Inheritance of 12 Microsatellite Loci in *Oncorhynchus mykiss*

W. R. Ardren, S. Borer, F. Thrower, J. E. Joyce, and A. R. Kapuscinski

Four full-sib families of *Oncorhynchus mykiss* were used to study the inheritance of allelic variation at 12 microsatellite loci. The loci examined were previously characterized in six different species from the family Salmonidae. Of the 48 genotypic ratios observed (12 loci × 4 crosses), disomic segregation of codominant autosomal alleles was established in 24 of 31 informative tests. The seven tests that did not conform to Mendelian ratios can be explained by the presence of null alleles (*One*μ14 and *Omy*77) and a locus (*One*μ1) that may be tandemly duplicated. Three significant pairwise linkage associations were observed between *One*μ1 and *Omy*207, *Ssa*85 and *One*μ11, and the two loci amplified by *One*μ1 primers, indicating these loci are not assorting independently. The presence of unexpected heteroduplex structures in *One*μ11 electropherograms in one cross prompted the sequencing of similar microsatellite electromorphs. Sequence differences revealed size homoplasy, that is, the electromorphs were identical in state but they were not identical by descent. These results demonstrate the need to conduct comprehensive species-specific inheritance studies for microsatellite loci used in population genetic or kinship analyses. Discovery of these four attributes by examining a small number of loci in four families suggests that they are common across microsatellite loci overall.

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Since their recent discovery, microsatellite markers have become increasingly appreciated for their utility in genome mapping (Weissenbach et al. 1992), kinship investigations (Queller et al. 1993), and studies of population structure (Goldstein and Pollack 1997). Microsatellites are highly polymorphic genetic markers and have gained the reputation of being the most powerful Mendelian markers ever found (Jarne and Lagoda 1996). Proper genetic interpretation of microsatellite data, and thus correct application of results, depends on testing four important attributes of microsatellite alleles. These include testing for (1) selective neutrality of alleles, (2) mode of inheritance of alleles (i.e., test for disomic segregation and independent assortment according to Mendel's law), (3) presence of null alleles, and (4) presence of size homoplasy.

Mendelian inheritance of a polymorphic genetic marker is a necessary requirement of many population genetic models and kinship analyses. Prior studies carried out on various diploid taxa suggest that microsatellites are generally neutral markers and that they are inherited in a Mendelian fashion (Queller et al. 1993). However, because salmonids have a tetraploid origin, it is imperative to verify Mendelian inheritance (Allendorf and Danzmann 1997). The complexity of the segregation ratios in salmonids is emphasized by loci in females only exhibiting disomic segregation ratios while a small proportion of loci in males show variable patterns of segregation ranging from disomic ratios in males from some populations to tetrasomic ratios in other populations (Allendorf and Waples 1996). The only way to discriminate disomic versus tetrasomic inheritance is to analyze breeding data (Allendorf et al. 1975).

Null alleles are nonamplifying alleles caused by mutations such as point mutations in the primer annealing site (Callen et al. 1993). Researchers have found null alleles at microsatellite loci in many taxa (Callen et al. 1993; Craighead et al. 1995; Pemberton et al. 1995) including *O. gorbuscha* (Spruell et al. 1999) and *O. kisutch* (Small et al. 1998). If null alleles are present but not accounted for, then any heterozygote bearing the null allele will be mistyped as a homozygote. This scoring error can cause an observed heterozygote deficiency in the population (Jones et al.

1998). Null alleles present at different frequencies in the sampled populations pose a potential problem when comparing levels of heterozygosity among populations. Null alleles can also cause the erroneous elimination of putative parents or errors in the degree of relatedness between individuals in kinship analysis.

Size homoplasy is the co-occurrence of alleles that are identical in state (PCR products of the same size) without being identical by descent (descent without mutation from the same ancestral allele). Not accounting for homoplasy, when it actually exists, leads to underestimation of the actual divergence between populations (Jarne and Lagoda 1996). Homoplasy is estimated by comparing DNA sequences or the heteroduplex structures formed by two alleles of the same size. In the only published report on size homoplasy of microsatellites, Estoup et al. (1995) found that homoplasy was only present among distantly related subspecies and between two species of bees. Thus the potential presence of homoplasy at the population level has been largely disregarded (Jarne and Lagoda 1996).

Heteroduplexes form during PCR amplification of a microsatellite because of the reannealing of alleles which have a high degree of sequence similarity but are noncomplementary strands due to base pair deletions, additions, or substitutions (Novy and Vorsa 1996). The two heteroduplex structures formed (determined by which allelic DNA strands anneal) generally migrate at different rates than the homoduplexes during electrophoresis on nondenaturing gels. This difference in migrational rate is due to conformational changes caused by incomplete homology between DNA strands. White et al. (1992) have found that 1 base pair differences between the DNA strands caused noticeable changes in the migration rate of heteroduplexes. The heteroduplexes almost always migrate more slowly than the homoduplexes and their phenotype is usually two distinct bands that migrate in a predictable fashion for every allelic combination. We and others (Dallas et al. 1995; Wilkin et al. 1993) use heteroduplexes as a diagnostic tool for identifying individuals who are heterozygous.

To date, genetic interpretation of microsatellite polymorphisms in *O. mykiss* has largely been based on population-level data and observations that allele frequencies conform to Hardy–Weinberg expectations. This approach is indirect and has a number of potential pitfalls because of the lack of statistical power (Fairbairn and Roff 1980; Lessios 1992). Inheritance studies involving controlled crosses provide the only direct verification of the electrophoretic phenotypes of each microsatellite locus (Stahl and Ryman 1982). In turn, this allows testing directly for Mendelian inheritance, the presence of null alleles, and an indication of homoplasy. Analysis of genetic data from controlled crosses should be performed for each microsatellite locus that will be used in salmonid genetic studies. This is particularly needed when genetic results will be used to guide management decisions. In this article we verify disomic segregation of seven microsatellite loci, report the presence of null alleles at two loci, identify a tandemly duplicated locus, identify pairwise linkage associations between loci, and uncover hidden levels of genetic variation in the form of size homoplasy.

Methods

Adult steelhead trout were mated in controlled crosses at the National Marine Fisheries Service laboratory in Little Port Walter, Alaska. Tissue samples were sent to the University of Minnesota where we extracted and analyzed DNA at microsatellite loci.

Controlled Crosses

In June 1996, four single-pair crosses were made with *O. mykiss* adults from the Sashin Creek drainage on Baranof Island in southeastern Alaska. Each dam and sire were only crossed once (i.e., four males and females were used to create four unique families, A–D). Juveniles ($N = 70$ for families A, C, and D or 61 for family B) were sampled from each full-sib family in March 1997 when they were approximately 10 months old. The survival from hatching (August 1, 1996) until sampling (March 1997) for these four groups averaged 92.5%.

Electrophoretic Methods

Whole juvenile fish and fin clips from the mated adults were preserved in 70% ethanol and shipped from Juneau, Alaska, to the Aquatic Genetics (AquaGen) Lab at the University of Minnesota for genetic analysis. DNA extractions and determinations of genotypes of all the parents and offspring at microsatellite loci were completed as described in Miller and Kapuscinski (1996). Briefly, the DNA was extracted using the chelex method and PCR amplification took place in 15 μl reactions using a Hybaid thermocycler. Each mixture contained 6 μl supernatant from the tissue sample, 7.5 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 1× Promega reaction buffer, and 0.5 units of *Taq* DNA polymerase (Promega). Temperature profiles consisted of an initial denaturing of 30 s at 94°C; the samples were cycled 38 times through the following steps: denaturing for 30 s at 94°C, annealing for 30 s at a primer-specific temperature, and elongation for 30 s at 72°C; we extended the final elongation step to 10 min. Each amplified microsatellite locus was then fractionated through an 8% nondenaturing polyacrylamide gel, stained with ethidium bromide, and visualized using UV light. We used three techniques to minimize scoring errors within and between gels. First, we determined the allele sizes of all the dams and sires on an ABI 377 automated sequencer. Second, 1 μg of 1 kb molecular weight standard was included in three lanes of each gel. Finally, progeny and parents from multiple crosses were run on the same gel to verify mobility patterns among individuals.

Segregation Studies

Using primers developed in six different species we tested for mode of inheritance and presence of null alleles at 12 microsatellite loci (Table 1) by observing genotypic proportions. Conformance of observed genotypic proportions to expected Mendelian proportions (1:1, 1:2:1, and 1:1:1:1) was tested via chi square (Zar 1984). The presence of duplicated loci was indicated by any observations of more than two segregating alleles using one pair of primers in one individual. Candidate electromorphs for size homoplasy were identified by the presence of different heteroduplex structures among individuals with the same apparent genotype.

Linkage Analysis

The computer program LINKMFEX (developed by Roy G. Danzmann, University of Guelph, Ontario) was used to execute the linkage analysis through a series of pairwise comparisons between loci. This program allowed the use of crosses between noninbred individuals for linkage analysis by considering the segregation of each parental complement of alleles separately. The linkage phase within the parents of these crosses was unknown so it was not possible to differentiate pseudolinkage from classical linkage between alleles inherited from the sire (Jackson et al. 1998). Log of odds ratio (LOD) scores were used

Table 1. **Informative families and individuals for examining the mode of transmission and segregation of the 12 (11 initially examined and 1 discovered duplicated locus used in this study) microsatellite loci**

Locus name	Number of alleles	Informative families for Mendelian ratio test[a]	Informative parents for linkage analysis[b] Dam	Sire	Species in which microsatellite primers were characterized	Reference for primer sequences
*Omy*77[c]	6	All	A,B,C	A,B,D	*Oncorhynchus mykiss*	Morris et al. 1996
*Omy*207[d]	7	All	All	A,C	*Oncorhynchus mykiss*	O'Connell et al. 1997
*One*µ1[d,e]	7	(B,C,D)[f]	(B,C,D)[f]	(B,C,D)[f]	*Oncorhynchus nerka*	Scribner et al. 1996
*One*µ11[c]	3	All	All	B	*Oncorhynchus nerka*	Scribner et al. 1996
*One*µ14[c]	3	A,B,C	A,B	A	*Oncorhynchus nerka*	Scribner et al. 1996
*Ots*1[c]	3	A,B,D	A	B,D	*Oncorhynchus tshawytscha*	Hedgecock D, personal communication
*Ssa*14[d]	3	A,B,C	B,C	A,C	*Salmo salar*	McConnell et al. 1995
*Ssa*85[d]	5	B,C,D	B,D	B,C,D	*Salmo salar*	O'Reilly et al. 1996
*One*µ8[c]	1	None	None	None	*Oncorhynchus nerka*	Scribner et al. 1996
*Sfo*8[d]	1	None	None	None	*Salvelinus fontinalis*	Angers et al. 1995
µ73[d]	1	None	None	None	*Salmo trutta*	Estoup et al. 1993

[a] One or both of the parents were heterozygous.
[b] The specified parent is heterozygous at this locus.
[c] PCR annealing temperature of 54°C.
[d] PCR annealing temperature of 50°C.
[e] Data revealed this primer pair to amplify tandemly duplicated loci.
[f] Only informative for *One*µ1 haplotypes.

to determine linkage. A minimum LOD score of 3.0 was interpreted as significant linkage (Botstein et al. 1980).

Microsatellite Sequencing

We excised the electromorph of interest from the 8% nondenaturing polyacrylamide gel and placed it into a 0.5 ml ependorf tube with 5 µl of water. We then placed the solution at 4°C overnight. Next, we exposed the solution in the tube to 12,771× G by placing it in a microfuge for 1 min. Then 2 µl of supernatant was used as a template for a second PCR amplification of the electromorph with the same primers and PCR reaction conditions used in the initial amplification. The PCR products were then fractionated as previously described and the same electromorphs were excised and placed in 0.5 ml ependorf tubes with 5 µl of water and allowed to stand overnight at 4°C. Finally, 2 µl of the supernatant was used as a template for sequencing by the Microchemical Facility at the University of Minnesota using an ABI 373 automated sequencer. Electropherograms of the sequences were viewed and compared using the Sequencer software (Gene Codes Corp.).

Results

Genotypes for each of 12 loci were determined for all eight parents and the 271 progeny. Of the 48 genotypic ratios observed (12 loci × 4 crosses), 18 came from crosses between homozygous parents and resulted in offspring identical to the parents or with the expected heterozygote phenotype; the only exception was cross C at *One*µ14 (Table 2). Six of the 30 remaining genotypic ratios (for which one or both parents were heterozygous) did not conform to expected Mendelian ratios because of unexpected genotypes (Table 2). The informative families for testing if the observed segregation ratios are consistent with Mendelian expectations are listed in Table 1.

Null Alleles Detected

Three of the seven genotypic ratios that did not conform to Mendelian expectations, because of unexpected genotypes, can be explained by the presence of null alleles at two loci (Table 3). These includ-

Table 2. **Segregation of microsatellite alleles at loci that did not conform to Mendelian expectations because of unexpected phenotypes**

Locus	Family	Parental phenotypes Dam	Sire	Progeny phenotypes[a]									χ^{2b}
*Omy*77				105/105	105/121	117/117	117/121	121/121	121/133	133/133			
	C	121/133	121/121					38 (35)	14 (35)	[18]			—
	D	121/121	105/117	[18]	22 (35)	[15]	15 (35)						—
*One*µ1[c]				113:141/113:141	113:141/117:135	113:141/115:135	113:141/117:135	113:141/119:135	113:141/123:135	115:135/117:135	117:135/123:135	117:135/119:135	
	A	113:141/113:141	115:135/115:135			70 (70)							—
	B	113:141/117:135	113:141/115:135	12 (15.25)		15 (15.25)	13 (15.25)			21 (15.25)			NS (3)
	C	119:135/119:135	113:141/117:135					33 (35)				37 (35)	NS (1)
	D	113:141/117:135	113:141/123:135	15 (17.5)	22 (17.5)				15 (17.5)		18 (17.5)		NS (3)
*One*µ14				148/148	148/156								
	C	148/148	156/156	[40]	30 (70)								—

[a] Upper value is the observed number; lower value in parentheses is the expected number; numbers of unexpected phenotypes in brackets.
[b] Degrees of freedom in parentheses.
[c] Data revealed this primer pair to amplify tandemly duplicated loci.

Table 3. Evidence for null alleles at two microsatellite loci in *O. mykiss*

Locus	Cross	Parental genotypes		Progeny phenotypes[a]				χ^{2b}
		Dam	Sire					
*Omy*77				121/Null or 121		121/133	133/Null	
	C	121/133	121/Null	38[c] (35)		14 (17.5)	18 (17.5)	NS (2)
*Omy*77				105/Null	105/121	117/Null	117/121	
	D	121/Null	105/117	18 (17.5)	22 (17.5)	15 (17.5)	15 (17.5)	NS (3)
*One*μ14				148/Null	148/156			
	C	148/148	156/Null	40 (35)	30 (35)			NS (1)

[a] First value is observed progeny phenotype frequency; value in parentheses is expected progeny genotype frequency according to Mendelian ratios.

[b] Degrees of freedom in parentheses.

[c] 121/Null heterozygotes cannot be distinguished from 121/121 homozygotes.

ed cross C at locus *Omy*77 and *One*μ14 and cross D at *Omy*77. Both loci fit the expected Mendelian ratios ($P > .05$) when accounting for the presence of a null allele.

A Duplicated Locus Discovered

The locus *One*μ1 had three or four alleles of different sizes for most individuals, suggesting that this primer set was amplifying a duplicated locus (Figure 1). Cosegregation between alleles at the duplicated loci indicated that they are closely linked (Table 2). Because we saw no recombination in the full sibs with at least one double heterozygous parent, we estimate that the loci are separated by less than 1 cM (Zanetto et al. 1996). Segregation of allele pairs at the linked loci conformed to Mendelian ratios (Table 2). Thus the segregation of these allele pairs indicates disomic inheritance of two tightly linked loci.

Homoplasy Detected at One Locus

Cross D gave three heteroduplex structures (only one heteroduplex phenotype was expected) during the amplification of *One*μ11, suggesting the presence of size homoplasy. Subsequent sequencing of 143 bp alleles at this locus confirmed the presence of size homoplasy within and among individuals in this population (Figure 2). The sequence data showed differences between the alleles in the repeat motif and flanking region.

Significant Pairwise Linkage Discovered

To include the tandemly duplicated *One*μ1 locus in the linkage analysis we treated each tightly linked pair of alleles as a haplotype. For example, the tandemly duplicated *One*μ1 alleles at 113 bp and 141 bp were always inherited together, indicating they were tightly linked loci on the same chromosome and were considered a haplotype in the linkage analysis. Two pairs of microsatellite loci showed linkage associations in sires with an LOD score greater than 3.0 (Table 4). None of the dams showed linkage associations with an LOD score greater than 3.0 in all pair wise tests. The informative parents for detecting pairwise linkage are listed by locus in Table 1.

Discussion

Genetic data from microsatellite loci are currently being used in research and genetic conservation applications that assume selectively neutral autosomal disomic inheritance of unlinked codominant alleles. Some examples of current and potential applications of microsatellite data to the management of *O. mykiss* include mixed-stock fisheries analysis (Utter and Ryman 1993), kinship analysis (Doyle et al. 1995), determination of population structure (Nielsen et al. 1994; O'Connell et al. 1997), marking of hatchery populations (Williams et al. 1996), genetic analysis of historical tissues (Rivers and Ardren 1998), estimation of effective population size (Waples 1989), broodstock management (Herbinger et al. 1996), and determination of evolutionarily significant units for listing under the Endangered Species Act (Moritz et al. 1995; Waples 1995). Our results address the assumptions underlying these various uses of microsatellite data in *O. mykiss.*

Mode of Inheritance

Before this study, salmonid geneticists typically relied on indirect evidence (conformance of allelic data to Hardy–Weinberg proportions or unpublished inheritance studies) to verify the assumptions that the inheritance of microsatellite alleles in steelhead trout was selectively neutral, codominant, autosomal, and disomic (Herbinger et al. 1996; Nielsen et al. 1994; O'Connell et al. 1997; Wenberg et al. 1996). In some recent studies (Nielsen et al. 1994; O'Connell et al. 1997; Wenberg et al. 1996), statistical power to detect deviations from Hardy–Weinberg was weakened by low sample sizes and allelic rich loci (Fairbairn and Roff 1980; Lessios 1992). The controlled crosses carried out in this study are the first to directly confirm disomic, codominant, and autosomal inheritance for all the observed electrophoretic variants at 7 of the loci examined and the first to screen all 12 loci for the presence of size homoplasy in *O. mykiss.* Because we only examined Mendelian segregation we could not test for selective neutrality. These results increase the confidence with which geneticists can use microsatellite data for the management, conservation, study of evolution, and aquaculture of *O. mykiss.*

Null Alleles

It is common for microsatellite loci to have null alleles. Researchers working with red deer found three loci segregating null alleles in the first 16 microsatellite loci screened (Pemberton et al. 1995). Null allele frequencies of up to 15% have been reported in black bears (Paetkau and Strobeck 1995), and they are found in up to 25% of loci examined in humans (Callen et al. 1993). Our findings are concordant with these values; we found null alleles at 16.67% of the loci examined and the frequencies of the null alleles were 12.5% and 6.25% for *Omy*77 and *One*μ14, respectively. No obvious pattern was observed in the occurrence of null alleles. The two microsatellite loci that segregated null alleles were first characterized in *O. mykiss* (*Omy*77) and *O. nerka* (*One*μ14), indicating that loci with null alleles are likely to occur in the first characterized species as well as in other species screened.

Not accounting for null alleles could lead to mistaken interpretations when estimating the level of inbreeding in the populations and comparisons of heterozygos-

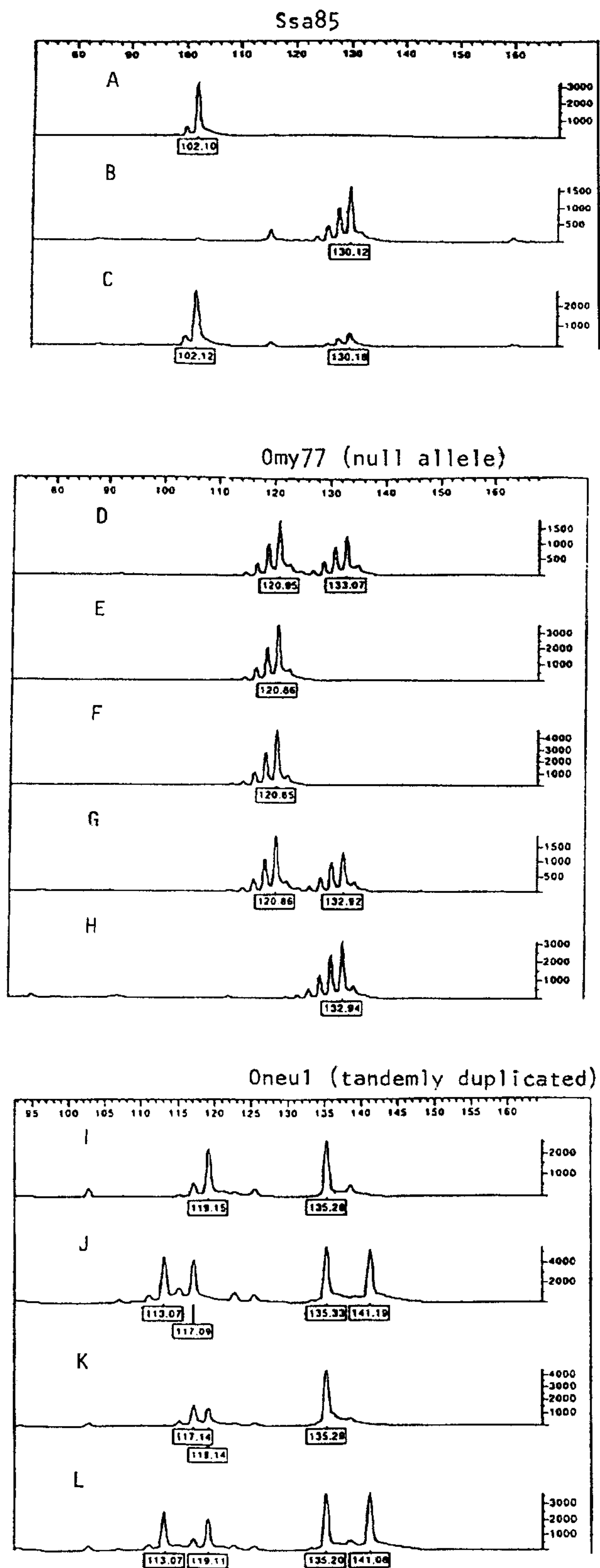


Figure 1. Segregation of three microsatellite loci through one generation for a normally segregating locus (*Ssa*85), a locus segregating a null allele (*Omy*77), and a tandemly duplicated locus (*One*μ1). DNA source: lanes A–C = cross A dam, sire, and one offspring; lanes D–H = cross C dam, sire, and two progeny with expected phenotypes and one progeny with a phenotype suggesting the segregation of a null allele; lanes I–L = cross C dam, sire, and the two progeny phenotypes observed for the tandemly duplicated locus.

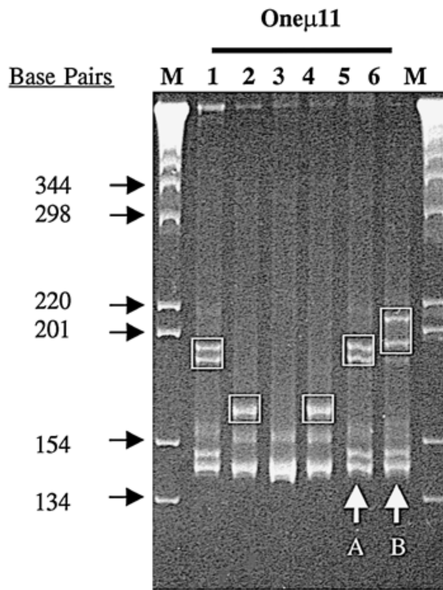
ities among populations that might differ in the frequency of null alleles. In fact, published data showing heterozygote deficiencies (Nielsen et al. 1994) might be due to the presence of null alleles. Researchers should also be careful in concluding that the nonamplification of a sample results from DNA degradation or other technical reasons rather than from the presence of null alleles.

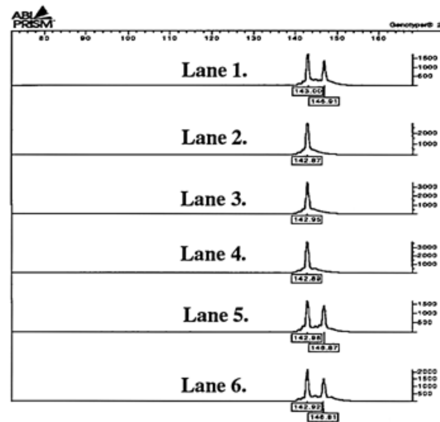
Without data from controlled crosses it is virtually impossible to estimate null allele frequencies in a wild population. Although it is possible to estimate null allele frequencies from a deficiency of heterozygotes (Brookfield 1996), such deficiencies are also consistent with a Wahlund effect or inbreeding. Because of limited funding, time, and facilities, it is impractical to carry out controlled crosses in every population examined and it may be possible to crudely differentiate the effects of null alleles from a Wahlund effect or inbreeding by the number of loci exhibiting the heterozygote deficiency. A population-level cause of heterozygote deficiency should affect the majority of loci, while the effect of null alleles would usually affect less than 25% of the loci. If a heterozygote deficiency was seen in less than 25% of the loci, it therefore is most likely caused by the segregation of null alleles at those loci (Bentzen et al. 1996).

Tandemly Duplicated Locus

The family Salmonidae have diverged from a single tetraploid ancestor in the last 25–100 million years (Allendorf and Thorgaard 1984). Many duplicated salmonid allozyme loci have been tested for joint segregation in at least one species or hybrid and none of these loci was found to be classically linked with its duplicate (Johnson et al. 1987; May et al. 1982). Johnson et al. (1987) speculated that the nonlinkage of duplicated loci is compelling evidence that the salmonid genomes arose from tetraploidy and not from tandem duplications.

We found one of nine (11%) informative loci revealed a duplicated locus. Others have apparently found duplicated loci in *O. mykiss.* O'Connell et al. (1997) speculated that loci *Omy*278 and *Omy*287 were duplicated; unfortunately the authors showed no data but reported that the inheritance patterns of these loci were unclear. Duplicated loci have also been reported in other species of *Oncorhynchus.* The locus *Fgt1* has been described as duplicated in *O. gorbuscha* (Spruell et al. 1999), *O. nerka* (Allendorf FW, personal





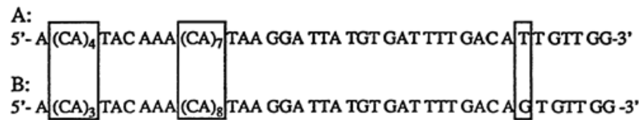


Figure 2. Comparison of the ability of nondenaturing versus denaturing electropherograms to detect the presence of size homoplasy. I. A nondenaturing electropherogram exhibiting the segregation of *One*μ11 through one generation in cross D: lanes 1 and 2 = dam and sire; lanes 3–6 = the four electromorph phenotypes exhibited by the progeny in a 1:1:1:1 ratio. The white boxes emphasize the heteroduplex structures that are diagnostic for the presence of size homoplasy. II. A denaturing electropherogram from an ABI automated sequencer showing the phenotypes of the samples in I. The samples are run in the same order with lane 1 in I = lane 1 in II and so on. III. Sequences of the A and B band. Sequence differences are indicated in the boxes (strand orientation 5′-3′).

communication), and in *O. mykiss* (Young et al. 1998). Young et al. (1998) showed that inheritance data for these loci could only be explained by tetrasomic inheritance. Scribner et al. (1996) found *One*μ18 to be duplicated in *O. tshawytscha* and *Salmo salar* but did not determine the mode of inheritance of these loci.

The duplicated loci amplified with the *One*μ1 primer pair appear to be tightly linked due to the cosegregation of alleles in the controlled crosses. Such tight linkage (<1 cM) may be the result of a tandem duplication of this locus in *O. mykiss* rather than residual ancestral tetraploidy (Allendorf and Danzmann 1997; Johnson et al. 1987). This duplication event has been observed in two other populations of *O. mykiss*; Knife River, a Lake Superior tributary (Ardren WR, unpublished results) and the Arlee strain located at the Jocko River State Trout Hatchery in Arlee, Montana (Knudsen KL, personal communication). The *One*μ1 locus also appears to be duplicated in bull trout (*Salvelinus confluentus*) (Spruell P, personal communication). These findings indicate that the duplication of the *One*μ1 locus is not a population or species-specific phenomenon. It is interesting to note that the *One*μ1 locus does not appear to be duplicated in six other species of *Oncorhynchus, Salmo salar, Salvelinus malma,* or two other populations of *O. mykiss* (Scribner et al. 1996; Wenburg et al. 1996). This could be because the populations or species examined had not undergone the duplication event, the individuals examined were not informative (i.e., had only one or two alleles), mutation precluded amplification of one locus, or there were scoring errors.

Duplicated loci can share alleles so that alleles cannot be unambiguously assigned to one locus or the other; when this occurs the loci are termed isoloci. It can be difficult to determine how many doses of each microsatellite allele are encoded by isoloci. Some authors, therefore, have been hesitant to use isoloci for population genetic analysis (Spruell et al. 1999; Wagner et al. 1994; but see May et al. 1997). Because we have confirmed Mendelian inheritance, it is possible to use this tandemly duplicated locus in other applications such as gene mapping, paternity studies, and kinship analysis in this population. Allendorf and Danzmann (1997) caution that while isoloci may be inherited in a Mendelian fashion, there still may be residual tetrasomy in other populations. However, we feel that the likelihood of residual tetrasomy in other populations is diminished by the tight classical linkage between these two loci.

Pairwise Linkage

The *O. mykiss* genome consists of 58–64 chromosomes with 104 chromosome arms (Thorgaard 1983) and contains approximately 2.4 × 10^9 base pairs (Ohno and Atkin 1966). The large number of haploid chromosome arms (52) found in *O. mykiss* have led researchers using a small number of microsatellite loci in population-level studies to discount the possibility of linkage between loci. Significant linkage between loci would violate the assumption of independent assortment and hence create a correlation between the information provided by those loci. This could bias the results of some genetic studies such as power analysis of parentage assessment (Estoup et al. 1998) and estimates of ef-

Table 4. Microsatellite loci exhibiting significant pairwise linkage in four *O. mykiss* full-sib families

Loci	Informative parent (family)	Observed progeny genotypes				LOD score	Recombination fraction ± SE
		AB	A′B	AB′	A′B′		
*One*μ11–*Ssa*85	Sire (B)	2	26	32	1	13.17	0.049 ± 0.028
	Dam (B)	21	15	14	11	0.032	0.48 ± 0.064
	Dam (D)	17	17	19	17	0.012	0.49 ± 0.06
*Omy*207–*One*μ1	Sire (C)	2	35	32	1	15.70	0.043 ± 0.024
	Dam (B)	20	16	6	19	1.04	0.36 ± 0.061
	Dam (D)	18	19	13	20	0.112	0.46 ± 0.06

LOD values greater than 3.0 are significant.

AB, A′B, AB′, A′B′ represent the four possible allelic combinations in the progeny inherited from the informative parent.

fective population size using the linkage disequilibrium method (Waples 1991).

In this study significant LOD scores were found only in sires. This difference in recombination rates between males and females is thought to be a consequence of the tetraploid ancestry of salmonids (Jackson et al. 1998; Wright et al. 1983; Young et al. 1998). In salmonids, recombination fractions are almost always lower in males than females. For example, three pair of loci that display random segregation in females show an average of only 10% recombination in males (May and Johnson 1990). This difference in recombination fractions between the sexes is thought to be caused by structural constraints imposed on crossing over by multivalent pairing, which only occurs in males. The linkage exhibited in males reported here could be due to classical or pseudolinkage (Wright et al. 1983).

The LOD scores of sires B and C indicate significant pairwise linkage of *One*μ1 to *Omy*207 and *Ssa*85 to *One*μ11. The LOD scores reported in females are not suggestive of pairwise linkage between these loci. It is difficult to predict the population-level effect of significant pairwise linkage found only in males. The degree of linkage may vary among populations (Allendorf and Dazmann 1997) and its effect on linkage disequilibrium depends on the proportion of males in the population and the number of generations in which the population has been randomly mating.

The recombination fraction of 0.0 between the duplicated *One*μ1 loci was present in both sexes. Because females also had no recombinants, we believe these two loci are tandemly duplicated on the same chromosome less than 1 cM apart. This very tight classical linkage means these loci essentially act as a single locus and may be advantageous for kinship and mapping studies because they double the number of possible polymorphisms. However, tandemly duplicated loci maybe difficult to use in population genetic studies because of problems with identification of doses at isoloci and assigning alleles to a locus (i.e., in this study it was not possible to assign the alleles amplified by the *One*μ1 primer pair to one or the other linked locus).

Size Homoplasy

Size homoplasy at microsatellite loci was first identified by sequence data of identical-size electromorphs between distantly related subspecies of honeybees (Estoup et al. 1995). These researchers found no size homoplasy in the same subspecies or closely related subspecies. We found homoplasy within a species (*O. mykiss*). When not taken into account, the presence of size homoplasy can cause erroneous conclusions in population genetic, kinship, gene mapping, and paternity analyses.

Direct sequencing of alleles is the best way to verify the presence of size homoplasy although the presence of multiple heteroduplex structures for individuals with the same genotype provides indirect support. The majority of methods used to visualize microsatellite electromorphs involve denaturing gels that eliminate the heteroduplex structures so that the occurrence of size homoplasy would go unnoticed (Figure 2). Not accounting for homoplasy when it is present leads to underestimation of genetic distances between populations. The magnitude of bias caused by homoplasy is proportional to the number of common-size alleles that differ in sequence between the examined populations (Estoup et al. 1995). Homoplasy can also cause errors in paternity, kinship, and hybridization analyses by erroneously determining relatedness based on identical-in-state electromorphs that are not identical by descent.

Conclusions

We have presented four factors that influence microsatellite genetic data that are currently not being adequately addressed and accounted for in the salmon and population/conservation genetics literature. The findings of size homoplasy, null alleles, pairwise linkage associations among loci, and a tandemly duplicated locus indicate that we still have much to learn about the evolution and characteristics of these highly informative genetic markers. The fact that these four attributes were found by initially examining only 11 loci in four families suggests that they are common across microsatellite loci overall. Yet the majority of the microsatellite loci examined to aid in fisheries management and conservation issues (e.g, Endangered Species Act listing) have not undergone inheritance tests. These results demonstrate the need to conduct detailed species-specific inheritance studies whenever possible.

This study also has implications for all species in which microsatellite data are used. Once identified the types of deviations from the expected inheritance of microsatellite markers we found in *O. mykiss* and other researchers have found in other taxa can be reasonably incorporated into the genetic analyses. Other genetic markers also have problems associated with them. Researchers using mitochondrial DNA must deal with the possibility of mistaking nuclear-integrated sequences as a cytoplasmic sequence (Zhang and Hewitt 1996). The use of RAPDs for conservation genetic and molecular genetic methods has suffered from difficulty in obtaining repeatable results (Queller et al. 1993). We therefore believe that it is dangerous to base conservation or management decisions on the results of one marker type. The most robust conservation genetic studies will be those that rely on the results from a variety of marker types.

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