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## GUEST EDITORIAL

# IMPROVING PRODUCTION OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* THROUGH COORDINATION OF GENETIC IMPROVEMENT PROGRAMS, RESEARCH, AND TECHNOLOGY TRANSFER ACTIVITIES

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## INTRODUCTION

The East Coast Shellfish Growers Association has been actively advocating for continued improvement of breeding infrastructure to support a vibrant and ecologically crucial industry. This white paper acknowledges these efforts and is meant to serve as a paradigm for oyster breeding on the East Coast. The vision is relatively straightforward; however, its execution—like most things—will be fraught with detail and inertia. Yet describing simply the idea without articulating some detail and potential roadblocks would be misleading. Instead, we present a picture of a breeding paradigm as it might play out across the entire range of a species. At this particular time, when there is increasing interest in oyster aquaculture and the breeding that supports it, and with incipient programs emerging in the Northeast and Gulf, this “plea” for a common approach is meant to help unify shellfish breeding.

A more unified approach would provide the following attributes:

- encourage progress in breeding across the entire range of the eastern oyster;
- ensure efficiencies in program expenses by establishing resource hub(s) for eastern oyster breeding;
- ensure program continuity through a multistate collaboration, superseding swings in academic focus;
- broaden and diversify the base of genetic material for characterization through scientific research, which will eventually inform practical breeding;
- share crucial data and information platforms among regions;
- support the efforts of core institutions, for example, USDA, ARS, and NOAA, on common platforms (e.g., genotyping and bioinformatics) and common research themes;
- provide a common approach across a broad geographical base for training a new generation of shellfish breeders;
- prepare the industry to address the future opportunities and challenges facing shellfish aquaculture, such as adapting to climate change, responding to emerging diseases, or implementing new state-of-the-art technologies (e.g., genomic selection), by establishing a unifying framework for responding to new exigencies.

## WHY THE CURRENT EFFORT IS UNCOORDINATED

At the first glance, the extensive range and plasticity of the eastern oyster suggest that there need to be regional approaches to breeding, arguably Northeast, mid-Atlantic, Southeast, Gulf, and perhaps southern Texas. These biogeographic zones are also genetic breaks for natural populations. Research comparing local strains across the Northeast and the mid-Atlantic confirms the need for lines that are regionally adapted and documents the benefits of selective breeding (Proestou et al. 2016). Breeding programs have been developed in some of these regions; in others, they are incipient. For extant programs, the approaches have been various, ranging from closing populations (domestication) to mass selection to family-based breeding, to marker-assisted selection of mass selected lines. For incipient programs, there are emerging questions about what approaches will be most effective and cost-efficient. This question about the appropriate approach is always couched by the availability of resources. The need to duplicate resources for each region along the East Coast is by far the most serious impediment to expanded breeding programs for oysters. Furthermore, current paradigms for funding breeding research and technology transfer are largely competitive, and thus discourage collaboration and integration of breeding activities.

Whereas different geographical regions may require specific lines, the desirable traits among all regions are the same, at least for now. All oyster breeders (on behalf of the commercial farmers) want high survival, rapid growth, and uniformity. These traits are easy to measure, albeit crude. Survival, for instance, is impacted by many factors that we know and at least as many that we do not. It is simple to count live and dead oysters but far more difficult to ascribe a specific cause that could be quantified as a genetic trait.

Other genetic traits are straightforward and directly related to value in the market, such as shape and meat content. Across regions, there will be, of course, huge variability in conditions contributing to these production traits, and this also defines the need for regional varieties of oysters.

A central unifying theme across genetic improvement programs for *Crassostrea virginica* would be the sharing of breeding infrastructure, information, and data resources to facilitate the development and implementation of state-of-the-art breeding technologies for any phenotype of economic or ecosystem service importance.

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### DEFINING A UNIFIED APPROACH

What if there were a way to relate the performance of oysters from one region to another to determine which traits were truly regionally specific and which traits were universal across the species? The family-based breeding program at the Aquaculture Genetics and Breeding Technology Center (ABC) at VIMS has been “test driving” family breeding for about 15 y. The ABC has seen the rich potential of this approach that could be expedited across other regions with centralized resources for critical features such as breeding advice and database management. We propose that a family-based breeding approach is applicable across the range of *Crassostrea virginica*.

Family breeding is an “industrial breeding” approach that enables the estimation of the breeding value for specific traits, the relationship (genetic correlation) among traits, and the relationship between traits and sites. Breeding values and relationships among traits and sites can be estimated simultaneously by testing common genetic material across numerous environments, with the capability of isolating those families that perform well regionally. These field tests of families are called sib tests. Estimations of breeding values and genetic relationships from the family-based approach can be further enhanced through genome tools and technologies. When layered with genomic information [such as using whole genome selection to calculate genomic breeding values (GEBV)], the accuracies for phenotypic improvement in successive generations are increased.

The Australian Seafood Industries (ASI) program in Australia is a poster child for the family breeding approach. Before the herpes virus outbreak several years ago, the ASI serviced the entire industry from a single hatchery base in Tasmania. Test sites across the entire southern part of the continent, including New South Wales, Tasmania, and South Australia, span a wide range of conditions: temperate estuarine, temperate subtropical, and oceanic subtropical. In the before-herpes era, commercial hatcheries were able to propagate regionally specific families to supply seed to these regions. After the herpes outbreak, hatchery production got more complicated because of biosecurity concerns, but the fact that the ASI program served the whole industry allowed for rapid response in creating herpes-resistant lines. The ASI program saved the industry from collapse. Other good examples include the New Zealand Cawthron Institute programs for mussels and Pacific oysters, also based on family breeding.

### DEVELOPING A UNIFIED APPROACH

Developing a family-based approach across the East and Gulf Coast regions will require some deliberate first steps, which probably represents a 3- to 5-y process. It is important to note that “3–5 y” is not specific to family breeding and any breeding approach would require setup time. The question is what is the optimum approach to set up?

- Each region will need to build a genetically diverse founder population ( $F_0$ ) using material from domesticated or wild populations from the region. For example, the family breeding program at the ABC began with mass-selected lines and a wide variety of wild founders from the Chesapeake Bay.
- Each region should develop breeding objectives based on industry input, but there should be a concerted effort to

develop interregional breeding objectives. For example, low- or high-salinity tolerance might be a regional objective, whereas fast growth might be shared.

- A process should be developed to deploy families across hubs to determine the benefits of gene flow among them and to establish correlations between sites and traits.
- During this 3- to 5-y period, genetic parameters for each regional breeding population should be estimated. These estimates will set breeding goals within each region and determine the potential for gene flow among regions.
- Importantly, a process for interaction among hubs should be developed to manage interregional genetic data.

### A UNIFIED APPROACH IN OPERATION

It is important to emphasize that the essence of the proposed “unified approach” is in fact an industrial breeding program, meaning this is not about the various genetics laboratories populating the East and Gulf coasts, rather it is its own entity centered on the priorities of the oyster aquaculture industry, developing and demonstrating the benefits of new technologies and providing on-the-ground breeding services and consultation. As such, a unified breeding program needs core infrastructure at each of several hubs, ostensibly, three hubs (Fig. 1). Each hub would need expertise and infrastructure to operate a family-based breeding program, including distribution of test groups for performance evaluations and phenotypic data collection. Distribution of seed for testing or of brood stock to hatcheries among regions along the East Coast states could be challenging, but efforts are underway to manage molluscan

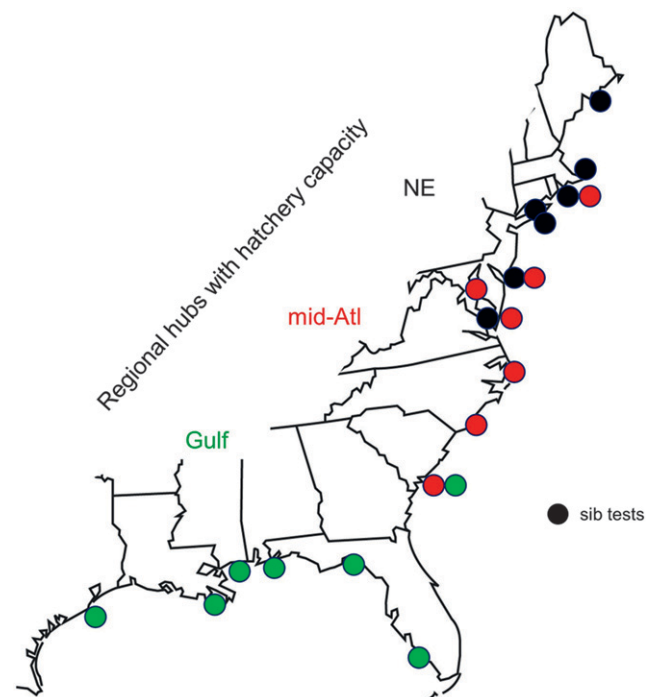


Figure 1. Three regions are shown in black, red, and green with hypothetical test sites represented by colored dots. Areas of regional overlap include the mid-Atlantic and the Southeast United States. Each region would be served by a hub with hatchery capacity.

diseases across regions with cogent biosecurity policies (Carnegie et al. 2016).

#### *Spawning Season*

Although it may seem so, a breeding program is not defined primarily by the hatchery effort. At most, the hatchery is a seasonal means to an end, the end being production of  $x$  number of pedigreed families to be deployed to field sites for testing. For the sake of discussion, assume that for a region,  $x$  equals 50 families, minimum, and an unselected control group to evaluate the impact of selection. A large magnificent hatchery is not required; however, the need for many small larval culture tanks, versus the usual *modus operandi* of large commercial-scale cultures, is a necessary accommodation. An itinerant, cleverly arranged hatchery space will do. The season lasts for a couple of months. Using an existing operating base, especially with algal culture ongoing, would be ideal, as the most onerous task will be providing high-quality algae for the larval cultures. An existing facility may also be able to provide brood stock conditioning services, which may be needed in one region or the other to ensure an early start in the year.

#### *Deployment Season*

The capacity to care for 50+ individual families in the nursery system, each having approximately 10,000 siblings, will be required, either at the hatchery base or nearby. The default position for family breeding is to deploy all families (with replicates) in individual grow-out units (first, upwellers, then grow-out baskets or bags). As genetic tools become more cost-effective, it may be possible to combine families for deployment and then assign them to parents retrospectively. Combining families will likely always be during some spat stage rather than the larval stage principally because of differential larval survival. Whether families will be deployed to individual units (the default) or combined in common grow out will be the major driver of the field effort.

It is axiomatic that the more field deployments there are, the more potential information on the environment  $\times$  genotype interaction there will be to inform breeding goals. In a program that encompasses the East Coast, there may need to be 6–7 field sites per hub, especially if sib tests overlap among regions (Fig. 1). These many field sites will require the cooperation of commercial growers, whereby test sites may consist of a combination of institutional (e.g., academic) and commercial farms.

#### *Due Diligence during the Field Season*

Far more important than the hatchery are the reliability and consistency of the field grow out. Field operations will be the most challenging aspect to manage because of multiple test sites. *A priori*, it will require reliable grow-out sites and careful husbandry. It is the field data and not the hatchery data that define the credibility of the genetic parameters and estimated breeding values.

#### *Progeny Evaluation Season*

When families reach the appropriate size for trait evaluation, another spate of work begins. When is evaluation stage? Not necessarily “market” size. At the beginning of the ABC program,

the correlation between family traits at 18 mo versus 30 mo was tested, and the correlation was extremely high, meaning breeding values were just as accurate if oysters were evaluated at 18 mo as at 30 mo. Similarly, it is possible that such evaluations could be carried out in only 12 mo if correlations with market size performance could be confirmed. Clearly, this phenomenon (age–age correlation) is trait-specific; therefore, priorities and methods for phenotypic data collection will be dictated by the region. In the Gulf, the sib test might be 9 mo. It is now 18 mo (but could be 12) in the mid-Atlantic; it may be 18 mo in the Northeast.

During evaluation of sib tests, all replicated families are measured for the pertinent traits. The mode of operation for each hub may vary, from working on the host farm to bringing samples back to a central location. The modality of data collection is unimportant, but modalities among regions (hubs) should be comparable to allow for data sharing.

#### *Data Entry and Analyses Season*

A purpose-built database is essential, and a shared database among regions is essential for a unified approach. Data are the fuel that drives the breeding program, and a well-designed data system allows the turnover of data before the spawning season begins. The data system effectively specifies the workflow and decreases the risk of disrupting the breeding program as personnel change. Whereas the bad news is that one needs to engage database services somewhere, the good news is that there need be only one database center for the eastern oyster. A database already exists for the ABC program, and adopting it is feasible in a unified approach. Managing the database on behalf of all the hubs will need to be a resource allocation.

Using the database, data files are generated to estimate the genetic parameters (i.e., heritabilities, genetic correlations, and genotype by environment effects), and these are used to inform strategic breeding decisions. Database reports are also used to generate files to calculate measures of genetic merit, or estimated breeding values (EBV), that are used for selection decisions. The EBV are typically calculated for each region in a program that encompasses a large geographic range, and the EBV of families may vary according to region. The degree to which they vary is one of the most important features of a family breeding program, dictating “generalists” versus “specialists,” as well as informing whether a trait should be treated as one trait or more than one. For example, in the ABC program, traits associated with growth are highly correlated between low and high salinity, but there is a distinct lack of correlation in survival at either low salinity or high salinity. Therefore, survival at high salinity is one trait and survival at low salinity is another, at least for ABC breeding purposes. Another way to look at testing across regions is that if a trait is correlated, information can flow across regions, informing all regions simultaneously. A lack of correlation in a trait among regions defines it as a separate, region-only trait.

#### *Spawning Season, again*

Armed with the breeding values, the breeding team sets up the hatchery space for another spawning season. The design for which families to cross is performed ahead of time by the breeding manager and the support cast (see the following), using an index for each breeding candidate that combines

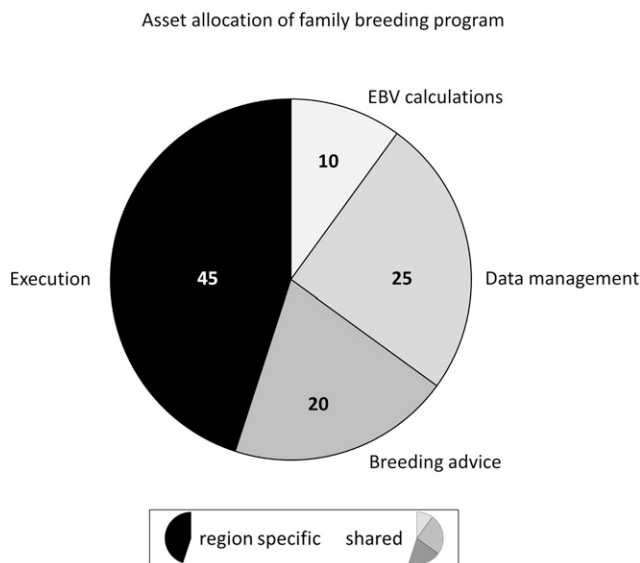


breeding values and appropriate economic weights for each trait. The trait weightings are determined by the breeding objective, which is defined at the program commencement and periodically reviewed. Of particular interest in this breeding design is the degree to which some families are produced to suit particular regions.

In addition to the families, the hatchery schedule will include the propagation of a multiplier population. There are several ways to go about this, but in essence, the top families—based on indexed breeding values—are spawned in a mass spawn to make a terminal “line” that can be distributed to hatcheries for propagation of genetically improved seed. The multiplier population, meant for commercial hatcheries, is produced each year from the best families in that year. There can be more than one multiplier line. One set of families may have high index values for the western Gulf and another for the eastern Gulf, or more likely, for the lower salinity farms versus the high-salinity farms. This agility to customize brood stock to several purposes is a powerful argument for a family breeding approach.

### THE SUPPORT CAST

The approximate allocation of resources to a family breeding program is represented in Figure 2. The outstanding thing about this allocation is that most of the effort for a family breeding program can be a shared resource. That is, the regional execution of the program, as described earlier, should be run from a regional hub. Once the data are acquired and strategic decisions are being made, a more centralized source could be invoked. Said another way, the ABC must engage all of the pie diagrams for its own program, but EBV calculation, data management, and ongoing industrial breeding advice could be shared for a coast-wide, unified approach.



**Figure 2.** Approximate resource allocation (%) for a family breeding approach, consisting of executing the hatchery and testing (execution), calculations of EBV, data management including database maintenance, and breeding advice.

### Region-Specific Execution

#### Breeding Program Manager

Breeding, family-based or not, can be a complex undertaking, and a manager of that complexity will be a necessity on a regional (hub) basis. An abridged list of responsibilities includes the following:

- coordinate the yearly breeding activities;
- manage the logistics of hatchery and field operations, after consulting with the relevant specialists (i.e., quantitative geneticist, hatchery manager, and field personnel);
- provide due diligence on execution of breeding strategy(ies);
- interface with other regional hub(s);
- manage and integrate the breeding program with research and development (see “Breeding Research Community”);
- oversee integrity of the data, with ultimate responsibility for data quality;
- interface with industry groups to ensure that target traits meet industry needs.

#### Assistance for Hatchery and Sib Test Sites

The needs for hatchery and fieldwork have been discussed. Although there are specific skills for each situation, these skills can easily be shared with a small crew that rotates among duties during the season: hatchery, nursery, deployment, maintenance, measurements, and data compilation. Thus, a hub could reasonably be populated by the breeding manager and two technicians. Were sib test sites to be located on commercial farms, one technician might be replaced by part-time help.

#### Shared Resources

##### Quantitative Genetics Capability

Using data (pedigrees or genotypes and sib test data) exported from the database, the calculations of EBV or GEBV by a competent quantitative geneticist are straightforward once a breeding strategy is developed and the program becomes fully operational. Presumably, a position at the ARS could easily provide these data analyses to extant breeding programs in exchange for research opportunities and shared publications.

##### Data System Management

A data management system is essential to enable seamless flows of data from the beginning (field data collection) to the end (spawning decisions and gains estimates). Core to this will be a database. The care and nurturing of a database to service one, two, or even three family-based breeding programs is one of the critical components of a successful program(s), and the ABC data system provides an example of how this can function. A well-designed data system will function across breeding nodes and will empower breeding managers and allow them to function without the need for programmers to intervene, doing things such as adding sib test sites and traits as required, and routinely generating data reports in a ready-to-use format. Furthermore, as the field moves further and further into genomic selection, the database will become far more complex and the need for specialized and robust data systems will intensify. It is not clear where or how such expertise might be obtained for

the unified approach advocated here. For the ABC family-based program, this service is outsourced.

#### Breeding Advice

Although the calculation of EBV and the like can be seen as routine, the truly creative role for a quantitative geneticist (e.g., possibly in an ARS position) is the understanding of the concept of industrial breeding and an appreciation by the incumbent for where the field is going, to coach oyster breeders in the same direction. Examples of “breeding advice” include interpreting the calculated genetic parameters, communicating outliers and discrepancies, making suggestions to each hub about adjusting their programs, and making suggestions as to how the overall, unified program should collaborate.

Breeding advice can diverge easily. One example is to do about tetraploids? The ABC has been working with their advisor(s) to develop a plan for domesticating and improving tetraploids through family breeding. Although the operations of a family-based tetraploid-breeding program are essentially the same as those in a diploid program, the traits, analyses, and management of genetic resources are all novel.

#### Breeding Research Community

There are a plethora of empirical questions that derive from the workman-like operations of an industrial breeding program. These questions feed academic, grant-based research and are often original. These can aptly be characterized by the phrase “genome to phenome” (Rexroad et al. 2019). The advantages of having the academic community tuned into the important biological questions of the industry are obvious. Also, there is great advantage in sharing expertise among regions, such as centralized disease challenges or experience with ploidy manipulation, which could accelerate progress everywhere.

#### CONTINUITY, COST, AND GOVERNANCE

Continuity and cost are the two most challenging aspects of running a breeding program. In the past, continuity has meant that an individual (academic) researcher was interested enough in the breeding side of genetics to sustain a string of grants to cobble together a program. Sometimes, institutions allocated resources that would assist this focus, and more rarely, there may have been a state allocation for some or all of the program operations. There is no getting around the issue of sustained funding. We posit that a unified approach to breeding that invokes collaboration coast-wide with other programs and with USDA scientists has a lot more credibility for leveraging ‘hard’ support than the ephemeral interests of individual faculty.

Thus, the important change for a family-based breeding program housed at an academic site would be the dissociation of industrial breeding from the academic expectations. For the ABC, it was the establishment of a center through a legislative initiative that allowed for the creation of a breeding program largely dissociated from the slings and arrows of grant funding. For the establishment of breeding hubs in other regions, the focus could be at a commercial company, such as in the Northeast. In another region, a breeding program could be based in an agricultural experiment station setting.

At the same time, if there is a breeding program—no matter how it is funded—there is a cost. In a world where financial resources are always limited, we argue that it is logical to spend those limited funds to mold the program to a unified approach.

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