Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*)

Jeff A. Rodzen and Bernie May

Abstract: Nine tetramer motif $(GATA)_n$ microsatellite systems were developed for use in the white sturgeon, *Acipenser transmontanus*. We report inheritance patterns for these nine systems, which range from one possible disomic system to tetrasomy and octosomy, with some systems containing null alleles. Because of the complex modes of inheritance underlying these systems and the highly duplicated nature of the genome, we propose each allele be scored as its own dominant marker, similar to AFLPs or RAPDs. The utility of this method is validated by the observation that individual alleles within a microsatellite system generally fit the expectation for independent transmission and fit the expected transmission frequency for single copy nuclear markers.

Key words: white sturgeon, *Acipenser transmontanus*, microsatellite, polyploid, inheritance, genetic markers.

Résumé : Neuf systèmes de marqueurs microsatellites à motif tétranucléotidique $(GATA)_n$ ont été développés chez l'esturgeon, *Acipenser transmontanus*. Les auteurs rapportent ici l'hérédité pour chacun de ces systèmes marqueurs, laquelle varie d'un cas possible de disomie à des cas de tétrasomie et d'octasomie, certains marqueurs ayant des allèles nuls. En vue de l'hérédité complexe de ces marqueurs et du haut degré de duplication du génome, les auteurs proposent que chaque allèle soit traité comme un marqueur dominant, comme cela se fait pour les marqueurs AFLP ou RAPD. L'utilité de cette approche est validée par l'observation que des allèles particuliers pour un système marqueur répondent adéquatement à l'attente de transmission indépendante et produisent les fréquences de transmission attendues pour des marqueurs nucléaires à simple copie.

Mots clés : esturgeon blanc, *Acipenser transmontanus*, microsatellite, polyploïdie, hérédité, marqueurs génétiques.

[Traduit par la Rédaction]

Introduction

Much uncertainty exists regarding the nature of the white sturgeon (*Acipenser transmontanus*) genome. There is evidence that a large amount of genome duplication has occurred and the species is suspected to be octoploid derived (Blacklidge and Bidwell 1993). Comparisons of DNA content and chromosome number across sturgeon species in the order Acipenseriformes worldwide show that genomes vary from 4*n* (e.g., Birstein et al. 1993; Fontana 1976) to possibly 16*n* (Birstein et al. 1993; Blacklidge and Bidwell 1993), with most appearing to be 4*n* (Birstein et al. 1997). Ludwig et al. (2001) elaborates on the three ploidy levels of sturgeon species, suggesting that species with about 120 chromosomes are functional diploids, those with around 250 chromosomes are functional tetraploids, and those with around 500 chromosomes (which includes the white sturgeon) are functional octoploids. Population and evolutionary genetics studies on white sturgeon have relied on mtDNA (Brown et al. 1992, 1993, 1996; Tagliavini et al. 1999) and sequencing of nuclear cytochrome and ribosomal RNA genes (Birstein and DeSalle 1998; Birstein et al. 1999). Van Eenennaam et al. (1998) suggested the process of diploidization to be complete in white sturgeon based on the absence of tetravalent or octovalent structures in meiotic karyotypes of white sturgeon spermatocytes. Since female gametes were not examined, this would not exclude the possibility of sex-specific residual polysomic inheritance. Van Eenennaam et al. (1998) is the only published work to date that examines cytological evidence of genome duplication in the white sturgeon.

More recently, microsatellite loci have been developed and characterized (May et al. 1997; McQuown et al. 2000, 2002). While polyploidy level in the white sturgeon is assumed to be 8*n*, there are no published definitive studies that determine and characterize the underlying mode of inheritance in this species. Ludwig et al. (2001) showed several *Acipenser fulvescens* microsatellite loci to be inherited disomically in the 120 chromosome species and tetrasomically inherited in the 250 chromosome species. However, the authors did note that there were exceptions to this rule, mainly that a smaller number of loci did not follow disomic or tetrasomic inheritance patterns in the 120 or 250 chromosome groups, but instead followed some higher order level of inheritance. Both Pyatskowit et al. (2001) and

Received 4 February 2002. Accepted 19 August 2002. Published on the NRC Research Press Web site at http://genome.nrc.ca on 18 October 2002.

Corresponding Editor: P.B. Moens.

J.A. Rodzen,[1,2] and B. May. University of California, Davis Department of Animal Science, Meyer Hall, One Shields Ave. Davis, CA 95616, U.S.A.

[1]Corresponding author (e-mail: jrodzen@davis.com).
[2]Present address: Wildlife Forensics Laboratory, California Department of Fish and Game, 1701 Nimbus Rd., Suite D, Rancho Cordova, CA 95670, U.S.A.

 DOI: 10.1139/G02-083

McQuown (2002) found a combination of disomically and tetrasomically inherited loci in *A. fulvescens*. In summary, it appears that the mode of inheritance may be locus-specific in a given species and cannot be assumed without inheritance data for that locus.

The salmonids are considered a model system for inheritance patterns and evolutionary "rediploidization" following ancestral genome duplication events (Johnson et al. 1987). Inheritance studies suggest homologous chromosomes pair as bivalents with loci on the homologs exhibiting Mendelian disomic segregation (Ardren et al. 1999; Spruell et al. 1999; Johnson et al. 1987). Meiotic pairing of homologs and homeologs has been shown to be sex-specific, with females exhibiting purely disomic inheritance, while males may exhibit residual tetrasomy (Allendorf and Danzmann 1997 and references therein). Based on inheritance studies of lake sturgeon and salmonids, we cannot make any predictions regarding the underlying mode of inheritance for a given locus in the white sturgeon genome.

In this study, we describe the inheritance patterns for nine newly developed white sturgeon microsatellite systems and make recommendations for their use in population and evolutionary genetics studies. To use nuclear markers for population and evolutionary genetic studies, the markers' underlying mode of inheritance must be known. Since most of the mathematical formulae used to quantify population differentiation assume disomic inheritance and Hardy–Weinberg genotypic frequencies, using marker systems that do not conform to these assumptions would provide spurious and misleading results in hypothesis tests of population differentiation.

Methods and materials

Primer design and PCR profiles

DNA was extracted from either fin tissue or whole sac fry using a digestion in TNES–urea extraction buffer (Asahida et al. 1996) followed by one extraction in 25:24:1 phenol – chloroform – isoamyl alcohol and one extraction in 24:1 chloroform – isoamyl alcohol. DNA was precipitated using 0.3 M sodium acetate and 2:1 isopropanol–supernatant followed by precipitation for 1 h at –80°C. Tubes were then centrifuged at 12 000 × g for 15 min, the supernatant discarded, and the pellet washed once with 70% ethanol. The pellet was resuspended in 100 μL 10 mM Tris–EDTA and stored at –4°C.

We tested primers from many studies, including 10 *A. fulvescens* primers (May et al. 1997), 140 shovelnose sturgeon (*Scaphirynchus platorynchus*) primers (McQuown et al. 2000), and five Atlantic sturgeon (*Acipenser oxyrinchus*) primers (King et al. 2001). The PCR profile using a 57ºC annealing temperature was as follows: (*i*) initial denaturing at 94ºC for 3 min, (*ii*) 30 cycles of denaturing at 94ºC for 1 min, annealing at 57ºC for 30 s, extension at 72ºC for 30 s, (*iii*) and final extention at 72ºC for 5 min. Magnesium chloride concentration was arbitrarily set at 2.5 mM, each dNTP concentration was 0.175 mM, and each primer was at a concentration of 0.4 mM. PCR products were mixed 1:2 v/v with a 98% formamide loading buffer and denatured at 95°C for 3 min and placed on ice for 5 min before gel loading. Electrophoresis was performed using 5% denaturing polyacrylamide gel electrohoresis (PAGE) and visualized using a Molecular Dynamics model 595 fluorimager.

White sturgeon amplifications that yielded polymorphic products without a lot of spurious products, such as stutter bands and nonspecific amplification, were excised from the electrophoresis gel. Bands were eluted overnight in 100 μL of 10 mM Tris–EDTA. We selected loci that yielded at least five or six alleles across six individuals, as we intended to develop microsatellite loci that would be used primarily for parentage testing and relatedness estimation. The supernatant was pipetted off and 1.0 μL of this supernatant was used as a source of DNA template in a 25 μL PCR reaction (see cycle profiles below). PCR products were purified using a QIAGEN QIAquik PCR Purification Kit (QIAGEN Inc., Valencia, Calif.). Sequencing reactions were performed on an ABI-377 sequencer by Davis Sequencing, Inc. (Davis, Calif.). The resulting DNA sequence information was white sturgeon specific, permitting design of white sturgeon specific PCR primers. DNA sequence information was analyzed using the DNAStar software package. Primer oligos were selected using Primer 3 online at the Whitehead Institute for Biomedical Research – MIT Center for Genome Research (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi).

White sturgeon specific PCR primers were optimized using $MgCl_2$ titrations (1.0–3.5 mM). Following optimization, PCR amplification was performed using either a 57°C or touchdown protocol (see Table 1). All reactions were performed in a volume of 25 μL using 1 μL of template DNA, 2.5 μL Gibco-BRL 10× PCR buffer, 0.175 mM of each dNTP, 0.4 mM of each primer, $MgCl_2$ concentrations as indicated in Table 1, 0.1 U Gibco-BRL recombinant *Taq* polymerase, and ddH_2O to 25 μL. The forward primer was 5′-labeled with fluoroscein to permit visualization and reduce background effects. As indicated above, two different thermal cycles were used, depending on the locus. All loci were initially screened for amplification at an annealing temperature of 54°C. Annealing temperature was later increased to either 57°C or a touchdown protocol. The touchdown protocol was used to eliminate stutter and artifact bands if these problems could not be corrected at 57°C. Both cycler profiles are detailed below.

The PCR profile using a 57°C annealing temperature was as follows: (*i*) initial denaturing at 94°C for 3 min, (*ii*) 30 cycles of denaturing at 94°C for 1 min, annealing at 57°C for 30 s, extension at 72°C for 30 s, and (*iii*) a final elongation at 72°C for 5 min. The touchdown PCR profile was as follows: (*i*) initial denaturing at 94°C for 5 min, 14 cycles of denaturing at 94°C for 30 s, annealing at 65°C for 1 min, extension at 72°C for 1 min, with a 0.5°C reduction in annealing temperature per cycle, (*iii*) 16 cycles of denaturing at 94°C for 30 s, annealing at 57°C for 1 min, extension at 72°C for 1 min, and (*iv*) hold at 4°C with no final extension.

Individuals were scored at each locus by loading an allelic ladder on the same gel and individual lanes were scored using Molecular Dynamics' FragmeNT Analysis software package. The results of the automated scoring were manually checked for errors, particularly in cases of gel "smiling".

Amplifying alleles in an individual were excised from the gel and sequenced in an attempt to detect flanking sequence

differences that might discriminate between duplicated loci. This approach was unsuccessful in that no patterns of flanking sequence divergence between alleles were detected. The pattern searched for was a dichotomy of insertions–deletions (indels) or sets of multiple base substitutions in alleles within an individual. A high level of flanking sequence similarity was observed across all alleles. Flanking sequence variability between alleles was limited to an occasional segregating single nucleotide polymorphism. In general, at least 50 bp of flanking sequence was returned on either side of the microsatellite.

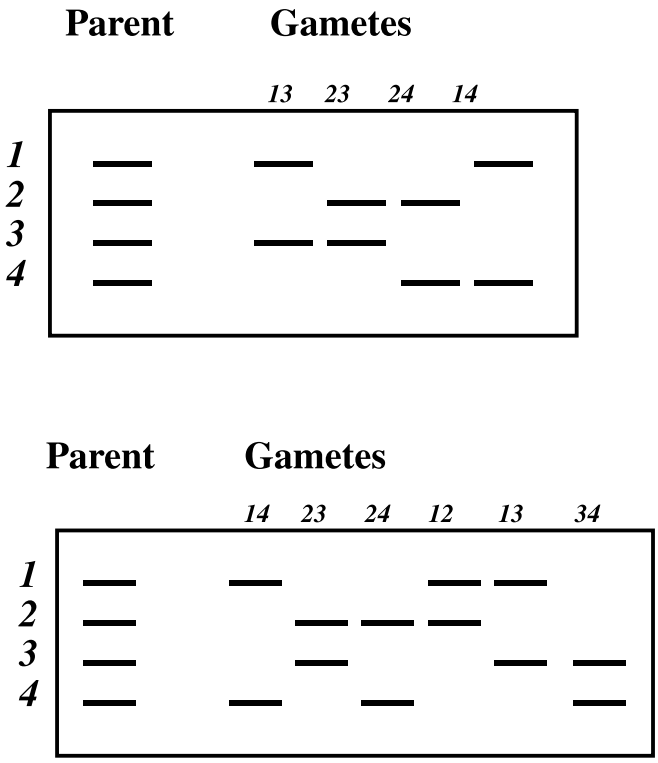
Methodology for determining modes of inheritance

Three randomly selected sires and three dams from broodstock held by a private aquaculture company were crossed factorially and the resulting families hatched in separate containers. Progeny were sampled 24–48 h post-hatch. Forty-eight fish per family were used to quantify the mode of inheritance for each locus. We were not able to genotype several sires and dams beforehand and then select ones that would make the most informative crosses, but were limited to using animals that were able to shed gametes during a certain time window.

We analyzed inheritance patterns using a combination of qualitative and quantitative genetics. The qualitative approach involves examining segregation patterns of pairs of alleles. Different patterns of pairwise combinations of alleles segregating in the progeny are expected if the alleles are being transmitted from two disomic loci versus a single tetrasomic locus. For example, Fig. 1 shows the patterns of hypothetical disomic and tetrasomic systems. The best evidence the four alleles are at two discrete disomic loci is an absolute absence of certain classes of pairwise combinations of alleles, while under tetrasomy all possible pairwise combinations are expected to be observed. In this paper we only consider tetrasomy under random assortment of chromatids from a quadrivalent or bivalent pairing; we did not consider double reduction.

The biometrical approach involved examining frequencies of pairwise combinations of alleles in the progeny. Consider a hypothetical system occurring in eight copies in the genome. If four disomic loci comprise the system, two possibilities exist for frequencies of pairwise combinations of alleles: 0 if at the same locus, or 1/4 if at separate loci. The expectation of 1/4 results from the random association of two elements (alleles). Each allele has a probability of 1/2 of being drawn (transmitted); thus the joint probability of drawing both elements is $(1/2)^2 = 1/4$. If two tetrasomic loci comprise the system, two possible frequencies for pairwise transmission are 1/6 if at the same locus or 1/4 if at separate loci. There are six possible pairs of alleles that can be transmitted from one tetrasomic locus; thus the expected frequency of observing a given pair is 1/6. If the alleles are at separate loci, each allele is expected to be transmitted 50% of the time; thus the probability of drawing both alleles is the product of their individual transmission frequencies (1/2), or 1/4. If the system is one octosomic locus, the probability is still 1/4, since each allele is transmitted 50% of the time and the chance of both alleles being transmitted is 1/2 × 1/2, or 1/4.

Fig. 1. Allelic segregation under disomic and tetrasomic inheritance. The gel on the top depicts the possible bands in the phenotypes for the gametes if alleles 1 and 2 are at one disomic locus and alleles 3 and 4 are at another disomic locus, noting that band combinations 12 and 34 are absent. The gel on the bottom depicts all possible band combinations if all four alleles are at one tetrasomic locus, resulting in transmission of all possible pairwise combinations of alleles in the gametes.



Single allele transmission frequencies were also used to examine inheritance patterns. Under any Mendelian inheritance model, a single copy allele is expected to be transmitted half of the time. The only difference is in the tetrasomic case when the allele is present in two copies, in which case at least one copy of the allele would be transmitted 5/6 of the time, leaving one to discriminate between transmission probabilities of 0.50 and 0.83.

Unfortunately, these frequencies become problematic because of the large sample size needed to discriminate between them. Approximately 450 samples are needed to classify the frequency of a pairwise combination as either 1/6 or 1/4 with a 95% level of confidence. The required sample size is large because the frequencies are so close (0.167 versus 0.250) and the 95% confidence interval (CI) for each frequency overlap.

Hypothesis tests

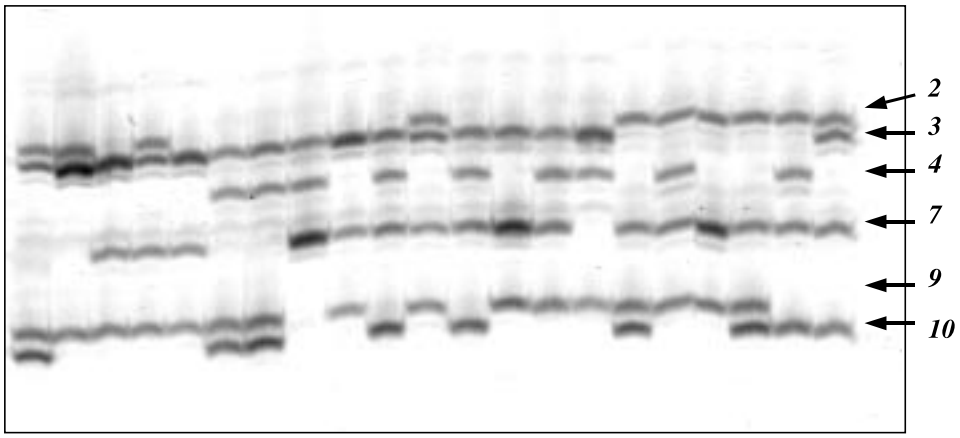
Hypothesis tests to differentiate transmission frequencies of individual alleles were formulated using the expected frequencies detailed above and applied against three alternative allele dosage hypotheses. Dosage of individual alleles in the parents was determined as 1, 2, or 2+ when the 95% CI of the transmission frequency overlapped 0.50, 0.83, or the lower confidence limit for the observed allele transmission frequency exceeded 0.83, respectively. The 95% CIs for ob-

Table 1. *Atr* primer sequences and reaction conditions.

Locus	Primer	Sequence 5′→3′	[$MgCl_2$] mM	Cycle profile	Clone size (bp)
Atr-100	Atr-100L	CGA CCA AAC AAA CAG TGA CA	2.5	57°C	150
	Spl-100R	TTT TCA CCA TTG GGT GTT CA			
Atr-1101	Atr-1101L	TAT CCC CTC CAC TGG AAA T	3.5	57°C	133
	Atr-1101R	GTT AAA CAT CCC TGC CTT CA			
Atr-105	Atr-105L	CGA TTT GAT TGG CTC TTG TA	1.5	Touchdown	180
	Atr-105R	TGC AAA TAA ATT GGA GCT GA			
Atr-107	Atr-107L	GGC AGG ATT ACA TCT CCT GA	3.0	Touchdown	233
	Atr-107R	TTA CTA ACT GCT AGA TAA TAC CTC TCT			
Atr-109	Atr-109L	ACC CGA GCT GTC ACA TTA CT	2.0	Touchdown	227
	Atr-109R	AAA ATA ACG CGA ATT CCT GA			
Atr-113	Atr-113L	TAA ACA AAT ACA AAA CTG CGT GTC	2.5	57°C	213
	Atr-113R	GGT TGG ATG AGA TCG GGA TA			
Atr-114	Atr-114L	GCA ATT CGT GTT ATG TTC ATT T	2.5	Touchdown	190
	Atr-114R	TGC ATT CAG AGA ATA ACC GA			
Atr-117	Atr-117L	TGC ATG ACA CAG GAC TTA CC	1.5	Touchdown	245
	Atr-117R	TGC CTC TCA ATA GCA ACA TC			
Atr-1173	Atr-1173L	ATC GGT TGT TGA ACT CCA CT	2.5	Touchdown	251
	Atr-1173R	TAT TAC AAG GTG GGT GGA CA			

Note: PCR conditions, cycle profile, and clone size for each microsatellite locus are provided. Cycle profiles are described in the text. For all loci, concentrations were 0.175 mM of each dNTP and 0.4 μM of each primer.

Fig. 2. *Atr-1173* allele segregation. This figure depicts allele segregation in 21 offspring within family 334×21, demonstrating the lack of artifact banding and consistency of amplification. Alleles 2, 3, 4, 7 and 9 amplify in dam 334, alleles 3, 7, 9, and 10 amplify in sire 21.



served transmission frequency were calculated using the binomial confidence interval $p \pm 1.96\{[p(1-p)]/n\}^{0.5}$ where n is the number of animals scored in the family in which the allele or pair of alleles is observed and p is the frequency of observing single alleles or pairwise combinations as indicated. A single copy allele is expected to be transmitted with a frequency of 0.50 in both disomic and tetrasomic systems. An allele present in two copies is expected to be transmitted with a frequency of 0.83 in a tetrasomic system of inheritance. If an allele is present in more than two copies and is still observed to segregate in the offspring, a level of duplication beyond tetrasomy is implied. The assumptions of this approach are that the loci behave consistently as disomic, tetrasomic, or "other" from one animal to another, such that a locus is not inherited in a tetrasomic manner in one animal and octosomic in another. Given the reviews of currently available scientific information on the ploidy levels of sturgeon species (e.g., Ludwig et al. 2001), we believe that a given locus will be either disomic, tetrasomic, or octosomic.

The co-segregation frequency of allele pairs in the progeny was examined when both alleles were specific to only one parent in the cross. The alternative co-segregation hypotheses are 0, 1/6, 3/14, and 1/4 for observed pairwise combinations of alleles. The null hypothesis was independent transmission of alleles comprising the pair, in which case the expected frequency of occurrence for the pair of alleles is the product of their individual transmission frequencies. The following assumptions apply: all alleles are present as a single copy in the parent, null alleles are absent, and loci do not share alleles with the same electrophoretic mobility.

Results

Of the approximately 150 primer pairs tested, only those from *S. platorynchus* primers (McQuown et al. 2000) were

Table 2. Allele sizes observed in parents.

Allele designation	*Atr-100*	*Atr-1101*	*Atr-105*	*Atr-107*	*Atr-109*	*Atr-113*	*Atr-114*	*Atr-117*	*Atr-1173*
1	137	155	152	242	306	194	222	283	299
2	141	151	148	234	298	198	214	252	295
3	149	143	144	230	286	206	210	248	291
4	153	139	136	217	278	210	208	240	281
5	157	135	132	213	274	214	206	236	277
6	101			205	270	242	204	230	273
7				201	266	262	200	224	269
8				197	262	266	197	222	265
9				193	258	274	190	220	253
10				189	254	282		216	249
11				185	250			210	
12					246			196	
13					242				
14					238				
15					234				
16					230				
17					222				
18					218				
19					214				

Note: This table lists the actual base pair sizes of the alleles observed in the parents at each *Atr* locus.

ultimately used. In total, we developed white sturgeon specific primers and optimized PCR conditions for nine microsatellite systems (Table 1). PCR and electrophoresis conditions were optimized to eliminate stutter bands. Several systems yielded more than two bands per individual, reflecting the polyploid nature of the white sturgeon genome. While this observation could lead one to suspect artifact banding, this was not an issue. No two bands were observed to co-segregate, as would be expected for stutter bands; thus we conclude the alleles scored are actual alleles and not artifact bands. The locus *Atr*-1173 shows the lack of artifact bands and clarity of phenotypes (Fig. 2).

Sizes (in base pairs) and numerical designations for each allele in each microsatellite system are cross-referenced in Table 2. Parental phenotypes are presented in Table 3. Data for individual microsatellite systems are presented in Tables 4 and 5. Transmission frequencies of individual alleles are calculated by dividing transmission of that allele (allele count) by the number of *n* progeny scored. Frequency of pairs of alleles are presented for some crosses as "allele-1/allele-2". Presumptive parental genotypes are presented for some systems.

Atr-100

This marker appears to amplify two loci; one invariant locus of unknown ploidy and one variable tetrasomic locus (Table 4). Six alleles were found among the parents. The 6 allele appeared in all but two of the amplified offspring (n = 299), and we believe it represents the invariant locus. Most alleles at the variable locus in the parents showed approximate 1:1 segregation in the offspring. Since allele dosage could not be visually detected in the gels, we relied on the segregation data to estimate parent dosage. Since alleles specific only to one parent (not shared between both parents) segregated 1:1, we believe those alleles to be present in single dose. One parent, 219, had three alleles at the presumed tetrasomic locus, and all three alleles segregated 1:1 and occurred in all possible pairwise combinations in the offspring. If the alleles had been divided between two disomic loci, all pairwise combinations would not be possible. Since all three alleles had equal segregation ratios and the three bands were of equal intensity, we assigned the parental genotype 1/2/3/Ø for the variable locus.

The system appears to contain null alleles. We amplified progeny we believe to be homozygous null for the variable system. Individuals were interpreted to be homozygous null when the 6 allele amplified but nothing amplified in the variable system. In addition, individuals occurred in some families, particularly those sired by sire 21, which received no bands from one or both parents. Sire 21 gave either allele 1 or nothing and transmitted allele 1 in a 1:1 ratio, which is accounted for if its genotype is 1/Ø/Ø/Ø. Also, dam 223 had a presumed genotype of 2/4/Ø/Ø. Progeny amplified allele *2*, 4, both, or none, which is expected if its genotype is 2/4/Ø/Ø, with the individual alleles segregating 1:1.

Atr-1101

Initially we suspected this system to be tetrasomic, as it has the characteristic dosage patterns of a tetrasomic system (Table 4). However, on further examination, two of the families had individuals with nontetrasomic dosages and some individuals had five alleles. Our ability to conclusively infer a mode of inheritance for this locus is hampered by a lack of allelic diversity in that parents had too many bands in common in the crosses. We include this locus in the paper solely to provide a primer sequence and PCR cycle reference to future investigators.

Atr-105

Atr-105 is presumptively tetrasomic, based on observed allele segregation patterns. The presumed genotypes of parents and the segregation ratios of alleles are presented in Ta-

Table 3. Parental phenotypes.

	Sires			Dams				
Locus	21	219	317	223	334	363	Total alleles	Presumptive inheritance
Atr-100	1 6	3 4 5 6	3 4 6	2 4 6	3 6	3 4 6	6	Tetrasomic
Atr-1101	1 3 5	1 2 3 5	4 5	5	1 2 3 4	4 5	5	Tetrasomic
Atr-105	1 2 3 4	2 4	2 3 4	2 3 4	2 4 5	2 4	5	Tetrasomic
Atr-107	1 8 10 11 11	2 3 10 11	1 2 4 7 9	5 6 8 9	1 8 10 11 11	5 6 8 9 10	11	Hexa- or octosomic
Atr-109	3 5 9 11 14 19	4 6 9 11	5 6 8 13 16 17	2 4 7 11 12 15	3 9 10 11 15	1 4 5 11 12 18	19	Octosomic
Atr-113	4 5	7	1 10	2 3 9	4 8	3 6 9	10	Unknown
Atr-114	3 4 10 8 9	2 4 6 7 9	3 4 9	1 4 7 9	4 8 9	1 2 4 5 7 9	10	Octosomic
Atr-117	3 6 9 11 12	3 6 12	1 4 6 8 12	2 3 5 6 7 8 10 12	3 6 9 11 12	2 3 5 6 7 8 10 12	12	Octosomic
Atr-1173	3 7 9 10	2 6 9	1 2 9	2 5 6 8 9	2 3 4 7 9	2 6 3 7 8 9	10	Hexa- or octosomic

Note: This table lists the alleles amplifying at each locus in each parent, the total number of alleles in all six parents, and the presumptive mode of inheritance for each system.

ble 4. Estimated parental genotypes coincide with observed transmission frequencies of presumed single copy alleles. Unlike *Atr-100*, some dosage in the gel was detectable, but not in all crosses. Amplification of the parents shows a dosage effect that suggests a tetrasomic genotype. However, this was not found in all crosses. For instance, amplification of the *1* allele was not favored equally and appeared "lighter" than the other bands in some individuals, even when all four bands were present in that individual. This was observed in families sired by 21. Whether this is evidence of partial null alleles or simply an artifact of this particular system is unknown. However, in family 363×219, there was an obvious dosage pattern, yielding genotypes of 2/4/4/4, 2/2/4/4, and 2/2/2/4 individuals. Detection of dosage appears to be quite sensitive to PCR conditions. Interestingly, allele 2 amplified in all 313 amplified offspring across all families. A possible alternative explanation is there are two disomic loci in this system, with one locus homozygous for the 2 allele on one or both parents in each cross; this would cause every offspring to have a 2 allele. Unfortunately, we did not have any parents that had four different alleles unique from another parent to which it was crossed. If that were the case, the absence of some pairwise allele combinations would indicate disomic inheritance.

Atr-107

This system appears to be a highly duplicated system, possibly between hexasomic and octosomic (Table 5). Transmission frequencies varied from 0.46 to 0.99 for the parental alleles, suggesting at least qualitatively these alleles occur in more than one dose in the parent. The maximum number of alleles observed in an individual in the progeny was eight, and occurred in one of 336 progeny; several had six or seven alleles.

No evidence of disomic inheritance was observed for any pairwise combinations, with one exception. In the 334×317 cross, each progeny received one of the two alleles 8 and 10 but no progeny received both alleles; however, only 24 individuals amplified, so this result should be interpreted with caution. The frequency of pairwise combinations generally fit the expectation for random association of alleles. The maximum number of alleles specific to a parent in a cross was four; in these cases the parent gave from one to four alleles to any given progeny. Evaluation of the true underlying level of duplication is hampered by the number of alleles shared by parents of the crosses; there were no cases where segregation of all alleles in one parent could be observed.

Atr-109

This system appears to occur in at least eight copies; however, dosages could not be read from the gels (Table 5). Parents used in the crosses amplified between four and six alleles with progeny having up to eight alleles, and in three cases, nine alleles. Since we could not read dosage visually, based on the transmission frequencies in Table 5, we estimate most alleles to be present in single copies in the parents, with some alleles obviously in higher dosages.

Two explanations are possible for observations seen in this system. First, the system is octosomically derived, and individuals with nine alleles are the result of unreduced ga-

Table 4. *Atr*-100, 105, and 1101 allele transmission frequencies.

Locus	Presumptive inheritance	Parent	Presumptive genotype	Allele	Allele count / *n*	Transmission frequency ± 95% CI	Estimated dosages
Atr-100	Tetrasomic	21	1/0/0/0	1	61/125	0.49±0.08	1
		219	3/4/5/0	3	17/38	0.44±0.17	1
				4	22/44	0.50±0.15	1
				5	53/105	0.50±0.09	1
				3/5	3/38	0.07±0.08	—
				4/5	12/44	0.27±0.13	—
		223	2/4/0/0	2	36/74	0.48±0.12	1
				4	21/36	0.58±0.16	1
				2/4	13/36	0.36±0.16	—
				0/0	17/36	0.47±0.17	—
		334	3/0/0/0	3	19/47	0.40±0.14	1
		363	3/4/0/0	3	21/42	0.50±0.15	1
				4	19/42	0.45±0.15	1
				3/4	11/42	0.26±0.13	—
				0/0	12/42	0.28±0.14	—
Atr-1101	Tetrasomic?	21	1/2/3/4	1	48/90	0.53±0.10	1
				3	54/90	0.60±0.10	1
				1/3	18/45	0.40±0.14	—
				1/3	10/45	0.22±0.12	—
		223	2/2/3/4	3	21/37	0.56±0.17	1
		317	2/2/3/4	3	30/61	0.49±0.13	1
			2/0/3/4				
		334	2/2/4/5	5	38/114	0.50±0.10	1
		363	2/2/4/4				
Atr-105	Tetrasomic	21	—	1	39/76	0.51±0.11	1
				3	62/76	0.82±0.08	2
				5	21/46	0.46±0.14	1
		219	—	1	39/83	0.47±0.11	1
				2	43/83	0.52±0.11	1
				3	40/83	0.48±0.11	1
				5	24/45	0.53±0.15	2
		317	—	5	21/23	0.91±0.12	2
		334	—	2	34/69	0.49±0.11	1
				3	12/23	0.52±0.21	1
				4	50/69	0.72±0.10	2
		363*	—	4	69/84	0.82±0.08	2

*Dam 363 did not have any alleles not shared with other sires at *Atr-105*.

metes. Another possibility is the system is duplicated beyond octosomy.

No disomic loci were detected in the system based on pairwise allele combinations. Pairwise allele combinations generally fit the expectation of random association for both alleles (the product of the two allele transmission frequencies). For alleles specific to one parent in a cross, each parent transmitted between zero and five alleles. Five alleles were transmitted together to progeny in low frequency (4 of 216 possible occurrences). We suspect transmission of more than four alleles may occur more often than is directly observable since progeny receiving five alleles from one parent did not always result in an individual with nine alleles.

Atr-113

This locus shows an interesting and perhaps sex-specific level of duplication (Table 5). One male, 219, amplified only one allele, which was transmitted 1:1 to the progeny, providing evidence of null alleles in the system. The other two males, 21 and 317, both amplified two alleles, each transmitted 1:1 in the progeny. Examining male 21 in more detail, it never passed both alleles 4 and 5 together, characteristic of a single disomic locus. Allele 4 was passed to 31 of 78 informative offspring (female 334 had the 4 allele and thus 334×21 was not informative), and allele 5 was passed to 70 of 124 offspring, where both of these ratios did not differ significantly from a 1:1 segregation ratio. Interestingly, each male always gave either allele, but never both, which is characteristic of disomic inheritance.

The females, conversely, are questionable. One female amplified two alleles while the other two females amplified three alleles. The female with two alleles transmitted zero, one, or two alleles. Considering the females with three alleles, progeny received either zero, one, two, or three alleles from the female, in all possible combinations. In all cases, alleles from the dam segregated 1:1 in the progeny, suggest-

Table 5. *Atr-107, 109, 113, 114, 117,* and *1173* allele transmission frequencies.

Locus	Presumptive inheritance	Parent	Allele	Allele count / *n*	Transmission frequency ± 95% CI	Estimated dosages
Atr-107	Hexasomic/octosomic	21	1	44/86	0.51±0.11	1
			10	20/39	0.51±0.16	1
			11	85/86	0.99±0.01	2+
		219	2	73/132	0.55±0.09	1
			3	70/132	0.53±0.09	1
			10	28/37	0.76±0.14	2
			11	43/84	0.51±0.11	1
		223	5	60/76	0.79±0.09	2
			6	57/76	0.75±0.10	2
			8	19/37	0.51±0.16	1
			9	63/76	0.83±0.08	2
		317	1	40/47	0.85±0.10	2
			2	36/71	0.51±0.11	1
			4	39/71	0.55±0.12	1
			7	35/71	0.49±0.12	1
			9	19/24	0.79±0.16	2
		334	1	39/48	0.81±0.11	2
			8	43/72	0.60±0.11	1
			10	11/24	0.46±0.20	1
			11	24/24	1.00	2+
		363	5	75/141	0.53±0.08	1
			6	74/141	0.52±0.09	1
			8	51/194	0.54±0.10	1
			9	59/194	0.63 + 0.10	Unknown
			10	40/47	0.85±0.10	2+
Atr-109	Octosomic	21	3	38/82	0.46±0.11	1
			5	48/84	0.57±0.11	1
			9	45/82	0.55±0.11	1
			14	63/129	0.49±0.08	1
			19	65/129	0.50±0.09	1
		219	4	39/48	0.81±0.11	2
			6	75/130	0.58±0.08	1
			9	42/82	0.51±0.11	1
		223	2	64/74	0.86±0.08	1
			4	21/38	0.55±0.16	1
			7	48/74	0.65±.011	Unknown
			12	39/74	0.53±0.11	1
			15	48/74	0.65±0.11	2
		317	5	18/24	0.75±0.17	2
			6	33/70	0.47±0.12	1
			8	37/70	0.53±0.12	1
			13	33/70	0.47±0.12	1
			16	37/70	0.53±0.12	1
			17	27/70	0.39±0.11	1
Atr-109		334	3	35/72	0.49±0.11	1
			9	15/24	0.63±0.20	1
			10	72/119	0.61±0.08	Unknown
			11	14/24	0.58±0.20	1
			15	58/119	0.49±0.09	1
		363	1	67/136	0.49±0.09	1
			4	51/90	0.57±0.10	1
			5	24/46	0.52±0.15	1
			11	23/46	0.50±0.14	1
			12	72/136	0.53±0.08	1
			18	68/136	0.50±0.08	1
Atr-113	Disomic (males only)	21	4	31/78	0.40±0.11	1

Table 5. (*continued*).

Locus	Presumptive inheritance	Parent	Allele	Allele count / *n*	Transmission frequency ± 95% CI	Estimated dosages
			5	70/124	0.56±0.11	1
			4/5	0/78	0	—
		219	7	68/130	0.52±0.09	1
		223	2	42/72	0.58±0.12	1
			3	30/72	0.42±0.11	1
			9	22/35	0.63±0.16	1
			2/3	13/72	0.18±0.09	—
			2/9	20/72	0.28±0.10	—
			3/9	18/72	0.25±0.10	—
		317	1	38/70	0.54±0.12	1
			10	32/70	0.46±0.11	1
			1/10	0/70	0	—
		334	4	39/71	0.55±0.12	1
			8	48/117	0.41±0.09	1
			4/8	8/71	0.11±0.08	—
		363	3	76/135	0.56±0.09	1
			6	74/135	0.55±0.08	1
			9	61/135	0.45±0.09	1
			3/6	34/135	0.25±0.08	—
			3/9	26/135	0.19±0.07	—
			6/9	23/135	0.17±0.06	—
Atr-114	Octosomic	21	3	76/133	0.57±0.09	1
			10	66/133	0.50±0.08	1
			8	64/86	0.74±0.10	2
		219	2	42/83	0.51±0.10	1
			6	65/129	0.50±0.09	1
			7	21/46	0.46±0.14	1
		223	1	69/76	0.91±0.06	2+
			7	22/39	0.56±0.16	1
		317	3	33/67	0.49±0.12	1
		334	8	40/70	0.57±0.12	1
		363	1	71/136	0.52±0.09	1
			2	44/90	0.49±0.10	1
			5	75/136	0.55±0.09	1
			7	46/90	0.51±0.10	1
Atr-117	Unknown	21	9	30/79	0.38±0.11	1
			11	46/79	0.58±0.11	1
		223	2	39/63	0.62±0.12	1
			5	32/63	0.51±0.12	1
			7	58/63	0.92±0.07	2+
			8	50/63	0.79±0.10	2
			10	38/63	0.60±0.12	1
			12	48/74	0.65±0.11	1–2
		317	1	21/48	0.44±0.58	1
			4	23/48	0.48±0.14	1
			8	13/24	0.50±0.20	1
		334	3	13/24	0.54±0.20	1
			9	37/72	0.51±0.12	1
			11	38/72	0.53±0.11	1
		363	2	60/116	0.52±0.09	1
			3	12/24	0.50±0.20	1
			5	65/116	0.56±0.09	1
			7	94/116	0.81±0.07	2
			8	49/92	0.53±0.10	1
			10	67/116	0.58±0.09	1
Atr-1173	Hexasomic/octosomic	21	3	43/84	0.51±0.11	1

Table 5. (*concluded*).

Locus	Presumptive inheritance	Parent	Allele	Allele count / *n*	Transmission frequency ± 95% CI	Estimated dosages
			7	21/38	0.55±0.16	1
			10	66/128	0.52±0.08	1
		219	6	19/37	0.51±0.16	1
		223	2	34/38	0.89±0.10	2
			5	47/75	0.63±0.11	1–2
			6	24/38	0.63±0.15	1
			8	43/75	0.57±0.12	1
		317	1	38/66	0.58±0.11	1
		334	2	17/44	0.39±0.14	1
			3	36/57	0.63±0.13	1–2
			4	59/101	0.58±0.10	1
			7	24/57	0.42±0.13	1
		363	2	18/46	0.39±0.14	1
			5	70/138	0.51±0.08	1
			6	50/92	0.54±0.11	1
			7	47/92	0.51±0.10	1
			8	47/92	0.51±0.10	1

Note: Allele 9 in dam 363 did not fit any expected transmission frequency at *Atr-107*. Allele 7 in dam 223 did not fit any expected transmission frequency at *Atr-109*. For *Atr-1173* allele transmission frequencies, alleles 5 in dam 223 and 3 in dam 334 fit expected transmission frequencies for both one and two dose alleles in a tetrasomic system.

ing the alleles occur in a single copy. Since dosage could not be read, we relied upon segregation ratios to estimate allele dosage in the parental genotypes.

Alternate interpretations of this system are possible. The simplest explanation is a male specific diploid locus with a higher level of ploidy in the females. The two informative males cleanly fit expectations under disomy, while the females are not disomic.

Atr-114

Presumed dosages are based on both band intensities and transmission frequencies (Table 5). This locus appears to reflect the octoploid level of duplication. No progeny had more than eight alleles, but several had six to eight alleles and received up to four alleles from one parent. Some dosage effect was seen, but visually scoring dosages to add up to eight copies was not possible. Only three of 329 offspring did not receive a 9 allele, and five of 329 did not receive a 4 allele; both alleles were present in every parent and visually appeared darker than other bands within each parent. Thus each parent was "nearly homozygous" for these alleles at some locus within the system. In general, darker bands showed a higher than 1:1 transmission ratio. Visual intensities were correlated approximately with segregation ratios. We found no evidence of underlying disomic loci. While the underlying mode of inheritance is unclear, the system as a whole appears to contain eight copies.

Atr-117

Allele sizes in this system did vary from the expected differences of 4 base pairs between alleles (Tables 2 and 5), suggesting there may also be a dimeric repeat motif amplifying and that *Atr-117* is a compound microsatellite. The data suggest a level of duplication beyond octosomy exists for this system. While dosage could not be determined visually, allele transmission frequencies suggest some alleles are present in multiple doses, even in parents with eight different alleles in their phenotype. In addition, when five alleles were specific to one parent in a cross, there were instances where that parent transmitted all five alleles to its progeny. This occurred in three families. Dam 223 passed five alleles to two of 32 progeny in the cross 223×21, to seven of 31 progeny in the cross 223×219, and dam 363 passed five alleles to eight of 45 progeny in cross 363×219. In crosses where less than four alleles were specific to a parent, there were instances where the parent transmitted none of the observed alleles to the offspring.

We found no evidence of disomic or tetrasomic inheritance patterns in this system. Pairwise allele combinations generally fit the expectation of random association of the two alleles. Analysis was hampered by crosses where alleles were shared by both parents, in which case we could not observe transmission of all combinations of alleles.

Atr-1173

This system appears to be highly duplicated, possibly hexasomically or octosomically derived, and dosage could not be visually determined (Table 5). Most observed parental alleles appeared to occur in a single copy based on transmission frequencies. The maximum number of alleles present in the progeny was seven; however, it must be noted that the maximum number of different alleles involved in any cross was eight. The maximum number of alleles specific to one parent in any cross was four; in these cases, the parent did transmit from one to four alleles to its progeny. If the system contained more than eight copies, parental phenotypes did not allow us to detect higher levels of dosage. Similar to other highly duplicated systems, we did not detect any underlying disomic or tetrasomic loci. Based on these observations, we tentatively believe this system is present in eight copies, while the underlying mode of inheritance remains unclear.

Discussion

Microsatellite loci have many uses in fisheries management and aquaculture. They have become common in studies investigating population subdivision via significant differences in allele frequencies, tracking gene flow between populations, and other hypotheses of relevance to fisheries managers (e.g., Ferguson and Danzmann 1998; Blouin et al. 1996). The hypervariability of microsatellite loci makes them an excellent choice for forensic applications, such as the identification of parents, estimation of sib groups, and individual identification. Their use in accurately rebuilding pedigrees on animals for which such information has been lost can be of much use when managing finite populations such as occur in hatcheries, where pedigree information can be used to avoid crossing parents and offspring or sibs. The use of genetic markers for parentage testing and pedigree reconstruction in aquaculture situations has been suggested by many authors (e.g., Davis and Hetzel 2000; Cunningham 1999; Ferguson and Danzmann 1998; Gjoen and Bentsen 1997).

The data provide the first insight into the nature of the white sturgeon genome from the perspective of Mendelian segregation. The white sturgeon is believed to be an octoploid-derivative (Blacklidge and Bidwell 1993) and the chromosome number for the species places it in the octoploid level of ploidy suggested by Ludwig et al. (2001). Van Eenannaam et al. (1998) suggested the diploidization process was complete based on the observation of bivalent pairing in males, though the pairing of chromatids in female white sturgeon has yet to be documented in the peer-reviewed literature. The mode of inheritance for microsatellite systems developed in this study appear to be locus-specific, with ploidy levels varying from disomy to at least octosomy.

Possible disomic inheritance was detected in one system, *Atr-113*. This system showed what is possibly a sex-specific level of duplication. Males segregated strictly as a single disomic locus, while females were clearly at a higher level of duplication. This locus would clearly be interesting to study further relative to sex determination in white sturgeon. Tetrasomic inheritance was observed in systems *Atr-100* and possibly *Atr-105*. In the latter system, parental genotypes did not permit us to distinguish between two disomic loci and a single tetrasomic locus. Higher duplication levels were observed in the remaining systems, with no evidence of disomy or tetrasomy, although distinguishing two tetrasomic loci from one octosomic locus was beyond our experimental design.

Occurrence of locus-specific levels of duplication within a species genome is not uncommon. Our results are consistent with previous inheritance studies on a closely related sturgeon species, *A. fulvescens* (Pyatskowit et al. 2001; McQuown et al.2002). Both studies showed a combination of locus-specific disomic and tetrasomic inheritance. Since *A. fulvescens* is a suspected tetraploid derivative, these results suggest that that species is in the process of diploidization. While the cytogenetic evidence in Van Eenannaam et al. (1998) shows bivalent pairing in males, our nuclear marker inheritance data suggest that the white sturgeon genome does not follow disomic inheritance, but rather that the mode of inheritance is locus-specific. These apparently contrasting results can be explained by suggesting that the observation of bivalents during meiosis does not mean that homologs at octosomic loci pair preferentially during meiosis, but perhaps that homologs form bivalents randomly. The main conclusion is that individual alleles themselves generally segregate one to one among progeny.

Applications to population genetic studies

We propose these systems not be scored as actual disomic, tetrasomic, or octosomic loci until their inheritance is further resolved or disomic loci can be isolated and characterized. Until then, each allele could be scored as a dominant nuclear marker, such as RAPD or AFLP bands. Lynch and Milligan (1994) provide a detailed discussion on the application of dominant marker frequencies to questions of population subdivision, genetic distance, and relatedness. We summarize their statistical procedures and assumptions below as applied to white sturgeon.

There are several assumptions behind scoring and interpreting each allele as its own locus. One assumption is that the allele behaves as a dominant marker. For a dominant marker to behave as a single locus, it should be present in either 50% or 100% of offspring derived from a parent amplifying the dominant marker, assuming the parent was heterozygous or homozygous, respectively. Our data show both cases occur in the white sturgeon. Most alleles specific to one parent in a cross did not significantly differ from the expected 1:1 segregation ratio in the progeny, or a 50% transmission rate. There were a few cases where alleles were passed to 100% of offspring, as if the parent was homozygous for the dominant marker. Not all alleles were inherited according to the inheritance assumptions of a dominant marker. This could potentially bias the interpretations of population differentiation statistics calculated using these loci and thus results derived therefrom should be interpreted with caution until true disomic loci are developed. In addition, see Lynch and Milligan (1994) for their recommendations on criteria for omitting loci from population genetic studies.

Another assumption is independence between loci, meaning individual loci are random and independent samples of genetic variability. We examined frequencies of pairwise allele combinations when possible. These frequencies generally fit the expected frequency for a pair of elements drawn independently with replacement, meaning the frequency of a pair of alleles did not differ significantly from the product of the transmission frequencies of both alleles independently. In fact, this observation led us to rule out both disomy and tetrasomy for many systems. One notable expection is *Atr-113*, which may be disomic in males.

Applying the method of dominant markers to these alleles works as follows. The parameter q where $1 - q = p$ is estimated by x, where x is the frequency of individuals of sample size N not displaying the allele (null homozygotes) via the formula $\hat{q} = \sqrt{x} \div (1 - \mathrm{var}(x) / 8x^2)$, where $\mathrm{var}(x) = x(1 - x)/N$ is the sample variance of the null homozygotes, and $\mathrm{var}(\hat{q}) = (1 - x)/4N$.

The authors recommend discarding loci from analysis where $x < 3/N$ to avoid biasing parameter estimates. While

this may seem a downfall for scoring alleles as dominant markers, one gains more markers by scoring each allele as its own locus. For instance, in this study, over 100 markers were observed across six individuals, which would yield over 100 markers before discarding low frequency alleles. Although information is lost by not scoring the genotypes of each microsatellite system as a unit, in the case where it is impossible to determine genotypes, one would have over 100 dominant markers as opposed to nine codominant loci, a substantial gain in information. Sample sizes should be equal to or greater than those used for codominant markers. Alternatively, one could average data first across alleles at a locus and then across loci, if one were concerned about the bias of loci with more alleles.

This system is not perfect, however, as some alleles were not transmitted at 50% or 100%, but somewhere in between. However, considering the highly duplicated nature of the genome and the fact that reliably reading dosages to add up to eight copies is difficult, scoring each allele as its own locus is more realistic and practical while providing a reasonable approximation to the assumptions of dominant markers.

Acknowledgements

This publication was supported in part by the National Sea Grant College Program of the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Grant No. NA06RG0142, project No. R/A-109, through the California Sea Grant College Program; and in part by the California State Resources Agency. The views expressed herein do not necessarily reflect the views of any of those organizations. J. Rodzen was supported on a Sea Grant traineeship and a USDA Biotechnology training grant. Eve McQuown and anonymous reviewers provided insightful and helpful comments in organizing this paper. Special thanks to Stolt Sea Farm California LLC for spawning the families used for the inheritance study and the use of their hatchery.

References

Allendorf, F.W., and Danzmann, R.G. 1997. Secondary tetrasomic segregation of *MDH-B* and preferential pairing of homeologs in Rainbow trout. Genetics, **145**: 1083–1092.

Ardren, W.R., Borer, S., Thrower, F., Joyce, J.E., and Kupuscinski., A.R. 1999. Inheritance of 12 microsatellite loci in *Oncorhynchus mykiss*. J. Hered. **90**: 529–536.

Asahida, T., Kobayashi, T., Saitoh, K., and Nakayama, I. 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fish. Sci. **62**: 727–730.

Birstein, V.J., and DeSalle, R. 1998. Molecular phylogeny of Acipenserinae. Mol. Phylogenet. Evol. **9**: 141–155.

Birstein, V.J., P. Doukakis, and R. DeSalle. 1999. Molecular phylogeny of Acipenserinae and black caviar species identification. J. Appl. Icthyol. **15**: 12–16.

Birstein, V.J., Poletav, A.I., and Goncharov, B.F. 1993. The DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry, **14**: 377–383.

Birstein, V.J., Hanner, R., and DeSalle, R. 1997. Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches. Environ. Biol. Fishes, **48**: 127–155.

Blacklidge, K.H., and Bidwell, C.A. 1993. Three ploidy levels indicated by genome quantification in Acipenseriformes of North America. J. Hered. **84**: 427–430.

Blouin, M.S., Parsons, M., Lacaille, V., and Lotz., S. 1996. Use of microsatellite loci to classify individuals by relatedness. Mol. Ecol. **5**: 393–401.

Brown, J.R., A.T. Beckenbach, and M.J. Smith. 1992. Mitochondrial DNA length variation and heteroplasmy in populations of white sturgeon (*Acipenser transmontanus*). Genetics, **132**: 221–228.

Brown, J.R., Beckenbach, A.T., and Smith, M.J. 1993. Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanus*). Mol. Biol. Evol. **10**: 326–341.

Brown, J.R., Beckenbach, K., Beckenbach, A.T., and Smith, M.J. 1996. Length variation, heteroplasmy and sequence divergence in the mitochondrial DNA of four species of sturgeon (*Acipenser*). Genetics, **142**: 525–535.

Cunningham, E.P. 1999. The application of biotechnologies to enhance animal production in different farming systems. Livestock Prod. Sci. **58**: 1–24.

Davis, G.P., and Hetzel, D.J.S. 2000. Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. Aquaculture Res. **31**: 3–10.

Ferguson, M.M., and Danzmann, R.G. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Can. J. Fish. Aquat. Sci. **55**: 1553–1563.

Fontana, F. 1976. Nuclear DNA content and cytometry of of erythrocytes of *Huso huso*, *Acipenser sturio*, and *Acipenser naccarii*. Caryologia, **29**: 127–138.

Gjoen, H.M., and Bentsen, H.B. 1997. Past, present, and future of genetic improvement in salmon aquaculture. ICES J. Mar. Sci. **54**: 1009–1014.

Johnson, K.R., Wright, J.E., and May, B. 1987. Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics, **116**: 579–591.

King T.L., Lubinski, B.A., and Spidle, A.P. 2001. Microsatellite DNA variation in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) and cross-species amplification in the Acipenseridae. Conserv. Genet. **2**: 103–119.

Ludwig, A., Belfiore, N.M., Pitra, C., Svirsky, V., and Jenneckens, I. 2001. Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser, Huso*, and *Scaphirynchus*). Genetics, **158**: 1203–1215.

Lynch, M., and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**: 91–99.

May, B., Krueger, C.C., and Kincaid, H.L. 1997. Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. Can. J. Fish. Aquat. Sci. **54**: 1542–1547.

McQuown, E.C., Sloss, B.L., Sheehan, R.J., Rodzen, J., Tranah, G.J., and May, B. 2000. Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirhynchus* and *Acipenser.* Trans. Am. Fish. Soc. **129**: 1380–1388.

McQuown, E., Gall, G.A.E., and May, B. 2002. Characterization and inheritance of six microsatellite loci in lake sturgeon (*Acipenser fulvescens*). Trans. Am. Fish. Soc. **131**: 299–307.

Pyatskowit, J.D., Krueger, C.C., Kincaid, H.L., and May, B. 2001. Inheritance of microsatellite loci in the polyploid derivative lake sturgeon (*Acipenser fulvescens*). Genome, **44**: 185–191.

Spruell, P., Pilgrim, K.L., Greene, B.A., Habicht, C., Knudsen, K.L., Lindner, K.R., Olsen, J.B., Sage, G.K., Seeb, J.E., and

Allendorf, F.W. 1999. Inheritance of nuclear DNA markers in gynogenetic haploid pink salmon. J. Hered. **90**: 289–296.

Tagliavini, J., Conterio, F., Gandolfi, G., and Fontana, F. 1999. Mitochondrial DNA sequences of six sturgeon species and phylogenetic relationships within Acipensiridae. J. Appl. Ichthyol. **15**: 17–22.

Van Eenennaam, A.L., Murray, J.D., and Medrano, J.F. 1998. Synaptonemal complex analysis in spermatocytes of white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Asipenseridae), a fish with a very high chromosome number. Genome, **41**: 51–61.