An underwater photograph showing a dense cluster of oyster shells and reef structures. The shells are various shades of brown, tan, and grey, with some showing signs of growth and attachment. The background is a hazy, greenish-blue water, suggesting an underwater environment. The overall scene is a natural, somewhat murky view of an oyster reef.

# **Genetic Considerations in Oyster Restoration**

**Ami E. Wilbur**

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**Department of Biology & Marine Biology**

**Center for Marine Science**

**University of North Carolina Wilmington**

# What do we mean by restoration?

- Variety of activities offsetting threats to shellfish populations
  - Can be categorized:
    - Stop destruction/depletion
    - Rebuild habitat
    - Redistribute natural recruitment
    - Supplementation with hatchery-produce shellfish
    - Supplementation with “designer” shellfish



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} Potential Genetic Issues



# Assumptions of supplementation...

- Stocked animals will survive and reproduce.
- Progeny of stocked animals will develop, metamorphose, grow, and survive to reproduce, contributing to the persistence/enhancement of the “population”



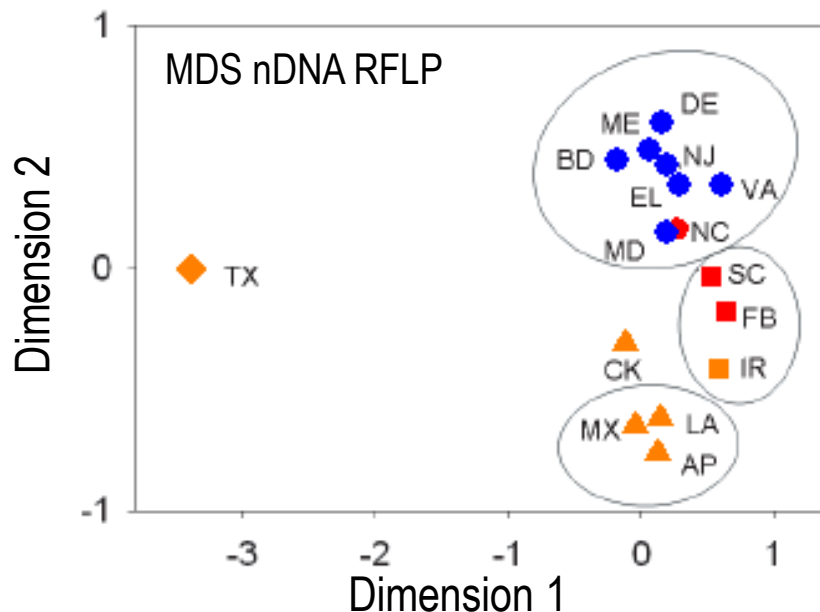
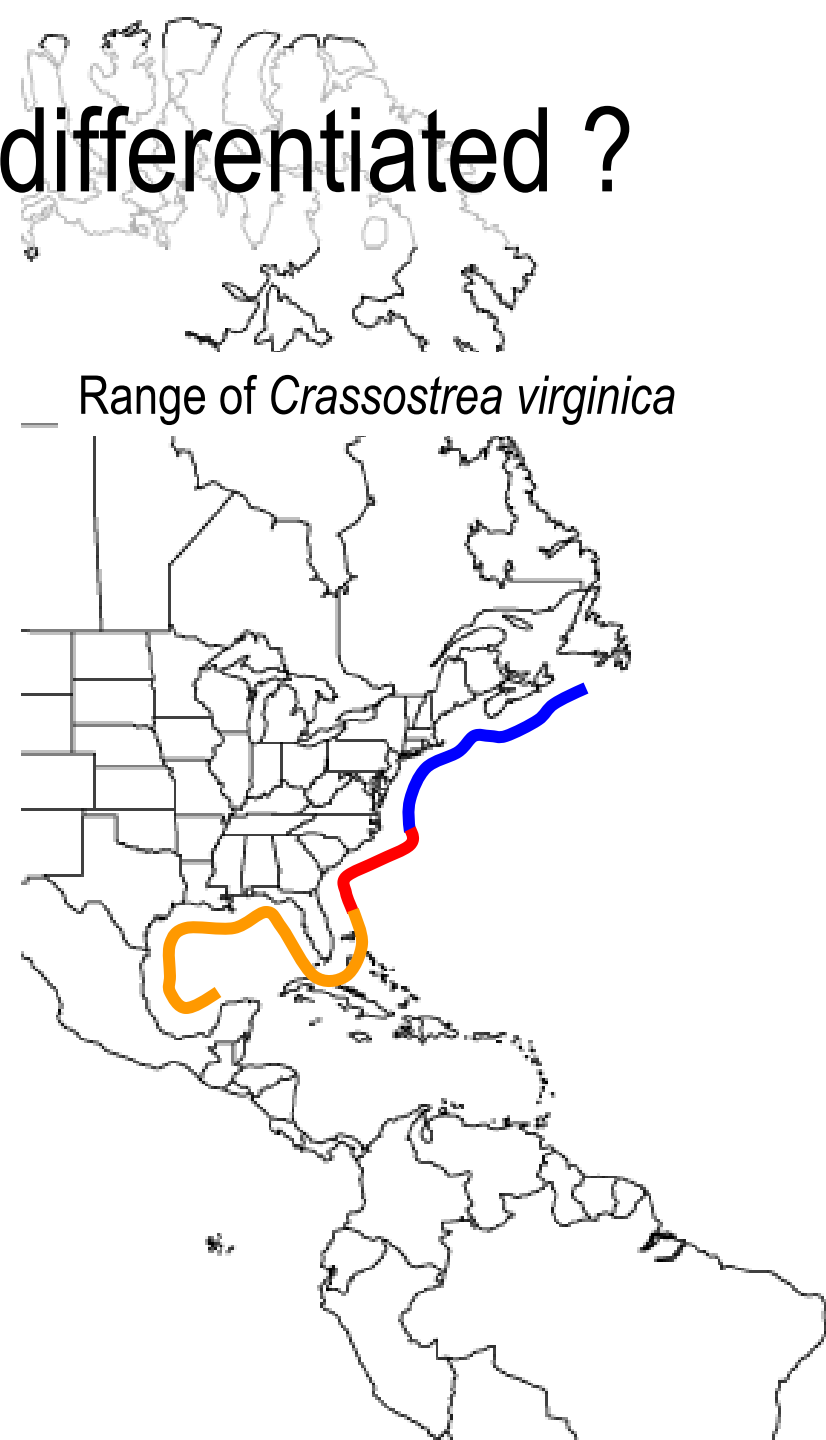
# ...lead to concerns about source of supplemented animals

- If sources exhibit genetic differentiation...
- And the existing levels and patterns of genetic variation are meaningful....
- Then supplementation with inappropriate genetic stocks could ...
  - Reduce fitness
  - Reduce genetic variation
  - Replace adapted wild genotypes
  - Reduce responsiveness to future challenges



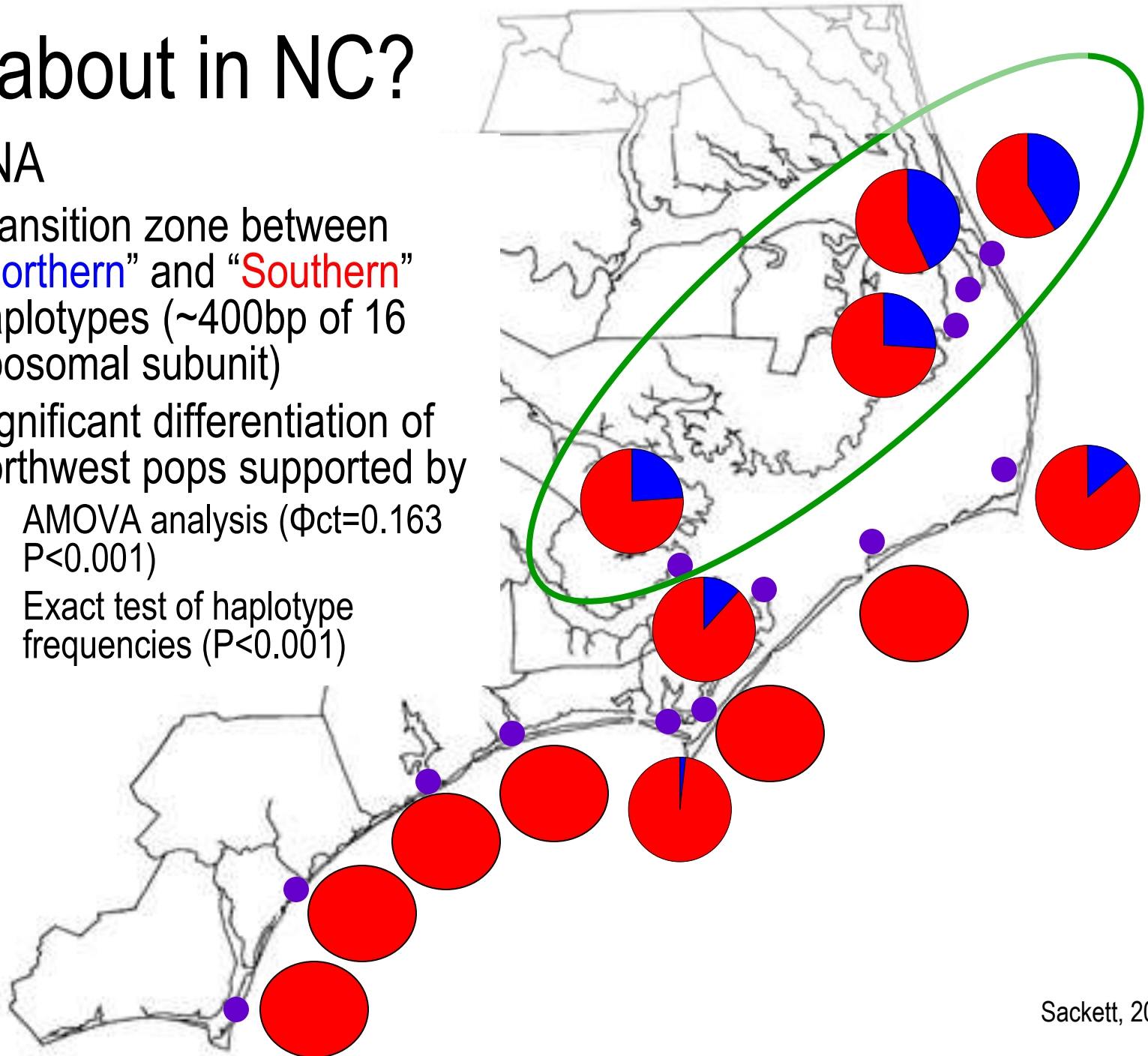
# Are oysters genetically differentiated ?

- Yes, regionally
  - mtDNA analysis suggests ~3 regional assemblages (Wakefield & Gaffney 1996, Gaffney 2006)
  - Nuclear DNA RFLP analysis supports geographic pattern (Hoover & Gaffney 2006)



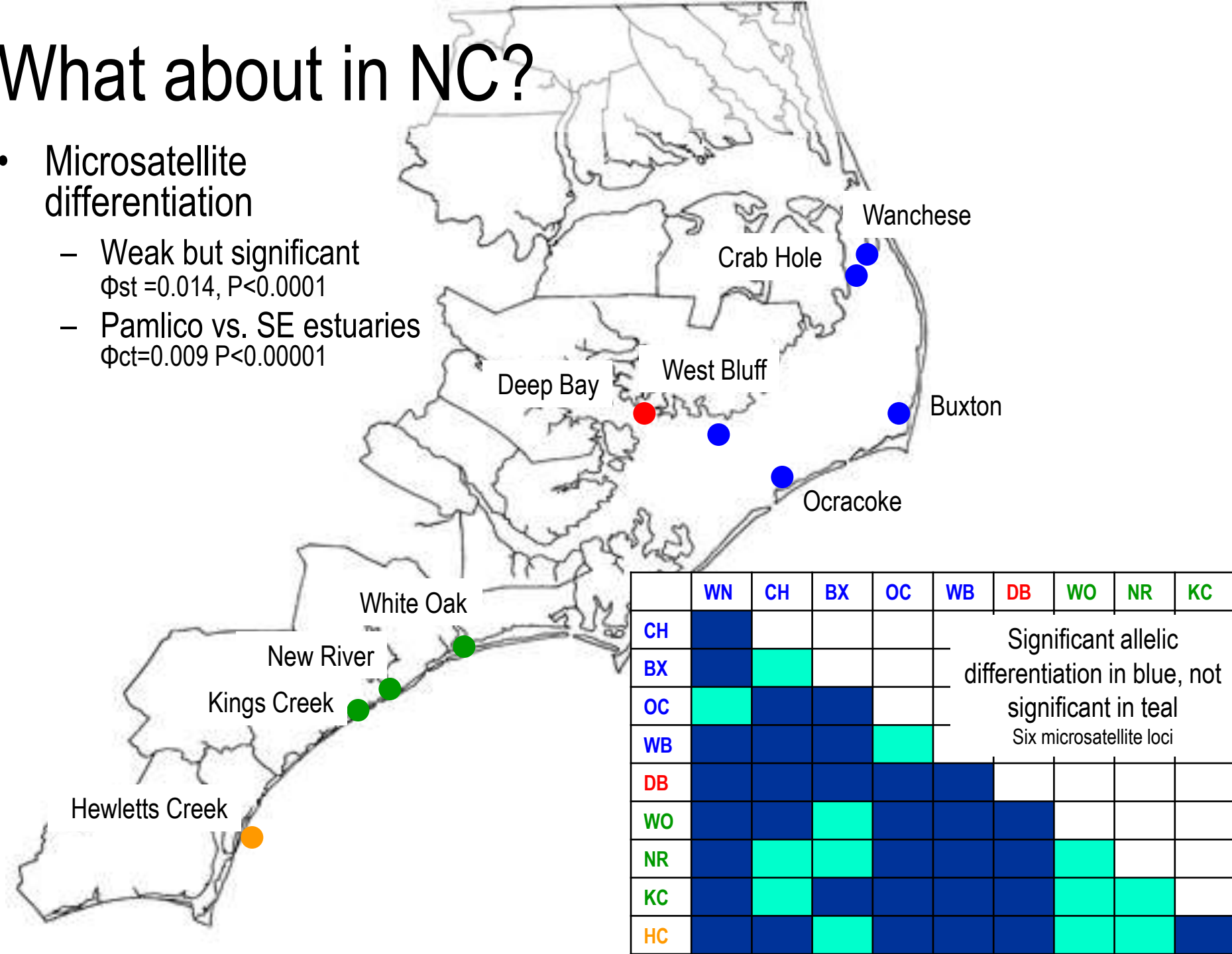
# What about in NC?

- mtDNA
  - Transition zone between “Northern” and “Southern” haplotypes (~400bp of 16 ribosomal subunit)
  - Significant differentiation of northwest pops supported by
    - AMOVA analysis ( $\Phi_{ct}=0.163$   $P<0.001$ )
    - Exact test of haplotype frequencies ( $P<0.001$ )



# What about in NC?

- Microsatellite differentiation
  - Weak but significant  
 $\Phi_{st} = 0.014$ ,  $P < 0.0001$
  - Pamlico vs. SE estuaries  
 $\Phi_{ct} = 0.009$ ,  $P < 0.00001$



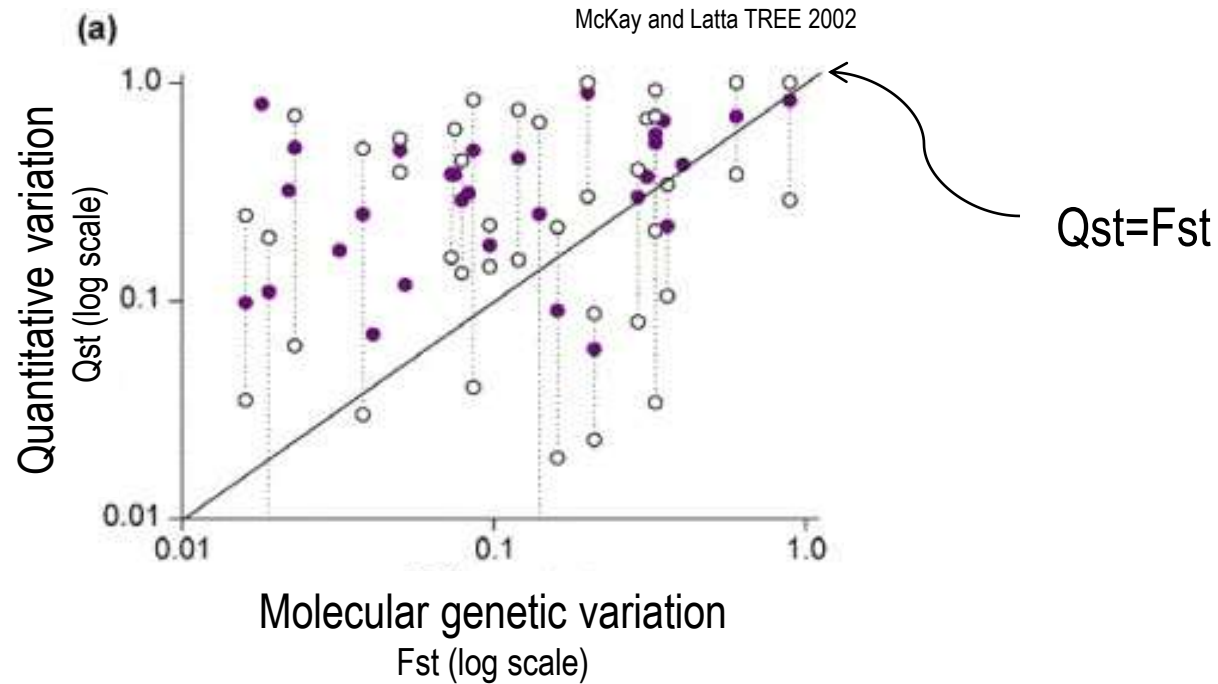


# Molecular genetic patterns exist but do they *matter*?

- What does “neutral” molecular variation tell us about the potential for successful supplementation?
  - Successful supplementation depends on growth, survival, reproduction, i.e. quantitative traits



# Molecular genetic patterns exist but do they *matter*?



- Analyses suggest that estimators of quantitative and molecular genetic variation are poorly correlated (although slightly positive Leinonen et al. J. Evol. Biol. 2008)
  - <4% quantitative variation explained by molecular variation (Reed and Frankham, Evolution 2001)

# What does this mean for restoration involving supplementation?

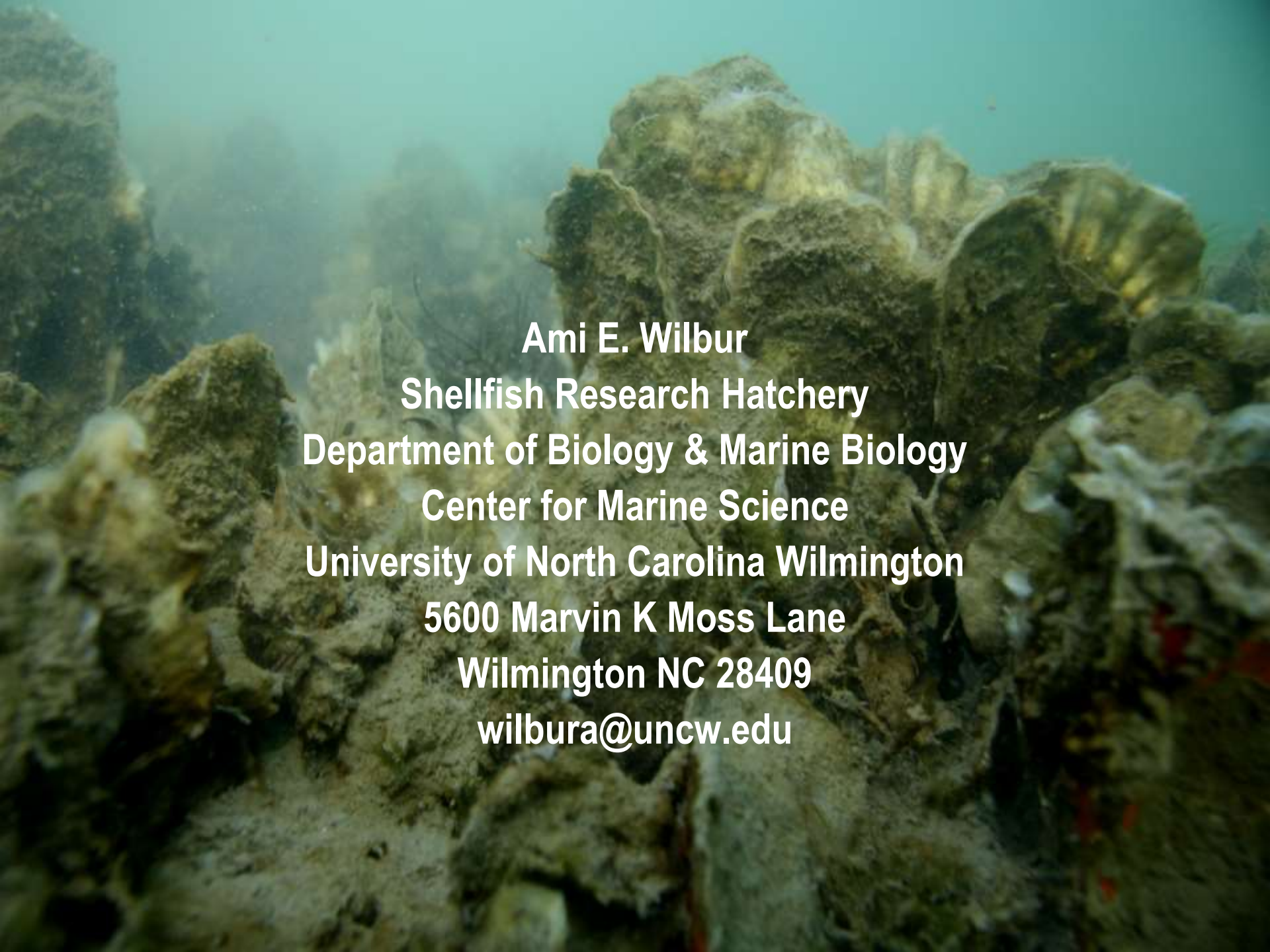
- Molecular information may not be predictive of differentiation among supplemental and recipient oyster populations
- What do we know about differentiation in quantitative traits?



# Genetic considerations restated...

- Largely an issue of source
- Paucity of information regarding differentiation on small spatial scales
- Is benefit > risk?



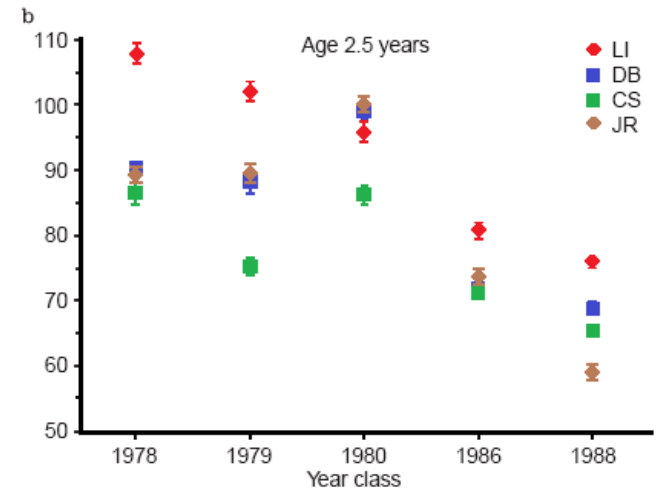
An underwater photograph of a rocky reef. The rocks are covered in various marine organisms, including oysters and sponges. The water is clear and blue-green. The text is overlaid in the center of the image.

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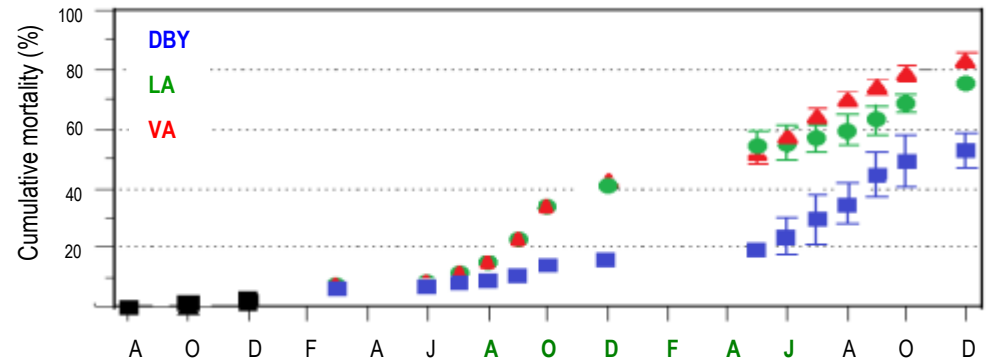
# What do we know about quantitative variation in oyster?

- Growth rate
  - Persistent differences in growth rate of strains maintained in common environment for 7 generations (Dittman et al. 1998)



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- Disease tolerance
  - Differences in cumulative mortality in strains with different geographic origin propagated in a common environment (Calvo et al. 2003)





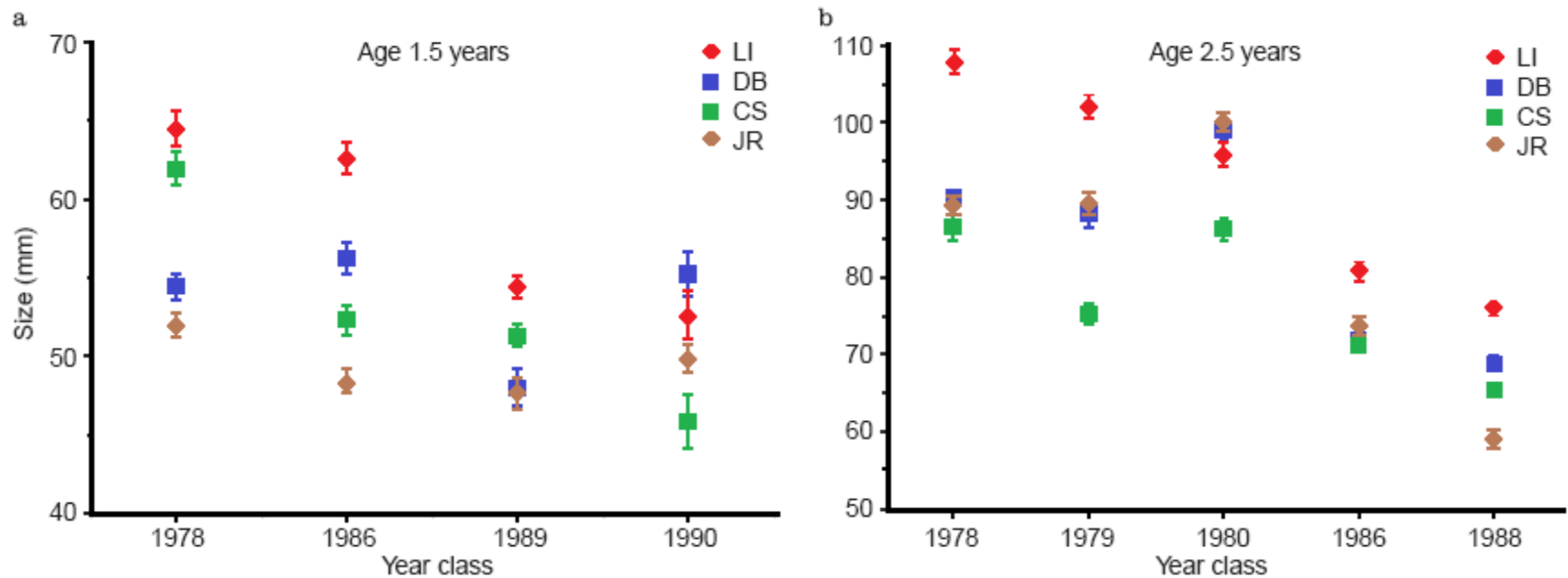
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- Reproductive timing
  - Persistent differences after 6 generations in common environment (Barber et al. 1991)

	Long Island	Delaware Bay
Maturation	May	June
Spawning	July	Late June to August
Spent	August	September

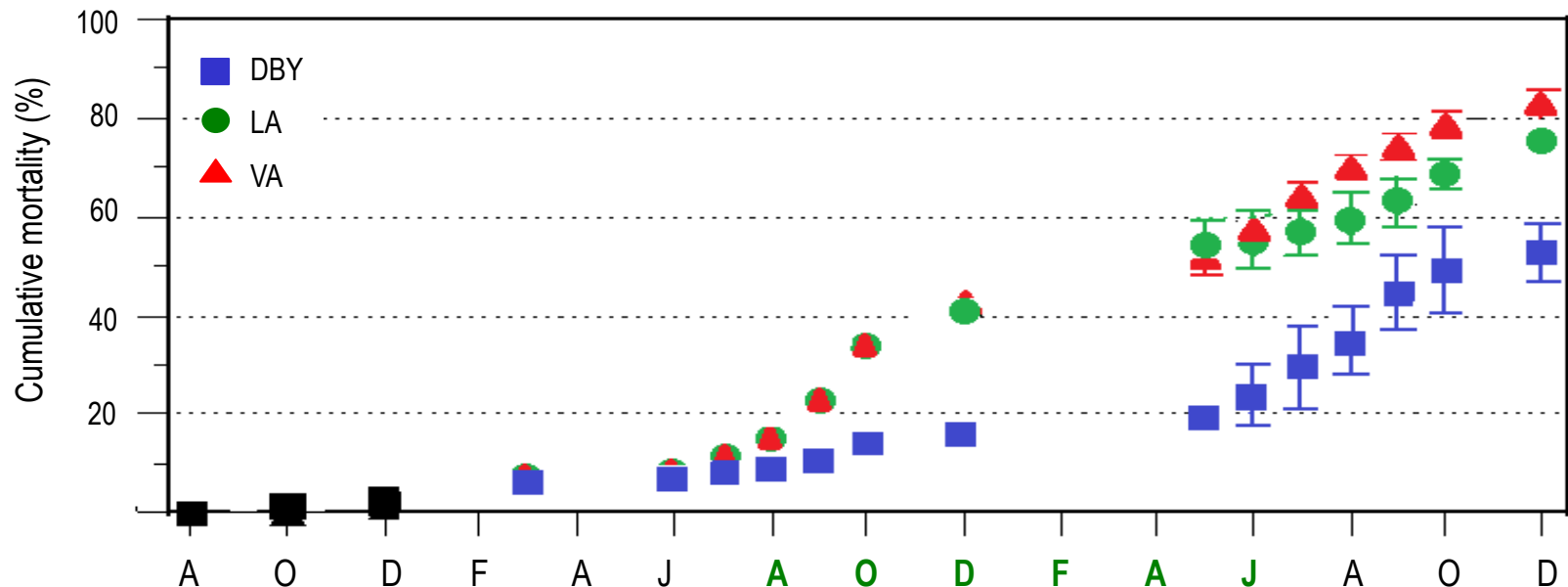
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Maturation	May	June
Spawning	July	Late June to August
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Does that mean those  
\$\$\$ molecular techniques  
are useless?



- NO!
  - Presence of differences are meaningful but a finding of homogeneity less informative
    - Need a better understanding of the distribution of quantitative variation in species targeted for restoration
- And molecular genetic assays are useful in
  - Maintaining/monitoring genetic variation in the hatchery and field
  - Assessing impacts

# How about oysters?

- J&B Aquafood manages 37 acres of bottom leases in Stump Sound, NC
  - 1.5 acre water column lease with ~70,000 “Gulf Coast” oysters
  - Gulf oysters stocked for ~ 3 years
- Diagnostic marker
  - 362bp of 16s ribosomal gene (mtDNA)
- Collect oysters in and around lease
  - N=75-100, 7 sites within Stump Sound, and culture stock

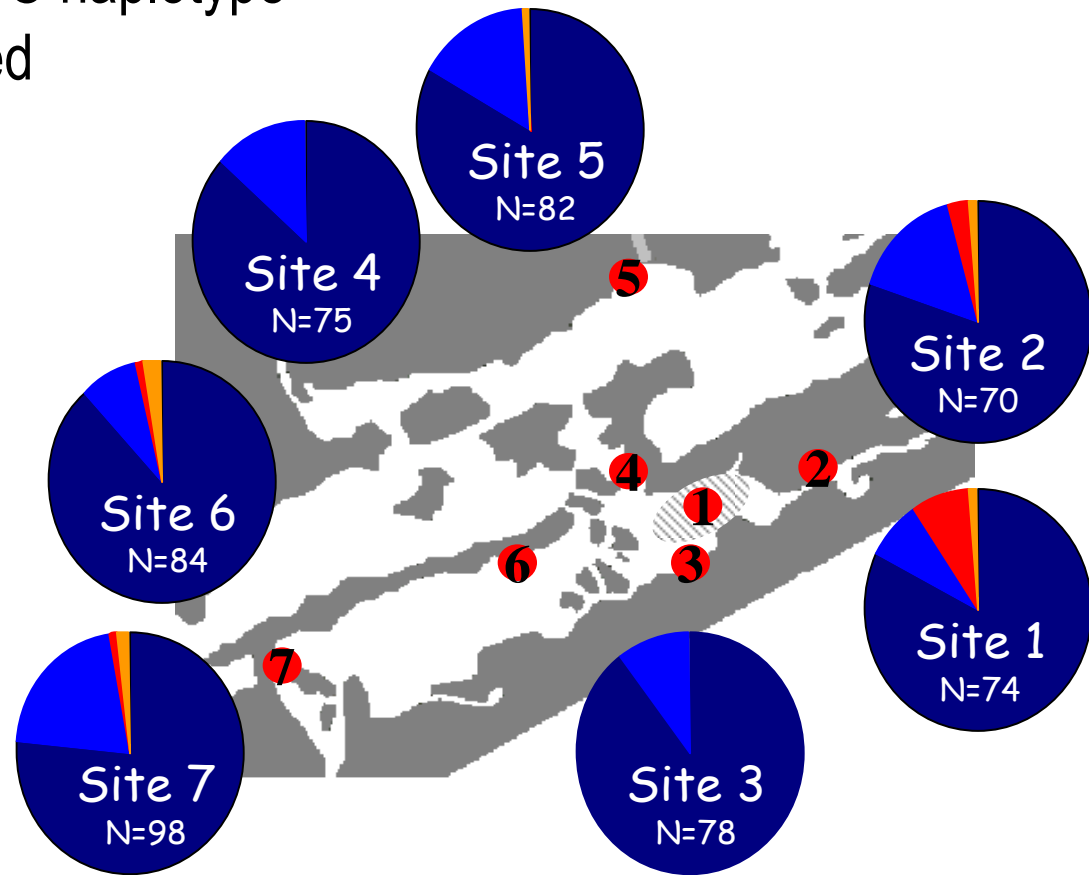


	Positions*			
	2096		2104	
NA	AATTACA	T	AAATTCT	A AC
SA	AATTACA	G	AAATTCT	A AC
GC	AATTACA	G	AAATTCT	G AC

\*GenBank AY905542

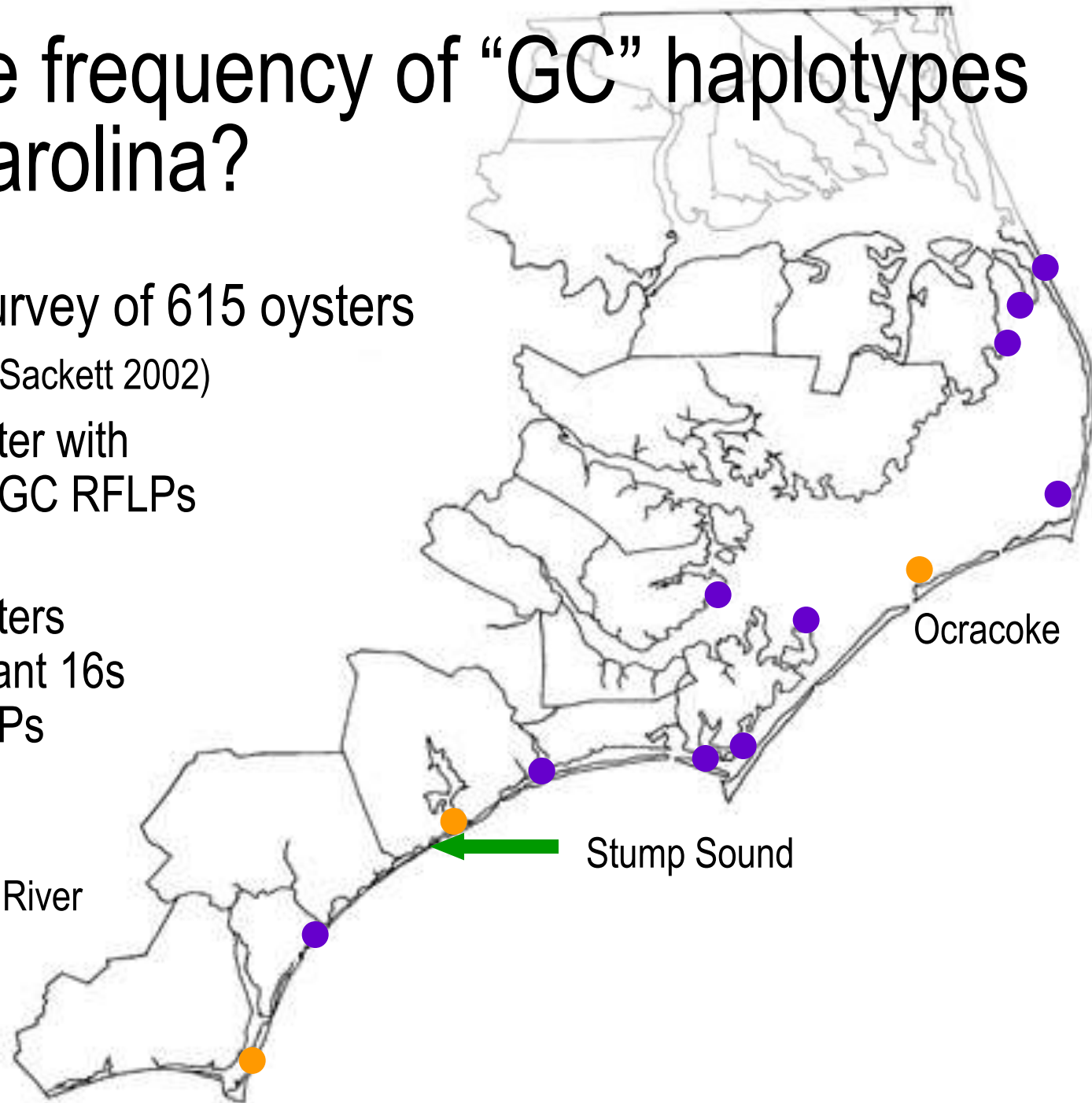
# Can Aquaculture provide Enhancement?

- Culture stock (N=30) fixed for GC haplotype
- Stump Sound oysters exhibited 34 haplotypes (562 oysters)
  - 84.5% **SA haplotype**
    - 12.5% **SA variants**
  - 1.8%(10) **GC haplotype**
    - 1.2%(7) **GC variant**
- Two additional markers
  - COI (~900bp)
  - Cyt B (~2.4kb) cut with *BsaH I*
- RFLP analysis revealed
  - Culture stock exhibited Gulf RFLP patterns
  - 10/17 Wild oysters (with GC 16s) exhibited Gulf RFLP patterns
    - 4 were identical to culture stock for both 16s and RFLPs (0.7% of total)



# What is the frequency of “GC” haplotypes in North Carolina?

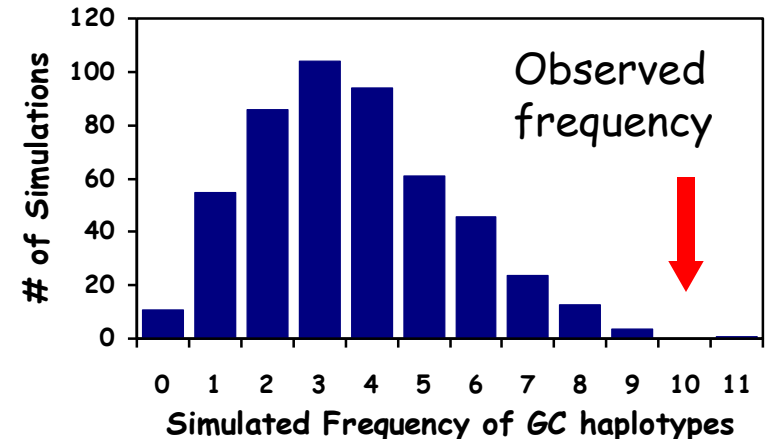
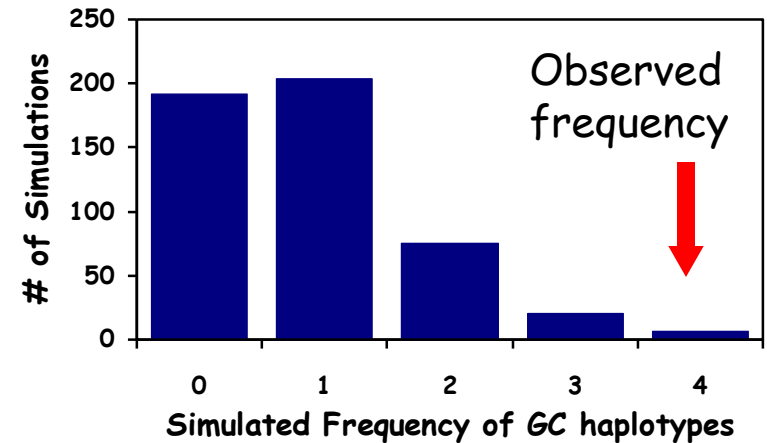
- 16s mtDNA survey of 615 oysters from 13 sites (Sackett 2002)
  - 1 (0.2%) oyster with GC 16s and GC RFLPs
    - Ocracoke
  - 3 (0.5%) oysters with GC variant 16s and GC RFLPs
    - Ocracoke
    - New River
    - Cape Fear River





# Are the Stump Sound frequencies significantly elevated?

- Yes!
- Simulation analyses
  - Consider only those oysters *identical* to the culture stock
    - 0.7% in Stump Sound vs 0.2% Statewide
    - Observed frequency seen in 7/500 simulated samples (P=0.014)
  - Consider all oysters exhibiting Gulf-like mtDNA haplotypes
    - 1.8% in Stump Sound vs 0.7% Statewide
    - Observed frequency seen 1/500 simulations (P=0.002)



# AUGUST-NOVEMBER 2013

- SHELL HEIGHT (NC ONLY)

- FARMS

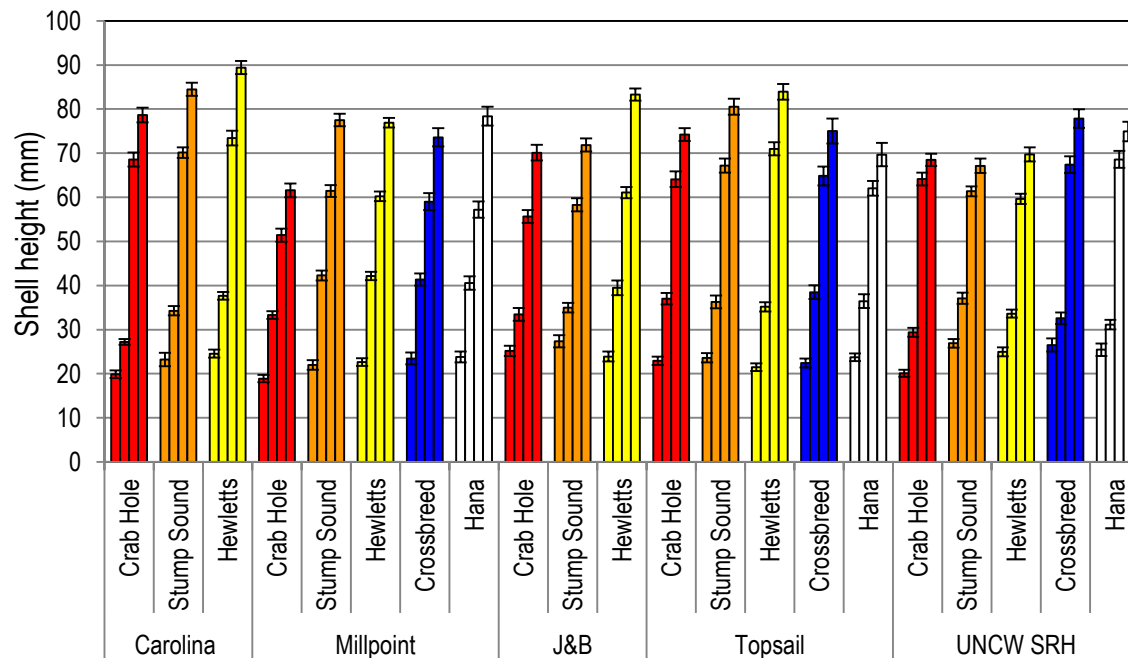
- CAROLINA>TOPSAIL>J&B>MILL POINT>SRH
- CAROLINA OYSTERS ~ 16MM LARGER THAN SRH OYSTERS

- LINES

- HEWLETTS>STUMPS>CRAB HOLES
- HEWLETTS OYSTERS ~ 10MM LARGER THAN CRAB HOLE

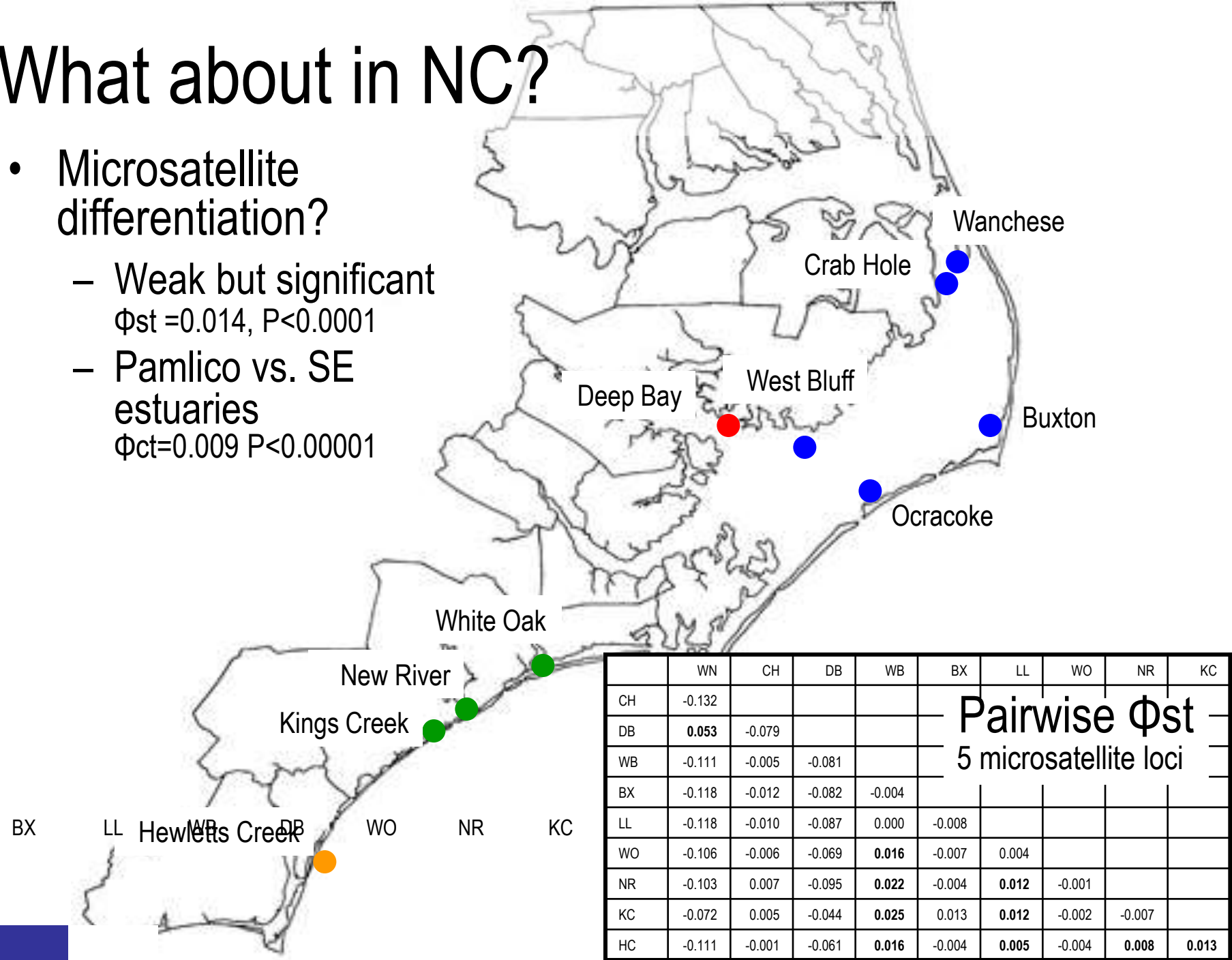
- NC vs VA

- NO SIGNIFICANT DIFFERENCE (BUT HEWLETTS ARE ~5MM LARGER THAN CROSSBREDS OR HANAS)



# What about in NC?

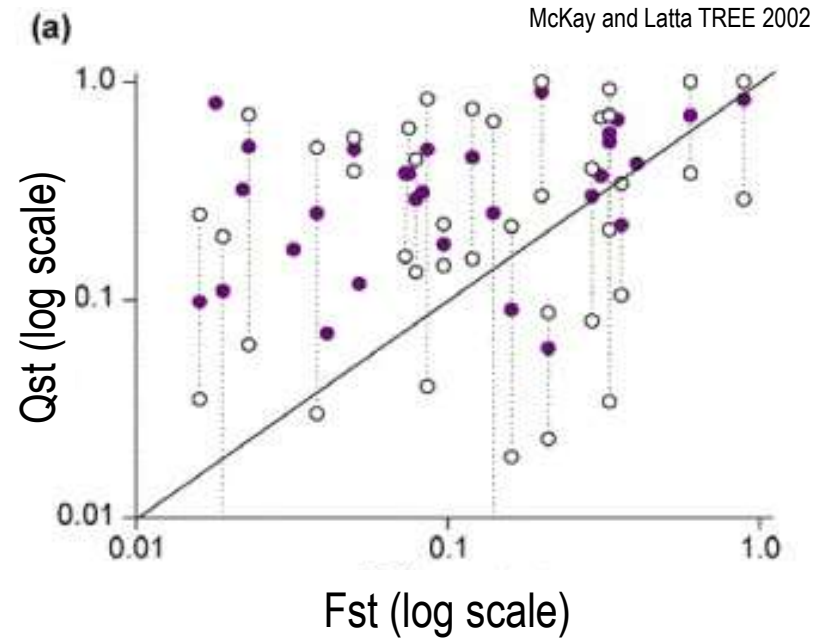
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Pairwise  $\Phi_{st}$   
5 microsatellite loci

# Patterns exist but do they matter?

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- Does neutral molecular variation tell us anything about quantitative variation?
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