8-2009

A Phylogenetic Analysis of Species Relationships in Hemlocks, the Genus *Tsuga* (Pinaceae).

Jordan David Baker
East Tennessee State University

Recommended Citation

Baker, Jordan David, "A Phylogenetic Analysis of Species Relationships in Hemlocks, the Genus *Tsuga* (Pinaceae)." (2009). *Electronic Theses and Dissertations.* Paper 1791. https://dc.etsu.edu/etd/1791

A Phylogenetic Analysis of Species Relationships in Hemlocks, the Genus *Tsuga* (Pinaceae)

A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Biological Sciences

---

by

Jordan Baker

August 2009

Timothy McDowell, Ph.D. - Chair

Foster Levy, Ph.D.

Christopher Liu, Ph.D.

Keywords: *Tsuga*, Phylogeny, Morphology, Seedlings, Seed, Leaf Cuticle, Phylogenetic Analysis, Morphological Characters

ABSTRACT

A Phylogenetic Analysis of Species Relationships in Hemlocks, the Genus *Tsuga* (Pinaceae)

by

Jordan Baker

The genus *Tsuga* is comprised of eight extant species found in North America and East Asia and four species represented by fossils from Europe and Japan. This study presents the first phylogenetic analysis based on structural, biochemical, and molecular sequence data. Characters obtained from published and unpublished literature were combined with new morphological characters from seeds, seedlings, and leaf cuticle material. Results from parsimony analyses of these characters differed from the published molecular based phylogeny. The non-molecular based phylogeny resolves two separate clades, a North American and an Asian, but did not group the western North American species, as in the molecular based analysis. Character states were traced on the trees to interpret character evolution. The combined analysis resulted in a phylogeny that differed from the previously published molecular tree by resolving a clade between *T. caroliniana* and *T. diversifolia* and placing *T. dumosa* outside of the Asian clade.

ACKNOWLEDGEMENTS

I would like to thank my committee for helping me develop and complete my project. I would also like to thank Susan Bentz at the U.S. Arboretum and Tom Ward at the Arnold Arboretum of Harvard University for sending me seeds for my analysis.

This project could not have been completed without the funding from the Marcia Davis Research Award, The ETSU Graduate School Research Award, and The Department of Biological Sciences.

Finally, I would like to thank my mom, dad, brother, and friends. I cannot thank them enough for the love and support they have given me.

CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

The genus *Tsuga*, the hemlocks, is in Pinaceae (Pine Family) and comprises eight or nine species of small to large trees distributed in East Asia and in western and eastern North America. Hemlocks are tolerant to shade, prefer moist sites, and are vulnerable to drought (Farjon 1990). They thrive in elevations ranging from 400-3300 meters and often form pure stands near rivers or streams. Eight species are currently accepted by the floras but up to as many as 24 species have been described in addition to some 50 fossil species (LePage 2003). Of the eight species, four species of *Tsuga* occur in North America, and four are found in Asia. Figure 1 and Table 1 show the distribution of the species. Current taxonomic treatments recognize two subgenera: *Tsuga* and *Hesperopeuce* (Farjon 1990). Seven species are placed in subgenus *Tsuga*; *T. sieboldii* Carriere*, T. caroliniana* Engelmann*, T. chinensis* (Franchet) E. Pritzel*, T. diversifolia* (Maxim) Mast*, T. canadensis* (Linnaeus) Carriere, *T. dumosa* (D. Don) Eichler, and *T. heterophylla* (Rafinesque) Sargent. Figure 2 shows mature branches of the eastern North American species. The subgenus *Hesperopeuce* is comprised of only one species*, T. mertensiana* (Bongard) Carriere. Lemmon placed *Tsuga mertensiana* in *Hesperopeuce* (Lemmon 1890) because of morphological differences (leaf shape, seed cone bracts) that suggested a close relationship to the genus *Picea* (the spruces) (Taylor 1972). Descriptions of all hemlock species, their habitats, and distributions are also presented in Farjon's monograph on the Pinaceae (Farjon 1990). *Tsuga forrestii*, a Chinese species recognized in Farjon (1990), was not included in this study because the recent taxonomic treatments have included it as a variety of *T. chinensis* (Flora of China 1999), a determination supported by molecular phylogenies (Havill et al. 2008). Thus, eight species are included in this study. There are two other genera comprising species that have often been included in *Tsuga*: *Cathaya* and *Nothotsuga*. These are now considered separate, monotypic genera (Flora of China 1999). *Cathaya* differs from *Tsuga* in its larger leaves and small erect cones, and *Nothotsuga* differs in its upright cones. Recent taxonomic treatments for all species in the genus are available in the Flora of North America (1993), the Flora of China (1999), and the Flora of Japan (JSPS 2008).

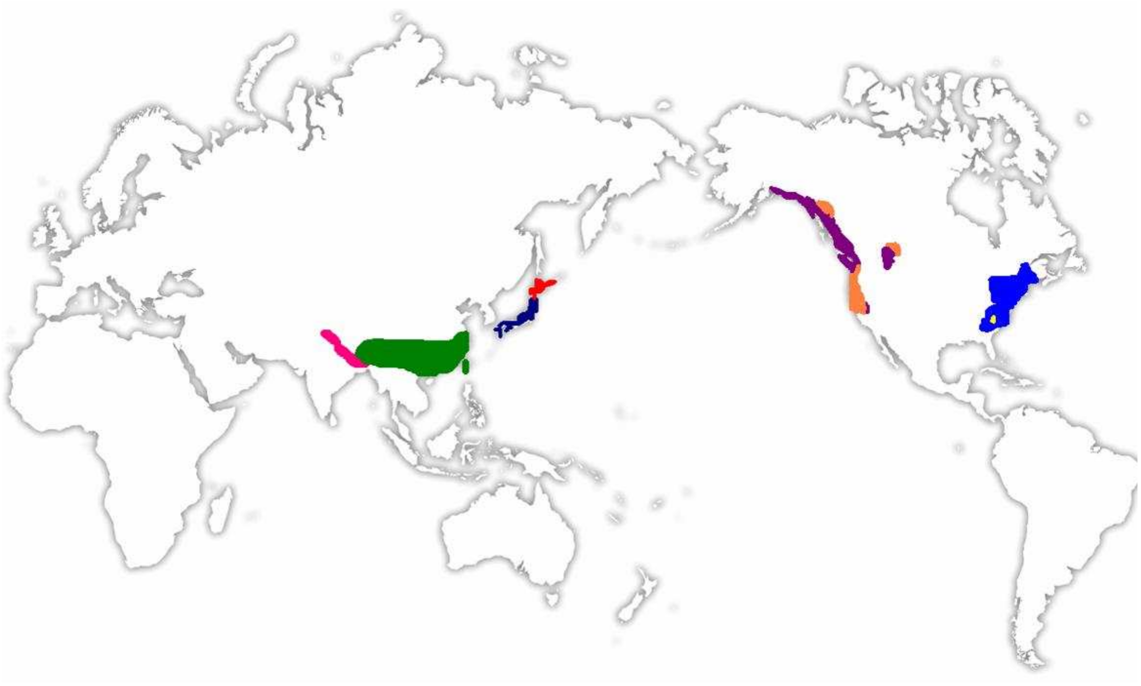


Figure 1 The global distribution of *Tsuga*

Species	Distribution	Color legend for Figure 1
Tsuga canadensis	Eastern North American	Blue
Tsuga caroliniana	Southern Appalachian Mtns.	Yellow
Tsuga chinensis	China, Taiwan	Green
Tsuga diversifolia	Northern Japan	Red
Tsuga dumosa	Himalayan Mtns.	Pink
Tsuga heterophylla	Western North America	Purple
Tsuga mertensiana	Western North America	Orange
Tsuga sieboldii	Southern Japan	Dark Blue

Table 1 Distribution of the extant species of *Tsuga* and color legend for Figure 1

Figure 2 Branches with mature seed cones of two species of *Tsuga* (L: *T. canadensis,* R: *T. caroliniana*)

Fossil Record

The fossil record for *Tsuga* was reviewed at length by LePage, who addressed aspects of the biogeography of extant and fossil species (LePage 2003). *Tsuga* fossils are recorded from Europe and from now Arctic regions of North America and from most Northern Hemisphere regions. Several other fossil species have been described on the basis of particular structures with comparisons to extant *Tsuga* species. These reports include description of the following fossil species and structures listed in Table 2.

Table 2 *Tsuga* fossil structures studied, their location, the age of the fossil, and reference

Species	Structure	Fossil Locality	Age	Literature
Tsuga shimokawaensis	Leaf	Japan	Middle Miocene	Matsumoto and Nishida. 1995
Tsuga canadensis fossil	Leaf, Cone, Seed	West Carpathian Mountains, Europe	Middle Pliocene	Szafer, 1949
Tsuga caroliniana fossil	Leaf, Cone, Seed	West Carpathian Mountains, Europe	Middle Pliocene	Szafer, 1949
Tsuga moenana	Cone, Leaf	Saxony	Lower Miocene	Kunzmann and Mai, 2005

Structural Features

Various authors have detailed aspects of the morphology, anatomy, and biochemistry of *Tsuga* species and for species of related genera. The morphological and anatomical characters will be known as structural characters. These studies describe characters related to tree habit and ecology, wood, cone, leaf morphology and anatomy, pollen characteristics, and leaf terpenoids. A brief review of these studies is given below.

Habit and Ecology

The habit, ecology, and several morphological characters were compiled by Farjon (1990), who covered many genera in Pinaceae. The genus *Tsuga* was broken down into a dichotomous key for easy identification. This monograph is highly referenced in several of the following studies that examine the species *Tsuga* and other members of the Pine family.

Wood

An examination of the wood of gymnosperms was compiled by Greguss (1955), which includes several pictures and descriptions. The data for many characters were shown in table form allowing for easy comparisons between genera. The monograph, however, does not include some *Tsuga* species and outgroups. *Tsuga sieboldii* and *T. caroliniana* were not included.

Cones

A detailed description of morphological features of cones and leaves of all extant and one fossil *Tsuga* species was presented by LePage (2003). Although a thorough examination of fossils was done in this study, the characters from each fossil were not listed, but the distribution of each was. An explanation of the evolution and the biogeography of the genus were described in this study and the conclusion was that the genus evolved out of Asia. LePage also stated that more work needed to be done to better understand the relationships between the species in the genus. There was a recommendation for more morphological and molecular analyses to be done to better understand the relationships.

Leaves

Leaf anatomy was studied by Matsumoto and Nishida (1995). They examined the leaves of all extant species and described several anatomical features along with a few morphological characters. In a cladistic analysis, the eight characters were ordered and they resulted in a parsimonious cladogram that showed that each species grouped with the other species in its geographic region (ENA, WNA, China, and Japan) with no sister-species disjunct across different regions. This does not correspond with the Havill et al. study (2008) that is described below, where some sister-species pairs or subclades include disjunct geographic regions.

Pollen

Pollen in *Tsuga* species was examined by Sivak (1973), who included all the currently recognized species except *T. mertensiana.* Measurements were provided for various aspects of the pollen and a description of the structures on both the distal and proximal surfaces. Sivak concluded that it was difficult to determine the relationships between the species based upon pollen features despite the fact that several characters were examined. Owens et al. (1998) examined pollination in the Pinaceae with descriptions of pollen characters from several *Tsuga* species. Owens concluded that *Tsuga mertensiana* was different from the other species in the genus *Tsuga* with a difference in the type of pollen and the pollination mechanisms.

Biochemical

Various terpenoids in the leaves were examined by Lagalante and Montgomery (2003) in an attempt to determine why the eastern species of *Tsuga* are being killed off by the hemlock woolly adelgid (*Adelges tsugae* Annand; HWA) while the other species were relatively resistant to the adelgid. They listed several terpenoids that differed among seven of the eight extant *Tsuga* species. All extant species except *Tsuga dumosa* were included in their study.

Molecular

A preliminary molecular DNA sequence study using ITS sequences was conducted by Vining but was not published (Vining, Ph.D. thesis 1999). In a recent publication (Havill et al. 2008), the authors presented a multi-gene molecular phylogeny for the genus *Tsuga* based upon chloroplast (matK, trnL-F, rpl16) DNA and nuclear (ITS) DNA regions.

Cuticle

My study includes new characters from seeds, seedlings, and cuticles of *Tsuga* species. A cuticle preparation by Cooke and Liu (unpublished) was examined to collect characters for my study. These characters add to the previous studies based on leaf morphology and anatomy (Matsumoto and Nishida 1995; LePage 2003). The cuticle characters are based on data from both the cuticle and the stomata of each species.

Seeds and Seedlings

The seeds and seedlings of the genus were examined in the other part of my study. Some seed characters have been published in LePage (2003) but more characters can be taken from the seeds. These characters add to the larger group of characters that already exist to allow for an even more comprehensive study of the genus *Tsuga.*

Phylogenetic Relationships

Phylogenetic relationships among the eight species of *Tsuga* have been uncertain. Several phylogenies based upon cladistic analyses of separate sets of morphological, anatomical, and molecular data have been published (Matsumoto and Nishida 1995; Havill et al. 2008). However, no study has used the combined data from all available sources in a cladistic analysis.

A previous study suggested removing *T. mertensiana* from the genus *Tsuga* (Lagalante and Montgomery 2003). *Tsuga mertensiana* differs from the other species because of differences in the leaves that are four sided, cones that are larger than those of the other species, and the bi-saccate pollen. The characters make *T. mertensiana* more basal than the other species in the genus because they are similar to the basal genera in Pinaceae. Another general conclusion places *T. caroliniana* as a sister group to the Asian species (Havill et al. 2008). This conclusion also notes that *T. caroliniana* is not close to the other eastern North American species, *T. canadensis*. *Tsuga caroliniana* differs from *T. canadensis* in having larger cones and seeds and leaves that are spirally arranged around the stem.

Investigations of hybridization between different *Tsuga* species have demonstrated strong reproductive barriers between the two eastern North American species (Bentz et al. 2002). The southern Appalachian endemic Carolina Hemlock (*T. caroliniana*) is often interfertile with most Asian *Tsuga* species, but the widespread eastern hemlock (*T. canadensis*) did not hybridize with either the Asian species or with the sympatric Carolina hemlock (Bentz et al. 2002). A naturally occurring hybrid is found among the western North American species (Swartley 1984). The Asian species also hybridize naturally (Swartley 1984).

The biochemical analysis by Lagalante and Montgomery (2003) shows support for the removal of *T. mertensiana* from the genus. Their analysis has *T. mertensiana* grouping separate and far away from the other species, which Lagalante and Montgomery (2003) say adds to the morphological evidence of segregating *T. mertensiana* from the other species.

The molecular phylogenetic study (Havill et al. 2008) does not support some of the phylogenetic relationships listed above. The results of a phylogenetic analysis from chloroplast DNA (matK, trnL-F, rpl16) and nuclear (ITS) DNA sequences are summarized in Figure 3. The western North American species *T. heterophylla* and *T. mertensiana* are sister species, and together are sister group to the remaining *Tsuga* species. The widespread eastern North American species *T. canadensis* is sister to the clade comprising the four Asian species plus the southern Appalachian *T. caroliniana*. Within this clade, the ITS and cpDNA topologies differ in the placement of the Chinese species *T. dumosa*, but both consensus trees show *T. caroliniana* and one Asian species as sister clades to a group consisting of the other Asian species, *T. diversifolia* and *T. sieboldii*.

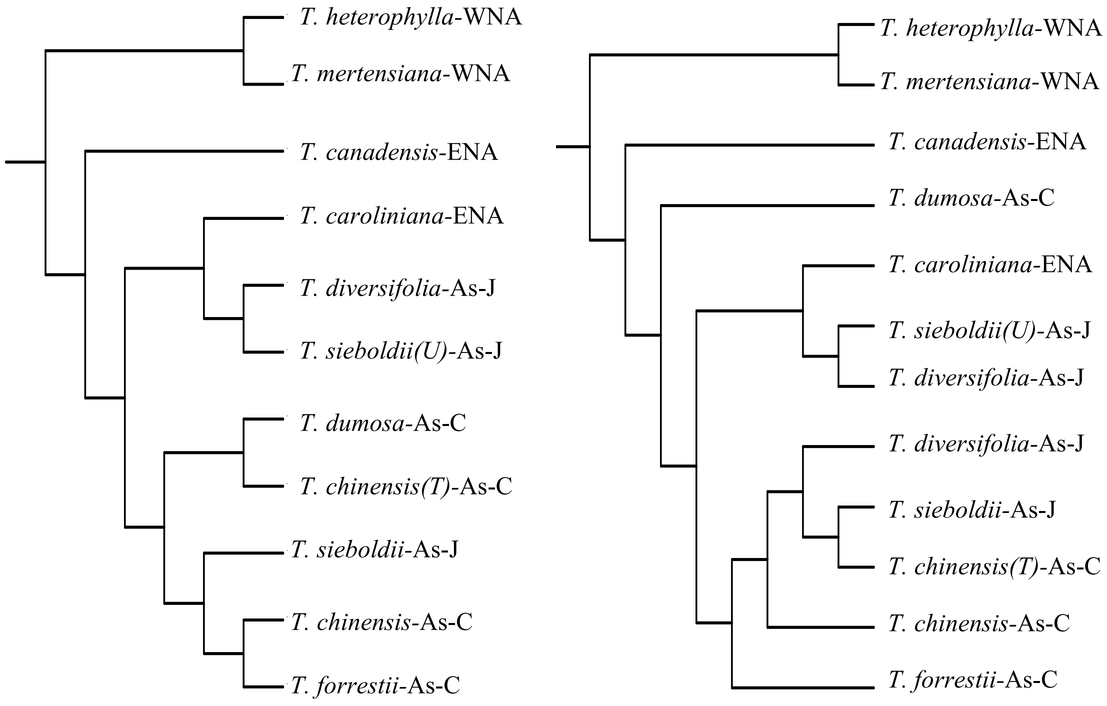


Figure 3 Bayesian 50% majority rule consensus trees of cpDNA and ITS (Havill et al. 2008). WNA-Western North America, ENA-Eastern North America, As-J-Asia (Japan), As-C-Asia (China, Himalayas, Korea)

An important conclusion was that the species from Asia or America do not group together in the phylogenies. Havill et al.(2008) notes that the ITS sequence had limited utility in resolving phylogenetic relationships among closely related taxa, but shows differences in the deeper roots of the tree (Havill et al. 2008).

Biogeography

This genus, like many other plant groups, presents a disjunct of east Asian-American distributions in which distantly separated species appear to be most closely related to one another. Many such "species pairs" have been studied using molecular phylogenetic analyses (Liston 1999; Wang 2000; Xiang et al. 2000; Campbell 2005). *Tsuga* provides a good example of an East Asia - Western North America - Eastern North America distribution. An analysis for divergence times and biogeography of *Tsuga* was examined by Havill et al. (2008) using the cpDNA phylogeny (Fig. 3). Havill et al. (2008) concluded that the genus was found all over the Northern Hemisphere and went extinct in locations that it can no longer be found today.

Outgroups

The determination of genera closely related to *Tsuga* is needed for the selection of appropriate outgroups for a phylogenetic analysis of *Tsuga*. These outgroups include *Keteleeria, Pseudolarix, Abies,* and *Nothotsuga* (Wang et al. 2000; Havill et al. 2008). These outgroups have similar characteristics to *Tsuga*. These outgroups were chosen from previous studies that examined the genus *Tsuga*. A DNA study (Havill et al. 2008) chose these four outgroups to be included in the analysis because they were the closest species from another study examining Pinaceae (Wang et al. 2000). *Abies* and *Nothotsuga* have flat needles with a rounded apex. *Abies* and *Keteleeria* are different in that they do not have cones that hang down from the branch, but they grow upwards and shatter when the seeds disperse. *Pseudolarix* differs from *Tsuga* and the other outgroups on a morphological level, as it is deciduous, and its needles radiate from short side shoots. *Pseudolarix* cones are small like those of *Tsuga* but have a pointed bract. Because several of these outgroups are rare species, some new characters were not obtained because there was a lack of material available.

Hemlock Woolly Adelgid

Tsuga is of special interest because of the immediate threat imposed by the HWA. This infestation in eastern North America is killing off many trees of both *T. canadensis* (eastern hemlock) and *T. caroliniana* (Carolina hemlock). This aphid-like pest was introduced to the eastern US in about 1950 from an infested Japanese species (Havill et al. 2006), and in the past 10 years, the HWA has spread into the southern Appalachian Mountains. This insect can infest and kill an entire tree within two to eight years. Currently, the HWA colonizes seven of the eight species of hemlocks but is only known to kill the two eastern North American species.

Considerable research is being done on these insects to find a way to control them. It is thought that the insects have only one genotype for the entire population in eastern North America (Havill et al. 2006). The insects survive on the trees sucking the sap from the petiole of the leaves causing the leaves to drop, which eventually causes the branch and then the tree to die. The treatments for HWA infestation are insecticides, soaps, sprays, and biological controls. These biological controls include various Asian beetles that eat the HWA, but the extent to which these beetles control the HWA populations in the wild is not yet established (Butin et al. 2002). The molecular study (Havill et al. 2008) of *Tsuga* shows that the Asian species group with the eastern North American species of *T. caroliniana.* This does not support the susceptibility of the species because the Asian species do not die from the HWA like the Carolina hemlock does.

Objectives

The purpose of this study was to investigate the phylogenetic relationships among the genus *Tsuga* using morphological, anatomical, biochemical, and molecular data. The use of new information on seeds, seedlings, and cuticle; and the use of published and unpublished literature allowed for both separate and combined phylogenetic analyses of the genus. The completed phylogenetic studies addressed the following questions:

- Do the results of phylogenetic analyses based upon morphological and biochemical characters coincide or conflict with the published molecular sequence phylogenies?
- What patterns of character evolution are revealed by tracking various traits onto the molecular, morphological, and combined data phylogenies?
- Has the vulnerability to the HWA evolved independently in the two eastern North American species, as indicated by the DNA phylogeny and suggested by some morphological characters?
- Can the phylogenetic relationships for some of the many fossil species be resolved based upon incomplete morphological data sets?

CHAPTER 2

METHODS

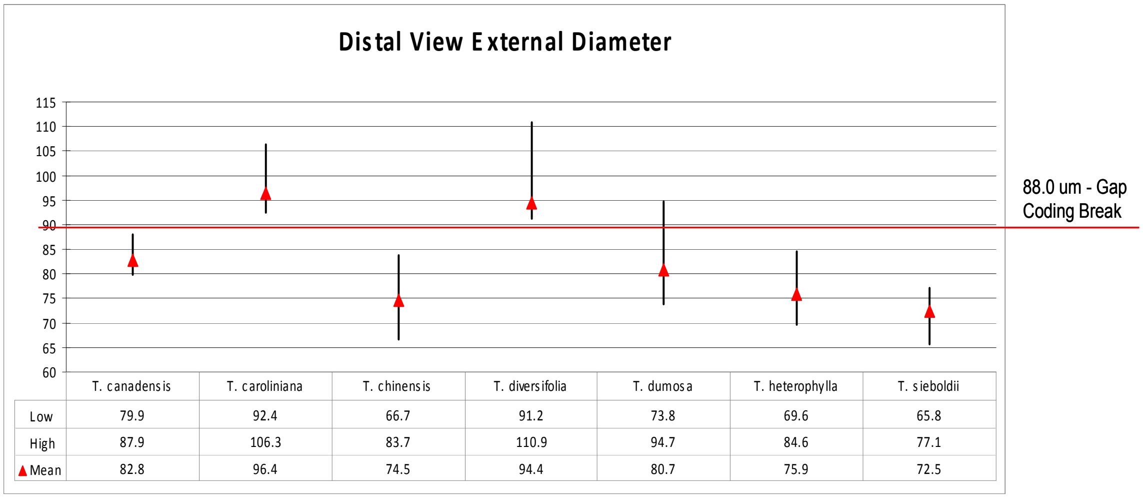
Taxonomic Sampling

The eight extant species of *Tsuga* were included in this study. Four fossil species of *Tsuga* were also included when sufficient characters for fossil and modern species were available. Seed material was acquired from seed vendors, arboreta, or local stands of hemlocks. Due to the lack of some material, a few species were omitted from the character analyses. The DNA sequences were taken from GenBank (Benson et al. 2005) and from data from Havill et al. (2008).

Four genera were chosen as outgroups (*Keteleeria, Pseudolarix, Nothotsuga, and Abies*) from previous phylogenetic studies (Wang et al. 2000; Havill et al. 2008). Their characters were included when available and were used to root the trees in the phylogenetic analyses.

Character Matrix

Characters were examined in all available species to make a data set comprising discrete characters for use in phylogenetic analyses. The structural, biochemical, and molecular characters examined included ecology, habit, wood, cone, seed, leaf, pollen, biochemical, and DNA characters. These characters were obtained from previous published and unpublished studies and monographs. These characters had to be re-evaluated to make sure that they were discrete characters and could be used in the analyses. The characters were examined to maintain low overlapping of numbers. When quantitative measurements were given they were broken down into character states so the range of measurements did not overlap. If the numbers overlapped a character state they were listed as polymorphic. Where a break was shown between values or morphological states, it was determined that the break would be the start of another character state. If data were not available for a particular species, it was noted as a question mark (?). The quantitative characters were broken down into discrete characters using the gap coding method (Schols et al. 2004). This method is based on distributions of mean values for a certain character, what a gap between means represents a change in the character state. An example of gap coding is shown below for a pollen character. Figure 4 shows where a gap is seen between means, but a range overlaps a character state is separated and *T. dumosa* is listed as polymorphic because its range overlaps the gap. The entire character matrix is included in Appendix C.



	T. canadensis	T. caroliniana	T. chinensis	T. diversifolia	T. dumosa	T. heterophylla	T. sieboldii
Low	79.9	92.4	66.7	91.2	73.8	69.6	65.8
High	87.9	106.3	83.7	110.9	94.7	84.6	77.1
▲Mean	82.8	96.4	74.5	94.4	80.7	75.9	72.5

Figure 4 Graph showing how quantitative characters were gap coded for discrete character states

Habit and Ecology

The genus *Tsuga* is made up of several species of trees that differ in size and shape. These trees also are found throughout the Northern Hemisphere and grow at several different altitudes from 500 meters up to high mountain peaks of 4000 meters. These characters were selected from Farjon (1990), and were confirmed by the Flora of North America (1993), the Flora of China (1999), and the Flora of Japan (JSPS 2008). Some species are considered polymorphic for these character sets and are shown in Table 3.

1. MAX TREE HEIGHT: (0) 20-30m (1) 40-50m (2) 60-70m
2. TREE D.B.H.: (0) .5-1m (1) 1.5-4.5m
3. TRUNK FORK AT BASE: (0) non-forked (1) forked
4. BRANCHES OF FIRST ORDER: (0) massive/horizontal (1) slender/ascending
5. CROWN: (0) drooping leader (1) flat (2) domed (3) pyramidal (4) conical
6. VEGETATIVE BUD SHAPE: (0) ovoid (1) conical (2) globular
7. ELEVATION OF RANGE OCCURENCE: (0) 500-1500m (1) 2000-4000m

	Max Tree height	Tree d.b.h	Trunk fork	Branches of first order	Crown	Vegetative bud shape	Elevation of range occurrence
Abies	0	0	0	0	3	0&2	0&1
Keteleeria	0&1	0&1	0	1	2	0&2	0&1
Nothotsuga	0	0	1	1	4	0	0&1
Pseudolarix	0&1	1	0	1	0	0	0
T. canadensis	0&1	1	0	1	0	1	0
T. caroliniana	0	0	0	1	0	0&1	0
T. chinensis	1	1	1	0	1	0&2	0&1
T. diversifolia	0	0	1	0	2	0&2	0&1
T. dumosa	1	1	1	1	1	0&2	1
T. heterophylla	2	1	0	1	0	0&1	0
T. mertensiana	0&1	0&1	0	0	0	0&1	0&1
T. sieboldii	0	1	0	0	0	0&1	0

Table 3 Habit and Ecology Character Matrix

Wood

Wood sections (radial, tangential, and transverse) were reported from a monograph on gymnosperms (Greggus 1955). The species have a wide range of tracheids and rays per square millimeter. Some species are considered polymorphic for these character sets and are shown in Table 4. Characters from *T. sieboldii*, and *T. caroliniana* were not included in the matrix because they were not published in the monograph.

8. APPROXIMATE NUMBER OF TRACHEIDS PER SQ. MM.: (0) 1500 – 2000 (1) 2000 – 3000 (2) 3000 – 4000 (3) > 5000

9. RAYS PER SQ. MM.: (0) 30 -45 (1) 50 – 60 (2) 65 – 80 (3) 85- 95 (4) >100

10. RAYS APPROXIMATE NUMBER OF CELLS PER SQ. MM.: (0) 200 – 300 (1) 300 – 400 (2) 400 – 500 (3) 500 – 600

11. RAYS CELLS WIDTH : (0) 4 – 8 um (1) 9 – 18 um

12. NUMBER OF PITS PER SQ. MICROMETER.: (0) 1-2 (1) 3-4

	Approx. number of tracheids per sq. mm	Rays per sq mm	Rays approx number of’ cells per sq mm	Ray cells width	Number of pits
Abies	3	4	2	0	0
Keteleeria	1	2	0	1	0
Nothotsuga	?	?	?	?	?
Pseudolarix	1	2	2	1	0&1
T. canadensis	1	0	0	0&1	0&1
T. caroliniana	?	?	?	?	?
T. chinensis	0	1	1	0&1	0&1
T. diversifolia	1	2	2	0	1
T. dumosa	1	2	0	0&1	0&1
T. heterophylla	0	0	1	0	0
T. mertensiana	2	3	3	1	0
T. sieboldii	?	?	?	?	?

Table 4 Wood Character Matrix

Cones

Cone characters were selected from a study by LePage (2003). The cones vary in size and shape. Some species are considered polymorphic for these character sets and are shown in Table 5. Figure 5 shows an example of the differences between cone scales found in *Tsuga.*

13. CONE SCALE SHAPE: (0) ovate (1) orbicular

14. CONE SCALE APEX: (0) rounded (1) entire/erose (2) obtuse

15. BRACT SHAPE: (0) denticulate (1) trilobate (2) truncate (3) ovate (4) lingulate (5) subspatulate (6) obcordate

Figure 5 Cone scales from *T. canadensis* (Left) and *T. caroliniana* (Right)

	Cone scale shape	Cone scale apex	Bract shape
Abies	0	1	6
Keteleeria	?	2	1
Nothotsuga	1	2	5
Pseudolarix	0	?	4
T. canadensis	0&1	0	0&2
T. caroliniana	0	0&1	1
T. chinensis	0	0	0
T. diversifolia	0&1	0&1	2
T. dumosa	0	0&1	3
T. heterophylla	0&1	0	1
T. mertensiana	0	0	4
T. sieboldii	1	0&1	0

Table 5 Cone Character Matrix

Seeds

Some seed characters were selected from LePage (2003). In that study, it was noted that more characters could be obtained from a more thorough examination of the seeds. In my study, I selected five characters to add to the three that LePage reported. Seeds were obtained from seed suppliers and arboreta. Due to the lack of seed material at the time of analysis, the number of seeds examined was low (n=5). The seeds were examined under the dissecting scope and light microscope at varying magnifications. Each seed was photographed on both sides and measurements were taken using the computer program ImageJ (Abramoff et al. 2004). Examples of methods for measurements are shown in Figures 6, 7, 8, 9, and 10. After measurements were taken, the seeds were used to grow seedlings. These character sets also include some species that are polymorphic and they have been noted in the matrix (Table 6).

16. SEED SHAPE: (0) cuneate (1) ovoid (2) oblong (3) triangular
17. SEED WING SHAPE: (0) ovate (1) oblong (2) cuneate (3) semitrullate
18. SEED WING LENGTH: (0) 4-8.5 mm (1) 9-14 mm (2) >15 mm

Figure 6 Whole seeds from *T. caroliniana* (left) and *T. diversifolia* (right)

19. SEED BODY LENGTH: (0) 3-3.9 mm (1) 4-5 mm (Measurements were taken from the seed apex to the farthest point opposite on a straight line, perpendicular to the seed body width measurement)

20. SEED BODY WIDTH: (0) 1-1.9 mm (1) 2-3 mm (Measurements were taken from the apex that attaches to the seed wing down to the farthest point at the base of the seed on a straight line, perpendicular to the seed body length measurement)

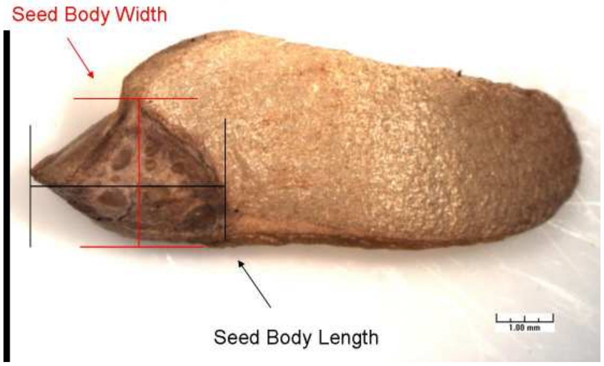


Figure 7 Measurement method for seed body length and width

21. SEED AREA: (0) 3-4 mm^2 (1) 5-6.9 mm^2 (2) 7.5-9 mm^2 (Measurements were taken using ImageJ, where the seed body was highlighted and an area measurement was taken)

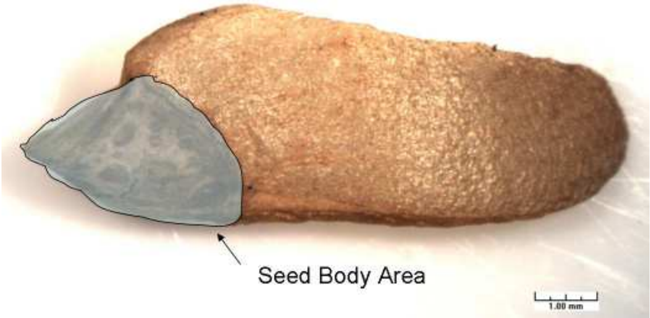


Figure 8 Measurement method for seed body area

22. RESIN SACS ON THE BRACT-SIDE OF SEED: (0) 2-7 (1) 9-12 (2) >23

Figure 9 Resin sacs (27) found on *Tsuga* seeds

23. SEED WING AREA: (0) 8-12mm^2 (1) 15-19mm^2 (2) >20mm^2 (Measurements were taken using Image J. An outline of the seed wing was drawn onto the photo and the area of the wing was measured.)

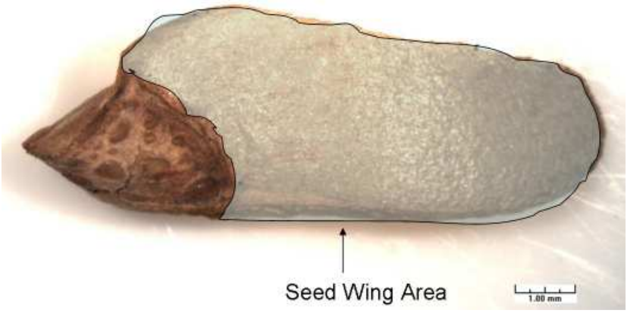


Figure 10 Measurement method for seed wing area

Seedlings

Seedlings were grown to examine differences between the species. Seeds were placed into room temperature water for 24 hours to soak. Then they were placed in a 50/50 mixture of vermiculite and potting soil moistened with a little water that was placed in a plastic sandwich bag for a period of 30 to 90 days for cold stratification. The bags were then placed in the refrigerator at 3 degrees Celsius for the determined period of time. After cold stratification, the seeds were removed from the refrigerator and plastic bags and placed into 4 inch pots in an incubator at temperatures alternated between 24^{o}C in light for 6 hours and 18^{o}C in dark for 18 hours. Seeds were watered when dry. Two measurements were taken from the seedlings. Two seedlings are shown in Figure 11. Some species are considered polymorphic for these character sets.

24. COTYLEDON NUMBER: (0) 3-4 (1) 5 – 6 (Several species of *Tsuga* showed a variation of the number of cotyledon changing only in one cotyledon from a base number.)
25. COTYLEDON LENGTH: (0) 5-7 mm (1) 8-11 mm (Cotyledon length was measured using calipers. The measurement was taken when the seedling was developed just before growing a new set of leaves.)

Figure 11 *T. mertensiana* seedling showing 5 cotyledons and *T. heterophylla* seedling showing 3 cotyledons

	Seed Shape	Seed wing shape	Seed wing length	Seed Body Length	Seed Body Width	Seed Body Area	Resin sacs on bract-side of seed	Seed wing area	Cotyledon Number	Cotyledon Length
Abies	0	2	0	?	?	?	?	?	?	?
Keteleeria	2	3	2	?	?	?	?	?	?	?
Nothotsuga	1	0&1	1	?	?	?	?	?	?	?
Pseudolarix	0&1	3	0&1	?	?	?	?	?	?	?
T. canadensis	1&2	0	0&1	0	1	1	0	1	0&1	0&1
T. caroliniana	1	1	0&1	1	1	2	2	1	?	?
T. chinensis	1&2	0	0	1	1	1	1	2	0	0
T. diversifolia	1	0	0&1	0	1	2	2	0	?	?
T. dumosa	1&2	0	0&1	0	0	1	0	0	0	0
T. heterophylla	1&2	0	0&1	0	0	0	0	1	0	0
T. mertensiana	0	1	0&1	1	1	2	1	2	1	1
T. sieboldii	1	0&1	0	0	1	1	1	0	?	?

Table 6 Seed and Seedling Character Matrix

Leaves

The characters from leaves were selected from studies of both the morphology and the anatomy of the leaves (Matsumoto and Nishida 1995; LePage 2003). Some species are considered polymorphic for these character sets and are shown in Tables 7 and 8. The character states determinations were confirmed by reference to the Flora of North America (1993), the Flora of China (1999), and the Flora of Japan (JSPS 2008).

26. LEAF APEX: (0) rounded (1) truncate/acute (2) mucrotate/obtuse

27. LEAF MARGIN: (0) entire (1) denticulate

28. LEAF ARRANGEMENT: (0) spiral around stem (1) planer (2) whorls

29. LEAF SHAPE: (0) 4 sided (1) spatulate (2) falcate-acuminate-obtuse

30. SIZE OF RESIN CANALS: (0) 40-70um (1) 75-100um (2) 110-140um

31. SIZE OF MESOPHYLL: (0) small (1) large

32. OCCURENCE OF HYPODERMIS: (0) all part except stomatal (1) partially present (2) absent

33. MASS OF HYPODERMIS CELLS AT LEAF MARGIN: (0) present (1) absent

	Leaf Apex	Leaf Margin	Leaf Arrangement	Leaf shape	Size of resin canals	Size of mesophyll	Occurrence of hypodermis	Mass of hypodermal cells at leaf margin
Abies	1	0	0	1	?	?	?	?
Keteleeria	2	0	0	2	?	?	?	?
Nothotsuga	1	0	0	2	?	?	?	?
Pseudolarix	1&2	0	2	2	?	?	?	?
T. canadensis	0	1	1	2	0&1&2	0	2	1
T. caroliniana	0	0	0	2	1&2	0	2	1
T. chinensis	1	0	1	2	0&1	1	1	0
T. diversifolia	1	0	0	2	1&2	0	1	0
T. dumosa	0&1	1	0	2	0	1	1	0
T. heterophylla	0&1	1	0	1	1	0	0	1
T. mertensiana	0&1	0	0	0	0&1	0	0	0
T. sieboldii	0&1	0	0	2	1&2	0	1	0

Table 7 Leaf Morphology and Anatomy Character Matrix

	Leaf Apex	Leaf Margin	Leaf shape	Occurrence of hypodermis	Stomatal Rows
T. shimokawaensis	?	0	1	0	0
Fossil *T. canadensis*	0	1	2	1	0
Fossil *T. caroliniana*	0	0	2	2	0
T. moenana	1	0	2	?	0&1

Table 8 Fossil Species Leaf Morphology and Anatomy Character Matrix

Leaf Cuticle

Cuticle characters have been obtained by examination of leaf specimens from material prepared by Cooke and Liu (unpublished). Additional observations were made for stomata row number, stomata dispersal, and stomata index (n=3). The character matrix is shown in Table 9. Figure 12 shows two examples of the leaf cuticle. For the preparation of the leaf cuticle, a small transverse section of the leaf was taken that included both sides of the leaf margins. This material was placed in a 10% aqueous chromium trioxide solution for 96 hours, then rinsed several times in distilled water, then stained with aqueous safranin O. It was then placed on a slide with a drop of glycerin and observed under light microscope. The stomata index was calculated using the equation, SI= (S/S+E)*100 where S is the number of stomata, and E is the number of epidermal cells in the field of view.

34. PERICLINAL WALLS: (0) Granular (1) Smooth (2) Acuminate spikes
35. STOMATA SHAPE: (0) Elliptical (1) Rectangular
36. STOMATA POLAR EXTENSION: (0) Developed (1) Undeveloped
37. CUTICULAR FLANGE BETWEEN GUARD CELLS: (0) Developed (1) Undeveloped
38. EXTRACUTICULAR WAX: (0) Absent (1) Present
39. FLORIN RING FURROW: (0) Undeveloped (1) Developed. A florin ring is a distinct raised ring around the stomata opening formed by raised subsidiary cells that are differentiated from other epidermis cells.

40. FLORIN RING GROOVE: (0) Undeveloped (1) Developed

41. STOMATA ROWS: (0) 4-7 (1) 8-11 (Stomatal rows were counted from one band of stomata on the underside of the leaf. *Tsuga* has two bands of stomata separated by a mid-rib.)

42. STOMATA DISPERSAL: (0) Stomata in a continuous chain (1) Stomata randomly spaced in each row (Stomata were counted as continuous if they had one or two cells separating each one. When there was a large gap show in each row in was determined to be randomly spaced.)

43. STOMATA INDEX: (0)14-16 (1)17-20

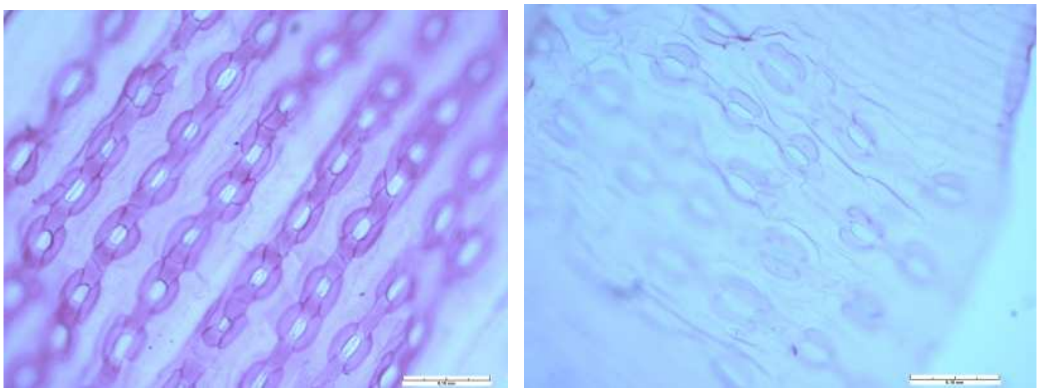


Figure 12 Cuticle structures of *T. chinensis* (8 stomata rows) and *T. mertensiana* (6 stomata rows) under light microscopy (20x)

	Periclinal walls	Stomata Shape	Stomata Polar Extension	Cuticular Flange between Guard Cells	Extracuticular Wax	Florin Ring Furrow	Florin Ring Groove	Stomata Rows	Stomata Dispersal	Stomata Index
Abies	?	?	?	?	?	?	?	0	?	?
Keteleeria	?	?	?	?	?	?	?	1	?	?
Nothotsuga	?	?	?	?	?	?	?	0&1	?	?
Pseudolarix	?	?	?	?	?	?	?	0	?	?
T. canadensis	0	0	1	0	1	0	1	1	0	1
T. caroliniana	1	0&1	1	0	1	1	1	0	0	1
T. chinensis	0	0&1	0	0	1	1	0	0&1	0	0
T. diversifolia	0	1	1	1	0	0	0	1	0	0
T. dumosa	0	0	0	0	1	1	0	1	0	1
T. heterophylla	0	?	1	0	1	?	?	0	1	0
T. mertensiana	2	0	0	0	1	1	1	0	0	1
T. sieboldii	0&1	1	0	1	0	0	0	1	0	0

Table 9 Leaf Cuticle Character Matrix

Pollen

These characters were taken from two studies done by Sivak (1975) and Owens (1998). The pollen characters include various measurements from pollen grains and pollination structures. Several drawings and photographs made by Sivak show the differences in the pollen between the species (Figure 13). This study, like several other studies, reports many characters but only a few characters have variations that can be broken down into discrete characters. Some species are considered polymorphic for these character sets and are shown in Table 10. *T. mertensiana* was not included in the Sivak (1975) study.

44. DISTAL VIEW EXTERNAL DIAMETER (Fig. 12 - Dd1): (0) 65-88 um (1) 87.7-111um
45. EKTEXINE WIDTH (Fig.12 - Ld): (0) 3.8-9.8um (1) >9.9um
46. PROXIMAL VIEW DIAMETER OF POLLEN CAP (Fig. 12 - Dp1): (0) 65-86.5um (1) >86.6
47. PROXIMAL VIEW INTERNAL DIAMETER (Fig. 12 - Dp2): (0) 60-70um (1) >78um
48. PROFILE VIEW HEIGHT OF POLLEN GRAIN (Fig. 12 - h): (0) 38-53.4um (1) >54um
49. PROFILE VIEW CENTER OF THE OPERCULUM LENGTH (Fig. 12 - E2): (0) 1.4-2.8um (1) >2.8um
50. PROFILE VIEW EDGE OF THE OPERCULUM LENGTH (Fig. 12 - E3): (0) .5-1.4um (1) >1.5um
51. PROXIMAL VIEW SURFACE STRUCTURES: (0) Pa (1) Pb (2) Pd (3) Pe
52. DISTAL VIEW SURFACE STRUCTURES: (0) Da (1) Db (2) Dc (3) Dd

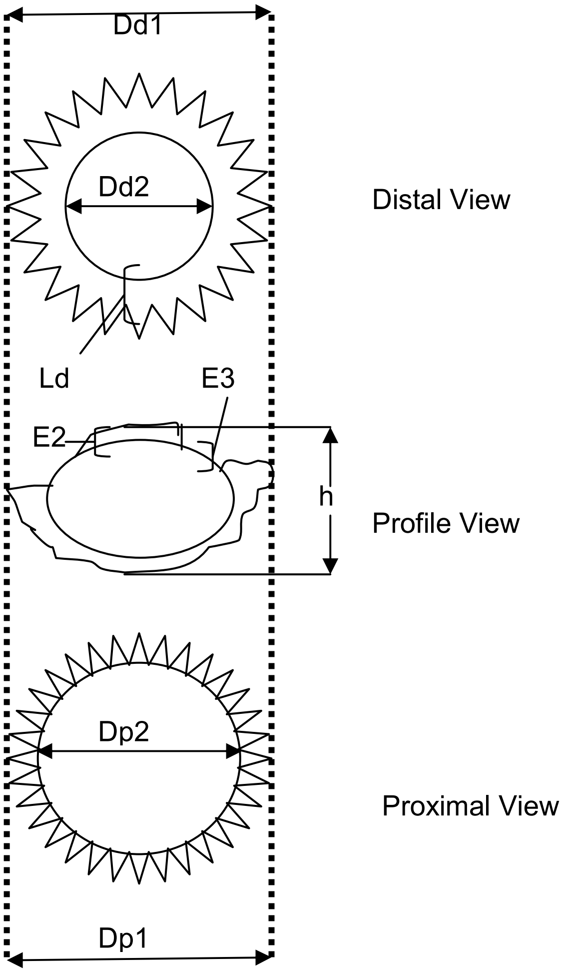


Figure 13 Measurement methods used by Sivak (1975) for *Tsuga* pollen grains

53. INTEGUMENT SHAPE: (0) funnel shaped (1) two-flaps

54. SACCATE POLLEN: (0) non-saccate (1) saccate

	DV - External Diameter	Bourrele Width	PV - Diameter of Cap	PV-Internal Diameter	PV- Height of grain	PfV - Center of the operculum	PfV - edge of the operculum	Proximal Structures	Distal Structures	Integument shape	Saccate Pollen
Abies	?	?	?	?	?	?	?	?	?	?	1
Keteleeria	?	?	?	?	?	?	?	?	?	?	1
Nothotsuga	?	?	?	?	?	?	?	?	?	?	0
Pseudolarix	?	?	?	?	?	?	?	?	?	?	1
T. canadensis	0	0	0	0&1	0&1	0	0	0	1	1	0
T. caroliniana	1	0	1	1	0&1	1	0&1	0	0	1	0
T. chinensis	0	1	0	0	0&1	0	0	2	3	1	0
T. diversifolia	1	1	0&1	0&1	0&1	0	0	1	3	1	0
T. dumosa	0&1	0&1	0	0&1	0&1	0	1	3	2	1	0
T. heterophylla	0	1	0	0	0	0	0	0	3	1	0
T. mertensiana	?	?	?	?	?	?	?	?	?	0	1
T. sieboldii	0	0	0	0	0&1	0&1	0&1	3	3	1	0

Table 10 Pollen Character Matrix

Biochemical

Terpenoid compositions were studied in the leaves of *Tsuga* species by Lagalante and Montgomery (2003). These terpenoids were selected to investigate possible effects on HWA susceptibility. The terpenoid compounds were identified using gas chromatography/ion-trap mass spectrometry. This allowed for the individual terpenoid amounts to be expressed as a percentage of all of the identified peaks in the chromatogram (Lagalante and Montgomery 2003) The character matrix is shown in Table 11. *T. dumosa* was not examined in this study.

55. TRICYCLENE: (0) 0-2.1 (1) 3.2-4.7

56. α-PINENE: (0) 9-21 (1) 26-28

57. CAMPHENE: (0) .3-.53 (1) 1.8-2.3 (2) >4.5

58. β-PINENE: (0) 1.2-2.6 (1) 3.8-5.1 (2) >6.5

59. MYRCENE: (0) .5-4 (1) >7

60. α-PHELLANDRENE: (0) .4-2.5 (1) 4-4.6 (2) >7
61. β-PHELLANDRENE: (0) 1.5-4.2 (1) 6.5-8 (2) >8
62. ISOBORNYL ACETATE: (0) 2.8-3.7 (1) 7.6-11.4 (2) 17.5-25 (3) >27
63. β-CARYOPHYLLENE: (0) .7-2 (1) 5.5-7.1 (2) >12.9
64. α-HUMULENE: (0) .5-.7 (1) 2.8-4.4 (2) 5.7-6.4 (3) >10.4
65. GERMACRENE D: (0) .1-1 (1) 3.9-4.8 (2) 10.3-10.7 (3) >20
66. γ-CADINENE: (0) 1.1-3 (1) 4.5-6.2
67. δ-CADINENE: (0) 2.1-4 (1) 6.9-10.7

	Tricyclene	a-pinene	camphene	B-pinene	Myrcene	a-phellandrene	B-phellandrene	isobornyl acetate	B-caryophyllene	a-humulene	germacrene D	y-cadinene	d-cadinene
Abies	?	?	?	?	?	?	?	?	?	?	?	?	?
Keteleeria	?	?	?	?	?	?	?	?	?	?	?	?	?
Nothotsuga	?	?	?	?	?	?	?	?	?	?	?	?	?
Pseudolarix	?	?	?	?	?	?	?	?	?	?	?	?	?
T. canadensis	1	0	2	0	0	0	0	3	0	1	0	0	0
T. caroliniana	0	0	2	0	1	1	0	3	0	1	1	0	0
T. chinensis	0	0	1	0	0	0	0	1	2	3	1	1	1
T. diversifolia	0	0	2	0	0	0	1	2	1	3	0	1	1
T. dumosa	?	?	?	?	?	?	?	?	?	?	?	?	?
T. heterophylla	1	0	2	0	0	0	0	3	1	3	0	0	0
T. mertensiana	0	1	0	2	0	2	2	0	0	0	3	0	0
T. sieboldii	0	0	2	1	0	0	0	2	1	2	2	0	1

Table 11 Biochemical Character Matrix

Molecular

An analysis of the genus *Tsuga* using DNA sequences from the gene regions ITS, matK, trnT-F, and rbl16 were reported by Havill et al. (2008). The DNA sequences were taken from GenBank (Benson et al. 2005) and TreeBASE (TreeBASE 2009). In the molecular phylogenetic investigations of Havill et al. (2008) many individuals per species were sampled. My analyses are based upon a single character set for each *Tsuga* species, so DNA sequences for each species were made from just one individual of those sampled by Havill et al. (2008). This representative sequence was selected by choosing the individual with the shortest branch from the basal node of the cluster of sampled members from each species. These representative sequences are identified in Table 12. A fourth chloroplast sequence, rbcL, was added to the molecular analysis. Accession numbers for the rbcL sequences of sampled species are listed in Table 13. These sequences were aligned in BioEdit and then analyzed with PAUP*.

68 - 1312. matK CODING REGION (ca. 1245 bp) (Variable Sites – 113) (Parsimony Informative Sites – 61)

1313 - 3074. ITS REGION (ITS1-5.8S-ITS2) (ca. 1762 bp) (Variable Sites; ITS1-561, 5.8S-27, ITS2-64) (Parsimony Informative Sites; ITS1-428, 5.8S-9, ITS2-38)

3075 - 4585. trnT-F REGION (ca. 1511 bp) (Variable Sites – 175) (Parsimony Informative Sites – 90)

4586 - 5263. rbl16 INTRON (ca. 678 bp) (Variable Sites – 68) (Parsimony Informative Sites – 38)

5264 - 6572. rbcL SEQUENCE (ca. 1309 bp) (Variable Sites – 87) (Parsimony Informative Sites - 53)

	ITS	**cpDNA**
T. canadensis	03 07 2	03 07
T. caroliniana	19447 9	15803
T. chinensis	02 09	04 55
T. diversifolia	04 46 13	18 37 77
T. dumosa	19924259 3a	02 50
T. heterophylla	22 94 14	03 03
T. mertensiana	03 03a 20	03 03a
T. sieboldii	04 347	04 33

Table 12 Species used from Havill et al. 2008 with GenBank and TreeBASE sequence accession number

	rbcL
T. canadensis	AY056581
T. caroliniana	-
T. chinensis	AF145462
T. diversifolia	-
T. dumosa	AF145460
T. heterophylla	X63659
T. mertensiana	AF145463
T. sieboldii	-

Table 13 Accession numbers for rbcL sequences used from GenBank.

Phylogenetic Analysis

The characters based upon the literature and new observations were placed into a character matrix. The data sets were analyzed individually for each data set and then combined to give a comprehensive analysis of the genus. The different analyses include: tree habit and ecology, wood, cone, seed, leaves, pollen morphology, biochemical and molecular. Combined analysis include: structural/biochemical, vegetative, reproductive, and a combined data analysis.

The program MacClade (Maddison and Maddison 2005) was used to construct the data matrix and to interpret and display the results of the phylogenetic analyses. The structural/biochemical and molecular data sets were analyzed using the Maximum Parsimony (MP) default settings in PAUP* (Swofford 2003). The heuristic search option for step-wise additions was changed to 100 random taxon additions to the sequence replicates. The MP analyses were conducted using unordered and equally weighted characters. Uninformative characters were excluded from all analyses. Bootstrap resampling (Felsenstein 1985) with 1000 replicates and decay analyses (Bremer 1994; Eriksson 1998) were used to estimate clade robustness.

Congruence between molecular and morphological data sets was tested with the incongruence length test (ILD; Farris el al. 1994) and Templeton test (Templeton 1993; Larson 1994) in PAUP*.

Character evolution was examined by using MacClade (Maddison and Maddison 2005) to trace states onto the final combined tree using ACCTRAN optimization. These characters were also analyzed using MINITAB (Minitab 2007) to check for character correlations. Character correlations within individual subsets and among subsets were examined for characters which are likely to be functionally related. (e.g. leaf and leaf cuticle).

CHAPTER 3

RESULTS

Seventeen new characters were added to the characters based upon the published literature on *Tsuga.* Ten new characters (character numbers 34-43) were obtained from the leaf cuticle structures prepared from a study by Cooke and Liu (unpublished), five new characters (character numbers 19-23) were obtained from *Tsuga* seeds, and two more new characters (character numbers 24-25) were obtained from *Tsuga* seedlings (Table 14). These new characters are described in the methods, along with their character states and how they were obtained. Due to the lack of material, there were missing data for *T. heterophylla* for the micromorphological cuticle investigation. Seedling data for *T. sieboldii*, *T. diversifolia*, and *T. caroliniana* are missing because no seedlings grew in the amount of time the analysis was being done. A matrix was compiled using the 67 total characters from morphological, anatomical, and biochemical data (henceforth known as the Structural/Biochemical Analysis) and the 6505 total characters from the molecular data. The complete matrix contained 6572 characters, of which 299 were informative (Table 15). The names and numbers for each character can be found above in the methods.

Two of four outgroups, *Abies veitchii,* and *Nothotsuga longibracteata,* were excluded from these analyses due to the lack of characters which may have caused them to be placed within the *Tsuga* clade.

The fossil characters from the four fossil species used in this study were also included in the 67 character matrix. Cone, seed, and leaf characters were added to the matrix from the published reports on fossils. Due to the lack of characters only the leaf characters from the fossils were used in a phylogenetic analysis.

	Resin Sacs	Seed Body Length *	Seed Body Width *	Seed Body Area **	Seed Wing Area**	Cotyledon Number	Cotyledon Length*
T. canadensis	6.8	3.92	2.02	5.7	15.5	3 to 4	5 to 10
T. caroliniana	24.4	4.67	2.34	7.6	18.4	-	-
T. chinensis	9.4	4.26	2.46	6.9	21.3	3	5 to 7
T. diversifolia	23.6	3.94	2.39	7.7	10	-	-
T. dumosa	2.4	3.67	1.87	5.7	12	3	6
T. heterophylla	4.2	3.39	1.57	3.6	18.6	3	5 to 7
T. mertensiana	11.6	4.86	2.59	8.7	20.7	4 to 5	8 to 12
T. sieboldii	11.4	3.37	2.13	5.99	8.5	-	-

Table 14 New character data that includes: number of resin sacs, seed body length, seed body width, seed body area, seed wing area, cotyledon number, and cotyledon length. (*measurements in mm, **measurements in mm^2, measurements reported as averages)

Structural and Biochemical

An analysis on all 67 structural and biochemical characters yielded six most parsimonious trees of 141 steps. A clade representing the Asian species was shown in all of the most parsimonious trees (Fig. 14). This clade places *T. chinensis* with *T. dumosa* as sister species and also shows the Japanese species, *T. diversifolia* and *T. sieboldii* as sister species. The strict consensus tree for these trees is shown in Figure 14. The eastern North American species *T. canadensis* and *T. caroliniana* and the western North American species *T. heterophylla* form a polytomy basal to the Asian clade. The analysis placed *T. mertensiana* basal in the genus *Tsuga*. Individual subsets of data showed higher resolution than this combined analysis and are described below.

	# of Taxa	# of Characters	# of Informative Characters	# MP Trees	Length MP trees	Consistency Index (CI)	Retention Index (RI)
Habit	10	7	5	92	32	0.906	0.727
Wood	8	5	4	41	18	0.944	0.800
Leaf	8	18	14	4	30	0.733	0.667
Cone and Seed	8	11	9	182	43	0.884	0.615
Pollen	7	11	3	193	31	0.966	0.750
Fossil	12	5	5	19	15	0.867	0.833
Biochemical	7	13	7	3	16	0.875	0.778
Structural	10	54	36	4	105	0.714	0.516
Structural/Biochemical	10	67	43	6	141	0.724	0.521
DNA	10	6505	256	2	420	0.848	0.827
Combined Data	11	6572	299	1	458	0.726	0.677

Table 15 Character data grouped by type: # of taxa, # of characters, # of informative characters, # of constant characters, # of most parsimonious trees, shortest number of steps, consistency index (CI), and retention index (RI)

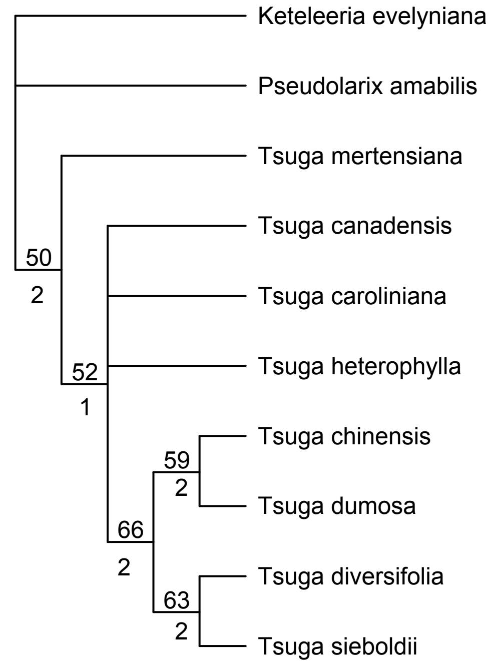


Figure 14 The strict consensus tree derived from an analysis of structural/biochemical data. Numbers above branches are bootstrap values, decay values are below

An analysis of structural characters yielded four most parsimonious trees of 105 steps. This analysis included the characters in the habit, ecology, wood, cones, seeds, seedlings, leaf, and leaf cuticle subsets. The strict consensus tree (Fig. 15) forms the same groups found in the structural/biochemical. The Asian clade has similar resolution as the structural/biochemical analysis.

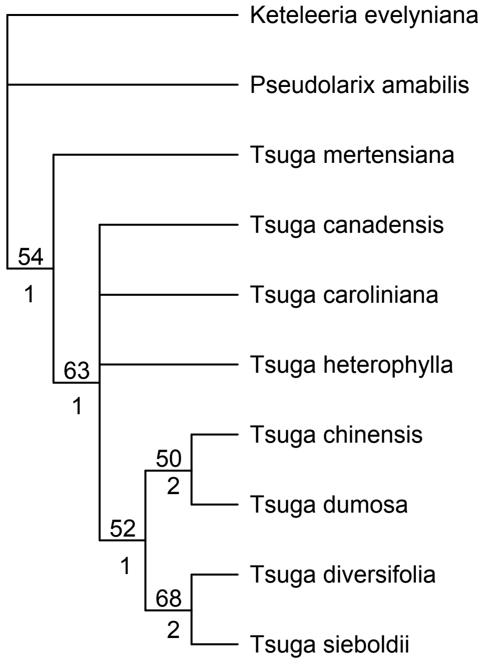


Figure 15 The strict consensus tree derived from an analysis of structural data. Numbers above branches are bootstrap values, decay values are below

An analysis of vegetative characters yielded 1 most parsimonious tree of 92 steps. The characters included in this analysis were from the habit, ecology, wood, leaf, and leaf cuticle subsets. This analysis resolved the same groups that have been found in the two previous analyses except for *T. mertensiana*. Little resolution is shown for the placement of this species among the ingroups and the branch collapses in a strict consensus tree.

An analysis of habit characters yielded 92 most parsimonious trees of 32 steps. All *Tsuga* species were included in this analysis with three outgroups because of the high availability of information. The consensus tree shows a grouping with *N. longibracteata* and *T. diversifolia* forming a sister group. This group is formed because it shares similar character states that include: max tree height, tree d.b.h., trunk fork, and the elevation of range occurrence. Another new sister group shown in this analysis is *T. mertensiana* and *T. sieboldii*. This group shares similar character states that include: trunk fork, branches of first order, crown, vegetative bud shape, and the elevation of growth. However, there is little support and resolution in the bootstrap analysis. *Tsuga chinensis* and *T. dumosa* form a sister group, also seen in previous analyses. The North American clade that was shown in the 67 character analysis is unresolved in this analysis.

An analysis based on wood characters yielded 41 most parsimonious trees of 18 steps. *Tsuga caroliniana*, *T. sieboldii,* and *N. longibracteata* were not included in this analysis because data were not available for these species. Little resolution was seen in this analysis with the only grouping shown was a sister group of *T. chinensis* and *T. heterophylla* with *T. canadensis* as a sister to that group.

An analysis based on leaf characters yielded four most parsimonious trees of 30 steps. The analysis based on leaf characters shows similar groupings to the structural/biochemical analysis (Figure 16). A clade representing the North American species of *T. canadensis*, *T. caroliniana*, and *T. heterophylla* was resolved in this analysis, forming a sister group between the three species. The other sister group from the analysis was the Japanese species *T. diversifolia* and *T. sieboldii.* There was little resolution for the other remaining three species, with *T. dumosa* and *T. chinensis* forming a sister group in one of the shortest trees but found to have no bootstrap support in the strict consensus tree. The resolution of the analysis based on structural/biochemical characters shows higher resolution based on several leaf characters (eg. stomatal shape, cuticular flange, and extracuticular wax).

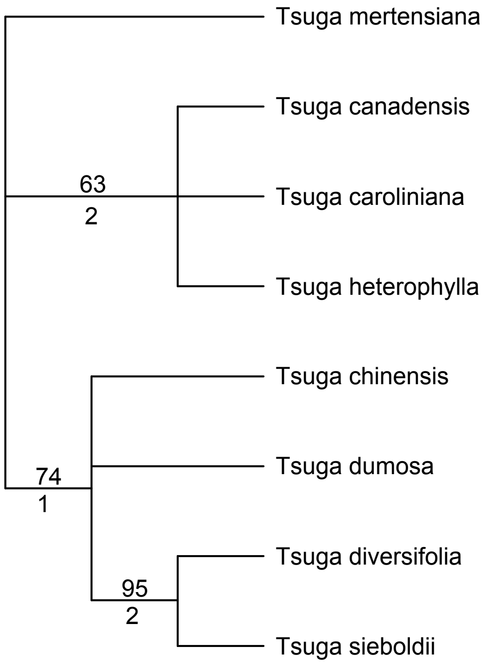


Figure 16 The strict consensus tree derived from an analysis of leaf data. Numbers above branches are bootstrap values, decay values are below

An analysis based on reproductive characters yielded 232 most parsimonious trees of 82 steps. The characters included in this study were cone, seed, and pollen. Two sister groups were resolved in the analysis, *T. caroliniana* and *T. diversifolia*, and *T. dumosa* and *T. sieboldii*. The *T. dumosa* and *T. sieboldii* sister group is supported by several pollen characters. The other grouping shares similar character states in both the seed and pollen data. No resolution when seen on the strict consensus tree with all of the ingroup species branches collapsing.

An analysis based on cone and seed characters yielded 182 most parsimonious trees of 43 steps. All extant species were included in this analysis. The analysis showed no resolution within the ingroup in the consensus tree. The low resolution for this analysis may be due to the many polymorphic characters in the cone data subset.

An analysis based on pollen characters yielded 193 most parsimonious trees of 31 steps. *T. mertensiana* and outgroups were not included in this analysis because of lack of data. The analysis showed one sister group on the consensus tree of *T. dumosa* and *T. sieboldii*. There was no resolution in the rest of the ingroup species.

Analysis of Extant Taxa and Fossils

An analysis based on leaf characters that included fossil leaves in the data matrix yielded 19 most parsimonious trees of 15 steps (Fig 17). This analysis includes four fossil species; *T. shimokawaenis* found in Asia, *T. moenana* found in Europe, and the fossils assigned to *T. canadensis* and *T. caroliniana* that were found in Europe. In the primary reports of fossil *T. canadensis* and *T. caroliniana*, the author chose not to rename the fossils because they appeared very similar to the extant species of *Tsuga* (Szafer 1949). Fossil *T. canadensis* and Fossil *T. caroliniana* grouped close to the NA clade but did not form a sister group with the extant species that have the same name. The other European fossil *T. moenana* formed a sister group with the Asian species, while the fossil from Japan *T. shimokawaenis* was not resolved in this analysis.

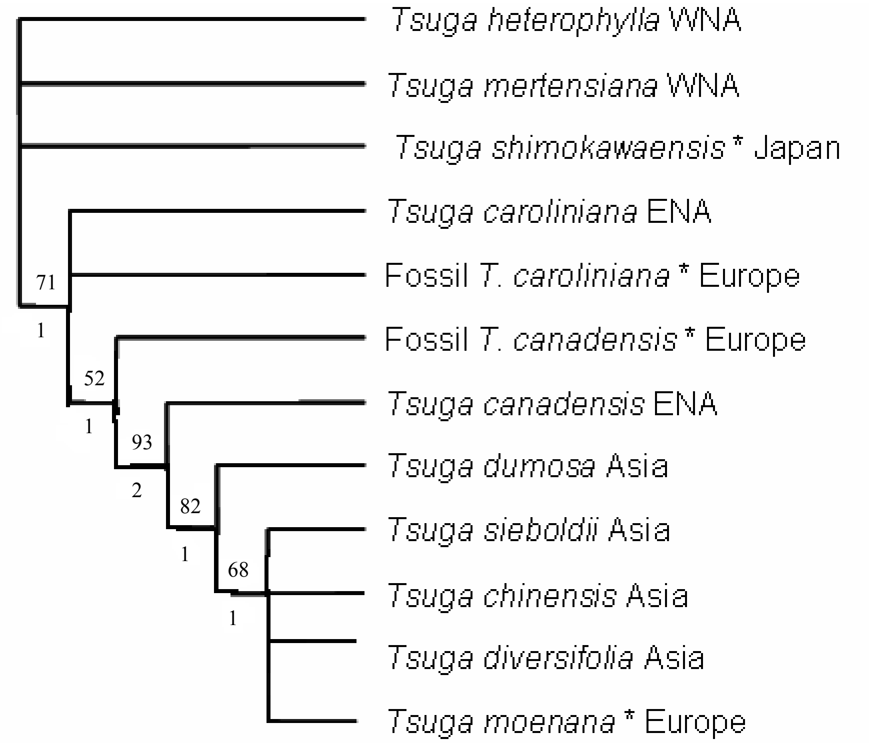


Figure 17 A 50% majority rule bootstrap consensus tree derived from an analysis of fossil leaf data. Numbers above are bootstrap values, decay values are below. (* marks the fossil species)

Biochemical

An analysis based on biochemical characters yielded three most parsimonious trees of 16 steps (Fig 18). *T. dumosa* was not included in this analysis due to the lack of data. An Asian clade was resolved, grouping *T. chinensis* and *T. diversifolia* as sister species with *T. sieboldii* as a sister species to that group. *T. heterophylla* resolved as a sister species to the Asian clade. The NA species *T. canadensis*, *T. caroliniana,* and *T. mertensiana* do not resolve.

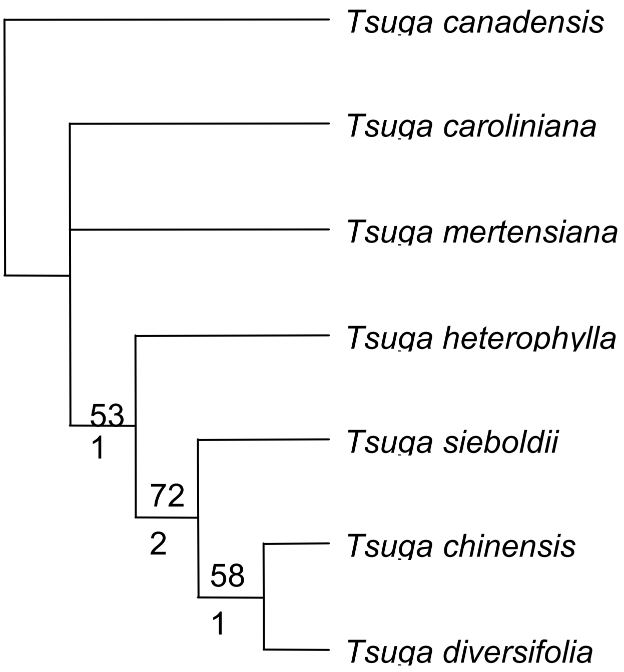


Figure 18 A 50% majority rule bootstrap consensus tree derived from an analysis of biochemical data. Numbers above branches are bootstrap values, decay values are below

Molecular

An analysis based on molecular characters yielded two most parsimonious trees of 420 steps (Fig. 19). The analysis included 6505 total characters from nuclear (ITS) and chloroplast (rbcL, rpl16, and trnT-F) data. Separate analysis on the two molecular data sets produced trees that were identical to the previously published analysis, even with the inclusion of the rbcL sequence (Fig. 3) (Havill et al. 2008). The combined molecular analysis produced similar groupings as the individual data set analyses. The western North American species *T. heterophylla* and *T. mertensiana* formed a sister group. A second clade includes both eastern NA species and the Asian species. *Tsuga canadensis* is basal in this clade. The sister group shown in that clade was *T. chinensis* and *T. sieboldii*. *Tsuga caroliniana* and *T. diversifolia* are sister species to that group but they themselves do not form a group. *Tsuga dumosa* is sister to that grouping, and *T. canadensis* is the sister to that clade. The results of the new analyses replicated the phylogeny of the ITS tree published by Havill et al. (2008). However, this analysis does not match the cpDNA tree published and described by Havill et al. (2008).

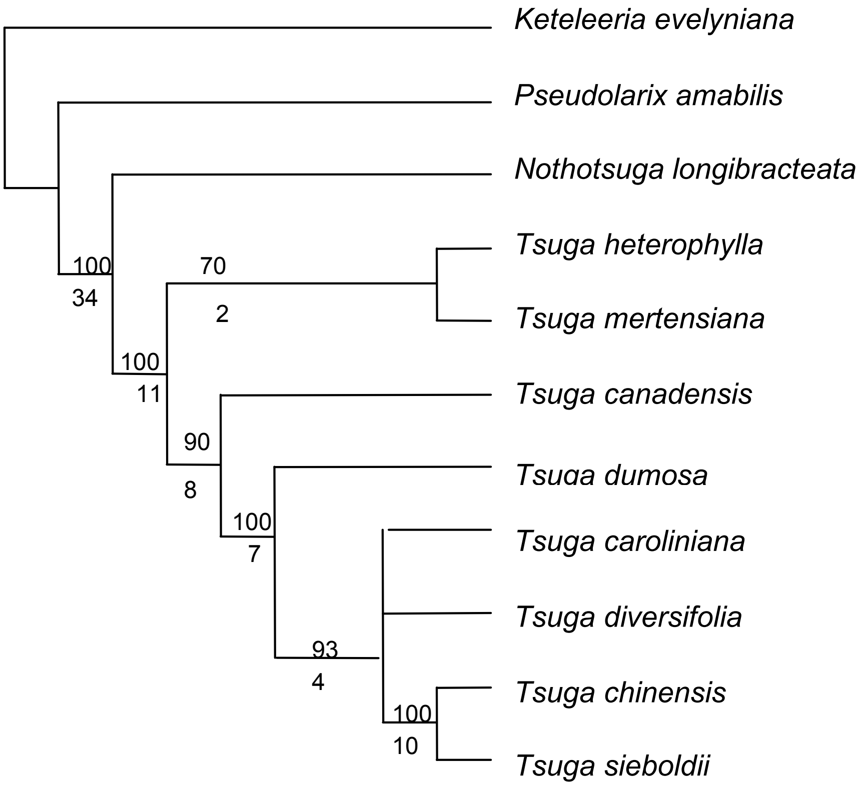


Figure 19 A 50% majority rule bootstrap consensus tree derived from an analysis of molecular data. Numbers above branches are bootstrap values, decay values are below

Combinability

A comparison between the structural/biochemical consensus tree and the molecular consensus tree showed no similar sister groupings between the two analyses. The ILD test (partition homogeneity test) between the structural/biochemical data and the molecular data showed weak heterogeneity (p= 0.01). The Templeton test to compare the 50% majority bootstrap consensus trees of the structural/biochemical data and the molecular data showed that the two trees were significantly different (p= 0.0001). These tests have been shown to have errors because they do not distinguish between alternate hypotheses for conflict (Weiblen 2000;

de Queiroz et al. 1995). Due to these tests having their faults and both groups having weak decay values and resolution for one or more species, the two analyses were combined.

Combined Data

An analysis of all the combined data yielded one most parsimonious tree of 458 steps (Fig. 20). The combined data consensus tree is identical in topology to the molecular consensus tree except for improved resolution for *T. caroliniana* and *T. diversifolia* and the increased support between the western North American species *T. heterophylla* and *T. mertensiana*.

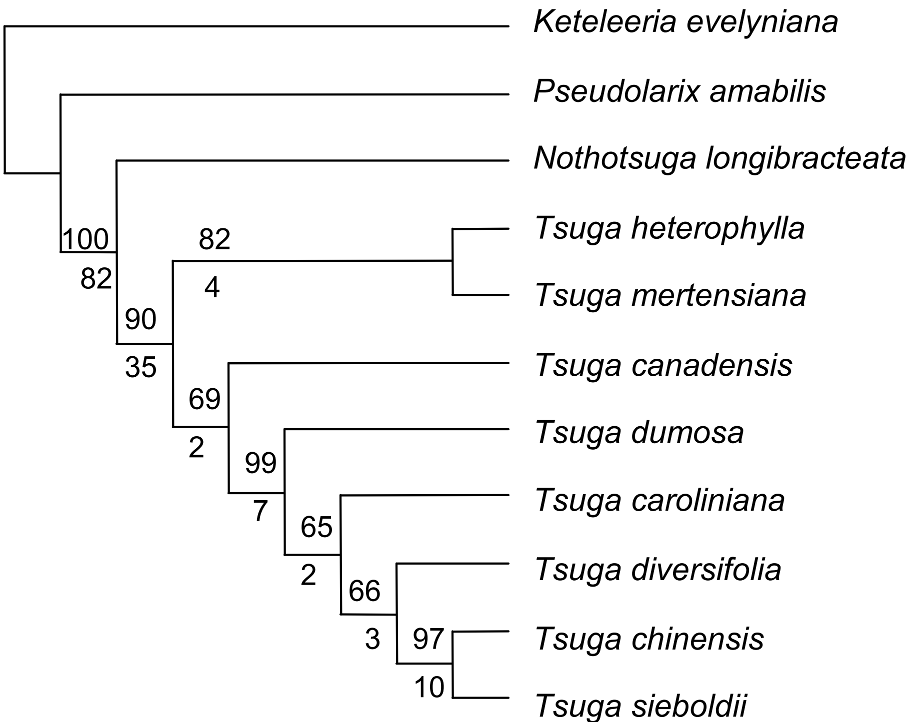


Figure 20 A 50% majority rule bootstrap consensus tree derived from an analysis of structural/biochemical and molecular data. Numbers above branches are bootstrap values, decay values are below

CHAPTER 4

DISCUSSION

The structural/biochemical data did not produce a well resolved phylogeny for relationship in *Tsuga*. Although the structural data-based phylogeny produced a resolved tree within *Tsuga*, none of the five internal nodes was supported by a bootstrap value greater than 70% or a decay index greater than 2. The structural/biochemical data-based phylogeny is less resolved and has lower bootstrap support.

There are several reasons for the low support for the topology produced from the analyses of structural/biochemical data. The quantity of the data is far less than in the molecular analysis. There are 43 informative characters for the structural/biochemical analysis compared to the 256 informative characters for the molecular analysis.

Also, the quality of the structural/biochemical characters appears weaker than that of the molecular sequence characters. The retention index for the molecular data set was 0.827 compared to a retention index of only 0.521 for the structural/biochemical data set. Many of the characters in the structural/biochemical data set are uninformative for the parsimony analyses. Several characters are autapomorphies that result in low phylogenetic signal for parsimony analyses. Six of the 13 biochemical characters were uninformative due to autapomorphies and multistate coding. Multistate characters were also included and provide little or no signal for the parsimony analyses. These multistate characters were usually included due in part to morphological variation in the outgroups, which have unique states for individual characters (e.g. bract shape).

Many characters were scored polymorphic (e.g. vegetative bud shape, wood characters, cone characters, and pollen characters.) This high degree of polymorphic states may be due to highly homoplastic characters, or may be an artifactual result of character state boundaries that determine these states. Leaf margin (#27) appears highly labile, with changes occurring several times in separate clades. Of the 11 pollen characters, 8 were parsimony uninformative, largely due to taxa coded as polymorphic. The extra states were used to keep unbiased gap coding, and to avoid using arbitrary character groupings, even though these groupings (lumping adjacent characters states) may have provided greater phylogenetic signal for parsimony analyses.

Missing data in the characters matrix also contributed to the low phylogenetic signal in the structural/biochemical analysis and reduced the resolution and branch support in the resulting phylogenetic analysis (Figure 21). There were many missing characters for the outgroups. This was due to the information and fresh material being unavailable in these rare or endangered species. Several characters were missing for the ingroup as well. There were missing data for fossil, wood, leaf, seedling, pollen, and biochemical data. The inclusion of these characters would lead to better resolution among the ingroup species.

The molecular analysis produced a well resolved tree with the placement of only two species, *T. caroliniana* and *T. diversifolia,* not resolved (Figure 21). The tree resolved seven out of eight nodes with each node having greater than 70% bootstrap support. Also, six of the seven resolved nodes had decay indexes that were greater than three. As explained above the high resolution was because the analysis included 256 informative characters and little missing data.

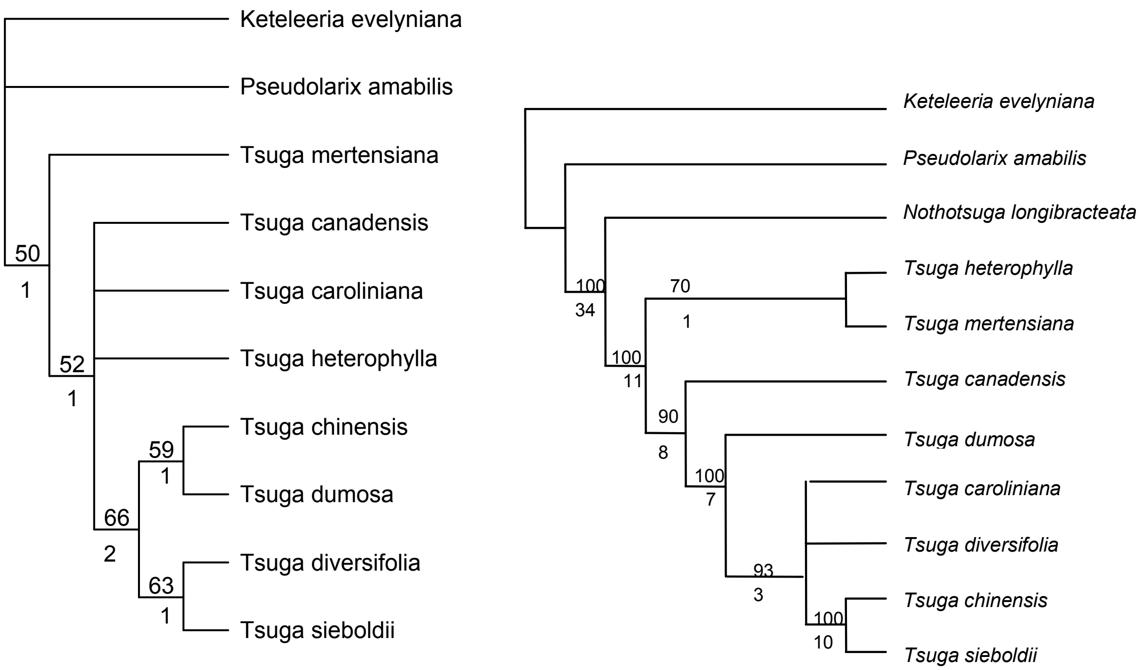


Figure 21 Phylogenetic trees based on structural/biochemical (left) and combined molecular data (right)

The structural/biochemical and molecular data was combined even though the ILD and Templeton test scores did not show combinability. Authors of previous studies have combined their data even if the test scores did not show combinability because several of these tests or either flawed (Lledo et al. 2004) or resolution was increased when the two data sets were combined (Kelly 1998; Chen et al. 1999; Torrecilla et al. 2003; Peterson et al. 2004; Bruyns et al. 2005; Hassan et al. 2005; Schneider et al. 2006). Other authors have found their data sets to be compatible and have combined their data sets (Olson 2002; Savage et al. 2004). The molecular data did provide most of the signal for the combined analysis but the combination of the molecular and the structural/biochemical data sets showed a fully resolved tree that was not seen in either of the separate analyses. *Tsuga caroliniana* and *T. diversifolia* were resolved in the combined analysis. The combined analysis did show some decrease in bootstrap and decay indexes for some nodes, but it also showed an increase in resolution for the western North American clade.

Character Evolution

To examine patterns of character evolution, the characters from the structural/biochemical analysis were mapped onto the combined data analysis tree. Relatively few of the 36 informative characters from the structural data set were found to be completely consistent with the combined total data phylogeny. Those few that did appear synapomorphic for clades in the combined total data tree were related to leaf anatomy and biochemistry (Figure 22). According to the combined total data tree, the rectangular stomata shape appears to have arisen a single time, and occurs in the crown group of *T. caroliniana* and the Asian species *T. diversifolia, T. chinensis* and *T. sieboldii*. No obvious function or ecological selective advantage can be inferred to explain the different states of the stomata shape. The terpenoid d-cadinene appears to have a higher relative concentration in the clade of three Asian species. This may have an ecological importance with respect to the defense against attacks by herbivores such as the HWA.

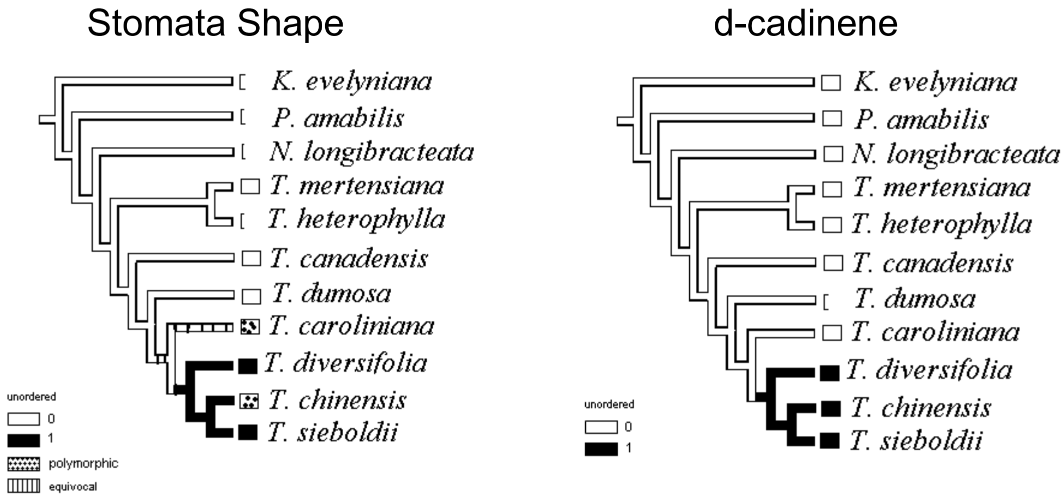


Figure 22 Synapomorphic characters reconstructions mapped on the combined analysis tree using MacClade

In addition to d-cadinene mentioned above, another secondary chemical compound appears to be present in differing concentrations in the Asian species which are not susceptible to HWA as compared to the eastern North American species which are fully susceptible to HWA (Figure 23).

The terpenoid a-humulene is low in the eastern North American species. The concentration is not as low as it is in *T. mertensiana*, but this species is an outlier in all of the concentrations, which is why the authors (Lagalante and Montgomery 2003) proposed it being removed from the genus. It is thought that perhaps a-humulene is a herbivore deterrent (Lagalante and Montgomery 2003) along with other terpenoids that the two eastern North American species have low concentrations (e. g. a-pinene).

The presence and thickness of the hypodermis also appears to coincide with resistance to the HWA. The eastern North American species *T. canadensis* and *T. caroliniana* that are susceptible to the HWA lack hypodermis cells (characters #32 & 33) and have less cell thickness in their leaves that may contribute to their higher vulnerability to the HWA (Figures 24 & 25).

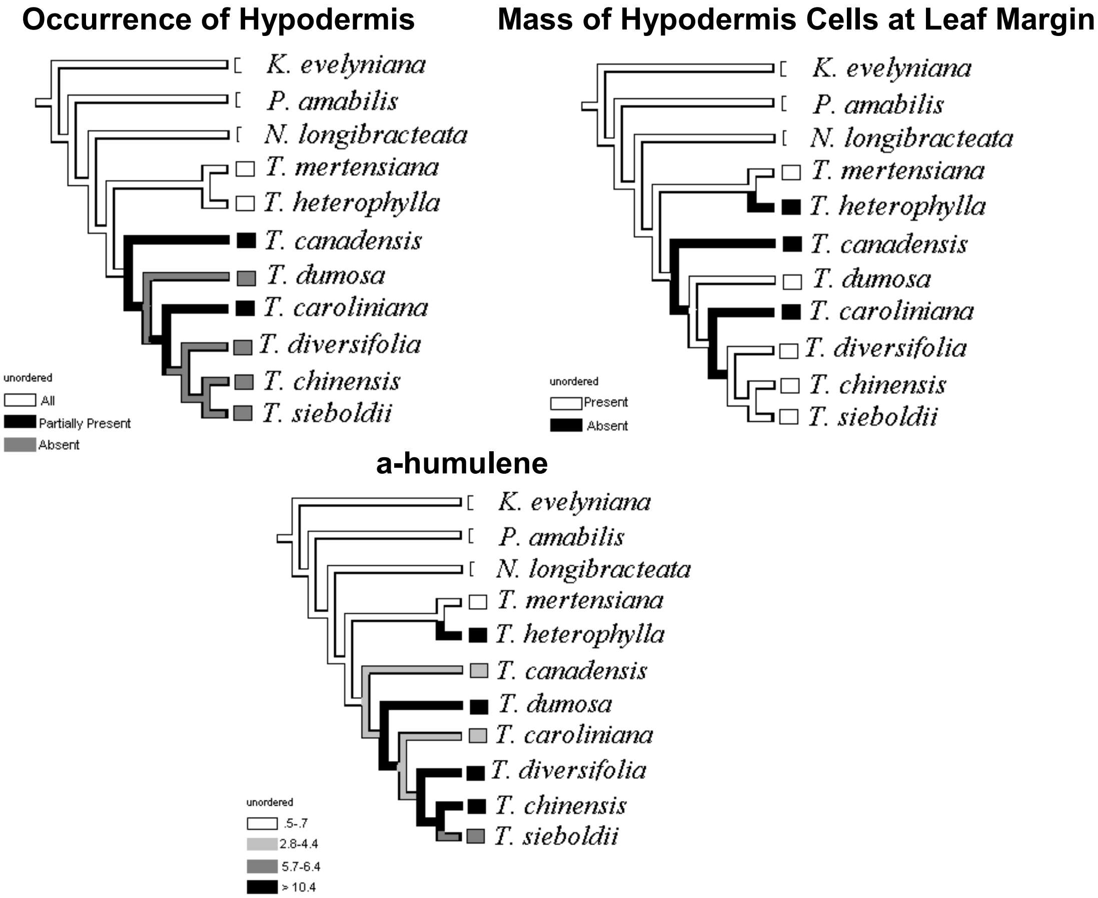


Figure 23 HWA associated character reconstructions mapped on the combined analysis tree using MacClade

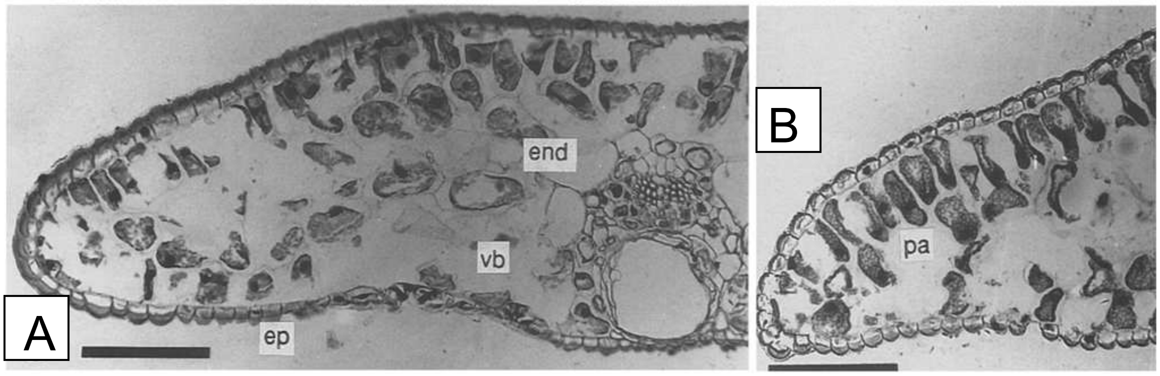


Figure 24 Transverse section of leaf showing the absence of hypodermis cells (A-*T. caroliniana,* B- *T. canadensis*) (Matsumoto and Nishida 1995)

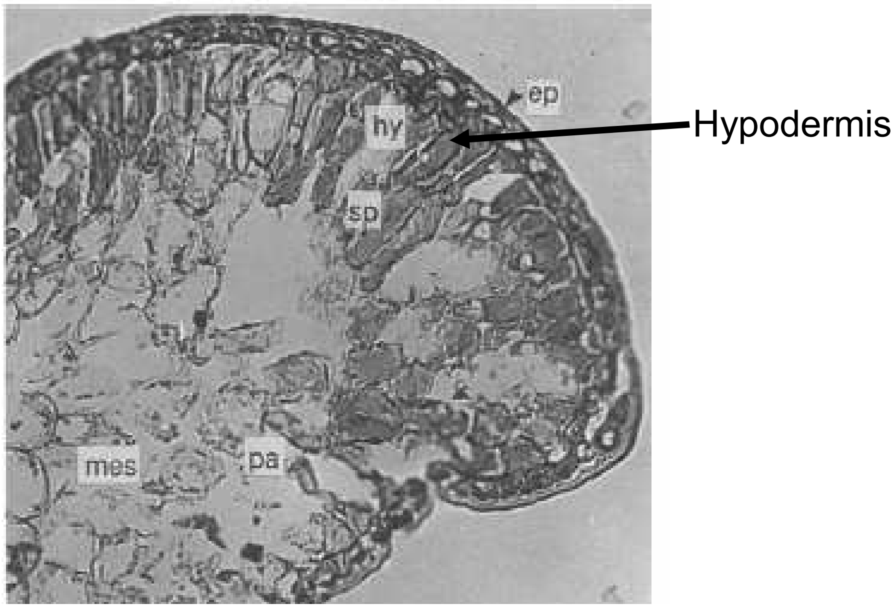
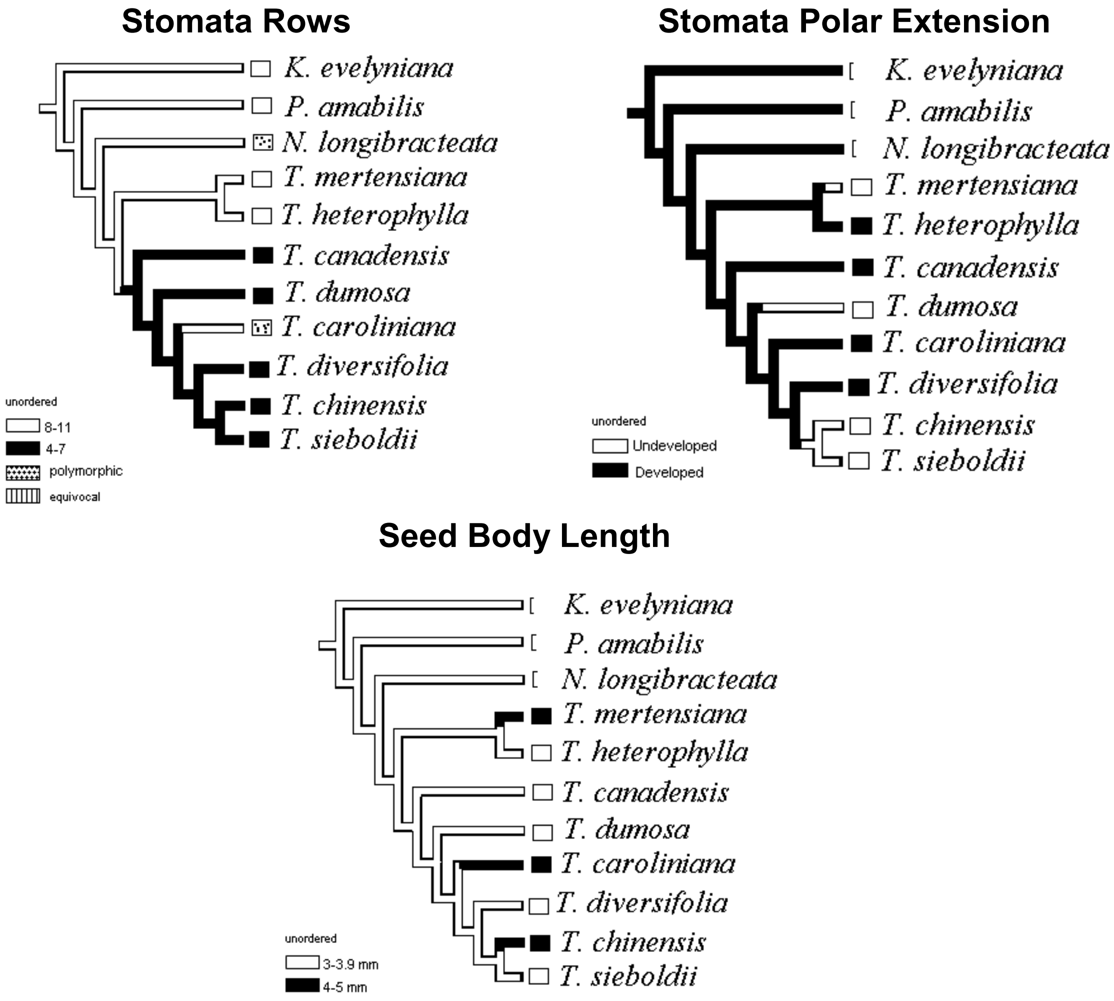


Figure 25 Transverse section of leaf showing the presence of hypodermis cells in *T. heterophylla* (Matsumoto and Nishida 1995)

Many homoplasious characters were seen among the species of *Tsuga*. These characters show either evolutionary reversals or multiple deviations among *Tsuga* species. Among the characters shown to be homoplasious in the genus include stomata rows, stomata polar extension, and seed body length (Fig. 26). Most of the structural/biochemical characters do not appear consistent with the combined data tree. No obvious function or ecological selective advantage can be inferred to explain the different states of stomata polar extension or seed body length. However, the stomata rows character may have a selective advantage. The gain in number of stomata rows in the crown Asian clade could be a response to less drought in the milder Asian climate compared to the North American climate. Asian winters tend to be less cold causing less desiccation and Asian summers are less hot and have less drought. Therefore, more stomata rows might be selected for when there is less drought stress.


Stomata Rows

Stomata Polar Extension

Seed Body Length

Figure 26 Homoplasious character reconstructions mapped on the combined analysis tree using MacClade

Character Correlation

A character correlation analysis was run in Minitab to test for correlations among structural and biochemical characters. Only a few subsets showed correlations among the characters found in them. Those subsets include seeds, pollen, and biochemical characters. The seed and pollen data show positive correlations indicating that as the size increases or decreases in one character it is going to do the same with the other characters in the subset (Table 16). The biochemical data however show a mix between both positive and negative correlation values showing a mix in the correlations between the separate characters (Table 17). The biochemical study authors (Lagalante and Montgomery 2003) note that a-pinene may function as a deterrent from HWA feeding, while isobornyl acetate may function as a HWA attractant. The negative correlation between the two characters is consistent with this supposition. These correlated changes in secondary compound concentrations could have allowed a selective advantage for the eastern North American species. Until about 50 years ago the eastern North American species did not have to protect themselves from the HWA. Making an herbivore deterrent could be energy inefficient if they did not need to protect themselves. Therefore, not having a high concentration of a-pinene or a-humulene could have been a selective advantage at one point in time.

	Seed Body Length	Seed Body Width	DV-External Diameter	PV- D of Cap	PV- Height of Grain
Seed Body Width	0.788 0.020				
Seed Body Area	0.827 0.011	0.956 0.000			
PV-D of Cap			0.984 0.000		
PV-Internal D			0.988 0.000	0.997 0.000	
PfV-edge of operculum					0.787 0.036

Table 16 Character Correlation Values for Seed and Pollen Characters (Pearson's correlation value on top, p-value below)

	tricyclene	a-pinene	Camphene	b-pinene	a-phellandrene	b-phellandrene	b-caryophyllene	y-cadinene
camphene	0.923 0.003							
isobornyl acetate	0.829 0.021	-0.872 0.010	0.830 0.021					
b-pinene		0.801 0.030						
germacrene D		0.751 0.052	-0.784 0.037	0.937 0.002	0.774 0.041	0.777 0.040		
b-phellandrene				0.792 0.034	0.882 0.009			
a-humulene							0.777 0.040	
y-cadinene							0.904 0.005	
d-cadinene							0.852 0.015	0.878 0.009

Table 17 Character Correlation Values for Biochemical Characters (Pearson's correlation value above, p-value below)

Fossils and Biogeography

The phylogenetic analysis based on leaf characters from extant and extinct species showed that Fossil *T. canadensis* and *T. caroliniana* did not form a sister group with *T. canadensis* or *T. caroliniana*. This may be because they were improperly identified. These fossils were found in Europe in the 1940s when little information was known about the Asian species of *Tsuga* (Szafer 1949). There are two differences between *T. canadensis* and fossil *T. canadensis*: the occurrence of the hypodermis and the number of stomata rows. Fossil *T. canadensis* has hypodermis cells unlike *T. canadensis* where the hypodermis cells are absent. There were no differences between *T. caroliniana* and fossil *T. caroliniana* but they did not resolve into a sister grouping in the consensus tree due to the low number of characters used in the analysis. Fossil *T. canadensis* may actually be closer to an Asian species, but due to the lack of Asian specimens available in Europe in 1949, the author only could compare these fossils to a species that he knew about.

The biogeography for the genus was investigated by Havill et al. (2008). Their analysis concluded that *Tsuga* once had a widespread distribution across the Northern Hemisphere (North America, Europe, and Asia) and its range was reduced over time. The range reduction caused the extinction of *Tsuga* about 15 million years ago. It is not known why the hemlocks are extinct from Europe. The fossil record for *Tsuga* reported by LePage (2003) did not include any fossils in China. Until recently, the only fossils from Asia were found in Japan. However, recently fossil wood from *Tsuga* cf *dumosa* was found in China dating to the late Pliocene (5.3 to 1.8 mya) (Yi et al. 2005). This information along with the large fossil record of *Tsuga* being found all over the Northern Hemisphere would support the already proposed biogeography.

Another range reduction could be possible with the Carolina hemlock (*T. caroliniana*). Due to the high susceptibility to the HWA and the already reduced range, if the HWA cannot be controlled the trees may all eventually die removing another species from the genus.

Hybridization

The combined data analysis is consistent with the hybridization study that tested crosses between eastern North American and Asian *Tsuga* species (Bentz et al. 2003). The sympatric species from eastern North America *T. caroliniana* and *T. canadensis* do not hybridize. However, *T. caroliniana* does hybridize with various Asian species. Species sharing more recent ancestors according to the combined data tree (*T. caroliniana, T. diversifolia, T. chinensis, T. sieboldii*) do hybridize. This is consistent with the traditional view that species which are more closely related are more likely to be interfertile. However, *T. canadensis* appeared unable to hybridize with the Asian species to which it was more distantly related than *T. caroliniana*. The hybridization of the western North American species was not tested but there are reports that they hybridize naturally forming a subspecies known as *Tsuga* X *jeffreyi* (Swartly 1984). Several Asian species also hybridize naturally with one another forming subspecies or varieties such as *T. forrestii*, which is a variety of *T. chinensis* (Flora of China 1999)

Conclusions

Overall, the structural/biochemical characters did not produce a fully resolved phylogeny for relationships within *Tsuga*. There were many homoplasious characters, and missing data for several species, that added to the low signal in the phylogenetic analyses. Character coding presented a serious challenge for use in the parsimony based analysis. The characters were coded to show an unbiased result which resulted in many multistate and polymorphic characters.

Mapping characters onto the combined data phylogeny revealed that several leaf and biochemical characters coincided with HWA susceptibility. This included the absence of hypodermis cells in the leaves, and a low concentration of proposed HWA-deterrent terpenoids. This information would allow for future research on biochemical and leaf anatomy characters that are more directed towards HWA susceptibility. Adding more characters and information to the already compiled matrix could provide a fuller picture of evolution within the genus *Tsuga*.

REFERENCES

Abramoff, M.D., P.J. Magelhaes, S.J. Ram. 2004. Image processing with ImageJ. Biophotonics International. 11 (7): 36-42.

Benson, Dennis A., Ilene Karsch-Mizrachi, David J. Lipman, James Ostell, and David L. Wheeler. 2005. GenBank. Nucleic Acids Research. 33: D34-D38.

Bentz, S.E., L. G. H. Riedel, M. R. Pooler and A. M. Townsend. 2002. Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. Journal of Arboriculture. 28(4): 200-205.

Bremer, K. 1994. Branch support and tree stability. Cladistics. 10: 295-304.

Bruyns, P.V., T. L. Nowell, and T. A. J. Hedderson. 2005. A revision and phylogenetic analysis of *Stapeliopsis* (Apocynaceae). Botanical Journal of the Linnean Society. 148: 125-155.

Butin, E., J. Elkinton, N. Havill, and M. Montgomery. 2003. Comparison of numerical response and predation effects of two coccinellid species on hemlock woolly adelgid (Homoptera: Adelgidae). Journal of Economic Entomology 96: 763-767.

Campbell, Christopher S. Wesley A Wright, Margaret Cox, et al. 2005. Nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) in *Picea* (Pinaceae): sequence divergence and structure. Molecular Phylogenetics and Evolution. 35 (1): 165-185.

Chen, Zhi-Duan, Steven R. Manchester, and Hai-Ying Sun. 1999. Phylogeny and evolution of the *Betulaceae* as inferred from DNA sequences, morphology, and paleobotany. American Journal of Botany. 86(8): 1168-1181.

Cooke, Graham and Yusheng Liu. Cuticular micromorphology of the leaves of *Tsuga.* Unpublished.

de Queiroz, Alan, Michael Donoghue, and Junhyong Kim. 1995. Separate versus combined analysis of phylogenetic evidence. Annual Review of Ecology and Systematics. 26: 657-681.

Eriksson, T., 1998. AutoDecay ver 4.0 (program distributed by author). Department of Botany, Stockholm University, Stockholm.

Farjon, A. 1990. *Pinaceae*: drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix*, and *Picea*. Königstein: Koeltz Scientific Books.

Farris, J.S., M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics. 10: 315-319.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 39: 783-791.

Frankis, M.P. 1988. Generic inter-relationships in Pinaceae. Notes RBG Edinb. 45(3): 527-548.

Flora of China Editorial Committee, eds. July 1999. *Flora of China. Vol. 4 (Cycadaceae through Fagaceae)*. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 12+ vols. New York and Oxford.

Greguss, P. 1955. Identification of living gymnosperms on the basis of xylotomy. Budapest (Rumania). Akademiai Kiadó. 263 p.

Hassan, N.S., J. Thiede, and S. Liede-Schumman. 2005. Phylogenetic analysis of *Sesuvioideae* (Aizoaceae) inferred from nrDNA internal transcribed spacer (ITS) sequences and morphological data. Plant Systematics and Evolution. 255: 121-143.

Havill, Nathan P, Michael E. Montgomery, Guoyue Yu, Shigehiko Shiyake, and Adalgisa Caccone. 2006. Mitochondrial DNA from Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Suggests Cryptic Speciation and Pinpoints the Source of the Introduction to Eastern North America. Annals of the Entomological Society of America. 99 (2): 195-203.

Havill, Nathan P.; Campbell, Christopher S.; Vining, Thomas F.; LePage, Ben; Bayer, Randall J.; Donoghue, Michael J. 2008. Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal ITS and chloroplast DNA sequence data. Systematic Botany. 33 (3): 478-489

Japan Society for Plant Systematists. 2008. Flora of Japan. http://foj.c.u-tokyo.ac.jp/gbif/

Kelly, Lawrence M. 1998. Phylogenetic relationships in *Asarum* (Aristolochiaceae) based on morphology and ITS sequences. American Journal of Botany. 85(10): 1454-1467.

Kunzmann, Lutz, Dieter Hans Mai. 2005. Conifers of the Mastixioideae-flora from Wiesa near Kamenz (Saxony, Miocene) with special consideration of leaves. Palaeontographica. Band 272; Part 1/6: 67-135

Lagalante, Anthony F., Michael E. Montgomery. 2003. Analysis of terpenoids from hemlock (*Tsuga*) species by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. J. Agric. Food Chem. 51: 2115-2120.

Larson, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. In B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle [eds.]. Molecular ecology and evolution: approaches and applications. 317-390. Birkhauser, Basel, Switzerland.

Lemmon, John Gill. 1890. 3d. Bienn. Rep. Cal. St. Bd. Forestry. p. 126.

LePage, B. A. 2003. A new species of *Tsuga* (Pinaceae) from the middle Eocene of Axel Heiberg Island, Canada, and an assessment of the evolution and biogeographical history of the genus. Botanical Journal of the Linnean Society. 141: 257-296.

Little, Damon P. 2006. Evolution and circumscription of the true cypresses (Cupressaceae: *Cupressus*). Systemic Botany. 31 (3): 461-480.

Liston, Aaron, William A. Robinson, Daniel Pinero, Elena R. Alvarez-Buylla. 1999 Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Molecular Phylogenetics and Evolution. 11(1): 95-109.

Lledo, M. D., A. P. Davis, M. B. Crespo, M. W. Chase, and M. F. Fay. 2004. Phylogenetic analysis of *Leucojum* and *Galanthus* (Amaryllidaceae) based on plastid matK and nuclear ribosomal spacer (ITS) DNA sequences and morphology. Plant Systematics and Evolution. 246: 223-243.

Maddison, W P. and D.R. Maddison. 2005. MacClade: a computer program for phylogenetic analysis. Version 3.0. http://macclade.org/index.html

Matsumoto, M., T. Ohsawa, and M. Nishida. 1995. *Tsuga shimokawaensis*, a new species of permineralized conifer leaves from the middle Miocene Shimokawa group, Hokkaido, Japan. *Journal of Plant Research* 108: 417-428.

Minitab. 2007. MINITAB 15. State College, PA

Olson, Mark E. 2002. Combining data from DNA sequences and morphology for phylogeny of *Moringaceae* (Brassicales). Systematic Botany. 27(1): 55-73.

Owens, John N. Tokushiro Takaso and C. John Runions. 1998. Pollination in conifers. Trends in Plant Science. 3 (12): 479-485.

Peterson, G., O. Seberg, and C. Baden. 2004. A phylogenetic analysis of the genus *Psathyrostachys* (Poaceae) based on one nuclear gene, three plastid genes, and morphology. Plant Systematics and Evolution. 249: 99-110.

Savage, Jade, Terry A. Wheeler, and Brian M. Wiegmann. 2004. Phylogenetic analysis of the genus *Thricops* Rondani (Dipter: Muscidae) based on molecular and morphological characters. Systematic Entomology. 29: 395-414.

Schneider, J. V., U. Swenson, R. Samuel, T. Stuessy, and G. Zizka. 2006. Phylogenetics of *Quiinaceae* (Malpighiales): evidence from trnL-trnF sequence data and morphology. Plant Systematics and Evolution. 257: 189-203.

Schols, P. C. D'hondt, K. Geuten, V. Merckx, S. Janssens, E. Smets. 2004. MorphoCode: coding quantitative data for phylogenetic analysis. PhyloInformatics. 4: 1-4.

Sivak, J. 1973. Observations nouvelles sur les grains de pollen de *Tsuga*. *Pollen et Spores* 15: 397-457.

Swartley, John C. 1984. The cultivated Hemlocks. Timber Press. Portland, Oregon.

Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Szafer, M. W. 1949. Studies on the genus *Tsuga* CARR. in the tertiary of Europe. Bull. Acad. Polon. Sci. Ser, Sci. Biol. 1949: 23-51

Taylor, Ronald. 1972. The relationship and origin of *Tsuga Heterophylla* and *Tsuga Mertensiana* based on phytochemical and morphological interpretations. Amer. J. Bot. 59 (2): 149-157.

Templeton, A. R. 1993. The "Eve" hypothesis: a genetic critique and reanalysis. American Anthropologist. 95: 51-72.

Torrecilla, P., J. A. Lopez Rodriquez, D. Stancik, and P. Catalan. 2003. Systematics of *Festuca* L. sects. *Eskia* Willk., *Pseudatropis* Kriv., *Amphigenes* (Janka) Tzvel., *Pseudoscariosa* Kriv. and *Scariosae* Hack. based on analysis of morphological characters and DNA sequences. Plant Systematics and Evolution. 239: 113-139.

TreeBASE. 2009. Retrieved February 11, 2009, from TreeBase Web site: http://www.treebase.org/treebase/index.html

Vining, T.F. 1999. Molecular phylogenetics of Pinaceae. Ph.D. dissertation, University of Maine, Orono, ME. 75 pp.

Wang, X. –Q., D. C. Tank, and T Sang. 2000. Phylogeny and divergence times in Pinaceae: evidence from three genomes. Mol. Biol. Evol. 17(5): 773-781.

Weiblen, George D. 2000. Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. American Journal of Botany. 87 (9): 1342-1357.

Xiang Qiao-Ping, Qiu-Yun Xiang, Aaron Liston, Li-Kuo Fu, De-Zhi Fu. 2000. Length variation of the nuclear ribosomal DNA internal transcribed spacer in the genus *Abies*, with reference to its systematic utility in *Pinaceae*. Acta Botanica Sinica. 42(9): 946-951.

Yi, Tie-Mie, Cheng-Sen Li, and Xiao-Mei Jiang. 2005. Conifer woods from the Pliocene age from Yunnan, China. Journal of Integrative Plant Biology. 47(3): 264-270.

APPENDICES

APPENDIX A

Species Names and First Publications

	Species Name, Author, and Publication	**Basionym, Author, and Publication**
Tsuga		
T. canadensis	*Tsuga canadensis* (Linnaeus) Carrière, 189. 1855.	*Pinus canadensis* Linnaeus, Sp. Pl. ed. 2, 2: 1471. 1763
T. caroliniana	*Tsuga caroliniana* Engelmann, Bot. Gaz. 6: 223. 1881	
T. mertensiana	*Tsuga mertensiana* (Bongard) Carrière, Traité Gén. Conif., ed. 2. 250. 1867.	*Pinus mertensiana* Bongard, Mém. Acad. Imp. Sci. St. Pétersbourg, Sér. 6., Sci. Math. 2: 163. 1832; *Abies hookeriana* A.Murray bis; *A . pattoniana* A.Murray bis; *Hesperopeuce mertensiana* (Bongard) Rydberg; *H . pattoniana* (A.Murray bis) Lemmon; *Picea* (*Tsuga*) *hookeriana* (A.Murray bis) Bertrand; *Pinus hookeriana* (A.Murray bis) McNab; *Pinus pattoniana* (A.Murray bis) Parlatore; *Tsuga crassifolia* Flous; *T . hookeriana* (A.Murray bis) Carrière; *T . pattoniana* var. *hookeriana* (A.Murray bis) Lemmon; *´Tsuga -Picea hookeriana* (A.Murray bis) M.Van Campo-Duplan and Gaussen.
T. heterophylla	*Tsuga heterophylla* (Rafinesque) Sargent, Silva. 12: 73, plate 605. 1898.	*Abies heterophylla* Rafinesque, Atlantic J. 1: 119. 1832
T. chinensis	*Tsuga chinensis* (Franchet) E. Pritzel, Bot. Jahrb. Syst. 29: 217. 1900.	
T. dumosa	*Tsuga dumosa* (D. Don) Eichler in Engler & Prantl, Nat. Pflanzenfam. 2(1): 80. 1887.	*Pinus dumosa* D. Don in Lambert, Descr. Pinus 2: 55. 1824; *Abies yunnanensis* Franchet; *Pinus brunoniana* Wallich; *Tsuga brunoniana* (Wallich) Carriere; *T. calcarea* Downie; *T. chinensis* (Franchet) E. Pritzel subsp. *wardii* (Downie) E. Murray; *T. dumosa* var. *yunnanensis* (Franchet) Silba; *T. dura* Downie; *T. intermedia* Handel-Mazzetti; *T. leptophylla* Handel-Mazzetti; *T. wardii* Downie; *T. yunnanensis* (Franchet) E. Pritzel; *T. yunnanensis* subsp. *dura* (Downie) E. Murray.
T. sieboldii	*Tsuga sieboldii* Carriere Trait{e'} G{e'}n. Conif. ed. 1: 186 (1855).	*Abies tsuga* Sieb. et Zucc., Fl. Jap. 2: 14, t. 106 (1842).
T. diversifolia	*Tsuga diversifolia* (Maxim) Mast , J. Linn. Soc. Bot. 18: 514 (1881).	*Abies diversifolia* Maxim. in Bull. Acad. Sci. St.-P{e'}t. 12: 229 (1868)
OUTGROUPS		
Nothotsuga longibracteata	*Nothotsuga longibracteata* (W. C. Cheng) Hu ex C. N. Page.	*Tsuga longibracteata* W. C. Cheng, Contr. Biol. Lab. Chin. Assoc. Advancem. Sci., Sect. Bot. 7(1): 1. 1932.
Pseudolarixamabilis	*Pseudolarix amabilis* (J. Nelson) Rehder, J. Arnold Arbor. 1: 53. 1919.	*Larix amabilis* J. Nelson, Pinaceae 84. 1866; *Abies kaempferi* Lindley; *Chrysolarix amabilis* (J. Nelson) H. E. Moore; *Laricopsis kaempferi* (Lindley) Kent; *Pseudolarix fortunei* Mayr; *P. kaempferi* Gordon; *P. pourtetii* Ferre.
Keteleeria evelyniana	*Keteleeria evelyniana* Masters, Gard. Chron., ser. 3. 33: 194. 1903.	*Keteleeria delavayi* Tieghem; *K. dopiana* Flous; *K. evelyniana* var. *pendula* Hsüeh
Abies veitchii	*Abies veitchii* Lindl., Gard. Chron. 1861:23	

APPENDIX B

Seed Analysis Data

	Resin Sacs	Avg	Seed Body Length (mm)	Avg	Seed Body Width (mm)	Avg	Seed Body Area (mm2)	Avg
Tcan1	8	6.8	3.675	3.92	2.14	2.02	5.72	5.7
Tcan2	7		3.872		2		6.43	
Tcan3	8		4.284		1.9		6.035	
Tcan4	4		3.799		1.939		5.01	
Tcan5	7		3.991		2.122		5.3	
Tcar1	25	24.4	4.575	4.67	2.279	2.34	7.2	7.57
Tcar2	25		4.702		2.253		7.5	
Tcar3	27		4.774		2.371		7.725	
Tcar4	22		4.758		2.45		7.65	
Tcar5	23		4.533		2.345		7.75	
Thet1	4	4.2	3.416	3.39	1.454	1.57	3.43	3.59
Thet2	6		3.527		1.703		4.1	
Thet3	4		3.302		1.716		3.72	
Thet4	3		3.423		1.376		3.24	
Thet5	4		3.306		1.624		3.45	
Tmert1	13	11.6	4.651	4.86	2.555	2.59	8.65	8.67
Tmert2	10		4.546		2.528		7.81	
Tmert3	12		4.913		2.594		7.84	
Tmert4	9		5.01		2.686		9.57	
Tmert5	14		5.188		2.568		9.47	
Tchi1	8	9.4	4.114	4.26	2.319	2.46	5.75	6.85
Tchi2	12		4.089		2.371		6.84	
Tchi3	6		4.28		2.961		7.42	
Tchi4	11		4.721		2.463		7.81	
Tchi5	10		4.09		2.201		6.45	
Tdum1	3	2.4*	3.332	3.67	2.04	1.87	6.272	5.65
Tdum2	3		3.82		1.68		6.46	
Tdum3	2		3.72		1.83		5.39	
Tdum4	2		3.57		1.62		4.43	
Tdum5	2		3.9		2.16		5.702	
Tdiv1	26	23.6	4.5	3.94	2.58	2.39	8.39	7.66
Tdiv2	27		4.1		2.6		8.35	
Tdiv3	26		4.36		2.21		8.32	
Tdiv4	15		3.25		2.15		6.2	
Tdiv5	24		3.51		2.42		7.02	
Tsie1	13	11.4	3.53	3.37	2.266	2.13	6.68	5.99
Tsie2	9		2.912		2.07		5.61	
Tsie3	9		3.3		2.16		5.33	
Tsie4	15		3.51		2.04		6.08	
Tsie5	11		3.6		2.1		6.23	

APPENDIX C

Complete PAUP* Matrix

```
BEGIN DATA;
DIMENSIONS  NTAX=12 NCHAR=6572;
FORMAT MISSING=? GAP=- INTERLEAVE;
FORMAT DATATYPE=DNA;
FORMAT SYMBOLS= ". 0 1 2 3 4 5 6";
OPTIONS  MSTAXA=POLYMORPH ;

CHARSTATELABELS
                                1  MTH,  2  TDBH,  3  TF,  4  BFO,  5  C,  6  VBS,
  7  EG,  8  NTPSM,  9  RPSM,  10  RNCPSM,  11  RCW,
  12  NP,  13  CSS,  14  CSA,  15  BS,  16  SS,
  17  SWS,  18  SWL,  19  SBL,  20  SBW,  21  SBA,
  22  RSBS,  23  SWA,  24  CN,  25  CL,  26  LA,
  27  LM,  28  LAR,  29  LS,  30  SRC,  31  SM,
  32  OH,  33  MHCL,  34  PW,  35  STS,  36  STPE,
  37  CFGC,  38  EW,  39  FRF,  40  FRG,  41  STR,
  42  STD,  43  STI,  44  DVED,  45  EKW,  46  PVDC,
  47  PVID,  48  PVHG,  49  □PFVCO,  50  PFVEO,  51  PRS,
  52  DIS,  53  IS,  54  SP,  55  TRI,  56  API,
  57  CAMP,  58  BPI,  59  MYR,  60  APHEL,  61  BPHEL,
  62  ISO,  63  BCAR,  64  AHUM,  65  GERM,  66  YCAN,
  67  DCAD;

MATRIX
[                               10        20        30        40        50        60      ]

Abies_veitchii                00003(02)(01)34200016020???????1001???????????0????????????1?????????????
Keteleeria_evelyniana□□□□□□     (01)(01)012(02)(01)12010?21232???????2022???????????1????????????1?????????????
Nothotusga_longibracteata     001140(01)?????1251(01)1???????1002???????????(01)????????????0?????????????
Pseudolarix_amabilis          (01)1010001221(01)0?4(01)3(01)???????(12)022???????????0????????????1?????????????
Tsuga_canadensis              (01)101010100(01)(01)(01)0(02)(12)0(01)01101(01)(01)0112(012)0210010101101(01)00(01)(01)0001101020000301000
Tsuga_caroliniana             00010(01)0?????0(01)111(01)11221??0002(12)0211(01)101110011011(01)1(01)00100020110301100
Tsuga_chinensis               11101(02)(01)011(01)(01)000(12)0011112001012(01)1100(01)00110(01)000100(01)0023100010000123111
Tsuga_diversifolia            00102(02)(01)12201(01)(01)210(01)01220??1002(12)010011100010011(01)(01)(01)0013100020001213011
Tsuga_dumosa                  11111(02)1120(01)(01)0(01)3(12)0(01)0010000(01)10201100000110101(01)(01)0(01)(01)013210?????????????
Tsuga_heterophylla            21010(01)000100(01)01(12)0(01)0000100(01)10110010?101??0100100000031010200003130 00
Tsuga_mertensiana             (01)(01)000(01)(01)2331000401(01)1121211(01)000(01)0002000111001?????????010102022000300
Tsuga_sieboldii               01000(01)0?????1(01)01(01)001110??(01)002(12)010(01)1010001000000(01)(01)(01)33100021000212201

Abies_veitchii             GCGACTAAGGTCTTTCACACATTTGAATGAAGTAGAAAACTCGTCGATACCATCGGTAAAGTTCGGAAGACTACGACT
Keteleeria_evelyniana      ..............................................................................
Nothotsuga_longibracteata ...............T.........................A....................................
Pseudolarix_amabilis       ...............T..............................................................
Tsuga_canadensis           ...............T..........................A....................T..............
Tsuga_caroliniana          ...............T..........................A....................T..............
Tsuga_chinensis            ...............T..........................A....................T..............
Tsuga_diversifolia         ...............T..........................A....................T..............
Tsuga_dumosa               ...............T..........................A....................T..............
Tsuga_heterophylla         ...............T........................A.A....................T..............
Tsuga_mertensiana          ...............T..........................A....................T..............
Tsuga_sieboldii            ...............T..........................A....................T..............

Abies_veitchii             GATCCTCGAAGTATAATGAACGGAAAAAATAGCATGTCGTTCCAAC----ATATGATCTTTATGATACCTTATATTAT
Keteleeria_evelyniana      ............................C.................----.....................G...G..
Nothotsuga_longibracteata ............................C.................----..................C..G......
Pseudolarix_amabilis       .........G..................C.................----..................T..G......
Tsuga_canadensis           ............................G.................----.....................G......
Tsuga_caroliniana          ............................G.................----.....................G......
Tsuga_chinensis            ............................G.................----.....................G......
Tsuga_diversifolia         ............................G.................----.....................G......
Tsuga_dumosa               ............................G.................----.....................G......
Tsuga_heterophylla         ............................G.................ATAC.....................G......
Tsuga_mertensiana          ............................G.................----.....................G......
Tsuga_sieboldii            ............................G.................----.....................G......

Abies_veitchii             TTTTAGGATACCTGATTGTATTCGATCAGGACCGGATCTTATTCAAGGAATCAAATAGGTGGGAAATGTCTATTATAT
Keteleeria_evelyniana      .......................................G............T...G.....................
Nothotsuga_longibracteata .......................................G................G.....................
Pseudolarix_amabilis       ......................................T...............T.G.....................
Tsuga_canadensis           .......................................G................G.....................
Tsuga_caroliniana          .......................................G................G.....................
Tsuga_chinensis            .......................................G................G.....................
Tsuga_diversifolia         .......................................G................G.....................
Tsuga_dumosa               .......................................G................G.....................
```

```
Tsuga_heterophylla          .......................................G................G.....................
Tsuga_mertensiana           .......................................G..A.............G.....................
Tsuga_sieboldii             .......................................G................G.....................



Abies_veitchii              ATTTAGATG-ATTTATAATATGCTCATCCTTTCAGATCAAATGTGATAATTGTGAATAGGATCGAATCAATTCACGAT
Keteleeria_evelyniana       .........G..G....................G..........................................G.
Nothotsuga_longibracteata   .........G.......................G.............................A....C.......G.
Pseudolarix_amabilis        .........G..G....................G............................................
Tsuga_canadensis            .........G.......................G..................................TC.....AG.
Tsuga_caroliniana           .........G.......................G..................................TC.....AG.
Tsuga_chinensis             .........G.......................G..................................TC.....AG.
Tsuga_diversifolia          .........G.......................G..................................TC.....AG.
Tsuga_dumosa                .........G.......................G..................................TC.....AG.
Tsuga_heterophylla          .........G.......................G..................................TC.....AG.
Tsuga_mertensiana           .........G.......................G..................................TC.....AG.
Tsuga_sieboldii             .........G.......................G..................................TC.....AG.

Abies_veitchii              TAAGTCGAATGAGTCAATGGAGAAAGTTATATGACTTGAATCATAGGTAAACCCAGAGGAACGAGCTTCTGTTCCTCG
Keteleeria_evelyniana       ..........................C...................................................
Nothotsuga_longibracteata   .....T....................C...................................................
Pseudolarix_amabilis        ..........................C...................................................
Tsuga_canadensis            .....T....................C..........T........................................
Tsuga_caroliniana           .....T....................C..........T........................................
Tsuga_chinensis             .....T....................C..........T........................................
Tsuga_diversifolia          .....T....................C..........T........................................
Tsuga_dumosa                .....T....................C..........T........................................
Tsuga_heterophylla          .....T....................C..........T........................................
Tsuga_mertensiana           .....T....................C..........T........................................
Tsuga_sieboldii             .....T....................C..........T........................................

Abies_veitchii              TTCCAATTAAACGAGCGAGGAATTCCCTTTACTGATCAGAAGTTAAGAGCATTTAGTACCCGATATCGGGAGGTTTT
Keteleeria_evelyniana       ...................................A.........................................
Nothotsuga_longibracteata   .....................................................C.......................
Pseudolarix_amabilis        ...........A.........................................C.......................
Tsuga_canadensis            .....................................................C.......................
Tsuga_caroliniana           .....................................................C.......................
Tsuga_chinensis             .....................................................C.......................
Tsuga_diversifolia          .....................................................C.......................
Tsuga_dumosa                .....................................................C.......................
Tsuga_heterophylla          ...........A.........................................C.......................
Tsuga_mertensiana           .....................................................C.......................
Tsuga_sieboldii             .....................................................C.......................

Abies_veitchii              CCCTGAGTAGATCAGGGATTTATACACTCACAATGAGTCCTAAGTACTCGA-TACAAATGTGTAAGATAAATAGTGTA
Keteleeria_evelyniana       ...................................................-...G......................
Nothotsuga_longibracteata   ...................................................T...G......................
Pseudolarix_amabilis        ...................................................G...G......................
Tsuga_canadensis            ...................................................G..........................
Tsuga_caroliniana           ...................................................G..........................
Tsuga_chinensis             ...................................................G..........................
Tsuga_diversifolia          ...................................................G..........................
Tsuga_dumosa                ...................................................G..........................
Tsuga_heterophylla          ...................................................G..........................
Tsuga_mertensiana           ...................................................G..........................
Tsuga_sieboldii             ...................................................G..........................

Abies_veitchii              CTGACCAGGTTGATTTATCCCATGAGTCAGGAGAGCGATCGGTCGCCAGGATAAAAGATTTCGTTCTTCCTCATGAC
Keteleeria_evelyniana       .......................................T.....................................
Nothotsuga_longibracteata   .................................................T...........................
Pseudolarix_amabilis        ...........................................................C..-..............
Tsuga_canadensis            .................................................T.......A...................
Tsuga_caroliniana           .................................................T.....T.A...................
Tsuga_chinensis             .................................................T.......A...................
Tsuga_diversifolia          .................................................T.......A...................
Tsuga_dumosa                .................................................T.......A...................
Tsuga_heterophylla          .................................................T.......A...................
Tsuga_mertensiana           .................................................T.......A...................
Tsuga_sieboldii             .................................................T.......A...................

Abies_veitchii              GAATGAGATCCTTGGGTAGTAAATCTGTTGAATTTAATATAGAATCTTAATATCCGGATATATATATTTTTTTC--CT
Keteleeria_evelyniana       ......A....................C....AG.............G.........................TTT..
Nothotsuga_longibracteata   ...........................C....AG...........A.G..............C..........--C..
Pseudolarix_amabilis        ...........................C....AG.............G.......--.................--..
Tsuga_canadensis            ...........................C....AG.............T....T.........C..........--C..
Tsuga_caroliniana           ...........................C....AG.............T....T.........C..........T-C..
Tsuga_chinensis             ...........................C....AG.............T....T.........C..........--C..
Tsuga_diversifolia          .........T.................C....AG.............T....T.........C..........--C..
Tsuga_dumosa                ...........................C....AG.............T....T.........C..........--C..
```

```
Tsuga_heterophylla        ........................C.....AG.........T..T....T.......C........--C..
Tsuga_mertensiana         ........................C.....AG.........T..T....T.......C........--C..
Tsuga_sieboldii           ........................C.....AG...........GT....T.......C........--C..

Abies_veitchii            ATCTTGTCCCGACTAGATCGCACCATGTATTATCTCAGAAATTAAGAGGTTATTCTCATGAACGAGGGACATAGCTGA
Keteleeria_evelyniana     .................................T............................C........
Nothotsuga_longibracteata .................T..........................................A.C........
Pseudolarix_amabilis      ..............................T...............................C........
Tsuga_canadensis          .................T..........................................A.C.G......
Tsuga_caroliniana         .................T..........................................A.C.G......
Tsuga_chinensis           .................T.................T........................A.C.G......
Tsuga_diversifolia        .................T..........................................A.C.G......
Tsuga_dumosa              .................T..........................................A.C.G......
Tsuga_heterophylla        .................T................................T.........A.C.G......
Tsuga_mertensiana         .................T..........................................A.C.G......
Tsuga_sieboldii           .................T..........................................A.C.G......

Abies_veitchii            GGTTTGAAAATGGATGAGTTCCATAGATACGGGAAGGAAGATAGCTCTTGGCAACAATGCTTTTTATATCCACTCTTT
Keteleeria_evelyniana     .............................A.........................................
Nothotsuga_longibracteata .....TC......................AA........................................
Pseudolarix_amabilis      .....T.......................A......................................C..
Tsuga_canadensis          .............................A.........................................
Tsuga_caroliniana         .............................A.........................................
Tsuga_chinensis           .............................A.........................................
Tsuga_diversifolia        .............................A.........................................
Tsuga_dumosa              .............................A.........................................
Tsuga_heterophylla        .....T.......................A.........................................
Tsuga_mertensiana         .............................A.........................................
Tsuga_sieboldii           .............................A.........................................

Abies_veitchii            TTCCAGGAAGATCTTTACGCAATTTATCATGATCATTATTTGGATGGATCAAGTTCCTCTGAATCGATGGAACATTTA
Keteleeria_evelyniana     .....A.................C..............................................T
Nothotsuga_longibracteata .......................C..................T..............C.............
Pseudolarix_amabilis      .......................C..................T..............C.............
Tsuga_canadensis          .......................C..................TC.............C.............
Tsuga_caroliniana         .......................C..................T..............C.............
Tsuga_chinensis           ..................T....C..................T..............C.............
Tsuga_diversifolia        .......................C..................T..............C.............
Tsuga_dumosa              ..................T....C..................T..............C.............
Tsuga_heterophylla        .......................C..................T..............C....---------
Tsuga_mertensiana         .......................C..................T..............C.............
Tsuga_sieboldii           ..................T....C..................T..............C.............

Abies_veitchii            AGTTCCAATGATCAATTCAGTTTCCTAACTGTAAAACGTTTGATTGGTCAAATACGTCAACAGAATAATTCAATTGTT
Keteleeria_evelyniana     ............................................................C..........
Nothotsuga_longibracteata ...............T............................................C.......T..
Pseudolarix_amabilis      ..C..........................................................C..........
Tsuga_canadensis          ............A....A.........T.........................A..............T..
Tsuga_caroliniana         ............A....A.........T........................................T..
Tsuga_chinensis           ...........GA....A.........T........................................T..
Tsuga_diversifolia        ............A....A.........T.........................A..............T..
Tsuga_dumosa              ............A....A.........T........................................T..
Tsuga_heterophylla        ---......A..A....A.........T........................................T..
Tsuga_mertensiana         ............A....A.........T........................................T..
Tsuga_sieboldii           ...........GA....A.........T........................................T..

Abies_veitchii            TTCTTTTGAATTGCGATCCAAATCCATTAGTTGATCGCAACAAGAGTTTCTATTATGAATCGGTACTAGAAGGACTT
Keteleeria_evelyniana     ..A.............T...........................C....C....................G
Nothotsuga_longibracteata .CA..CG.....................................C....C....................G
Pseudolarix_amabilis      ..A..CG.................................T...C....C.....................
Tsuga_canadensis          ..A...G.......................A..A..........C....C....................G
Tsuga_caroliniana         ..A...G..........................A..........C....C......A.............G
Tsuga_chinensis           ..A...G..........................A..........C....C......A.............G
Tsuga_diversifolia        ..A...G..........................A..........C....C......A.............G
Tsuga_dumosa              ..A...G..........................A..........C....C......A.............G
Tsuga_heterophylla        ..A...G..........................A..........C....C....................G
Tsuga_mertensiana         .CA...G..........................A..........C....C....................G
Tsuga_sieboldii           ..A...G..........................A..........C....C......A.............G

Abies_veitchii            ACATTGGTCCTGGAAGTTCCGTTCTCTATACGGTCGAAATATTCTGTGGAAGGGATGAATGAATGGAAGAGTTTCCGA
Keteleeria_evelyniana     ......................................................................G
Nothotsuga_longibracteata .....................T..........A.....................................G
Pseudolarix_amabilis      ................................A.................C...................G
Tsuga_canadensis          ................................A..................T..................G
Tsuga_caroliniana         ................................A..................T.......T..........G
Tsuga_chinensis           ................................A..................T..................G
Tsuga_diversifolia        ................................A..................T..................G
Tsuga_dumosa              ................................A..................T..................G
Tsuga_heterophylla        ................................A..................T..................G
Tsuga_mertensiana         ................................A......T...........T..................G
```

```
Tsuga_sieboldii            ...................................A....................T....................G

Abies_veitchii             TCGATCCATTCAATATTTCCCTTCTTGGAAGATAAATTCCCGCATTCTAATTATATATTAGATACACGAATACCC
Keteleeria_evelyniana      ................................G..........................................
Nothotsuga_longibracteata  ...............................................A................A..........
Pseudolarix_amabilis       ......................................T........A...................A.......
Tsuga_canadensis           ...............................................A....................C......
Tsuga_caroliniana          ..........................T....................A....................C......
Tsuga_chinensis            ...............................................A....................C......
Tsuga_diversifolia         ...............................................A....................C......
Tsuga_dumosa               ...............................................A....................C......
Tsuga_heterophylla         ...............................................A....................C......
Tsuga_mertensiana          ...............................................A............T.......C......
Tsuga_sieboldii            ...............................................A....................C......

Abies_veitchii             ------------------------------------------------------------------------------
Keteleeria_evelyniana      ------------------------------------------------------------------------------
Nothotsuga_longibracteata  ACAGTGGGGGATCAT-CGATCCCCTCTT-----GTGTGGGGGCGTCGATGGCC-GTGGCCCATGCCCACTGGAAGGGT
Pseudolarix_amabilis       ------------------------------------------------------------------------------
Tsuga_canadensis           ......A...CCT..A..GC..T.CTCCTA---.........A..T....C.T-...A......T.AGAA........
Tsuga_caroliniana          T........ATCT..A.....A..C.CCTTAGCA........A..T....C.TC...A........AGAA........
Tsuga_chinensis            T.........TCT..A........C.CCTTAGCA........A..T....C.TC............AGAA........
Tsuga_diversifolia         ..........TCT..A........C.CCTTAGCA........A..T....C.TC...A........AGAA........
Tsuga_dumosa               .........ATCT..A.....A..C.CCTTAGCA........A..T....C.TC...A........AGAA........
Tsuga_heterophylla         ......A..ATCT.-A......T.CTCCT-ATT...C.....A.CG....C.T-..TA........AGAA........
Tsuga_mertensiana          .........ATCT.-A......T.CTCCT-ATT...C.....A.......C.T-..TA........AGAA........
Tsuga_sieboldii            T.........TCT..A........C.CCTTAGCA........A..T....C.TC............AGAA........

Abies_veitchii             ------------------------------------------------------------------------------
Keteleeria_evelyniana      ------------------------------------------------------------------------------
Nothotsuga_longibracteata  GCTCGGATTCGTCGTGG-GTGGCGGAGCTGTGTCATCCTGCCGGTCGGGAGGTGTAGCCTCGGGCATGGCCTGCGGCC
Pseudolarix_amabilis       ------------------------------------------------------------------------------
Tsuga_canadensis           ......C.....T....-......A..A...........TTGC.CG......C........C..TG...GT..T..G.
Tsuga_caroliniana          ......C.....T..TTT......A..A...........TTGC.CG......C.C......C..TG...GT..T..G.
Tsuga_chinensis            ......C.....T..TTT......A..A...........TTGC.CG......C........C..TG...GT..T..G.
Tsuga_diversifolia         ......C.....T..TTT......A..A...........TTGC.CG...............C..TG...GT..T..G.
Tsuga_dumosa               ......C.....T..TTT......A..A...........TTGC.CG......C........C..TG...GT..T..G.
Tsuga_heterophylla         ......C..........-......A..TC..........TTGC.CG......C........C..T....GT..A..GT
Tsuga_mertensiana          ......C.....T....-......A..T...........TTGC..G......C........C..T....GT..A..G.
Tsuga_sieboldii            ......C.....T..TTT......A..A...........TTGC.CG......C........C..TG...GT..T..G.

Abies_veitchii             ------------------------------------------------------------------------------
Keteleeria_evelyniana      ------------------------------------------------------------------------------
Nothotsuga_longibracteata  TGTCGGCCC-TCTCTACAATG-TTTTTGGCATGTGCT-TGTGTTTGTTGGCTCGGTGGGCTCTCCTCTTATCGCCCCC
Pseudolarix_amabilis       ------------------------------------------------------------------------------
Tsuga_canadensis           AT.....T.-......T..AA-.G............C....A.C...TT.......A..........GC........
Tsuga_caroliniana          ACC..T.T.-....C...TAA-.G............C......C...TT......TT..........GC..A.....
Tsuga_chinensis            ATC..A.T.-....C.T.TAA-.G............A......C...TT.....C.A..........GC........
Tsuga_diversifolia         ATC..T.T.-....C.T.TAA-.G............C......C...TT.......A..........GC........
Tsuga_dumosa               AT.....T.C......T.TAA-.G..C..........A......C...TT.......A..........GC........
Tsuga_heterophylla         G......T.-C...G.T.TAAG.G............T......C....T.......A..........GC........
Tsuga_mertensiana          G......T.-....G.T.TAA-.G............T......C....T.......A..........GC........
Tsuga_sieboldii            ATC....T.-....C.T.TAA-.G............C......C...TT.....C.A..........GC........

Abies_veitchii             ------------------------------------------------------------------------------
Keteleeria_evelyniana      ------------------------------------------------------------------------------
Nothotsuga_longibracteata  CAA-GGGGGGAGATGCAAGTTTCGCGGCCTTGCATGCCTC-GGCCTTCCGCAATGTGCATTTGTCGGAGAGTGATCCA
Pseudolarix_amabilis       ------------------------------------------------------------------------------
Tsuga_canadensis           A---..A...G..G...T....T.........T......T-................T.........G........GG
Tsuga_caroliniana          A.--.........G...T....T.........T......T-................T.........G........GG
Tsuga_chinensis            A.--.........G...T....T.........T......T-................T.........G........GG
Tsuga_diversifolia         A.--.........G...T....T.........T......T-........................T.G........GG
Tsuga_dumosa               A.--.........G...T....T.........T......T-................T.........G........GG
Tsuga_heterophylla         A..A...T.....G...T....T.........T......TT.......T........T.........G........GG
Tsuga_mertensiana          A.--...C.....G...T....T.......C.T......T-.......T........T........AG........GG
Tsuga_sieboldii            A.--.........G...T....T.........T......T-................T.........G........GG

Abies_veitchii             ------------------------------------------------------------------------------
Keteleeria_evelyniana      ------------------------------------------------------------------------------
Nothotsuga_longibracteata  TGGATGGATGAAAGCGCGGTGAAAACCGCGGGGCTGGCCACCCTAGTCCTCCT----GGTTGCTCTCTGCGGTTCTCT
Pseudolarix_amabilis       ------------------------------------------------------------------------------
Tsuga_canadensis           .............A.......C......T....................C.TCCC--.A.C....G....AA......
Tsuga_caroliniana          GC...........A.A.....C......T..A.................C...CCC-.A.C....G....AA......
Tsuga_chinensis            G............A.......C......T....................C...CCC-.A.C....G....AA......
Tsuga_diversifolia         G............A.......C......T....................C...CCC-.A.C....G....AA......
Tsuga_dumosa               ....G........A.......C......T....................C..CCCCC.A.C....G....AA......
Tsuga_heterophylla         .............A.A.....C......T........................CCC-...C....A....AA......
Tsuga_mertensiana          .............A.......C......T........................CCC-...C....G....AA......
Tsuga_sieboldii            G............A.......C......T....................C...CCC-.A.C....G....AA......
```

```
Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata TGCGGAGGCTTGGCGTTCAGGGTTTTGAGGAGTTCTAT------------------CGCATGGTTGTCGGCTTGTG
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          .....G....G.A...G..................C.CGATGGGTTGGAGTTGCTCT.........C......C...
Tsuga_caroliniana         .....G..T.G.A...G.............T....C.CGATGGGTTGGATCTGCTCT.........C......C...
Tsuga_chinensis           .....G..T.G.A...G..................C.CGATGGGTTGGAGCTGCTCT.........C......C.C.
Tsuga_diversifolia        .....G..T.G.A...G...........AA......C.CGATGGGTTGGAGCTGCTCT.........C......C...
Tsuga_dumosa              .....G..T.G.....G..................C.CGATGGGTTGGAGCTGCTCT..A......C......C...
Tsuga_heterophylla        .....G.C..G.A...G..................C.--GTGGGTTGGA---GATCT..............AC....
Tsuga_mertensiana         .....G.C..G.A...G..A................C.--GTGGGTTGGA---GCTCT....................
Tsuga_sieboldii           .....G..T.G.A...G..................C.CGATGGGTTGGAGCTGCTCT.........C......C...

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata ATGGTTGTCTTGGTTGAGCTCTCCTCTACCCACCCCCAAG--GGGGGGGAGGCATGTTTTGCGGCCTTGTGTGCCTCG
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          T.C......CC...-............T.T.G........--.....A..........CC................T.
Tsuga_caroliniana         T.C....C.CC...-............T.T.G........--.....A..........CC..........A.....T.
Tsuga_chinensis           T.C....C.CC...-............T.T.G........--.....A..........CC..........A.....T.
Tsuga_diversifolia        T.C....C.CC...-............T.T.G........--.....A..........CC..........A.....T.
Tsuga_dumosa              T.C....C.CC...-............T.T.G........--....TA..........CC..........A.....T.
Tsuga_heterophylla        T.C.......C...-....AA......T.T.T.......AAC.....T..........CC..........A.....T.
Tsuga_mertensiana         T.C......CC...-....AA......T.T.G........--.....T..........CC.........GA.....T.
Tsuga_sieboldii           T.C....C.CC...-............T.T.G........--.....A..........CC..........A.....T.

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata GTCTTCCGCAATGTGTA-TTTGTCGGGGAGTGATCACTGGATGGATGAAAACGCGGTGCAACACCCGCGGGGCTGGCC
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          .C....T..........-....G..C....C....GT...................G.....--..............
Tsuga_caroliniana         .C....T......C...-.................GT...................G.....--...T..........
Tsuga_chinensis           .C....T......A...-.................GT...................G.....--..............
Tsuga_diversifolia        .C....T......C...A.................GT...................G.....--..............
Tsuga_dumosa              .C....T......C...-............C....GT...................G.....--....T.........
Tsuga_heterophylla        .C....T......C...-.................GT.............G.....G.....--..............
Tsuga_mertensiana         .C..-.T......C...-.................GT.............G.....G.....--..............
Tsuga_sieboldii           .C....T...G..A...-.................GT...................G.....--........T.....

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata ACCCTAGTCC-TCCTGGTCCCTCACTGCAATTCTCTTGCGGAGGTCGGGCATTCAGGGTTTTGAGGAGT--GCTCATG
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          ..........-........G........G..............ACT.............C.........--T...TC.
Tsuga_caroliniana         ..........C...C....G........G..............ACT............C..........--T...TC.
Tsuga_chinensis           ..........C...C....G........G..............ACT............C..........--T...TC.
Tsuga_diversifolia        ..........C...C....G........G..............ACT............C..........--T...TC.
Tsuga_dumosa              ..........C...C....G........G..............ACT.........T..C..........GTT...TC.
Tsuga_heterophylla        ......A...-........G........G..........A...ACA............C..........--T...TC.
Tsuga_mertensiana         ..........-........G........G..............ACT............C..........--T...TC.
Tsuga_sieboldii           ..........C...C....G........G..............ACT............C..........--T...TC.

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata CTGGGTTGTTGGAGCTCTCGCATGGTTGTCGGCTTTTTTTGCATGTGCGTGCACGATGGGGGCGTGGATCGGAAAGGG
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          T..---C..C.A......T...C.T............C..-........................T........G...
Tsuga_caroliniana         T..---C..C.A......T...C...C.............-........................T............
Tsuga_chinensis           T..---C..C.A......T...C.............C..-........................T............
Tsuga_diversifolia        T..---C..C.A......T...C.................-........................T............
Tsuga_dumosa              ...---C..C.A......T...C.................-........................T............
Tsuga_heterophylla        T..---CA.C........T.............C...G...........................T............
Tsuga_mertensiana         T..---CT.C........T............TCC.-G...........................T............
Tsuga_sieboldii           T..---C..C.A......T...C.............C..-........................T............

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata CTTGCGCCCTCTATGCCCGTCGGTTCTCTCGAGCTTGTTGCATCCCGTGGGTTGTCCAGTGCTGGTTGCCGTTGCCTG
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          ..........T......T-..............GC.....TGC........C.A.....C....AC..........A.
Tsuga_caroliniana         ..........T.G....T-.T............GCC.....GC........C.A..........TCA........T..
Tsuga_chinensis           ............G....T-.T............GCC.....GC........C.A..........TCA........T..
Tsuga_diversifolia        ..........T.G....T-.T............GCC....TGC........C............TCA........T..
Tsuga_dumosa              ..........T.G....T-.T............GCC..C.TGC........C.A...........C.........TC.
Tsuga_heterophylla        ..........T..C...T-..............GC......GC........C.A...........C............
Tsuga_mertensiana         ..........T..C...T-.T............GC......GC........C.A...........C............
Tsuga_sieboldii           ............G....T-.T............GCC.....GC........C.A..........TCA........T..

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
```

```
Nothotsuga_longibracteata ACTTCTTCGCCTTGCCGTGATTGCTGTCGCGGGCTTCGGCACTGGAAGGTTGACG---TGTTGTTCTCGTGGGTGATT
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          ......C..A.CC.T......................A....C....A..C..T.GGC.A..C...............
Tsuga_caroliniana         ......CT...CC..................A.....A....C....A..C....GGC.A..C...............
Tsuga_chinensis           ......CT...CC........................A....C....A..C....GGC.A..C...............
Tsuga_diversifolia        ......CT...CC........................A....C....A..C....GGC.A..C...............
Tsuga_dumosa              ......CT....C.T......................A....C....A..C....GGC.A..C...............
Tsuga_heterophylla        ......CT...CC........................A........T..CC.G..GGC.A..C...............
Tsuga_mertensiana         ......CT...CC........................A........TA.C.....GGC.A..C...............
Tsuga_sieboldii           ......CT...CC........................A....C....A..C....GGC.A..C...............

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata GATCG----TTGGAGGGAGCGGACCCTGCCTCGCCGATGCGCTCTTCGGAAGCCTCGATGTCCCTTGACGTTTTGCAC
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          .....ATCG........T....T.....T..T................A...................T...CC..C.
Tsuga_caroliniana         .....----........T....T.....T...................A...................T...C...C.
Tsuga_chinensis           .....----........T....T.....T...................A...................T...CC..C.
Tsuga_diversifolia        .....----........T....T.....T...................A...................T...C...C.
Tsuga_dumosa              .....----........T....T.....T...................A...................T...CC..C.
Tsuga_heterophylla        .....----........T....T.....T...................A.....C..T.A........T..CCCA.CT
Tsuga_mertensiana         .....----........T....T.....T...................A.........T.........T..CCC..CG
Tsuga_sieboldii           .....----........T..T.T.....T.......T...........A...................T...CC..C.

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata GTGTGTCGCGGTTGTTGGGTAT-CTCAGCGGCGCTGCTTTCCTGACCGGTGCGCCTCTCTTTCCCAAGGGGAATCTAG
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          T..C......A.C.........-..........T.A.....TC......................A..........T.
Tsuga_caroliniana         T.........A.CC........-..........T.A.....TC..T.....T.............A..........T.
Tsuga_chinensis           T...........CC........-..........T.......TC..T.....T.............A..........T.
Tsuga_diversifolia        T...........CC.......AA..........T.A.....TC..T.....T.............A..........T.
Tsuga_dumosa              T..C........CC........-..........T.A.....TC......C.T.............A..........T.
Tsuga_heterophylla        ..........C.CC........-.......T....A.....TC....T...T.............A.......T.....
Tsuga_mertensiana         ..........C.C.........-.......C....A.....TC....T.................A............
Tsuga_sieboldii           T...........CC........-..........T.......TG..T.....T.............A..........T.

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata CCAGTGGATTGACATGGTTTGGACGGATGAAACGCGGCGCAAGCCGTTGGGTTGGCCACCCTAGTCCGTTCTCCATGC
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          TTG.G.......T---.GC............A.....................................CG.......
Tsuga_caroliniana         TTG.G.......T---.G.............A......................................C.......
Tsuga_chinensis           TTG.G.......T---.G.............A......................................C.......
Tsuga_diversifolia        TTG.G.......T---.G.............A......................................C.......
Tsuga_dumosa              TTG.G.......T---.G.............A......................................C.......
Tsuga_heterophylla        TTG.A......CT---.G.............T......................................C.......
Tsuga_mertensiana         TTG.G.A.C...T---.G.............T......................................C.......
Tsuga_sieboldii           TTG.G..G....T---.G.............A......................................C.......

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata TGTCCACTGCTGTTCTCCTCGGCGAGAGGGGCCTTCCGGGAAGGCCGCTGCTTGTCCCAGTCGGTTCGGCCATGGATT
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          .A.T.T.CA.A......G.T..T............A.............................C....--......
Tsuga_caroliniana         .A.T.T....A...T..G.T..T.G..........G.......T................C....CG....G......
Tsuga_chinensis           .ACT.T....A......G.T..T............G.......T..T............TC....CG....G......
Tsuga_diversifolia        .A.T.T.C..A......G.T..T............G.......T................C....CG....G......
Tsuga_dumosa              .A.T.T....A......G.T..T............G.......T................C....CG....G......
Tsuga_heterophylla        ...T.T....A......G.T..T............G.......T.....................CG.........A.
Tsuga_mertensiana         ...T.T.C..A......G.T..T............G.......T.....................CG.........A.
Tsuga_sieboldii           .A.T.C.C..A......G.T..T............G.......T..T............TC....CG....G...C.

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata TTCATGGGCGTCGGGTTTATACATCGATTGTGCGCATATGCACCCCCCCCCTTTCTTCCCTGGGTGCCTTGTGCATCG
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          G......C...................G....A.......C.-------A.G.G.TC.T..C................
Tsuga_caroliniana         G...............................A.......C.-------A.G.G.TC.T.TC................
Tsuga_chinensis           G...............................A.......C.-------A.G.G.TC.T..C................
Tsuga_diversifolia        G...............................A.......C.-------A.G.G.TC.T..C................
Tsuga_dumosa              G............C..................A.......C.-------A.G.G.TC.T..C................
Tsuga_heterophylla        G......CA..........C............A.........G.-----A.G...TC.T..C................
Tsuga_mertensiana         G......CA............................G....G.-----A.G.G.TC.T..C................
Tsuga_sieboldii           G........C......................A.......C.-------A.G.G.TC.T..C................

Abies_veitchii            --------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata GGGTGACTGTCCTGTGCCTCGTGGACCCCTTGCCGCGTGGTTTTGGGTTTGCAGGCAAC-GTTTAAAAGCAATGACTC
Pseudolarix_amabilis      ------------------------------------------------------------------------------
```

```
Tsuga_canadensis          ...C..T...T.......................TG................G......-AC.......A........
Tsuga_caroliniana         ......T...T...........A...........TG................G......-.C.......A........
Tsuga_chinensis           ...C..T...T...........A...........TG................G......-.C.......A........
Tsuga_diversifolia        ......T...T...........A...........TG.C..............GT.....-.C.......A........
Tsuga_dumosa              ......T...T.......................TG................G......-.C.......A........
Tsuga_heterophylla        ......T...T..C......C.............G.................G.....AA.C....G..A........
Tsuga_mertensiana         ......T...T........T.C.T..........G.................G.....AA.C.......A........
Tsuga_sieboldii           ...C..T...T...........A...........TG................G......-.C.......A........

Abies_veitchii            ------------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata TCGGCAACGGATATCTCGGCTCTCGTTACGATGAAGAACGTAGCGAAATGCGATACTTAGTGTGAATTGCAGAATCCC
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          .........................................................C....................
Tsuga_caroliniana         ..............................................................................
Tsuga_chinensis           ..............................................................................
Tsuga_diversifolia        ..............................................................................
Tsuga_dumosa              ..............................................................................
Tsuga_heterophylla        ..............................................................................
Tsuga_mertensiana         ..............................................................................
Tsuga_sieboldii           ................A.............................................................

Abies_veitchii            ------------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata GTGAATCATCAAGTCTTTGAACGCAATTTGCGCCCGAGGCCTCGGCTGAGGGCACGTTTGTCTGGGCGTCGCATACAA
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          ....................................G....T....................................
Tsuga_caroliniana         .........................................T....................................
Tsuga_chinensis           .........................................T....................................
Tsuga_diversifolia        .........................................T....................................
Tsuga_dumosa              .........................................T....................................
Tsuga_heterophylla        .........................................T....................................
Tsuga_mertensiana         .........................................T....................................
Tsuga_sieboldii           .......................G.................T....................................

Abies_veitchii            ------------------------------------------------------------------------------
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata TATACGCCCTCTCCGCACGGTAGCGGGGAGGAGCGGAGATGGTCGTCCGTGCCCAGCGTGGTGCGGTTGGCTGAAATG
Pseudolarix_amabilis      ------------------------------------------------------------------------------
Tsuga_canadensis          ...........TT.......................A.................A.......................
Tsuga_caroliniana         ...........TT.......................A.................A.......................
Tsuga_chinensis           C..........TT..G....................A.................A..C....................
Tsuga_diversifolia        ...........TT.......................A.................A.......................
Tsuga_dumosa              ...........TT.......................A.................A.......................
Tsuga_heterophylla        ............T.......................AT................A..............A........
Tsuga_mertensiana         ........A...T.......................A.................A..C....A......A........
Tsuga_sieboldii           ...........TT..G....................A.................A.......................

Abies_veitchii            ----------------------------------------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------------------------------------
Nothotsuga_longibracteata CATTCGATGTTGTGTGCTTTGCATGGGCTAGCGGTGGCCTTGTCCCCCCTTCGGCTGTGGGCGAGCCGGCGTTTGCCA
Pseudolarix_amabilis      ----------------------------------------------------------------------------
Tsuga_canadensis          .............C.........C..C......................T..T..C.......T...........C
Tsuga_caroliniana         .............C..C......C..C......................T..T..C.......T...........C
Tsuga_chinensis           .............C.........C..C......................T..T..G.......T...........C
Tsuga_diversifolia        .............C.........C..C......................T..T..G.......T...........C
Tsuga_dumosa              .........C...C...C.....C..C......................T..T..C.......T...........C
Tsuga_heterophylla        .............C.........C..C.........................T..G.......T.T.........C
Tsuga_mertensiana         .............C.........C..C......................T..T..........T.T.........C
Tsuga_sieboldii           ......G......C.........C..C......................T..T..G.......T...........C

Abies_veitchii            ----------------------------------------------
Keteleeria_evelyniana     ----------------------------------------------
Nothotsuga_longibracteata GTGCGAGGCGTGCTCGGTGTCGTATGAACTTTGTTTGGGCAGCATC
Pseudolarix_amabilis      ----------------------------------------------
Tsuga_canadensis          A.......T....C..AC.......................CT...
Tsuga_caroliniana         A.......T.....T.AC.......................CT...
Tsuga_chinensis           A.......T.C.....AC.......................CTG..
Tsuga_diversifolia        A.......T.C.....AC.......................CT...
Tsuga_dumosa              A.......T.......AC.......................CT...
Tsuga_heterophylla        A...T...T....C..AC.......................CT...
Tsuga_mertensiana         A...T...T....C..AC.......................CT...
Tsuga_sieboldii           A.......T.C.....AC.......................CT...

Abies_veitchii            CATTACAAATGCGATGCTCTAACCTCTGAGCTAAGCAGGCTCAATGGAATATAACTCCTCATTTCATTGGCGTGAGAT
Keteleeria_evelyniana     ..............................................................................
Nothotsuga_longibracteata ..............................................................................
Pseudolarix_amabilis      .......................................................................TA.....
Tsuga_canadensis          ..............................................................................
Tsuga_caroliniana         ..............................................................................
```

```
Tsuga_chinensis           ..............................................................................
Tsuga_diversifolia        ..............................................................................
Tsuga_dumosa              ..............................................................................
Tsuga_heterophylla        .............................................C................................
Tsuga_mertensiana         ..............................................................................
Tsuga_sieboldii           ..............................................................................

Abies_veitchii            CCGTAGATTCTTTTGGAATCCTAACAATTATAGCGCGAATCAGATTCAATGACGCAATCCAGATTACAATTACAA-TG
Keteleeria_evelyniana     ..............................................T.....T......T...............-C.
Nothotsuga_longibracteata .....T......................................C.................T............-CT
Pseudolarix_amabilis      ...........................................................................-C.
Tsuga_canadensis          .....T...........................T..........C................AT............AC.
Tsuga_caroliniana         .....T...........................T..........C................AT............AC.
Tsuga_chinensis           .....T...........................T..........C................AT............AC.
Tsuga_diversifolia        .....T...........................T..........C................AT............AC.
Tsuga_dumosa              .....T...........................T..........C................AT............AC.
Tsuga_heterophylla        .....T...........................T..........C................AT............AC.
Tsuga_mertensiana         .....T...........................T..........C................AT............AC.
Tsuga_sieboldii           .....T...........................T..........C................AT............AC.

Abies_veitchii            GAACATCACCTGAATGTAGATTAAAGGACAGAAAAGGGAAGTAAGGAAGGGTCAATTGATAT---CGATAATTTGAC
Keteleeria_evelyniana     ......................C.....A.....A...........................---....CG...T..
Nothotsuga_longibracteata ......A...............C.....A............................T....CAA....CG...T..
Pseudolarix_amabilis      ......................C.....A........................C........---....CG...T..
Tsuga_canadensis          ......................C.....A.C..........................T....AAA....CG...T..
Tsuga_caroliniana         ......A...............C.....A.C..........................T....AAA....CG...T..
Tsuga_chinensis           ......A...............C.....A.C..........................T....AAA....CG...T..
Tsuga_diversifolia        ......A...............C.....A.C.-----....................T....AAA....CG...T..
Tsuga_dumosa              ......A...............C.....A.C..........................T....AAA....CG...T..
Tsuga_heterophylla        ......................C.....A.C........................C.T....CAA....CG...T..
Tsuga_mertensiana         ........T.............C.....A.C..........................T....CAA....CG...T..
Tsuga_sieboldii           ......A...............C.....A.C..........................T....AAA....CG...T..

Abies_veitchii            TCACTATTCCAAATCGACTAGAGGAGGATAATAACATTGCATTGAAAATGCAGAAATAATATAATGATTCCCAGTC--
Keteleeria_evelyniana     ..............G..............C..............................................--
Nothotsuga_longibracteata ..............T.......A..................................................G..GT
Pseudolarix_amabilis      .....................G......................................................--
Tsuga_canadensis          ......................A....................TC............................G..GT
Tsuga_caroliniana         ......................A....................TC............................G..GT
Tsuga_chinensis           ......................A..T.................TC............................G..GT
Tsuga_diversifolia        ......................A....................TC............................G..GT
Tsuga_dumosa              ......................A..T.................TC............................G..GT
Tsuga_heterophylla        ......................A....................TC............................G..GT
Tsuga_mertensiana         ......................A....................TC............................G..GT
Tsuga_sieboldii           ......................A..T.................TC............................G..GT

Abies_veitchii            -ACATTCGATTGGGGTAGAGATAGAGATGGCGAGAGAGGGGGAGT----AGGAGAGGATATTTCTTCCACCCAATATT
Keteleeria_evelyniana     -.T..........A..A............................GAGT............T.C..............
Nothotsuga_longibracteata C.T....................A.....T.............A.----..C.........T.C..............
Pseudolarix_amabilis      -.T..............................A...........----..............C..............
Tsuga_canadensis          C.T..........A........A.....T............A.A.----...A.T......A.C...T..........
Tsuga_caroliniana         C.T..........A........A.....T............A.A.----.....T........C..............
Tsuga_chinensis           C.T.........AA........A.....T............A.A.----.....T......A.C..............
Tsuga_diversifolia        C.T..........A........A.....T............A.A.----.....T......A.C..............
Tsuga_dumosa              C.T..........A........A.....T............A.A.----.....T......-----------------
Tsuga_heterophylla        C.T..........A........A.....T..............A.----.....T......A.C..............
Tsuga_mertensiana         C.T..........A........A.....T..............A.----.....T......A.C..............
Tsuga_sieboldii           C.T..........A........A.....T............A.A.----.....T......A.C..............

Abies_veitchii            GAGCAAATATCCAATGAATAACGCTGATGGAGATTACATAATAGGATTAGATTAAGGTATGATCTCGTTCTCCGAGAA
Keteleeria_evelyniana     ............................................A.................................
Nothotsuga_longibracteata ...........T..C......A.............................G..........................
Pseudolarix_amabilis      ..............G.....................................C.........................
Tsuga_canadensis          ...........T..C......A..........................T...................--------..
Tsuga_caroliniana         ...........T..C......A..........................T.............................
Tsuga_chinensis           ...........T..C......A..........................T....................A........
Tsuga_diversifolia        ...........T..C......A..........................T.............................
Tsuga_dumosa              -----------------------------------------------------.........................
Tsuga_heterophylla        ...........T..C......A..........................T.............................
Tsuga_mertensiana         ...........T..C......A..........................T.............................
Tsuga_sieboldii           ...........T..C......A..........................T.............................

Abies_veitchii            GGGGATATGGCGAAATCGGTAGACGCTACGGACTTGATCGAATTGAGCCTTGGTATGGAAACCTACCAAGTGATAGCT
Keteleeria_evelyniana     ..............................................................................
Nothotsuga_longibracteata ..............................................................................
Pseudolarix_amabilis      ..............................................................................
Tsuga_canadensis          ..............................................................................
Tsuga_caroliniana         ..............................................................................
Tsuga_chinensis           ..............................................................................
Tsuga_diversifolia        ..............................................................................
```

```
Tsuga_dumosa              ......................................................................
Tsuga_heterophylla         ......................................................................
Tsuga_mertensiana          ......................................................................
Tsuga_sieboldii          ......................................................................

Abies_veitchii            TCCAAATCCAGGGAACCCTGGGATATTTTGAATGGGTAATCCTGAGCCAAATCCGGTTCATAGAGAAAAGGGTTT---
Keteleeria_evelyniana     ..................................................................C..T.....---
Nothotsuga_longibracteata .......T..........................................................C..T.....---
Pseudolarix_amabilis      ..................................................................C..T.....---
Tsuga_canadensis          .......T.........C................................................C..T.....---
Tsuga_caroliniana         .......T.........C................................................C..T.....---
Tsuga_chinensis           .......T.........C................................................C..T.....---
Tsuga_diversifolia        .......T.........C................................................C..T.....GTT
Tsuga_dumosa              .......T.........C................................................C..T.....---
Tsuga_heterophylla        .......T.........C................................................C..T.....---
Tsuga_mertensiana         .......T.........C................................................C..T.....---
Tsuga_sieboldii           .......T.........C................................................C..T.....---

Abies_veitchii            -CTCTCCTTCTCCTA----AGGAAAGGGATAGGTGCAGAGACTCAATGGAAGCTATTCTAACGAATGAATCTCATTTG
Keteleeria_evelyniana     -..T....AGGAT------.....G.....................................................
Nothotsuga_longibracteata -..T....AGGAT------.....G.....................................................
Pseudolarix_amabilis      -..T....AGG....GGAT.....G.....................................................
Tsuga_canadensis          -..T....AGGAT------.....G..............................................T......
Tsuga_caroliniana         -..T....AGGAT------.....G..............................................T......
Tsuga_chinensis           -..T....AGGAT------.....G..............................................T......
Tsuga_diversifolia        T..T....AGGAT------.....G..............................................T......
Tsuga_dumosa              -..T....AGGAT------.....G..............................................T......
Tsuga_heterophylla        -..T....AGGAT------.....G..............................................T......
Tsuga_mertensiana         -..T....AGGAT------.....G..............................................T......
Tsuga_sieboldii           -..T....AGGAT------.....G..............................................T......

Abies_veitchii            GGGTCCAATACTCTATTTATAGAACGTTCTATTT--------------ACACCTCGAAAGTAGGAATGTTATATAAC
Keteleeria_evelyniana     .......G......---------------------------------------------------------------
Nothotsuga_longibracteata ..........................C.T.....--------------............G.......T.....A
Pseudolarix_amabilis      ..................................--------------............G.......G.....
Tsuga_canadensis          ..........................C.T.....--------------............G.......G.....A
Tsuga_caroliniana         ..........................C.T.....--------------............G.......G.....A
Tsuga_chinensis           ..........................C.T.....ACACCTCGAAAATTT............G.......G.....A
Tsuga_diversifolia        ..........................C.T.....--------------............G.......G.....A
Tsuga_dumosa              ..........................CGT.....--------------............G.......G.....A
Tsuga_heterophylla        .A........................C.T.....--------------............G.......G.....A
Tsuga_mertensiana         ..........................C.T.....--------------............G.......G.....A
Tsuga_sieboldii           ..........................C.T.....--------------............G.......G.....A

Abies_veitchii            ATCAAACAAAACTCGCGATCAGAACTTGAATTGTTCCAAGCATTTTATTCGTAAAATAGATGCCAGATTCGAGTTGAA
Keteleeria_evelyniana     ------------------------------------------------------------------------------
Nothotsuga_longibracteata ..A...A......T................C.....................G..A.........T.......
Pseudolarix_amabilis      ..A.........................C.....................G......................
Tsuga_canadensis          .........C....................C...............T...G..A.........T........
Tsuga_caroliniana         ..A......C....................C...T............T...G.G.A.........T........
Tsuga_chinensis           ..A......C.............T......C...T............T...G..A.........T........
Tsuga_diversifolia        ..A......C....................C...T............T...G.G.A.........T........
Tsuga_dumosa              ..A......C....................C...T.........G...T...G.G.A.........T........
Tsuga_heterophylla        ..A......C................C...C................T...G.G.A.........TT.......
Tsuga_mertensiana         .........C................G...C................T...G.G.A.........T........
Tsuga_sieboldii           ..A......C....................C...T............T...G..A.........T........

Abies_veitchii            GTAATAATTTTACATTAAGTAATCAAATTATGAACTT-ATCTACTCTAGATAGGGAGTTGAATCAGTTTTTGGAATAA
Keteleeria_evelyniana     -------------------------------------....................A.................
Nothotsuga_longibracteata .....T....G............CC...........-C................G.......T.............
Pseudolarix_amabilis      ..C..G.................C............-.......................................
Tsuga_canadensis          .....G....G............C............TC......................................
Tsuga_caroliniana         .....G....G............C............TC...................................G..
Tsuga_chinensis           .....G....G............C............TC...................................G..
Tsuga_diversifolia        .....G....G............C............TC...................................G..
Tsuga_dumosa              .....G....G............C............TC......................................
Tsuga_heterophylla        .....G....G............C............-C......................................
Tsuga_mertensiana         .....G....G............C............-C......................................
Tsuga_sieboldii           .....G....G............C............TC...................................G..

Abies_veitchii            ATGA-----TTGGACGAGGATAAAGATAGAGTCCAATTCTACGTGTCAATGTAAACAACAATGCAAATTGCAGTAGAA
Keteleeria_evelyniana     ....AATGA.........................................C.......................G.
Nothotsuga_longibracteata ....-----.........................................C.......................G.
Pseudolarix_amabilis      ....-----.........................................C.......................G.
Tsuga_canadensis          ..T.-----.................G.......................C.......................G.
Tsuga_caroliniana         T.T.-----.........................................C.......................G.
Tsuga_chinensis           ..T.-----.........................................C.......................G.
Tsuga_diversifolia        ..T.-----.........................................C.......................G.
Tsuga_dumosa              ..T.-----.........................................C.......................G.
Tsuga_heterophylla        ....-----.........................................C.......................G.
```

```
Tsuga_mertensiana         ....-----..........................................C......................G.
Tsuga_sieboldii          ..T.-----..........................................C......................G.

Abies_veitchii           GGAAAATCCGTTGGCTTTATAGACCGTGAGGGTTCAAGTCCCTCTATCCCCACCCAGGTTCATTCCCGAACGACTGAT
Keteleeria_evelyniana     ...........................................................C..............
Nothotsuga_longibracteata ...............................-....................T.......G...............
Pseudolarix_amabilis      ....................................................T..----.G...............
Tsuga_canadensis          ....................................................T.......G...............
Tsuga_caroliniana         ....................................................T.......G...............
Tsuga_chinensis           ....................................................T.......G...............
Tsuga_diversifolia        ...................................................AT.......G..T............
Tsuga_dumosa              ....................................................T.......G...............
Tsuga_heterophylla        ....................................................T.......G...............
Tsuga_mertensiana         ....................................................T.......G...............
Tsuga_sieboldii           ....................................................T.......G...............

Abies_veitchii           CTATTTTATCCAATTCCGTTAGTTCAAATCCATTCTCACTTCTCTTTGACCTCACTATTTCATTTATTCATGTTTATT
Keteleeria_evelyniana     .C......................TG................--------.T.T.C.....CC.CAAT..-......
Nothotsuga_longibracteata .C......................TG.................T.A.----..TT.CACC..CC.CACT..-......
Pseudolarix_amabilis      ....C....................G................--------.T.T.C.....CC.CACT..-------
Tsuga_canadensis          .C......................TT...T............T.A.----..TT.CAC...CC.CACT..-......
Tsuga_caroliniana         .C......................TT...T............T.A.----..TT.CAC...CC.CACT..-......
Tsuga_chinensis           .C......................TT...T............T.A.----..TT.CAC...CC.CACT..-......
Tsuga_diversifolia        .C......................TT...T............T.A.----.TTT.CAC...CC.CACT..-......
Tsuga_dumosa              .C......................TT...T............T.C.----..TT.CAC...CC.CACT..-..----
Tsuga_heterophylla        .C......................TT...T............T.A.----..TT.CACCT.CC.CACT..-..C...
Tsuga_mertensiana         .C...................A..TG...T............T.A.----..TT.CACCT.CC.CACT..-......
Tsuga_sieboldii           .C......................TT...T............T.A.----..TT.CAC...CC.CACT..-......

Abies_veitchii           TATTCATGAAGAGAAGAAATGGGAACATGAATCTTTCCATCTTATGACAAGTTGAGTTGATCAGTGGATCAATTCATT
Keteleeria_evelyniana     .....................AT.....T...................................T............
Nothotsuga_longibracteata ..C..................AA....G.................T..................T............
Pseudolarix_amabilis      -----................AT....G....A...A...........................T............
Tsuga_canadensis          .....................AA....G....................................T............
Tsuga_caroliniana         .....................AA....G....................................T............
Tsuga_chinensis           ............A........AA....G....................................T............
Tsuga_diversifolia        .....................AA....G....................................TT...........
Tsuga_dumosa              ------------------------------------------------------------------------------
Tsuga_heterophylla        .....................AA....G....................................T............
Tsuga_mertensiana         .....................AA....G.............................T......T............
Tsuga_sieboldii           ............A........AA....G....................................T............

Abies_veitchii           TTGTCATATATGATCCACATAGATGTGATCATTTGGAAATTATTCGATCGCAGTCGATTTTTTA--TCGTATTAGTTA
Keteleeria_evelyniana     .........................A.............................C.......C--..A.........
Nothotsuga_longibracteata ....G....................A.........A........T..........C.......TTC..A.........
Pseudolarix_amabilis      .........................A...A..............T........CTT........--..A.........
Tsuga_canadensis          ....G....................A..................T..........C.......T-C..A.........
Tsuga_caroliniana         ....G....................A..................T..........C.......T-C..A.........
Tsuga_chinensis           ....G....................A..................T..........C.......T-C..A.........
Tsuga_diversifolia        ....T....................A..................T..........C.......T-C..A.........
Tsuga_dumosa              --..G....................A..................T..........C.......T-C..A.........
Tsuga_heterophylla        ....G....................A..................T..........C.......T-C..A.........
Tsuga_mertensiana         ....G....................A..........C......T..........C.......T-C..A.........
Tsuga_sieboldii           ....G....................A..................T..........C.......T-C..A.........

Abies_veitchii           TTTCCAGATCGAAAAGAATAAAGATCATTCTAAAAACTGGGA------------AAAATCCATTTCTTCCTTATTTTT
Keteleeria_evelyniana     C............................A.C..........------------....GAAT............C...
Nothotsuga_longibracteata C.........C....T...............C...-------------------------------------------
Pseudolarix_amabilis      C..............T...............C..........------------.......T................
Tsuga_canadensis          C..............T.........A................------------.......T.......T........
Tsuga_caroliniana         C..............T.........A................------------.......T.......T........
Tsuga_chinensis           C..............T.........A...A............------------.......T.......T........
Tsuga_diversifolia        C..............T.........A...........AAAATCCTTTTCTTCTT.......T.......T........
Tsuga_dumosa              C..............T.........A................------------.......T.......T........
Tsuga_heterophylla        C..............T.........A................------------.......T.......T---.....
Tsuga_mertensiana         C........T.....T.........A................------------.......T.......T........
Tsuga_sieboldii           C..............T.........A................------------.......T.......T........

Abies_veitchii           AGTTGACACGAGTTAAAACCCTGTACCAGGATGATCCACAGGAAAGAGCCGGGATAGCTCAGTTGGTAGAGCAGAGGA
Keteleeria_evelyniana     .........A.T..C.........G..........A..........................................
Nothotsuga_longibracteata ---------------------.........................................................
Pseudolarix_amabilis      .........A....................................................................
Tsuga_canadensis          .........A....................................................................
Tsuga_caroliniana         .........A....................................................................
Tsuga_chinensis           .........A....................................................................
Tsuga_diversifolia        .........A....................................................................
Tsuga_dumosa              .........A....................................................................
Tsuga_heterophylla        .........A....................................................................
Tsuga_mertensiana         .........A....................................................................
Tsuga_sieboldii           .........A.................................G..................................
```

```
Abies_veitchii            CTGAAAATCCTCGTGTCACCAGTTCAAAT
Keteleeria_evelyniana     .............................
Nothotsuga_longibracteata .............................
Pseudolarix_amabilis      .............................
Tsuga_canadensis          .............................
Tsuga_caroliniana         .............................
Tsuga_chinensis           .............................
Tsuga_diversifolia        .............................
Tsuga_dumosa              .............................
Tsuga_heterophylla        .............................
Tsuga_mertensiana         .............................
Tsuga_sieboldii           .............................

Abies_veitchii            ATTTTGATCTTTCGTGAAGCGAGAAAGGTGTTTGGTAATGCGGAAAAA------GAAAAAAAAAAAAGAAGAAGGAGA
Keteleeria_evelyniana     .....TT.........................................------..........---------.....
Nothotsuga_longibracteata .....TC.........................................-----------G..........---.....
Pseudolarix_amabilis      ............................T...................------................---.....
Tsuga_canadensis          .....T..............................T...........CGGAAAA.G.............---.....
Tsuga_caroliniana         .....T..............................T...........-------G..............---.....
Tsuga_chinensis           .....T..............................T...........----------G...........---.....
Tsuga_diversifolia        .....T..............................T...........-------------G.....A..---.....
Tsuga_dumosa              .....TC.............................T...........----------G...........---.....
Tsuga_heterophylla        .....T..........................................----------G...........---.....
Tsuga_mertensiana         .....TC.........................................----------G...........---.....
Tsuga_sieboldii           .....T..............................T...........-------------G.....A.----.....

Abies_veitchii            ATTGAAATCTTCTCCCAATATTGTATGGTTAATTAATTACCCTCCGAAAAGCAGTGTGATAAAGCATAAGAATCATCC
Keteleeria_evelyniana     .A............................C.........T.....................................
Nothotsuga_longibracteata .A.............-..............T..............A................................
Pseudolarix_amabilis      .A............................C--------.G.....................................
Tsuga_canadensis          .A.T...........-..............C..............A................................
Tsuga_caroliniana         .A.TC..........-..............C..............A................................
Tsuga_chinensis           .A.T.........T.-..............C..............A................................
Tsuga_diversifolia        .A.TC..........-..............C..............A................................
Tsuga_dumosa              ...T.........T.-..............C..............A................................
Tsuga_heterophylla        .A.............-..............C..............A................................
Tsuga_mertensiana         .A.TC..........-..............C..............A................................
Tsuga_sieboldii           .A.T...........-..............C..............A................................

Abies_veitchii            TGATTCATTCAAGGAAGATTGAAGGGATTCAGTTTATGAAGGAGAAAGAGCTTCGGATCGATGGAAACTAAGGAAATT
Keteleeria_evelyniana     ...C........C...........................................G.....................
Nothotsuga_longibracteata .....T......C..A........................................G.....................
Pseudolarix_amabilis      ............C...........................................G.....................
Tsuga_canadensis          .....T.....AC..A........................................G.....................
Tsuga_caroliniana         .....T.....AC..A........................................G.....................
Tsuga_chinensis           .....T.....AC..A........................................G.....................
Tsuga_diversifolia        .....T.....AC..A........................................G.....................
Tsuga_dumosa              .....T.....AC..A........................................G.....................
Tsuga_heterophylla        .....T......C..A........................................G.....................
Tsuga_mertensiana         .....T......C..A........................................G.....................
Tsuga_sieboldii           .....T.....AC..A........................................G.....................

Abies_veitchii            GATTCCATAACAGATGAAAAGAAAGCTCCATTGCAGAGGTAAGACCTGGGCATTTAGATAGAAGCTATGGAACGATGG
Keteleeria_evelyniana     ......G...........................G...G......................................
Nothotsuga_longibracteata ......G...........................G...G......................................
Pseudolarix_amabilis      ......G................................G......................................
Tsuga_canadensis          ......G...........................G...G.......A..............................
Tsuga_caroliniana         ......G...........................G...G.......A..............................
Tsuga_chinensis           ......G...........................G...G.......A..............................
Tsuga_diversifolia        ......G...........................G...G.......A..............................
Tsuga_dumosa              ......G...........................G...G.......A..............................
Tsuga_heterophylla        ......G...........................G...G.......A..............................
Tsuga_mertensiana         ......G...........................G...G.......A..............................
Tsuga_sieboldii           ......G...........................G...G.......A..............................

Abies_veitchii            AACCTATGACTGCATAAGATCATATAGGAATCTTAATCATTCATTGGATAGGATGGCGAAATAAACCAAAAACGAATT
Keteleeria_evelyniana     ....................................................................G.........
Nothotsuga_longibracteata ........................................C...........................G.........
Pseudolarix_amabilis      .....................T..............................................G........A
Tsuga_canadensis          ........................................C...........................G.........
Tsuga_caroliniana         ....G...................................C.C.........................G.........
Tsuga_chinensis           ........................................C...........................G........A
Tsuga_diversifolia        ........................................C...........................G.........
Tsuga_dumosa              ....C...................................C...........................G.........
Tsuga_heterophylla        ........................................C...........................G.........
Tsuga_mertensiana         ...........................A............C...........................G........G
Tsuga_sieboldii           ........................................C...........................G.........

Abies_veitchii            AATCTCATTGGATGGAGTCTATAAGTA-----AGTCGACCCCATGGAGCAAATACCGAAAGAGTCCATATTCGCCTGT
```

```
Keteleeria_evelyniana     .....A......----....C.G....-----.........................G.......A............
Nothotsuga_longibracteata ............----...........-----...T............T...............AA............
Pseudolarix_amabilis      ...A........----......G....GGTCA.........................G......AA............
Tsuga_canadensis          ............----...........-----........................AG.......A............
Tsuga_caroliniana         ............----...........-----........................AG.......A............
Tsuga_chinensis           ............----...........-----........................AG.......A............
Tsuga_diversifolia        ............----...........-----........................AG.......A............
Tsuga_dumosa              ............----...........-----........................AG.......A............
Tsuga_heterophylla        ............----...........-----.........................G.......A............
Tsuga_mertensiana         ............----...........-----.......T.................G......AA............
Tsuga_sieboldii           ............----...........-----........................AG.......A............

Abies_veitchii            GAAATTATTTATTAAGAGTCTCGTTGGATTGAAA-T-------------AAAGAGTTATTCGTCCCATAAATTAGATT
Keteleeria_evelyniana     ..........C...........A...........-G-------------........T........G...........
Nothotsuga_longibracteata ......................A...........TATTGGATTGAAATA.........................T...
Pseudolarix_amabilis      ........-----....A...AA...........TA-------------.................G......CT...
Tsuga_canadensis          ......................A...........TA-------------...A.............G...........
Tsuga_caroliniana         .........A............A...........TA-------------...A.............G...........
Tsuga_chinensis           .........A............A...........TA-------------...A.............G...........
Tsuga_diversifolia        .........A............A...........TA-------------...A.............G...........
Tsuga_dumosa              .........A............A...........TA-------------...A.............G...........
Tsuga_heterophylla        ...T..................A.......TC..TA-------------...A.............G...........
Tsuga_mertensiana         ......................A.......T...TA-------------...A.............G...........
Tsuga_sieboldii           .........A............A...........TA-------------...A.............G...........

Abies_veitchii            CTATTTATGAT----ATGCGTAATGCGTAGATATAGACTCATTTATATATA-------------------ACTAATAA
Keteleeria_evelyniana     ....C......----....A...............................T-----------------A........
Nothotsuga_longibracteata .......C...----....................................---------TATATA---T........
Pseudolarix_amabilis      T......C...CGAT....................................-------------------........
Tsuga_canadensis          .......C...----....................................-TAAATATATATATA--TA........
Tsuga_caroliniana         .......C...----....A...............................-T--ATATATATATA--TA........
Tsuga_chinensis           .......C...----....................................-----TA----TA----TA........
Tsuga_diversifolia        .......C...----....................................ATATATATATATA----TA........
Tsuga_dumosa              .......C...----....................................-----TAA-TATA----TA........
Tsuga_heterophylla        .......C...----................................C....---------TATAGA--TA........
Tsuga_mertensiana         .......C...----................................C...C-TATATATATATATA--TA........
Tsuga_sieboldii           .......C...----....................................ATATATATATATATATATA........

Abies_veitchii            CTAACTACACATTGTCCAATTTGATATAGATTCTTCACAAGGA-----------
Keteleeria_evelyniana     .........T..............C..................GCCGGATGAGA
Nothotsuga_longibracteata ......................T.C.......T..-------------------
Pseudolarix_amabilis      ........................C..................GCCGGATGAG-
Tsuga_canadensis          ......................T.C...--------------------------
Tsuga_caroliniana         ......................T.C.......T....-----------------
Tsuga_chinensis           ......................T.C.......T..........GCCGG------
Tsuga_diversifolia        ......................T.C.......T.........------------
Tsuga_dumosa              ......................T.C.......T..........GC---------
Tsuga_heterophylla        ......................T.C.......T..........GCC--------
Tsuga_mertensiana         ......................T.C.......T.....----------------
Tsuga_sieboldii           ......................T.C.......T......---------------

Abies_veitchii            CTGGTGTTAAAGATTACAGATTAACTTATTATACTCCTGAATATCAGACCAAAGATACGGATATCTTGGCGGCATTCC
Keteleeria_davidiana      ................T.............................................................
Nothotsuga_longibracteata ..........G.....T...C.................................................A.......
Pseudolarix_amabilis      .................C....................................................A.......
Tsuga_canadensis          ..........G.....T.....................................................A.......
Tsuga_caroliniana         ------------------------------------------------------------------------------
Tsuga_chinensis           ..........G.....T.........G..G........................................A.....A.
Tsuga_diversifolia        ------------------------------------------------------------------------------
Tsuga_dumosa              ..........G.....T.........G..G........................................A.....A.
Tsuga_heterophylla        ..........G.....T.....................................................A.......
Tsuga_mertensiana         ..........G.....T.....................................................A.......
Tsuga_sieboldii           ------------------------------------------------------------------------------

Abies_veitchii            GAGTAACTCCTCAACCTGGGGTGCCGCCCGAGGAAGCGGGAGCAGCAGTAGCTGCTGAATCTTCCACCGGTACATGGA
Keteleeria_davidiana      .......C......................................................................
Nothotsuga_longibracteata ................C..A.........................................G................
Pseudolarix_amabilis      ................C........A....................................................
Tsuga_canadensis          ................C..A.........................................G................
Tsuga_caroliniana         ------------------------------------------------------------------------------
Tsuga_chinensis           ................C..A.........................................G.....T..........
Tsuga_diversifolia        ------------------------------------------------------------------------------
Tsuga_dumosa              ................C..A.........................................G.....T..........
Tsuga_heterophylla        ................C..A.........................................G................
Tsuga_mertensiana         ................C..A.........................................G................
Tsuga_sieboldii           ------------------------------------------------------------------------------

Abies_veitchii            CCACTGTTTGGACCGATGGACTTACCAGTCTTGATCGTTACAAAGGGCGATGCTATGACATCGAGCCCGTTGCTGGAG
Keteleeria_davidiana      ...........................................G...........................C......
Nothotsuga_longibracteata ............................................G....................A......C......
```

```
Pseudolarix_amabilis      ...........................................G.................T.........C......
Tsuga_canadensis          ...........................................G....................A......C......
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           .T........................................G...........................C......
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              .T........................................G...........................C......
Tsuga_heterophylla        ...........................................G....................A......C......
Tsuga_mertensiana         ...........................................G....................A......C......
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            AGGAAAGTCAATTTATTGCCTATGTAGCTTACCCCTTAGACCTTTTCGAAGAAGGTTCTGTTACTAACTTGTTCACTT
Keteleeria_davidiana      .A............................................................................
Nothotsuga_longibracteata .A..G.................................................................A.......
Pseudolarix_amabilis      ....G..C......................................................................
Tsuga_canadensis          .A..G.................................................................A.......
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           .A..G.................................................................A.......
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              .A..G.................................................................A.......
Tsuga_heterophylla        .A..G.................................................................A.......
Tsuga_mertensiana         .A..G.................................................................A.......
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            CCATTGTAGGTAATGTATTTGGATTCAAGGCCCTACGGGCTTTACGTTTGGAAGATTTGCGGATTCCCCCTGCTTATT
Keteleeria_davidiana      ..........................................C....................................
Nothotsuga_longibracteata .....................................A...C....................................
Pseudolarix_amabilis      ..........................................C....................................
Tsuga_canadensis          ..........................................C....................................
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           .........................................C.............................T......
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              .........................................C.............................T......
Tsuga_heterophylla        ..........................................C....................................
Tsuga_mertensiana         ..........................................C....................................
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            CCAAAACTTTTCAAGGTCCACCTCATGGTATCCAAGTTGAAAGAGATAAATTGAACAAATATGGCCGTCCTTTGTTGG
Keteleeria_davidiana      ...............................CT................................T............
Nothotsuga_longibracteata ...................G............................................T............
Pseudolarix_amabilis      .............................................................................
Tsuga_canadensis          ...................G.........................................C..T............
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           ...............T...G.................TC.....................C..T............
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              ...............T...G.................TC.....................C..T............
Tsuga_heterophylla        ...................G.........................................C..T............
Tsuga_mertensiana         ...................G.........................................C..T............
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            GATGTACTATCAAACCAAAATTGGGTCTATCGGCTAAGAACTATGGTAGAGCAGTTTACGAATGTCTTCGTGGTGGAC
Keteleeria_davidiana      ................................A...................................C..........
Nothotsuga_longibracteata ..........................................................T........C..........
Pseudolarix_amabilis      ...................................................................C..........
Tsuga_canadensis          ..........................................................T........C..........
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           ..........A................................................T........C..........
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              ..........................................................T........C..........
Tsuga_heterophylla        ..........................................................T........C..........
Tsuga_mertensiana         .......G..A...............................................T........C..........
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            TCGATTTTACCAAGGATGATGAGAACGTAAATTCCCAACCATTCATGCGCTGGAGAGATCGTTTTGTCTTTTGTGCGG
Keteleeria_davidiana      .........................................................C.....................
Nothotsuga_longibracteata ...............................C.............................................
Pseudolarix_amabilis      .T............................................................................
Tsuga_canadensis          ...............................C.................T............................
Tsuga_caroliniana         -----------------------------------------------------------------------------
Tsuga_chinensis           ..A............................C.............................................
Tsuga_diversifolia        -----------------------------------------------------------------------------
Tsuga_dumosa              ..C............................C.............................................
Tsuga_heterophylla        ...............................C.................T............................
Tsuga_mertensiana         ...............................C.................T............................
Tsuga_sieboldii           -----------------------------------------------------------------------------

Abies_veitchii            AAGCAATTAATAAGGCTCAGGCTGAGACGGGTGAAATTAAAGGACATTACTTGAATGCTACTGCAGGTACATGTGAAG
Keteleeria_davidiana      .....C........................................................................
Nothotsuga_longibracteata .....C..T.....................................................................
Pseudolarix_amabilis      ........T.....................................................................
Tsuga_canadensis          .....C..T.....................................................................
```

```
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             .....C..T.....................................................................
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                .....C..T.....................................................................
Tsuga_heterophylla          .....C..T.....................................................................
Tsuga_mertensiana           ..............................................................................
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              AAATGATGAAAAGGGCAATATTTGCAAGAGAATTAGGAGTTCCTATCGTCATGCATGACTATCTGACGGGAGGTTTTA
Keteleeria_davidiana        ......................................................................G.......
Nothotsuga_longibracteata   ..............................................................................
Pseudolarix_amabilis        .G............................................................................
Tsuga_canadensis            .................G................................T...........................
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             .................G..............................A.............................
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                .................G...NN........................A..........N..........T........
Tsuga_heterophylla          .................G................................T...........................
Tsuga_mertensiana           .................G............................................................
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              CCGCAAATACTAGTTTGGCTCATTATTGCCGAGACAATGGTCTACTTCTTCACATTCACCGCGCGATGCATGCAGTTA
Keteleeria_davidiana        .T......................................C.....................................
Nothotsuga_longibracteata   ........................................CT....................................
Pseudolarix_amabilis        .T......................................C.....................................
Tsuga_canadensis            ............C...........................C.....................................
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             ........................................C.....................................
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                ........................................C........N............................
Tsuga_heterophylla          ............C...........................C.....................................
Tsuga_mertensiana           ........................................C.....................................
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              TCGACAGACAAAGAAATCATGGTATGCATTTCCGTGTACTGGCTAAAGCATTGCGTATGTCCGGTGGAGATCATGTTC
Keteleeria_davidiana        .T........................................................................A...
Nothotsuga_longibracteata   .T.........................T...T..........................................A...
Pseudolarix_amabilis        .T........................................................................A...
Tsuga_canadensis            .T..............................T.........................................A...
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             .T.......................A.TG..T................C.........................A...
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                .T.......................A.TG..T................C.........................A...
Tsuga_heterophylla          .T..............................T.........................................A...
Tsuga_mertensiana           .T..............................T.........................................A...
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              ACGCCGGTACTGTAGTAGGTAAACTTGAAGGGGAACGAGACGTCACTTTAGGGTTTGTTGATCTACTGCGTGATGATT
Keteleeria_davidiana        ..............................................................................
Nothotsuga_longibracteata   ...............................A..............................................
Pseudolarix_amabilis        ...............................A..............................................
Tsuga_canadensis            ...............................A........A.................................A...
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             ..T............................A........A..........T..........................
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                ..T............................A........A..........T..........................
Tsuga_heterophylla          ...............................A........A.................................A...
Tsuga_mertensiana           ...............................A........A.....................................
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              TTATCGAAAAAGATCGAAGTCGTGGTATTTACTTCACTCAAGATTGGGTATCTATGCCAGGTGTCCTGCCCGTAGCTT
Keteleeria_davidiana        ....T.........................................................................
Nothotsuga_longibracteata   ................................................................T.....G.......
Pseudolarix_amabilis        ................................................................T.............
Tsuga_canadensis            A...............................................................T.............
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             ................................................................T.............
Tsuga_diversifolia          ----------------------------------------------------------------------------
Tsuga_dumosa                ................................................................T.............
Tsuga_heterophylla          A...............................................................T.............
Tsuga_mertensiana           ................................................................T.............
Tsuga_sieboldii             ----------------------------------------------------------------------------

Abies_veitchii              CAGGAGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGATCTTTGGAGATGATTCCGTACTACAGTTTGGTGGGG
Keteleeria_davidiana        ..............................................................................
Nothotsuga_longibracteata   ..........................................................T...................
Pseudolarix_amabilis        ..........................................................T...................
Tsuga_canadensis            ..........................................................T...................
Tsuga_caroliniana           ----------------------------------------------------------------------------
Tsuga_chinensis             ..........................................................T.................T.
```

```
Tsuga_diversifolia          -----------------------------------------------------------------------------
Tsuga_dumosa                .........................................................T.................T.
Tsuga_heterophylla          .........................................................T...................
Tsuga_mertensiana           .........................................................T...................
Tsuga_sieboldii             -----------------------------------------------------------------------------

Abies_veitchii              GAACTTTGGGACACCCTTGGGGAATGCACCTGGTGCAGTAGCTAATCGGGTTGCTGTAGAAGCTTGTGTACAAGCTC
Keteleeria_davidiana        .............................................................................
Nothotsuga_longibracteata   .......................................................C.....................
Pseudolarix_amabilis        .......................................................C.....................
Tsuga_canadensis            .......................................................C.C...................
Tsuga_caroliniana           -----------------------------------------------------------------------------
Tsuga_chinensis             .......................................................C.....................
Tsuga_diversifolia          -----------------------------------------------------------------------------
Tsuga_dumosa                .......................................................C.....................
Tsuga_heterophylla          .......................................................C.C...................
Tsuga_mertensiana           .......................................................C.C...................
Tsuga_sieboldii             -----------------------------------------------------------------------------

Abies_veitchii              GTAATGAAGGACGTGATCTTGCTCGTGAAGGTAATGAAGTGATCCGTGAAGCTTGTAAATG
Keteleeria_davidiana        .....................................................GC......
Nothotsuga_longibracteata   ......................................A.T..T..........C......
Pseudolarix_amabilis        .....................................................A.......
Tsuga_canadensis            ........................................TG.T.................
Tsuga_caroliniana           -------------------------------------------------------------
Tsuga_chinensis             ........................................T..T.................
Tsuga_diversifolia          -------------------------------------------------------------
Tsuga_dumosa                ........................................T..T.................
Tsuga_heterophylla          ........................................TG.T.................
Tsuga_mertensiana           ..........................C.............T..T.................
Tsuga_sieboldii             -------------------------------------------------------------
```

VITA

JORDAN D. BAKER

Personal Data:	Date of Birth: August 10, 1984 Place of Birth: Lewisburg, PA Marital Status: Single
Education:	Public Schools, Lewisburg, Pennsylvania B.S. Biology, Lenoir-Rhyne University, Hickory, NC 2006 M.S. Biology, East Tennessee State University, Johnson City, Tennessee 2009
Professional Experience:	Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 2006-2009 Lecturer, Biology (1010, 1020), East Tennessee State University, College of Arts and Sciences, 2008-2009
Publications:	Levy, Foster, Jordan Baker, Ke Chen, Graham Cooke, Yu-Sheng Liu, Elaine S. Walker, and Tim McDowell. Patterns of Spread of Hemlock Wooly Adelgid in Carolina Hemlock Populations. Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States. 2008.
Honors and Awards:	The Marcia Davis Research Award for Graduate Research in Conservation of Natural Resources, East Tennessee State University.