EFFECTS OF TEMPERATURE ON CROSSING OVER IN *NEUROSPORA*¹

AGNES M. TOWE AND D. R. STADLER

Department of Genetics, University of Washington, Seattle

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WE should like to know the nature of crossing over. What chromosomal materials are involved, and how are the structures formed by these materials altered in the crossover event? One way to pursue this problem is to observe the effects on crossing over of those physical and chemical agents having known effects on specific biological materials. The present report deals with crossing over in *Neurospora* as detected by segregation patterns for an ascospore-color mutant. The effects of various environmental treatments on the frequency of crossing over have been recorded. The greatest effect, resulting from change of temperature of incubation, has been studied in further detail.

The methods employed in this study have been described elsewhere (STADLER and TOWE 1962). All crosses were segregating for the mutant *asco* (37402, lysine-requiring, colorless ascospores); each mature ascus had four black (wild-type) spores and four colorless (mutant) spores. The *asco* locus is centromere-linked, so counts of first-division and second-division segregation patterns for spore color provided a quick measure of crossing over in the *asco*-centromere interval. Three other markers in this interval were also segregating: *cys-2* (80702, cysteine-requiring), *γlo* (Y30539y, yellow conidia), and *ad-1* (3254, adenine-requiring). The order of the marked loci is *asco-cys-γlo-ad*-centromere. In asci segregating for *asco* in the second division, it was possible to determine which of the four included intervals was the site of crossing over by dissecting these asci and then testing the germinating black spores (the colorless ascospores fail to germinate under all conditions used in these experiments).

Control crosses were incubated on crossing medium (WESTERGAARD and MITCHELL 1947) at 25°C. The *asco*⁺ strain (*asco*⁺ *cys* *γlo* *ad* *A*) served as protoperithecial parent and was grown five days before being fertilized with conidia of the *asco* (*asco* *cys*⁺ *γlo*⁺ *ad*⁺ *a*) parent. These same parental strains were used for all crosses with the exception of the backcross, in which case the protoperithecial parent was an *asco*⁺ *cys* *γlo* *ad* *A* which was one of the progeny of the original cross. The crosses grown at 25° were mature ten days after fertilization; at lower temperatures maturation was somewhat slower.

(Some of the data reported here have previously appeared in abbreviated form in the Microbial Genetics Bulletin [Number 16], a mimeographed nonpublication.)

RESULTS

When *asco a* was crossed to *cys γlo ad A* at 25°C under standard conditions, the frequency of second-division segregation patterns for *asco* was 26.8 percent. The crossing medium was altered by the addition or omission of various substances; frequencies of recombination between *asco* and the centromere in these cases are

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recorded in Table 1. While some of these chemical alterations of the crossing medium (omission of Ca and Mg, addition of versene or formalin) resulted in significant increases in the frequency of recombination, no further study was made of crosses raised under these conditions.

The various physical treatments of the parent strains before or during development of the cross which produced no significant change in the recombination frequency included heat shock of fertilizing conidia (60° for 60, 120, 180 sec), ultraviolet irradiation of the fertilizing conidia, and X-irradiation (680r/min up to 8160r) of the developing perithecia at various stages (0 to 6 days after fertilization). One physical alteration had a marked effect on recombination between *asco* and the centromere. When the cross was incubated at 18°C from the time of fertilization until the mature asci were examined, the frequency of segregation for *asco* in the second division rose from 26.8 percent to 38.4 percent.

The cross was incubated at various other temperatures and analyzed for crossing over in the *asco*-centromere region. The results (Table 2) indicate a peak at temperatures in the neighborhood of 18°C with less crossing over at lower or higher temperatures. There is an indication of a second rise in the frequency at temperatures above 25°C, but it was difficult to get accurate data above 28°C, because few of the spores matured.

It has been demonstrated (STADLER and TOWE 1962) that inbreeding results in an increase in crossing over in the *asco*-centromere region; a backcross to *asco a* of one of the *cys ylo ad A* progeny from the parental cross gave 37 percent second division segregation at 25°C. The same temperature series was performed with this cross (Table 2). The results are similar to those for the parental cross at

TABLE 1

Effects of chemically altering the crossing medium on the frequency of second-division segregation of asco. Parental strains were identical for all these crosses

Alteration of crossing medium	1st-division segregation	2nd-division segregation			Frequency 2nd-division segregation	Standard error
		alternate	symmetrical	total		
None	3548	658	641	4847	0.268	0.00635
Calcium deficient (CaCl ₂ replaced by NaCl)	800	165	180	1145	0.301	0.0135
Magnesium deficient (MgSO ₄ replaced by K ₂ SO ₄)	956	201	201	1358	0.296	0.0124
Ca and Mg deficient	920	207	203	1330	0.308	0.0126
Plus 10 ⁻⁴ M versene	317	78	66	461	0.312	0.0216
Plus 10 ⁻³ M versene	145	27	26	198	0.268	0.0315
Plus 10 ⁻³ M hydrogen peroxide	100	19	15	134	0.254	0.0376
Plus 10 ⁻² M hydrogen peroxide	86	17	22	125	0.312	0.0415
Plus 10 ⁻¹ M hydrogen peroxide	67	12	15	94	0.287	0.0466
Plus 10 ⁻¹ M formalin	349	85	80	514	0.321	0.0206
Plus 10 ⁻² M formalin	371	82	87	540	0.313	0.0200

TABLE 2

Effect of temperature of incubation on the frequency of second-division segregation for asco

Temperature	1st-division segregation	2nd-division segregation			Frequency 2nd-division segregation	Standard error
		alternate	symmetrical	total		
Parental cross						
15°	531	123	148	802	0.338	0.0167
18°	1086	349	329	1764	0.384*	0.0116
21°	485	99	101	685	0.292	0.0174
25°	3548	658	641	4847	0.268†	0.00635
28°	554	129	114	797	0.305	0.0163
Backcross						
15°	316	104	101	521	0.393	0.0214
18°	595	218	222	1035	0.425	0.0154
21°	384	126	113	623	0.384	0.0195
25°	1115	322	332	1769	0.370‡	0.0115
28°	326	75	94	495	0.341	0.0213

* This represents counts from seven cross tubes. The sample variance is 18.65×10^{-4} .† This represents counts from 22 cross tubes. The sample variance is 6.56×10^{-4} .‡ This represents counts from 11 cross tubes. The sample variance is 14.17×10^{-4} .

temperatures of 25°C and below, except that the magnitude of temperature effects is somewhat diminished. In the backcross, the frequency of crossing over continues to drop in the 25° to 28°C range; however, the sample at 28° was limited and the counts were made difficult by the fragility of the asci.

Asci with segregation for *asco* in the second division were dissected and scored for the other segregating markers in order to determine the locations of the cross-over and to construct a crossover map for the four adjacent marked intervals. Such an analysis was previously reported (STADLER and TOWE 1962) for the parental cross and the backcross at 25°C. Those results, plus more recent data, are totalled in Table 3 along with the analysis of the same two crosses grown at 18°C. Some multiple crossovers are detectable and they are found to occur with a low frequency. Two-strand and four-strand double crossovers result in first-division segregation for *asco*, and the analysis of a sample of these asci (Table 4) reveals that such events do occur. The crossing-over maps in Table 5 are based on all detected crossing over in first-division and second-division segregation asci.

DISCUSSION

Studies in *Drosophila* (PLOUGH 1917; STERN 1926; GRAUBARD 1934; SMITH 1936) indicate that a minimum amount of crossing over occurs at temperatures between 22°C and 25°C, with higher frequencies occurring if the temperature is either raised or lowered. The analysis of crossing over in *Neurospora* reported here (Table 2) is in general agreement with these studies and with the recent findings in *Neurospora* by MCNELLY and FROST (1963).

A similar pattern was observed by WHITE (1934) in chiasma counts in three species of *Acridiidea* at various temperatures. However, ELLIOTT (1955), in experiments with *Locusta migratoria* and *Endymion nonscriptus*, observed that the

TABLE 3

Analysis of asci with second-division segregation for asco

Cross	Number of asci analyzed	Single crossovers				Multiple crossovers	
		Region I <i>asco-cys</i>	Region II <i>cys-ylo</i>	Region III <i>ylo-ad</i>	Region IV <i>ad-cent.</i>	Number of asci	Type
Parental (25°C)	100	42	25	29	3	1	I, II, IV triple
Parental (18°C)	98	28	36	30	3	1	I, II double
Backcross (25°C)	102	38	25	32	2	5	III, III, IV triple I, III double I, II double I, II double II, III double
Backcross (18°C)	108	27	31	43	3	4	II, III double I, II double II, III double I, III double

TABLE 4

Analysis of asci with first-division segregation for asco

Cross	Number of noncrossovers	Double crossovers	
		Number	Type
Parental (25°C)	37	0	
Parental (18°C)	37	5	2-strand II, III; II, III; II, IV; I, III 4-strand I, II
Backcross (25°C)	46	3	2-strand II, III; II, III; II, III
Backcross (18°C)	44	3	2-strand I, IV; I, III; I, III

TABLE 5

Map distances based on dissected asci

Cross	Region I <i>asco-cys</i>	Region II <i>cys-ylo</i>	Region III <i>ylo-ad</i>	Region IV <i>ad-centromere</i>	Total <i>asco-centromere</i>
Parental (25°C)	5.76	3.48	3.89	0.54	13.67
Parental (18°C)	7.36	10.58	8.38	1.42	27.74
Backcross (25°C)	7.44	7.14	8.60	0.54	23.72
Backcross (18°C)	7.67	6.69	10.36	1.24	25.96

Numbers are crossover map units.

variation in chiasma frequency between temperatures was not significant when compared with the variation between replicate individuals at the same temperature. In *Hyacinthus orientalis*, ELLIOTT observed a significant *lowering* of chiasma frequency at temperatures below 20°C, but he interpreted this to result from failure of chromosome pairing prior to pachytene. In the present study, the variations in frequency of second division segregation between cross tubes incu-

bated at the same temperature were small compared to those between tubes incubated at different temperatures. With regard to the possibility that the lower crossover rates at 25°C result from a failure of meiotic pairing, such an effect might be expected to also give rise to frequent nondisjunction and resulting spore abortion. There was no indication of such an effect in our material.

RIFAAT (1959), studying crossing over in linkage group I of *Neurospora*, observed a minimum frequency at the lowest temperature tested (17°C) with increasing frequencies up to the highest temperature tested (30°C). His experiments, and those performed with *Drosophila*, indicated that temperature effects on crossing over were most pronounced in regions of the chromosome close to the centromere. The same localization is observed in the present study. In the parental cross, crossing over in the *cys-ylo-ad*-centromere region is increased nearly threefold at 18°C (Table 5), while there is little increase in the adjacent region on the distal side. It must be remembered, however, that crossing-over interference may distort the pattern of effects of environmental changes on crossing-over frequency. It was previously reported (STADLER and TOWE 1962) that inbreeding caused no increase in crossing over in the very small region between *ad* and the centromere, and it appeared then that the increase in crossing over at 18°C did not occur in that region either. However, the subsequent analysis of first-division segregation asci from the 18°C parental cross (Table 4) resulted in an increase in the *ad*-centromere map distance, even though there was only one crossover in that interval among the asci examined. It is clear that much larger numbers of progeny would have to be scored to get reliable measurements of crossing over in an interval as short as this.

Either genetic factors (in the selected backcross) or temperature of incubation can cause a marked increase in crossing over, but the two conditions together (backcross at 18°C) are only slightly more effective than either one alone. This suggests that only a fraction of the meiotic cells have the potentiality for crossing over in the marked region and that this fraction is being largely exhausted by either condition alone.

The frequency of crossing over between *asco* and its centromere under standard conditions (synthetic crossing medium at 25°C) is among the lowest observed under any of the conditions tested. Several of the chemical alterations resulted in an increased rate, but none caused a significant decrease. Several temperatures of incubation gave higher rates than 25°C, but none gave a lower rate (with the dubious exception of the limited sample of fragile material from the backcross at 28°C). Standard conditions are those which were arrived at in the attempt to get the maximum yield of fertile spores in the shortest time. The various alterations of these conditions which have been imposed here result in failure of spore maturation or slowing of maturation or both.

The correlation of increasing temperature with decreasing crossing over and with faster maturation could mean that the critical event in crossing over has a lower temperature coefficient than does the duration of the stage at which this event may occur. However, this would not account for the drop in frequency below 18°C (Table 2).

The negative correlation between yield of mature spores and frequency of crossing over could be interpreted in several ways. Some product of growth in suboptimal conditions might influence crossing over frequency directly (by stimulating or causing the crossover event) or indirectly (by selectively killing non-crossover progeny). Selection could operate against a particular combination of genes in the *asco*-centromere region of one of the parents, or it could select for crossover products in some more general way. A general correlation between suboptimal conditions and crossing over would presumably have value in natural selection, regardless of the mechanism underlying the correlation.

The hope of identifying the basic process involved in crossing over by studies of the type reported here rests on finding a spectrum of biological activity (frequency of crossing over) in the various conditions tested which parallels the activity spectrum for a known chemical or physical event. The current findings fall short of such an aim. The multiphase curve which results if one plots crossing-over frequency against temperature of incubation is not characteristic of the kinetics of any simple process. This suggests that crossing over is a complex process, and studies of the type attempted here may be successful only when the process can be resolved into its simpler elements. MITCHELL (1955) demonstrated several years ago that meiotic recombination can no longer be accounted for exclusively by single, reciprocal exchanges between homologous chromosomes, and it has been suggested (FREESE 1957) that the event which results in crossing over may involve a series of exchanges in a very short interval. If this is correct, then we can study single exchanges only in a cross in which the two segregating loci are extremely closely linked.

In a cross between two cysteine-requiring mutants which are functionally allelic, the frequency of prototrophic recombinants at 25°C is about one per thousand. Preliminary studies of the effect of temperature on this recombination event (STADLER and TOWE, unpublished) show a rise in frequency with increasing temperature. The plot of recombinant frequency against temperature (15°C, 18°C, 21°C, 25°C) approximates a straight line, with the rate at 25°C somewhat over twice that at 15°C. It may be hoped that we are dealing here with a simple recombination event and observing a temperature response characteristic of a simple process. Even so, it would probably require studies of the effects of chemical treatments to recognize any *unique* properties of the basic process involved in this recombination event.

SUMMARY

Counts of segregation patterns of an ascospore mutant which is centromere-linked have been used to assay the effects of environmental changes on the frequency of crossing over in *Neurospora*. A number of different chemical alterations of the standard crossing medium were observed to cause a slight increase in crossing over. The temperature of incubation affects the crossing over frequency with the rate at 18°C considerably higher than the rate at 25°C. The effect of temperature is more pronounced in a region close to the centromere than in a

more distal region. The relationship of temperature to crossing over frequency is complex; this suggests that crossing over is a complex process and that the simple components of this process are not likely to be identified until they can be studied individually.

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