

Florida Panther

Phylogeography/Molecular Taxonomy



- Taxonomy (species and subspecies level)
- Phylogeography (identify ESUs and MUs)

Melanie Culver – Florida Panther Recovery Meeting May 19, 2015

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?

Subspecies Criteria

- Share unique geographical habitat
- Share unique natural history
- Share phylogenetically concordant characters
- Probably, share recognizable genetic differences in the absence of gene flow

Modern and
 museum puma
 samples collected,
 total of 315



Molecular Methods Used

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

Characteristics of Mitochondrial DNA

- Haploid genome
- Maternally inherited
- 37 genes and one non-coding region
- Rapid evolutionary rate, relative to nuclear genes

Mitochondrial DNA Haplotypes

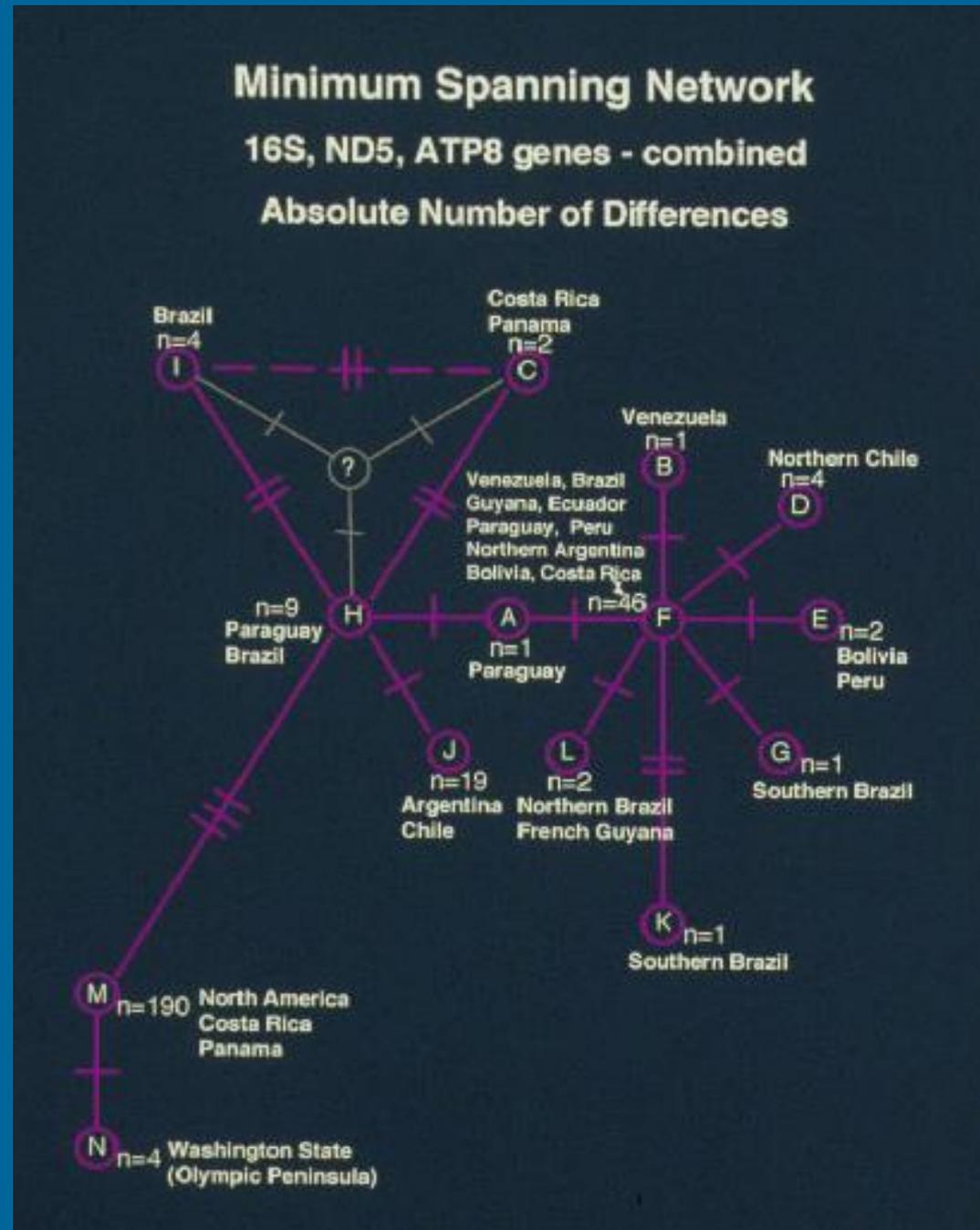
mtDNA haplotypes found in each subspecies, listed north to south



-Ancestral haplotypes

-2 historical radiations

-NA is most recently founded population



Characteristics of Microsatellite DNA

- Variation in number of repeat units
 - Highly polymorphic
- Non-coding DNA regions
 - Very rapid evolutionary rate

Microsatellite Alleles

Allele distribution across subspecies for microsatellite locus FCA008

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	8											12											
	* olympus	4											8											
	" californica	23											43							3				
	" kaibabensis	3											5							1				
	" hippolestes	12											19							5				
	" cougar	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			
NSA	bangsi	5	2		1					1	3	2									1			
	* concolor	5	1	1					1		3	2												
	* soderstromi	2							1			1	4	2										
	* incarum	5		1									3	2		1								
	* osgoodi	5		4									4	1		1			3					
CSA	cabreriae	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	2										3						2				5	
	" pearsoni	4											1	1					3			1	2	

-Geographic clustering
of individuals

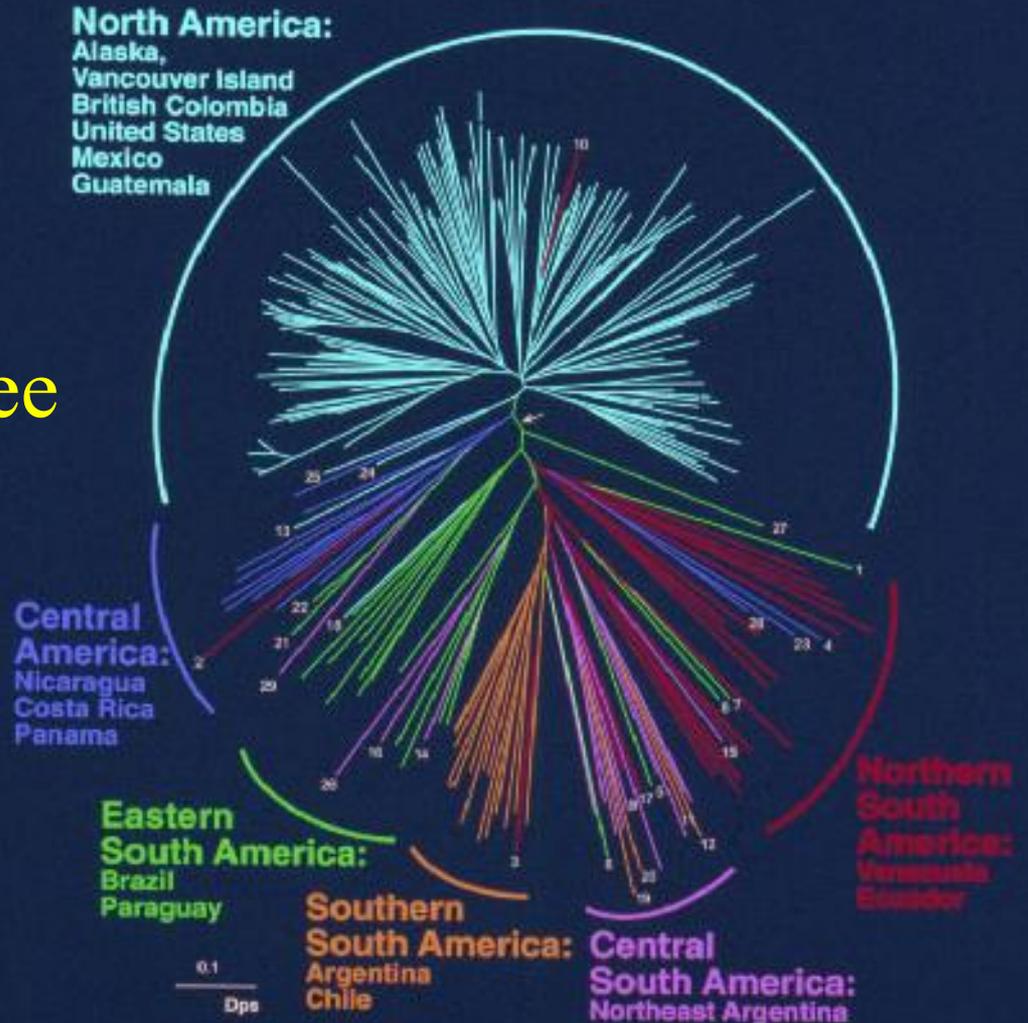
~Six groups identified

2 distance methods agree

Minimum Evolution Neighbor-Joining Tree

10 Microsatellites and 262 individuals

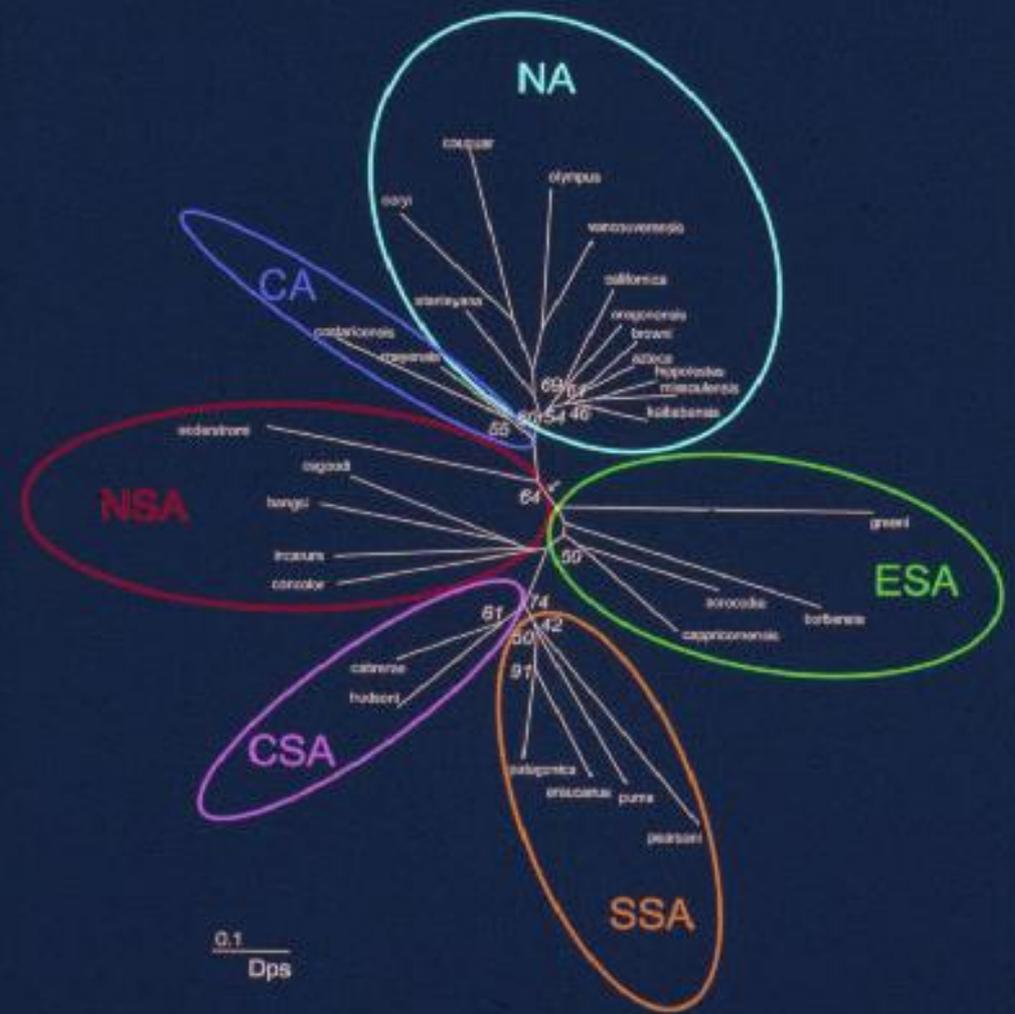
Proportion of Shared Alleles distance



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

Criteria for Phylogeographic Unit

- Phylogenetic clustering of mtDNA haplotypes and microsatellite alleles
- Spatial distribution of mtDNA haplotypes and microsatellite alleles
- Statistical measures which describe population structure

Spatial Clustering

- 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?



Wright's F_{ST} 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	-

Subspecies and Unique Characters for each Phylogeographic Group

Group	Subspecies (oldest available name)	No. unique mtDNA sites	No. unique mtDNA haps	No. unique micrisat. alleles
NA	<i>P. c. azteca</i>	1	1	5
	<i>P. c. browni</i>	(site 12908)	(N)	(104 at FCA043)
	<i>P. c. californica</i>			(252 at FCA082)
	<i>P. c. coryi</i>			(95,127 at FCA090)
	<i>P. c. cougar</i> (Kerr 1792)			(152 at FCA117)
	<i>P. c. hippolestes</i>			
	<i>P. c. improcera</i>			
	<i>P. c. kalbabensis</i>			
	<i>P. c. mayensis</i>			
	<i>P. c. missoulensis</i>			
	<i>P. c. olympus</i>			
	<i>P. c. oregonensis</i>			
	<i>P. c. schorgeri</i>			
	<i>P. c. stanleyana</i>			
<i>P. c. vancouverensis</i>				
CA	<i>P. c. costaricensis</i> (Merriam 1901)	1 (site 8630)	1 (C)	2 (236 at FCA082, 223 at FCA166)
ESA	<i>P. c. acrocodia</i>	3	5	5
	<i>P. c. borbensis</i>	(site 3063)	(A,H,G,I,K)	(140,146,150,164 at FCA008)
	<i>P. c. capricornensis</i> (Merriam 1901)	(site 12723)	**including both	(120 at FCA043)
	<i>P. c. greeni</i>	(site 12819)	ancestral types	
NSA	<i>P. c. anthonyi</i> *	2	2	5
	<i>P. c. bangsi</i>	(site 12834)	(B,E)	(162 at FCA008)
	<i>P. c. concolor</i> (Linne 1771)	(site 12840)		(124 at FCA035)
	<i>P. c. incarum</i>			(142 at FCA043)
	<i>P. c. osgoodi</i>			(221 at FCA166)
	<i>P. c. soderstromi</i>			(251 at FCA249)
CSA	<i>P. c. cabreræ</i> (Pocock 1940)	0	0	1
	<i>P. c. hudsoni</i>			(176 at FCA008)
SSA	<i>P. c. araucanus</i>	1	1	1
	<i>P. c. patagonica</i>	(site 12809)	(D)	(239 at FCA249)
	<i>P. c. pearsoni</i>			
	<i>P. c. puma</i> (Molina 1782)			

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

Future studies.....

- Next Generation Genetic Markers

- What is a Single Nucleotide Polymorphism (SNP)?

- What are important features

- Smaller in size (number of base pairs analyzed)
- More numerous in genome - analyze a few or >1 million in one reaction
- Easily comparable across labs

Bob	CATGCTAGCTAGCTGTCATCGACTGTAC
Melanie	CATGCTAGCTAGCTGTCATCGACTGTAC
Ashwin	CATGCTAGCTAGCTGACATCGACTGTAC



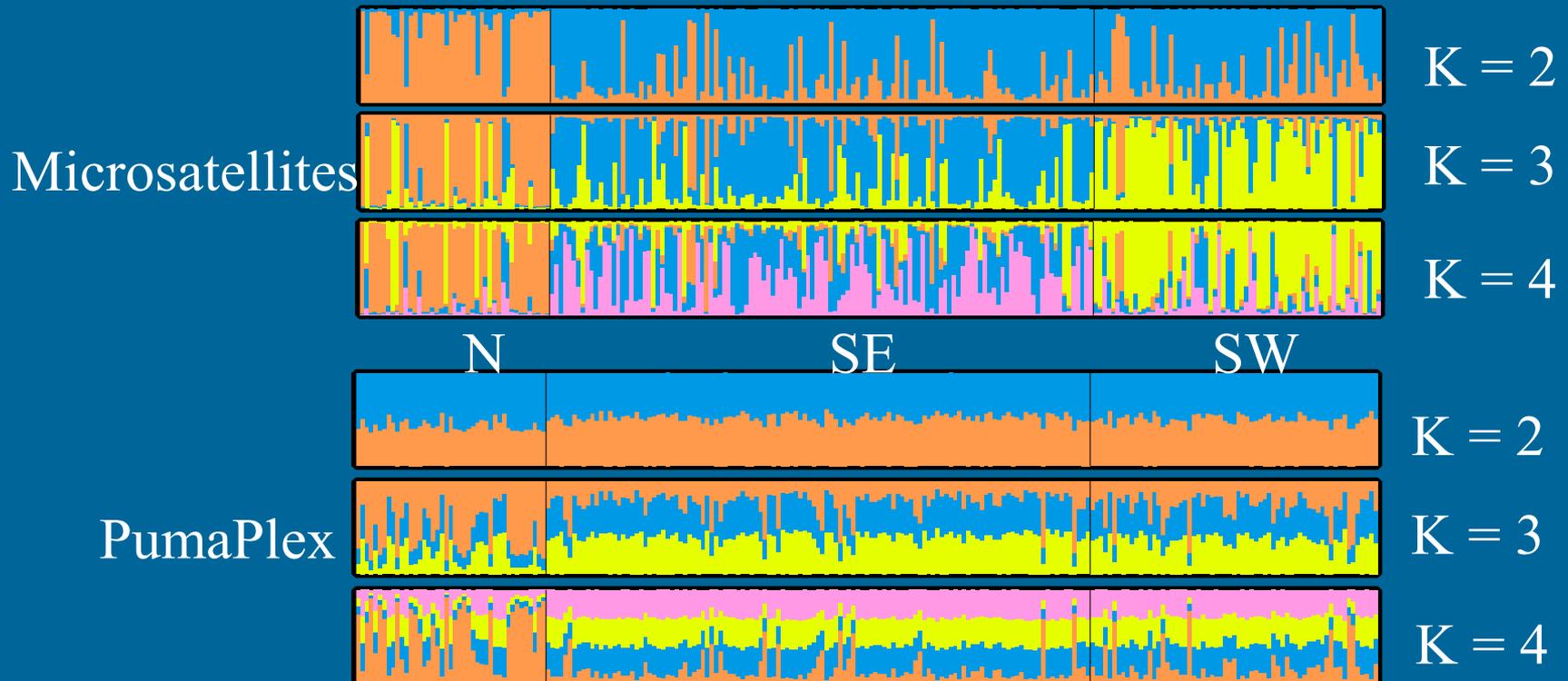
SNP

Goals for PumaPlex SNP set

- Develop a genetic tool for pumas that is:
 - Fast, high-throughput, and inexpensive
 - As powerful as current genetic analyses
 - Genotyping success and population genetic statistics
 - Especially in scat
 - Comparable across laboratories

Fine Scale Population Structure

- Bayesian structure analyses at $k = 2-4$ populations
- With 27 SNPs, PumaPlex lacks fine scale resolution
- Now have ~ 80 SNPs, aiming for 120 SNPs



What are noninvasive samples?

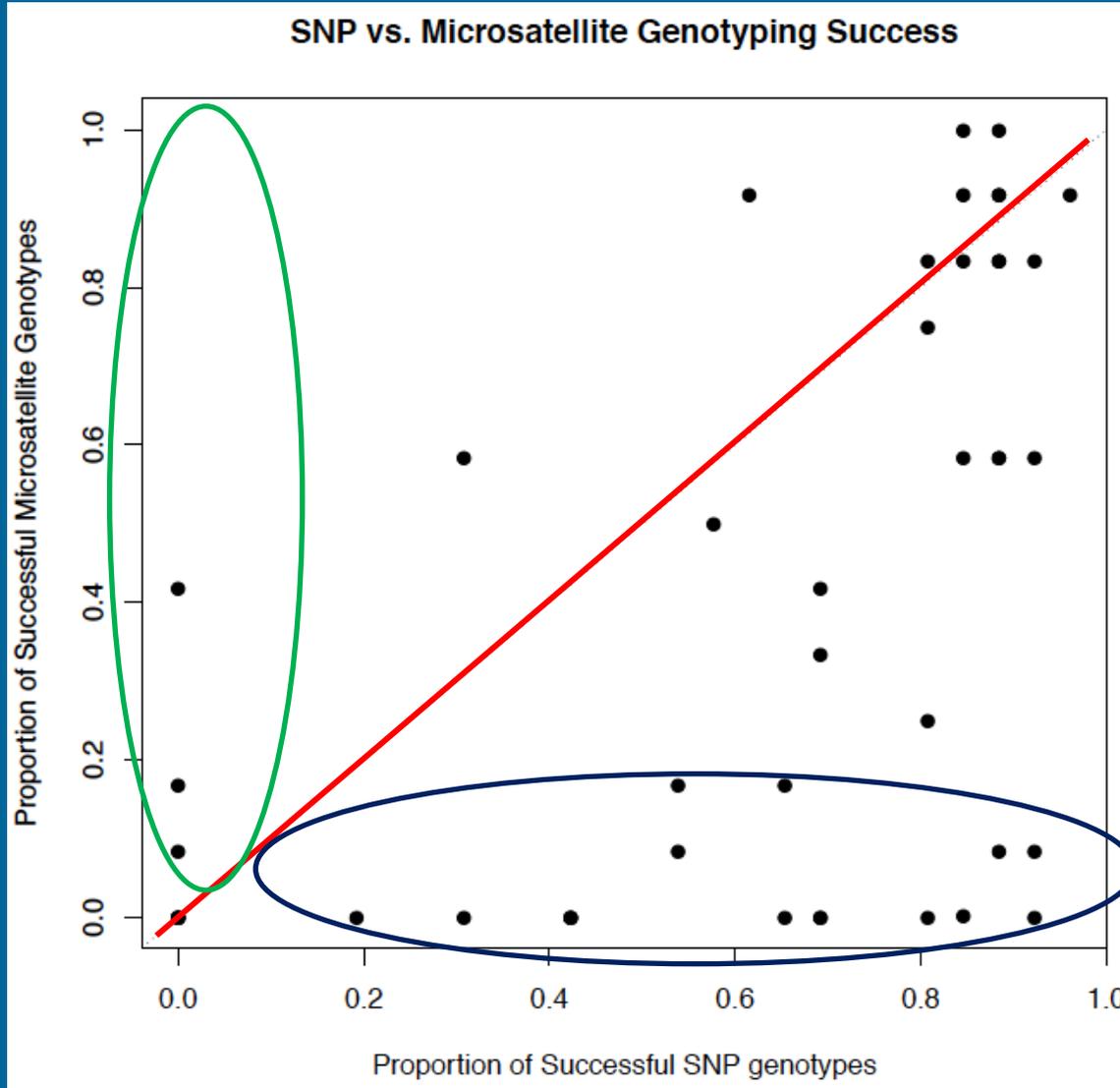


Traditional genetic markers for noninvasive samples

- Mitochondrial DNA
 - High amplification success
 - but not very polymorphic
- Microsatellites
 - Highly polymorphic
 - But reduced amplification success
 - Also difficult to compare across labs
- SNPs.....?

Genotyping Success of SNPs vs Microsatellites in Scats

Genotyped
More
Successfully
with
Microsats



Genotyped
More
Successfully
with SNPs

Efficiency

- Speed
 - Microsatellites: months
 - PumaPlex: 2 day - week
- Costs (including labor)
 - Microsatellites: ~\$120/sample for scat
 - PumaPlex: ~\$10/sample (\$40 once expanded)

Questions?

