Using Principles of Conservation Genetics to Inform Propagation

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Use of Propagation for Conservation

- Save species from extinction
- Reintroduce extirpated populations
- Augment extant populations
- Provide individuals for research / education

Modified from IUCN (1987)

Goals of Propagation

Create populations that:

- survive in captivity
- have a high probability of survival in the wild
- retain evolutionary potential

Problem

How do we

maximize “good” variation

increased survival in the wild

maintenance of evolutionary potential

while

minimizing “bad” variation?

loss of variation

adaptation to captivity

Population Genetics Refresher

Gene: nucleotide sequence coding for a protein

Allele: one version of a gene

- uniparental: haploid
- biparental: diploid
- homozygous vs. heterozygous

Locus: physical location on a chromosome

Population Genetics Refresher

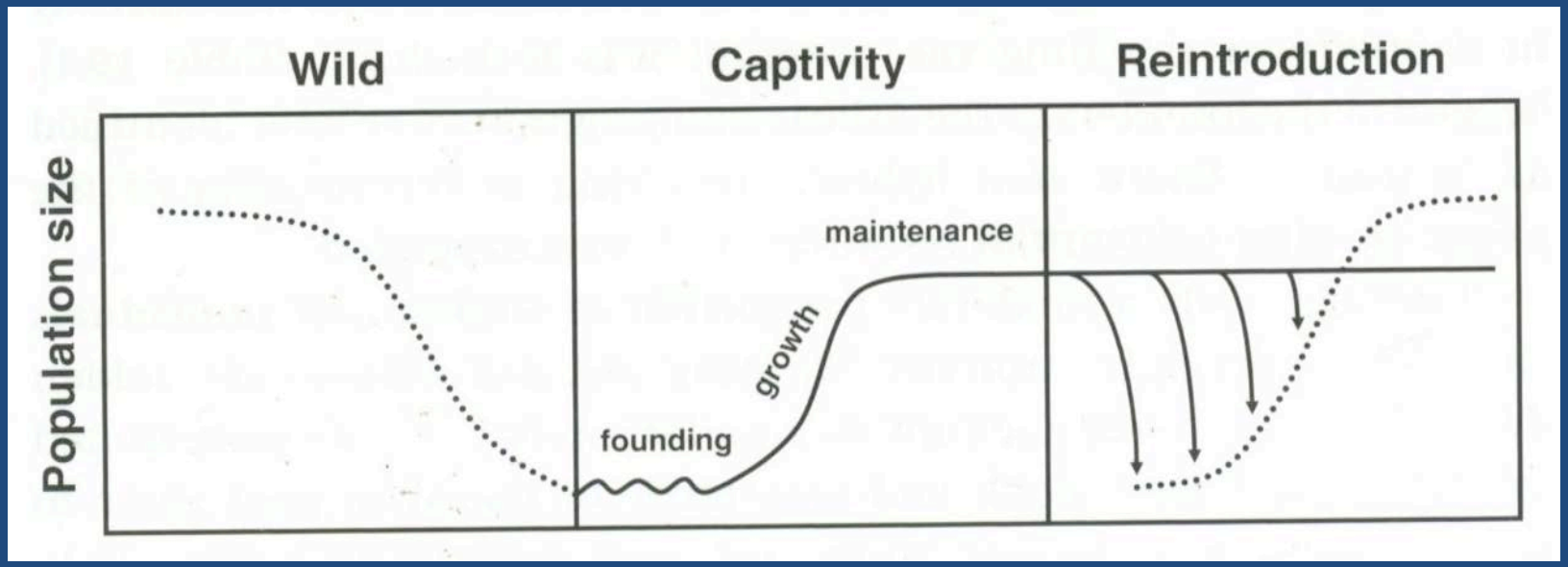
Mutation: change in nucleotide sequence

Population Genetics Refresher

Evolution: change in allele frequency over time

Hardy-Weinberg equilibrium = no change IF

- No mutation
- Infinite population size
- Random mating
- Closed population
- No selection



Modified from Frankham *et al.* (2010), page 433

Six stages of captive breeding

1. Observe decline, characterize wild population(s)
2. Found captive population(s)
3. Expand captive population(s)
4. Manage captive population(s) over generations
5. Choose individuals for reintroduction
6. Manage reintroduced population(s)

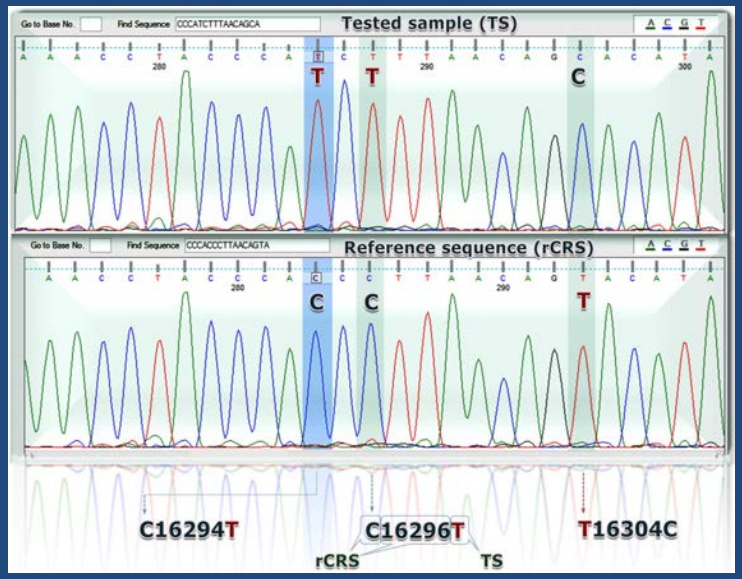
1. Characterize variation

Total variation within a population is important

Variation among populations must be maintained

Common Markers

DNA sequences



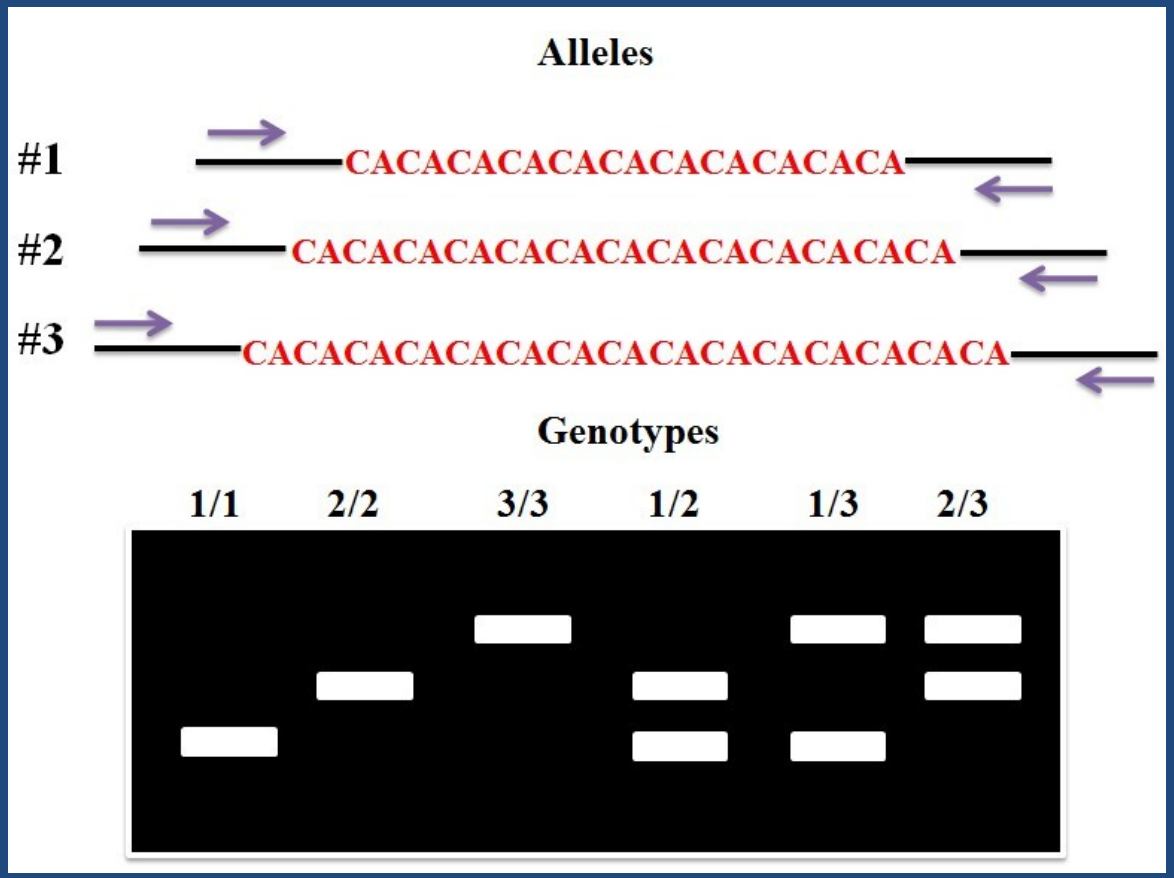
http://www.mtdnatest.com/reagents-kits/mtdnatest-human/

- Mitochondrial DNA
  - haploid, maternal, neutral, 1 locus
- Nuclear DNA
  - ~ haploid, neutral or under selection, ≥ 1 locus

Common Markers

Microsatellites

- diploid
- neutral
- 10s of loci



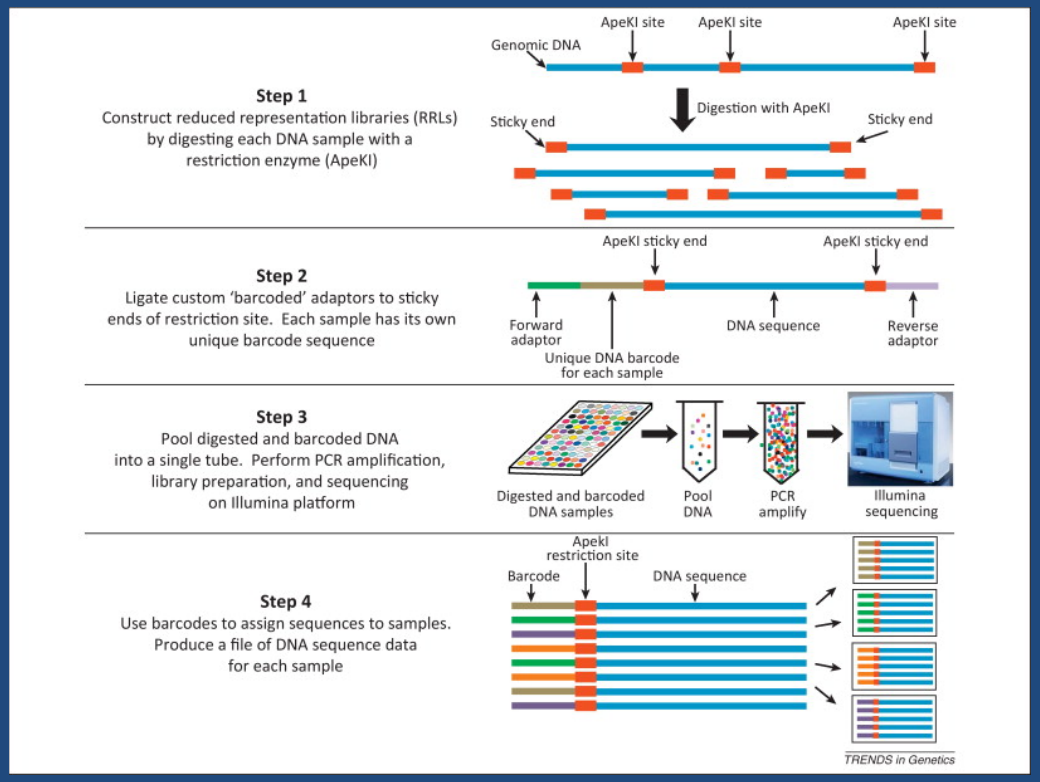
http://genomics.cafs.ac.cn/ssrdb/index.php?do=about

Future Markers

- SNPs (genomics)

  diploid, neutral or under selection

  1000s of loci



Measures of within-population variation

- Microsatellites

  N_A = allelic richness

  H = heterozygosity (H_O and H_E)

  N_e = genetically effective population size

- Sequences

  Number of haplotypes

  k = average number of nucleotide differences

Genetically Effective Population Size

Target population with census size (N_c) = x has genetic variation equal to an “ideal” population of size y.

Constant N_c

50:50 sex ratio

Small, random variation in family size

Measures of among-population variation

- Microsatellites

  F_{ST} = (pooled variation – avg. variation)/pooled variation

  variation among populations within rivers

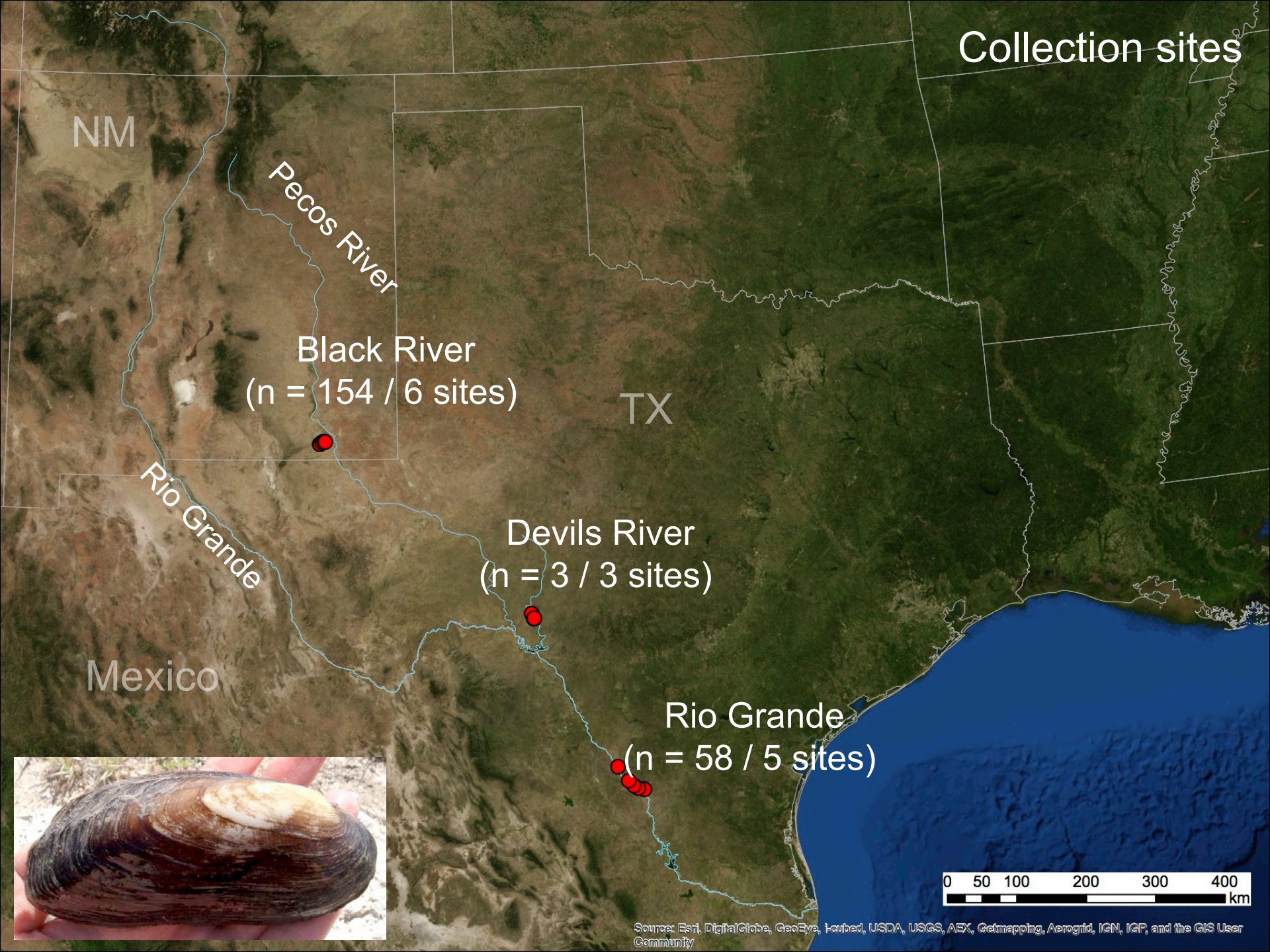
  variation among rivers within regions

  variation among regions

  Assignment tests

- Sequences

  Φ_{ST} = F_{ST} analogue, accounting for sequence similarity


Collection sites
NM
Pecos River
Black River
(n = 154 / 6 sites)
TX
Rio Grande
Devils River
(n = 3 / 3 sites)
Mexico
Rio Grande
(n = 58 / 5 sites)
0 50 100 200 300 400
km
Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, and the GIS User Community

18 loci 2 populations 20 individuals per population *Popenaias popeii* (Texas hornshell)

		Locus																																		
Sample	Population	Tetra19-V		Tetra17-N		Tetra24-F		Tetra30-F		Tetra09-F		Tetra01-V		Tetra36-N		Tetra14-P		Tetra03-F		Tetra02-V		Tetra23-N		Tetra22-P		Tetra40-V		Tetra31-N		Tetra05-P		Tetra33-F		Tetra08-V		Tetra15-N		Individual H
BR-1	BR	251	263	156	168	133	137	275	279	424	428	259	259	191	191	151	167	332	332	203	207	212	216	279	279	132	200	309	313	284	284	201	209	357	357	153	161	0.67
BR-2	BR	263	263	156	168	133	133	275	295	428	428	259	259	191	191	151	167	332	332	199	203	212	212	279	279	132	140	313	313	264	264	201	225	357	357	153	161	0.39
BR-3	BR	251	263	164	168	133	133	275	275	420	432	259	259	191	191	151	159	332	332	199	203	212	216	279	279	132	200	309	313	272	276	225	225	357	369	157	157	0.56
BR-4	BR	263	263	156	164	133	133	275	275	424	424	255	259	191	191	151	167	328	328	203	203	212	216	279	279	200	200	309	313	264	276	201	201	357	357	157	157	0.33
BR-5	BR	251	263	164	168	133	133	275	275	428	428	259	259	191	191	159	167	328	332	211	211	212	212	279	279	132	132	313	313	264	272	201	201	357	357	153	157	0.33
BR-6	BR	263	263	156	156	133	133	295	295	424	424	259	259	183	191	159	159	328	332	207	211	212	212	279	279	168	168	309	313	276	276	201	209	357	369	153	153	0.33
BR-7	BR	263	263	164	164	133	133	275	275	428	428	259	259	183	191	159	159	332	332	203	203	212	244	279	279	128	200	309	313	272	276	201	201	357	369	157	161	0.39
BR-8	BR	263	263	168	168	133	133	275	275	420	420	259	259	191	191	151	159	328	332	211	211	212	212	279	279	140	200	305	309	272	284	201	201	357	385	157	157	0.33
BR-9	BR	263	263	156	168	133	133	275	275	428	428	259	259	183	191	151	159	332	332	203	211	212	212	279	279	132	200	305	313	264	272	201	225	357	369	157	161	0.56
BR-10	BR	259	263	156	164	133	133	275	275	424	428	255	259	191	191	151	151	328	328	207	207	212	212	279	279	200	200	313	313	264	272	201	201	357	369	161	161	0.33
BR-11	BR	263	263	168	168	133	133	275	275	420	420	259	259	191	191	151	159	328	332	207	211	212	212	279	279	140	200	313	313	272	284	201	201	357	369	157	157	0.33
BR-12	BR	263	263	164	168	133	137	275	275	428	428	259	259	191	191	159	167	332	332	203	215	212	244	279	279	132	200	309	313	268	272	201	201	369	369	153	161	0.50
BR-13	BR	251	251	156	168	133	133	275	295	424	428	259	259	191	191	159	159	328	328	203	211	212	216	279	279	200	200	309	309	264	272	201	225	357	365	153	157	0.50
BR-14	BR	263	263	168	168	133	137	275	279	428	432	259	259	191	191	159	159	328	332	203	215	212	212	279	279	132	200	313	313	276	276	201	209	357	357	153	153	0.39
BR-15	BR	251	251	168	168	133	133	275	295	420	432	259	259	191	191	159	159	328	332	203	211	212	212	279	279	132	132	313	313	272	276	201	225	357	357	153	161	0.39
BR-16	BR	263	263	168	168	133	137	279	279	428	432	255	259	191	191	159	167	332	332	203	211	212	216	279	279	132	164	309	313	272	276	201	201	357	369	153	153	0.56
BR-17	BR	263	263	156	168	133	137	275	279	428	432	259	259	191	191	159	167	328	332	203	211	212	216	279	279	132	200	305	309	276	276	209	209	357	357	153	157	0.61
BR-18	BR	251	251	168	168	133	133	275	295	420	424	259	259	191	191	151	159	332	332	203	203	212	216	279	279	132	200	309	313	272	276	201	209	357	369	153	157	0.55
BR-19	BR	251	263	156	168	133	133	275	275	424	428	255	259	191	191	159	167	328	328	203	203	212	216	279	279	200	200	309	309	264	276	201	225	357	357	153	153	0.44
BR-20	BR	251	263	168	168	133	133	275	279	420	432	243	259	187	191	151	159	332	332	203	203	212	212	279	291	132	140	309	313	272	276	201	225	357	357	153	153	0.61
Fall21	RG	231	231	144	148	133	133	243	259	428	444	247	247	183	191	167	171	332	340	191	215	264	292	275	291	164	168	305	325	256	292	205	225	353	353	137	157	0.78
Fall22	RG	271	271	132	144	133	141	263	263	400	420	247	251	179	195	111	111	344	344	227	231	236	260	287	303	160	164	301	321	228	268	265	265	341	369	161	173	0.72
Fall23	RG	239	271	124	140	101	145	247	247	408	428	219	219	191	191	111	159	324	340	251	251	240	240	275	283	132	152	301	353	260	272	193	217	365	369	161	165	0.72
Fall24	RG	259	263	128	136	129	141	267	283	412	416	239	247	199	199	111	171	316	328	211	227	256	272	275	291	132	160	321	337	240	308	201	201	353	381	165	189	0.89
Fall25	RG	267	267	136	140	113	153	247	271	408	412	231	255	183	187	163	175	328	336	219	283	248	252	259	291	156	160	301	321	272	300	177	177	365	369	185	193	0.89
Fall26	RG	255	275	144	164	129	145	263	263	388	412	215	215	183	187	139	171	340	340	211	219	236	264	267	267	132	152	301	321	244	244	261	265	349	361	133	165	0.72
Fall27	RG	247	259	112	140	121	121	263	263	388	412	215	255	191	191	151	163	320	344	211	219	240	260	263	267	136	156	289	301	256	308	209	253	353	377	153	173	0.83
Fall28	RG	255	267	140	152	121	149	259	287	412	416	231	231	199	199	163	167	344	344	215	227	232	260	275	275	152	160	329	361	296	308	205	205	369	381	157	177	0.72
Fall29	RG	251	271	132	140	125	141	251	263	400	400	247	263	183	191	111	147	328	328	191	215	252	264	267	291	168	172	289	301	276	312	197	221	361	361	133	177	0.83
Fall30	RG	247	263	124	128	133	153	243	283	392	392	247	255	187	191	147	163	328	340	211	219	244	252	291	311	152	160	293	301	244	268	201	205	365	373	157	161	0.94
Fall31	RG	263	271	136	144	133	153	251	283	392	436	239	275	183	191	135	175	324	364	227	227	260	264	267	275	132	136	345	349	236	284	205	217	353	385	133	181	0.94
Fall32	RG	255	255	136	140	129	129	259	263	408	408	243	263	179	179	151	155	316	328	231	251	264	268	263	275	128	152	289	361	256	256	173	217	369	373	161	189	0.72
Fall33	RG	243	259	140	152	121	153	263	279	380	436	227	267	179	195	167	167	324	336	215	231	232	252	283	295	132	160	301	321	264	304	217	217	373	397	177	189	0.89
Fall34	RG	267	271	132	132	153	165	243	251	368	388	247	251	195	199	151	159	304	324	211	255	216	252	287	323	140	156	301	321	244	252	193	261	357	361	177	189	0.94
Fall35	RG	271	271	128	152	125	137	283	287	408	420	263	263	187	199	127	139	344	356	199	227	216	244	259	287	128	168	289	337	224	252	213	217	369	373	133	177	0.89
Fall36	RG	247	255	124	136	141	145	247	251	368	428	215	215	187	187	111	159	344	364	223	227	248	260	267	267	156	168	293	305	264	268	213	213	349	369	185	193	0.78
Fall37	RG	263	263	132	140	137	149	243	251	408	432	251	275	179	187	111	111	332	344	207	215	256	260	263	275	136	156	301	301	260	272	213	213	369	381	173	189	0.78
Fall38	RG	259	263	132	144	129	145	251	287	408	424	247	251	191	195	111	111	320	328	211	235	244	276	267	271	144	152	325	345	272	308	213	213	369	381	173	189	0.89
Fall39	RG	255	259	112	132	141	145	275	287	408	424	263	263	183	195	147	171	380	384	239	239	240	244	279	327	152	156	309	337	224	224	277	277	377	381	157	181	0.78
Fall40	RG	279	283	132	136	129	157	279	279	412	412	231	263	191	191	155	163	320	320	215	227	252	256	275	291	152	160	317	373	256	304	209	209	349	385	173	189	0.72

BR = Black River, Eddy County, NM
RG = Rio Grande, Webb County, TX
Each entry is a diploid genotype at a locus, consisting of two alleles each defined by the number of 4-nucloetide repeats (i.e., 251 = 251 repeats of a four nucleotide sequence)

Tetra36-N		BR		RG	
	Allele	#	freq.	#	freq.
	179	0	0.000	5	0.125
	183	3	0.075	6	0.150
	187	1	0.025	7	0.175
	191	36	0.900	11	0.275
	195	0	0.000	5	0.125
	199	0	0.000	6	0.150

Within-population Variation

Statistic	BR	RG	
N_A	3.33	12.78	total # alleles/#loci
H_O	0.46	0.82	mean # heterozygous loci
H_E	0.47	0.88	
F_{IS}	0.04	0.07	

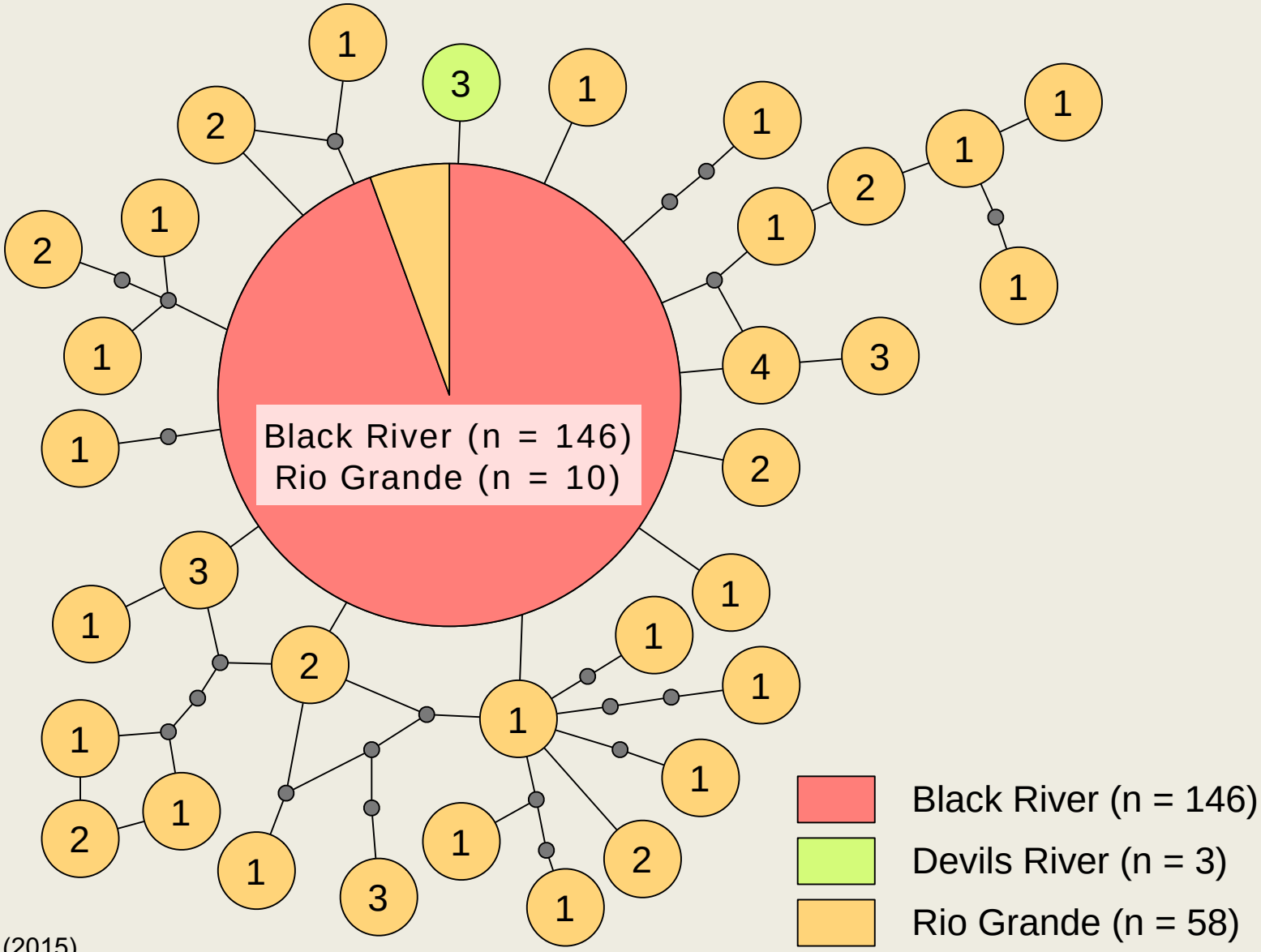
Among-population Variation

Statistic	
F_{ST}	0.257

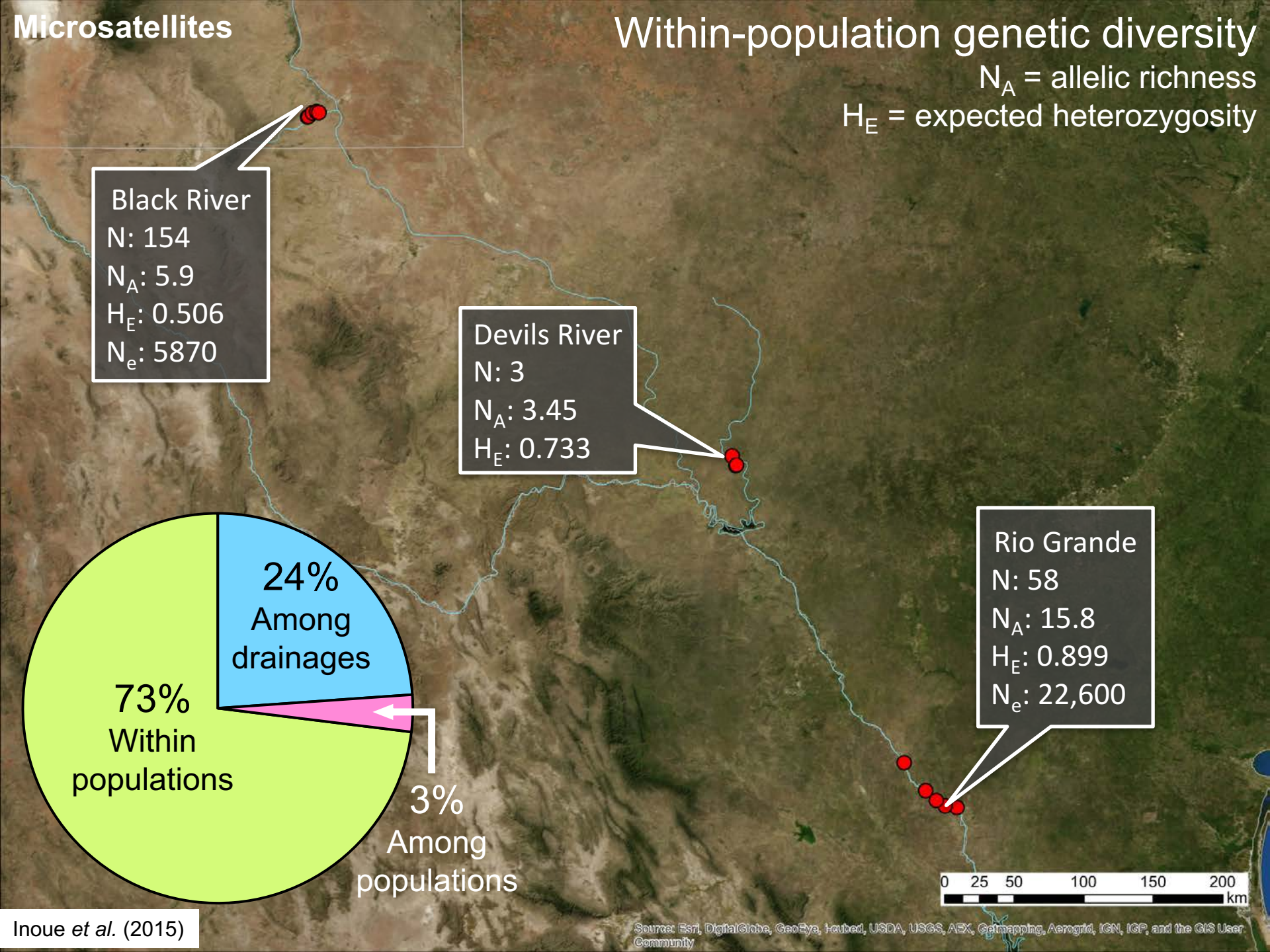
mtDNA COI

Parsimony Network of COI

$N_{haplotype} = 34$



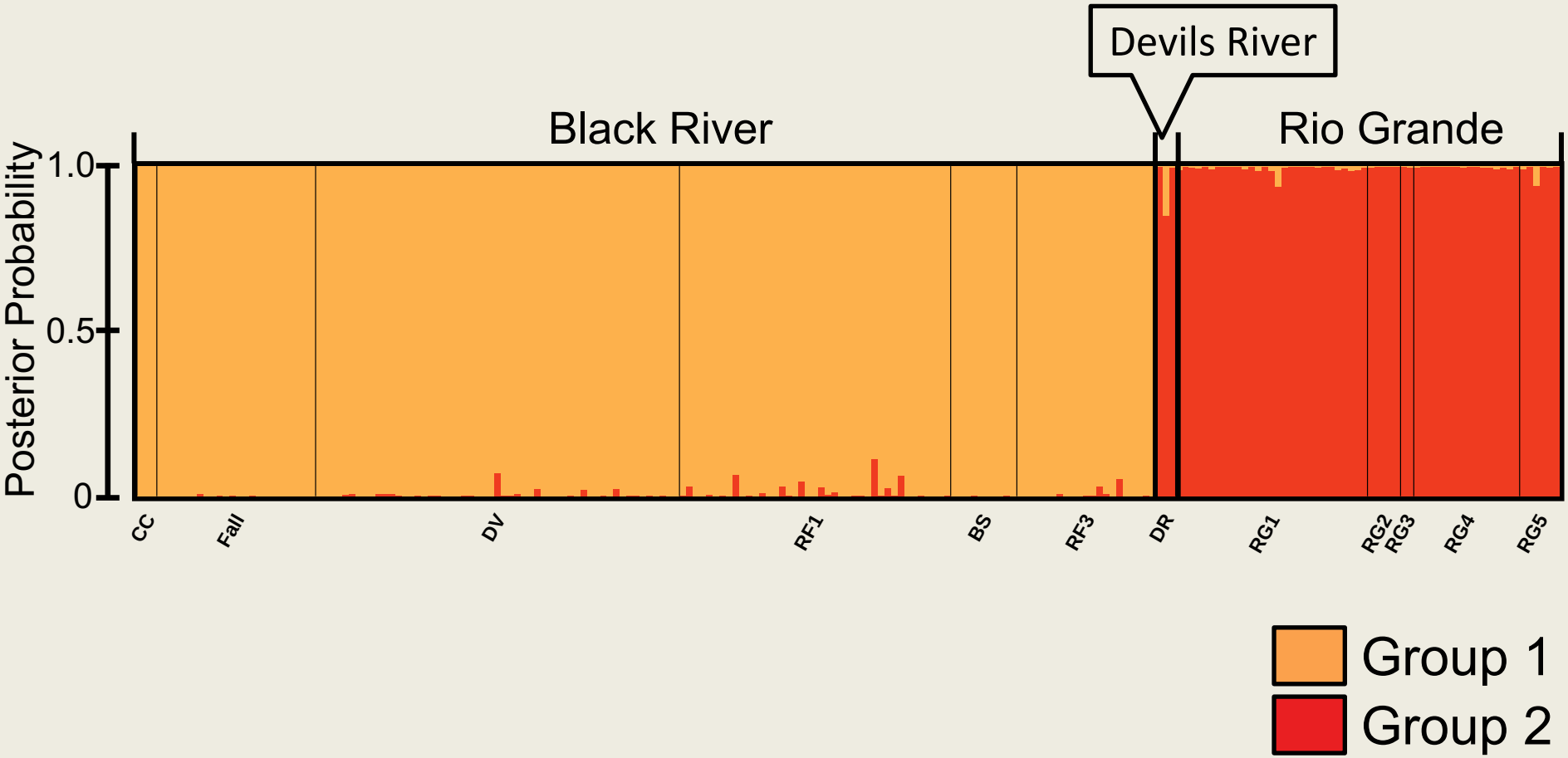
Inoue *et al.* (2015)



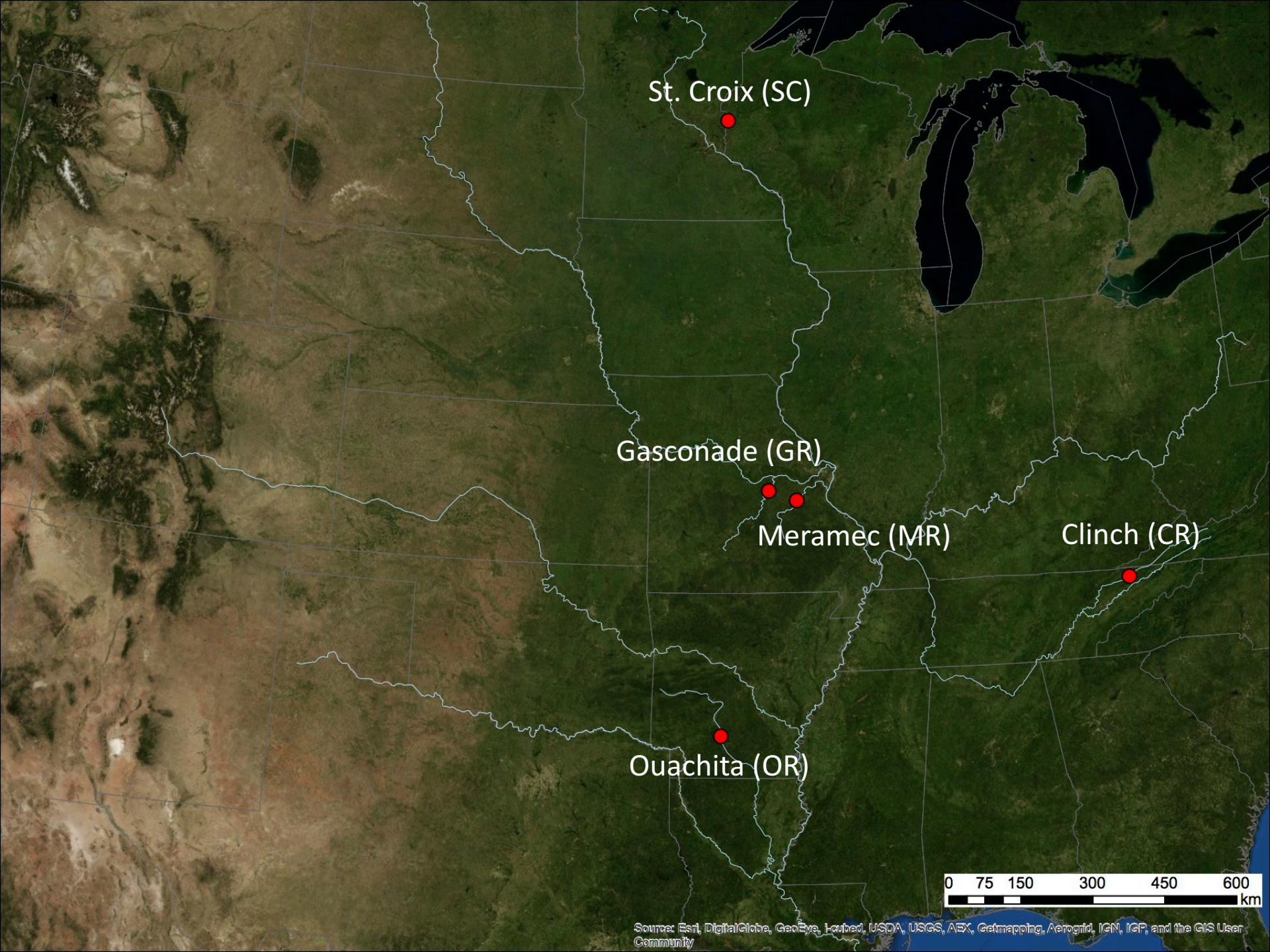
Inoue *et al.* (2015)

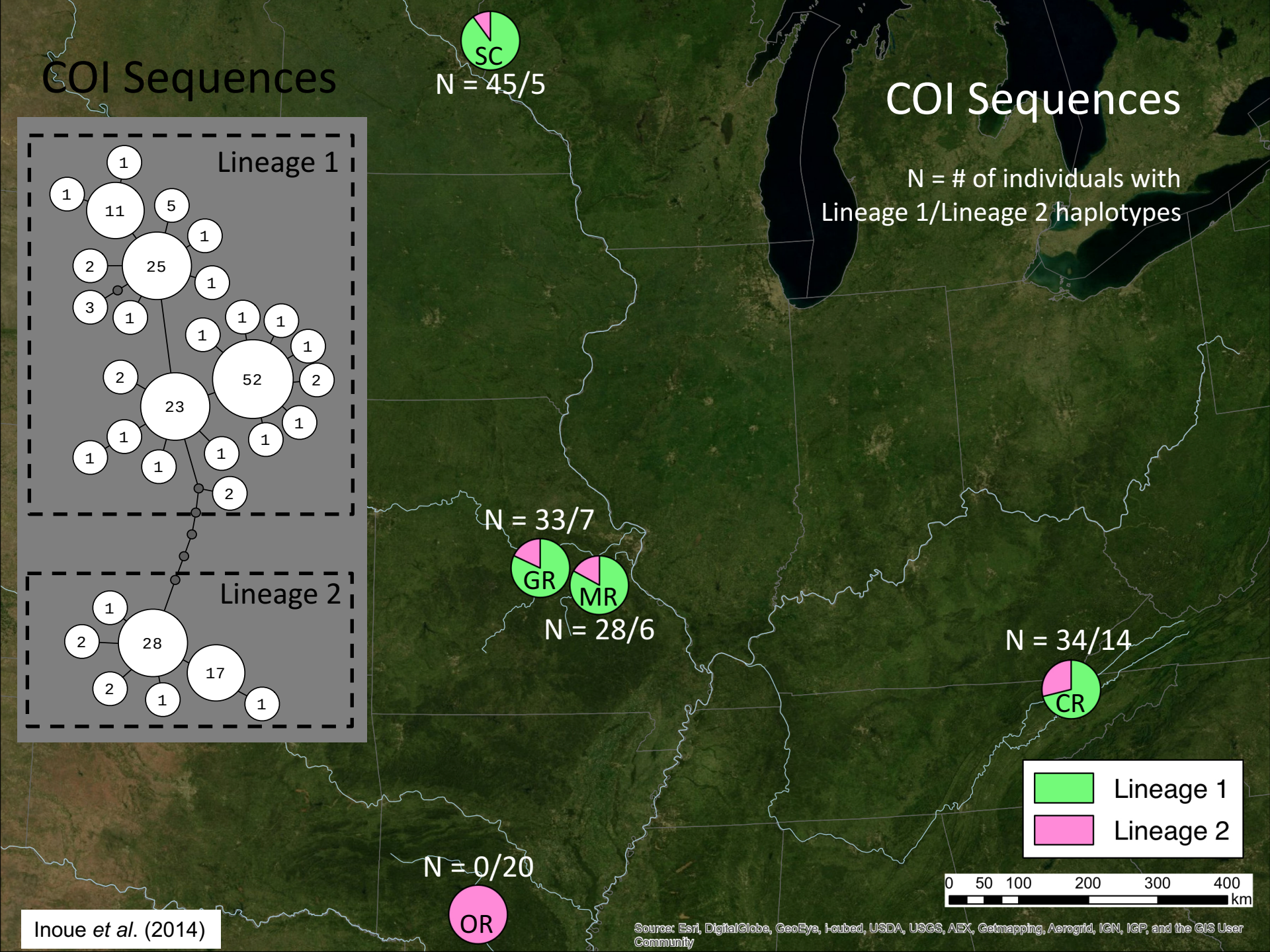
Microsatellites

Structure
2 distinct clusters (k = 2)

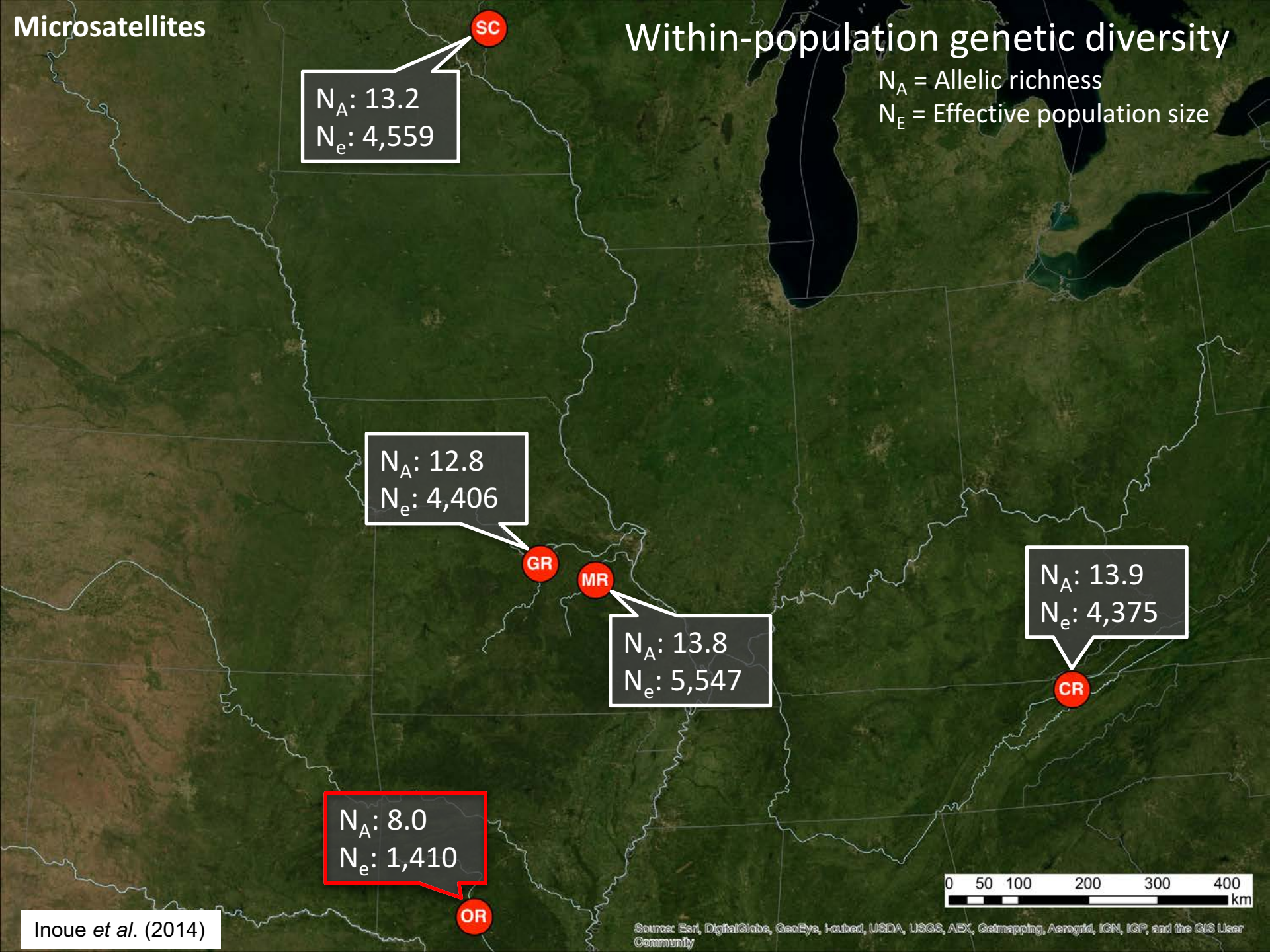


Inoue *et al*. (2015)


St. Croix (SC)
Gasconade (GR)
Meramec (MR)
Clinch (CR)
Ouachita (OR)
0 75 150 300 450 600
km
Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, and the GIS User Community



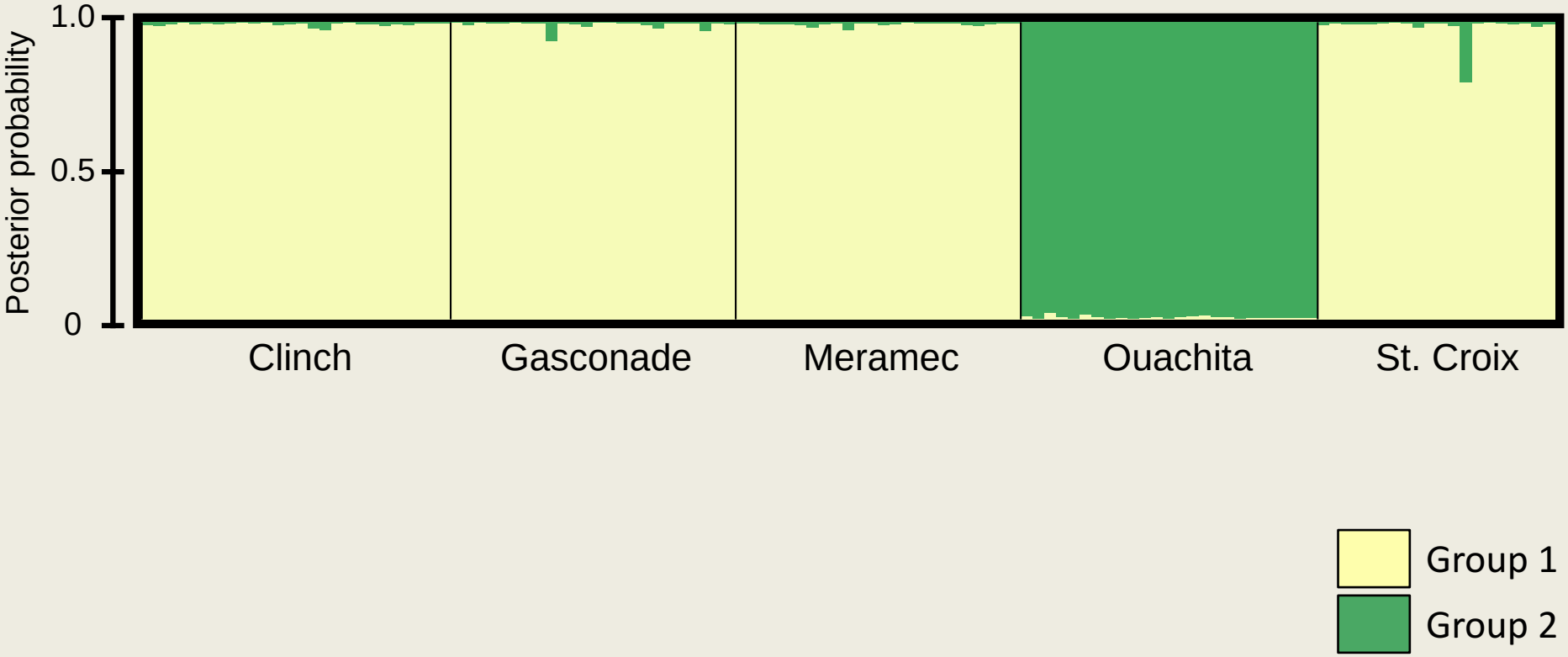
Inoue *et al*. (2014)



Inoue *et al*. (2014)

Structure
k = 2

Microsatellites



Inoue *et al*. (2014)

Popenaias popeii

- Rio Grande has much greater within-population variation than the Black River
- Significant among-river variation

Manage as separate units

Cumberlandia monodonta

- Ouachita population much lower variation
- Low among-population variation except for Ouachita

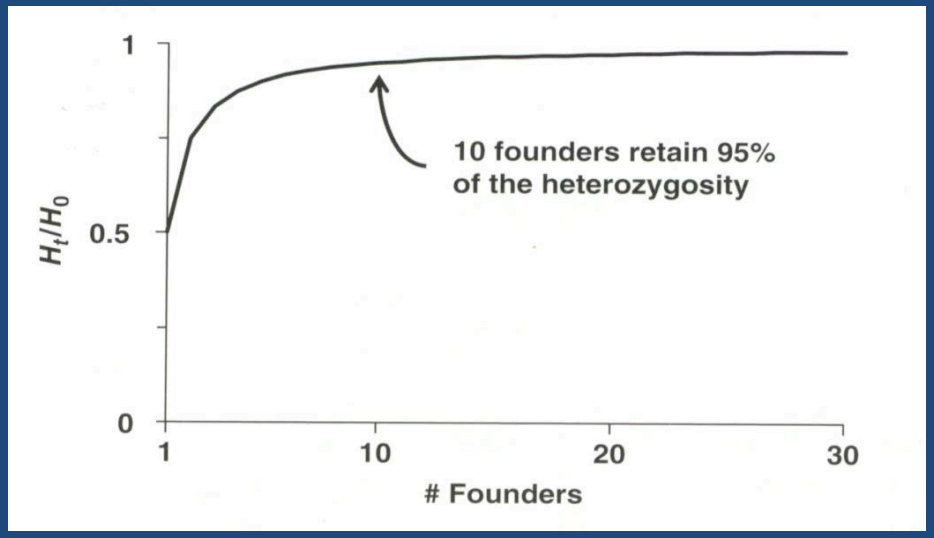
Manage as two units, one covering large area

2. Found captive population(s)

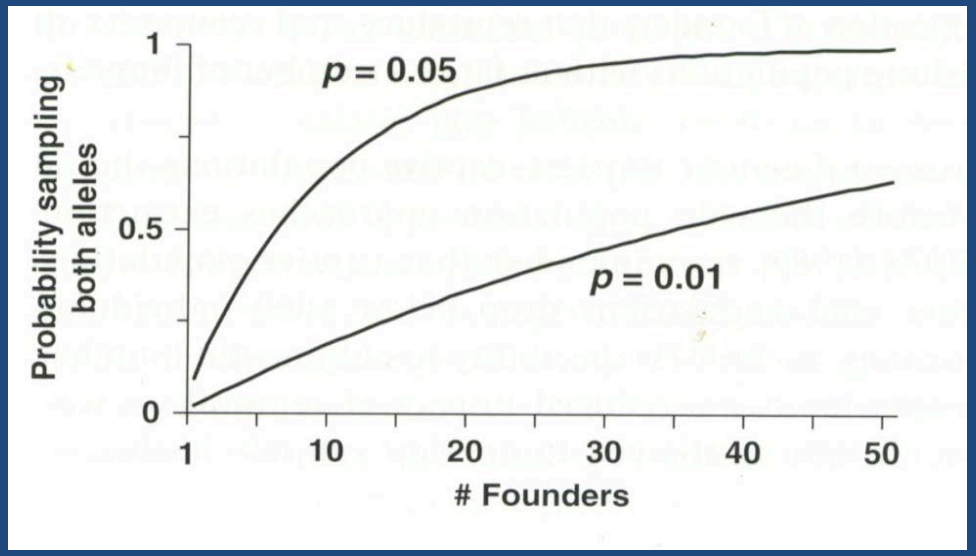
Number of founders determines within-population variation

Demographic features are important

Size of founding population

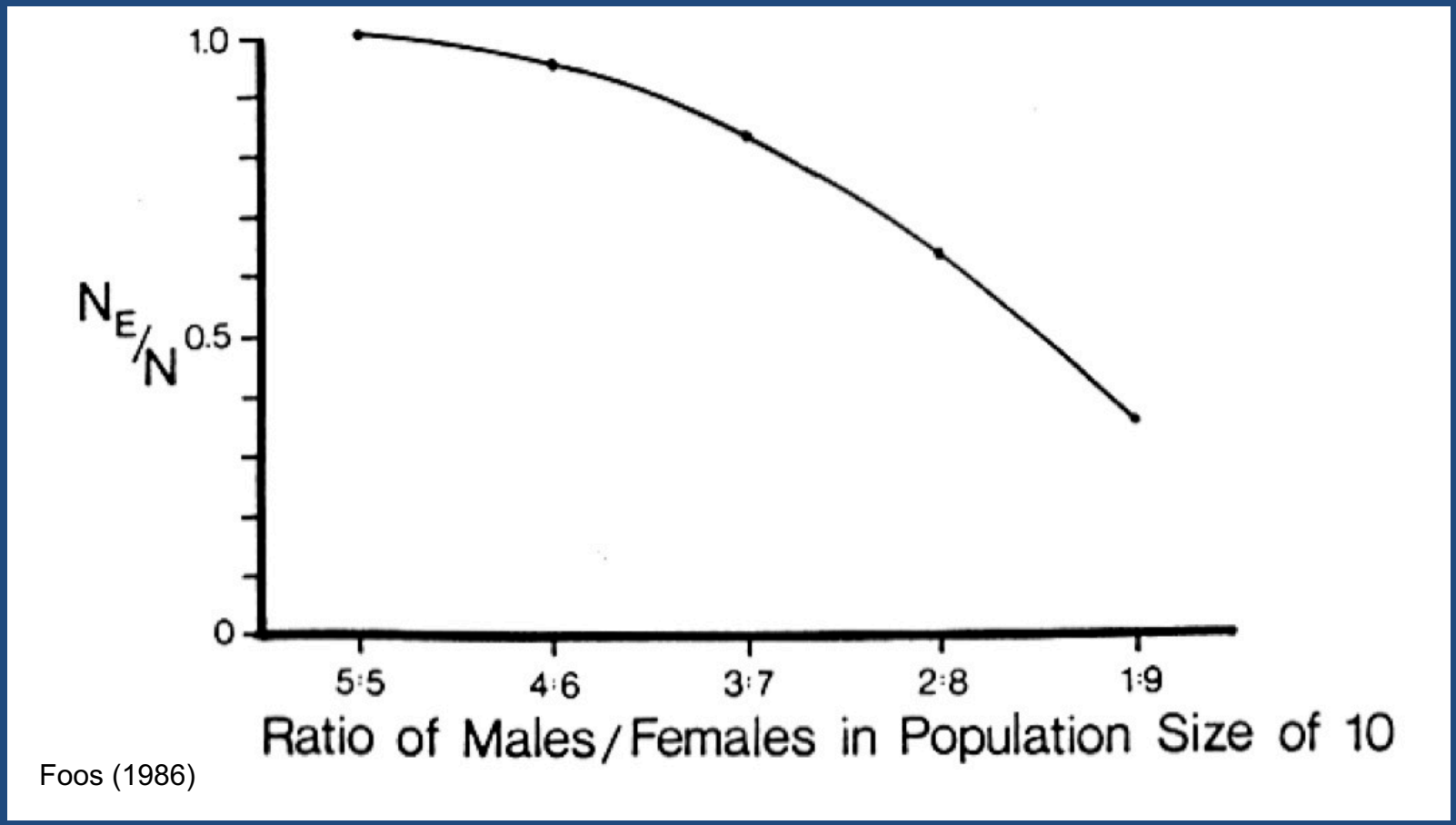


Frankham *et al.* (2010), page 436



$N_e = N_c$, when

50:50 sex ratio with random mating



Foos (1986)

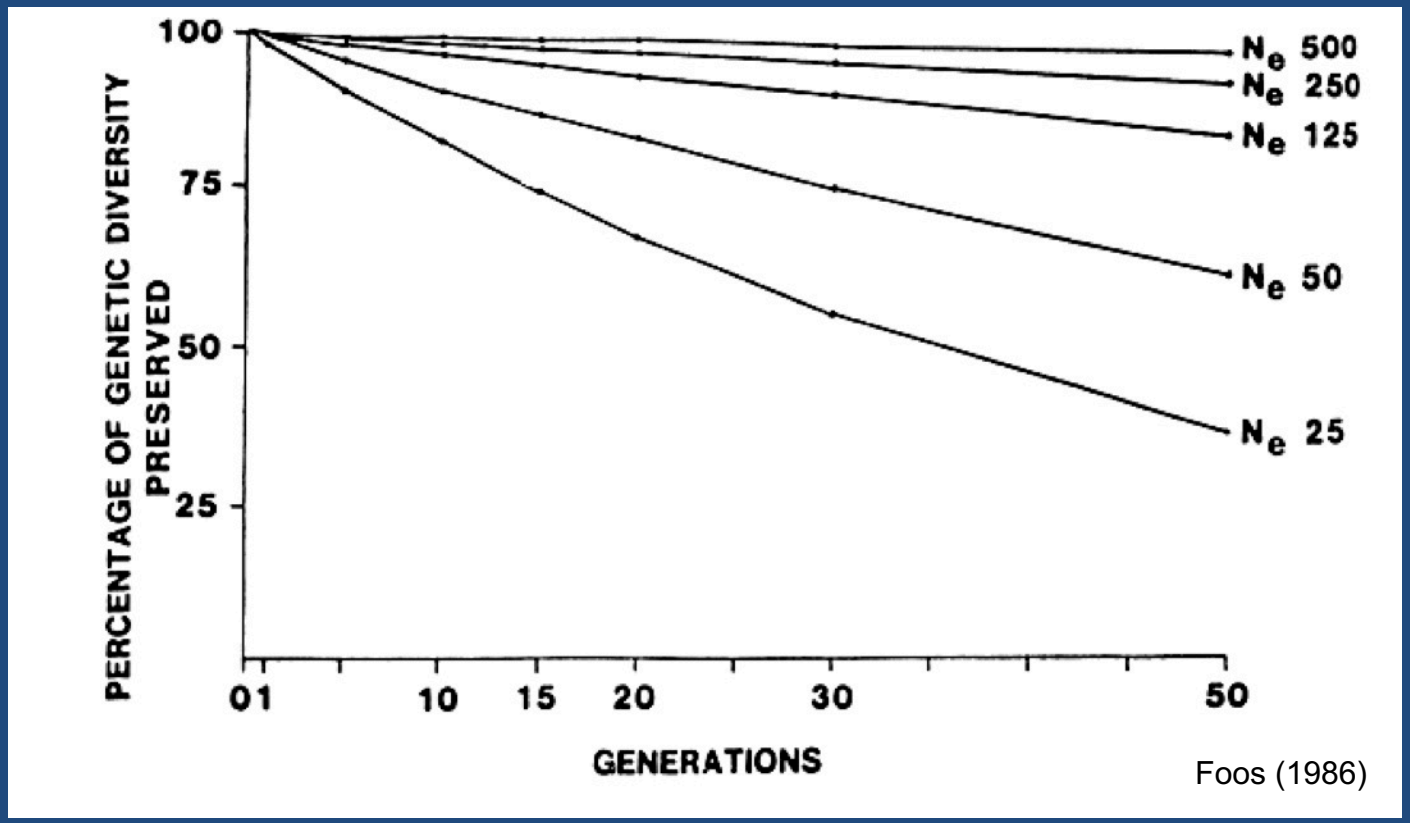
3. Expand captive population(s)

Genetic drift = loss of genetic variation

Variance in reproduction must be random

Genetic diversity is lost over time

Rate of loss depends on N_e



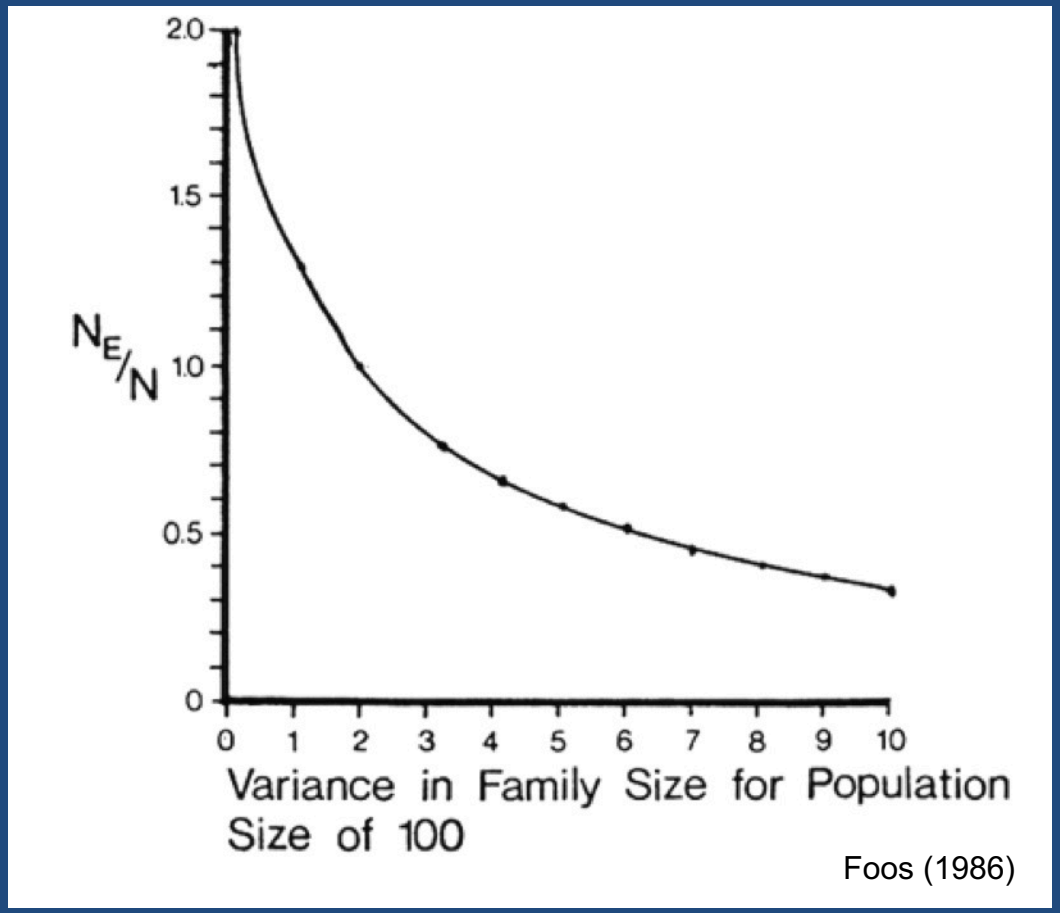
Foos (1986)

$N_e = N_c$ when

50:50 sex ratio with random mating

AND

variance in reproduction is random



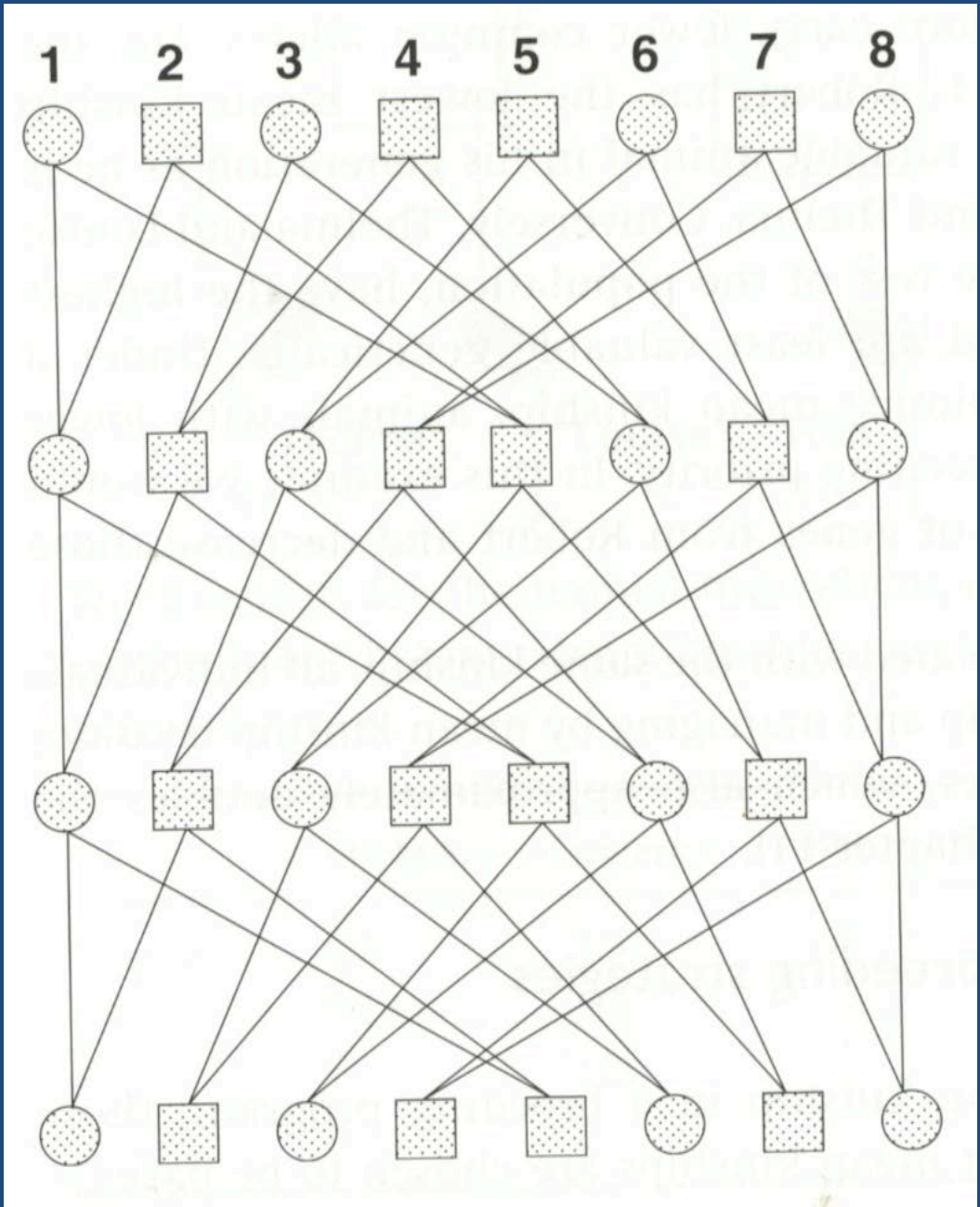
4. Manage captive population(s)

Minimize inbreeding

Maintain N_e

Minimize adaptation to captivity

Maximum avoidance of inbreeding



Frankham *et al.* (2010), page 442

$N_e = N_c$, when

50:50 sex ratio with random mating

AND

variance in reproduction is random

AND

population size is constant

N_e is *harmonic* mean of N over time

long-term $N_e \sim t / \Sigma(1/N)$

N_e and population size

Time	Population Size
1	5000
2	100
3	1000
4	3000
5	6000

$N_e = 427$

Selection

Selection is on heritable traits

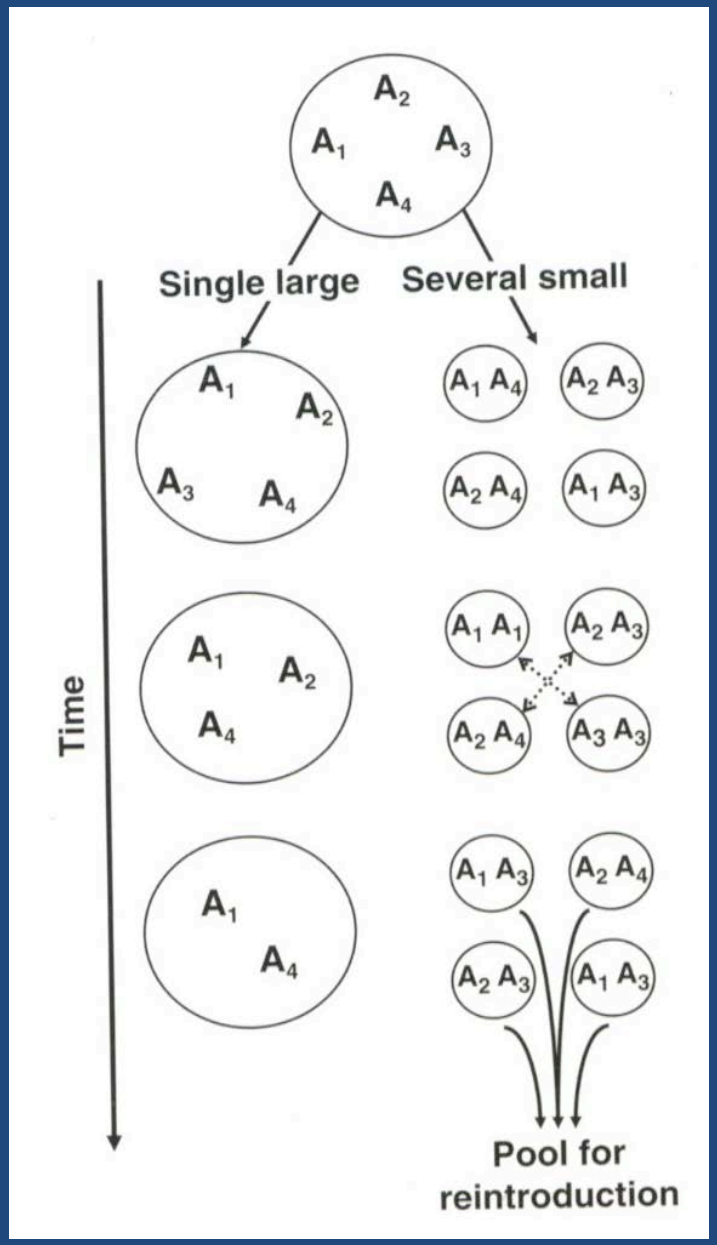
Selection is on the phenotype:

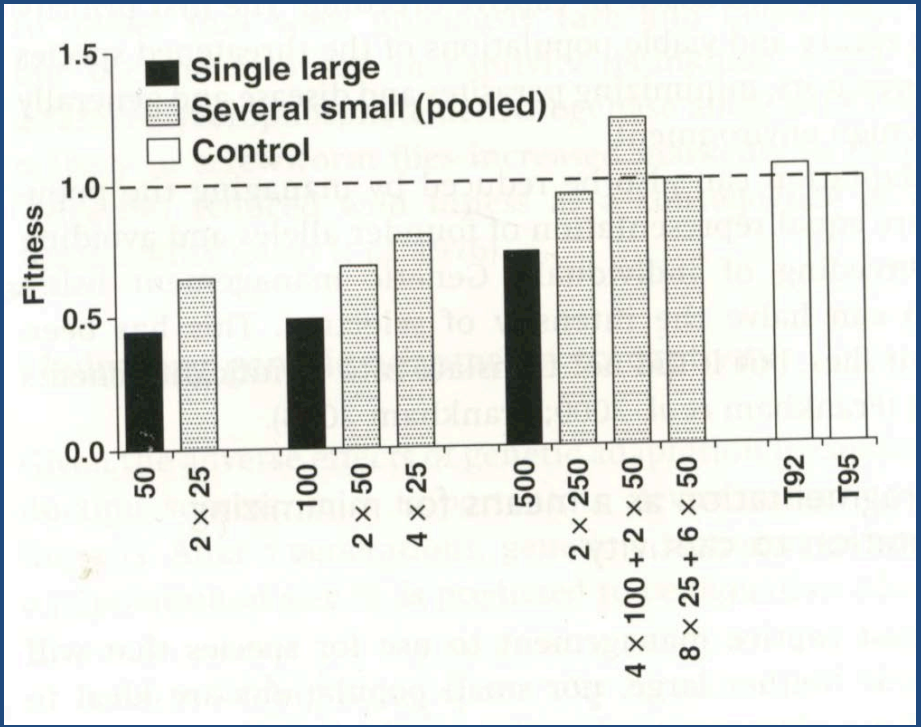
genotype + *environment*

Selection varies with environment

natural vs. artificial selection

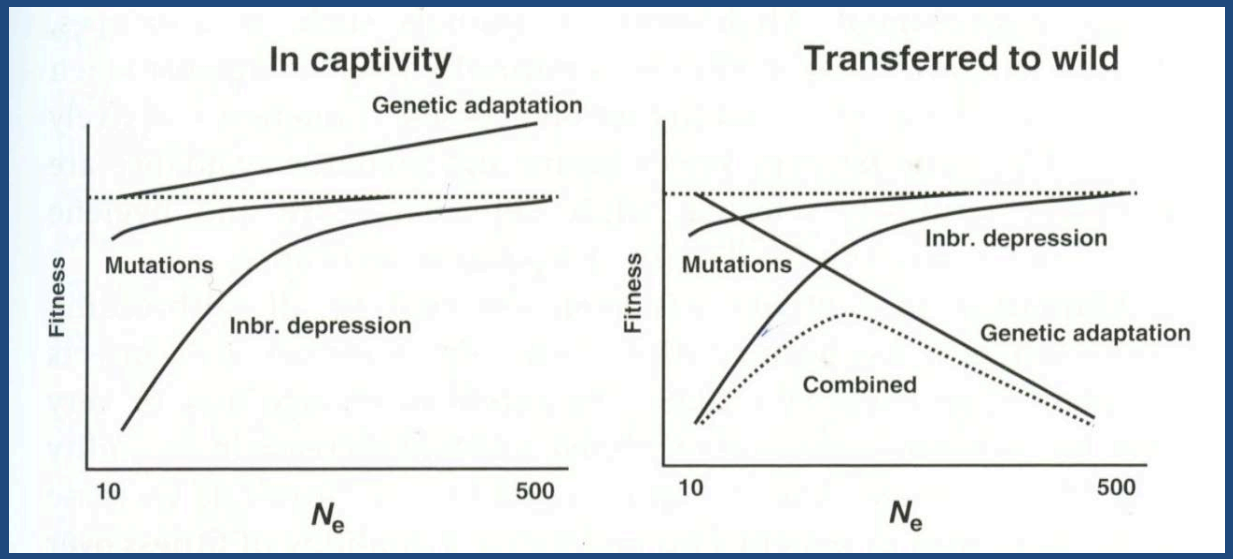
SLOSS: Single large or several small populations?



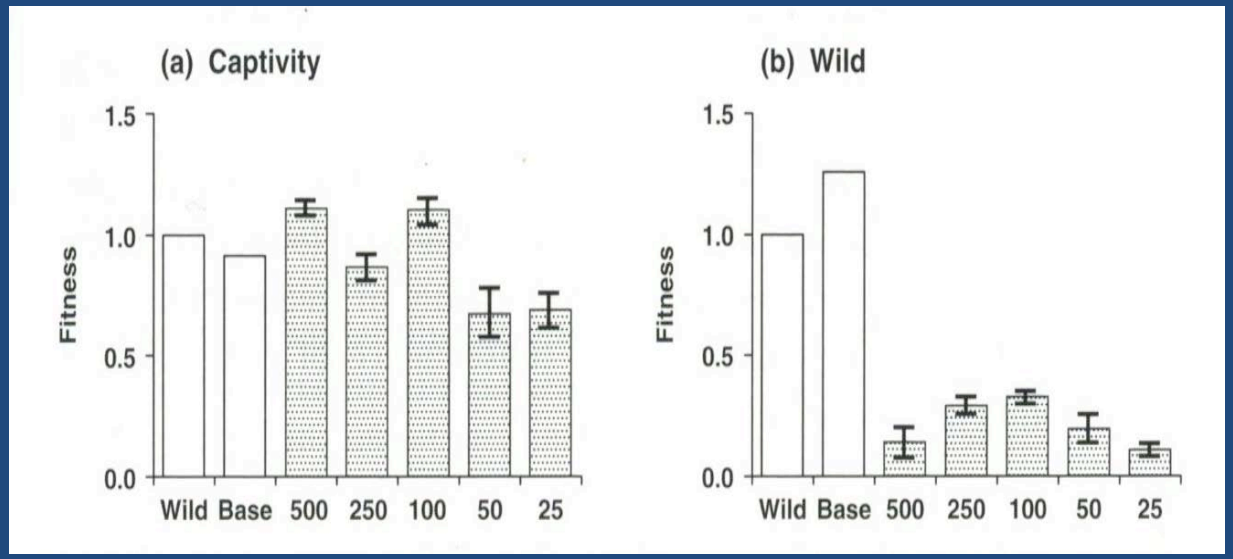


Frankham *et al.* (2010), page 460

Traits under positive selection in captivity can be deleterious in the wild



Genetic deterioration in *Drosophila*



Frankham *et al.* (2010), page 457

5. Choose individuals to reintroduce

Distribute evenly among families / lineages

If augmenting, do not swamp out natives

Maintain geographic boundaries

Destroy / repurpose excess individuals

N_e / N_c

Population	N_e	N_c	N_e / N_c
Black River	5,870	48,006	0.12
Rio Grande	22,600	280,000	0.08

Burlakova & Karatayev (2013)
Inoue *et al.* (2015)

6. Manage reintroduced population(s)

Protect from abnormal mortality, allow mortality from “normal” sources

Maintain natural habitat

Maintain geographic integrity

Let evolution happen

Table 15.1 How large must populations be to retain genetic 'health'? Various estimates of the required effective population size (N_e) are given, along with the times to recover normal levels of genetic diversity following complete loss of diversity

Goal	N_e	Regeneration time (generations)	Reference
Avoid inbreeding depression	50		1, 2
Retain evolutionary potential	500	10^2–10^3	1, 3
	5000		4
	570–1250		5
Retain single-locus genetic diversity	10^5–10^6	10^5–10^7	3
Avoid accumulating deleterious mutations	1000		4
	100		6
	12		7

References: 1, Franklin (1980); 2, Soulé (1980); 3, Lande & Barrowclough (1987); 4, Lande (1995); 5, Franklin & Frankham (1998); 6, Lynch *et al.* (1995a); 7, Charlesworth *et al.* (1993).

Frankham *et al.* (2010), page 340

N_e is key!

No evolution in culture is the goal:

1. No mutation
2. Infinite population size
3. Random mating
4. No gene flow
5. No selection

- Maximizing N_e will retain evolutionary potential
- Reintroduce and let nature take its course

Thanks to the following

Curt Elderkin, Kentaro Inoue, Emy Monroe, Ashley Walters

Brian Lang (*in memorium*)

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