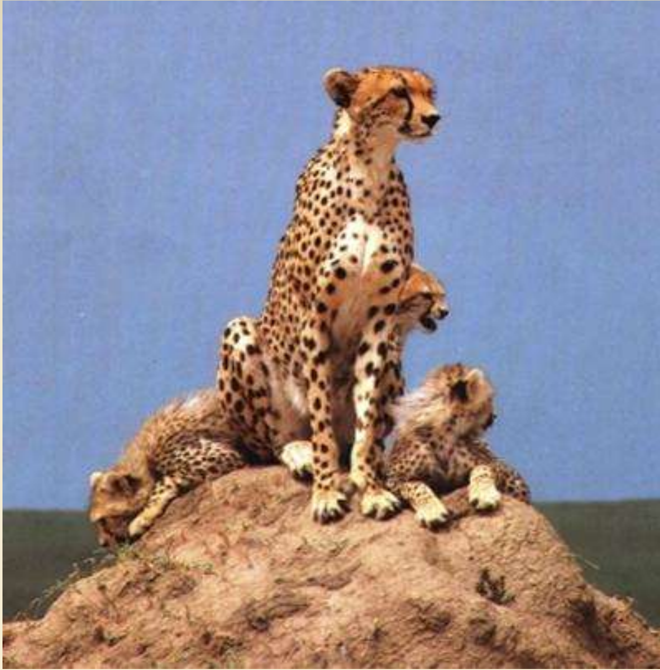
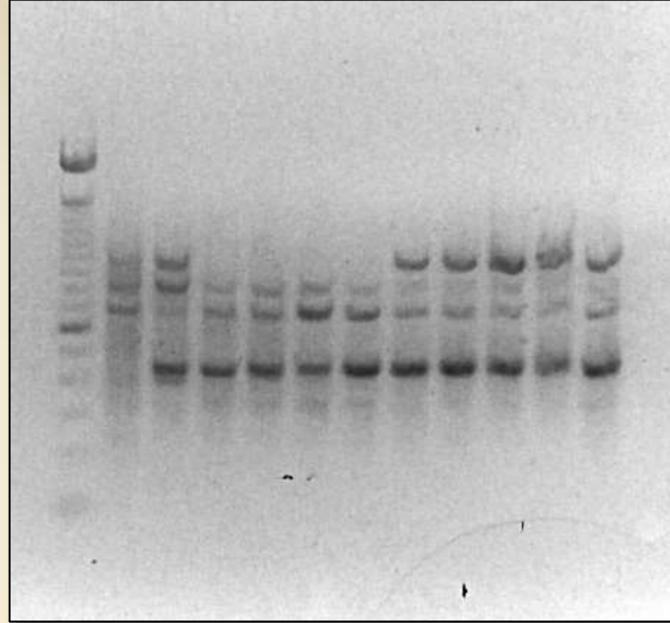
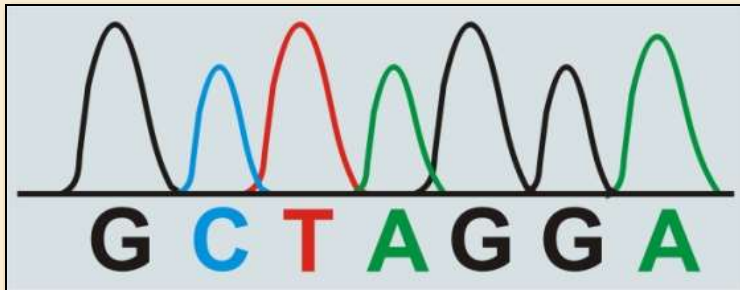
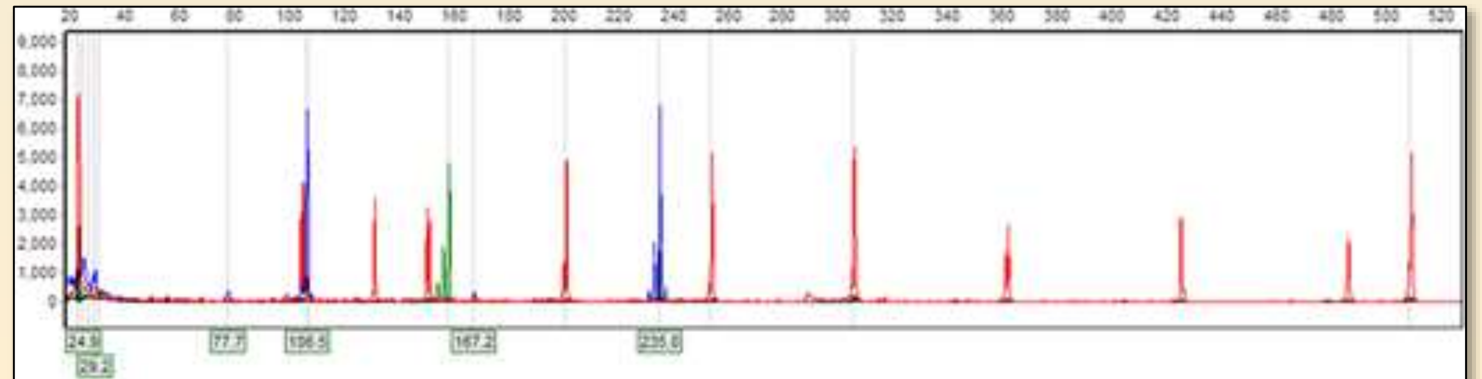
Conservation Biology and Genetics





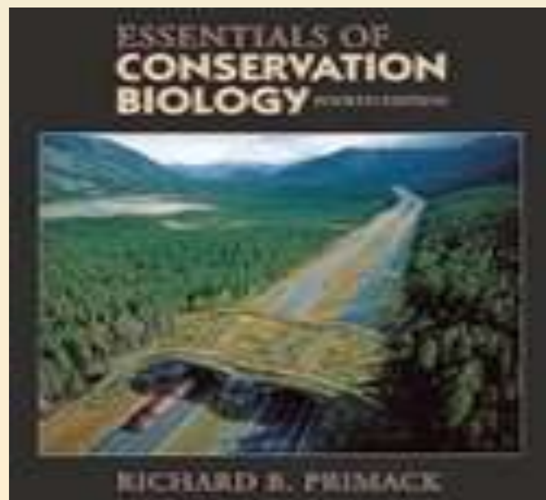


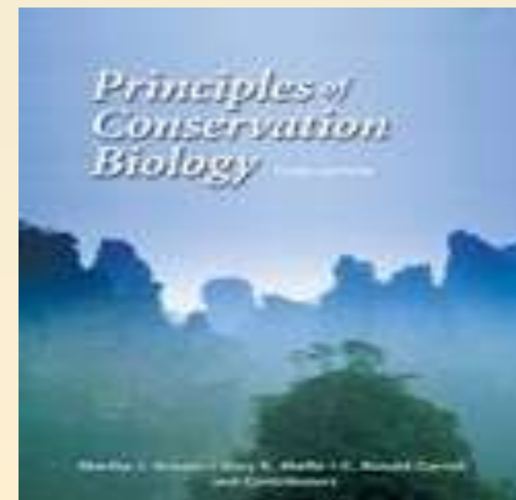


What is Conservation Biology?

Primack (2006): Conservation Biology “carries out research on biological diversity, identifies threats to biological diversity, and plays an active role in the preservation of biological diversity”

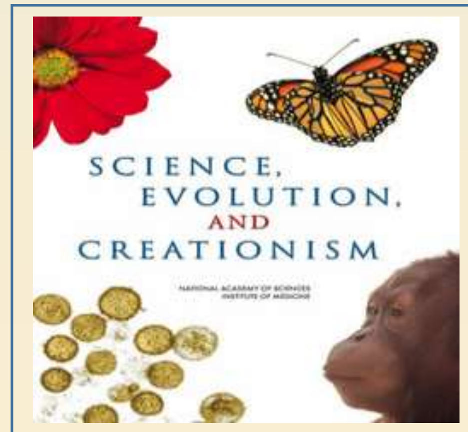
Groom et al. (2006): “An integrative approach to the protection and management of biodiversity…”





Conservation Biology is grounded in **Science**

"The use of evidence to construct testable explanations and predictions of natural phenomena, as well as the knowledge generated through this process"



Definition of "Science" extracted from *Science, Evolution & Creationism* (2008) – published by (and freely available through) the National Academy of Sciences and Institute of Medicine of the U. S. National Academies

Conservation Biology draws from many disciplines

For ethical, practical & theoretical considerations

Biology
- Biogeography
- Genetics
- Ecology *
- Evolution
- Fisheries Science
- Forestry
- Physiology
- Wildlife Biology

Anthropology

Chemistry

Economics

History

Philosophy

Physics

Political Science

Religion

Sociology

Etc.

* “*We should not conflate ecology with environmentalism…*”
(Kingsland, 2005, *The Evolution of American Ecology: 1890-2000*, pg. 4)

Conservation Biology Central Issue:

Loss of habitat to agriculture, forestry, and urbanization.

Underlying cause is increase in human population, expected to reach 8-12 billion this century. Most of this growth will be in the tropics where most of the biological diversity is.

Not much can be done about it really. Politics, corruption.

The only effective solution is establishment of large reserves, try to save remnant ecosystems and species.

Even in the developed countries there are many problems with loss of habitat and diversity.

Fragmentation of the habitat disrupts movement, reduces effective population.

Human Population

Billions of People

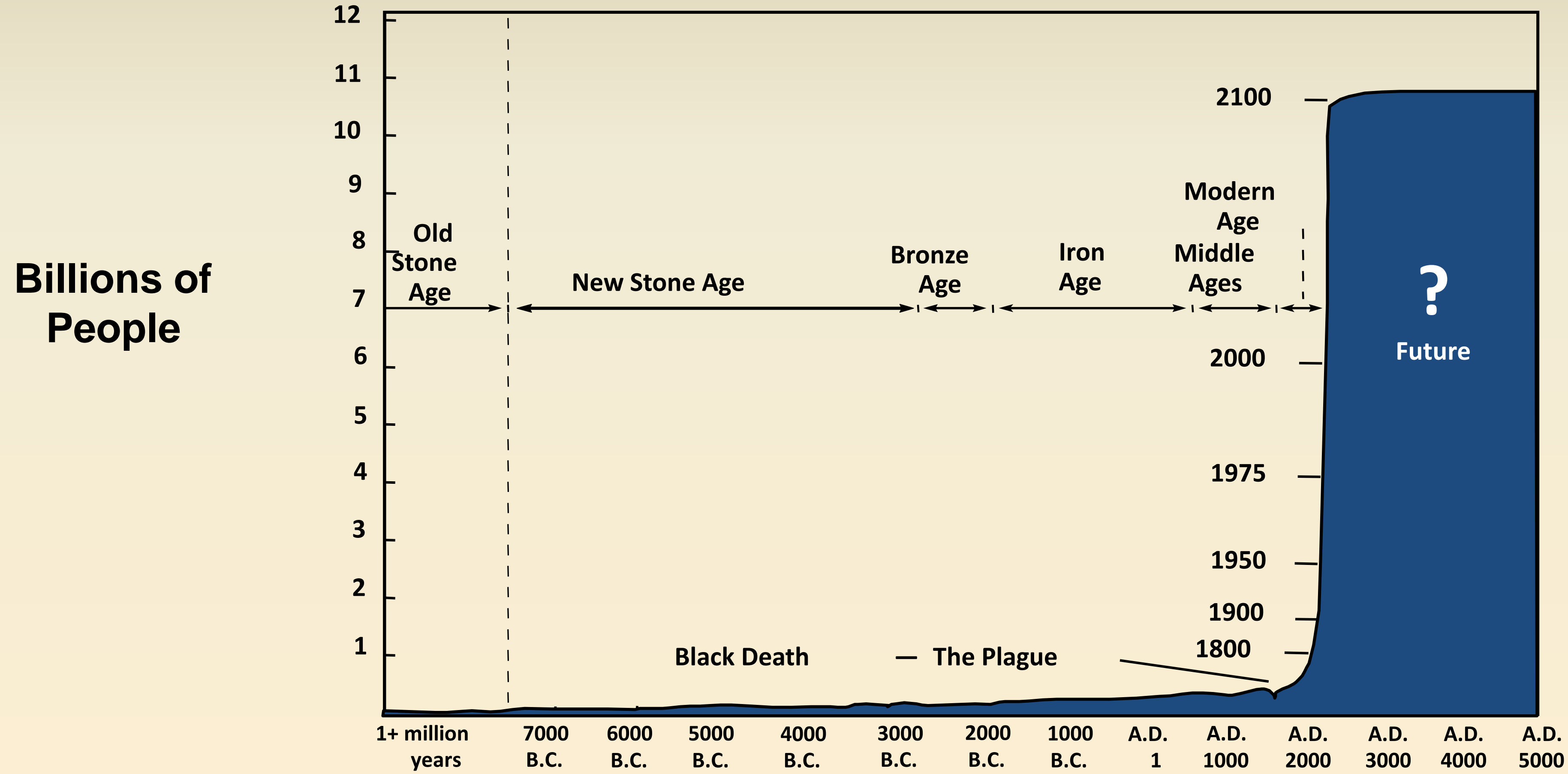


Image from the Population Reference Bureau © 2006

Wilderness, what we started out with …High diversity, genetically rich

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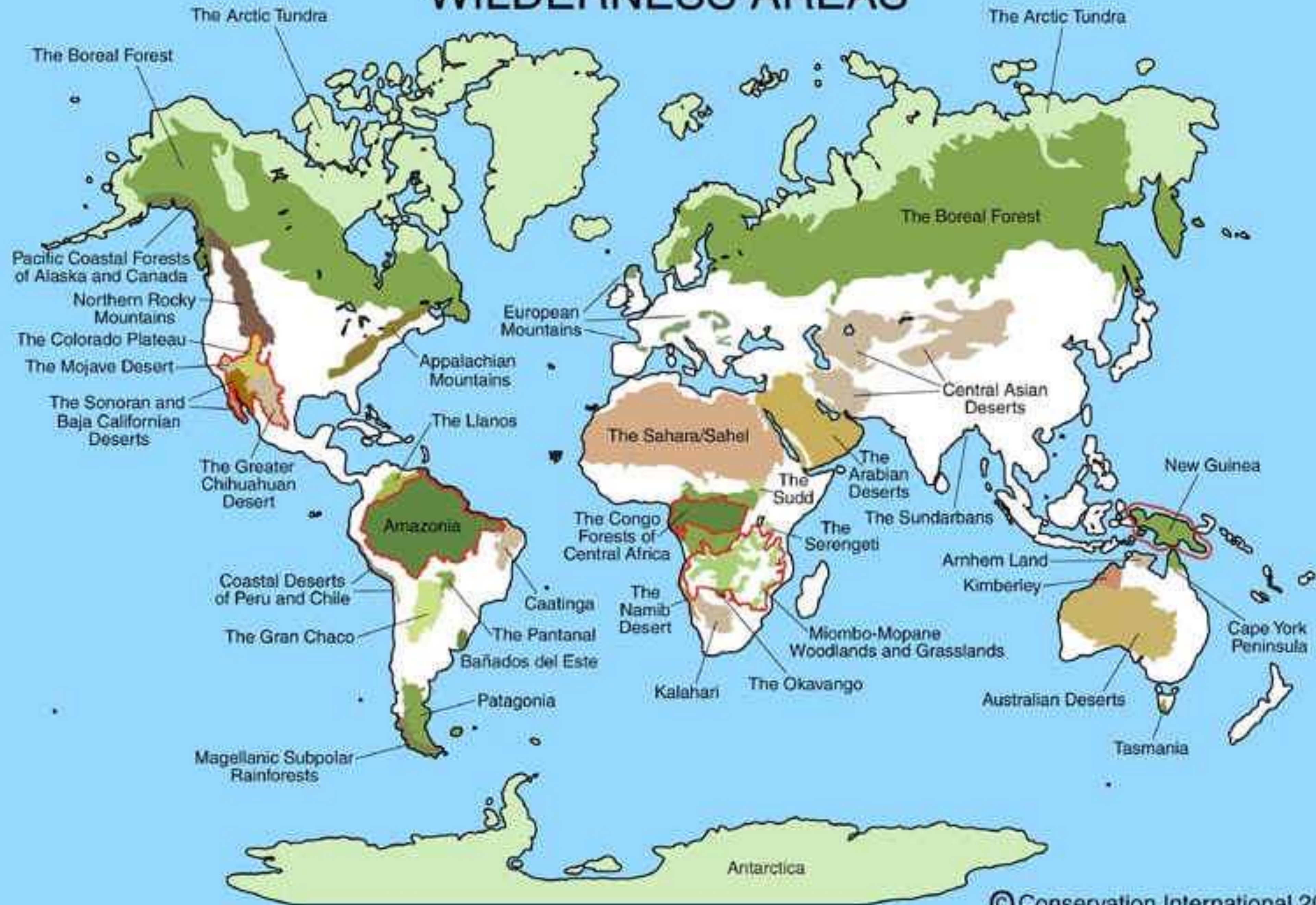
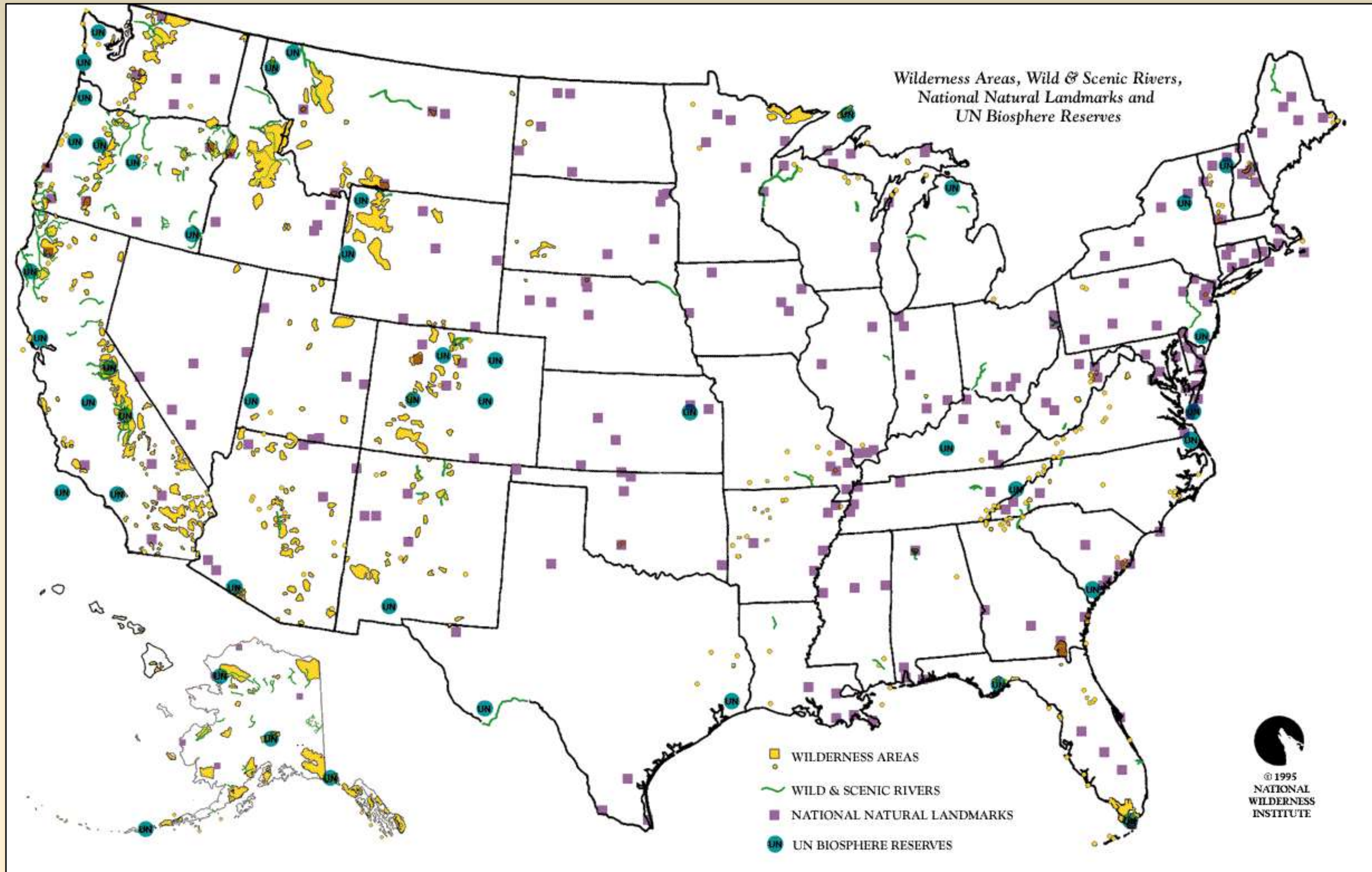
Fragmented landscapes, what we have now…. Lower diversity, genetically poorer

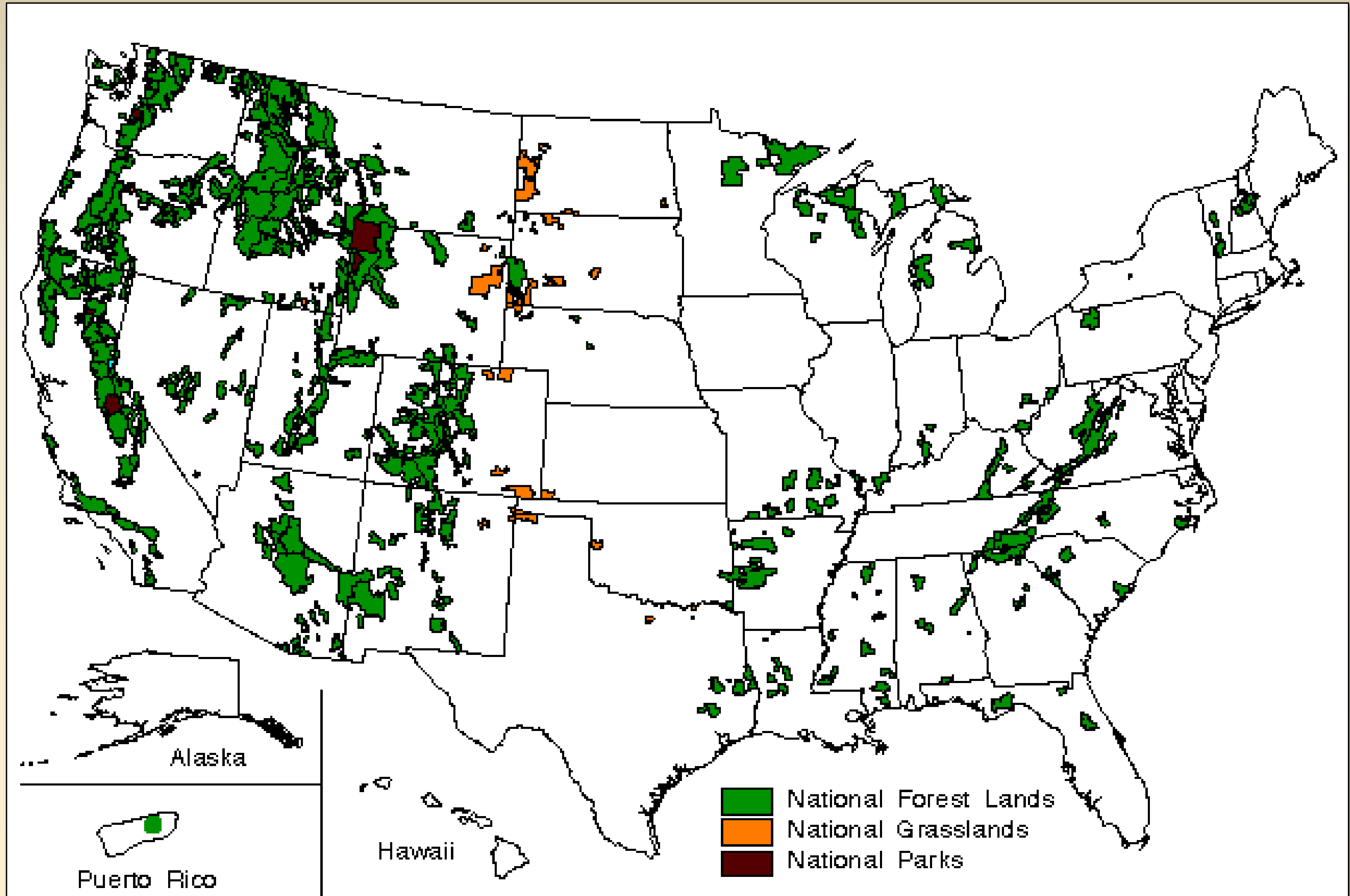

WILDERNESS AREAS
The Arctic Tundra
The Boreal Forest
Pacific Coastal Forests of Alaska and Canada
Northern Rocky Mountains
The Colorado Plateau
The Mojave Desert
The Sonoran and Baja Californian Deserts
The Greater Chihuahuan Desert
Appalachian Mountains
The Llanos
Amazonia
Coastal Deserts of Peru and Chile
The Gran Chaco
Caatinga
The Pantanal
Bañados del Este
Patagonia
Magellanic Subpolar Rainforests
European Mountains
The Sahara/Sahel
The Sudd
The Congo Forests of Central Africa
The Serengeti
The Namib Desert
Miombo-Mopane Woodlands and Grasslands
Kalahari
The Okavango
The Arabian Deserts
Central Asian Deserts
The Sundarbans
New Guinea
Arnhem Land
Kimberley
Cape York Peninsula
Australian Deserts
Tasmania
Antarctica
© Conservation International 2002

Wilderness Areas, Biosphere Reserves, Scenic Riverways

(610 Biosphere Reserves spanning 117 countries)

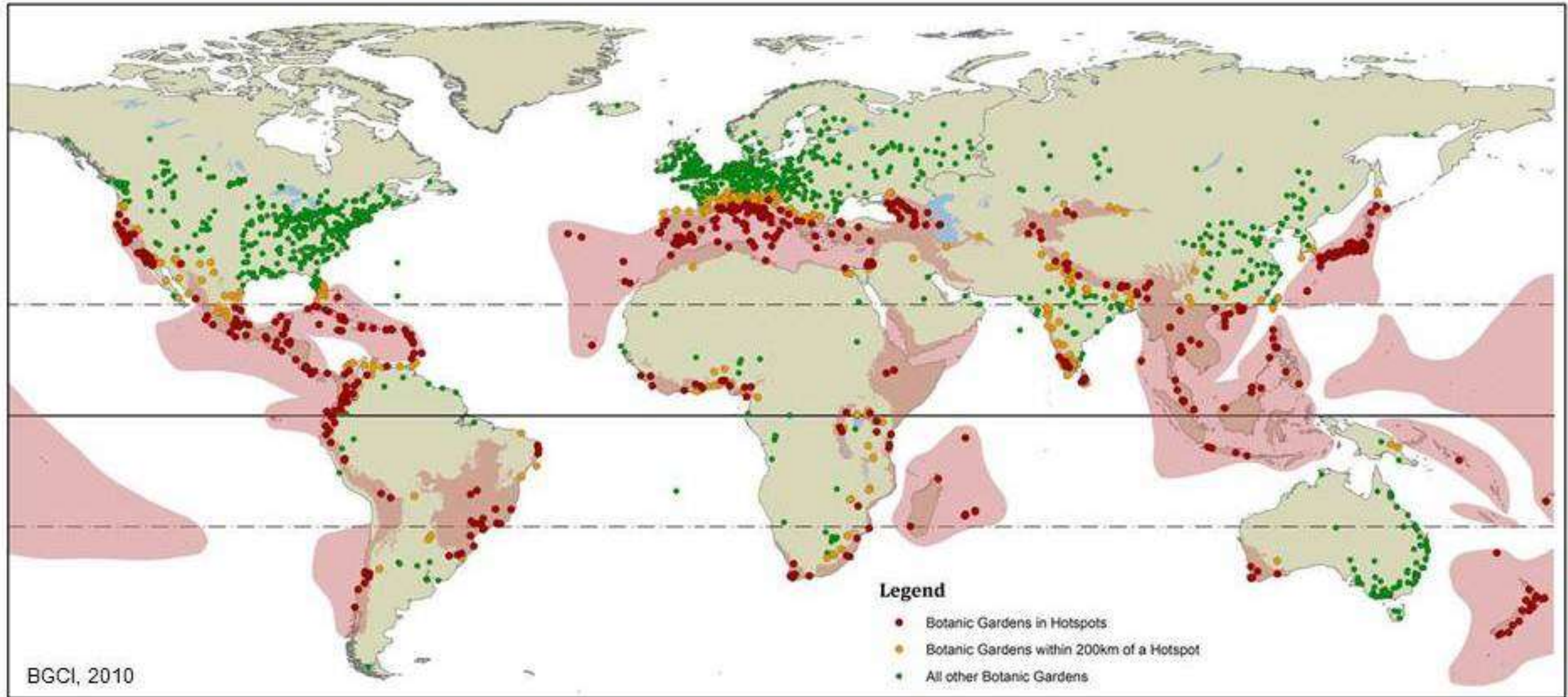


National Forests, National Grasslands, and National Parks



Unesco Biosphere Reserves

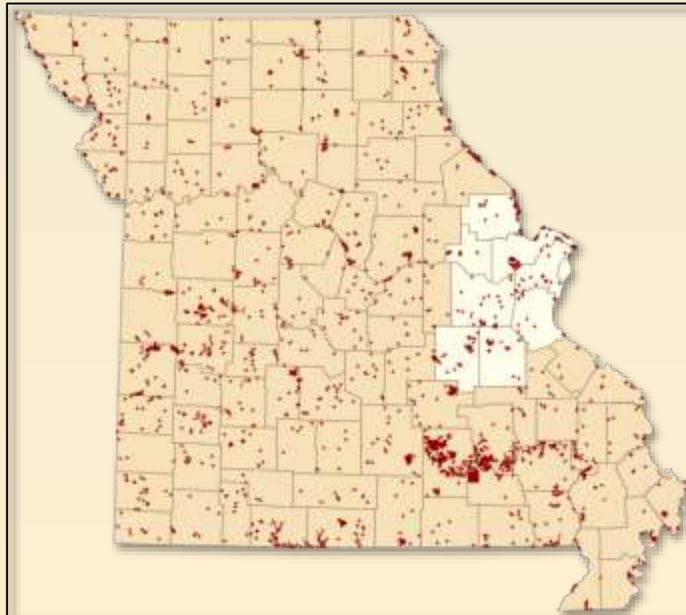
Botanic gardens and biodiversity hotspots



Missouri Natural Features Inventory - MDC/TNC

Missouri National Forest, Wilderness Areas

Missouri Dept. Conservation Lands



Mo-Ka Prairie

Fragmentation and Edge Effects = loss of diversity

interior species
edge species
fragmentation
interior habitat
edge habitat
interior habitat and species decrease
edge habitat and species increase

Genetic consequences of Habitat Loss and Fragmentation

1. Smaller populations.
2. Barriers to gene flow.
3. Loss of allelic diversity through genetic drift.
4. Increase in homozygosity through forced inbreeding, creates genetic problems.
5. Reduced ability to respond to selection.

Genetic diversity is generally considered healthy.

Solutions:

Protection in reserves. Probably the best solution, but often decisions have to be made about which populations to protect. Can't protect all of them. Protect the ones with the greatest diversity? The biggest populations? SLOSS debate - single large or several small reserves. Problem of connecting up reserves to enable gene flow.

Reintroduction. Plants or animals can be taken into captivity or gardens, reproduce, eventually reintroduce back into the wild. Seedbanks. Must be careful about reintroducing genotypes that are adapted to the local conditions. Avoid reintroducing progeny of just a few parents, introducing an instant "bottleneck".

Ex situ preservation. Protect in gardens and zoos. Important, but not the best long term solution. Growing sense in botanical gardens and zoos about maintaining genetic variation. MBC populations of palms and cycads.

General agreement that information about genetic variation, breeding systems is very important in conservation biology.

Conservation Biology - Preserve Design

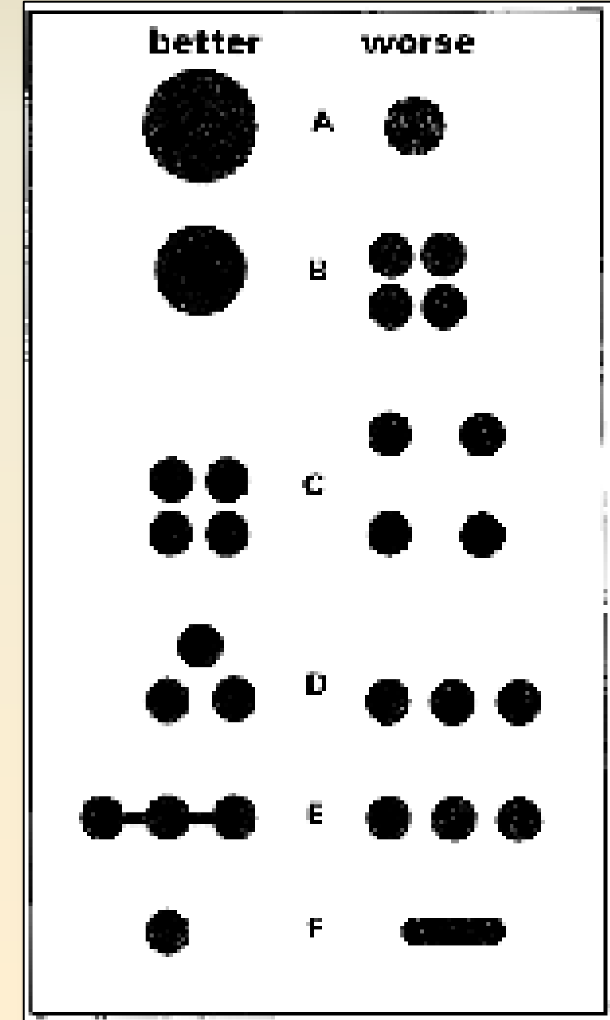
UNESCO Biosphere Reserves

Structure of a model biosphere reserve.

Core Area
Buffer Area
Transition Area
Human Settlement
R Research
E Education / Training
T Tourism / Recreation

SLOSS

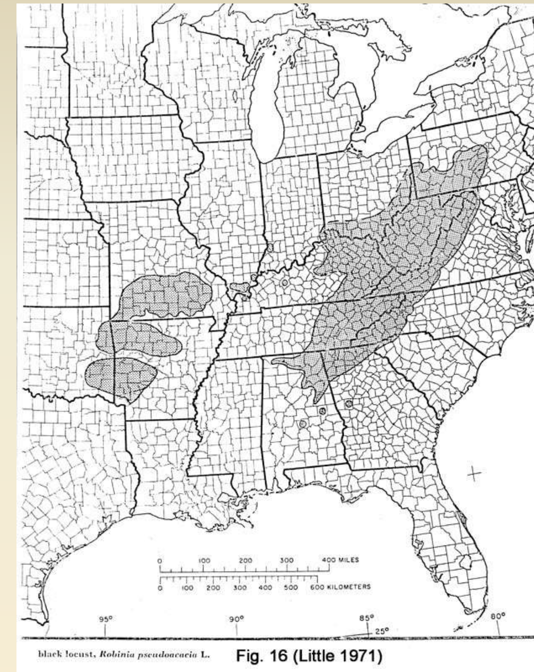
Single Large Or Several Small



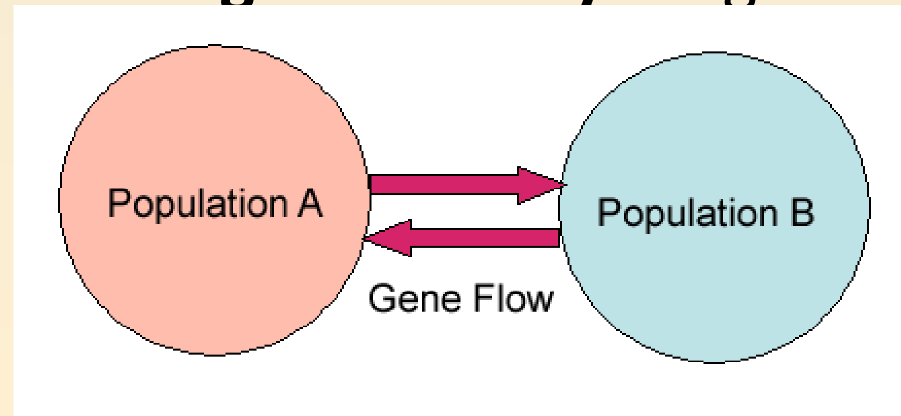
Conservation Genetics and Populations

Geocarpon minimum

Robinia pseudoacacia

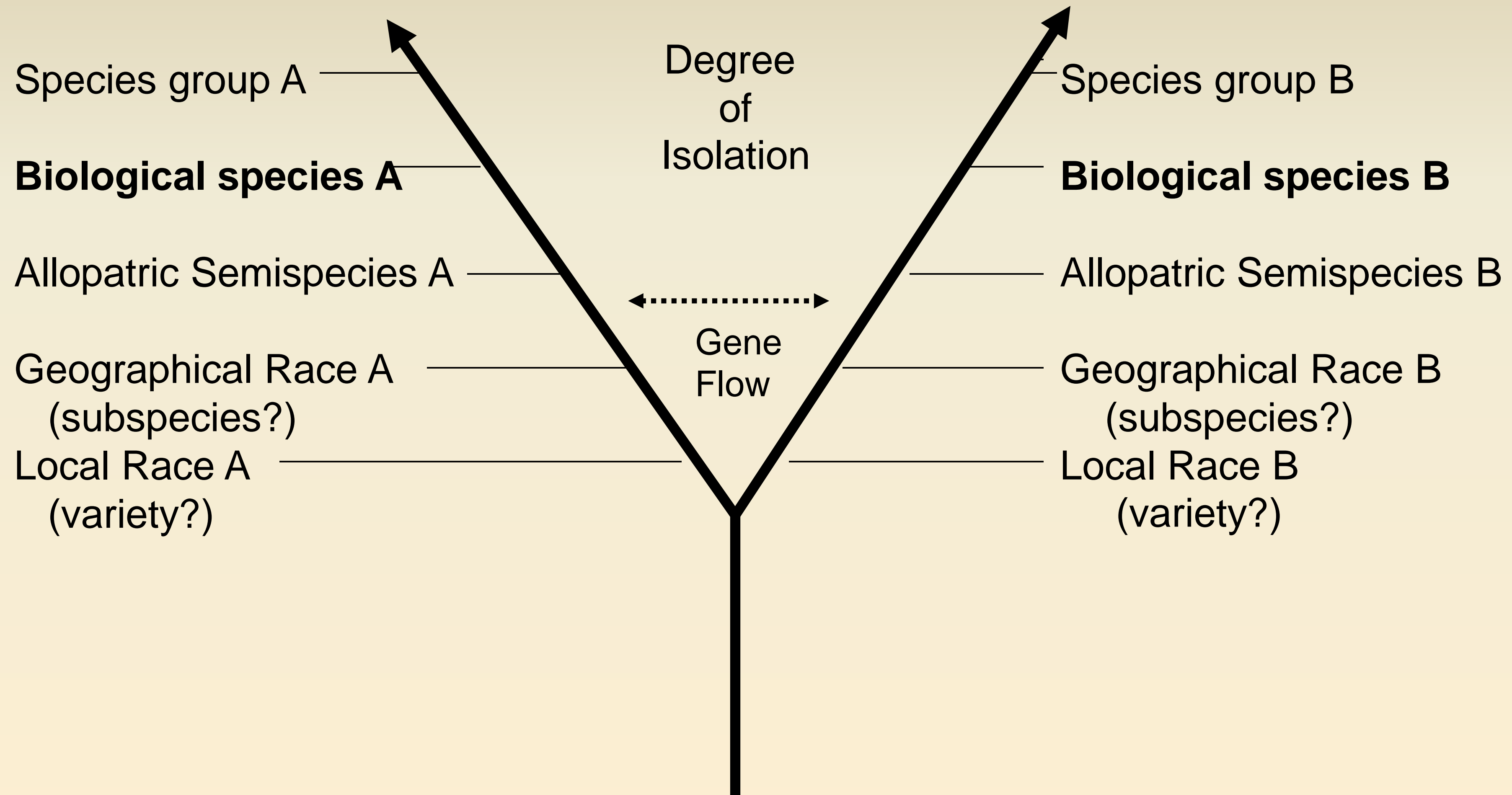


How did the distribution get that way? Is gene flow interrupted?



Stages in Divergence Leading to Biological Species

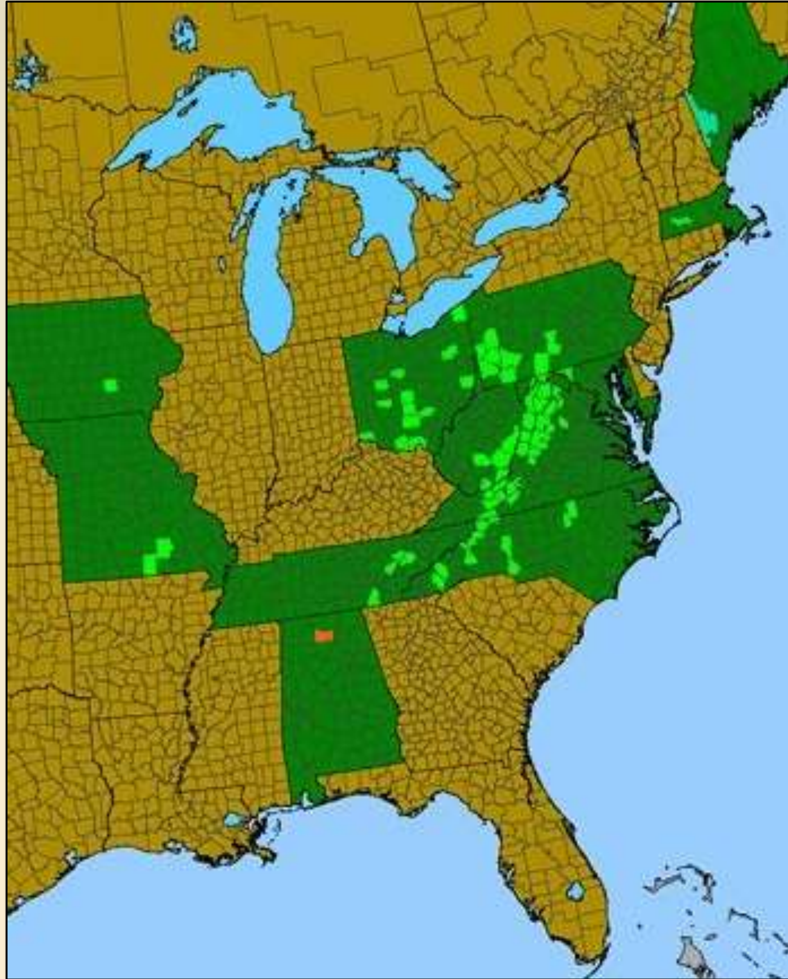
from V. Grant, 1981



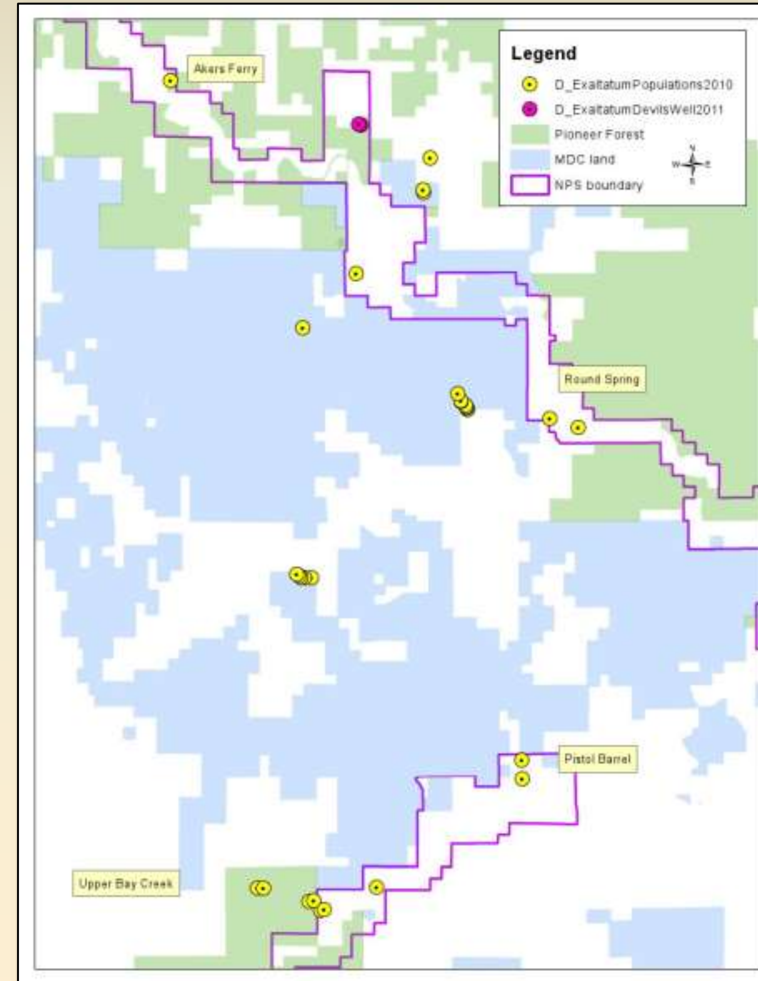
Conservation Genetics and Populations

Delphinium exaltatum

U.S. Distribution



Shannon Co., Missouri



Scale, Populations and Metapopulations

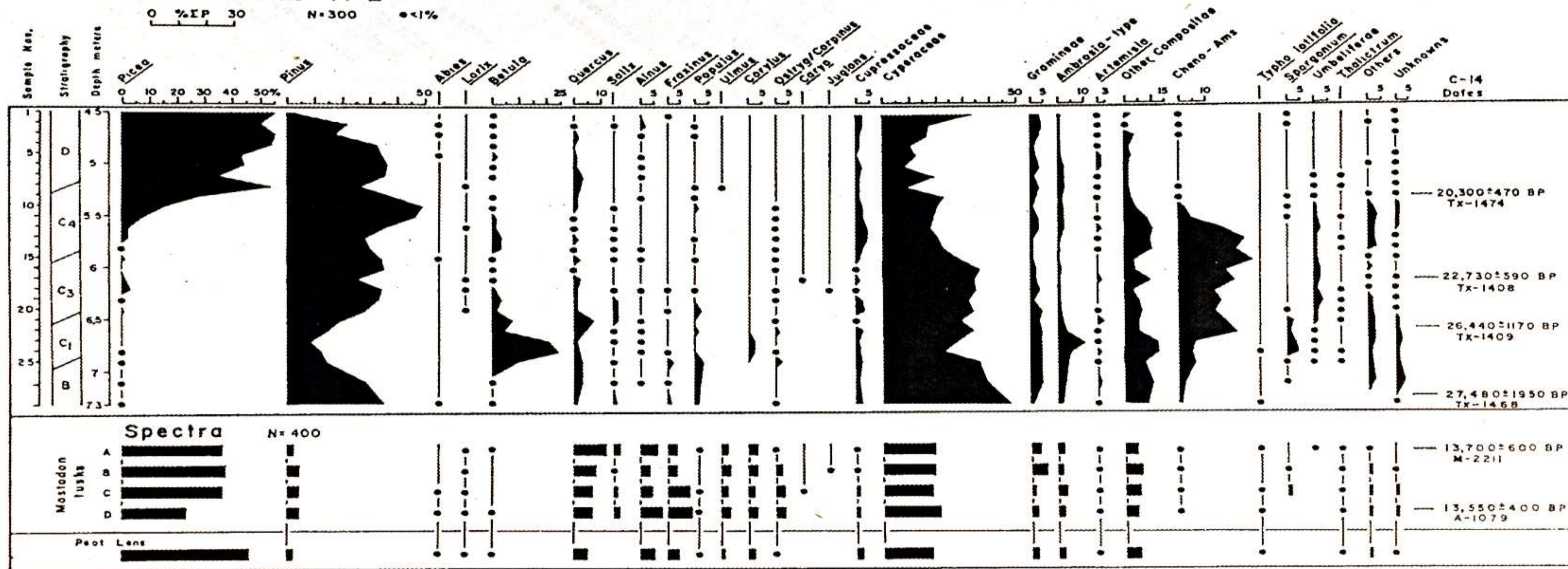


FIG. 16. Boney Spring pollen profile 71-I and miscellaneous pollen spectra. Only those radiocarbon dates associated with the profile are shown; the others are listed in Fig. 6 and 7. Other taxa include (sample 2) Polemoniaceae; (6) Polygonaceae; (9) Ranunculaceae; (10) Malvaceae, Onagraceae; (11) 3% Ranunculaceae; (12) *Myriophyllum*, Portulacaceae; (13) Liliaceae, Ranunculaceae, *Ribes*, Rosaceae; (14) *Myriophyllum*, Polygonaceae, *Potamogeton*, Ranunculaceae; (16) Leguminosae, Polygonaceae, Rosaceae; (17) Polygonaceae, Rosaceae; (18) Rosaceae; (19) Rosaceae; (20) *Potamogeton*, Rosaceae; (21) Leguminosae, Polygonaceae;

Missouri Pollen Cores

40,000 BP – non-arboreal, Cyperaceae, Pinus – open pine parkland
25,000 BP – full glacial, pollen shifts to Picea (spruce)
18,000 BP – retreat of glaciers, shift to oak, maple, willow, ash, elm, sedges and grasses
9,000 BP – oak-hickory forest
8,000 - 4,000 BP – Xerothermic, higher tempeatures, much open prairie
600-120 BP (1400-1880 AD) - Little Ice Age, wetter, cooler
Recent - oak-hickory again became dominant in the Ozarks

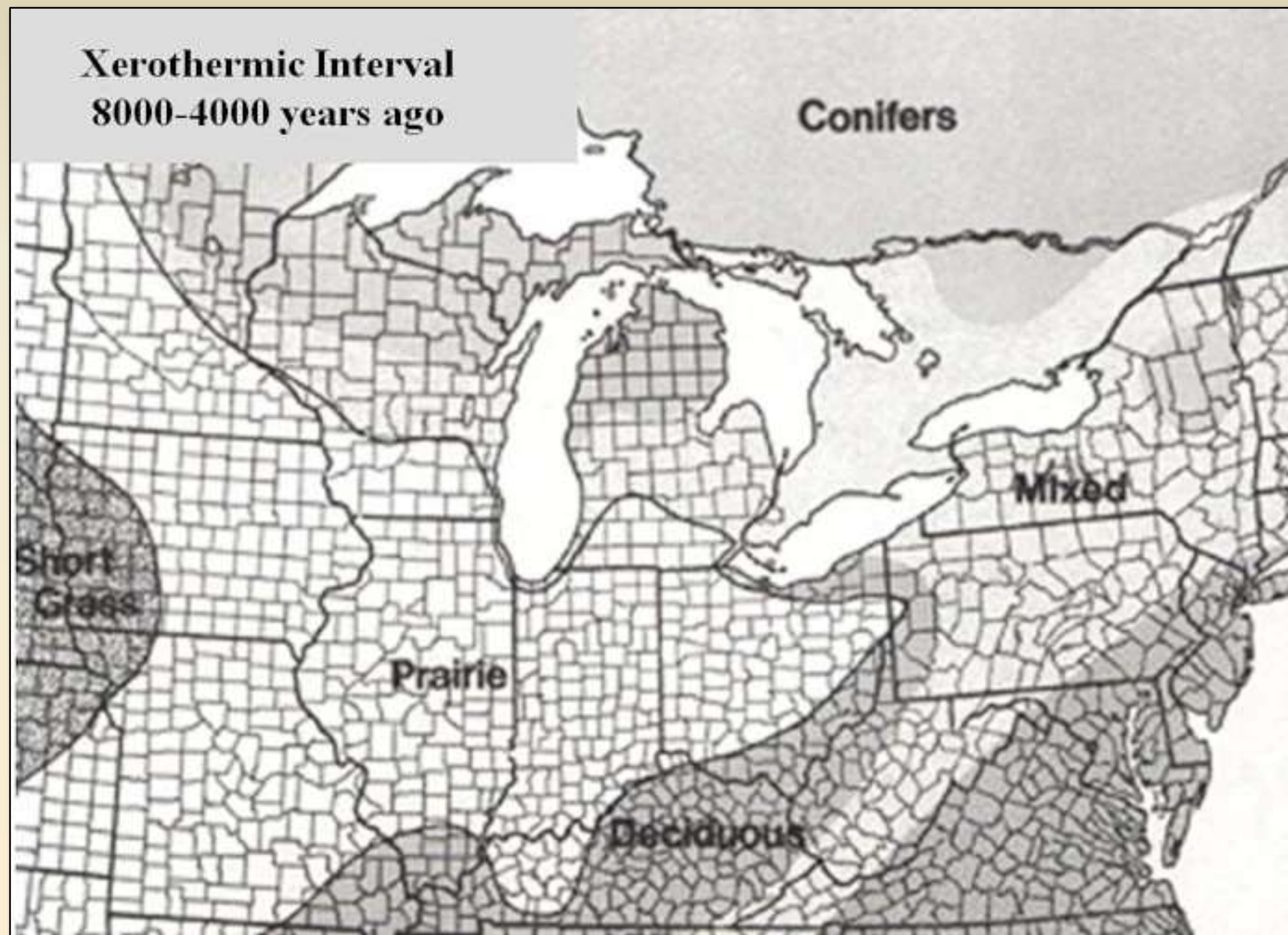
Pleistocene Glaciation Missouri

Pleistocene Relicts in the Ozarks?

Campanula rotundifolia

Trautvetteria caroliniensis

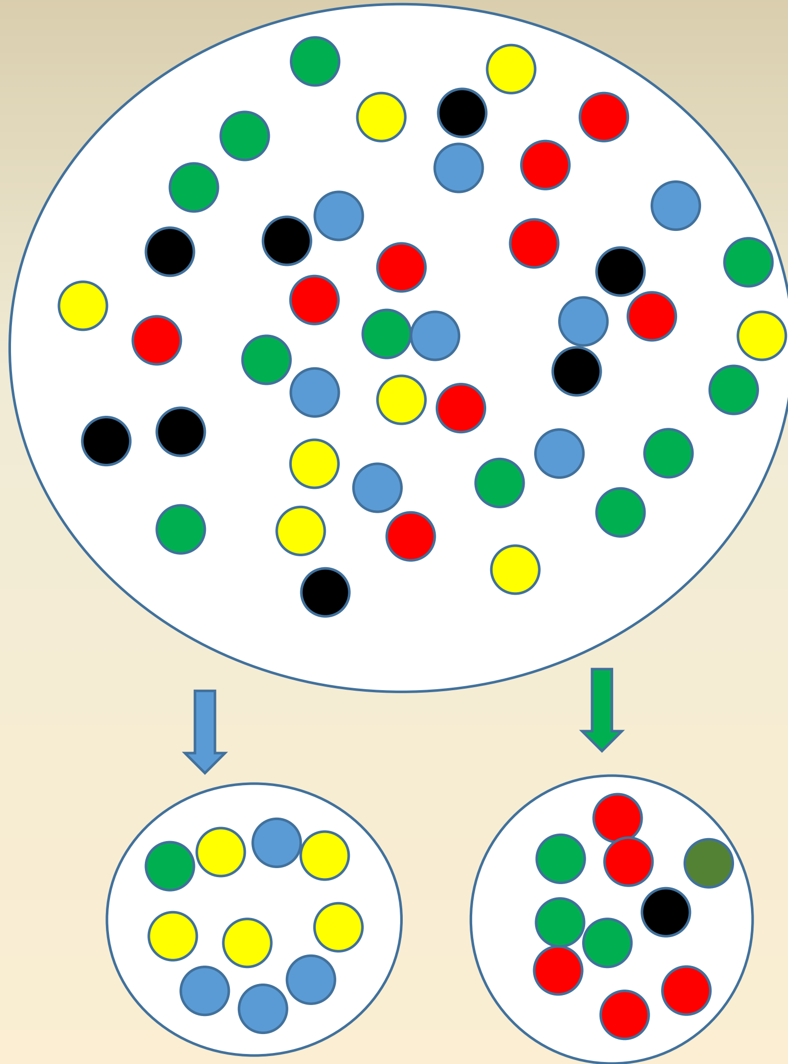
Prairie Peninsula During the Xerothermic

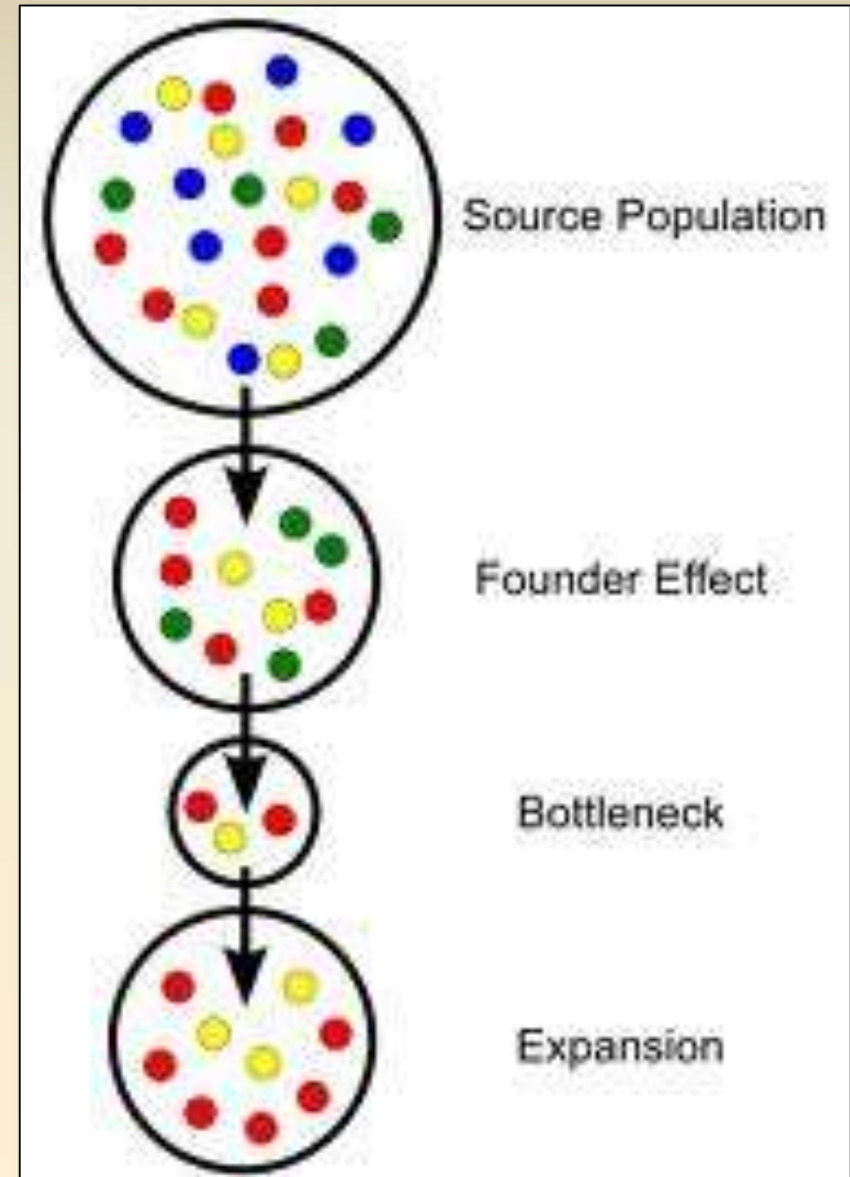


Transeau (Stucky, 1981)

Missouri Glades, Prairies, Savannas

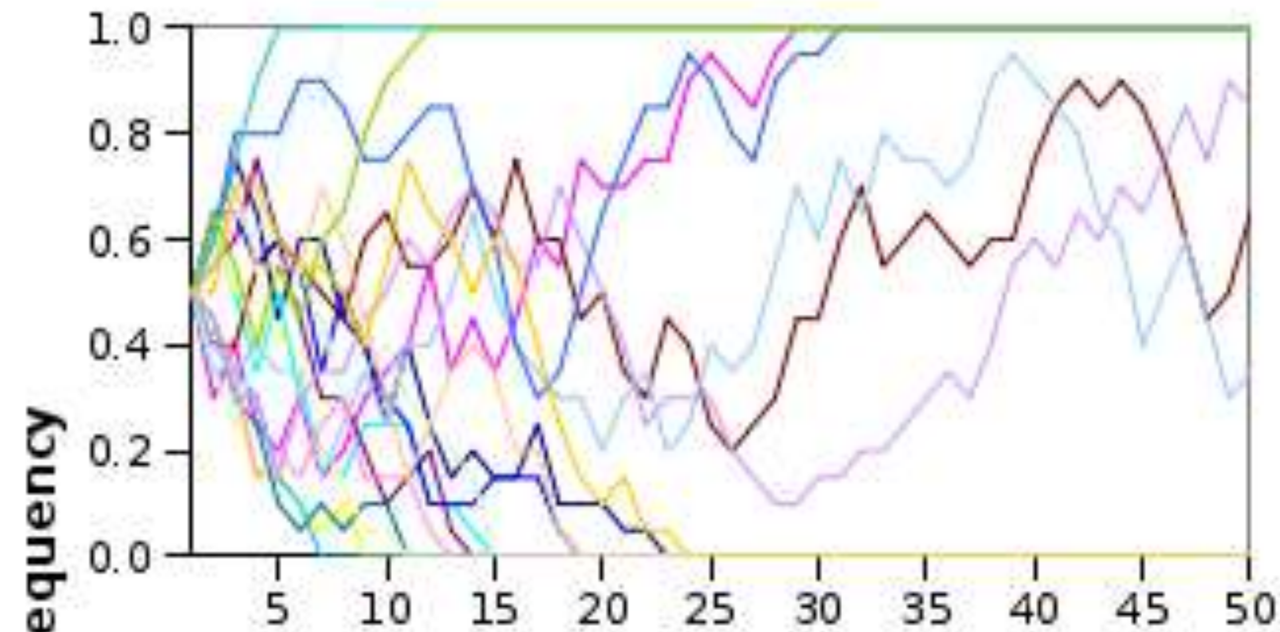
Effect of Population Size on Genetic Variation





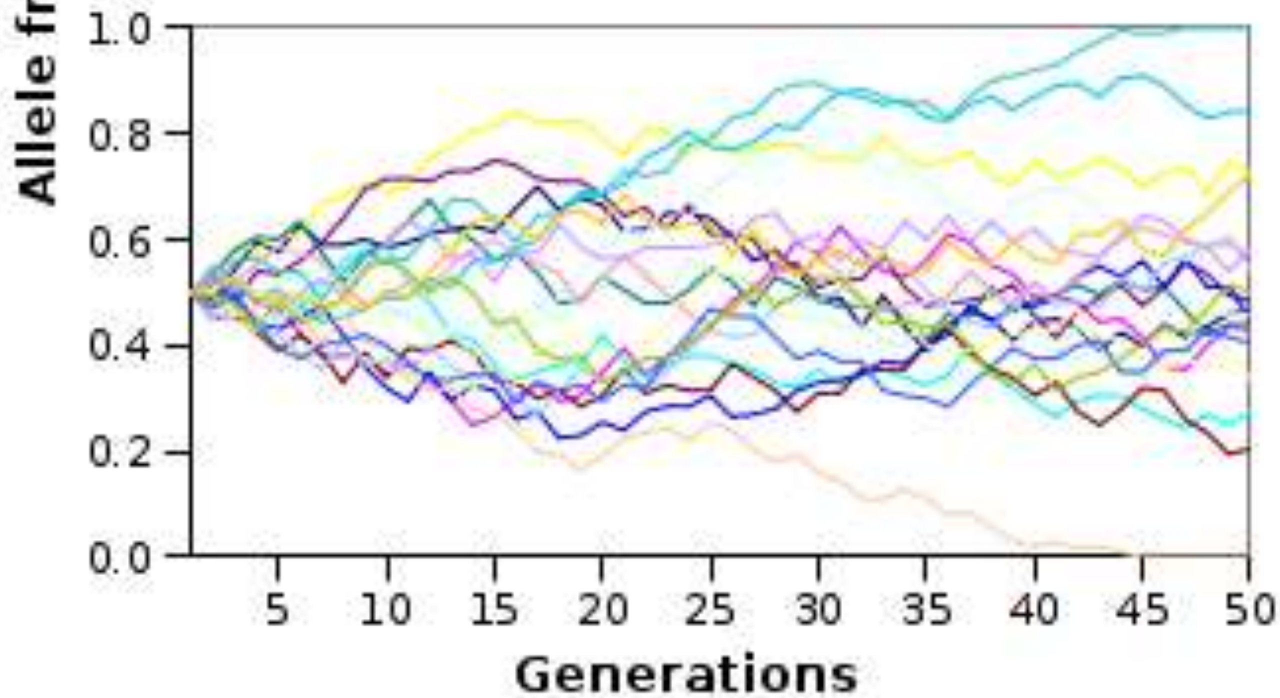
Genetic Drift, Mutation, Migration, Inbreeding
Loss of genetic variation

Population Size and Drift



N = 10

drift to fixation faster, loss of alleles



N = 100

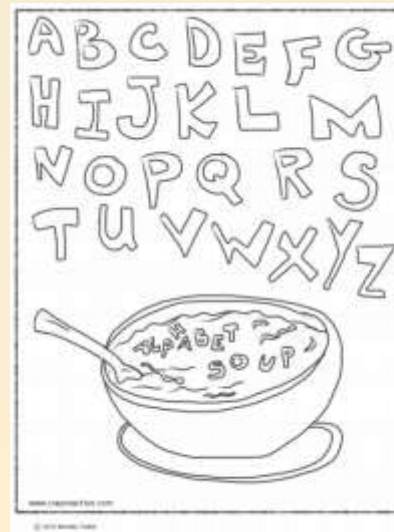
General Conservation Genetics Questions

1. What patterns of variation are present in the populations?
2. How do landscape features and distance impact population structure and migration?
3. How has habitat fragmentation influenced this variation?
4. How are the populations related to each other?
5. How much gene flow occurs between near and distant population?
6. Are widely disjunct populations sufficiently differentiated to be considered separate species or subspecies?
7. Did the population structure or connectivity change in the recent past?
8. Have small populations become genetically differentiated due to drift, inbreeding, and or selection?
9. What kind of management will decrease, increase, or maintain levels of genetic variation?

Selected Population Genetic Markers

Sequences – DNA coding and non-coding regions
Allozymes – different forms of proteins (enzymes)
RAPD – Random Amplified Polymorphic DNA
ISSR – Inter Simple Sequence Repeat
AFLP – Amplified Fragment Length Polymorphism
SSR – Simple Sequence Repeats
SNP – Single Nucleotide Polymorphism

Considerations
Cost
Time
Reproducibility
Genetic relatedness
Information needed

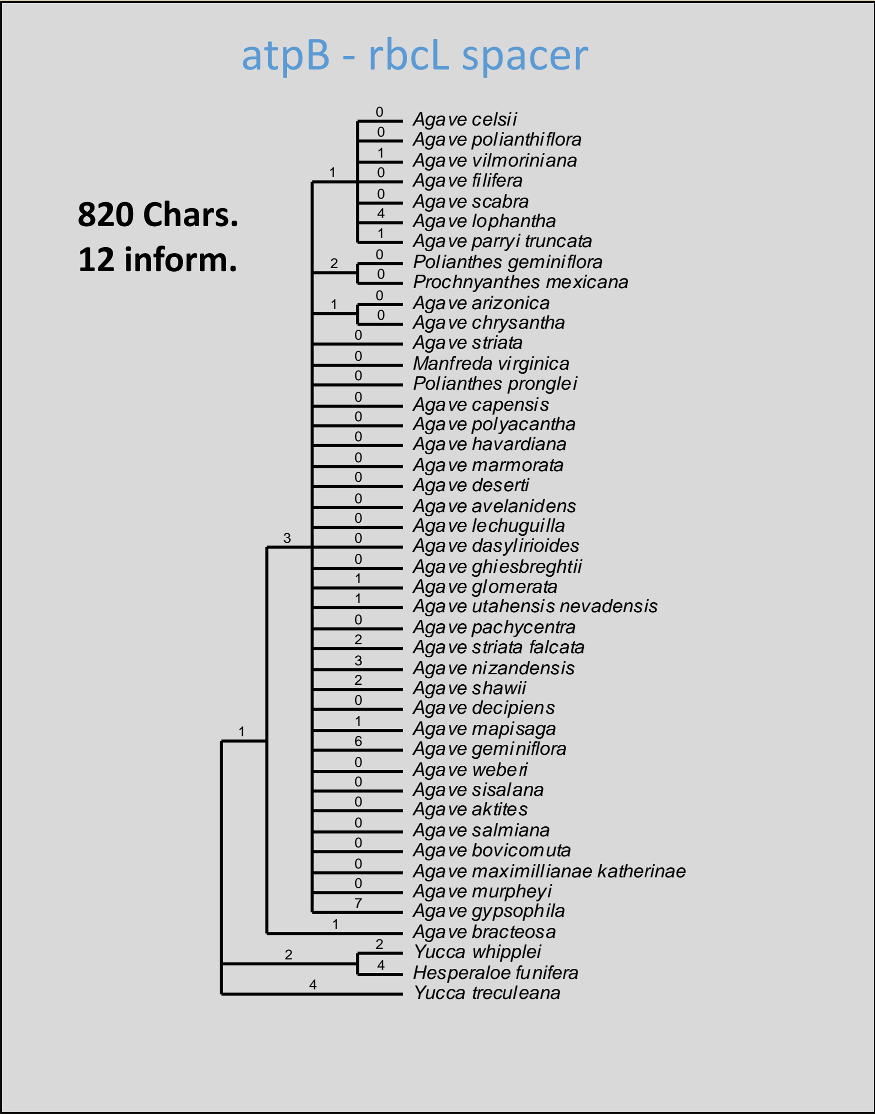


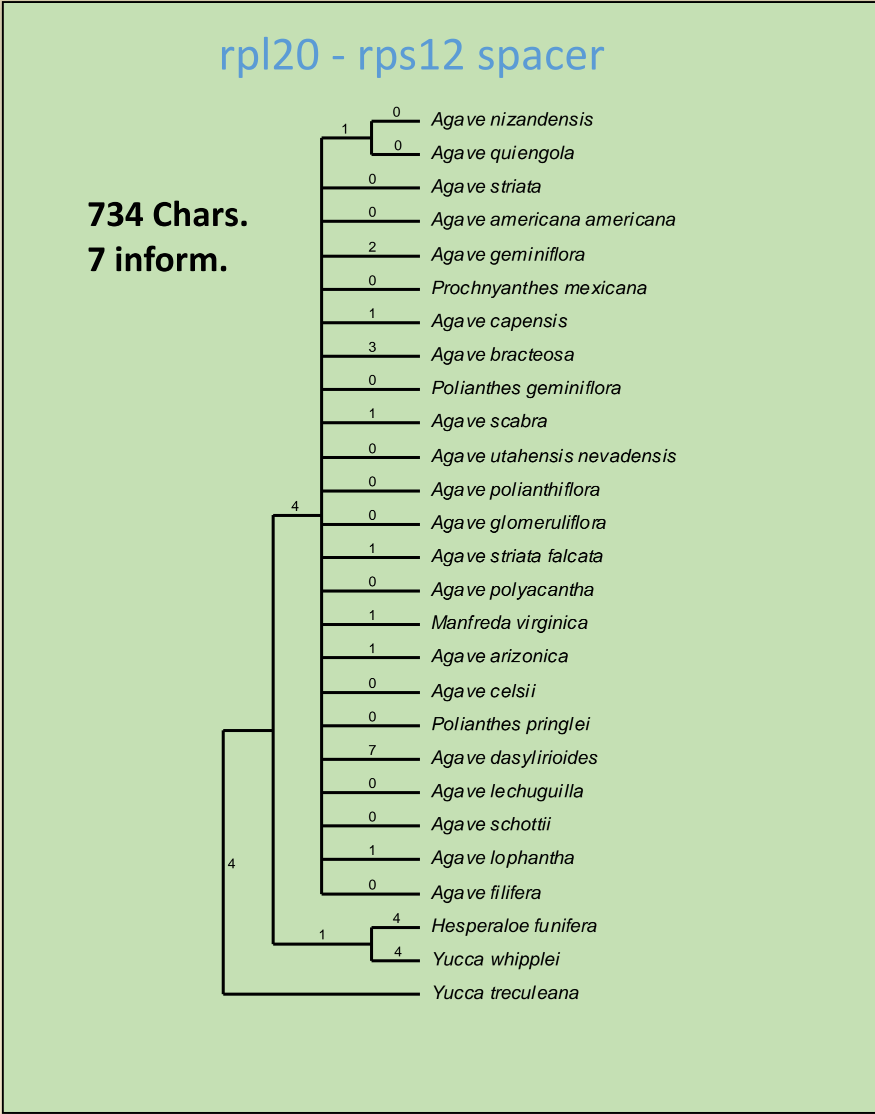
The Perfect Genetic Marker:

1. Highly polymorphic.
2. Co-dominant - allows us to discriminate homo- and heterozygotic states in diploid organisms.
3. Frequent occurrence in the genome.
4. Even distribution throughout the organism.
5. Selectively neutral behavior.
6. Easily accessible - fast procedures, kits, common reagents.
7. Easy and fast assay - amenable to automation.
8. High reproducibility.
9. Easy exchange of data between laboratories.

No marker has all these characteristics.

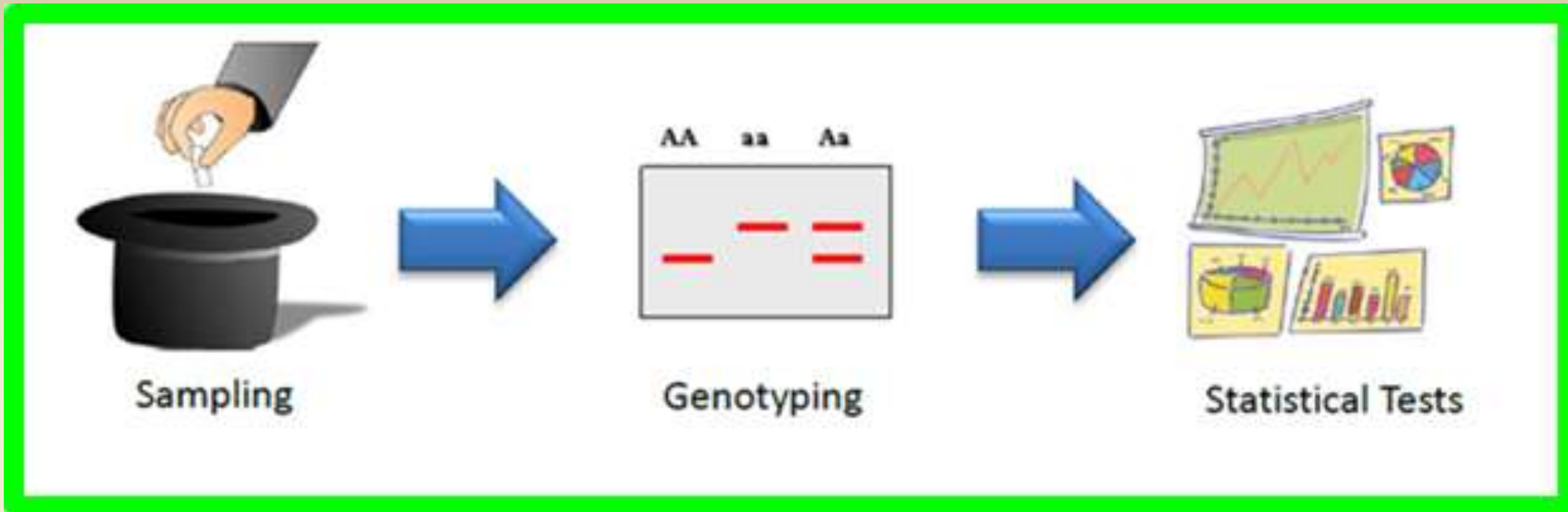
Sequence Markers - Chloroplast Gene Spacers in *Agave*





Usually not enough variation to resolve relationships!

General Protocol for Most Genetic Studies



Populations
Individuals
~20 – 30 best

Extract DNA
Amplify DNA with Primers
RAPD, ISSR,
AFLP, SSR
PCR
Electrophoresis
Score Data

Similarity
Distance
Heterogeneity
F-stats

Allozymes:

Different alleles produce slightly different proteins which migrate differently on an electrically charged starch gel.

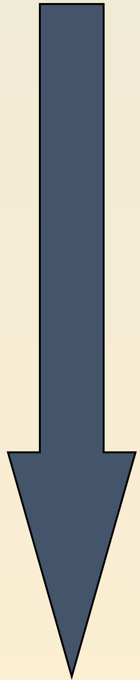
Gives presence/absence of enzyme types

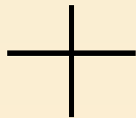
Reveals the number of loci for an enzyme, the state of homozygosity or heterozygosity (2 alleles of a gene = heterozygous).

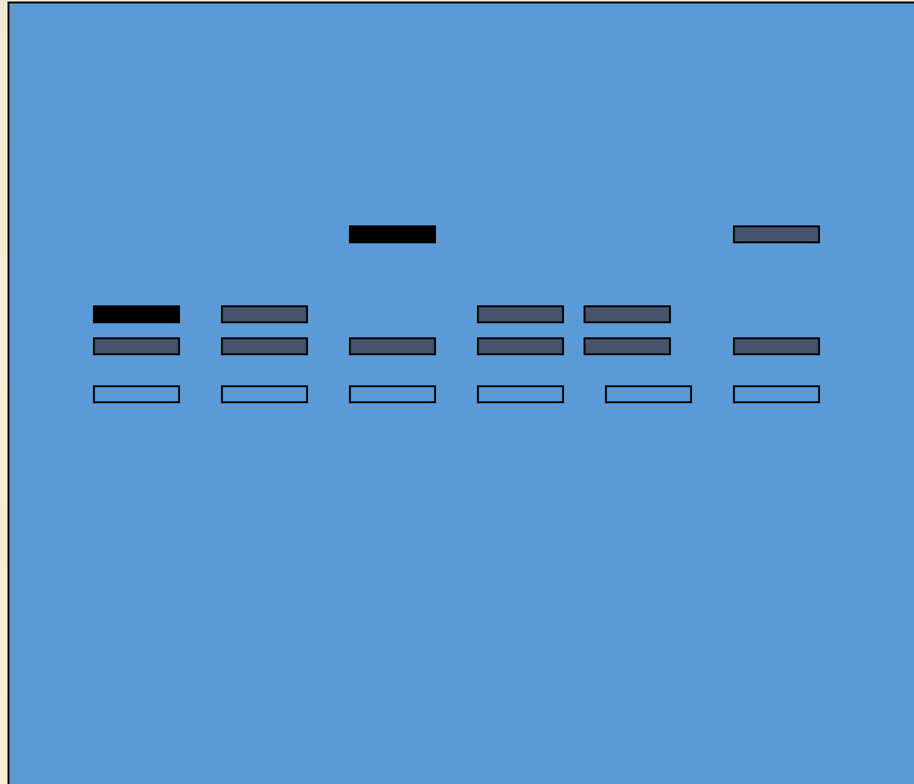
Data used to **measure genetic diversity, heterozygosity**, in populations.

Easy, but messy and uses some dangerous stains. Used a lot in the past frequently, now largely replaced by DNA methods.

Allozymes







Different forms (alleles) of the same enzyme

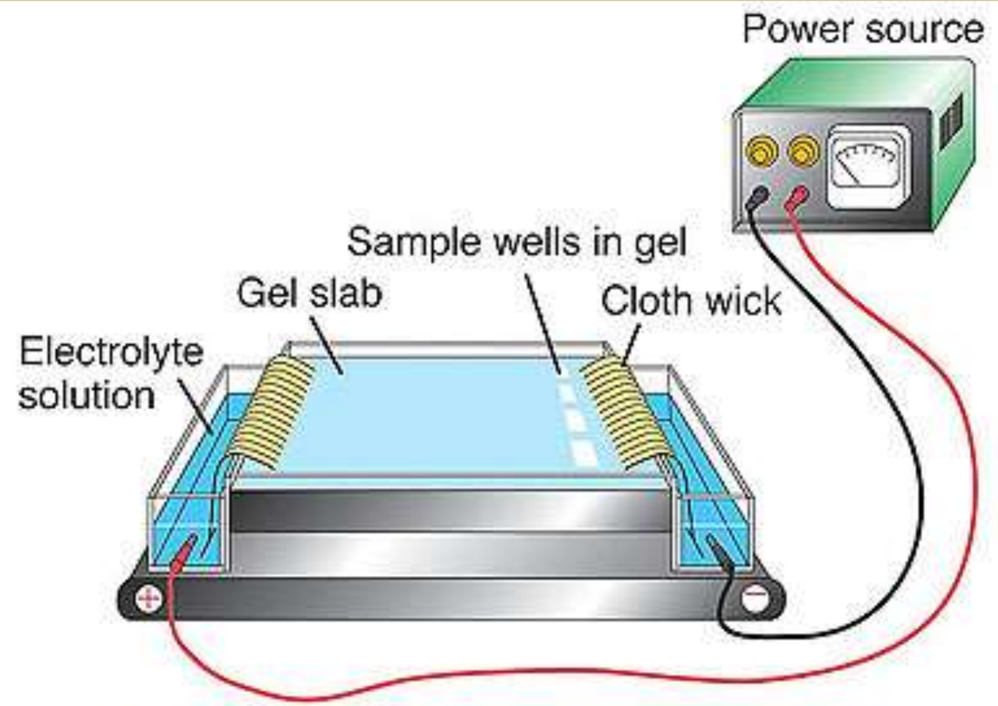
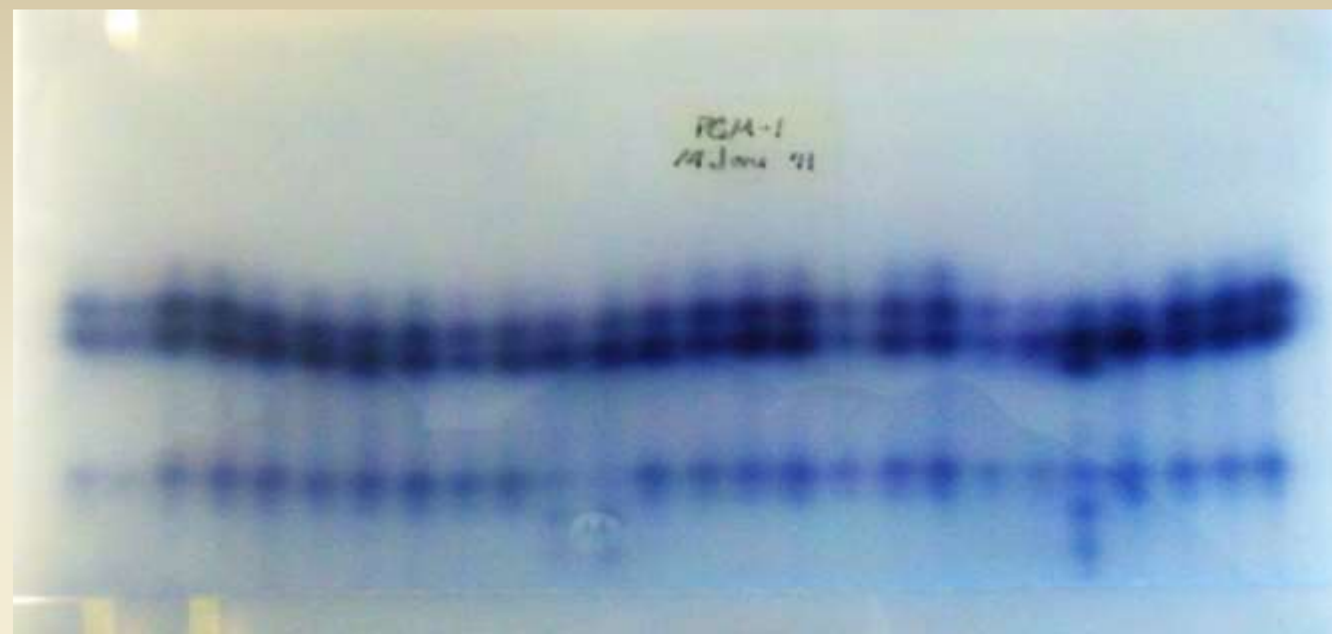
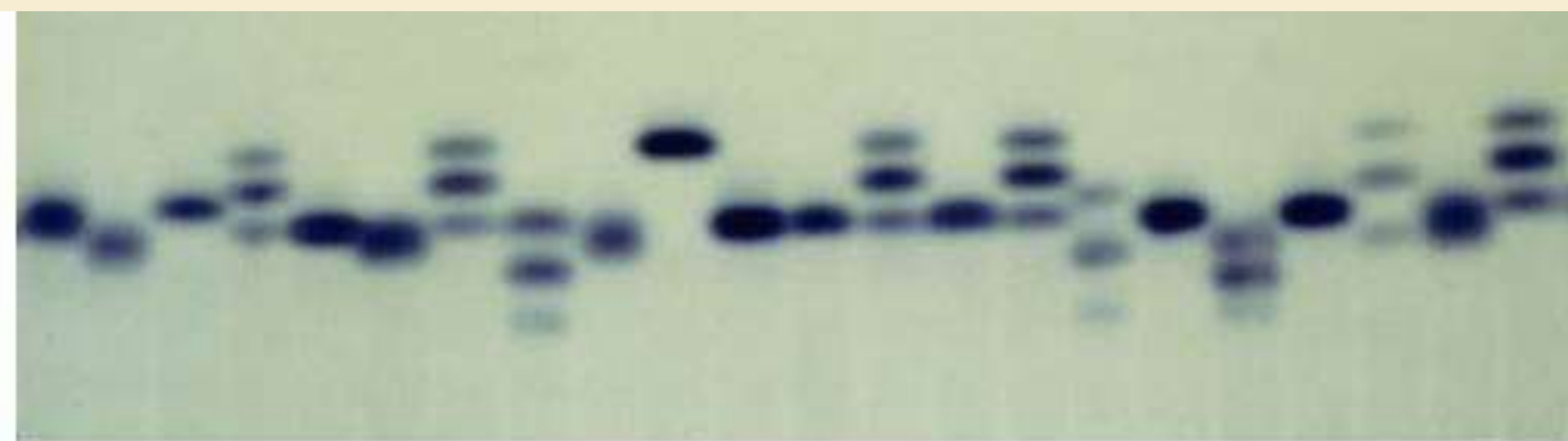
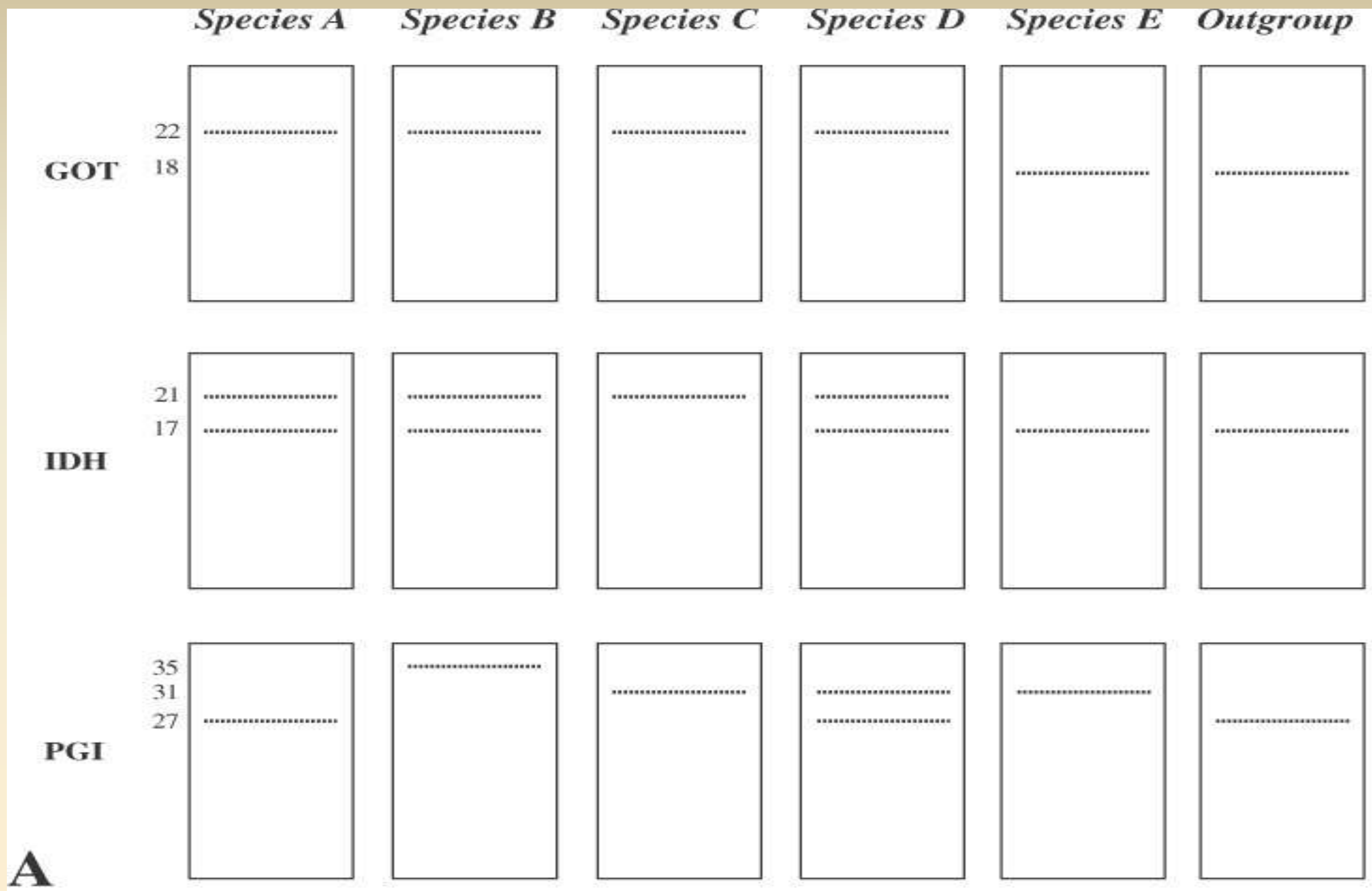


Figure 4: Schematic of devices used in protein electrophoresis (*http://www.mun.ca/biology*)







Allozyme Data

RAPDs – Randomly Amplified Polymorphic DNA

Simple technique

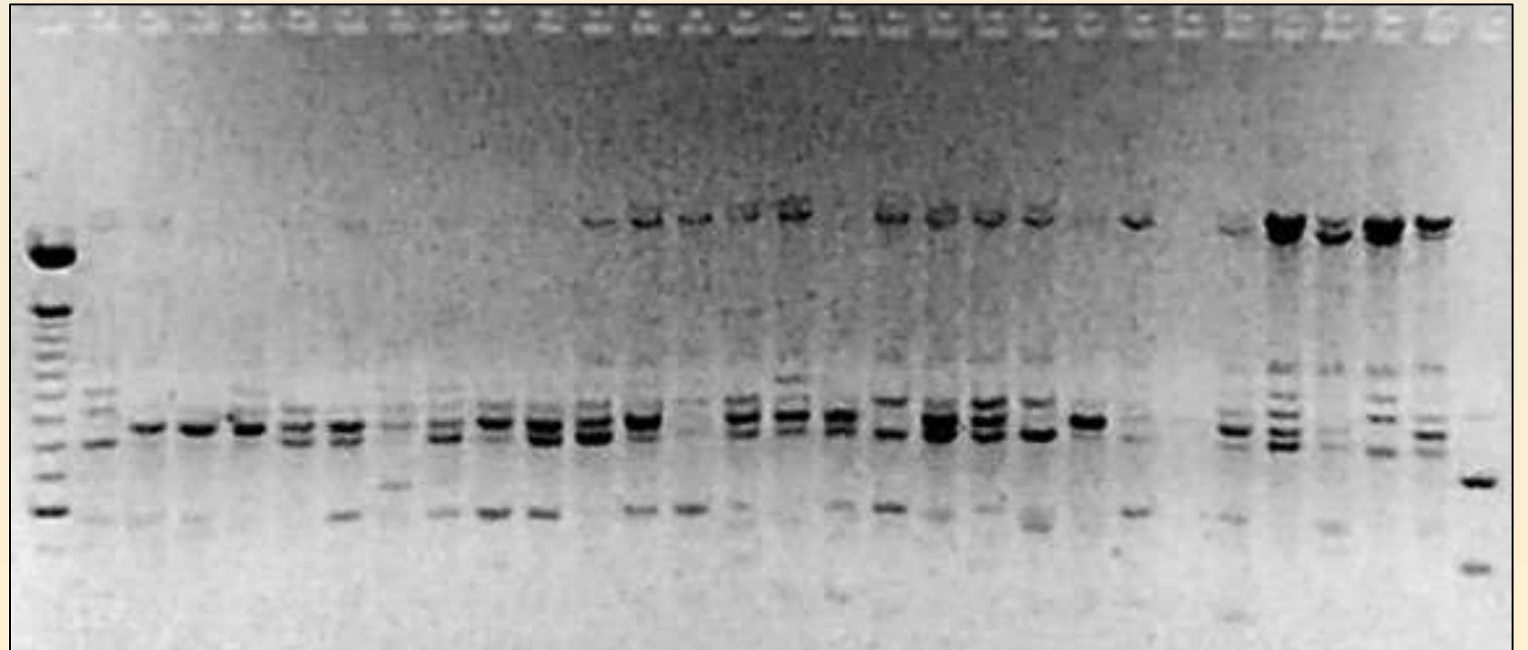
Amplify DNA using a single, short (10 bp) primer
Separate fragments on agarose gel
Visualize with transilluminator, photograph.
Score bands 1 or 0
Make matrix
Calculate statistics, distance

Advantages

Universal primers
Fast
Inexpensive
No special equipment

Disadvantages

Sensitivity to conditions
Reproducibility
Markers are dominant



Microsatellites

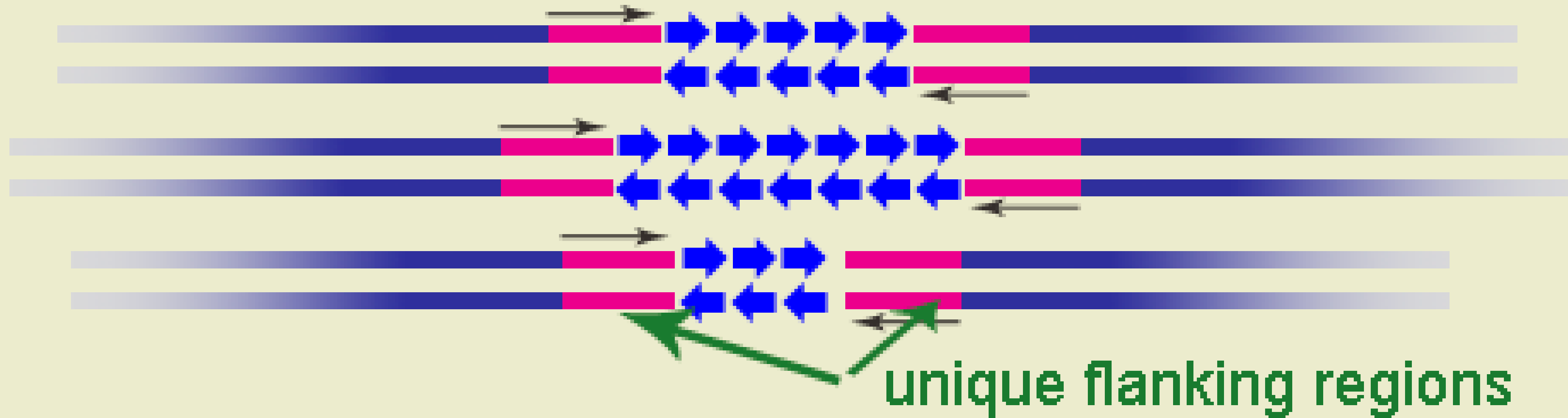
SSR – Simple Sequence Repeats
STR – Simple Tandem Repeats

Short repeating units (e.g. CA, GTG, TGCT etc)
arranged in tandem – usually 2-5 bp
Frequent, scattered throughout the genome
Function unknown, may be involved with gene expression
Highly polymorphic
High mutation rate
Form by unequal crossing over.
Primers designed on short flanking regions.

Advantages
High variability
Codominant
Rapidly genotyped using automated DNA sequencing.
Disadvantages
Need to develop new primers for each group of species.
Development of microsatellites is laborious and expensive

SSRs - Simple Sequence Repeats = Microsatellites

Short repeating sequences scattered throughout the genome,
e.g..GTGTGTGTGTGT, or CATCATCATCATCAT
The number of SSRs is highly variable among individuals



Microsatellites

Microsatellites: Length polymorphism

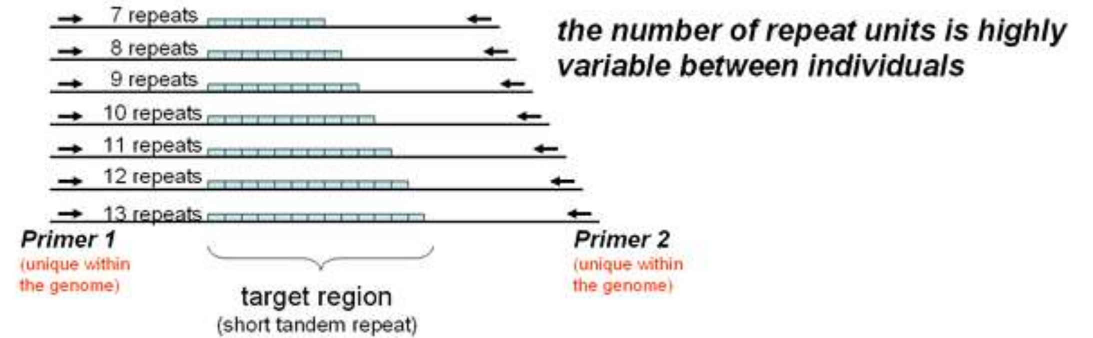
- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

Short tandem repeat (STR) = Microsatellites = simple sequence repeat (SSR)

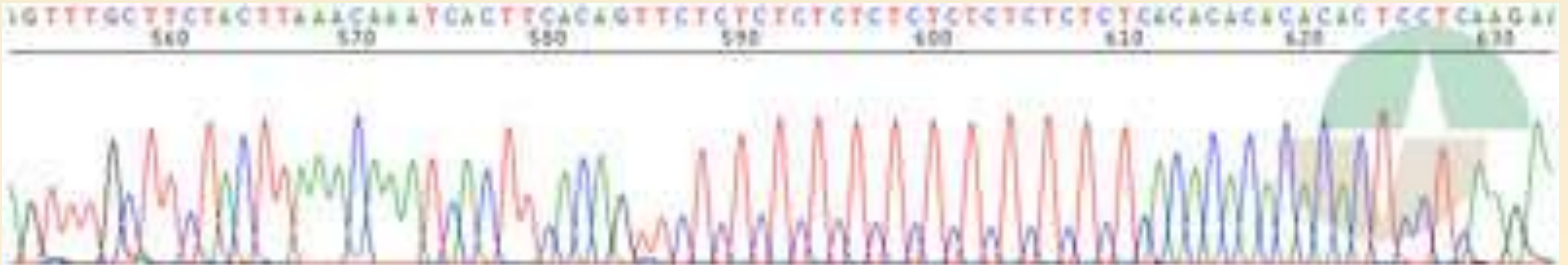
similar to accordion- DNA-sequences between genes

TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACA
GGTG**GATAGATAGATAGATAGATAGATAGATAGATAGATAGA**
TAGATAGATATCATTGAAAGACAAAACAGAGATGGATGATAGAT
ACATGCTTACAGATGCACAC

= 12 GATA repeats



the number of repeat units is highly variable between individuals



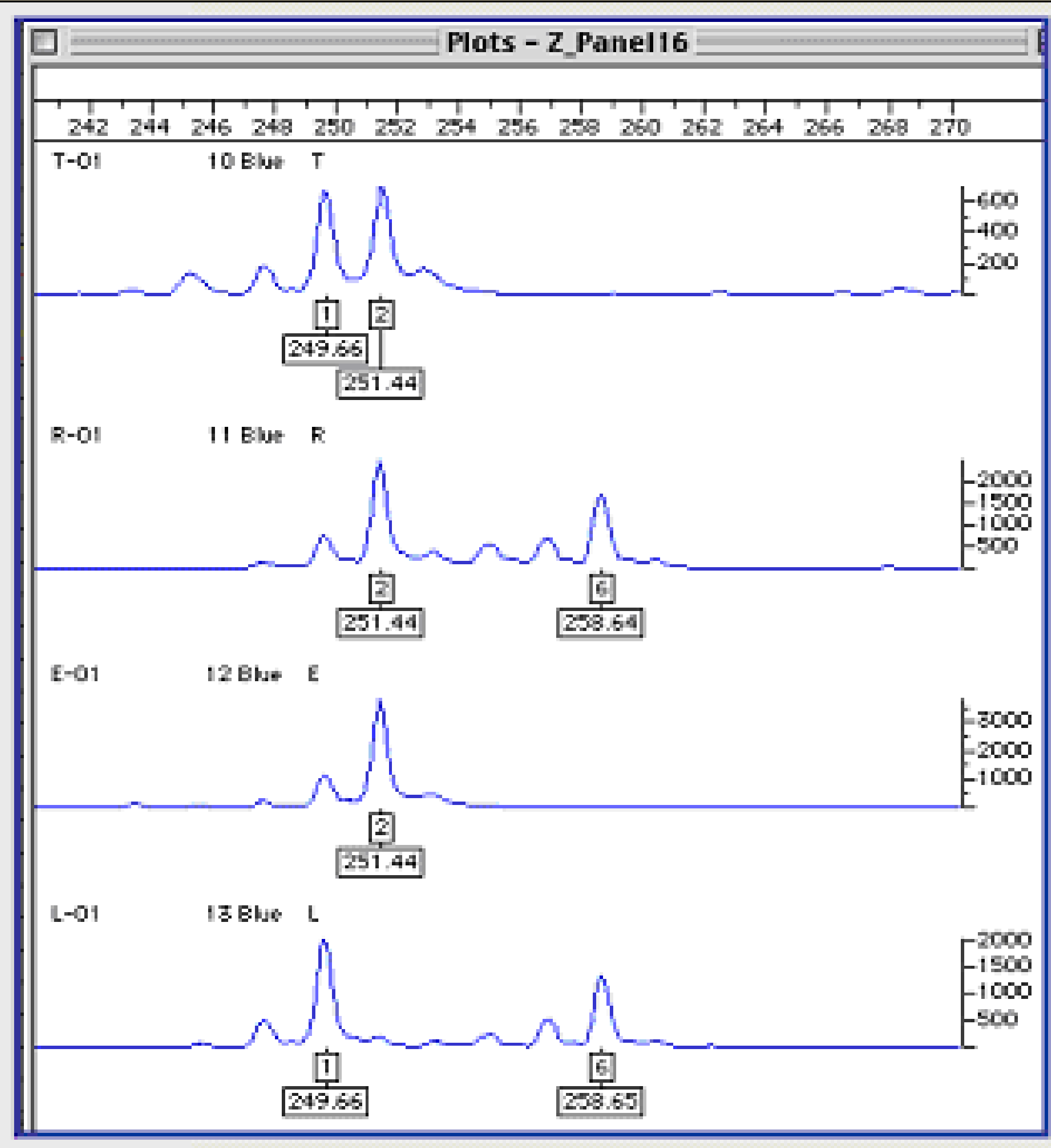
Microsatellites are Codominant – Show Heterozygotes

Father alleles of sizes 249.66 and 251.44 (heterozygous)

Mothers alleles of sizes 251.44 and 258.64 (heterozygous)

Child 1 alleles both of Size 251.44 (homozygous)

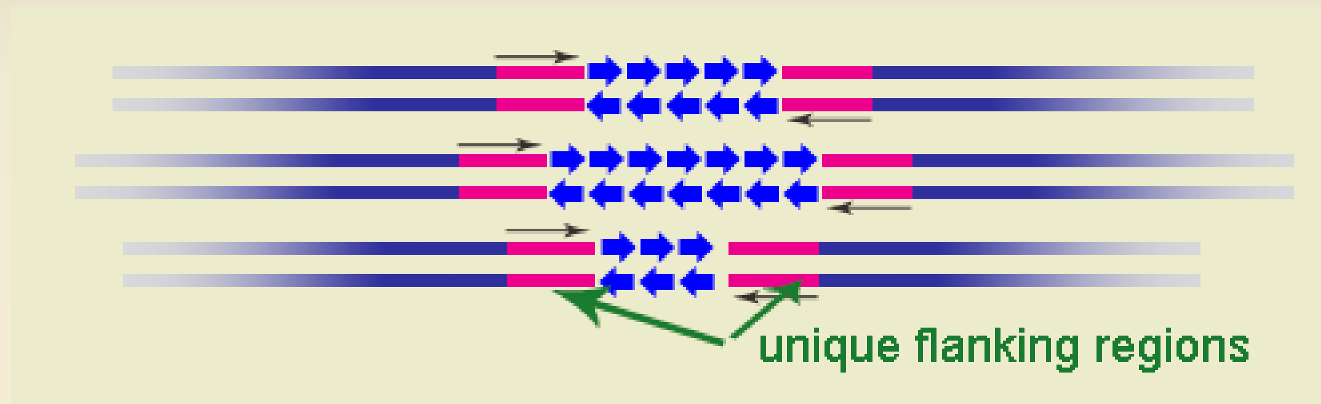
Child 2 alleles of sizes 249.66 and 258.65 (heterozygous)



SSRs - Simple Sequence Repeats (= Microsatellites)

Short repeating sequences scattered throughout the genome, e.g..GTGTGTGTGTGT, or CATCATCATCATCAT

The number of SSRs is highly variable among individuals



Two Kinds of Markers Use SSRs

Microsatellites - Flanking regions used to amplify SSR repeating unit

ISSRs – Inter-Simple-Sequence-Repeats - Repeating unit used as a primer to amplify region in between SSRs. e.g. CTCTCTCTCTCTCTG

ISSRs - Inter Simple Sequence Repeats

Simple Technique

Amplify with single primer based on SSR, e.g. CACACACACACACAG

Regions between SSRs are amplified

Very similar to RAPDs, generates many bands. Analysis the same.

Annealing temperatures used are higher than those used for RAPD markers.

Advantages

Does not require sequence information.

Variation found at several loci simultaneously.

Fast, easy, inexpensive.

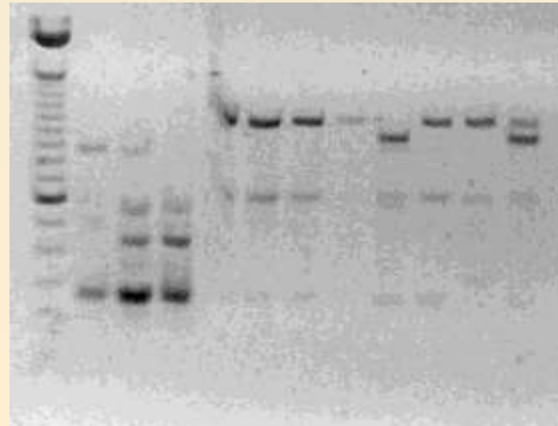
Disadvantages

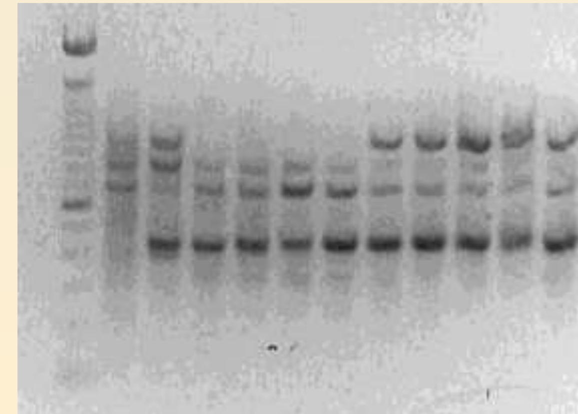
Dominant markers

Band staining can be weak

Reproducibility

Pseudophoenix ISSR Gels





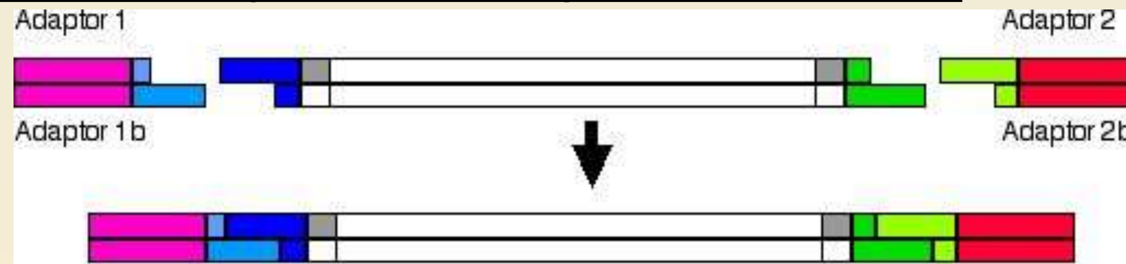
AFLP - Amplified Fragment Length Polymorphism

1. Cut DNA into fragments with restriction enzyme

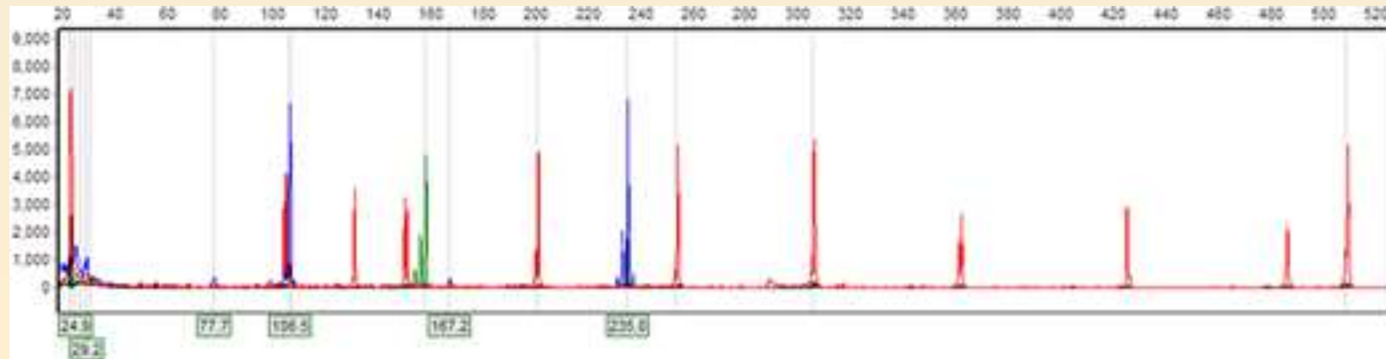
GTAAGAATTCTTTAGAATTCCGCCATTATCGAATTCAGGATCTTAC
CATTCTTAAGAAATCTTAAGGCGGTAATAGCTTAAGTCCTAGAATG

GTAAG AATTCTTTAG AATTCCGCCATTATCG AATTCAGGATCTTAC
CATTCTTAA GAAATCTTAA GGCGGTAATAGCTTAA GTCCTAGAATG

2. Attach special adapters to ends



3. Amplify fragments, separate in capillary sequencer



AFLP - Amplified Fragment Length Polymorphism

Technique

Break DNA into fragments

Attach special adapters to ends.

Amplify fragments

Separate fragments on sequencer.

Advantages

Generates many fragments

High resolution separation

Reproducible

Multiplexing, 4 dyes per sample

Disadvantages

Technically demanding.

Dominant markers.

Scoring and interpretation

Expensive

Plant Breeding

Identify cultivars

Relatedness

Linkage maps

Population Genetics

Structure

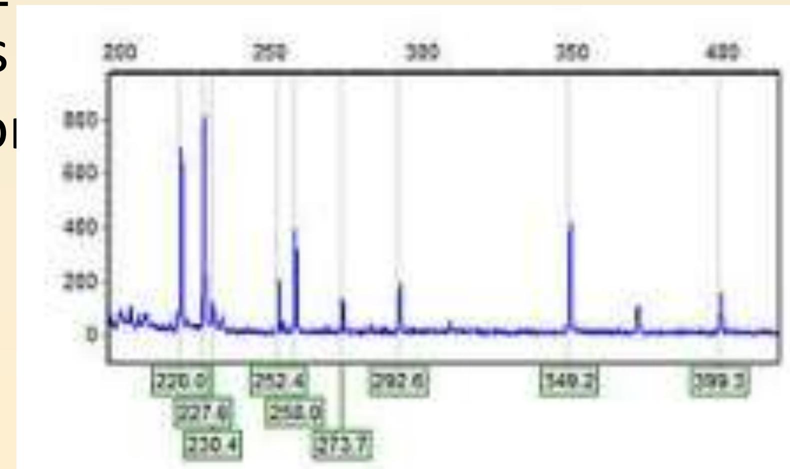
Genetic diversity

Paternity

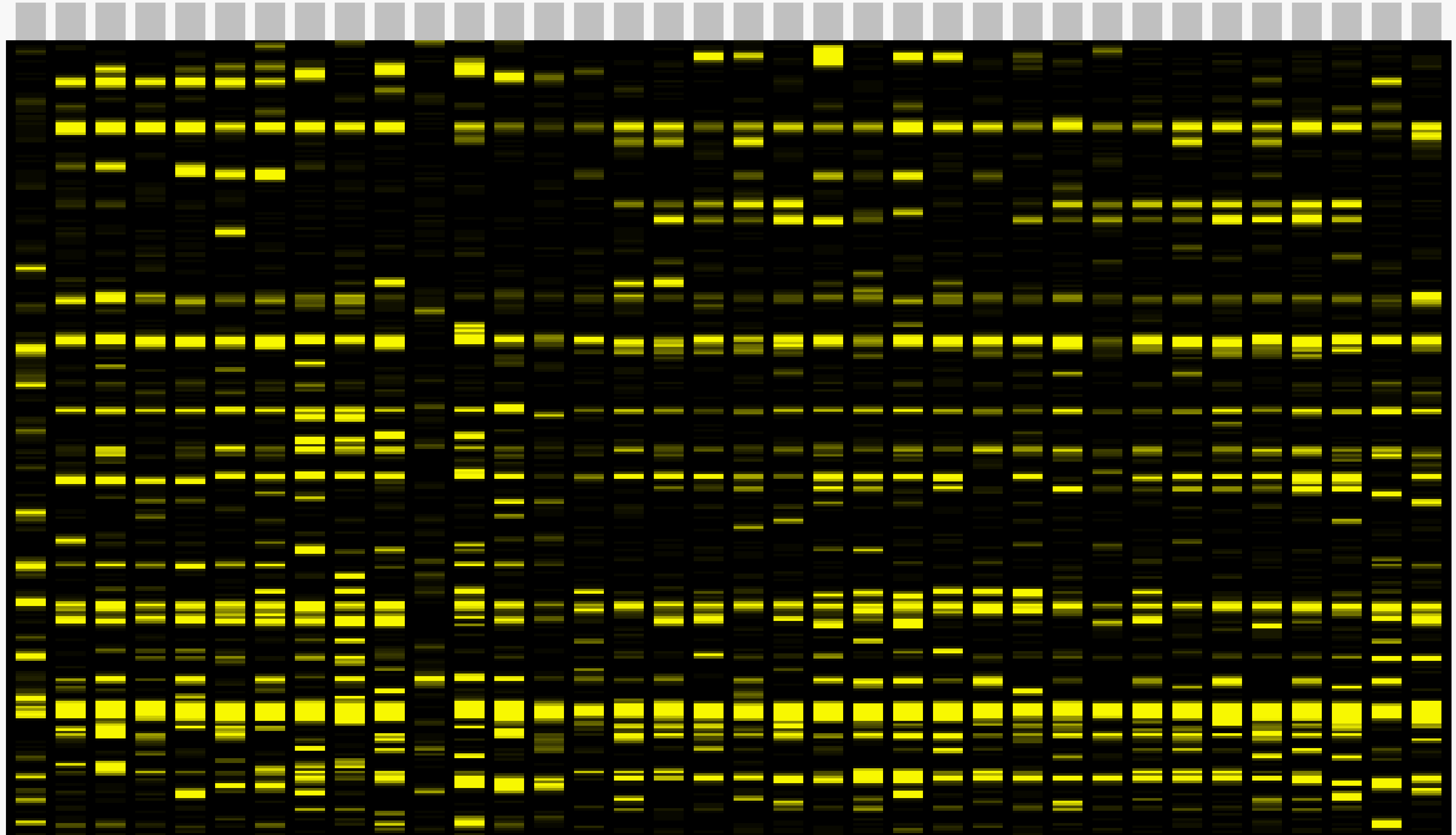
Systematics

Relatedness

Hybridizatio



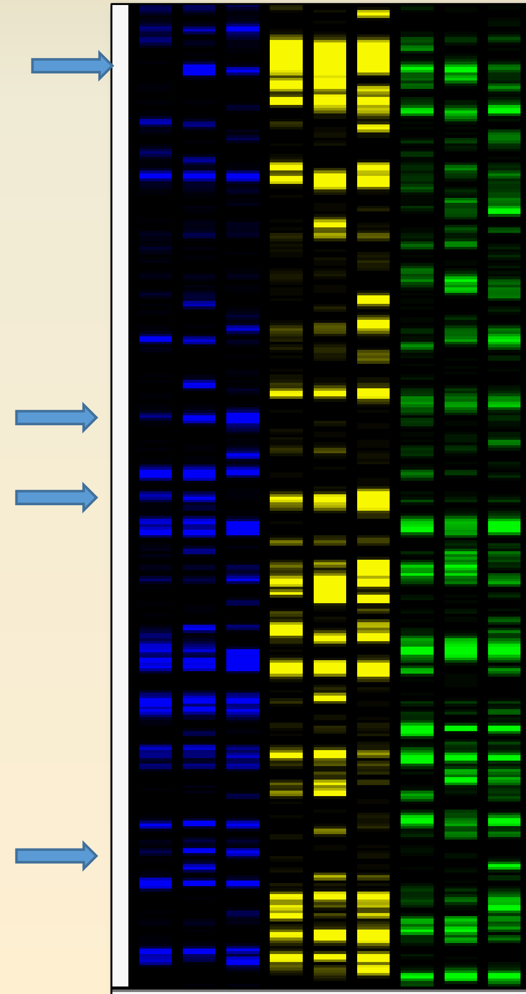
Dasylirion AFLP Data: EcoRI-AAC, MseI-CTA

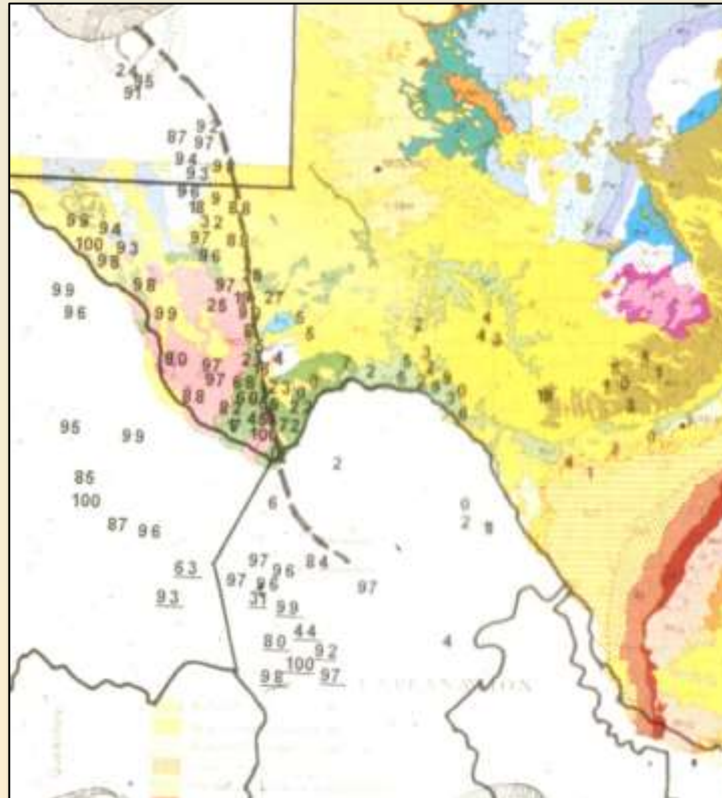


Hybrids between *Dasylirion wheeleri* and *D. leiophyllum* in west Texas?

1. *D. wheeleri* - Organ Mtns.
2. *D. wheeleri/leio.* - Hueco Tanks
   Putative hybrid
3. *D. leiophyllum* - Chinati Mtns.

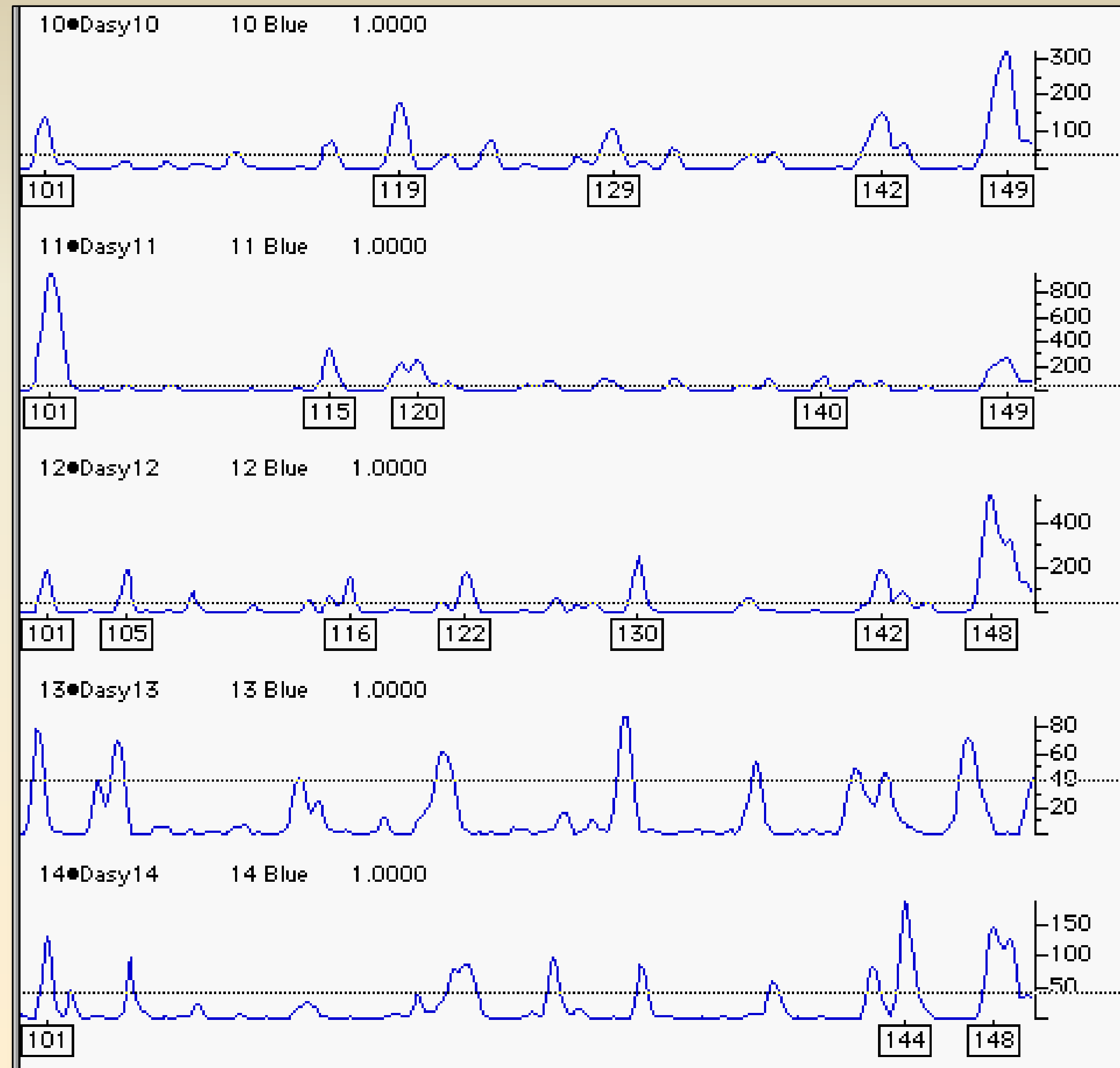
1 2 3 1 2 3 1 2 3





Need to look at larger sample size

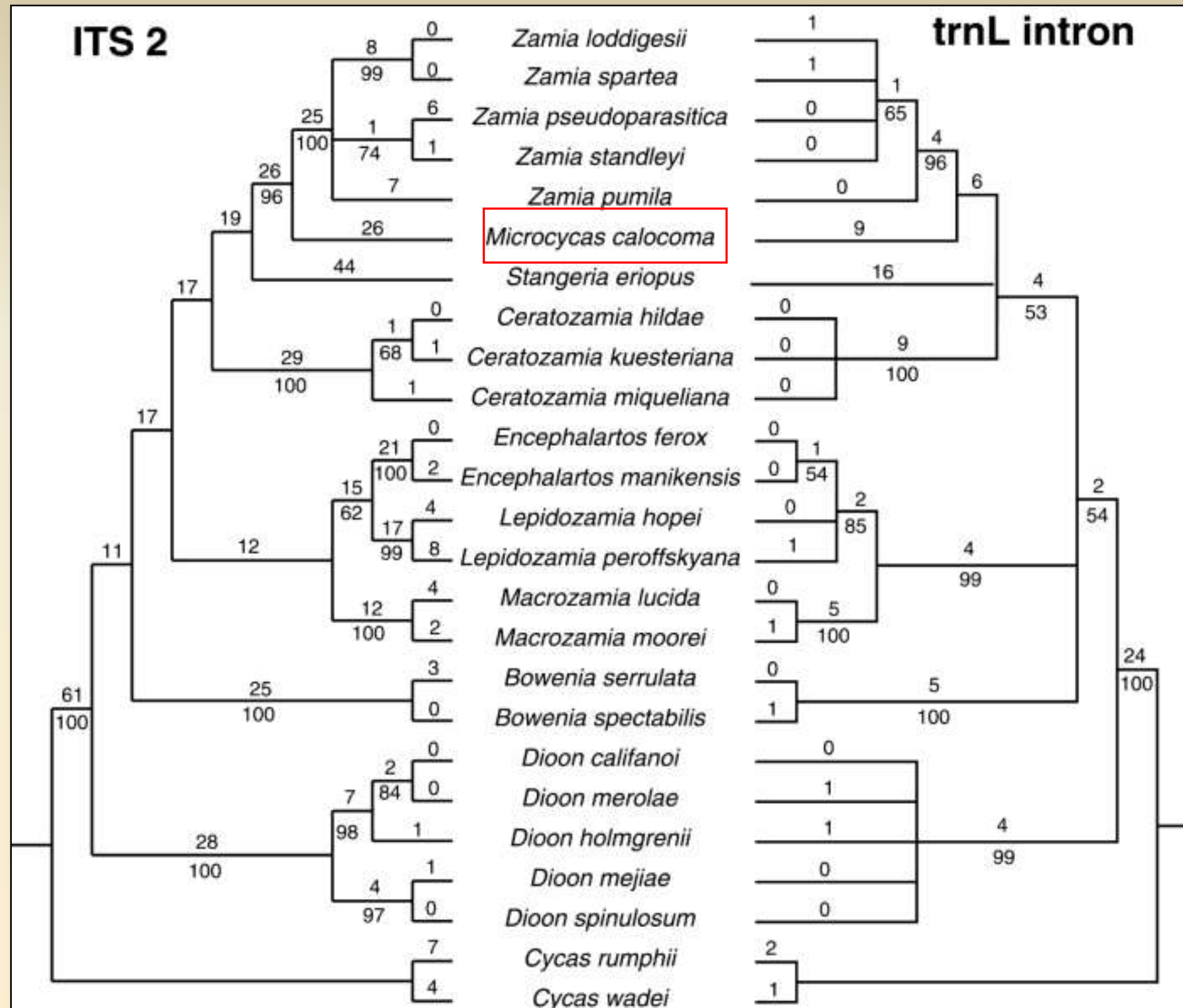
Automated AFLP Analysis with Genotyper (now GeneMapper)



Microcycas calocoma

from David Jones, Cycads of the World

Cycad Phylogeny



Bogler & Francisco-Ortega. 2004. Bot. Rev.: 70.

REVOLUCION
somos todos

hava

Vinales, with Mogotes

Esperanza Pena Garcia
Cycad Conservation Specialist

Microcycas Conservation Efforts

In Situ - wild populations

Protected areas - Mil Cumbres
Protected status
Education
Hand pollination of females
Reproductive biology/pollinaton
Monitoring

Ex Situ - off-site collections

Hand pollination
Pollen banks
Seed propagation and distribution
Tissue and embryo culture
Molecular genetics

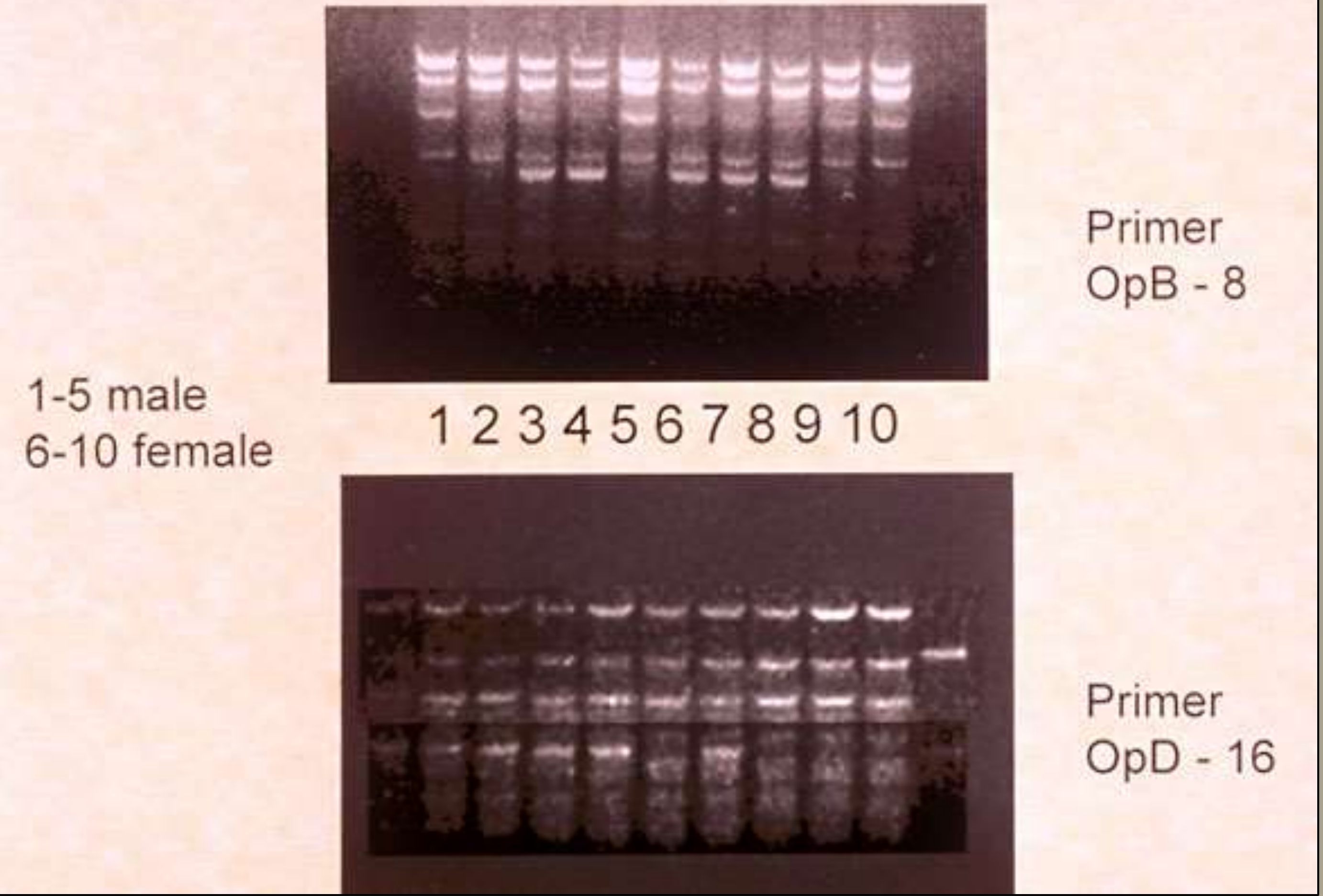
Issues in *Microcycas* Conservation Genetics

Sex Determination in Cycads

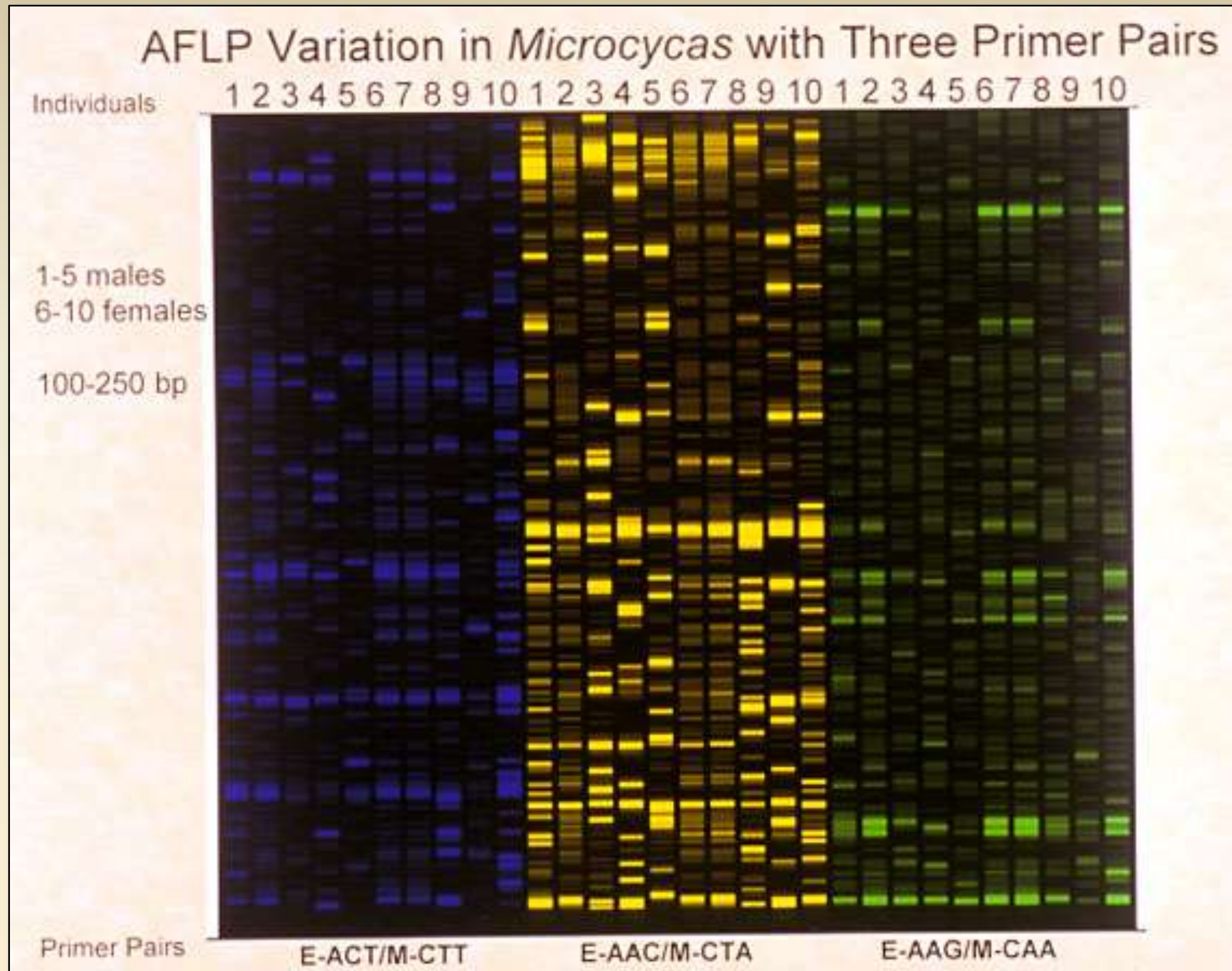
- Unbalanced sex ratios
- Reintroduction of seedlings

Levels of Genetic Variation

- Within populations
- Between populations
- Ex situ collections
- Pollination and reintroduction efforts

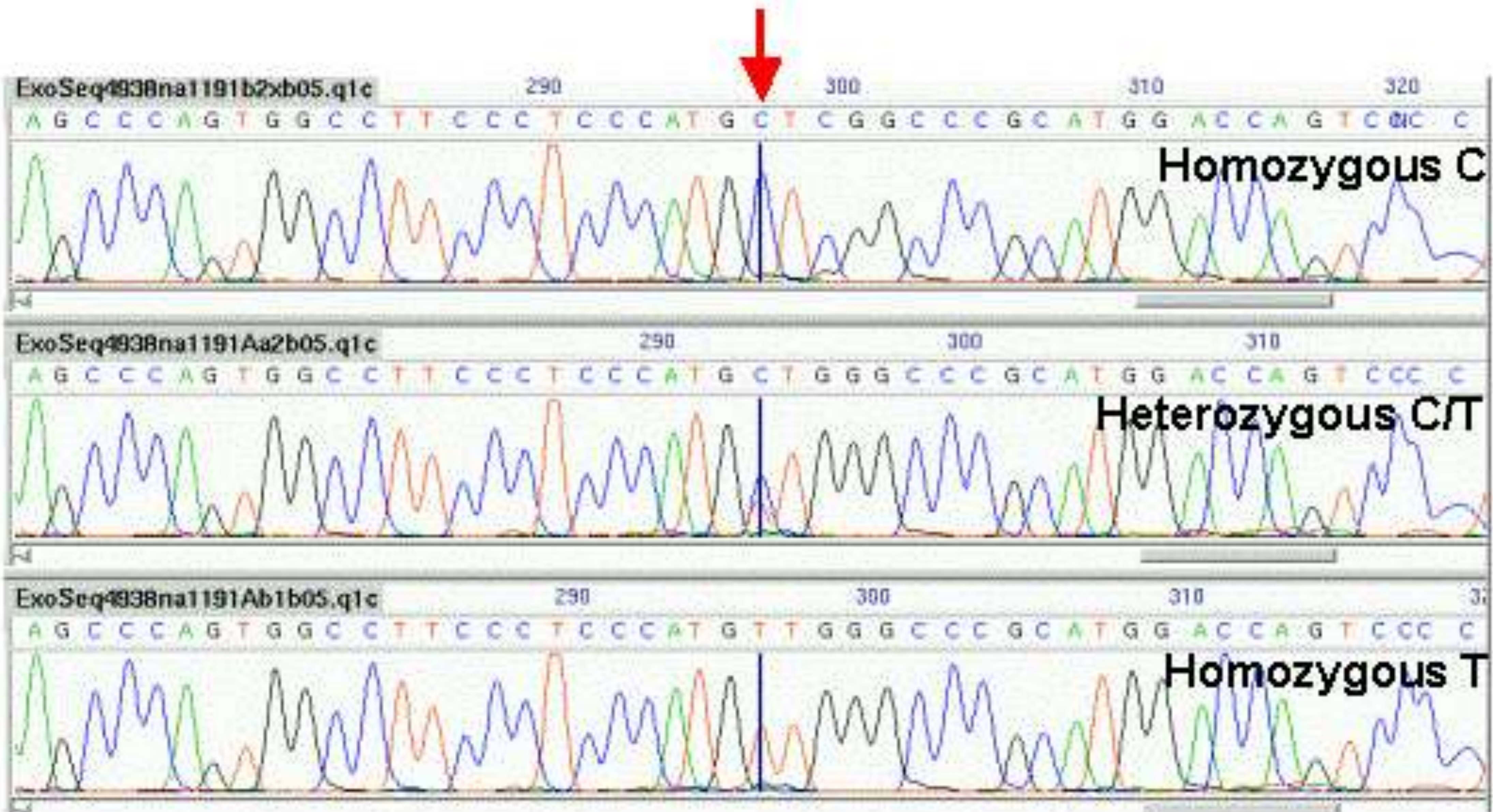


We screened 80 RAPD primers => No sex-linked loci



We screened 18 AFLP primer pairs => No sex-linked loci

SNPs - Single Nucleotide Polymorphisms

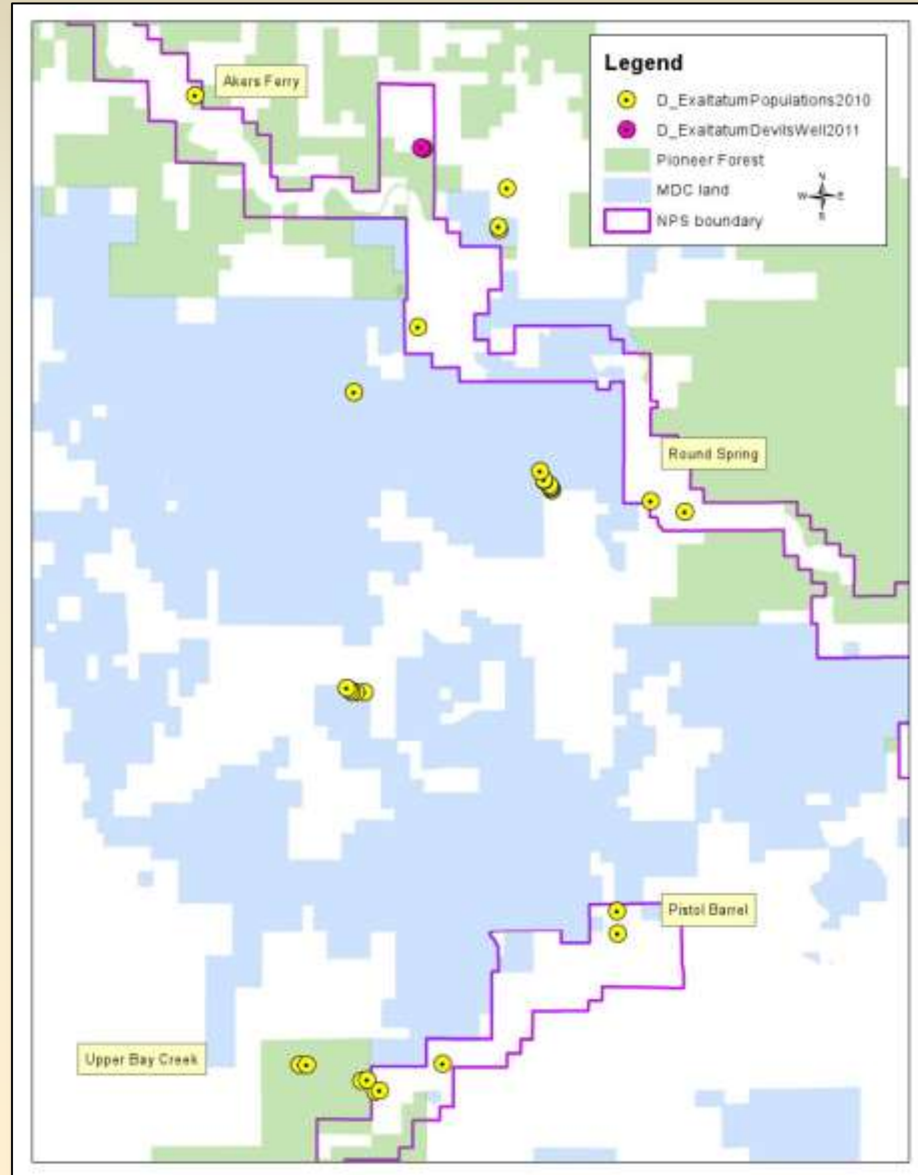


Conservation Genetics of Tall Larkspur (*Delphinium exaltatum*)

Conservation Genetics of Tall Larkspur (*Delphinium exaltatum)*

U.S. Distribution

Shannon Co., Missouri



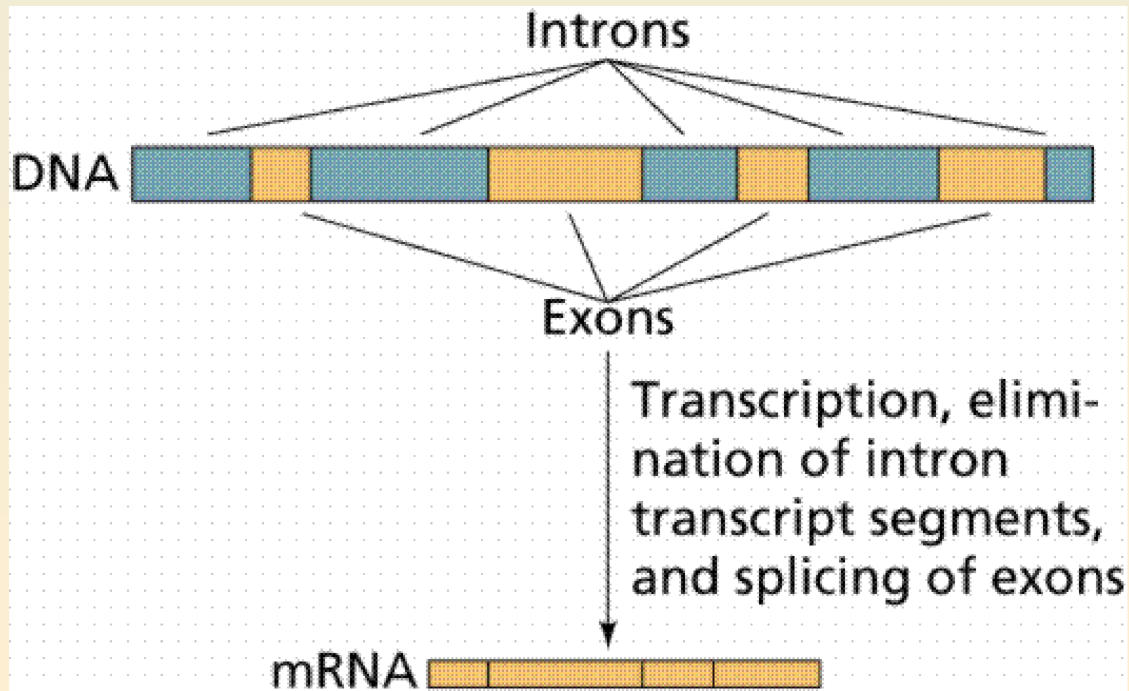
PEP Carboxylase Gene Introns

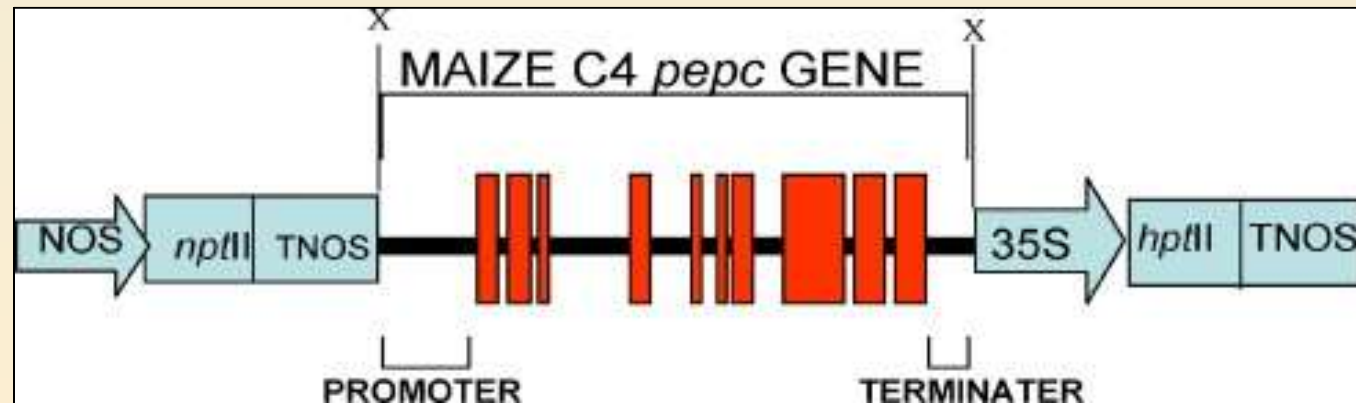
Enzyme with role in C4 cycle photosynthesis

Coded by nuclear gene

PEPC Intron 4 used in other population studies, primers from Gaskin and Schaal 2002 (Tamarix))

Provides resolution at the population level





Summary – Picking the right tool for the job.

RAPDs

pros: quick, inexpensive, informative, good student projects, identify cultivars, no sequence knowledge needed, minimal equipment.

cons: sensitive, must check reproducibility, dominant markers.

ISSRs

pros: quick, inexpensive, more bands, good for identifying cultivars.

cons: sensitive to conditions, reproducibility, dominant markers.

F-ISSRs – fluorescence-tag, multiplexing, fast, automated.

AFLPs

pros: powerful, generates lots of data, automated scoring, reproducible,..

cons: expensive kits, technical, scoring issues, dominant markers

SNPs, nuclear gene introns

pros: phylogenetic signal, co-dominant markers

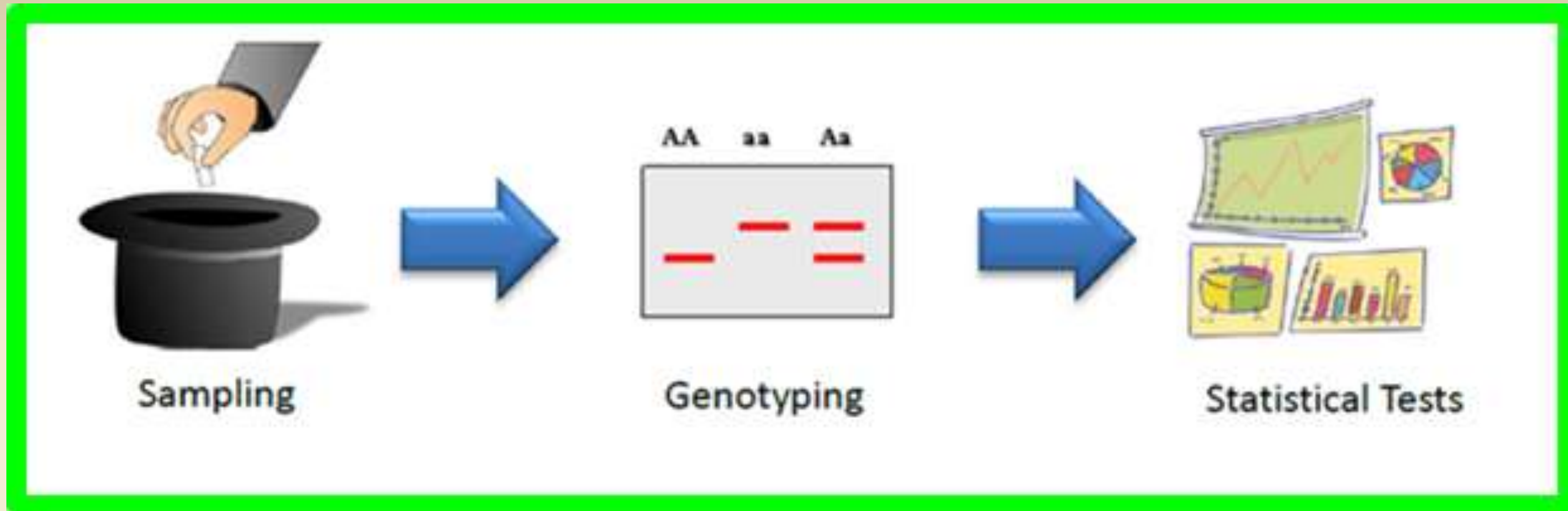
cons: multiple gene copies may be present

Microsatellites (SSR)

pros: highly variable, co-dominant markers, good for population and evolutionary studies

cons: need to find regions and develop primers for each group.

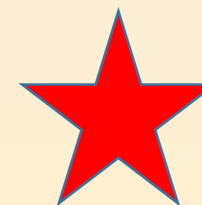
General Protocol for Most Genetic Studies



Populations
Individuals
~20 – 30 best

Extract DNA
Amplify DNA with Primers
RAPD, ISSR,
AFLP, SSR
PCR
Electrophoresis
Score Data

Similarity
Distance
Heterogeneity
F-stats



Genetic Levels of Analyses

Individual - identifying parents & offspring– very important in zoological circles – identify patterns of mating between individuals. In fungi, it is important to identify the "individual" -- determining clonal individuals from unique individuals that resulted from a single mating event.

Families – looking at relatedness within colonies (ants, bees, etc.)

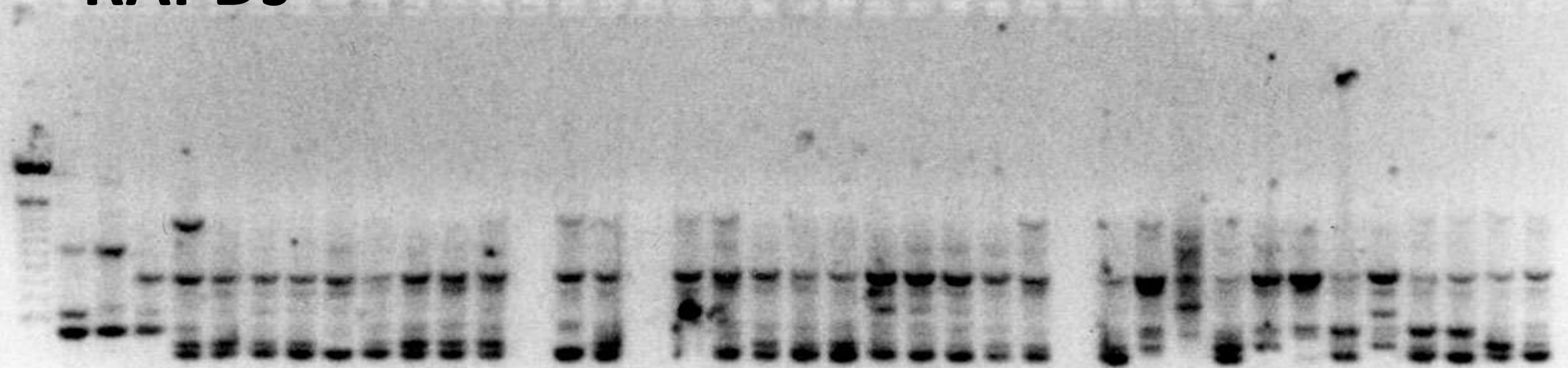
Population – level of variation within a population.

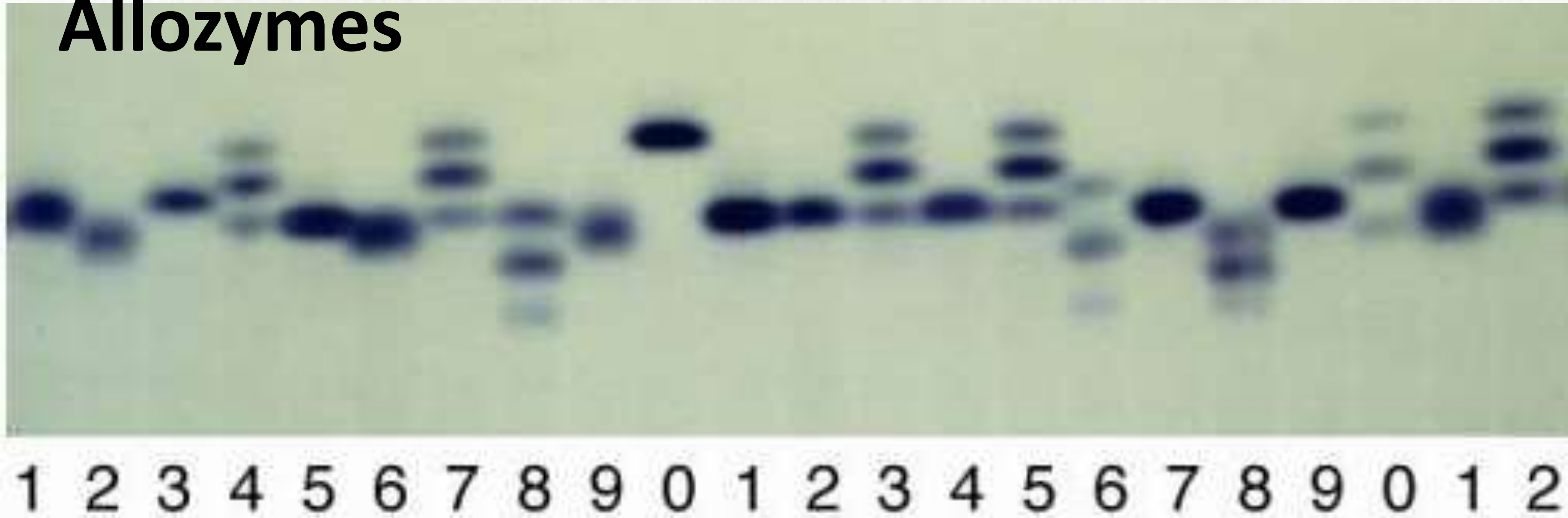
Dispersal - indirectly estimate by calculating migration

Conservation and Management - looking for founder effects (little allelic variation), bottlenecks (reduction in population size leads to little allelic variation)

Species – variation among species = what are the relationship between species.

Family, Order, ETC. = higher level phylogenies


RAPDs


Allozymes
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2

Proportion of polymorphic loci - P

The number of polymorphic loci divided by the total number of loci (polymorphic and monomorphic):

P = n-p/n-total

It expresses the percentage of variable loci in a population.
Its calculation is based on directly counting polymorphic and total loci.
It can be used with codominant markers and, very restrictively, with dominant markers

Proportion of polymorphic loci - P

P = n-p/n-total

e.g. 20 loci, 4 polymorphic, P = 0.2

Not precise - The number of variable loci observed depends on how many individuals are examined. If we examine more individuals we might identify more polymorphisms and the measure tends to increase.

Population Genetics - Analytical Techniques

Hardy-Weinberg Equilibrium

- $p^2 + 2pq + q^2 = 1$
- Departures from non-random mating

Wright's F-Statistics

- measures of genetic differentiation in populations

Inbreeding Index

Clustering Techniques

- UPGMA
- Structure
- AMOVA

Hardy-Weinberg Equilibrium

- $p^2 + 2pq + q^2 = 1$
- Departures from non-random mating

Homozygotes – alleles are the same (AA, aa)

Heterozygotes - alleles are different (Aa)

Heterozygosity - the percentage of heterozygotes in a population.

Population Heterozygosity - H

The average frequency of heterozygous individuals per locus.

Calculated by first obtaining the frequency of heterozygous individuals of each locus and then averaging these frequencies over all loci.

Example Heterozygosity

Locus	**Heterozygotes in sample**	**Total population**	**Heterozygosity (Hobs)**
1	40	100	0.4
2	20	100	0.2
3	35	100	0.35
			0.32 = H

Departures from HW Equilibrium

Check Gene Diversity = Heterozygosity

Heterozygosity High

- different genetic sources due to high levels of migration

Heterozygosity Low

- Inbreeding , mating system "leaky" or breaks down allowing mating between siblings
- Restricted dispersal - local differentiation leads to non-random mating

Population Substructure

Many species naturally subdivide themselves into herds, flocks, colonies, schools etc.

Patchy environments can also cause subdivision

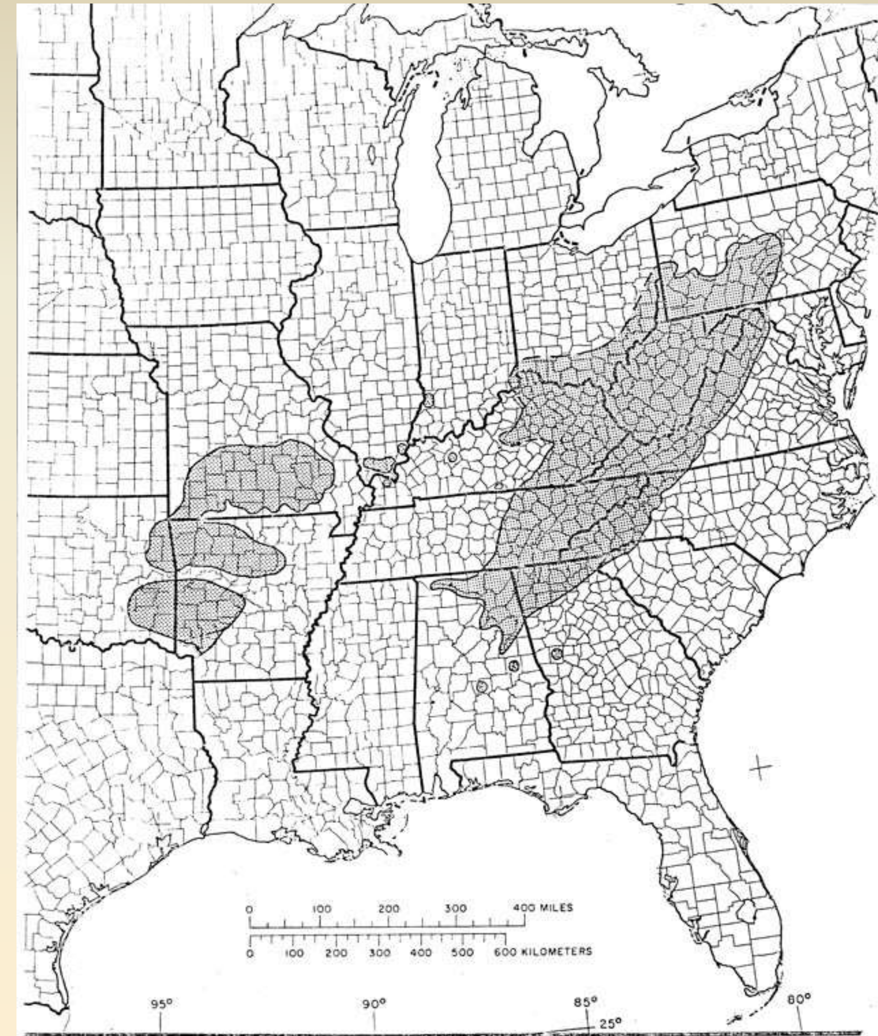
Human – caused habitat fragmentation results in subdivision and subpopulations

Subdivision decreases heterozygosity and generates genetic differentiation via:

- Natural selection
- Genetic drift

Geocarpon minimum

Robinia pseudoacacia



Wright's Fixation Index (F_{ST}) - Subpopulation Variation

Important to know the degree to which specific subpopulations are different

Subpopulation can evolve from other populations

- Genetic drift
- Selection
- Mutation
- Migration
- Recombination

Compares the ratio of a value for a subsection of population to the value for the whole population

Wright's Fixation Index - F_{ST}

The Fst statistic was designed by Sewall Wright to measure the amount of genetic variation found among **s**ubpopulations relative to the **t**otal population (hence, the subscript "**st**")

$$F_{ST} = (H_T - H_S)/ H_T$$

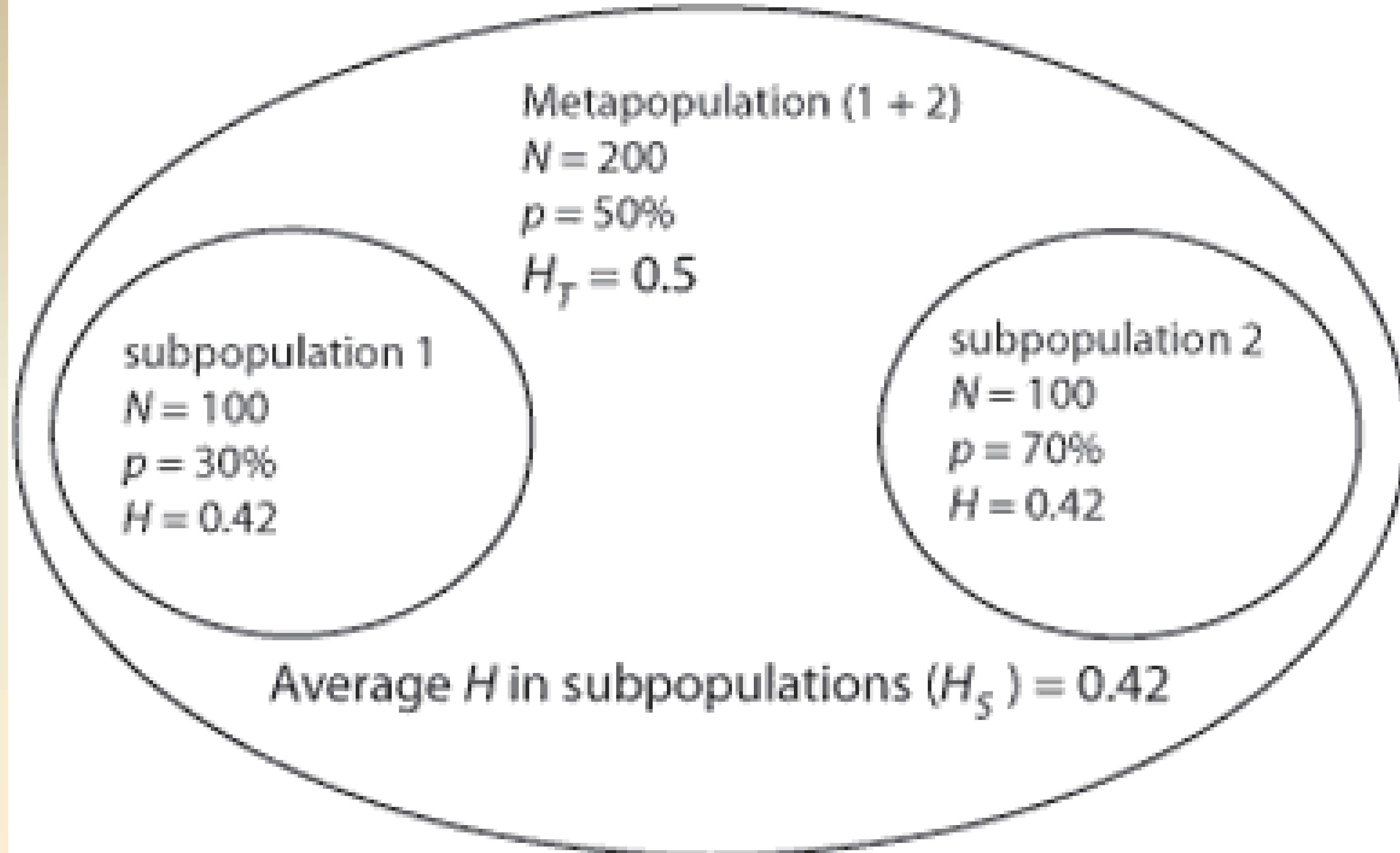
The greater the reduction of heterozygotes in a subpopulation the larger the value of F_{ST}

Heterozygosity = mean percentage of heterozygous individuals per locus

Calculate mean heterozygosities at each population level

Assuming H-W, heterozygosity (H) = 2pq where p and q represent mean allele frequencies

$\mathbf{H_S}$ = sum of all subpopulation heterozygosities divided by the total number of subpopulations



$$F_{ST} = \frac{H_T - H_S}{H_T} = \frac{0.5 - 0.42}{0.5} = 0.16$$

Interpreting F_{ST}

HT: proportion of the heterozygotes in total population

HS: average proportion of heterozygotes in subpopulations

If HT is nearly equal to HS, then subpopulations are similar

If HS is less in subpopulations, the subpopulations are different

Can range from 0 to 1

0 (no genetic differentiation) to

1 (fixation of alternative alleles).

How can *FST* be interpreted?

Wright suggestions:

FST = 0.00 – 0.05 = little genetic divergence

FST = 0.05 – 0.15 = moderate degree of genetic divergence

FST = 0.15 – 0.25 = great degree of genetic divergence

FST > 0.25 = very great degree of genetic divergence

These are suggestions!

Fst should be balanced against what the researcher actually knows about a population

Conservation Implications – save the most diversity?

F_{ST} for various organisms

Organism	Number of Populations	Number Loci	Ht	Hs	Fst
Human (major races)	3	35	0.13	0.121	**0.069**
Yanomama Indian Villages	37	15	0.039	0.036	**0.077**
House mouse	4	40	0.097	0.086	**0.113**
Jumping rodent	9	18	0.037	0.012	**0.676**
Fruit fly	5	27	0.201	0.179	**0.109**
Horseshoe crab	4	25	0.066	0.061	**0.076**
Lycopod plant	4	13	0.071	0.051	**0.282**

Measuring Inbreeding

Recall that inbreeding decreases the number of heterozygotes in the population: each generation of selfing decreases the number of heterozygotes by 1/2.

By comparing the number of heterozygotes observed to the number expected for a population in H-W equilibrium, we can estimate the degree of inbreeding.

A measure of inbreeding in the “inbreeding coefficient”, F.

F = 1 - (Hobs) / (Hexp)

If F = 0, the observed heterozygotes is equal to the expected number, meaning that the population is in H-W equilibrium.

If F = 1, there are no heterozygotes, implying a completely inbred population.

Thus, the higher F is, the more inbred the population is.

Inbreeding Example – California Wild Oats

Wild oats is a common plant in California, the cause of the golden-brown hillsides all summer out there. Wild oats can pollinate itself, but the pollen also blows in the wind so it can cross fertilize. The task is to estimate the relative proportions of these two types of mating.

Inbreeding Example – California Wild Oats

Data for the phosphoglucomutase (Pgm) gene:

- 104 AA, 9 AB, 42 BB = 155 total individuals

observed heterozygotes = 9

<u>H-W calculations:</u>

- freq of A = 104 + 1/2 * 9 = 108.5 / 155 = 0.7
- freq of B = 1 – freq (A) = 0.3

exp heterozygotes = 2pq = 2 * 0.7 * 0.3 = 0.42 (freq) * 155 = 65.1

- F = 1 -(Hobs) / (Hexp) = 1 - 9 / 65.1 = 1 - 0.14
- **F = 0.84**

This is a very inbred population: most matings are from self pollination.

Inbreeding Depression and Genetic Load

For most species, including humans, too much inbreeding leads to weak and sickly individuals, as seen in this example of mice inbred by brother-sister matings.

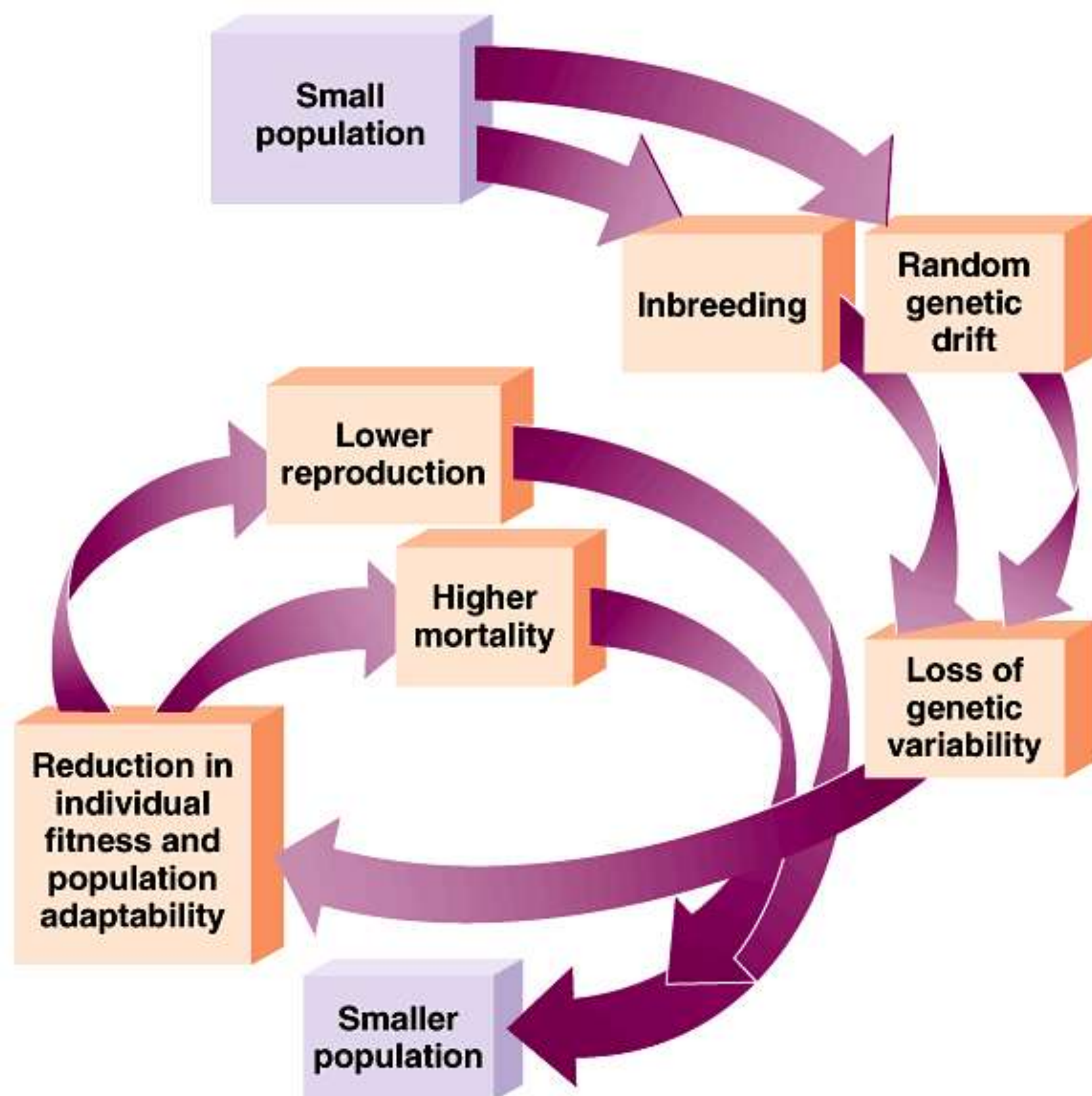
Inbreeding depression is caused by homozygosity of genes that have slight deleterious effects. It has been estimated that on the average, each human carries 3 recessive lethal alleles. These are not expressed because they are covered up by dominant wild type alleles. This concept is called the **"genetic load".**

However, it has been argued that some amount of inbreeding is good, because it allows the expression of recessive genes with positive effects. The level of inbreeding in the US has been estimated (from Roman Catholic parish records) at about F = 0.0001, which is approximately equivalent to each person mating with a fifth cousin.

gen	litter size	% dead by 4 weeks
0	7.50	3.9
6	7.14	4.4
12	7.71	5.0
18	6.58	8.7
24	4.58	36.4
30	3.20	45.5

Extinction Vortex

Inbreeding depression can potentially contribute to a so-called extinction vortex, in which decline reduces fitness which in turn hastens the decline, increasing both inbreeding depression and vulnerability to stochastic events in a destructive feedback loop.



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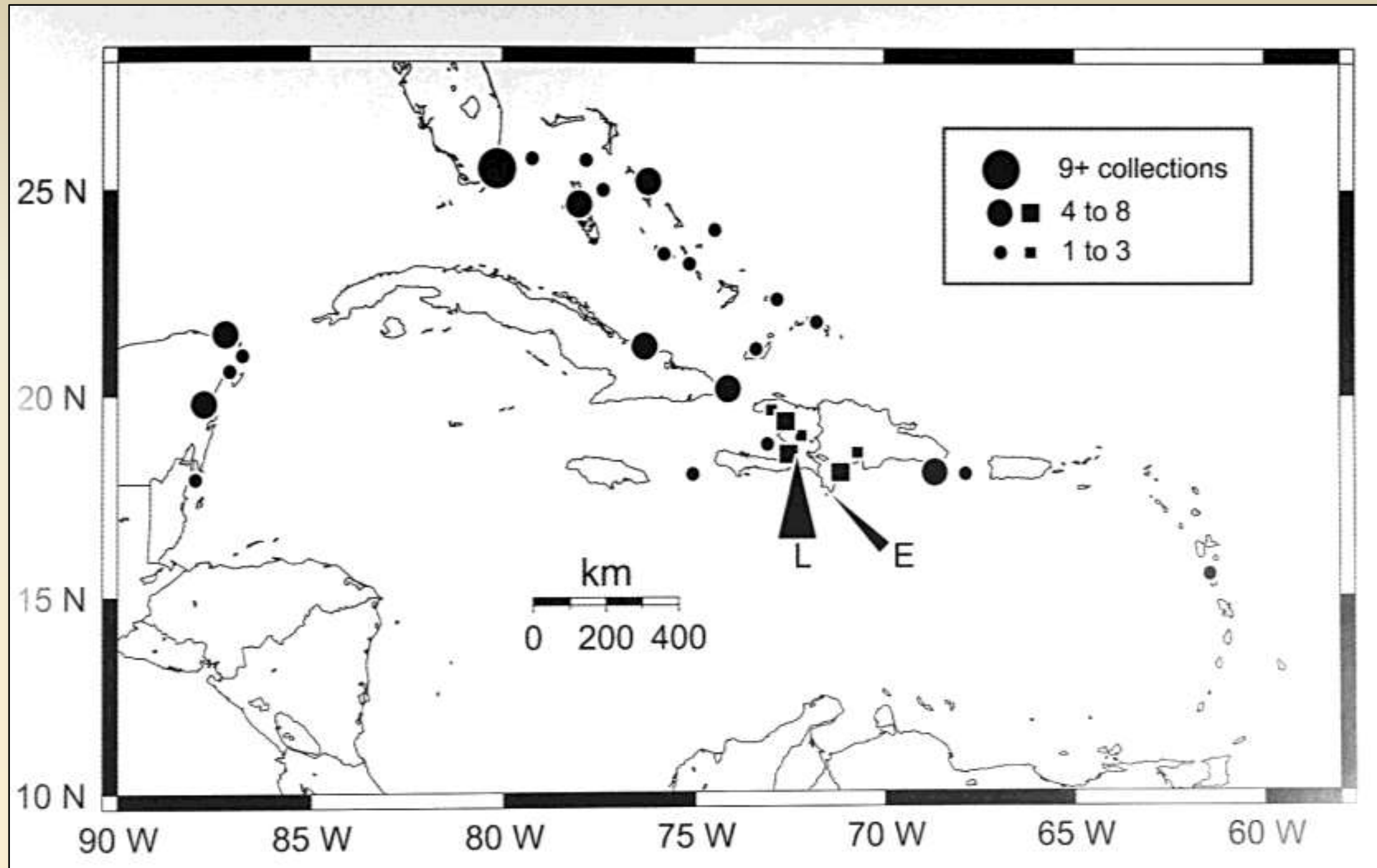
Pseudophoenix

P. lediniana, P. sargentii, P. vinifera, P. ekmanii

***Pseudophoenix lediniana* - Haiti**

***Pseudophoenix vinifera* - Domincan Republic**

Pseudophoenix Distribution – Scott Zona, 2002



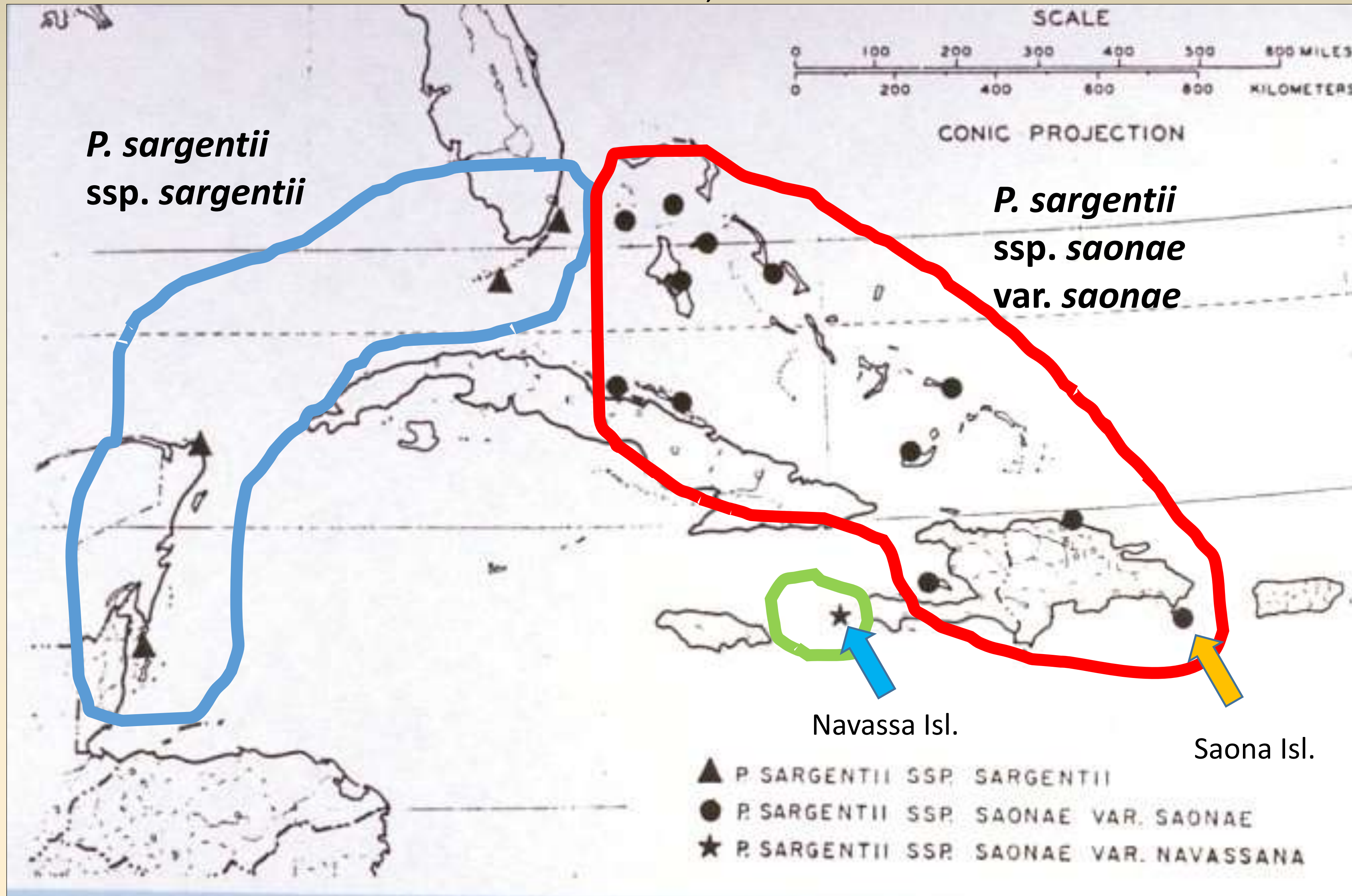
Did not recognize subspecies or varieties

Pseudophoenix ekmannii - Hispaniola

Pseudophoenix sargentii - Cherry Palm

Distribution of *Pseudophoenix sargentii*

from Read, 1968



The last remaining *P. sargentii* on Navassa Island?
Scott Zona, 2002

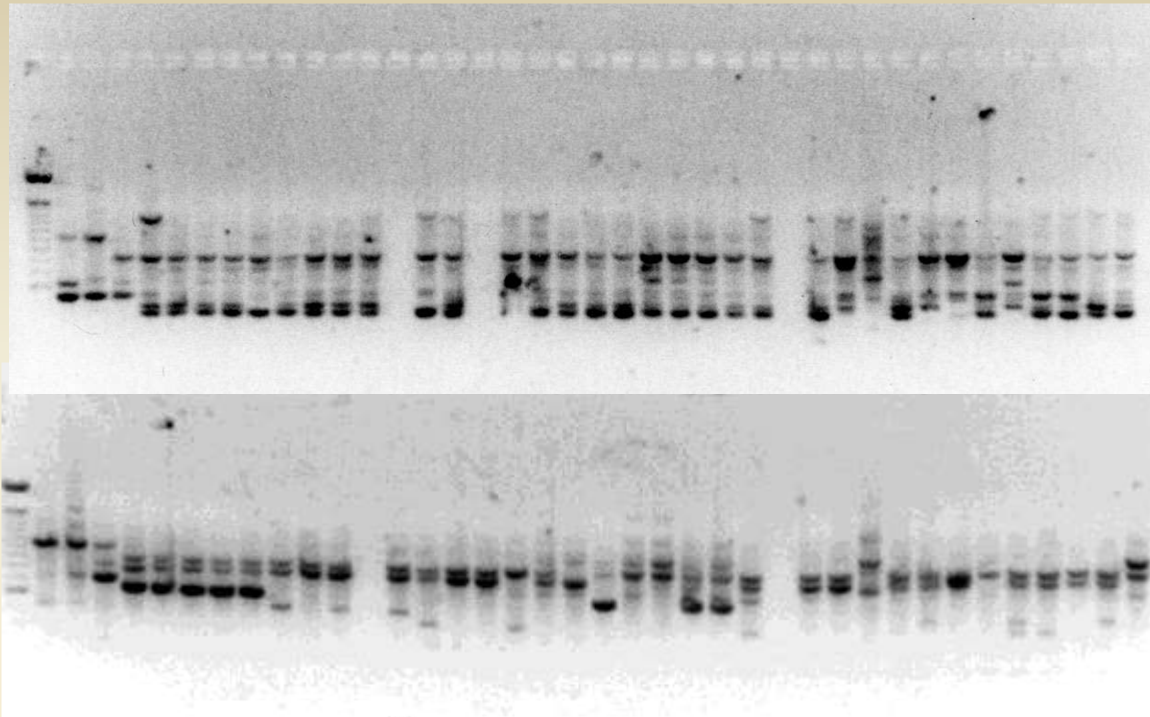
Hurricane Andrew, 1992

Eleuthera, Bahamas

Quintana Roo, Mexico

Pseudophoenix sargentii – Elliot Key, Florida

Pseudophoenix RAPDs

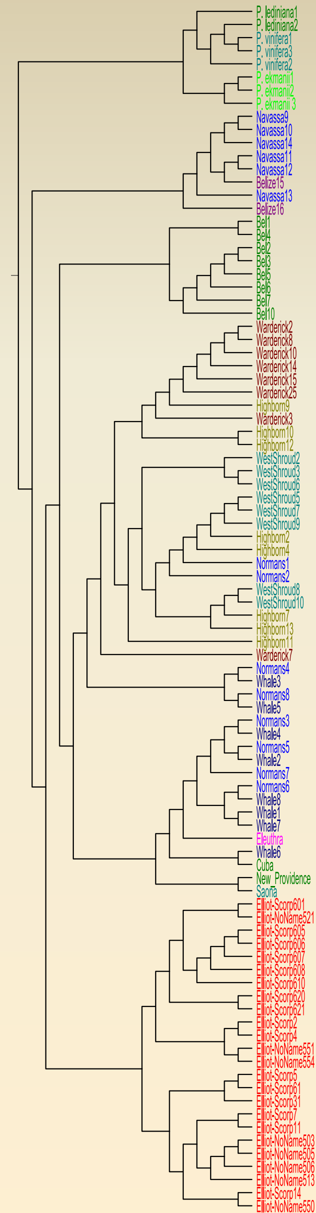


Primers
Opa 7
Opa 8
Opa 9
Opa 11
Opb 1

27 loci

PsNav01 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 1 1 1 0 1 0 0 1 0 0 1 0 0
PsNav01 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 1 1 1 0 1 0 0 1 0 0 1 0 0
PsNav02 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 1 1 1 0 1 0 0 1 0 0 1 0 0
PsNav02 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 1 1 1 0 1 0 0 1 0 0 1 0 0
PsNav03 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 0 1 1 0 1 0 0 1 0 0 1 0 0
PsNav03 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 0 1 1 0 1 0 0 1 0 0 1 0 0
PsNav04 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 0 1 1 0 1 0 0 1 0 0 1 0 0
PsNav04 1 1 0 0 1 1 1 0 1 0 0 1 1 0 1 0 1 1 0 1 0 0 1 0 0 1 0 0

UPGMA Clustering



Outgroup

P. lediniana, P. vinifera, P. ekmannii

Navassa Island

Belize

7% Polymorphic

Belize

14% Polymorphic

Bahamas

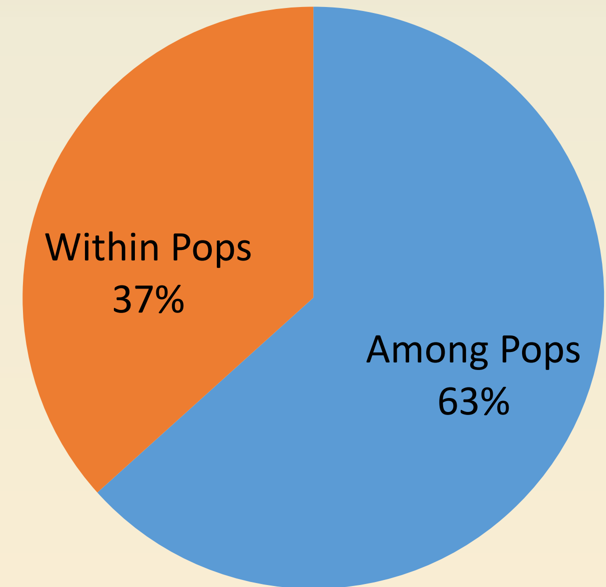
33% Polymorphic

Florida Keys

22% Polymorphic

Analysis of Molecular Variance AMOVA

Percentages of Molecular Variance



Within Populations 37%

Among Populations 63%

AMOVA (Analysis of Molecular Variance)

Method of estimating population differentiation directly from molecular data (e.g. RFLP, direct sequence data, or phylogenetic trees)

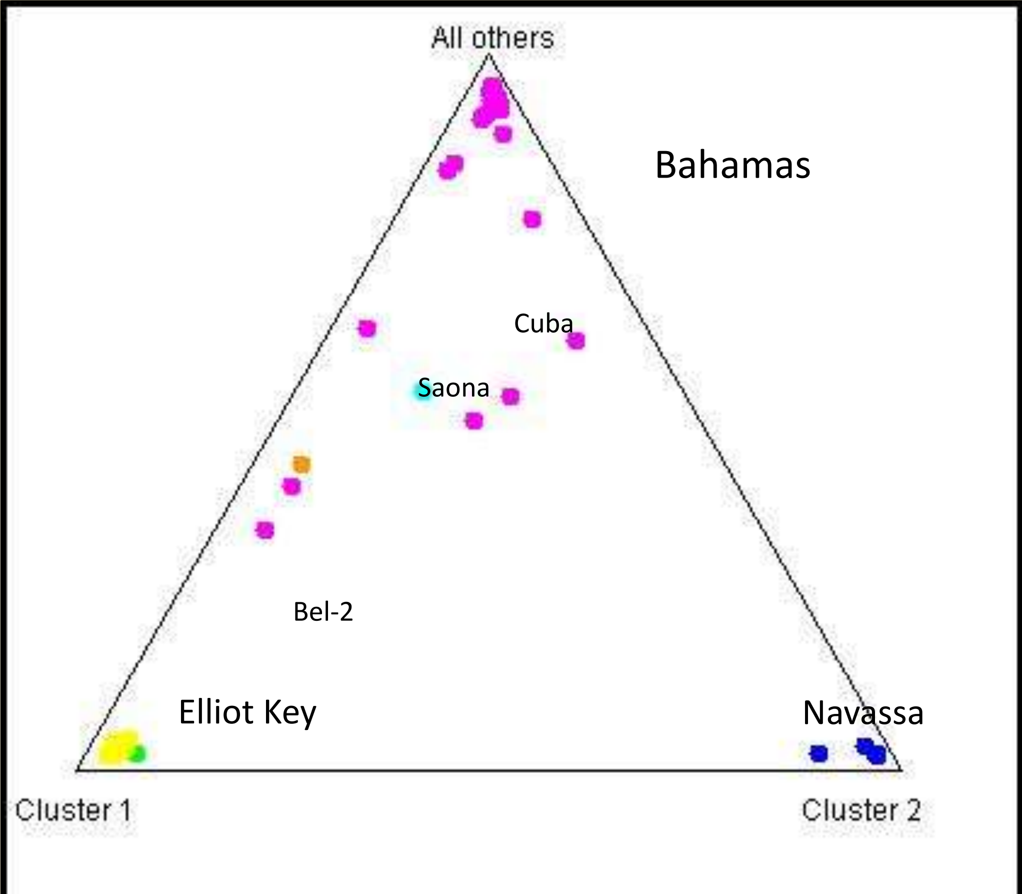
The variance components are used to calculate phi-statistics which are analogous to Wright's F-statistics

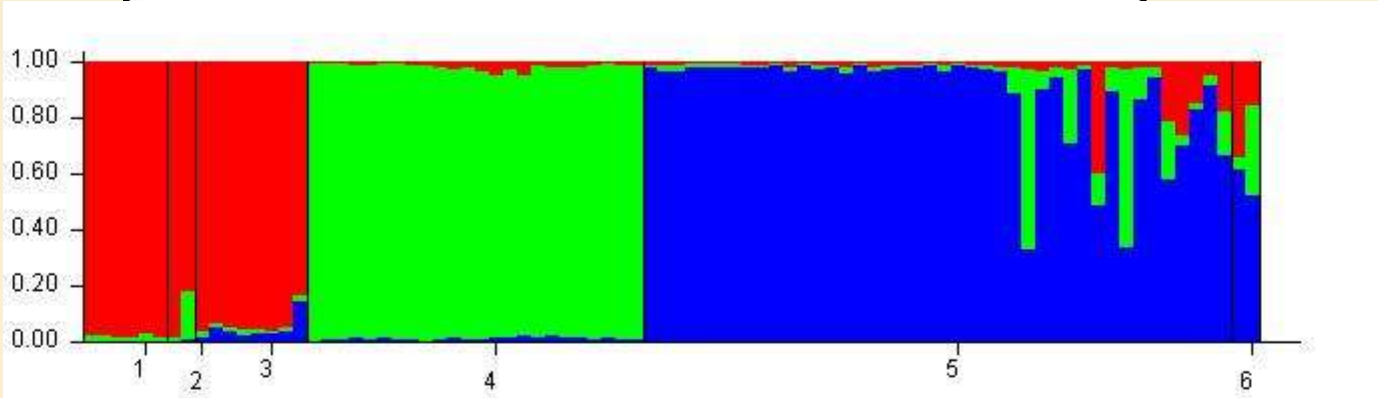
$$\Phi_{ST} = (\sigma^2_a + \sigma^2_b)/\sigma^2_T$$

Clustering Programs

Structure

K=3





Navassa Bel-1 Bel-2 Elliot Key Bahamas Cuba, Saona

Pseudophoenix sargentii
Summary RAPD Study

1. Population clusters are identified.
2. Subspecies do not match clusters.
3. Belize has a mixture of populations.
4. Bahamas populations most variable.
5. Elliot Key populations distinct.
6. Variation evenly distributed.

Next steps:
ISSR pilot study
AFLP pilot study
Develop microsatellite primers

Effective Population Size (N_e)

Effective population size gives a crude estimate of the average number of contributors to the next generation (N_e).

Always a fraction of the total population.

Some individuals will not produce offspring due to age, sterility, etc.

Of those that do, the number of progeny may vary.

A variety of ways of estimating (N_e) have been formulated.

Effective Population Size (N_e)

One that accounts for unequal sex ratios among breeding adults is:

$$N_e = \frac{4(N_M * N_F)}{N_M + N_F}$$

where N_M = number of males

N_F = number of females

Effective Population Size (N_e)

What is the effective population size (N_e) of one with 100 females and 10 males?

- Remember:

$$N_e = \frac{4(N_M * N_F)}{N_M + N_F}$$

where N_M = number of males

N_F = number of females

Effective Population Size (N_e)

What is the effective population size (N_e) of one with 100 females and 10 males?

$$N_e = \frac{4(10 * 100)}{10 + 100} = \frac{4000}{110} = 36$$

- Remember:

$$N_e = \frac{4(N_M * N_F)}{N_M + N_F}$$

where N_M = number of males

N_F = number of females

Microcycas calocoma in Natural Habitat

End