

# The Genetics of Colonization: Evidence for a Recent Range Expansion in *Hyla cinerea*

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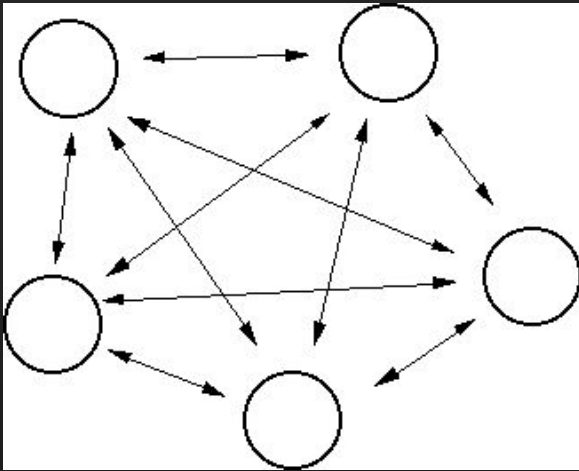
**MONTCLAIR STATE**  
UNIVERSITY

# The Genetics of Colonization

- Understanding the genetics of colonization is important for a variety of reasons:
  - Speciation
  - Migration patterns
  - Invasion biology
    - Determination of genetic front and geographic location
    - Do introduced individuals all originate from the same source? Is it possible to determine that source?

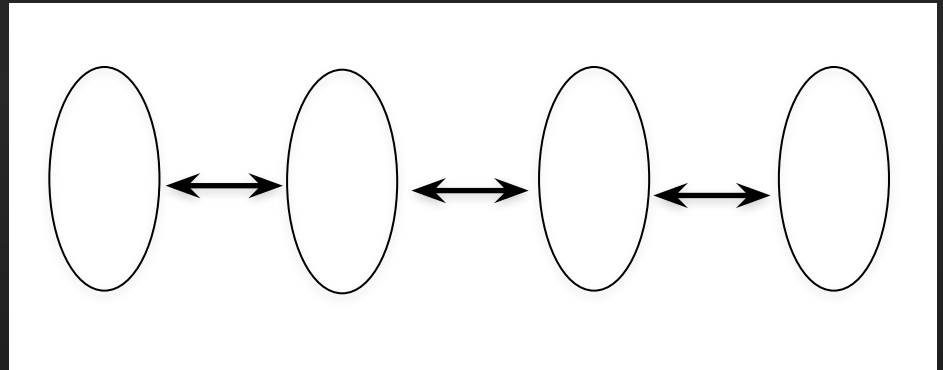
# The Genetics of Colonization

- Traditionally, 2 models:
- Infinite island



- Diminished founder effect

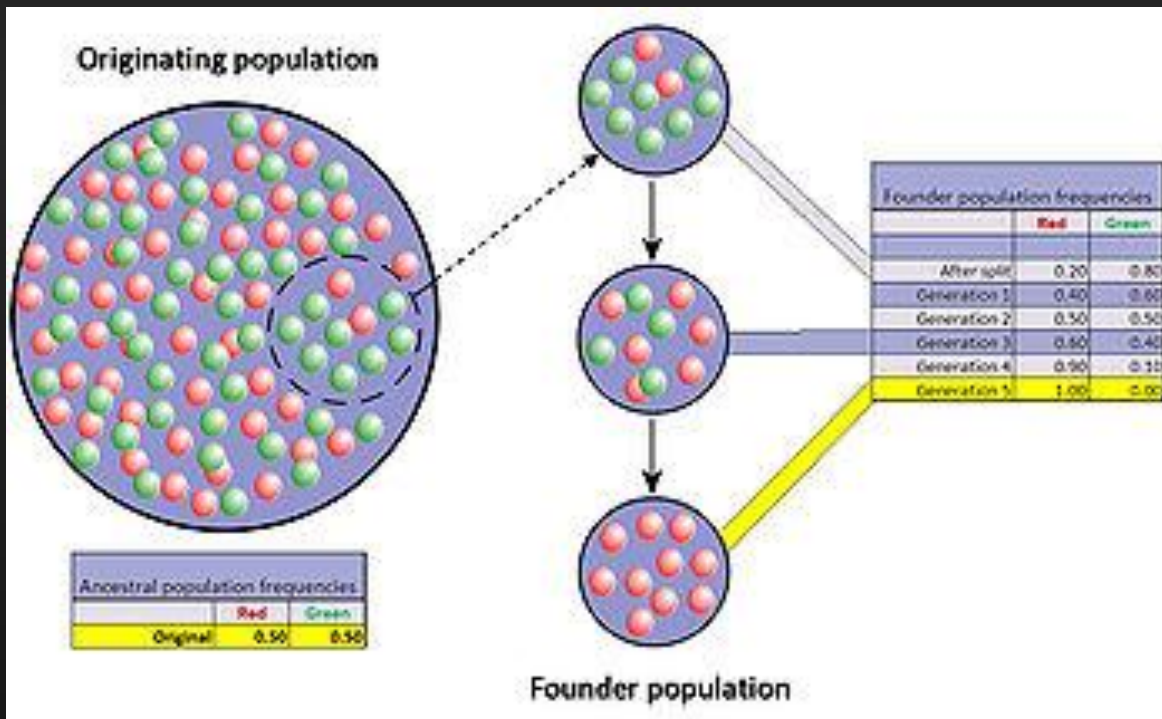
- Stepping stone



- Potentially strong founder effect

# The Genetics of Colonization

- **Decrease in genetic diversity indication of colonization event** (Wright 1942, Mayr 1954, 1963, 1970, 1976, 1982, Austerlitz et al. 1997, Excoffier 2009, Frankham 1997, Stone and Sunnucks 1993, Johnson 1988, Klopstein 2005, LeCorre 1998, Slatkin 1987, Templeton 1980, 1998)



- Due mainly to founder effect in stepping stone model (Templeton 1980, Slatkin 1987)

- Examination of actual, recent range expansions to determine what is biologically realistic

# The American Green Tree Frog (*Hyla cinerea*)



- SVL of 32-64 mm (1.25 to 2.5”)
- Lateral stripe white or yellow, variation within and between populations
- Diet - indiscriminate and opportunistic feeder (insects, snails, spiders)



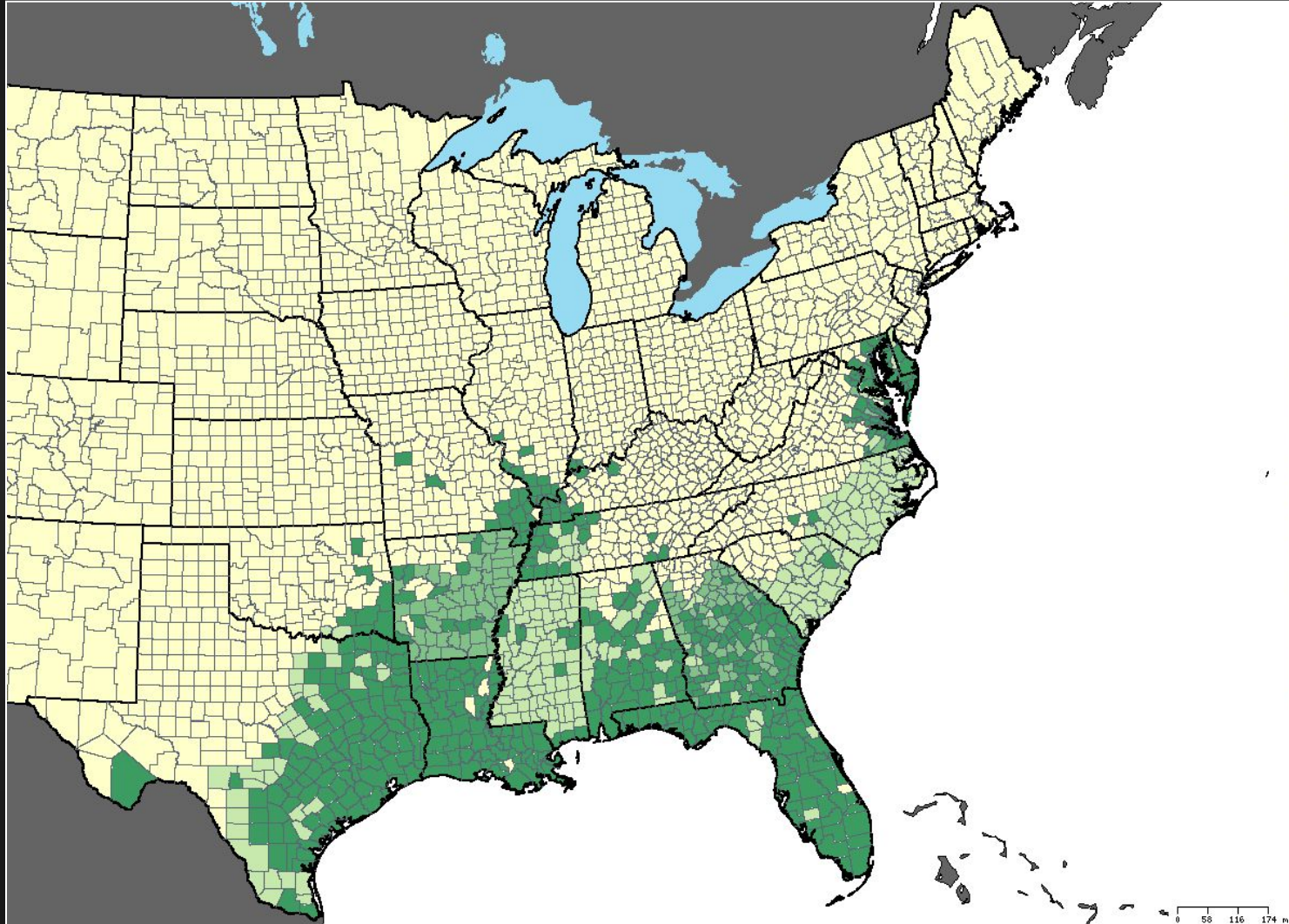
# The American Green Tree Frog (*H. cinerea*)



- Habitat: tidal marshes, ponds, lakes, swamps, with emergent vegetation
- Frequently found around humans; use eaves of buildings as retreats, found around house lights foraging for insects
- Popular in pet trade

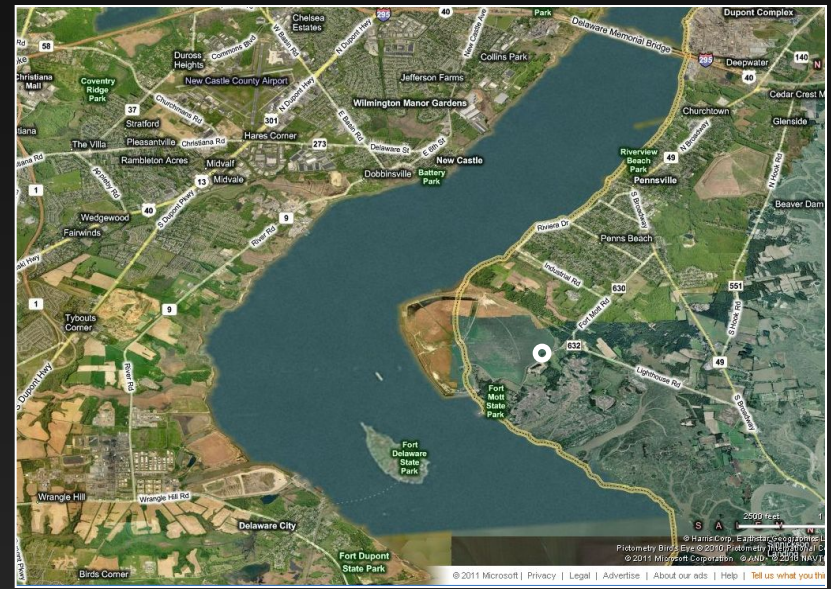


# Geographic Distribution (2005)



[http://www.nwrc.usgs.gov/sc\\_armi/frogs\\_and\\_toads/hyla\\_cinerea.htm](http://www.nwrc.usgs.gov/sc_armi/frogs_and_toads/hyla_cinerea.htm)

# Killcohook National Wildlife Refuge...New Jersey



- Initially located by its loud chorus, then confirmed through individual ID
- Freshwater tidal marsh along the Delaware River
- Other species present, Leopard Frog *spp.* and Bullfrog

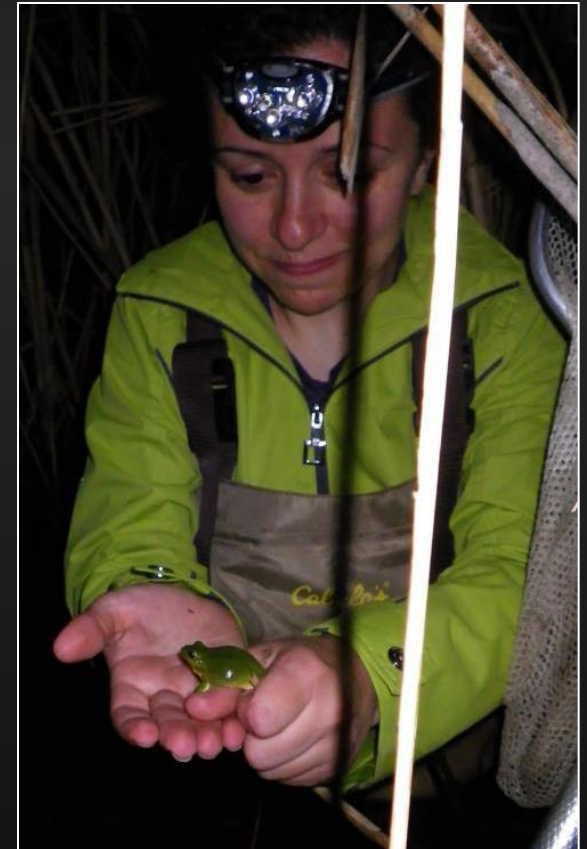
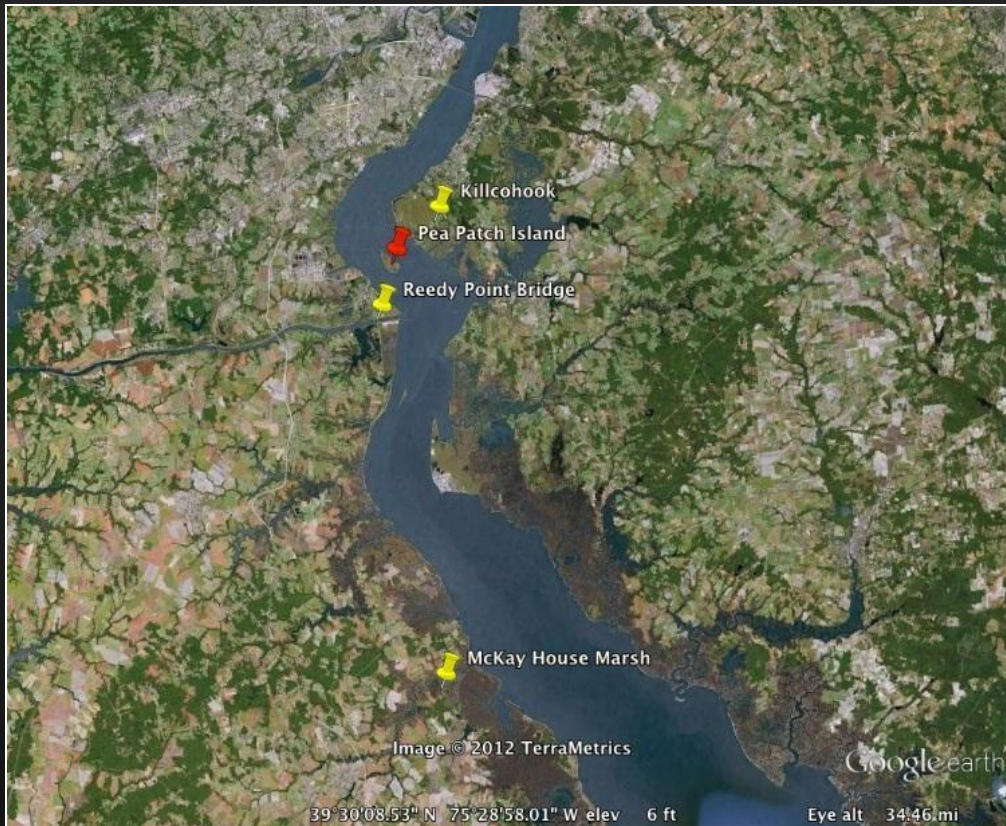


# Objectives

1. To determine the most likely source population for the *Hyla cinerea* that colonized New Jersey
2. To begin to document the current range of *Hyla cinerea* in New Jersey

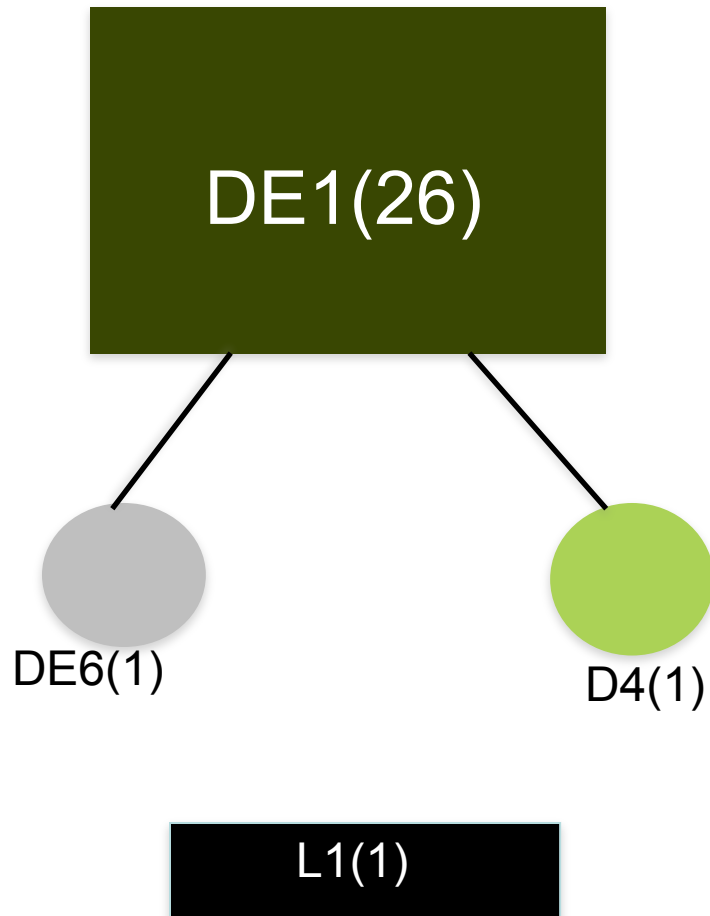
# Methods

- Toe clips for DNA from Killcohook site and 2 sites in Delaware
- DNA extraction via alkaline lysis and silica spin column using QIAmp DNA Mini Kit (QIAGEN)
- PCR, Sequence analysis on ABI sequencer



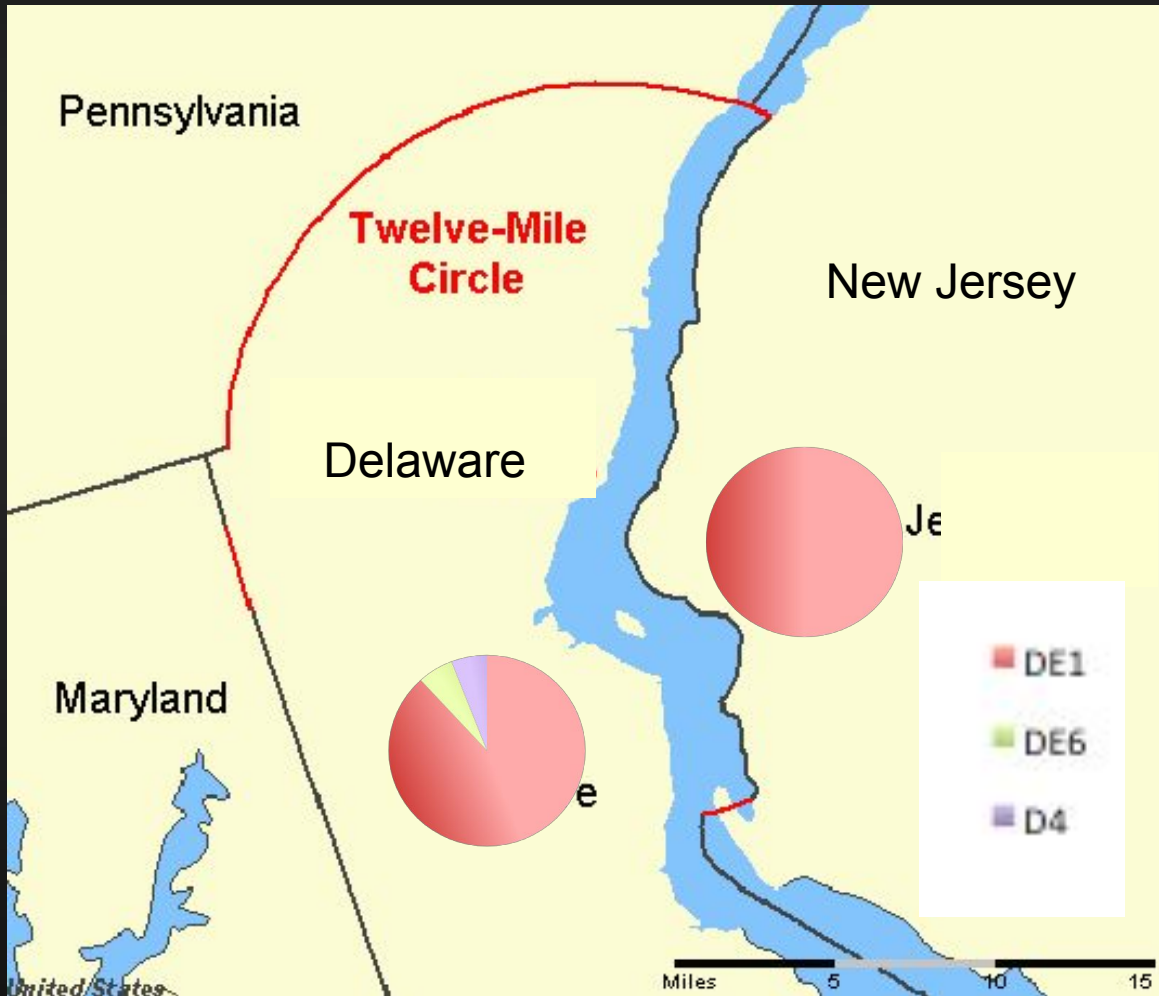


# Results - TCS



- Statistical parsimony network --> graphical representation of relationship among sequences
- 4 haplotypes, with all NJ samples being identical to DE1 sequence
- Most common haplotype is DE1
- Louisiana sequence (from GenBank) --> unrelated

# Estimates of Genetic Diversity



- $F_{st} = -0.025$
- Mean number of pairwise differences = 1.310 (+/- 0.840)
- Average gene diversity over loci = 0.003 (+/- 0.002)
- Haplotype diversity = 0.200 (+/- 0.098)
- $S = 19$

# Results - CLUSTALW2

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DE1      GTATTATACAAACCATAACCTGATTATTTATGACTTAGTCTCAATAAACACTCAACCTATT 180
DE6      GTATTATACAAACCATAACCTGATTATTTATGACTTAGTCTCAATAAACACTCAACCTATT 180
D4       GTATTATACAAACCATAACCTGATTATTTATGACTTAGTCTCAATAAACACTCAACCTATT 180
L1       GTATTATGCAAACCATAACCCGATTATTTATGACCTAGTCTCAATAAACACTCAACCTACT 180
*****↑*****↑*****↑*****↑*****

DE1      ATTTCCCTCAAGACAAGACTAGTTAATGTAGCAAATCTGGTTTTGCAAAGACCT-AAA 239
DE6      ATTTCCCTCAAGACAAGACTAGTTAATGTAGCAAATCTGGTTTTGCAAAGACCT-AAA 240
D4       ATTTCCCTCAAGACAAGACTAGTTAATGTAGCAAATCTGGTTTTGCAAAGACCT-AAA 239
L1       ACCTCTCTCAAGACAAGACTAGTTAATGTAGCAAATCTGGTTTTGCAAAGACCT-AAA 239
**↑↑**↑*****↑*****↑*****↑*****

DE1      CCCTTCTATAGAGGTTCAAATCCTCTCATTAACTTTGAACCTAGTCCAACCTATTCTCC 299
DE6      CCCTTCTATAGAGGTTCAAATCCTCTCATTAACTTTGAACCTAGTCCAACCTATTCTCC 300
D4       CCCTTCTATAGAGGTTCAAATCCTCTCATTAACTTTGAACCTAGTCCAACCTATTCTCC 299
L1       CCCTTCTATAGAGGTTCAAATCCTCTCATTAACTTTGAACCTAATCCAACCTATTCTTC 299
*****↑*****↑*****↑*****

DE1      CCCTTTTATATATTGCTCCGATCCTTCTGTCAGTTGCCCTTCCTCACCCTTATTGAACGCA 359
DE6      CCCTTTTATATATTGCTCCGATCCTTCTGTCAGTTGCCCTTCCTCACCCTTATTGAACGCA 360
D4       CCCTTTTATATATTGCTCCGATCCTTCTGTCAGTTGCCCTTCCTCACCCTTATTGAACGCA 359
L1       CCCTTTTATATATTGCCCGATCCTTCTGTCAGTTGCCCTTCCTCACCCTTATTGAACGCA 359
*****↑*****

DE1      AAGTGCTTGGTTATATACAACACCGCAAAGGCCCAACGTCAGTCGGCCCCACAGGCCTCC 419
DE6      AAGTGCTTGGTTATATACAACACCGCAAAGGCCCAACGTCAGTCGGCCCCACAGGCCTCC 420
D4       AAGTGCTTGGTTATATACAACACCGCAAAGGCCCAACGTCAGTCGGCCCCACAGGCCTCC 419
L1       AAGTGCTTGGCTATATACAACATCGCAAAGGCCCAACGTCAGTCGGCCCCACAGGCCTTC 419
*****↑*****↑*****↑*****

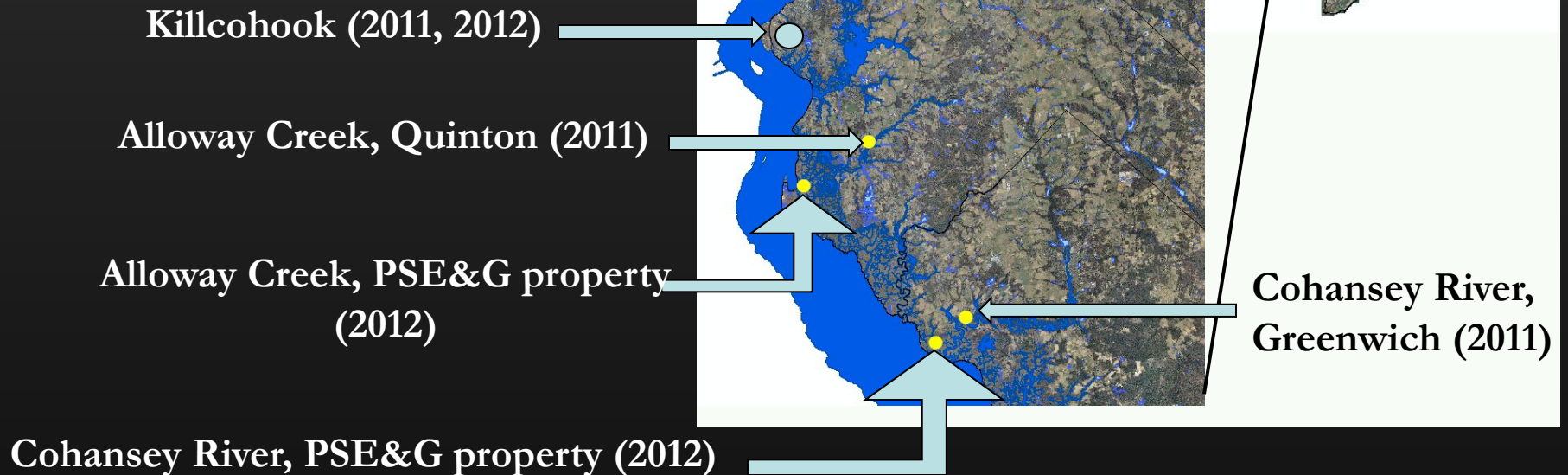
DE1      TTCAGCCAATCGCTGATGGGGTAAACCTTTCATTAAAGAACCATCCGACCATCAAATT 479
DE6      TTCAGCCAATCGCTGATGGGGTAAACCTTTCATTAAAGAACCATCCGACCATCAAATT 480
D4       TTCAGCCAATCGCTGATGGGGTAAACCTTTCATTAAAGAACCATCCGACCATCAAATT 479
L1       TTCACCAATCGCTGATGGAGTAAACTCTTCATTAAAGAACCATCCGACCATCAAATT 479
****↑*****↑*****↑*****

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- Aligned 4 unique haplotypes using CLUSTALW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) to visualize 19 polymorphic sites
- Arrows represent loci that differ between L1 and all other sequences
- Rectangles represent loci that differ between DE sequences

# Results of Range Survey

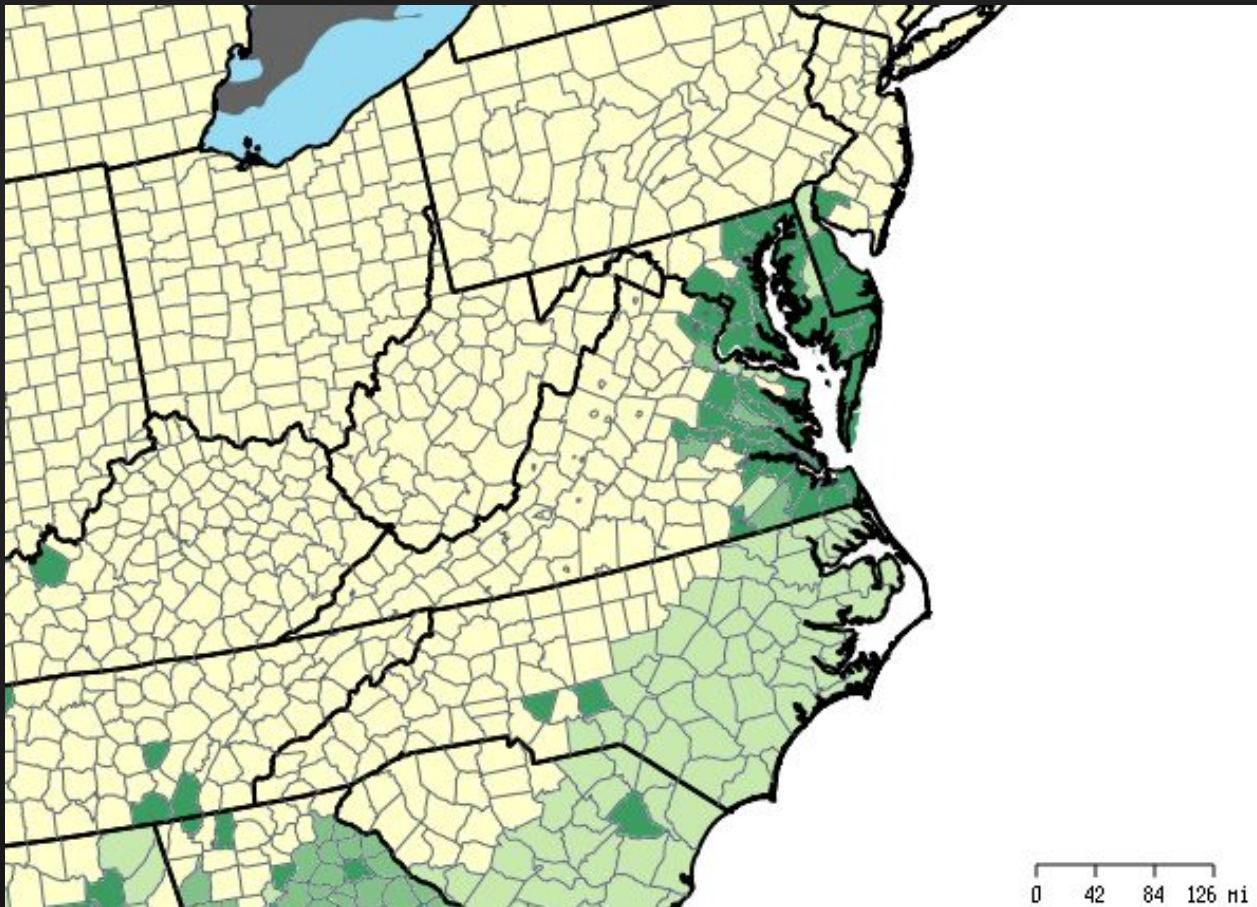
- Local Reports and Acoustic Surveys
- Awaiting results of 2012 Calling Amphibian Monitoring Program (CAMP)





# Conclusions and Significance

- DE population possible source for NJ *Hyla cinerea*



- Genetic impoverishment in newly colonized population  
predominant haplotype from founding population
- Genetic impoverishment and  $F_{st}$  that is essentially zero suggests very recent colonization



# Climate Change and Amphibians

- Models predict significant climate-induced changes in amphibian species composition in central and eastern US
- Amphibian susceptibility to regional climate changes due to:
  - dependence on microhabitat
  - dependence on hydrology
  - limited dispersal ability
- Earlier calling phenology of frogs documented in New York state --> species calling 10-13 days earlier over the past 100 years



# Climate Change and Amphibians

- *H. cinerea* most recent range expansion --> Illinois, moved 110 km north of previous northernmost population in central US
- NOAA climate data show:
  - temperature in NJ has already increased an average of 2°F since 1900, with winter temps increasing 4°F since 1970
  - rainfall has also increased 5-10%
  - predicted 2°-8°F increase in temperature will see regional climate of southern NJ comparable to current climate of DE



# Conclusions and Significance

- Genetic distinctness at periphery (and therefore in colonized population as well) could represent evolutionary potential



- How to treat species that cross political boundaries?
- Monitor colonization?

# Implications for Native Species and Ecosystems

- Florida --> natural hybrids between *H. cinerea* and *H. andersonii*
  - hybrids showed no developmental abnormalities but were infertile
  - 2 consecutive years of drought may have limited suitable breeding habitat
- *H. cinerea* known to hybridize with *H. gratiosa* --> no known current population in NJ
- No records of hybridization with *H. chrysoscelis* or *H. versicolor*
- *H. cinerea* has demonstrated dietary plasticity outside of native range

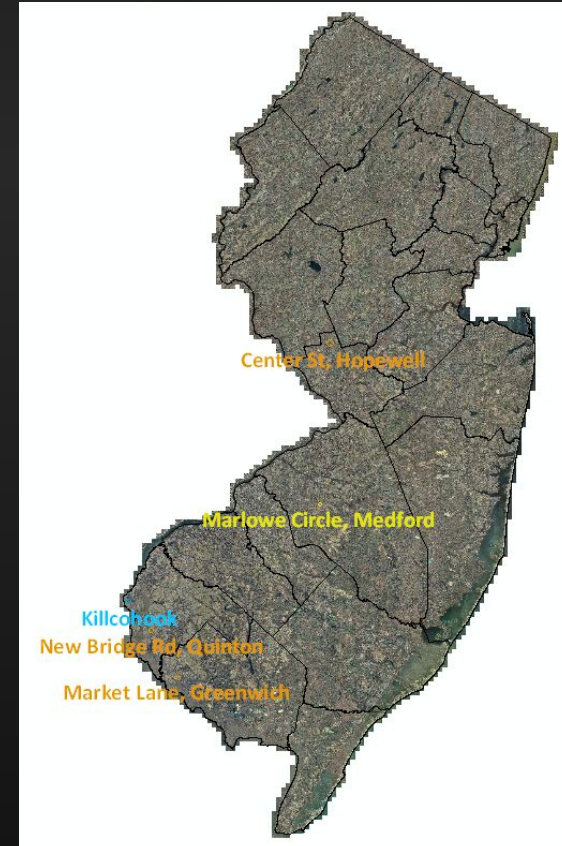


# Future Directions



- Requesting museum specimens of *Hyla cinerea* collected throughout its range in order to understand species-wide diversity

- Obtain DNA from other NJ populations, to determine whether all NJ *Hyla cinerea* are from the same source
- Calling surveys to understand the extent of *Hyla cinerea* range in NJ
- Use nuclear DNA markers to explore patterns not present in mtDNA



# Acknowledgements

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