Genet. Res., Camb. (1974), **23**, *pp.* 201–206
Printed in Great Britain

A new method of detecting centromere linkage in homothallic yeast

By A. P. JAMES

Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

(*Received* 9 *January* 1974)

SUMMARY

A method is described for detecting centromere linkage in *Saccharomyces cerevisiae* using a simple monohybrid for the locus in question. The method utilizes the two-spored asci produced by a normal homothallic diploid and depends upon a selective elimination of specific chromatids during spore formation. The pattern of selective elimination was not detected in a heterothallic strain but was found to be a dominant trait in an inter-strain cross. It was shown that gross chromosome aberrations are probably not responsible for the two-spored asci produced by either the heterothallic or homothallic strains.

1. INTRODUCTION

Three different methods for detecting centromere linkage in yeast have been reported. These involve either the use of strains which produce asci with linearly arranged spores (Hawthorne, 1955), strains that are polyploid (Roman, Phillips & Sands, 1955), or strains that are heterozygous for loci already known to be centromere-linked (Hawthorne & Mortimer, 1960). This report describes a fourth independent method. It utilizes the two-spored asci of a normal homothallic strain of *S. cerevisiae*, and for its operation depends on the fact that the spores of such asci usually if not always contain non-sister nuclei.

2. MATERIALS AND METHODS

The homothallic strains under test were derived from a series of crosses involving the homothallic *Saccharomyces cerevisiae* (var. *ellipsoideus*) (B67) and two heterothallic strains of *S. cerevisiae* (X1687-16C and X2928-7D). The latter were obtained from R. K. Mortimer, University of California, Berkeley. The derived lines sporulated well and produced asci which contained one, two, three, or four spores in frequencies of about 0·1, 0·3, 0·4 and 0·2 respectively. Such frequencies are usual in laboratory strains of yeast (Takahashi, 1962).

Genetic markers were *trp1*, *ade1*, *ade2*, and disomy for chromosome I. The loci for *trp1* and *ade1* are centromere-linked, that for *trp1* being located about one centi-morgan from the centromere of chromosome IV, and that for *ade1* about 5 centi-morgans from the centromere of chromosome I. The locus of *ade2* on

chromosome XV is independent of its centromere (Mortimer & Hawthorne, 1966). Disomy for chromosome I was radiation-induced (James, 1973).

The strains that were trisomics for chromosome I were phenotypically near normal and were genetically stable in both meiosis and mitosis. These lines were homothallic, and for this reason their spores germinated to produce colonies that were either normal diploids or tetrasomic. The latter were easily identified by their ragged appearance when inspected microscopically after incubation for 24 h.

To produce spores for tetrad analysis, cells were incubated overnight on a presporulation medium consisting of 5% dextrose, 1% yeast extract, 2·3% nutrient agar, 2% Bacto-peptone, and 0·5% agar. They were then replica-plated to a sporulation medium that contained 1% potassium acetate, 0·1% dextrose, 0·25% yeast extract and 2% agar. Sporulation was adequate after incubation for 2 days at 30 °C. A loopful of this culture was then suspended in a 2·5% solution of Glusulase (Endo Laboratories, Inc., Garden City, N.Y.) for 5–6 minutes. The suspension was handled as gently as possible to prevent disruption of spore clusters, and was then spotted with a loop onto a YEPD slab for dissection.

Other procedures were routine.

3. RESULTS AND DISCUSSION

Initial evidence that two-spored asci can be used to detect centromere-linkage was obtained by tetrad analysis of trisomic lines of genotype *ADE1 ade1 ade1 TRP1 trp1*.

A four-spored ascus produced by a cell of this genotype is expected to yield a 2:2 segregation for each of the three characters disomy, adenine and tryptophan. Exceptions may be caused by gene conversion in the case of adenine and tryptophan, and by chromosome loss in the case of disomy.

The situation is a little more complex in the case of two-spored asci. Here the two spores of an ascus may be phenotypically like (+, + or −, −) or unlike +, −) for any of the three characters. The expected frequency of unlike spore pairs is 0·67 if the two existing segregants are a random sample of the four meiotic products. In fact, the frequency of two-spored asci of this type exceeded 0·90 for each character (Table 1). The deviations from expected were highly significant, and it is thus evident that the two spores are not usually a random sample of the four meiotic products.

An acceptable explanation of this non-randomness is that spores containing non-sister nuclei are selectively retained. An alternative explanation, based on selective survival of individual alleles, can be discarded because the overall ratio, + : −, was close to the expected 1:1 for each of the three characters.

Precise information about the extent of this non-random retention of non-sister nuclei was not provided by the data relating to discrete loci because the nuclei of an unlike spore pair could be either sister or non-sister, depending on whether recombination had or had not occurred between the locus and its centromere. The data for disomy, on the other hand, provided more exact information; the two nuclei of an unlike spore pair must be non-sister with very few exceptions because

Table 1. *The segregations for disomy, adenine requirement and tryptophan requirement in two-spored asci produced by cells of genotype* ADE1 ade1 trp1/ade1 TRP1 (*S304*) *and* ade1 ade1 TRP1/ADE1 trp1 (*S303*)

	Disomy			Adenine			Tryptophan		
	+, +	−, −	+, −	+, +	−, −	+, −	+, +	−, −	+, −
Actual	7	3	121	4	9	118	5	5	121
Expected	22	22	87	22	22	87	22	22	87
χ^2		39·9			33·5			39·6	
Probability		$\ll$ 0·01			$\ll$ 0·01			$\ll$ 0·01	

recombination between homologous chromosomes would not affect the disomic phenotype. Exceptions would be a consequence of equational division of the supernumerary chromosome at the first meiotic division and these occur only rarely (James, to be published). Thus, the data for disomy (Table 1) indicate that 92% (121/131) of two-spored asci contain non-sister nuclei. It seems likely that the actual frequency with which this selective retention operates is even higher than the data imply because some of the paired spores might have been false in the sense that they were a result of disruption of the spore clusters produced by complete or three-spored asci.

The 121 spore pairs which contained non-sister nuclei were available for determining whether the amount of recombination is normal among such asci. The numbers of spore pairs that were like for adenine and tryptophan were 6 and 1 respectively. These are expected to represent half the second division segregations. Thus, for *ade1* the estimated frequency of second division segregation is $(2 \times 6)/121 = 0{\cdot}10$. For *trp1* the corresponding value is $(2 \times 1)/121 = 0{\cdot}017$. These are close to the values of 0·10 and 0·009 obtained by other methods (Mortimer & Hawthorne, 1966). It is clear that the amount of crossing over is normal where two-spored asci are concerned. The frequency of unlike spore pairs can thus be used to obtain information on centromere linkage since the closer a locus is to its centromere the more likely it is that segregation will occur at the first meiotic division.

The unusual pattern of segregant elimination in two-spored asci cannot be attributed to the fact that the test strains were trisomic. This was demonstrated by tetrad analysis of two-spored asci produced by a normal homothallic diploid of genotype *TRP1 trp1 ADE2 ade2*. The data are summarized in Table 2. For *trp1* the frequency of unlike spore pairs was 0·90 (122/136), a value nearly identical to that provided by the data of Table 1. In the case of *ade2*, on the other hand, the frequency of unlike spore pairs was only 0·68 (92/136). This value, expected under circumstances of non-linkage, reinforced the conclusion that the frequency of meiotic recombination is essentially normal in cells which produce two-spored asci.

Heterothallic strains of yeast do not display selective spore eliminations. This has been demonstrated by the data of Takahashi (1962), which shows a good fit of actual to expected frequencies of segregant types, for each of ten loci. However,

Table 2. *Tetrad analysis of two-spored asci produced by a homothallic diploid (S316) of genotype* TRP1 trp1 ADE2 ade2

	Tryptophan			Adenine		
	+, +	−, −	+, −	+, +	−, −	+, −
Actual	5	9	122	24	20	92
Expected	23	23	90	23	23	90
χ^2		33·99			0·47	
Probability		$\ll$ 0·01			> 0·3	

Table 3. *Tetrad analysis of two-spored asci produced by heterothallic cells (X2928-7D × XY222-1D*) heterozygous at seven centromere-linked loci*

	Loci								
	ade1	*ura3*	*trp1*	*his2*	*gal1*	*leu1*	*asp5*	Total	Expt
SDS†	0·100	0·102	0·009	0·365	0·133	0·049	0·271	—	—
+, +	11	7	8	6	8	6	5	51	43
−, −	5	8	4	7	5	6	5	40	43
+, −	21	22	25	24	24	25	27	168	173

* Obtained from R. K. Mortimer.

† Second-division segregations (Mortimer & Hawthorne, 1966).

Table 4. *Tetrad analysis of two-spored asci produced by the hybrid resulting from a cross of a heterothallic strain (X2928X-7D) and a homothallic strain (B67)*

	Loci				
	ura3	*trp1*	*his2*	*leu1*	*asp5*
SDS	0·102	0·009	0·365	0·049	0·271
+, +	17	6	5	8	15
−, −	5	4	15	5	7
+, −	45	57	47	54	45
Freq., +, −	0·76	0·85	0·70	0·81	0·67
Expected	0·67	0·67	0·67	0·67	0·67
Probability	> 0·5	< 0·01	< 0·5	< 0·05	< 0·5

only three of the loci used by Takahashi were centromere-linked. Tetrad analysis involving seven centromere-linked genes were therefore carried out with two-spored asci. The results (Table 3) fully corroborated the earlier data; the mean frequency of unlike spore pairs was 0·65.

The non-random retention of non-sister nuclei in two-spored asci of the homothallic strain is a dominant trait. This was shown in the results of a genetic analysis of the two-spored asci from a hybrid produced by crossing heterothallic and homothallic strains (Table 4). The frequency of unlike spore pairs was significantly greater than expected in the data relating to the two loci, *trp1* and *leu1*, most tightly linked to their centromeres. The evidence of linkage is weaker than that produced by the previous data, suggesting that the trait is only partially dominant. However, the spores produced by the first-generation hybrid did not germinate well

and only 63 % of the two-spored asci produced two viable spore colonies. Furthermore, as may be noted from the data of Table 4, the auxotrophic segregants were at a selective disadvantage in most instances. It therefore seems likely that the lower sensitivity of the analysis was due to a bias in the data.

A reliable estimate of the actual frequency of paired non-sister nuclei in the data of Table 4 can be obtained from a consideration of the frequency distribution of spore pairs which contained unlike alleles for 5, 4, 3, 2, 1 or 0 of the five loci under consideration. This distribution was 25, 25, 6, 0, 5 and 6. It is evident and 84 % (56/67) of the spore pairs contained non-sister nuclei.

Six different meiotic defects have been considered by Takahashi (1962) to explain the production of two-spored asci. He was able to discard all but two of these: asynapsis of one pair of homologous chromosomes at the first meiotic division with subsequent elimination of one pair of sister chromatids, and non-splitting of some chromosomes at one pole in the second anaphase. The data of Tables 1 and 3 can be used to test the validity of these two explanations. Both predict preferential survival of sister nuclei. If such is the case, then the frequency of unlike spore pairs should be significantly less than 67 % for centromere-linked loci. This is not so, and it thus appears that the production of two-spored asci is not attributable to gross chromosome mutation in either the heterothallic or homothallic strains. It seems more likely that spore elimination occurs in response to physiological conditions rather than in response to a specific type of aberrant chromosome behaviour. Nevertheless, it is evident that a genetic component of some kind is influential in the homothallic strain.

It seemed possible that the genetic component responsible for non-random segregant retention exists as a tendency for precocious spore formation such that spore walls envelop two sister chromatids. These chromatids might be present either in one diploid nucleus or as two haploid nuclei which would eventually fuse to produce a diploid in a manner typical of a homothallic strain. This was shown not to be so. It was reasoned that if each spore did in fact contain two sister chromatids, then adenine-positive segregants (data of Table 2) would sometimes be heterozygous (*ADE2 ade2*). Such segregants, when themselves sporulated, would yield 2:2 segregations for adenine. With this possibility in mind, 46 adenine-positive segregants from 23 like spore pairs were subjected to tetrad analysis. In addition, ten adenine-positive spore colonies derived from unlike spore pairs and ten adenine-positive spore colonies from asci in which the segregants had been like for tryptophan were sporulated. About eight four-spored asci from each line were dissected. Spore germination was good (99 %), but none of the 2090 segregant spore colonies was adenine-negative. It was evident that the spores of two-spored asci are seldom other than haploid.

The method outlined here for detecting centromere linkage has obvious advantages due to its simplicity, in particular to the fact that detection is possible using a simple monohybrid for the locus in question. If the trait affecting non-random spore survival is not invariably associated with homothallism, then it should be possible to incorporate it into heterothallic laboratory strains of yeast.

REFERENCES

HAWTHORNE, D. C. (1955). The use of linear asci for chromosome mapping in *Saccharomyces*. *Genetics* **40**, 511–518.

HAWTHORNE, D. C. & MORTIMER, R. K. (1960). Chromosome mapping in *Saccharomyces*: centromere-linked genes. *Genetics* **45**, 1085–1110.

JAMES, A. P. (1973). Lethal sectoring in yeast. *Genetics Supplement* **73**, 165–166.

MORTIMER, R. K. & HAWTHORNE, D. C. (1966). Genetic mapping in *Saccharomyces*. *Genetics* **53**, 165–173.

ROMAN, H., PHILLIPS, M. M. & SANDS, S. M. (1955). Studies of polyploid *Saccharomyces*. I. Tetraploid segregation. *Genetics* **40**, 546–561.

TAKAHASHI, T. (1962). Genetic segregation of two-ascus in *Saccharomyces*. *Bulletin of Brewing Science* **8**, 1–9.