A STUDY OF IRREGULAR QUADRUPLETS IN SACCHAROMYCES

HAIG P. PAPAIZIAN¹ AND CARL C. LINDEGREN

University of Southern Illinois, Carbondale, Illinois

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AN attempt will be made to introduce the term quadruplet or quad for the four cells produced by meiotic division or the cultures derived therefrom. The term "tetrad", then, defines its original object: the chromosomes at a certain stage of meiosis.

The fact that genes do not always segregate 2:2 in a quad has been confirmed both in microorganisms (PONTECORVO 1958) and higher plants (REIMANN-PHILIPP 1955). Whereas the first papers that drew attention to this phenomenon (LINDEGREN 1953) were mainly concerned with the elimination of explanations in terms of polyploidy or other classical phenomena, more recently attention has been focused on correlations with crossing over (STADLER 1959).

In the present study irregular segregation, or conversion, and crossing over of four linked genes have been analyzed in four different crosses or families. These crosses also included several other markers with which we are not here concerned.

Crosses were made by mixing two haploid populations of opposite mating type and then isolating and dissecting asci at room temperature. This allows little, if any, division of diploid cells before sporulation.

All four markers in all four families were linked, having a Parental:Non-parental ditype ratio greater than 1:1 at a one percent level of significance.

RESULTS

The genes *ch*, *hi*, *is* and *an*, requiring the amino acids choline, histidine, isoleucine and anthranilic acid, respectively, were examined in Family Nos. 99, 106, 107 and 108. Other mutants in these crosses are described in LINDEGREN, LINDEGREN, SCHULT and DESBOROUGH (1959). Mating media and dissection technique are described in LINDEGREN 1949 and media for testing requirements in MIDDLEKAUFF, HINO, YANG, LINDEGREN, and LINDEGREN 1956.

The best order of the four genes is the same in all four families; recombination distances with confidence limits at a five percent level are indicated in Figure 1. Confidence limits were calculated using half the number of spores to allow approximately for the correlation between spores of a quad (PAPAIZIAN 1952).

The complete description of each quad containing a converted gene is given in Table 1. In order to compress this information and entertain the reader, a simple code has been used.

A description of a quad consists of a matrix of four rows for the four spores

¹ Present address: 169 Cold Spring Street, New Haven, Conn.

FIGURE 1.—Maps showing intervals between four genes in four families. Figures between letters give percentage recombination followed by total number of spores in brackets. Below are five percent confidence limits of each interval. The centromere lies to the left of CH (LINDEGREN *et al.* 1959).

TABLE 1
*Irregular quads and their crossover patterns**

Quad no.	Segregation	Conversions	Simplest crossovers
#99	$P_0 = +hi + an$ (<i>ur, arg, a</i>) $P_1 = \overline{ch} + is +$ (<i>th, cu, my, mg, dx, pa, act, a</i>)		
21-696	1114	AN 1.	$\frac{1}{5}$
704	1446	<i>is</i> 4.	$\frac{5}{1}$
708	1156	<i>hi</i> 4, <i>an</i> 1.	$\frac{3}{5}$
716	1152	<i>hi</i> 3.	$\frac{3}{5}$
720	1222	IS all.	$\frac{1}{1}$
744		(CH, HI, IS, AN) all.	
768	1112	AN 1.	$\frac{5}{1}$
772	1555	<i>is</i> 3.	$\frac{1}{1}$
776	1226	<i>hi</i> 4.	$\frac{1}{5}$

TABLE 1—Continued

*Irregular quads and their crossover patterns**

	Quad no.	Segregation	Conversions	Simplest crossovers
#106	$P_0 = +hi + an (ur, cu, \alpha)$			
	$P_1 = ch + is + (t, dx, a)$			
	22-175	1155	CH 3.	3
	183, 439	1114	CH all.	 5
	187	1155	CH all.	3
	199, 247	1113	CH all.	
	203	1113	CH 4.	5
	207	1113	CH 3.	5
	211, 311	1426	CH 3.	5 3 1
	215, 367, 387, 395, 411, 423, 455, 459	1111	CH all.	
	239	1144	CH all.	 3
	255, 271, 463, 471	1444	CH 3.	 1
	259, 299, 399, 431, 435, 443	1111	CH 3.	
	291, 351	1115	CH 3.	5
	307	1666	CH 3, HI 3.	2 1
	319	1444	CH 3, IS all, an 3.	 1
	331	1111	CH 3, IS 4, AN 2.	
	347	1144	CH 3.	 3
	355, 475	1446	CH 3.	5 1
	391, 415	1121	CH all.	3, 5
	403	1114	CH 3.	 5
	419	1112	CH all, IS 3.	 5
	427	1112	CH all.	 5
	451	1111	CH all, HI 2, AN 2.	

TABLE 1—Continued
*Irregular quads and their crossover patterns**

	Quad no.	Segregation	Conversions	Simplest crossovers
#107	$P_0 = + \text{hi is an } (arg, ur, my, mg, me, dx)$			
	$P_1 = ch + + + (g, ma, th)$			
	22-537, 639	1111	CH 3.	$\frac{1}{1}$
	553, 603	1444	CH 3.	$\frac{1}{1}$
	565	1111	(CH, IS, AN) all.	...
	635	1422	CH 3.	$\frac{3}{1}$
	643	1144	CH 3.	$\frac{1}{1}$
#108	$P_0 = ch + + + (th, as, \alpha)$			
	$P_1 = + \text{hi is an } (arg, ur, pa, cu, a)$			
	22-719	1122	IS 4, an 1.	$\frac{3}{1}$
	727	1222	is 1.	$\frac{1}{1}$
	743	1233	AN 1.	$\frac{3}{4}$
	755	1222	AN 4.	$\frac{3}{3}$
	779	1462	CH 2.	$\frac{3}{1}$
	783	1116	(HI, IS) all.	$\frac{1}{3}$
	803	1221	is 3.	$\frac{1}{1, 5}$
	879	1344	CH 2.	$\frac{1}{3}$
	919	1555	CH 2.	$\frac{1}{1}$
	947	1336	HI 3.	$\frac{5}{1}$
	995	1111	CH 2, HI 2.	
	23-005	1111	CH 2, HI 4, IS 4, AN 4.	
	243	1553	CH 1.	$\frac{1}{5}$
	247	1152	HI 3.	$\frac{3}{5}$
	255	1111	CH 2, HI 3.	
	426	1222	HI 4.	$\frac{1}{1}$
	442	1111	IS 3.	
	406	1333	CH 2.	$\frac{1}{1}$

* Explanation in text.

and, in the present case, four columns for the four genes. The six possible orders of two alleles in each column of such a matrix have been given numbers as follows:

P ₀	P ₀	P ₁	P ₀	P ₁	P ₁
P ₀	P ₁	P ₀	P ₁	P ₀	P ₁
P ₁	P ₀	P ₁	P ₁	P ₀	P ₀
P ₁	P ₁	P ₀	P ₀	P ₁	P ₀
1	2	3	4	5	6

where P₀ represents the alleles derived from one parent and P₁ those from the other. The four columns of any matrix can then be represented by four numbers. Successive numbers represent the columns for genes *ch*, *hi*, *is* and *an* in that order.

The quads from yeast are unordered and therefore arbitrary; in the table the spore order in each quad has been arranged so that the first gene, *ch*, is in order 1.

Conversions are represented by two letters denoting the allele that is irregular and a number representing the row of the matrix in a 3:1 quad or the word "all" in a 4:0 quad.

The crossovers which can account for each quad are represented by a new convention, borrowed from games theory. In a two by two matrix the first and second columns represent the genetic strands 1 and 2, respectively, and the top and bottom rows represent strands 3 and 4, respectively. Thus, a crossover between strands 2 and 3 is represented by a figure in the top right-hand quadrant. To identify the interval in which a crossover occurs two numbers are reserved for each: 1 and 2 for the first interval, 3 and 4 for the second, 5 and 6 for the third. Quad 21708 entered as "1156 *hi* 4, *an* 1, $\frac{3}{5}$ " is:

<i>CH</i>	<i>hi</i>	<i>is</i>	(<i>an</i>)	and the crossover pattern is:	<i>CH</i>	<i>hi</i>	<i>IS</i>	<i>an</i>
<i>CH</i>	<i>hi</i>	<i>IS</i>	<i>AN</i>		<i>CH</i>	<i>hi</i>	<i>IS</i>	<i>an</i>
<i>ch</i>	<i>HI</i>	<i>IS</i>	<i>an</i>		<i>ch</i>	<i>HI</i>	<i>is</i>	(<i>an</i>)
<i>ch</i>	(<i>hi</i>)	<i>is</i>	<i>an</i>		<i>ch</i>	(<i>hi</i>)	<i>is</i>	<i>AN</i>

where the converted genes are in brackets.

It will be noticed that two-strand double crossovers are represented by figures in the same quadrant, i.e., in both the same row and column; three-strand doubles by figures in either the same row or column; four-strand doubles by figures in neither the same row or column.

Only four-spored asci were used in this work. three-spored asci accounted for less than five percent of the total asci. Two-spored asci were very rare, and no single-spored asci were seen. The number of asci dissected in Families 99, 106, 107 and 108 were 34, 94, 120 and 188, respectively. The asci in which one or more spores failed to germinate amounted to 9, 20, 42, and 23 percent, respectively. The genotype of the ungerminated spore, where the three others are known, has been inferred for the purpose of map distances in Figure 1.

Discrepancies in the total spores shown in Figure 1 and in the total quads which

can be inferred from Table 2 are accounted for by the cases in which cultures were lost or could not be classified. Loci with 4:0 segregation were not used for estimating map distances in Figure 1.

DISCUSSION

It is assumed that the conversions enumerated were not due to merely phenotypic changes or due to errors of classification but represent genotypes. This assumption is partly justified by the detailed analysis of similar conversions (LINDEGREN 1953).

Two genetic models are poor candidates to account for these conversions. The first is polyploidy or polysomy; this can give 3:1 or 4:0 quads and with two crossovers can allow genes on either side to segregate regularly; it cannot account for four recessives and no dominants in a quad, but we do not have such in the present data. If we assume that single crossovers are more frequent than doubles in the intervals under consideration, then this model would predict that more often than not a series of adjacent linked genes would be converted simultaneously. This is not the case in the present data (see below). Again it can be shown that if trisomy is used to account for single conversions, then the strand bearing the conversion will also be a crossover strand. Furthermore, spores from irregular quads would tend to be polysomic themselves and have irregular quads in their progeny. These conditions, as shown below, do not fit the present data.

The second model is somatic crossing over during division, if any, of the diploid cell. This could account for 4:0 or 3:1 quads depending upon the multiplicity of the chromosomes when crossing over takes place. Two crossovers could give conversions with regular segregation of genes on either side, but again if single crossovers are more frequent than two in adjacent intervals, then simultaneous adjacent conversions should be more frequent than single conversions.

In quads 21744 (*ME* segregated regularly), 22307, 22331, 22451, 22565, 22719, 23005 and 23255 an allele of one parent was in excess at one locus and an allele of the other parent was in excess at another locus. Such an occurrence is impossible on a trisomic model and with somatic crossing over three or more crossovers would be required. In balanced tetrasomics the allele in excess must always be the dominant which contradicts quads like 22719.

The frequencies of conversion are given in Table 2. The four genes in each

TABLE 2
*Frequencies of conversion**

Family	<i>ch</i> $\rightleftharpoons$ <i>CH</i>	<i>hi</i> $\rightleftharpoons$ <i>HI</i>	<i>is</i> $\rightleftharpoons$ <i>IS</i>	<i>an</i> $\rightleftharpoons$ <i>AN</i>	Total
99	2 = 1.7%	2-3 = 4.6%	4-2 = 5.5%	4-1 = 4.8%	18 = 4.0%
106	61 = 22.3%	2 = 0.7%	4 = 1.5%	2-1 = 1.0%	70 = 6.7%
107	8 = 8.0%	0	2 = 2.0%	2 = 2.0%	12 = 3.0%
108	8 = 1.3%	9 = 1.3%	5-2 = 1.0%	3-1 = 0.6%	28 = 1.1%
Total	79 = 7.2%	16 = 1.2%	19 = 1.6%	14 = 1.1%	128 = 2.7%

* In each column the first and positive number is the number of genes converted to wild type and the second, negative, number, the number converted to the recessive allele. Both figures are considered positive in calculating percentages. Marginal percentages represent average conversions per gene, per Family and the grand average which is the average percentage genes converted per total genes observed.

family and any particular gene in each of the four families is converted either to the dominant or to the recessive with a frequency lying, for the most part, between one percent and five percent. One notable exception is the gene *ch* in Family 106. Conversion to the wild type is more frequent than to the recessive with the exception of gene *hi* in Family 99.

If the distribution of quads with 0, 1, 2, 3 conversions is compared with a Poisson, there are too few quads with a single converted gene and too many with multiple conversions. This is not entirely due to the five quads with four conversions and the one quad with eight conversions. The effect is significant at a five percent level of probability but not at a one percent level.

The segregation of the quads from which the parents came does not seem to affect the rate of conversion. One parent of Family 108 was a spore from Family 107 in which only the gene *mg* was converted to wild type. The other parent was the first row of quad 21708 in which *AN* was converted to *an* and *HI* to *hi*, but only the former conversion was on the strand used as parent; yet Family 108 is not exceptional as regards either of these genes. One parent of Family 107 was the first row of quad 21716 in which *HI* was converted to *hi* but on a different strand.

Conversion and crossing over: There is no evidence in this data of a correlation between the occurrence of conversion and of crossing over in a quad. This can be seen in Table 3 where the sum of crossovers in all three intervals is expressed as a mean number of crossovers (chiasmata) per quad (meiotic division). The distribution of crossovers in Family 108, the only family large enough to warrant such analysis, agree with a Poisson both for regular and irregular quads. This shows, incidentally, that there is no chromosome interference over this region. In quads in which there is a crossover and a conversion, there is no marked tendency for the crossover to occur in an interval adjacent to the conversion. In quads in which there is a conversion and a crossover in an adjacent interval, the strand carrying the converted gene should be involved in the crossover in half the cases if the two events are independent. Quad 21708, illustrated above, is an example of two conversions and two crossovers in adjacent intervals without the same strand being involved in either case. In the present data there are altogether 29 quads with a conversion and an adjacent crossover, and in only six of these does the crossover involve a strand carrying a conversion. This is significantly different (at a one percent level) from one half, but the effect is almost entirely due to the numerous

TABLE 3

Comparison of crossovers in regular and irregular quads

Family	Total crossovers per quad, in quads with and without converted genes		Significance (P)
	With	Without	
99	1.5	1.5	
106	1.3	2.4	0.025
107	0.7	1.5	0.21
108	1.3	1.1	0.35

quads, especially in Family 106, in which *ch* segregates irregularly. If these are removed, then the ratio becomes four out of ten, which is very near to one half.

In all the foregoing, the minimum number of crossovers that will account for the distribution of alleles has been taken. The same distributions could result from a more complex pattern of crossovers, and it is only through lack of information that the simplest pattern is assumed. This assumption may well be erroneous since there is some evidence (PONTECORVO 1958) that crossovers occur in clusters over small regions. This may account for the fact that in studies of conversion over very small intervals there seems to be a correlation with crossing over not found in the present data.

SUMMARY

From this data it can only be said that alleles, in the heterozygous state, mutate to or are replaced by other alleles at a rate much higher than the spontaneous mutation rate. In particular genes and particular families, for instance *ch* in Family 106, this can reach over 20 percent.

It would also appear that the change is not random in the way that mutation is, but that the new allele is the same as a parental allele. This is indicated in the present data by the fact that conversions to wild type are much more frequent than to the recessive, and it is supported by other data (PAPAIZIAN 1958) in which conversion affected a multiple allele system and in which conversion was not random to any allele of the series but always to the particular allele present in the heterozygote. The data indicates that neither polyploidy, polysomy, or somatic crossing over is involved.

Regular meiotic crossing over appears to be an independent phenomenon not correlated in any way with conversion.

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