

# Applications of Genetics to Conservation Biology

## Molecular Taxonomy

Populations, Gene Flow, Phylogeography

Relatedness - Kinship, Paternity, Individual ID



# Conservation Biology

- Population biology
- Physiology
- Island biogeography
- Hazard evaluation
- Veterinary medicine
- Environmental monitoring
- Social science
- Natural Resources
- Policy
- Management
- Genetics

# Conservation Genetics

- Evolution (M,M,S,D)
- Systematics (Taxonomy)
- Small populations
- Population structure
- Inbreeding/Outbreeding
- Hybridization
- Genetic diversity
- Genetic management
- Reintroduction
- Species biology
- Forensics

# How can genetics minimize extinction?

- Understanding species biology
  - Relatedness (kinship, paternity, individual ID)
  - Gene flow (migration, dispersal, movement patterns)
- Molecular Systematics
  - Resolve taxonomic uncertainty
  - Resolve population structure (phylogeography)
  - Define management units
  - Identify populations of concern

# How can genetics minimize extinction?

## (continued)

- Detect and minimize inbreeding and loss of genetic diversity
- Detect and minimize hybridization
- Non-intrusive (non-invasive) sampling
- Identify best population for reintroduction
- Forensics

# Molecular Taxonomy

Using molecules (ie. DNA based techniques)  
for systematic study  
or to define taxonomic units  
(species, subspecies, ESUs and MUs)

# Molecular Taxonomy: Molecules versus Morphology

- Cryptic species (sibling species)
- Morphological variation without genetic variation

# Molecular Taxonomy: Conservation Relevance

- Unrecognized species may go extinct
- Incorrect species recognition
  - Non-optimal partition of management resources
  - Problems with hybridization
- Incorrect subspecies or population recognition
  - Not optimal partition of management resources
  - Problems with introgression

# Populations, Gene Flow, Phylogeography

- Compare genetic traits among populations
  - Resolve substructure among populations
- Infer movement patterns among individuals
  - Infer historical events for species

# Population, Gene Flow, Phylogeography: Conservation Relevance

- Determine units for management
- Heterozygosity estimates
  - Population bottlenecks
  - Hardy-Weinberg assumptions (mutation, migration, selection, drift, inbreeding)
- PVA (Population Viability Assessment)
- MVP (Minimum Viable Population Size)
- Effective population size
  - Number of breeding individuals

# Relatedness (Kinship, Paternity and Individual ID)

Application of molecular genetic techniques, using hypervariable, repetitive DNA (ie. microsatellites, minisatellites) to questions of kinship, paternity or individual ID

# Kinship, Paternity, Individual ID:

- Infer relatedness among individuals
  - First order, second order, etc.
- Infer paternity (maternity)
- Reproductive success (male, female)
- Interpret reproductive strategies
  - Monogamy, harem, female choice, etc.
- Interpret behaviors
  - Dispersal (male, female), care giving, others
- Individual ID
  - Populations size estimates
  - Forensics

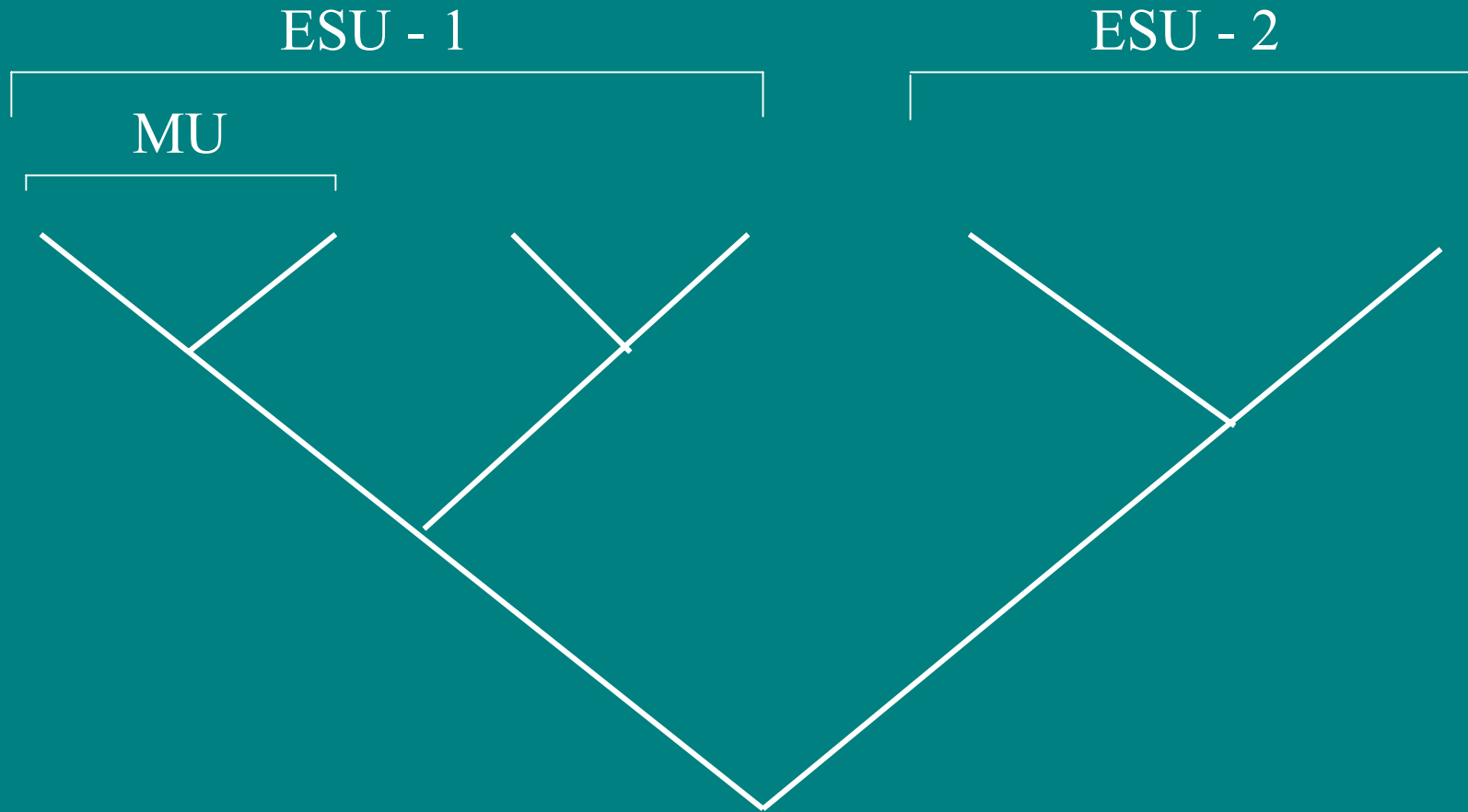
# Kinship, Paternity, Individual ID: Conservation Relevance

- Knowledge to aid management
  - Family structure
  - Reproductive strategy
  - Behavior
  - Dispersal
  - Inbreeding
  - Forensics/law enforcement

# Important Techniques: Phylogenetic Analysis

- Resolve evolutionary relationships (species, subspecies, populations, individuals)
- Tool used to determine Evolutionarily Significant Units (ESUs)
  - Also resolves Management Units (MUs)

# Phylogenetic tree with ESUs and MUs

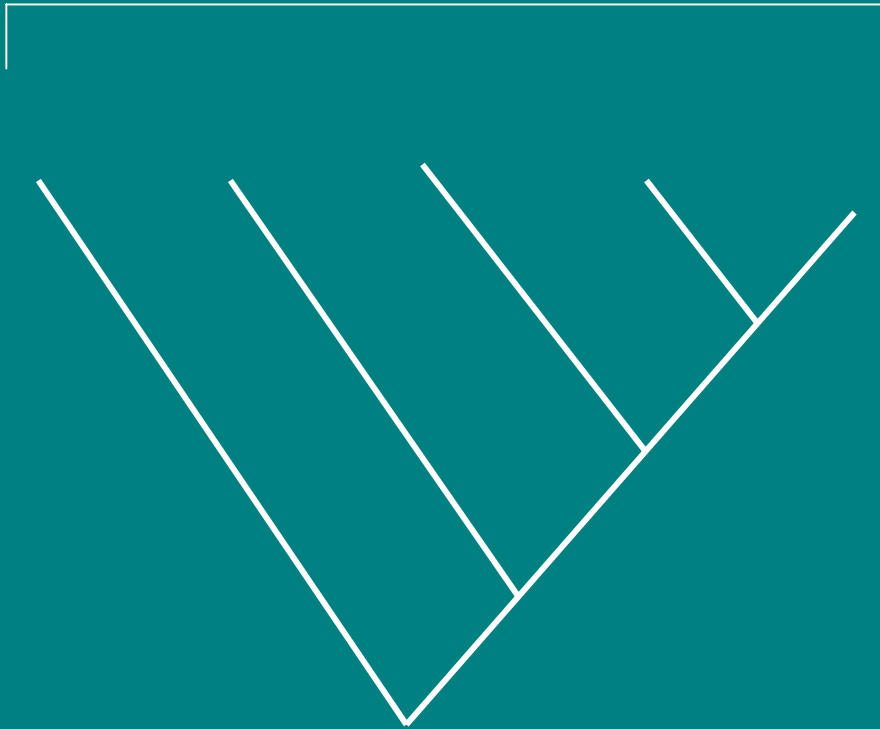


# Phylogenetic Analysis: Phylogeography

- Combines phylogeny with geographical locations of populations
- Three likely outcomes for geographical populations
  - Equally different branches of tree
  - Shallow structure
  - Monophyletic group

# Phylogenetic Analysis: Phylogeography

Equally Divergent



Shallow and Monophyletic



# Important Techniques: Non-Invasive Sampling

Samples are collected without  
disturbing the individual  
(includes scat, hair, feather etc.)

# Non-Invasive Sampling

- Allows sampling without disturbance to individual
- Rare or hard to capture species
- Examples (hair, scat, feathers, saliva/cheek swab, regurgitated pellets, dried blood, biopsy dart, museum tissues)

# Non-Invasive Sampling: Applications

- Species ID
- Individual ID
- Sex Determination (Sex Ratio)
- Gene Flow
- Previously described applications for genetics in conservation biology

# Non-Invasive Sampling: Pros and Cons

- Allow studies that are not possible by other methods
- Error/failure rates associated with having very minimal amount of DNA
- Use fresh tissues when possible to avoid potential errors

# Examples

- Taxonomy, Population Subdivision, Gene Flow, Phylogeography
  - Puma (cougar, mountain lion)
- Kinship and Paternity
  - Madagascar Fish-Eagle

# Subspecies Taxonomy, Phylogeography, Gene Flow: Puma (cougar, mountain lion)



32 *Puma*  
subspecies,  
as of the early  
1900s



# Objectives

- Does current population differentiation reflect
  - Trinomial descriptions?
  - Physical or ecological barriers?
  - Isolation by distance?
- Are current levels of genetic variation the same within each population?
- Does population structure and genetic variation reflect
  - Historic migrations?
  - Historic dispersals?
  - Historic bottlenecks?

Modern and  
 museum puma  
 samples collected,  
 total of 315



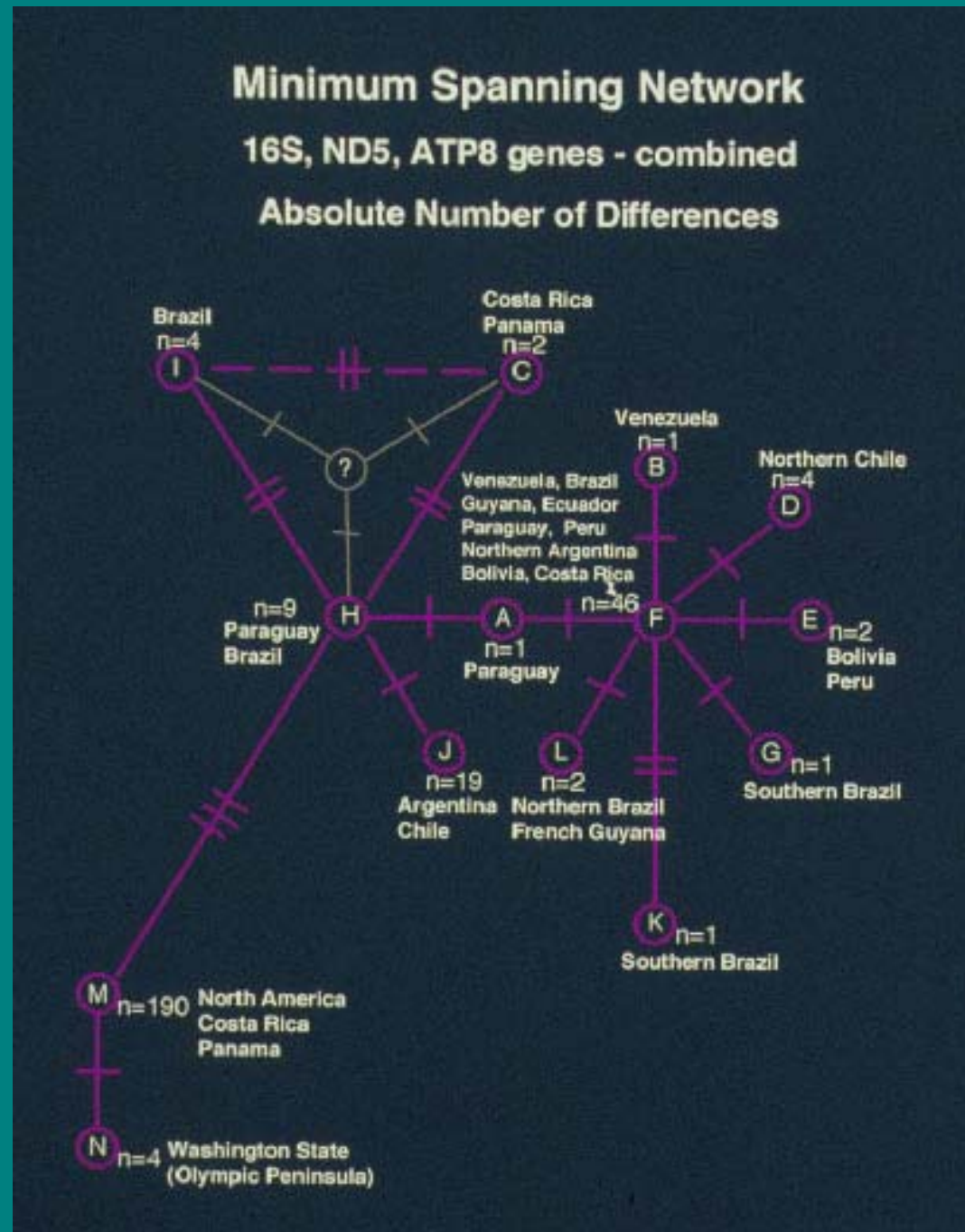
# Molecular Methods Used

- Mitochondrial gene sequencing
  - 16SrRNA
  - NADH-5
  - ATPase8
- Nuclear microsatellite length determination
  - 10 domestic cat microsatellite loci

# Mitochondrial DNA Haplotypes (in a geographical cline)



- Ancestral haplotypes
- 2 historical radiations
- NA is most recently founded population



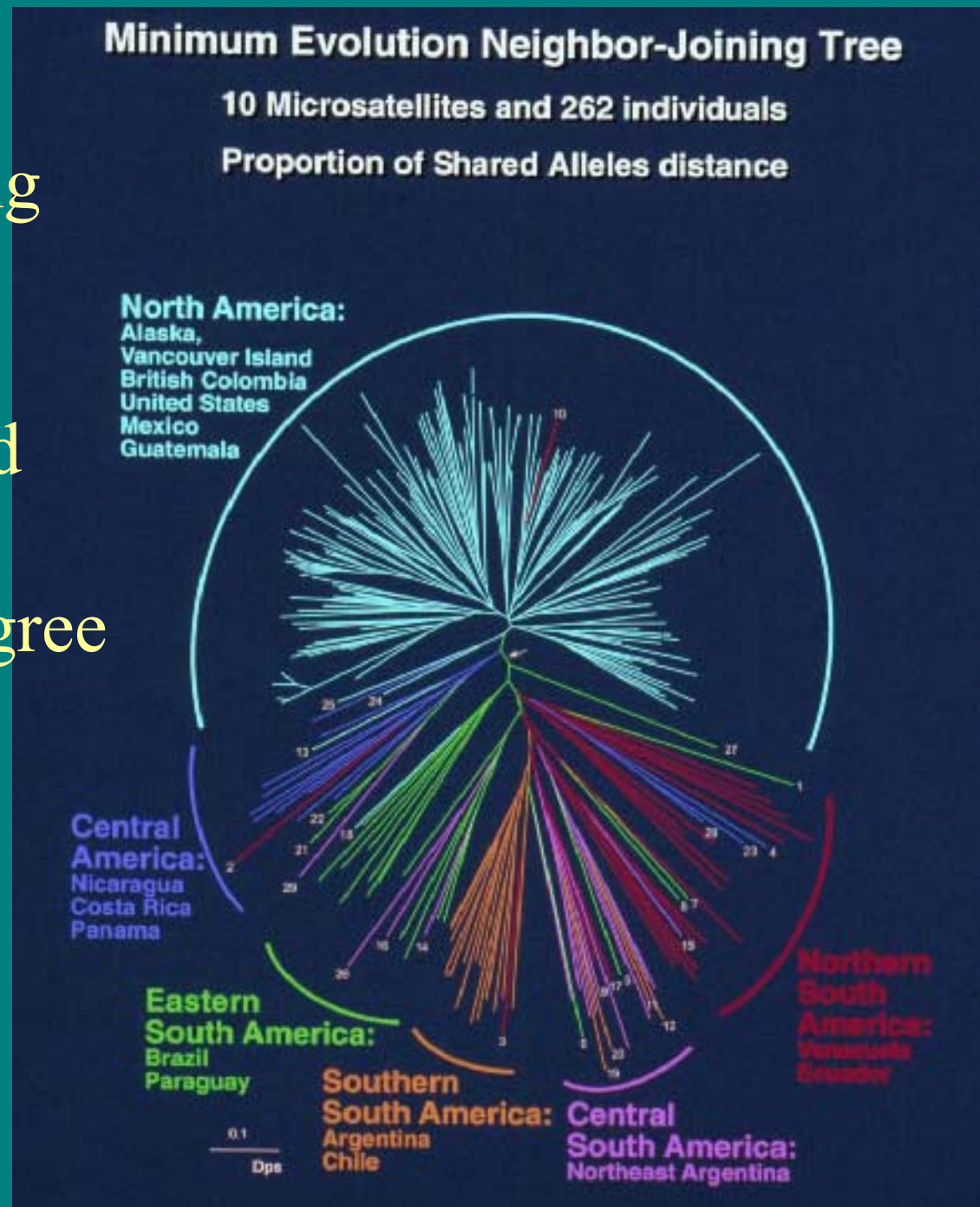
# Microsatellite Alleles at FCA008

Group	Subspecies	Tot no individ	Allele size																					
			134	136	138	140	142	144	146	148	150	152	154	156	158	160	162	164	166	168	170	172	174	176
NA	missoulensis	21											25						16	1				
*	oregonensis	14											26						2					
*	vancouverensis	6											12											
*	olympus	4											8											
*	californica	23											43						3					
*	kalibabensis	3											5						1					
*	hippolestes	12											19						5					
*	couguar	1											2											
*	browni	17											30						4					
*	azteca	33											59	1					6					
*	stanleyana	10											20											
*	coryi	6											12											
*	mayensis	11	3										18						1					
CA	costaricensis	13	9								1	7							3	3	1	2		
ESA	borbensis	2											1				1					2		
*	greeni	1												1					1					
*	acrocodia	12	1		1	2	2			1	1		6	6	1				1			2		
*	capricornensis	8	3		1				1		1	1	2	5	1				1			1		
NSA	bangsi	5	2			1				1	3	2									1			
*	concolor	5	1	1						1		1	4	2										
*	soderstromi	2											3			1								
*	incarum	5		1									4	1			1			3				
*	osgoodi	5		4									2	1					1	1		1		
CSA	cabreræ	10	2	3	1								2	6					4			1		1
*	hudsoni	7		1										4					3	2		2	2	
SSA	puma	3											1	1					1		2	1		
*	araucanus	4											1						1		1	3	2	
*	patagonica	6	2										3							2			5	
*	pearsoni	4											1	1					3			1	2	

-Geographic clustering  
of individuals

~Six groups identified

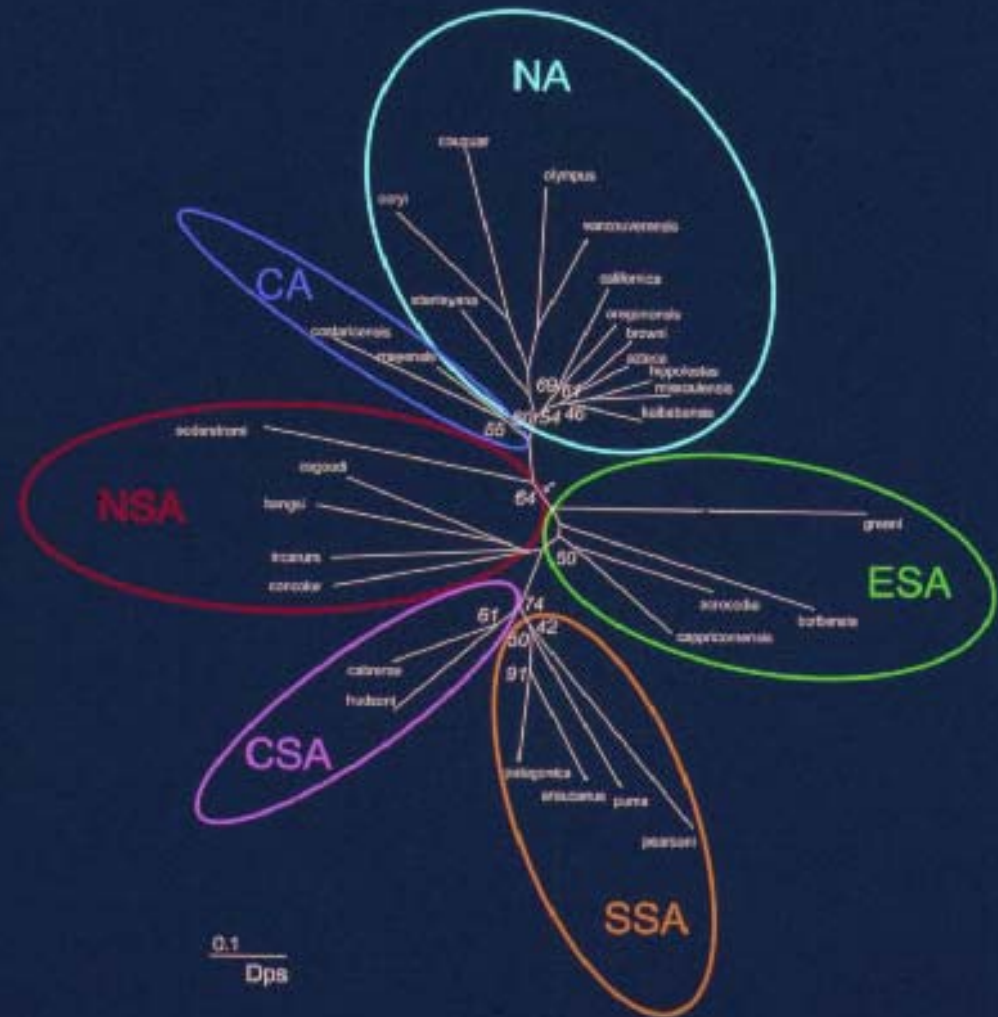
2 distance methods agree



# Minimum Evolution Neighbor-Joining Tree

10 Microsatellites and 29 Subspecies

Proportion of Shared Alleles distance



-Subspecies associate into same 6 groups

-Statistical support from bootstrap values

-2 distance methods agree

-6 groups identified using microsatellites

-mtDNA haplotypes overlaid onto map, supports 6 groups

-Location of 2 ancestral haplotypes



# Wright's Fst Estimates and Slatkin's Migration Estimates

## mtDNA

	NA	CA	ESA	NSA	CSA	SSA
NA	-	0.1	0.1	0.02	0.03	0.1
CA	*0.784	-	8.3	0.5	1.6	1.6
ESA	*0.815	0.057	-	0.8	2.3	2.2
NSA	*0.958	*0.492	0.384	-	4.2	0.5
CSA	*0.935	0.233	*0.177	*0.107	-	1.3
SSA	*0.835	0.240	*0.186	*0.526	*0.281	-

## microsatellites

	NA	CA	ESA	NSA	CSA	SSA
NA	-	4.0	4.4	8.0	2.2	0.9
CA	*0.110	-	2.3	3.5	3.5	1.2
ESA	*0.103	*0.179	-	15.7	4.8	1.0
NSA	*0.059	*0.126	*0.031	-	6.0	1.1
CSA	*0.186	*0.126	*0.094	*0.077	-	2.4
SSA	*0.367	*0.288	*0.330	*0.316	*0.172	-

Major restrictions  
to gene flow:

- Amazon River
- Rio Parana
- Rio Negro
- Andes?



# Fossil Record versus Molecular Divergence Estimates

- Oldest fossils in North and South America date to 0.2-0.3 Mya
- From mtDNA mutation rate of 1.15%/My, divergence for extant puma lineages is 390,000 years ago
- From mutation rate of  $5 \times 10^{-9}$ /yr for microsatellite flanking regions, pumas are less than 230,000 years old

# Historical Inferences

- Extant pumas originated in Brazilian Highlands (ancestral haplotypes)
- Fossil record suggests dispersal to NA soon after the common origin in Brazil
- 2 historical radiation events occurred

-Ancestor to puma crosses  
land-bridge ~2-3 Mya

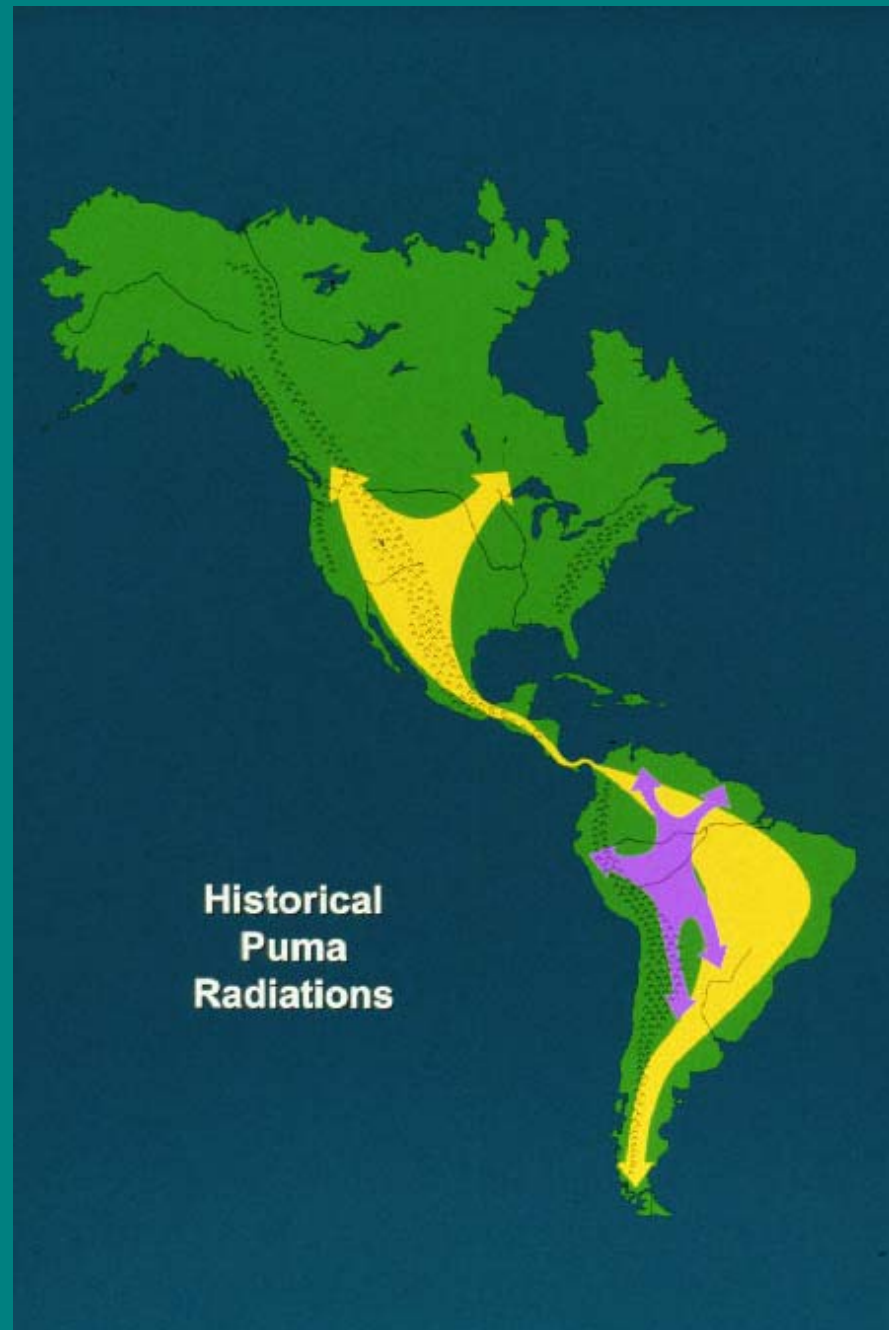
-Puma origin in Brazillian  
Highlands ~300,000 ya



2 Major historical radiations

-One locally distributed

-One broad ranging



# Puma Bottlenecks

- Subspecies-level
  - North America low overall genetic variation
- Population-level
  - Florida monomorphic at 8/10 microsatellite loci
  - Olympic Peninsula and Vancouver Island, monomorphic at 5/10 microsatellite loci

# Puma Conclusions

- Pumas originated in Brazil approximately 300,000 years ago
- Possible extirpation and recolonization in North America (Pleistocene age?)
- Molecular data does not support 32 subdivisions, instead 6 groups
- Pumas are fairly panmictic within 6 groups

# Conservation Implications

- Maintain habitat connectivity within 6 large groups
- Management should consider effects of bottlenecked populations
- Eastern cougar, Florida panther and Yuma puma management take into account revised subspecies

# Paternity Application: Madagascar Fish-Eagle

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# Antsalova wetland region of western Madagascar



# Background and Methods

- Endangered eagle in Madagascar
- 2-3 males, and one female, attend each nest (cooperative breeding)
- Dominance hierarchy among males at nest
- Multi-locus DNA fingerprinting used to infer potential fathers, and estimate adult relatedness among and between nests



# Conclusions

- At all nests with young ( $n=3$ ), subordinate males fathered all offspring
- Dominant males have higher energy investment
- Dominant male may be first-order relative to adult female
- One dominant male may have full-sib within nest

# Conservation Implications

- Preliminary results, more samples needed
- Advantageous to raise young of full-sib
- Conservation management may consider
  - Adult relatedness in area
  - Number of males that successfully breed