Segregation models for disomic, tetrasomic and intermediate inheritance in tetraploids: A general procedure applied to Rorippa (yellow cress) microsatellite data

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ABSTRACT

Tetraploid inheritance has two extremes: disomic in allotetraploids and tetrasomic in autotetraploids. The possibility of mixed, or intermediate, inheritance models has generally been neglected. These could well apply to newly formed hybrids or to diploidizing (auto)tetraploids. We present a simple likelihood-based approach that is able to incorporate disomic, tetrasomic, and intermediate inheritance models and estimates the double-reduction rate. Our model shows that inheritance of microsatellite markers in natural tetraploids of Rorippa amphibia and R. sylvestris is tetrasomic, confirming their autotetraploid origin. However, in F1 hybrids inheritance was intermediate to disomic and tetrasomic inheritance. Apparently, in meiosis, chromosomes paired preferentially with the homolog from the same parental species, but not strictly so. Detected double-reduction rates were low. We tested the general applicability of our model, using published segregation data. In two cases, an intermediate inheritance model gave a better fit to the data than the tetrasomic model advocated by the authors. The existence of inheritance intermediate to disomic and tetrasomic has important implications for linkage mapping and population genetics and hence breeding programs of tetraploids. Methods that have been developed for either disomic or tetrasomic tetraploids may not be generally applicable, particularly in systems where hybridization is common.

POLYPLOIDY is considered to be a major evolutionary force in both plants and animals (Otto and Whitton 2000; Soltis and Soltis 2000; Mable 2004; Soltis et al. 2004). Recent genome analyses indicate that many extant diploids are actually ancient (diploidyized) polyploids (Wolfe and Shields 1997; Wolfe 2001; McLysaght et al. 2002; Bowers et al. 2003). A common mechanism of polyploidization is through fusion of unreduced gametes (Karpechenko 1927; Harlan and Dewey 1975; Bretagnolle and Thompson 1995) from the same or from different species, termed autotetraploidy and allotetraploidy, respectively (see Ramsey and Schemske 1998). However, Stebbins (1947) already recognized that autopolyploidy and allopolyploidy are the extreme ends of a range and introduced the term segmental allopolyploidy for intermediate cases. In extreme autotetraploids, each chromosome has four homologous versions (denoted \(A_1 A_2 A_3 A_4\)). Each chromosome may then pair randomly with any of its homologs in bivalents or quadrivalents during meiosis. This leads to tetrasomic inheritance; \(i.e.,\) all possible allelic combinations are produced in equal frequencies (Muller 1914), which is generally considered indicative of autotetraploidy (Soltis and Soltis 1993). Approaches have been developed to account for the complexities of tetrasomic inheritance in population genetic analyses (Moody et al. 1993; Ronfort et al. 1998; Luo et al. 2006b) and linkage mapping (e.g., Luo et al. 2004, 2006a).

In extreme allotetraploids, there are two homologous sets consisting of two homologous chromosomes each (denoted \(A_1A_2B_1B_2\)). If a chromosome exclusively pairs with its homolog, this leads to disomic inheritance, which is generally considered indicative of allotetraploidy (Soltis and Soltis 1993; Ramsey and Schemske 2002). This often surfaces as fixed heterozygosity in genetic analyses. Variation can be analyzed with the standard population genetic and linkage mapping tools developed for diploid organisms (Soltis and Soltis 1993; Cao et al. 2005).

Inheritance may shift from disomic to tetrasomic (or vice versa). In (tetrasomic) autotetraploids the four initially homologous chromosomes can differentiate into two sets of preferentially pairing chromosomes, resulting in (cyto)genetic diploidization (Sybenga 1969; Soltis and Soltis 1993; Wolfe 2001; Ramsey and Schemske 2002). In (disomic) allotetraploids meiotic pairing may not always be strictly preferential (Sybenga...
1996) so that crossing over between homoeologous chromosomes (e.g., Udall et al. 2005) can homogenize the genome (Sybenga 1996). A shift in inheritance pattern may take several generations with intermediate inheritance. Intermediate inheritance may also be expected in fertile interspecific hybrids, since their parents are usually related and therefore are expected to possess some degree of chromosomal homology, but on the other hand have diverged enough to earn their species status. Thus, particularly in systems where hybridization is common, individuals characterized by intermediate pairing preferences (i.e., characterized by inheritance intermediate between disomic and tetrasomic) may not be exceptional (e.g., Ramsey and Schemske 2002). The exact mode of inheritance greatly affects the segregation of variation in the offspring of such plants and is therefore of great interest, both from an evolutionary perspective and for breeding purposes. Moreover, neither the standard nor the tetrasomic-specific methods for population genetics and linkage mapping (see above) may be appropriate for tetraploids with intermediate inheritance.

In segregation studies, normally only the completely disomic and tetrasomic inheritance models have been considered, thereby discounting the possibility of intermediate pairing preferences. Several studies suggested intermediate pairing preferences as an explanation for inheritance patterns intermediate to disomic and tetrasomic, but lacked a method to evaluate this hypothesis statistically (Hickok 1978a, b; Danzmann and Bogart 1982, 1983; Marsden et al. 1987; Allendorf and Danzmann 1997). In a highly polyploid sugarcane hybrid lineage (2n ≈ 115), Janno et al. (2004) showed that pairing affinities among hom(e)ologous linkage groups ranged from 0 to 40%, leading to complex mixtures of disomic and polypolistic inheritance. In this article, we propose a similar, likelihood-based approach to statistically evaluate whether disomic, tetrasomic, or intermediate inheritance models best explain the segregation of genetic markers in tetraploids and test whether the homologous alleles show preferential pairing in hybrids.

We apply this approach to the perennial tetraploids Rorippa amphibia and R. sylvestris and their hybrid R. x anceps. The species form a polyploid complex with mainly diploids and tetraploids in R. amphibia and mainly tetraploids and hexaploids in R. sylvestris (Jonsell 1968). The natural hybrids are mostly tetraploids (our unpublished data). In R. amphibia, diploids are indistinguishable from tetraploids with respect to leaf morphology (Jonsell 1968), and other diploid close relatives are absent (Bleeker et al. 2002). This suggests an autotetraploid origin and the expectation to find tetrasomic inheritance. Diploids are absent in R. sylvestris, impeding speculations on the origin of tetraploids in this species and thus about the mode of inheritance.

We also study the mode of inheritance in artificial hybrids R. x anceps to evaluate whether the cytological divergence between the two species leads to a mostly disomic, tetrasomic, or intermediate pattern of inheritance. At the tetraploid level, the species can be crossed easily and F1 hybrids readily backcross with both parental species. We intend to use the increased segregation variance of hybrids (Lexer et al. 2003) for mapping of traits associated with flooding tolerance. The choice of linkage mapping tools depends on the exact mode of inheritance of the parental species and the hybrids (Cao et al. 2005; Luo et al. 2006a). We expect that intermediate inheritance models could very well apply to the Rorippa F1 hybrids, as their parents are closely related (also given the occurrence of fertile backcrossing hybrids in nature), while at the same time genomic differences exist that underlie the parental species’ distinct morphologies (Jonsell 1968) and habitat preference (Stift et al. 2008). Moreover, the two species differ in DNA content by ~16% (Stift 2007).

Despite the importance of exact knowledge of the mode of inheritance of tetraploids for evolutionary, genetic, and linkage analysis (Ronfort et al. 1998; Cao et al. 2005; Luo et al. 2006a, b) this is—to our knowledge—the first approach that accounts for the possibility of inheritance intermediate to disomic and tetrasomic and for double reduction. We specifically tested the general applicability of our approach by analyzing microsatellite segregation in tetraploid Rorippa species and F1 hybrids and reanalyzing some published tetraploid segregation data sets for which only the extreme (i.e., disomic and/or tetrasomic) inheritance models had been tested and compared.

MATERIALS AND METHODS

Plant material and crosses: During the growing seasons of 2003–2005, root and shoot fragments of tetraploid Rorippa amphibia (denoted AAAA) and R. sylvestris (denoted SSSS) were collected from several locations throughout Europe and grown in a greenhouse environment (Table 1). In the summer of 2004, we made reciprocal crosses between two independent wild-collected pairs of AAAA and SSSS (Table 1) to create first-generation (F1) hybrids. From each of the four resulting F1 hybrid seed families, we germinated ~50 seeds on filter paper moistened with 2 ml of a 3-μM gibberellic acid solution. Seedlings were transferred to soil and further grown in a common greenhouse environment. In the summer of 2005, one individual of each of the four F1 hybrid seedling families was backcrossed with an unrelated, wild-collected plant (Table 1) to create first-generation backcrosses (BC1). From each of the four resulting BC1 seed families, we again germinated ~50 seeds on 2 ml of a 3-μM gibberellic acid solution. Seedlings were transferred to soil and further grown in a common greenhouse environment.

DNA extraction and analysis of microsatellite loci: DNA was extracted from fresh leaves using a modified CTAB protocol (Doyle and Doyle 1987). We genotyped the wild-collected plants and the backcrossed F1 hybrids for 12 microsatellite loci (Stift et al. 2006). On the basis of this initial screening, we selected the most informative loci for each cross. Thus, ideally, each parent possessed four different alleles (i.e.,
fully heterozygous) and shared no alleles with its crossing partner. We genotyped each of the four F1 and the four BC1 offspring families for the selected loci. Offspring with genotypes that could be explained only by mutation or contamination (i.e., alleles observed that were not present in the parents) or nondisjunctions (i.e., more than two alleles observed from one of the parents) were excluded from the analyses. Such anomalous genotypes were found for two loci—RS44 (five times) and RS101 (eight times)—and never constituted >4% of the offspring within one family.

**Testing for reciprocal differences**: From the genotypes observed in the offspring of the experimental crosses, we reconstructed the parental gamete frequencies. In cases where the two parents had alleles in common, we worked with the observed genotype frequencies in the offspring. We used the likelihood G-test for contingency tables (Sokal and Rohlf 1995) to test whether the observed parental gamete frequencies differed between the reciprocal crosses.

**Gamete formation model**: Consider a tetraploid where each chromosome is marked by a different allele (e.g., ABCD). Under complete tetrasomic inheritance, assuming no double reduction, gametes carrying the allelic combinations AB, AC, AD, CD, and BC will occur in equal proportions (1/6) (Müller 1914). The maximum frequency of double reduction (α) is 1/6 which can be reached if quadrivalents always form at meiosis, one effective crossover occurs between the locus and its centromere, and the recombined chromosomes migrate to the same pole at anaphase 1 (adjacent orientation, Mather 1935). Under this scenario, the allelic combinations AB, AC, AD, CD, and BC will still occur in equal proportions (1/6), and there will be double-reduction gametes (AA, BB, CC, and DD) each at an expected frequency of 1/6.

Preferential (bivalent) pairing in meiosis leads to expected gamete frequencies characteristic of disomic inheritance. If alleles A and B mark homologous chromosomes that pair exclusively with each other, alleles A and B will never end up in the same gamete, and likewise for C and D marked chromosomes. This AB/CD pattern of pairing thus produces gametes with the allelic combinations AC, AD, BC, and BD in equal proportions (1/4). Double reduction is not possible with bivalent formation. The other possible pairings, namely of AC/BD and AD/BC; result in gametes AB, AD, BC, CD and AB, AC, BD, CD, respectively. The expected proportions (probabilities) of all possible gametes produced by an individual ABCD is calculated by the formulas:

\[
p(AB) = \frac{1}{6} \tau - \frac{1}{6} \beta \tau + (1 - \tau)\left(\frac{1}{4} \delta_2 + \frac{1}{4} \delta_3 \right)
\]

\[
p(AC) = \frac{1}{6} \tau - \frac{1}{6} \beta \tau + (1 - \tau)\left(\frac{1}{4} \delta_1 + \frac{1}{4} \delta_3 \right)
\]

\[
p(AD) = \frac{1}{6} \tau - \frac{1}{6} \beta \tau + (1 - \tau)\left(\frac{1}{4} \delta_1 + \frac{1}{4} \delta_2 \right)
\]

\[
p(CD) = \frac{1}{6} \tau - \frac{1}{6} \beta \tau + (1 - \tau)\left(\frac{1}{4} \delta_2 + \frac{1}{4} \delta_3 \right)
\]

\[
p(BC) = \frac{1}{6} \tau - \frac{1}{6} \beta \tau + (1 - \tau)\left(\frac{1}{4} \delta_1 + \frac{1}{4} \delta_2 \right)
\]

or in matrix notation.
These equations define a set of nonlinear equations with four unknown parameters (by definition $\delta_1 + \delta_2 + \delta_3 = 1$). The "tetrasomic" parameter ($\tau$) indicates the proportion of gametes formed by random meiotic chromosome associations (i.e., random bivalent or quadrivalent pairing) and can take values from zero (full disomic) to 1 (full tetrasomic). In the latter case, the entire last (disomic) part of the equation cancels out.

Without such a constraint, there would be an ambiguity in the calculation of the DR rate ($\beta$). The LRT was estimated; (2) three constrained intermediate models, in which the proportion of random segregation ($\tau$) and the DR rate ($\beta$) were estimated, while the disomic parameters were fixed at $\delta_1 = 1$, $\delta_2 = 1$, or $\delta_3 = 1$, respectively; and (3) three unconstrained intermediate models, in which $\tau$, $\beta$, and two of the disomic parameters were estimated, while the third was set to zero.

For partially informative cross-locus combinations (of type $ABCD$ or $AABC$) with some alleles shared with the crossing partner for that particular locus it is not possible to unambiguously reconstruct the parental gamete frequencies from the observed offspring genotypes. Therefore, we worked the other way around and calculated the expected offspring genotype frequencies from the expected gamete frequencies of the parents under the following parameter settings. We assumed full tetrasomic inheritance for one parent ($i.e., \tau = 1$) and let the $\tau$ of the other parent decrease from 1 (full tetrasomic) to 0 (full disomic) in steps of 0.01, at $\delta_1 = 1$, $\delta_2 = 1$, or $\delta_3 = 1$, respectively.

For each of these parameter settings and their expected offspring genotype frequencies we calculated the log-likelihood of the observed frequencies and identified the parameter settings that gave the largest likelihood ($i.e.,$ the best fit). This procedure was executed as a (more tedious) spreadsheet algorithm (Microsoft Excel) scanning the parameter space, rather than as a nonlinear regression problem.

For easier comparison across independent analyses ($e.g.,$ different loci, different crosses, different plants) and with published studies using a chi-square-based approach or $G$ goodness-of-fit test, we calculated the likelihood deviance $G = 2 \sum_{i=1}^{n} \text{obs} \ln(\text{obs}/\text{exp})$, corrected for use of discrete data (Williams' correction) (Sokal and Rohlf 1995) for the best-fit and null models rather than working with the likelihood scores directly.

We then evaluated whether intermediate models (with $\tau$ estimated 0 < $\tau$ < 1) provided a significantly better fit than the tetrasomic null model ($\tau = 1$) through a likelihood-ratio test (LRT). The LRT follows a chi-square distribution in which the degrees of freedom (d.f.) correspond to the difference in degrees of freedom between the two models compared (Sokal and Rohlf 1995). However, since the parameter value $\tau$ of the null model lies at its upper theoretical boundary ($i.e., \tau = 1$), the LRT has to be adapted to become one-tailed and should be tested against a compound distribution of $\chi^2_{\delta_1} + \chi^2_{\delta_2}/2$ (Self and Liang 1987). Essentially, this means that the $P$-value of a conventional $\chi^2$-test should be halved. Statistical comparisons of intermediate models (0 < $\tau$ < 1) with disomic null
TABLE 2
Fitting inheritance models on segregation of microsatellite loci in progeny of crosses involving wild-collected tetraploid *R. sylvestris* (SSSS) and *R. amphibia* (AAAA)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Locus</th>
<th>Genotype*</th>
<th>n</th>
<th>G</th>
<th>Pairing alleles</th>
<th>T</th>
<th>G</th>
<th>LRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSSS1</td>
<td>RS10</td>
<td>A A B C</td>
<td>99</td>
<td>6.73</td>
<td>—</td>
<td>1.00</td>
<td>6.73</td>
<td>0.00</td>
</tr>
<tr>
<td>SSSS1</td>
<td>RS44</td>
<td>A C I N</td>
<td>100</td>
<td>5.23</td>
<td>AN/GI</td>
<td>0.93</td>
<td>4.95</td>
<td>0.28</td>
</tr>
<tr>
<td>SSSS1</td>
<td>RS46</td>
<td>B B C C</td>
<td>99</td>
<td>1.81</td>
<td>BC/BC</td>
<td>0.64</td>
<td>0.23</td>
<td>1.58</td>
</tr>
<tr>
<td>SSSS1</td>
<td>RS64</td>
<td>A A B C</td>
<td>99</td>
<td>2.12</td>
<td>AA/BC</td>
<td>0.91</td>
<td>1.71</td>
<td>0.41</td>
</tr>
<tr>
<td>SSSS2</td>
<td>RA12</td>
<td>D E E E</td>
<td>102</td>
<td>17.41</td>
<td>DF/EE</td>
<td>0.83</td>
<td>16.71</td>
<td>0.71</td>
</tr>
<tr>
<td>SSSS2</td>
<td>RA13</td>
<td>A B B D</td>
<td>98</td>
<td>1.59</td>
<td>AD/BB</td>
<td>0.95</td>
<td>1.49</td>
<td>0.10</td>
</tr>
<tr>
<td>SSSS2</td>
<td>RS44</td>
<td>B D E M</td>
<td>108</td>
<td>8.77</td>
<td>BD/EM</td>
<td>0.78</td>
<td>5.98</td>
<td>2.79*</td>
</tr>
<tr>
<td>SSSS2</td>
<td>RS60</td>
<td>A A C E</td>
<td>106</td>
<td>4.66</td>
<td>AA/CE</td>
<td>0.92</td>
<td>4.39</td>
<td>0.27</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS44</td>
<td>A G H J</td>
<td>114</td>
<td>8.33</td>
<td>AJ/GH</td>
<td>0.88</td>
<td>7.43</td>
<td>0.90</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS44</td>
<td>A G H J</td>
<td>115</td>
<td>3.10</td>
<td>AG/HJ</td>
<td>0.86</td>
<td>1.94</td>
<td>1.16</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS60</td>
<td>B D D F</td>
<td>116</td>
<td>8.77</td>
<td>—</td>
<td>1.00</td>
<td>8.77</td>
<td>0.00</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS89</td>
<td>B B C E</td>
<td>115</td>
<td>40.14</td>
<td>BC/BE</td>
<td>0.95</td>
<td>40.12</td>
<td>0.02</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS101</td>
<td>C D E K</td>
<td>108</td>
<td>6.13</td>
<td>CK/DE</td>
<td>0.78</td>
<td>3.27</td>
<td>2.86*</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS101</td>
<td>C D E K</td>
<td>113</td>
<td>59.96</td>
<td>CD/KE</td>
<td>0.92</td>
<td>59.67</td>
<td>0.29</td>
</tr>
<tr>
<td>AAAA1</td>
<td>RA12</td>
<td>A B E G</td>
<td>100</td>
<td>16.68</td>
<td>AB/EG</td>
<td>0.70</td>
<td>12.22</td>
<td>4.46*</td>
</tr>
<tr>
<td>AAAA1</td>
<td>RS44</td>
<td>F K L L</td>
<td>100</td>
<td>5.00</td>
<td>FL/KL</td>
<td>0.59</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>AAAA2</td>
<td>RA12</td>
<td>C E E H</td>
<td>102</td>
<td>17.41</td>
<td>CF/EH</td>
<td>0.83</td>
<td>16.68</td>
<td>0.74</td>
</tr>
<tr>
<td>AAAA2</td>
<td>RS30</td>
<td>B D F C</td>
<td>104</td>
<td>7.12</td>
<td>BF/DC</td>
<td>0.84</td>
<td>5.72</td>
<td>1.40</td>
</tr>
<tr>
<td>AAAA2</td>
<td>RS44</td>
<td>L L N O</td>
<td>108</td>
<td>1.65</td>
<td>LL/NO</td>
<td>0.89</td>
<td>0.97</td>
<td>0.68</td>
</tr>
<tr>
<td>AAAA3</td>
<td>RS89</td>
<td>A D D G</td>
<td>121</td>
<td>52.36</td>
<td>AD/DG</td>
<td>0.96</td>
<td>52.34</td>
<td>0.02</td>
</tr>
<tr>
<td>AAAA3</td>
<td>RS101</td>
<td>G H I I</td>
<td>101</td>
<td>13.11</td>
<td>GH/II</td>
<td>0.71</td>
<td>10.70</td>
<td>2.41</td>
</tr>
</tbody>
</table>

Comparison of fit (G-test statistic) is shown of a tetrasomic model of inheritance (null model) and the best-fitting intermediate model. For intermediate models, \( \tau \) indicates the proportion of random meiotic pairings (approximate tetrasomy); if \( \tau = 1 \), the alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of \( \frac{1}{2} \chi^2_0 + \frac{1}{2} \chi^2_1 \) (see MATERIALS AND METHODS for further explanation). Values are significant at the indicated level (*P < 0.05).

*Letters indicate allele lengths (A represents the longest allele). Underlined letters indicate alleles shared between parents.

models (\( \tau = 0 \) and \( \delta_1 = 1, \delta_2 = 1, \text{or} \delta_3 = 1 \)) are not informative, because the log-likelihood of disomic null models becomes infinitely small due to observations in classes where the expectation is zero.

Evaluation of model performance on tetraploid segregation data from the literature: We selected some specific crosses and loci from the literature to test the general applicability of our gamete formation model (specified in Table 6). We specifically included cases in which tetrasomic inheritance could not be statistically rejected in the original study, but to which we suspected that intermediate inheritance models might apply. Also, we included a case where the observed patterns were clearly disomic (PAIRON and JACQUEMART 2005).

RESULTS

Reciprocal differences: There were no significant differences between observed female and male gamete frequencies of the same individual (data not shown). Therefore, in subsequent analyses the reciprocal data were pooled.

Inheritance in natural tetraploids: For the wild-collected tetraploid *R. sylvestris* (genotypes SSSS1, SSSS2, and SSSS3), the estimated value of \( \tau \) of the best-fitting intermediate inheritance models varied from \( \tau = 0.64 \) to \( \tau = 1 \) for the different loci (Table 2). In none of these cases was the fit significantly better than the null model of full tetrasomic inheritance \( \tau = 1 \) (Table 2). The likelihood of intermediate models typically decreased asymptotically upon approaching \( \tau = 0 \) (reflected in an asymptotically increasing deviance \( G \), Figure 1), flattened out around the minimum, and increased again toward \( \tau = 1 \) (leading to a decreasing deviance \( G \), Figure 1). For the wild-collected tetraploid *R. amphibia* (genotypes AAAA1, AAAA2, and AAAA3), the estimated value of \( \tau \) of the best-fitting intermediate model varied from \( \tau = 0.59 \) to \( \tau = 0.96 \) for the different loci (Table 2). For three loci (locus RS44 in SSSS2, locus RS101 in SSSS3, and locus RA12 in AAAA1) an intermediate model provided a significantly better fit than the full tetrasomic null model with estimates of \( \tau = 0.70 \) (RA12) and \( 0.78 \) (RS44, RS101) (Table 2).

Inheritance in artificial \( F_1 \) hybrids: For the artificial \( F_1 \) hybrids (genotypes AASS1, AASS2, SASS1, and SASS2), the estimated value of \( \tau \) of the best-fitting (constrained) intermediate models varied from 0.29 to 1 for the different loci (Table 3). Similar to the observations in the parents, the likelihood of intermediate models typically decreased asymptotically upon approaching \( \tau = 0 \) (leading to an asymptotically increasing deviance \( G \), Figure 2), flattened out around the minimum, and increased again toward \( \tau = 1 \) (leading to
a decreasing deviance $G$, Figure 2). In 9 of the 12 cases
the fit was significantly better than the null model of
tetrasomic inheritance $\tau = 1$ (Table 3). In 7 of these 9
cases the preferential pairing involved chromosomes
that originated from the same parental species, i.e.,
preferential pairing of the homologous chromosomes
(Table 3). In the two other cases, the preferential pairing
involved chromosomes that originated from different
parental species, i.e., preferential pairing of homeologous
chromosomes (AASS2, locus RS60; SSA2, locus RS44).
An unconstrained intermediate inheritance model never
provided a significant improvement in fit over a con-
strained intermediate model (data not shown).

### Double reduction:

For loci RA13, RS10, and RS64 no
double-reduction (DR) gametes were detected. For
locus RS30 null alleles prevented DR gamete identifica-
tion. For all other loci we detected DR gametes (Table
4). With only 1 observed DR in $>1000$ offspring ana-
alyzed, the DR rate for locus RS44 was the lowest. With 16
observed DRs in 641 offspring, locus RS101 had the
highest DR rate (Table 4). The total number of DRs
was higher in female meioses (17 vs. 10). In four cases
the parental genotypes had no alleles in common, so
that iterative estimation of the DR parameter ($\delta$) was
possible. For the cases involving full heterozygotes (i.e.,
plants of type $ABCD$), the observed and estimated DR
rates were equal. For the two remaining genotypes (of
type $AABC$), the estimated DR rates were higher than
the observed rate (Table 5).

### Evaluation of model performance on tetraploid
segregation data from the literature:

For the allozyme inheritance data of tetraploid
Centaraea jacea (HARDY et al. 2001), the estimated values of $\tau$ of the best-fitting
intermediate inheritance models varied from $\tau = 0.71$ to
$\tau = 0.98$ (Table 6). In one case the fit was significantly
better than the null model (and Hardy et al.’s conclu-
sions) of full tetrasomic inheritance $\tau = 1$ (Table 6) and
included 29% preferential pairing (i.e., $\tau = 0.71$) of
the chromosomes marked by alleles $A–C$ and $B–D$ (i.e.,
$\delta = 1$). For the allozyme inheritance data of tetraploid
Tolmioa menziesii (SOULIS and SOULIS 1988), the esti-
imated value of $\tau$ of the best-fitting intermediate model
varied from $\tau = 0.27$ to $\tau = 0.96$ (Table 6). In one case
the fit was significantly better than the null model (and

### TABLE 3

Fitting inheritance models on segregation of microsatellite loci in progeny of crosses involving first-generation hybrids

<table>
<thead>
<tr>
<th>Plant</th>
<th>Locus</th>
<th>Origin alleles</th>
<th>Null model ($\tau = 1$):</th>
<th>Best intermediate model</th>
<th>Model comparison:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\text{AAAS}^a$</td>
<td>$\text{SSSS}^b$</td>
<td>$n$</td>
<td>$G$</td>
</tr>
<tr>
<td>AASS1</td>
<td>RS44</td>
<td>FL CN</td>
<td>113</td>
<td>34.98</td>
<td>FF/CN</td>
</tr>
<tr>
<td></td>
<td>RS60</td>
<td>FF CE</td>
<td>116</td>
<td>25.45</td>
<td>FF/CE</td>
</tr>
<tr>
<td></td>
<td>RS101</td>
<td>J FHH</td>
<td>109</td>
<td>7.11</td>
<td>FHI/HJ</td>
</tr>
<tr>
<td>AASS2</td>
<td>RS44</td>
<td>LO BE</td>
<td>121</td>
<td>14.09</td>
<td>LO/BE</td>
</tr>
<tr>
<td></td>
<td>RS60</td>
<td>CF AE</td>
<td>121</td>
<td>8.19</td>
<td>AC/EF</td>
</tr>
<tr>
<td></td>
<td>RS89</td>
<td>CG EF</td>
<td>121</td>
<td>52.36</td>
<td>CG/EF</td>
</tr>
<tr>
<td>SSA1</td>
<td>RS44</td>
<td>LL IN</td>
<td>115</td>
<td>37.13</td>
<td>LL/IN</td>
</tr>
<tr>
<td></td>
<td>RS89</td>
<td>AH DE</td>
<td>115</td>
<td>40.14</td>
<td>AH/DE</td>
</tr>
<tr>
<td></td>
<td>RS101</td>
<td>GJ EF</td>
<td>112</td>
<td>60.00</td>
<td>EF/GJ</td>
</tr>
<tr>
<td>SSA2</td>
<td>RS44</td>
<td>NO BE</td>
<td>101</td>
<td>7.54</td>
<td>BO/EN</td>
</tr>
<tr>
<td></td>
<td>RS46</td>
<td>BE BD</td>
<td>100</td>
<td>3.76</td>
<td>BE/BD</td>
</tr>
<tr>
<td></td>
<td>RS101</td>
<td>GG AD</td>
<td>101</td>
<td>13.11</td>
<td>AG/DG</td>
</tr>
</tbody>
</table>

Comparison of fit ($G$-test statistic) is shown of a tetrasomic model of inheritance (null model) and the best-fitting intermediate
model. For intermediate models, $\tau$ indicates the proportion of random meiotic pairings (approximate tetrasomy); if $\tau < 1$, the
alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of $\sum \chi^2$ (see Mate-
rials and Methods for further explanation). Values are significant at the indicated level ($*P < 0.05; **P < 0.005; ***P < 0.0005$).

$^a$Letters indicate allele lengths ($A$ represents the longest allele).
DISCUSSION

In this article, we propose a likelihood-based approach to estimate the parameters of a general tetraploid inheritance model that best fits observed segregation data. The model incorporates full disomic inheritance, tetrasomic inheritance, and the whole range of intermediate inheritance. In addition, it estimates the rate of double reduction.

We applied the approach to establish whether the perennial tetraploids _R. amphibia_ and _R. sylvestris_ most likely have an auto- or an allotetraploid origin and to pave the road for future studies of population genetics and linkage mapping in these species. We analyzed the segregation of microsatellites for six tetraploid plants (three each of _R. amphibia_ and _R. sylvestris_). Only for two of the loci analyzed an intermediate inheritance model provided a significantly better explanation for the observed progeny ratios than a tetrasomic null model. This provides strong evidence for an autotetraploid origin of both _R. amphibia_ and _R. sylvestris_. This is in concordance with the morphological resemblance of diploid and tetraploid _R. amphibia_ (Jonsell 1968). In contrast, for most of the loci analyzed for artificial F1 hybrids between _R. amphibia_ and _R. sylvestris_, an intermediate model (i.e., including some degree of preferential pairing) explained the observed segregation ratios significantly better than the disomic and tetrasomic null models. This appears to be the first published example of tetraploids with intermediate inheritance, i.e., inheritance of single loci significantly deviating from both disomic and tetrasomic predictions.

Our approach calculates a measure of preferential pairing between chromosomes that allows direct predictions of the expected gamete frequencies. It provides a straightforward method to statistically evaluate whether disomic, tetrasomic, or intermediate inheritance models best explain the segregation of genetic markers and that is generally applicable to any marker segregation data set. Wu _et al._ (2001) developed a likelihood-based model that estimates the meioic preferential pairing factor (Sybenga 1994; Jackson and Jackson 1996). As such, their model may offer the advantage of allowing predictions on the expected ratio of bivalent/multivalent formation and may provide a direct link to traditional approaches dealing with meioic configurations. However, such predictions would be based on the assumption that a quadrivalent frequency of $<\frac{3}{8}$ (i.e., a 1:2 bivalent: quadrivalent ratio) is always the consequence of preferential pairing (Sybenga 1994). This assumption appears to be violated in many cases (see Ramsey and Schemske 2002). For example, colchicine-induced autotetraploid Arabidopsis thaliana lines had quadrivalent frequencies beyond the expected theoretical maximum of $\frac{3}{8}$, whereas established lines were often cytogenetically diploidized.
in that they formed a relatively high number of bivalents (Santos et al. 2003). Tetrasomic inheritance was associated with exclusively bivalent pairing in *Lotus corniculatus* (Fjellstrom et al. 2001), *Vaccinium darrowii*, and *V. corymbosum* (Qu et al. 1998) and in colchicine-induced autotetraploid *Brassica oleracea* (Jenczewski et al. 2002).

In Rorippa hybrids, chromosomes derived from the same parental species paired more frequently than heterospecific chromosomes. This gives rise to the question of whether the observed intermediate pairing affinities in the F1 hybrids are stable or whether recombination between homologous and homeologous chromosomes will homogenize the genome and result in a shift to tetrasomic segregation in future generations (Stebbins 1950; Sybenga 1996).

Loci that were analyzed in more individuals did not always follow the same model of inheritance, and the model of inheritance was also not always consistent across loci analyzed for the same individual. This underscores that segregation in tetraploid hybrids is not always predictable. Our approach also estimates the DR rate. Double reduction can play a role in the purging of deleterious mutations through gametophytic selection (Butruille and Boiteux 2000). Double reduction can occur if the recombined chromosomes move to the same pole (i.e., adjacent orientation). It requires multivalent formation and further depends on the frequency of crossing over and the distance of the locus from the centromere. We observed a large variation in the DR rate among individuals. Although the sample sizes presented

### Table 5

Observe the number of double-reduction (DR) gametes, observed DR rate, and estimated DR rate per plant per locus

<table>
<thead>
<tr>
<th>Plant</th>
<th>Locus</th>
<th>Genotype*</th>
<th>n</th>
<th>Observed DR frequency</th>
<th>Observed DR rate</th>
<th>Estimated DR rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSSS3</td>
<td>RS44</td>
<td>A G H J</td>
<td>114</td>
<td>1</td>
<td>0.0088</td>
<td>0.0088</td>
</tr>
<tr>
<td>SSSS3</td>
<td>RS101</td>
<td>C D E K</td>
<td>108</td>
<td>3</td>
<td>0.028</td>
<td>0.028</td>
</tr>
<tr>
<td>AASS1</td>
<td>RS101</td>
<td>F H H J</td>
<td>109</td>
<td>4</td>
<td>0.037</td>
<td>0.068</td>
</tr>
<tr>
<td>SSAA2</td>
<td>RS46</td>
<td>B B D E</td>
<td>100</td>
<td>1</td>
<td>0.010</td>
<td>0.016</td>
</tr>
</tbody>
</table>

*Letters indicate allele lengths (A represents the longest allele)

### Table 6

Fitting inheritance models on published tetraploid segregation ratios

<table>
<thead>
<tr>
<th>Species, marker</th>
<th>Locus genotype*</th>
<th>n</th>
<th>Data source</th>
<th>Null model (τ = 1):</th>
<th>Best disomic model</th>
<th>Model comparison:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Centauraea jacea</em>, alzyme</td>
<td>Pgd-2</td>
<td>148</td>
<td>HARDY et al. (2001): Table 3 (cross 1)</td>
<td>7.81</td>
<td>AC/BD</td>
<td>6.59*</td>
</tr>
<tr>
<td>abc</td>
<td>60</td>
<td>HARDY et al. (2001): Table 3 (cross 2)</td>
<td>3.39</td>
<td>AC/BD</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>abc</td>
<td>64</td>
<td>HARDY et al. (2001): Table 4 (cross 2)</td>
<td>0.26</td>
<td>BB/CD</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>abc</td>
<td>37</td>
<td>SOLTIS and SOLTIS (1988): Table 2 (1398-24 × 1398-20)</td>
<td>6.78</td>
<td>AC</td>
<td>3.46*</td>
<td></td>
</tr>
<tr>
<td>abc</td>
<td>37</td>
<td>SOLTIS and SOLTIS (1988): Table 2 (1398-24 × 1398-20)</td>
<td>6.78</td>
<td>BC</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>abc</td>
<td>72</td>
<td>SOLTIS and SOLTIS (1988): Table 2 (1398-24 × 1398-20)</td>
<td>8.29</td>
<td>AB/CC</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>abc</td>
<td>369</td>
<td>SOLTIS and SOLTIS (1988): Table 2 (VANC × 1392-1)</td>
<td>3.43</td>
<td>BB/CE</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Prunus serotina, microsatellite</td>
<td>M4c</td>
<td>36</td>
<td>PAINON and JACQUEMART (2005): Table 3</td>
<td>29.88</td>
<td>AB/CD</td>
<td>28.28***</td>
</tr>
<tr>
<td>abc</td>
<td>36</td>
<td>PAINON and JACQUEMART (2005): Table 3</td>
<td>28.97</td>
<td>EE/FG</td>
<td>28.53***</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of fit (G-test statistic) is shown of a tetrasomic model of inheritance (null model) and the best-fitting intermediate model. For intermediate models, τ indicates the proportion of random meiotic pairings (approximate tetrasomy); if τ < 1, the alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of $\chi^2_1 + \chi^2_1$ (see materials and methods for further explanation). Values are significant at the indicated level (*P < 0.05; ***P < 0.0005).

*Allele coding according to original articles (A represents the longest allele for microsatellites).
in this article are probably not sufficient for an accurate estimation of the DR rate, this suggests that double reduction may be individual specific. Cases that we considered the result of double reduction (e.g., an AA gamete from an ABCD parent) could theoretically also stem from a mutation event of B, C, or D to A. Our model without mutation may then overestimate the double-reduction rate. However, mutations appear to be rare in our data: only one nonparental allele was found in all offspring screened. Our data further suggested a higher prevalence of double reduction in female meioses. In two cases (both partial heterozygotes of DR rates and those estimated by our model were exactly zero), the DR rate estimated by the model was constrained to 1. Boxes specify species, individual/cross, and locus name as used in the original publication.

Chi-square or other goodness-of-fit approaches are often used to test whether observed segregation ratios fit either a disomic or a tetrasomic model of inheritance. Four outcomes are possible. First, neither disomic nor tetrasomic inheritance may be rejected, in which case power/sampling size appears to be insufficient. Second, tetrasomic inheritance may be rejected, and one of the possible disomic models may not (e.g., Quiros 1982; Marsdens et al. 1987; Soltis and Soltis 1988; Hardy et al. 2001). This type of outcome is normally regarded as evidence for tetrasomic inheritance resulting from random bivalent or quadrivalent pairing in meiosis. However, this may overestimate the importance of tetrasomic inheritance, since it disregards the possibility of intermediate inheritance models. In fact, disomic inheritance is already rejected if only one (nonartifactual) observation is made in a class with an expectancy of zero, because the test statistic becomes infinite, impeding any formal testing. Fourth, both disomic and tetrasomic inheritance may be rejected. This can be explained only by the existence of intermediate pairing preferences in meiosis, resulting in an inheritance intermediate between disomic and tetrasomic (e.g., Hickok 1978b; Danzmann and Bogart 1982, 1983). This type of outcome raises the question of what is the relative importance of disomic and tetrasomic inheritance (or preferential vs. random chromosome pairing) for a particular tetraploid genome. Our approach allows addressing this question and showed that intermediate inheritance is more likely than 100% tetrasomic inheritance in two published cases where tetrasomic inheritance could not be rejected using conventional methods. A model including disomic inheritance (29 and 58%, respectively) significantly better explained segregation of allozyme locus Pgd-2 in tetraploid C. jacea (Hardy et al. 2001) and of allozyme locus Fe-I in T. menziesii (Soltis and Soltis 1988). This may reflect different scenarios regarding the history of the chromosomes on which these loci are located. First, the chromosomes may have been homologous at the time of the polyploidization event (i.e., an autopolyploidization event), and now are differentiating into two homologous sets (i.e., diplodizing), so that inheritance is shifting to disomic inheritance. Second, the chromosomes may have been similar, but not completely homologous at the time of the polyploidization event (i.e., allopolyploidization). This could mean that recombination is homogenizing the chromosomes and that the intermediate inheritance is shifting to tetrasomic inheritance. In T. menziesii (Soltis and Soltis 1988), our approach showed that the likelihood surface was extremely flat for segregation of locus Fe-I in several crosses for which only very few individuals had been analyzed. This means that the intermediate models (including 73–53% disomic inheritance) could not be distinguished from full tetrasomic inheritance (in all but one case, see above). Larger sample sizes would be needed to elucidate whether locus Fe-I has an exceptional inheritance or that the observed patterns simply reflect random noise. All other loci supported tetrasomic inheritance (Soltis and Soltis 1988).

Summarizing, our approach showed that intermediate inheritance models provided a significantly better fit than the tetrasomic model of inheritance in first-generation hybrids between R. amphibia and R. sylvestris.
and in tetraploids of *C. jacea* and *T. menziesii*. The existence of inheritance patterns intermediate to disomic and tetrasomic inheritance has important repercussions for population genetics and mapping in tetraploids. In *Rorippa*, and in any system where hybridization plays a role, any wild-collected tetraploid individual may exhibit different pairing preferences, depending on the locus under study and the ancestry of the individual. This means that the methods that have been developed for linkage mapping and population genetics of tetrasomic tetraploids (*Ronfort et al. 1998; Cao et al. 2005; Luo et al. 2006a, b*) may not be generally applicable in these systems. In the case of *Rorippa*, an assumption of tetrasomic inheritance may be legitimate only if the individuals under study have been collected from locations where hybridization is known to be absent.

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**LITERATURE CITED**


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