

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 partitioning of management resources
 - Problems with hybridization
- Incorrect subspecies or population recognition
 - Not optimal partitioning 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

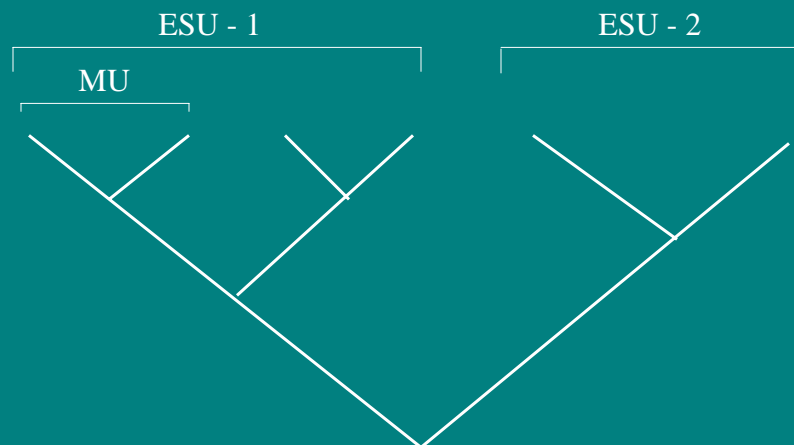
Kinship, Paternity, Individual ID: Conservation Relevance

- 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)

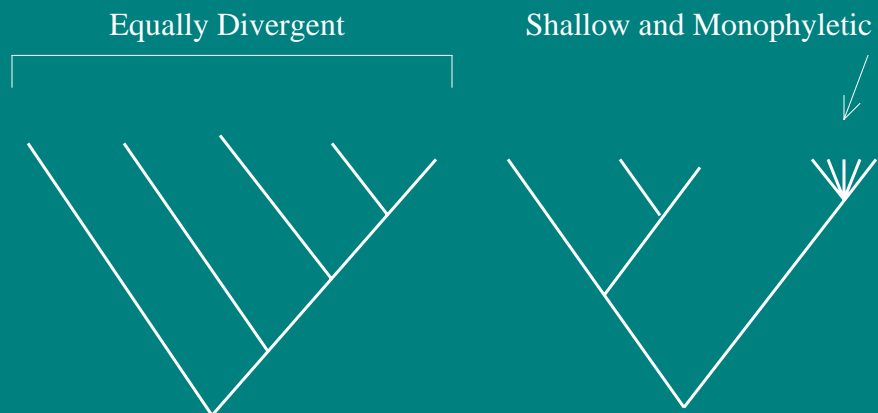
Phylogenetic tree with ESUs and MUs



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



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
- Species Inventory
 - Mammals at SNP using non-invasive methods

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



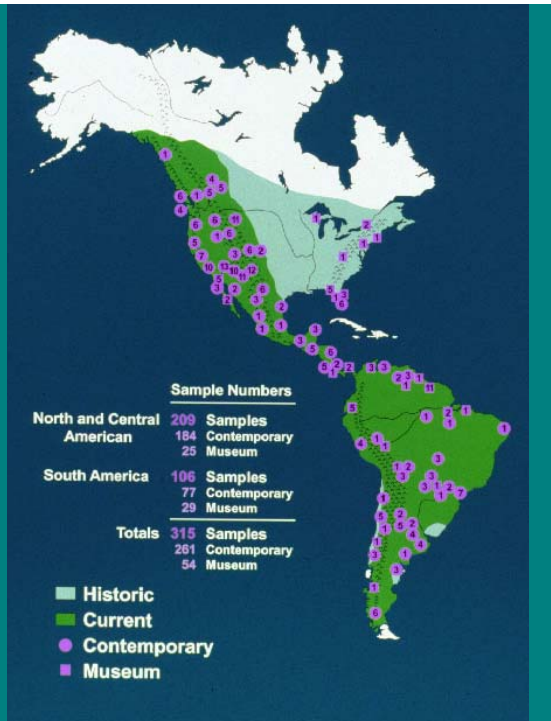
32 Puma subspecies,
as of the early
1900s



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?

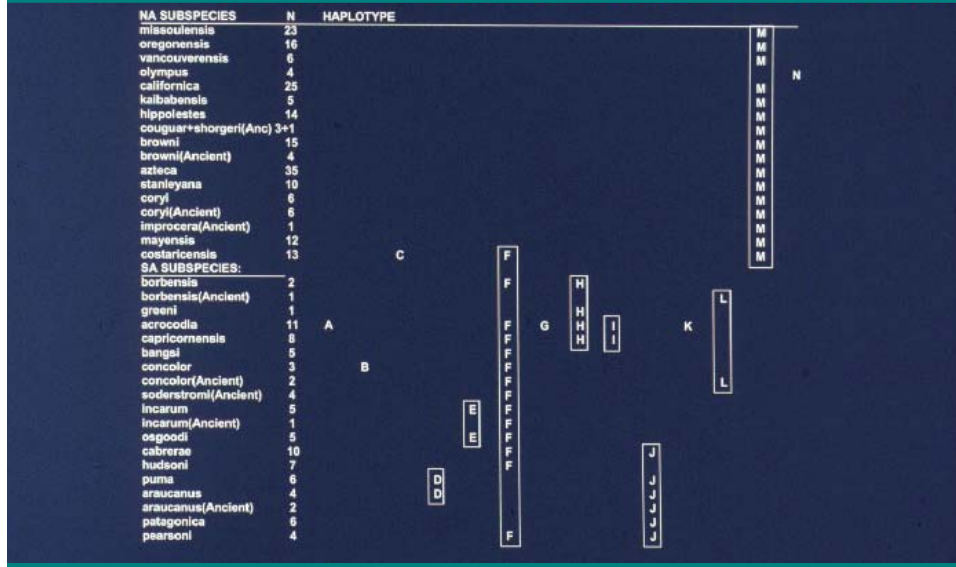
Modern and
museum puma
samples collected,
total of 315



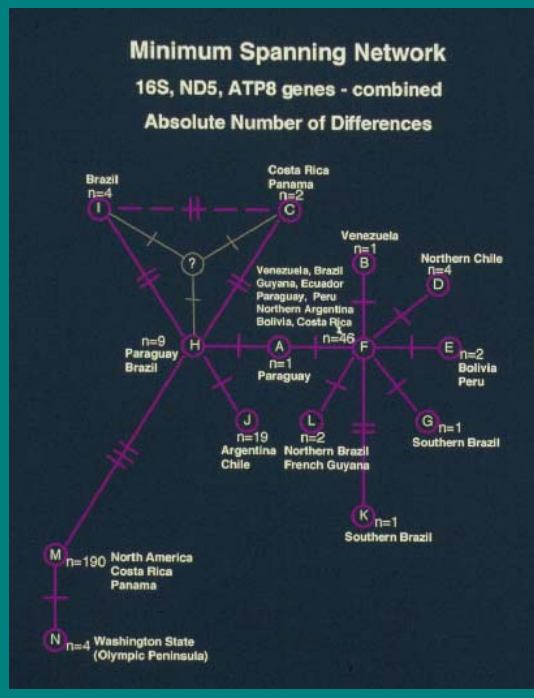
Molecular Methods Used

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

Mitochondrial DNA Haplotypes (in a geographical cline)



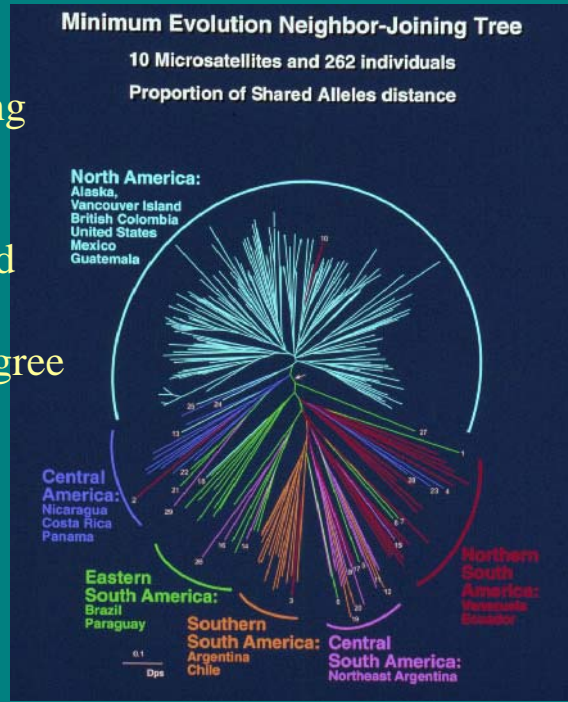
- Ancestral haplotypes
- 2 historical radiations
- NA is most recently founded population



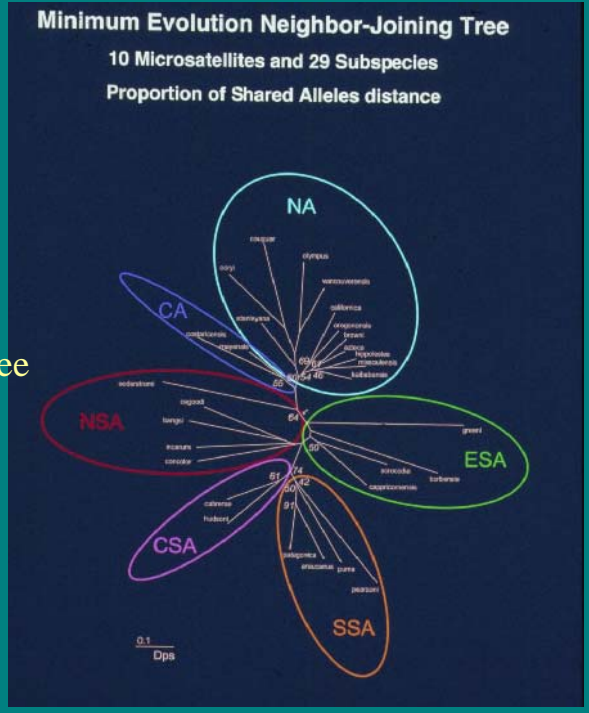
Microsatellite Alleles at FCA008

Group	Subspecies	Tot no individu	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	5											12											
	* olympus	4											8											
	* californica	23											43						3					
	* kalibabensis	3											5						1					
	* hippolestes	12											19						5					
	* cougar	1											2											
	* brownii	17											30						4					
	* azteca	33											59	1					6					
	* stanleyana	10											20											
* coryi	6											12												
* mayensis	11	3										18							1					
CA	coastaricensis	13	9									1	7						3	3	1	2		
ESA	borbensis	2												1				1				2		
	* greenii	1												1				1						
	* acrocodia	12	1		1	2	2			1	1	1		6	6	1		1				2		
	* capricornensis	8	3		1				1		1	1		2	5			1				1		
NSA	bangsi	5	2			1															1			
	* concolor	5	1	1									4	2										
	* soderstromi	2							1		3	2												
	* incarum	5			1								3		1									
	* osgoodi	5		4									2	1		1			3	1	1		1	
CSA	cabrerae	10	2	3	1								2	6					4			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											3						2		1	3	5	
	* pearsoni	4		2									1	1					3			1	2	

- Geographic clustering of individuals
- ~Six groups identified
- 2 distance methods agree



- Subspecies associate into same 6 groups
- Statistical support from bootstrap values
- 2 distance methods agree



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	-

Summary:

- 6 groups identified using microsatellites
- mtDNA haplotypes overlaid onto map, supports 6 groups
- Location of 2 ancestral haplotypes

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 5×10^{-9} /yr for microsatellite flanking regions, pumas are less than 230,000 years old

Historical Inferences

- Extant pumas originated in Brazillian 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

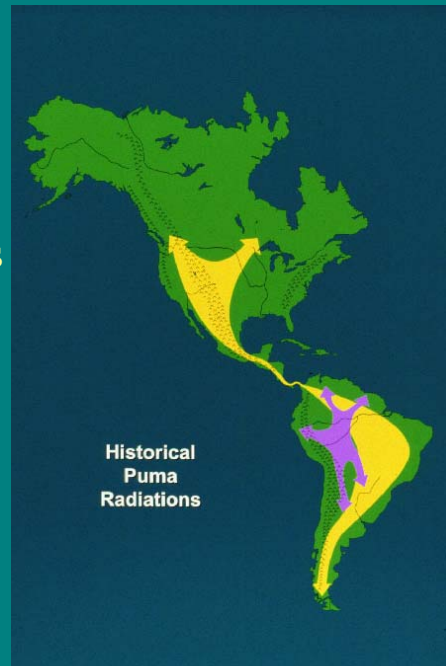
-Puma origin in Brazillian Highlands ~300,000 ya



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

DNA Fingerprinting Alleles at 4 nests (6 representative bands out of 34)

INDIVIDUAL #	ANK 4			BEFO 2				SOAM 2				BEFO 3					
	F	αM	βM	F	αM	βM	NSL	F	αM	βM	γM	NSL	NSL	F	$\alpha M?$	$\alpha M?$	JUV
	113	31	34	121	118	8	47	103	5	136	30	68	00	6	150	48	128
BANDS																	
H 16.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H 10.7				+			+				+	+					+
H 8.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H 7.3			+	+	+	+	+	+	+	+	+	+		+	+		+
H 6.5			+			+	+							+			+
H 6.0			+			+	+		+								+

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

Species Inventory at Saguaro NP

Assess presence of mammals by
utilizing DNA found in scat samples
and
compare with remote camera data

Methods

- Remote cameras to capture photographic evidence of mammals present
 - Drawback is small mammals
- Collect scat along several transects in park
 - Extract DNA from surface of scat
- If scat is from a carnivore, collect bone samples from scat
 - Extract DNA from bones

Results

- Attained species ID from 50% of the scats
- Attained species ID from 75% of bones
- Added many small mammals to the inventory made from photographic data, larger mammals were captured accurately in photographs

Conservation Implications

- Aid the assessing biodiversity present
 - Area not examined yet
 - Assessment after habitat perturbation
- Diet information from various carnivores

