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

Stift, M.; Berenos, C.; Kuperus, P.; van Tienderen, P.H.

DOI
10.1534/genetics.107.085027
Publication date
2008

Published in
Genetics

DOI: 10.1534/genetics.107.085027

Segregation Models for Disomic, Tetrasomic and Intermediate Inheritance in Tetraploids: A General Procedure Applied to Rorippa (Yellow Cress) Microsatellite Data

Marc Stift,[1] Camillo Berenos,[2] Peter Kuperus and Peter H. van Tienderen

Experimental Plant Systematics, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 1090 GB Amsterdam, The Netherlands

Manuscript received December 22, 2007
Accepted for publication May 25, 2008

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 F_1 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 (diploidized) 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 Dewet 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 $A_1A_2A_3A_4$). 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 homeologous 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

[1]*Corresponding author:* Division of Environmental and Evolutionary Biology, Graham Kerr Bldg., University of Glasgow, Glasgow, Scotland G12 8QQ. E-mail: m.stift@bio.gla.ac.uk

[2]*Present address:* ETH Zürich, Experimental Ecology, Institute of Integrative Biology, CHN J14, 8092, Zürich, Switzerland.

1996) so that crossing over between homeologous 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 ($2n \approx 115$), Jannoo *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 polysomic 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 F_1 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 F_1 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 F_1 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 (F_1) hybrids. From each of the four resulting F_1 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 F_1 hybrid seedling families was backcrossed with an unrelated, wild-collected plant (Table 1) to create first-generation backcrosses (BC_1). From each of the four resulting BC_1 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 F_1 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.*,

TABLE 1

Characteristics of the tetraploid genotypes of *R. amphibia* (*AAAA*), *R. sylvestris* (*SSSS*), and F_1 hybrids that were used to generate F_1 and BC_1 offspring

Plant code	Origin	Latitude and longitude	Crossed with
AAAA1	Vecht, Dalfsen, The Netherlands	North: 52°30′09″ East: 06°15′36″	*SSSS1*
AAAA2	Zwarte Water, Zwartsluis, The Netherlands	North: 52°37′30″ East: 06°04′48″	*SSSS2*
AAAA3	Lake Balaton, Balatongyörök, Hungary	North: 46°46′13″ East: 17°22′04″	*AASS2* *SSAA2*
SSSS1	Waal, Millingerwaard, The Netherlands	North 51°52′48″ East: 06°00′17″	*AAAA1*
SSSS2	Stour, Child Okeford, United Kingdom	North: 50°5′05″ West: 02°15′01″	*AAAA2*
SSSS3	Elbe, Darchau, Germany	North: 53°14′01″ East: 10°54′18″	*AASS1* *SSAA1*
AASS1	F_1 hybrid *AAAA1* × *SSSS1*		*SSSS3*
SSAA1	F_1 hybrid *SSSS1* × *AAAA1*		*SSSS3*
AASS2	F_1 hybrid *AAAA2* × *SSSS2*		*AAAA3*
SSAA2	F_1 hybrid *SSSS2* × *AAAA2*		*AAAA3*

Plant codes, crossing partners, and for wild-collected plants the specifics of origin (river, closest town, country) and the WGS84 coordinates of the exact sampling location are given. For F_1 hybrids, the parents are indicated.

fully heterozygous) and shared no alleles with its crossing partner. We genotyped each of the four F_1 and the four BC_1 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*, *BD*, and *BC* will occur in equal proportions ($\frac{1}{6}$) (Muller 1914). The maximum frequency of double reduction (α) is $\frac{1}{6}$, which can be reached if quadrivalents are always formed at meiosis, one effective crossover occurs between the locus and its centromere, and the recombined chromosomes migrate to the same pole at anaphase I (adjacent orientation, Mather 1935). Under this scenario, the allelic combinations *AB*, *AC*, *AD*, *CD*, *BD*, and *BC* will still occur in equal proportions ($\frac{1}{6} - \frac{1}{6}\alpha$), and there will be double-reduction gametes (*AA*, *BB*, *CC*, and *DD*) each at an expected frequency of $\frac{1}{4}\alpha$.

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 ($\frac{1}{4}$). 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

$$p(AA) = \frac{1}{4}\beta\tau$$

$$p(BB) = \frac{1}{4}\beta\tau$$

$$p(CC) = \frac{1}{4}\beta\tau$$

$$p(DD) = \frac{1}{4}\beta\tau$$

$$p(AB) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 2 + \frac{1}{4}\delta 3\right)$$

$$p(AC) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 1 + \frac{1}{4}\delta 3\right)$$

$$p(AD) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 1 + \frac{1}{4}\delta 2\right)$$

$$p(CD) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 2 + \frac{1}{4}\delta 3\right)$$

$$p(BD) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 1 + \frac{1}{4}\delta 3\right)$$

$$p(BC) = \frac{1}{6}\tau - \frac{1}{6}\beta\tau + (1-\tau)\left(\frac{1}{4}\delta 1 + \frac{1}{4}\delta 2\right)$$

or in matrix notation

$$\begin{pmatrix} p(AA) \\ p(BB) \\ p(CC) \\ p(DD) \\ p(AB) \\ p(AC) \\ p(AD) \\ p(CD) \\ p(BD) \\ p(BC) \end{pmatrix} = \begin{pmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ \frac{1}{6} \\ \frac{1}{6} \\ \frac{1}{6} \\ \frac{1}{6} \\ \frac{1}{6} \\ \frac{1}{6} \end{pmatrix} \tau + \begin{pmatrix} \frac{1}{4} \\ \frac{1}{4} \\ \frac{1}{4} \\ \frac{1}{4} \\ -\frac{1}{6} \\ -\frac{1}{6} \\ -\frac{1}{6} \\ -\frac{1}{6} \\ -\frac{1}{6} \\ -\frac{1}{6} \end{pmatrix} \beta\tau + (1-\tau) \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ \mathbf{0} & \frac{1}{4} & \frac{1}{4} \\ \frac{1}{4} & \mathbf{0} & \frac{1}{4} \\ \frac{1}{4} & \frac{1}{4} & \mathbf{0} \\ \mathbf{0} & \frac{1}{4} & \frac{1}{4} \\ \frac{1}{4} & \mathbf{0} & \frac{1}{4} \\ \frac{1}{4} & \frac{1}{4} & \mathbf{0} \end{pmatrix} \begin{pmatrix} \delta 1 \\ \delta 2 \\ \delta 3 \end{pmatrix}.$$

These equations define a set of nonlinear equations with four unknown parameters (by definition $\delta 1 + \delta 2 + \delta 3 = 1$). The "tetrasomic" parameter (τ) 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. If $\tau < 1$, the expected gamete proportions depend on the setting of three "disomic" parameters ($\delta 1$, $\delta 2$, and $\delta 3$) that indicate the respective degree of preferential pairing of *AB/CD*, *AC/BD*, and *AD/BC* marked chromosomes (respectively) in the nonrandom meiotic chromosome associations. Each can take values from 0 (no pairing) to 1 (obligate pairing) with the constraints that $\delta 1 + \delta 2 + \delta 3 = 1$ and $\delta 1 \times \delta 2 \times \delta 3 = 0$ (*i.e.*, one of the disomic parameters must be zero). The latter constraint guarantees that random bivalent or quadrivalent meiotic configurations are exclusively expressed in the parameter τ. Without such a constraint, there would be an alternative solution for each parameter setting with $\tau > 0$ (*e.g.*, $\tau = 1$ would be equivalent to $\tau = 0$ with $\delta 1 = \delta 2 = \delta 3 = \frac{1}{3}$). Finally, the "double-reduction" parameter β represents the frequency of double reductions relative to the total frequency of random (quadrivalent or random bivalent) meiotic associations, from which the frequency of double reduction as used in the literature (α) can be calculated as $\alpha = \beta\tau$.

Parameter estimation: The likelihood of multinomial data can be calculated as the sum of expected frequencies raised to the power corresponding to the observed counts (Kalbfleisch 1985): $L(x_1, x_2, \ldots, x_k) = \binom{n}{x_1\ x_2\ \ldots\ x_k} p_1^{x_1} p_2^{x_2} \ldots p_k^{x_k}$, in which n is the sample size, x_k is the number of observations of event k, and p_k is the probability of that event k. An event in our case is an observed gamete with two specific parental alleles. The total log-likelihood over all observations can be calculated as

$$\begin{aligned} L(\text{total data}) = \sum x_i \ln(p_i) = {} & x_{AA} \ln(p_{AA}) + x_{BB} \ln(p_{BB}) \\ & + x_{CC} \ln(p_{CC}) + x_{DD} \ln(p_{DD}) \\ & + x_{AB} \ln(p_{AB}) + x_{AC} \ln(p_{AC}) \\ & + x_{AD} \ln(p_{AD}) + x_{CD} \ln(p_{CD}) \\ & + x_{BD} \ln(p_{BD}) + x_{BC} \ln(p_{BC}), \end{aligned}$$

with x_{AA} the frequency of gamete type *AA*, and p_{AA} its probability given the model of inheritance under scrutiny, and so forth for all gamete types. We used the constrained nonlinear regression (CNLR) function as implemented in SPSS to estimate the parameter values that gave the best fit to the data. The model was specified with the COMPUTE PRED_ command as (n*(t*TAU+bt*BETATAU+(1-TAU)*(d1*DI1+d2*DI2+d3*DI3)).

Initial starting values for the iterations were set through the MODEL PROGRAM command. The constrained nonlinear regression (*i.e.*, the actual parameter estimation) was performed with the default iterative procedure (sequential quadratic programming), with the negative log-likelihood $(-1)(\text{obs})\ln(\text{PRED}/n)$ as the loss function to be minimized. Within the CNLR command, the values or boundaries for the parameters were specified using the BOUNDS/ subcommand. The supplemental material provides the syntax used (including a user guide) and two examples with data (observed gamete counts); model coefficients; parameter estimates for TAU (τ), BETATAU ($\beta\tau$), DI1 ($\delta 1$), DI2 ($\delta 2$), and DI3 ($\delta 3$); and the corresponding expected frequencies and likelihoods for the example data.

Application of the gamete formation model: For plant-locus combinations of the type *ABCD* or *AABC* with no alleles shared with the crossing partner, we deduced the parental gamete frequencies from the observed offspring genotypes. Then we used SPSS (constrained nonlinear regression, see specifications above) to obtain the parameter values that gave the largest log-likelihood for the following situations: (1) the full tetrasomic null model ($\tau = 1$), in which only the double-reduction rate ($\beta\tau$) was estimated; (2) three constrained intermediate models, in which the proportion of random segregations (τ) and the DR rate ($\beta\tau$) 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 τ, $\beta\tau$, 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 τ of the other parent decrease from 1 (full tetrasomic) to 0 (full disomic) in steps of 0.01, at $\delta 1 = 1$, $\delta 2 = 1$, $\delta 3 = 1$, 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 $G = 2\sum_i \text{obs}_i \ln(\text{obs}_i/\text{exp}_i)$, 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 τ 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 τ 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 $\frac{1}{2}\chi_0^2 + \frac{1}{2}\chi_1^2$ (Self and Liang 1987). Essentially, this means that the *P*-value of a conventional χ_1^2-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*)

				Null model ($\tau = 1$):	Best intermediate model			Model comparison:
Plant	Locus	Genotype[a]	n	G	Pairing alleles	T	G	LRT
SSSS1	*RS10*	*A A B C*	99	6.73	—	1.00	6.73	0.00
	RS44	*A C I N*	100	5.23	*AN/CI*	0.93	4.95	0.28
	RS46	*B B C C*	99	1.81	*BC/BC*	0.64	0.23	1.58
	RS64	*A A B C*	99	2.12	*AA/BC*	0.91	1.71	0.41
SSSS2	*RA12*	*D E E F*	102	17.41	*DF/EE*	0.83	16.71	0.71
	RA13	*A B B D*	98	1.59	*AD/BB*	0.95	1.49	0.10
	RS44	*B D E M*	108	8.77	*BD/EM*	0.78	5.98	2.79*
	RS60	*A A C E*	106	4.66	*AA/CE*	0.92	4.39	0.27
SSSS3	*RS44*	*A G H J*	114	8.33	*AJ/GH*	0.88	7.43	0.90
	RS44	*A G H J*	115	3.10	*AG/HJ*	0.86	1.94	1.16
	RS60	*B D D F*	116	8.77	—	1.00	8.77	0.00
	RS89	*B B C E*	115	40.14	*BC/BE*	0.95	40.12	0.02
	RS101	*C D E K*	108	6.13	*CK/DE*	0.78	3.27	2.86*
	RS101	*C D E K*	113	59.96	*CD/EK*	0.92	59.67	0.29
AAAA1	*RA12*	*A B E G*	100	16.68	*AB/EG*	0.70	12.22	4.46*
	RS44	*F K L L*	100	5.00	*FL/KL*	0.59	3.00	2.00
AAAA2	*RA12*	*C E F H*	102	17.41	*CF/EH*	0.83	16.68	0.74
	RS30	*B D F -*	104	7.12	*BF/D-*	0.84	5.72	1.40
	RS44	*L L N O*	108	1.65	*LL/NO*	0.89	0.97	0.68
AAAA3	*RS89*	*A D D G*	121	52.36	*AD/DG*	0.96	52.34	0.02
	RS101	*G H I I*	101	13.11	*GH/II*	0.71	10.70	2.41

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 $\tau < 1$, the alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of $\frac{1}{2}\chi_0^2 + \frac{1}{2}\chi_1^2$ (see MATERIALS AND METHODS for further explanation). Values are significant at the indicated level (*$P < 0.05$).

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

models ($\tau = 0$ and $\delta 1 = 1$, $\delta 2 = 1$, 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 τ 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 τ 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 τ of 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*, *SSAA1*, and *SSAA2*), the estimated value of τ 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

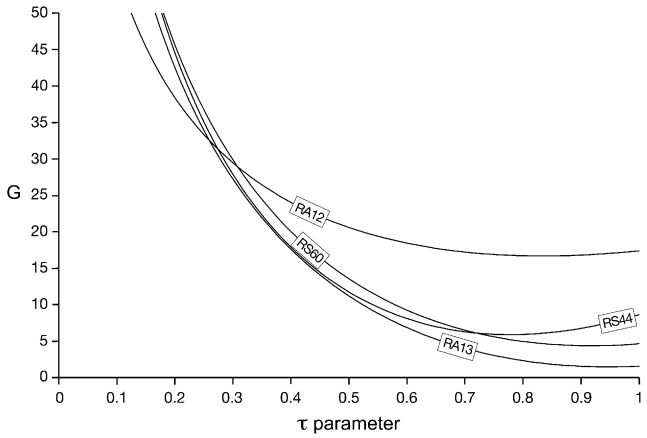

FIGURE 1.—Fit (deviance *G*) of observed segregation of microsatellite loci in a tetraploid *R. sylvestris* (*SSSS2*) to inheritance models ranging from complete disomic ($\tau = 0$) to complete tetrasomic inheritance ($\tau = 1$). The "disomic" parameter of the most likely disomic model was constrained to 1. Boxes specify locus names.

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*; *SSAA2*, locus *RS44*). An unconstrained intermediate inheritance model never provided a significant improvement in fit over a constrained 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 identification. For all other loci we detected DR gametes (Table 4). With only 1 observed DR in >1000 offspring analyzed, 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 ($\beta\tau$) 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 *Centaurea jacea* (HARDY *et al.* 2001), the estimated values of τ 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 conclusions) 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.*, $\delta 2 = 1$). For the allozyme inheritance data of tetraploid *Tolmiea menziesii* (SOLTIS and SOLTIS 1988), the estimated value of τ 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 *R. amphibia* × *R. sylvestris* (*AASS*) and *R. sylvestris* × *R. amphibia* (*SSAA*)

		Origin alleles			Null model ($\tau = 1$):	Best intermediate model			Model comparison:
Plant	Locus	*AAAA*[a]	*SSSS*[a]	*n*	*G*	Pairing alleles[a]	τ	*G*	$LRT_{[1]}$
AASS1	*RS44*	*FL*	*CN*	113	34.98	*FL/CN*	0.53	21.10	13.88***
	RS60	*FF*	*CE*	116	25.45	*FF/CE*	0.48	8.77	16.68***
	RS101	*J*	*FHH*	109	7.11	*FH/HJ*	0.72	6.05	1.06
AASS2	*RS44*	*LO*	*BE*	121	14.09	*LO/BE*	0.69	8.17	5.92*
	RS60	*CF*	*AE*	121	8.19	*AC/EF*	0.74	4.01	4.18*
	RS89	*CG*	*EF*	121	52.36	*CG/EF*	0.58	40.79	11.57***
SSAA1	*RS44*	*LL*	*IN*	115	37.13	*LL/IN*	0.29	1.18	35.95***
	RS89	*AH*	*DE*	115	40.14	*AH/DE*	0.42	18.68	21.46***
	RS101	*GJ*	*EF*	112	60.00	*EF/GJ*	0.58	50.30	9.70**
SSAA2	*RS44*	*NO*	*BE*	101	7.54	*BO/EN*	0.74	4.03	3.51*
	RS46	*BE*	*BD*	100	3.76	*BE/BD*	0.96	3.74	0.02
	RS101	*GG*	*AD*	101	13.11	*AG/DG*	0.94	13.07	0.04

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 $\tau < 1$, the alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of $\frac{1}{2}\chi_0^2 + \frac{1}{2}\chi_1^2$ (see MATERIALS 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).

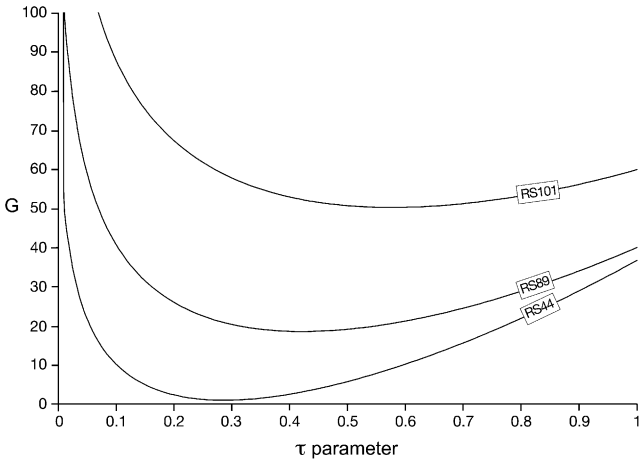

FIGURE 2.—Fit (deviance *G*) of observed segregation of microsatellite loci in a tetraploid F_1 hybrid *R. sylvestris* × *R. amphibia* (*SSAA1*) to inheritance models ranging from complete disomic ($\tau = 0$) to complete tetrasomic inheritance ($\tau = 1$). The "disomic" parameter of the most likely disomic model was constrained to 1. Boxes specify locus names.

Soltis and Soltis's conclusions) of full tetrasomic inheritance, suggesting 58% preferential pairing (*i.e.*, $\tau = 0.42$) of the chromosomes marked by alleles *A–C* (*i.e.*, $\delta 2 = 1$). For the microsatellite inheritance data of tetraploid *Prunus serotina* (PAIRON and JACQUEMART 2005), in agreement with Pairon and Jacquemart's conclusions, the fit of disomic inheritance was better than any intermediate model and significantly better than the tetrasomic null model (Table 6). The likelihood of the disomic model decreased from $\tau = 0$ to $\tau = 1$ in an almost linear fashion (reflected in a linear increase of the deviance *G*, Figure 3).

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 F_1 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.

TABLE 4

Overview of the observed number and rate of double-reduction (DR) gametes per locus per type, for female and male gametes (parental and hybrid data pooled)

Locus	Parental type	DR in female meiosis	DR in male meiosis	*n*	Observed rate
RA12	*AABC*	1	2	304	0.010
RA13	*AABC*	0	0	98	0.000
RS10	*AABC*	0	0	99	0.000
RS30	*ABC-*	No information due to null allele			
RS44	*AABC*	0	0	323	0.000
RS44	*ABCD*	1	0	772	0.001
RS46	*AABC*	1	0	199	0.005
RS60	*AABC*	4	0	338	0.012
RS60	*ABCD*	0	0	121	0.000
RS64	*AABC*	0	0	99	0.000
RS89	*AABC*	0	1	236	0.004
RS89	*ABCD*	0	1	236	0.004
RS101	*AABC*	5	1	311	0.019
RS101	*ABCD*	5	5	333	0.030
	Total	17	10		

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 meiotic 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 meiotic configurations. However, such predictions would be based on the assumption that a quadrivalent frequency of $< \frac{2}{3}$ (*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 $\frac{2}{3}$, whereas established lines were often cytogenetically diploidized,

TABLE 5

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

Plant	Locus	Genotype[a]	*n*	Observed DR frequency	Observed DR rate	Estimated DR rate
SSSS3	*RS44*	*A G H J*	114	1	0.0088	0.0088
SSSS3	*RS101*	*C D E K*	108	3	0.028	0.028
AASS1	*RS101*	*F H H J*	109	4	0.037	0.068
SSAA2	*RS46*	*B B D E*	100	1	0.010	0.016

[a] Letters indicate allele lengths (*A* represents the longest allele)

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 darrowi*, 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 F_1 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 6

Fitting inheritance models on published tetraploid segregation ratios

Species, marker	Locus genotype[a]	*n*	Data source	Null model ($\tau = 1$): *G*	Best disomic model: Pairing alleles[a]	τ	*G*	Model comparison: LRT
Centaurea jacea, allozyme	*Pgd-2* *abcd*	148	Hardy *et al.* (2001): Table 3 (cross 1)	7.81	*AC/BD*	0.71	1.22	6.59*
	Pgd-2 *abcd*	60	Hardy *et al.* (2001): Table 3 (cross 2)	3.39	*AC/BD*	0.80	2.16	1.23
	Lap-1 *bbcd*	64	Hardy *et al.* (2001): Table 4 (cross 2)	0.26	*BB/CD*	0.98	0.26	0.01
Tolmiea menziesii, allozyme	*Fe-1* *aacc*	37	Soltis and Soltis (1988): Table 2 (1398-24 × 1398-20)	6.78	*AC*	0.42	3.31	3.46*
	Fe-1 *bbcc*	37	Soltis and Soltis (1988): Table 2 (1398-24 × 1398-20)	6.78	*BC*	0.27	5.67	1.11
	Fe-1 *abcc*	72	Soltis and Soltis (1988): Table 2 (VANC × 1352-1)	8.29	*AB/CC*	0.47	6.22	2.07
	Pgi-2 *bbce*	369	Soltis and Soltis (1988): Table 2 (1347-5 × 1347-3)	3.43	*BB/CE*	0.96	3.33	0.10
Prunus serotina, microsatellite	*M4c* *abcd*	36	Pairon and Jacquemart (2005): Table 3	29.88	*AB/CD*	0.00	1.60	28.28***
	M4c *eefg*	36	Pairon and JacqueMart (2005): Table 3	28.97	*EE/FG*	0.00	0.44	28.53***

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 $\tau < 1$, the alleles that preferentially pair are indicated. LRT values were evaluated against a compound distribution of $\frac{1}{2}\chi_0^2 + \frac{1}{2}\chi_1^2$ (see materials and methods for further explanation). Values are significant at the indicated level (*$P < 0.05$; ***$P < 0.0005$).

[a] Allele coding according to original articles (*A* represents the longest allele for microsatellites).

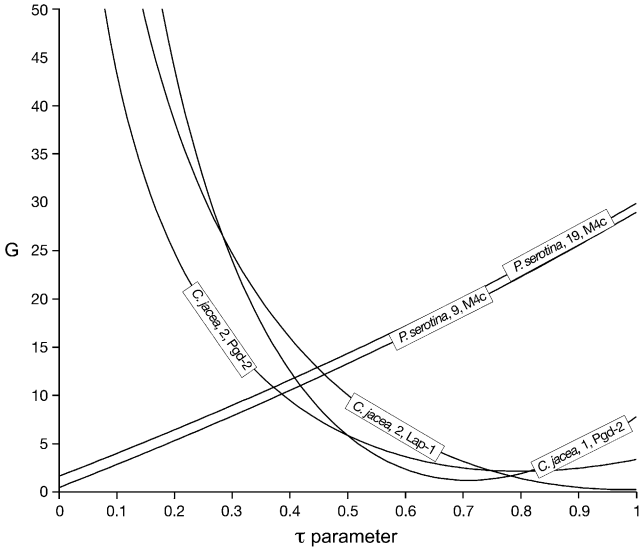

FIGURE 3.—Fit (deviance *G*) of observed segregation of microsatellite loci in tetraploid *Centaurea jacea* (HARDY *et al.* 2001) and *Prunus serotina* (PAIRON and JACQUEMART 2005) to inheritance models ranging from complete disomic ($\tau = 0$) to complete tetrasomic inheritance ($\tau = 1$). The "disomic" parameter of the most likely disomic model was constrained to 1. Boxes specify species, individual/cross, and locus name as used in the original publication.

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 full heterozygotes of type *ABCD*), the observed DR rates and those estimated by our model were exactly the same. In two other cases (both partial heterozygotes of type *AABC*), the DR rate estimated by the model was higher than the observed rate. This pattern makes sense since not all double reductions can be observed in partial heterozygotes. Both the observed and the estimated DR rates were always much closer to its theoretical minimum (*i.e.*, zero), than to its maximum ($\frac{1}{6}$). Even the largest estimated DR rate was still $< \frac{1}{12}$ (Table 4).

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.*, PAIRON and JACQUEMART 2005). This type of outcome is regarded as evidence for disomic inheritance. Third, disomic inheritance may be rejected, and tetrasomic may not (*e.g.*, QUIROS 1982; MARSDEN *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-1* 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 homeologous sets (*i.e.*, diploidizing), 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-1* 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-1* 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.

The authors thank Rob Bregman for sowing and seedling care; Harold Lemereis, Ludek Tikovsky, and Thijs Hendrix for growing and maintaining the plants; Jan van Arkel for help with the figures; Emiel van Loon, Paul Johnson, Daniel Haydon, and Richard Reeve for statistical advice; Andrew Tedder for testing the SPSS syntax; and Jaap Sybenga, Hans de Jong, Sarah Otto, and two anonymous referees for their helpful comments on drafts of the manuscript.

LITERATURE CITED

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

Bleeker, W., C. Weber-Sparenberg and H. Hurka, 2002 Chloroplast DNA variation and biogeography in the genus *Rorippa* Scop. (Brassicaceae). Plant Biol. **4:** 104–111.

Bowers, J. E., B. A. Chapman, J. K. Rong and A. H. Paterson, 2003 Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. Nature **422:** 433–438.

Bretagnolle, F., and J. D. Thompson, 1995 Tansley review no-78—gametes with the somatic chromosome-number—mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol. **129:** 1–22.

Butruille, D. V., and L. S. Boiteux, 2000 Selection-mutation balance in polysomic tetraploids: impact of double reduction and gametophytic selection on the frequency and subchromosomal localization of deleterious mutations. Proc. Natl. Acad. Sci. USA **97:** 6608–6613.

Cao, D. C., B. A. Craig and R. W. Doerge, 2005 A model selection-based interval-mapping method for autopolyploids. Genetics **169:** 2371–2382.

Danzmann, R. G., and J. P. Bogart, 1982 Evidence for a polymorphism in gametic segregation using a malate dehydrogenase locus in the tetraploid treefrog *Hyla versicolor*. Genetics **100:** 287–306.

Danzmann, R. G., and J. P. Bogart, 1983 Further evidence for a polymorphism in gametic segregation in the tetraploid treefrog *Hyla versicolor* using a glutamate oxaloacetic transaminase locus. Genetics **103:** 753–769.

Doyle, J. J., and J. L. Doyle, 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19:** 11–15.

Fjellstrom, R. G., P. R. Beuselinck and J. J. Steiner, 2001 RFLP marker analysis supports tetrasomic inheritance in *Lotus corniculatus* L. Theor. Appl. Genet. **102:** 718–725.

Hardy, O. J., M. De Loose, X. Vekemans and P. Meerts, 2001 Allozyme segregation and intercytotype reproductive barriers in the polyploid complex *Centaurea jacea*. Heredity **87:** 136–145.

Harlan, J., and J. M. J. deWet, 1975 On Ö. Winge and a prayer: the origins of polyploidy. Bot. Rev. **41:** 361–390.

Hickok, L. G., 1978a Homoeologous chromosome pairing and restricted segregation in the fern Ceratopteris. Am. J. Bot. **65:** 516–521.

Hickok, L. G., 1978b Homoeologous chromosome pairing: frequency differences in inbred and intraspecific hybrid polyploid ferns. Science **202:** 982–984.

Jackson, R. C., and J. W. Jackson, 1996 Gene segregation in autotetraploids: prediction from meiotic configurations. Am. J. Bot. **83:** 673–678.

Jannoo, N., L. Grivet, J. David, A. D'Hont and J. C. Glaszmann, 2004 Differential chromosome pairing affinities at meiosis in polyploid sugarcane revealed by molecular markers. Heredity **93:** 460–467.

Jenczewski, E., F. Eber, M. J. Manzanares-Dauleux and A. M. Chevre, 2002 A strict diploid-like pairing regime is associated with tetrasomic segregation in induced autotetraploids of kale. Plant Breed. **121:** 177–179.

Jonsell, B., 1968 Studies in the north-west European species of *Rorippa* s. str. Symb. Bot. Ups. **19:** 1–221.

Kalbfleisch, J. G., 1985 *Probability* (Probability and Statistical Inference, Vol. 1). Springer-Verlag, New York/Berlin/Heidelberg, Germany/Tokyo.

Karpechenko, G. D., 1927 The production of polyploid gametes in hybrids. Hereditas **9:** 349–368.

Lexer, C., R. A. Randell and L. H. Rieseberg, 2003 Experimental hybridization as a tool for studying selection in the wild. Ecology **84:** 1688–1699.

Luo, Z. W., R. M. Zhang and M. J. Kearsey, 2004 Theoretical basis for genetic linkage analysis in autotetraploid species. Proc. Natl. Acad. Sci. USA **101:** 7040–7045.

Luo, Z. W., Z. Zhang, L. Leach, R. M. Zhang, J. E. Bradshaw *et al.*, 2006a Constructing genetic linkage maps under a tetrasomic model. Genetics **172:** 2635–2645.

Luo, Z. W., Z. Zhang, R. M. Zhang, M. Pandey, O. Gailing *et al.*, 2006b Modeling population genetic data in autotetraploid species. Genetics **172:** 639–646.

Mable, B. K., 2004 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. Biol. J. Linn. Soc. **82:** 453–466.

Marsden, J. E., S. J. Schwager and B. May, 1987 Single-locus inheritance in the tetraploid treefrog *Hyla versicolor* with an analysis of expected progeny ratios in tetraploid oirganisms. Genetics **116:** 299–311.

Mather, K., 1935 Reductional and equational separation of the chromosomes in bivalents and multivalents. J. Genet. **30:** 53–78.

McLysaght, A., K. Hokamp and K. H. Wolfe, 2002 Extensive genomic duplication during early chordate evolution. Nat. Genet. **31:** 200–204.

Moody, M. E., L. D. Mueller and D. E. Soltis, 1993 Genetic variation and random drift in autotetraploid populations. Genetics **134:** 649–657.

Muller, H. J., 1914 A new mode of segregation in Gregory's tetraploid Primulas. Am. Nat. **48:** 508–512.

Otto, S. P., and J. Whitton, 2000 Polyploid incidence and evolution. Annu. Rev. Genet. **34:** 401–437.

Pairon, M. C., and A. L. Jacquemart, 2005 Disomic segregation of microsatellites in the tetraploid *Prunus serotina* Ehrh. (Rosaceae). J. Am. Soc. Hort. Sci. **130:** 729–734.

Qu, L. P., J. F. Hancock and J. H. Whallon, 1998 Evolution in an autopolyploid group displaying predominantly bivalent pairing at meiosis: genomic similarity of diploid *Vaccinium darrowi* and autotetraploid (Ericaceae). Am. J. Bot. **85:** 698–703.

Quiros, C. F., 1982 Tetrasomic segregation for multiple alleles in Alfalfa. Genetics **101:** 117–127.

Ramsey, J., and D. W. Schemske, 1998 Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu. Rev. Ecol. Syst. **29:** 467–501.

Ramsey, J., and D. W. Schemske, 2002 Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. **33:** 589–639.

Ronfort, J. L., E. Jenczewski, T. Bataillon and F. Rousset, 1998 Analysis of population structure in autotetraploid species. Genetics **150:** 921–930.

Santos, J. L., D. Alfaro, E. Sanchez-Moran, S. J. Armstrong, F. C. H. Franklin *et al.*, 2003 Partial diploidization of meiosis in autotetraploid *Arabidopsis thaliana*. Genetics **165:** 1533–1540.

Self, S. G., and K.-Y. Liang, 1987 Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J. Am. Stat. Assoc. **82:** 605–610.

Sokal, R. R., and F. J. Rohlf, 1995 *Biometry. The Principles and Practice of Statistics in Biological Research*, Ed. 3. W. H. Freeman, New York.

Soltis, D. E., and P. S. Soltis, 1988 Electrophoretic evidence for tetrasomic segregation in *Tolmiea menziesii* (Saxifragaceae). Heredity **60:** 375–382.

Soltis, D. E., and P. S. Soltis, 1993 Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. **12:** 243–273.

Soltis, P. S., and D. E. Soltis, 2000 The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. USA **97:** 7051–7057.

Soltis, D. E., P. S. Soltis and J. A. Tate, 2004 Advances in the study of polyploidy since Plant speciation. New Phytol. **161:** 173–191.

Stebbins, G. L., 1947 Types of polyploids: their classification and significance. Adv. Genet. **1:** 403–429.

Stebbins, G. L., 1950 *Variation and Evolution in Plants*. Columbia University Press, New York/London.

Stift, M., 2007 Polyploidy and hybridisation in the *Rorippa x anceps* hybrid complex. Ph.D. Dissertation, University of Amsterdam, Amsterdam.

Stift, M., P. Kuperus and P. H. Van Tienderen, 2006 Development of highly conserved primers for 12 new polymorphic microsatellite loci for the genus *Rorippa* Scop. (Brassicaceae), yellow-cress. Mol. Ecol. Notes **6:** 1129–1131.

Stift, M., P. C. Luttikhuizen, E. J. W. Visser and P. H. van Tienderen, 2008 Different flooding responses in *Rorippa amphibia* and *R. sylvestris*, and their modes of expression in F1 hybrids. New Phytol. (in press).

Sybenga, J., 1969 Allopolyploidization of autopolyploids I. Possibilities and limitations. Euphytica **18:** 355–371.

Sybenga, J., 1994 Preferential pairing estimates from multivalent frequencies in tetraploids. Genome **37:** 1045–1055.

Sybenga, J., 1996 Chromosome pairing affinity and quadrivalent formation in polyploids: Do segmental allopolyploids exist? Genome **39:** 1176–1184.

Udall, J. A., P. A. Quijada and T. C. Osborn, 2005 Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics **169:** 967–979.

Wolfe, K. H., 2001 Yesterday's polyploids and the mystery of diploidization. Nat. Rev. Genet. **2:** 333–341.

Wolfe, K. H., and D. C. Shields, 1997 Molecular evidence for an ancient duplication of the entire yeast genome. Nature **387:** 708–713.

Wu, R. L., M. Gallo-Meagher, R. C. Littell and Z. B. Zeng, 2001 A general polyploid model for analyzing gene segregation in outcrossing tetraploid species. Genetics **159:** 869–882.

Communicating editor: O. Savolainen