



Phylotranscriptomics Reveals the Genetic Basis of Climate Adaptation in *Acers*

Mengmeng Lu
Jan 14, 2024

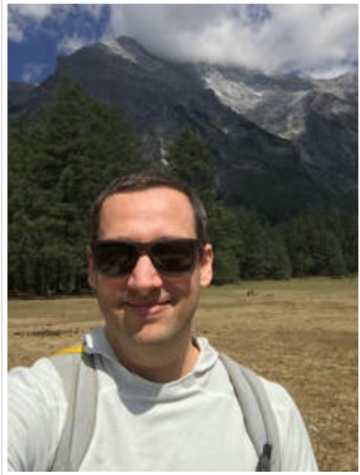
Principal Investigator

Nate Swenson

Gillen Director of the University of Notre Dame Environmental Research Center
Professor of Biology

CV


Google Scholar



National
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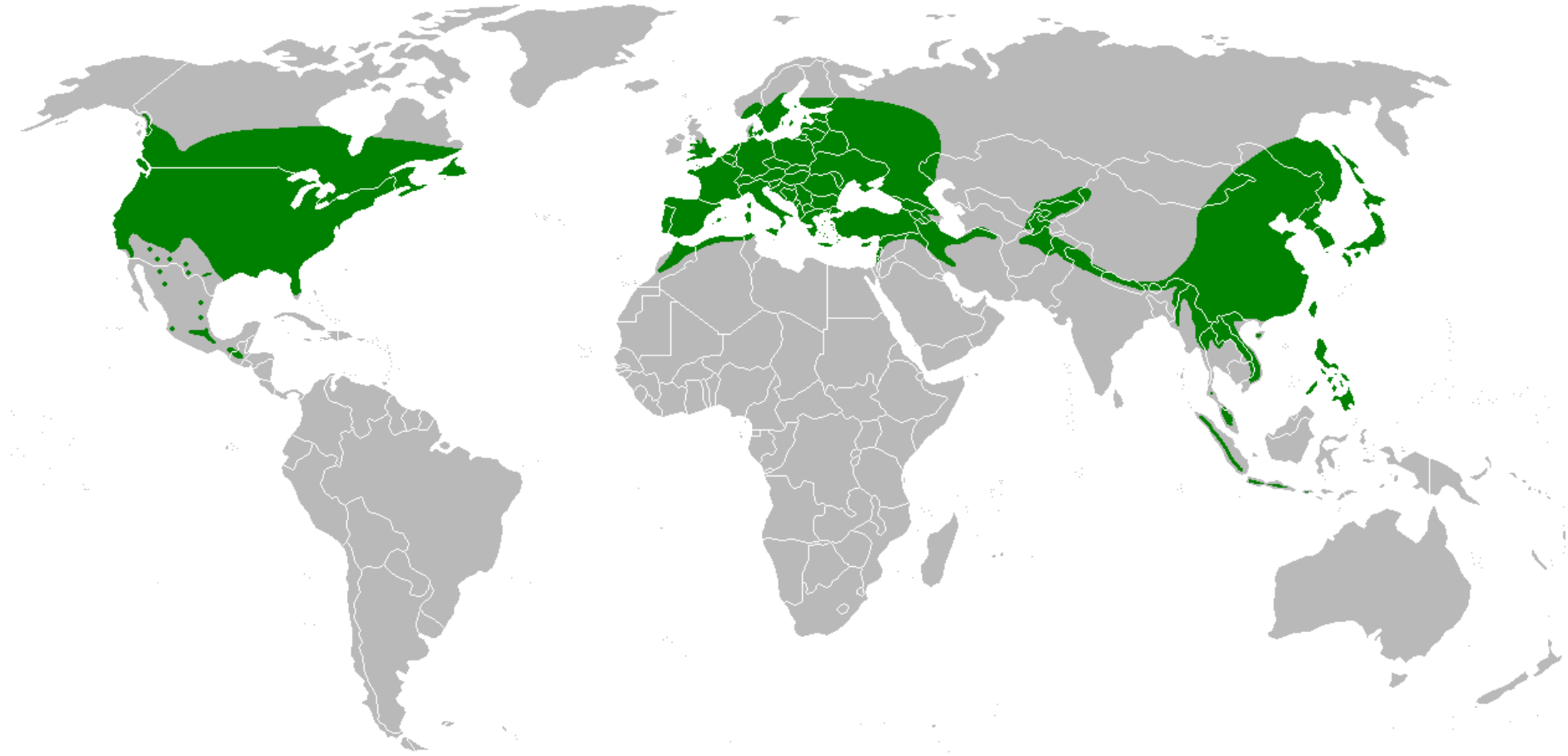
**Pristine
Environments.
World-Changing
Research.**

Promoting environmental
research and education



Distribution of *Acer* (maples)

- ~132 extant species of trees or shrubs
- Most are native to Asia



From <https://en.wikipedia.org/wiki/Maple>

Acer (maples)



From <https://www.thoughtco.com/the-national-flag-of-canada-508080>

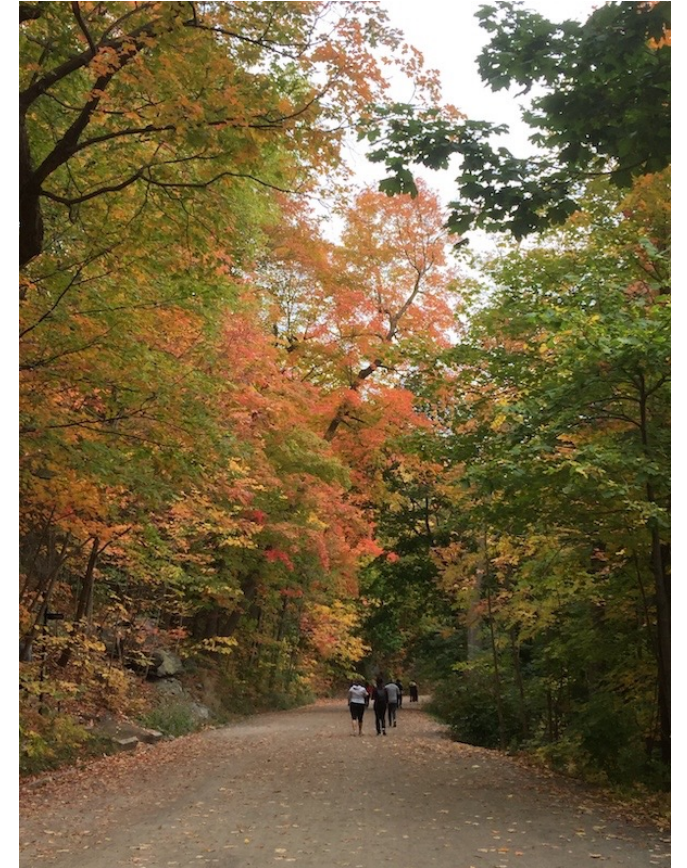


From <https://www.cbc.ca/news/canada/montreal/maple-syrup-grade-producers-sugar-shack-1.6375742>



From <https://www.etsy.com/listing/1484227236/84-soft-maple-hardwood-lumber-top-grade?gpla=1&gao=1&>

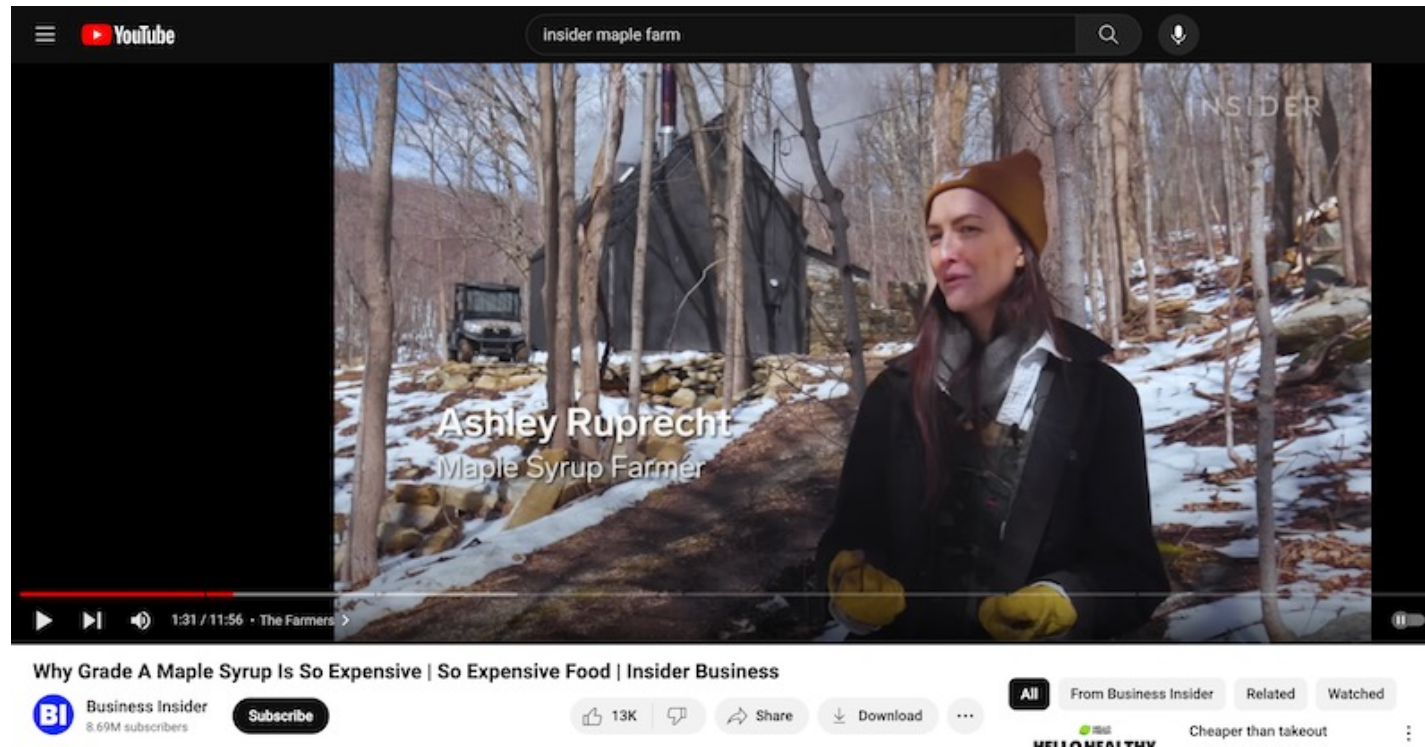
Acer (maples)



Mount Royal, Montreal,
October, 2021

Acer under climate change

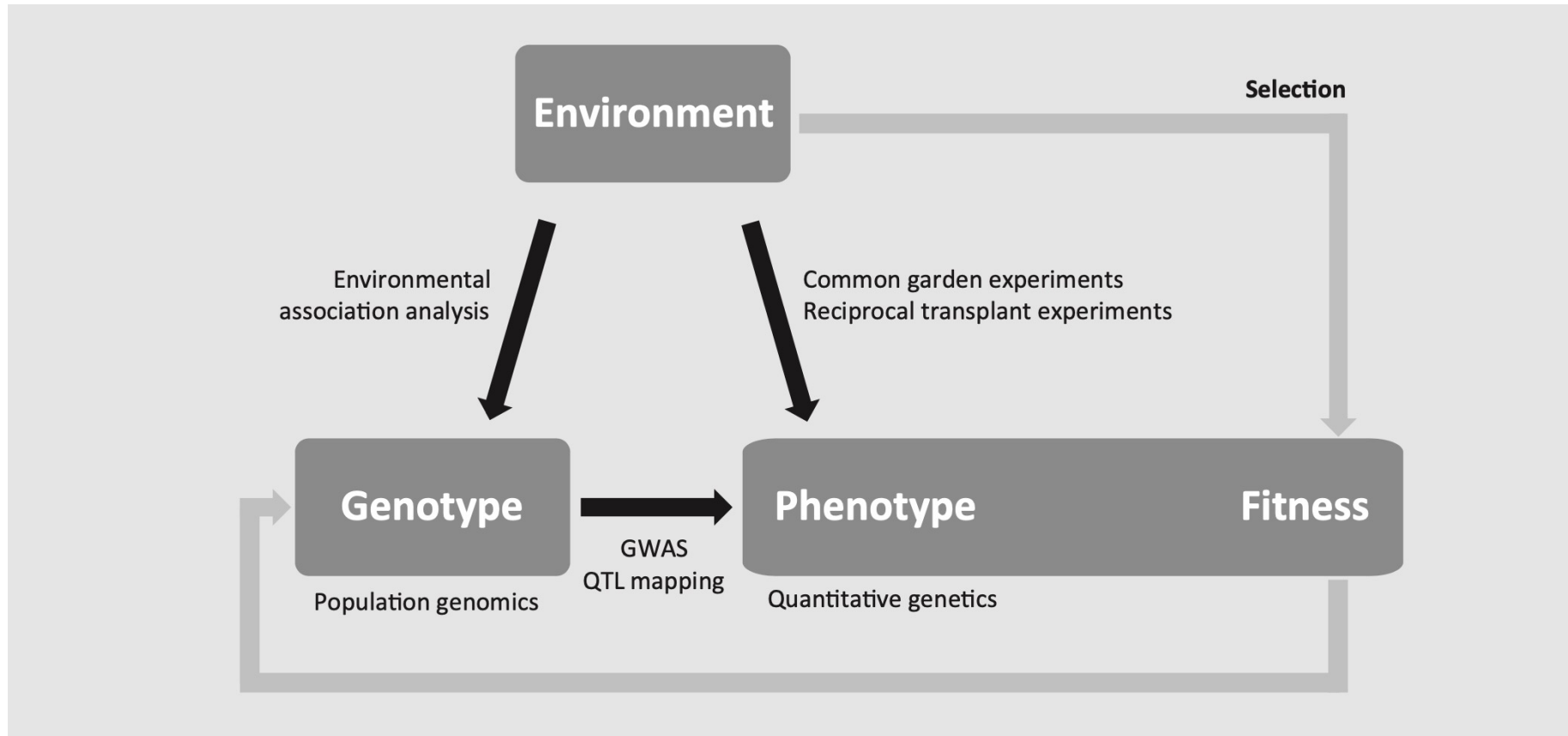
Warming temperatures and changes in freeze and thaw cycles have affected maple trees and syrup production and quality



“... long periods of freezing in the middle of the season are really hard...and sap is not flowing...”

“...A few weeks later it’s really warm and the trees just stop producing sap...”

To study the genetic basis of local adaptation



MOLECULAR ECOLOGY

Molecular Ecology (2015) 24, 4348–4370

doi: 10.1111/mec.13322

INVITED REVIEWS AND SYNTHESSES

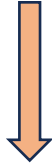
A practical guide to environmental association analysis in landscape genomics

CHRISTIAN RELLSTAB,* FELIX GUGERLI,* ANDREW J. ECKERT,† ANGELA M. HANCOCK‡ and ROLF HOLDEREGGER*§

*WSL Swiss Federal Research Institute, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland, †Department of Biology, Virginia Commonwealth University, Richmond, VA 23284, USA, ‡Faculty of Molecular Biology, Max F. Perutz Laboratories and University of Vienna, Oskar-Morgenstern-Platz 1, 1090 Vienna, Austria, §ETH Zürich, Institute of Integrative Biology, Universitätstrasse 16, 8092 Zürich, Switzerland

Phylotranscriptomics-based method to study the genetic basis of climate adaptation

Step 1
Performing ortholog search
and phylogeny construction



Step 2
Detecting correlation
between phylogeny and
climate distance



Step 3
Identifying genes underlying
climate adaptation

RNA-Seq



De novo transcriptome
assembly



Phylogeny

Phylogenetic signal
in climate variables

Pairwise ecological
niche similarities

Correlations between pairwise
phylogenetic distance and
climate variables

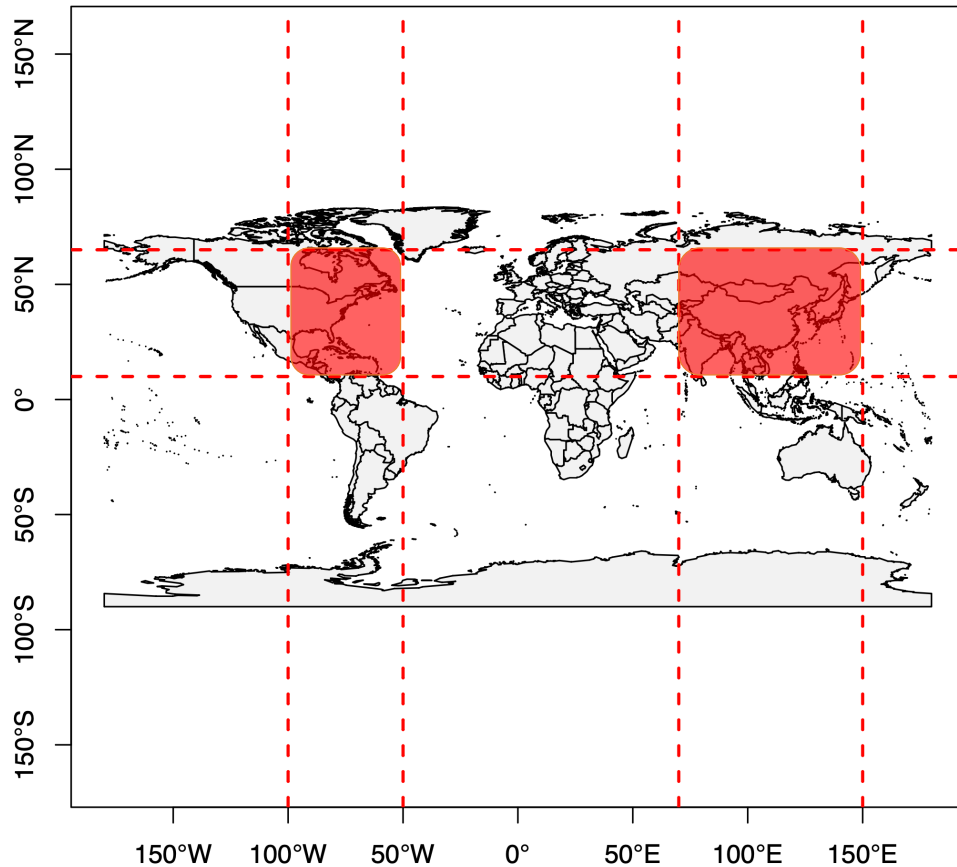
Selecting ortholog groups highly
correlated with climate variables

Detecting genes for positive
selection and selection intensity

Studied species

North America

- Acer rubrum* (red maple)
- Acer saccharum* (sugar maple)
- Acer spicatum* (mountain maple)



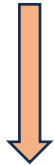
Asia

- Acer amplum*
- Acer barbinerve* (bearded maple)
- Acer buergerianum*
- Acer caesium* (Himalayan maple)
- Acer caudatum* (candle-shape maple)
- Acer chingii*
- Acer cordatum*
- Acer franchetii*
- Acer ginnala* (Amur maple)
- Acer henryi*
- Acer heptalobum* (Japanese maple)
- Acer kuomeii*
- Acer laevigatum* (smooth maple or Nepal maple)
- Acer mandshuricum* (Manchurian maple)
- Acer mono* (painted maple or mono maple)
- Acer oblongum* (Himalayan maple, evergreen maple or Kashmir maple)
- Acer paxii*
- Acer pectinatum*
- Acer robustum*
- Acer sinense*
- Acer stachyophyllum*
- Acer tegmentosum* (Manchurian stripedbark maple or Manchu striped maple)
- Acer triflorum* (three-flowered maple)
- Acer truncatum* (Shandong maple or purpleblow maple)
- Acer wilsonii* (Three Gorges maple)
- Acer flabellatum*

- Dipteronia sinensis*

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Step 1

Performing ortholog test and phylogeny construction

To find one-to-one orthogroups (OGs)

- *De novo* transcriptome assembly
- Predicting the longest peptide sequence
- Using OrthoFinder to predict the preliminary OGs
- Filtering OGs with sequences absent in one or more samples
- Aligning OGs and selecting conserved blocks with at least 100 amino acids
- Removing the remaining paralogs and contamination

Step 1

Performing ortholog test and phylogeny construction

➤ 211 one-to-one orthogroups (OGs)

Sequence format

- Amino acid
- CDS
- CDS (1st +2nd)



- Method 1 Coalescence analysis
Generating gene trees ---→ inferring species tree using gene trees
- Method 2 Maximum likelihood (ML) analysis using concatenated sequences
- Method 3 Maximum likelihood (ML) analysis using partitioned sequences
- Method 4 Bayesian inference (BI) using concatenated sequences
- Method 5 Bayesian inference (BI) using partitioned sequences

- Finally, we yielded 15 phylogenetic trees.

Step 1

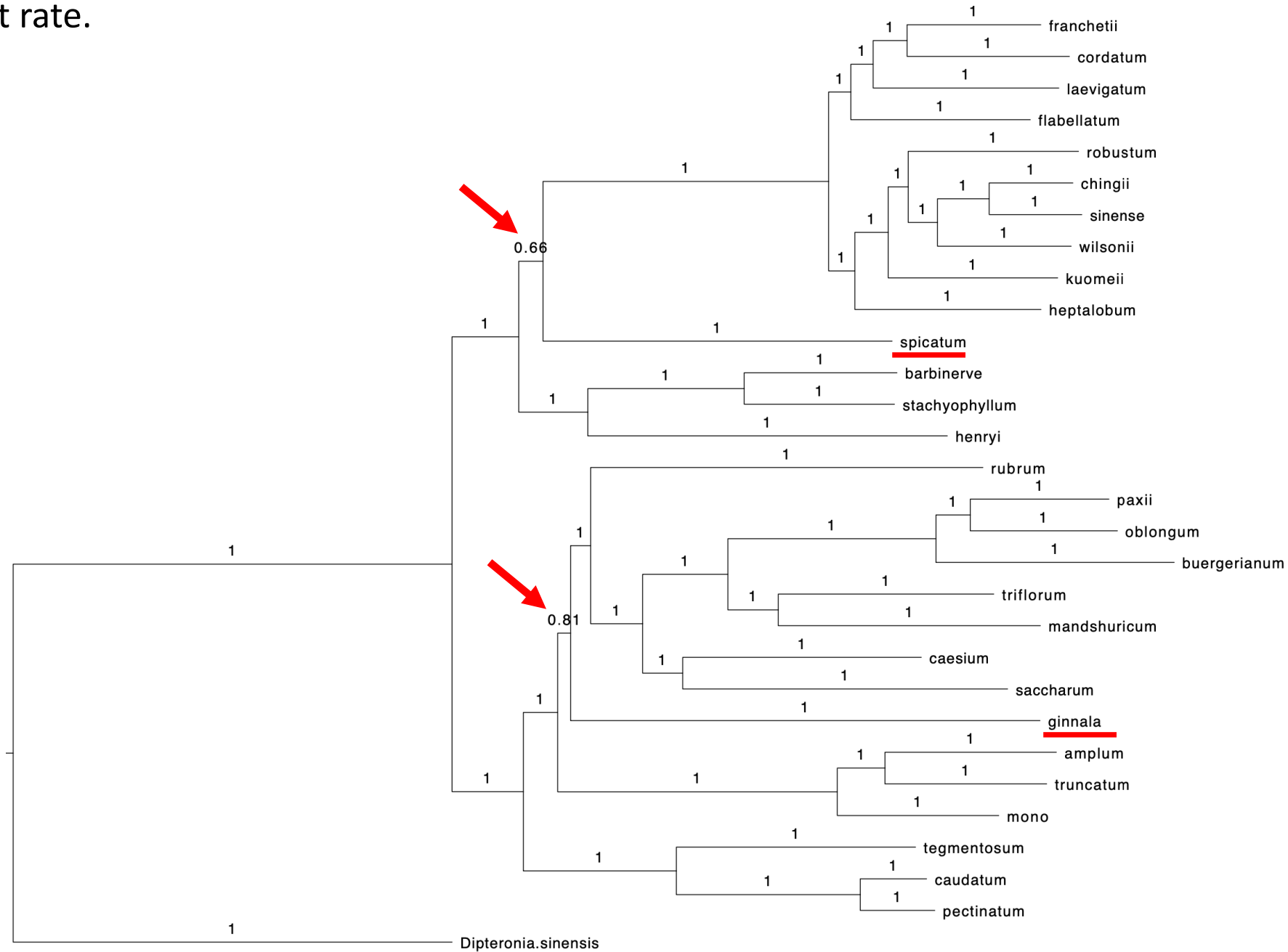
Performing ortholog test and phylogeny construction

➤ These 15 phylogenetic trees are almost the same except for the placement of *Acer ginnala* & *Acer spicatum*.



A species tree inferred using 210 gene trees labeled with branch local posterior probability.

A consensus BI tree of *Acers* reconstructed from the partitioned alignment of CDS (1st +2nd) of 211 OGs labeled with support rate.



Removing *A. spicatum* & *A. ginnala*, the final phylogeny includes 27 species. () contains the section names.

List of *Acer* species 13 languages

From Wikipedia, the free encyclopedia

There are over 160^[1] species in the genus *Acer*. Species with evergreen foliage are tagged #. Species and sections that are extinct are tagged with †.

Species listed by section and series [edit]

Infrageneric classification of extant species follows The Maple Society (E. Davis), 2021. ^[3]^[clarification needed]

Scientific classification

Kingdom: [Plantae](#)

Clade: [Tracheophytes](#)

Clade: [Angiosperms](#)

Clade: [Eudicots](#)

Clade: [Rosids](#)

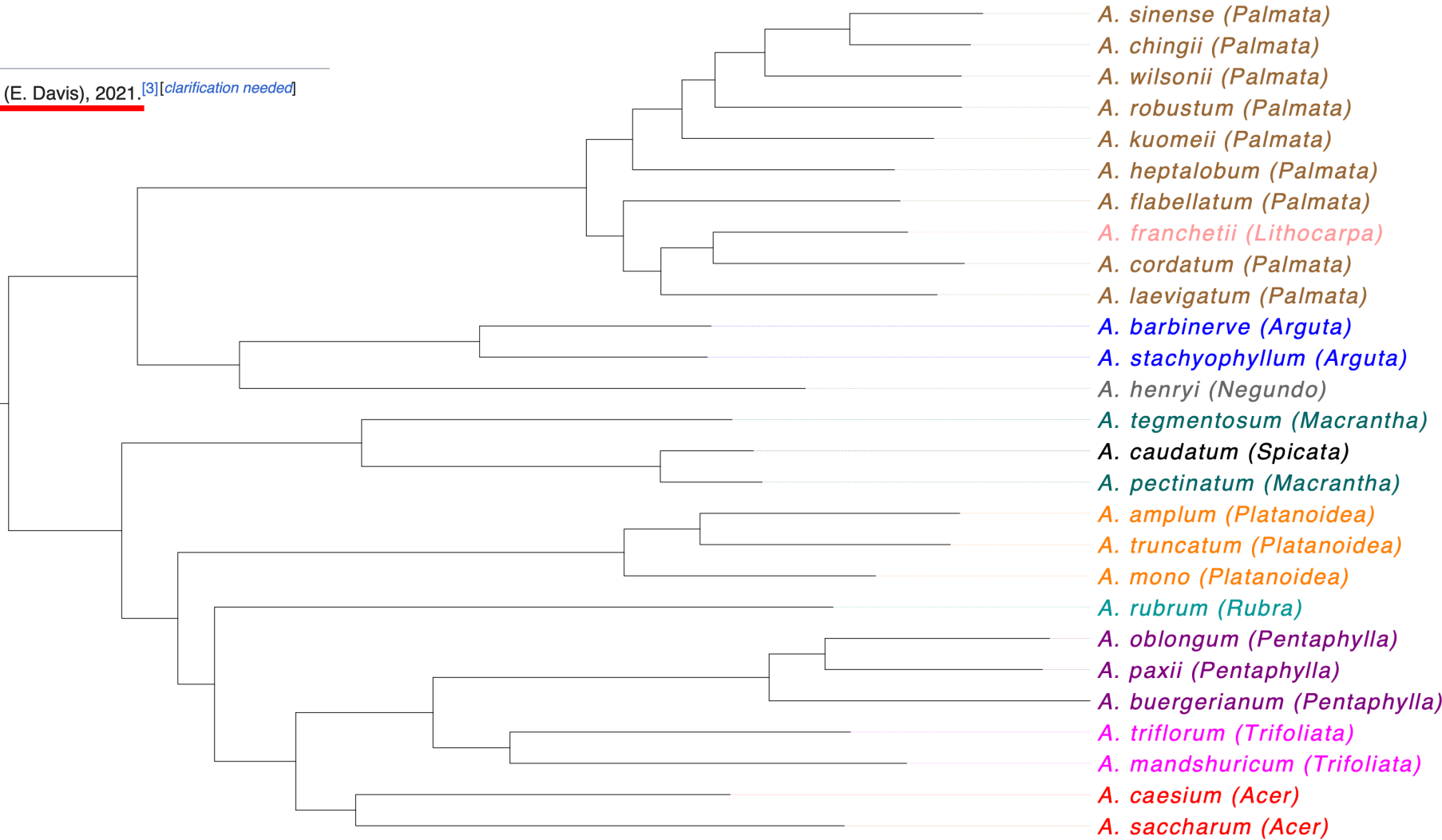
Order: [Sapindales](#)

Family: [Sapindaceae](#)

Genus: *Acer*

Section: *Acer* sect. *Rubra*

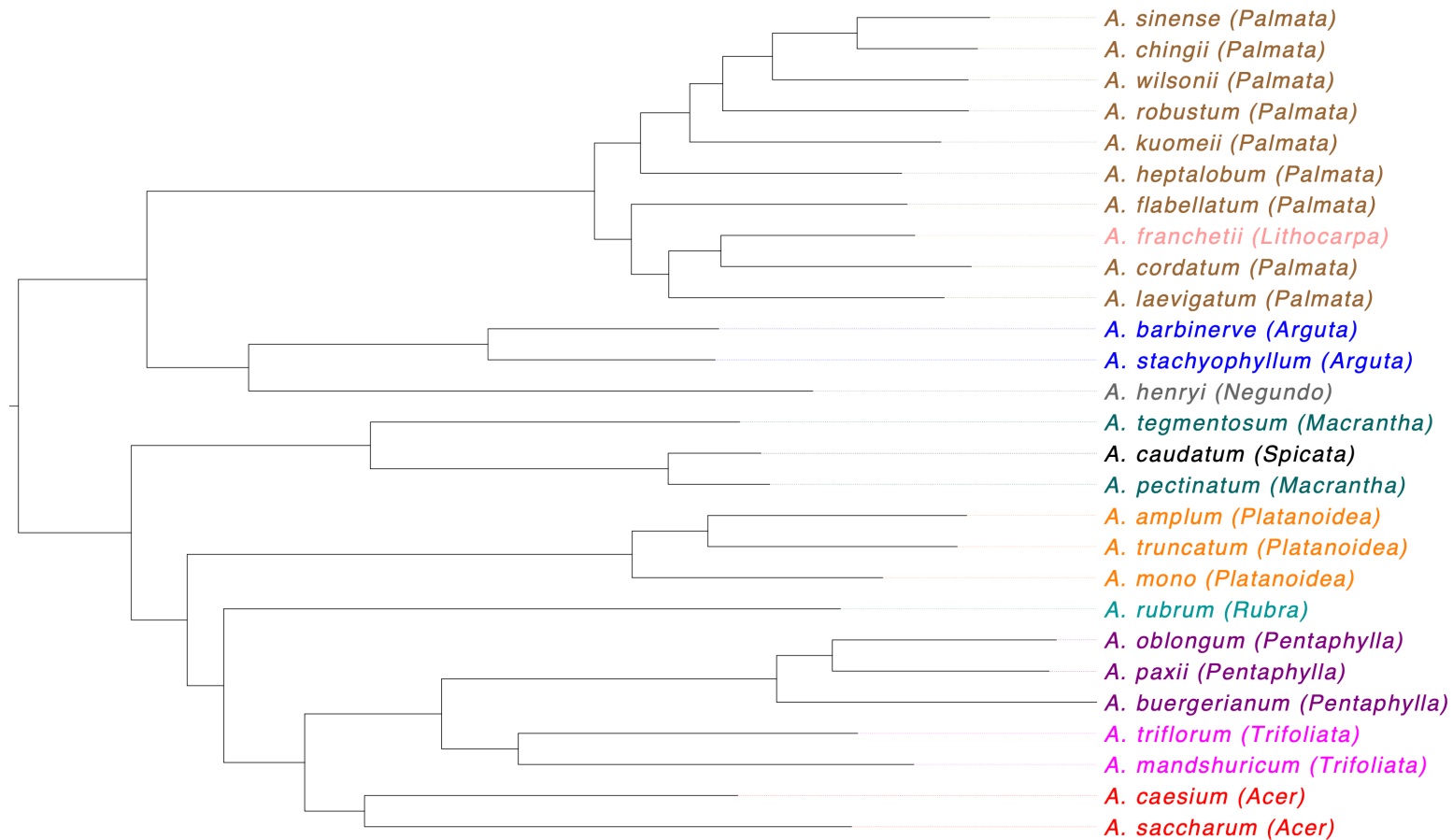
Species: ***A. rubrum***



➤ Clustering in this phylogenetic tree is consistent with section classification of *Acers*.

Step 1

Performing ortholog test and phylogeny construction



- This is the final phylogenetic (species) tree.
- Derive phylogenetic distance from this tree.

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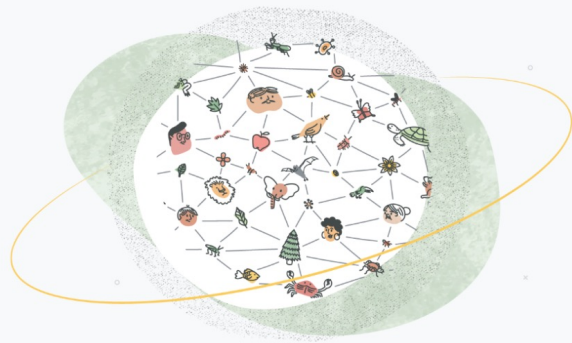
Detecting genes for positive
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Step 2

Detecting correlation between phylogeny and climate distance

- Download GBIF presence occurrence data for each of the 27 species in the phylogeny
- Plot occurrence distribution maps

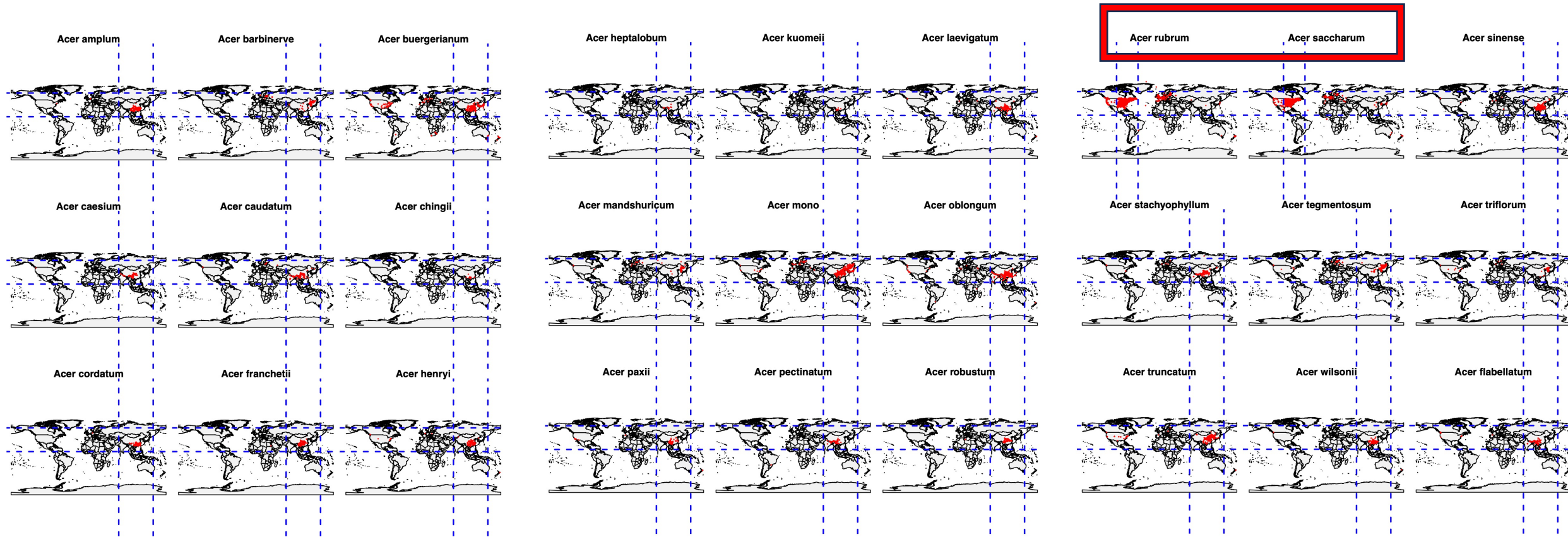
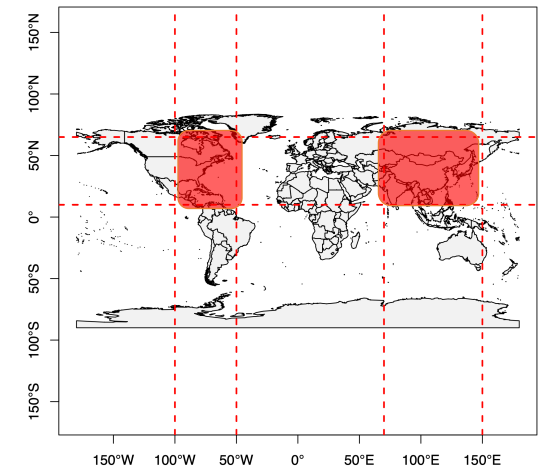
What is GBIF?



GBIF—the Global Biodiversity Information Facility—is an international network and data infrastructure funded by the world's governments and aimed at providing anyone, anywhere, open access to data about all types of life on Earth.

▶ [Video](#) [Learn more](#)

- Download GBIF presence occurrence data for each species
- Plot occurrence distribution (red spots) maps

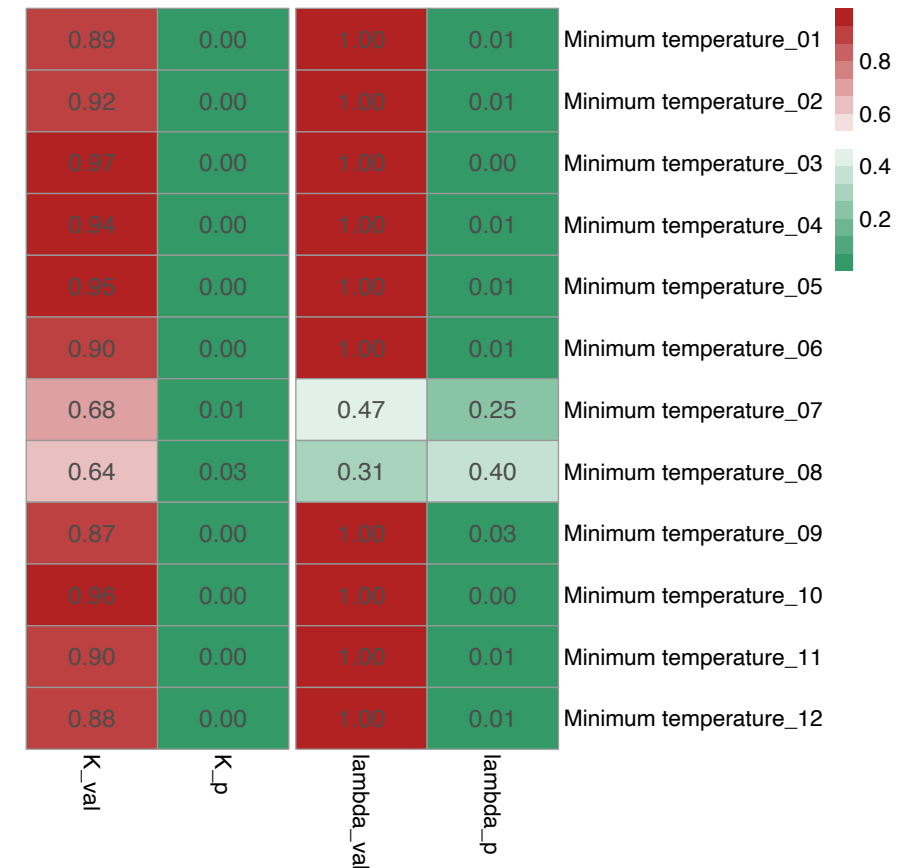
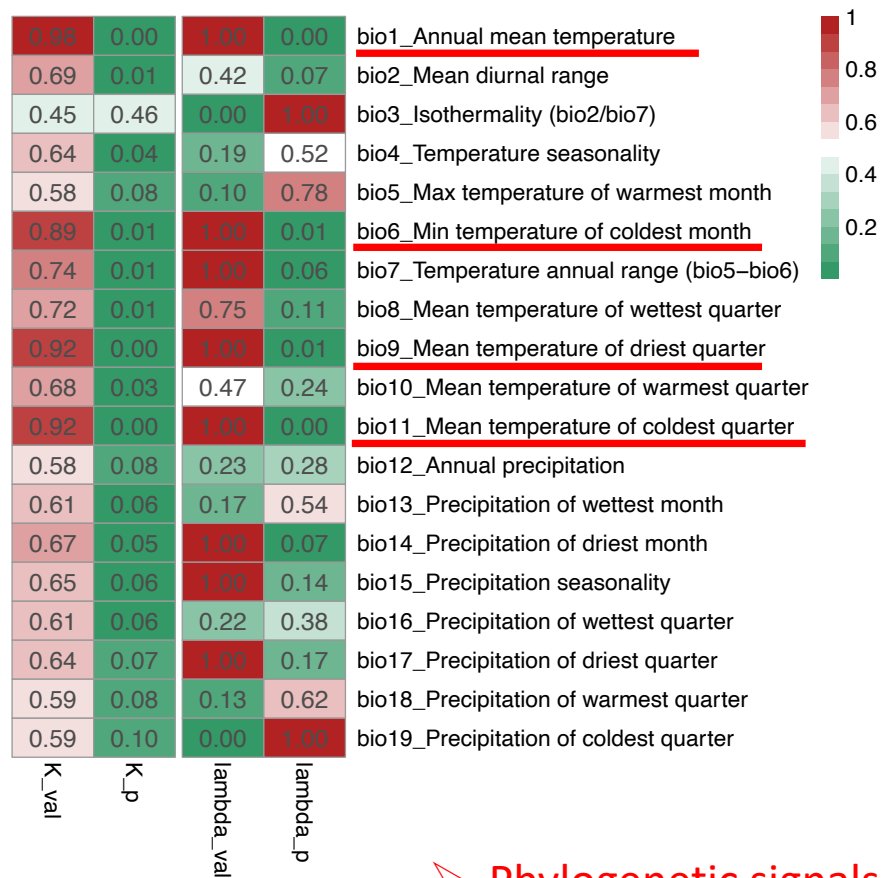


- Calculate the median values of each climate variable (WorldClim v2.1) within the natural range

Step 2 Detecting correlation between phylogeny and climate distance

1) Phylogenetic signal in climate variables

- Blomberg's K and Pagel's lambda are two measures of phylogenetic signals
- Calculate K and lambda in the median values of each climate variable (From WorldClim v2.1) in the natural range



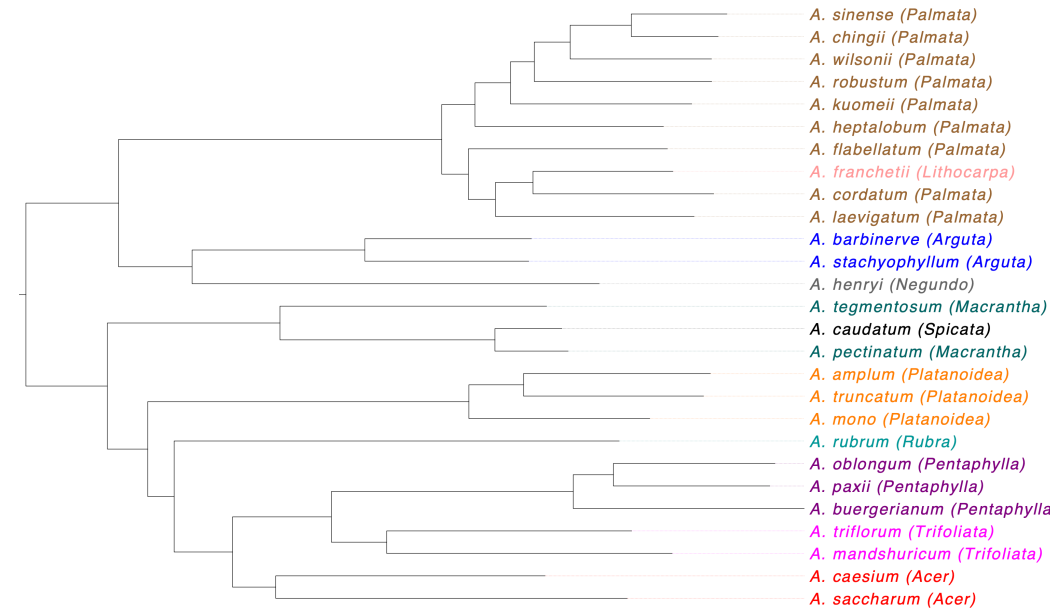
- Phylogenetic signals exists in climate variables related with minimum temperature of cold months.

Step 2 Detecting correlation between phylogeny and climate distance

2) Correlations between pairwise phylogenetic distance and climate variables

For each pair of the 27 species in the phylogenetic tree,

- Calculate the pairwise phylogenetic distances using its branch lengths
- Calculate the pairwise Euclidean distances in the 11 temperature BIOs and the 12 monthly minimum temperature
- Run Mantel test

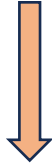


	Temperature BIOs vs. phylogenetic distance	Monthly minimum temperature vs. phylogenetic distance
Mantel coefficient (r)	0.098	0.154
One-tailed p-value (null hypothesis: $r \leq 0$)	0.067	0.018

- Phylogenetic distance is correlated with monthly minimum temperature distance.

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Step 3 Identifying genes underlying minimum temperature adaptation

1) Selecting ortholog groups (OGs) highly correlated with climate variables

- Ortholog assignment including at least 10 species
- Align OGs and select the conserved alignment
- Remove paralogs
- Construct gene trees for each OG, and calculate the phylogenetic distance between each pair of species
- Calculate the pairwise Euclidean distances in the 12 monthly minimum temperature
- Run Mantel test between phylogenetic distance and climate distances
- Find out the OGs significantly correlated with climate distances

141 OGs ($r > 0.5$ & $p\text{-value} \leq 0.05$) are highly correlated with pairwise distance in minimum temperature

Step 3 Identifying genes underlying minimum temperature adaptation

1) Selecting ortholog groups highly correlated with climate variables

141 OGs ($r > 0.5$ & $p\text{-value} \leq 0.05$) are highly correlated with pairwise distance in minimum temperature

Check the GO term enrichment in these highly correlated OGs.

- Biological process
 - DNA geometric change
 - response to unfolded protein
 - DNA duplex unwinding
 - acyl-CoA biosynthetic process
 - tRNA N1-guanine methylation
 - endoplasmic reticulum unfolded protein response
 - thioester biosynthetic process
- Molecular function
 - ubiquitin-specific protease binding
- Cellular component
 - Hrd1p ubiquitin ligase ERAD-L complex

Epigenetic regulation is involved in cold adaptation.

- ❖ Activation or suppression of transcription factors control target gene expression.
- ❖ Chromatin changes are essential for the binding of transcription factors.

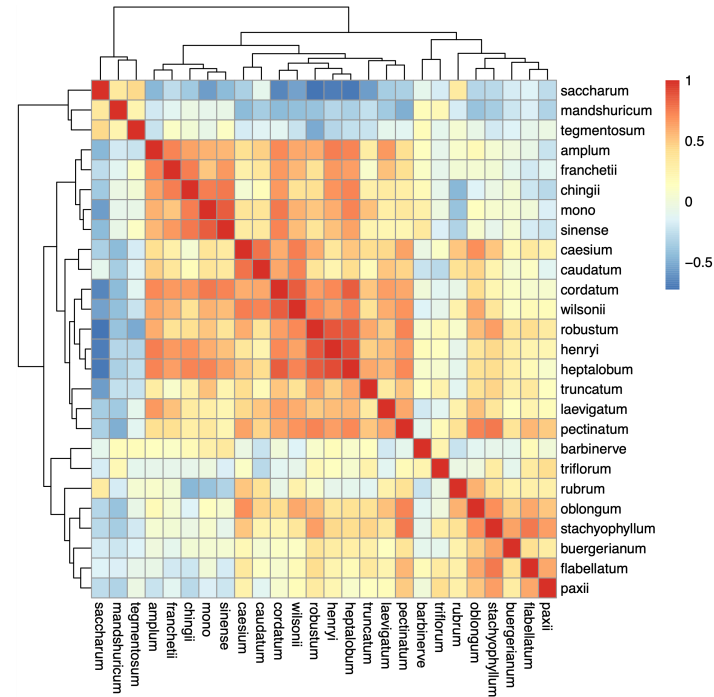
Epigenetic switch from repressive to permissive chromatin in response to cold stress

Junghoon Park^{a,b,1}, Chae Jin Lim^{a,b,1}, Mingzhe Shen^{b,1}, Hee Jin Park^{a,c}, Joon-Yung Cha^b, Elisa Iniesto^d, Vicente Rubio^d, Tesfaye Mengiste^e, Jian-Kang Zhu^f, Ray A. Bressan^f, Sang Yeol Lee^g, Byeong-ha Lee^g, Jing Bo Jin^h, Jose M. Pardoⁱ, Woe-Yeon Kim^b, and Dae-Jin Yun^{a,2}

^aDepartment of Biomedical Science and Engineering, Konkuk University, 05029 Seoul, South Korea; ^bDivision of Applied Life Science (BK21 plus Program), Plant Molecular Biology and Biotechnology Research Center, Institute of Agriculture and Life Science, Gyeongsang National University, 52828 Jinju, Republic of Korea; ^cInstitute of Global Disease Control, Konkuk University, 05029 Seoul, Republic of Korea; ^dPlant Molecular Genetics Department, Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain; ^eDepartment of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907; ^fDepartment of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907; ^gDepartment of Life Science, Gyeongsang National University, 52828 Jinju, South Korea; ^hInstitute of Botany, Chinese Academy of Sciences, Beijing 100093, China; ⁱCentro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

Step 3 Identifying genes underlying minimum temperature adaptation

2) Detecting genes for positive selection and selection intensity



Set “*A. saccharum*, *A. mandshuricum*, *A. tegmentosum*” as foreground and the other species as background,

- Test selection intensity using RELAX
k>1 indicates that selection strength has been intensified;
k<1 indicates that selection strength has been relaxed
- Test positive selection using PAML

Set “*A. saccharum*, *A. mandshuricum*, *A. tegmentosum*” as foreground and the other species as background,

- Test selection intensity using RELAX
- Calculate genome-wide median of K (stringency of selection) value for each species
- Run PGLS between K and BIOs

bio_var	PGLS_pval
bio1_Annual mean temperature	0.05
bio2_Mean diurnal range	0.27
bio3_Isothermality (bio2/bio7)	0.43
bio4_Temperature seasonality	0.06
bio5_Max temperature of warmest month	0.8
bio6_Min temperature of coldest month	0.02
bio7_Temperature annual range (bio5-bio6)	0.05
bio8_Mean temperature of wettest quarter	0.69
bio9_Mean temperature of driest quarter	0.01
bio10_Mean temperature of warmest quarter	0.51
bio11_Mean temperature of coldest quarter	0.02
bio12_Annual precipitation	0.71
bio13_Precipitation of wettest month	0.95
bio14_Precipitation of driest month	0.9
bio15_Precipitation seasonality	0.56
bio16_Precipitation of wettest quarter	0.86
bio17_Precipitation of driest quarter	0.87
bio18_Precipitation of warmest quarter	0.7
bio19_Precipitation of coldest quarter	0.73

tmin_var	PGLS_pval
Minimum temperature_01	0.02
Minimum temperature_02	0.02
Minimum temperature_03	0.04
Minimum temperature_04	0.08
Minimum temperature_05	0.13
Minimum temperature_06	0.18
Minimum temperature_07	0.57
Minimum temperature_08	0.6
Minimum temperature_09	0.09
Minimum temperature_10	0.03
Minimum temperature_11	0.03
Minimum temperature_12	0.02

- Cold adaption is correlated with relaxation of selection (all median $K < 1$, 0.21~0.29).

“ Relaxation of selective strength can drive evolutionary innovation ... ”

“ Selection can be relaxed from the removal of an existing selective constraint”

---- Wertheim et al., 2014

RELAX: Detecting Relaxed Selection in a Phylogenetic Framework

Joel O. Wertheim,^{*1} Ben Murrell,¹ Martin D. Smith,² Sergei L. Kosakovsky Pond,¹ and Konrad Scheffler^{*1,3}

¹Department of Medicine, University of California, San Diego

²Bioinformatics and Systems Biology Graduate Program, University of California, San Diego

³Department of Mathematical Sciences, Stellenbosch University, Stellenbosch, South Africa

***Corresponding author:** E-mail: jwertheim@ucsd.edu; kscheffler@ucsd.edu.

Associate editor: Beth Shapiro

Abstract

Relaxed selection is a precursor to the evolution of phenotypic plasticity

Brendan G. Hunt^{a,1}, Lino Ometto^{b,1,2}, Yannick Wurm^{b,c,3}, DeWayne Shoemaker^d, Soojin V. Yi^a, Lau and Michael A. D. Goodisman^{a,4,5}

^aSchool of Biology, Georgia Institute of Technology, Atlanta, GA 30332; ^bDepartment of Ecology and Evolution, University of Lausanne, Switzerland; ^cVital-IT Group, Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland; and ^dCenter for Medical, Entomology, United States Department of Agriculture- Agricultural Research Service, Gainesville, FL 32608

Edited by Michael Lynch, Indiana University, Bloomington, IN, and approved August 16, 2011 (received for review March 30, 2011)

Phenotypic plasticity allows organisms to produce alternative phenotypes under different conditions and represents one of the most important mechanisms for adaptation to environmental change. It is often associated with differences in expression of genes, and these differences are often associated with differences in expression of genes (10, 16–19). However, few studies have investigated the evolution of differentially expressed genes in response to environmental change.

Trends in Genetics

CellPress
REVIEWS

Opinion

Relaxed Selection and the Rapid Evolution of Reproductive Genes

Amy L. Dapper^{1,2,3,*} and Michael J. Wade²

Evolutionary genomic studies find that reproductive protein genes, those directly involved in reproductive processes, diversify more rapidly than most other gene categories. Strong postcopulatory sexual selection acting within species is the predominant hypothesis proposed to account for the observed pattern.

Highlights

Reproductive protein genes evolve more rapidly than almost any other gene category.



Evolutionary Biology
Plant Biology

Positive selection and relaxed purifying selection contribute to rapid evolution of male-biased genes in a dioecious flowering plant

Lei Zhao, Wei Zhou, Jun He, De-Zhu Li, Hong-Tao Li

Germplasm Bank of Wild Species, Yunnan Key Laboratory of Crop Wild Relatives Omics, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China; Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650201, China

https://en.wikipedia.org/wiki/Open_access

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Reviewed Preprint

Revised by authors after peer review.

About eLife's process

Reviewed preprint version 2
October 31, 2023 (this version)

Reviewed preprint version 1
September 1, 2023

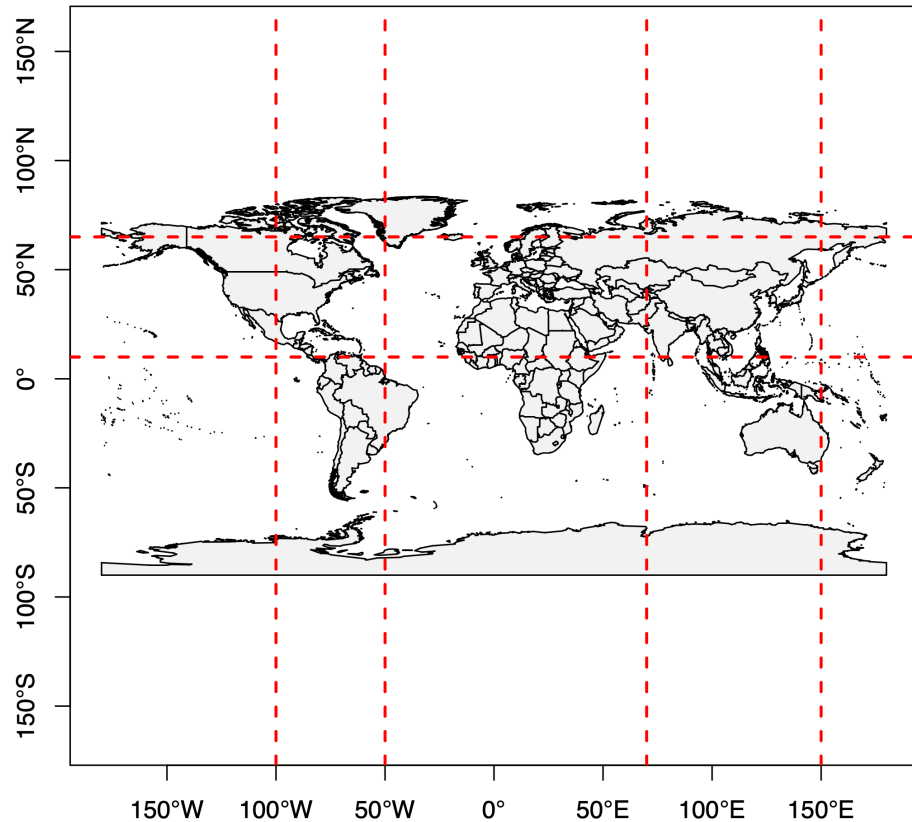
Set “*A. saccharum*, *A. mandshuricum*, *A. tegmentosum*” as foreground and the other species as background,

➤ Test relaxation of selection using RELAX

$k > 1$ indicates that selection strength has been intensified;

$k < 1$ indicates that selection strength has been relaxed

Cold adaption is correlated with relaxation of selection (all median $K < 1$, $0.21 \sim 0.29$).



My interpretation:

- *A. saccharum*, *A. mandshuricum*, *A. tegmentosum* were originated in other regions, possibility warm/hot regions.
- At some historical point, these species migrated from their ancient niche to their current habitat, so the original constraint were released.
- Thus, relaxation of selection was applied to help cold adaptation.

Step 3 Identifying genes underlying minimum temperature adaptation

2) Detecting genes for positive selection and selection intensity

- 155 out of 2828 OGs have significant **relaxed selection** ($K < 1$) on test lineages

Check the GO term enrichment in these identified OGs.

Biological process

- positive regulation of flower development
- tRNA 3'-end processing
- serine family amino acid biosynthetic process
- L-serine metabolic process

Molecular function

- 3'-tRNA processing endoribonuclease activity
- calmodulin-dependent protein kinase activity
- calcium-dependent protein serine/threonine
- monocarboxylic acid transmembrane transp

Cellular component

- mitochondrial respiratory chain complex I
- protein phosphatase type 2A complex

Epigenetic regulation

Epigenetic regulation is involved in cold adaptation.

- ❖ Activation or suppression of transcription factors control target gene expression.
- ❖ Chromatin changes are essential for the binding of transcription factors.

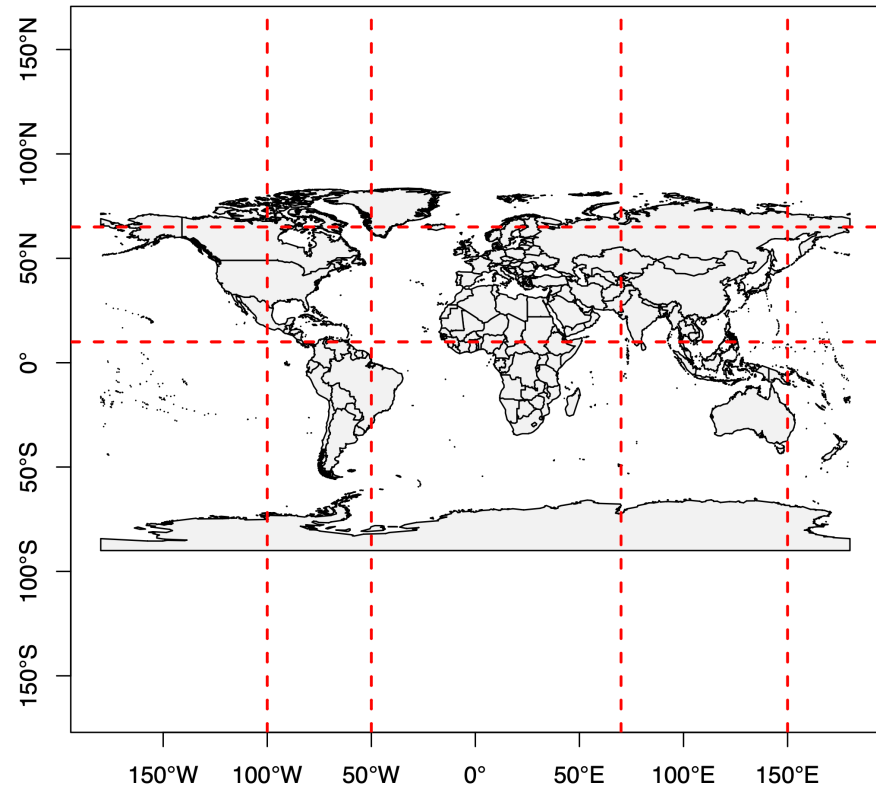
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- At some historical point, these species migrated from their ancient niche to their current habitat, so the original constraint were released.
- Thus, relaxation of selection was applied to help cold adaptation.

➤ **Relaxation of selection involves genes controlling epigenetic regulation:**

DNA and histone modifications and higher-order chromatin structures through alternative polyadenylation (mRNA 3'-End Processing) in flower development genes.

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2) Detecting genes for positive selection and selection intensity

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➤ Test relaxation of selection using RELAX

$k > 1$ indicates that selection strength has been intensified;

$k < 1$ indicates that selection strength has been relaxed

➤ Test positive selection using PAML

➤ 155 out of 2828 OGs have significant relaxed selection ($K < 1$) on test lineages

➤ 137 out of 2828 OGs have significant intensified selection ($K > 1$) on test lineages

➤ 109 out of 3279 OGs were tested positive selection on test lineages.

Check the GO term enrichment in the identified intensively selected and positively selected OGs.

Step 3 Identifying genes underlying minimum temperature adaptation

2) Detecting genes for positive selection and selection intensity

GO term enrichment

Intensified selection

Positive selection

Biological process

- transcription by RNA polymerase I
- glucose metabolic process
- purine-containing compound metabolic process
- ATP transport
- intracellular protein transport
- nitrogen compound transport

- endoplasmic reticulum unfolded protein response
- cellular response to unfolded protein
- immune response
- defense response
- glucose metabolic process
- intracellular chemical homeostasis
- phosphate ion transport

Molecular function

- ATP:ADP antiporter activity
- RNA polymerase I activity
- electron transfer activity

- monosaccharide binding

Cellular component

- RNA polymerase III complex
- RNA polymerase I complex

- Mitochondrial inner membrane complex
- heterotrimeric G-protein complex
- extrinsic component of membrane
- nitrite reductase complex

Step 3 Identifying genes underlying minimum temperature adaptation

2) Detecting genes for positive selection and selection intensity

GO term enrichment

Intensified selection

Positive selection

Biological process



Sugar accumulation and transport genes are enriched in intensified and positive selection genes,

❖ Energy and carbon sources

❖ Defense response to abiotic and biotic stress factors, as priming molecules, metabolic signaling molecules

Molecular function

Cellular component

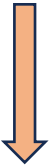
- RNA polymerase III complex
- RNA polymerase I complex

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- extrinsic component of membrane
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Summary

Phylotranscriptomics-based method to study the genetic basis of climate adaptation in *Acers*

Step 1
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Step 2
Detecting correlation
between phylogeny and
climate distance



Step 3
Identifying genes underlying
climate adaptation

RNA-Seq



De novo transcriptome
assembly



Phylogeny

Phylogenetic signal
in climate variables

Pairwise ecological
niche similarities

Correlations between pairwise
phylogenetic distance and
climate variables

Selecting ortholog groups highly
correlated with climate variables

Detecting genes for positive
selection and selection intensity

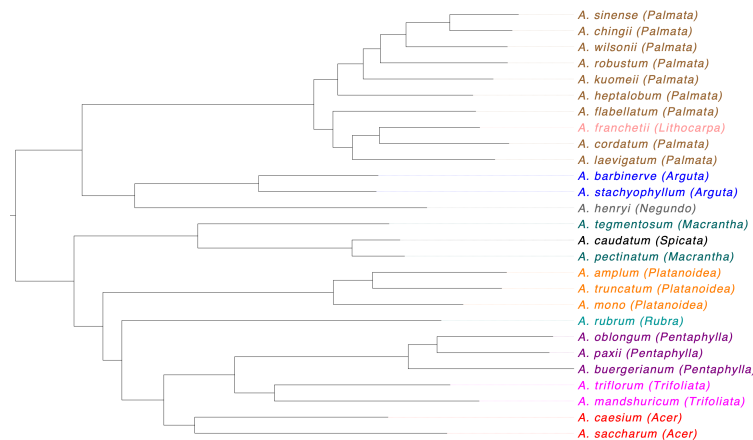
Summary

Phylotranscriptomics-based method to study the genetic basis of climate adaptation in *Acers*

Step 1
Performing ortholog search
and phylogeny construction

Step 2
Detecting correlation
between phylogeny and
climate distance

Step 3
Identifying genes underlying
climate adaptation



- Phylogenetic signals exists in climate variables related with minimum temperature of cold months.
- Cold adaption is correlated with relaxation of selection (all median $K < 1$, $0.21 \sim 0.29$).
- Relaxation of selection involves genes controlling epigenetic regulation.
- Sugar accumulation and transport genes are enriched in intensified and positive selection genes.

Take-home

- Phylogeny-based method is efficient to identify orthologs related to climate adaptation.
- Relaxation of selection in genes involved in epigenetic regulation is the main mechanism for cold adaption in *Acers*.
- **Caution:** Genetic variation that arise from gene duplication (paralogs) were not considered in the present study.

Thank you!

