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Preservation of Genetic Variation within Aquaculture Stocks of White Sturgeon

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OVERVIEW OF PROGRESS TOWARDS ACHIEVING OBJECTIVES

The research project "Preservation of Genetic Variation within Aquaculture Stocks of White Sturgeon" has been essentially sub-divided into four main areas of emphasis. These were:

1. Identification and inheritance testing of microsatellite markers

2. Sire and dam effects on growth rate; heritability of growth rate and caviar characteristics

3. Utility of microsatellite markers in parentage analysis

4. Estimation of relatedness within year classes of aquaculture stocks

The overall intent of this project was to develop a breeding plan for white sturgeon, using Stolt Sea Farm California LLC as a model system for broodstock management and selection using molecular markers. While the title of this project implies the function of the breeding plan to be of maintaining existing genetic variation as inferred from nuclear DNA markers, it is in the best interest of those parties involved in commercial production of this species to evaluate the feasibility of directed selection for economic traits such as growth rate. Thus, this project involves the estimation of quantitative genetic variation in addition to developing specific applications of molecular markers to broodstock management. Recommendations for their integration into a breeding plan and an overview of our research will be summarized in a manuscript being prepared (Rodzen et al. in prep[4]).

IDENTIFICATION AND INHERITANCE OF MICROSATELLITE MARKERS

In the fall of 1998, 110 possible microsatellite markers were screened in white sturgeon as part of collaborative effort between the Genomic Variation Laboratory at UC Davis and Southern Illinois University.   These microsatellite markers had been developed from an dimer- and tetramer-enriched genomic library made from Pallid / Shovelnose sturgeon DNA. Roughly 30% of these microsatellites cross-primed in the White sturgeon. Since that time, each of the loci that successfully cross-primed and appeared to be disomic or at least tetrasomic in nature have been evaluated in more detail in the white sturgeon. This has involved the excision and subsequent sequencing of informative bands / alleles that were amplified with the Pallid/Shovelnose (Spl) primers. The sequencing effort has allowed us to make new primers from White sturgeon DNA sequence information, greatly refining the stringency of the PCR

reaction for each locus. In addition to using the Spl primers, we have also been using this procedure on Lake sturgeon and, most recently, Atlantic sturgeon primers. To date we have nine loci that have been refined for use in the White sturgeon.

Another major focus in the development of microsatellite markers in this species is determining the mode of inheritance for each locus. Since white sturgeon are a suspected octoploid derivative, this has proven to be the most challenging aspect of developing new microsatellites. For instance, one of the Lake sturgeon primers amplifies up to seven alleles per individual even after new white sturgeon primers were made. To answer the inheritance question, we extracted DNA from 50 fish from each family for 9 different families (total N = 450) resulting from a 3 by 3 cross and amplified each fish for all nine microsatellite loci.

We describe the inheritance of nine microsatellite loci Rodzen et al. (in prep.[1]), providing the first insight into the nature of the white sturgeon genome from the perspective of Mendelian segregation. Although the white sturgeon genome is suspected to be an ancestral octoploid, it has been suggested by other investigators that the diploidization process to be complete in this species, based on the observation of bivalent homolog paring during meiosis. Rodzen et al. (in prep.[1]) reported the mode of inheritance of microsatellite systems to be locus-specific, with levels of ploidy varying from disomy to octosomy. While a couple loci are possibly tetrasomic, most loci, better termed "systems", show higher levels of duplication, reflective of the suspected octoploidy condition of the white sturgeon genome.

In Rodzen et al. (in prep.[1]), we proposed these systems not be scored as actual disomic, tetrasomic, or octosomic loci until further resolved, but rather each allele be scored as a dominant nuclear marker, such as with RAPD or AFLP bands. Instead, we cite mathematical methods papers which provide a detailed discussion of applying data from dominant marker frequencies to questions of population subdivision, genetic distance, and relatedness.

SIRE AND DAM EFFECTS ON GROWTH RATE;
HERITABILITIES OF GROWTH RATE AND CAVIAR CHARACTERISTICS

The basis for testing sire and dam effects is to estimate the heritability of growth rate, as measured by body length and weight. Heritability is a parameter that is used in nearly all breeding calculations, such as predicting rate of genetic gain and choosing the optimal method for estimating breeding values. The estimation of heritability is accomplished by crossing multiple sires and dams to create full-sib (FS) and half-sib (PHS and MHS) families, allowing for the estimation of genetic variance components. Variance components were estimated using the linear mixed model:

$$y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + e_{l(ijk)}$$

where A_i = sire, B_j = dam, and C_k = site effects.

Variance components were estimated using the MTDFREML computer package, which iterates for variance components using derivative-free restricted maximum likelihood. This approach is much less sensitive to unequal family sizes than the ANOVA method of variance component estimation.

Several sires and dams were crossed to create several full-sib and half-sib families, which were replicated across two of Stolt Sea Farm's facilties. Each family was reared in its own tank, with sires and dams thus being replicated across tanks. In attempt to consolidate research objectives, we also made a factorial cross of three sires and three dams and pooled them into the same tank. These fish were grown out at both farm sites and 300+ fish were measured for body weight, length, and K-factor about 6 months post-hatch, at which time each individual was fin-clipped to permit DNA extraction and subsequent reassignment to family in the lab using microsatellite markers.

We also made 16 families (8 sires by 2 dams) which were reared in separate tanks with each family being replicated twice. In May 1999, we made another 8 families. Unfortunately, these families were lost after a disease outbreak in the hatchery. In replacement, we made an additional 18 families in early March of this year. These fish were measured for length, weight, and K-factor in late summer.

Heritability estimates for body length and weight at six months post hatch (0.17 and 0.14 respectively) were at the low end of the published range of heritabilities for these traits in a variety of species. Heritability estimates for these traits at the early age of six months were made with the assumption that there are positive genetic and phenotypic correlations between body length and weight at an early age with these two traits at harvest time. This assumption is based on reviews of several studies. This observation, coupled with heritable variability in early body size in this species, suggests that grading and culling at early ages may have a beneficial effect on increasing the body size of individuals at harvest.

We also estimated the heritabilities of several caviar characteristics: caviar yield, caviar weight, and female adult body weight. This was done by sampling approximately 200 fish processed for caviar from two year classes in summer 2000. Trait data and a tissue sample was collected from each animal. Pedigree information on the animals was created by genotyping these animals using our nine microsatellite loci and estimating relatedness from the genetic profile data to identify sib groups.

In addition, we examined the association of other caviar characteristics (grade, color, egg size, and egg firmness) with other non-genetic factors, including spawn cycle, growout site, and year class. Heritability estimates were not made for these traits as they were not normally distributed and scored as di- or trichotomous traits. Fixed effects of growout site, year class, and spawn cycle on each of these traits was

tested with with only ovary fat demonstrating a lack of independence with all three of these effects. However, the other traits generally did not differ significantly with growout site, year class, or spawn cycle. Body weight at harvest was highly heritable ($h^2 = 0.42$), while both caviar weight and caviar yield had low heritabilities ($h^2 = 0.05$ and 0.08, respectively). Regarding estimated genetic correlations, caviar weight and fish weight were positively correlated, while percent caviar yield was negatively correlated with these two traits. This suggests selection for heavier fish should also produce more caviar, although the percent yield, a measure of efficiency of caviar production, should decrease. Results of this study are presented in Rodzen et al. (in prep[2]).

UTILITY OF MICROSATELLITE MARKERS IN PARENTAGE ANALYSIS / RELATEDNESS ESTIMATION

A major component of the breeding plan includes developing specific applications for the microsatellite loci we developed. One application is their utility in assigning individuals from mixed family lots back to their parents when the genotypes of the parents are known. This technique will bring several advantages to commercial white sturgeon aquaculture because it permits families to be mixed together into a common environment should a combination of between- and within-family selection scheme become the optimal method for achieving desired selection gains. This has the effect of standardizing the environment in which the animals are being evaluated. Once animals are selected, they can be physically tagged and reassigned to families using their genetic profiles so that the breeder can avoid accidentally crossing full- or half-sibs.

As mentioned in the section concerning the growth rate study, fish from the 3x3 pooled cross were sampled and reassigned to their correct families so the data on these animals can be included in the parameter estimation procedure. Genotype data from the animals used in the inheritance study was used to determine the most optimal combination of microsatellite loci for conducting parentage analysis. Some of the factors analyzed were robustness against mutations and scoring errors, percentage of correct parentage assignment, and how effectively each locus excludes fish that are not the true parents. We also tested a relatedness estimator and the use of a clustering algorithm to identify sib groups from a matrix of pairwise relatedness estimates.

We tested the utility of our microsatellite loci in estimating parentage and relatedness in the white sturgeon. The accuracy of assignment to parent-pairs, individual parents, and identification of sib groups using relatedness and clustering algorithms was evaluated. Both the mathematical, gel scoring, and data manangement procedures applied in that study may be unique to white sturgeon. However, the high percentages of correct parental assignment and identification of sib groups from relatedness estimates suggest these approaches to be robust.

Parent-pair test crosses were limited to a three by three factorial cross, reflective of the mating scheme used in white sturgeon aquaculture. Since a 99% correct reassignment rate was achieved with only three loci, the authors suggested a high percentage of parental reassignment in a mating system using several more sires and dams could be obtained if more loci were employed. This assumption is supported by results in similar studies. Our parentage results are comparable to results in current literature. Previous studies demonstrated greater than a 90% accuracy of reassignment to parent-pairs in a variety of mating schemes. Many of these studies tested for correct reassignment to hundreds of possible parental crosses, reflective of the mating schemes used in production of the respective species.

In a manuscript detailing our results (Rodzen et al. in prep[3]), we demonstrated the approach of scoring microsatellite alleles as dominant loci still resulted in correct estimation of the full sib groups from a test data set of eight families. We also presented some caveats, notably that many alleles are needed, in their case approximately 80 alleles were required to correctly regroup full sibs. In addition, they suggest the approach is appropropriate when the levels of genetic relatedness in the group of individuals being analyzed is known. While pedigree information on individual animals is not known, it is important to know if there are full sibs, half sibs, and unrelated individuals in the pool of animals for which relatedness estimates are being made.

RELATEDNESS WITHIN AND BETWEEN YEAR CLASSES

The objective of this phase of the project was to determine how accurately the genetic make-up of a tank or year-class can be estimated when genetic data on the parents is unavailable. This is important information for the producers in that they currently have no way of estimating relationship for a given group of fish since multiple families are pooled at hatching. Since the producers want to avoid unintentional inbreeding, it will be crucial to know if year-classes tend to become more homogenous over time, which would increase the risk of unintentional inbreeding. The estimation of relatedness is accomplished by sampling fish across several year classes to establish a baseline level of genetic diversity and then comparing that with the observed amount of genetic variation within the given year-class or tank. Four year classes were selected for relatedness estimation per request of Stolt Sea Farm. We testing for an overall decline in genetic diversity with increasing age of the year-class.

Four year classes of fish were sampled from the 1992 (n=50), 1993 (n=50), 1995 (n=100), and 1996 (n=100) year classes at a Stolt Sea Farm California facility. In addition, 157 broodstock animals were sampled from Stolt Sea Farm California, representing all currently existing broodstock. Genotyping protocols and parentage and relatedness estimation methodologies were as described in Rodzen et al. (in prep[3]).

Using these methods, the number and relative sizes of full- and half-sib groups were estimated for each year class and for the broodstock. Given the relative family sizes and numbers of families comprising a year class, we calculated the mean expected inbreeding coefficient of the offspring if two fish at random were crossed from within the same year class. Since there was some family structuring within the broodstock, we also calculated the mean inbreeding coefficient of the offspring if two random broodstock fish were crossed.

The estimated number of full-sib groups comprising a year class varied from 10 in the 1992 year class to 20 in the 1996 year class. However, since each year class originates from a different number of spawnings and, thus, full sib families, it is difficult to hypothesize if the year classes tend to lose families over time. The mean expected inbreeding coefficients varied from 0.041 (1996 year class) to 0.057 (1993 year class). We estimate there to be 26 full-sib groups comprising the broodstock. Given the sexes of the animals in each family and the pedigree structure, the mean expected inbreeding coefficient of the offspring is only 0.013. Results are summarized in Rodzen et al. (in prep[4]).

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