Secondary Tetrasomic Segregation of MDH-B and Preferential Pairing of Homeologues in Rainbow Trout

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ABSTRACT

We examined the inheritance of allelic variation at an isozyme locus, MDH-B, duplicated by ancestral polyploidy in salmonid fishes. We detected only disomic segregation in females. Segregation ratios in males were best explained by a mixture of disomic and tetrasomic inheritance. We propose a two-stage model of pairing in male meiosis in which, first, homologous chromosomes pair and recombine in the proximal region of the chromosome. Next, homeologous chromosomes pair and recombine distally. We suggest that this type of tetrasomic inheritance in which centromeres segregate disomically should be referred to as "secondary tetrasomy" to distinguish it from tetrasomy involving entire chromosomes (i.e., "primary tetrasomy"). Differences in segregation ratios between males indicate differences between individuals in the amount of recombination between homeologous chromosomes. We also consider the implication of these results for estimation of allele frequencies at duplicated loci in salmonid populations.

GENETIC analysis of autopolyploids is extremely complicated relative to the elegant simplicity of disomic Mendelian inheritance in diploids. Unlike disomic ratios, tetrasomic ratios are affected by differential pairing affinities among chromosomes, multivalent formation, frequency of crossovers, and the position of a locus in relation to the centromere. The complexities of tetrasomic inheritance have been discussed in detail by many authors (e.g., Matther 1936; Little 1945; Marsden et al. 1987; Solts and Solts 1989); the most comprehensive treatment is that of Burnham (1962).

An autotetraploid lineage is expected to demonstrate tetrasomic chromosome segregation immediately following genome duplication. Over time, however, diploidization of the genome occurs, and disomic segregation becomes prevalent (Waines 1976). Studies of isozymes have presented evidence that such a diploidization process is occurring in the recent autopolyploid treefrog, Hyla versicolor (Danzmann and Bogart 1983; Marsden et al. 1987). Tetrasomic, disomic, and intermediate ratios have all been found at some loci in some individuals of this species.

Twelve genera containing some 72 species of the family Salmonidae (Nelson 1994) have all diverged from a single tetraploid ancestor in the last 25–100 million years (Allendorf and Thorgaard 1984). Studies of the inheritance of isozyme encoding loci in several species of the tetraploid derivative salmonid fishes have revealed a number of exceptions to disomic segregation in males (reviewed in Wright et al. 1983; Allendorf and Thorgaard 1984).

This paper presents results of an intensive effort to understand the pattern of inheritance of a pair of loci encoding the cytosolic form of malate dehydrogenase (EC 1.1.1.37; MDH-B, Bailey et al. 1970) in the rainbow trout (Oncorhynchus mykiss). MDH-B is encoded by two iso loci (i.e., loci that share alleles so that alleles cannot be unambiguously assigned to one locus or the other; Allendorf and Thorgaard 1984) and have been shown to exhibit partial tetrasomic segregation in males (Max et al. 1982; Allendorf and Thorgaard 1984). Those authors, however, reported data from only two crosses with appropriate genotypes to detect partial tetrasomic inheritance in females. The purpose of the present paper is to test for any evidence of partial tetrasomy in additional females, to test for evidence of double-reduction division in males, and to test for differences in the frequency of tetrasomy in individual males.

MATERIALS AND METHODS

Electrophoresis: MDH-B phenotypes were examined from white skeletal muscle by horizontal starch gel electrophoresis for 3 hr at 200 V, as described by Allendorf et al. (1977) with an amino-propyl morpholine buffer pH 6.6 (Clayton and Tretiak 1972).

Inheritance experiments: Experimental crosses were made in late November or December with the Arlee strain of rainbow trout. The origin and maintenance of this strain is described in Leary et al. (1983). The initial letter of the cross designation indicates the year the cross was made; the first was 1979, G-crosses, and the last year was 1983, K-crosses. Gametes were removed from 160 ripe fish each year and stored at 3°C while phenotypes were scored on parental fish. Parents of specific crosses were then selected on basis of their phenotype, and crosses were made within 36 hr after removal.

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of gametes. The developing eggs were held in incubating racks at 8°, and the fish were raised at 9°.

**Nomenclature:** Allozymes were described on the basis of their electrophoretic mobility relative to the most frequent allele in rainbow trout, which is designated *100 in accordance with the recommendations of Shaklee et al. (1984). Two allelic alternatives to the *100 allele were detected in this study, *83 and *74. For purposes of brevity and clarity in identifying genotypes, the *100 allele is called *1, the *83 allele is called *2, and the *74 allele is called *3.

**RESULTS**

**Models of inheritance and terminology:** The opaque-ness of tetrasomic inheritance is increased by a bewildering array of terms used in papers describing segregation in autopolyploids. We have tried to choose our terminology carefully using two major criteria: historical accuracy and simplicity (Table 1). For example, genotypes containing two copies of one allele and two copies of another (e.g., AAna) have historically been referred to as “duplex” (King and Stansfield 1985). Some recent papers have referred to these individuals as being “symmetrical heterozygotes” (Danzmann and Bogart 1983; Marsden et al. 1987); this is especially confusing because such symmetrical heterozygotes may in fact be homozygous at two disomic loci (Table 1).

We have previously published an electropherogram of phenotypes for MDH-B (Allendorf and Thorgaard 1984; also see Clayton et al. 1975). As indicated in Table 1, we refer to these phenotypes as nulliplex, simplex, duplex, triplex, and quadriplex. These terms were defined in the array of terms used in papers describing segregation allelic systems so that the prefix described the number of phenotypes for polyploidy. We have tried to choose our terminol-ogy carefully using two major criteria: historical accuracy and simplicity (Table 1).

**TABLE 1**  
Phenotypic and genotypic designations at an isolocus

<table>
<thead>
<tr>
<th>Tetrasomic genotype</th>
<th>Phenotypic designations</th>
<th>Tetrasomic genotype</th>
<th>Disomic genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>aaaa</td>
<td>0, nulliplex</td>
<td>*11 *11</td>
<td>*11 *11</td>
</tr>
<tr>
<td>Aaaa</td>
<td>I, simplex</td>
<td>*1122 *1122</td>
<td>*11 *12</td>
</tr>
<tr>
<td>AAaa</td>
<td>II, duplex</td>
<td>*1122 *1122</td>
<td>*11 *22</td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
<td>*12 *12</td>
</tr>
<tr>
<td>AAAa</td>
<td>III, triplex</td>
<td>*1222 *1222</td>
<td>*12 *22</td>
</tr>
<tr>
<td>AAAA</td>
<td>IV, quadriplex</td>
<td>*2222 *2222</td>
<td>*22 *22</td>
</tr>
</tbody>
</table>

**Segregation in females:** Segregation ratios from all 11 crosses between duplex (*1122) females and nulliplex (*1111) or simplex (*1112) males were compatible with disomic segregation. Four females produced only *12 gametes (Table 2) and are therefore assumed to be homozygous at both loci; one additional female (family J04) was homozygous at one locus (*11) and heterozygous (*23) at the other. Six females produced *11, *12, and *22 gametes (Table 3) and are assumed to be heterozygous at both loci. All of the crosses fit expected disomic ratios (P > 0.05), with exception of G05 (P = 0.03). However, this result is not significant.
when we take into account the six independent tests (Rice 1989).

Tetrasomic segregation ratios could be rejected ($P < 0.01$) in four crosses involving heterozygous duplex females; these deviations remain significant when we take into account the six independent tests. Segregation ratios in two crosses (G08 and J28) are compatible with both disomic and tetrasomic ratios. However, as discussed by Marsden et al. (1987), extremely large sample sizes are required to distinguish between disomic and tetrasomic ratios in progeny from heterozygous duplex parents. We conclude that the MDH-B isoloci segregate disomically in female rainbow trout from this strain.

**Double-reduction in males:** Twenty-three crosses produced by simplex males were examined for evidence of double-reduction divisions (Table 4). There were no double-reduction gametes in 22 of these crosses. However, in cross H04 (*1111 x *1112) two out of 152 progeny were *1222, as would be expected with double-reduction. It is unlikely that both double-reduction gametes would be produced by the same male by chance alone (exact $P < 0.06$). It is, therefore, possible that there is something unusual about segregation in this male.

Nevertheless, double-reduction is extremely rare. We estimated an overall rate of double-reduction by combining results from simplex males crossed with either nulliplex or simplex females. The total number of double-reduction gametes in simplex males is four times the number observed because we can only detect double-reduction division of the chromosome carrying the *2 allele. All double-reduction gametes (*22) will be observable in crosses with a nulliplex female. However, only one-half of the double-reduction gametes produced by males will produce a unique progeny phenotype (*1222) in crosses with simplex females. Thus, the

### TABLE 2

<table>
<thead>
<tr>
<th>Parents</th>
<th>Progeny</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1111</td>
<td>*1112</td>
</tr>
<tr>
<td>H13</td>
<td>*1122</td>
<td>*1111</td>
</tr>
<tr>
<td>H17</td>
<td>*1122</td>
<td>*1111</td>
</tr>
<tr>
<td>J03</td>
<td>*1122</td>
<td>*1112</td>
</tr>
<tr>
<td>J04</td>
<td>*1123</td>
<td>*1111</td>
</tr>
<tr>
<td>J31</td>
<td>*1122</td>
<td>*1111</td>
</tr>
</tbody>
</table>

*** $P < 0.001$.

* Expected numbers of progeny with disomic inheritance in parentheses and with tetrasomic inheritance in brackets.

* Degrees of freedom in parentheses.
TABLE 3

Frequency of progeny phenotypes in families from heterozygous duplex females

<table>
<thead>
<tr>
<th>Family</th>
<th>Parents</th>
<th>Progeny</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>G05</td>
<td>*1122   *1222</td>
<td>20/30/30/6</td>
<td>8.99* (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[7] [36] [36] [7]</td>
<td>22.91*** (3)</td>
</tr>
<tr>
<td>G06</td>
<td>*1122   *1111</td>
<td>32/57/19</td>
<td>2.94 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(27) [54] [27]</td>
<td>13.06** (2)</td>
</tr>
<tr>
<td>G08</td>
<td>*1122   *1222</td>
<td>20/66/87/25</td>
<td>4.08 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[25] [74] [74] [25]</td>
<td>7.72 (3)</td>
</tr>
<tr>
<td>I21</td>
<td>*1122   *1111</td>
<td>20/39/20</td>
<td>0.01 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20) [40] [20]</td>
<td>10.53** (2)</td>
</tr>
<tr>
<td>I22</td>
<td>*1122   *1111</td>
<td>13/40/25</td>
<td>3.68 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20) [39] [20]</td>
<td>13.73** (2)</td>
</tr>
<tr>
<td>J28</td>
<td>*1122   *1112</td>
<td>7/37/46/10</td>
<td>4.76 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[12] [38] [38] [12]</td>
<td>1.46 (3)</td>
</tr>
</tbody>
</table>

*Expected numbers of progeny with disomic inheritance in parentheses and with tetrasomic inheritance in brackets.

Degrees of freedom in parentheses.

****P < 0.05, 0.01, 0.001.

The estimate of \( \alpha \) for these data is the number of double-reduction gametes observed divided by the number of progeny from nulliplex females plus one-half the progeny from simplex females:

\[
\alpha = \frac{8}{(2,598)} = 0.003
\]

**Duplex males:** Fifteen crosses between duplex males and nulliplex females were examined to determine the mode of segregation in males. The distribution of \(*1112\) progeny in these crosses clearly separates the males into two distinct groups. In one group, over 91% of all progeny were \(*1112\); in the other group, from 40 to 65% of the progeny were \(*1112\). (Figure 2).

Duplex males with >90% \(*12\) gametes have been interpreted previously to be homozygotes that occasionally produce \(*11\) or \(*22\) gametes by homeologous exchanges (May et al. 1982; Allendorf and Thorgaard 1984). Contingency chi-squared analysis indicates no evidence of differences among these six males in the proportion of \(*12\) gametes produced (Table 5; \( \chi^2 = 8.50, 5 \text{ d.f.} \)). The average proportion of tetrasomic segregation in these six males is \( \Theta = 0.184 \).

Nine males produced 40–65% \(*12\) gametes and these males are apparently heterozygous at both loci (Table 6). The proportions of \(*12\) gametes produced in these crosses are highly heterogeneous (\( \chi^2 = 36.36, 8 \text{ d.f.}, P < 0.001 \)). We tested segregation ratios in these crosses with three different sets of expectations: disomic inheritance, tetrasomic inheritance, and a mixture of disomic and tetrasomic (\( \Theta = 0.2 \), based upon the average of the six homozygous duplex males from Table 5). The two degrees of freedom associated with segregation ratios in these families can be partitioned into one degree of freedom that tests for equality of the \(*11\) and \(*22\)

**TABLE 4**

Families used to test for double-reduction division in males

<table>
<thead>
<tr>
<th>Parents</th>
<th>No. families</th>
<th>No. progeny</th>
<th>No. DR progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1111   *1112</td>
<td>16</td>
<td>1961</td>
<td>0</td>
</tr>
<tr>
<td>*1111   *1112</td>
<td>1</td>
<td>152</td>
<td>2</td>
</tr>
<tr>
<td>*1112   *1112</td>
<td>6</td>
<td>970</td>
<td>0</td>
</tr>
</tbody>
</table>

DR, double-reduction.
TABLE 5
Frequency of progeny phenotypes in families from nulliplex females and apparently homozygous duplex males

<table>
<thead>
<tr>
<th>Parents</th>
<th>Progeny</th>
<th>χ² (1 d.f.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fam</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>H06</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>H18</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>I15</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>I20</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>I24</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>K37</td>
<td>*1111</td>
<td>*1122</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Proportion of heterozygous (*1112) progeny.

gametes, and one degree of freedom that tests for the ratio between the sum of these gametes and the *112 gamete. There are no significant differences in numbers of *11 and *22 gametes in these families. Therefore, we have tested for the mode of segregation by testing the ratio of the *112 gamete and the sum of the *11 and *22 gametes (1 d.f.).

The segregation ratios in these crosses do not fit any single segregation model. It is very difficult to distinguish between disomic segregation ratios (θ = 0.0) and the mixed model (θ = 0.2). Two crosses either have too few progeny (H14) or have intermediate ratios that are not informative (H19). Several crosses have ratios in which pure tetrasomic inheritance (θ = 1.0) is extremely unlikely, but either of the other two models fit the data (H05, H07, H12, and K36). Two crosses show tetrasomic ratios (K33 and K35) that are highly unlikely even with the mixed model of inheritance (P < 0.002 and P < 0.0001).

DISCUSSION

Disomy in females: The persistence of disomy in females indicates faithful pairing and segregation of homologues in both sexes. If tetrasomic inheritance in males involved an entire chromosome, then inclusion of two copies of the same homologue in a male gamete would produce an unbalanced zygote. That is, the zygote would contain one copy of one homologue and three copies of the other homologue. The resulting individuals could not segregate disomically because they would not possess the two copies of each homologue necessary for disomic pairing. Frequent homologous pairing in one sex should thus quickly restore tetrasomic segregation in the entire population. Therefore, the tetrasomic segregation occurring in males must not involve an entire chromosome.

Secondary tetrasomy in males: The persistence of disomic segregation in females indicates that the tetrasomic ratios in males apparently result from distal recombination between homologues (WRIGHT et al. 1983). This can be explained conceptually by a two-stage model of pairing in which, first, homologous chromosomes pair and recombine in the proximal region of the chromosome. Next, homologous chromosomes pair and recombine distally (Figure 3). If the crossover nearest the centromere determines the pattern of disjunction (BURNHAM 1962), then each gamete would receive one copy of each homologue, allowing persistent disomic segregation in females.

TABLE 6
Frequency of progeny phenotypes in families from nulliplex females and apparently heterozygous duplex males

<table>
<thead>
<tr>
<th>Family</th>
<th>Female</th>
<th>Male</th>
<th>*1111</th>
<th>*1112</th>
<th>*1122</th>
<th>he*</th>
<th>θ</th>
<th>χ² (1 d.f.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H05</td>
<td>*1111</td>
<td>*1122</td>
<td>52</td>
<td>92</td>
<td>58</td>
<td>0.455</td>
<td>0.000</td>
<td>1.60</td>
</tr>
<tr>
<td>H07</td>
<td>*1111</td>
<td>*1122</td>
<td>49</td>
<td>127</td>
<td>46</td>
<td>0.572</td>
<td>0.452</td>
<td>4.61*</td>
</tr>
<tr>
<td>H12</td>
<td>*1111</td>
<td>*1122</td>
<td>15</td>
<td>22</td>
<td>16</td>
<td>0.415</td>
<td>0.000</td>
<td>1.53</td>
</tr>
<tr>
<td>H14</td>
<td>*1111</td>
<td>*1122</td>
<td>5</td>
<td>11</td>
<td>4</td>
<td>0.550</td>
<td>0.300</td>
<td>0.920</td>
</tr>
<tr>
<td>H19</td>
<td>*1111</td>
<td>*1122</td>
<td>38</td>
<td>107</td>
<td>37</td>
<td>0.588</td>
<td>0.528</td>
<td>5.62*</td>
</tr>
<tr>
<td>K33</td>
<td>*1111</td>
<td>*1122</td>
<td>41</td>
<td>163</td>
<td>54</td>
<td>0.632</td>
<td>0.392</td>
<td>17.92***</td>
</tr>
<tr>
<td>K34</td>
<td>*1111</td>
<td>*1122</td>
<td>24</td>
<td>60</td>
<td>13</td>
<td>0.619</td>
<td>0.714</td>
<td>5.45*</td>
</tr>
<tr>
<td>K35</td>
<td>*1111</td>
<td>*1122</td>
<td>67</td>
<td>289</td>
<td>65</td>
<td>0.644</td>
<td>0.864</td>
<td>30.86***</td>
</tr>
<tr>
<td>K36</td>
<td>*1111</td>
<td>*1122</td>
<td>25</td>
<td>38</td>
<td>25</td>
<td>0.432</td>
<td>0.000</td>
<td>1.63</td>
</tr>
</tbody>
</table>

* Chi-squared values for expected disomic ratios, tetrasomic ratios, and a mixture of disomic and tetrasomic ratios (θ = 0.2).
We have no direct chromosomal evidence for this model in salmonids. However, DARLINGTON (1929) and LAWRENCE (1931) first suggested that genetically similar bivalents pair secondarily during meiosis in polyploids. RILEY (1960) provided the first cytological evidence for secondary pairing of bivalents using hexaploid wheat (Triticum aestivum). He found that homeologous bivalents tended to be adjacent to one another during metaphase I. RILEY suggested that homeologous pairs are attracted together following bivalent formation because of their genetic similarity.

We suggest that this type of tetrasomic inheritance in which centromeres segregate disomically should be referred to as secondary tetrasomy to distinguish it from tetrasomy involving entire chromosomes (i.e., primary tetrasomy). WRIGHT et al. (1983) have described meiotic models of chromosome pairing based upon cytological evidence and segregation data to explain residual tetrasomy in salmonids. Their models also assume that segregation of homologues is based upon pairing of proximal regions and that tetrasomic segregation ratios result because of distal recombination between homeologues. Their models, therefore, would also be considered examples of secondary tetrasomic segregation.

**Double-reduction:** Our results indicate that double-reduction division is extremely rare for MDH-B in rainbow trout. This is in agreement with MAY et al. (1982) who found no evidence of double-reduction for these loci in 354 rainbow trout from two crosses between simplex males and multiplex females. In contrast, JOHN-SON et al. (1987) have reported a high frequency of double-reduction gametes at an isolocus (PALB-1.2) that is syntenic with MDH-B. WRIGHT et al. (1983) observed that double-reduction appears to be rare in general in salmonid fishes.

Double-reduction (i.e., the production of gametes containing two copies of an allele carried on sister chromosomes) will result only when three things occur (BURNHAM 1962, page 186): (1) formation of multivalents, (2) crossovers between the locus under study and the centromere, and (3) passage of the chromatid products of such crossovers to the same pole in anaphase I, followed by random chromatid segregation in anaphase II. Requirements numbers 1 and 2 do occur in males, as demonstrated by occurrence of residual tetrasomic inheritance. Therefore, homeologous chromosomes that cross over in males must pass to opposite poles. Experiments with Drosophila and maize show that chromosomes that cross over do not pass to opposite poles (BURNHAM 1962).

Only two individuals in one cross (H04) showed evidence of double-reduction. Double-reduction can occur even if homeologous recombinant chromosomes pass to opposite poles if an additional crossover occurs (BURNHAM 1962, p. 186). In addition, double-reduction is not the only possible explanation for the two aberrant phenotypes in H04. For example, aneuploidy so that a chromosome (or part of a chromosome) was lost could produce a *112 genotype that would be extremely difficult to distinguish phenotypically from the phenotype of the *1122 genotype that would be produced by double-reduction.

**Differences between males:** The significant differences in segregation ratios between males (Table 6) suggest that there are differences between individuals in the amount of homeologous recombination. These differences could result from differential pairing affini-
ties (Little 1945). Experiments with a variety of plant and animal species have shown differential pairing affinities based upon homologies and degree of similarity (reviewed in Santos et al. 1983). For example, Grell (1961) has described preferential segregation in triploid D. melanogaster with two normal and one rearranged chromosome. The normal chromosomes tend to pair and segregate from one another so that the rearranged homologue tends to be found in diploid gametes. Diter et al. (1988) described preferential segregation of similar chromosomes in artificially constructed tetraploid male and female rainbow trout.

The Arlee hatchery strain used in the present matings were derived by crossing two different populations of rainbow trout that differed in allele frequencies at many loci some 10–12 generations ago (Leary et al. 1983). The telomeric regions in the chromosomes bearing MDH-B would be expected to be divergent between these previously isolated populations. The frequency of secondary pairing may be affected by the amount of sequence similarity between homeologues. Wright et al. (1983) have provided evidence that aberrant segregation ratios following interspecific hybridization in salmonids result from preferential secondary pairing affinities of chromosomal segments from the same species (i.e., preferential “homogenetic associations” in the sense of Stebbins 1947).

For example, males with low frequency of tetrasomic segregation (e.g., the homozygous duplex males in Table 5) may possess homeologues with distal segments that are derived from different populations, resulting in low frequency of secondary pairing. In contrast, males showing high frequency of tetrasomy (e.g., crosses K33 and K35, Table 6) may possess distal segments of homeologues that are derived from the same population.

If, as we believe (Leary et al. 1983), the two populations contributing to this strain had different allele frequencies for MDH-B, then we would expect the *1 and *2 alleles to be associated with the differences between homeologues. That is, we would expect residual nonrandom associations between the MDH-B loci and other loci (i.e., linkage disequilibrium). Thus, we would expect homozygous duplex males, based upon MDH-B genotypes, to tend to show less secondary pairing and tetrasomic segregation. However, this association should not be complete. It is likely that both alleles (*1 and *2) were present in at least one of the two populations; in addition, recombination since the origin of this strain should cause decay of any linkage disequilibrium. This would result in differences between males as seen in Table 6.

The question arises as to why no homozygous duplex males were detected with a high frequency of tetrasomic segregation. The answer can be seen with Figure 1. A homozygous duplex male with a high Θ (say 0.80) is indistinguishable from a heterozygous duplex male with high Θ. There is a bias in detection, homozygous duplex males with low Θ are the only ones that can be identified by segregation ratios. Thus, some of the males listed in Table 6 as showing disomic segregation assuming a heterozygous state may actually be homozygous with high tetrasomic segregation.

**Evolutionary implications:** A comparison of MDH-B from other salmonid species suggests that homeologous exchanges have continued to occur in all species and that they are not artifacts of hybridization associated with hatchery culture of salmonids. The MDH-B loci are isoloci in all salmonid species that we have tested (F. W. Allendorf, unpublished results). These include other closely related trout, salmon, and char species, as well as more distantly related species from the two other salmonid subfamilies (whitefish and grayling). Thus, the same pattern of divergence has occurred for the chromosomes bearing MDH-B in species that may have been separate lineages for as long as 25 million years or more (Allendorf and Thorgaard 1984).

The problem of the frequency of homeologous exchanges needed to prevent divergence between loci is analogous to effects of genetic exchange (gene flow) between populations on genetic divergence. The two MDH-B loci can be thought of as a single locus in two populations with homeologous exchanges acting analogously to gene flow between populations. Wright (1969) has shown that a single “migrant” per generation is sufficient to ensure that the same alleles will be shared by populations over long periods of evolutionary time. This does not mean that allele frequencies will be the same in all populations, but rather that the same alleles will be found in all populations (see discussion page 1508, Allendorf and Phelps 1981). We recognize that this is a somewhat crude analogy; a more realistic consideration of this problem would have to consider that the two MDH-B loci themselves occur in many different partially isolated populations within a species. Nevertheless, a surprisingly small frequency of homeologous exchange between MDH-B loci may be sufficient to maintain them as isoloci.

**Population implications:** A comprehensive treatment of estimation of allele frequencies at isoloci has been developed by Waples (1988). He assumed disomic inheritance and developed a maximum likelihood procedure to “identify the set of allele frequencies at the individual gene loci with the highest probability of producing the observed phenotypic distribution.” These methods provide important rigor to describe genetic divergence at isoloci among populations. Nevertheless, there are still serious problems. As pointed out by Waples (1988), there is a tendency for these methods to estimate equal allele frequencies at the isoloci. Even more importantly, there is no way to distinguish between homeologous loci in different populations. For example, Waples (1988) has adopted the convention that the locus with the lower estimated allele frequency of the common allele is arbitrarily designated as locus...
one \((L_1)\); however, as pointed out by Waples (1988), this does not imply homology with \(L_1\) in another population.

The present paper raises another set of problems with estimating allele frequencies at isoloci in salmonids. These statistical techniques assume disomic inheritance at both isoloci. This is not a valid assumption in salmonids because these loci have remained isoloci because of residual tetrasomy. The problems become statistically intractable when models incorporate differences in segregation between males and females, as well as differences in segregation between males. Furthermore, segregation analysis of \(MDH-B\) in another population of rainbow trout indicated disomic inheritance in both males and females (Allendorf and Thorgaard 1984). Thus, there are likely to be differences between populations in the occurrence and frequency of tetrasomic ratios.

The patterns of inheritance we have described do not appear to be unique to \(MDH-B\) in rainbow trout. Residual tetrasomy has also been described for aspartate aminotransferase (\(AAT; EC 2.6.1.1\)) in brook trout (Wright et al. 1980) and brown trout (Johnson et al. 1987), and a pair of eye-specific paralumin loci in rainbow trout that are syntenic to \(MDH-B\) (Johnson et al. 1987).

The most conservative approach is to make no assumptions about inheritance and simply estimate allele frequencies by a direct count of the number of alleles expressed by each individual in a population sample; this is computationally equivalent to treating the isoloci as a single tetrasomic locus (Leary et al. 1987). This requires being able to determine the number of doses of each allele present in each individual. The shortcoming of this approach is that these data are not amenable to use with standard indices of genetic divergence (Waples 1988). However, this shortcoming results from the biology of the situation and not inadequate statistical treatment of the data. That is, in many cases, isoloci are not two genetically distinct disomic loci. In the absence of segregation data for each population, we believe this is the best approach.

Some of these difficulties are illustrated by an analysis of \(MDH-B\) phenotypes that we have observed from Arlee rainbow trout (Table 7). We first estimated allele frequencies and expected genotypic proportions assuming a single tetrasomic locus; the observed phenotypic proportions do not differ significantly from expected tetrasomic proportions in any year. There is remarkable stability in the estimate of the frequency of the most common allele \((P)\), which varied only between 0.86 and 0.90 over 10 years. The values \(p1\) and \(p2\) are the estimates of allele frequencies assuming two disomic loci and using the method of Waples (1988). These allele frequency estimates varied considerably from year to year. This method appears to be extremely sensitive to the presence or absence of the triplex \((III)\) phenotype. Whenever this phenotype was present, the allelic variation was assigned equivalently to both loci.

The difficulties in estimating allele and genotype frequencies for isoloci detected with protein electrophoresis (isozymes) will also be a problem with nuclear DNA (nucDNA) markers detected with PCR. In fact, the problems may be worse for such nucDNA markers. It will be difficult to determine how many doses of each allele are present because the amount of PCR product may not accurately reflect the number of allelic doses present (Wagner et al. 1994). In addition, the presence of heteromeric isozymes also aids in estimating doses for enzymes (Allendorf et al. 1975; Waples 1988).

**Evolution of disomy**: The genetic basis of “diploidization” of an autopolyplloid genome has received surprisingly little attention in the plant genetics literature in view of the frequency of occurrence of polyploid plant species. The genetic basis of the mechanism for the evolution of homologous pairs in which only homologous pairs is largely unknown (e.g., Waines 1976). It is thought that diploidization occurs by gradual loss of genetic similarity through small changes until partial tetrasomy is replaced by functional disomy (Shaver 1963).

We are using the term autopolyplloid in the sense of Stebbins (1950, p. 150); that is, there are multivalents at meiosis and tetrasomic inheritance ratios. The mosaic of disomy and tetrasomy at various loci in salmonids is also consistent with segmental allopolyploidy. Nevertheless, polyploidy in the salmonids is likely to have involved some type of intersubspecific hybridization. Schultiz (1969) has proposed that tetraploidy could arise in an animal by a two-step process that includes (1) the origin of a unisexual triploid strain, followed by (2) fertilization by a normal haploid gamete of an unreduced gamete produced by the unisexual triploids.

The models of secondary tetrasomy discussed in this paper provide a mechanism for the gradual evolution of disomy. Sequence differentiation should gradually accumulate among homologous chromosomes. This differentiation will be greatest proximally because crossovers between chromosomes will exchange distal segments and thus delay accumulation of differentiation. As differentiation increases, it should continue to extend more and more distally because of reduced homologous crossovers. Eventually sufficient differentiation would accumulate so that only homologous pairing and crossovers occur.

There are alternatives to this model of gradual evolution of disomy. Structural chromosomal rearrangements may provide a rapid basis for distinction of homologues in meiosis. In addition, major loci have been discovered in many plant species that suppress pairing of homeologues and thus accelerate the diploidization process (Waines 1976). The \(Ph\) locus in wheat (\(Triticum aestivum\)) is the best genetically defined example of these pairing-promoting genes (Riley 1986). Lu et
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tion of the genetic control of duplicate loci through inheritance studies and the examination of populations, pp. 415–431 in Iso-
zymes IV: Genetics and Evolution, edited by C. L. MARKERT. Aca-
demic Press, New York.

alleles were pooled in this analysis.

al. (1996) have shown that this locus regulates chro-

some pairing by processing homology along the entire length of the chromosome.

Reviews of the literature describing segregation ratios in tetraploid plants (LITTLE 1958; SOLTIS and SOLTIS 1993) do not present any ratios intermediate between tetrasomy and disomy. This may partly result from the inclusion of many older studies in which gene doses could not be determined because of the use of recessive/dominant morphological markers. Even recent examinations of segregation ratios in tetraploid plants using isozyme loci often do not take gene dosages into account (e.g., QUIROS 1982; QUIROS and MCHALE 1985).

MARSDEN et al. (1987) have discussed difficulties of distinguishing segregation ratios in tetraploids. They analyze statistical difficulties and sample sizes needed to distinguish between the disomic 1:2:1 and tetrasomic 1:4:1 ratios. They conclude that there is only one definitive method of distinguishing disomic and tetrasomic ratios; the detection of duplex individuals that produce only heterozygous gametes can only be explained by disomic inheritance (e.g., Table 2). Similarly, detecting ratios intermediate between disomy and tetrasomy also relies upon segregation ratios from such homozygous duplex parents (e.g., Table 5). Unfortunately, many studies in plants have relied upon distinguishing between 1:2:1 and 1:4:1 ratios (MARSDEN et al. 1987).

The paucity of reports of persistent tetrasomic segregation or secondary tetrasomic inheritance in plants may result from a lack of studies using suitable experimental design to address these issues. As pointed out by SOLTIS and SOLTIS (1993), there were very few studies or reports of tetrasomic inheritance in plants before the last few years. This view is supported by work with the fern Ceratopteris thalictroides that provides evidence of homeologous segregation in homozygous duplex parents resulting in segregation ratios intermediate between disomy and tetrasomy (HICKOK 1978). Differences in frequency of homeologous pairing also pro-
vided evidence for differential pairing affinity based upon genetic similarity of the chromosomes.

Inheritance studies with the recent autotetraploid treefrog (H. versicolor) provide results similar to those found with salmonids (DANZMANN and BOGART 1982, 1983; MARSDEN et al. 1987). Some loci segregated disomically in some families and tetrasomically in others. In addition, some crosses yielded ratios that were intermediate between disomy and tetrasomy. These results with a fern and a treefrog suggest that the mechanisms operating in salmonids may be general in autopolyploids.

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LITERATURE CITED


ALLENDORF, F. W., F. M. UTTER and B. P. MAY, 1975 Gene duplica-
tion within the family Salmonidae. 2. Detection and determina-
tion of the genetic control of duplicate loci through inheritance studies and the examination of populations, pp. 415–431 in Iso-
zymes IV: Genetics and Evolution, edited by C. L. MARKERT. Aca-
demic Press, New York.

ALLENDORF, F. W., N. J. MITCHELL, N. RYMAN and G. STAHL, 1977 Isozyme loci in brown trout (Salmo trutta): detection and inter-

BAILEY, G. S., A. C. WILSON, J. HALVER and C. JOHNSON, 1970 Multi-


CLAYTON, J. M., D. N. TRETIAK, B. N. BILECK and P. IHSSEN, 1975

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*The *74 and *83 alleles were pooled in this analysis.


Lawrence, W. G. C., 1931 The genetics and cytology of Dablia variabilitas. J. Genet. 24: 257–324.


Communicating editor: W. F. Sheridan