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
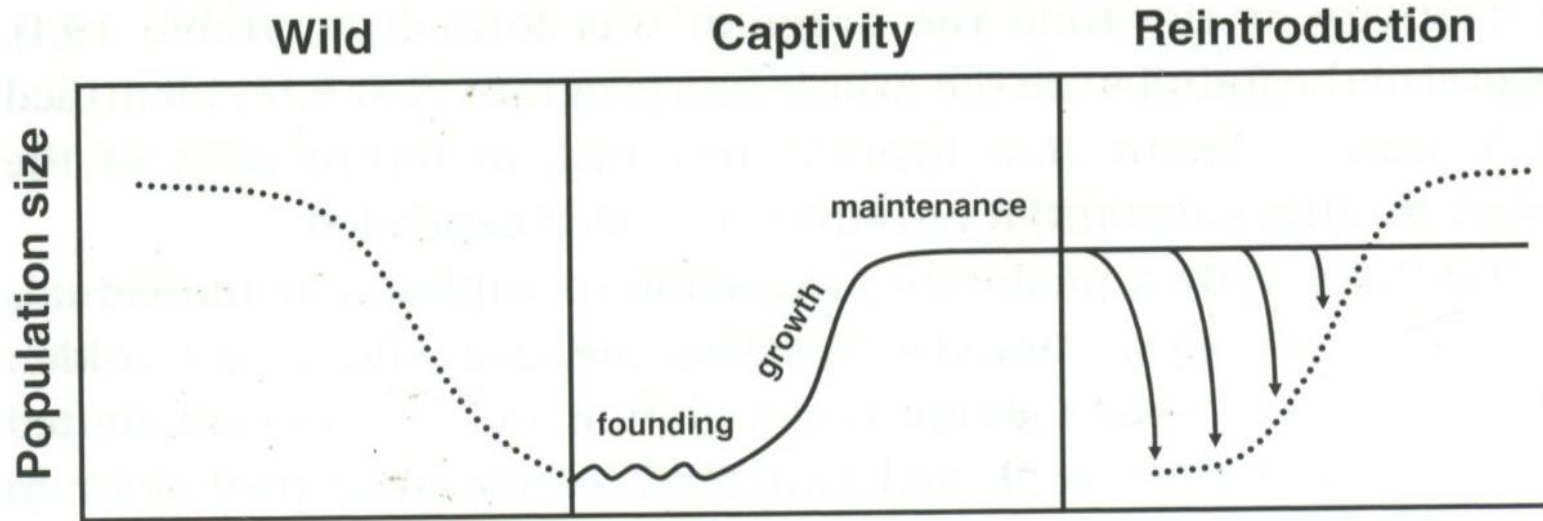
Population Genetics Refresher

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
  - Mitochondrial DNA
    - single copy, maternally inherited
    - neutral
  - Nuclear DNA
    - two copies (maternal & paternal)
    - neutral or under selection

- Microsatellites
  - two copies
  - neutral

Measures of within-population variation

- Microsatellites

  N_A = allelic richness

  A_R = rarified number of alleles; standardized

  H = heterozygosity (H_O and H_E)

  N_e = genetically effective population size

- Sequences

  Number of haplotypes

  k = average number of nucleotide differences

  $\pi = k$ / sequence length; standardized

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

  $\Phi_{ST} = F_{ST}$ analogue, accounting for sequence similarity

Collection sites

NM
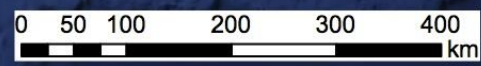
Pecos River

Black River
(n = 154 / 6 sites)
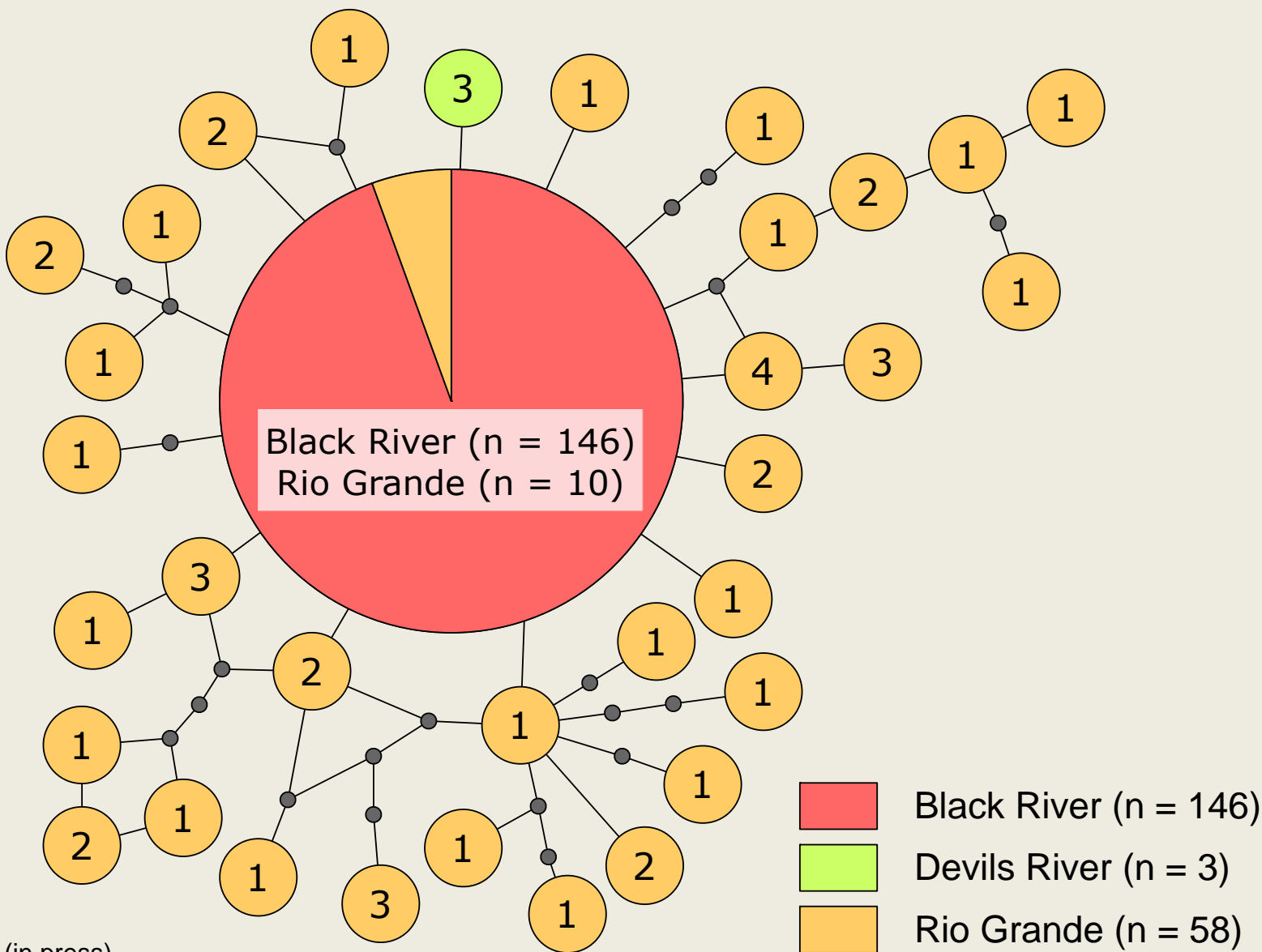
TX

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	332 332	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	133 137	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	133 137	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	275 275	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	428 428	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	168 168	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-nucleotide repeats (i.e., 251 = 251 repeats of a four nucleotide sequence)

Tetra36-N	Allele	#	BR freq.	#	RG freq.	Within-population Variation Statistic	BR	RG		Among-population Variation Statistic	
	179	0	0.000	5	0.125	N_A	3.33	12.78	total # alleles/#loci	F_{ST}	0.257
	183	3	0.075	6	0.150	H_O	0.46	0.82	mean # heterozygous loci		
	187	1	0.025	7	0.175	H_E	0.47	0.88			
	191	36	0.900	11	0.275	F_{IS}	0.04	0.07			
	195	0	0.000	5	0.125						
	199	0	0.000	6	0.150						

mtDNA COI

Parsimony Network of COI
$N_{haplotype} = 34$

Black River (n = 146)
Rio Grande (n = 10)

Black River (n = 146)
Devils River (n = 3)
Rio Grande (n = 58)

Inoue *et al.* (in press)

Microsatellites
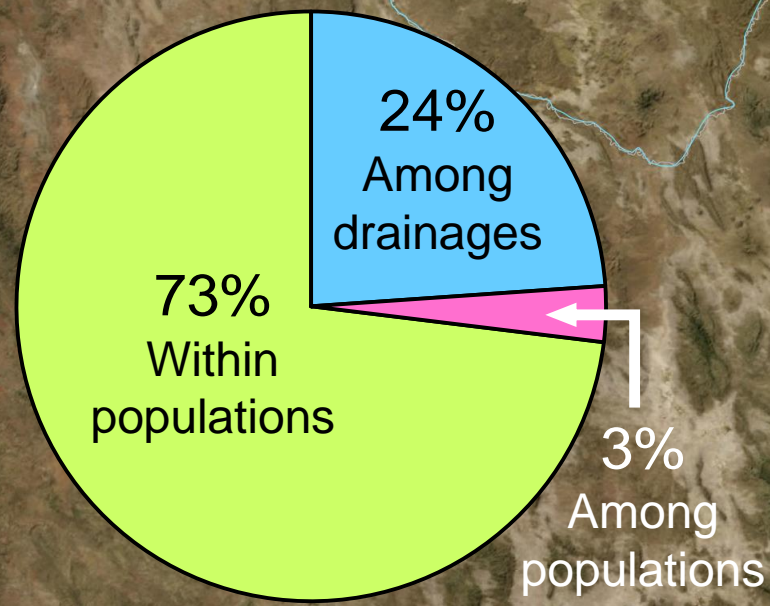
Within-population genetic diversity
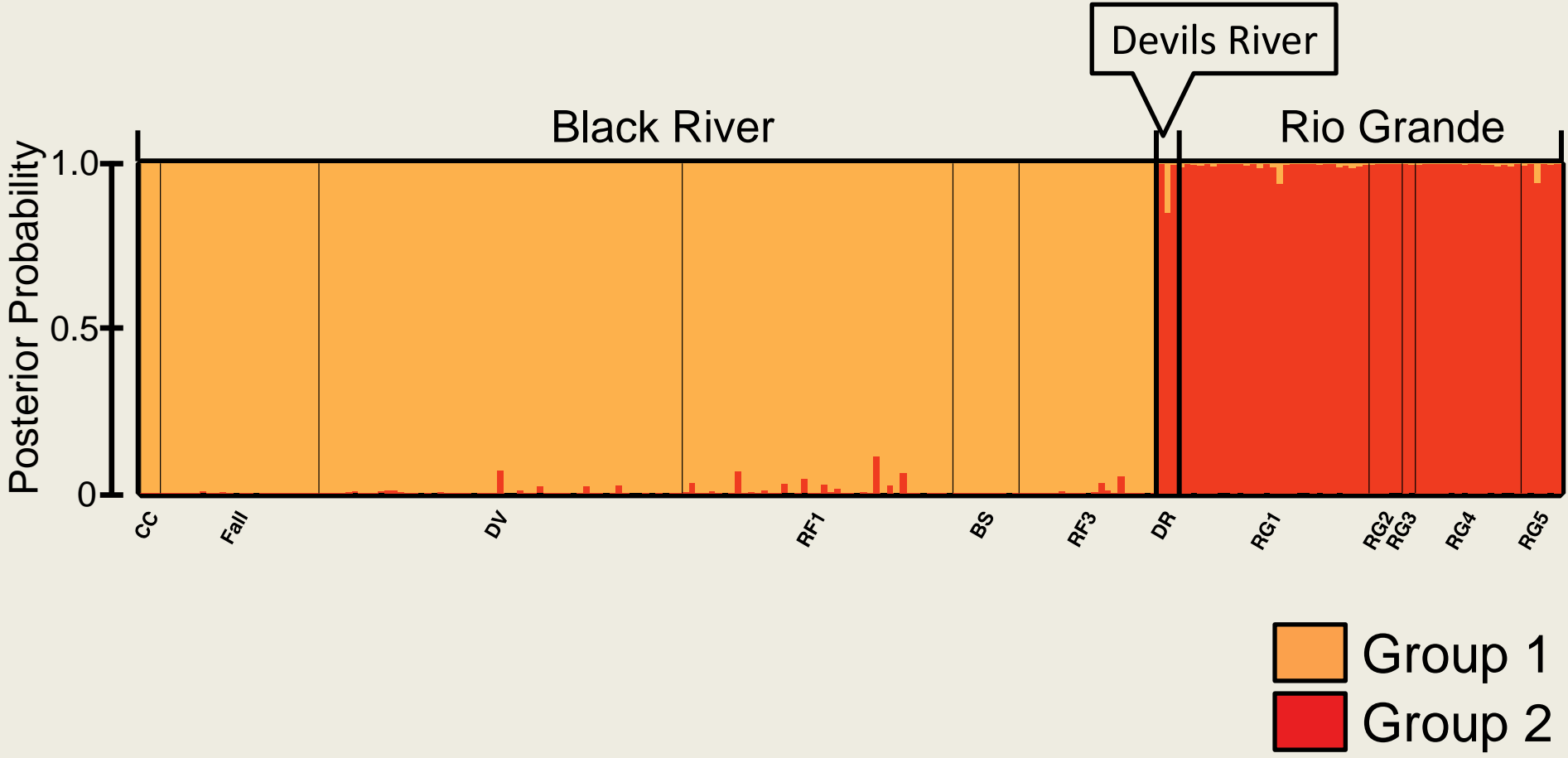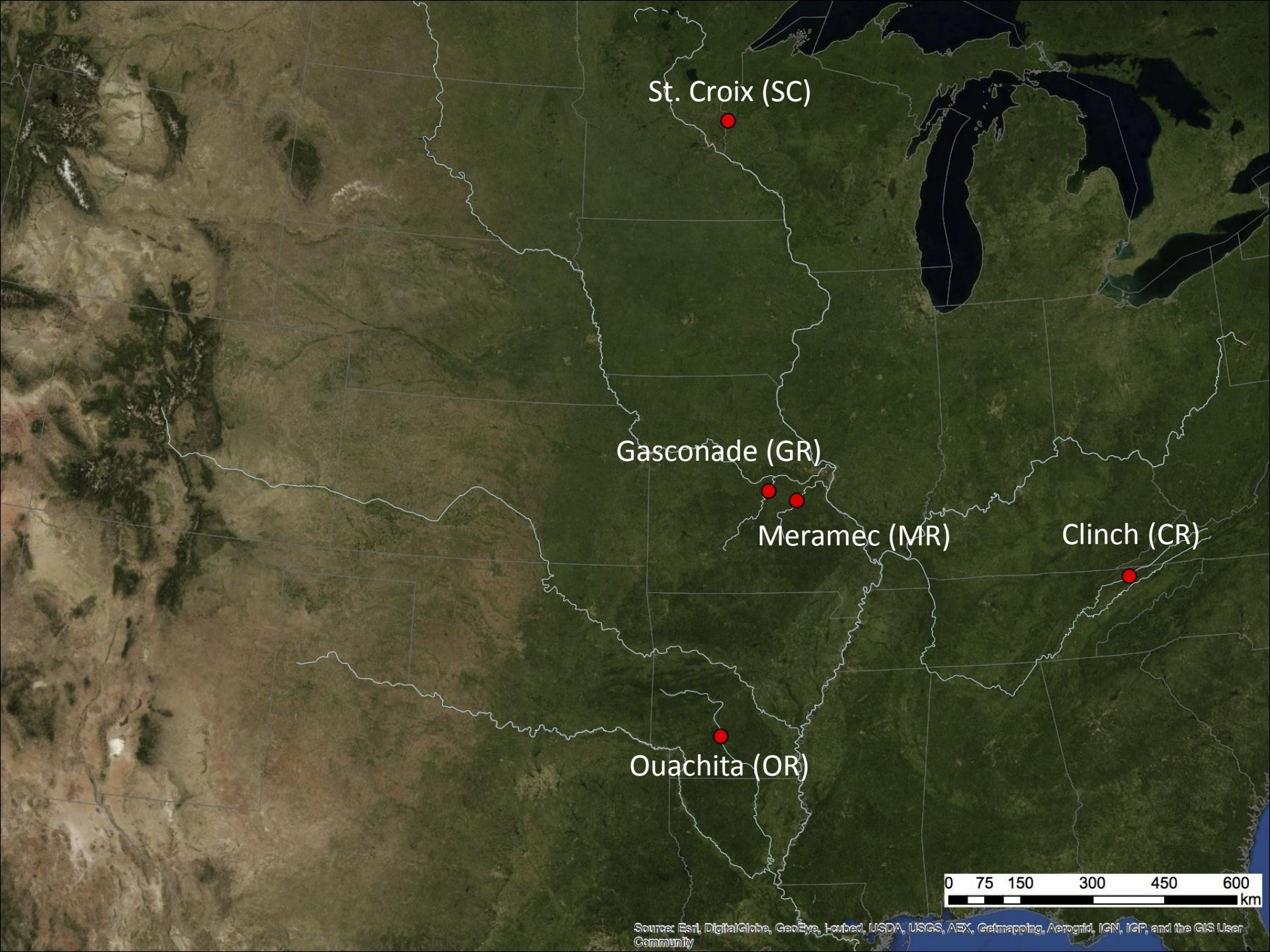N_A = allelic richness
H_E = expected heterozygosity

Black River
N: 154
N_A: 5.9
H_E: 0.506
N_e: 5870

Devils River
N: 3
N_A: 3.45
H_E: 0.733

Rio Grande
N: 58
N_A: 15.8
H_E: 0.899
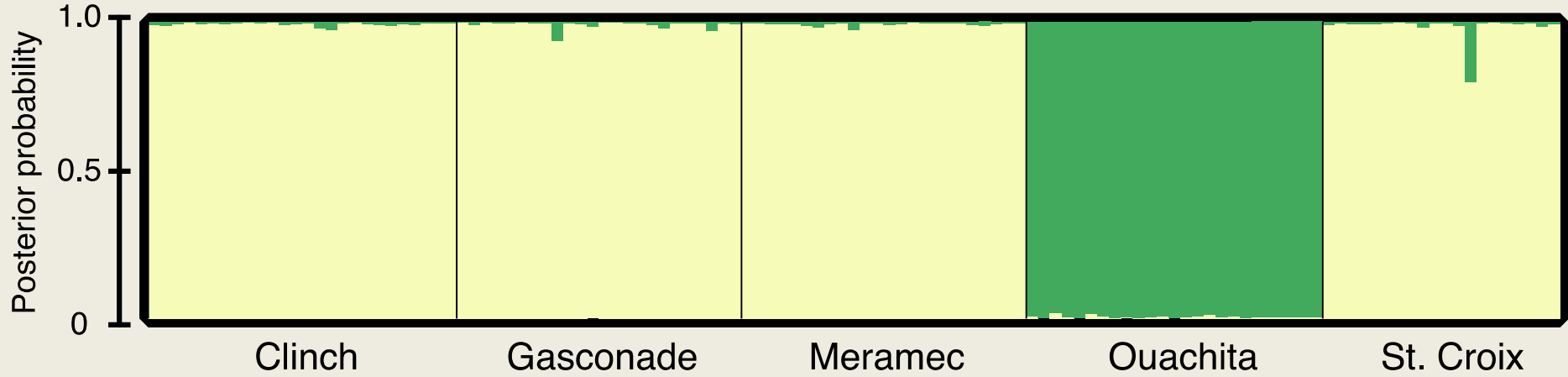N_e: 22,600

24%
Among
drainages

73%
Within
populations

3%
Among
populations

Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, and the GIS User Community

Inoue *et al.* (in press)

Microsatellites

Structure
2 distinct clusters ($k = 2$)

Devils River

Black River

Rio Grande

Posterior Probability

1.0

0.5

0

CC Fall DV RF1 BS RF3 DR RG1 RG2 RG3 RG4 RG5

Group 1
Group 2

Inoue *et al.* (in press)
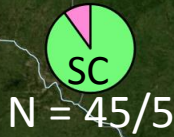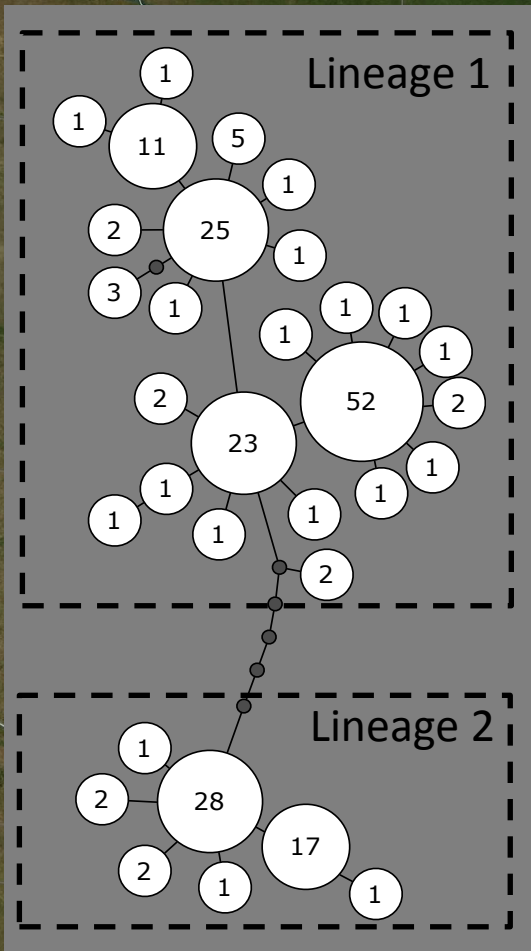
COI Sequences

COI Sequences

N = # of individuals with
Lineage 1/Lineage 2 haplotypes

SC
N = 45/5

Lineage 1

1
1
11
5
1
2
25
1
3
1
1
1
1
1
2
52
2
23
1
1
1
1
1
2

Lineage 2

1
2
28
2
17
1
1

N = 33/7
GR
MR
N = 28/6

N = 34/14
CR

Lineage 1
Lineage 2

N = 0/20
OR

0   50 100   200   300   400
km
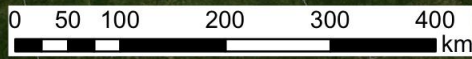
Inoue *et al*. (2014)

Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, and the GIS User Community

Microsatellites

Within-population genetic diversity

N_A = Allelic richness
N_E = Effective population size

SC
N_A: 13.2
N_e: 4,559

GR
N_A: 12.8
N_e: 4,406

MR
N_A: 13.8
N_e: 5,547

CR
N_A: 13.9
N_e: 4,375

OR
N_A: 8.0
N_e: 1,410

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

Inoue *et al.* (2014)

Microsatellites

Structure
$k = 2$

Posterior probability: 1.0, 0.5, 0

Clinch   Gasconade   Meramec   Ouachita   St. Croix

Group 1
Group 2

Inoue *et al.* (2014)
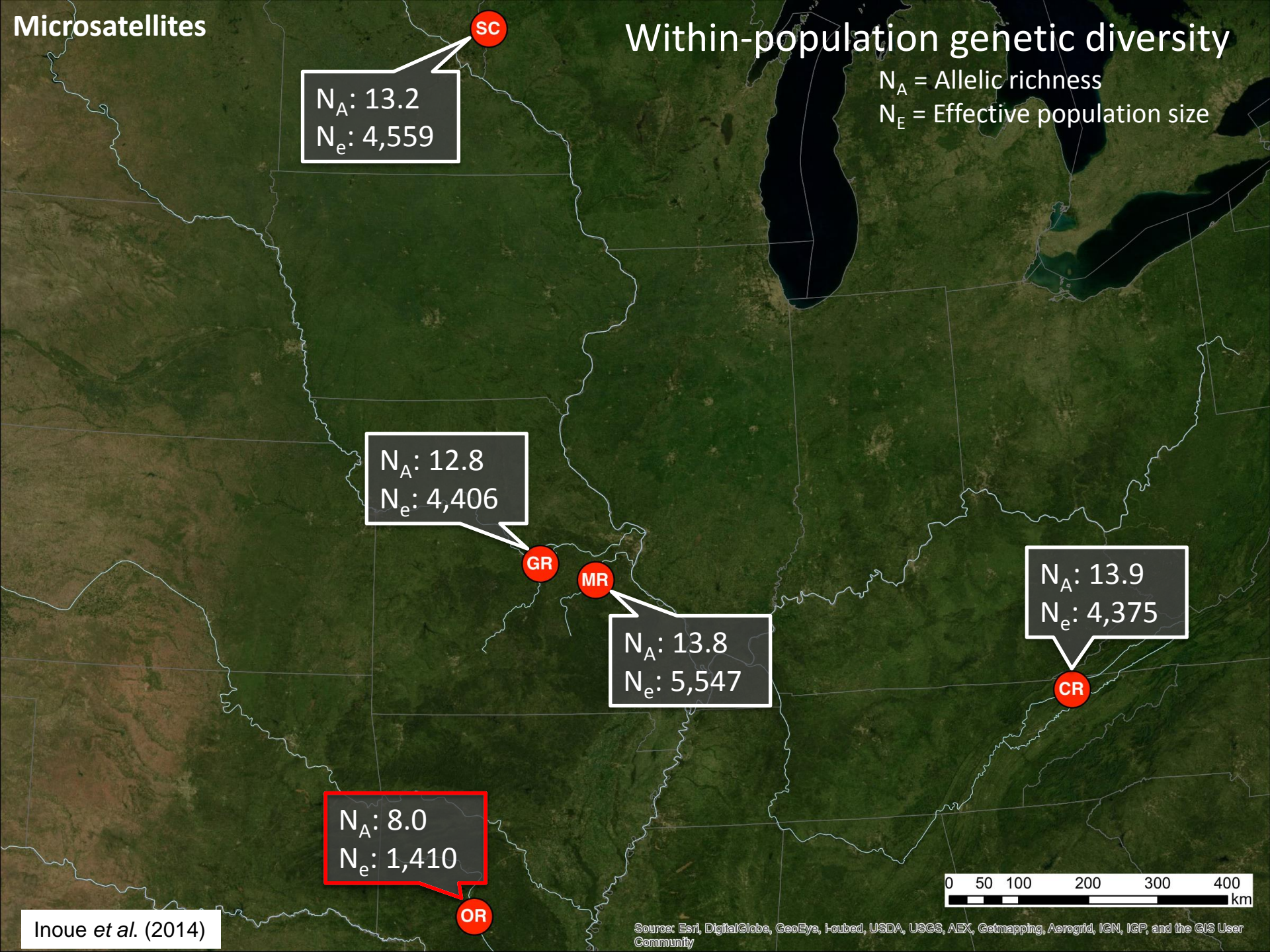
Popenaias popeii

  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

10 founders retain 95% of the heterozygosity
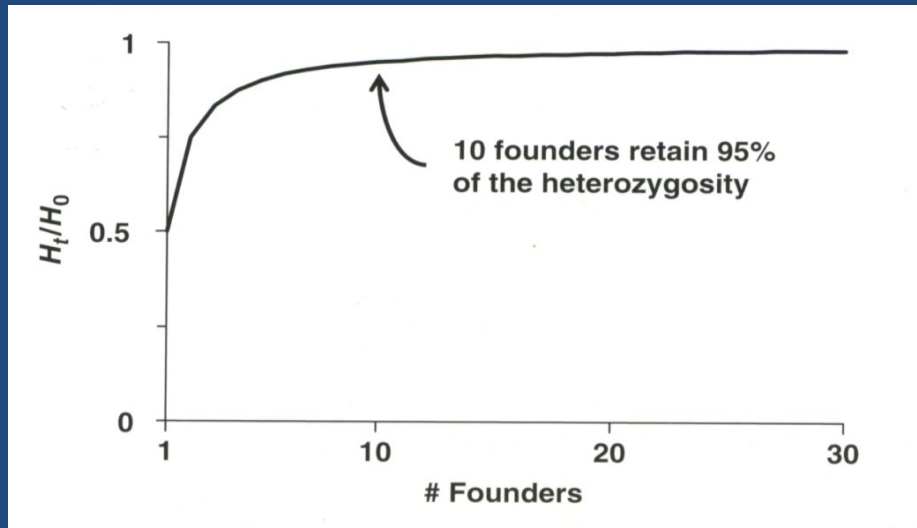
Frankham *et al.* (2010), page 436
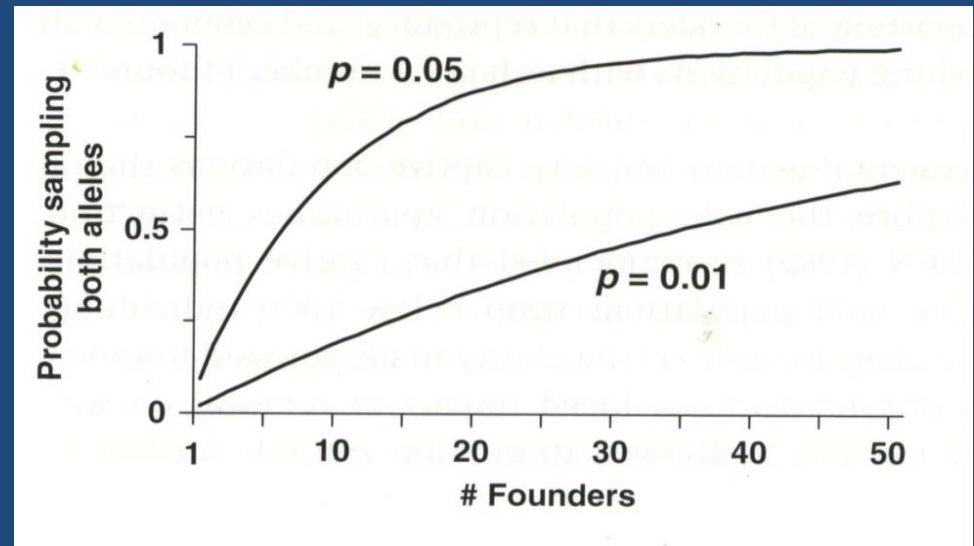
$N_e = N_c$, when
50:50 sex ratio with random mating
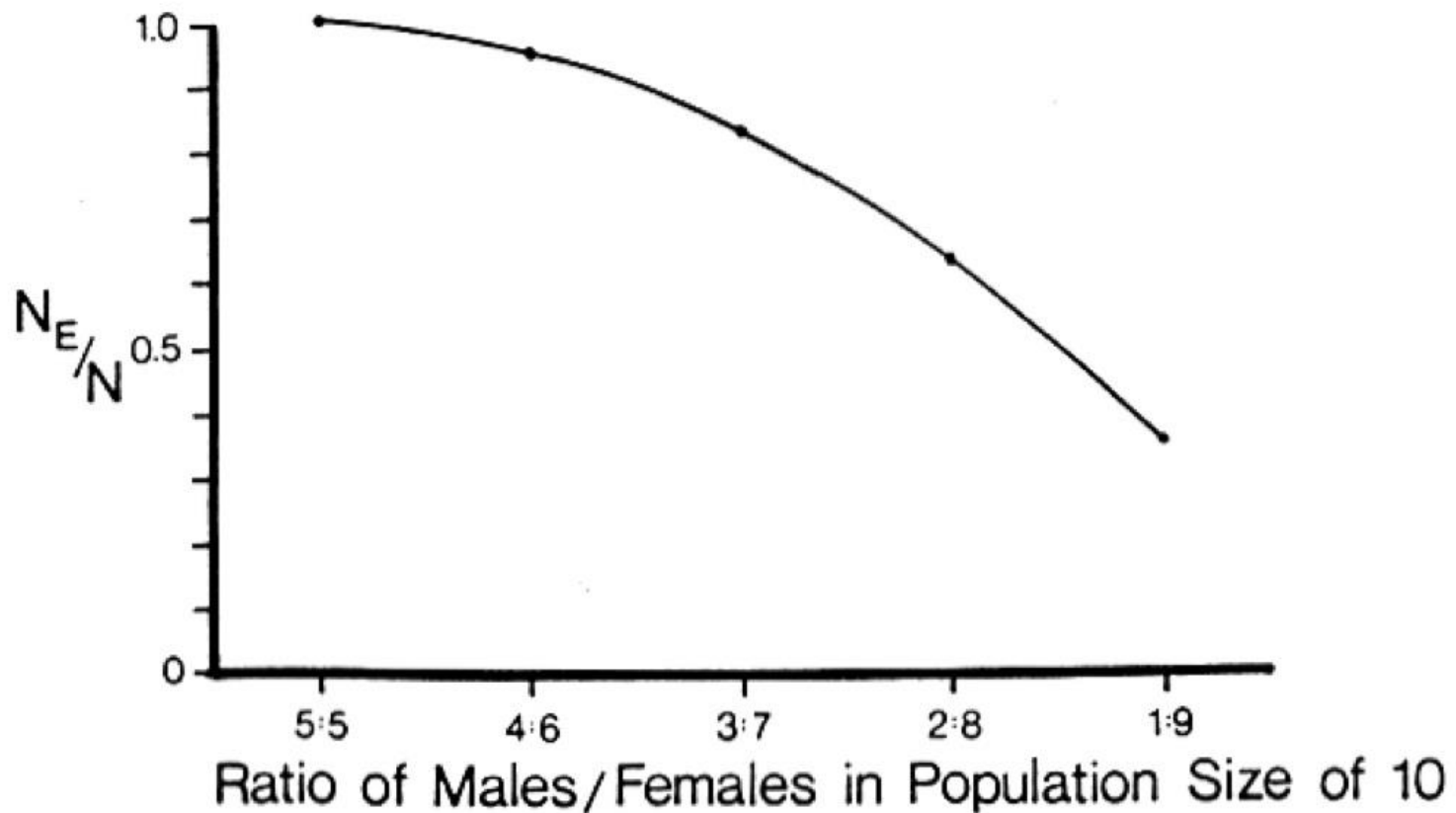
Foos (1986)

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
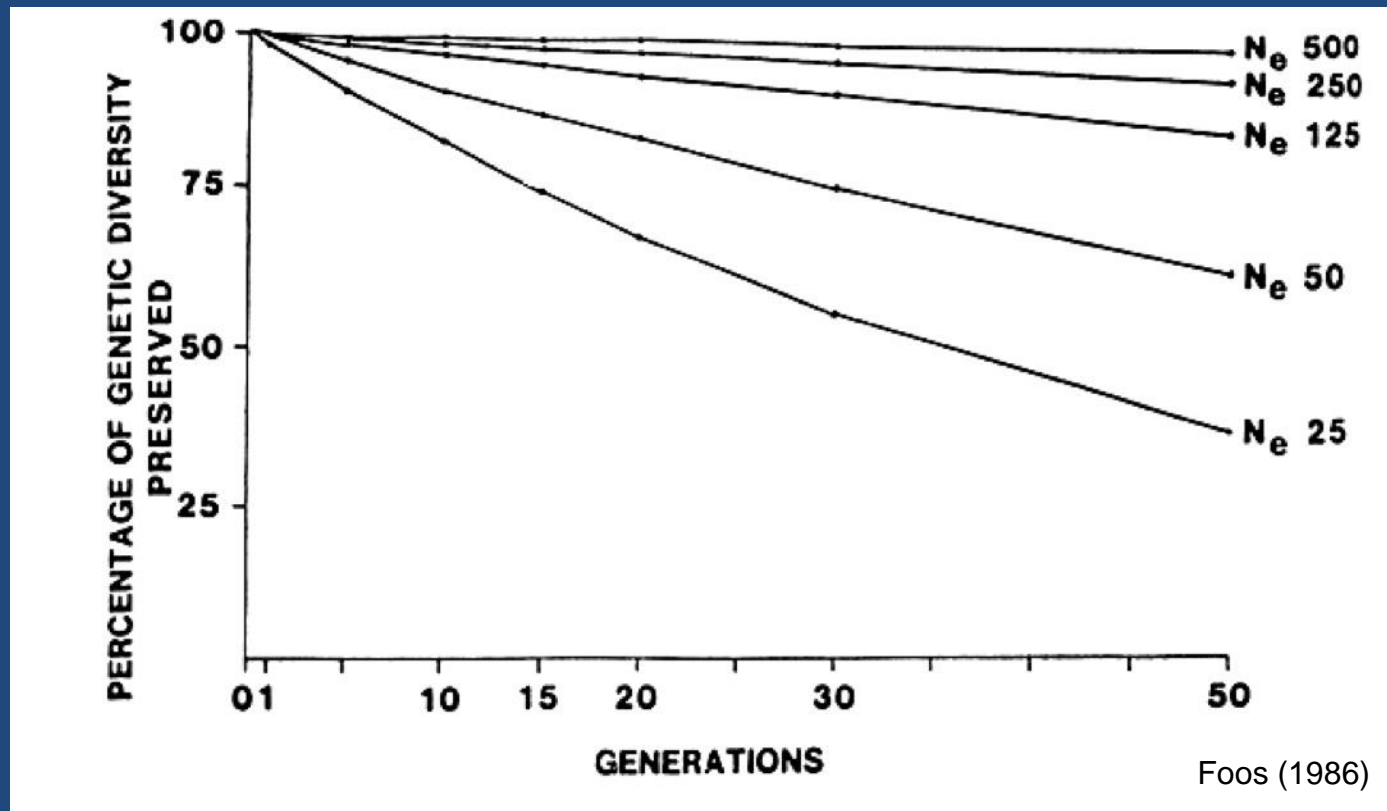
variance in reproduction is random
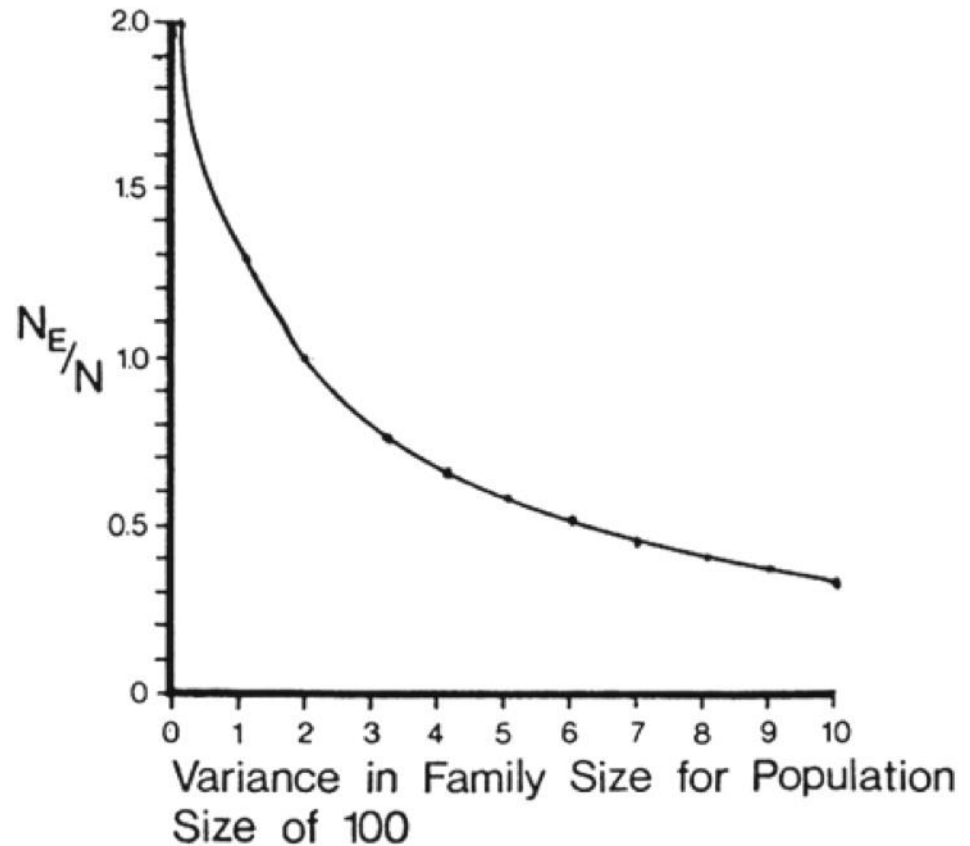
Foos (1986)

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
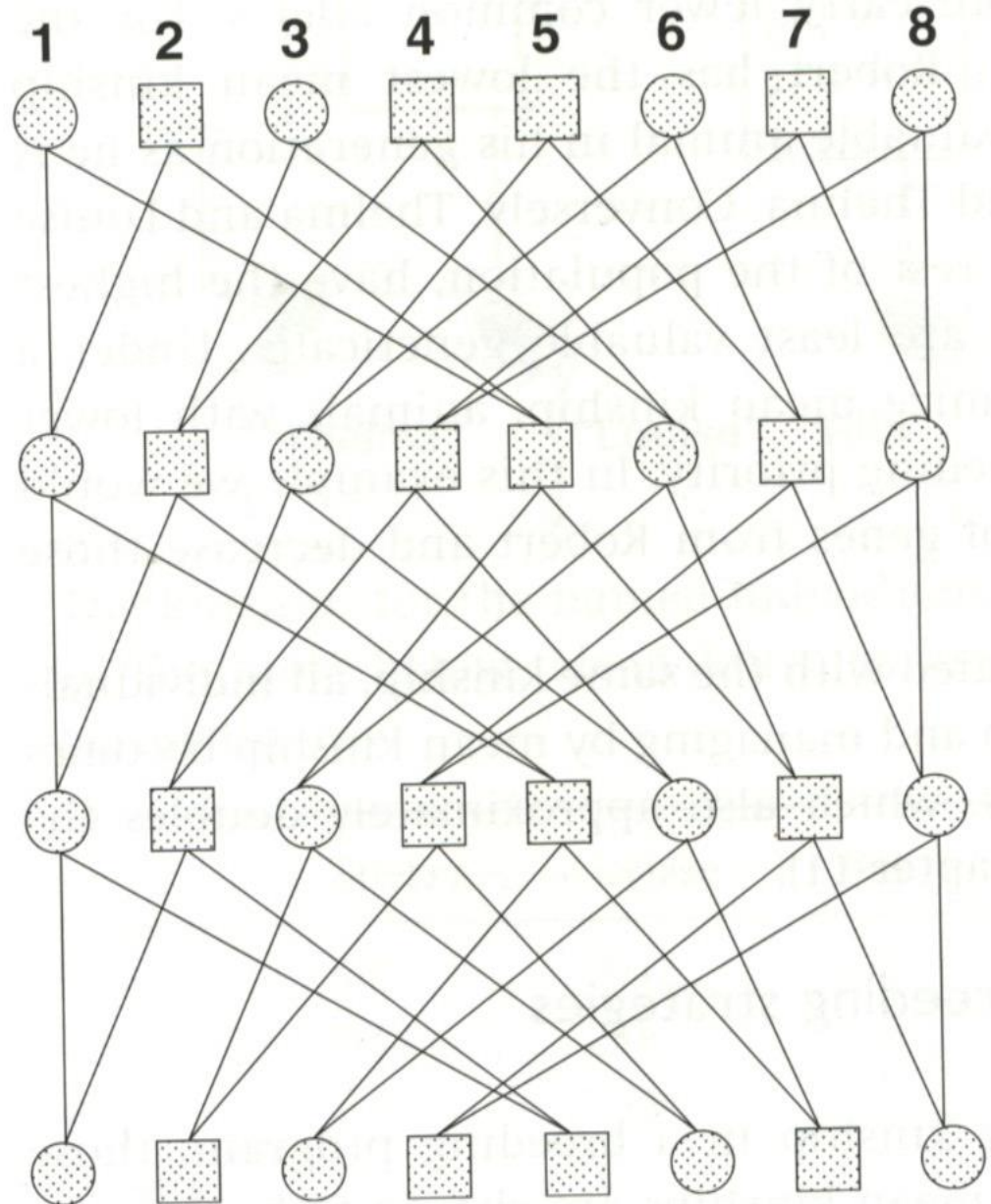
variance in reproduction is random

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
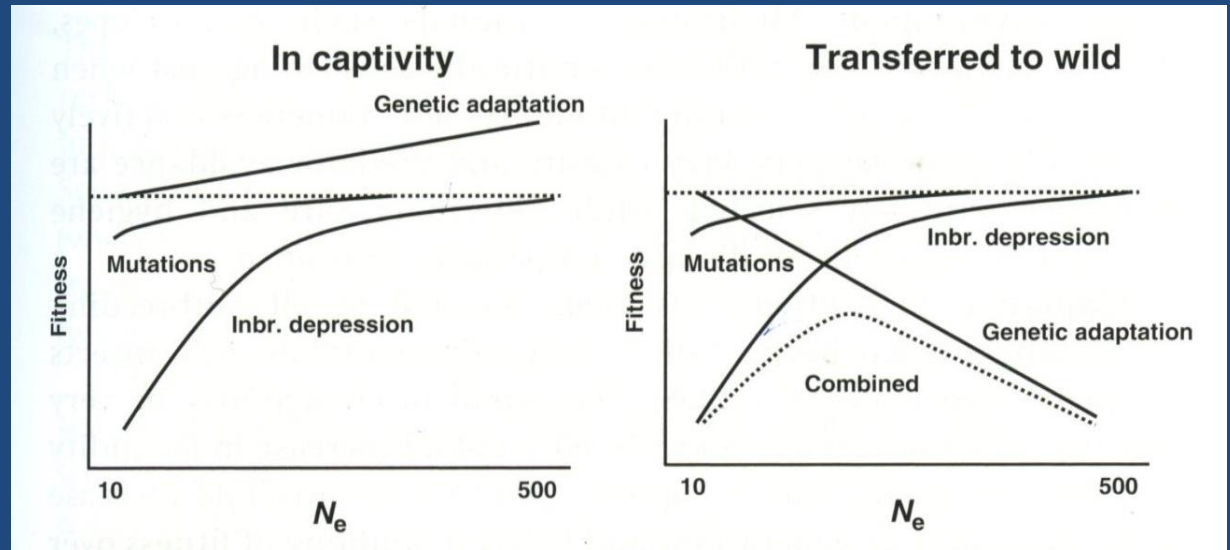
Selection is on the phenotype:
genotype + environment

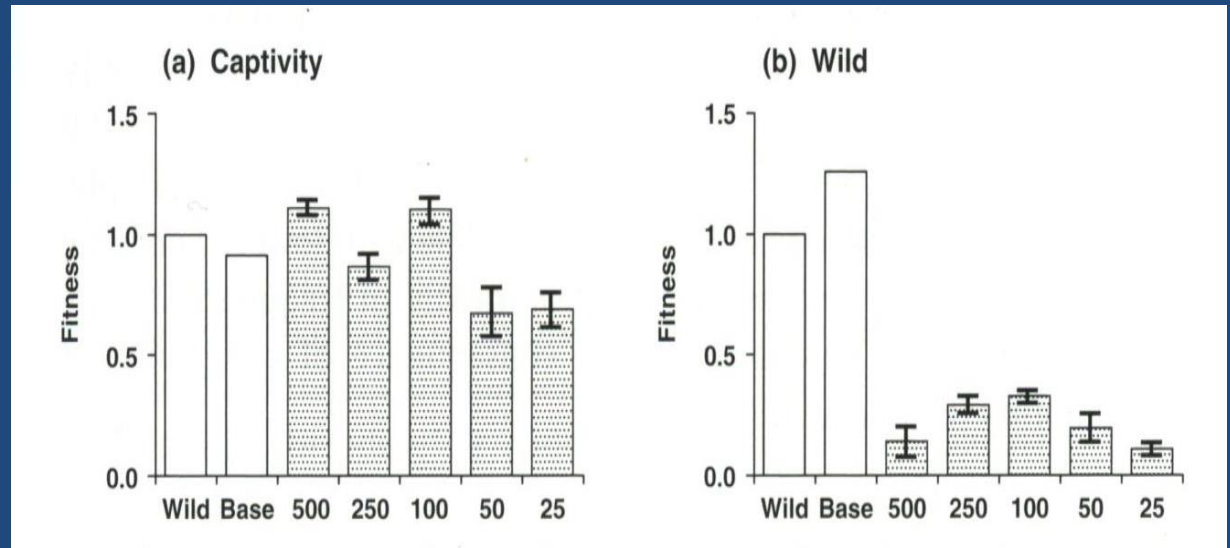Selection varies with environment
natural vs. artificial selection

SLOSS: Single large or several small populations?

Frankham *et al.* (2010), page 460
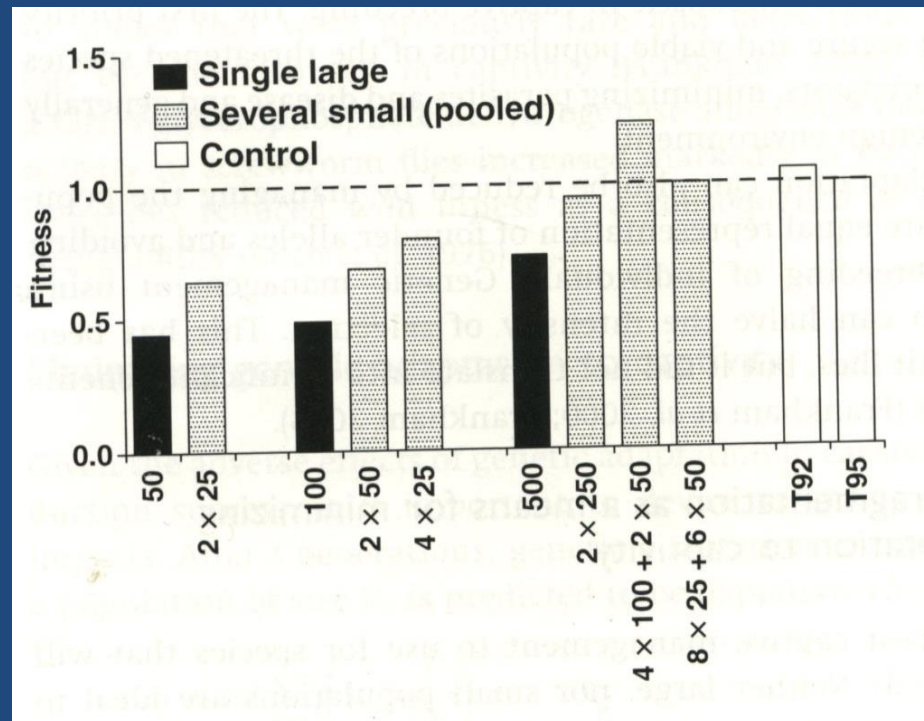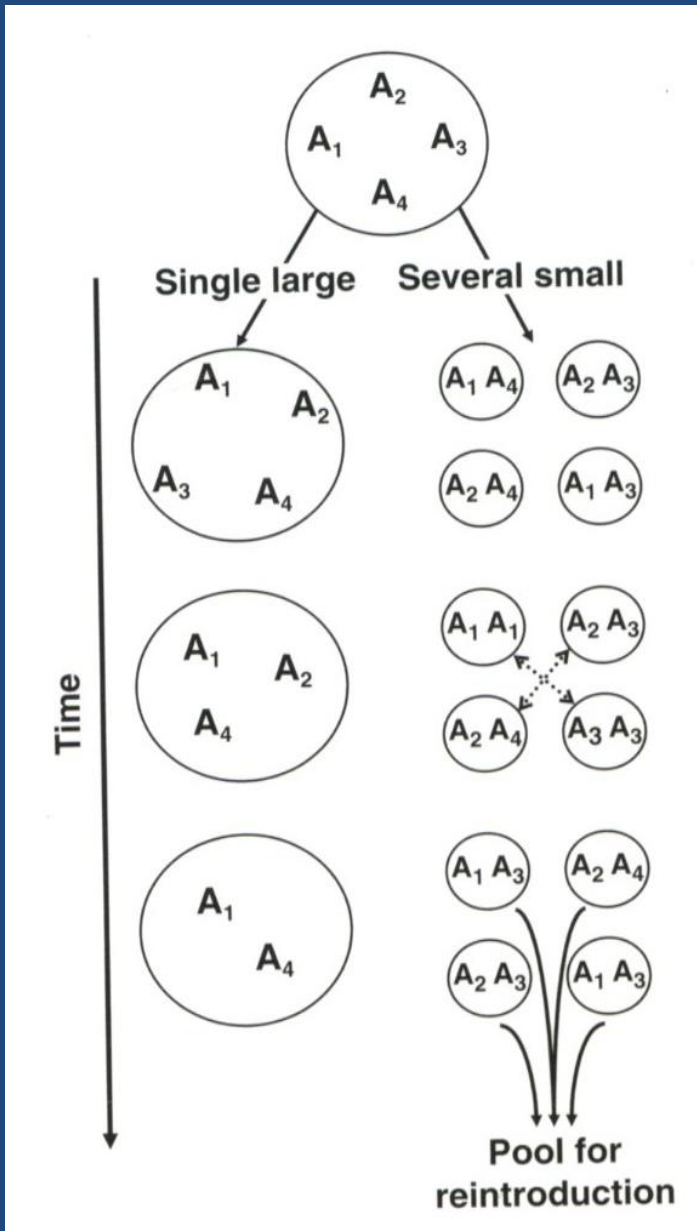
Traits under positive selection in captivity can be deleterious in the wild

Genetic deterioration in *Drosophila*

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.* (in press)

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

Citations

Burlakova, L. E., A. Y. Karatayev (2013) Survey of Texas hornshell populations in Texas. *Interim Performance Report for Project* #419446. Texas Parks & Wildlife Department, Austin.

Foos, T. J. (1986) Genetics and demography of small populations. *The Przewalski Horse and Restoration to Its Natural Habitat in Mongolia.* Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/docrep/004/ac148e/AC148E00.htm#TOC

Frankham, R., J. D. Ballou, D. A. Briscoe (2010) *Introduction to Conservation Genetics*, 2nd Ed. Cambridge University Press, England.

Inoue, K., B. K. Lang, D. J. Berg (in press) Past climate change drives current genetic structure of an endangered freshwater mussel. *Molecular Ecology*

Inoue, K., E. M. Monroe, C. L. Elderkin, D. J. Berg (2014) Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide-ranging, but endangered, freshwater mussel. *Heredity* 112: 282-290.

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