest that many loci coding for various kinds of enzymes should be surveyed to ensure a reliable estimate of the amount of genetic variation within a species.

Inter-population variation in the level of heterozygosity of a given locus is another common observation in the study of isozyme polymorphisms and may possibly result from the differences in natural selection and random genetic drift among populations.

4.4.2 Genetic Differentiation Among Populations

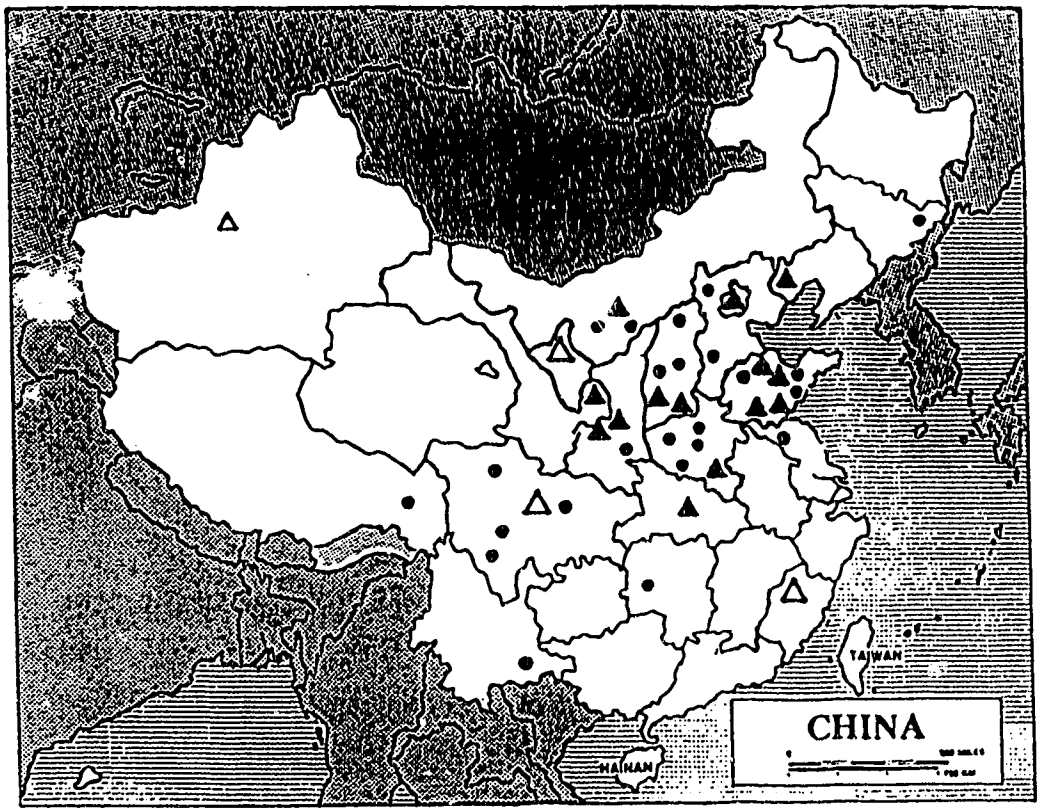
Although conifers are one of the most genetically variable groups of species, evidence from isozyme studies indicates that differentiation among populations of these species commonly is weak. The amount of genetic differentiation reported so far in conifers ranges from a low of 0.0% in *Pinus resinosa* (Fowler and Morris, 1977) to a high of 16.2% in *Pinus radiata* (Moran *et al.*, 1988). Compared with studies involving large numbers of populations sampled from wide geographical ranges and using large samples of loci, the estimated G_{ST} value (14.1%) in the present study ranks at the high end of the range of those estimates and about twice the mean (Table 4.12). The actual level of population differentiation in *Thuja orientalis* may be much higher, since many remote or marginal populations

Table 4.12 Results of Genetic Differentiation studies in some Conifers

Species	No. of Pop.	No. of Loci.	G_{ST}	Geographical Range Lat. (Degree)	Long. (Degree)	Ele. (Meter)	References
Picea sitchensis	10	24	0.079	46-58	124-134	0-137	Yeh and El-Kassaby, 1980.
Pinus contorta	28	42	0.032	37-65	107-138	-	Wheeler and Guries, 1982.
Pinus jeffreyi	14	20	0.138	31-43	115-124	505-2465	Furnier and Adams, 1986.
Pinus rigida	11	21	0.023	37-45	70-83	5-915	Guries and Ledig, 1982.
Pinus radiata	5	31	0.162	-	-	5-1220	Moran *et al.*, *1988.*
Larix laricina	36	19	0.042	40-60	53-160	-	*Cheliak et al.*, 1983.
Thuja orientalis	14	26	0.141	31-41	109-118	50-1200	present study.

were not included in the present study (Figure 4.8). Great interpopulation variation in quantitative traits of *Thuja orientalis* has also been revealed by provenance tests. Highly significant variation among populations was detected for 12 morphological characters of maternal trees and for 14 morphological and physiological traits of the seedlings surveyed (Wu, 1985).

The observed differentiation pattern in *Thuja orientalis* may be better understood by examining the location of the sampled populations and the natural range of the species (Figure 4.7). Populations 15 and 2 are marginal populations, located near the northern and southern boundaries, respectively, of the species' range. They are separated from the rest of the populations by relatively great distances, and therefore frequent gene exchange between those two populations and the others is unlikely. Since they are distributed in two different climatic and edaphic regions, adaptation to the local environments may further lead to differentiation from each other and the other populations. The local climate of population 6 has been found to be unique even from that of its nearest neighbor, population 7 (Yao and Shen, 1984). Great differences in morphological characters including cone length, seed size, seed germination rate, and height, root length and biomass of young seedlings have been observed between this population and nearby populations in the climatic region where population 7 is located (Wu, 1985).



▲: sampled natural population, △: sampled plantation,
●: non-sampled natural population.

Figure 4.7 Locations of the Sampled and Non-sampled Populations of *Thuja orientalis* Linn.

Thus, adaptation to local climate seems to be an important mechanism in determining the observed strong differentiation between this population and the others. Population 13 is located in the central range of the species and relatively close to its neighbor populations. Climatic differences do not seem sufficient to explain the observed differentiation. One of its nearest neighbors, population 12, belongs to the same climatic region (Yao and Shen, 1984) but is highly differentiated from population 13. From Table 4.2 we can see that 9 out of 24 rare alleles observed in population 12 were not present in population 13, and the frequencies of the remaining rare alleles are quite different between these two populations. Possible explanations to the observed differentiation of population 13 are the restriction of gene flow between this and the other populations due to some physical barriers, adaptation to some unknown distinct environmental factors, and past or present disturbance to the population by disease, insects, and human activities. Differentiation analysis using all 18 populations revealed that population 3 is also well differentiated from the others. This artificial population was most likely established from the nearby natural populations in the same province (i.e. Shichuan province). If this is true, the observed strong differentiation of this population is not surprising, since those populations in Shichuan province as a group are separated from the rest of the species by a long distance and by the Chinling Mountain range, which is the

watershed between the northern and southern parts of China. In addition, both the climatic and edaphic conditions in Shichuan province are quite different from those in the central range of the species. The differentiation of populations 18 and 1 is fairly weak considering the great geographical distances between these two populations and the others, and their distinct environmental conditions. This might suggest that they were established from those populations located in the central range and with a genetic structure nearly representative for this species in China. Such a suggestion seems reasonable since there is no local natural population nearby.

The observation that geographically nearby populations are not genetically more similar than geographically distant populations and that environmentally more distant populations are genetically more differentiated regardless of the geographic distance suggests that populations of *Thuja orientalis* have been well isolated and adapted to their local environments. Although I do not exactly know how long the discontinuous distribution of *Thuja orientalis* has prevailed, it is unlikely a recent phenomenon. Li Daw-Yuan, a Chinese geographer of the fifth century A.D., investigated the vegetation of the Yellow River valley, and the tree species he noted included elm, willow, pines, *Juniperus*, and other hardwoods (Wang, 1961). Most of those species are still common in this region at the present. The Yellow River valley is in the central range of *Thuja orientalis* (Chapter

1); if there had been large continuous populations of *Thuja orientalis* Li should not have missed them. In other words, *Thuja orientalis* probably was distributed as relatively small and isolated populations at that time, fifteen centuries ago. Historical changes of environment may be important causes resulting in the scattered distribution of *Thuja orientalis*. However, human activity should also be regarded as an important factor. The Yellow River valley is known as the cradle of the Chinese civilisation, and has been densely inhabited for many thousands of years. Continuous wars, clearing for agriculture, cutting for fuel, logging, etc. may have further divided large populations into small and isolated stands. Long-term isolation and adaptation to their local environments thus could lead to a relatively high level of population differentiation.

Differences in the extent of genetic differentiation among populations at different loci may suggest that the degree of adaptive sensitivity of allelic variation to the environmental differencies existing among the populations is not identical among loci. However, such individual-locus patterns of inter-population differentiation may also be related to the molecular structures of the enzymes coded by the loci. Through examination of the patterns of inter-population differentiation in *Drosophila* species, Koehn and Eanes (1978) found that loci coding for large subunit enzymes show higher levels of inter-population differentiation.

4.4.3 Maintenance of the Observed Genetic Variation

One of the central problems in molecular population genetics today is the determination of mechanisms maintaining genetic variation in natural populations. Two major theories have been proposed to explain the observed genetic variation at the molecular level. One is called neutralism and the other selectionism. The former states that the majority of the observed polymorphisms is maintained by a balance between neutral mutation and random genetic drift, while the latter argues that those polymorphisms are basically a consequence of various balancing forms of natural selection. While it is difficult to falsify one or the other theories based on the limited information available, the suggestion of natural selection as an important force in maintaining the observed genetic variation could be drawn from the following analysis.

According to the relationship of the effective number of electrophoretically detectable alleles (n_e) and the effective population size (N_e) given by Ohta and Kimura (1973) ($n_e=(1+8N_e\mu)^{0.5}$), and using the most commonly cited mutation rate to neutral alleles ($\mu=10^{-7}$), I calculated the effective population sizes for two populations (population 11 and 2) with known sizes (3.46 ha and 1.00 ha, respectively). The estimated value of N_e is 61,050 for population 11 and 31,800 for population 2. Based on the crown densities and sizes of the trees in those two populations (the crown density is 0.5 for population 11 and

0.3 for population 2; and the average crown sizes are not smaller than 2 m^2 in these two populations), the actual number of individual trees is hardly over 9,000 in population 11 and 1,500 in population 2. In other words, the estimated values of N_e are far too large to be realistic. One may argue that migration among populations increased the effective population sizes of these two populations. From Figure 4.7 and Table 4.7 we can see that the horizontal distances between these two populations and their nearest neighbors (populations 10 and 4, respectively) are 174 km and 326 km, respectively. It is unlikely there could be any effective gene exchange between these two populations and the others over such great distances. Another argument is that the estimates of n_e, and μ are not accurate and the populations are not at equilibrium. This might be true since the estimates of n_e can be affected by the sample size, the loci surveyed and the experimental conditions used; μ may deviate from 10^{-7} for a different set of loci; and the populations may not be at equilibrium due to various causes. While such disagreement can not reject the neutrality hypothesis because of the above argument, it does suggest that natural selection may be an important force in maintaining the observed genetic polymorphisms.

Indirect evidence of the impact of natural selection on the observed genetic variation in *Thuja orientalis* can be drawn from the detected significant correlations between the common allele frequencies and climate factors, as well as

the correlations between average heterozygosity and climatic factors. The observed correlations may result either from close linkages of those isozyme loci to some other unidentified loci on which natural selection was acting or real relationships between allozymes and the climatic factors. Some of the correlations detected in this study have been well documented before. For example, clinal variation patterns of *Adh* allele frequencies along a temperature gradient were detected in *Drosophila melanogaster* (Vigue and Johnson, 1973), and differential survival of the *Adh* phenotypes of the flies under different temperatures was observed (Johnson and Powell, 1974). It was suggested that *Adh* phenotypes of *Drosophilia melanogaster* are targets of temperature-induced selection (Johnson and Powell, 1974). It has been demonstrated that those climatic factors tested here are also the most important ones in affecting the evolution of quantitative traits of *Thuja orientalis* (Wu, 1985). Of the 25 morphological characters of maternal trees and seedlings, 17 showed significant correlations with annual mean temperature, 16 with relative humidity, and 16 with annual precipitation. The numbers of signicant correlations detected for those climatic factors rank among the top three of the 10 climatic factors surveyed. Since only three climatic factors were tested in this study, significant correlations were detected only at seven loci. Correlations likely would have been detected at more loci if more environmental factors were involved.

The presence of significant amounts of multilocus association within populations may also suggest the operation of natural selection, since such associations could be generated from natural selection favoring some particular allelic combinations at different loci (linked or unlinked). The intensities of multilocus association in the 18 populations of *Thuja orientalis* were estimated from Brown *et al.*'s (1980) model, and significant amounts of multilocus association were detected in two of them (Table 4.13). Genetic hitchhiking and migration may not be important in generating the observed association, since the loci surveyed are not tightly linked (see chapter 2) and the selfing rates are not high (see chapter 3), as well as the fact that the populations are well isolated. Since these two populations, Gansu Huexian and Liaoling Chaoyan, are marginal ones (Figure 4.1), it seems reasonable to infer that tha detected multilocus associations are due to natural selection favoring some particular allelic combinations at different loci.

In summary, although genetic drift may have made a significant contribution to the maintenance of the observed genetic variation in *Thuja orientalis*, my data do suggest that natural selection is an important force in maintaining such variation. The most likely situation may be that some alleles at some loci are selectively neutral, while other alleles at other loci are selectively sensitive.

Table 4.13 Intensity of Multilocus Association in 18 Populations of *Thuja orientalis* Linn.

Population	No. of seeds	σ_k^2	s_k^2	X(2)	L
Fujiang Nanping	80	0.352	0.354	0.004	0.443
Hubei Jingmen	80	0.296	0.299	0.009	0.877
Sichuan Mianyang	60	0.710	0.751	0.057	0.978
Henan Queshan	80	0.593	0.531	-0.105	0.768
Shannxi Huangling	80	0.793	0.797	0.005	1.037
Shandong Changshan	80	0.320	0.335	0.049	0.402
Shandong Zaozhuang	60	0.548	0.515	-0.061	0.744
Shanxi Jieshan	80	1.412	1.265	-0.105	1.872
Shanxi Jinchang	80	1.443	1.373	-0.048	1.894
Shannxi Chunhua	80	0.772	0.585	-0.242	1.017
Gansu Huexian	80	1.746	3.120	0.787	2.291*
Shandong Boshan	60	0.743	0.691	-0.071	1.017
Shandong Lichang	80	0.412	0.457	0.110	0.519
Ningxia Yinchuan	80	1.299	1.334	0.028	1.718
Liaoling Chaoyan	80	0.433	0.721	0.664	0.596*
Beijing Miyun	90	0.633	0.745	0.176	0.829
Neimong Wulashan	80	0.310	0.313	0.009	0.383
Xinjiang Yili	80	0.524	0.488	-0.068	0.673

note:

σ_k^2=expected variance of the number of heterozygous loci in two randomly chosen gametes;

s_k^2=observed variance of the number of heterozygous loci in two randomly chosen gametes;

$X(2)=(s_k^2/\sigma_k^2)-1$=intensity of multilocus association;

L=the upper 95% confidence limit for s_k^2;

*: multilocus association is significant at 5% level.

4.5 Conclusion

The genetic structure of *Thuja orientalis* reported in the present study is similar to the general picture of that of other conifers, that is, the majority of genetic variation is maintained within populations and only a small proportion of total genetic variation can be attributed to among-population differences. However, compared with other conifer species, *Thuja orientalis* exhibited relatively low within-population variation and high among-population differentiation. Small and relatively isolated populations scattered in a large geographical range with diverse environmental conditions seem to be the most probable cause for the observation. The variation pattern revealed is generally consistent with that derived from provenance tests, and the same climate factors have been found to have significant influences on both patterns. Information from this and other related studies suggests that natural selection is an important force in maintaining the observed genetic variations in *Thuja orientalis*.

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5. Practical Implications

As indicated in chapter one, studies on the mating system and genetic structure of a species are not only theoretically important, but also practically significant. Information on the mating system and genetic structure of a species is essential for many aspects of tree improvement practice. In this chapter, some practical implications of the results obtained in this study are briefly discussed.

5.1 Gene Conservation

The aim of gene conservation is to preserve as many different gene complexes as possible. Allozyme alleles can be regarded as markers of gene complexes; therefore, strategies that maximize the number of allozyme alleles in *in situ* reserves or *ex situ* collections should be considered (Adams, 1981; Marshall and Brown, 1975). As we observed earlier, population 11 contains the largest proportion (85%) of alleles detected in the species (table 4.2). Thus, if the other conditions affecting the efficiency of *in situ* conservation are the same for all the studied populations, this population should be the first candidate for *in situ* gene conservation of the species. Since significant genetic differences among populations were detected by both this isozyme study and other provenance/progeny tests (Wu, 1985), preservation of several populations distributed in different environmental regions is suggested.

The best strategy for *ex situ* collections is to sample as many alleles as possible with minimal effort. Since the ability to sample alleles depends on both their presence in a population and their frequencies, I divided the observed alleles into four arbitrary classes following Marshall and Brown's (1975) suggestion:

1) common and widespread alleles: alleles with frequency equal to or higher than 0.05 and occuring in more than 25% of the populations;

2) common and localized alleles: alleles with frequrncy equal to or higher than 0.05 and occuring in less than 25% of the populations;

3) rare and widespread alleles: alleles with frequency lower than 0.05 and occuring in more than 25% of the populations; and

4) rare and localized alleles: alleles with frequency lower than 0.05 and occuring in less than 25% of the populations.

Alleles in the first class are the easiest to sample and inevitably will be collected no matter what sampling strategy is employed. Sampling of the class 3 alleles depends only on the total number of trees taken, rather than how those trees are distributed within and among populations. Therefore, sampling of those alleles is also largely independent of sampling strategy. Alleles in class 4 are the most difficult to sample, and the probability of including such alleles in a sample with limited size is very

small (formulas have been developed to calculate the number of trees required to minimize losses of alleles with different frequencies and under different situations by Namkoong *et al.* (1980)). Obviously, it is the second class of alleles that are of greatest importance in terms of sampling strategy. Since they are locally common, the way to increase the probability of including them in a collection of limited size is to sample more populations at the cost of fewer individuals in each population. However, population sampling is more expensive and time-consuming than individual tree collecting. Thus the choice of this sampling strategy basically depends on the quantity of class 2 alleles in the target species. From table 4.2, the proportion of the alleles in each of the four classes are 66%, 17%, 5% and 12% for class 1, 2, 3 and 4, respectively. It is apparent that class 2 alleles make up a significant proportion of the alleles in *Thuja orientalis*. Therefore, the more effecient strategy for *ex situ* collections of *Thuja orientalis* would be to sample fewer trees from a larger number of populations located in various environments. In population sampling, those populations that show great differentiation should be particularly targeted.

5.2 Seed Production

In recognition of the important value of *Thuja orientalis* in afforestation, a large number of planting activities are underway in China. The seeds currently used

in afforestation are those collected from phenotypically superior individual trees or stands. Since those trees or stands were selected on the basis of their untested phenotypes, genetic improvement through such selection would be very limited. Information on the genetic structure and mating system of *Thuja orientalis* obtained in the present study could be very useful in the selection of seed-production trees and stands. Seed produced by those selected trees and stands with high outcrossing rates would be genetically superior in terms of high germination rate, low seedling mortality and high vigour. The populations established from the seeds produced by the stands with high levels of genetic variability would have a broad genetic base. The present study indicated that both the level of genetic variability and outcrossing vary considerably from stand to stand. In order to broaden the genetic base and reduce the impact of inbreeding on the resultant populations, seed should be collected from those stands with high levels of genetic variability and outcrossing. In addition, significant tree-to-tree variation in outcrossing rate was detected in all the studied populations. It would be wise, therefore, to avoid collecting seed from those trees with low outcrossing levels.

A widely used method to obtain genetically improved seed in early tree improvement programs is to establish seed production areas. A seed production area is established by roguing the poor phenotypes from a natural stand and leaving

the good trees to intermate (Zoble and Talbert, 1984). Although the levels of inbreeding would be lowered and the ranking of individual trees by the extent of inbreeding might be changed after roguing due to the destruction of the family structures within the stand, variation in inbreeding level among the "leave" trees may still exist due to their genetic differences in selfing ability. Therefore, it may be worthwhile to follow a two-step roguing procedure; first, remove poor phenotypes and keep the number of phenotypically acceptable trees larger than the optimum number; second, rogue those trees with high inbreeding levels that are estimated after the first roguing, and let the number of trees left in the stand equal the optimum number.

All the seed production methods mentioned above are only for meeting immediate seed needs, since the genetic improvement, if any, by such methods is likely to be minimal. The standard method of achieving genetic gain and producing genetically improved seed in operational quantities is to use the seed orchard approach (Andersson, 1960). In establishing a seed orchard, it is important to select proper parent trees from which clones or seedlings are used as the planting materials for the seed orchard. Significant genetic differentiation and tree-to-tree variation in the level of outcrossing suggest that the parent trees should be selected from a large number of populations located in various environments and should have high levels of outcrossing. Since *Thuja orientalis* seems to

have high selfing ability, minimizing the probability of inbreeding should be of particular concern in seed orchard design. This could be done by increasing the number of clones (or families) in a seed orchard, by maximizing the distance between ramets (or family members) of the same clone (or family), and by using equal numbers of ramets (or seedlings) of each clone (or family).

We should note that since *Thuja orientalis* exhibits a high level of inbreeding, a relatively large proportion of seeds produced by open-pollination is inbred. In nursery production of seedlings, optimal environments for germination and growth are provided, thus genetic inferiority of those inbreds could not be expressed and the inbred seedlings could not be removed. However, when they are outplanted, they will face intense natural selection and, consequently, show severe inbreeding depression. This suggests that caution should be exercised in the use of seeds produced by open-pollination, especially those collected from natural stands and individuals for nursery production of seedlings for afforestation. However, since high quality seeds such as those from intensively managed seed orchards are not available, open-pollinated seeds collected from natural stands or individuals are currently used. In order to ensure the success of afforestation, higher initial planting density or planting 2 or 3 seedlings in each planting hole and thinning at later stage might be recommended.

5.3 Progeny Testing and the Estimation of Genetic Parameters

A progeny test is the best way to evaluate the genetic worth of selected parents. Such tests enable us to tell whether the phenotypic superiority of the selected parents has resulted from growth in a good environment or from good genotypes. However, if open-pollinated progeny are used, the ranking of parent trees for measurable traits based on the performance of their open-pollinated progeny may simply reflect ephemeral differences in outcrossing rate, rather than genotypic superiority. The large, more than six-fold, differences in outcrossing rate among individual trees in *Thuja orientalis* strongly indicate the need of caution when drawing inference of genetic superiority of selected parent trees from the performance of their open-pollinated progeny. Caution is also needed in provenance comparisons due to the significant heterogeneity of outcrossing rate among provenances.

Genetic parameters, such as heritability and genetic gain, are of great importance to tree breeders. Those parameters are functions of additive genetic variance and, therefore, the reliablity of their estimates depends in part on the precision of additive genetic variance estimation. Open-pollinated progeny, being relatively cheap and easy to obtain, are frequently used in estimating additive genetic variance. In practice, it is commonly assumed that open-pollinated progeny are half-siblings from a random mating population (see Sorensen and White (1988) for

references) and the expected coefficient of additive genetic variance is 1/4. However, this assumption does not seem to be valid for the populations of *Thuja orientalis*, since significant amounts of inbreeding have been detected. The presence of inbreeding would bias the estimate of additive genetic variance and, consequently, the estimates of heritability and genetic gain. Such a bias can be corrected when information on the level of inbreeding is available. The expected coefficient of additive genetic variance (K) under a certain level of inbreeding can be calculated by:

$$K=1/2((1+F)/2),$$

where F is the inbreeding coefficient.

When mating is completely random, F=0 and K=1/4. However, when mating is completely selfing (in such a case, the open-pollinated progeny are self full-siblings), F=1 and K=1/2. It is therefore apprent that assuming mating between parents is completely random (F=0) when it actually involves inbreeding (F>0) would bias the estimate of additive genetic variance upwards and the rate of such a bias is F/(1+F)X100%. With the estimated equilibrium inbreeding coefficients presented in Chapter 3, the expected rates of overestimation are 9.5%, 16.0%, 9.5%, and 15.5% for Shandong Changqin, Shanxi Changzhi, Shanxi Houxian, and Beijing Miyun, respectively.

It should be pointed out, however, that whether these potential consequences of inbreeding are actually realized, and to what extent thay are realized in a progeny test and in the estimation of genetic parameters, depend on the survival of the inbreds under experimental conditions at the time of test or estimation. Therefore, adjustments should be made based on the proportion of inbred offspring surviving under such conditions at that time.

5.4 References

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