GENETIC CONCEPTS FOR SELECTIVE BREEDING PROGRAMS

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TECHNICAL BULLETIN
USSEC SEA 06/2021
AQ2021-04

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Abstract

Selective breeding has embraced background knowledge of genetics and population genetics as its foundation successfully guiding the way to achieve breeding goals and maintain selected breeds. Traits that are heritable can be selected to increase in frequency from generation to generation. Several key concepts in genetics are important for successfully starting and managing a breeding program. The advance in molecular genetic technologies has changed the way we conduct selective breeding from conventional breeding to what is known as "molecular breeding". Genetic markers are used to evaluate genetic relationship among breeding individuals, and in many cases to assist in selection of genetically best fit individuals for breeding. Recently, genomic selection, in which genome-wide markers are used to predict the breeding values of candidate individuals, has been implemented in many breeding programs including aquaculture species. In practice, genotyping costs and effective ways to utilize genetic data are important considerations to determine the extent to which genetic tools will be invested into a breeding program. Collaborations among breeding programs would not only help with cost sharing, but would also potentially increase prediction accuracy from the pooled large sample size.

Introduction

Mankind started to create breeds through selective breeding since ancient times, long before our understanding of how phenotypes are determined and inherited. Selective breeding (or artificial selection) has shown to cause dramatic changes in the appearances of many domesticated animals and cultivated plants through numerous generations of selection. Charles Darwin recognized this process as resembling of how natural selection has created diversity of life over evolutionary courses (Darwin, 1859). Our understanding of how phenotypes are determined and how selection causes changes in a population is a foundation for selective breeding (Gjedrem & Baranski, 2009; Oldenbroek & Waaij, 2014), and has helped us to be more efficient in managing breeding programs. As technologies in molecular genetics have advanced, selective breeding has changed from conventional breeding to molecular breeding. Genetic markers are used as tools to evaluate relationships among selected individuals instead of pedigree records, facilitating the management of the inbreeding rate in a breeding program. Recently, new technologies have enabled us to discover and genotype numerous genetic markers throughout genome, making the application of genome-wide markers in selective breeding programs easier. For example, genome-wide markers can be used to perform genetic association studies, such as genome-wide association study (GWAS), in order to identify markers that are linked to the alleles with large effect on the desired traits (quantitative trait loci – QTLs). The results from GWAS can be used to perform marker-assisted selection, by which genotypes of the identified markers or QTLs are incorporated into the estimation of breeding values. For complex traits that are influenced by a large number of genes with small effects, genome-wide markers can be employed to perform genomic selection, by which genomic breeding values (GEBV) are estimated based on the summed effect of all markers across the entire genome. Genomic selection has been implemented in several aquaculture species, and was shown to provide more accurate prediction of the traits than prediction based solely on pedigree information, emphasizing its potential to increase the rate of genetic improvement (Zenger et al., 2019). However, there are practical requirements to consider for implementing genomic selection. Moreover, analysis of cost and benefit as well as profitability of implementing genomic selection in the breeding program should be evaluated (Zenger et al., 2019). Here, we visit key concepts in genetics that are important for successfully starting and managing a breeding program. We also summarize recent applications of genetic tools in selective breeding, and encapsulate

considerations for investing in utilization of genetic tools in a breeding program.

Traits and Heritability

Traits are phenotypic characteristics of an organism, which are determined by either gene(s) or environment, or for most traits by a combination of both. In selective breeding, only variations of traits that are determined by gene(s) would respond to a selection pressure as the responsible allele(s) would be selected and inherited to the offspring in the next generation. We can simply categorize types of traits as single gene traits or multifactorial traits. Single gene traits are traits that are determined by a single gene, whereas a multifactorial traits (also known as a polygenic trait or a complex trait) are traits that are determined by many genes and typically also influenced by environment. Multifactorial traits are therefore often found to have great variations in populations. The extent of the phenotypic variations of a multifactorial trait caused by genes as opposed to environment can be reflected as heritability – a statistic that estimates the proportion of phenotypic variations in a population that are explained by genetic variations between individuals in that population (Wray, 2008). Heritability can be estimated from the observed and expected resemblance between relatives such as the correlation of offspring and parental phenotypes, the correlation of full or half siblings phenotypes, or the correlation between phenotypic and genetic similarity within families. In selective breeding, traits with high heritability are likely to respond to selection pressures faster than those with low heritability (Gjedrem, 1983). It should be noted that heritability could vary from population to population, species to species, or even at different developmental stages of the same individuals. Nevertheless, many traits were found to have fairly consistent heritability across populations and species (Visscher et al., 2008). Many economically important traits such as growth rate, food conversion efficiency, age at maturation were found to have moderate to high heritability, indicating possibilities to achieve genetic improvement on those traits through selective breeding (Gjedrem, 1983; Hamzah et al., 2017; Khang et al., 2018).

Genetic Variations, Selection, and Inbreeding

Any breeding program has a goal to increase the proportion of the desired trait(s) in a population. However, as traits are determined by genes, it is more precise to state that the ultimate goal of a breeding program is to increase the frequencies of alleles that affecting the desired traits to "fixation" (where all member of population carry these alleles) while maintaining diversity of genetic background in the population. This ultimate goal consists of two important components:

The first component is to increase frequencies of alleles that have effects on the desired traits over successive generations. Genetic improvement can occur only if the population contains genetic variations that affect the desired traits. If there are no such genetic variations in the population, there will be no selection response. Therefore, it is very important that the established base population contain high genetic diversity in order to create possibilities of there being genetic variations that contribute its effect to the desired traits.

The second component is to maintain diversity of genetic background even though those genetic variations do not have any effect on the desired traits. The reasons for this are that the population would have adaptability to respond to future changes of breeding objectives, and that inbreeding depression can be prevented. A captive population tends to lose genetic diversity over generations due to its small population size, where both random genetic drift and selection can cause dramatic changes in allele frequencies. Moreover, as the circle of selection is kept going, by which a proportion of individuals is selected as parents to produce offspring and a proportion of those offspring is selected to produce offspring in the following generation and so on, eventually individuals in the population would all be related and inbreeding is inevitable. Therefore, to maintain genetic variations in the breeding program, one needs to control and manage the effective population size (the

number of parents that contribute their alleles to the next generation), selection intensity, and inbreeding rate.

Large effective population size is preferable not only because the effect of random genetic drift would be smaller, but also because the inbreeding rate would be lower. Most aquaculture species produce many offspring per female. The effective population size is usually limited by the capacity of the facility to hold the animals. Therefore, restrictions in the number of offspring per parent should be put in place.

Selective breeding aims to make an increase in genetic gain in each generation, the degree of which depends on selection accuracy and selection intensity. Selection accuracy is the accuracy of the genetically best animals being selected for breeding. Selection intensity is the proportion of population that is used for breeding; the smaller number of individuals is selected, the higher selection intensity is. Given that selection accuracy is high, strong selection intensity would result in a large increase in genetic gain in a successive generation. However, strong selection intensity also has adverse consequences. As selection is acting on the affecting alleles, the genetic variations that are located nearby or linked to the affecting alleles would increase in their frequencies along with the affecting alleles. This phenomenon is called "genetic hitchhiking" (Smith & Haigh, 1974). The significance of this phenomenon in breeding programs is that genetic gain is likely to rely on additive effects from combination of alleles at many loci and therefore, population will need time (generations of sexual reproduction) for recombination between additive alleles to happen. If selection is too strong with few individuals being selected, other additive alleles may be lost before recombination between them happens. Moreover, strong selection intensity can cause a sudden reduction in genetic diversity due to the small effective population size, which would consequently lead to a higher inbreeding rate. In practice, decisions on what level of selection intensity should be set depends on the consideration of genetic gain versus rate of inbreeding.

In a captive population, inbreeding is unavoidable. However, rate of inbreeding can be controlled. In addition to having a large effective population size, mating between individuals with less genetic similarity can slow the rate of inbreeding. For conventional breeding, the information on genetic similarity is obtained from pedigree records. For molecular breeding, genetic markers are employed to estimate genetic relationship between individuals. Mating between siblings or closely related individuals reduces genetic diversity in the population because it results in an increase in homozygosity. The undesirable consequence of inbreeding is inbreeding depression. Inbreeding depression occurs as a result of recessive genetic defects. The signs of inbreeding depression include deformities, dwarfism, reduction in reproduction and health, etc.

New genetic variations are created through mutations in every cycle of cell divisions. Therefore, each newborn individual would carry many new mutations in its genome. Mutations could have advantageous effects that increase fitness of the individual, but some could have deleterious effects that reduce fitness of the individual. In addition, some mutations could be neutral, having no effect on individual fitness. Strong deleterious mutations are rare in the population. This is because strong deleterious would cause serious health problems and the individual that carries these mutations would die even before it has a chance to pass on those mutations to the next generation. These strong deleterious mutations would therefore be quickly eliminated from the population.

However, many mutations are slightly deleterious and are recessive, which do not reduce fitness of the individuals unless they present as homozygous. Mating between siblings or relatives would increase chances that the mating pair may carry the same deleterious recessive alleles and would produce offspring with homozygous recessive genetic defects. Information on genetic relationships between breeding individuals can be used to as a guideline to avoid mating between very closely related individuals. By this way, rate of

inbreeding will be slower, diminishing chances of inbreeding depression.

Genetic Tools for Breeding Programs

In selective breeding, genetic markers are employed for two main purposes; 1) to evaluate levels of genetic diversity and genetic relationship between breeding individuals (and/or parentage analysis) for better controlling the rate of inbreeding, and 2) to increase selection accuracy where the genetically best individuals are correctly chosen for breeding. Since the invention of polymerase chain reaction "PCR" (Mullis, 1990), the investigation of DNA variations has become much more feasible. Employing PCR, many techniques have been developed to detect genetic variations in the genome. However, over the last 25 years, microsatellite markers have proven the most powerful tool for evaluating genetic diversity and genetic relationship between individuals. Microsatellites are DNA regions that contain short tandem repeated nucleotide sequences, typically with 2 to 10 nucleotides per repeat unit. The addition or deletion of the repeat units in microsatellites occurs at high rates, making microsatellites highly polymorphic with various number of repeat units per locus. Microsatellites are genotyped by PCR amplification using primers located in the flanking regions that are conserved. The polymorphic alleles are then discriminated by their length through electrophoresis. By this, both alleles of a microsatellite locus of an individual can be detected, and the homozygous and heterozygous state can be distinguished from each other. These features facilitate the usefulness of microsatellites to evaluate genetic diversity, genetic relationship, and parentage analysis. Microsatellites were quickly adopted and proposed as a genetic tool for pedigree auditing in animal breeding (Crawford *et al.* 1991), and has been widely used in many plant and animal breeding programs (de Leon et al., 1998; Miah et al., 2013; Teneva et al., 2018).

Recent advances in next generation sequencing technologies (NGS) now offer an alternative approach to evaluate genetic variations in the genome. NGS is DNA sequencing technologies that perform sequencing of millions of DNA fragments in parallel, providing massive sequencing data of the genome from a single run. NGS technologies offer a diverse range of applications, which are not limited to model organisms. These include reference genome construction, gene expression profiles, and single nucleotide polymorphism (SNP) detection (Ekblom & Galindo, 2011; Egan et al., 2012; Lee et al., 2013). Due to the improvement of biochemistry, high throughput, cost, accessibility, and computing power, NGS has become an effective tool to study genomes in a greater depth, allowing us to gain better understanding of how genetic variations underlie phenotypes (Goodwin et al., 2016). In selective breeding, NGS technologies opens opportunities for breeders to discover and implement genome-wide SNPs for estimating genomic relationship, carrying out genome-wide association studies, and performing genomic selection. The fact that SNPs are abundant in the genome and that they can be genotyped with high accuracy and high throughput have made an increase in use of SNP markers in recent years (Vieira et al., 2016; Flanagan & Jones, 2019).

Genetic markers have been used for mapping loci involved in quantitative traits (QTL). Once identified, the genetic markers that are tightly linked with the desired traits can be incorporated with phenotype data to more accurately infer breeding values of the individuals, assisting in selection strategies for specific breeding objectives – the technique is referred to as marker-assisted selection (Dekkers & Hospital, 2002). The merit behind marker-assisted selection is that selection can be at the genotype level, which is extremely useful in case that phenotypic recording is difficult, expensive, or can only be obtained in later stages. If QTLs are correctly identified, marker-assisted selection would help increase the selection accuracy of the program and potentially yield higher and faster genetic gain. Now genome-wide markers can be discovered through NGS, and massive number of SNPs can be genotyped either through array chip technologies and/or NGS via targeted sequencing or amplicon sequencing. Mapping loci involved in quantitative traits can be performed at a genome-wide scale – genome-

wide association studies (GWAS) – offering more complete investigation of the genetic architectures which underlie quantitative traits (Dekkers, 2012). Although QTLs with large effects have been successfully identified for some economically important traits, accumulated research on genome-wide association studies have shown a norm of there being large number of QTLs with small effects on most complex traits (Hayes & Goddard, 2010). This has led to a rapid gain of interest in an alternative approach of molecular breeding called genomic selection. Genomic selection employs high density SNPs throughout genome to capture all QTLs, and the breeding values are predicted using the sum of the effect of these SNPs across the entire genome (Meuwissen et al., 2001; Hayes & Goddard, 2010). The potential of genomic selection to achieve high selection accuracy has created an interest in implementing genomic selection in several high-value aquaculture species, some of which have shown promising prediction accuracies (Zenger et al., 2019). Furthermore, genome-wide SNPs can also be used to estimate genomic relationships for better management of inbreeding (Hayes & Goddard, 2010; Vandeputte & Haffray, 2014).

Considerations for Investing Genetic Tools in Breeding Programs

It is clear that genetic markers can provide substantial benefits to breeding programs, especially for a long-term development of breeding lines. The question is to what extent we should invest or implement genetic tools in our breeding programs? Considerations include cost, practical requirements, and obstructive challenges. We can simply consider the implementation of genetic markers in breeding programs at two different scales/purposes. Firstly, the genetic markers are to be used for managing inbreeding rate. In this case, a small number of microsatellite markers or a small number of SNPs can be employed. Although the exact number of markers to be used would depend on the polymorphic levels of the markers and the diversity of the populations, the recommended number of microsatellites at 30 loci (FAO, 2011) or 100 - 500 SNPs (Flanagan & Jones 2019) has been proposed. Microsatellite markers have been proven to be powerful, but there are several drawbacks such as the need of marker development and validation for non-model species and the high error calling rates (Vieira et al., 2016; Flanagan & Jones, 2019). On the other hand, SNPs can now be discovered in any non-model species using NGS, but a higher number of loci would be required to retain the same statistical power as microsatellites. Genotyping cost and turnaround time are factors to be considered. The genotypic data should be obtained before breeding decisions have to be made. The benefits of implementing genetic markers for controlling inbreeding are widely recognized. In practice, challenges such as asynchronous spawning behavior and unavailability of artificial insemination can diminish the usefulness of genotypic data, as specific mating pairs could not be arranged. Nevertheless, genetic markers are still useful for auditing changes in the level of genetic diversity and inbreeding rate in the populations.

Secondly, genetic markers are to be used for improving selection accuracy. In theory, genomic selection requires genome-wide markers that are evenly distributed throughout genome to maximize the possibility of markers being linked with all QTLs. For this, commercial SNP panels have been developed, but only for a handful of high-value cultured species. However, various methods of NGS have recently been used to discover and genotype SNPs in order to demonstrate the potential of implementing genomic selection in non-model species (Palaiokostas et al., 2016; Nguyen et al., 2018). To perform genomic selection, a reference population, by which individuals are both genotyped and phenotyped, is required. The information from the reference population is then used to derive a predicted model to estimate breeding values of the breeding candidates in the selection population, by which individuals are genotyped but not necessary phenotyped (Hayes & Goddard, 2010). Simulation studies showed that prediction accuracy of genomic selection could achieve as high as 0.85 accuracy (Meuwissen et al., 2001). However, empirical studies on genomic selection in aquaculture species showed a wide range of the prediction

accuracy from 0.1 – 0.8, with an average of 0.6 (Zenger et al., 2019). The prediction accuracy of genomic selection depends on marker density, the size of the reference population, and genetic relationship between the reference population and the selection population (Norman et al., 2018). For example, prediction accuracy increases with an increase in size of the reference population, and higher density markers would be required if genetic relationship between the reference population and the selection population is low. The cost of genotyping a high density of markers in a large number of samples would be too high for routinely implementing genomic selection. This leads to ongoing research of how to use low-density markers that are well represented or can be imputed for genomic selection (Goddard & Hayes, 2009; Zenger et al., 2019). Another practical requirement of implementing genomic selection is the establishment of precise phenotypic records in the reference population. The effect of each genetic marker on the quantitative traits is all calculated into the predicted model. Therefore, the more precise the phenotypic data is, the more accurate the prediction would be.

NGS technologies are a large capital investment, and data analyses require computational resources and bioinformatic skills. Many companies around the world are now offering NGS services that also include basic data analyses. In general, the cost of the service per sample reduces dramatically with an increase of number of samples. This is because the multiplexing and high throughput technologies allow a huge reduction in cost on reagents, time, and manpower. It would be very interesting if collaborations among breeding programs could be established. Samples from each program could be pooled together for genotyping service in order to bring the cost per sample down. Moreover, with a well-designed study plan, data from each breeding program could be analysed together to strengthen the statistical power by expanding the samples size. Collaboration would not only help with cost sharing but would also potentially increase prediction accuracy of genomic selection.

About the Author

James Collins is founder and president of Infini-SEA LLC, which conducts research and selective breeding of Pacific White Shrimp *L. vannamei* at its facilities in Vero Beach, FL. He started his career with pioneering domesticated shrimp developer Shrimp Improvement Systems first in the Americas and later as a consultant in Asia and South East Asia providing technical services and project planning for various aquaculture projects in shrimp and fish production and facilities planning across the globe.
James graduated from the Florida Institute of Technology in 1996 with Biological Science degrees in Marine Biology and Aquaculture. Then working for Harbor Branch Oceanographic Institute, in marine science and aquaculture Divisions with marine fin fish culture, inland low salinity "zero exchange" shrimp production projects, and practical lab training in the Aquaculture Center for Training Education and Development (ACTED).

In 2000 he began working with Shrimp Improvement Systems (SIS) on shrimp selective breeding and genetic improvement and providing technical assistance to clients throughout the Americas and later in the Asia-Pacific region.

In late 2003 James pursued a master's of Science degree in Marine Affairs and Policy at the University of Miami's Rosensteil School for Marine and Atmospheric Science (RSMAS) building a commercial pilot scale marine fin fish hatchery and conducting environmental impact studies for open ocean, high value, marine fin fish cage culture projects in the Caribbean.

James was based in Thailand from 2005-2017 and consulting throughout Southeast Asia on myriad projects, including expansion and development of selective breeding facilities, programs, and protocols for *P. vannamei* with Shrimp Improvement Systems and later with selective breeding production systems and nutrition for SyAqua and the Gold Coin Group.

Infini-SEA has also conducted a selective breeding project for Barramunidi "Genetic Improvement and Selective Breeding of Asian Seabass *Lates Calcarifer*" for the US Soy Export Council. Today Infini-SEA works with groups interested in assistance with their selective breeding strategies in a variety of species and is employing the latest generation of genomic selection tools to make these technologies more accessible to programs of all sizes.

Infini-SEA LLC Projects

2017-present
Aqquua LLC
Advanced Grouper Farm
Chantaburi, Thailand
Design and construction of 2000m3/hr intake treatment system, advanced pond design and waste treatment systems. Pond culture protocol development and training, and environmental compliance guideline development and implementation.

2017
US Soy Export Council
Genetic Improvement and Selective Breeding of Asian Seabass *Lates Calcarifer*
Thailand, Indonesia, Philippines, Vietnam
Genetic analysis of Asian seabass from Commercial hatcheries in the Asia-Pac regions and development and advisement on strategies for development of selective breeding programs for commercial seabass producers.

2010-2016
SyAqua Siam LLC
Production Manager Hatchery and broodstock production operations

Technical Service Manager
Feeds Innovation Group
Conducted hatchery operations and broodstock production and selection for SyAqua Siam and various technical service roles and feeds innovation team positions for the Gold Coin Group. Client Farm and Hatchery troubleshooting and problem solving through Asia-Pac region, India, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia, Vietnam, and China.

2007-2009
Aquabounty Technologies Inc
Therapeutics Research and Development
Conducted trials of novel immunostimulants for use in shrimp feeds, and review of regulatory requirements, and university and industry collaborations. Projects focused on Thailand, and China.

2005-2007
Shrimp Improvement Systems LLC.
Technical services consultation for implementation of best management practices for farms and hatcheries in, China, Indonesia, and Malaysia, establishment and training of staff for the breeding and selectin program field trials in China and Indonesia. Refinement of operating systems for broodstock growout in Singapore, and Florida.

Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and "advanced soy proteins" such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

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