INFLUENCE OF TEMPERATURE ON RECOMBINATION IN YEAST

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1. INTRODUCTION

THE effect of temperature upon recombination frequency has been the object of numerous investigations since the pioneer work of Plough (1917). The result obtained by Plough with *Drosophila melanogaster*, namely a U-shaped temperature function of crossing over with maxima around 9° C. and 32° C. and a minimum around 22° C. for certain chromosome regions (Smith, 1936), has since been substantially confirmed (for example, Stern 1926; Kikkawa, 1934; Hayman and Parsons, 1960). When organisms other than *Drosophila* have been examined, however, no consistent picture has been obtained. For instance, crossing over in *Habrobracon* appears independent of temperature (Clark, 1943) and in *Ustilago hordei*, a minimum frequency at 8° C. has been found (Hüttig, 1931). In *Neurospora crassa*, various results have been obtained (Rifaat, 1959; Towe and Stadler, 1964; McNelly-Ingle, Lamb and Frost, 1966) and it appears likely that genetic background exerts an important influence (Lawrence, 1963). In general, a change of recombination frequency with temperature has appeared to be confined to regions close to the centromere. In both *Drosophila* (Lawrence, 1958) and *Neurospora* (McNelly-Ingle, Lamb and Frost, 1966), however, certain regions removed from the centromere have shown an increased frequency of recombination at lower temperatures. The purpose of the experiments reported here was to investigate the effect of different temperatures upon recombination in a number of regions in hybrid *Saccharomyces*, in which there is a stable diplophase and little effect upon viability and growth rate when cultures carry many mutant markers.

2. MATERIALS AND METHODS

(i) Strains

The diploid *Saccharomyces* hybrids investigated had the genotypes †

$$\text{X764} \quad \frac{a}{a} \quad \frac{ur_3}{UR_3} \text{ —o— } \frac{thr_3}{THR_3} \quad \frac{tr_5}{TR_5} \quad \frac{le_1}{LE_1} \text{ —o— } \frac{ad_6}{AD_6} \quad \frac{ma_1}{MA_1} \quad \frac{o\,ar_4}{oAR_4} \quad \frac{hi_6}{HI_6} \text{ —o— } \frac{ly_1}{LY_1}$$

$$\text{XJ119} \quad \frac{\text{—o—GA}_1}{\text{—o—ga}_1} \quad \frac{LY_2}{ly_2} \quad \frac{TY_1}{ty_1} \text{ —o—} \frac{a}{a} \quad \frac{ur_3}{UR_3} \text{ —o— } \frac{thr_3}{THR_3} \quad \frac{tr_2}{TR_2} \quad \frac{le_1}{LE_1} \text{ —o— } \frac{ad_6}{AD_6} \quad \frac{ma_1}{MA_1}$$

$$\frac{\text{—o— ar}_4}{\text{—o—AR}_4} \quad \frac{thr_1}{THR_1} \quad \frac{cu_1}{CU_1} \quad \frac{\text{—o— ser}_1}{\text{—o—SER}_1} \quad \frac{hi_8}{HI_8}$$

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† The symbols *a* and α represent the complementary mating type alleles; ad, ar, hi, le, ly, thr, ser, tr, ty and ur represent ability (upper case) or inability (lower case) to grow in the absence of adenine, arginine, histidine, leucine, lysine, threonine (thr_1) or threonine plus

(ii) *Media and Procedure*

Sporulation was achieved by growing cultures for two days at 30° C. on slopes of pre-sporulation medium (5 per cent. glucose, 2·3 per cent. Difco Nutrient Agar) and transferring to slopes of sporulation medium (0·3 per cent. sodium acetate, 0·02 per cent. raffinose). The latter were incubated at either 25° C., 15° C. or 12° C.

Four-spored asci were dissected after digestion of ascus walls by snail juice (Johnston and Mortimer, 1959) and tetrad analysis of asci with four viable spores performed by replica plating (Lederberg and Lederberg, 1952). Omission, fermentation and copper sensitivity media were as previously described (Hawthorne and Mortimer, 1960).

3. RESULTS AND DISCUSSION

Sporulation was slower at 15° C. and 12° C. (completion of all asci by 10-12 days) than at 25° C. (completion by 3-5 days). The final yield of asci was also less at the lower temperatures (10-15 per cent.) than at 25° C. (20-25 per cent. asci). Tetrads were classified into parental ditype (PD), non-parental ditype (NPD), and tetratype (T), for the segregation of pairs of linked genes, and into first division segregation (FDS) and second division segregation (SDS) for the segregation of genes with their centromeres. Centromere segregation was determined for each ascus from an examination of the segregation of the different centromere-linked markers in the crosses, *i.e.* ur_3, le_1, ar_4 and hi_6 for X764, and ga_1, ur_3, le_1, and ar_4 for XJ119 (Howe, 1956; Hawthorne and Mortimer, 1960). The results determined at the different temperatures are presented for both hybrids in tables 1 and 2. The map distance in centimorgans was calculated for each region and temperature. The results were tested for the statistical significance of differences of recombination at the different temperatures.

These experiments permit the following conclusions. Firstly, the recombination frequency of many chromosome regions of yeast appears to be independent of temperature. These regions include some which are close to centromeres (c-ur_3, c-ar_4) and others which are distal (ly_2-ty_1, thr_3-tr_2, ar_4-thr_1, thr_1-cu_1). Other chromosome regions in which recombination appears independent of temperature contain lengths both close to and considerably removed from centromeres (c-thr_3 and c-ly_1). In these regions, however, an effect of temperature upon a short length adjacent to the centromere could be masked by the greater but unchanged recombination frequency of the remaining length removed from the centromere. Additional markers more closely linked with respective centromeres are required for further investigation.

Other regions of these strains, however, show a significant response to the variation of sporulation temperature. The effect observed is consistently a reduction of recombination frequency at 15° C. or

methionine (thr_s), serine, trytophan, tyrosine plus phenylalanine, and uracil. ma/MA and ga/GA represents inability/ability to ferment maltose and galactose respectively. cu/CU represents inability/ability to grow in the presence of 60 μg./ml. of copper. The mapping of these genes has been described (Hawthorne and Mortimer, 1960; Mortimer and Hawthorne, 1966).

TABLE 1

Tetrad analysis and map distances of hybrid X764

Region	Chromosome	12° C.			15° C.			25° C.				
		PD:NPD / T	FDS:SDS	Map distance (CM)*	PD:NPD / T	FDS:SDS	Map distance (CM)	FDS:SDS	Map distance (CM)	X² 25-12 P	X² 25-15 P
c-ur₃	V	...	116 : 17	6·39	...	54 : 12	9·09	158 : 17	4·85	2·39 > 0·1	3·24 > 0·05
c-thr₃	V	...	59 : 74	27·81	...	23 : 43	32·57	60 : 115	32·85	2·42 > 0·1	0·007 > 0·9
tr₅-le₁	VII	100 : 0 / 25	...	10·00	49 : 0 / 18	...	13·43	117 : 0 / 51	15·17	4·00 < 0·05	0·280 > 0·5
c-le₁	VII	...	123 : 3	1·19	...	67 : 0	0·00	159 : 9	2·67	1·63 > 0·2	2·43 > 0·1
c-ad₆	VII	...	77 : 49	19·44	...	48 : 19	14·17	67 : 101	30·05	12·9 < 0·01	19·09 < 0·01
c-ar₄	VIII	...	109 : 19	7·42	...	59 : 8	5·97	148 : 21	6·21	0·73 > 0·3	0·118 > 0·7
c-ly₁	IX	...	76 : 42	17·79	...	39 : 22	18·03	102 : 54	17·30	0·028 > 0·8	0·040 > 0·8

* The map distance x, in centimorgans, was calculated from the formulae:

$$x = \frac{100}{2}\left(\frac{SDS}{FDS+SDS}\right)$$

or

$$x = \frac{100}{2}\left(\frac{T+6NPD}{PD+NPD+T}\right) \quad \text{Perkins (1953)}$$

The first equation does not take into account the effect of multiple crossing over on second division segregation frequency and is not reliable for regions greater than approximately 30 centimorgans.

TABLE 2

Tetrad analysis and map distances of hybrid XJ119

Region	Chromosome	15° C.			25° C.			X² P
		PD : NPD / T	FDS : SDS	Map distance (CM)*	PD : NPD / T	FDS : SDS	Map distance (CM)	25-15
c-ga₁	II	...	104 : 9	3·98	...	76 : 15	8·24	3·524 > 0·05
ga₁-ly₂	II	23 : 5 / 87	...	50·86	33 : 6 / 52	...	48·35	8·17 < 0·02
ly₂-ty₁	II	38 : 1 / 76	...	35·65	35 : 1 / 56	...	33·69	1·92 > 0·3
c-ur₃	V	...	104 : 12	5·17	...	81 : 10	5·49	0·009 > 0·9
c-thr₃	V	...	46 : 69	30·00	...	28 : 62	34·44	1·728 > 0·1
thr₃-tr₂	V	62 : 1 / 53	...	25·43	41 : 0 / 48	...	26·96	2·09 > 0·3
c-le₁	VII	...	102 : 10	4·46	...	84 : 8	4·34	0·003 > 0·95
c-ad₆	VII	...	68 : 48	20·68	...	33 : 57	31·66	9·776 < 0·01
ad₆-MA₁	VII	23 : 12 / 81	...	65·94	17 : 16 / 57	...	85·00	2·80 > 0·2
c-ar₄	VIII	...	93 : 19	8·48	...	71 : 17	9·65	0·184 > 0·5
ar₄-thr₁	VIII	74 : 0 / 34	...	15·74	65 : 0 / 26	...	14·28	0·199 > 0·5
thr₁-CU₁	VIII	63 : 0 / 40	...	19·41	53 : 0 / 36	...	20·22	0·055 > 0·8
c-ser₁	Frag. I	...	33 : 81	35·53	...	27 : 62	34·83	0·046 > 0·8
ser₁-his₈	Frag. I	96 : 0 / 17	...	7·62	61 : 0 / 29	...	16·11	8·423 < 0·01

* See footnote for table 1.

$12°$ C. compared with $25°$ C. The most striking effect is seen in the region c-ad$_6$ of linkage group VII, in which the frequency of recombination is reduced to 30-50 per cent. of its value at $25°$ C. in both hybrids. The result is doubly interesting in that c-ad$_6$ is a relatively long region and it is unlikely therefore that the effect of temperature is confined to a length closely linked to the centromere. Another marked region of chromosome VII may be affected in similar fashion since crossing over is apparently reduced at $12°$ C. in the region le$_1$-tr$_5$ of hybrid X764. There is, however, no evidence of a corresponding reduction in region c-le$_1$ of either hybrid. The increase in recombination frequency observed between $15°$ C. and $12°$ C. for the region c-ad$_6$ is not statistically significant and therefore apparently does not indicate an inflexion point between $25°$ C. and $12°$ C.

In XJ119, recombination in the centromere region c-ga$_1$ of chromosome II is reduced approximately twofold at $15°$ C. Although this difference is not statistically significant $(P > 0.05)$, it becomes so $(\chi^2 = 6.25, P < 0.02)$, if 8·24 CM is taken as the standard map distance at $25°$ C. This procedure seems justified by the supporting data of Hawthorne and Mortimer (1960), who give a SDS value of 15·3 per cent. to region c-ga$_1$. Another approximately twofold reduction in crossing over at $15°$ C. is obtained in XJ119. This effect is observed in region ser$_1$-hi$_8$ which is well removed from the centromere. This result and the reduction in recombination in the extended region c-ad$_6$, demonstrate that the effects of temperature upon crossing over in yeast are not confined to regions close to a centromere. In this respect these findings in yeast confirm those of Lawrence (1958, 1963) in *Drosophila* and of McNelly-Ingle, Lamb and Frost (1966) in *Neurospora*.

Other experiments with *Drosophila* (Plough, 1921; Graubard, 1934; Politzer, 1940), *Neurospora* (Rifaat, 1959; Towe and Stadler, 1964) and *Podospora* (Rizet and Engelmann, 1949) have suggested that the effect of temperature upon crossing over is concentrated in regions close to a centromere.

In the regions sensitive to a lowered temperature, the effect in yeast is opposite to that found in *Drosophila* (Plough, 1917; Smith, 1936), in which lower temperatures increase the degree of crossing over in sensitive regions. This effect in yeast is similar, however, to that in *Ustilago hordei* (Hüttig, 1931) and to certain findings in *Neurospora* (Rifaat, 1959), in which some crosses showed less recombination at $17°$ C. than at $25°$ C. Other experiments with *Neurospora* (Lawrence, 1958, 1963; Towe and Stadler, 1964; McNelly-Ingle, Lamb and Frost, 1966), however, in which reduced temperatures of $15°$ C. and $18°$ C. were used, have given results similar to those with *Drosophila*, *i.e.* increased recombination frequency at lower temperature. Compared with these results, yeast shows an opposite effect to *Neurospora*. One possible explanation of these variations is that there exists genetic control over the response of recombination frequency to changes in temperature. Such control is indicated in the different results between

crosses of *Neurospora* (Rifaat, 1959) and *Podospora anserina* (Rizet and Engelman, 1949). The above experiments with yeast do not permit any conclusion to be drawn in this respect.

4. SUMMARY

1. Two diploid hybrids of *Saccharomyces cerevisiae* with marked regions on several linkage groups have been sporulated at 25° C. and 15° C., and one of them at 12° C. also. Asci have been subjected to tetrad analysis and recombination frequencies compared for the different temperatures.

2. The frequency of crossing over in a majority of regions is not significantly different at these temperatures.

3. The regions unaffected by a lower temperature include those close to, and distant from a centromere.

4. Recombination frequency of a few regions is approximately halved by sporulation at reduced temperature. This effect is not confined to regions in the vicinity of a centromere.

5. The opposite effect has been found in *Drosophila*. Similar and different results have been obtained with *Neurospora crassa*.

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