

Series on
Genetics, Genomics and Breeding of Crop Plants

Series Editor
Chittaranjan Kole, Clemson University, Clemson, SC, USA

Genetics, Genomics and Breeding of Conifers

Editors
Christophe Plomion • Jean Bousquet
Chittaranjan Kole



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GENETICS, GENOMICS AND BREEDING OF CONIFERS

Genetics, Genomics and Breeding of Crop Plants

Series Editor

Chittaranjan Kole
Department of Genetics and Biochemistry
Clemson University
Clemson, SC
USA

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GENETICS, GENOMICS AND BREEDING OF CONIFERS

Editors

Christophe Plomion

INRA

UMR BIOGECO

Cestas

France

Jean Bousquet

Centre d'étude de la forêt

Université Laval

Québec

Canada

Chittaranjan Kole

Department of Genetics and Biochemistry

Clemson University

Clemson, SC

USA



CRC Press
Taylor & Francis Group
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www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK



Science Publishers
Jersey, British Isles
Enfield, New Hampshire

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Taylor & Francis Group
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Preface to the Series

Genetics, genomics and breeding has emerged as three overlapping and complimentary disciplines for comprehensive and fine-scale analysis of plant genomes and their precise and rapid improvement. While genetics and plant breeding have contributed enormously towards several new concepts and strategies for elucidation of plant genes and genomes as well as development of a huge number of crop varieties with desirable traits, genomics has depicted the chemical nature of genes, gene products and genomes and also provided additional resources for crop improvement.

In today's world, teaching, research, funding, regulation and utilization of plant genetics, genomics and breeding essentially require thorough understanding of their components including classical, biochemical, cytological and molecular genetics; and traditional, molecular, transgenic and genomics-assisted breeding. There are several book volumes and reviews available that cover individually or in combination of a few of these components for the major plants or plant groups; and also on the concepts and strategies for these individual components with examples drawn mainly from the major plants. Therefore, we planned to fill an existing gap with individual book volumes dedicated to the leading crop and model plants with comprehensive deliberations on all the classical, advanced and modern concepts of depiction and improvement of genomes. The success stories and limitations in the different plant species, crop or model, must vary; however, we have tried to include a more or less general outline of the contents of the chapters of the volumes to maintain uniformity as far as possible.

Often genetics, genomics and plant breeding and particularly their complimentary and supplementary disciplines are studied and practiced by people who do not have, and reasonably so, the basic understanding of biology of the plants for which they are contributing. A general description of the plants and their botany would surely instill more interest among them on the plant species they are working for and therefore we presented lucid details on the economic and/or academic importance of the plant(s); historical information on geographical origin and distribution; botanical origin and evolution; available germplasms and gene pools, and genetic and cytogenetic stocks as genetic, genomic and breeding resources; and

basic information on taxonomy, habit, habitat, morphology, karyotype, ploidy level and genome size, etc.

Classical genetics and traditional breeding have contributed enormously even by employing the phenotype-to-genotype approach. We included detailed descriptions on these classical efforts such as genetic mapping using morphological, cytological and isozyme markers; and achievements of conventional breeding for desirable and against undesirable traits. Employment of the *in vitro* culture techniques such as micro- and megaspore culture, and somatic mutation and hybridization, has also been enumerated. In addition, an assessment of the achievements and limitations of the basic genetics and conventional breeding efforts has been presented.

It is a hard truth that in many instances we depend too much on a few advanced technologies, we are trained in, for creating and using novel or alien genes but forget the infinite wealth of desirable genes in the indigenous cultivars and wild allied species besides the available germplasms in national and international institutes or centers. Exploring as broad as possible natural genetic diversity not only provides information on availability of target donor genes but also on genetically divergent genotypes, botanical varieties, subspecies, species and even genera to be used as potential parents in crosses to realize optimum genetic polymorphism required for mapping and breeding. Genetic divergence has been evaluated using the available tools at a particular point of time. We included discussions on phenotype-based strategies employing morphological markers, genotype-based strategies employing molecular markers; the statistical procedures utilized; their utilities for evaluation of genetic divergence among genotypes, local landraces, species and genera; and also on the effects of breeding pedigrees and geographical locations on the degree of genetic diversity.

Association mapping using molecular markers is a recent strategy to utilize the natural genetic variability to detect marker-trait association and to validate the genomic locations of genes, particularly those controlling the quantitative traits. Association mapping has been employed effectively in genetic studies in human and other animal models and those have inspired the plant scientists to take advantage of this tool. We included examples of its use and implication in some of the volumes that devote to the plants for which this technique has been successfully employed for assessment of the degree of linkage disequilibrium related to a particular gene or genome, and for germplasm enhancement.

Genetic linkage mapping using molecular markers have been discussed in many books, reviews and book series. However, in this series, genetic mapping has been discussed at length with more elaborations and examples on diverse markers including the anonymous type 2 markers such as RFLPs, RAPDs, AFLPs, etc. and the gene-specific type 1 markers such as EST-SSRs, SNPs, etc.; various mapping populations including F_2 , backcross,

recombinant inbred, doubled haploid, near-isogenic and pseudotestcross; computer software including MapMaker, JoinMap, etc. used; and different types of genetic maps including preliminary, high-resolution, high-density, saturated, reference, consensus and integrated developed so far.

Mapping of simply inherited traits and quantitative traits controlled by oligogenes and polygenes, respectively has been deliberated in the earlier literature crop-wise or crop group-wise. However, more detailed information on mapping or tagging oligogenes by linkage mapping or bulked segregant analysis, mapping polygenes by QTL analysis, and different computer software employed such as MapMaker, JoinMap, QTL Cartographer, Map Manager, etc. for these purposes have been discussed at more depth in the present volumes.

The strategies and achievements of marker-assisted or molecular breeding have been discussed in a few books and reviews earlier. However, those mostly deliberated on the general aspects with examples drawn mainly from major plants. In this series, we included comprehensive descriptions on the use of molecular markers for germplasm characterization, detection and maintenance of distinctiveness, uniformity and stability of genotypes, introgression and pyramiding of genes. We have also included elucidations on the strategies and achievements of transgenic breeding for developing genotypes particularly with resistance to herbicide, biotic and abiotic stresses; for biofuel production, biopharming, phytoremediation; and also for producing resources for functional genomics.

A number of desirable genes and QTLs have been cloned in plants since 1992 and 2000, respectively using different strategies, mainly positional cloning and transposon tagging. We included enumeration of these and other strategies for isolation of genes and QTLs, testing of their expression and their effective utilization in the relevant volumes.

Physical maps and integrated physical-genetic maps are now available in most of the leading crop and model plants owing mainly to the BAC, YAC, EST and cDNA libraries. Similar libraries and other required genomic resources have also been developed for the remaining crops. We have devoted a section on the library development and sequencing of these resources; detection, validation and utilization of gene-based molecular markers; and impact of new generation sequencing technologies on structural genomics.

As mentioned earlier, whole genome sequencing has been completed in one model plant (*Arabidopsis*) and seven economic plants (rice, poplar, peach, papaya, grapes, soybean and sorghum) and is progressing in an array of model and economic plants. Advent of massively parallel DNA sequencing using 454-pyrosequencing, Solexa Genome Analyzer, SOLiD system, Heliscope and SMRT have facilitated whole genome sequencing in many other plants more rapidly, cheaply and precisely. We have included

extensive coverage on the level (national or international) of collaboration and the strategies and status of whole genome sequencing in plants for which sequencing efforts have been completed or are progressing currently. We have also included critical assessment of the impact of these genome initiatives in the respective volumes.

Comparative genome mapping based on molecular markers and map positions of genes and QTLs practiced during the last two decades of the last century provided answers to many basic questions related to evolution, origin and phylogenetic relationship of close plant taxa. Enrichment of genomic resources has reinforced the study of genome homology and synteny of genes among plants not only in the same family but also of taxonomically distant families. Comparative genomics is not only delivering answers to the questions of academic interest but also providing many candidate genes for plant genetic improvement.

The 'central dogma' enunciated in 1958 provided a simple picture of gene function—gene to mRNA to transcripts to proteins (enzymes) to metabolites. The enormous amount of information generated on characterization of transcripts, proteins and metabolites now have led to the emergence of individual disciplines including functional genomics, transcriptomics, proteomics and metabolomics. Although all of them ultimately strengthen the analysis and improvement of a genome, they deserve individual deliberations for each plant species. For example, microarrays, SAGE, MPSS for transcriptome analysis; and 2D gel electrophoresis, MALDI, NMR, MS for proteomics and metabolomics studies require elaboration. Besides transcriptome, proteome or metabolome QTL mapping and application of transcriptomics, proteomics and metabolomics in genomics-assisted breeding are frontier fields now. We included discussions on them in the relevant volumes.

The databases for storage, search and utilization on the genomes, genes, gene products and their sequences are growing enormously in each second and they require robust bioinformatics tools plant-wise and purpose-wise. We included a section on databases on the gene and genomes, gene expression, comparative genomes, molecular marker and genetic maps, protein and metabolomes, and their integration.

Notwithstanding the progress made so far, each crop or model plant species requires more pragmatic retrospect. For the model plants we need to answer how much they have been utilized to answer the basic questions of genetics and genomics as compared to other wild and domesticated species. For the economic plants we need to answer as to whether they have been genetically tailored perfectly for expanded geographical regions and current requirements for green fuel, plant-based bioproducts and for improvements of ecology and environment. These futuristic explanations have been addressed finally in the volumes.

We are aware of exclusions of some plants for which we have comprehensive compilations on genetics, genomics and breeding in hard copy or digital format and also some other plants which will have enough achievements to claim for individual book volume only in distant future. However, we feel satisfied that we could present comprehensive deliberations on genetics, genomics and breeding of 30 model and economic plants, and their groups in a few cases, in this series. I personally feel also happy that I could work with many internationally celebrated scientists who edited the book volumes on the leading plants and plant groups and included chapters authored by many scientists reputed globally for their contributions on the concerned plant or plant group.

We paid serious attention to reviewing, revising and updating of the manuscripts of all the chapters of this book series, but some technical and formatting mistakes will remain for sure. As the series editor, I take complete responsibility for all these mistakes and will look forward to the readers for corrections of these mistakes and also for their suggestions for further improvement of the volumes and the series so that future editions can serve better the purposes of the students, scientists, industries, and the society of this and future generations.

Science publishers, Inc. has been serving the requirements of science and society for a long time with publications of books devoted to advanced concepts, strategies, tools, methodologies and achievements of various science disciplines. Myself as the editor and also on behalf of the volume editors, chapter authors and the ultimate beneficiaries of the volumes take this opportunity to acknowledge the publisher for presenting these books that could be useful for teaching, research and extension of genetics, genomics and breeding.

Chittaranjan Kole

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Preface to the Volume

Conifers are woody plants, the great majority being trees. They represent 650 species, some ranking as the largest, tallest, and longest living non-clonal terrestrial organisms on Earth. They are of immense ecological importance, dominating many terrestrial landscapes and representing the largest terrestrial carbon sink. They are evolutionary distinct from angiosperm trees on many accounts and with their extraordinary large genomes, they provide a different view of plant genome biology and evolution. They are also of great economic importance, as they are primarily used for timber and paper production worldwide. Domestication of some of these species was started about 60 years ago through traditional genetic improvement programs. It has resulted in advances in overall growth, wood quality, pest resistance and adaptation, but breeding still remains a slow process because of long generation intervals typical of most conifers and because most traits cannot be correctly evaluated at an early stage.

During the past 20 years, more and more sophisticated genomics tools have been developed to describe the extreme plasticity and variability of these species at different levels of integration (from genes up to phenotypes) and are now being integrated into breeding to accelerate the domestication process by a more precise exploitation of genetic diversity. Application of genomic-based science is also playing an important role in understanding the evolution, patterns of nucleotide variation and the molecular basis of quantitative traits and adaptation. Altogether, this new knowledge is also expected to help delineate more efficient gene conservation strategies.

This book will give the reader an in-depth review of the current state-of-the-art of genetic and genomic research conducted in conifers. Each chapter is the product of specialists in their field. Their goal was to report on the latest trends and findings and at the same time, promote awareness and make this knowledge accessible to the vast majority. Accordingly, the chapters are well documented and illustrated. Their contribution is greatly appreciated.

The book begins with an exhaustive description of the conifers in terms of classification, geographical distribution, life history and ecology, morphology and fossil history as well as phylogenetics (Chapter 1). It is followed by a chapter devoted to their economic importance and the

development of conifer breeding programs worldwide, which lead to significant improvement of productivity and quality (Chapter 2). Chapter 3 deliberates on various classical and molecular cytogenetical tools useful to elucidate evolution, integrate physical and genetic maps, conserve species and assist in marker-based breeding. Chapter 4 describes the applications of neutral genetic markers from the perspectives of conservation genetics, phylogeography and gene flow studies. In Chapter 5, research efforts on linkage mapping, emerging gene maps as well as QTL detection and architecture are reviewed. An exhaustive review of investigations on candidate genes is provided in Chapter 6, from estimates of nucleotide diversity and recombination to new-generation selection signatures studies and the development of association mapping and outlier detection approaches. The ever-increasing applications of molecular markers into breeding from the management operations to selection strategies are considered in Chapter 7. Switching to more functional aspects, Chapters 8 and 9 review the current status of our understanding of transcriptome, proteome and metabolome modifications in responses to developmental changes and environmental constraints. The rapid advances in sequencing and cataloging the conifer gene space are also reported (Chapter 8). As a prerequisite for the sequencing of a conifer genome, insights into the characteristics of the large conifer genomes, especially with respect to the composition and evolution of transposable elements, are provided in Chapter 10. The book ends with refreshing views on the challenges faced by the conifer genomics community and how the pace of rapid advancement of the “omic” sciences might affect our understanding of conifer biology and the future use of conifer genetic resources (Chapter 11).

This book is a testimony to the substantial progress made in the field of conifer genetics and genomics and the definite value of conifers as a model system. Although the tools and concepts that are presented will continue to evolve rapidly, we hope this volume will provide a solid foundation for further development in conifer and more generally in forest tree genetics, genomics and breeding.

Christophe Plomion
Jean Bousquet
Chittaranjan Kole

Contents

<i>Preface to the Series</i>	v
<i>Preface to the Volume</i>	xi
<i>List of Contributors</i>	xv
<i>Abbreviations</i>	xxiii
1. The Conifers (Pinophyta)	1
<i>David S. Gernandt, Ann Willyard, John V. Syring and Aaron Liston</i>	
2. Economic Importance, Breeding Objectives and Achievements	40
<i>T.J. Mullin, B. Andersson, J.-C. Bastien, J. Beaulieu, R.D. Burdon, W.S. Dvorak, J.N. King, T. Kondo, J. Krakowski, S.J. Lee, S.E. McKeand, L. Pâques, A. Raffin, J.H. Russell, T. Skrøppa, M. Stoehr and A. Yanchuk</i>	
3. Cytogenetics	128
<i>M. Nurul Islam-Faridi and C. Dana Nelson</i>	
4. Neutral Patterns of Genetic Variation and Applications to Conservation in Conifer Species	141
<i>Francesca Bagnoli, Bruno Fady, Silvia Fineschi, Sylvie Oddou-Muratorio, Andrea Piotti, Federico Sebastiani and Giovanni G. Vendramin</i>	
5. Genetic Mapping in Conifers	196
<i>Kermit Ritland, Konstantin V. Krutovsky, Yoshihiko Tsumura, Betty Pelgas, Nathalie Isabel and Jean Bousquet</i>	
6. Patterns of Nucleotide Diversity and Association Mapping	239
<i>González-Martínez SC, Dillon S, Garnier-Géré PH, Krutovsky KV, Alía R, Burgarella C, Eckert AJ, García-Gil MR, Grivet D, Heuertz M, Jaramillo-Correa JP, Lascoux M, Neale DB, Savolainen O, Tsumura Y and Vendramin GG</i>	
7. Integration of Molecular Markers in Breeding	276
<i>Rowland D. Burdon and Phillip L. Wilcox</i>	

8. Transcriptomics	323
<i>John J. Mackay and Jeffrey F. D. Dean</i>	
9. Recent Advances in Proteomics and Metabolomics in Gymnosperms	358
<i>Rebecca Dauwe, Andrew Robinson and Shawn D. Mansfield</i>	
10. Toward the Conifer Genome Sequence	389
<i>Michele Morgante and Emanuele De Paoli</i>	
11. Future Prospects	404
<i>Jeffrey F.D. Dean</i>	
<i>Index</i>	439
<i>Color Plate Section</i>	449

List of Contributors

R. Alía

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain.

Email: alia@inia.es

B. Andersson

Skogforsk (Sävar), Box 3, S-918 21 Sävar, Sweden.

Email: bengt.andersson@skogforsk.se

Francesca Bagnoli

Plant Protection Institute, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy.

Email: bagnoli@ipp.cnr.it

J.-C. Bastien

INRA—Centre de Recherche d'Orléans, 2163, Avenue de la Pomme de Pin, CS 400001 ARDON, F-45075 Orléans Cedex 2, France.

Email: jean-charles.bastien@orleans.inra.fr

J. Beaulieu

Natural Resources Canada, P.O. Box 10380, Stn. Sainte-Foy, Québec, QC G1V 4C7, Canada.

Email: jeanbeau@nrcan-rncan.gc.ca

Jean Bousquet

Centre d'étude de la forêt, Université Laval, Québec, Québec G1V 0A6, Canada.

Email: jean.bousquet@sbflaval.ca

R.D. Burdon

Scion (NZ Forest Research Institute Ltd.), Private Bag 3020, Rotorua 3010, New Zealand.

Email: rowland.burdon@scionresearch.com

C. Burgarella

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain.

Email: concettaburgarella@hotmail.com

Rebecca Dauwe

Department of Wood Science, Faculty of Forestry, 4030-2424 Main Mall,
Vancouver, BC, V6T 1Z4, Canada.

Email: rebecca.dauwe@u-picardie.fr

Jeffrey F.D. Dean

Warnell School of Forestry and Natural Resources, University of Georgia,
Athens, GA 30602, USA.

Email: jeffdean@uga.edu

Tel: +1-706-542-1710

Emanuele De Paoli

Istituto Agrario di San Michele all'Adige, Vie E. Mach 1, 38010 San Michele
all'Adige, Italy.

Email: emanuele.depaoli@iasma.it.

S. Dillon

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

Email: shannon.dillon@csiro.au

W.S. Dvorak

North Carolina State University, Campus Box 8008, Raleigh, NC 27695-
8008, USA.

Email: w_dvorak@ncsu.edu

A.J. Eckert

Section of Evolution and Ecology and Center for Population Biology,
University of California at Davis, Davis, CA 95616, USA.

Email: ajeckert@ucdavis.edu

Bruno Fady

INRA, UR629, Ecologie des Forêts Méditerranéennes, Domaine Saint Paul,
Site Agroparc, 84914 Avignon, France.

Email: bruno.fady@avignon.inra.fr

Silvia Fineschi

Plant Protection Institute, CNR, Via Madonna del Piano 10, 50019 Sesto
Fiorentino (FI), Italy.

Email: fineschi@ipp.cnr.it

M.R. García-Gil

Umeå Plant Science Center, Swedish University of Agricultural Science, SE
901 83 Umeå, Sweden.

Email: m.rosario.garcia@genfys.slu.se

P.H. Garnier-Géré

INRA, UMR1202 Biodiversity Genes & Communities, 69 route d'Arcachon,
33612 Cestas Cedex, France.

Email: pauline@pierroton.inra.fr

David S. Gernandt

Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-233, México Distrito Federal 04510, Mexico.

Email: dgernandt@ibiologia.unam.mx

S.C. González-Martínez

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain.

Email: santiago@inia.es

Tel: +34 913471499

D. Grivet

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain.

Email: dgrivet@inia.es

M. Heuertz

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain.

and

Université Libre de Bruxelles, Faculté des Sciences, Behavioural and Evolutionary Ecology cp160/12, av. F.D. Roosevelt 50, 1050 Brussels, Belgium.

Email: mheuertz@ulb.ac.be

Nathalie Isabel

Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn Sainte-Foy, Québec, Québec G1V 4C7, Canada.

Email: nisabel@cfl.forestry.ca

Nurul Islam-Faridi

Forest Tree Molecular Cytogenetics, Southern Institute of Forest Genetics, US Forest Service.

and

Dept. of Ecosystem Science & Management, Texas A&M University, College Station, TX 77843, USA.

Email: nfaridi@tamu.edu

J.P. Jaramillo-Correa

Department of Forest Ecology and Genetics, Center of Forest Research, INIA, 28040 Madrid, Spain; and

Department of Evolutionary Ecology, Ecology Institute, Universidad Nacional Autónoma de México, Ciudad Universitaria, Tercer circuito Exterior, Apartado Postal 70-275, México, D.F.

Email: jaramillo@miranda.ecologia.unam.mx

J.N. King

British Columbia Forest Service, PO Box 9519 Stn Prov Govt, Victoria, B.C.
V8W 9C2, Canada.

Email: john.king@gov.bc.ca

T. Kondo

Forest Tree Breeding Centre, 3809-1 Ishi, Juo, Hitachi, Ibaraki 319-1301,
Japan.

Email: kotei@affrc.go.jp

J. Krakowski

British Columbia Ministry of Forests and Range, Box 335, Mesachie Lake,
B.C. V0R2N0, Canada.

Email: Jodie.Krakowski@gov.bc.ca

Konstantin V. Krutovsky

Department of Ecosystem Science and Management, Texas A&M University,
College Station, Texas 77843-2138, USA.

Email: k-krutovsky@tamu.edu

M. Lascoux

Program in Evolutionary Functional Genetics, Evolutionary Biology Centre,
Uppsala University, 75326 Uppsala, Sweden.

Email: Martin.Lascoux@ebc.uu.se

S.J. Lee

Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY,
Scotland.

Email: steve.lee@forestry.gsi.gov.uk

Aaron Liston

Department of Botany and Plant Pathology, 2082 Cordley Hall, Oregon
State University, Corvallis, Oregon 97331, USA.

Email: listona@science.oregonstate.edu

John J. Mackay

Center for Forest Research, Laval University, Québec City, Québec, Canada,
G1V 0A6.

Email: John.mackay@sbfi.ulaval.ca

Shawn D. Mansfield

Department of Wood Science, Faculty of Forestry, 4030-2424 Main Mall,
Vancouver, BC, V6T 1Z4, Canada.

Email: shawn.mansfield@ubc.ca

S.E. McKeand

North Carolina State University, Campus Box 8002, Raleigh, NC 27695-8002, USA.

Email: steve_mckeand@ncsu.edu

Michele Morgante

Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy; and

Istituto di Genomica Applicata, Parco Scientifico e Tecnologico di Udine, Via Linussio 51, 33100 Udine, Italy.

Email: michele.morgante@uniud.it

T.J. Mullin

BioSylve Forest Science NZ Limited, 45 Krokoro Road, Lower Hutt 5012, New Zealand.

Email: tim.mullin@biosylve.com

D.B. Neale

Department of Plant Sciences, University of California at Davis, Davis, CA 95616, USA; and

Institute of Forest Genetics, Pacific Southwest Research Station, US Department of Agriculture Forest Service, Placerville, CA 95667, USA.

Email: dbneale@ucdavis.edu

C. Dana Nelson

USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, 23332 Success Road, Saucier, MS 39574, USA.

Email: dananelson@fs.fed.us

Sylvie Oddou-Muratorio

INRA, UR629, Ecologie des Forêts Méditerranéennes, Domaine Saint Paul, Site Agroparc, 84914 Avignon, France.

Email: sylvie.oddou@avignon.inra.fr

L. Pâques

INRA—Centre de Recherche d'Orléans, 2163 Avenue de la Pomme de Pin, CS 400001 ARDON, F-45075 Orléans Cedex 2, France.

Email: luc.paques@orleans.inra.fr

Betty Pelgas

Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 10380, Stn Sainte-Foy, Québec, Québec G1V 4C7, Canada.

Email: betty.pelgas@RNCa-n-NRCa-n.gc.ca

Andrea Piotti

Department of Environmental Sciences, University of Parma, Viale Usberti 11 / A, 43100 Parma, Italy.

Email: andrea.piotti@nemo.unipr.it

A. Raffin

INRA (Pierroton), 69 route d'Arcachon, 33612 CESTAS Cedex, France.

Email: annie.raffin@pierroton.inra.fr

Kermit Ritland

Department of Forest Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.

Email: kermit.ritland@ubc.ca

Andrew Robinson

Department of Wood Science, Faculty of Forestry, 4030-2424 Main Mall, Vancouver, BC, V6T 1Z4, Canada.

Email: andrewrobinsonnz@gmail.com

J.H. Russell

British Columbia Ministry of Forests and Range, Box 335, Mesachie Lake, B.C. V0R2N0, Canada.

Email: john.russell@gov.bc.ca

O. Savolainen

Department of Biology, University of Oulu, 90014 Oulu, Finland.

Email: outi.savolainen@oulu.fi

Federico Sebastiani

Plant Genetics Institute, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy.

Email: federico.sebastiani@unifi.it

T. Skrøppa

Norwegian Forest and Landscape Institute, Høgskoleveien 8, 1432 Ås, Norway.

Email: tore.skrøppa@skogoglandskap.no

M. Stoehr

British Columbia Ministry of Forests, PO Box 9519, Stn Prov Govt, Victoria, B.C. V8W 9C2, Canada.

Email: michael.stoehr@gov.bc.ca

John V. Syring

Department of Biology, Linfield College, 900 SE Baker St., McMinnville, Oregon 97128, USA.

Email: jsyring@linfield.edu

Yoshihiko Tsumura

Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan.

Email: ytsumu@ffpri.affrc.go.jp

Giovanni G. Vendramin

Plant Genetics Institute, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy.

Email: giovanni.vendramin@igv.cnr.it

Phillip L. Wilcox

Scion: New Zealand Forest Research Institute Ltd, Private Bag 3020, Rotorua 3046, New Zealand.

Email: phillip.wilcox@scionresearch.com

Ann Willyard

Biology Department, Hendrix College, 1600 Washington Ave, Conway, Arkansas 72032, USA.

Email: willyard@hendrix.edu

A. Yanchuk

British Columbia Ministry of Forests, PO Box 9519, Stn Prov Govt, Victoria, B.C. V8W 9C2, Canada.

Email: alvin.yanchuk@gov.bc.ca

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Abbreviations

μ	Mutation rate
2-D	Two-dimensional
2-DE	Two-dimensional electrophoresis
ABC	Approximate Bayesian computation
AFA	Adaptive Force Acoustics
AFLP	Amplified fragment length polymorphism
AGP	Arabinogalactan protein
ANOVA	Analysis of variance
AT	Adenine-Thymine
ATRS	<i>Arabidopsis</i> -type telomere repeat sequence
BAC	Bacterial artificial chromosome
BHT	Butylated hydroxytoluene
BIC	Bayesian information criterion
BLUP	Best Linear Unbiased Prediction
bp	Base pairs
CAPS	Cleaved amplified polymorphic sequence
CCA	Canonical correlation analysis
CDA	Canonical discriminate analysis
cDNA	Complementary-DNA
CDS	Complete coding sequences
Ch	Chromosome
ChIP-Seq	Chromatin ImmunoPrecipitation coupled with next-generation sequencing
CID	Carbon isotope discrimination
cM	CentiMorgan
CMA	Chromamycin A ₃
COLD-PCR	CO-amplification at lower denaturation temperature-PCR
COS	Conserved orthologous set
cpDNA	Chloroplast-DNA
cpSSR	Chloroplast-SSR
DAPI	4', 6-Diamidino -2-phenylindole
DaRT	Diversity array technology
DIGE	Differential in gel electrophoresis

DOE	Department of Energy (US)
DOP	Degenerate oligonucleotide primed
DUF	Domains of unknown function
EBV	Estimated breeding value
ECD	Electrochemical detector
eQTL	Expression-QTL
EST	Expressed sequence tag
ESTP	Expressed sequence tag polymorphism
ESU	Evolutionary significant units
FA	Factor analysis
FID	Flame ionization detector
FISH	Fluorescent/ <i>in situ</i> hybridization
FL-cDNA	Full length-cDNA
Fst	Fixation index
GA	Gibberellin
GAB	Gene-assisted breeding
GAS	Gene-assisted selection
Gbp	Giga base pair
GBV	Genomic breeding value
GC	Gas chromatography
GC	Guanine-Cytosine
GC/MS	Gas chromatography-mass spectroscopy
GDP	Gross domestic product
GIS	Geographic information system
GLM	General linear model
GNP	Gross national product
GS	Genome Selection
GS	Genomic selection
Gst	Average amount of differentiation observed over multiple loci
G_{st}	Population differentiation statistic
GWS	Genome-wide selection
GWS	Genome-wide scan
HCA	Hierarchical cluster analysis
H_e	Expected heterozygosity statistic
HMM	Hidden Markov model
HMPR	Hypomethylated partial restriction
H_o	Observed heterozygosity statistic
HPLC	High pressure liquid chromatography
HSD	Honestly significant difference
HSP	heat-shock protein
HTS	High-throughput sequencing
IBF	Identity by function

ICAT	Isotope-coded affinity tags
IE	Isoelectric point
IEF	Isoelectric focusing
IHGSC	International Human Genome Sequencing Consortium
Indel	Insertion/deletion
INTA	Instituto Nacional de Tecnología Agropecuaria (Argentina)
IR	Inverted repeat
IS	Importance sampling
ISSR	Inter-simple sequence repeat
iTRAQ	Isobaric tag for relative and absolute quantitation
ITS	Internal transcribed sequence
IUFRO	International Union of Forestry Research Organizations
JGI	Joint Genome Institute (US)
Kb	Kilobase(s)
Kbp	Kilo base pair
LC ESI-MS/MS	Liquid chromatography electrospray ionization tandem mass spectrometry
LC/ESI/MS	Liquid chromatography electrospray ionization mass spectrometry
LC/MS	Liquid chromatography-mass spectrometry
LD	Linkage disequilibrium
LDD	Long-distance dispersal
LG	Linkage group
LGM	Last glacial maximum
LSC	Large region of single copy genes
LTR	Long terminal repeats
MAB	Marker-assisted backcrossing
MAF	Minimum allele frequency
MALDI-TOFMS	Matrix-assisted laser desorption/ionization time of flight mass spectrometry
MARG	Marker-assisted recovery of genotypes
MAS	Marker-aided/assisted selection
MAS	Magic angle spinning
Mbp	Mega base pair
MCMC	Markov Chain Monte Carlo
MDA	Multiple discriminate analysis
MFA	Microfibril angle
miRNA	Micro-RNA
MLM	Mixed linear model
MOE	Modulus of elasticity
MPB	Mountain pine beetle
MPK	Mitogen-activated protein kinase

Mr	Relative molecular weight
mRNA	Messenger-RNA
MS/MS	Mass spectrometry /mass spectrometry or Tandom mass spectrometry
MS ⁿ	Mass spectrometry to the “n” th power
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
mtDNA	Mitochondrial-DNA
mtSSR	Mitochondrial-SSR
MudPIT	Multidimensional protein identification technology
MW	Molecular weight
MY	Million years
Mya	Million years ago
N	Census number
NCA	Nested clade analysis
NCBI	National Center for Biotechnology Information (US)
NCPA	Nested clade phylogeographic analysis
ncRNA	Non-coding RNA
nDNA	Nuclear-DNA
N_e	Effective population size
$N_e m_e$	Effective number of migrants per generation
NGS	Next-generation sequencing
NHGRI	National Human Genome Research Institute
NMR	Nuclear magnetic resonance spectroscopy
NRC	National Research Council (Canada)
nrITS	Nuclear ribosomal-ITS
N_s	Status number, a measure of effective population size
nSSR/nucSSR	Nuclear-SSR
ORF	Open reading frame
PAC	Product of approximating conditionals
PAGE	Polyacrilamide gel electrophoresis
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCSR	Proximal CMA band-specific repeat
PET	paired-end tag
PGI	Plant Gene Indices
pI	Isoelectric point
PLSR	Partial least squares regression
PMF	Peptide mass fingerprinting
PUT	Putative transcripts
PVPP	Polyvinylpyrrolidone
QCI	Queen Charlotte Islands
QTL	Quantitative trait loci
QTN	Quantitative trait nucleotide

R&D	Research and development
RAPD	Random(ly) amplified polymorphic DNA
rDNA	Ribosomal-DNA
RFLP	Restriction fragment length polymorphism
RNA-Seq	Whole-transcriptome shotgun sequencing
RT-PCR	Reverse transcriptase-PCR
SAGE	Serial analysis of gene expression
SAMT	S-Adenosylmethionine transferase
SCAR	Sequence characterized amplified region
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Somatic embryogenesis
SFS	Site frequency spectrum
SGS	Spatial genetic structure
SILAC	Stable isotope labeling by amino acids in cell culture
siRNA	Small-interfering RNA
siRNA	Short interfering RNA
SNP	Single nucleotide polymorphism
SPF	Spruce-Pine-Fir lumber specification
SPME	Solid-phase microextraction
SSC	Small region of single copy genes
SSR	Simple sequence repeat
STS	Sequence tagged site
tasiRNA	Trans-acting siRNA
TBR	Tree bisection reconnection
TDT	Transmission disequilibrium test
TLP	TUBBY-like protein
TOF	Time-of-flight
U-HPLC	Ultra-HPLC
USD	US Dollar
USDA	United States Department of Agriculture
UTR	Untranscribed region
UV/Vis	Ultraviolet-visible spectrophotometry
VGN	Crucifer Genome Network
WGS	Whole-genome sequencing/shotgun

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The Conifers (Pinophyta)

David S. Gernandt,^{1,*} Ann Willyard,² John V. Syring,³ and Aaron Liston⁴

ABSTRACT

Conifers (Pinophyta) are woody trees or shrubs with simple leaves, simple pollen cones, and compound or reduced ovulate cones. Despite their dominance in many terrestrial landscapes, the 670 species of extant conifers make up less than 0.3% of the species diversity of modern land plants. The fossil record of conifers, which extends to the Carboniferous, indicates that a much greater diversity is now extinct. Conifers occur on six of the seven continents and include both widely distributed, dominant species that form vast forests and narrow endemics. They rank as the largest, tallest, and longest living non-clonal terrestrial organisms on the Earth. *Pinus* is the largest extant genus with approximately 20 species distributed throughout the Northern Hemisphere. It is rivaled in diversity in the Southern Hemisphere and the tropics by *Podocarpus*, with approximately 105 species. Genetic diversity is often high in conifers, promoted by large population size, outcrossing reproductive systems, high mutation rates, and long distance dispersal of pollen and sometimes seeds. Estimates of ages and mutation rates in the group are expected to improve greatly as conceptual advances related to fossil interpretation converge with the enormous quantities of new sequence data being generated by genetic and phylogenetic studies of living species. Contrasting patterns of organellar and nuclear inheritance

¹Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-233, México Distrito Federal 04510, Mexico; e-mail: dgernandt@ibiologia.unam.mx

²Biology Department, Hendrix College, 1600 Washington Ave, Conway, Arkansas 72032, USA; e-mail: willyard@hendrix.edu

³Department of Biology, Linfield College, 900 SE Baker St., McMinnville, Oregon 97128, USA; e-mail: jsyring@linfield.edu

⁴Department of Botany and Plant Pathology, 2082 Cordley Hall, Oregon State University, Corvallis, Oregon 97331, USA; e-mail: listona@science.oregonstate.edu

*Corresponding author

make conifers an important system for studying pollen and seed flow, hybridization, lineage sorting, and gene coalescence.

Keywords: conifers; ecology; fossils; molecular clock; phylogeny; Pinophyta

1.1 Conifer Diversity

1.1.1 Classification and Phylogeny

1.1.1.1 Are Conifers Monophyletic?

Conifers are classified with seed plants, which include five living groups: conifers, cycads, *Ginkgo*, gnetophytes and angiosperms. The first four groups comprise the gymnosperms, which expose their ovules during pollination. There is wide acceptance for the rank of order Coniferales (also called Pinales), and conifers have often been recognized at the higher taxonomic ranks of class (Coniferae, Coniferopsida or Pinopsida) and division (Coniferophyta or Pinophyta). Living conifers are grouped in six families, 71 genera (Fig. 1-1), and ca. 670 species. In a recent global checklist (Farjon 2001), 69 genera and 630 species were recognized; we treat *Callitropsis* and *Xanthocyparis* as separate from *Cupressus* and recognize more species in Pinaceae and Cupressaceae.

Despite intensive study, the phylogenetic relationships among the major lineages of living and extinct seed plants remain ambiguous, with some DNA sequence analyses indicating that gnetophytes (*Ephedra*, *Gnetum*, and *Welwitschia*) are derived from conifers, rendering the conifers paraphyletic. Cladistic analyses of morphological characters (Crane 1985; Doyle and Donoghue 1986; Nixon et al. 1994) have recovered the gnetophytes and extinct gymnosperm groups like Bennettitales as more closely related to angiosperms than to other extant gymnosperms, thus supporting the Anthophyte hypothesis (Arber and Parkin 1907). Shared characters uniting these groups include “flower-like” reproductive structures, double fertilization (Friedman 1994), and the presence of vessels in their wood. In contrast, most molecular phylogenetic studies reject this hypothesis, placing gnetophytes either as sister to the conifers, the “gnetifer” hypothesis (Chaw et al. 1997), or sister to Pinaceae, within the conifers, the “gnepine” hypothesis (Bowe et al. 2000; Chaw et al. 2000). A close relationship between conifers and gnetophytes is supported by morphological characters such as simple leaves, compound ovulate cones, and wood anatomical characters that are also shared with *Ginkgo* such as tracheids with helical sculpturing intercalated with circular bordered pits, and the presence of a torus suspended by margo threads maintaining separation of the pits (Carlquist

1996). Nevertheless, results from molecular data have shown striking sensitivity to the choice of analytical method, characters, and taxonomic sampling (reviewed by Mathews 2009).

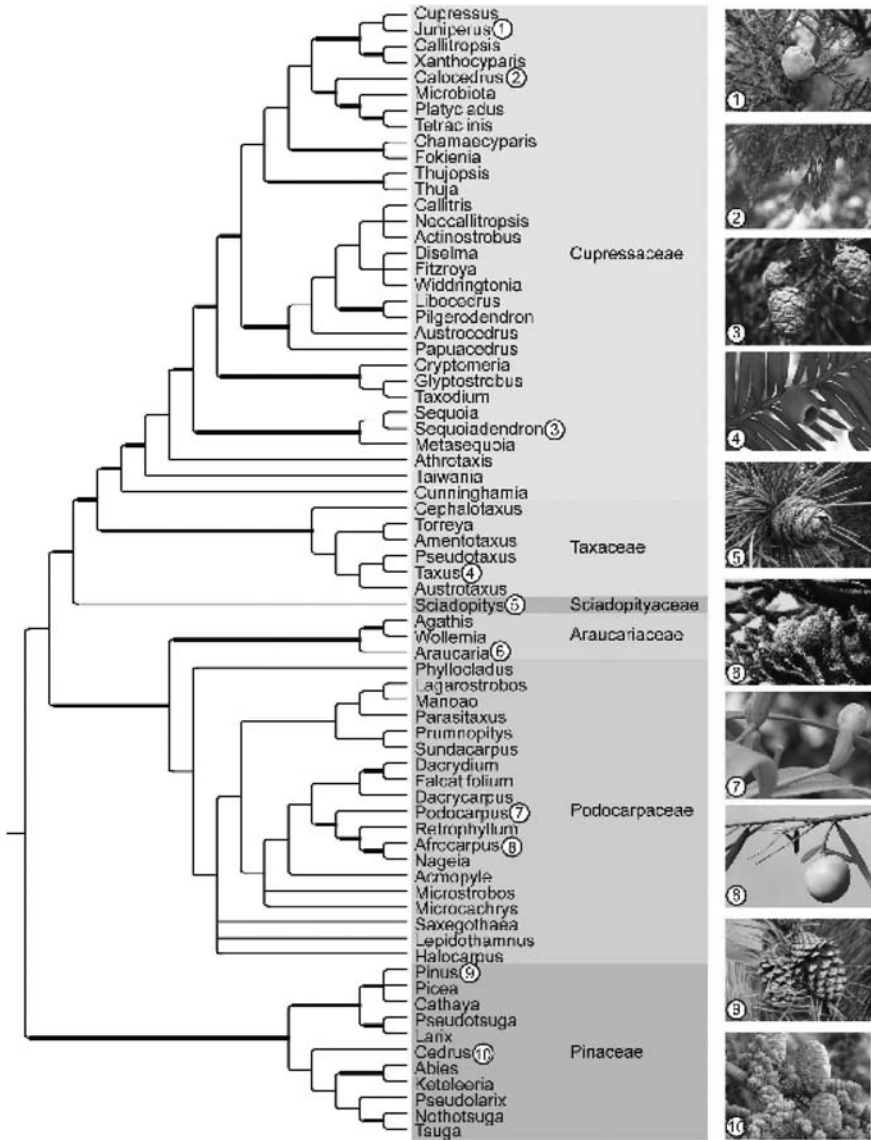


Figure 1-1 Conifer phylogenetic tree. A representation of our current understanding of intergeneric relationships.

Color image of this figure appears in the color plate section at the end of the book.

A recent phylogenetic analysis of 14 kbp of cpDNA for 38 taxa including 22 conifers representing all families failed to recover an association between Gnetales and conifers (Rai et al. 2008). This study included Sciadopityaceae and both subfamilies of Pinaceae, the most comprehensive taxonomic sampling of conifers to date. The chloroplast genome sequence of *Welwitschia* (McCoy et al. 2008) confirms that Gnetales possess the large inverted repeat that is present in most seed plants but lacking in conifers (Strauss et al. 1988; Wakasugi et al. 1994; Hirao et al. 2008). However, the *Pinus* “remnant” inverted repeat (495 bp including a duplicated *trnI*-CAU and partial *psbA*) could be derived from the inverted repeat of Gnetales (McCoy et al. 2008). The repeated *trnI*-CAU in *Cryptomeria* may be derived in a similar fashion (Hirao et al. 2008). These results are inconsistent with the hypothesis of a Gnetales–Pinaceae clade, but do not reject a sister group relationship between Gnetales and Pinophyta (the gnetifer hypothesis). For the purpose of this book, we exclude Gnetales from the conifers.

Another contentious issue in conifer classification has been the phylogenetic placement of the Taxaceae, most of whose members lack recognizable ovulate cones. Taxaceae has usually been considered a conifer family (Pilger 1926; Page 1990), but some botanists (Sahni 1920; Florin 1948) argued that it should be treated as a separate order, Taxales, principally because its ovules are borne terminally on lateral shoots rather than in cones. However, evidence from wood and leaf anatomy, embryological characters, and chloroplast, mitochondrial, and nuclear DNA sequences all unambiguously place Taxaceae within the conifers (Hart 1987; Chaw et al. 1993; Chaw et al. 2000; Quinn et al. 2002; Doyle 2006; Rai et al. 2008).

1.1.1.2 Relationships at the Level of Family and Genus

The six extant families of conifers are Pinaceae, Podocarpaceae, Araucariaceae, Sciadopityaceae, Taxaceae, and Cupressaceae (Table 1-1). Relationships among families and genera have become much clearer in recent years (Fig. 1-1). Molecular sequence data from the nuclear and chloroplast genomes have recovered Pinaceae as monophyletic and in a sister position to all other conifer families (Chaw et al. 1997; Stefanović et al. 1998; Quinn et al. 2002; Rai et al. 2008). The result is also supported by the loss of an intron in the mitochondrial *nad1* gene (Gugerli et al. 2001b) and in a morphological analysis of conifer genera (Hart 1987). Podocarpaceae and Araucariaceae have been recovered as sister groups consistently and with high branch support using nuclear and chloroplast data, but not with morphology (Hart 1987; Doyle 2006). Nuclear and chloroplast data strongly support a sister relationship between Taxaceae and Cupressaceae with Sciadopityaceae successively sister to them, which contrasts with previous morphological evidence uniting Cupressaceae and Sciadopityaceae (e.g., Hart 1987).

Table 1-1 Conifers of economic and/or ecological importance. This table is representative of conifer diversity, and not comprehensive.

Family	# Gen- era	Representative Genera or Subgenera	# Species	Representative Species	Native Range	Common Names	Notes	Genetic Resources
Pinaceae	11	<i>Abies</i>	50	<i>A. alba</i>	Europe	silver fir	forestry in Europe	nSSR
				<i>A. balsamea</i>	E Canada, NE USA	balsam fir	forestry in Canada, E USA	
		<i>Cedrus</i>	4	<i>C. atlantica</i>	NW Africa	Atlas cedar	forestry in NW Africa, horticulture	
		<i>Larix</i>	10	<i>L. decidua/L. sibirica/L. gmelinii</i>	N Eurasia	larch, Siberian larch, Dahurian larch	forestry in Europe & Russia	
				<i>L. laricina</i>	Canada, N USA	tamarack	forestry in Canada, E USA	
		<i>Picea</i>	34	<i>P. abies</i>	Europe	Norway spruce	forestry in Europe	nSSR, EST
				<i>P. glauca</i>	Canada, N USA	white spruce	forestry in Canada	EST
				<i>P. mariana/P. rubens</i>	E Canada, NE USA	black spruce, red spruce	forestry in Canada, E USA	
				<i>P. sitchensis</i>	W Canada, NW USA	Sitka spruce	forestry in Canada, W USA, NZ	EST
		<i>Pinus</i> subg. <i>Pinus</i>	ca. 80	<i>P. banksiana/P. contorta</i>	Canada, N USA	jack pine, lodgepole pine	forestry in Canada, Scandinavia	
				<i>P. brutia/P. halepensis</i>	Mediterranean	Brutia pine, Aleppo pine	forestry in Mediterranean, other arid zones	nSSR

Table 1-1 contd....

Table 1-1 contd....

Family	# Gen- era	Representative Genera or Subgenera	# Species	Representative Species	Native Range	Common Names	Notes	Genetic Resources
				<i>P. caribaea</i>	C America, Caribbean	Caribbean pine	subtropical forestry	nSSR
				<i>P. densiflora</i>	China, Korea, Japan	Japanese red pine	forestry in Japan, Korea	EST
				<i>P. elliottii</i>	SE USA	slash pine	forestry in SE USA, S Africa	nSSR
				<i>P. kesiya</i>	SE Asia		forestry in SE Asia	
				<i>P. massoniana</i>	China	Chinese red pine	forestry in SE Asia	
				<i>P. merkusii</i>	SE Asia	Sumatran pine	forestry in SE Asia	
				<i>P. nigra/P. thunbergii</i>	Eurasia, Japan	black pine, Japanese black pine	horticulture	
				<i>P. pinaster</i>	Mediterranean	maritime pine	forestry in Mediterranean, other arid zones	nSSR, EST
				<i>P. pinea</i>	Mediterranean	Italian stone pine	forestry in Mediterranean, other arid zones	EST
				<i>P. oocarpa</i>	Mexico		subtropical forestry	
				<i>P. patula</i>	Mexico	Mexican weeping pine	subtropical forestry	
				<i>P. ponderosa</i>	W Canada, W USA	ponderosa pine	forestry in W USA	

<i>Pinus</i> subg. <i>Strobos</i>	ca. 40	<i>P. radiata</i>	California, USA	Monterey pine, radiata pine	forestry in Australia, NZ, Chile, S Africa	nSSR, EST
		<i>P. resinosa</i>	E Canada, NE USA	red pine	forestry in E USA	
		<i>P. sylvestris</i>	Europe	Scots pine	forestry in Europe, Russia	EST
		<i>P. tabuliformis/P. yunnanensis</i>	China, Korea	Chinese pine, Yunnan pine	forestry in China	
		<i>P. taeda</i>	SE USA	loblolly pine	forestry in SE USA, Australia	nSSR, EST
		<i>P. armandii</i>	China, Japan	Armand pine	forestry in China	
		<i>P. cembroides/P. edulis/P. monophylla</i>	W USA, N Mexico	pinyon pine	local forestry, ecological genomics	
		<i>P. albicaulis/P. lambertiana/P. monticola</i>	W Canada, W USA	whitebark pine, sugar pine, western white pine	pathogen induced decline	
		<i>P. cembra/P. sibirica/P. pumila</i>	N Eurasia, Japan	stone pine	local forestry	
		<i>P. krempfii</i>	Vietnam	Krempf's pine	only pine with flat needles	
<i>Pseudotsuga</i>	4	<i>P. longaeva</i>	W USA	Great Basin bristlecone pine	oldest living tree	
		<i>P. menziesii</i>	Canada, W USA	Douglas-fir	forestry in Canada, USA, NZ	nSSR, EST
<i>Tsuga</i>	9	<i>T. canadensis</i>	E Canada, NE USA	Eastern hemlock	pest-induced dieback	

Table 1-1 contd....

Table 1-1 contd....

Family	# Gen- era	Representative Genera or Subgenera	# Species	Representative Species	Native Range	Common Names	Notes	Genetic Resources
Araucariaceae	3	<i>Agathis</i>	23	<i>T. heterophylla</i>	W Canada, W USA	Western hemlock (Canadian pine, Australia)	forestry in Canada	nSSR
				<i>A. australis</i>	New Zealand	kauri	historical forestry in NZ	
				<i>A. damarra</i>	SE Asia	East Indian kauri	forestry in SE Asia	
			19	<i>A. angustifolia</i>	Brazil, Paraguay, Argentina	Parana pine	forestry in Brazil	
				<i>A. araucana</i>	S Argentina, S Chile	monkey-puzzle tree	local forestry	
				<i>A. bidwillii</i>	NE Australia	bunya-bunya tree	forestry in Australia, horticulture	
			1	<i>A. cunninghamii</i>	NE Australia, New Guinea	hoop pine	local forestry	
				<i>A. heterophylla</i>	Norfolk Island	Norfolk Island pine	horticulture	
Podocarpaceae	19	<i>Wollemia</i>	6	<i>W. nobilis</i>	SE Australia	Wollemi pine	discovered in 1994, horticulture	
				<i>A. falcatus/A. gracilior</i>	E Africa, S Africa	yellow-wood	local forestry	

		<i>Nageia</i>	6	<i>N. nagi</i>	SE Asia	broad-leaved podocarpus	local forestry	
		<i>Parasitaxus</i>	1	<i>P. usta</i>	New Caledonia	corail	only parasitic gymnosperm	
		<i>Podocarpus</i>	105	<i>P. totara</i>	New Zealand	totara	forestry in NZ	
Sciadopityaceae	1	<i>Sciadopitys</i>	1	<i>S. verticillata</i>	Japan	koyamaki, umbrella pine	horticulture	
Taxaceae (includes Cephalotaxaceae)	6	<i>Taxus</i>	10	<i>T. baccata/T. cuspidata</i>	Europe, China, Korea, Japan	yew, Japanese yew	local forestry, horticulture, pharmaceuticals	EST
Cupressaceae	31	<i>Callitris</i>	16	<i>C. glaucophylla</i>	E Australia	white cypress pine	forestry in Australia	
		<i>Callitropsis</i>	18	<i>C. lusitanica</i>	Mexico, C America	Mexican cypress	forestry in C America, horticulture	
				<i>C. macrocarpa</i>	California, USA	Monterey cypress	horticulture	
				<i>C. nootkatensis</i>	W Canada, NW USA	Alaska yellow- cedar	forestry in Canada	
		<i>Chamaecyparis</i>	5	<i>C. obtusa</i>	S Japan, Taiwan	hinoki cypress	forestry in Japan, Taiwan	nSSR, EST
				<i>C. lawsoniana</i>	NW USA	Port Orford cedar	historical forestry, pathogen induced decline	
		<i>Cryptomeria</i>	1	<i>C. japonica</i>	China, Japan	sugi	forestry in Japan, China	nSSR, EST

Table 1-1 contd....

Table 1-1 contd....

Family	# Gen- era	Representative Genera or Subgenera	# Species	Representative Species	Native Range	Common Names	Notes	Genetic Resources
		<i>Cunninghamia</i>	1-2	<i>C. lanceolata</i>	China, Vietnam, Laos	China fir	forestry in China	EST
		<i>Cupressus</i>	12	<i>C. sempervirens</i>	Mediterranean	Italian cypress	horticulture	
		<i>Fitzroya</i>	1	<i>F. cupressoides</i>	S Argentina, S Chile	alerce	local forestry; tetraploid	
		<i>Juniperus</i>	67	<i>J. virginiana</i>	E Canada, E USA	Eastern redcedar	local forestry	
				<i>J. communis</i>	Circumboreal	common juniper	horticulture, flavoring (gin)	nSSR
		<i>Metasequoia</i>	1	<i>M. glyptostroboides</i>	China	dawn redwood	discovered in 1944, horticulture	
		<i>Platycladus</i>	1	<i>P. orientalis</i>	China, Korea, E Russia	Chinese arbor- vitae	forestry in China	
		<i>Sequoia</i>	1	<i>S. sempervirens</i>	California, USA	coast redwood	forestry in USA, tallest trees; hexaploid	
		<i>Sequoiadendron</i>	1	<i>S. gigantea</i>	California, USA	giant sequoia	largest trees, horticulture	
		<i>Taiwania</i>	1	<i>T. cryptomerioides</i>	China	Taiwania	local forestry	EST
		<i>Taxodium</i>	2	<i>T. distichum</i> / <i>T. mucronatum</i>	USA, Mexico, Guatemala	baldcypress	local forestry, horticulture	EST
		<i>Thuja</i>	5	<i>T. plicata</i>	W Canada, NW USA	western red cedar	forestry in Canada, USA	

Pinaceae is comprised of 10–11 genera (the separation of *Nothotsuga* from *Tsuga* is not universally accepted), and there is moderate support from all three genomes (Wang et al. 2000) and from morphology (Hart 1987; Gernandt et al. 2008) for dividing the family into two subfamilies, Pinoideae and Abietoideae. Relationships among the genera of subfamily Abietoideae (*Cedrus*, *Abies*, *Keteleeria*, *Tsuga*, *Nothotsuga*, and *Pseudolarix*) are not robust and the subfamily may actually be paraphyletic to Pinoideae (Wang et al. 2000; Gernandt et al. 2008).

Relationships among the approximately 19 genera of Podocarpaceae are less certain than in other families. Morphological, embryological, and molecular evidence indicate that *Podocarpus sensu lato* is not monophyletic (Page 1989; Kelch 1997, 1998; Conran et al. 2000; Sinclair et al. 2002; Barker et al. 2004). *Podocarpus* is now restricted to ca. 105 species, with the designation of the genera *Afrocarpus*, *Dacrycarpus*, *Nageia*, *Parasitaxus*, *Prumnopitys*, *Retrophyllum*, and *Sundacarpus*. The genus *Phyllocladus* sometimes has been recognized as the separate family, Phyllocladaceae, based on the presence of cladodes and reduced, scale-shaped leaves. It also lacks an epimatium (a fleshy structure subtending the ovule probably homologous to the ovuliferous scale; Tomlinson and Takaso 2002). However, the epimatium is absent in other Podocarpaceae genera (e.g., *Microstrobus*) and the sister relationship between *Phyllocladus* and the rest of Podocarpaceae is not robust; recognition of the separate family Phyllocladaceae is thus unsupported.

Araucariaceae includes three extant genera. Early studies of phylogenetic relationships based on *rbcL* sequences recovered *Wollemia* as sister to *Agathis* and *Araucaria* (Setoguchi et al. 1998), but more recent studies with longer cpDNA data sets and more taxa have recovered *Wollemia* as sister to *Agathis* (Quinn et al. 2002; Knapp et al. 2007; Rai et al. 2008).

Cephalotaxus has often been separated from Taxaceae because its ovules are borne in recognizable cones. Molecular evidence has shown that it is the sister group to the other five genera of Taxaceae (Cheng et al. 2000; Quinn et al. 2002; Rai et al. 2008). Although recognition of Cephalotaxaceae would not render Taxaceae paraphyletic, its relatively modest genetic and morphological differentiation from Taxaceae are considered insufficient for recognition of a separate family. Its sister relationship with remaining members of the family is consistent with the hypothesis that the absence of compound ovulate cones in the other genera is due to a secondary loss. The remaining five genera of Taxaceae were divided into tribes Taxeae and Torreyaee, and this division is reflected in two clades inferred from DNA (Cheng et al. 2000) but not by morphology (Hart 1987).

Taxodiaceae (nine genera) was formerly recognized as separate from Cupressaceae, but the morphological differences are minor (alternate vs. opposite leaves in four ranks or whorled) and they possess similar cone morphology and karyotypes (Eckenwalder 1976). *Sciadopitys* was often

classified in Taxodiaceae, despite its unique dimorphic shoots, presence of cladodes (photosynthetic branchlets) in place of leaves, and a chromosome number of $2n = 20$ (Farjon 2005). Molecular evidence has demonstrated that all Taxodiaceae genera except *Sciadopitys* are paraphyletic to Cupressaceae (Brunsfeld et al. 1994; Gadek et al. 2000; Kusumi et al. 2000; Quinn et al. 2002; Rai et al. 2008). *Sciadopitys* is now recognized as a monotypic family and the Cupressaceae has been expanded to include the other genera previously placed in Taxodiaceae. The 31 genera of Cupressaceae have been divided into seven subfamilies (Gadek et al. 2000).

The paraphyly of the genera formerly classified in Taxodiaceae with Cupressaceae clarifies the interpretation of ancestral states for this family. For example, *Cunninghamia*, the sister group to all other Cupressaceae, has three inverted ovules on each bract, while *Taiwania*, which is successively sister to the rest of Cupressaceae, has two ovules. This suggests that the proliferation of ovules on each bract-scale complex, the erect orientation of ovules in Cupressaceae and the reduced number of ovules per scale in some species of *Juniperus*, are more recently derived innovations (Farjon and Ortiz Garcia 2003).

1.1.2 Geographic Distribution

The natural range of conifers is from 55° south latitude on Tierra del Fuego in South America (*Pilgerodendron uviferum* Florin) (Veblen et al. 1995), to 75° north latitude deep within the Arctic Circle in Siberia (*Larix gmelinii* (Rupr.) Kuzen) (Farjon 2003). Many occur in extreme environments typified by high altitudes, high latitudes, and/or ecosystems with nutrient-poor soil (Stopes and Kershaw 1910; Richardson and Rundel 1998; Coomes et al. 2005). Between these extremes, ecological limitations on conifer distribution appear to be predominantly controlled by their ability to compete with angiosperms (Bond 1989; Coomes et al. 2005).

Although mostly absent from deserts, conifers are often found in environments with relatively high levels of evaporative stress, such as high light—low temperature (alpine tree line), high light—high temperature (semi-desert pinyon-juniper woodlands), and in temperate ecosystems with summer drought and winter rain where they compete well with deciduous angiosperms. The most extensive coniferous region in the world is the northern boreal forest, where *Picea*, *Abies*, *Pinus*, and *Larix* (Pinaceae) are dominant genera (Richardson and Rundel 1998). Conifer-dominated ecosystems are more frequent in the Northern Hemisphere, while in the Southern Hemisphere conifers are typically found either individually or as associates in mixed hardwood-conifer forests (Ogden and Stewart 1995). Geographic ranges of the species vary widely, from continent-wide (e.g., *Pinus sylvestris* Mill.) to narrow endemics only recently discovered

(e.g., *Wollemia nobilis* W.G. Jones, K.D. Hill & J.M. Allen). Many species are rare and/or threatened with extinction (Farjon et al. 1999). In *Pinus*, geographic ranges have been shown to decrease with increasing proximity to the equator (Stevens and Enquist 1998), while species diversity increases dramatically along this same gradient (Farjon et al. 1993).

Overall, the Northern Hemisphere contains about 70% of total conifer diversity (Farjon 2001). Regions with high species diversity include California, Mexico, the Chinese provinces of Sichuan and Yunnan, and the eastern Himalayas, Japan, Taiwan, and New Caledonia (Farjon 2001). Pinaceae comprises 11 genera and 238 species distributed throughout Eurasia, North Africa, the Himalayas, and North and Central America. *Pinus*, with approximately 120 species, is the largest genus. The only member of Pinaceae that occurs naturally in the Southern Hemisphere is *Pinus merkusii* Jungh. & de Vriese, with a distribution that crosses the equator in Sumatra.

Podocarpaceae, with approximately 19 genera and 190 species, and Araucariaceae, with three genera and approximately 42 species, are distributed across the Southern Hemisphere and the tropics. Podocarpaceae occurs in Africa, South America, Australia, South and East Asia, Indonesia, and numerous other islands of the South Pacific. Other Podocarpaceae taxa occur north of the equator in East Africa, Japan, China, Central America, and Mexico. *Podocarpus*, with ca. 105 is the largest genus, and once better studied, may eventually be shown to be more diverse than *Pinus* (Farjon 2001, 2003). Araucariaceae occurs in South America, South and East Asia, Australia, and on islands throughout the South Pacific. The largest genera are *Agathis* (ca. 23 species) and *Araucaria* (19 species).

Sciadopitys verticillata Siebold & Zucc., the sole representative of Sciadopityaceae, is endemic to southern Japan. Taxaceae (6 genera and 24 species) occurs primarily in the Northern Hemisphere (North and Central America, Eurasia, and the Himalayas), but *Taxus sumatrana* Miquel de Laub. occurs south of the equator and the monotypic genus *Austrotaxus* is endemic to New Caledonia. *Taxus*, with 10 species, is the largest genus. Cupressaceae, with approximately 31 genera (approximately 18 monotypic) and 165 species (Little 2006), occurs on every continent except Antarctica. *Juniperus*, with ca. 67 species, is the largest genus.

1.1.3 Life History and Ecology

Most conifers are monopodial trees, and include the largest and longest living non-clonal organisms on Earth (Waring and Franklin 1979). Western North American ecosystems provide a striking array of the world's tallest and largest trees, including *Sequoia*, the tallest (maximum height 115 m), and *Sequoiadendron*, the most massive (>1,400 m³). Other genera that

attain extraordinary size in western North America include *Pseudotsuga*, *Picea*, *Abies*, *Pinus*, *Thuja*, and *Chaemaecyparis*. However, this habit is not geographically limited; *Agathis australis* Steud. and *Dacrycarpus dacrydioides* (A. Rich) de Laub. (New Zealand), *Fitzroya cupressoides* I.M. Johnst. (South America), *Cryptomeria japonica* D. Don (Japan) and *Taxodium mucronatum* Ten. (Mexico and Guatemala) are all remarkable. Other conifers are shrubs either throughout their range (e.g., *Microcachrys tetragona* Hook.f.) or at their altitudinal extremes where they may take on a Krummholz form (e.g., *Pinus albicaulis* Engelm., *Athrotaxis selaginoides* D. Don). *Parasitaxus usta* Vieill. de Laub. (Podocarpaceae) is the only parasitic conifer; it obtains carbon from the roots of *Falcatifolium taxoides* (Brongn. & Gris) de Laub. (Podocarpaceae) via a vesicular-arbuscular mycorrhizal association (Feild and Brodribb 2005).

Conifer forests achieve dominance in a variety of environments through a suite of structural characters (Waring and Franklin 1979). The leaves of most conifers are evergreen (retained for years, sometimes decades), and possess several modifications that reduce water loss while conducting photosynthesis under a wider range of conditions than most angiosperms. Conifer leaves are typically needle-like (Araucariaceae, Pinaceae, Podocarpaceae, Sciadopityaceae, and Taxaceae) or scale-like (Cupressaceae), conferring a high surface area to volume ratio and maximizing the diffusion of heat. The conical crowns, the separation between branch layers, the arrangement, density, and orientation of leaves on branches, the thickness of the cuticle covering the epidermis, and the distribution and degree to which the stomata are sunken in the epidermis are important in enhancing photosynthesis and limiting environmental stress (Smith and Brewer 1994). Roughly 20 species in five genera are deciduous (Pinaceae: *Larix*, *Pseudolarix*; Cupressaceae: *Glyptostrobus*, *Metasequoia*, and *Taxodium*).

Loehle (1988) estimated a typical life span of North American conifers at 400 years, while Enright and Ogden (1995) estimate 525 years for all Southern Hemisphere conifers. This is in stark contrast to the 250 years calculated for angiosperm trees (Loehle 1988). *Pinus longaeva* D.K. Bailey is the oldest recorded, non-clonal living organism in the world, with one living individual aged at ca. 4,700 years. A 9,550-year old *Picea abies* (L.) H. Karst has been recently reported from Sweden, but awaits publication in a peer-reviewed journal. There are a number of species with the potential to exceed 2,000 years (e.g., *Sequoiadendron giganteum* (Lindl.) J. Buchholz, *Lagarostrobos franklinii* (Hook.f.) Quinn, *Fitzroya cupressoides* I.M. Johnst.) (Lanner 2002).

Conifers have unisexual reproductive structures, with ovulate and pollen cones either on the same (monoecious) or different plants

(dioecious). Other gymnosperm groups—Cycadales, Ginkgoales and Gnetales—are dioecious (only rarely monoecious), and dioecy occurs in genera of the families Araucariaceae, Podocarpaceae, Taxaceae, and Cupressaceae (Coulter and Chamberlain 1917; Sporne 1965). Only Pinaceae and Sciadopityaceae are exclusively monoecious. The reproductive cycle of most conifers is one to three years (Owens et al. 1998). The minimum age to first seed set is highly variable, but in natural populations of *Pinus*, ranges between ca. 10–25 years (Mirov 1967; Lanner 1998). However, the first seed crops and the seed from early producers are likely to be minimal in number with reduced viability (Lanner 1998). This makes it difficult to establish generation times, complicating calculations of per-generation mutation rates and effective population size.

While certainly less common than in angiosperms, conifers display a wide range of asexual forms of reproduction that allow them to maintain dominance at a site (Ogden and Stewart 1995). These include resprouting from basal lignotubers in *Sequoia sempervirens* Endl., from the root collar in *Pinus rigida* Mill., from epicormic buds on buried stems in *Actinostrobus acuminatus* Parl., and vegetative layering in *Picea* and *Phyllocladus aspleniifolius* (Labill.) Hook.f.

Outcrossing in conifers is promoted through dioecy, monoecy, and physical separation of the sexes on the plant. Self-fertilization is possible, but the effects of inbreeding depression are pronounced (Mirov 1967), leading to a reduction in seed set and growth (Keeley and Zedler 1998; Sorensen 2001). Nevertheless, it is possible that facultative selfing has proved beneficial by providing a means for conifers to disperse across the landscape, taking advantage of landscape disturbances and responding to changing climates. Prezygotic isolating mechanisms in conifers are limited (Williams et al. 2001), allowing for the potential of interspecific hybridization. However, in *Pinus*, the ability to hybridize is generally restricted to members of the same subsection, suggesting that barriers develop through time. Even amongst closely related species, some pairings never yield any progeny (Critchfield 1986). While studies documenting potential hybrid speciation exist (Ma et al. 2006), most interspecific hybridization is geographically restricted to regions of sympatry. Even so, introgression at the local level may prove important in the maintenance of intraspecific heterozygosity (Mirov 1967; Ledig 1998).

Seed dispersal most commonly occurs via wind, as in the dry, winged seeds of most Pinaceae, or a combination of birds and small mammals as in seeds surrounded by arils or epimatia (Taxaceae or Podocarpaceae), or the dry, wingless seeds of the “stone pines”. In *Juniperus*, the unit of dispersal is the fleshy cone. Bird dispersal is more predominant in the

Southern Hemisphere due to the prevalence of the Podocarpaceae (Enright et al. 1995). Seed transport in excess of 22 km has been reported for bird dispersal in *Pinus* (Lanner 1998; Ledig 1998), while the range of pollen dispersal can be on the order of tens to hundreds of kilometers (Burczyk et al. 2004). Widespread distribution of the pollen acts mainly as a cohesive force reducing population differentiation, while occasional long-distance dispersal of the seed provides a means for species migration and population establishment (Ledig 1998). In at least some conifers, migration rates have been shown to be among the fastest of all tree species (Ledig 1998; Sannikov and Sannikova 2008).

Conifers are found in ecosystems that can exhibit tremendous biomass accumulation and some of the highest worldwide productivities (Franklin and Halpern 2000). Given their propensity to attain great heights, they are commonly canopy emergents. Conifers are generally early successional, light-demanding species unable to regenerate in mass under dense canopies. However, due to varying degrees of shade tolerance (Enright and Ogden 1995), some species occur in late successional forests where they are able to regenerate in the understory (e.g., members of Taxaceae, *Tsuga canadensis* Carrière, *Prumnopitys ferruginea* (D. Don) de Laub.) (Enright and Ogden 1995). Through periods of episodic recruitment following disturbance, coupled with their tremendous longevity, “relictual” conifer stands or individuals of early successional species can be found in mixed hardwood-conifer forests.

Disturbance is an integral component of succession for many conifers. Most shade-intolerant species have evolved strategies to take advantage of a variety of disturbance regimes (Agee 1998; Enright and Ogden 1995). Fire has probably been the most thoroughly studied disturbance (Veblen et al. 1995; Agee 1998) and has been an intensive selective force in the evolution of conifer life-history strategies. Fire strategies vary by species, and adaptations include cone serotiny and flammable foliage (e.g., *Pinus contorta* Dougl. ex Loudon), resprouting (e.g., *Widdringtonia cupressoides* Endl., *Sequoia sempervirens*, *Pinus rigida*), insulating bark (e.g., *Pinus ponderosa* Douglas ex P. Lawson & C. Lawson), and the seedling grass stage of several species of pines (e.g., *Pinus devoniana* Lindl., *P. palustris* Mill., *P. merkusii* Jungh. & de Vriese) (Keeley and Zedler 1998). Many conifers lacking these specific life history features are adapted to reinvade burned sites through the production of light, wind born seeds (Barnes 1991; Larson and Franklin 2005).

1.1.4 Cytology and Genetics

Conifer basic chromosome numbers vary from nine in Podocarpaceae to 22 in Pinaceae (*Pseudolarix*). The ancestral condition is likely to be 12

chromosomes (Flory 1936; Page 1990), however this has not been examined in a phylogenetic framework. Numbers can be conserved within genera, as in *Pinus* ($n = 12$), or they can be highly variable, as in *Dacrydium* or *Podocarpus* (Page 1990). Polyploidy has played a minor role in the evolution of conifers, the only naturally occurring cases are tetraploid *Fitzroya cupressoides* and hexaploid *Sequoia sempervirens* (Ahuja 2005).

Genetic diversity in conifers is generally high, promoted by large population sizes, long life spans, outcrossing reproductive systems, high mutation rates, and long distance dispersal of pollen, and sometimes seeds (Hamrick et al. 1992; Ledig 1998). Hamrick et al. (1992) estimated an average of 71.1% polymorphic loci and 16.9% expected heterozygosity across representative gymnosperms heavily favoring conifers. Ledig (1998) recognizes *Pinus* as one of the most variable of organisms with an average of 70.4% polymorphic loci and typical expected heterozygosity of 13 to 16%. Quiroga and Premoli (2007) reported 57.0% polymorphic loci and an expected heterozygosity of 14.8% in *Podocarpus parlatorei* Pilg., values within the reported range for other conifers. Some conifers do have low levels of genetic diversity. Most known examples are narrow endemics, including *Pinus torreyana* Carrière (Provan et al. 1999), *Picea chihuahuana* Martínez (Ledig et al. 1997), and *Picea omorika* (Pančić) Purk. (Ballian et al. 2006). In contrast, *Pinus resinosa* Aiton has low genetic diversity but a wide geographic distribution in eastern North America (Walter and Epperson 2005). Due to their outcrossing reproductive system, the ability of pollen to travel vast distances, and occasional long-distance seed dispersal, most species of conifers show little among population differentiation (Ledig 1998). Exceptions occur where drift is acting on small, fragmented populations (Ledig et al. 1997; Ge et al. 1998; Ballian et al. 2006).

As a result of their life history traits, conifers will generally have large effective population sizes (N_e), though variation by species is expected according to individual history (Syring et al. 2007a, b). Across *Pinus*, N_e estimates range from 1.7×10^4 in *P. flexilis* James to 1.2×10^5 in *P. lambertiana* Dougl. (Syring et al. 2007b). Values for three species of *Picea* are on the same order of magnitude as the higher *Pinus* estimates ($1.2\text{--}1.5 \times 10^5$) (Bouillé and Bousquet 2005). For comparison, reports from both inbreeding and outcrossing angiosperm species are typically less than 1.0×10^4 (Schoen and Brown 1991; Reusch et al. 2000). Large N_e promotes the retention of allelic diversity and has implications for phylogenetic analyses (see below). Because conifer species are less likely to form large, contiguous populations in the Southern Hemisphere (Enright 1995), it is tempting to assume that N_e will be larger for Northern Hemisphere species. However, geographic range is known to be a poor predictor of N_e (Syring et al. 2007b). Future estimates of N_e would prove informative.

1.2 Morphology and Fossil History

1.2.1 Morphology

Conifers are woody trees or shrubs with resin canals and single-veined simple leaves reduced to needles, scales, or blades. They have unisexual simple pollen cones and compound ovulate cones. Different interpretations have been proposed for the morphological and embryological characters that unite conifers (Hart 1987; Loconte and Stevenson 1990; Rothwell and Serbet 1994; Doyle 2006). Some characters are present in other plant groups, living and extinct, including uniseriate rays in the wood, also in *Ginkgo*, a torus in the tracheid pits, also in *Ginkgo*, *Gnetum*, and *Ephedra*, and simple needle-like leaves, also in *Ephedra*. The resin canals that are present in almost all conifers, variously distributed in leaves, shoots, roots, and/or seed coats, have been considered a synapomorphy (shared derived character); but the mucilage canals of *Ginkgo* are similar (Hart 1987). Other characters thought to be synapomorphies, such as the compound ovulate cone with inverted ovules, are lost, reduced, or otherwise modified in some genera (see below). Simple pollen cones with helically arranged scale-like microspophylls bearing free sporangia abaxially, are prevalent, but in Cupressaceae the microsporophylls are cyclical and borne terminally on lateral shoots (Stewart and Rothwell 1993). Characters such as five or fewer free nuclear divisions during embryogenesis and a stratified or tiered proembryo system are considered conifer synapomorphies (Hart 1987; Loconte and Stevenson 1990), but our knowledge of these characters is lacking in many living and fossil species.

Ovulate cones. The ovulate cone of conifers is generally interpreted as a compound inflorescence that includes a central axis that gives rise to fertile axillary shoots, often reduced to ovuliferous scales (Florin 1951). Cones of Cupressaceae, Sciadopityaceae, Araucariaceae, and Pinaceae have bract-scale complexes that are bilateral and dorsiventrally compressed. In Araucariaceae and some Cupressaceae, the bract-scale complex shows varying degrees of fusion, and in some taxa it is difficult to identify these structures (Tomlinson and Takaso 2002; Farjon and Ortiz Garcia 2003).

Independent, extreme reduction of the cone scale complex has taken place in Podocarpaceae and Taxaceae. The Podocarpaceae cone is composed of one or two ovules subtended by a scale that is often modified into an epimatium, which is in turn subtended by a bract (Tomlinson and Takaso 2002). Exceptionally, up to 15 ovule bearing complexes per cone can be present (*Prumnopitys*). In Taxaceae, the ovule is born terminally on its axis. In *Taxus*, the terminal ovule and aril are produced on a short secondary axis subtended by bracts, occasionally with indeterminate growth, while in *Torreya* they are produced on a primary axis (Tomlinson and Takaso

2002). In *Juniperus* (Cupressaceae), the cone scales are fleshy and fused into a bird dispersed “berry-like” structure. Multiple lineages of *Pinus* (Pinaceae) have cones with relatively few scales and enlarged, functionally wingless, bird-dispersed seeds. One of the two seeds per cone scale often aborts, presumably allowing for the more extensive growth of the surviving seed.

Wood. The exceptional size and height of many conifers with respect to other living organisms is due in part to the strength of their wood (secondary xylem), which is composed of thick walled vertical tracheids with bordered pits and lacks vessels. In addition to conducting water and nutrients, these cells provide much greater mechanical support than thin-walled parenchyma cells (Greguss 1955). The type of pitting, together with the arrangement of the horizontal rays, is diagnostic for conifer families. The horizontal rays have also undergone specialization, from homogeneous, thin walled parenchyma as seen in cycads, *Ginkgo*, and fossil conifer woods similar to modern Araucariaceae and Podocarpaceae, to heterogeneous, with variously pitted ray parenchyma and ray tracheids. Heterogeneous rays are found in two separate lineages, Cupressaceae (*Sequoia* and *Metasequoia*) and Pinaceae.

Pollen morphology and ovule orientation. The pollen grains of many conifers have air bladders, or sacci. The presence of air bladders facilitates pollen dispersal by wind, although their primary function is probably to orient pollen grains on pollen drops exuded on the micropyle of the ovulate cone, allowing germination towards the nucellar chamber (Doyle 1945; Tomlinson 1994). In many conifers, fertilization is facilitated by the absorption of the pollen drop, which draws the pollen inside the nucellus. Pollen drops appear to be functionally linked to ovule inversion and the presence of pollen sacs (Tomlinson and Takaso 2002). Families with ovules that are inverted during pollination (Pinaceae and Podocarpaceae) tend to have saccate pollen, and families with erect ovules during pollination (Araucariaceae, Sciadopityaceae, Taxaceae, and Cupressaceae) have nonsaccate pollen.

1.2.2 Fossil Record

Conifers have a rich fossil history, and evidently the living species represent only a fraction of past diversity (reviewed in Stockey 1982; Alvin 1988, Miller 1988; Rothwell and Scheckler 1988; Rothwell et al. 2005; Stockey et al. 2005). Gymnosperms were morphologically diverse during the Carboniferous (Pennsylvanian; ca. 300 Mya) and Permian (ca. 250 Mya). The sister group to conifers may be the Cordaitales, a diverse lineage of small woody shrubs or trees with large, helically arranged strap-shaped leaves and compound, monosporangiate ovulate and pollen cones known from Pennsylvanian