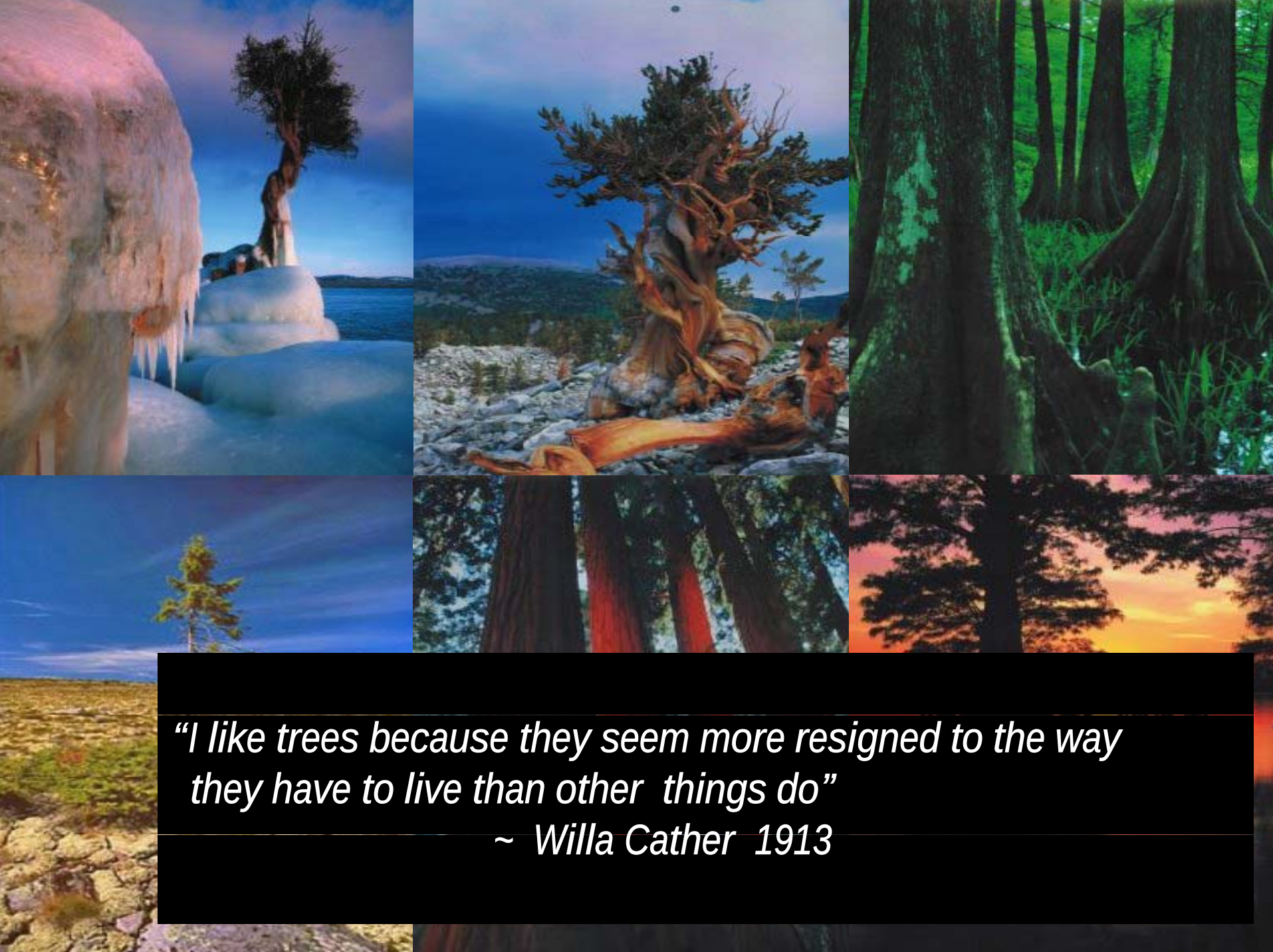
[redacted]

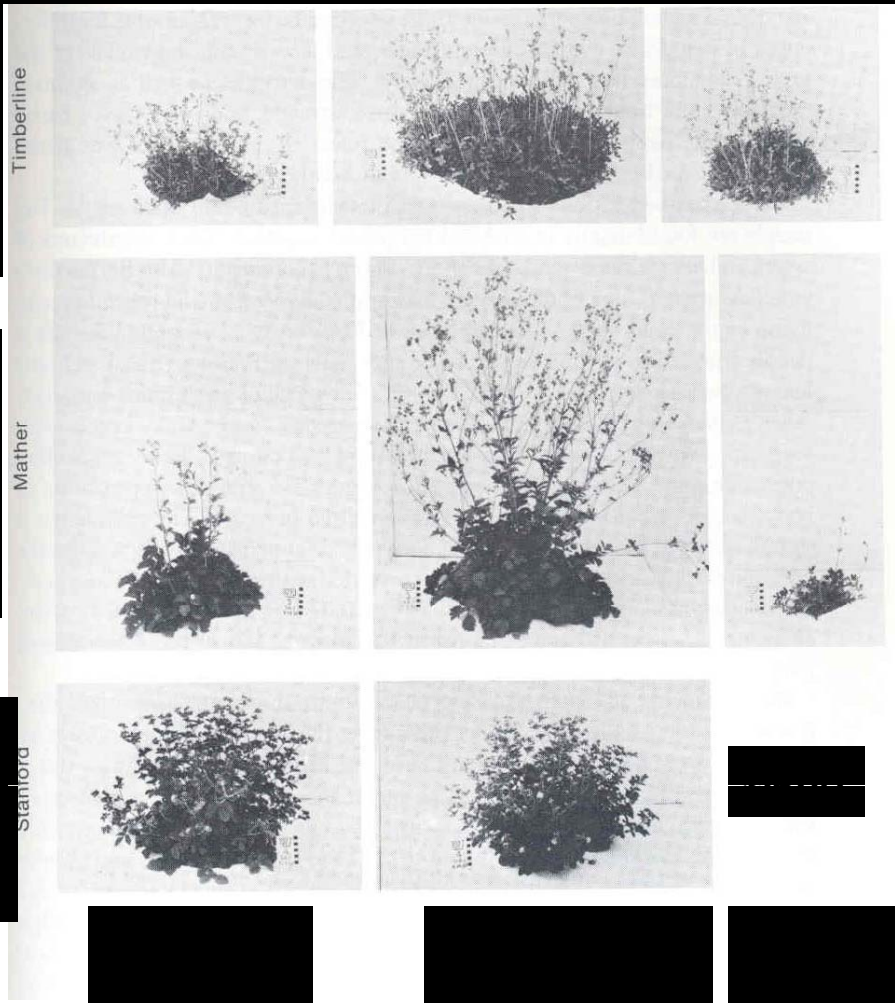


“I like trees because they seem more resigned to the way they have to live than other things do”

~ Willa Cather 1913

Potentilla glandulosa from three different elevations planted at three different elevations (from Clausen, Keck and Hiesey 1940)

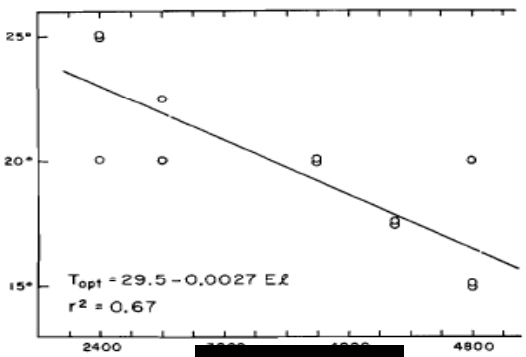
Native to



Grown at

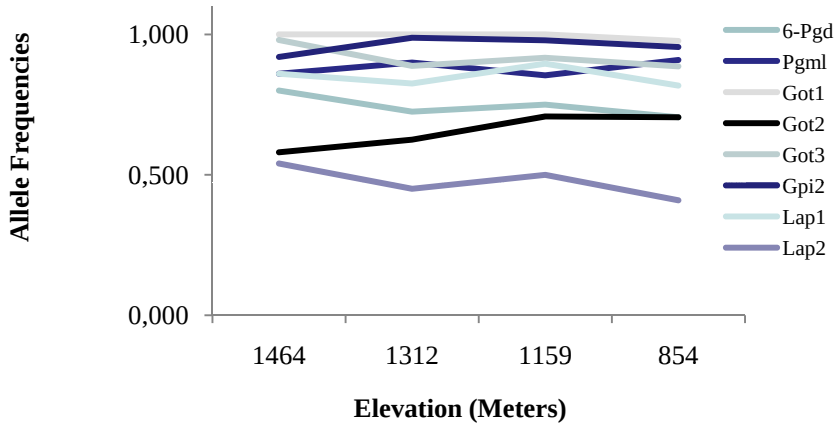
Clinal Patterns of Adaptation in Balsam Fir

Relationship of temperature optimum for net photosynthetic CO2 uptake to elevational origin of balsam fir seedlings. Points represent determinations on individual pots.



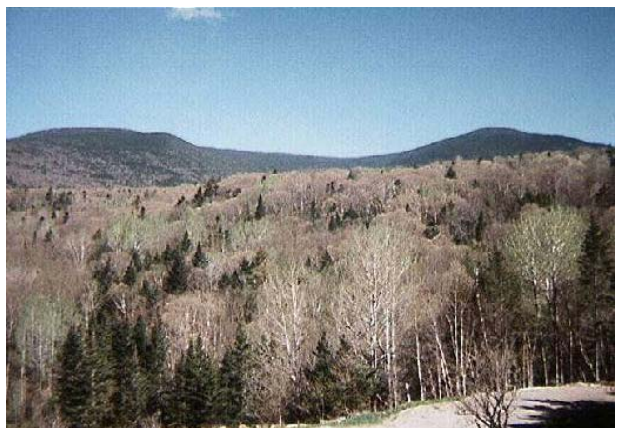
[redacted]

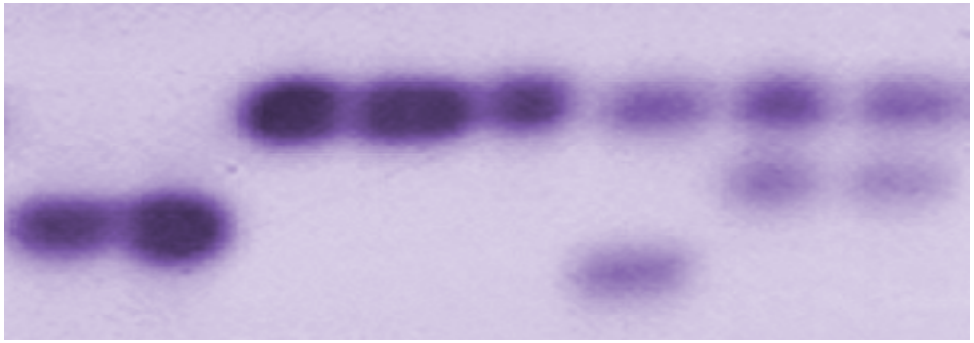
Estimated allele frequencies for eight allozyme loci in four subpopulations of balsam fir on Mt. Moosilauke, New Hampshire

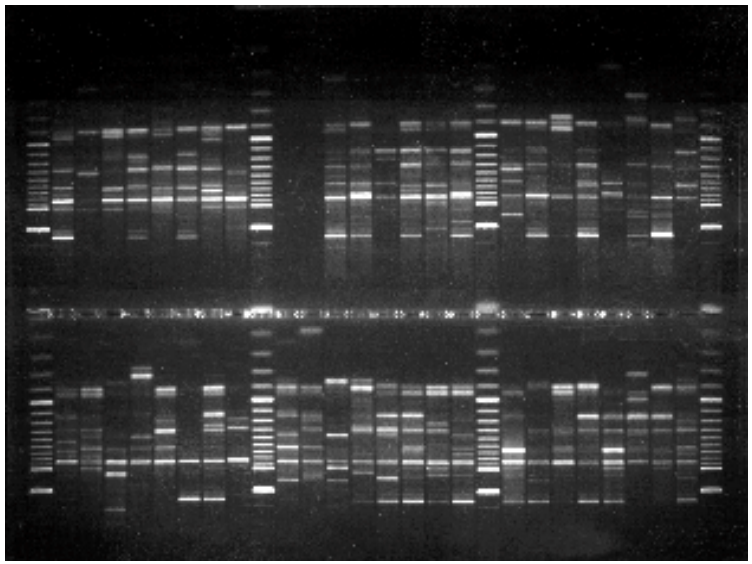


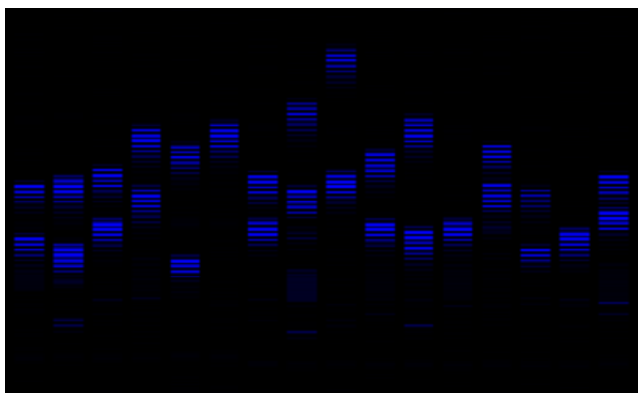
[redacted]

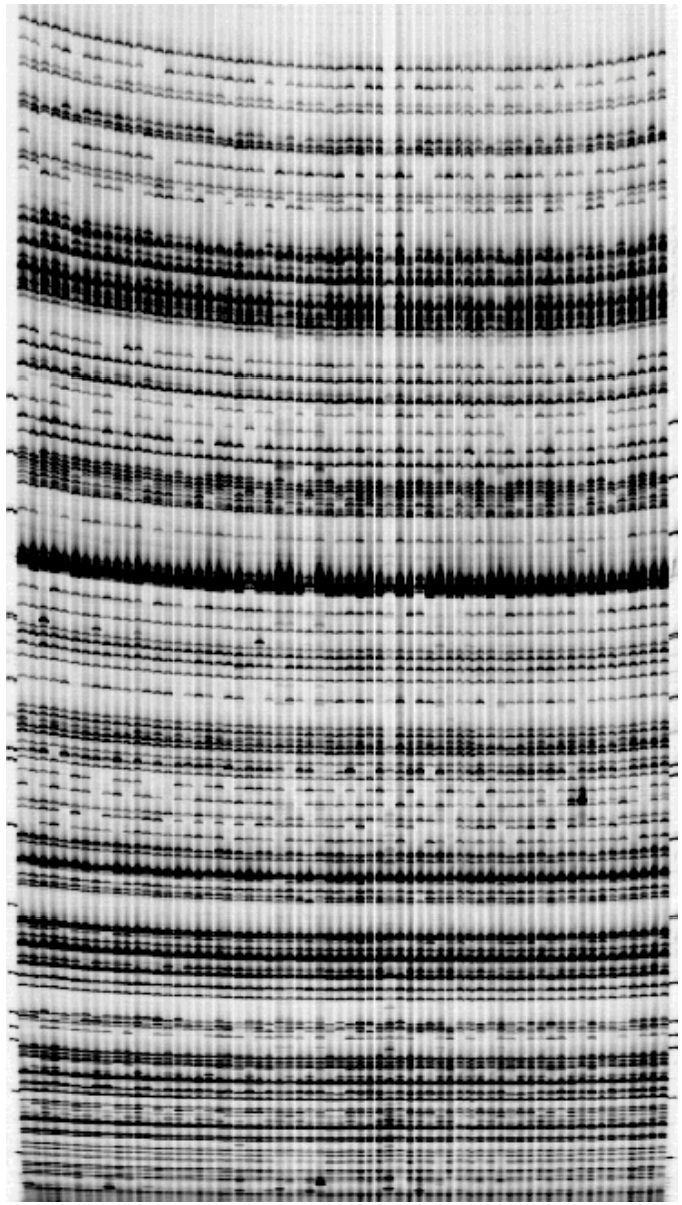
elevational transect. Canadian Journal of Botany 63, 2448-2453.









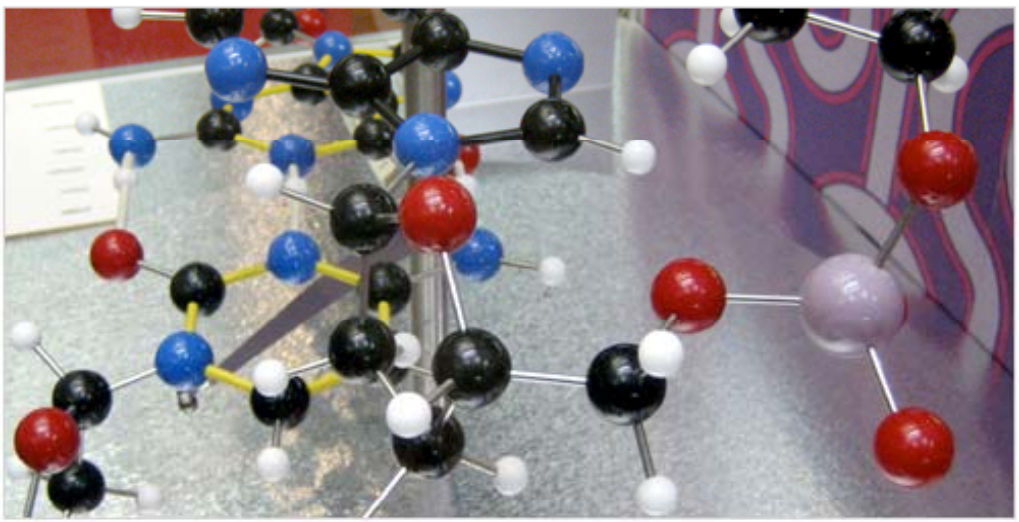



Science
16 February 2001
15 February 2001
nature
8 December 2005 | www.nature.com/nature | $10
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
nature
1 September 2005 | www.nature.com/nature | $10
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
14 December 2000
$10.00
BSE in France
Infected beef in the food chain?
Fluid dynamics
The physics of flapping
Coral bleaching
Shielded by the glow
15 September 2006 | $10
Science
AAAS
5 April 2002
Vol. 296 No. 5565
Pages 1–204 $9

Traits that are Controlled by Single Genes

Mapping / Positional Cloning Disease Resistance Genes

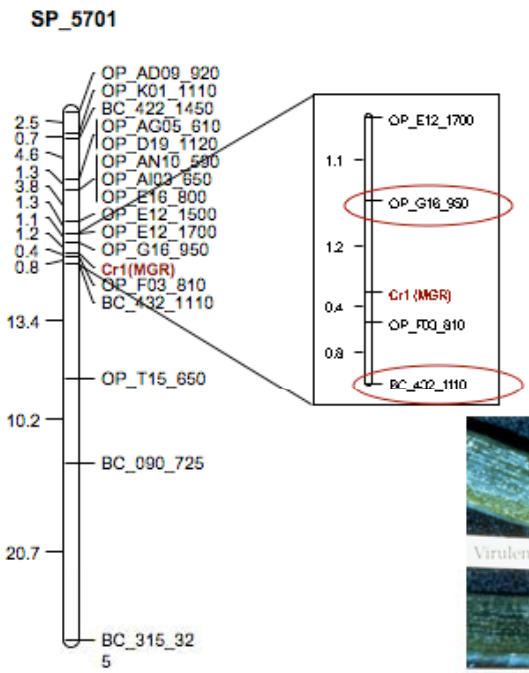
Eye on DNA | How will it change your life?

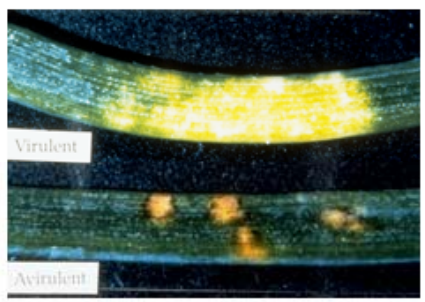


What happens after a positive breast and ovarian cancer (BRCA) genetic test?

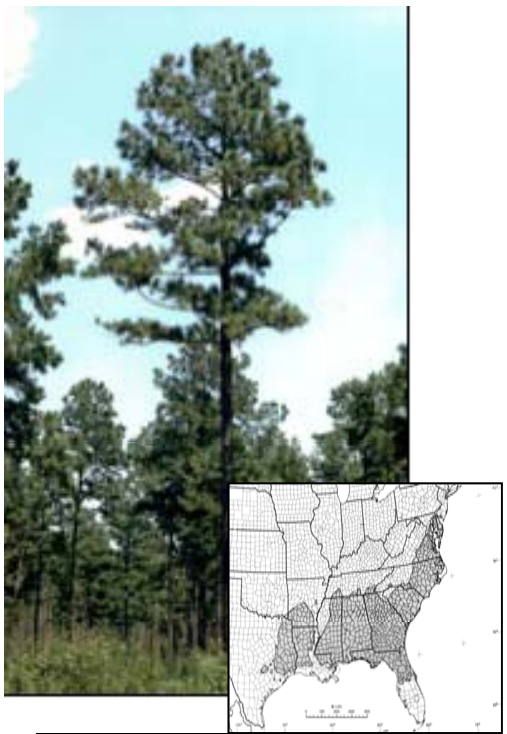
by Dr. Hsien-Hsien Lei

Posted August 15, 2007 in DNA Testing, DNA and Disease

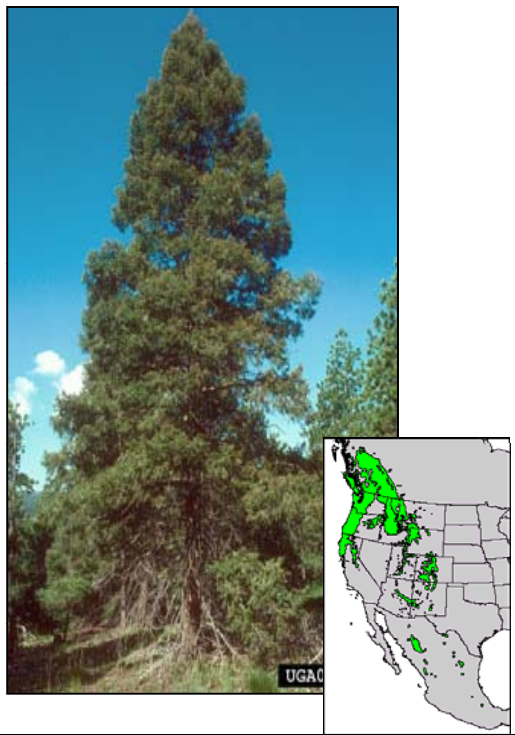




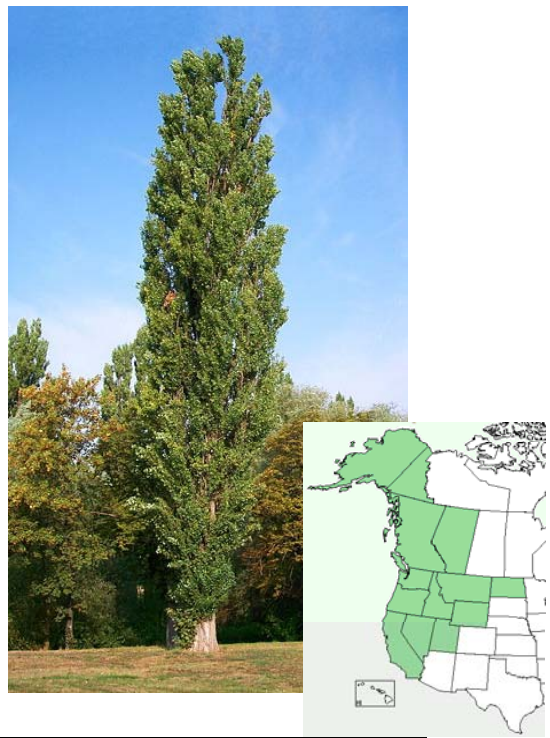
Genomic Approaches to Complex Trait Dissection



Pinus taeda
(loblolly pine)

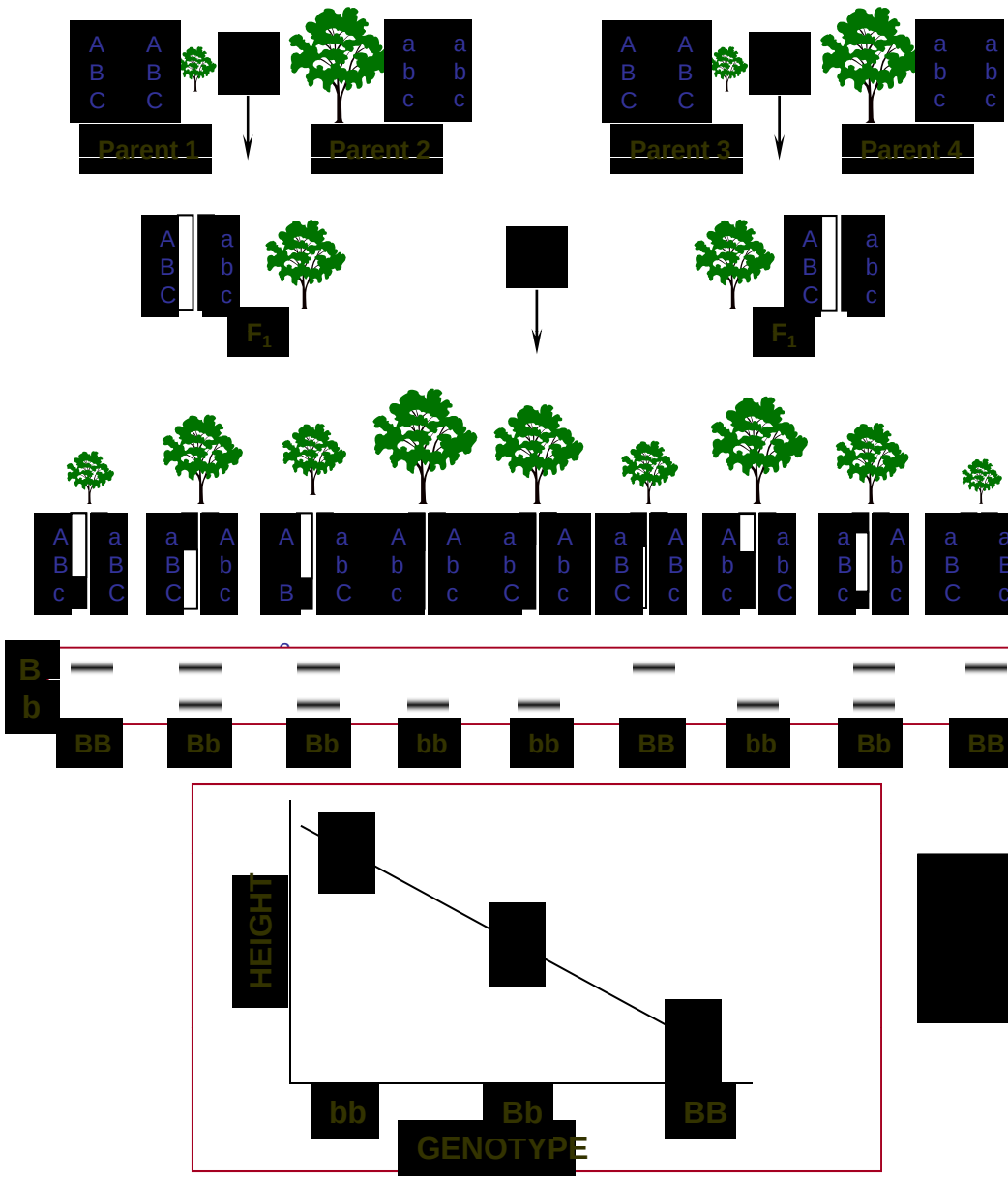


Pseudotsuga menziesii
(Douglas-fir)



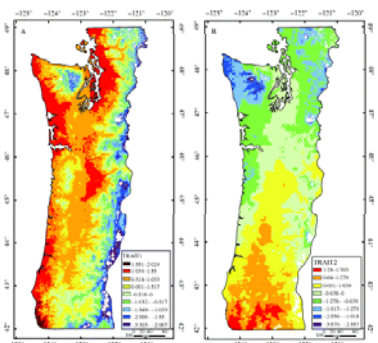
Populus trichocarpa
(black cottonwood)

[redacted]bing



[redacted]

Genecology of Phenology and Cold-hardiness in Coastal Douglas-fir

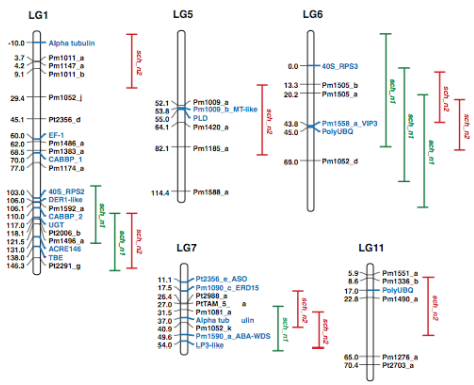


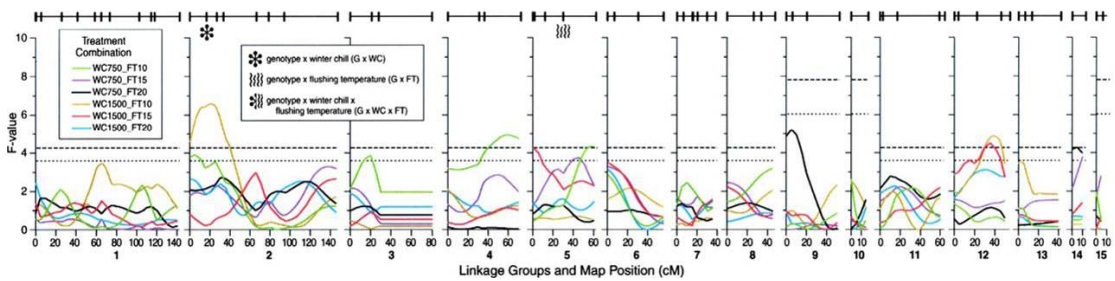
Trait	h^2
Bud flush	0.87
Bud set	0.70
Second flushing	0.45
Fall hardiness	0.19
Winter hardiness	0.11
Spring hardiness	0.77
Spring frost damage	0.56

[redacted]

Phenology and Cold-hardiness QTLs in Douglas fir

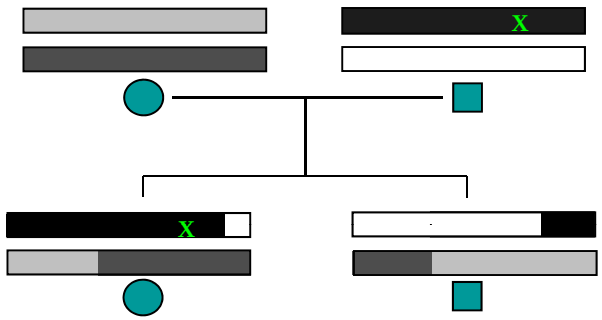
Publication	Phenotype	No. QTLs
Jermstad et al. (2001, *TAG* 102:1142-1151)	Bud flush timing	33
Jermstad et al. (2001, *TAG* 102:1152-1158)	fall/spring cold-hardiness	11/15
Jermstad et al. (2003, *Genetics* 165:1489-1506)	many	4-11 QTL x environment
Wheeler et al. (2005, *Mol. Breed.* 15:145-156)	spring cold-hardiness	10 (assoc. of 17 candidate genes to QTLs)

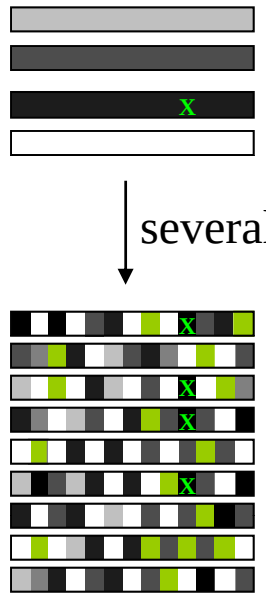




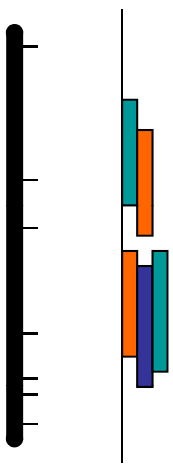
Linkage versus Association

A favourable mutation





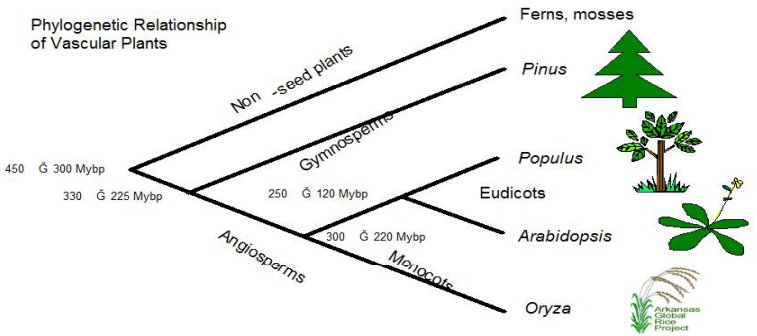
LG

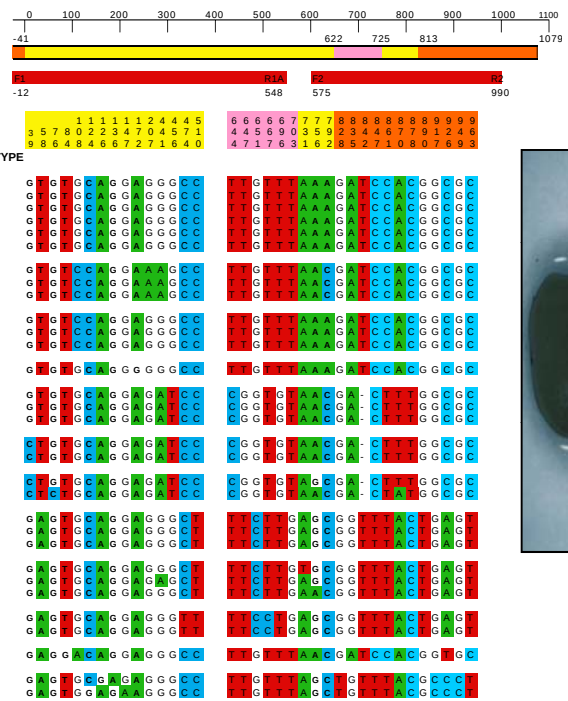


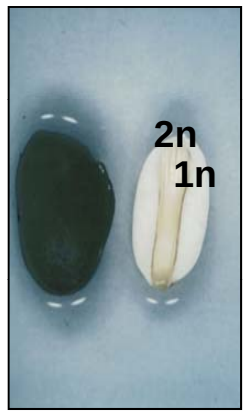
Mapping pedigree

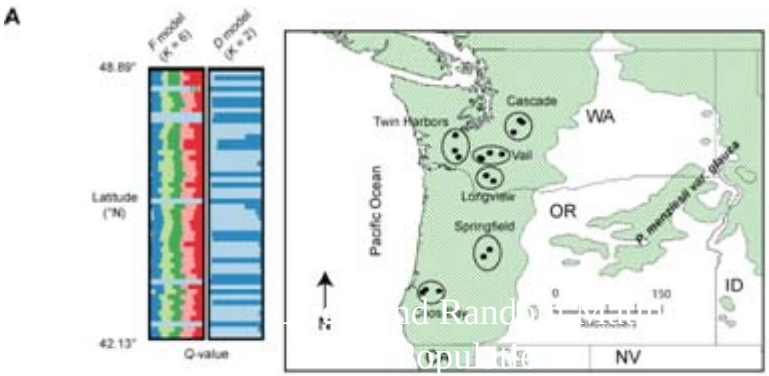
Natural population (= multiple genetic backgrounds)

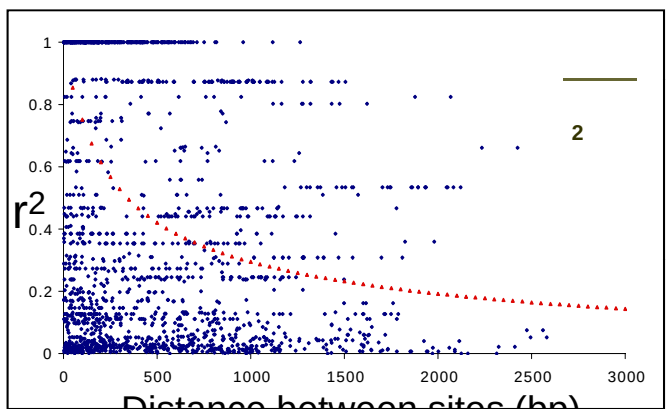
Association Genetics in Conifers











Cold-induced Proteins in Douglas-fir EST Libraries

- Candidate genes in *Arabidopsis* from Lee et al. (2005, *Plant Cell* 17: 3155-3175)

939

- Total with tBLASTx scores < e^{-10} from Douglas fir EST libraries

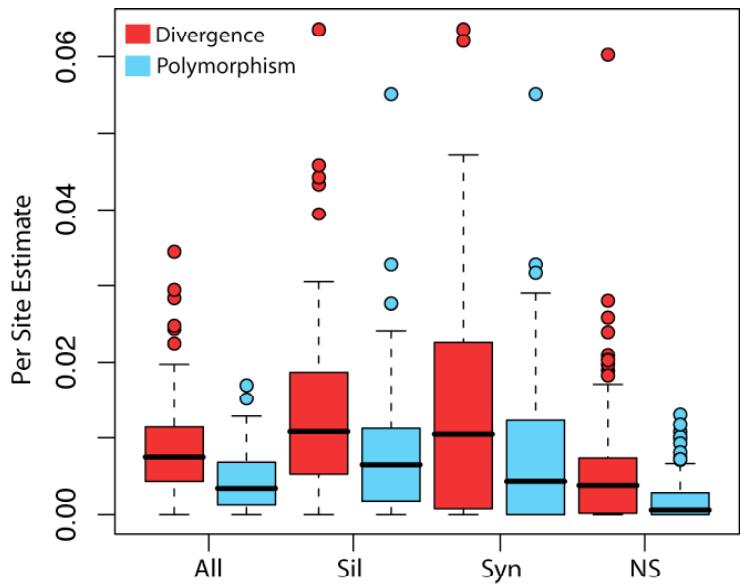
553

- Automated and manual primer design for Sanger resequencing

378

- Final selection

121



Eckert et al. 2009. *Genetics* 183: 289-298

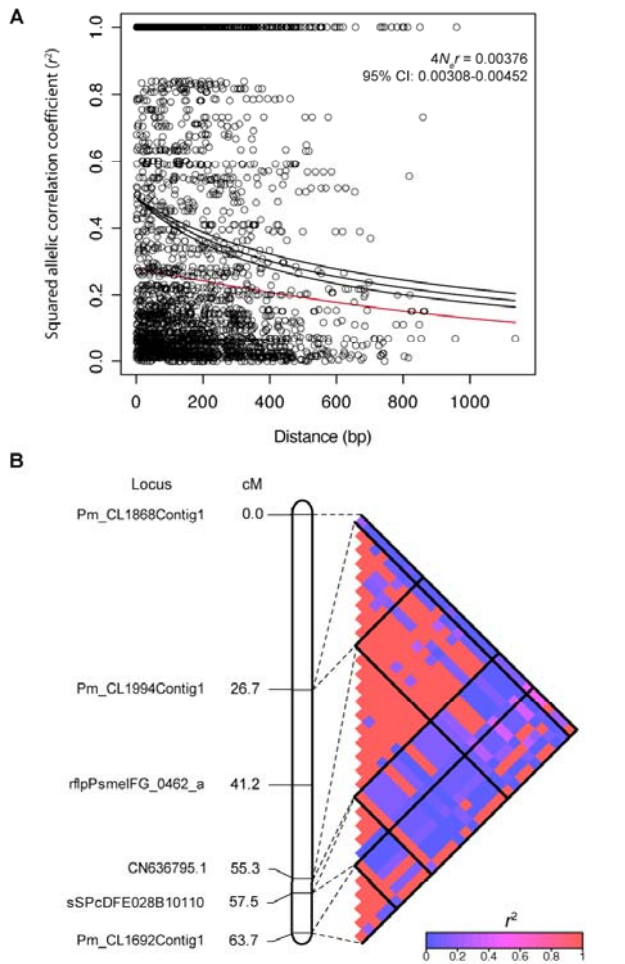
Patterns of Linkage Disequilibrium

Intragenic LD:

1. Extends upwards of 1 kb
2. Higher than previously reported in other conifers

Intergenic LD:

1. Prevalent among genes on the same linkage group.
2. Limited to proximal genes.



Eckert et al. 2009. *Genetics* 183: 289-298

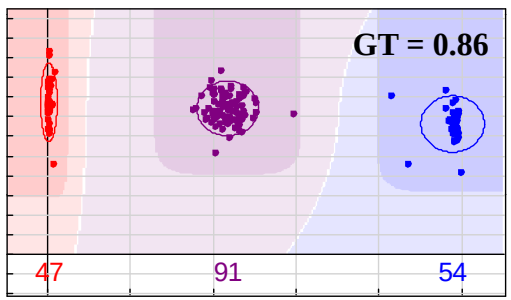
Candidate Genes Consistent with Selective Sweeps

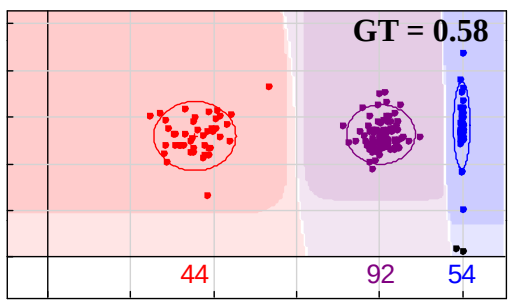
Gene Product	Result
Compound *DHEW* test	
GRAM-containing/ABA-responsive protein	$P_D = 0.001$, $P_H < 0.001$, $P_{EW} = 0.080$
cold-regulated plasma membrane protein	$P_D = 0.009$, $P_H = 0.050$, $P_{EW} = 0.035$
dehydrin-like protein	$P_D = 0.072$, $P_H = 0.083$, $P_{EW} = 0.042$
luminal binding protein	$P_D = 0.034$, $P_H = 0.148$, $P_{EW} = 0.076$
Polymorphism-to-divergence	
cyclosporin A-binding protein	$k = 0.32$
GRAM-containing/ABA-responsive protein	$k = 0.58$
transcription regulation protein	$k = 0.41$
Ka/Ks	
thaumatin-like protein	Ka/Ks = 14.45
auxin-responsive family protein	Ka/Ks = 5.97
bicoid-interacting 3 domain containing protein	Ka/Ks = 4.94
pentatricopeptide (PPR) containing protein	Ka/Ks = 4.27

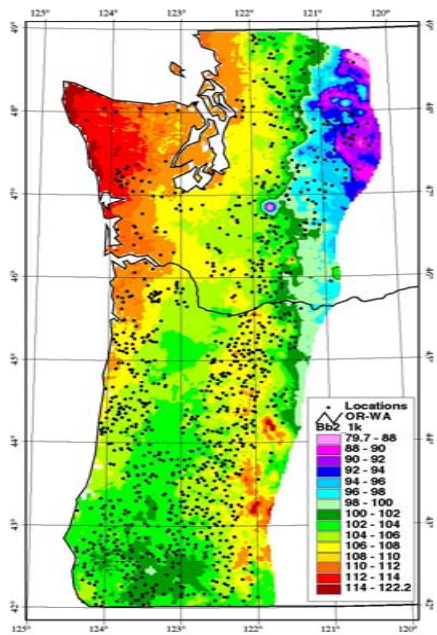
Eckert et al. 2009. *Genetics* 183: 289-298

SNP Genotyping

Application	SNPs	*n*	GT (>0.25)	CR	CG
Linkage mapping	384	192	295	0.96	37
Association mapping	384	706	277	0.92	94



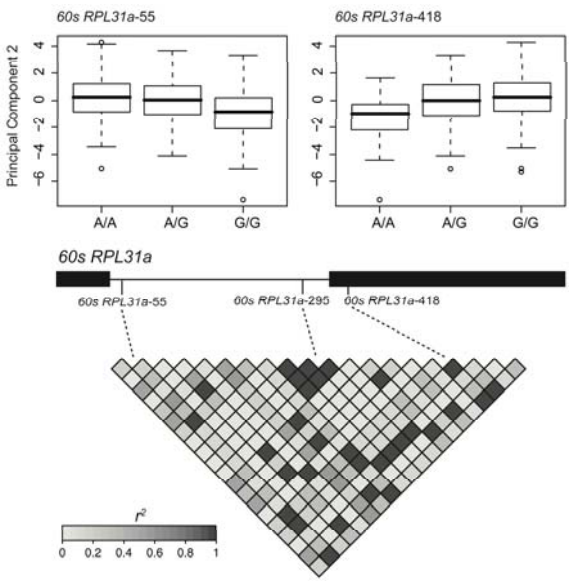


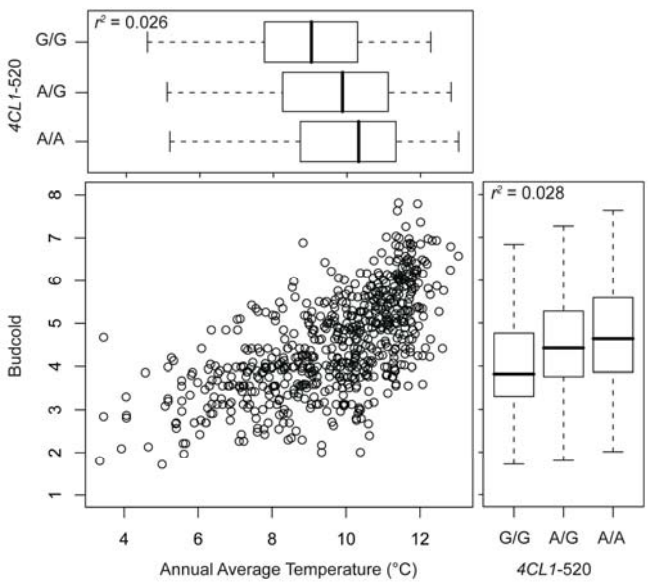


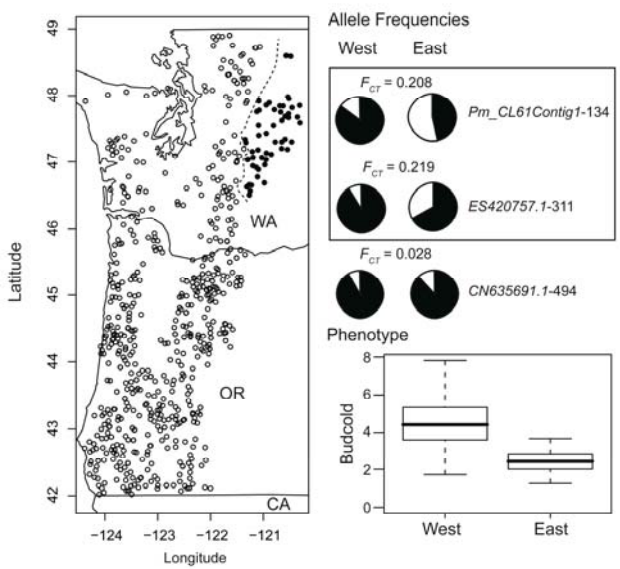
Association Genetics

- 30 associations
- 12 candidate genes
- Mostly additive effects

- SNP genotypes track environmental gradients in the same way as the phenotypes to which they are associated.
- 7 genes with strong allele frequency differentiation across the Cascade Crest
- Direct relation to phenotypes is confounded with structure

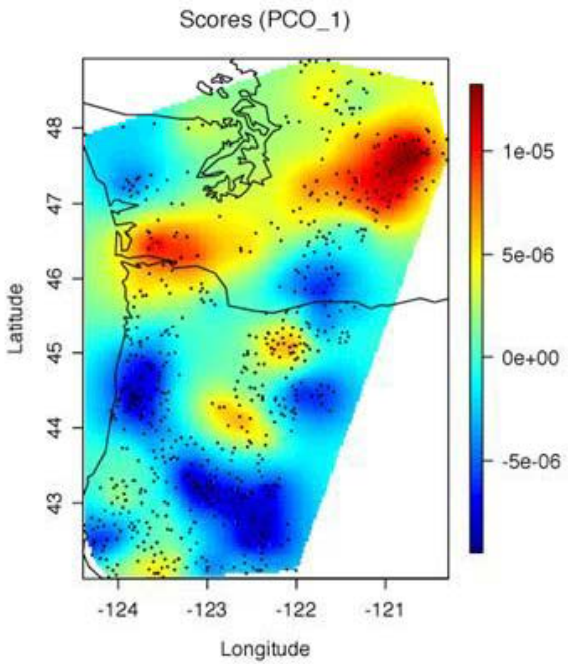




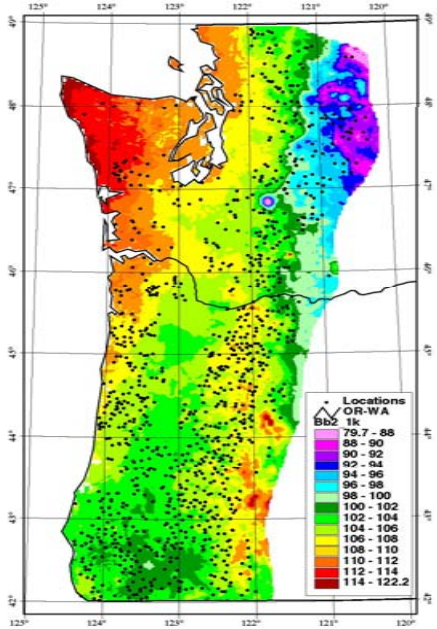


Eckert et al. 2009. *Genetics* 182: 1289-1302

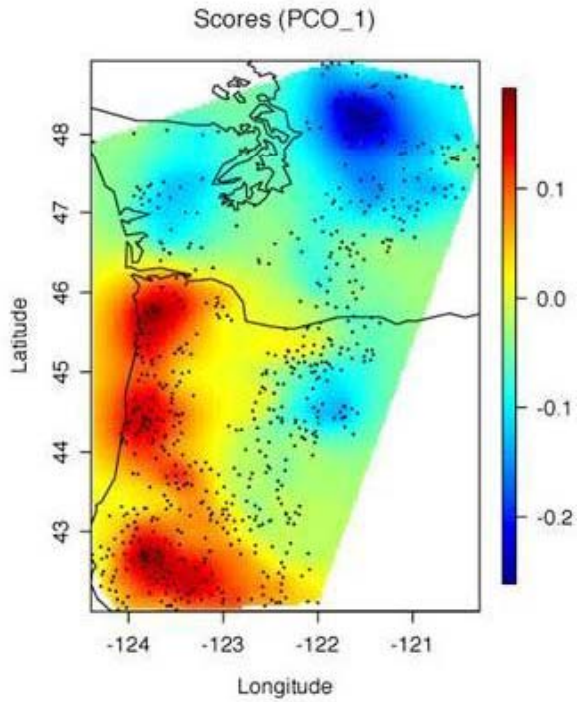
Patterns of Adaptive Molecular Genetic Diversity



Neutral Genotype



Phenotype

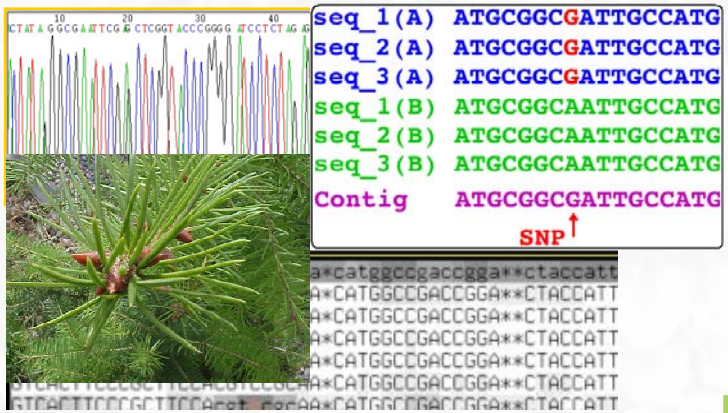


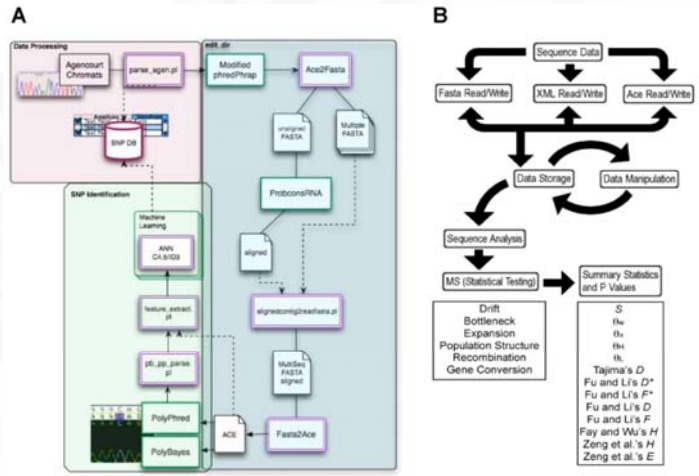
Non-neutral and associated with phenotype

ADEPT 2

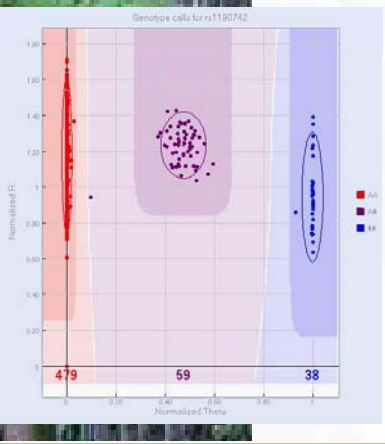
Allele Discovery for Economic Pine Traits

Resequencing and SNP discovery (7,424 genes)

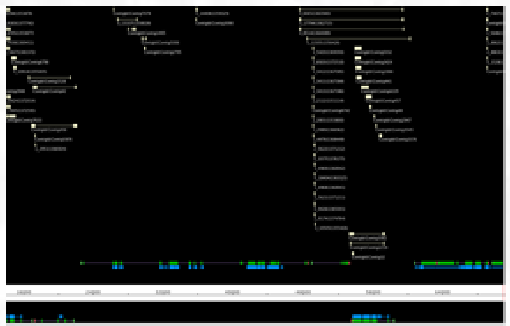




High-throughput computational solutions developed for bioinformatic SNP determination and sequence analysis



Genotype 1-2 SNPs per Candidate Gene on a 7600 Illumina Infinium chip



Sequencing, Assembly, & Analysis of 10 BAC clones

Phenotyping:
Wood Quality
Disease Resistance
Drought-Tolerance
Gene Expression
Metabolites

UC DAVIS PLANT SCIENCES

NSF

Texas A&M University

NC STATE UNIVERSITY

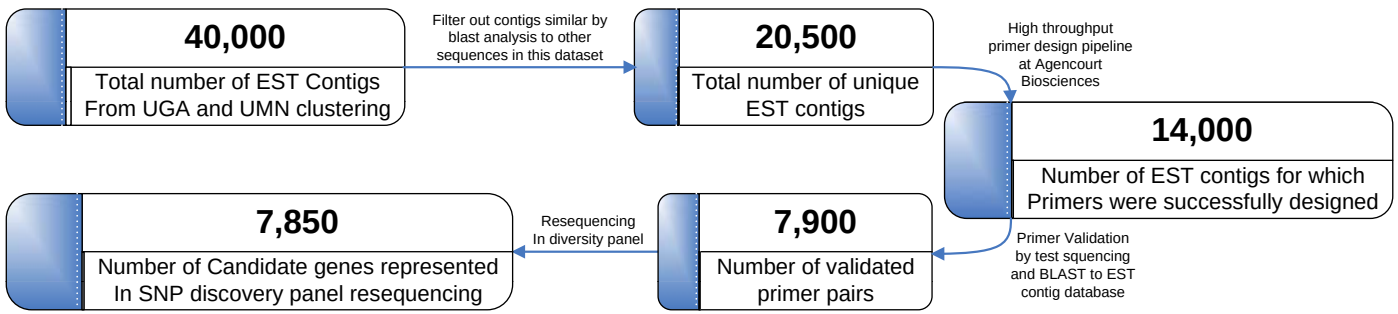
UF UNIVERSITY of FLORIDA

ADEPT2 SNP discovery

Materials and Methods

- Range-wide diversity panel of 18 megagametophytes + 5 primer validation samples
- Sanger sequencing (Agencourt Biosciences)
- Sequence Analysis and SNP calling (PineSAP, cf. Wegrzyn et al. 2009, *Bioinformatics*)

Bioinformatic assessment



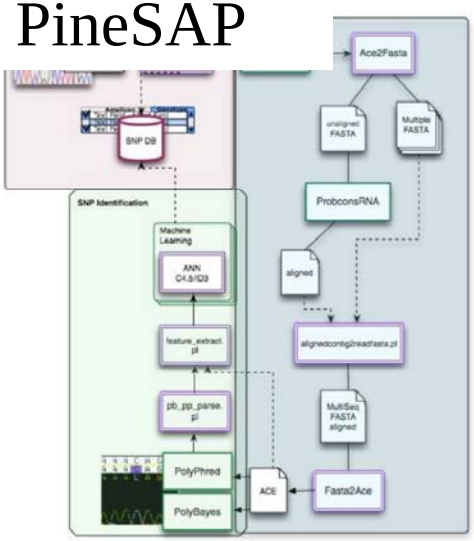
Sequence analysis assessment

Description	Count	Outgroup
Core Set	4861	----------
Outgroup-1	2975	Radiata pine (*P. radiata*)
Outgroup-2	719	Sugar pine (*P. lambertiana*)

Analysis tools

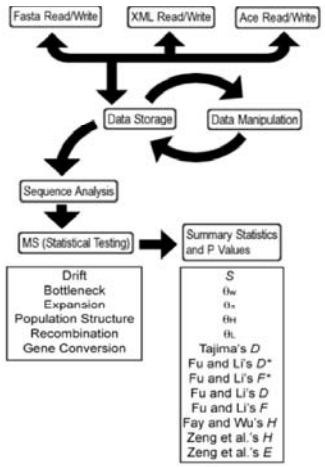
Database and web tools

PineSAP



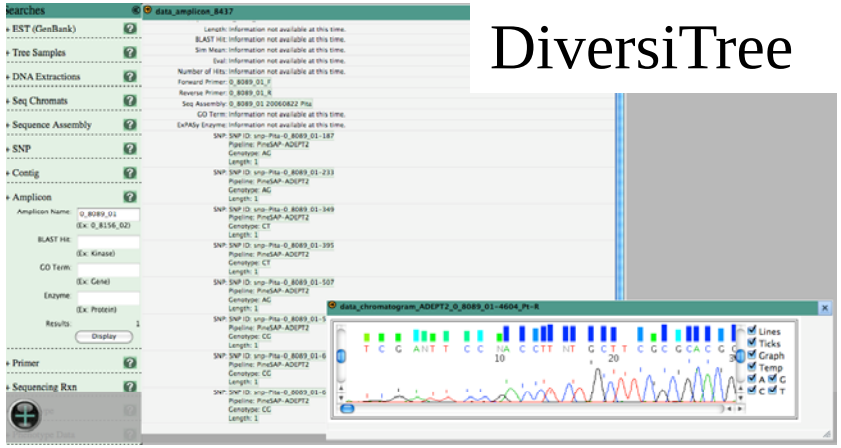
Wegrzyn et al. 2009. *Bioinformatics*

DnaSAM



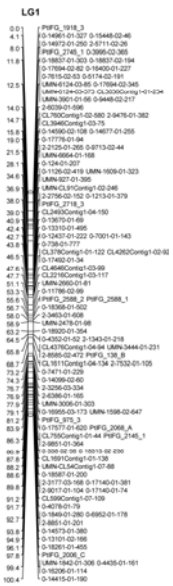
Eckert et al. 2010. *Mol. Ecol. Res.*

DiversiTree



http://dendrome.ucdavis.edu/interface/

Data sets



Linkage maps:

(*Pinus taeda*)

~1800 gene loci mapped

~1900 cM total map distance

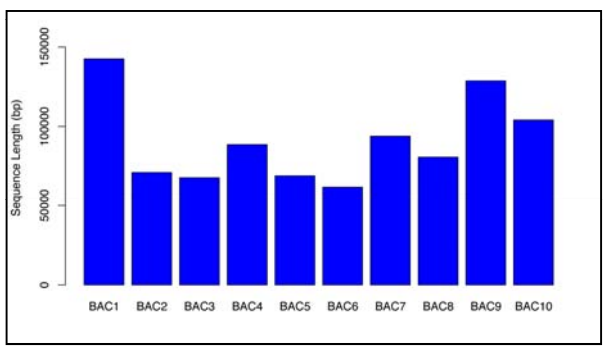
~0.80 loci/cM (range 1-10+)

Loblolly pine BAC assemblies:

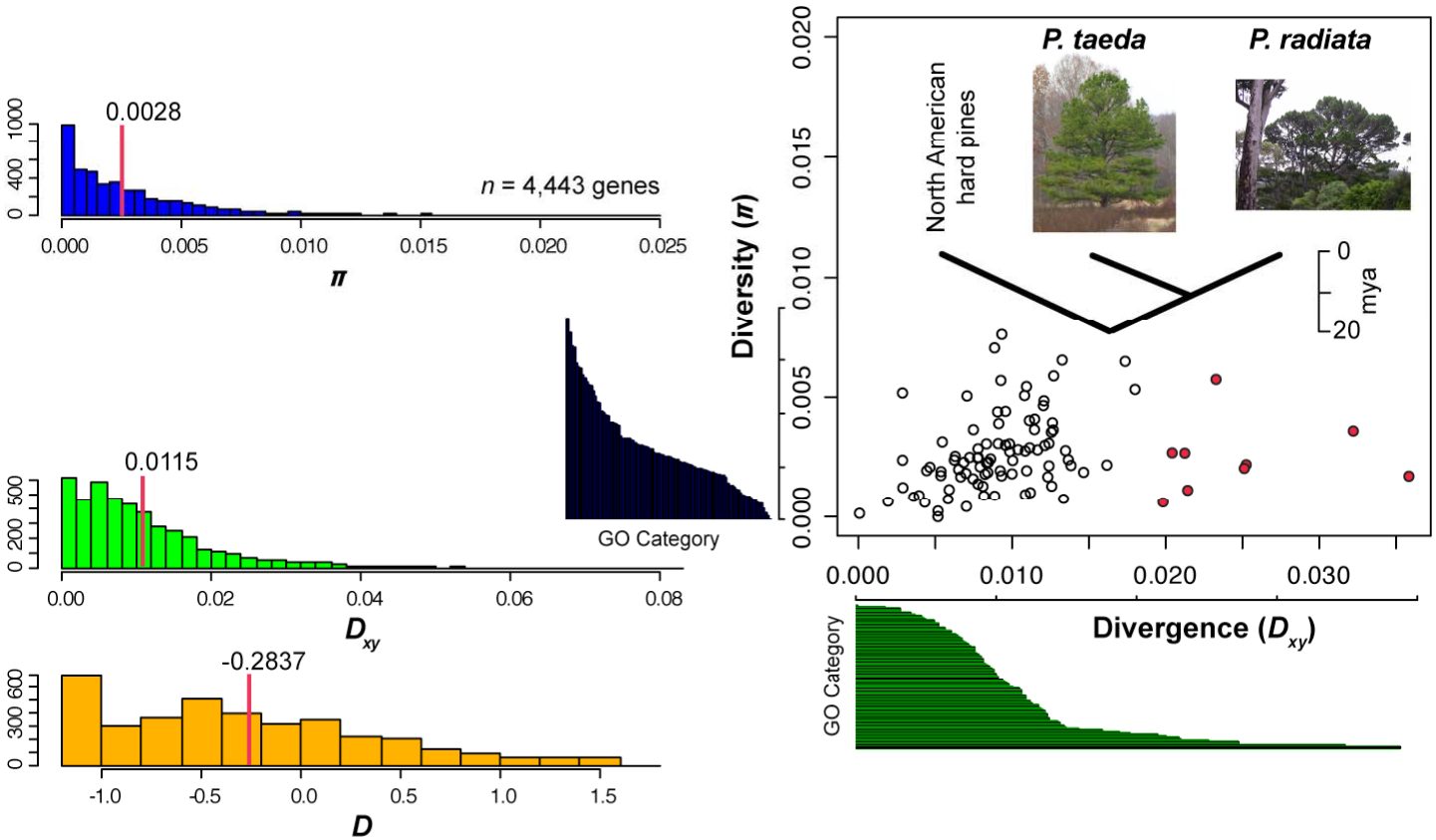
10 BACs

Lengths: ~60000-150000 bp

Total Length: ~950000 bp



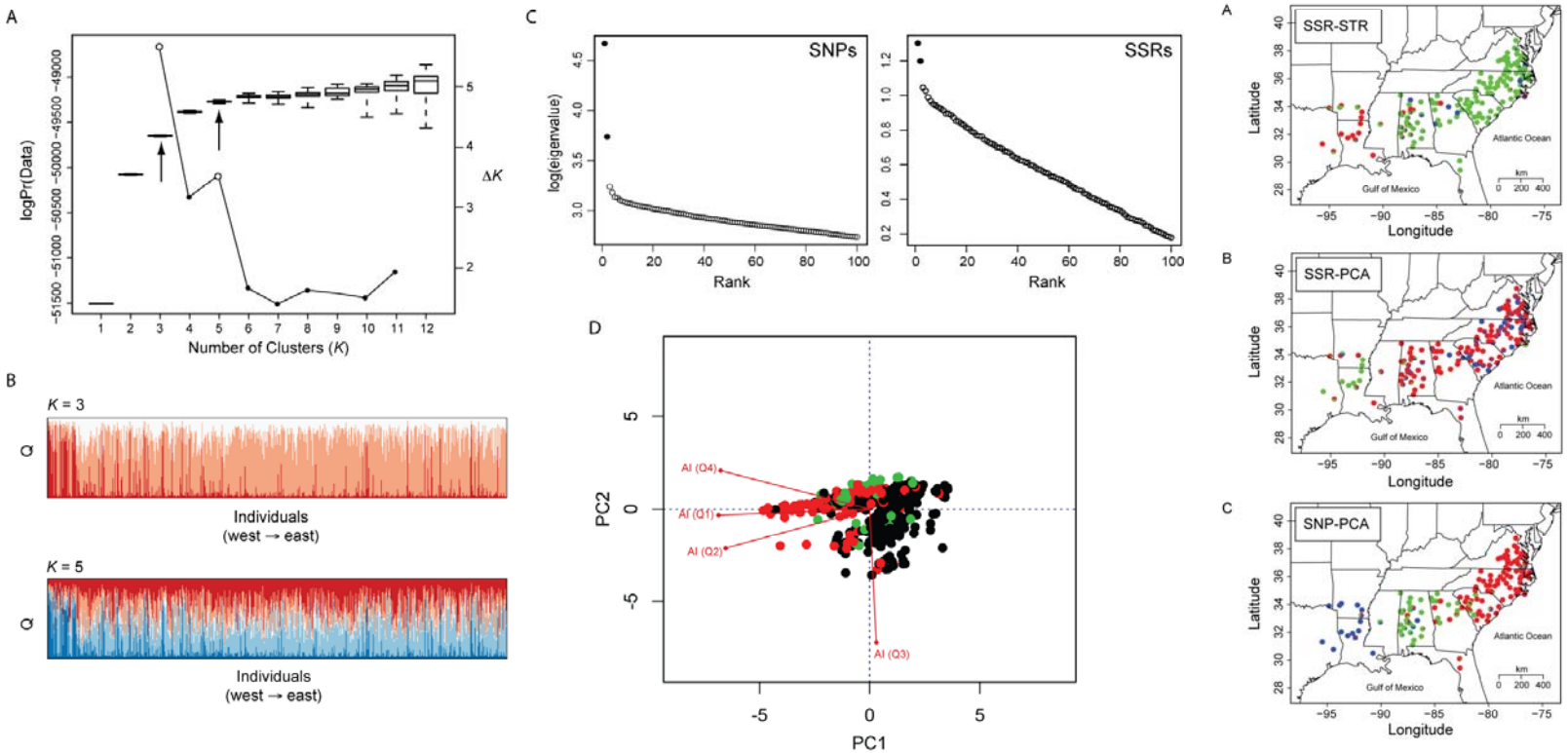
Loblolly pine diversity and divergence



GO Molecular Function Category	GO identifier	Genes
Amine oxidase activity	GO:0008131	3
Enzyme inhibitor activity	GO:0004857	2
Chitin binding	GO:0008061	2
Fructose-bisphosphatase activity	GO:0042132	3
Flavenoid 3'-monooxygenase activity	GO:0016711	3
Phosphate transmembrane transporter	GO:0005315	4
Chintinase activity	GO:0004568	2
Iron-sulfur cluster binding	GO:0051539	2
Cation-transporter	GO:0008324	10

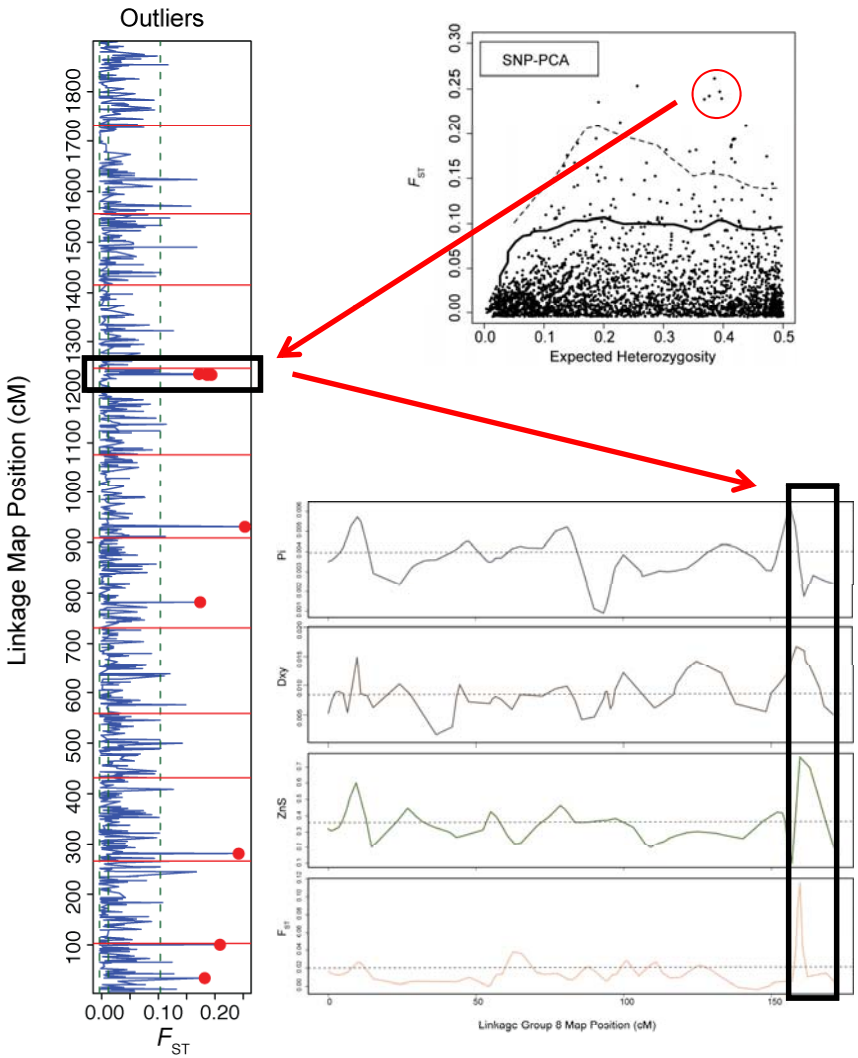
Population structure in loblolly pine

Using genotypes at 3900 SNPs and 23 nuclear microsatellites for 907 range-wide collections in combination with the Bayesian clustering algorithms (STRUCTURE) and PCA 3 main genetic clusters were identified:



Eckert et al. in press. *Genetics.*

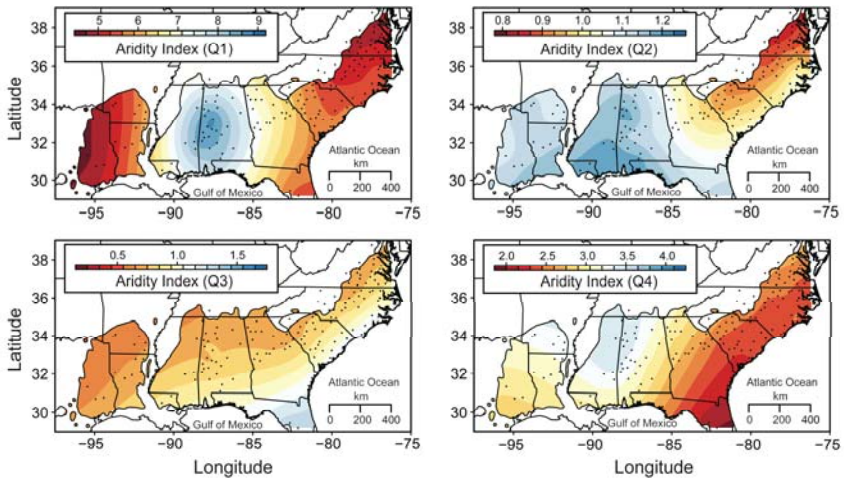
Deviations from neutrality in loblolly pine



- Moderate levels of diversity
- Rapid decay of LD
- Little population differentiation
- Signs of selective sweeps:
  - LG8
  - 5-10 genes.
  - Putative functions: abiotic and biotic stress response

Eckert et al. in press. *Genetics.*

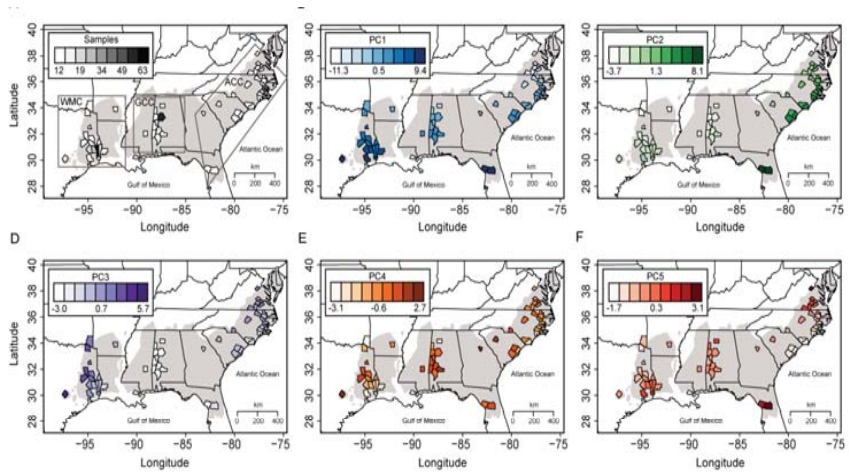
Molecular Basis of Adaptive Genetic Diversity



Eckert et al. in press *Genetics*.

Which SNPs have unusual correlations to aridity gradients (i.e. ratio of precipitation to potential evapotranspiration)?

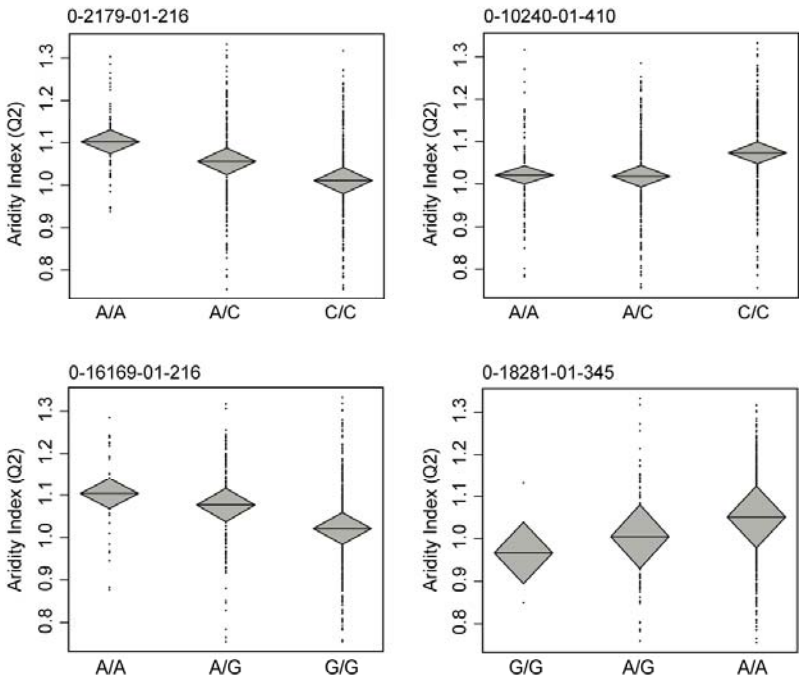
Which SNPs have unusual correlations to multivariate climate gradients corresponding to temperature, growing degree-days, precipitation and aridity?



Eckert et al. in press. *Mol. Ecol.*

Models Incorporating Population Structure Corrections

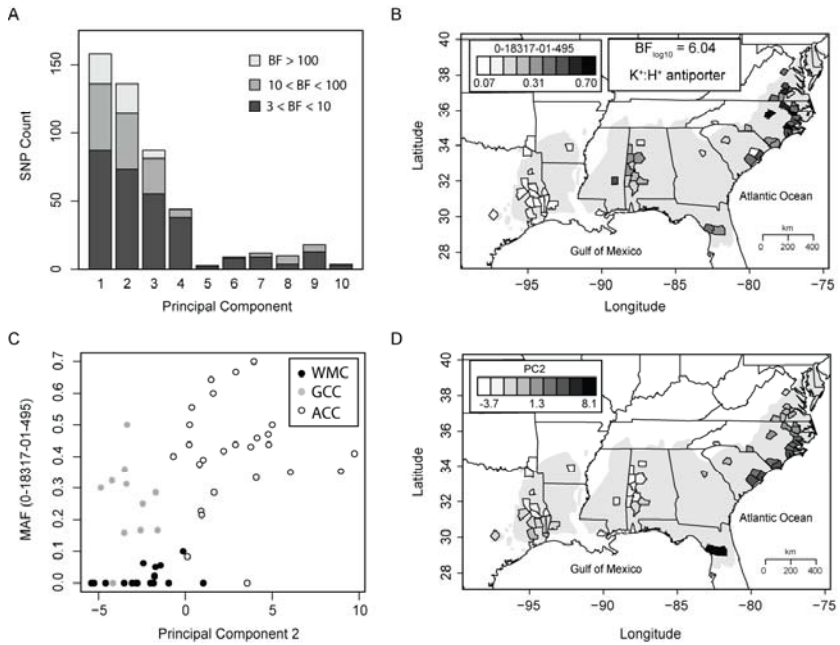
PCA – GLM:



Eckert et al. in press. *Genetics.*

Six highly significant associations (after Bonferroni corrections) to aridity. Four are shown here. This is out of 3,938 SNPs tested.

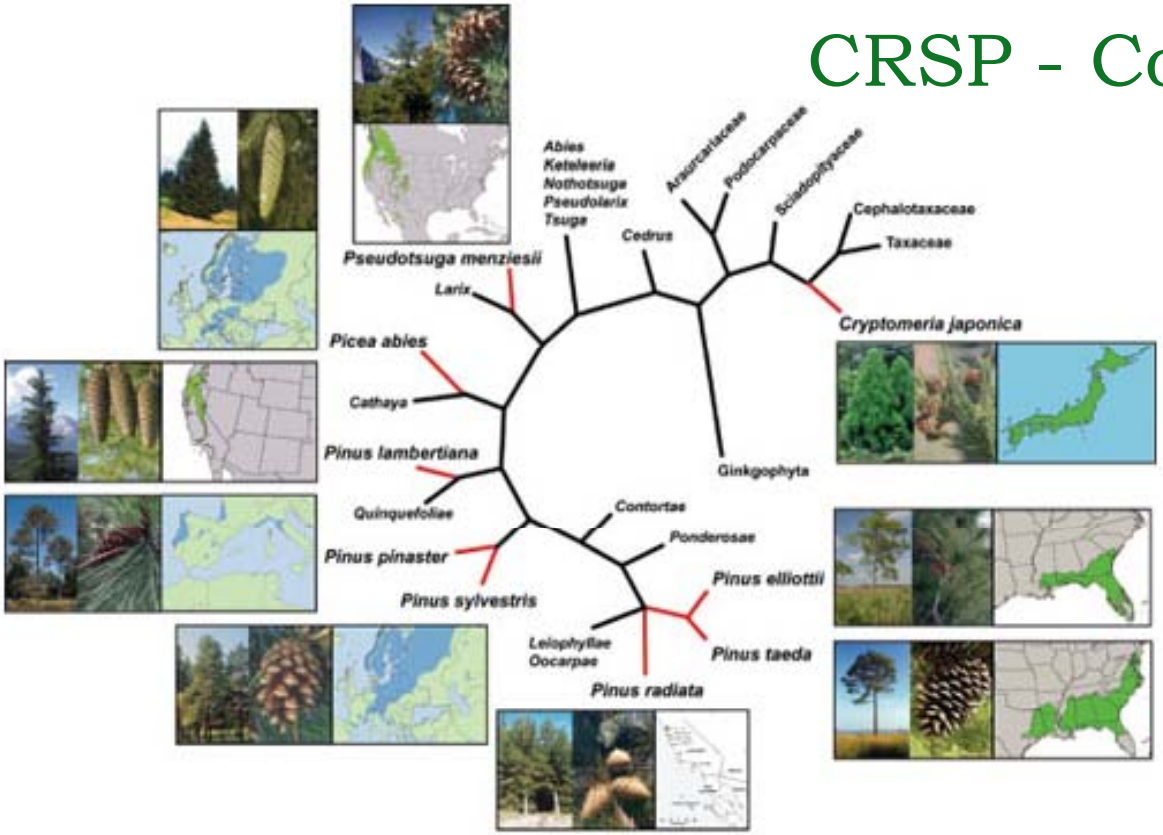
Bayesian GLMM:



Eckert et al. in press. *Mol. Ecol.*

Core set of 48 SNPs correlated to temperature and precipitation with Bayes factors > 100. This is out of 1,730 tested.

CRSP - Comparative Re-Sequencing in Pinaceae



Scientific Name	Common Name	Genome Size(Mb)	SNPs Identified	Investigators
Pinus ellottii	Slash Pine	24,200	2,879	Dudley Huber (University of Florida)
Pinus radiata	Monterey Pine	26,500	2,474	Shannon Dillon (CSIRO)
Pinus pinaster	Maritime Pine	30,300	2,981	Santiago Gonzalez Martinez & Delphine Grivet (ICIFOR - INIA)
Pinus sylvestris	Scots Pine	24,600	3,803	Outi Savolainen & Sonja Kujala (University of Oulu)
Pinus lambertiana	Sugar Pine	33,500	4,238	Kathie Jermstad (IFG)
Pseudotsuga menziesii	Douglas Fir	18,700	2,491	Kathie Jermstad (IFG)
Picea abies	Norway Spruce	18,200	3,417	Marta Scalfi (IASMA)

WHISP - White Pine Re-Sequencing Project

Goal: SNP Discovery in North American members of the white pines to investigate evolutionary relationships among eleven species

200 genes will be resequenced in the following white pines:

Pinus strobus

Pinus strobiformis

Pinus albicaulis

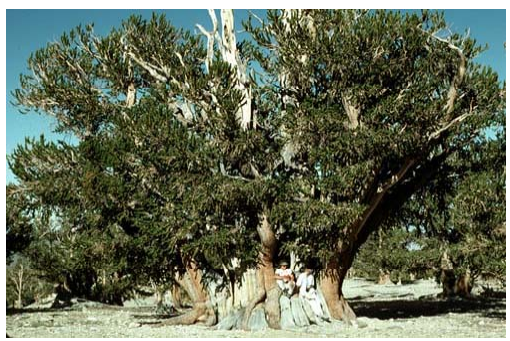
Pinus monticola

Pinus aristata

Pinus ayacahuite

Pinus monophylla

Pinus longaeva



Pinus balfouriana

Pinus lambertiana

Pinus flexilis

Alpine Ecosystems in Changing Environments: Biodiversity Sensitivity and Adaptive Potential

Larix decidua

Pinus abies

Pinus cembra

Pinus mugo

Centro di Ecologia Alpina

il portale della
PROVINCIA AUTONOMA
DI TRENTO

ISTITUTO AGRARIO DI SAN MICHELE ALL'ADIGE
Fondazione Edmund Mach

Abies alba

Resequencing of the candidate genes

- **4 species**:

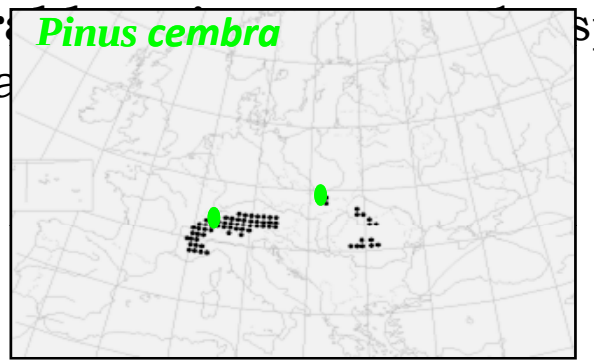
Pinus cembra PICE *Pinus mugo* PIMG

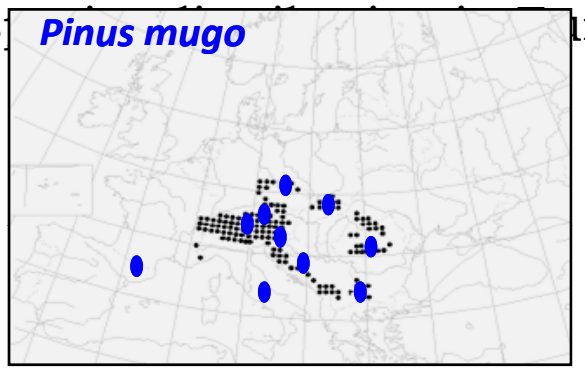
Larix decidua LADE *Abies alba* ABAL

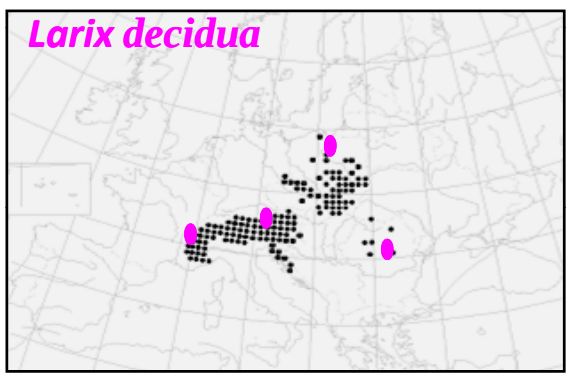
- **12 individuals** per species (megagametophytes)
- **735 genes** assigned to 3 categories according to their putative function:

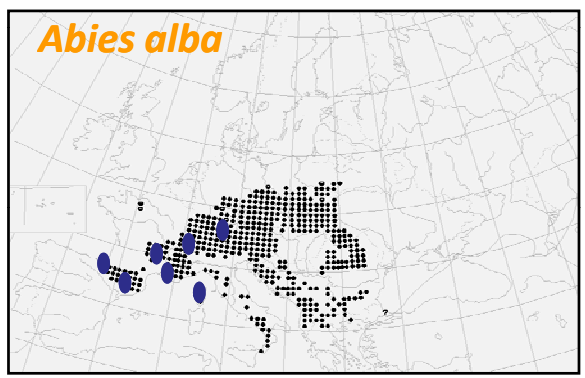
candidate control demographic

- **sever** ... ropean Mounta



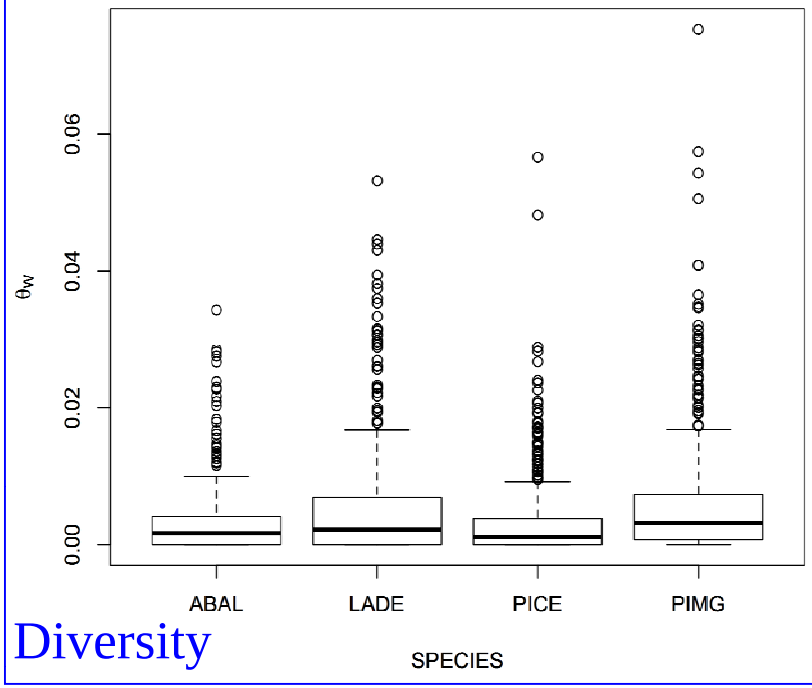






All genes

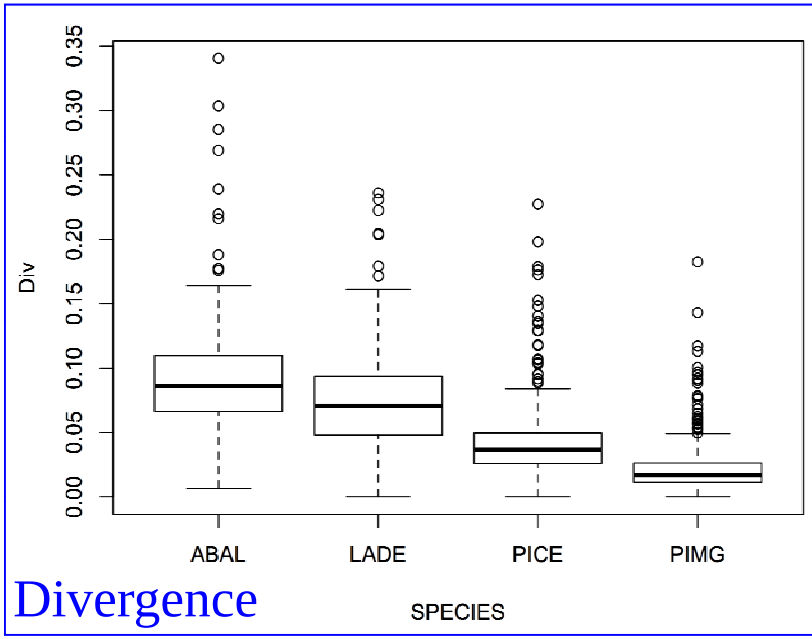
	ABAL	LADE	PICE	PIMG
Genes #	260	298	501	532
Polym. #	178	200	304	410
SNPs #	1044	1625	1748	3522
SNP freq.	106	79	118	63



Diversity

Gene categories

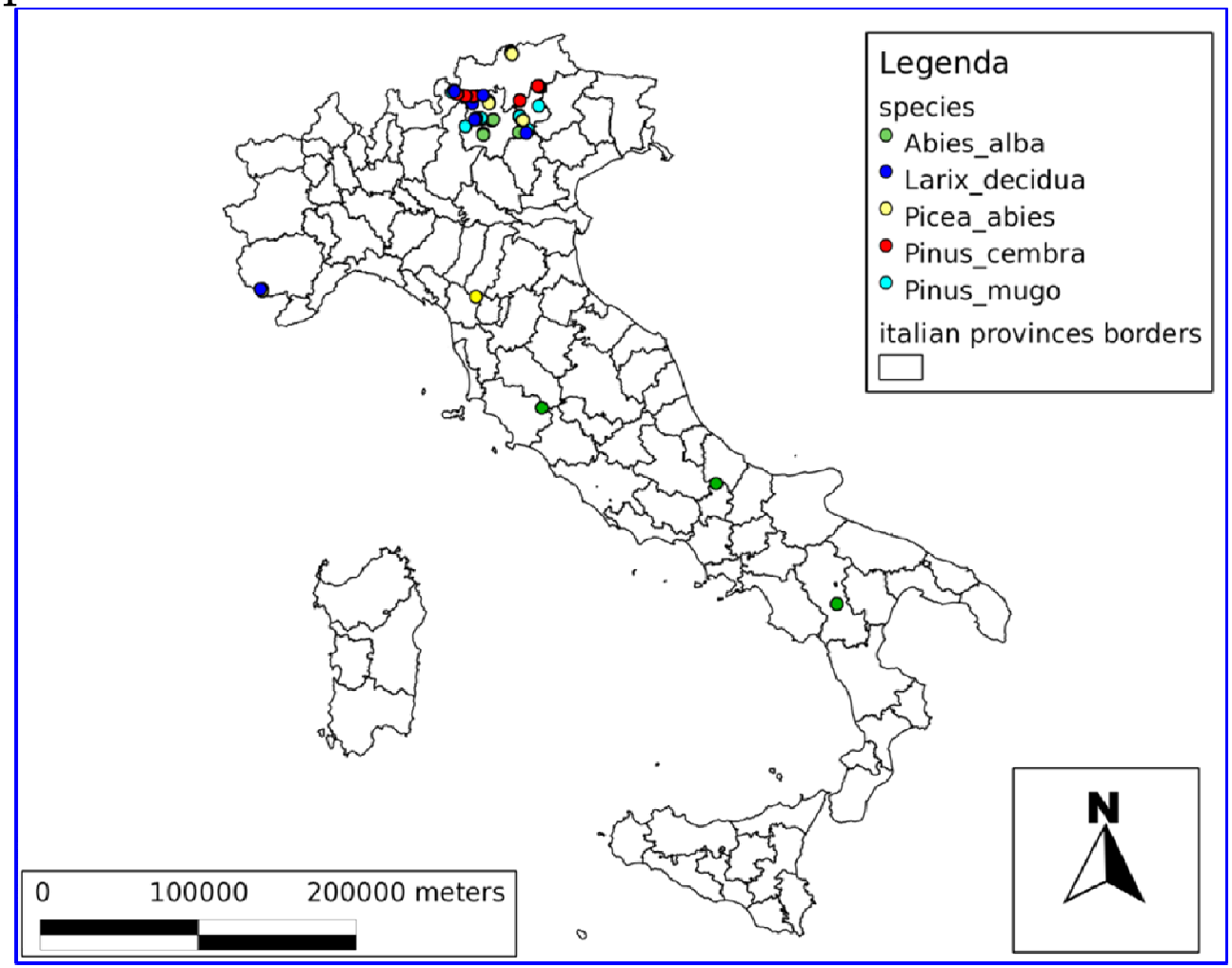
	ABAL	LADE	PICE	PIMG
Candidate	196	192	317	335
Control	70	82	154	165
Demographic	21	24	30	42



Divergence

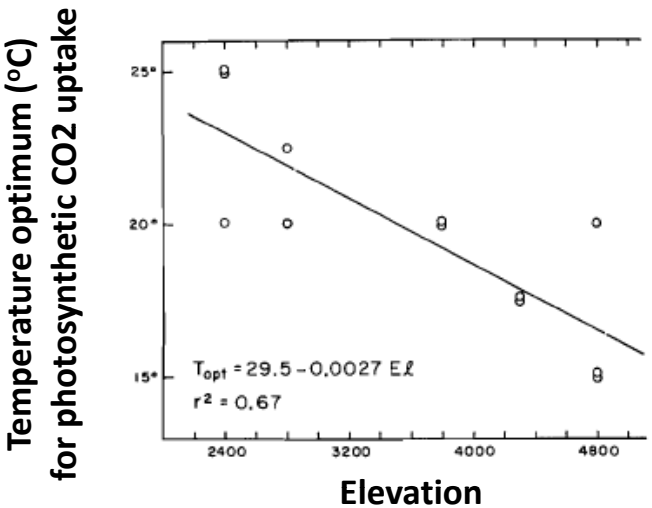
Sampling across Alpine Mountains

- 3 sampling levels: from geographic level to local one
- 5 species: *Abies alba; Larix decidua;*
  Pinus cembra; Pinus mugo; Picea abies
- 800 trees per species
- 2 gradients:
  altitudinal
  soil type



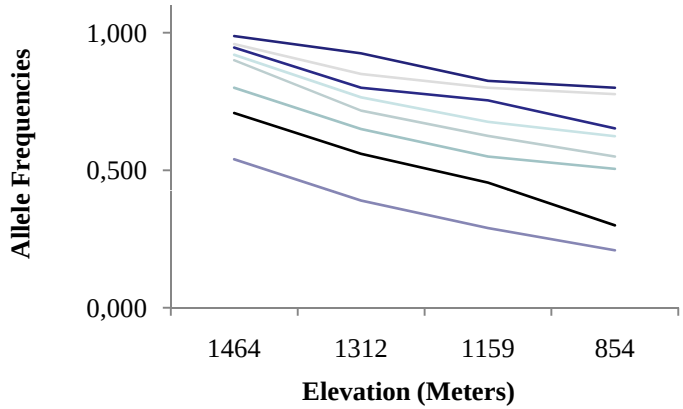
Clinal Patterns of Adaptation in Balsam Fir

Relationship of temperature optimum for net photosynthetic CO2 uptake to elevational origin of balsam fir seedlings. Points represent determinations on individual pots.

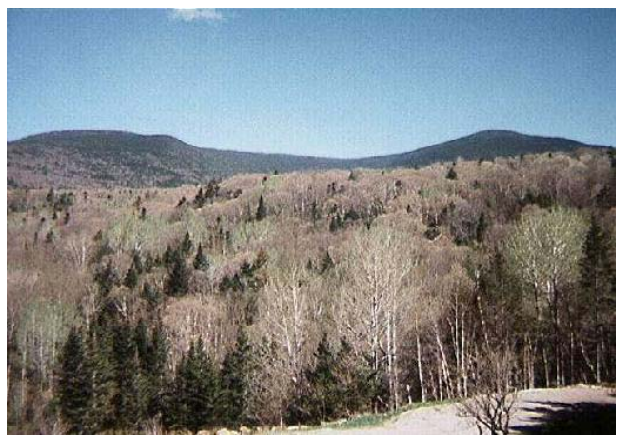


Fryer JH, Ledig FT (1972) Microevolution of photosynthetic temperature optimum in relation to elevational complex. Canadian Journal of Botany 50, 1231-1235.

Estimated allele frequencies for eight allozyme loci in four subpopulations of balsam fir on Mt. Moosilauke, New Hampshire



Neale DB, Adams WT (1985) Allozyme and mating-system variation in balsam fir across a continous elevational transect. Canadian Journal of Botany 63, 2448-2453.



Acknowledgments

UC DAVIS
UNIVERSITY OF CALIFORNIA

David Neale
Jill Wegrzyn
Andrew Eckert
Kathleen Jermstad
John Liechty
Katie Tsang
Ben Figueroa
Elly Chen
Alex Voong

Chuck Langley
Kristian Stevens

Dana Nelson
Brad St. Clair

NC STATE UNIVERSITY

Barry Goldfarb
Nick Wheeler
Bialian Li
Patrick Cumbie

Steve McKeand
Ross Whetten
Fikret Isik
JB Jett

The University of Georgia

Jeff Dean

UNIVERSITY OF FLORIDA

Gary Peter
John Davis
Matias Kirst
Dudley Huber
George Cassela

Agencourt® Bioscience Corporation
A Beckman Coulter Company

Betty Wolf
Wei Tao
Marc Rubenfield
Kerri Stelatto
Patricia Harris

Carol Loopstra
Tom Byram
Konstantin Krutovsky

OSU Oregon State University College of Forestry

Glenn Howe
David Harry
Nick Wheeler

MichiganTech

Chung-Jui Tsai

NREL

Mark Davis

GreenWood Resources

Brian Stanton

Funding: National Science Foundation WHERE DISCOVERIES BEGIN; Chevron; USDA; CSREES; NRI