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SHORT COMMUNICATION

Inheritance in tetraploid yeast revisited: segregation patterns and statistical power under different inheritance models

M. STIFT*, R. REEVE*† & P. H. VAN TIENDEREN‡

*Division of Ecology and Evolutionary Biology, University of Glasgow, Glasgow, UK
†Boyd Orr Centre for Population and Ecosystem Health, University of Glasgow, Glasgow, UK
‡Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

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Abstract

In their recent article, Albertin *et al.* (2009) suggest an autotetraploid origin of 10 tetraploid strains of baker's yeast (*Saccharomyces cerevisiae*), supported by the frequent observation of double reduction meiospores. However, the presented inheritance results were puzzling and seemed to contradict the authors' interpretation that segregation ratios support a tetrasomic model of inheritance. Here, we provide an overview of the expected segregation ratios at the tetrad and meiospore level given scenarios of strict disomic and tetrasomic inheritance, for cases with and without recombination between locus and centromere. We also use a power analysis to derive adequate sample sizes to distinguish alternative models. Closer inspection of the Albertin *et al.* data reveals that strict disomy can be rejected in most cases. However, disomic inheritance with strong but imperfect preferential pairing could not be excluded with the sample sizes used. The possibility of tetrad analysis in tetraploid yeast offers a valuable opportunity to improve our understanding of meiosis and inheritance of tetraploids.

Introduction

Inheritance patterns in tetraploids are still poorly documented. Convincing cases of tetrasomic inheritance are especially rare (reviewed in Soltis & Soltis, 1993), as analyses require detailed marker data and extensive crossing experiments (Soltis & Soltis, 1993; Stift *et al.*, 2008). Tetraploid yeasts offer an elegant system to study tetraploid inheritance: meiosis in ascomycetes is not inherently different from meiotic processes in other organisms but unique because the tetrads that form after meiosis II do not disintegrate into separate meiospores. By micromanipulation and generating monosporic clones of the four meiospores of a tetrad (meiospores being the equivalent for fungi and plants of what gametes are in an animal system), products of meiosis can be genotyped directly. A recent study by Albertin *et al.* (2009) makes use of this technique to assess inheritance in tetraploid strains of *Saccharomyces cerevisiae* (baker's yeast). The authors support an autotetraploid origin, given several observations of microsatellite loci with four alleles found only in *S. cerevisiae*, and tetraploid karyotypes similar to that of a diploid *S. cerevisiae* reference strain. The authors also claim that their segregation patterns support tetrasomic inheritance, as could be expected because autopolyploidy and tetrasomic inheritance are often (but not always) coupled (reviewed in Soltis & Soltis, 1993; Ramsey & Schemske, 2002). However, in the two examples of segregations for which the data were provided in Albertin *et al.* (2009), we noted that particular allelic combinations in meiospores were missing, which cannot easily be reconciled with tetrasomy. In this brief communication, we clarify the exact probabilities for all possible allelic combinations in meiospores and meiospore combinations in tetrads, given scenarios of strict disomic and strict tetrasomic inheritance. We also perform a power analysis to find the sample sizes required to distinguish between models with different degrees of preferential pairing. We do this both with and without recombination between focal locus and centromere, depending on the location of the locus.

Correspondence: Marc Stift, Division of Ecology and Evolutionary Biology, University of Glasgow, Glasgow G12 8QQ, UK.
Tel.: +44 141 330 6637; fax: +44 141 330 5971;
e-mail: m.stift@bio.gla.ac.uk

Expectations are tailored to tetrad analysis in yeast, but general expectations of tetraploid meiospore frequencies can also be derived from this. We then re-examine the inheritance data of Albertin *et al.* (2009), discuss scenarios of tetraploid evolution that could underlie the observed patterns and present ideas how these hypotheses could be tested by complementing the data presented in Albertin *et al.* (2009).

Disomic inheritance

Consider a tetraploid that has four different alleles (denoted A, B, C, D) for a single-copy co-dominant marker (e.g. a microsatellite). If it is a classical allotetraploid, it will have two diploid sets of chromosomes, derived from each of the diploid ancestors. Let us assume that A and B are markers for homologous chromosomes from one parental ancestor and C and D from the other. Under strict disomic inheritance, chromosomes originating from the same species are expected to pair in bivalents and segregate to separate poles in meiosis I (Fig. 1). Effectively, the segregation pattern is that of a two-locus model.

Disomic inheritance, without crossing over

If there is no crossing over between the locus and the centromere (or in fact any even number of crossovers between the locus and centromere), the homologous A and B alleles will segregate to opposite poles in meiosis I and likewise for C and D. This results in two different tetrads in a 1 : 1 ratio: A and C migrating to the same pole in meiosis II gives a ditype tetrad with meiospores AC-AC-BD-BD, A and D migrating to the same pole gives a ditype tetrad AD-AD-BC-BC (Fig. 1, Table 1). The homologous A and B and C and D alleles are never in the same meiospore, and the expected frequency of each of the four possible meiospores (AC, BD, AD, BC) is ¼. Hence, the absence of two types of allele combination is characteristic for disomic inheritance.

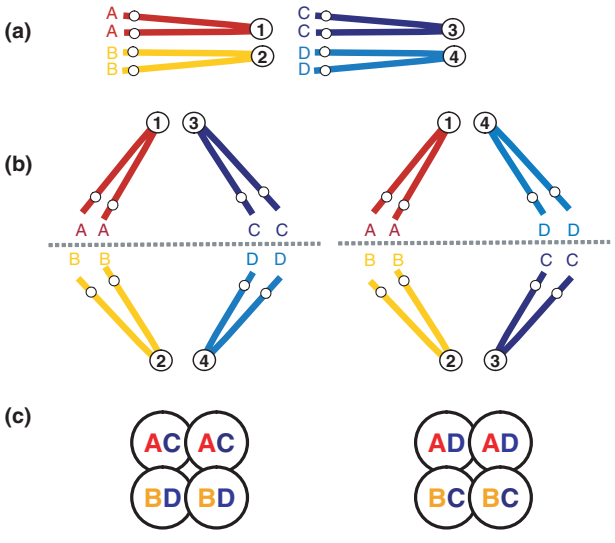

Fig. 1 Disomic inheritance, no crossovers. Chromosomes marked by A and B are homologous, as are those marked by C and D. (a) Chromatids have replicated, homologous chromosomes pair into bivalents: no crossing over assumed. (b) Chromosomes segregating, homologous chromosomes migrate to opposite poles in meiosis I, in two possible combinations. (c) Meiospore genotypes expected within tetrads after chromatids split and migrate to separate poles in meiosis II. Thus, two types of tetrads are expected in a 1 : 1 ratio, each containing two pairs of meiospores ('ditype').

Disomic inheritance, with crossing over

For telomeric loci, crossing over is possible after pairing of homologues in meiosis I (Fig. 2). If A and B, and C and D are again markers for the two ancestral species, crossing over unites A and B (and likewise C and D) on the same chromosome arm on different chromatids (Fig. 2). The chromatids bearing A and B are separated in meiosis II, when the chromatids migrate to separate poles. With one crossover (in fact with any odd number of crossovers) per chromosome arm, this results in three different types of tetrads in a 1 : 2 : 1 ratio (Fig. 2, Table 1). Note that there is only one tetratype tetrad, with the four nonhomologous allelic combinations (Table 1). The overall meiospore frequencies are not affected by crossing over, and again two types of meiospores (AB and CD) are absent.

Tetrasomic inheritance (Mendelian or random segregation)

A classical autotetraploid will have one tetraploid set of chromosomes, and no preferential pairing is to be expected in meiosis. This can lead either to random bivalent pairing, the formation of quadrivalents or to a combination of both [see (Sybenga, 1994) for a detailed view on models explaining meiotic configurations in autotetraploids]. We derive the expectations under random bivalent pairing and exclusive quadrivalent pairing.

Random bivalent pairing, without crossing over

If there is no crossing over between the locus and the centromere (or with any even number of crossovers between the locus and centromere), with four equivalent chromosomes, there are three alternative bivalent pairing configurations that are expected with equal frequencies. If A and B (and hence C and D) pair, the two possible tetrads are AC-AC-BD-BD and AD-AD-BC-BC (see above). If A and C (and hence B and D) pair, the two tetrads are AB-AB-CD-CD or AD-AD-BC-BC. If A and D (and hence B and C) pair, the two tetrads are AB-AB-CD-CD or AC-AC-BD-BD. The three possible tetrads are expected in a 1 : 1 : 1 ratio, and each tetrad has two

Table 1 Predictions of meiotic outcomes (tetrads and meiospores) of a tetraploid set of chromosomes under different inheritance and meiotic pairing models (disomic inheritance with bivalent pairing between homologues, tetrasomic inheritance with random bivalent pairing, tetrasomic inheritance with quadrivalent pairing). Each of the four chromosomes is marked by a specific allelic variant of a marker locus (A, B, C or D). For each model, the expected allelic combinations in meiospores within tetrads and the expected tetrad frequencies are presented given different assumptions regarding the total number of crossovers taking place (even, odd involving a single chromosome pair or odd involving two chromosome pairs). In models where crossing over is assumed, the chromosomes involved in the crossing over are connected by a cross in the schematic meiotic configuration (e.g. Quad 1×2–3×4 indicates that the chromosomes form a quadrivalent with crossing over between the chromosomes 1 and 2 marked by A and B and between 3 and 4 marked by C and D. Biv: Bivalent or Quad: Quadrivalent. For each configuration in meiosis I, the expected proportions are given for each unique tetrad that can be formed after the second meiotic division, followed by their meiospore composition. For each meiospore, the expected proportions in the total meiospore pool are given for each model and its assumptions (i.e. for an even or odd number of crossovers, in the latter distinguishing between models involving crossover between a single or two chromosome pairs, assuming that crossing over is equally likely between any pair of chromosomes).

Inheritance model	Cross-over assumption	Configuration in meioisis I (frequency)	Tetrad frequency	Tetrad meiospore composition									
				AB	CD	AC	BD	AD	BC	AA	BB	CC	DD
Disomic	No (or even) number or	Biv 1–2 & 3–4 (1)	1/2	–	–	–	–	2	2	No double reduction meiospores expected with bivalent pairing			
1 homologous to 2	crossovers	(cf. Fig. 1)	1/2	–	–	2	2	–	–				
3 homologous to 4				–	–	¼	¼	¼	¼				
	Odd number of crossovers	Biv 1–2 & 3–4 (1)	1	–	–	1	1	1	1				
	involving one pair			–	–	¼	¼	¼	¼				
	Odd number of crossovers	Biv 1–2 & 3–4 (1)	1/4	–	–	2	2	–	–				
	involving two pairs	(cf. Fig. 2)	1/2	–	–	1	1	1	1				
			1/4	–	–	–	–	2	2				
				–	–	¼	¼	¼	¼				
Tetrasomic	No (or even) number or	Biv 1–2 & 3–4 (1/3)	1	–	–	–	–	2	2				
Random bivalent	crossovers	Biv 1–3 & 2–4 (1/3)	1	–	–	2	2	–	–				
pairing*		Biv 1–4 & 2–3 (1/3)	1	2	2	–	–	–	–				
				1/6	1/6	1/6	1/6	1/6	1/6				
	Odd number of crossovers	Biv 1×2 & 3–4 (1/3)	1	–	–	1	1	1	1				
	involving one pair	Biv 1×3 & 2–4 (1/3)	1	1	1	–	–	1	1				
		Biv 1×4 & 2–3 (1/3)	1	1	1	1	1	–	–				
				1/6	1/6	1/6	1/6	1/6	1/6				
	Odd number of crossovers	Biv 1×2 & 3×4 (1/3)	1/4	–	–	2	2	–	–				
	involving two pairs		1/2	–	–	1	1	1	1				
			1/4	–	–	–	–	2	2				
		Biv 1×3 & 2×4 (1/3)	1/4	2	2	–	–	–	–				
			1/2	1	1	–	–	1	1				
			1/4	–	–	–	–	2	2				
		Biv 1×4 & 2×3 (1/3)	1/4	2	2	–	–	–	–				
			1/2	1	1	1	1	–	–				
			1/4	–	–	2	2	–	–				
				1/6	1/6	1/6	1/6	1/6	1/6				

Table 1 (Continued)

Inheritance model	Cross-over assumption	Configuration in meioisis I (frequency)	Tetrad frequency	Tetrad meiospore composition									
				AB	CD	AC	BD	AD	BC	AA	BB	CC	DD
Tetrasomic Quadrivalent pairing*	No (or even) number or crossovers	Quad 1–2–3–4 (1)	1/3	–	–	–	–	2	2	No double reduction meiospores expected without crossing over			
			1/3	–	–	2	2	–	–				
			1/3	2	2	–	–	–	–				
				1/6	1/6	1/6	1/6	1/6	1/6				
	Odd number of crossovers involving one pair	Quad 1×2–3–4 (1/6)	2/3	–	–	1	1	1	1	–	–	–	–
			1/3 * 1/2	–	2	–	–	–	–	1	1	–	–
			1/3 * 1/2	2	2	–	–	–	–	–	–	–	–
		Quad 1×3–2–4 (1/6) (cf. Fig. 3)	2/3	1	1	–	–	1	1	–	–	–	–
			1/3 * 1/2	–	–	–	2	–	–	1	–	1	–
			1/3 * 1/2	–	–	2	2	–	–	–	–	–	–
		Quad 1×4–2–3 (1/6)	2/3	1	1	1	1	–	–	–	–	–	–
			1/3 * 1/2	–	–	–	–	–	2	1	–	–	1
			1/3 * 1/2	–	–	–	–	2	2	–	–	–	–
		Quad 2×3–1–4 (1/6)	2/3	1	1	1	1	–	–	–	–	–	–
			1/3 * 1/2	–	–	–	–	2	–	–	1	1	–
			1/3 * 1/2	–	–	–	–	2	2	–	–	–	–
		Quad 2×4–1–3 (1/6)	2/3	1	1	–	–	1	1	–	–	–	–
			1/3 * 1/2	–	–	2	–	–	–	–	1	–	1
			1/3 * 1/2	–	–	2	2	–	–	–	–	–	–
		Quad 3×4–1–2 (1/6)	2/3	–	–	1	1	1	1	–	–	–	–
			1/3 * 1/2	2	–	–	–	–	–	–	–	1	1
			1/3 * 1/2	2	2	–	–	–	–	–	–	–	–
				11/72	11/72	11/72	11/72	11/72	11/72	1/48	1/48	1/48	1/48
	Odd number of crossovers involving two pairs	Quad 1×2–3×4 (1/3)	2/3 * 1/4	–	–	2	2	–	–	–	–	–	–
			2/3 * 1/4	–	–	–	–	2	2	–	–	–	–
			2/3 * 1/2	–	–	1	1	1	1	–	–	–	–
			1/3 * 1/4	–	–	–	–	–	–	1	1	1	1
			1/3 * 1/4	–	2	–	–	–	–	1	1	–	–
			1/3 * 1/4	2	–	–	–	–	–	–	–	1	1
			1/3 * 1/4	2	2	–	–	–	–	–	–	–	–
		Quad 1×3–2×4 (1/3) (cf. Fig. 4)	2/3 * 1/4	2	2	–	–	–	–	–	–	–	–
			2/3 * 1/4	–	–	–	–	2	2	–	–	–	–
			2/3 * 1/2	1	1	–	–	1	1	–	–	–	–
			1/3 * 1/4	–	–	–	–	–	–	1	1	1	1
			1/3 * 1/4	–	–	–	2	–	–	1	–	1	–
			1/3 * 1/4	–	–	2	–	–	–	–	1	–	1
			1/3 * 1/4	–	–	2	2	–	–	–	–	–	–

Table 1 (Continued)

Inheritance model	Cross-over assumption	Configuration in meiosis I (frequency)	Tetrad frequency	Tetrad meiospore composition									
				AB	CD	AC	BD	AD	BC	AA	BB	CC	DD
Tetrasomic Quadrivalent pairing* *continued*	Odd number of crossovers involving two pairs *continued*	Quad 1×4–2×3 (1/3)	2/3 * 1/4	2	2	–	–	–	–	–	–	–	–
			2/3 * 1/4	–	–	2	2	–	–	–	–	–	–
			2/3 * 1/2	1	1	1	1	–	–	–	–	–	–
			1/3 * 1/4	–	–	–	–	–	–	1	1	1	1
			1/3 * 1/4	–	–	–	–	–	2	1	–	–	1
			1/3 * 1/4	–	–	–	–	2	–	–	1	1	–
			1/3 * 1/4	–	–	–	–	2	2	–	–	–	–
				5/36	5/36	5/36	5/36	5/36	5/36	1/24	1/24	1/24	1/24

*All chromosomes homologous to each other, crossing over assumed to be equally likely between any pair of chromosomes.

identical copies of two meiospore genotypes (ditype tetrads, see Table 1). Overall, this results in six possible meiospore genotypes (AB, AC, AD, CD, BD, BC) in equal frequency (1/6) for random chromosome pairing and segregation (Muller, 1914).

Random bivalent pairing, with crossing over

With crossing over, the expectations under a tetrasomic model again depend on the pattern of bivalent formation. In addition to the ditype tetrads (see previous paragraph), each meiotic pairing combination can now also produce a tetratype tetrad (with four different meiospore genotypes) as a result of crossing over. If A and B pair in meiosis I, three different tetrads are expected in a 1 : 2 : 1 ratio: AC-BD-AC-BD, AC-BD-AD-BC and AD-BC-AD-BC, respectively. If A and C pair, the three expected tetrads (1 : 2 : 1) are AB-CD-AB-CD, AB-CD-AD-BC or AD-BC-AD-BC. If A and D pair, the three expected tetrads (1 : 2 : 1) are AB-CD-AB-CD, AB-CD-AC-BD and AC-BD-AC-BD. Overall, this leads to six different tetrads (three ditype, three tetratype) in equal frequencies (Table 1). The overall expectations for the expected meiospore frequencies are the same as for the model without crossing over, all 1/6.

Quadrivalent pairing, without crossing over

Unless quadrivalent formation leads to uneven segregation and aneuploid formation, it does not alter the expectations with respect to a model of random bivalent pairing in the absence of crossing over (Table 1). This is also true for cases where there is an even number of crossovers between locus and centromere.

Quadrivalent pairing, with crossing over

With crossing over, quadrivalent formation alters the expectations under random bivalent formation with crossing over because of the possibility of double reduction (sister chromatids ending up in the same meiospore). Suppose that an odd number of crossovers occurs between the arms of chromosome 1 and 3, involving telomeric marker alleles A and C (Fig. 3a). In meiosis I, chromosomes migrate to opposite poles in three possible and equally likely segregations: 1 and 2 can migrate to the same pole, 1 and 3, or 1 and 4. Both the first and last give rise to a single tetratype tetrad: AB-BC-AD-CD (Fig. 3b,c). Double reduction only occurs in one case, when chromosomes involved in crossing over go to the same pole (the middle case in this example, Fig. 3d). This then results in homozygous meiospores of a fully heterozygous parent (Fig. 3e, Table 1).

If each chromosome recombines through an odd number of crossovers with only one other chromosome arm, this results in what is called maximum equational

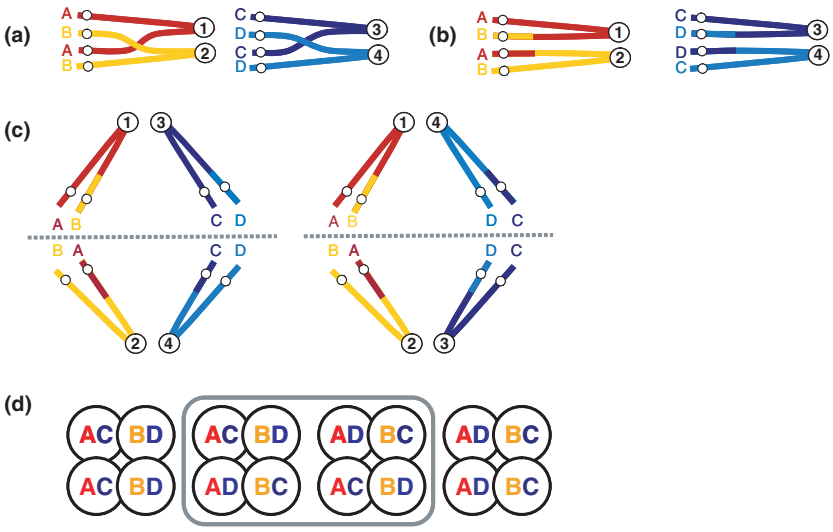

Fig. 2 Disomic inheritance, odd number of crossovers between two chromosome pairs. Chromosomes marked by A and B are homologous, as are those marked by C and D. (a) Chromatids have replicated, homologous chromosomes pair into bivalents: one crossover initiated between the arms of each chromosome pair. (b) Crossing over completed. (c) Chromosomes segregating, homologous chromosomes migrate to opposite poles in meiosis I, in two possible combinations that differ in centromere but not in the marker segregation. (d) Meiospore genotypes expected within tetrads after chromatids split and migrate to separate poles in meiosis II. Thus, one type of tetrads is expected with four different meiospores ('tetratype').

segregation (Mather, 1935). Suppose that such recombination occurs both between the arms of chromosome 1 and 3 and between the arms of chromosome 2 and 4 (Fig. 4a). Again, there are three possible scenarios for segregation in meiosis I (Table 1): 1 and 2 can migrate to the same pole, 1 and 3, or 1 and 4. Both the first and last give rise to three different tetrads in a 1 : 2 : 1 ratio: AC-BD-AC-BD, AC-BD-AD-BC and AD-BC-AD-BC (Fig. 4b,c). Only if 1 and 3 (and hence 2 and 4) migrate to the same poles can the second meiotic division lead to double reduction meiospores (Fig. 4d). This gives four different tetrads in equal frequency: AA-CC-BB-DD, AA-CC-BD-BD, AC-AC-BB-DD and AC-AC-BD-BD (Fig. 4e). The frequency of tetrads containing double reduction meiospores is 3/12, the frequency of ditype tetrads is 5/12, and the frequency of tetratype tetrads is 4/12. At the meiospore level, this results in 10 possible meiospore genotypes (AA, BB, CC, DD, AB, AC, AD, CD, BD, BC) of which the four double reduction meiospores occur in equal frequency (1/24 each, so 1/6 in total). This corresponds to theoretical maximum frequency 1/6 of double reduction derived by Mather (1935) under maximum equational segregation. Note that the frequencies of the normal six types of meiospores are not all equal in the aforementioned example because of the starting assumption that recombination occurred between the arms of chromosome 1 and 3 (alleles A and C) and between the arms of chromosome 2 and 4 (alleles B and D). If other crossover configurations between chromosomes are as likely (1×2 and 3×4, 1×4 and 2×3), the overall frequencies would be 5/36 for the six normal meiospores (Table 1).

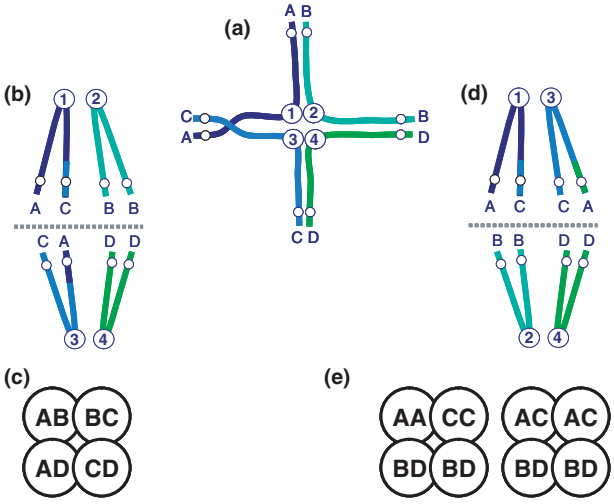

Fig. 3 Tetrasomic inheritance, odd number of crossovers involving arms of two chromosomes. Chromosomes marked by A, B, C and D are all homologous. (a) Chromatids have replicated, chromosomes pair into quadrivalents: one crossover initiated involving the arms of chromosomes 1 and 3. (b) Chromosomes segregating, in this case (1 and 2) and (3 and 4). (c) One type of tetrad is formed with four different meiospores (tetratype); note that chromosome segregation (1 and 4) and (2 and 3) gives exactly the same marker results. (d) Chromosomes (1 and 3) and (2 and 4) segregating, i.e. the combinations that were involved in crossing over now migrate to the same pole. (e) Meiospore genotypes expected within tetrads after chromatids split and migrate to separate poles in meiosis II. Two types of tetrads are expected, and 2/8 of the meiospores are double reduction homozygotes. As configuration (d) is one of the three possible centromere segregations, the overall expected frequency, assuming an odd number of crossovers involving two chromosomes, is $1/3 \times 1/4 = 1/12$.

Preferential pairing of chromosomes and power analysis

Strict disomic and strict tetrasomic inheritance can be considered as the extremes of a spectrum of possible patterns of chromosome pairing (Stebbins, 1947). Stift *et al.* (2008) presented a model that accommodates intermediate inheritance, depending on a parameter τ

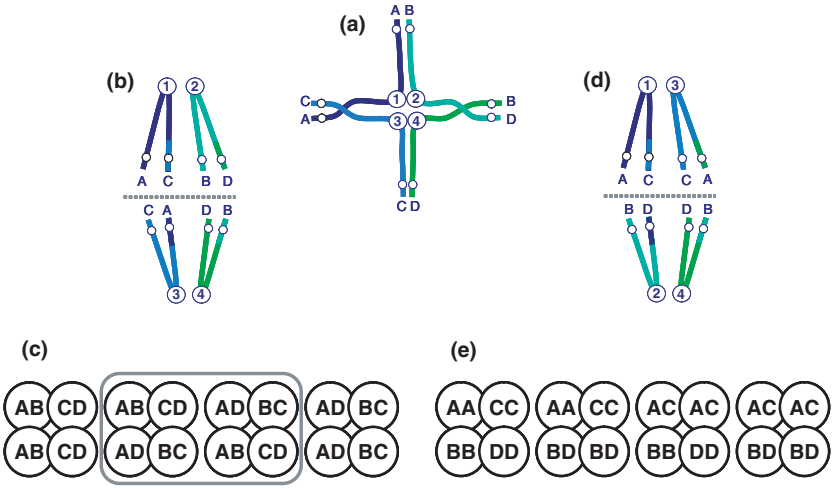

Fig. 4 Tetrasomic inheritance, odd number of crossovers between each of two chromosome combinations. Chromosomes marked by A, B, C and D are all homologous. (a) Chromatids have replicated, chromosomes pair into quadrivalents: one crossover initiated between chromosome 1 and 3 and one between 2 and 4. (b) Chromosomes segregating, in this case (1 and 2) and (3 and 4). (c) Three types of tetrads are formed, two ditype and one tetratype tetrad that is twice as frequent; note that chromosome segregation (1 and 4) and (2 and 3) gives exactly the same marker results. (d) Chromosomes (1 and 3) and (2 and 4) segregating, i.e. the combinations that were involved in crossing over migrate to the same pole. (e) Meiospore genotypes expected within tetrads after chromatids split and migrate to separate poles in meiosis II. Four types of tetrads are expected after separation of chromatids, and 8 of the 16 meiospores are double reduction homozygotes. Because configuration (d) is one of three possible centromere segregations (see b), the overall expected frequency of double reduction meiospores, assuming an odd number of crossovers between the arms of each of two chromosome combinations, is $1/3 \times 1/2 = 1/6$.

that ranges from complete preferential pairing, i.e. strict disomic inheritance ($\tau = 0$), to complete tetrasomic inheritance ($\tau = 1$). Based on a modification of this model to accommodate tetrad analysis (see Appendix S1 in Supporting Information), we derived exact probabilities of all possible outcomes based on the multinomial distribution, for values of τ from 0 to 1 at 0.1 intervals, without recombination (R-scripts available upon request from RR). We set $\alpha < 0.05$ and calculated the power of the analysis (i.e. the number of observations with a probability that fell within the 5% least probable outcomes of the distribution under the null hypothesis). The minimum sample sizes required for at least 80% power of distinguishing the alternative hypotheses are summarized in Table 2. Sample sizes above 10 are needed to have reasonable power to reject the null model of tetrasomic inheritance ($\tau = 1$) in favour of a strictly disomic model ($\tau = 0$).

We repeated the analysis assuming one recombination event between each chromosome pair in the absence of double reduction and using Monte Carlo simulation rather than the exact probabilities that were too complex to derive for sample sizes above 60. The model with crossing over has slightly more power than the one without recombination, thus requiring somewhat lower sample sizes to achieve the same power (Table 2). Crossing over results in more tetrads that are unique to the tetrasomic null model, so there is more information to distinguish between hypotheses. Still, sample sizes below 9 should be avoided if reasonable power is desired to reject the null model of tetrasomic inheritance ($\tau = 1$) in favour of a fully disomic model ($\tau = 0$). Obviously, irrespective of the recombination model assumed, power is lower for models with intermediate τ's, as the alternative hypothesis (pairing of bivalents more random and less preferential) becomes increasingly similar to the null hypothesis of tetrasomic inheritance ($\tau = 1$). For instance, sample sizes of 66 and 43 are required for models without and with

Table 2 Sample size required to reject the null hypothesis (H_0) of tetrasomic inheritance ($\tau = 1$) if the alternative hypothesis H_1 (with a lower τ) is in fact true, with a power of 80% ($\beta = 0.2$), and a Type I error rate $\alpha = 0.05$.

τ of H_1	Model without recombination	Model with recombination
0	11	9
0.1	16	12
0.2	22	15
0.3	30	21
0.4	43	30
0.5	66	43
0.6	108	68
0.7	197	120
0.8	> 200	> 200
0.9	> 200	> 200

recombination, respectively, for testing the alternative hypothesis of $\tau = 0.5$ (50% preferential pairing) with a power of 80%.

Re-examination of yeast segregation data in Albertin *et al.* (2009)

The raw segregation data underlying Albertin *et al.* (2009) were kindly provided by the authors and consisted of gels representing microsatellite fragment analysis of tetraploid yeast strains and their diploid meiospores. First, we genotyped the samples on the gels such that the longest allele was coded A, the second-longest B and so on (summarized in Supplementary Table S1). The maternal strains were fully heterozygous in all cases (10 strains) and hence genotyped as ABCD. Strict disomic inheritance could be rejected in all but one case, either because all possible meiospores were observed or because double reduction meiospores were observed. Hence, most observations are compatible with a tetrasomic model; however, as explained earlier, given the sample size, there is no power to distinguish a tetrasomic model from other models with preferential pairing.

The observation of tetratype tetrads – used by the authors as an additional argument for tetrasomic inheritance – is possible both with disomic and with tetrasomic inheritance. For example, assuming an odd number of crossovers involving two chromosome pairs, the probability of such tetratype tetrads is ½ for both a disomic and a tetrasomic model (Table 1). Tetratypes are not expected for loci tightly linked to the centromere (no crossing over), irrespective of the mode of inheritance. Hence, the frequency of tetratypes is an indication of the distance to the centromere, not of the mode of inheritance.

Conclusions

Different lines of evidence suggested an autotetraploid origin of tetraploid strains of baker's yeast (Albertin *et al.*, 2009). However, the current mode of inheritance of these strains can only be partly clarified by the segregation analysis of the data. Indeed, in most cases, strict disomic inheritance could be rejected, but there is insufficient power to distinguish slightly different models with a strong but imperfect preferential pairing from the tetrasomic null model. Such intermediate situations are not unlikely, especially when there is a transition from tetrasomic to disomic inheritance. The high frequency of tetrads containing double reduction meiospores suggests multivalent formation and tetrasomic inheritance. However, it has been noted that even in (disomic) allotetraploids meiotic pairing may not always be strictly preferential (Sybenga, 1996) and can lead to crossing over between homologous chromosomes (e.g. Udall *et al.*, 2005).

We conclude that it may be worthwhile to consider intermediate models of inheritance in this system. Because meiotic pairing behaviour could vary among chromosomes, it would be commendable to analyse inheritance for several loci per strain and increase sample sizes to shed further light on the current mode of inheritance in tetraploid baker's yeast. Clearly, the possibility of tetrad analysis in tetraploid yeast offers exciting opportunities to test not only models of inheritance but also underlying models of meiotic configurations (Sybenga, 1994). We hope that our comprehensive account of the possible outcomes and power considerations will help the design and interpretation of such studies.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Summary of data underlying the segregation analysis presented by Albertin *et al.* (2009).

Appendix S1 Matrix representation of non-linear models of probabilities of tetrads resulting from a tetraploid with genotype ABCD (models are derived from Stift *et al.*, 2008).

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