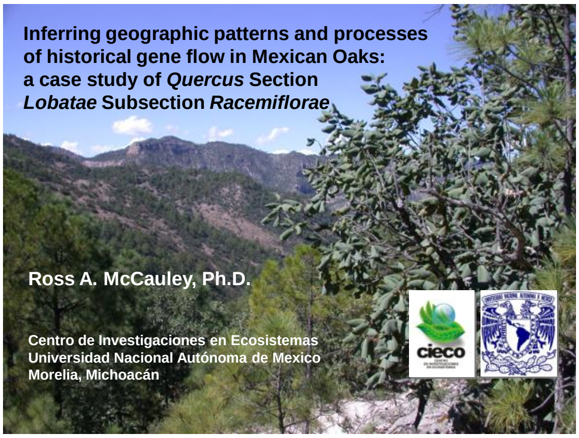
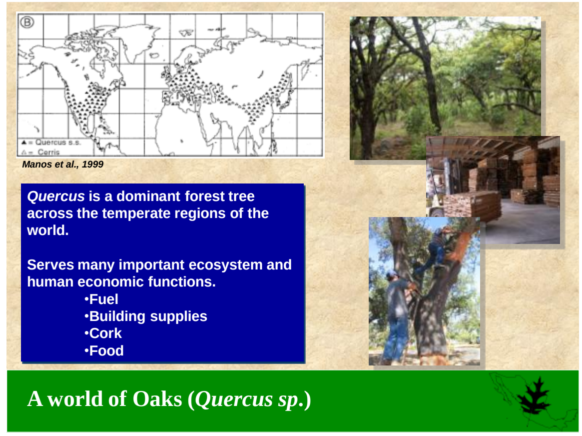
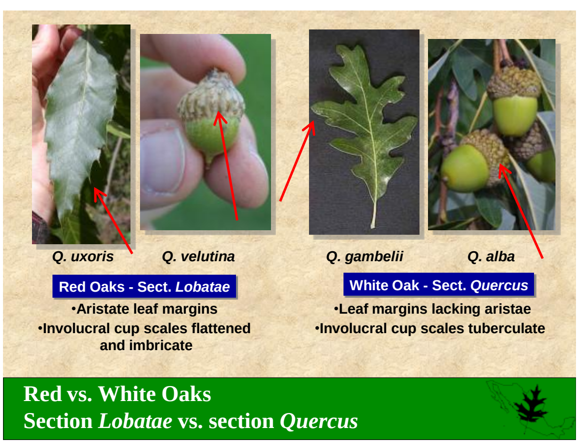
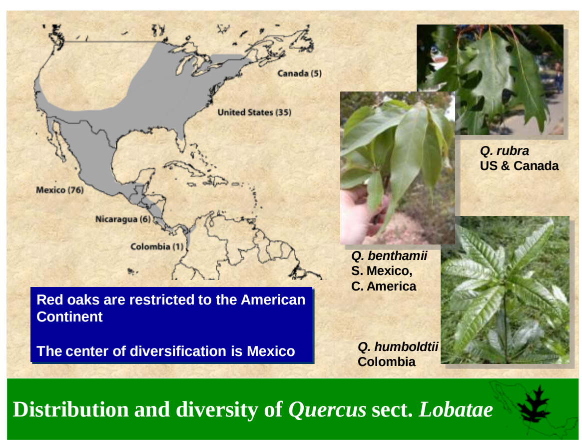

Inferring geographic patterns and processes
of historical gene flow in Mexican Oaks:
a case study of *Quercus* Section
Lobatae Subsection *Racemiflorae*
Ross A. McCauley, Ph.D.
Centro de Investigaciones en Ecosistemas
Universidad Nacional Autónoma de Mexico
Morelia, Michoacán
cieco

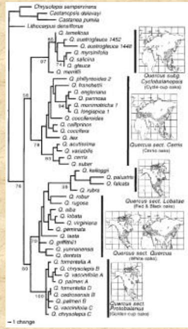

Manos et al., 1999
Quercus is a dominant forest tree across the temperate regions of the world.
Serves many important ecosystem and human economic functions.
•Fuel
•Building supplies
•Cork
•Food
A world of Oaks (*Quercus sp.*)


Dominant feature across the mountain ranges of the country
Approximately 20% of national territory is covered by oak or oak/pine forests
Rzedowski, 1978
Distribution of *Quercus* in Mexico


Mexico is a primary center of oak diversity
- 161 species


Q. uxoris
Q. velutina
Q. gambelii
Q. alba
Red Oaks - Sect. *Lobatae*
•Aristate leaf margins
•Involucral cup scales flattened and imbricate
White Oak - Sect. *Quercus*
•Leaf margins lacking aristae
•Involucral cup scales tuberculate
Red vs. White Oaks
Section *Lobatae* vs. section *Quercus*


Canada (5)
United States (35)
Mexico (76)
Nicaragua (6)
Colombia (1)
Q. rubra
US & Canada
Q. benthamii
S. Mexico,
C. America
Q. humboldtii
Colombia
Red oaks are restricted to the American Continent
The center of diversification is Mexico
Distribution and diversity of *Quercus* sect. *Lobatae*



Phylogeny of sectional groups in Quercus
Manos & Stanford, 2001

A phylogeny for major sections of *Quercus* was already present.

Given the high diversification in Mexico a phylogeny to understand species relationships would be valuable.

I used a series of chloroplast genes to construct a phylogeny of *Lobatae*.

But….

It didn't work!!

Phylogeny of *Quercus* sect. *Lobatae*

Oaks are wind pollinated and the pollen can move very large distances

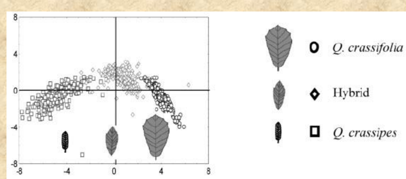
Seeds often produced in great numbers – however they are large and not dispersed far

Reproduction in *Quercus*

Oaks show low barriers to cross-pollination

Hybrid oaks are well known in areas where species of the same section have overlapping ranges

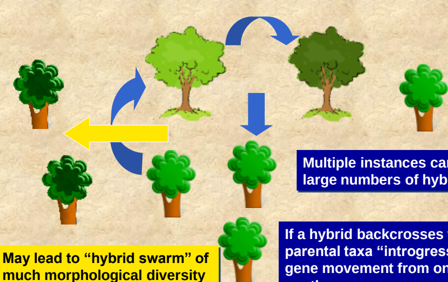
Hybrid individuals usually show intermediate characters between parental species



Tovar-Sánchez and Oyama, 2004

Hybridization and Introgression in *Quercus*

Initial hybridization between different species results in hybrid

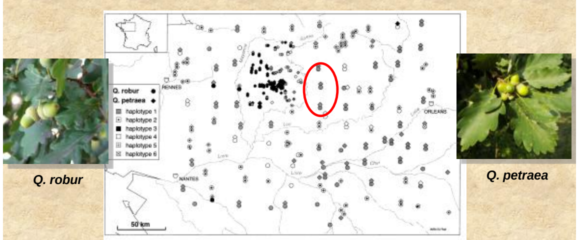


Multiple instances can result in large numbers of hybrids

May lead to "hybrid swarm" of much morphological diversity

If a hybrid backcrosses with one of the parental taxa "introgression" leading to gene movement from one taxon to another

Genetic basis of hybridization and introgression



Q. robur

Q. petraea

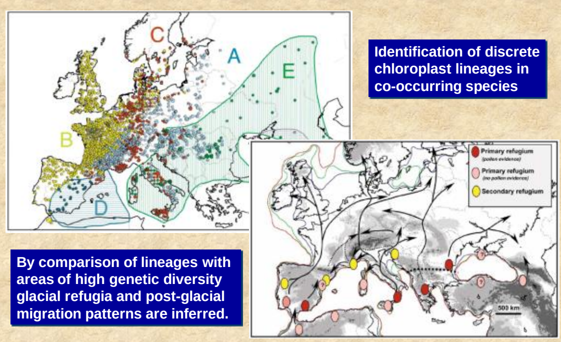
Co-occurring species in France share their chloroplast genomes

Suggests that hybridization and gene flow have served to homogenize the genetic components of species

Petit et al., 1997

Continental scale studies in European white oaks

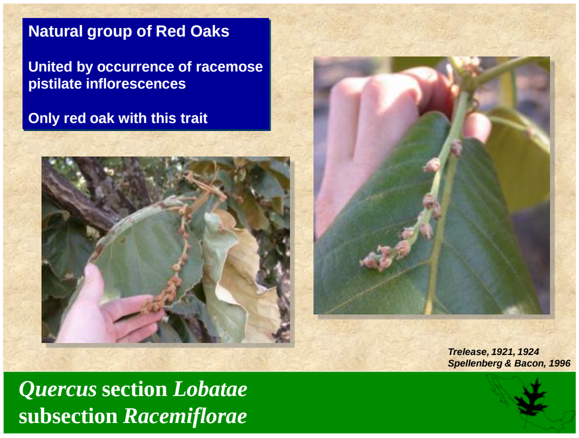
Identification of discrete chloroplast lineages in co-occurring species

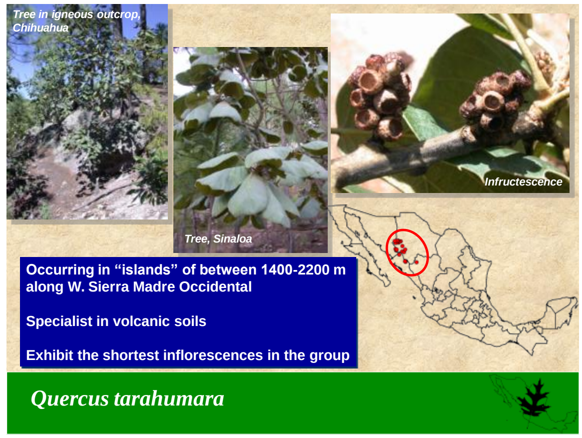


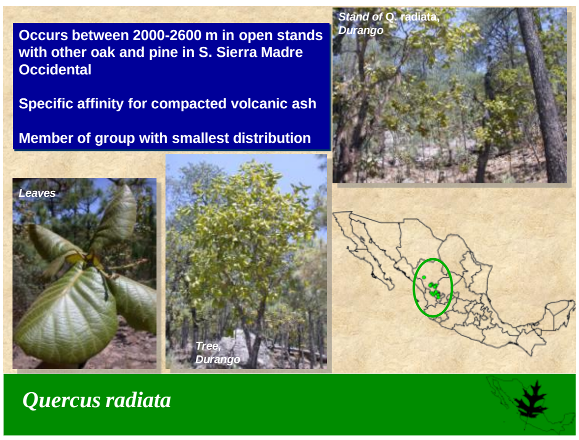
By comparison of lineages with areas of high genetic diversity glacial refugia and post-glacial migration patterns are inferred.

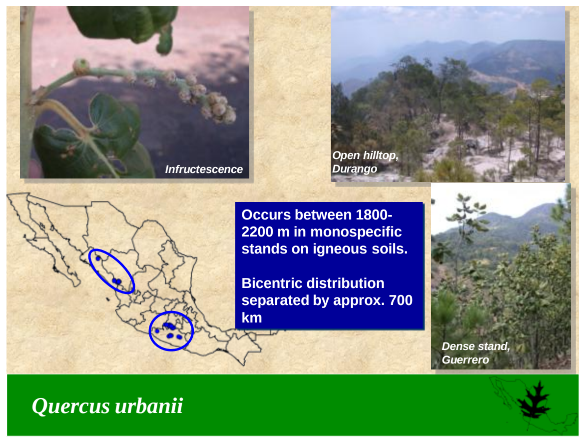
Petit et al., 2002

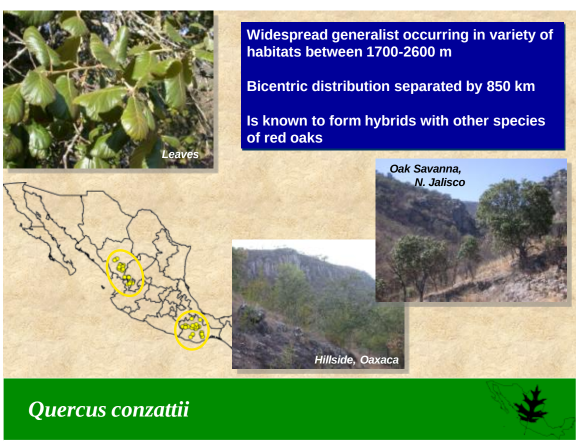
Continental scale studies in European white oaks

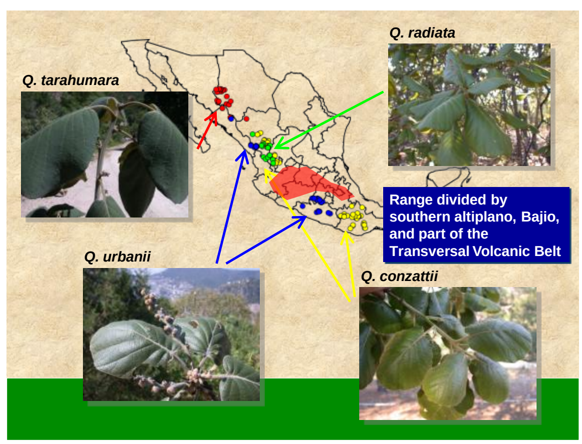

Natural group of Red Oaks
United by occurrence of racemose pistilate inflorescences
Only red oak with this trait
Trelease, 1921, 1924
Spellenberg & Bacon, 1996
Quercus section Lobatae subsection Racemiflorae

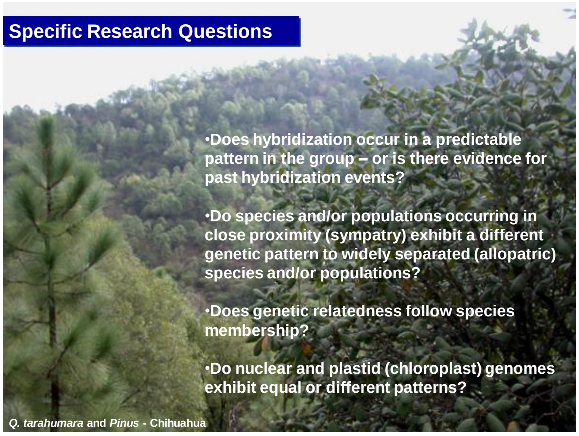

Tree in igneous outcrop, Chihuahua
Tree, Sinaloa
Infructescence
Occurring in "islands" of between 1400-2200 m along W. Sierra Madre Occidental
Specialist in volcanic soils
Exhibit the shortest inflorescences in the group
Quercus tarahumara

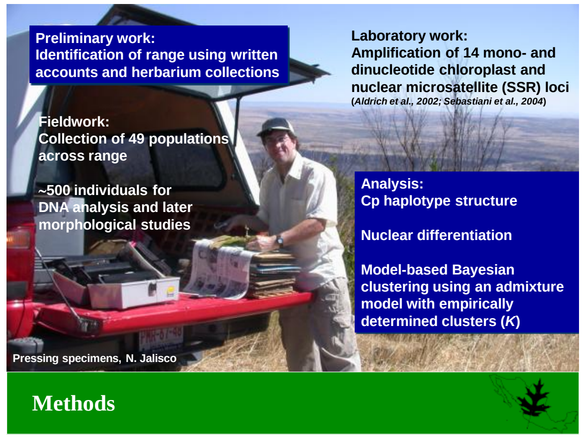

Occurs between 2000-2600 m in open stands with other oak and pine in S. Sierra Madre Occidental
Specific affinity for compacted volcanic ash
Member of group with smallest distribution
Stand of Q. radiata, Durango
Leaves
Tree, Durango
Quercus radiata

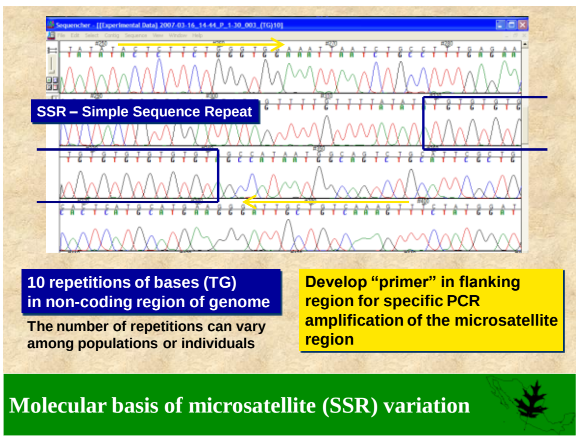

Infructescence
Open hilltop, Durango
Occurs between 1800-2200 m in monospecific stands on igneous soils.
Bicentric distribution separated by approx. 700 km
Dense stand, Guerrero
Quercus urbanii

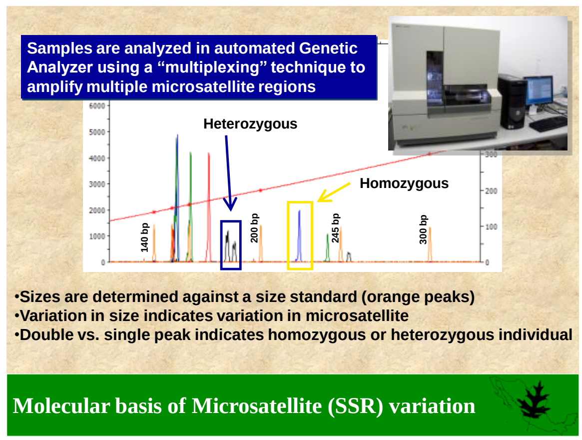

Leaves
Widespread generalist occurring in variety of habitats between 1700-2600 m
Bicentric distribution separated by 850 km
Is known to form hybrids with other species of red oaks
Oak Savanna, N. Jalisco
Hillside, Oaxaca
Quercus conzattii

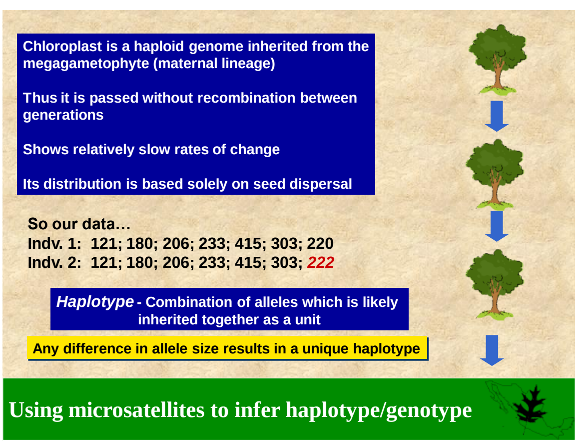

Q. tarahumara
Q. radiata
Range divided by southern altiplano, Bajio, and part of the Transversal Volcanic Belt
Q. urbanii
Q. conzattii

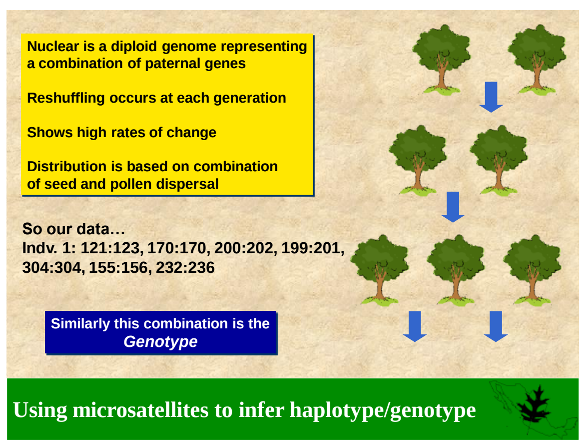

Specific Research Questions
•Does hybridization occur in a predictable pattern in the group – or is there evidence for past hybridization events?
•Do species and/or populations occurring in close proximity (sympatry) exhibit a different genetic pattern to widely separated (allopatric) species and/or populations?
•Does genetic relatedness follow species membership?
•Do nuclear and plastid (chloroplast) genomes exhibit equal or different patterns?
Q. tarahumara and Pinus - Chihuahua

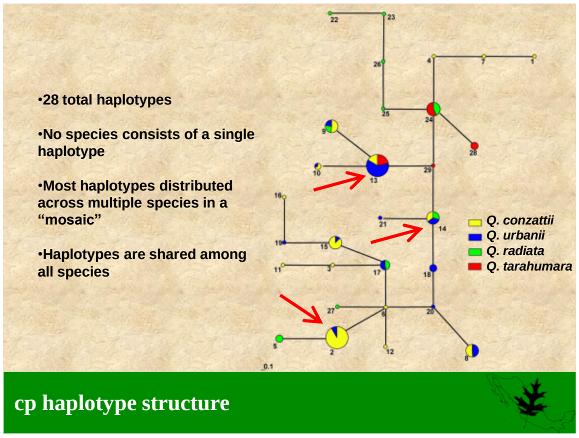

Preliminary work:
Identification of range using written accounts and herbarium collections
Laboratory work:
Amplification of 14 mono- and dinucleotide chloroplast and nuclear microsatellite (SSR) loci
(Aldrich et al., 2002; Sebastiani et al., 2004)
Fieldwork:
Collection of 49 populations across range
~500 individuals for DNA analysis and later morphological studies
Analysis:
Cp haplotype structure
Nuclear differentiation
Model-based Bayesian clustering using an admixture model with empirically determined clusters (K)
Pressing specimens, N. Jalisco
Methods

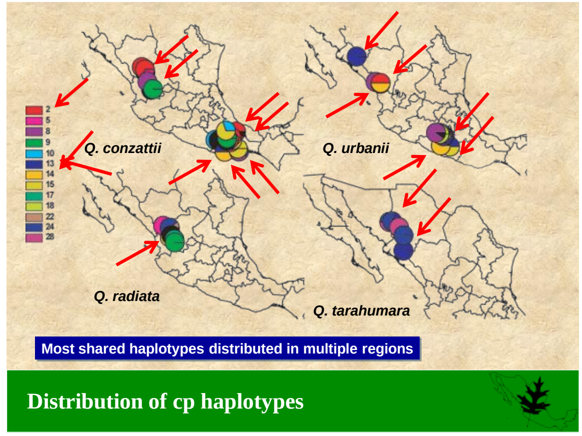

SSR – Simple Sequence Repeat
10 repetitions of bases (TG) in non-coding region of genome
The number of repetitions can vary among populations or individuals
Develop "primer" in flanking region for specific PCR amplification of the microsatellite region
Molecular basis of microsatellite (SSR) variation

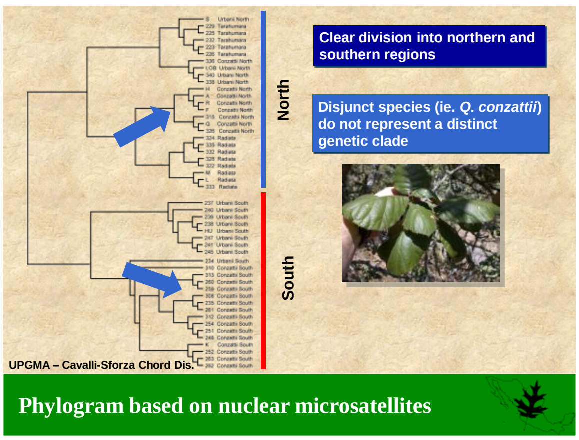

Samples are analyzed in automated Genetic Analyzer using a "multiplexing" technique to amplify multiple microsatellite regions
Heterozygous
Homozygous
140 bp
200 bp
245 bp
300 bp
•Sizes are determined against a size standard (orange peaks)
•Variation in size indicates variation in microsatellite
•Double vs. single peak indicates homozygous or heterozygous individual
Molecular basis of Microsatellite (SSR) variation

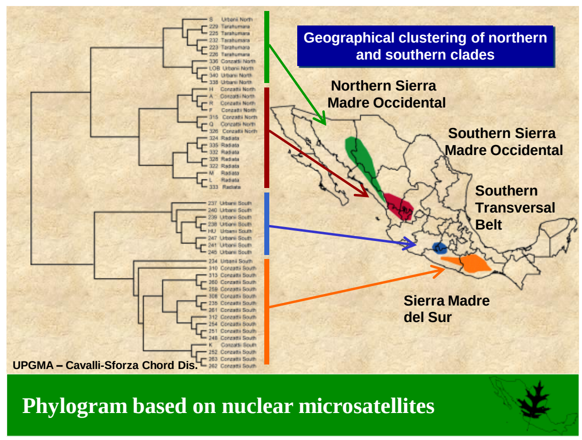

Chloroplast is a haploid genome inherited from the megagametophyte (maternal lineage)
Thus it is passed without recombination between generations
Shows relatively slow rates of change
Its distribution is based solely on seed dispersal
So our data…
Indv. 1: 121; 180; 206; 233; 415; 303; 220
Indv. 2: 121; 180; 206; 233; 415; 303; 222
Haplotype - Combination of alleles which is likely inherited together as a unit
Any difference in allele size results in a unique haplotype
Using microsatellites to infer haplotype/genotype

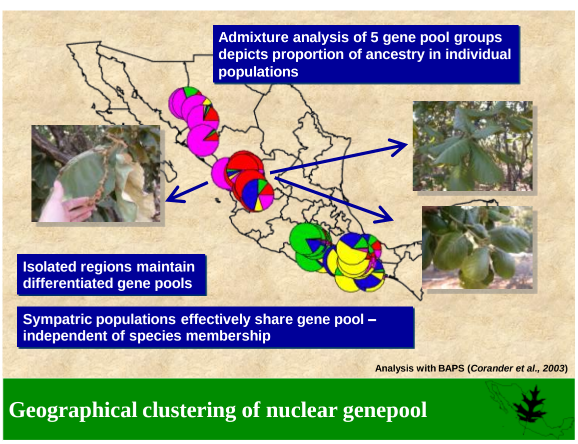

Nuclear is a diploid genome representing a combination of paternal genes
Reshuffling occurs at each generation
Shows high rates of change
Distribution is based on combination of seed and pollen dispersal
So our data…
Indv. 1: 121:123, 170:170, 200:202, 199:201, 304:304, 155:156, 232:236
Similarly this combination is the Genotype
Using microsatellites to infer haplotype/genotype

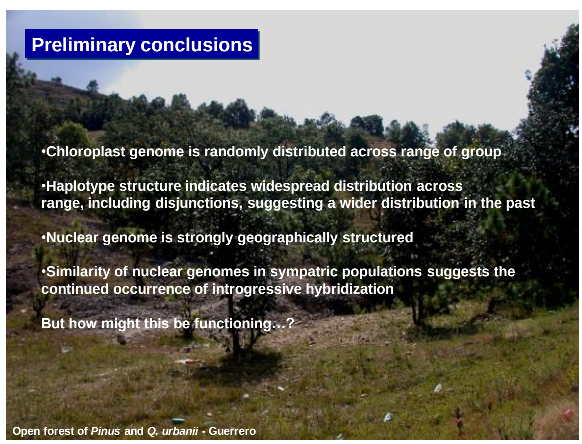

•28 total haplotypes
•No species consists of a single haplotype
•Most haplotypes distributed across multiple species in a “mosaic”
•Haplotypes are shared among all species
Q. conzattii
Q. urbanii
Q. radiata
Q. tarahumara
cp haplotype structure

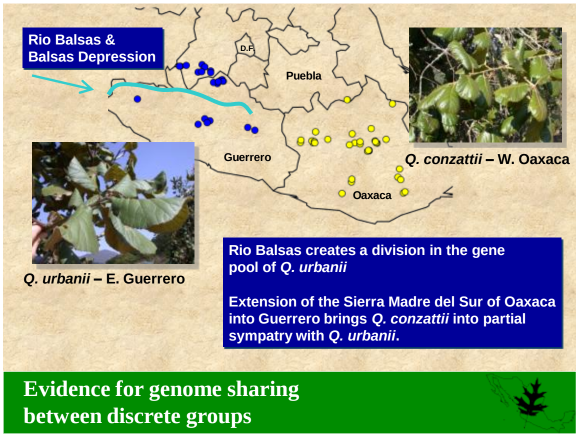

Q. conzattii
Q. urbanii
Q. radiata
Q. tarahumara
Most shared haplotypes distributed in multiple regions
Distribution of cp haplotypes

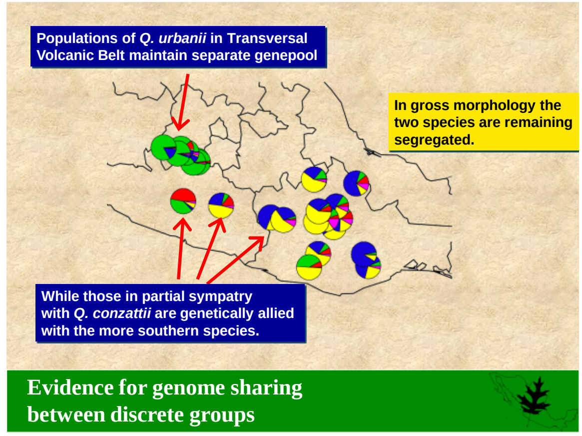

North
South
Clear division into northern and southern regions
Disjunct species (ie. Q. conzattii) do not represent a distinct genetic clade
UPGMA – Cavalli-Sforza Chord Dis.
Phylogram based on nuclear microsatellites

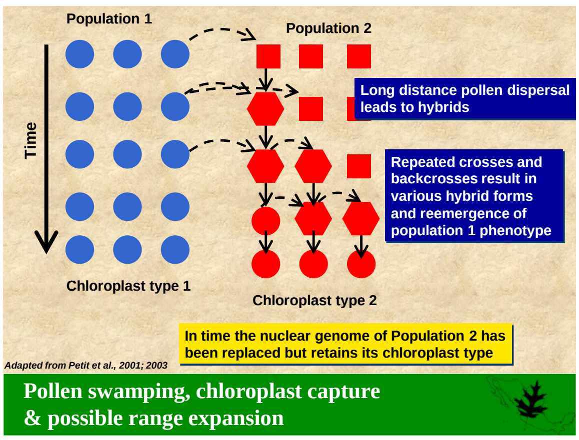

Geographical clustering of northern and southern clades
Northern Sierra Madre Occidental
Southern Sierra Madre Occidental
Southern Transversal Belt
Sierra Madre del Sur
UPGMA – Cavalli-Sforza Chord Dis.
Phylogram based on nuclear microsatellites

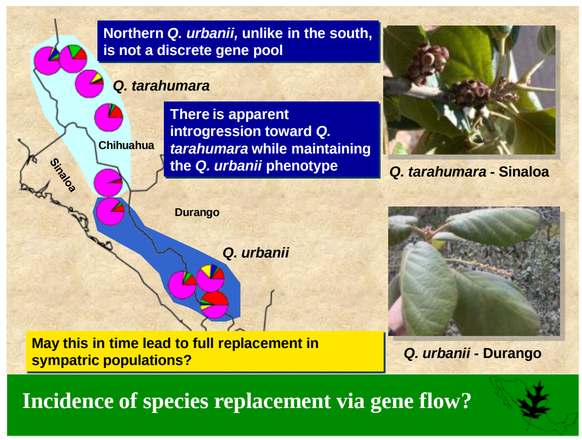

Admixture analysis of 5 gene pool groups depicts proportion of ancestry in individual populations
Isolated regions maintain differentiated gene pools
Sympatric populations effectively share gene pool – independent of species membership
Analysis with BAPS (Corander et al., 2003)
Geographical clustering of nuclear genepool

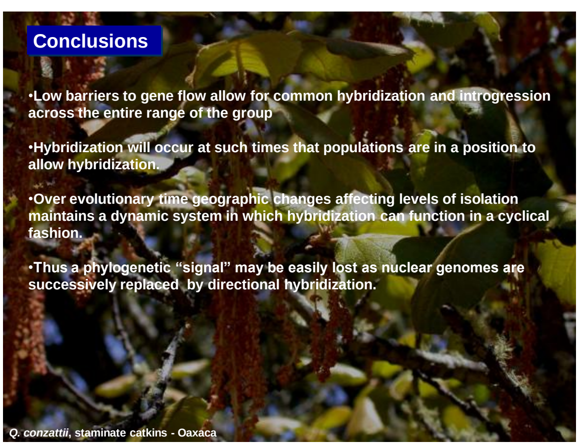

Preliminary conclusions
•Chloroplast genome is randomly distributed across range of group
•Haplotype structure indicates widespread distribution across range, including disjunctions, suggesting a wider distribution in the past
•Nuclear genome is strongly geographically structured
•Similarity of nuclear genomes in sympatric populations suggests the continued occurrence of introgressive hybridization
But how might this be functioning…?
Open forest of Pinus and Q. urbanii - Guerrero


Rio Balsas &
Balsas Depression
D.F.
Puebla
Guerrero
Oaxaca
Q. conzattii – W. Oaxaca
Q. urbanii – E. Guerrero
Rio Balsas creates a division in the gene pool of Q. urbanii
Extension of the Sierra Madre del Sur of Oaxaca into Guerrero brings Q. conzattii into partial sympatry with Q. urbanii.
Evidence for genome sharing
between discrete groups


Populations of Q. urbanii in Transversal Volcanic Belt maintain separate genepool
In gross morphology the two species are remaining segregated.
While those in partial sympatry with Q. conzattii are genetically allied with the more southern species.
Evidence for genome sharing
between discrete groups


Population 1
Population 2
Time
Long distance pollen dispersal leads to hybrids
Repeated crosses and backcrosses result in various hybrid forms and reemergence of population 1 phenotype
Chloroplast type 1
Chloroplast type 2
In time the nuclear genome of Population 2 has been replaced but retains its chloroplast type
Adapted from Petit et al., 2001; 2003
Pollen swamping, chloroplast capture
& possible range expansion


Northern Q. urbanii, unlike in the south, is not a discrete gene pool
Q. tarahumara
There is apparent introgression toward Q. tarahumara while maintaining the Q. urbanii phenotype
Chihuahua
Sinaloa
Durango
Q. urbanii
Q. tarahumara - Sinaloa
Q. urbanii - Durango
May this in time lead to full replacement in sympatric populations?
Incidence of species replacement via gene flow?


Conclusions
•Low barriers to gene flow allow for common hybridization and introgression across the entire range of the group
•Hybridization will occur at such times that populations are in a position to allow hybridization.
•Over evolutionary time geographic changes affecting levels of isolation maintains a dynamic system in which hybridization can function in a cyclical fashion.
•Thus a phylogenetic "signal" may be easily lost as nuclear genomes are successively replaced by directional hybridization.
Q. conzattii, staminate catkins - Oaxaca


Acknowledgments
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DGAPA-PAPIIT, UNAM
•Postdoctoral Funding
DGAPA, UNAM
Q. urbanii
Sierra de Nanchititla, Estado de México