The effect of temperature on recombination frequency in *Neurospora crassa*

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1. INTRODUCTION

The effect of temperature on recombination frequency has been studied by several workers. Plough (1917, 1921, with corrections by Smith, 1936) using *Drosophila melanogaster* showed a negative correlation between temperature and recombination frequency below 25°C. but a positive correlation at higher temperatures. This effect was observed only in close proximity to the centromere. These observations were confirmed by Stern (1926), Graubard (1934) and Pollitzer (1940). However, Lawrence (1958, 1963) has found that in some crosses the temperature effect may be detected in more distal regions of the chromosome while absent in the centromeric region.

Studies using micro-organisms have shown some variation. Hüttig (1931), using a measure probably related to the second division segregation frequency of mating-type in *Ustilago hordei*, obtained a U-shaped curve with a minimum at 8°C. Rizet & Engelmann (1949), using *Podospora anserina*, reported either increases or decreases in recombination frequency with a rise in temperature of incubation depending on the mutant pair crossed. No consistent relationship between temperature and recombination frequency was observed in *Sordaria fimicola* by Olive (1956).

Different relationships between recombination frequency and temperature have been reported for *Neurospora crassa* by Rifaat (1959) and by Towe & Stadler (1964). The former, using markers in Linkage Group I, found a positive correlation over the range 17° to 30°C. but no such effect when the experiment was repeated using progeny from this cross. The latter, however, report a minimum frequency of second division segregation at 25°C. and a maximum at 18°C. for markers in Linkage Group VI. Similar results were obtained using progeny from this cross.

The study reported here, using *Neurospora crassa*, was aimed at clarifying some of the points raised by the work summarized above. The relationship between recombination frequency and temperature has been examined in several crosses over the maximum range (15° to 30°C.) at which the sexual cycle can be completed (McNelly-Ingle & Frost, 1965). Markers have been used in both Linkage Groups I and VI to determine if the temperature relationship is common or specific for

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the linkage group under test. The markers were selected to span distal as well as proximal regions in the two linkage groups. In addition an attempt has been made to determine the time at which temperature affects the processes leading to recombination.

2. MATERIALS AND METHODS

The following strains of *N. crassa* were used:

Lindegren 25*a* (L25*a*). Wild-type.

Abbott 4*a* (A4*a*). Wild-type re-isolate derived from progeny from the cross Abbott 4*A* × Lindegren 25*a* backcrossed to Abbott 4*A* seven times.

asco(37402). Lysine requiring and showing ascospore abortion. Four re-isolates of *asco* from a cross to Lindegren 1*A* wild-type. Linkage Group VI.

cr(F945)*A*. Crisp morphological mutant. Re-isolated from a cross to L25*a*. Linkage Group I.

his-2, *nic*-2(C94, 43002)*a*. Double mutant requiring histidine and nicotinamide. Re-isolated from a cross to A4*a*. Linkage Group I.

cr,me-6,*aur*(F945, 35809, 34508)*A*. Triple mutant requiring methionine and with aurescent conidia. Derived from mutants backcrossed six times to Lindegren 1*A*. Linkage Group I.

ylo, *tryp*-2(Y30539, 75001)*A*. Double mutant with yellow conidia and requiring tryptophane. Linkage Group VI.

ad-1(3254)*a*. Adenine requiring. Linkage Group VI.

Crosses were made on the minimal medium of Westergaard & Mitchell (1947) adjusted to pH 6·7 and supplemented according to the strains used. The proto-perithecial parent, the first strain listed below in each cross, was incubated for 14 days at 15°C. but for 7 days at higher temperatures before the paternal strain was added as a conidial suspension in suitably supplemented distilled water. The conidiating parent was initially grown at 25°C. in the light and stored at 4°C. before use. The crosses of *asco* were usually analysed when spores were first visible on the wall of the tube. Random spore isolates were made 3 weeks after conidiation but 6 weeks in the case of crosses incubated at 15°C. Mating-type was determined on Petri dishes using Lindegren 1*A* and Lindegren 1*a* (a backcrossed derivative of L1*A*) as the standard tester strains.

The determination of the second division segregation frequency of *asco* was made by visual scoring of the asci mounted in a solution of sucrose. Remarkably high frequencies of second division segregation of *asco* resulted. Accordingly, a comparison of estimates using water or sucrose as the mounting fluid was made over the range of temperatures used. An increased frequency using sucrose instead of water was observed at all temperatures tested. Compared with water as the mounting fluid, the increases with sucrose ranged from 14 to 28% and were significantly different at the 1–2% level of probability. Thus from crosses incubated at 27·5°C., using water as mounting fluid the percentage second division segregation out of 1264 asci scored was 15·4 ± 1·0 while in 1 M sucrose the percentage out of

1449 asci was 19.7 ± 1.1 ($2 \times 2\chi^2 = 8.93$, $P = < 0.01$). From crosses incubated at 15°C., in water the percentage second division segregation out of 1055 asci scored was 39.8 ± 3.7 but in 2 M sucrose the percentage out of 2435 asci was 45.3 ± 2.5 ($2 \times 2\chi^2 = 9.15$, $P = < 0.01$). Using the same mounting fluid, duplicate crosses showed good agreement. Potassium chloride solution had the same effect as sucrose but the handling of the delicate asci was far easier in the viscous sucrose solutions. At the lower temperatures tested, higher concentrations of sucrose were needed to reduce the preferential bursting of second division asci which occurs in water. These effects of mounting fluids were not discovered until after the initial experiments had been completed; in the later experiments two molar sucrose solution was used throughout as mounting fluid for crosses at all temperatures tested as this concentration was found to minimize the preferential bursting of second division asci.

3. RESULTS

The cross between *asco* and a wild-type strain (A4*a*) was the only one found in which sexual reproduction would occur over the range 15° to 30°C. The range of temperatures over which some wild strains will produce fertile perithecia is more limited than for other wild-types and in addition several mutant strains are sterile at temperatures other than 25°C. or near (McNelly-Ingle & Frost, 1965).

The second division segregation frequency of *asco* in crosses incubated from 15° to 27·5°C. is shown in Table 1 and from 15° to 25°C. in Table 2. A change of 2·5°C. in incubation temperature produced a change in second division segregation frequency significant at the 1% level of probability in most cases. The frequency showed a minimum value at 25°C.; an increasing frequency occurred towards either the highest or lowest temperature tested. Two replicates were made at each temperature tested and there was no significant difference between replicates at the 10% level of probability.

Table 1. *The effect of temperature on the frequency of second division segregation of* asco(37402)

Cross: Abbott 4*a* × *asco*(37402)-35*A*

Temp. °C.	Conc. of sucrose mounting fluid	Total asci	2nd div. seg. frequency: Uncorrected	2nd div. seg. frequency: Corrected[†]	$\chi^2_{2 \times 2}$ for temp. intervals, D.F. = 1
27·5	1 M	1449	19·7	20·6	
25·0	1 M	1109	11·3	12·0	33·4*
20·0	1 M	921	31·9	34·0	131·0*
17·5	2 M	1515	39·3	42·5	13·6*
15·0	2 M	2435	45·3	49·5	13·7*

† Corrected by means of the mapping function curve of Barratt, Newmeyer, Perkins & Garnjobst (1954). $\chi^2_{2 \times 2}$ for temperature intervals have been calculated from the uncorrected values.

* Heterogeneity χ^2 significant at $P = 0.01$.

M = molar.

Table 2. *The effect of temperature on the second division segregation frequency of asco(37402). Repeat experiments*

Cross: Abbott 4a × asco(37402)-35A

Temp. °C.	Total asci	2nd div. seg. frequency:		$\chi^2_{2\times2}$ for temp. intervals, D.F. = 1
		Uncorrected	Corrected†	
25·0	2239	14·6	15·2 ⎫	
22·5	2066	16·8	17·6 ⎬ ⎫	4·08*
20·0	2153	20·9	21·9 ⎫ ⎬	11·1**
17·5	2049	26·0	27·4 ⎬ ⎫	15·3**
15·0	2120	33·7	35·8 ⎭	29·9**

† Corrected by the mapping function curve of Barratt *et al.* (1954). $\chi^2_{2\times2}$ for temperature intervals have been calculated from the uncorrected data.
* Heterogeneity χ^2 significant at $P = 0·05$.
** Heterogeneity χ^2 significant at $P = 0·01$.
2 M sucrose used as mounting fluid for scoring asci throughout.

The initial experiment (Table 1) was later repeated but with differences of medium (e.g. pH 6·5) and physiological states of the inoculum in that fresh material incubated in the light for 7 days at 25°C. was used as conidial parent for all crosses (Table 2). In addition, the asci were mounted in 2 M sucrose solution throughout and scored by a different observer. In this repeat series, three separate experiments
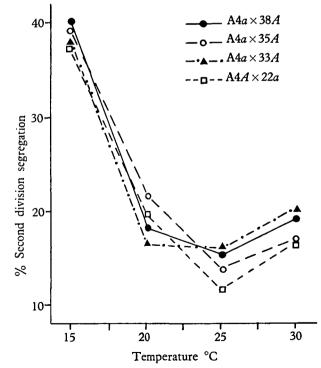

Fig. 1. The effect of temperature on the frequency of second division segregation of *asco* in four re-isolates of *asco*(37402) crossed to Abbott 4.

were made with a total of eight replicates. There was no significant difference between the replicates at the 10% level of probability. The results of the initial and repeat experiments showed a similar response to temperature (Tables 1 and 2) and the slopes of the logarithmic plot of second division segregation frequency against reciprocal of temperature of the two sets of data (Fig. 2) were similar over the range 15° to 20°C. However, the percentage second division segregation of *asco* differed between the two series of experiments, due probably to the different conditions described above and to differences in the maturity of the asci at the time of scoring which can affect the observed second division segregation frequency (Lamb, 1964).

The possibility that the segregation of heritable factors gives rise to variation between crosses in the effect of temperature on recombination frequency was suggested by the work of Rifaat (1959). A limited investigation of this possibility was made using four re-isolates of *asco*. The results, shown in Fig. 1, give no clear evidence of a differing response of second division segregation frequency to temperature between the four re-isolates. Sucrose was used as the mounting fluid for crosses incubated at the higher temperatures as the full implications of

Table 3. *The effect of temperature on the frequency of second division segregation of* asco(37402) *in reciprocal crosses of Abbott* 4a *with* asco-35A

	asco-35A × Abb. 4*a*		% 2nd div. seg.*:		$\chi^2_{2\times2}$ reciprocal crosses	% 2nd div. seg. both phases*
Temp. °C.	Total asci	% 2nd div. seg.	*asco-35A*♀	Abbott 4*a*♀†		
25·0	2144	13·3	14·0	15·2	1·68	14·6 ± 0·4
22·5	2119	15·9	16·8	17·6	0·75	17·2 ± 0·4
20·0	1817	20·2	21·0	21·9	0·26	21·5 ± 0·6
17·5	2064	25·8	27·0	27·4	0·01	27·2 ± 0·7
15·0	2021	31·4	33·3	35·8	2·50	34·5 ± 0·7

    * Corrected by the mapping function curve of Barratt *et al.* (1954). Heterogeneity χ^2 values have been calculated from the uncorrected data for the reciprocal crosses and none is significant at $P = 0·10$ (D.F. = 1 throughout).
    † Primary data given in Table 2.
    2 M sucrose used as mounting fluid for scoring asci throughout.

the preferential bursting of second division segregation asci in water were not known at the time these experiments were performed. In addition, reciprocal crosses between A4*a* (derived from Abbott 4*A* wild-type) and *asco-35A* (re-isolated from a cross to Lindegren 1*A* wild-type) showed no clear evidence of maternally inherited differences giving rise to variation in the effect of temperature on the frequency of second division segregation of *asco* (Table 3). However, the A4*a*♀ × *asco* crosses consistently gave a higher, but not significantly higher, frequency of second division segregation at each temperature compared with the reciprocal crosses (Table 3). Seven replicates were made at 20°C. and eight at the other temperatures. In no case were there significant differences between the replicates

at the 10% level of probability. It should be noted that these experiments confirm the general pattern of the effect of temperature on second division segregation frequency found in the previous crosses.

Further crosses were made to determine the effect of temperature on recombination frequency; the analyses were carried out by means of random spore isolates. Markers other than *asco* were chosen to include intervals in Linkage Groups I and VI and to delimit distal as well as proximal regions. As several of the mutant strains would not form fertile perithecia at the temperature extremes (McNelly-Ingle & Frost, 1965), the range of temperatures it was possible to test was more limited than in the experiments with *asco* above. The results are presented in Tables 4 and 5 where it can be seen that if a change in recombination frequency with temperature was detected then this change followed the pattern established with the *asco* crosses. The ratios of the two parental classes and of the mutant to non-mutant alleles showed no deviation from the expected $1:1$ ratios at the 5% level of probability. In no case did the germination percentage fall below 70% and replicate crosses were in good agreement, all being above the 10% level of probability.

Cross 1 (Tables 4 and 5) involved markers spanning the centromere and extending a short distance into the right arm of Linkage Group I. The data obtained from crosses incubated continuously at 30°C. and those obtained from crosses in which protoperithecia were allowed to form at 25°C. but the temperature of incubation raised to 30°C. at the time of addition of the conidiating parent, showed good agreement (Interval 1, $\chi^2 = 0.12$, $P = 0.70-0.80$; Interval 2, $\chi^2 = 0.001$, $P = 0.90-0.95$; Interval 3, $\chi^2 = 2.07$, $P = 0.10-0.20$) and have been combined for the

Table 4. *The effect of temperature on the recombination frequency between markers in Linkage Groups I and VI from the analysis of random spore isolates*

Cross No.[†]	Temp. °C.	% Germ.	Parentals	Recombinants (crossover intervals):						
				I	II	III	I & II	I & III	II & III	I, II & III
1	30	90·3	171	56	24	8	7	5	0	0
1	25→30*	89·7	159	59	23	16	7	4	0	1
1	25	80·0	554	105	29	26	4	2	0	0
1	20	84·4	275	78	19	17	7	5	0	0
2	25→30*	87·4	274	72	34	41	4	6	4	2
2	25	84·0	165	37	25	18	3	1	2	1
2	15	85·5	76	13	20	36	2	5	7	2
2	15††		187	—	52	88	—	—	15	—
3	25	90·4	364	16	68	—	4	—	—	—
3	15	78·9	121	26	64	—	4	—	—	—

† Cross 1: $A,+,+,cr \times a,his\text{-}2,nic\text{-}2,+$ (Group 1); Cross 2: $a,+,+,+ \times A,cr,me\text{-}6,aur$ (Group 1); Cross 3: $ylo,+,tryp\text{-}2 \times +,ad\text{-}1,+$ (Group VI). Crossover intervals numbered from left to right in each cross.

†† Not tested for Interval 1.

* Indicates protoperithecia were developed at 25°C. but transferred to 30°C. after conidiation.

Table 5. *The frequency of recombination between markers in Linkage Groups I and VI at different temperatures of incubation*

Cross No.†	Temp. °C.	Marker	% Recomb.	Marker	$\chi^2_{2\times2}$ D.F.=1	% Recomb.	Marker	$\chi^2_{2\times2}$ D.F.=1	% Recomb.	$\chi^2_{2\times2}$ D.F.=1	Marker
1	30	mt	(C) 25·7	his-2	20·7**	11·5	nic-2	21·1**	6·3	3·82	cr
	25		15·4		8·64**	4·6		1·86	3·9	1·54	
	20		22·4			6·5			5·5		
2	25–30††	mt	(C) 19·2	cr	0·70	10·1	me-6	0·82	12·1	1·90	aur
	25		16·7		0·12	12·3		5·60*	8·7	39·9**	
	15		13·7			19·6			30·1		
3	25	ylo	4·4	ad-1 (C)	7·99**	15·9	tryp-2	3·65			
	15		9·5			21·3					

† Crosses as listed in Table 4.
†† Protoperithecia developed at 25°C. and cross transferred to 30°C. on conidiation.
* Heterogeneity χ^2 significant at $P = 0.05$.
** Heterogeneity χ^2 significant at $P = 0.01$.
C = centromere.

comparison with data obtained from crosses incubated continuously at 25°C. A minimum recombination frequency was detected at 25°C. for all three intervals. Interval 1, spanning the centromere, showed a significant increase in recombination frequency at the highest and the lowest temperatures tested compared with the 25°C. data. Interval 2, to the right of the centromere, showed an increase at both temperature extremes but significant only at 30°C. Interval 3, extending a short distance into the right arm, showed a small but non-significant increase at both temperature extremes.

Cross 2 (Tables 4 and 5) included the same interval as Cross 1 but extended some considerable distance further into the right arm. It involved a strain of different ancestry from Cross 1. Furthermore although the maternal parent used formed protoperithecia at 30°C., no fertile perithecia were produced at this temperature after addition of the conidiating parent. Contrary to expectations from Cross 1, the data from the cross at 15°C. compared with 25°C. showed no significant increase in the centromeric region (Interval 1); instead the two distal regions in the right arm (Intervals 2 and 3) showed significant increases in recombination frequency, the most distal region which is far removed from the centromere showing the most highly significant increase of all (Table 5). The crosses in which protoperithecia were formed at 25°C. and the cross transferred to incubation at 30°C. after conidiation, showed no significant difference in recombination frequencies in any of the intervals from crosses incubated continuously at 25°C. However, the temperature at which the protoperithecia develop may have some subsequent effect and influence the relationship between temperature and recombination frequency.

Cross 3, involving markers spanning the centromere in Linkage Group VI, showed increases in recombination frequencies when incubated at 15°C. compared with incubation at 25°C. The increase was barely significant in the centromeric region but highly significant in the region immediately to the left of the centromere (Table 5).

The possibility that the temperature at which protoperithecia develop may subsequently influence the relationship between temperature and recombination frequency was investigated further using the mutant, *asco*. The maternal strain was allowed to form protoperithecia at a given temperature and then immediately after addition of the conidiating parent the cross was transferred to incubation at either a higher or a lower temperature. The number of replicates made for each temperature treatment varied from three to seven and there was no significant difference between replicates at the 10% level of probability. In all cases the temperature of incubation prior to conidiation influenced the frequency of second division segregation of *asco* (Table 6). The actual frequency found was intermediate between the frequencies resulting from continuous incubation at the higher and the lower temperature. Thus development of protoperithecia at 25°C. produced a reduction in the second division segregation frequency of *asco* when the cross was transferred to either 20° or 27·5°C. immediately after conidiation compared with the frequencies resulting from continuous incubation at either 20° or 27.5°C. These changes in the frequency of second division segregation were not significant in

Table 6. *The effect of a change of temperature at conidiation and before fertilization on the frequency of second division segregation of* asco(37402)

Temp. change °C.	Total asci	% 2nd div. div. seg.	$\chi^2_{2\times2}$ with 25°C. data	$\chi^2_{2\times2}$ with 27·5°C. data	$\chi^2_{2\times2}$ with 20°C. data
25→30*	1286	25·1	53·8**	—	—
27·5	964	21·5	—	—	—
27·5→25*	1626	19·7	16·9**	1·12	—
25→27·5*	2019	16·6	3·58	10·2**	—
25	1630	14·4	—	—	—
25→20*	1359	22·1	30·1**	—	9·86**
20→25*	1702	21·4	—	—	28·5**
20	1092	27·6	—	—	—
15→20*	1856	28·5	—	—	0·30
15→25*	1607	18·5	10·1**	—	—

* 25→30 indicates that protoperithecia formed at 25°C. and cross transferred to 30°C. after conidiation. Other temperature changes indicated in a similar manner.
** Heterogeneity χ^2 significant at $P = 0.01$. D.F. = 1 throughout.

every case tested but such a trend is evident throughout (Table 6). The reduced effect at 15°C. may have been due to the immaturity of the protoperithecia at the time of transfer to a higher temperature. It should be remembered that while these *asco* crosses showed a marked effect in most cases, Cross 2 described above showed no effect on recombination frequency when protoperithecia were developed at 25°C. and the cross transferred to incubation at 30°C. after conidiation.

In order to investigate further the time at which temperature affects recombination frequency, series of crosses were transferred from incubation at 25°C. to incubation at 15°C. for a given number of days during a known stage of perithecial

Table 7. *The effect on the frequency of second division segregation of* asco(37402) *of a change in temperature from* 25°C. *to* 15°C. *during certain periods of perithecial development*

Period at 15°C. (days after conidiation)	Growth stages affected	Meioses affected (phases of asci)	Total asci	% 2nd div. seg.	$\chi^2_{2\times2}$ with 25°C. data, D.F. = 1
None	—	—	1525	12·7	—
2–6	Ascogenous hyphae to young asci	1st	1298	11·9	0·39
2–10	Ascogenous hyphae to spore delimitation	1st, 2nd & 3rd	2454	16·5	10·5**
4–8	Young asci to spore delimitation	2nd & 3rd	3305	15·0	4·57*

* Heterogeneity χ^2 value significant at $P = 0.05$.
** Heterogeneity χ^2 value significant at $P = 0.01$.
1 M sucrose used as mounting fluid for scoring asci throughout.

development. The stage of development was determined by fixing and staining samples of perithecia at the time of transfer. Observations on perithecial development (McNelly, 1962) had indicated that the asci developed in several phases and within each phase the majority of the asci were at the same stage of meiosis. The first phase consisted of only a few asci; the second and third phases involved most of the asci found in the mature perithecium; in some crosses a fourth phase was also present. The stage of development of the perithecium and the phases of asci together with the corresponding second division segregation frequency of *asco* are given in Table 7. Two replicate crosses were made for each treatment and there was no significant difference between replicates at the 10% level of probability.

Treatment at 15°C. during the second to sixth day after conidiation, which affected the developmental stages up to the first phase of asci and therefore prior to the majority of the asci undergoing meiosis, produced no significant change in the frequency of second division segregation of *asco* compared to continuous incubation at 25°C. Treatment at 15°C. during the fourth to the eighth days after conidiation produced a significant increase while treatment from the second to the tenth days produced a highly significant increase in the frequency of second division segregation of *asco* compared with continuous incubation at 25°C. These differences are probably due to the fact that the majority of the asci in the perithecia were undergoing meiosis during the longer temperature treatment. This suggests that temperature may have some direct effect on the processes resulting in recombination. However, it should be pointed out that the change in second division segregation frequency with treatment for a few days at a different temperature compared to continuous incubation at the same temperature is smaller than the changes resulting from the other types of temperature treatment detailed above (cf. Tables 1, 6 and 7).

4. DISCUSSION

Seven different crosses involving markers in two separate linkage groups have demonstrated a consistent relationship between temperature and recombination frequency over distances of several map units. A minimum frequency was found at the temperature at which *N. crassa* is normally incubated (25°C.) which is in agreement with the reports for this and other organisms by Plough (1917, 1921), Hüttig (1931) and Towe & Stadler (1964) but in contrast to the work of Rifaat (1959). These phenomena may be of some significance to wild populations for it would appear that at least in some organisms the temperature at which meiosis normally occurs in the wild is also the temperature which gives rise to the minimum recombination frequency.

The second decrease in recombination frequency below 18°C. noted by Towe & Stadler (1964) using the *asco* mutant of *N. crassa* was not observed in this work. It is possible that their decrease below 18°C. is related to the observations reported here, namely that when asci are mounted in water there is a preferential bursting of asci showing second division segregation for *asco* especially at temperatures

below 20°C. This phenomenon was discovered by the first author (McNelly, 1962) and the nature and mechanism of preferential bursting are under further investigation. In addition, Lamb (1964) found that the observed frequency of second division segregation for *asco* decreased as the asci matured. Towe and Stadler's finding of a lower frequency of second division segregation at 15°C. than at 18°C., in contrast to the results of the present study, might be explained if their cultures at 15°C. were scored in a more mature condition than those at 18°C.

Limitation of a change in recombination frequency with temperature to intervals spanning or adjacent to the centromere have been recorded several times in both *Neurospora* and *Drosophila*. In this work, all of the crosses except one demonstrated this effect. The exceptional cross (Cross 2, Tables 4 and 5), involving four markers in Linkage Group I spanning the centromere and extending far out into the right arm, showed no effect of temperature on recombination frequency in the centromeric region but a threefold increase in the most distal region far removed from the centromere. Lawrence (1958, 1963) reported a similar phenomenon in *Drosophila*.

Several *Neurospora* workers have suggested that recombination frequency is under genetic control and that this control varies between the different wild strains. Rifaat (1959) suggested that differences in the effect of temperature on recombination frequency between crosses differing in ancestry might be due to the segregation of these heritable factors which might show differences in temperature sensitivity. The only evidence in the present work supporting this suggestion is given by Cross 2 compared with the *asco*-35*A* cross. These crosses differ in ancestry. In the former, when protoperithecia were developed at 25°C. and the cross transferred to 30°C. on conidiation, no increase in recombination frequency occurred compared with continuous incubation at 25°C.; in the latter a significant increase was detected. The present work provides supporting evidence, however, for the suggestion that heritable factors influencing recombination frequency in specific regions of each chromosome also show differences in temperature sensitivity. Hence in Crosses 1 and 2 above, which differed in ancestry, temperature affected the recombination frequency in different intervals.

The time at which temperature is effective is of interest in relation to the time(s) at which recombinational events may occur. The experiments reported here indicate that temperature does have an effect during the meiotic cycle. In addition, an effect of temperature prior to fertilization and meiosis was detected by the experiments involving temperature pretreatment of the protoperithecia, suggesting that the state of the nucleus and cytoplasm prior to meiosis may subsequently affect the recombination frequency. The relationship between temperature and recombination frequency in these experiments was the same as that observed for continuous incubation at a given temperature, namely a minimum frequency at 25°C. The effect, however, was not detected in every case where it might have been expected to occur. Mitchell (1957) reported a somewhat similar effect. Conidia treated with a heat shock of 60°C. for up to 4 min. and then used to conidiate protoperithecia showed, compared with controls, an increase in gene conversion

frequency but not, however, in recombination frequency over an interval of about 5 map units.

The effect of different temperatures of incubation is expected to be complex as many aspects of the metabolism of the organism may be affected and these in turn may affect recombination frequency. Therefore it is rather surprising that a well-defined relationship between recombination frequency and temperature should be found in such unrelated organisms as *Neurospora* and *Drosophila*. On this basis it is suggested that the temperature curve may be indicative of processes closely associated with recombinational events. The most striking feature is the negative relationship observed over the range 15° to 22·5°C. in both *Neurospora* and *Drosophila*. Dr B. F. Folkes has suggested that one of the possible interpretations of this negative relationship is that in some stage in chromosome replication two competing processes occur, one of which results in recombination. These two processes could have different heats of activation. The following example shows how this might apply in one such possible reaction system.

Suppose that reaction 1 is the stabilization of DNA strands against breakage and that reaction 2 is the breakage of unstabilized strands. If these reactions follow Arrhenius kinetics then:

$$(1) \quad k_1 = C_1 \, e^{-E_1/RT}$$

and

$$(2) \quad k_2 = C_2 \, e^{-E_2/RT}$$

where k_1 and k_2 are transfer coefficients for the two reactions, C_1 and C_2 are constants which include steric factors and E_1 and E_2 are the energies of activation of the two reactions. Let b be the number of breaks produced per unit length of nucleotide chain, then:

$$(3) \quad b \propto \frac{k_2}{k_1}$$

Substituting (1) and (2) in (3) and taking logarithms, it follows that:

$$(4) \quad \log_{10} b \propto \log_{10} \frac{C_2}{C_1} + \frac{E_1 - E_2}{2 \cdot 303 \, RT}$$

If the observed recombination frequency reflects the number of breaks per unit length of nucleotide chain, a plot of $\log_{10}$ recombination frequency against reciprocal of temperature would, on the basis of these kinetics, give a straight line of slope $(E_1 - E_2)/2 \cdot 303 \, R$. The difference in the energies of activation of the two reactions could be calculated from the slope obtained. If E_1 is greater than E_2 the slope will be positive (as in Fig. 2) in contrast to the negative slope, usually obtained with simple chemical systems, which show a positive relationship with temperature. When the data for second division segregation frequency for *asco* in *Neurospora* are plotted in this manner, a linear negative relationship is obtained as is the case for Plough's data for *Drosophila* (Fig. 2).

The results reported here and those of previous workers, have examined crossing-over at a relatively gross level. Towe & Stadler (1964) have briefly reported on investigations of the effect of temperature on recombination frequency over very short intervals. A positive relationship was observed over the range 15° to 25°C. This is in contrast to the negative relationship reported above and suggests that
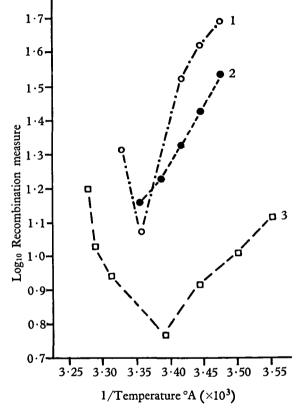

Fig. 2. Log plot of recombination measure against reciprocal of temperature for certain marked intervals in *Neurospora* and *Drosophila*. 1. Percentage second division segregation of *asco*(37402) in *Neurospora crassa* (data from Table 1). 2. Percentage second division segregation of *asco*: repeat experiments (data from Table 3). 3. Percentage recombination in the *b–pr* interval of chromosome II in *Drosophila melanogaster*. Data from Plough (1917) as corrected by Smith (1936).

either recombinational events over very short distances involve a different reaction system or that recombination frequencies over long distances may not reflect simply the result of recombinational events over very short distances.

SUMMARY

In *Neurospora crassa*, a change of 2·5°C. in incubation temperature produced highly significant changes in the second division segregation frequency of *asco* (Linkage Group VI). A minimum value was found at 25°C. consistently increasing towards higher and lower temperatures over the range 15° to 30°C.

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Where recombination frequencies in proximal and distal marked regions in Linkage Groups I and VI showed a change, this followed the same pattern as for *asco* with proximal intervals usually affected; but in one cross a distal region, instead of the proximal, showed a significant change. This behaviour supports the suggestion that heritable factors influencing recombination frequency in specific regions of each chromosome may show differences in temperature sensitivity.

Temperature treatments during protoperithecial development prior to meiosis as well as during meiosis had significant effects on recombination frequencies in several cases. Protoperithecia incubated at one temperature and then transferred to a different temperature after conidiation, gave second division segregation frequencies of *asco* intermediate in value between those obtained with continuous incubation at either of the two temperatures alone.

In the present work with *Neurospora*, the similarity in the relationship between temperature and recombination frequency with the reported results in *Drosophila* is discussed and suggests that the common temperature/recombination frequency curve is indicative of a possible direct effect of temperature on the recombination processes. Present knowledge of these processes is insufficient to permit any detailed explanation of the temperature effects found but the observed negative relationship between recombination frequency and temperature over the range 15° to 22·5°C. is discussed in kinetic terms.

The second division segregation frequency of *asco* was found to be increased at all temperatures tested when the asci were mounted for scoring in strong sucrose solution compared to mounting in water.

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