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Rare Occurrence of the Tetratype Tetrads in *Saccharomycodes ludwigii*

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Genetic data suggesting the absence of crossover in *Saccharomycodes ludwigii* have been described. Tetrad data obtained from 888 asci from 60 pairs of genes with 22 genetic markers showed the absence of tetratype asci, except for 5 asci in which a single pair of alleles showed tetratype segregation to the other genetic markers in each ascus. Spore arrays in the linear asci showed that the $+--+$ and $+---+$ (or $-++-$) asci occurred at almost equal frequencies. The two coherent spores at each end of an ascus were always marked with different alleles of a gene.

In the pioneering genetic studies of Winge and Laustsen (12), a characteristic life cycle was demonstrated in *Saccharomycodes ludwigii*. Their original strain was a heterothallic diploid. On sporulation, each cell became an ascus containing two pairs of spores, one pair at each end. Normally, each pair of spores copulates just as the spore germinates and produces diploid cells. Hence, there is no opportunity for haplophase multiplication in the natural habitat. However, they obtained haploid vegetative cells by single spore isolation. By tetrad dissection, they observed that two pairs of genes, *L/l* and *N/n*, which originally were marked in their strain, never showed tetratype segregation, whereas almost equal frequencies of the parental ditype and nonparental ditype asci occurred. Lindegren (6) explained this phenomenon by assuming that the location of the gene pair *L/l* was on a different chromosome from *N/n*, with both loci being sufficiently close to the centromeres on their respective chromosomes. One of two coherent spores at one end of the ascus always carried one allele for the *N* and *L* genes; the second carried the other allele. Since adjacent spores fuse at germination, the resultant diploids are again heterozygous for both the *L* and *N* loci and are probably also heterozygous for the mating-type alleles, or any other heterozygous gene in the original diploid.

In a previous series of studies, we observed heterothallism in two independent strains of *S. ludwigii*, O-17 and O-81, and observed two mating (agglutination) types, **a** and α, with mutual agglutination in the spore cultures from these strains (10). These two mating types are probably controlled by a pair of alleles, **a** and α, because all the haploid segregants in the tetrads so far examined showed 2**a**:2α segregation. (Previous symbols for the phenotype of the mating types [agglutination types], X and Y [10], and for the respective genotypes, h^+ and h^- [18], have been respectively revised as **a** and α.) Diploid formation by cell-to-cell fusion between haploid vegetative cells of opposite mating types was carried out (14), as well as the normal spore-to-spore fusion occurring at germination. Many auxotrophic mutants were isolated from the haploid strains after ultraviolet mutagenesis (18). During these studies, the occurrence of a polyploid series of cells was suggested by the deoxyribonucleic acid content of the cells, the cell weight, and by genetic analysis of some diploid derivatives (15-17).

The present communication deals with an investigation of the segregation patterns of the auxotrophic markers in the asci. We observed that two coherent spores at either end of an ascus were marked with different alleles for all the 22 genes tested. In 60 pairs of loci among these genes in the 888 asci tested, there were no tetratype asci, except for 5 asci in which a single pair of alleles in each ascus showed tetratype segregation to the other genetic markers.

MATERIALS AND METHODS

Organisms and genetic symbols. The strains used were descendants of successive crosses between mutants that had been isolated by ultraviolet irradiation (18) of the haploid wild-type strains $IIA_{11}S_1$ (ATCC 26617; **a**) and $IIA_{11}S_2$ (ATCC 26618; α). These two wild-type strains are heterothallic haploid segregants obtained by single-spore isolation from the *S. ludwigii* strain O-81, which was isolated from grape musts in the pilot winery of Yamanashi University by Ohara et al. (9). Strains $IIA_{11}S_1$ and $IIA_{11}S_2$ are of the arbitrarily designated complementary mating types, **a**

and α, respectively, and have been used as standard strains for the determination of mating types.

The symbols and nomenclature used for genetic markers follow the proposal made by the Genetic Nomenclature Committee for Yeast (R. C. von Borstel, personal communication and manuscript in press). Auxotrophic markers used in the present study are those listed in Table 1. Each was shown to be a single mutation as it always showed 2+:2− segregation in the asci from a heterozygous diploid.

Media and cultivation methods. General cultivation of this yeast was performed in malt extract (Blg. 15°) prepared by the method of Lodder and Kreger-van Rij (7). Sock cultures were kept at 4 C in diluted malt extract (Blg. 5°). For the mating of two haploid clones of opposite mating types, the synthetic nutrient medium described by Lindegren (6), to which 1% NaCl had been added to accelerate agglutination (10, 13), was used. Synthetic minimal medium was prepared according to the procedure of Wickerham (11). For testing auxotrophic genetic markers, the replica plating procedure was employed with plates containing Wickerham medium supplemented with nutrients (50 μg each of amino acids and 10 μg each of adenine, guanine, and uracil per ml, if required). The modified medium of McClary and co-workers (8) was selected for spore formation, with the glucose concentration increased from 1 to 10 g/liter (10). To prepare solid media, 2% agar (Difco) was added to the medium. All incubation, except for sporulation at 25 C, was performed at 30 C by letting cultures stand or shaking them on a reciprocal shaker.

Genetic techniques. Mating types were determined by observing an agglutination reaction similar to that described in *Hansenula wingei* (1) or by the ability to form zygotes (10) with strains $IIA_{11}S_1$ (**a**) and $IIA_{11}S_2$ (α) as standard. Diploid hybrids were prepared by isolating a zygote with a micromanipulator. Tetrad dissection was performed under a microscope with the aid of a micromanipulator after treatment of the asci with zymolyase (4) (purchased from Kirin Brewery Co. Ltd.).

RESULTS

Tetrad distributions of genetic markers. Tetrad distributions in 60 pairs of genes among

TABLE 1. *Pooled data of tetrad distribution for 60 different gene pairs*

Gene pair	Tetrad distribution[a]			Gene pair	Tetrad distribution[a]		
	PD	NPD	T		PD	NPD	T
α-ade1	159	173	0	*ade1-lys2*	52	0	0
α-ade2	43	43	0	*ade1-met1*	28	43	1
α-ade3	38	25	1	*ade1-pro1*	15	21	0
α-arg1	52	61	1	*ade1-trp1*	8	0	0
α-cys1	21	22	0	*ade1-ura1*	2	7	0
α-gua1	148	0	0	*ade1-ura2*	3	5	0
α-gua2	64	59	0	*ade2-his2*	40	40	0
α-his1	36	23	0	*ade2-lys1*	32	41	0
α-his2	185	0	0	*ade2-ura1*	41	33	0
α-his3	55	68	1	*ade3-gua2*	22	26	1
α-his4	4	4	0	*ade3-his3*	26	31	1
α-his5	3	2	0	*arg1-gua1*	36	47	1
α-ilv1	37	44	0	*arg1-ilv1*	6	0	0
α-ilv2	12	8	0	*arg1-met1*	6	0	0
α-lys1	58	65	0	*arg1-ura2*	28	41	1
α-lys2	52	54	0	*gua1-his2*	61	0	0
α-met1	66	83	2	*gua1-ura2*	32	38	0
α-pro1	24	21	0	*gua2-his3*	22	38	0
α-trp1	13	9	0	*his1-ilv1*	24	30	0
α-ura1	60	57	0	*his1-lys2*	26	26	0
α-ura2	43	46	0	*his1-met1*	23	29	0
ade1-arg1	8	4	0	*his2-ilv1*	6	5	0
ade1-cys1	22	0	0	*his2-lys1*	34	39	0
ade1-gua2	31	29	0	*his2-met1*	4	4	1
ade1-his1	27	27	0	*his2-ura1*	34	39	0
ade1-his2	4	2	0	*ilv1-lys2*	19	33	0
ade1-his3	23	22	1	*ilv1-met1*	52	0	0
ade1-ilv1	25	35	0	*lys1-ura1*	51	51	0
ade1-ilv2	5	9	0	*lys2-met1*	52	52	0
ade1-lys1	7	7	0	*trp1-cys1*	12	0	0

[a] Tetrad distribution data showing the occurrence of the parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci for a pair of genes were collected from unordered tetrads, except for the linear asci listed in Table 3.

21 loci for auxotrophic traits and the mating-type locus from the multiply marked diploids were tested (Table 1). None of the tetrads from analyses of 888 asci showed tetratype segregation, except for 1 ascus in each of five families. In these exceptional asci, the tetratype ascus occurred due to the unique segregation of a certain marker, i.e., the *his3* gene in one of 46 asci from the diploid prepared by the M-1 (**a** *ade1*) × M-11 (*α his3*) cross; *met1* in 9 and 20 asci from the M-18 (**a** *met1*) × M-10 (*α his2*) and the M-18 (**a** *met1*) × M-2 (*α ade1*) crosses, respectively; *arg1* in one of 70 asci from the R-48 (**a** *arg1 gua1*) × M-5 (*α ura2*) cross; and *ade3* in one of 49 asci from the M-36 (**a** *gua2*) × R-51 (*α ade3 his3*) cross (Table 2).

Another striking observation is that only parental ditype segregations were seen for the mating-type, *gua1*, and *his2* genes, and also for the *arg1*, *ilv1*, and *met1* genes. The same situation was suggested for the *ade1*, *lys2*, *trp1*, and *cys1* genes, though some possible combinations of gene pairs have not yet been tested. These groups of genes, showing a parental ditype segregation, are most probably located on the same chromosome.

Spore arrays in linear asci. A characteristic arrangement of tetrads in each ascus was strongly suggested from the copulation of coherent spores and the segregation of *L/l* and *N/n* traits, as described by Winge and Laustsen (12). With the linear asci of *Saccharomyces cerevisiae*, it has been reported that the + − + − spore array is of the noncrossover class (3). Two alternative arrays of spores in each linear ascus, i.e., the + − + − (type I) and the + − − + or − + + − (type II), with respect to the centromere segregation in *S. ludwigii* may occur. For example, if the mating-type locus is linked closely enough to the centromere, as suggested by Lindegren (6), either type I or type II spore array will ensure that the two coherent spores at one end of the ascus are of opposite mating type, thus permitting copulation and restoration of the diploid state.

To test this possibility, four-spored linear asci from diploids of three different combinations, i.e., the M-1 (**a** *ade1*) × R-36 (*α gua2*), M-1 (**a** *ade1*) × M-11 (*α his3*), and R-52 (**a** *lys1 ura1*) × R-40 (*α ade2 his2*) crosses in which the linear asci were sufficiently formed for tetrad dissection, were carefully dissected, and the tetrad spores from 10 asci of each were collected in their original order in the ascus. None of the genes showed linkage to another in these crosses (Table 1). Two types of segregation occurred, types I and II, with almost equal frequencies (Table 3), whereas no ascus showing the + + − − or − − + + array ever appeared. It must be emphasized that all the genetic traits showed the same type of spore array (type I or II), and no mixture of type I and type II arrays for different gene pairs in the same ascus occurred. Thus, any pair of genes should have showed either parental or nonparental ditype segregations. However, in a tetratype ascus (Table 2) the mixed occurrence of both types of segregation was strongly suggested; e.g., *his3* in the M-1 × M-11 cross showed type II segregation, whereas the other alleles, **a**/*α* and *ade1/ADE1*, were segregated as type I or vice versa. However, this could not be verified, because the data listed in Table 2 came from unordered tetrads. Further analyses of spore arrays in many more asci from these three families were studied by testing the phenotype of two coherent spores where the ordering of the spores in each pair was not identified precisely. Results (Table 4) showed that all the coherent spore pairs were marked with different alleles for all the tested markers.

DISCUSSION

The concept of nuclear migration at meiosis in *S. ludwigii* was suggested by Guilliermond in 1903 (2). He posited that daughter nuclei of the primary nucleus move to opposite ends of the ascus, and then both divide on the spot. If Guilliermond's description were correct, then the two coherent spores should contain sister nuclei.

TABLE 2. *Segregation of genetic markers in asci showing tetratype segregation*

Cross (genotype)	No. of asci dissected[a]	Phenotype of spore[b]			
		A	B	C	D
M-1 (**a** *ade1*) × M-11 (*α his3*)	1/46	**a** *ade his*	*α* wild	**a** *ade*	*α his*
M-18 (**a** *met1*) × M-10 (*α his2*)	1/9	**a** *met*	*α his*	**a** wild	*α met his*
M-18 (**a** *met1*) × M-2 (*α ade1*)	1/20	**a** *ade met*	*α* wild	**a** *ade*	*α met*
R-48 (**a** *arg1 gua1*) × M-5 (*α ura2*)	1/70	**a** *arg gua*	*α ura*	**a** *gua*	*α arg ura*
M-36 (**a** *gua2*) × R-51 (*α ade3 his3*)	1/49	**a** *ade gua*	*α his*	**a** *gua*	*α ade his*

[a] Number of tetratype asci per total number of asci dissected.
[b] Spores in each ascus were not set in array.

TABLE 3. *Tetrad data of the linear asci from the three different diploids*

Segregant		Cross and phenotypic segregation in asci[a]										
		M-1 (**a** *ade1*) × R-36 (α *gua2*)			M-1 (**a** *ade1*) × M-11 (α *his3*)			R-52 (**a** *lys1 ura1*) × R-40 (α *ade2 his2*)				
Ascus	Spore[b]	**a**/α	*ade*	*gua*	**a**/α	*ade*	*his*	**a**/α	*ade*	*his*	*lys*	*ura*
1	A	**a**	+	+	α	−	−	α	−	−	−	−
	B	α	−	−	**a**	+	+	**a**	+	+	+	+
	C	α	−	−	**a**	+	+	α	−	−	−	−
	D	**a**	+	+	α	−	−	**a**	+	+	+	+
2	A	**a**	−	−	**a**	+	−	α	+	−	+	+
	B	α	+	+	α	−	+	**a**	−	+	−	−
	C	α	+	+	**a**	+	−	**a**	−	+	−	−
	D	**a**	−	−	α	−	+	α	+	−	+	+
3	A	**a**	+	−	**a**	−	−	α	+	−	+	−
	B	α	−	+	α	+	+	**a**	−	+	−	+
	C	α	−	+	**a**	−	−	α	+	−	+	−
	D	**a**	+	−	α	+	+	**a**	−	+	−	+
4	A	α	−	+	**a**	+	+	α	+	−	−	+
	B	**a**	+	−	α	−	−	**a**	−	+	+	−
	C	**a**	+	−	α	−	−	α	+	−	−	+
	D	α	−	+	**a**	+	+	**a**	−	+	+	−
5	A	α	−	+	**a**	−	−	α	+	−	−	+
	B	**a**	+	−	α	+	+	**a**	−	+	+	−
	C	**a**	+	−	**a**	−	−	Dead				
	D	α	−	+	α	+	+	α	+	−	−	+
6	A	**a**	−	+	**a**	+	+	Dead				
	B	α	+	−	α	−	−	**a**	+	+	+	−
	C	**a**	−	+	**a**	+	+	**a**	+	+	+	−
	D	α	+	−	α	−	−	α	−	−	−	+
7	A	**a**	−	+	**a**	−	−	Dead				
	B	α	+	−	α	+	+	α	−	−	+	−
	C	**a**	−	+	α	+	+	**a**	+	+	−	+
	D	α	+	−	**a**	−	−	α	−	−	+	−
8	A	**a**	+	+	α'	−	−	**a**	−	+	+	−
	B	α	−	−	**a**	+	+	α	+	−	−	+
	C	α	−	−	**a**	+	+	**a**	−	+	+	−
	D	**a**	+	+	α	−	−	Dead				
9	A	**a**	−	+	α	−	+	Dead				
	B	α	+	−	**a**	+	−	α	−	−	−	+
	C	**a**	−	+	**a**	+	−	α	−	−	−	+
	D	α	+	−	α	−	+	Dead				
10	A	**a**	+	−	α	+	+	α	−	−	+	+
	B	α	−	+	**a**	−	−	Dead				
	C	α	−	+	α	+	+	Dead				
	D	**a**	+	−	**a**	−	−	α	−	−	+	+

[a] +, Growth; −, no growth without the addition of the indicated nutrient.
[b] Spores A, B, C, and D were aligned in that order in each ascus.

Winge and Laustsen (12) negated Guilliermond's scheme of nuclear migration by their observations of the chracteristic segregation patterns for the genetic traits, *L/l* and *N/n*, and by their cytological studies. Since they noted that the two coherent spores could copulate just

TABLE 4. *Phenotypic combination of the two coherent spores in unordered asci from the three different diploids*

Cross (genotype)	Total asci dissected	Phenotype	Phenotype of the two coherent spores	
			+− or −+	++ or −−
M-1 (**a** *ade1*) × R-36 (*α gua2*)	37	**a**/*α*	37	0
		ade	37	0
		gua	37	0
M-1 (**a** *ade1*) × M-11 (*α his3*)	25	**a**/*α*	25	0
		ade	25	0
		his	25	0
R-52 (**a** *lys1 ura1*) × R-40 (*α ade2 his2*)	14	**a**/*α*	14	0
		ade	14	0
		his	14	0
		lys	14	0
		ura	14	0

as the spore germinates, the same segregation pattern for the mating types was suggested. They stated that the second meiotic division in the ascus takes place with the spindle parallel to the longitudinal axis of the ascus, but stipulated no final alignment of the nuclei other than that the two coherent spores contained nonsister nuclei. Lindegren (6) supported this view by assuming that both the *N* and *L* loci are sufficiently near the centromere of their respective chromosomes so that crossing over between the loci and the respective centromere is very rare. Our observations based on genetic study coincide well with their scheme and further suggested the possibility that the two coherent spores at one end of an ascus may be arranged independently of the pair of the opposite end of the ascus, as both +−+− (type I) and +−−+ or −++− (type II) asci occurred with the same frequency. The possible nuclear migration at the first and the second meiotic divisions may be explained by the scheme described by Winge and Laustsen (12) or that by Lindegren (6) (Fig. 1). After the meiotic division, two nonsister nuclei might lie at each end of the cell in either order by slippage (Fig. 1, stage D), and then the spores containing these nuclei may stand side by side in +− or −+ alignment at the maturation of the ascus. Hence the four types of spore array, +−+−, +−−+, −++−, and −+−+, with respect to the centromere alignment of a pair of homologous chromosomes, will be expected with the same frequency, as observed in the present study.

Though it is possible to expect copulation between the inner two spores in the type I ascus on germination, as instances are known in which spores of different coherent pairs may conjugate (2), this might be prohibited in general by the four spores being separated, more or less, into two pairs that lie one at each end of the ascus. Furthermore, the fact observed by Kreger-van Rij (5), that the *S. ludwigii* has a round spore with a narrow but distinct ledge and that the two coherent spores lie in a brim-to-brim position, may also facilitate the copulation between two coherent spores at each end but not between the inner two spores of the type I ascus.

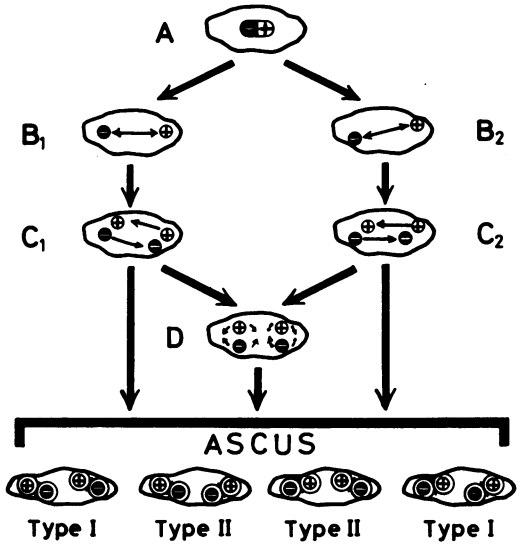

FIG. 1. *Diagram of possible meiosis in S. ludwigii. The schemes of nuclear migration at the first and second meiotic divisions were adopted from that of Winge and Laustsen (A → B_1 → C_1) (12) and that of Lindegren (A → B_2 → C_2) (6). + and −, Centromeres of a pair of homologous chromosomes.*

The absence or very low frequency of a tetratype ascus is not explained by the mode of nuclear migration at meiosis. Lindegren's speculation (6) on the absence of a tetratype ascus is

also unlikely, because it is difficult to envisage that all of the 22 loci, some of which might be located on the same chromosomes, are centromere markers. Since the frequencies of sporulation and spore viability for all the diploids so far tested were high and recombinant clones in nonparental ditype tetrads were indistinguishable from the parental clones with respect to their growth characteristics on nutrient or any other medium tested, it is unlikely that the absence of tetratype asci could be explained by postulating that recombination produces a lethal effect on the spore. Thus, it is most probable that the absence or very low frequency of tetratype asci may be due to the absence of crossing over in *S. ludwigii*. The mechanisms that prevent crossing over and that give rise to the rare tetratype asci have yet to be determined.

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