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A mathematical model of meiotic segregation in trisomics of yeast

BY STUART J. MACMARTIN AND A. P. JAMES
Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6

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SUMMARY

Segregations at five loci on the left arm of chromosome VII and other data from published sources were used to test a new mathematical model of meiotic segregation in trisomics of the yeast *Saccharomyces cerevisiae*. Results support this model which predicts that non-centromere-linked genes in trivalent complexes segregate at random. The data indicate that bivalent–univalent complexes are infrequent in trisomics and that recombination between all three homologues is frequent in regions close to the centromere. A test of homologue interference produced no evidence that a crossover between two homologues has any influence on the probability that the same two homologues will be involved in an adjacent crossover.

1. INTRODUCTION

Yeast is well suited to studies of the meiotic behaviour of aneuploids not only because it is open to tetrad analysis and is well mapped genetically (Mortimer & Hawthorne, 1973) but also because strains are available in which aneuploid segregants can be detected with ease and efficiency by their colony morphology (Seligy & James, 1977; Duck & James, 1976). However, studies of chromosome pairing and crossover behaviour in these strains have been hampered by the lack of a truly realistic yet simple mathematical model to describe the segregations expected of certain major cytological configurations.

To the present, in models devised to predict the segregations expected of trisomy it has been assumed, for the sake of mathematical simplicity, that in any meiosis only two of the homologues recombine between the centromere and an observed locus, even if the three homologous chromosomes associate as a trivalent. These models have been sufficiently accurate to demonstrate that trivalent complexes are indeed prevalent in trisomics (Shaffer *et al.* 1971; Culbertson & Henry, 1973). There are data though, some of them extensive, for which the model is grossly inadequate (Duck & James, 1976). This suggests the existence of an unusual interference pattern or an unrecognized form of pairing. Alternatively, the simplifying assumption that one chromosome does not undergo recombination may be sufficiently invalid to affect the expected results significantly. These possibilities are examined here, and a new model describing trisomic segregations is presented.

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2. MATERIALS AND METHODS

Three strains of homothallic yeast were used to produce the main body of data. These strains were heterozygous for *his*6 on chromosome IX and for six markers, *leu*1, *trp*5, *cyh*2, *met*13, *lys*5, and *ade*5,7, on the left arm of chromosome VII. Two of the strains, S528-3 and S529-2, were trisomic for chromosome VII. The trisomic constitution was $+/+/-$ at all loci except that of *leu*1, at which it was $+/-/-$. The other strain, S528-1, was normal diploid.

Meiotic segregations of a trisomic cell yields two normal haploid spores and two disomic spores. In the case of a homothallic strain the individual spores normally germinate to produce cells which diploidize, and such a strain thus gives rise, on tetrad analysis, to two diploid spore colonies and two tetrasomic spore colonies. Spore colonies that are tetrasomic for chromosome VII can be differentiated quickly and efficiently from normal diploid spore colonies; diploidization is delayed in the aneuploid and this can be detected by inspection with a microscope at 24 h.

A marker associated with trisomy ($++-$) is expected to yield three phenotypic classes of tetrads and four genotypic types. The phenotypic classes are $4+:0-$, $2+:2-$, and $3+:1-$. The genotypic types are $+-/+-/+/+$ ($4+:0-$), $++/++/-/-$ ($2+:2-$) and, for the $3+:1-$ phenotype, either (*a*) $++/+-/+/-$ or (*b*) $++/--/+/+$. (The $+--$ trisomic produces analogous types.) The ability to identify aneuploid segregants, as described in the preceding paragraph, is of great advantage in a tetrad analysis since it permits one to distinguish at a glance between the 3:1 (*a*) and the 3:1 (*b*) genetic types.

In tetrad analyses of trisomics it is important that the data are not distorted by the breakdown of the aneuploid system within the cells of a culture. Chromosome loss during or before the first meiotic division can lead to the production of four diploid segregants, and loss of a chromosome at the second meiotic division can lead to the production of one aneuploid and three diploid segregants. The ability to identify aneuploid segregants is thus advantageous in this respect also since it permits all deviant tetrads to be discarded from the data. In fact the two trisomic strains were very stable; fewer than 2% of the asci produced four diploid segregants, and fewer than 1% contained three diploid and one aneuploid segregant. The data from the two trisomic strains did not differ in any apparent way and were combined for analysis.

The diploid was used to check map distances and to provide an estimate of the frequency of gene conversion which cannot be detected in every instance in the trisomic. The amount of gene conversion was less than 3% at all loci except that of adenine. Here the frequency was so high (20%) that all data dealing with that locus in the trisomics were discarded.

The techniques of sporulation and tetrad analysis were routine.

3. RESULTS AND DISCUSSION

In the trisomic, it is generally accepted that there are two likely configurations of the three homologous chromosomes during meiosis I: bivalent–univalent and trivalent. In the case of bivalent–univalent configurations only two homologues pair; these recombine and segregate as in the normal diploid while the third homologue is expected to move to either pole at anaphase I with equal probability. In the case of trivalent configurations all three chromosomes are associated and all may be involved in recombination. Subsequent segregation of the homologues at anaphase I is random with two chromosomes moving to one pole and one to the other.

If it is assumed, as a first approximation, that only two of the three homologues can recombine even if the three homologues are paired as a trivalent, and if it is also assumed that these two recombine as if they were in a diploid, then the expected frequencies of ascal classes from a $+/+/-$ trisomic can be related to

Table 1. *Expected frequencies of ascal classes from a* $+/+/-$ *trisomic assuming no three-chromosome recombinants*

Ascal class	Configuration	Biv.–univ.	Trivalent	Combined
4:0	+ − + − + +	$2/3-x/3$	$2/3-x/3$	$2/3-x/3$
2:2	+ + + + − −	$1/3-x/3$	$1/3-2x/9$	$1/3-x/3+xz/9$
3:1 (*a*)	+ + + − + −	$2x/3$	$4x/9$	$2x/3-2xz/9$
3:1 (*b*)	+ + − − + +	0	$x/9$	$xz/9$

x = frequency of second-division segregation in the diploid. z = frequency of trivalent formation.

Table 2. *Diploid segregation of chromosome VII markers*

Gene pair	Total tetrads	Parental ditype	Non-parental ditype	Tetratype	x*
*leu*1–*trp*5	295	213	0	82	0·28
*leu*1–*cyh*2	295	56	31	208	0·71
*leu*1–*met*13	295	46	44	205	0·69
*leu*1–*lys*5	295	49	56	190	0·64
Exp., random	295	49	49	197	0·67

* Using *leu*1 as a chromosome marker (x = 0·05; Mortimer & Hawthorne, 1966).

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the frequency of second-division segregation (x) observed for the diploid (Shaffer *et al.* 1971; Culbertson & Henry, 1973; Duck & James, 1976). These expected frequencies are given in Table 1, and values of x obtained for markers on chromosome VII are given in Table 2. The values of x obtained for *trp*5 and *lys*5 do not differ significantly from the values (0·326 and 0·618, respectively) recorded by Mortimer & Hawthorne (1966). Genes segregating independently of their centromeres produce expected x values of 0·67 (1/6 parental ditype:2/3 tetratype:1/6 non-parental ditype) with respect to a centromere marker such as *leu*1, the x value of which is 0·05 (Mortimer & Hawthorne, 1966). Values of x as high or higher than 0·67 may also be obtained for loci where linkage is indicated by the relative frequencies of parental and nonparental ditypes.

The inadequacy of the model presented in Table 1 is evident in the results of a tetrad analysis of four of the markers on the left arm of chromosome VII. The summarized data produced by $+/+/-$ trisomics are given in the upper section of Table 3 together with comparable data, already published by this laboratory, for three markers on chromosomes I or XVII. Accompanying the data are values expected under the model for the two cases of 100% trivalent formation and 100% bivalent-univalent formation. It was found that no value for the frequency of trivalent formation (z) could be chosen for which the model would account for any of the data to the 0·05 level of significance, using the χ^2 test. A goodness of fit test of the model calls for estimating z using the data and performing the χ^2 calculations, losing 1 degree of freedom. Clearly such a test would reject the model for each of the markers on chromosomes VII, I and XVII. In particular there is a marked deficiency of 4:0 and 2:2 segregations in favour of 3:1 except in the case of *ade*1.

The lower section of Table 3 contains comparable data, already published by other laboratories, for six genes on chromosomes III and XI. Although most of these data provide an acceptable fit to the model, they do not provide an adequate test of its validity. In the case of *met*14, the locus is so close to its centromere that crossovers should rarely occur between it and its centromere under any circumstances, so the data should fit many models, including one based on trivalent formation only or one based on bivalent–univalent formation only. The data relating to *trp*3, *ura*1, *leu*2, and to a lesser extent *his*4, are too few to distinguish between a high frequency of trivalent formation and a high frequency of bivalent–univalent formation, a fact which suggests that some other model might also fit these data.

The poor fit of the more extensive data is indicative of some sort of interference, but the failure of the model to consider the effects of recombination involving all three chromosomes may also be responsible. In fact, it is possible to detect tetrad types in the data relating to the left arm of chromosome VII which could only have resulted from such three-chromosome recombination, barring the occurrence of a false ascus or a gene conversion. All three chromosomes must have been involved in recombination if a tetrad includes a 3:1 (b) at one locus and also one of a *leu*1-tetrasome tetratype ($+-, --, +, -$), or at some other locus, either a 2:2

Table 3. *Observed and expected segregations of + + − trisomics**

Data from:	Chrom.	Marker	x†	4:0 Obs.	4:0 $E_{triv.}$	4:0 $E_{biv./univ.}$	2:2 Obs.	2:2 $E_{triv.}$	2:2 $E_{biv./univ.}$	3:1(*a*) Obs.	3:1(*a*) $E_{triv.}$	3:1(*a*) $E_{biv./univ.}$	3:1(*b*)‡ Obs.	3:1(*b*)‡ $E_{triv.}$	Total	Acceptable§ range for z
This paper	VII	*trp*5	0·33	433	529·0	529·0	172	247·3	213·0	294	137·4	206·0	49	34·3	948	None
		*cyh*2	0·71	283	407·7	407·7	63	166·4	91·6	499	291·1	448·7	103	74·8	948	None
		*met*13	0·69	299	414·0	414·0	58	170·6	98·0	498	290·7	436·0	93	72·7	948	None
		*lys*5	0·62	253	436·1	436·1	56	185·4	120·1	514	261·2	391·8	125	65·3	948	None
James & Inhaber (1974)	I	*ade*1	0·10	158	127·3	127·3	41	62·5	60·3	2	11·2	13·4	—	—	201	None
Duck & James (1976)	XVII	*met*4	0·70	114	168·1	168·1	19	69·0	38·8	209	120·7	181·1	46	30·2	388	None
		*pha*2	0·66	143	206·8	206·8	27	86·4	52·5	232	135·8	203·7	61	34·0	463	None
Shaffer *et. al.* (1971)	III	*leu*2	0·13	29	31·8	31·8	17	15·5	14·8	5	3·7	4·4	—	—	51	$0 \leqslant z \leqslant 1$
		*his*4	0·42	52	64·8	64·8	32	29·5	23·8	39	28·7	34·4	—	—	123	$0 \leqslant z \leqslant 0{\cdot}86$
		*thr*4	0·67	17	25·7	25·7	3	10·7	6·4	38	21·6	25·9	—	—	58	None
Culbertson & Henry (1973)	XI	*met*14	0·03	45	46·0	46·0	23	22·9	22·7	2	1·1	1·3	—	—	70	$0 \leqslant z \leqslant 1$
		*ura*1	0·67	9	11·1	11·1	5	4·6	2·8	11	9·3	11·1	—	—	25	$0 \leqslant z \leqslant 1$
		*trp*3	0·69	9	10·9	10·9	5	4·5	2·6	11	9·6	11·5	—	—	25	$0 \leqslant z \leqslant 1$

* Segregation ratios are + : − except for *cyh*2, for which they are − : +. $E_{triv.}$ = expected from 100 % trivalent formation. $E_{biv./univ.}$ = expected from 100 % bivalent–univalent formation.

† Second-division segregation frequencies for *cyh*2 and *met*13 are from Table 1. All others are from Mortimer & Hawthorne (1966) except for *pha*2, which uses the combined data of Duck & James (1976) and Mortimer & Hawthorne (1966).

‡ Included with 3:1(*a*) if blank.

§ To the 0·05 level of significance.

(+ +, + +, −, −) or a 3:1(*a*). These tetrad types can be expected to include only a fraction of the instances of three-chromosome recombination between a centromere and a given locus, especially if only a few markers are being observed in this region. Nevertheless, in the interval between *leu*1 and *trp*5, six such tetrads (0·6 %) were found. The number to *cyh*2 was 56 (6 %), to *met*13, 102 (11 %), and to *lys*5, 173 (18 %). Thus there can be no doubt that three-chromosome recombination does occur with a high frequency and that its effect on segregation ratios may be large.

(i) *Non-centromere-linked genes*

Since it is likely that three-chromosome recombination occurs often in trivalent complexes, there is a possibility that enough crossovers occur between the centromere and a given locus for the alleles at that locus to become randomly positioned over all six chromatids whenever trivalent pairing occurs. This would result in the frequencies of ascal classes presented in Table 4, again using z to denote the frequency of trivalent pairing. Naturally, the further a locus is from its centromere, the closer this model should approximate the true distribution.

Table 4. *Expected frequencies of ascal classes from a* +/+/− *trisomic assuming random segregation in the trivalent*

Ascal class	Biv.–univ.	Trivalent	Combined
4:0	$2/3-x/3$	$4/15$	$2/3-x/3-6z/15+xz/3$
2:2	$1/3-x/3$	$1/15$	$1/3-x/3-4z/15+xz/3$
3:1(*a*)	$2x/3$	$8/15$	$2x/3+8z/15-2xz/3$
3:1(*b*)	0	$2/15$	$2z/15$

x = frequency of second-division segregation in the diploid. z = frequency of trivalent formation.

This model was tested against the data of each of the 13 markers by determining an estimate of z under the model and performing a χ^2 test against the model with that value of z. The results are recorded in Table 5. The estimate used for z was the maximum likelihood estimate, which is that value of z which maximizes the probability of observing the data actually observed. That is, if N_1 asci were observed to be 4:0, N_2 were 2:2, N_3 were 3:1(*a*) and N_4 were 3:1(*b*), and if p_1, p_2 p_3 and p_4 are the probabilities that a particular tetrad is in, respectively, ascal class 4:0, 2:2, 3:1(*a*) and 3:1(*b*), then the maximum likelihood estimate ($\hat{z}$) of z maximizes

$$L(z) = cp_1^{N_1}p_2^{N_2}p_3^{N_3}p_4^{N_4},$$

where c is a constant. Since $\hat{z}$ also maximizes $\log L(z)$, it is a solution of

$$\frac{\partial}{\partial z}\log L(z) = 0,$$

$$\text{i.e. } \sum_{i=1}^{4}\frac{N_i}{P_i}\frac{\partial P_i}{\partial z} = 0,$$

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Table 5. *Expected segregation of + + − trisomics and test of fit assuming that markers segregate at random in the trivalent*

				4:0		2:2		3:1 (*a*)		3:1 (*b*)[4]				
Chrom.	Marker	x[2]	$\hat{z}$[1]	Obs.[2]	Exp.[3]	Obs.[2]	Exp.[3]	Obs.[2]	Exp.[3]	Obs.[2]	Exp.[3]	χ^2	P	0·01 likelihood interval
VII	*trp*5	0·33	0·33	433	437·0	172	163·1	294	306·2	49	41·7	2·29	> 0·25	0·24 ⩽ z ⩽ 0·43
	*cyh*2	0·71	0·82	283	280·6	63	68·3	499	495·8	103	103·3	0·45	> 0·75	0·64 ⩽ z ⩽ 1·0
	*met*13	0·69	0·76	299	292·0	58	71·1	498	482·2	93	95·7	2·81	0·25	0·58 ⩽ z ⩽ 0·99
	*lys*5	0·62	1·0	253	252·8	56	63·2	514	505·6	125	126·4	0·98	0·95	0·85 ⩽ z ⩽ 1·0
I	*ade*1	0·10	0	158	127·3	41	60·3	2	13·4	—	—	23·3**	⩽ 0·005	—
XVII	*met*4	0·70	0·91	114	109·3	19	27·0	209	204·6	46	47·1	2·69	> 0·25	0·63 ⩽ z ⩽ 1·0
	*pha*2	0·66	0·91	143	131·0	27	32·8	232	243·0	61	56·2	2·83	> 0·10	0·67 ⩽ z ⩽ 1·0
III	*leu*2	0·13	0·02	29	31·4	17	14·6	5	5·0	—	—	0·58	> 0·25	0 ⩽ z ⩽ 0·31
	*his*4	0·42	0·08	52	62·2	32	22·5	39	38·3	—	—	5·70*	< 0·02	0 ⩽ z ⩽ 0·41
	*thr*4	0·67	0·95	17	16·0	3	4·0	38	38·0	—	—	0·3	> 0·50	0·05 ⩽ z ⩽ 1·0
XI	*met*14	0·03	0·01	45	45·7	23	22·4	2	1·9	—	—	0·022	> 0·75	0 ⩽ z ⩽ 0·17
	*ura*1	0·67	0	9	11·1	5	2·7	11	11·2	—	—	2·4	> 0·10	0 ⩽ z ⩽ 1·0
	*trp*3	0·69	0	9	10·9	5	2·6	11	11·5	—	—	2·3	> 0·10	0 ⩽ z ⩽ 1·0

(1) z = maximum-likelihood estimate of z. (2) Data from Table 3. (3) Expected under model using $\hat{z}$. (4) Included under 3:1 (*a*) where blank.
* Significant at the 0·05 level. ** Significant at the 0·005 level.

provided that this has a solution between zero and one, since z is a probability. Otherwise $\hat{z}$ is either zero or one. The above argument can be used for finding the maximum likelihood estimates in the case where the 3:1 (a)'s and the 3:1 (b)'s are indistinguishable; if N_3 is now used to denote the observed number of 3:1's, and if $p_3 = 2x/3 + 2z/3 - 2xz/3$ denotes the probability that a tetrad is 3:1, then the maximum likelihood estimate satisfies

$$\sum_{i=1}^{3} \frac{N_i}{P_i} \frac{\partial P_i}{\partial z} = 0,$$

if this has a solution between zero and one. In particular, for markers which segregate randomly in the diploid we have $x = 2/3$ and this last equation can be solved to obtain

$$\hat{z} = \begin{cases} (9N_3)/(2n) - 2 & \text{if } 4/9 \leqslant N_3/n \leqslant 2/3, \\ 1 & \text{if } N_3/n \geqslant 2/3, \\ 0 & \text{if } N_3/n \leqslant 4/9, \end{cases}$$

where n is the total number of observations.

For all thirteen markers, the maximum likelihood estimates of z were found using the computer when necessary, and are given in Table 5 together with the results of the goodness of fit tests. Clearly the model provides an acceptable fit for all markers except *ade*1, and *his*4. Also included for these markers giving an acceptable fit are the 0·01 likelihood intervals, which contain all but highly improbable values of z and are defined by those values of z for which $L(z)/L(\hat{z}) > 0{\cdot}01$, where $L(z)$ is as previously defined. It is clear from Table 5 that although the model provided an acceptable fit to all the data with the exception of *ade*1 and *his*4, the values of z obtained from data of markers close to the centromere are inconsistent with the values found using data of markers more distant from the centromere. This suggests that although the model may be adequate to confirm trisomy using data of markers near the centromere, it is not good enough to be used to estimate the actual frequency of trivalent pairing using those data, and in fact should give an underestimate. The most reliable estimates of the frequency of trivalent pairing should come from markers which segregate randomly in the diploid. Thus for chromosome VII, the most reliable estimate uses the data of *lys*5, a marker whose segregations do not deviate significantly from random in the diploid (cf. Table 2). For this chromosome, it is highly unlikely that the frequency of trivalent formation is less than 0·85. If for *trp*5 the values of $\hat{z}$ are constrained to the range $0{\cdot}85 \leqslant \hat{z} \leqslant 1$, then the model in Table 4 is rejected by the χ^2 test, as expected.

Interval estimates of z made in terms of variance were not calculated. The variances of the maximum likelihood estimates of z are complicated by the fact that the estimates are constrained to the interval from zero to one and also by the fact that x has a sampling variance. However, if the constraint on the estimators is removed and if x is assumed to be two-thirds, it can be shown that for large samples the variance of the maximum likelihood estimator of z is decreased to at most three-quarters of its original value if the 3:1 class is split. Thus for large

sample sizes, it is desirable to use all four ascal classes whenever possible, as was done in the calculations for chromosomes VII and XVII.

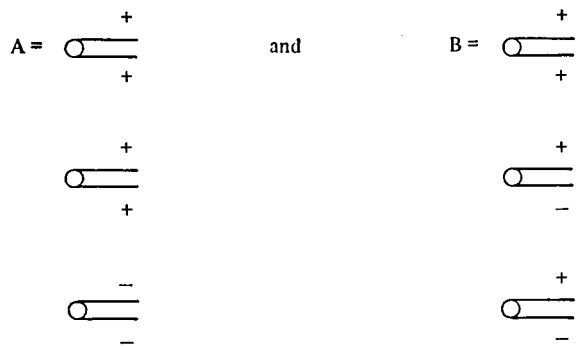
Reasonable estimates of z cannot be obtained for chromosomes III and XI from the data of Table 5 due to small sample sizes, but a further chromosome XI trisomy study using complementing *fas*1 alleles (Culbertson & Henry, 1973) gives a range for z of 0·62–1·0 using a random segregation model.

The fit of the data for all centromere-unlinked loci to those expected of the random segregation model is very good. We conclude that segregation of such loci is random in a trivalent, and that the frequency of trivalent formation itself is close to 1. Furthermore it is evident that deviations of the data from the ratios expected of the previously used model (Table 1) can be explained in terms of three-chromosome recombination without invoking interference or any additional pairing configurations of the homologues.

(ii) *Centromere-linked genes*

The random segregation model described above cannot be expected to properly describe the segregations of centromere-linked genes such as *trp*5 ($x = 0.33$), nor does it suggest a method for measuring homologue interference, a term used here to describe the influence of a crossover on subsequent crossingover between the same two homologues of a trivalent. Homologue interference would in fact influence the segregation of such genes but its extent cannot be measured by using only the segregation data of one marker without first obtaining information on the probability distributions for the number of crossovers expected between the centromere and the markers observed. However, an expected relationship of ascal classes for asci resulting from trivalent formation can be determined assuming only that the first crossover occurs at random between any two homologues.

To obtain the relationship between ascal classes for the $+/+/-$ trivalent, consider separately the segregation configurations



If the probability of A is $(1-r)$ and of B is r, and if, for example, the probability that a given tetrad is in ascal class 4:0 is denoted $P(4:0)$, then

$$P(4:0) = (2/3)(1-r)+(1/6)r;$$
$$P(2:2) = (1/3)(1-r);$$
$$P(3:1a) = (2/3)r;$$
$$P(3:1b) = (1/6)r.$$

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The equations simplify to

$$P(4:0) = (2/3) - 3P(3:1b);$$
$$P(2:2) = (1/3) - 2P(3:1b);$$
$$P(3:1a) = 4P(3:1b).$$

Both the original model presented in Table 1 and the random model presented in Table 4 are special cases of this relationship for asci resulting from trivalent formation.

Even without considering the effect of bivalent–univalent pairing, this relationship satisfactorily accounts for the data at the *his*4 locus. Using the observed frequency of 3:1's for five times the probability of 3:1(*b*)'s gives a χ^2 value of 2·46, which is acceptable at the 0·1 level of significance. On the other hand, this relationship cannot be used to explain the segregations observed at the *ade*1 locus. In both this trivalent relationship and the bivalent–univalent model in Tables 1 and 4, the probability of ascal class 4:0 is less than two thirds no matter what the value of x. However, the number of asci in ascal class 4:0 was found to be significantly greater than two-thirds the total number of asci observed ($\chi^2 = 12{\cdot}9$, d.f. = 1, $P = < 0{\cdot}001$). Hence, the data indicate that there must have been non-random segregation of the chromosomes at anaphase I, as was proposed by James & Inhaber (1974).

The relationship suggests a test for the amount of trivalent formation (z) using centromere-linked genes. Let w be the conditional probability of obtaining ascal class 3:1(*b*) in one meiosis, given that trivalent pairing has occurred. Then considering both pairing types, we have

$$p_1 = P(4:0) = \frac{2}{3} - \frac{x}{3} + \left(\frac{x}{3} - 3w\right) z;$$
$$p_2 = P(2:2) = \frac{1}{3} - \frac{x}{3} + \left(\frac{x}{3} - 2w\right) z;$$
$$p_3 = P(3:1a) = \frac{2x}{3} + \left(4w - \frac{2x}{3}\right) z;$$
$$p_4 = P(3:1b) = wz.$$

Note that if $w = x/9$ these reduce to the combined probabilities of Table 1, and if $w = 2/15$ these reduce to the combined probabilities of Table 4. Making the substitutions $k = x/3$ and $v = wz$, we get

$$p_1 = \tfrac{2}{3} - k + kz - 3v;$$
$$p_2 = \tfrac{1}{3} - k + kz - 2v;$$
$$p_3 = 2k - 2kz + 4v;$$
$$p_4 = v.$$

Again using the maximum likelihood technique, we wish to find $\hat{z}$ and $\hat{v}$ to maximize

$$L(z, v) \propto p_1^{N_1} p_2^{N_2} p_3^{N_3} p_4^{N_4}.$$

Setting the partial derivatives of $\ln L(z,v)$ to zero gives

$$\sum_{i=1}^{4} \frac{N_i}{p_i} \frac{\partial p_i}{\partial z} = 0$$

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and

$$\sum_{j=1}^{4} \frac{N_j}{p_j} \frac{\partial p_j}{\partial v} = 0.$$

That is,

$$\frac{kN_1}{p_1} + \frac{kN_2}{p_2} - \frac{2kN_3}{p_3} = 0$$

and

$$\frac{-3N_1}{p_1} - \frac{2N_2}{p_2} + \frac{4N_3}{p_3} + \frac{N_4}{p_4} = 0,$$

which simplify to

$$\frac{N_1}{p_1} + \frac{N_2}{p_2} - \frac{2N_3}{p_3} = 0 \tag{1}$$

and

$$\frac{N_1}{p_1} - \frac{N_4}{p_4} = 0. \tag{2}$$

Equation (2) gives

$$v = \{(\tfrac{2}{3} - k + kz)N_4\}/\{N_1 + 3N_4\}.$$

Using this value in eqn (1) gives a quadratic equation in z which is easily solved on the computer. For *trp5*, $\hat{z} = 0{\cdot}541$, $\hat{w} = \hat{v}/\hat{z} = 0{\cdot}096$, and the 0·01 relative likelihood interval defined by those z for which $L(z, v)/L(\hat{z}, \hat{v}) > 0{\cdot}01$ for some v is $0{\cdot}12 \leqslant z \leqslant 1{\cdot}0$, which is consistent with the range for z found using the unlinked loci ($z \geqslant 0{\cdot}85$). However, substantial data would be needed to get a reasonable estimate of z using centromere-linked genes.

One refinement of the model is of interest, since it can be used to describe the probable rate of convergence to the case of random segregation. This refinement assumes that homologue interference is constant along the entire length of the chromosome, and is developed using basic Markov chain theory. In the trivalent, consider crossovers as occurring consecutively. At any given time, then the $+/+/-$ trivalent complex is either in the configuration A or in B, as defined above. Consider a Markov chain whose states are AA, BA, AB, BB_1, and BB_2, where the last letter refers to the configuration after a certain number of crossovers, the first letter refers to the configuration exactly one crossover before this, and any subscript refers to the number of chromosomes which were involved in this last crossover and also carried a '$-$' allele (Fig. 1). Let the probability that a double crossover involves exactly two chromosomes be s; that is, the probability that a crossover involves the same two chromosomes as the immediately previous crossover is s. This is an indicator of homologue interference, with the absence of homologue interference corresponding to $s = 1/3$. Assuming no chromatid interference, the occurrence of one additional crossover will hange the states as follows:

$$\begin{aligned}
&AA \to sAA + (1-s)\,AB;\\
&BA \to (1-s)/2\,AA + (s + (1-s)/2)\,AB:\\
&AB \to s/2\,BA + (1-s)\,BB_1 + s/2\,BB_2;\\
&BB_1 \to (1-s)/4\,BA + (s + (1-s)/2)\,BB_1 + (1-s)/4BB_2;\\
&BB_2 \to s/2\,BA + (1-s)\,BB_1 + s/2\,BB_2.
\end{aligned}$$

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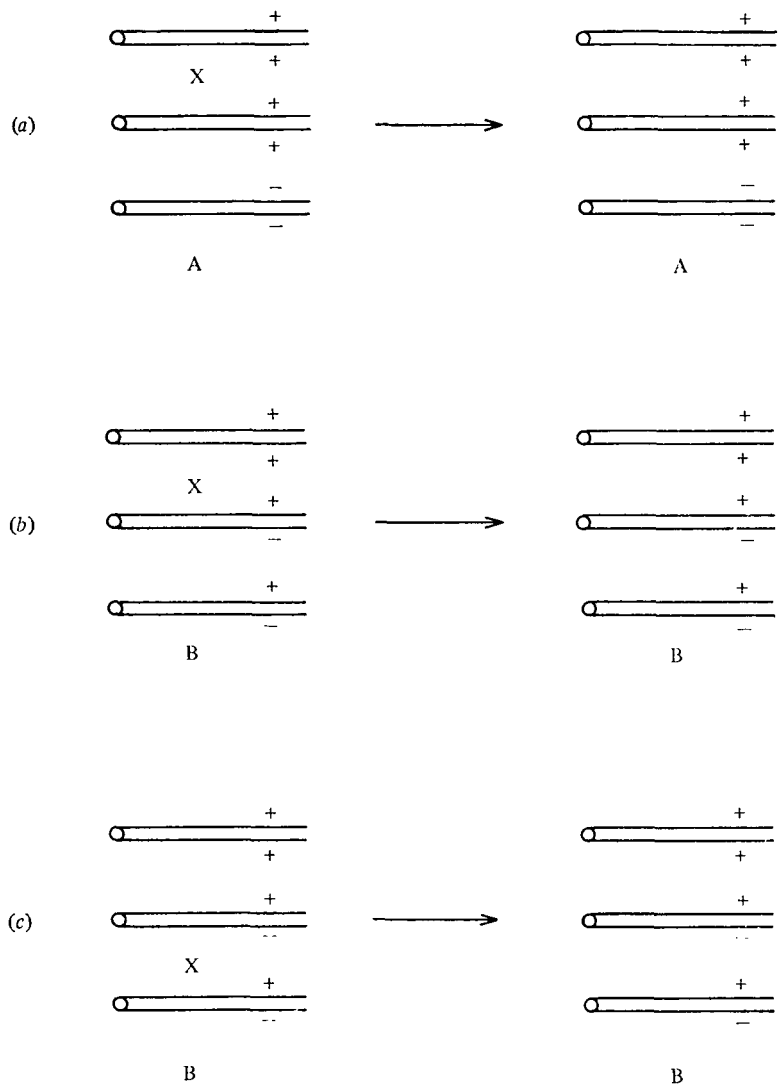

Fig. 1. Three of the Markov chain states. (*a*) State AA. Both before and after the crossover, the chromosomes are in configuration A (*b*) State BB_1. The position of the one (−) allele may or may not be changed by the crossover. (*c*) State BB_2. Both chromosomes involved carry a (−) allele; either these are interchanged by the crossover, or else neither is involved.

Even though it is only necessary to look at the effects of a few crossovers, calculations become much easier if this is represented by the matrix

$$S = \begin{pmatrix} s & 0 & (1-s) & 0 & 0 \\ (1-s)/2 & 0 & (1+s)/2 & 0 & 0 \\ 0 & s/2 & 0 & (1-s) & s/2 \\ 0 & (1-s)/4 & 0 & (1+s)/2 & (1-s)/4 \\ 0 & s/2 & 0 & (1-s) & s/2 \end{pmatrix}.$$

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After the first crossover, the distribution (AA, BA, AB, BB_1, BB_2) is $V_1 = (1/3, 0, 2/3, 0, 0)$ assuming that the position of the first crossover is random. If

$$T = \begin{pmatrix} 2/3 & 1/3 & 0 & 0 \\ 2/3 & 1/3 & 0 & 0 \\ 1/6 & 0 & 2/3 & 1/6 \\ 1/6 & 0 & 2/3 & 1/6 \\ 1/6 & 0 & 2/3 & 1/6 \end{pmatrix}.$$

then it can easily be shown that, if the probability of an ascal class (i) after exactly n crossovers is written $P_n(i)$, $P_n = (P_n(4:0), P_n(2:2), P_n(3:1a), P_n(3:1b),$ $= V_1 S^{n-1} T$ for $n \geqslant 1$. Values of P_n for a few values of s are given in Table 6. If there is negative or no homologue interference ($s \geqslant 1/3$) then the probability of an ascal class does not oscillate with increasing numbers of crossovers, but increases or decreases monotonically, at least after the second crossover. Also, if $s \geqslant 1/3$, the expected number of asci in an ascal class should be between the values predicted by the two-chromosome-recombination-only model and by the random model if no other factor is operating. This observation is essentially true for all markers in Table 3 with the notable exception of *ade*1. In fact, the calculations indicate that, in the absence of homologue interference, the segregation of a centromere-linked marker will be indistinguishable from random whenever those asci which had fewer than two crossovers between the marker and its centromere are rare. Since this is true of all markers which are unlinked to their centromeres in the diploid, the random segregation model should apply to such loci, and resultant estimates of z should be reliable.

A test for interference between homologues ($5 \neq 1/3$) can be made provided information is available concerning the segregations at two loci within a region. If an ascus resulting from trivalent pairing of a doubly marked ($++/++/--$) trisomic segregates 2:2 at the locus nearest the centromere, then a 4:0 or a 3:1b segregation at the other locus signals a multiple crossover between the two markers. The latter segregation involves recombination between all three homologues; the former need not. By referring to Table 6 it can be seen that if $s = 1/3$ then the ratio $(4:0)/(3:1b)$ is expected to be two, but if there is negative interference ($s > 1/3$) the ratio will be greater than 2. On the other hand, if there is positive interference ($s < 1/3$), then the ratio will be less than two provided that most asci have fewer than four crossovers between the two markers.

A correction can be made for the presence of bivalent–univalent complexes as follows: The frequency of 2:2's arising from such complexes as opposed to trivalent complexes is, for markers near the centromere, $1-z$ since if very few crossovers ever occur between a locus and its centromere, both pairing types render the same probability of 2:2's. For markers distant from the centromere the proportion of 2:2's which arise from bivalent–univalent pairing increases to

$$\{(1-z)/9\}/\{(1-z)/9+z/15\} = \frac{1-z}{1-0{\cdot}4z},$$

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Table 6. *Expected segregations from a + + − trivalent after a given number of crossovers and for differing values of s**

No. Crossovers	$S = 0{\cdot}25$				$S = 0{\cdot}35$				$S = 0{\cdot}45$				$S = 0{\cdot}65$				$S = 1$			
	4:0	2:2	3:1(*a*)	3:1(*b*)	4:0	2:2	3:1(*a*)	3:1(*b*)	4:0	2:2	3:(*a*)	3:1(*b*)	4:0	2:2	3:1(*a*)	3:1(*b*)	4:0	2:2	3:1(*a*)	3:1(*b*)
0	0·667	0·333	0	0	0·667	0·333	0	0	0·667	0·333	0	0	0·667	0·333	0	0	0·666	0·333	0	0
1	0·333	0·111	0·444	0·111	0·333	0·111	0·444	0·111	0·333	0·111	0·444	0·111	0·333	0·111	0·444	0·111	0·333	0·111	0·444	0·111
2	0·250	0·056	0·556	0·139	0·283	0·078	0·511	0·128	0·317	0·100	0·467	0·117	0·383	0·144	0·378	0·094	0·500	0·222	0·222	0·056
3	0·260	0·063	0·542	0·135	0·270	0·069	0·528	0·132	0·284	0·078	0·511	0·128	0·320	0·103	0·462	0·115	0·417	0·167	0·333	0·083
4	0·267	0·067	0·533	0·133	0·268	0·067	0·532	0·133	0·275	0·072	0·522	0·131	0·312	0·097	0·473	0·118	0·458	0·194	0·278	0·069
5	0·267	0·067	0·533	0·133	0·267	0·067	0·533	0·133	0·270	0·069	0·529	0·132	0·294	0·085	0·497	0·124	0·438	0·181	0·306	0·076
10	0·267	0·067	0·533	0·133	0·267	0·067	0·533	0·133	0·267	0·067	0·533	0·133	0·271	0·069	0·528	0·132	0·445	0·185	0·296	0·074

* s = probability that a double crossover involves only two chromosomes.

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since $x = 2/3$ for markers randomly segregating in the diploid and since the probabilities in Table 5 hold for those markers. If few asci had three or more crossovers in the observed region, then of the asci in which two or more such crossovers have occurred, one quarter of the bivalent–univalent complexes are expected to produce a 4:0 at the second locus, whereas one-sixth of the trivalent complexes are expected to produce either a 4:0 or a 3:1*b* there. As a result, if N is the total number of asci with 2:2's at the first locus and with either a 4:0 or a 3:1(*b*) at the second, and if the markers are near the centromere,

$$N \times \{(1-z)/4\}/\{(1-z)/4+z/6\} = \left\{\frac{1-z}{1-z/3}\right\} N$$

asci are expected to be 4:0's from bivalent–univalent pairing, and these should be subtracted. Bivalent–univalent pairing does not produce 3:1(*b*)'s. If the markers are distant from the centromere, a larger amount should be subtracted, namely

$$\left\{\frac{1-z}{1-0{\cdot}6z}\right\} N.$$

Expected ratios for various values of s, obtained by using Markov chain theory, are presented in Table 7. Clearly the test is most sensitive if the region under

Table 7. *Effect of crossingover on the ratio* (4:0)/(3:1*b*) *among asci segregating* 2:2 *at a preceding locus*

	s									
Crossovers	0·05	0·15	0·25	0·35	0·45	0·55	0·65	0·75	0·85	0·95
2	1·11	1·35	1·67	2·08	2·64	3·44	4·71	7·00	12·33	39·00
3	1·85	1·88	1·94	2·02	2·13	2·32	2·61	3·17	4·48	11·13
4	2·20	2·07	2·01	2·00	2·07	2·22	2·52	3·10	4·53	11·79
5	2·04	2·02	2·01	2·00	2·03	2·10	2·27	2·62	3·48	7·92
6	1·96	1·99	2·00	2·00	2·01	2·06	2·19	2·49	3·26	7·26
10	2·00	2·00	2·00	2·00	2·00	2·01	2·04	2·15	2·53	4·70

observation is small enough to exclude triple crossovers. Of the data from this investigation, those relating to the region between *trp*5 and *cyh*2 are most appropriate for such a test. Among 2:2 segregations for *trp*5, the number of 4:0 segregations for *cyh*2 was 26 whereas the number of 3:1*b* segregations for the same locus was 13. Correction for bivalent–univalent complexes led to values of 20 and 13, a ratio of 1·5. This ratio does not differ significantly from 2. We conclude that there is no evidence of homologue interference in the region near the *trp*5 locus. Certainly it is very unlikely that $s \geqslant 0{\cdot}55$.

The test described above permits s to be measured along the length of a chromosome arm. However, if the markers are very close together, and this is a prerequisite for high sensitivity, many dissections are required. Present data covering the region between *cyh*2 and *lys*5 were too few for this purpose even though nearly 1000 asci were analysed.

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