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Genetic variability of selected populations of yellow perch over six generations of commercial-scale marker-aided cohort selection for growth

Han-Ping Wang, Muhammad Luqman Aslam, Hong Yao, Paul O'Bryant, and Dean Rapp
Aquaculture Genetics and Breeding Laboratory, Ohio State University South Centers, Piketon, Ohio, USA

ABSTRACT: In total 3,318 broodfish and 600 random fish from two overlapping lines (L-1 and L-2) consisting six generations of selected populations and a random population were genotyped using eight microsatellite markers to investigate genetic structure and diversity among and within these generations. Global heterozygosity values of L-1 and L-2 generations ranged from 0.76-0.80 and 0.85-0.88, respectively. Cohort-specific heterozygosity ranged from 0.53-0.95, suggesting it was well maintained during the course of selection. Global inbreeding coefficient values (F_{is}) for L-1 (0.03) and L-2 (0.04) were found close to zero, while generation specific F_{is} values were less than zero ranging from -0.18 to -0.08, indicating heterozygote abundance and clarifying that inbreeding was avoided over six generations of selection. Effective population size (N_e) ranged from 1,013.7 to 394.2 in all broodfish generations. The genetic distance among the 7 populations ranged from 0.002 to 0.228.
Keywords: Yellow perch; Cohort selection; Genetic variability

Introduction

Success and sustainability of breeding programs depends on how breeders maximize genetic gain while maintaining genetic diversity using effective and novel selection strategies. In reality, selected strains through breeding programs tend to lose genetic diversity and variation compared to wild populations due to excessive genetic drift and unavoidable inbreeding during selection (Cruz et al., 2004). The decrease in genetic diversity could result in an overall decline in the fitness of the genetically improved fish, which would compromise genetic gain of selected fish (Ferguson et al., 1995). Therefore, monitoring genetic variation over generations and the effect of selection on the genetic structure of selected populations with molecular markers is crucial to control inbreeding and maintain genetic gain for a selective breeding program. The information can help breeders to make right decisions over time in terms of whether needing to refine selection strategy and when and how to introduce new or wild populations.

Yellow perch *Perca flavescens* is a particularly important aquacultural and ecological species in the Great Lake Region (GLR) and the Midwest USA. The demand for yellow perch has remained very high in the GLR since they are the traditional fish species used in local restaurants, social organizations, and the Friday night fish fry dinners that are a staple in many Great Lakes states. The health benefits of yellow perch and its history of consumer fidelity in the market place present significant marketing opportunities for fish farmers in the Great Lake region. One reason in particular hindering expansion has been relatively slow growth of currently cultured populations of this species.

We have been conducting commercial-scale (more 100 families/year) selective breeding of yellow perch using marker-aided cohort selection (MACS) (Wang et al., 2011) So far, three rounds of selection have been conducted with six generations being gone through. In the first 3-round selection, a marker-aided cohort selection has been developed and tested to establish an effective selection method, which was designed to be easily adapted by industry, maximize genetic gain and minimize loss of genetic variation for the breeding program. On-farm and on-station tests on three sites at different latitudes showed that our three rounds of selection using the marker-aided cohort selection and breeding strategy enabled us to get significantly higher production, survival and growth rate over non-selected local commercial strains of yellow perch. On an average, our improved fish exhibited 27.6 - 42.1% higher production, and 25.5 - 32.0% higher growth rates in the condition of having 12.3 - 27.8% higher survival than local strains across the three sites (Wang at al. unpublished data). The objective of current study was to examine if the MACS breeding strategy could avoid a significant depletion in genetic variability of selected populations.

Methods and Materials

Selection strategy and establishment of base and selected generations. In the first three rounds of selection, we have employed the following specific strategies for testing : 1) using founder stocks with high variability; 2) maintaining large effective population sizes (>100 families) in each generation; 3) keeping about 1:1 sex ratio; 3) diversifying mating strategy, including factorial mating, multi-pair nest mating and single-pair mating; 4) communal rearing to reduce environmental effects and conserve hatchery/pond resources; 5) molecular fingerprinting to reconstruct the pedigrees of communally-reared individuals and identify genetic relatedness for mating unrelated individuals; 6) using cohort strategy; and 7) overlapping generations or lines.

Founder animals or base populations (YC-2004 and YC-2005) for production of the base generations (YC-2006 and YC-2007) originated from eight different populations. During the winter 2003 and spring 2004, four broodstock strains were obtained from geographically disparate wild populations: North Carolina, Pennsylvania, New York and Maine; two additional broodstock groups were derived from captive populations held in Michigan and Ohio State University South Centers. In spring 2005, two strains were obtained from Wisconsin and Nebraska. In both starting years (YC-2004 and YC-2005) five cohorts were established and cross-breeding was performed among the individuals from different cohorts. Using performance records and the genotypic information, approximately 1500 fast-growing and least-related broodfish candidates constituting more than 100 families were selected from progeny of previously established 5 cohorts as base generations (YC-2006 and YC-2007). For each subsequent generation, approximately 800 - 1000 breeding candidates (top 5-10% and ~200 fish from each cohort) were selected from progeny of previous cohorts, which were produced by cross-breeding of, or breeder-exchange between, five cohorts from previous generation. Among the 800 - 1000 fish, at least 150 pairs of the largest and least-related fish were mated to reconstruct new 4-5 cohorts based on their cohort origin and pedigree to make sure that there were at least 100 families in each generation. That means the selection lines were created by pairmating at least 30 pairs within each cohort to find the next generation of improved cohort lines. These breeding candidates in each generation were genotyped using 8 microsatellites. If the constraint on the rate of inbreeding could not be achieved, another batch of fish was genotyped and included in the total number of candidates. To increase genetic diversity, a part of founder populations was used in 2008 and 2009, and new wild NC population was introduced in 2010 and 2011.

Sampling and microsatellite genotyping. Approximately 100 fast-growing broodfish of each sex from the six generations (parallel generations, YC-2006, YC-2008, YC-2010 vs. YC-2007, YC-2009, YC-2011) were taken at year-2 harvest during 2006-2013. Six hundred samples of random fish from the YC-2006 were also collected to compare genetic diversity between random fish and selected broodfish. A non-lethal biopsy (fin clip) was taken from each selected or sampled fish and preserved immediately in 95% ethanol for DNA analyses.

In total 3,318 broodfish and 600 random fish were genotyped in this study. Genomic DNA was extracted from fin tissues of the yellow perch using the method described by Li et al.(2007), and parents and progeny were genotyped with 4-8 highly polymorphic microsatellite loci (YP30, YP41, YP49, YP60, YP73, YP78, YP96 and YP109).

Data analysis. Basic statistics were calculated for each generation/population using the genetic analysis package PowerMarker ver. 3.25; Diversity measurements at each microsatellite locus, including gene diversity/expected heterozygosity (H_e), observed heterozygosity (H_o) and polymorphism information content (PIC) were estimated using mentioned program. Population specific structure was evaluated by the hierarchical F statistics–F_{is}, F_{st}. The equations for the estimation of above parameters (H_e, H_o, PIC, F_{is} and F_{st}) are given in the PowerMarker software manual. PHYLIP software, version 3.69 was used to calculate pairwise Nei's genetic distance among all populations/generations. Pairwise genetic distance analyses were based on marker data that the individuals had in common, because PHYLIP is unable to deal with missing data. Mega 5.0 was used for hierarchical clustering using Neighbour-joining (NJ) algorithm on the genetic distance matrix for all the populations. Effective population size (Ne) was calculated for each generation of L-1 and L-2 populations and compared with the heterozygosity (H_o) of subsequent generation.

Results and Discussion

Genetic variation across generations of broodfish. Comparing two parallel Yellow Perch populations L-1 vs. L-2, overall global values for heterozygosity (H_o) were found higher, 0.85 in L-2 while L-1 showed slightly lower heterozygosity of 0.79. Digging into these parallel populations, average heterozygosity values of L-1 and L-2 generations (each with three generations) ranged from 0.76-0.80 and 0.85-0.88, respectively. The generation YC-2011 from L-2 showed the highest while YC-2010 from L-1 showed the lowest heterozygosity. The levels of observed microsatellite-based heterozygosity (H_o = 0.75 to 0.88) in broodstock populations of current study are somewhat greater than observed for several other commercially produced aquaculture fish species.

Genetic variation within each generation of broodfish. Genotyping data on 30 cohorts of 7 populations (6 broodfish and 1 random, each population had 4 cohorts, except YC-2008 and YC-2010 which had 5 cohorts.) were analyzed separately to estimate cohort-specific genetic variation. Results on cohort-specific heterozygosity showed a range of 0.53-0.95 with the highest and the lowest value accompanied by YC-2010 under the cohort 1 and cohort 2, respectively. Cohorts of L-2 showed comparatively narrower range of heterozygosity, 0.82-0.92 with the highest and the lowest value from cohort 2 of YC-2007 and the YC-2009 respectively.

Genetic variation between random fish and broodfish. Results on comparative heterozygosity analysis on random population from year class 2006 vs. broodfish populations of L-1 and L-2 showed the lowest observed heterozygosity of 0.75 in random population. Cohort-specific heterozygosity in random population ranged from

0.72-0.79, which falls within the heterozygosity range of L-1 broodfish generations.

F-statistics, F_{is} and F_{st}. Comparative population structure analyses for L-1 vs. L-2 and the underlying generations are presented in table 1 and figure 2. Global value for genetic differentiation fixation index (F_{st}) was slightly higher in L-1 compared to L-2, (0.05 vs. 0.04, respectively), while global inbreeding coefficient values (F_{is}) were found close to zero, 0.03 and -0.04, respectively. Digging into these two L-1 and L-2 parallel populations, L-1 generations showed higher values for F_{st} ranging from 0.17-0.22 while L-2 generations showed lower values ranging from 0.02-0.09. YC-2011 presented the lowest value of F_{st}, 0.02 while YC-2010 showed the highest value 0.22. Estimated values of F_{is} for all generations was less than zero ranging from -0.18 to -0.08 with the highest value in YC-2010 and the lowest value in YC-2009.

Effective population size. The N_e ranged from 1,013.7 to 394.2 in all broodfish generations with the highest N_e in YC-2008 (bred with 5 cohorts) and the lowest N_e in YC-2011. Our results slightly disagree with the expectation, probably because the number of males and females counted in N_e calculation are the expected numbers, which were selected for breeding in different YC. There is an uncertainty if all counted individuals (Males and females) really contributed to the next progeny. There is a chance that in a particular YC some of the parents did not spawn at all or they did spawn, but produced a few number of offspring and they did not survive etc.

Genetic diversity. Microsatellite marker genotyping data was also used to calculate Nei's pair wise genetic distances among different Yellow Perch populations/generations. The genetic distance among these populations ranged from 0.002 to 0.228. The un-rooted phylogenetic neighbor-joining tree based on Nei's genetic distance for the 7 Yellow Perch populations presents their genetic relationships. Among these populations, random and the YC-2006 broodfish populations showed the lowest, while random and the YC-2011 broodfish populations showed the highest genetic distance. Two separate clusters of Yellow Perch populations were observed in dandrogram. Among the L-1 generations, YC-2008 and YC-2010 clustered together while YC-2006 clustered separately with YC-2007 and YC-2009 which are L-2 generations.

Conclusion

The result of well-maintained heterozygosity through eight years of breeding infers that MACS strategy in our yellow perch breeding program has been working well for controlling the levels of inbreeding. MACS strategy not only worked well for elevating genetic gain, but also maintaining genetic diversity for yellow perch breeding program. Our findings suggest the levels of variation are appropriate to proceed with a long-term selective breeding program in yellow perch. MACS can be easily adapted by industry and should be an effective breeding method for other aquaculture species.

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