Physiographic and Anthropogenic Influences on the Genetic Structure of Timber Rattlesnake Populations

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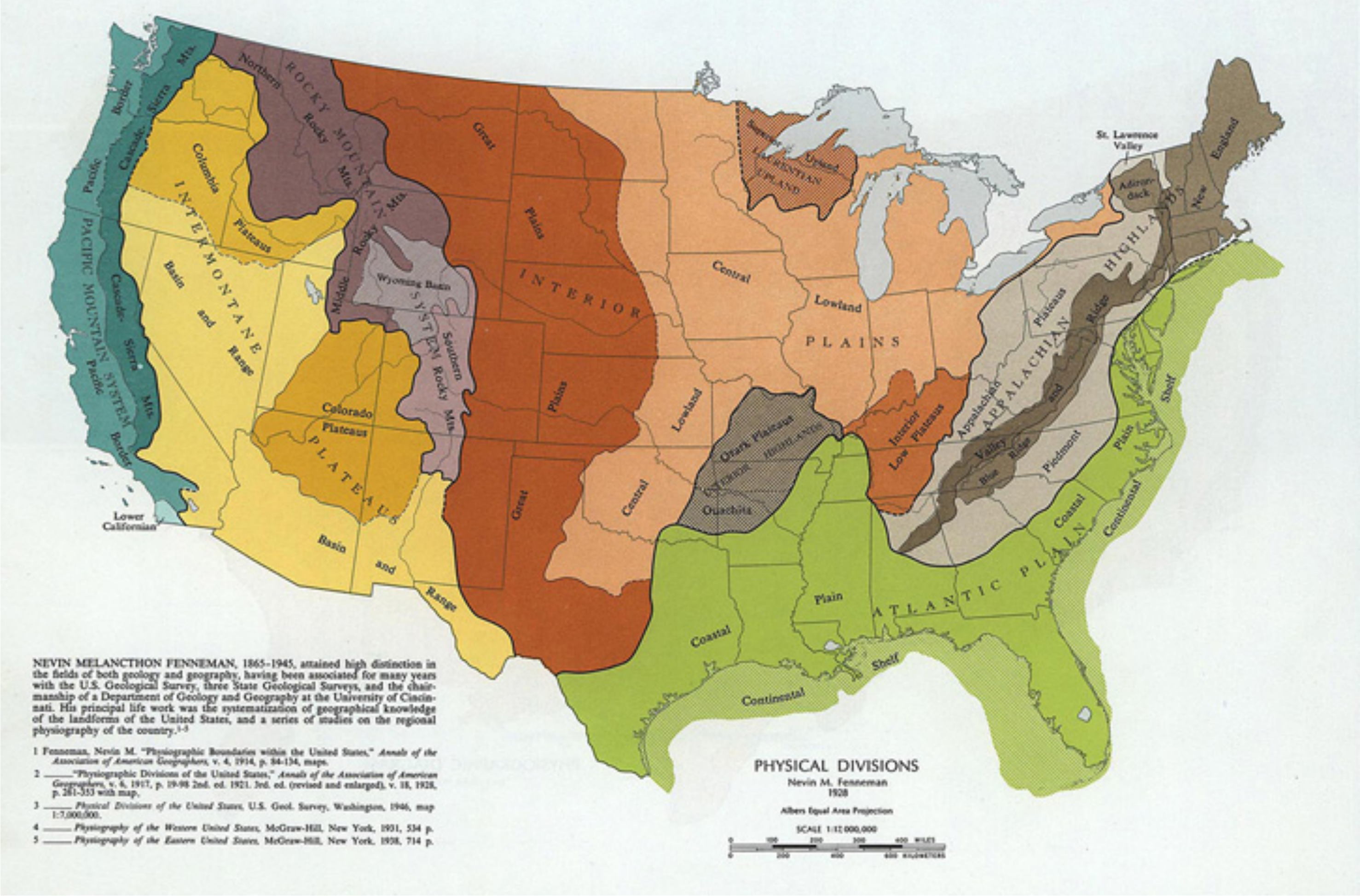
Physiographic regions: Landform description based on geologic morphology and history



NEVIN MELANCTHON FENNEMAN, 1865–1945, attained high distinction in the fields of both geology and geography, having been associated for many years with the U.S. Geological Survey, three State Geological Surveys, and the chairmanship of a Department of Geology and Geography at the University of Cincinnati. His principal life work was the systematization of geographical knowledge of the landforms of the United States, and a series of studies on the regional physiography of the country.[1-5]

1 Fenneman, Nevin M. "Physiographic Boundaries within the United States," *Annals of the Association of American Geographers*, v. 4, 1914, p. 84-134, maps.
2 ——— "Physiographic Divisions of the United States," *Annals of the Association of American Geographers*, v. 6, 1917, p. 19-98 2nd. ed. 1921. 3rd. ed. (revised and enlarged), v. 18, 1928, p. 261-353 with map.
3 ——— *Physical Divisions of the United States*, U.S. Geol. Survey, Washington, 1946, map 1:7,000,000.
4 ——— *Physiography of the Western United States*, McGraw-Hill, New York, 1931, 534 p.
5 ——— *Physiography of the Eastern United States*, McGraw-Hill, New York, 1938, 714 p.

PHYSICAL DIVISIONS
Nevin M. Fenneman
1928

Albers Equal Area Projection

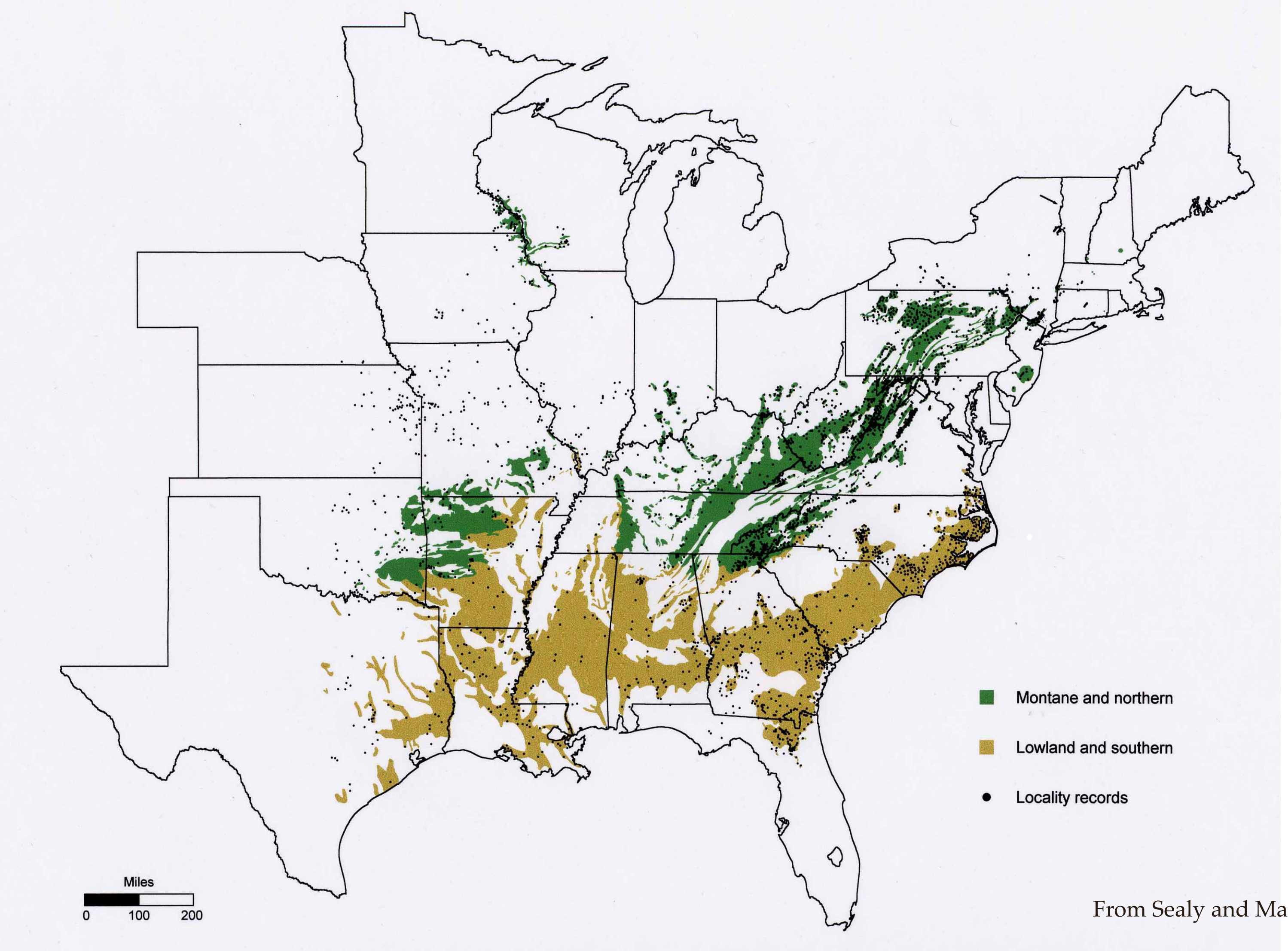
SCALE 1:12,000,000

Anthropogenic: Resulting from human activity

The Timber Rattlesnake: a species at risk

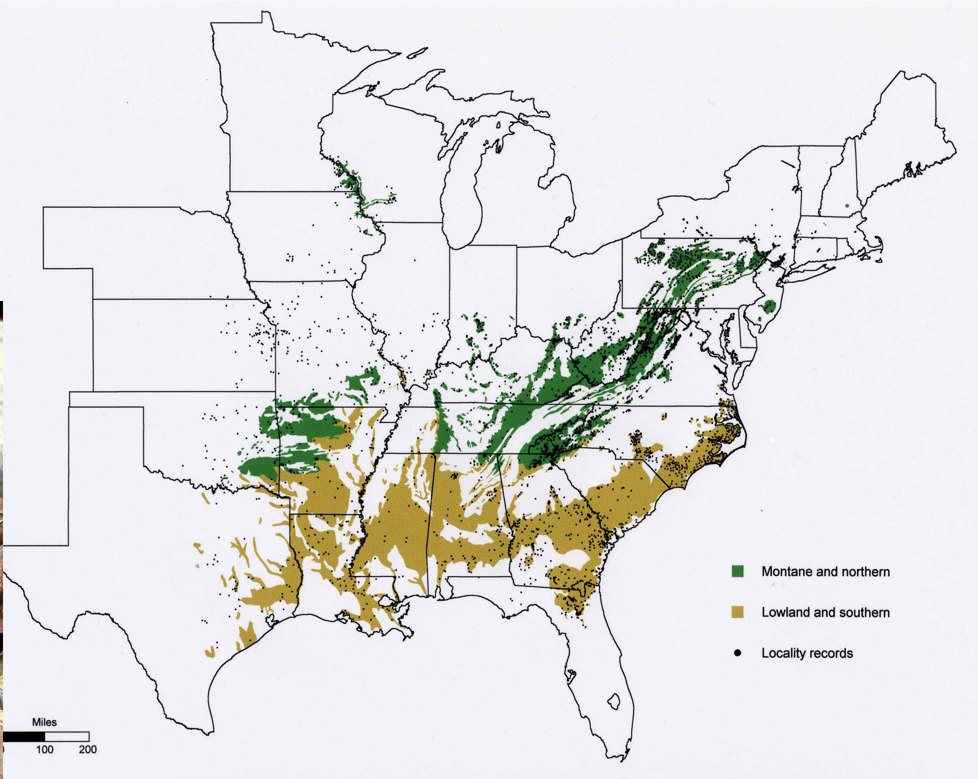
- **Endangered:**
  - Connecticut, Massachusetts, New Hampshire, New Jersey, Ohio, Vermont, and Virginia
- **Threatened:**
  - Illinois, Indiana, New York, and Texas
- **Species of Special Concern:**
  - Minnesota, Pennsylvania, West Virginia, Wisconsin

Distribution of the Timber Rattlesnake



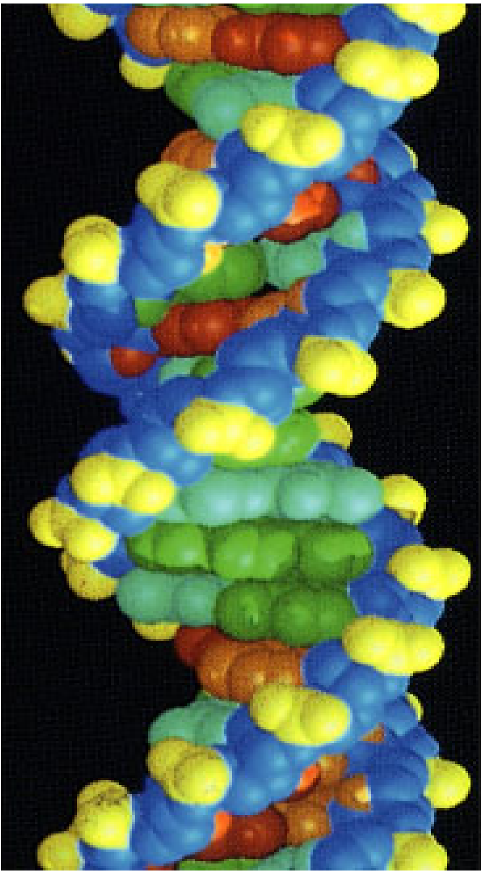
From Sealy and Martin

We use genetic analyses to study Timber Rattlesnake populations

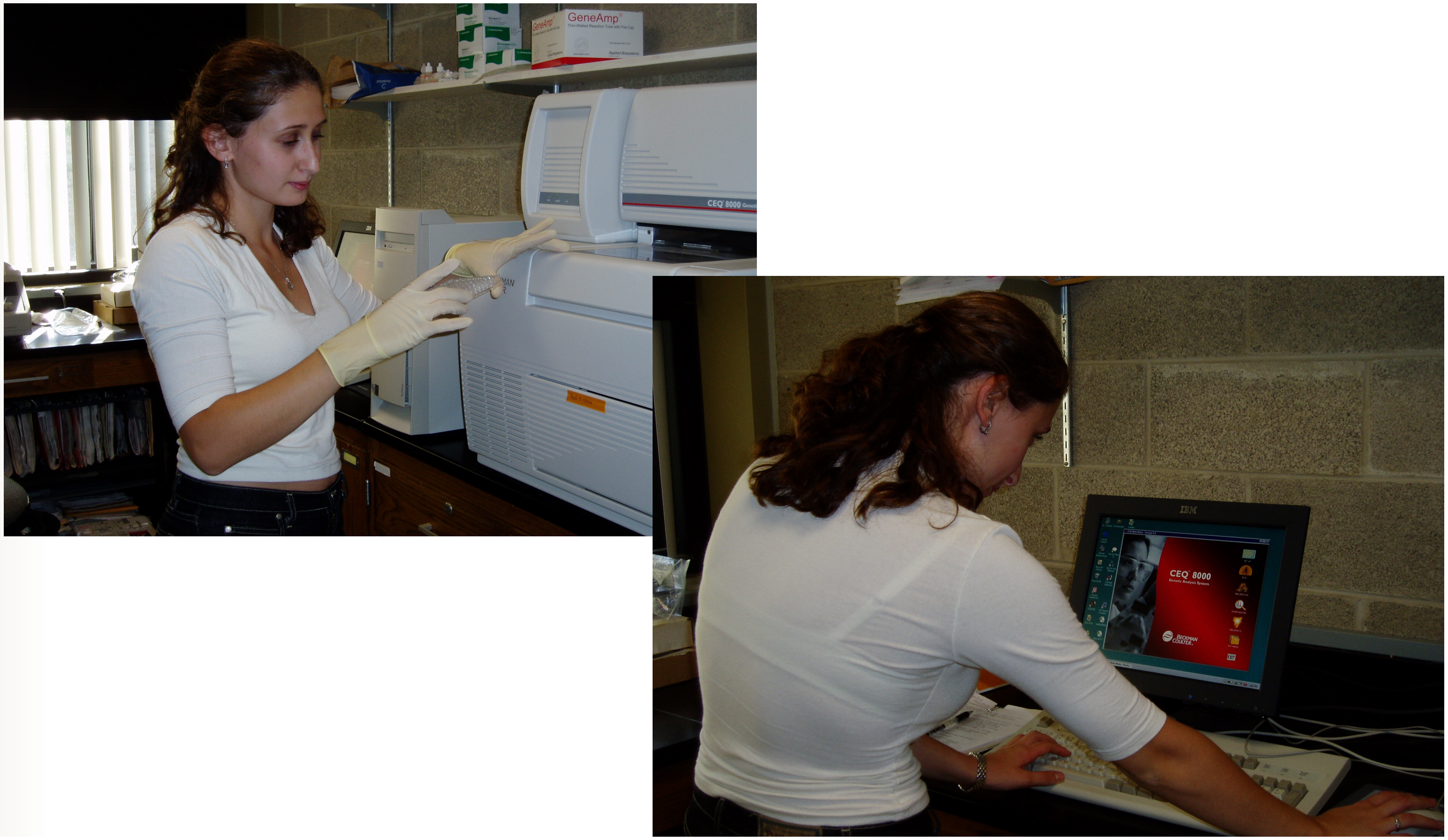


From Sealy and Martin

DNA is purified from whole blood



Microsatellite loci (a type of genetic marker) are used to look at relationships among Timber Rattlesnakes

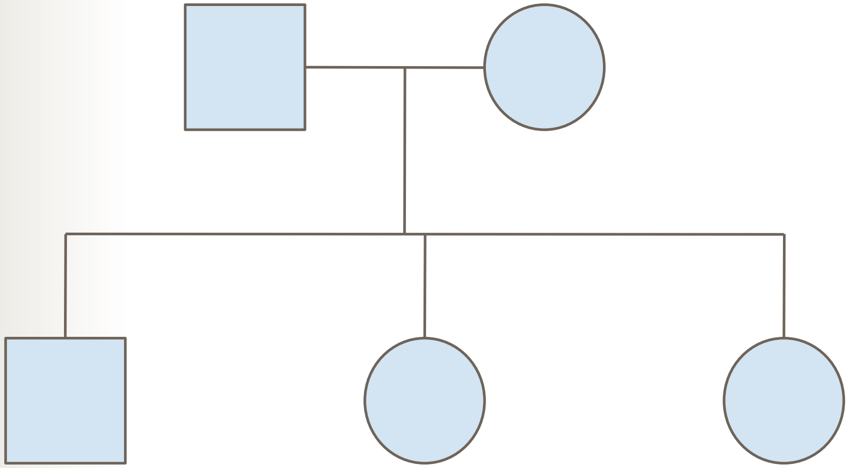


Genes encode traits

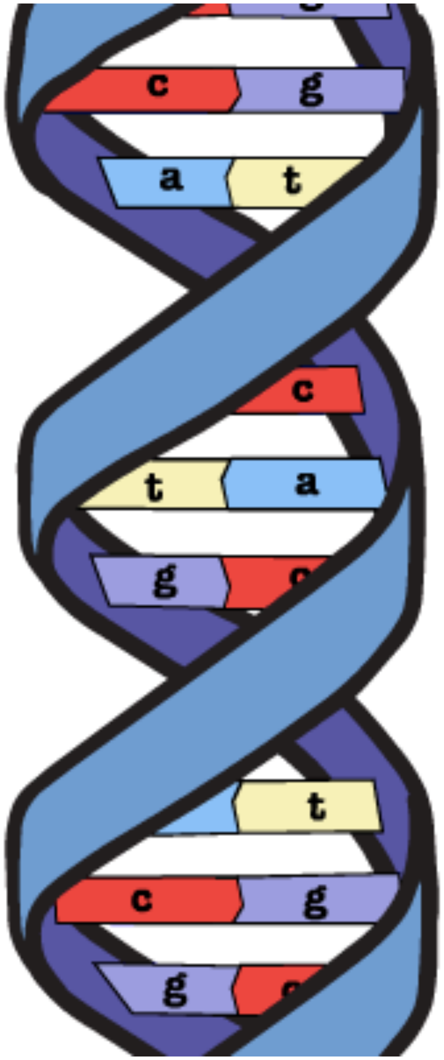
- For example, hair color or eye color
- We have two genes for each trait
  - Alleles: different forms of the same gene

Blue/Blue

Brown/Brown



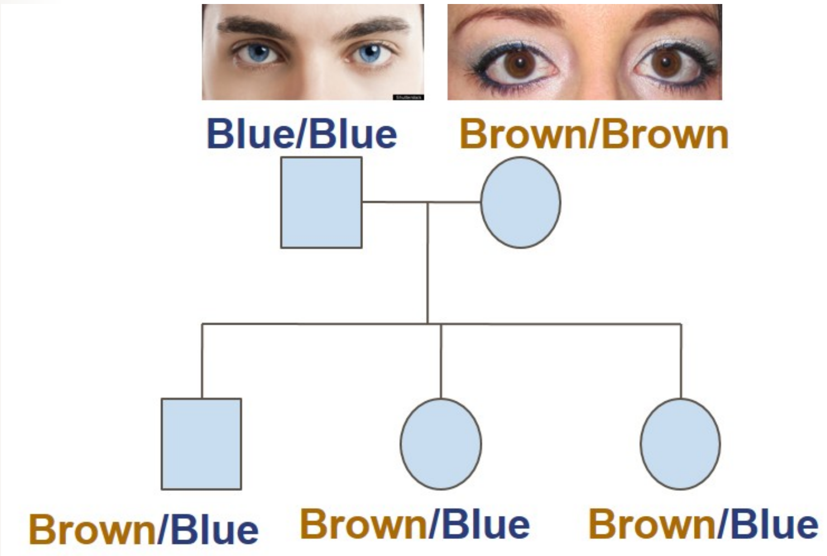
Brown/Blue **Brown/Blue** **Brown/Blue**

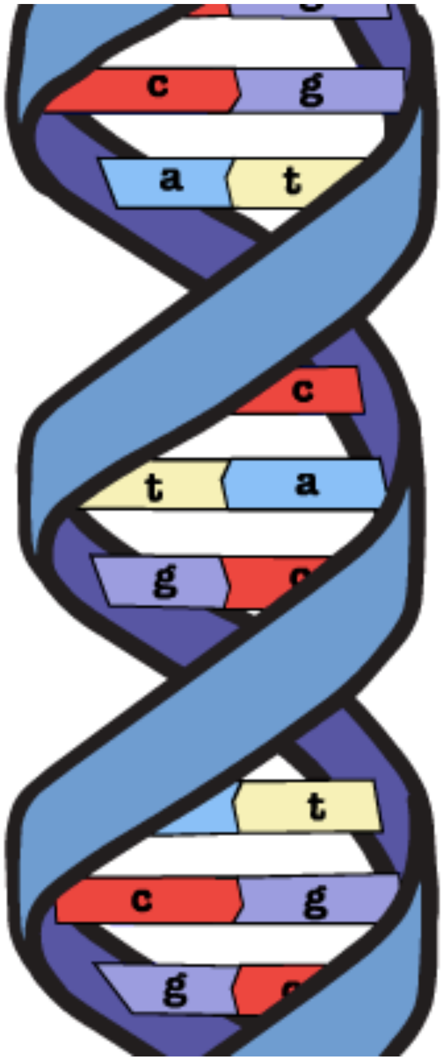


http://venturebeat.com/

Genetic information is in **DNA**

- DNA consists of four "bases"
    - Adenine (A)
    - Cytosine (C)
    - Guanine (G)
    - Thymine (T)
- Sequence of bases spells out the geneti information

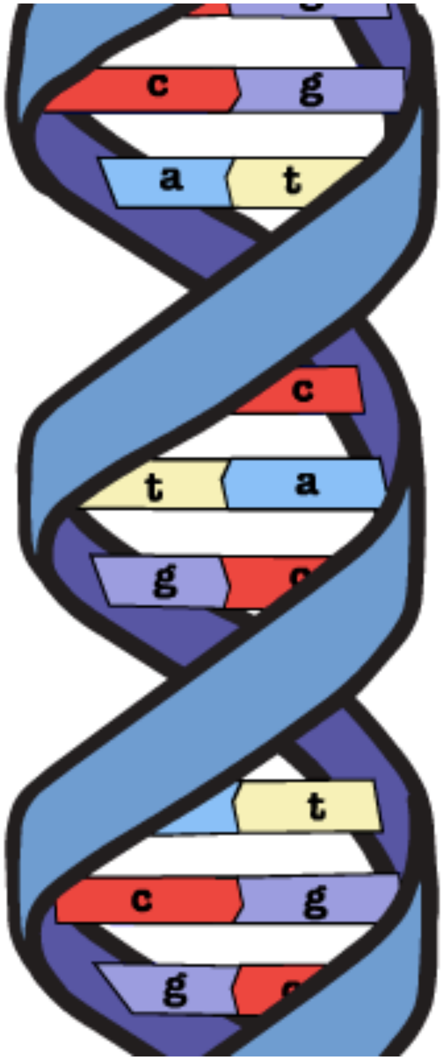




http://venturebeat.com/

Microsatellites

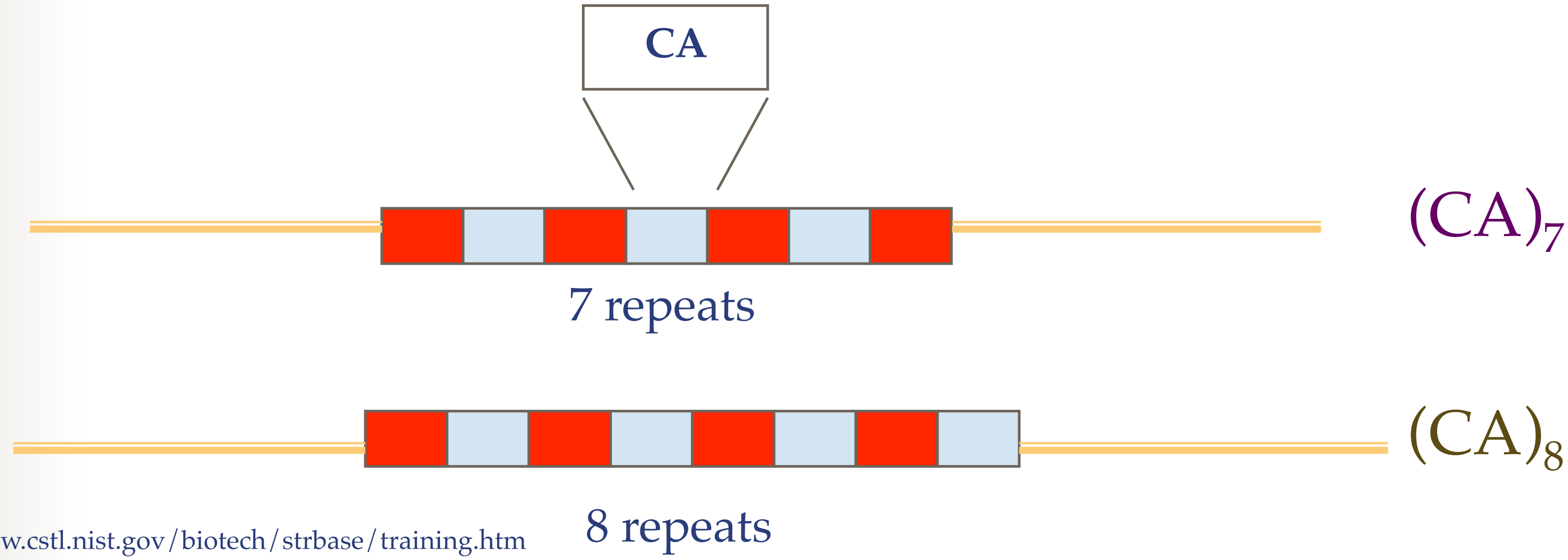
- Noncoding
  - Not subject to natural selection
  - Tend to be more variable than coding sequences
- Microsatellites are short tandemly repeated sequences of bases in the DNA
  - CACACACA = $(CA)_4$



http://venturebeat.com/

Microsatellites

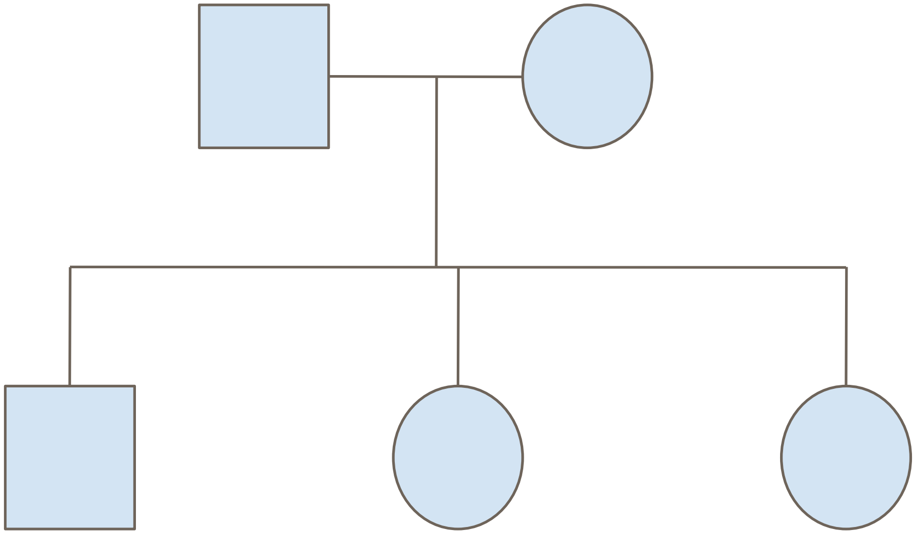
- Alleles (different forms of the same gene) differ by the number of tandem repeats
  - CACACACA = $(CA)_4$
  - CACACACACACA = $(CA)_6$
  - CACACACACACACA = $(CA)_7$
  - CACACACACACACACA = $(CA)_8$



http://www.cstl.nist.gov/biotech/strbase/training.htm

Microsatellites

- Used for:
  - Paternity testing
  - Identity testing



$(CA)_5/(CA)_4$ $(CA)_6/(CA)_3$

$(CA)_5/(CA)_3$ $(CA)_6/(CA)_4$ $(CA)_6/(CA)_5$

Microsatellites

- Identical twins share the exact same genes
- Siblings share about half of their genes
- Parents and children share about half their genes

$(CA)_5$ / $(CA)_3$

$(CA)_5$ / $(CA)_4$

Microsatellites

- Identical twins share the exact genes
- Siblings share about half of their genes
- Parents and children share about half their genes
- Grandparents and grandchildren share about ¼ of their genes

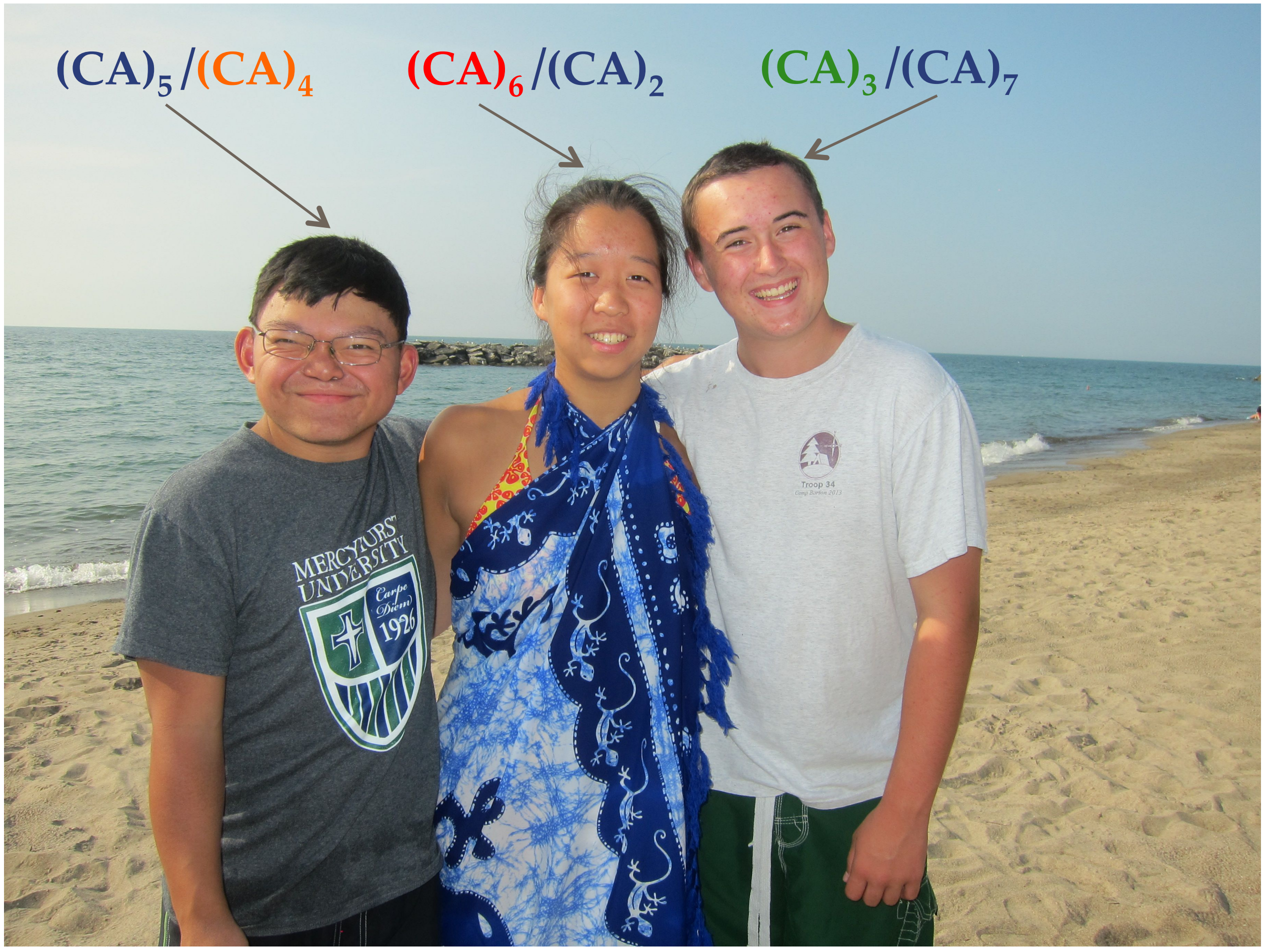
$(CA)_5$ /$(CA)_7$

$(CA)_5$ /$(CA)_3$

$(CA)_6$ /$(CA)_4$

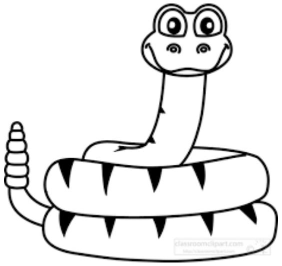
Microsatellites

- Genetically unrelated individuals share very few genes

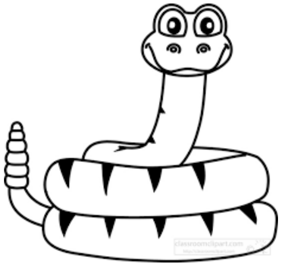


Microsatellites

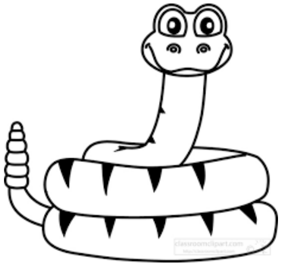
- Used to determine the relationships among Timber Rattlesnake populations



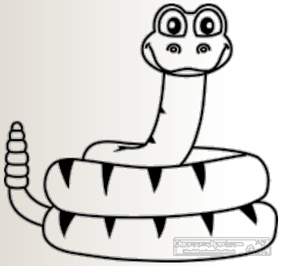
$(\mathrm{CA})_6$ /$(\mathrm{CA})_4$



$(\mathrm{CA})_5$ /$(\mathrm{CA})_5$



$(\mathrm{CA})_6$ /$(\mathrm{CA})_5$



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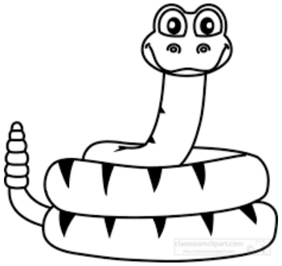

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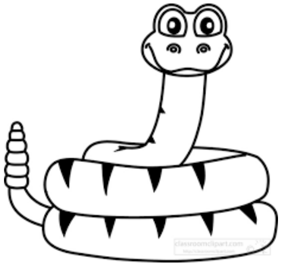

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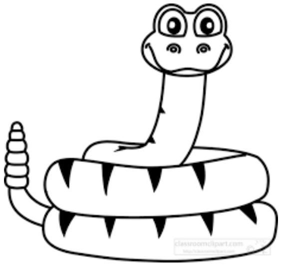
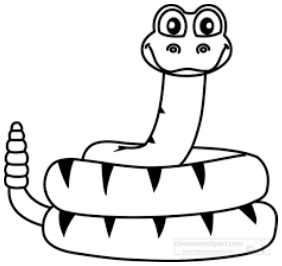


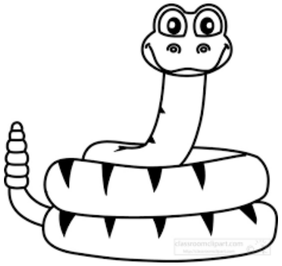
$(\mathrm{CA})_6$ /$(\mathrm{CA})_5$

Microsatellites

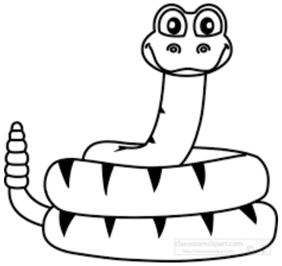
- Individuals in the same population have many of the same genes because they are mating with each other



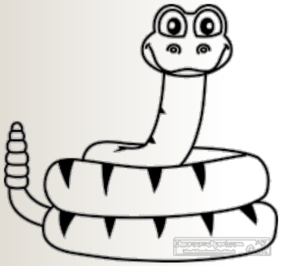
$(CA)_6$ /$(CA)_4$



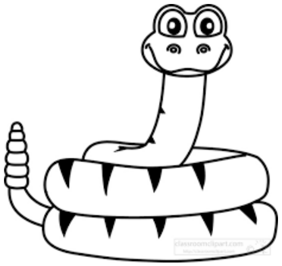
$(CA)_5$ /$(CA)_5$



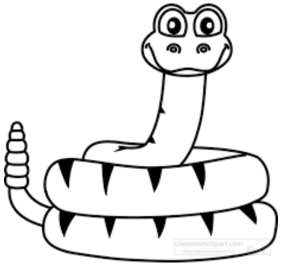
$(CA)_6$ /$(CA)_5$



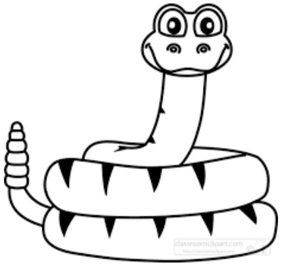
$(CA)_6$ /$(CA)_4$



$(CA)_5$ /$(CA)_4$



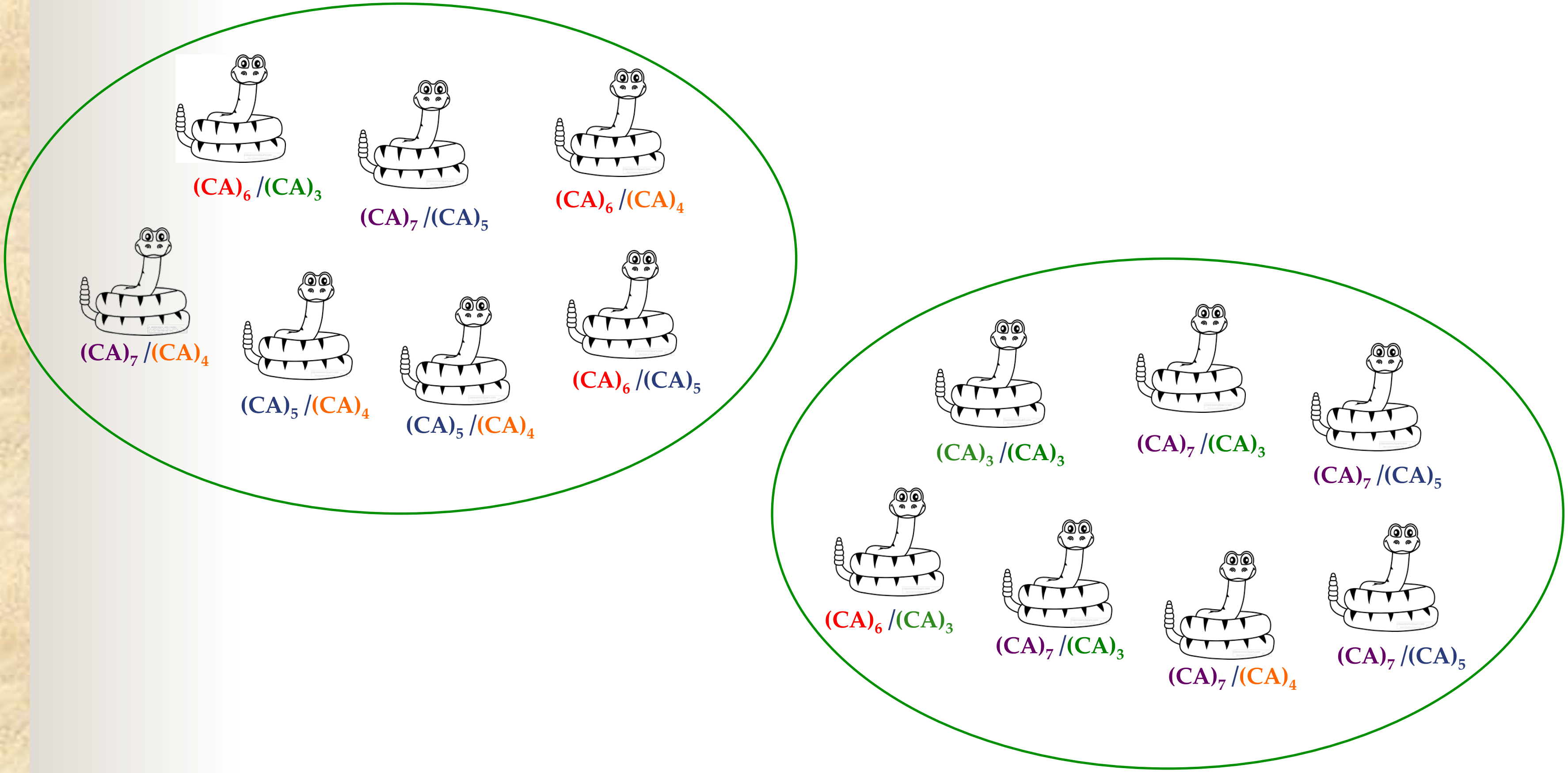
$(CA)_5$ /$(CA)_4$



$(CA)_6$ /$(CA)_5$

Microsatellites

- Individuals in two nearby populations will share some genes as long as some individuals can migrate between the populations in order to mate



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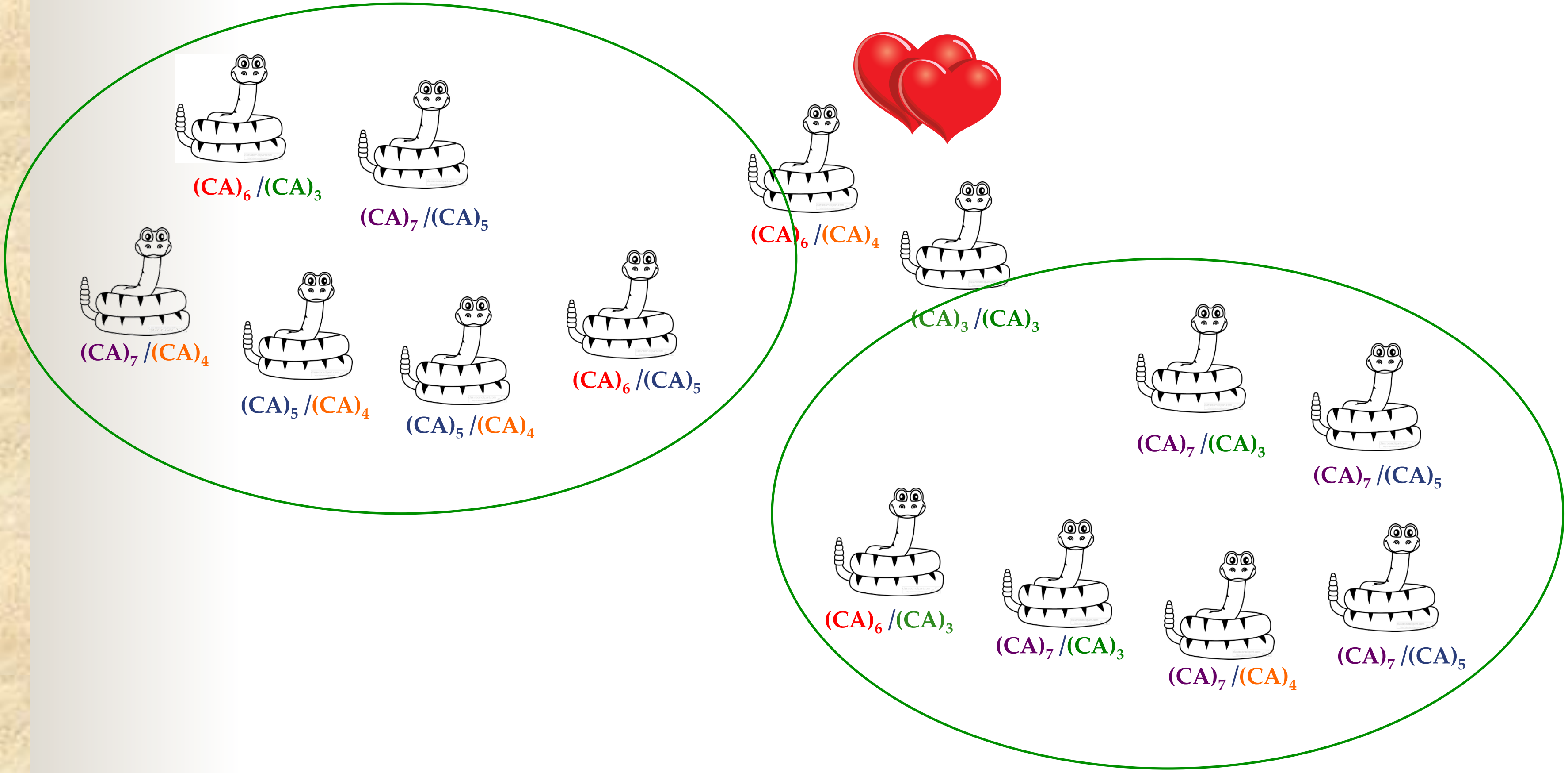

$(CA)_6/(CA)_3$

$(CA)_7/(CA)_5$

$(CA)_6/(CA)_4$

$(CA)_3/(CA)_3$

$(CA)_7/(CA)_4$

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$(CA)_5/(CA)_4$

$(CA)_5/(CA)_4$

$(CA)_7/(CA)_3$

$(CA)_7/(CA)_5$

$(CA)_6/(CA)_3$

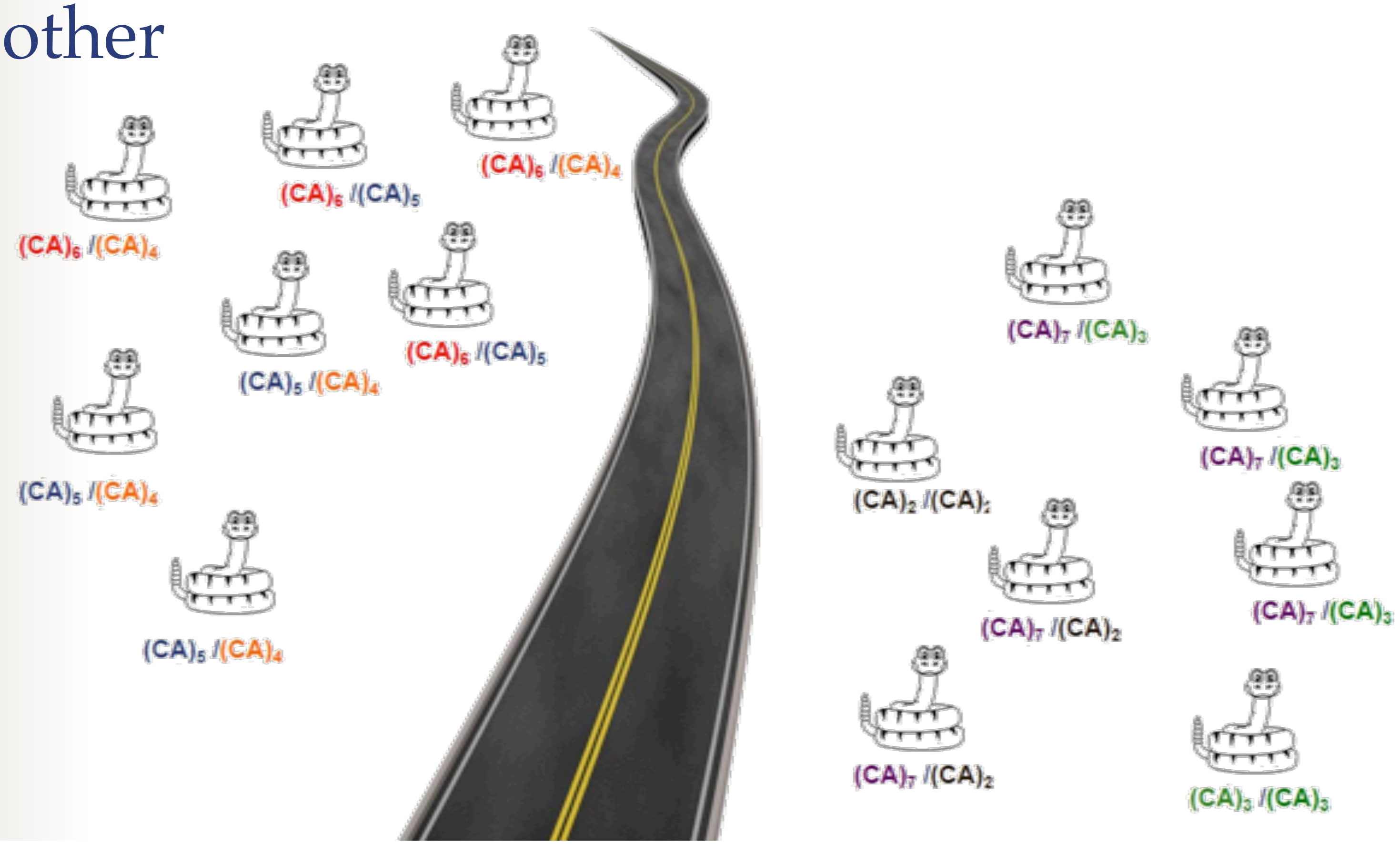
$(CA)_7/(CA)_3$

$(CA)_7/(CA)_4$

$(CA)_7/(CA)_5$

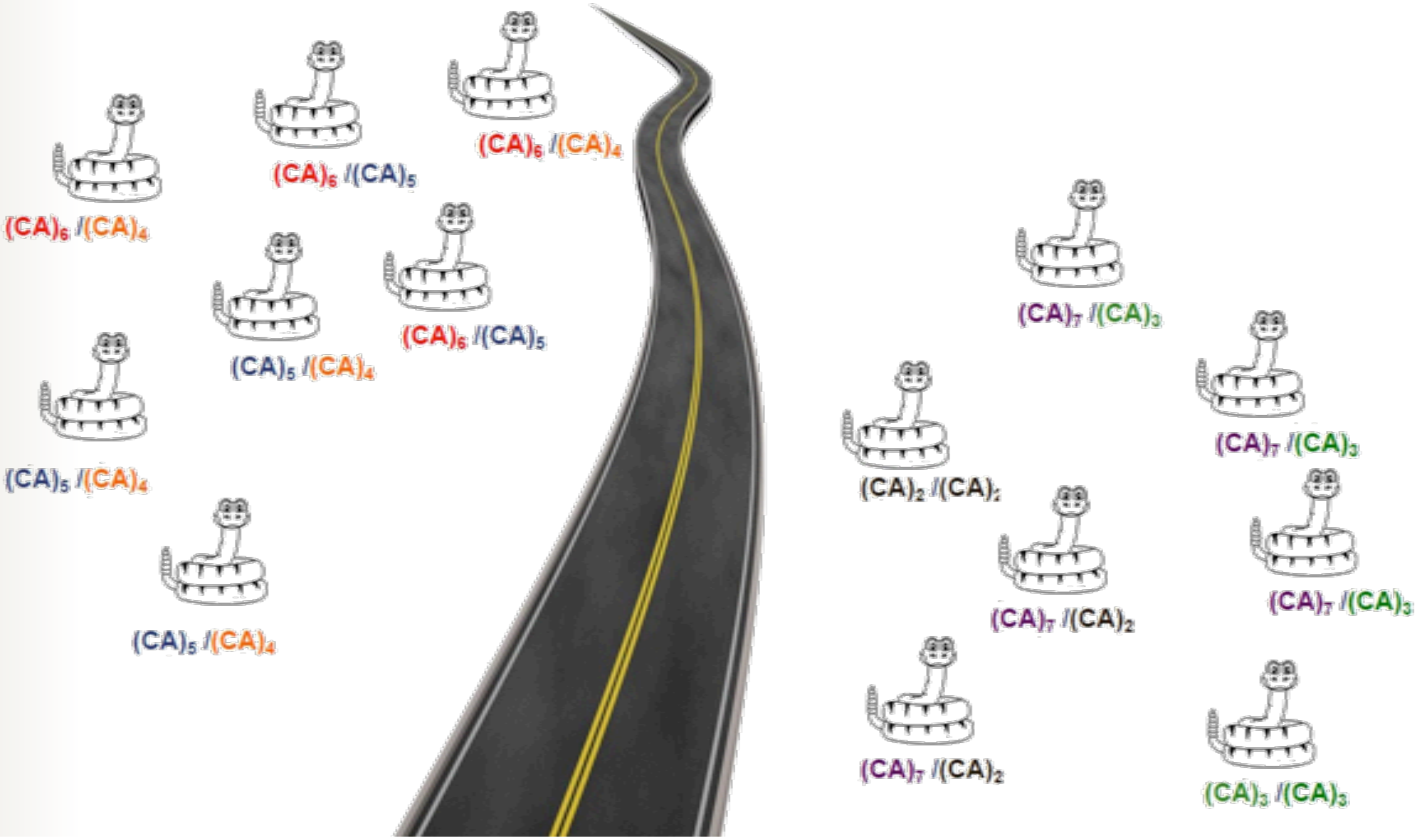
Microsatellites

- Over time, individuals in two populations isolated by distance or other barriers will share few genes because they never mate with each other

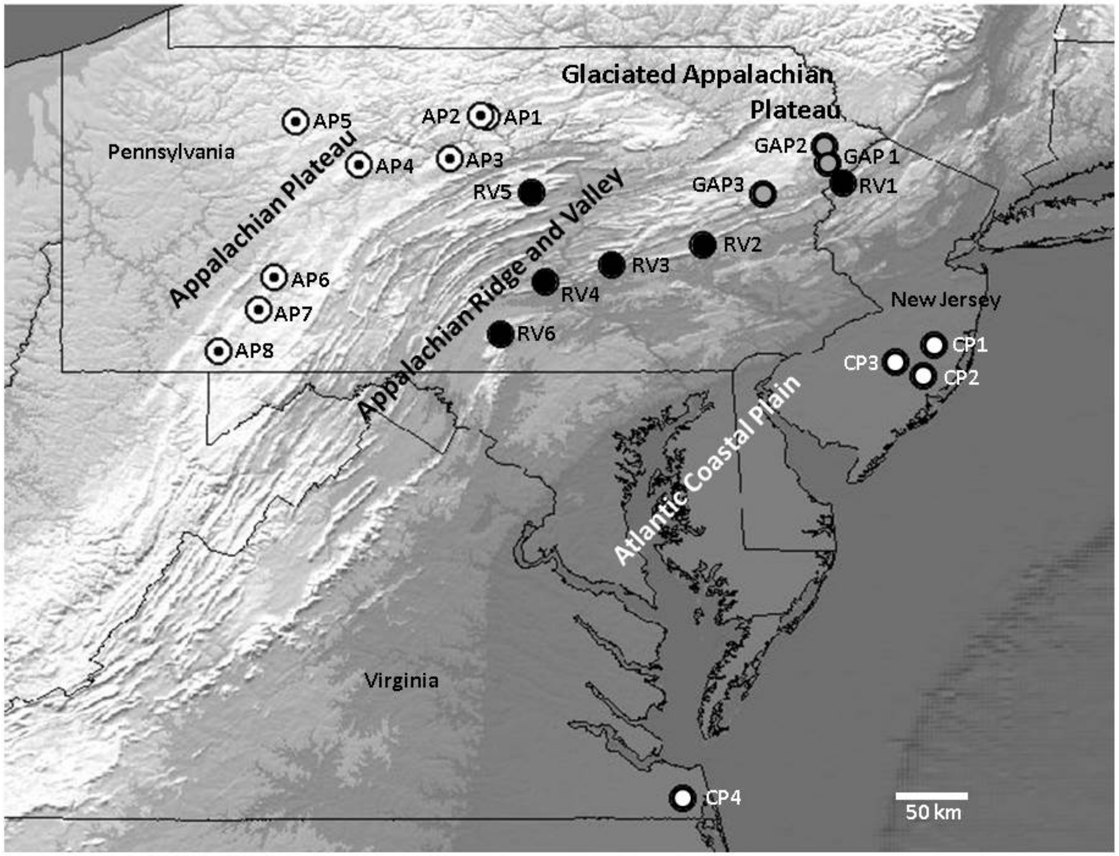


Microsatellites

- So the number of genes shared between populations can be used as a measure of the **genetic distance** between populations



We used microsatellite loci to analyze the genetic relationships among Timber Rattlesnakes from Pennsylvania, New Jersey, and Virginia

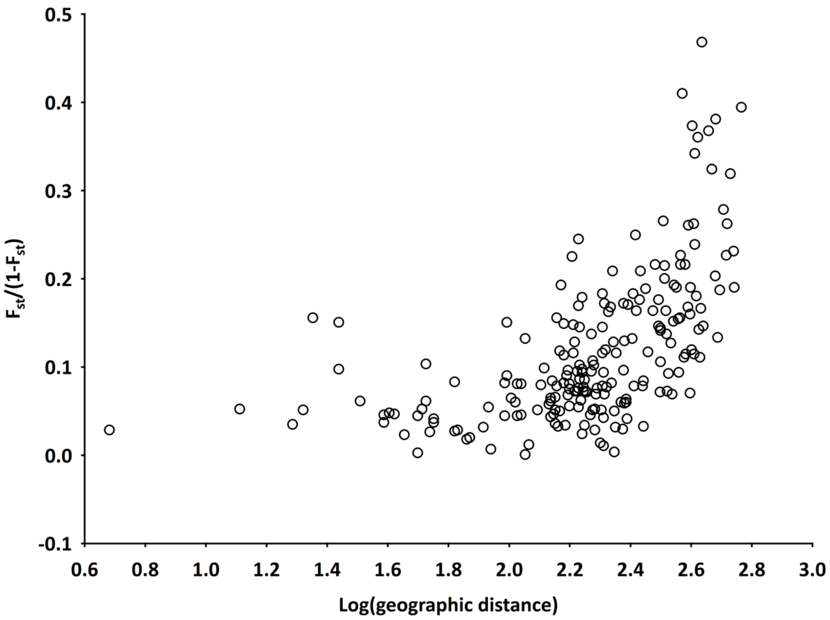


Bushar et al. Copeia 2014:694-706

Isolation by distance

- Timber Rattlesnake populations that are far apart geographically tend to be more distant genetically
    - $Z = 59.2345$, $r = 0.56$, $n = 210$, $P < 0.001$

- However, only a small amount of variation in genetic distance could be explained by the geographic distance between populations
    - $r^2 = 0.31$



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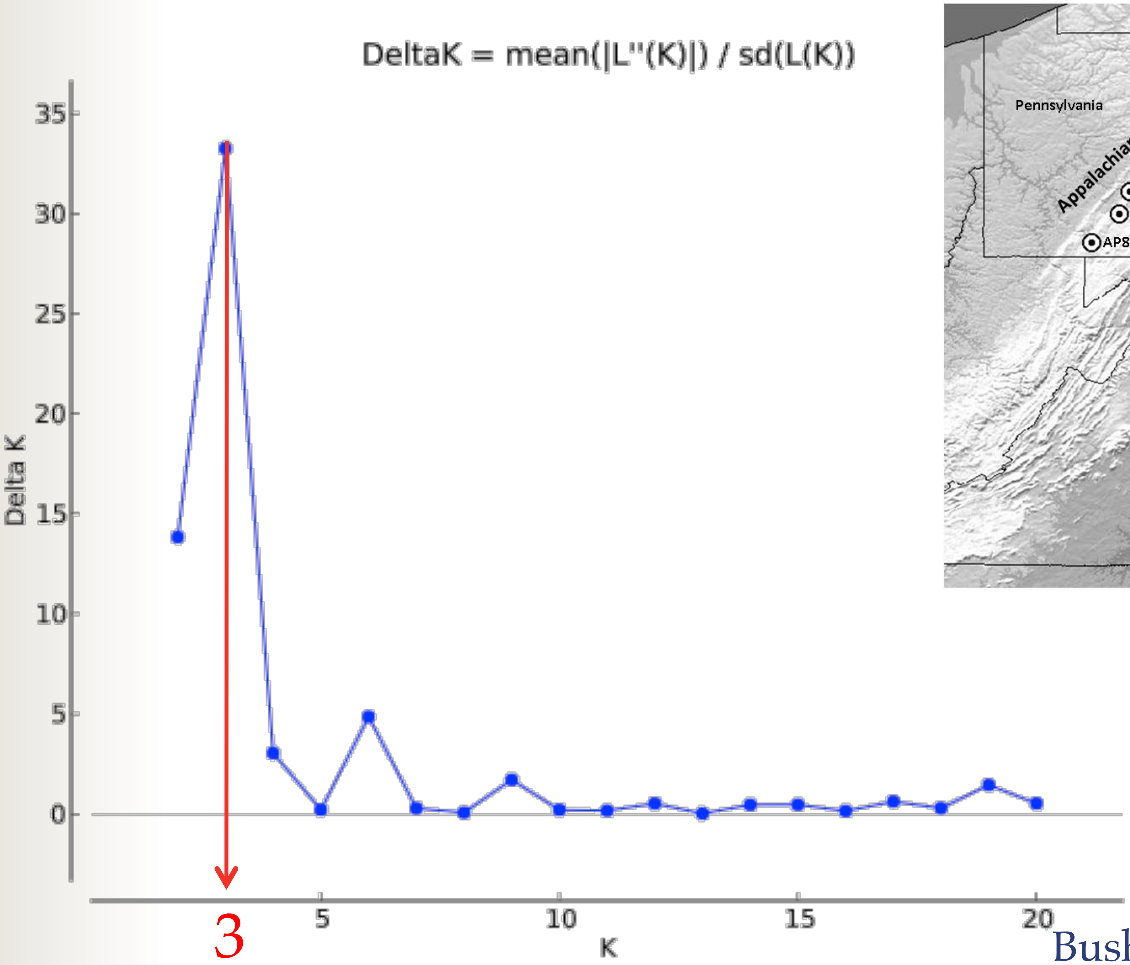
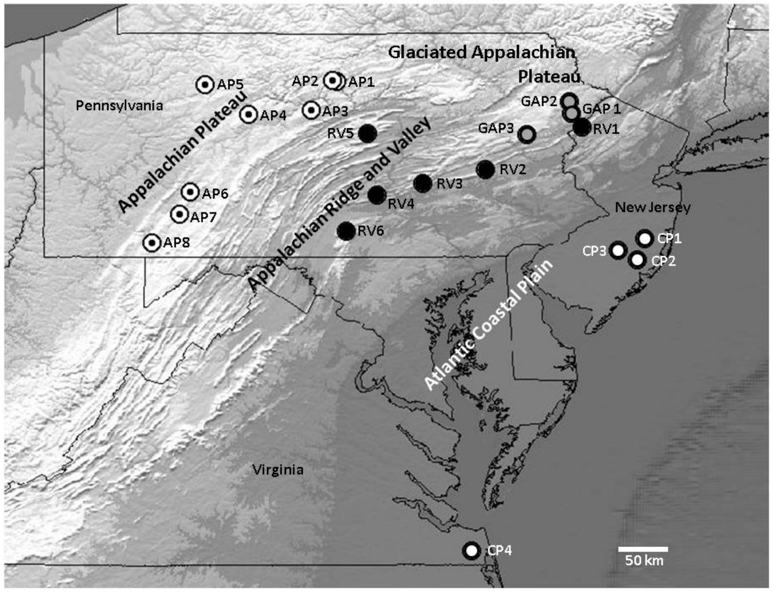
A Bayesian cluster analysis estimated that the snakes comprise three genetically different groups





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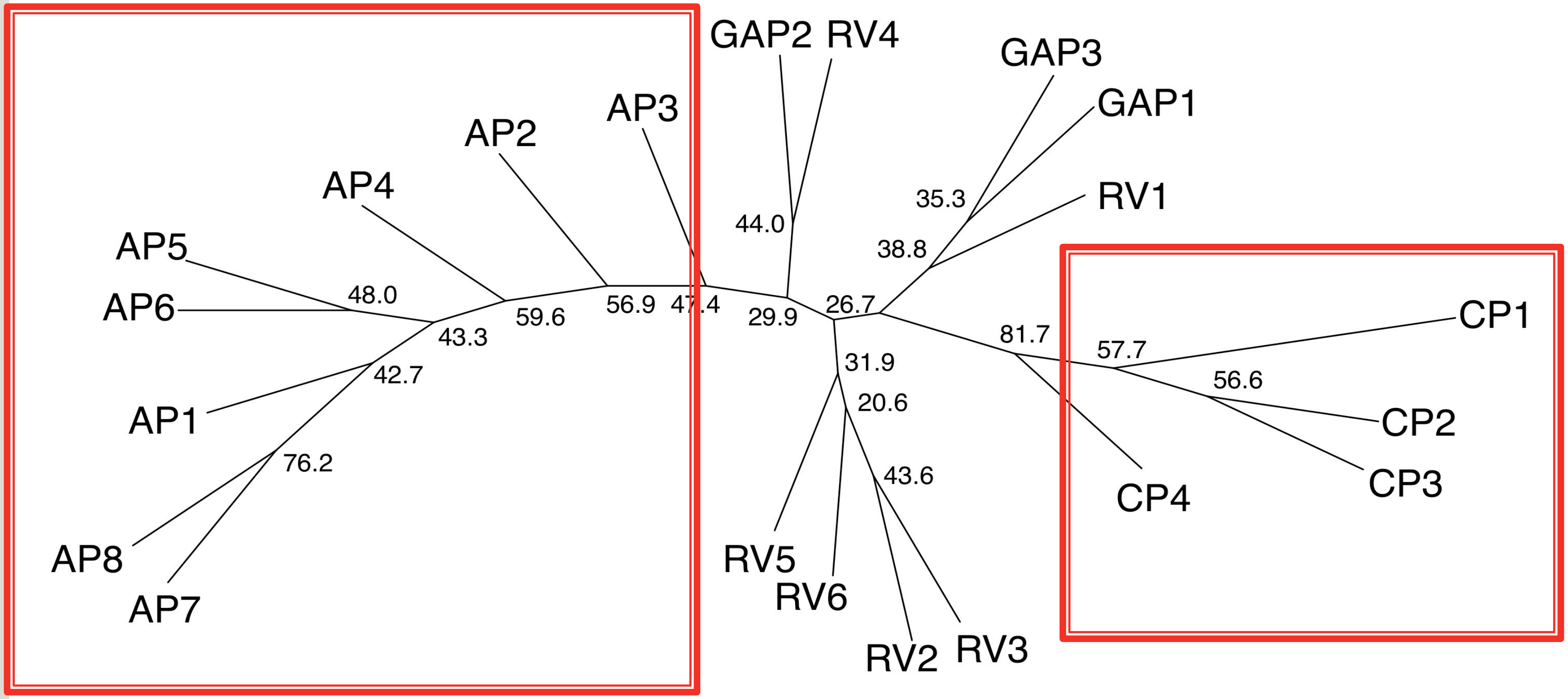
Genetic distance tree showed three genetically different groups



Cavalli-Sforza chord distance (Cavalli-Sforza and Edwards.1967. Am J Hum Gen 19:233-257)

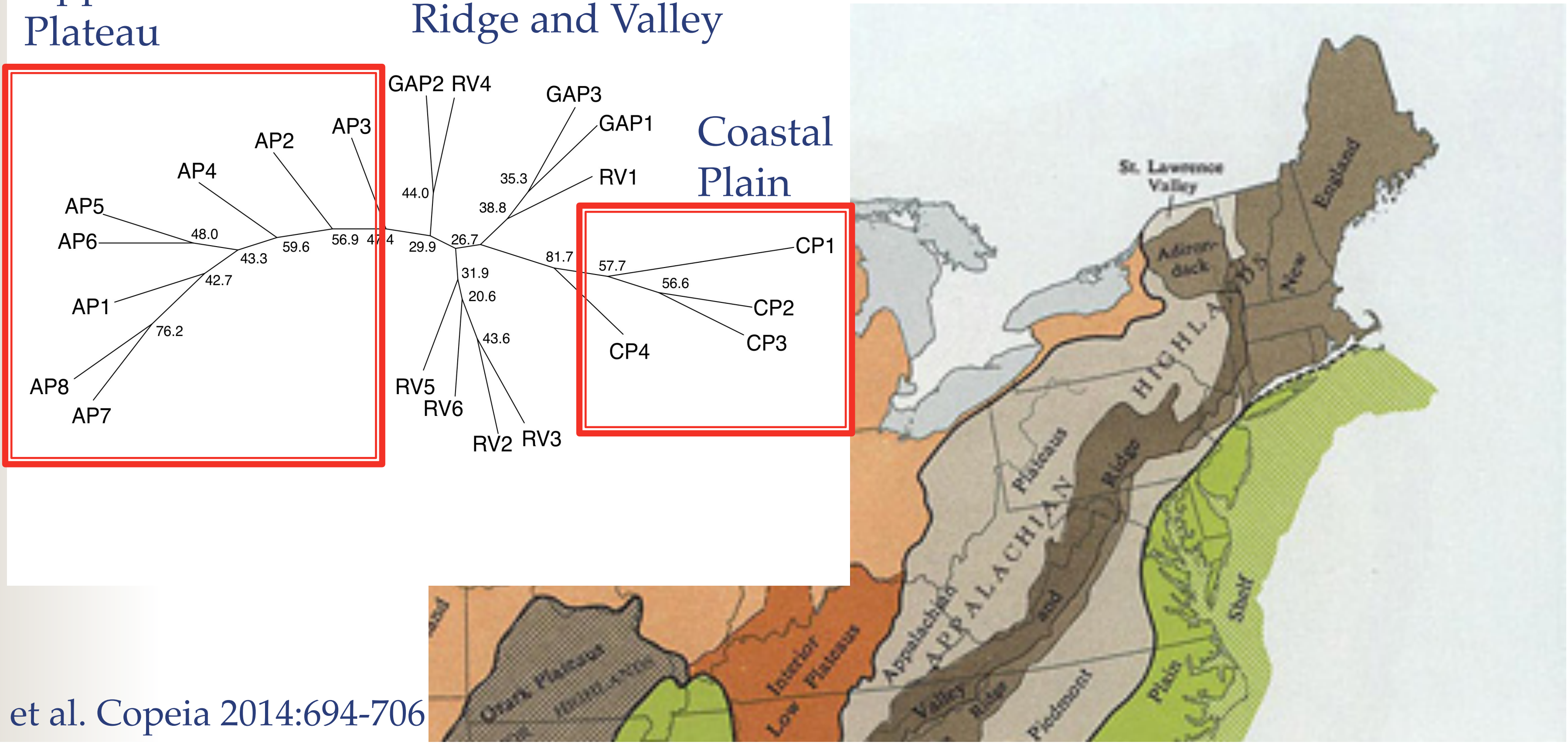
Bushar et al. Copeia 2014:694-706

The three genetic groups correspond to physiographic regions

Appalachian Plateau

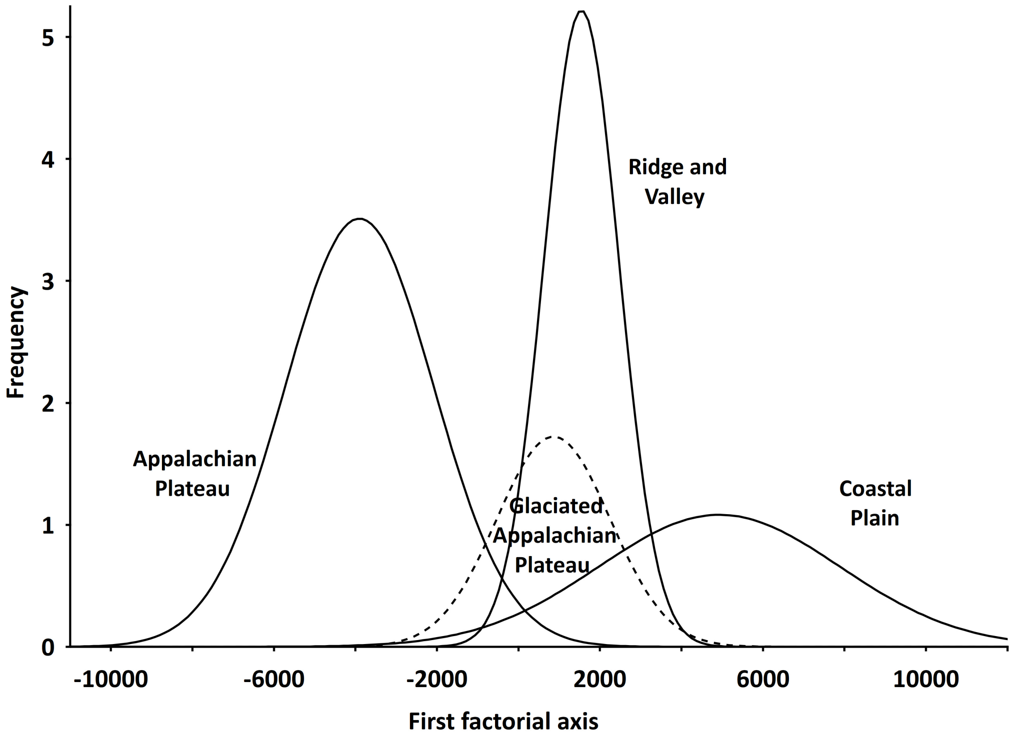
Appalachian Ridge and Valley

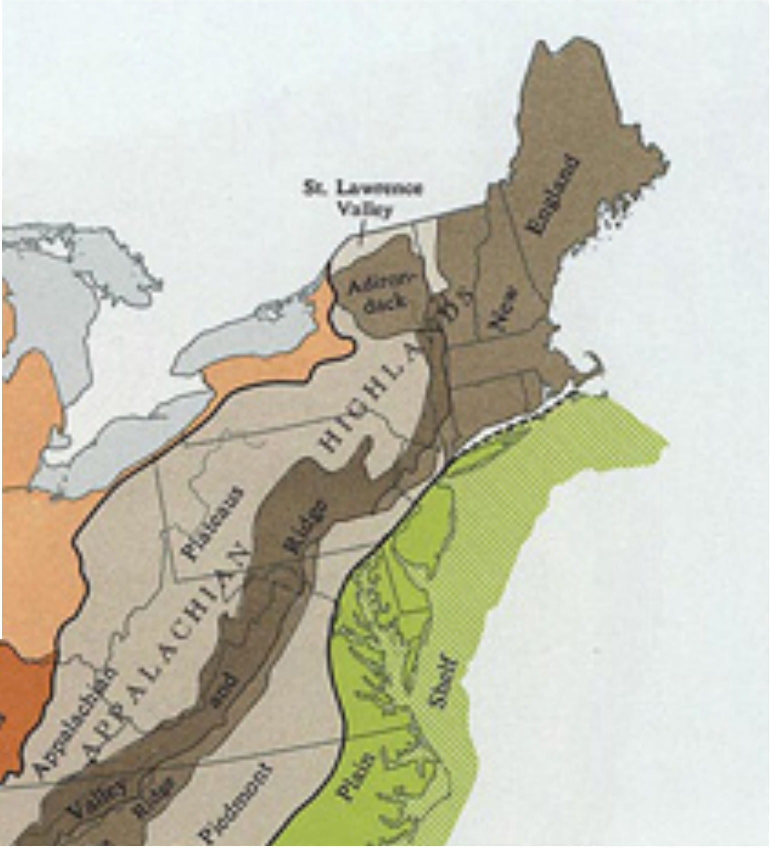
Coastal Plain



Bushar et al. Copeia 2014:694-706

Factorial correspondence analysis of the frequency of microsatellite alleles showed three genetic groups that corresponded with physiographic regions

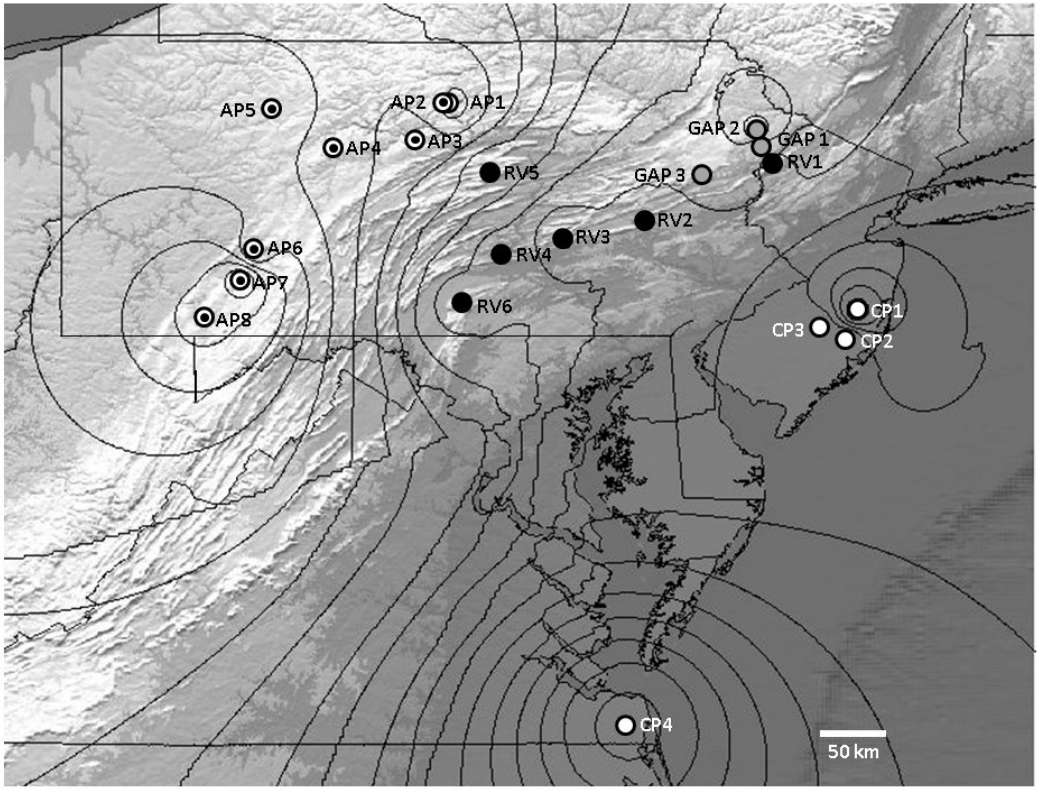




Bushar et al. Copeia 2014:694-706

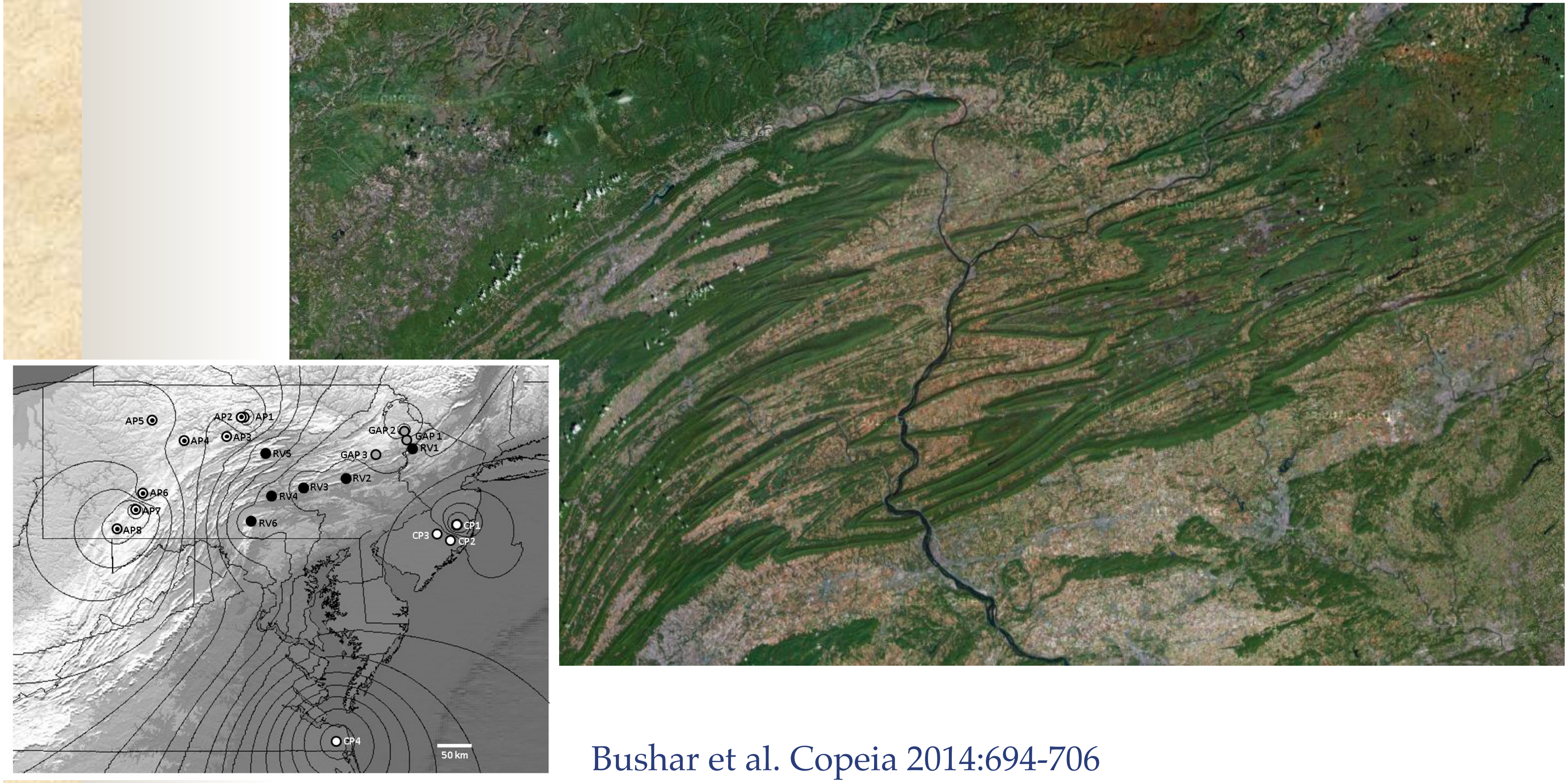
The differences in frequency of microsatellite alleles was georeferenced to a map of landform topography

- Sharp changes suggest barriers to gene flow



Bushar et al. Copeia 2014:694-706

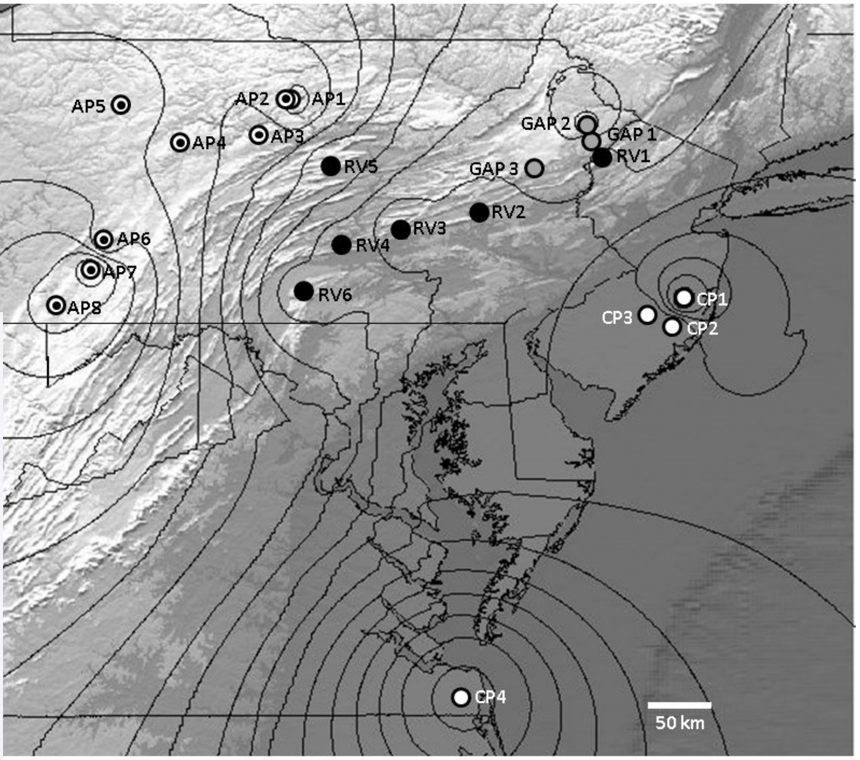
The ridges and valleys of the Appalachian Mountains as well as rivers provide obvious barriers to migration between populations

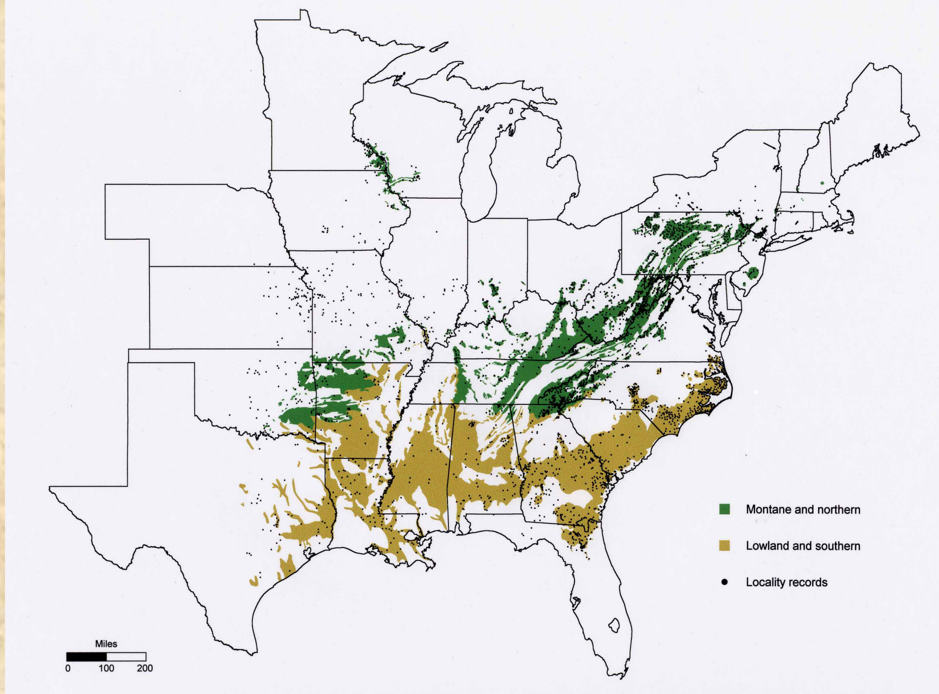


Bushar et al. Copeia 2014:694-706

The Timber Rattlesnakes along the Coastal Plain were isolated from all other populations

- Isolation is often associated with reduced genetic variation





Bushar et al. Copeia 2014:694-706

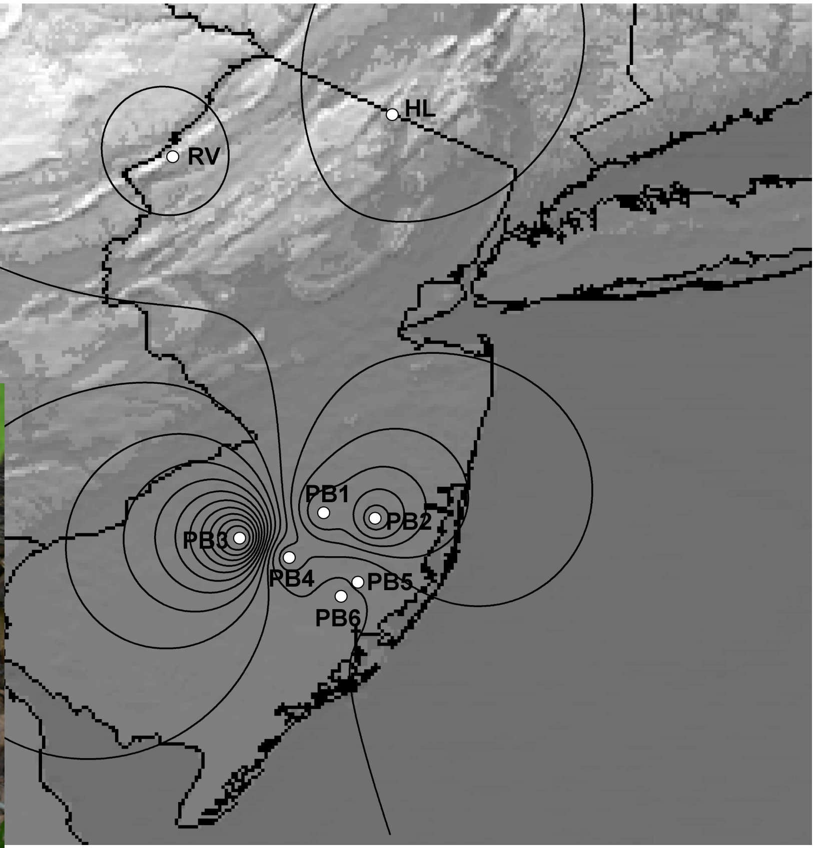
The Timber Rattlesnakes in the New Jersey Pine Barrens have lower levels of genetic variation than other Timber Rattlesnakes

Group	Mean number of alleles	Mean allelic richness	Mean observed heterozygosity	Mean expected heterozygosity	Mean total number of alleles
Atlantic Coastal Plain	3.85^{a} ± 0.406 (N=20)	3.41^{a} ± 0.317 (N=20)	0.44^{a} ± 0.053 (N=20)	0.47^{a} ± 0.048 (N=20)	19.2^{a} ± 0.85 (N=4)
Appalachian Ridge and Valley	5.02^{b} ± 0.304 (N=45)	4.45^{b}± 0.254 (N=45)	0.58^{b}± 0.030 (N=45)	0.61^{b} ± 0.029 (N=45)	25.1^{b} ± 1.19 (N=9)
Appalachian Plateau	4.68^{b} ± 0.319 (N=40)	4.23^{b} ± 0.263 (N=40)	0.56^{b} ± 0.036 (N=40)	0.63^{b} ± 0.032 (N=40)	23.4^{b} ± 1.16 (N=8)
Overall	4.67 ± 0.197 (N=105)	4.17 ± 0.163 (N=105)	0.54 ± 0.022 (N=105)	0.59 ± 0.020 (N=105)	23.3 ± 0.82 (N=21)

Genetic variation

- Essential for the long term viability of wildlife populations
- Reduced levels of genetic variation have been associated with
  - Reduced fertility
  - Increased disease susceptibility
  - Morphological abnormalities

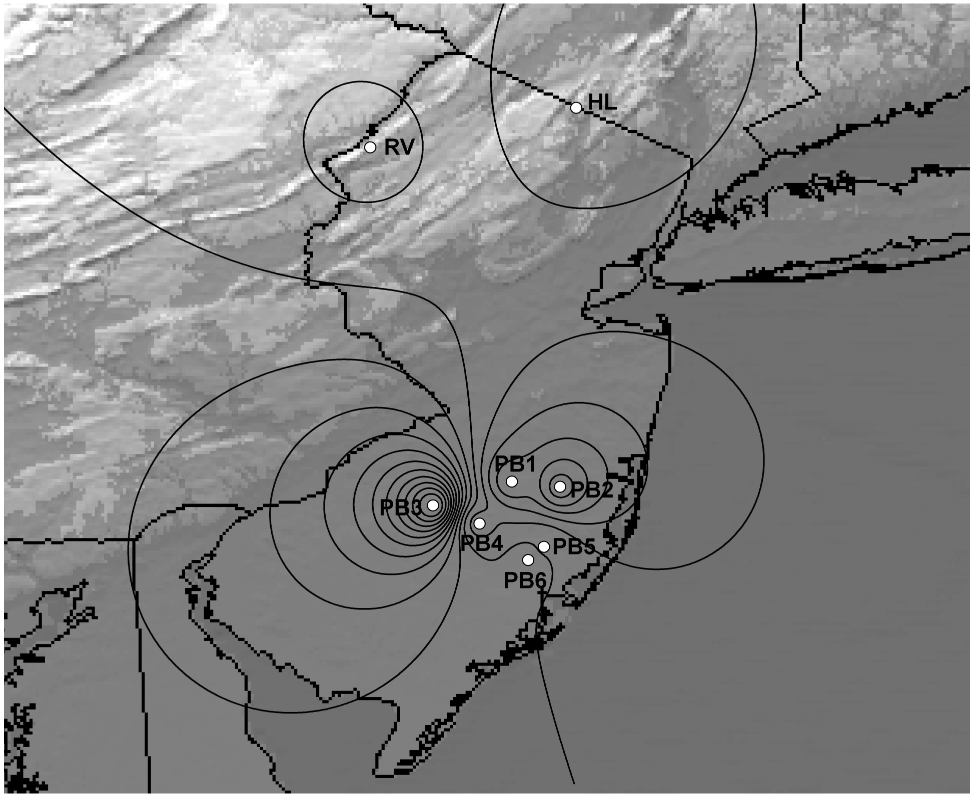
We used microsatellite loci to analyze the relationships among Timber Rattlesnakes in the Pine Barrens of New Jersey



Bushar et al. Herpetologica. In press

Isolation by distance

- There was no relationship between geographic and genetic distance
  - $Z = 8.588$, $r = -0.1734$, $n = 28$, $P = 0.658$



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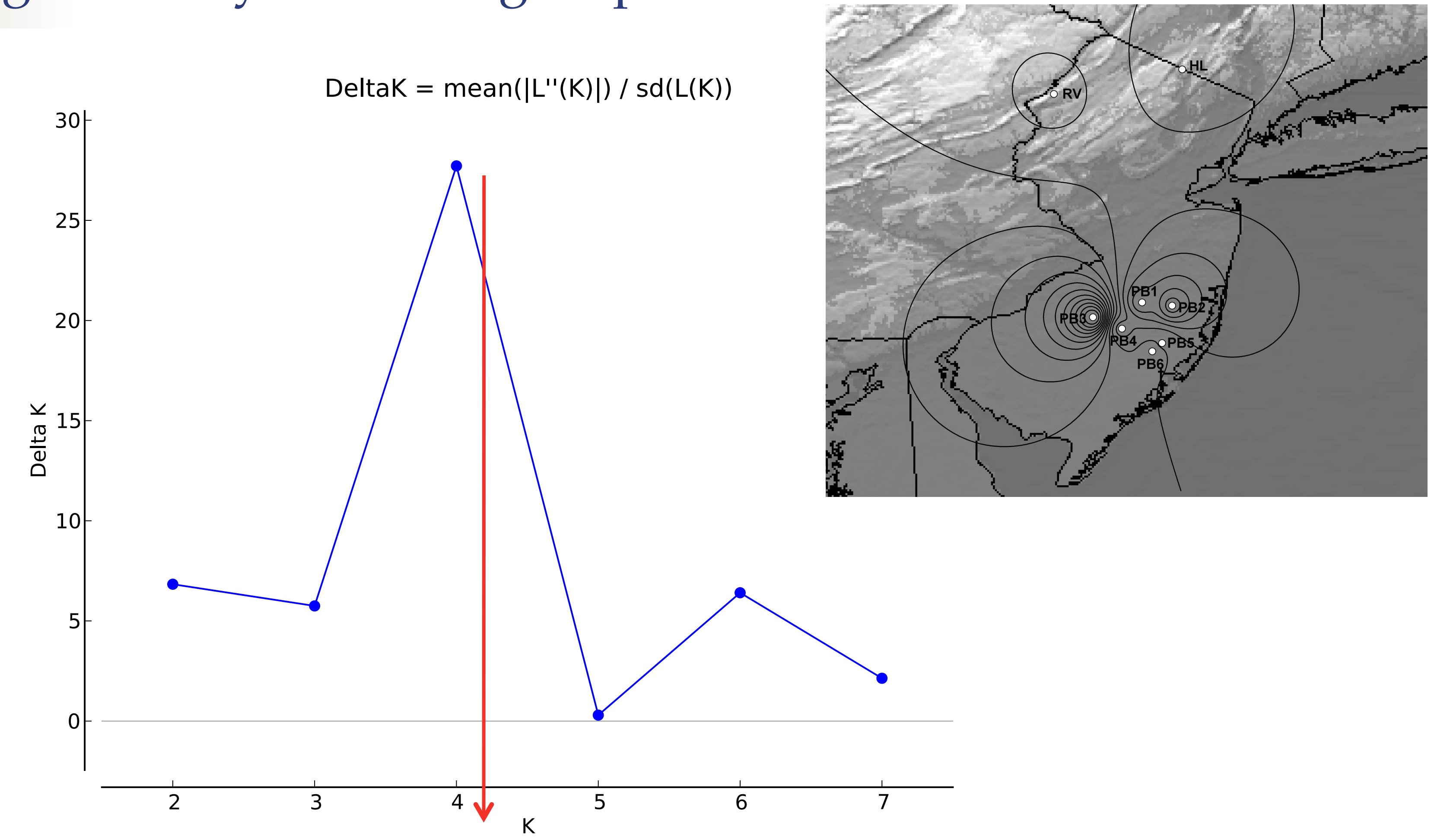

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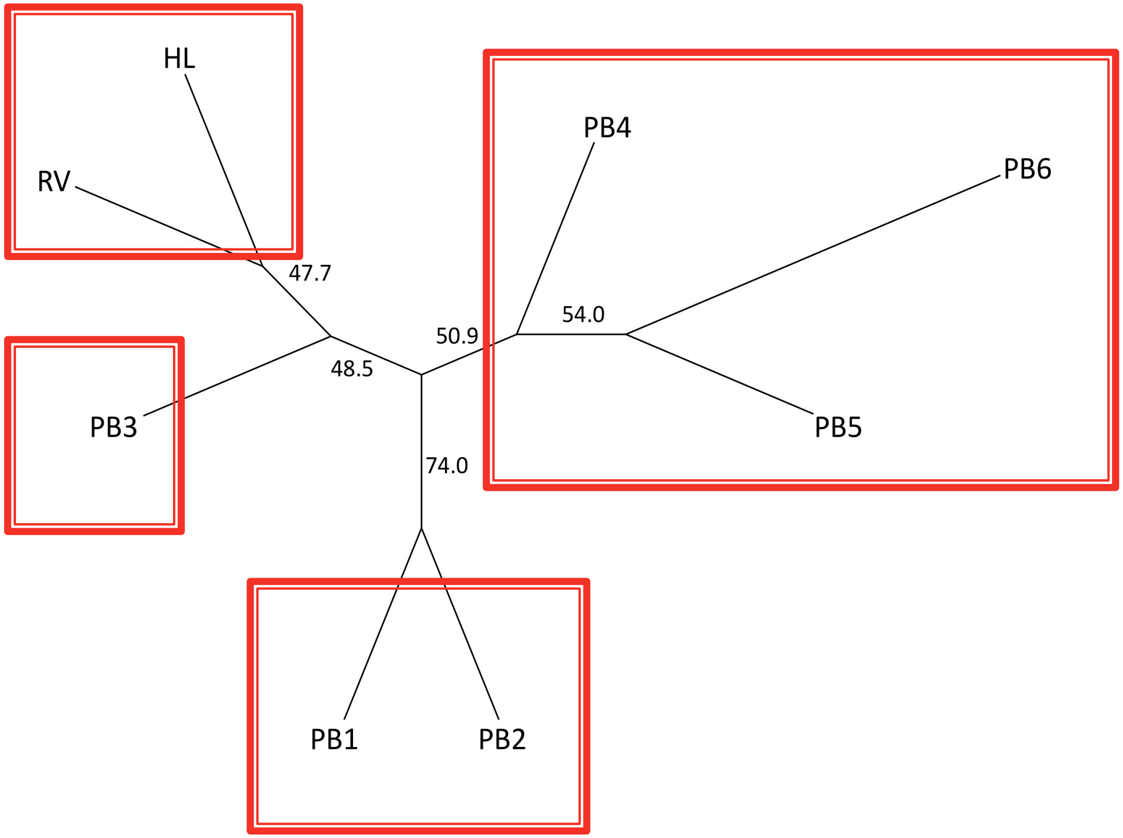

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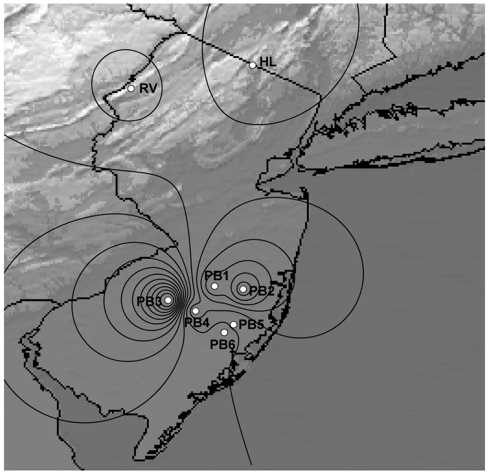

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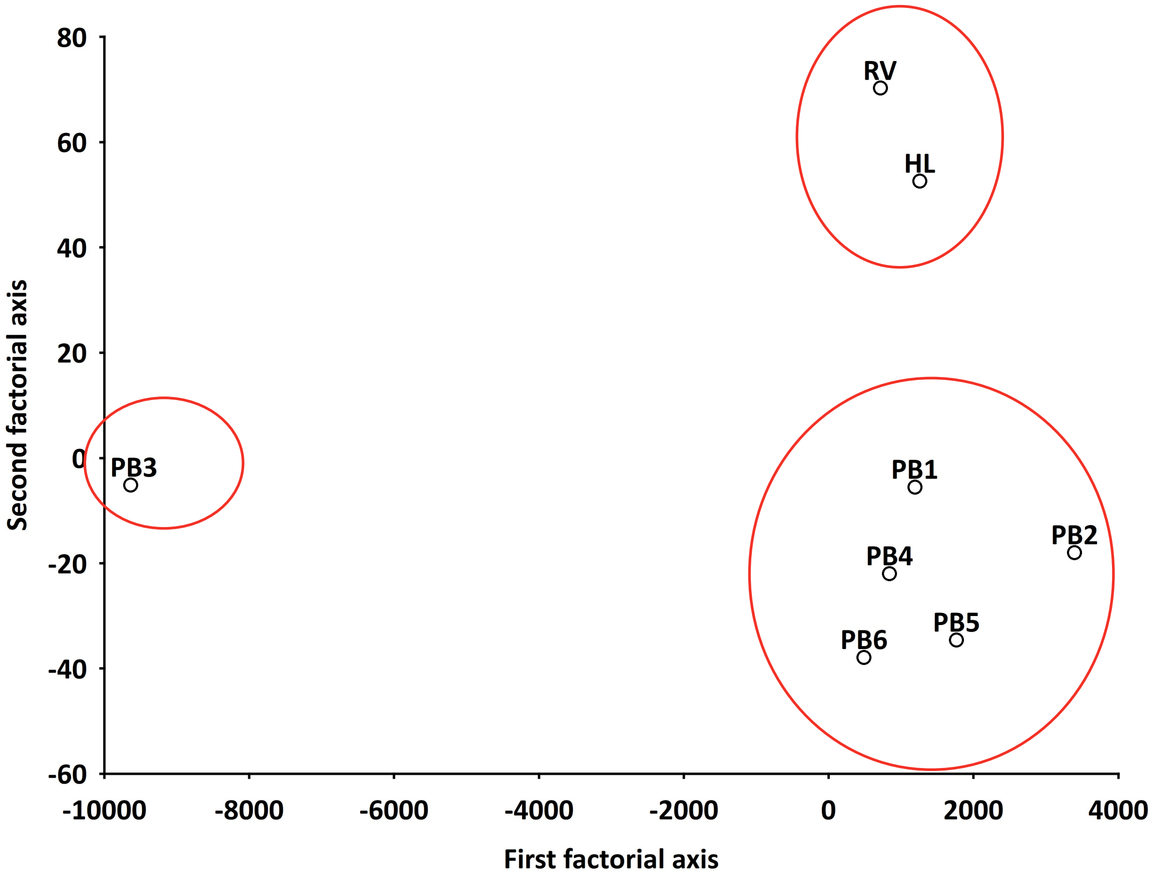
Factorial correspondence analysis of the frequency of microsatellite alleles showed three genetic groups



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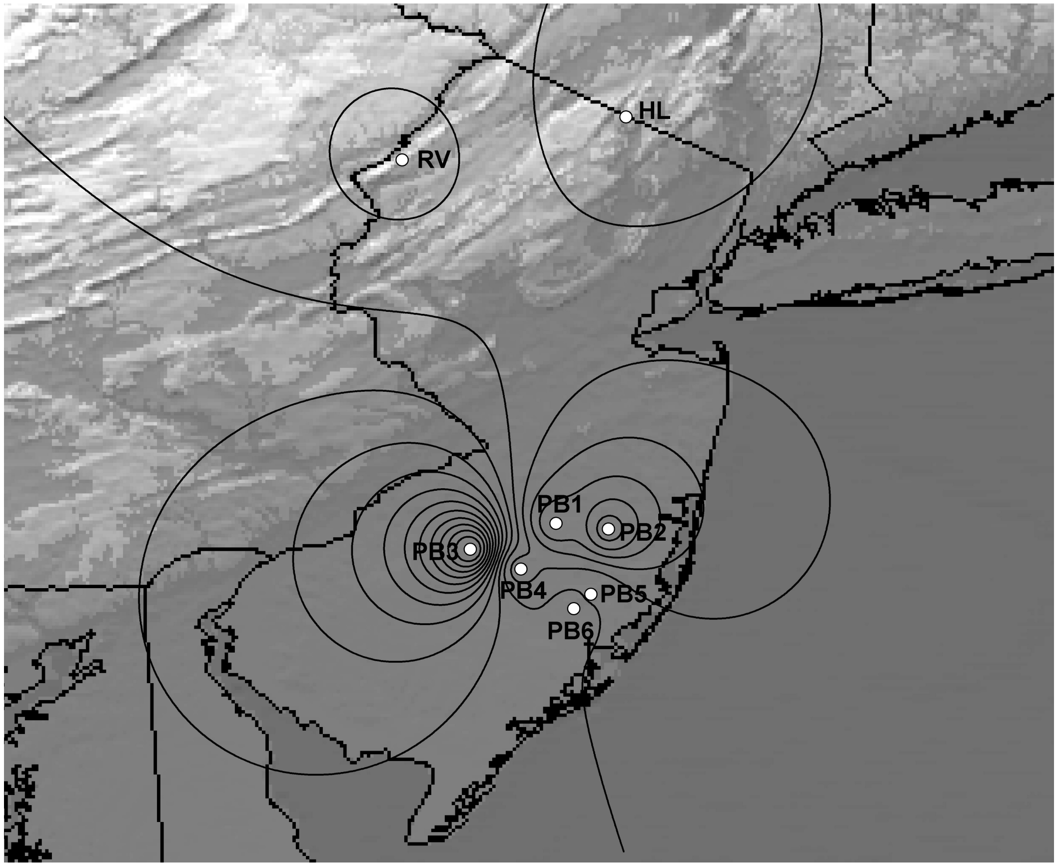
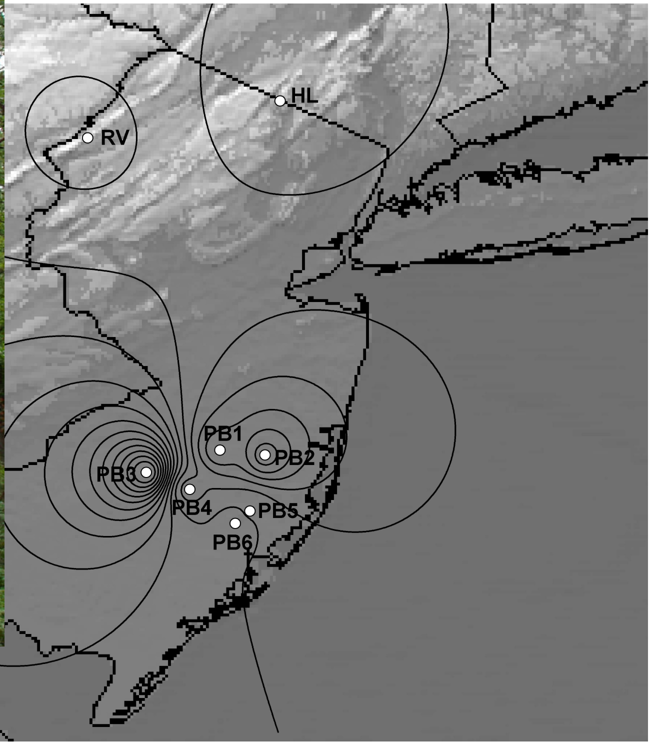
- The differences in frequency of microsatellite alleles was georeferenced to a map of landform topography
  - Sharp changes suggest barriers to gene flow



Bushar et al. Herpetologica. In press

These major contour gradients within the Pine Barrens were not associated with any obvious topographic features



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We looked for factors that could explain this isolation

- Number of paved roads between populations
- Number of unpaved roads between populations

	PB2	PB3	PB4	PB5	PB6
PB1	2/13	35/14	6/9	4/31	4/36
PB2		17/25	3/14	3/17	3/22
PB3			15/7	16/30	17/17
PB4				2/29	2/10
PB5					0/5

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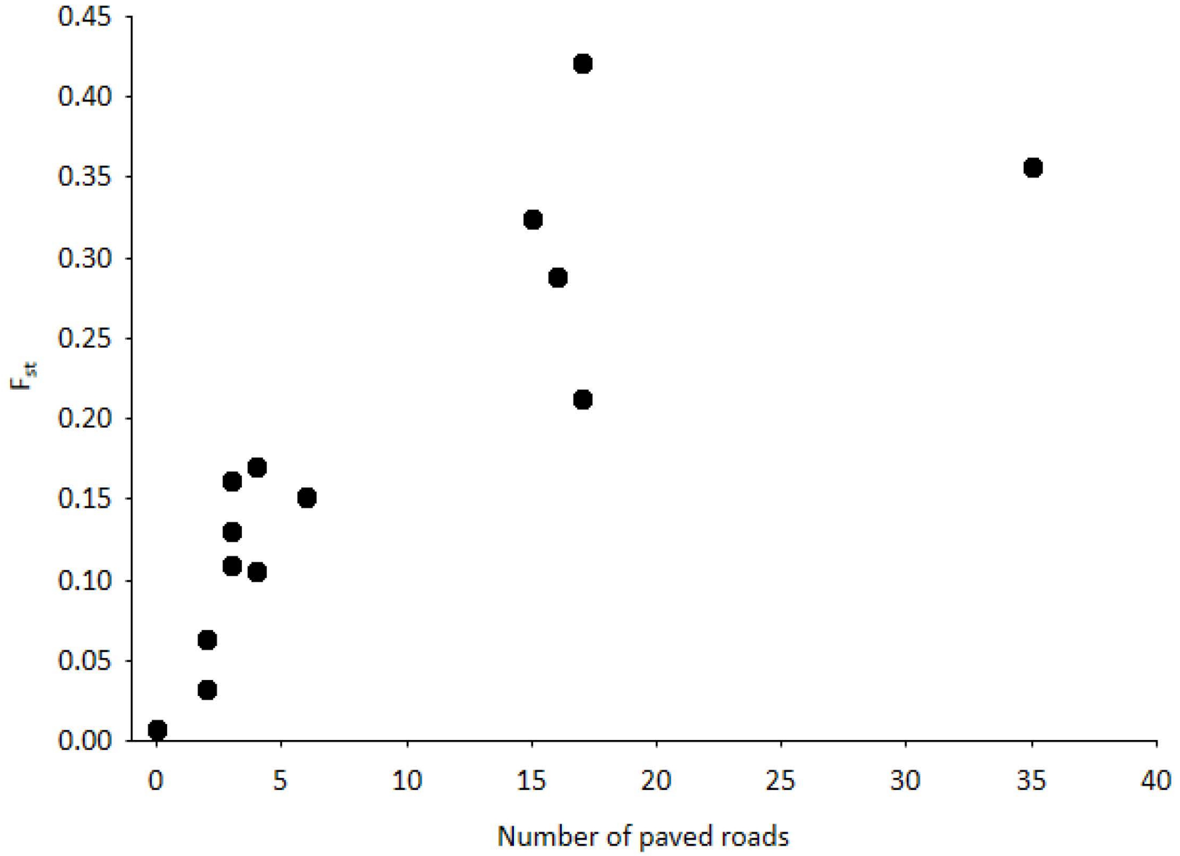
There was a strong correlation between the number of paved roads and the genetic distance between populations

- $r = 0.84$, $n = 15$, $P = 0.0001$



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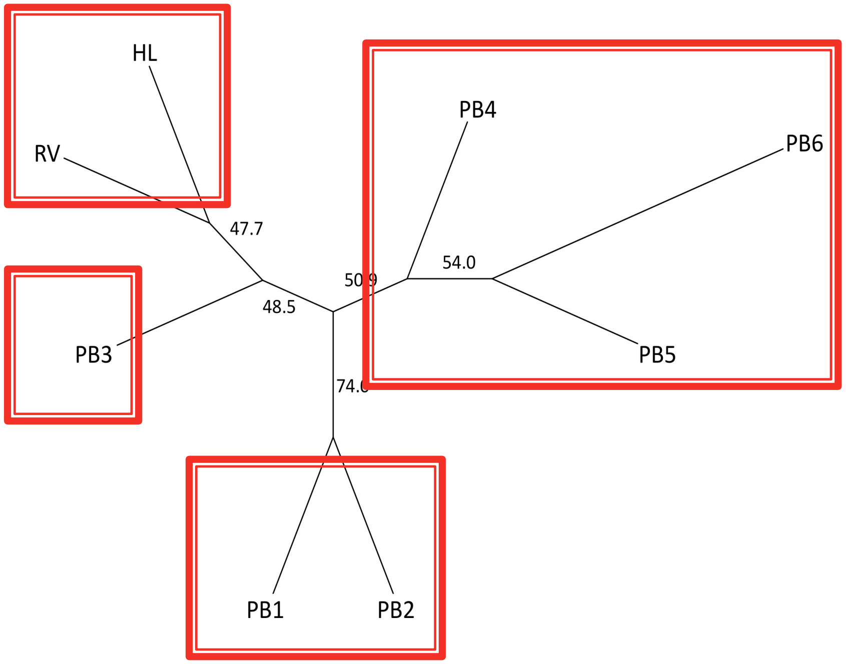
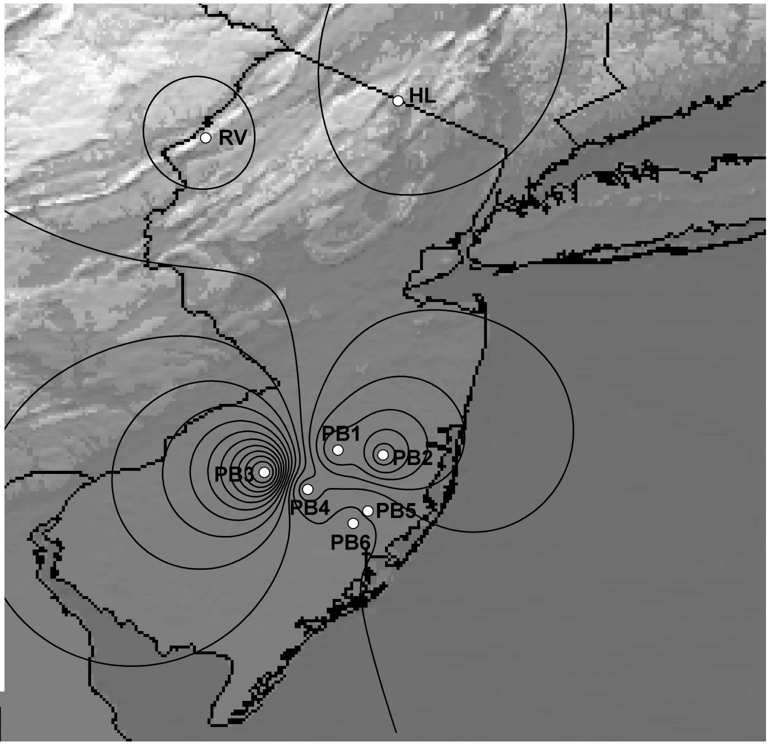
- PB1 and 2 are separated from PB4, 5, and 6 by NJ Routes 70 and 72
- PB3 is separated from the other Pine Barrens populations by US Route 206





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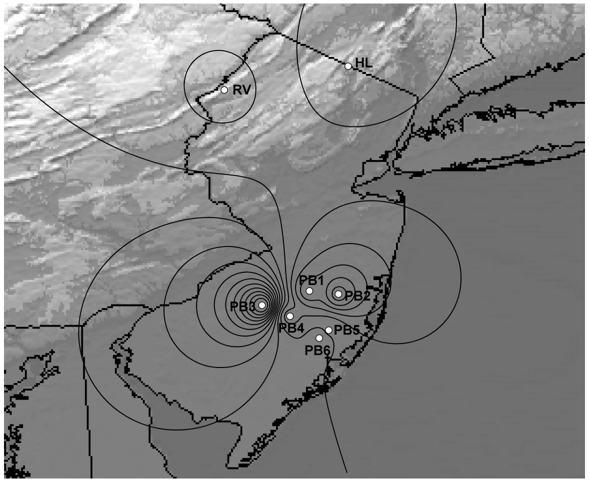
- Traffic densities ≥ 9000 vehicles/day
  - 100% probability of mortality for Timber Rattlesnakes crossing roads
    - K. M. Andrews and J. W. Gibbons (Copeia 2005:772-782)
- US Route 206 and NJ Routes 70 and 72
  - 10,672 to 18,479 vehicles/day
    - http://www.state.nj.us/transportation/refdata/roadway/traffic_counts



There was no relationship between the number of unpaved roads and the genetic distance between populations

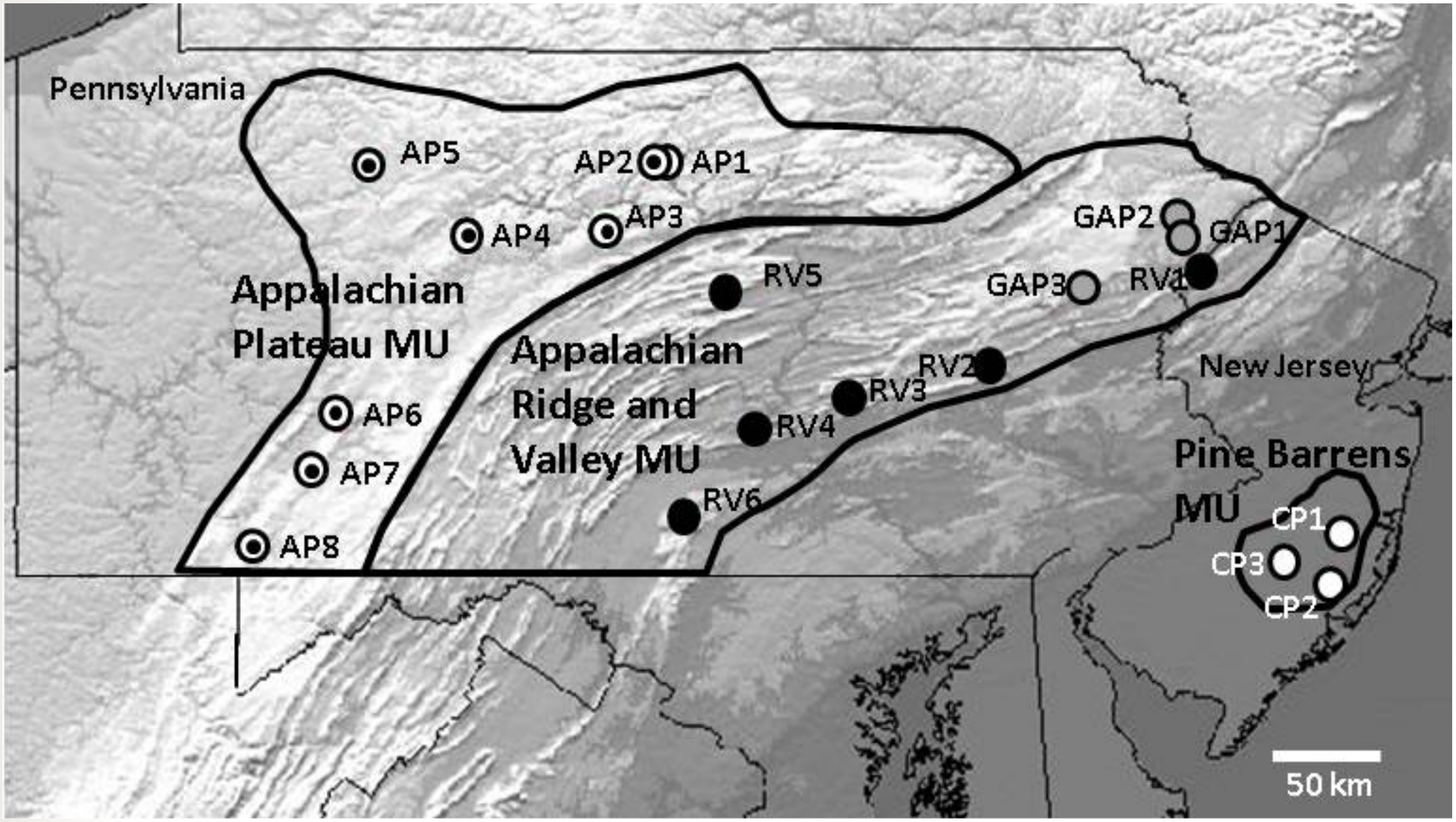
- $r = 0.14$, $n = 15$, $P = 0.65$

Conclusions

- The Timber Rattlesnakes in Pennsylvania, New Jersey and Virginia represent three large genetically different groups
  - These groups correspond with physiographic regions
    - Atlantic Coastal Plain
    - Appalachian Plateau
    - Appalachian Ridge and Valley

Recommendations

These three genetic groups should be considered separate **conservation management units** (MUs)

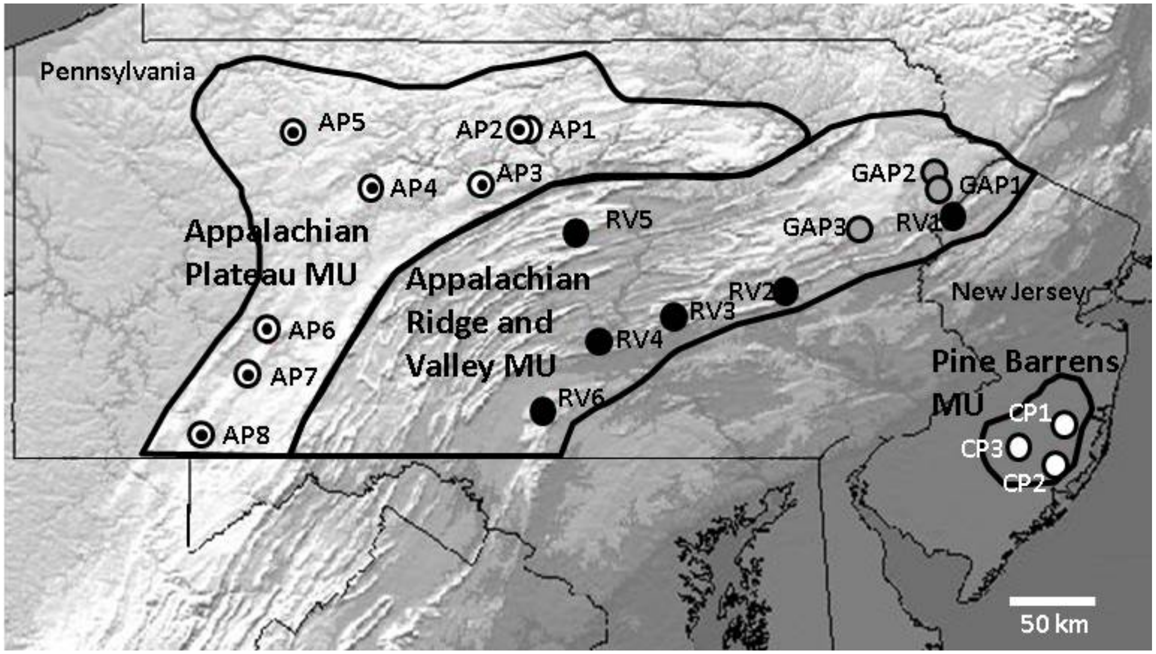


Bushar et al. Copeia 2014:694-706

Recommendations

In selected areas within each management unit

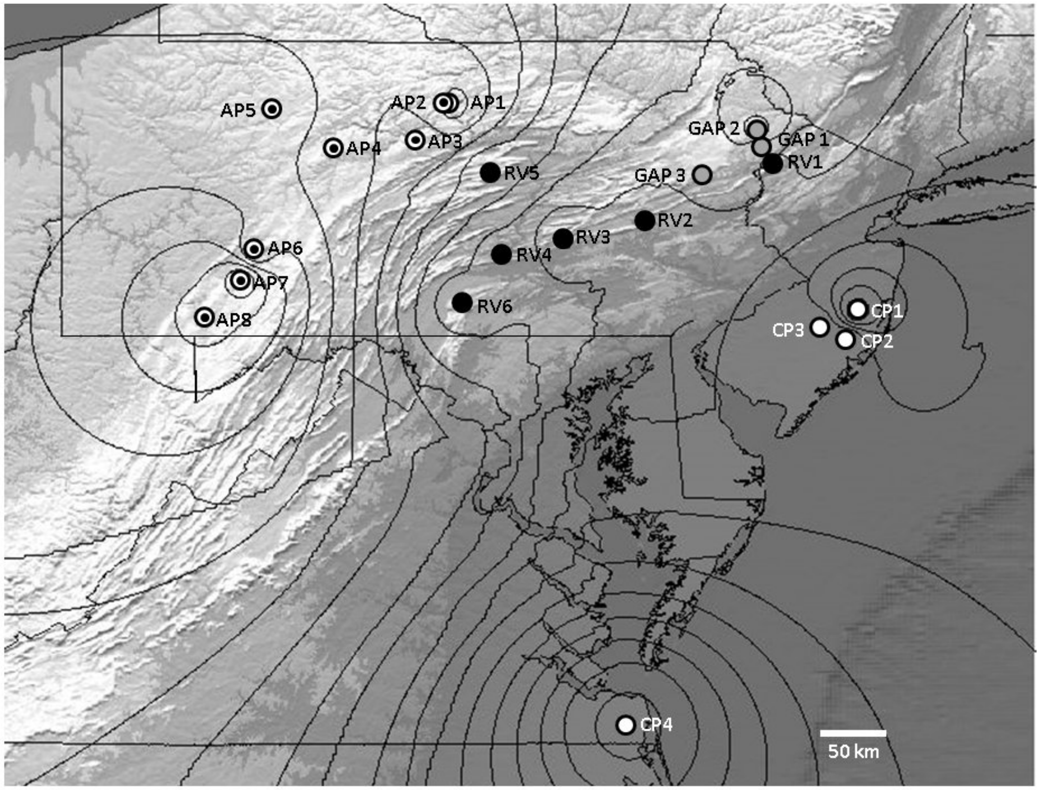
- Protect
  - High density populations
  - High quality habitat
- Monitor
  - Threats
  - Populations



Bushar et al. Copeia 2014:694-706

Conclusions

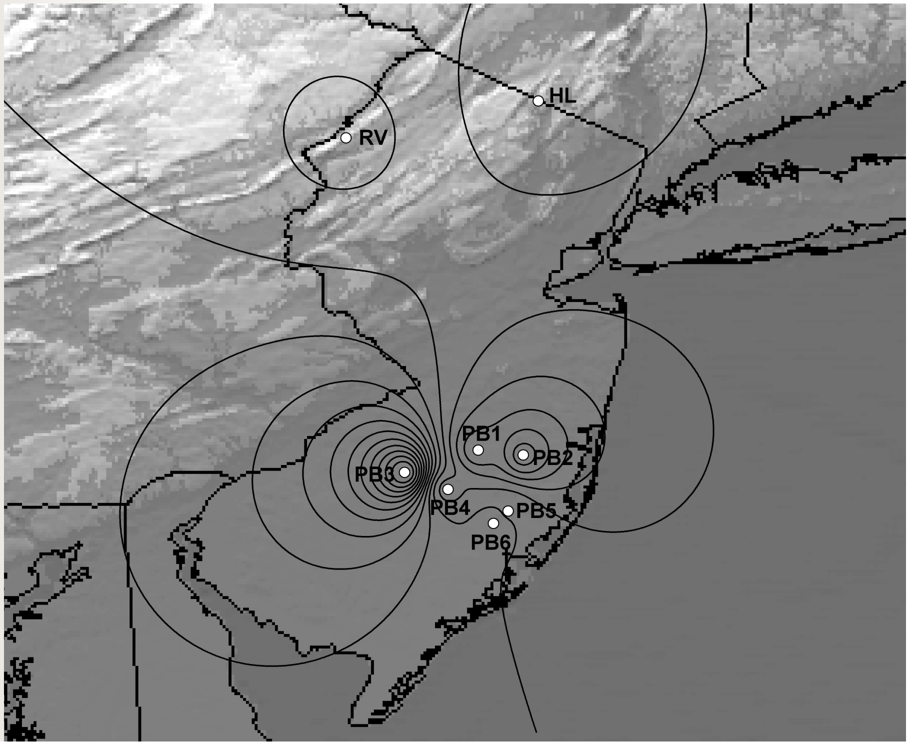
- Barriers that isolate populations include
  - Ridges and Valleys of the Appalachian Mountains
  - Major Rivers



Bushar et al. Copeia 2014:694-706

Conclusions

- Barriers that isolate populations include
  - Ridges and Valleys of the Appalachian Mountains
  - Delaware and Susquehanna Rivers
  - Major roadways



Bushar et al. Herpetologica. In press

Recommendations

Population connectivity should be encouraged

- Install
  - Wildlife culverts
  - Barriers
  - Underpasses
  - Overpasses
- Close roads

http://www.aco-wildlife.com/

http://www.wildlifeandroads.org/

http://barkingriversideproject.blogspot.com/

Recommendations

Population connectivity is especially important in the Pine Barrens

- Maximal possible dispersal is potentially greater than 20 km
- Interbreeding between all Pine Barrens populations would be possible if other dispersal barriers (roads) were overcome

http://transportationfortomorrow.com/

Large packs of Timber Wolves once roamed our woodlands

They disappeared from much of the Northeast by the late 1800s

Eastern Mountain Lion populations were decimated by the early 1900s

Many other species could not survive the changes in Northeastern forests

The Timber Rattlesnake is still here

It is our responsibility to protect it

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Questions?