Better Shellfish Restoration through Genetics?

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The problem

Original population

Current population: reduced $N$, $N_e$
lower genetic diversity?
altered life history traits

habitat alteration
selective overfishing
The solution?

Restored population:
- increased N
- altered $N_e$?
- lower genetic diversity?
- restored life history traits?

Current population:
- reduced N, $N_e$
- lower genetic diversity?
- altered life history traits

habitat restoration
hatchery supplementation
From initial optimism

Moav et al. (1978) *Genetic improvement of wild fish populations*. Science 201:1090: “Harvested wild organisms can be preserved by hybridization with ‘tailor-made’ selected breeds”.

Rationale:
1) Heavily harvested natural populations are selected for reduced size and earlier reproduction.
2) Domesticated populations often display improved growth rate and/or disease resistance but lowered hardiness in the wild.
3) F1 hybrids of wild (W) x domestic (D) strains show heterosis when grown in natural environments.
4) Stocking natural populations with WxD F1 hybrids would counter the negative impacts of overfishing, increasing yield and restoring desired genotypes.
To current pessimism

Heath et al. (2003, Science 299:1738) “Unintentional selection in captivity can lead to rapid changes in critical life-history traits that may reduce the success of supplementation or reintroduction programs.”

Naylor et al. 2000 (Nature 405:1017) “Increasing evidence suggests that farm escapees may hybridize with and alter the genetic makeup of wild populations of Atlantic salmon which are genetically adapted to their natal spawning grounds. Such genetic alterations could exacerbate the decline in many locally endangered populations of wild Atlantic salmon.”

Ryman & Laikre (1991), Conservation Biol. 5:325) “…supportive breeding may result in a drastic increase in the rate of loss of genetic heterozygosity through reduction of the effective population number.”
Genetic concerns in shellfish restoration

- Identifying and using the correct genetic material (germ plasm)
- Maintaining genetic variability in hatchery stocks
- Maintaining $N_e$ in the target (wild) population
Germ Plasm Diversity

- Geographic distribution of genetic variation: how different are populations? Which ones should be used for production and selective breeding?
  - Taxonomic uncertainty at the species level
  - Human-mediated gene flow superimposed on historical population structure
Crassostrea virginica ranges from maritime Canada to the Yucatan Peninsula.
PCR-RFLP analysis of five mitochondrial regions (>5500 bp total) shows deep separation of Gulf and Atlantic haplotype clusters as well as modest structure within each region.

(Gaffney et al. in prep)
Direct sequencing of mtDNA confirms phylogeographic structure

[685 bp; ND2/Arg/His/ND4]
Nonmetric multidimensional scaling of genetic distances among eastern oyster populations, based on PCR-RFLP analysis of four nuclear genes (Hoover & Gaffney 2005)

4 amplicons, 17 restriction sites
Multidimensional scaling of genetic similarities (Nei 1972) in *C. virginica*. 6 SNPs in 5 amplified gene fragments (arginine kinase, chitinase, thymosin beta (2 SNPs), RP L27A, RAN) were scored in ≈20 individuals from 12 sites.
Multidimensional scaling of genotypic similarities (allele-sharing) based on >350 SNPs in *C. virginica*. Nine gene fragments (200-700 bp) were sequenced for 12 individuals representing the geographic distribution of the species.
C. gigas: 10 Type I loci, 164 SNPs
nonmetric multidimensional scaling of genotypic similarity

Loci:
ALAT
NDK
TIMP
BTG
GAL-8
PRO
EF1a
RP L13
ApN
Δ9D
Germ Plasm Diversity

- Geographic distribution of genetic variation: how different are populations?
  - Current genetic methods provide good resolution of population structure

- Which ones should be used for production and selective breeding?
  - To be determined empirically
Unlocking genetic potential from the wild

A. wild tomato stays green.
B. left: modern cultivar. right: after introgression of QTL from wild species
C. top left: another wild species. top right: modern cultivar bottom: after introgression of QTL from wild species

Maintaining genetic variability in hatchery stocks

- Mass spawning results in \( N_e < N_b \)
- Equalizing parental contributions (pooling families or small mass spawns) can cause \( N_e > N_b \)
- Pedigree monitoring with genetic markers recommended to evaluate \( N_e \) (and detect contamination)

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## Allelic Diversity is Reduced in Disease Resistance Selected Stocks Compared to Natural Populations

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K. Reece, VIMS

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C. gigas: 10 Type I loci, 164 SNPs
mean SNP heterozygosity

Source population

Mean heterozygosity

Portugal  Kyushu  Korea  Hokkaido  Domestic
Maintaining $N_e$ in the wild population

- Ryman-Laikre effect: supplementation of wild stocks with hatchery-bred animals may boost total numbers but result in a lowered $N_e$
- Is this a concern for shellfish restoration?
  - Critical parameters
    - Relative contribution of hatchery stock
    - $N_e$ in the wild population vs. hatchery stock
Ryman-Laikre effect, $N_{e(h)} = 200$
Virginia Basin Oyster Populations
_Crassostrea virginica_

CBOPE Program
www.vims.edu/mollusc/cbope/

Estimated number of oysters

Estimated oyster biomass (g dry tissue)

Year


Maryland Department of Natural Resources Sarbanes Cooperative Oxford Laboratory
University of Maryland Marine Estuarine and Environmental Studies Program
Virginia Institute of Marine Science Department of Fisheries Science Molluscan Ecology Program
Virginia Marine Resources Commission Conservation and Replenishment Division
Crassostrea virginica restoration

- Estimated census number (N)
  - Chesapeake Bay: $\approx 2-3 \times 10^9$
  - Delaware Bay: $\approx 1-2 \times 10^9$

- Estimated $N_e << N$
  - For Dabob Bay $C. gigas$, $N_e \approx 400$, $N = 10^8$
  - Applying $N_e/N$ from $C. gigas$:
    - Chesapeake Bay: $N_e \approx 10^4$
    - Delaware Bay: $\approx 5 \times 10^3$
Wild population $N = 10^9$, $N_{e(w)} = 10^5$
Wild population $N = 10^9$, $N_{e(w)} = 10^4$
Wild population $N = 10^9$, $N_{e(w)} = 5 \times 10^3$
Wild population $N = 10^9$, $N_{e(w)} = 10^3$
This scenario would apply to enhancement efforts in the Chesapeake Bay 'basins'.

Wild population $N = 10^8$, $N_{e(w)} = 500$
Is $N_{e(w)}$ at risk from hatchery supplementation?

- Even for severely depleted populations, census numbers are large ($>10^9$), dwarfing the numbers of hatchery stock planted.

- The impact of restoration planting on $N_{e(w)}$ depends on its original value:
  - For low $N_{e(w)}$, planting hatchery seed should increase $N_{e(t)}$ even when $N_{e(h)}$ is low.
  - For high $N_{e(w)}$, planting hatchery seed will reduce $N_{e(t)}$ especially when $N_{e(h)}$ is low.
How can genetics help restoration efforts?

- Informed selection of germ plasm for creation of restoration lines
  - Use genetically diverse sources to make synthetic populations, followed by selection (for disease resistance, growth, etc.)
  - Cross hatchery ♂♂ x wild ♀♀ to produce F₁ for outplanting
How can genetics help restoration efforts?

- Maintain adequate $N_e$ in hatchery lines
  - spawning protocols (pooling multiple families or small group spawns, equalizing numbers)
  - regular pedigree monitoring to detect bottlenecks and contamination
How can genetics help restoration efforts?

- Maintain $N_e$ in the wild population
  - obtain better estimates of $N_e/N$ in wild populations
  - use $N_{e(h)}$ large enough to prevent substantial drop in $N_e$ of wild population (Ryman-Laikre model)
How can genetics help restoration efforts?

- Genetic improvement of degraded wild populations
  - Selection in hatcheries for high-growth, disease-resistant lines
  - Supplement wild population with $F_1$ DxW hybrids
    - Superior performance (heterosis)
    - Introgression of favorable alleles into wild population, reversing anthropogenic negative selection
How can genetics help restoration efforts?

- Monitoring effectiveness of restoration efforts with genetic markers
  - High-throughput DNA assays are needed to sample seeded populations
    - Milbury et al. (2004) used Pyrosequencing to screen >4500 oyster spat for a diagnostic mitochondrial DNA polymorphism to detect reproduction by Gulf Coast oysters planted in Chesapeake Bay
RESULTS

N = 4538 spat screened from three spat sets (1999, 2000, 2001)

5 spat with Louisiana haplotype detected

RFLP confirmation confirms 4 spat as Gulf Coast, one a rare Atlantic

Conclusions – the answer is yes

- For shellfish populations, the risks of ‘genetic swamping’, ‘genetic pollution’ and reduced genetic diversity in the wild population resulting from restoration projects appear negligible.

- Well-designed enhancement programs using genetically improved lines may restore degraded wild populations.

- Genetic tools will enhance selective breeding programs and allow monitoring of restoration efforts.
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