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With 2 text-figures

Some details and effects of the premeiotic controls of recombination frequencies in *Neurospora crassa*

By B. C. LAMB

Department of Botany, Imperial College, London, S.W.7

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SUMMARY

Recombination data from crosses made at a single constant temperature of incubation were compared with those from crosses transferred to a different temperature at either the time of conidiation of protoperithecia by the strain of opposite mating-type, or after fertilization when crozier stages were first visible. Results were also compared from reciprocal crosses, from crosses made in different ways and from crosses in which protoperithecia were conidiated at different stages of maturity.

Different temperature regimes during vegetative growth and protoperithecial development had highly significant effects on subsequent meiotic recombination, while temperature differences during later premeiotic stages (between conidiation of protoperithecia and the crozier stage) had no or little effect. It was found that premeiotic controls could have as great, or greater, effects on meiotic recombination than those operating directly during meiosis. The possible adaptive significance of this is discussed.

Recombination frequencies were affected by the method of making a cross (joint-inoculation of strains of opposite mating-type, or conidiation of protoperithecia), and by protoperithecial age at the time of conidiation by the opposite mating-type. Differences in recombination between reciprocal crosses were obtained and were dependent on temperature of incubation and age of protoperithecia at the time of conidiation. Recombination was not affected by different lysine concentrations in the medium. Genetic differences in premeiotic effector-production between the strains used were inferred.

1. INTRODUCTION

Recombination frequencies in *Neurospora crassa* (McNelly-Ingle, Lamb & Frost, 1966) and recombination and conversion frequencies in *Sordaria fimicola* (Lamb, 1969*b*) have been shown to be partly controlled by conditions occurring well before meiosis takes place. The present study was made to elucidate further details of the premeiotic controls of recombination, using *Neurospora crassa*.

Experiments were made as follows: (i) to identify developmental stages at which temperature affected subsequent meiotic recombination; (ii) to determine the relative importance of meiotic and pre-meiotic controls of recombination frequencies; (iii) to determine whether the method of making a cross, or the age of protoperithecia at crossing, affected subsequent meiotic recombination; (iv) to determine whether reciprocal crosses gave identical recombination frequencies.

When studying the premeiotic controls of recombination, it is essential to distinguish meiotic recombination from any mitotic recombination which might be induced by the particular treatments used. In the present study, recombination was scored in meiotic tetrads using ordered segregation patterns for a single allele-pair, which are unaffected by previous mitotic recombination. Although recombination was studied in only one interval, in linkage group VI, the work of McNelly-Ingle *et al.* (1966) comparing temperature effects in different intervals suggests that the current findings are likely to be of general application. In addition, Landner (1970) found evidence of premeiotic effects of temperature in linkage groups I and II as well as in the group VI *asco*-centromere interval as studied here.

2. METHODS AND TERMINOLOGY

The two strains of *N. crassa* used were kindly supplied by Dr L. C. Frost. They have the following origins: (1) Abbott 4*a*; (+*a*), a wild-type reisolate derived from progeny of the cross Abbott 4*A* × Lindegren 25*a* backcrossed seven times to Abbott 4*A*; (2) *Asco* (37402) 33*A* (*asco A*), a reisolate of *asco* from a cross of *asco* (37402)*a* to Lindegren 1*A* wild-type.

The mutation *asco* autonomously affects ascospore pigmentation, so segregation patterns in crosses with wild-type can be scored by direct observation of the intact ascus. The frequency of second-division segregation patterns is proportional to twice the recombination frequency in the *asco*-centromere interval. Precautions against particular scoring biases were taken as previously described (references in Lamb, 1969*a*).

General methods and media were those of Lamb (1966). Inocula from cultures stored at 4 °C were grown on glucose minimal medium at 25° for 7 days to provide inocula for protoperithecial and conidial cultures. Conidial cultures for fertilizing protoperithecia were grown for 7 days at 25° on glucose minimal medium.

Recombination frequencies in many organisms are affected by temperature of incubation (see references in McNelly-Ingle *et al.* 1966). Use was made of this here to determine when the premeiotic controls of recombination operate. Slopes of minimal reproductive medium inoculated with one strain were incubated at one of two temperatures, 18 or 29 °C, for 6 days to produce protoperithecial (♀) cultures. Conidia from the other strain, grown as described above, were suspended in water, poured on to the ♀ cultures, left for 5 min, then were poured off. One third of these 'conidiated' ♀ cultures were transferred immediately to incubation at the other of the two temperatures, 29 or 18 °C respectively, for the remainder of their development ('conidiation transfers'). Another third of the conidiated ♀ cultures was incubated at the same temperature as before conidiation until cytological examination (method as in Lamb, 1969*b*) showed crozier stages to be present in the perithecia, when they were transferred to the other temperature for the remainder of their development ('crozier-transfers'). It is within these croziers on the ascogenous hyphae that fusion of the parental nuclei finally occurs, immediately before

meiosis. The last third of the ♀ cultures was incubated throughout its development at one temperature ('constant-temperature crosses').

If there are no premeiotic controls of recombination, the expectation is that results from crozier and conidiation transfers will be identical to those from crosses incubated throughout at the temperature used after transfer, because meiosis will have occurred at the same temperature in all three types of cross. The difference between the two temperatures of a transfer, 11 °C, was rather small to cause any temperature-shock effects, but as a control, 'brief transfers' were made. In these, crosses were transferred after conidiation to the second temperature for 3 h (more than long enough for equilibration), then were returned to the first temperature for the remainder of their development.

In the third series of experiments (to determine whether the method of making a cross, or the age of protoperithecial cultures at the time of crossing, affected recombination) slopes of minimal reproductive medium were inoculated with conidia of one strain. In the 'mixed inoculation' crosses, a loopful of conidia of the other strain was added immediately; in other crosses, the inoculated slopes were incubated for 3, 6, 13, 20 or 24 days and then were conidiated in the usual way. All ♀ cultures and crosses were incubated at a constant temperature: three sets of experiments were made using 18°, 25° and 29° respectively.

The fourth aim was achieved by making most of the crosses already described in two ways, using the reciprocal crosses $+a$ ♀ (protoperithecial parent) × *asco A* ♂ (conidial parent) and *asco A* ♀ × $+a$ ♂. Because *asco* strains require lysine and are normally grown on media supplemented with lysine (at 0·5 g/l), control crosses were made at 18 °C with different lysine concentrations, using mixed-inoculation crosses and the two reciprocal crosses conidiated 6 days after inoculation.

In all experiments each treatment was tried with at least two replicates and each experiment was repeated at least twice. The symbol → indicates a transfer. For example, 18 → 29 °C indicates a cross in which the ♀ culture was grown at 18° and then transferred, either at conidiation or the crozier stage, to 29° for the remainder of its development.

3. RESULTS AND DISCUSSION

In all experiments, replicates and repeat experiments gave very similar results: the few cases of significant heterogeneity are marked in the appropriate tables.

The expected equalities amongst the two first-division, and amongst the four second-division, segregation classes of ordered tetrad were obtained in these crosses, showing that second-division segregation frequencies were not biased by a differential bursting of asci (Lamb, 1967) nor by spindle overlap at the second division of meiosis.

(i) *Identification of developmental stages at which temperature affects subsequent meiotic recombination*

The data are shown in Table 1 and Fig. 1. Recombination results from the transferred crosses, 18 → 29 °C and 29 → 18 °C, were quite different from those incubated at constant temperature, either at 18 or 29 °C. The second-division

segregation frequencies of the transfer-crosses (including reciprocal crosses, crozier and conidiation transfers) differed significantly at the $P = 0{\cdot}01$ level in all eight crosses from those incubated at the pre-transfer temperature, and in seven out of eight crosses from those incubated at the post-transfer temperature. These differences are unlikely to have arisen from any temperature-shock during the transfer because the control 'brief-transfer' result was homogeneous with the constant temperature result for that cross (Table 1). Crozier-transfer results were very similar to conidiation-transfer results: corresponding results from the two kinds of transfer did not differ even at the $P = 0{\cdot}1$ level of significance.

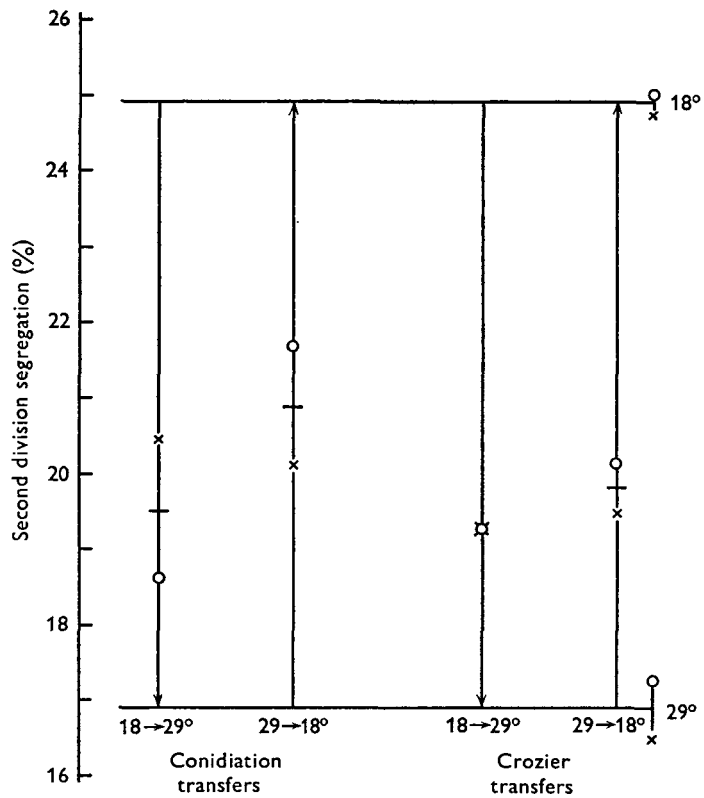

Fig. 1. Effect of temperature-transfers at the conidiation and crozier stages on second-division segregation frequencies. ○, Value from $+a\,♀ \times asco\,A\,♂$; ×, value from $asco\,A\,♀ \times +a\,♂$; —, mean of the two reciprocal cross values; ——— (long), mean of the two reciprocal cross values (shown at extreme right) from continuous incubation at temperature stated on right; →, direction of transfer.

The highly significant differences between results from the transfer crosses and those of crosses incubated only at the post-transfer temperature show clearly that recombination is affected by conditions prior to the transfers; that is, prior to meiosis in the crozier-transfers and prior even to contact with and fertilization by the opposite mating type in conidiation-transfers. The similarity between the conidiation and crozier-transfer results strongly suggests that developmental

stages between conidiation and the beginning of crozier formation are unimportant for determination of meiotic recombination frequencies. It is therefore likely that the premeiotic determinants of recombination are produced during vegetative mycelial growth or at the protoperithecial stages. These determinants, which have been called 'effectors' (Lamb, 1969*b*), are presumably stable through the post-fertilization stages to meiosis.

Table 1. *Recombination data from crosses incubated at constant temperature and crosses transferred to a different temperature at conidiation or the crozier stage*

	+ *a* ♀ crosses				*asco A* ♀ crosses			
	Asci		$\chi^2 2 \times 2$		Asci		$\chi^2 2 \times 2$	
Temperature (°C) Constant	Total	% 2nd div.	With 18° data	With 29° data	Total	% 2nd div.	With 18° data	With 29° data
18	9559	25·1	—	—	6377	24·8	—	—
29	9917	17·3	—	—	9307	16·5	—	—
Transfers								
Conidiation								
18 → 29	5060	18·6	81·0**	3·8	4986	20·4†	30·8**	33·1**
29 → 18	4494	21·7	19·8**	39·9**	5442	20·1	35·8**	31·2**
Crozier								
18 → 29	8166	19·3	91·4**	11·5**	5941	19·3	53·1**	19·7**
29 → 18	3074	20·2	31·6**	13·2**	2075	19·5	24·4**	10·6**
Brief								
18 ↔ 29	6266	25·3	0·04	—	—	—	—	—

** Significant at $P = 0{\cdot}01$.
† Repeats heterogeneous at $P = 0{\cdot}05$.

It has been suggested (Lamb, 1963, 1969*b*) that the various response patterns of recombination to temperature in different organisms may be evolved ones of adaptive significance. If this is so, it would provide obvious reasons for the operation of pre-meiotic controls of recombination during the main growth stages (hyphal development in *Neurospora*), not just during late sexual development. Effectors could be considered as conveying information and adaptive instructions from the growth stages to meiosis.

In these experiments, highly significant differences in recombination were obtained between transferred crosses and crosses incubated throughout at the pre-transfer temperature, so post-transfer conditions affect recombination. The post-transfer period includes meiosis, some stages of which have been shown in other organisms to be sensitive to temperature effects on recombination (Lu, 1969, and references in Lamb, 1969*b*), so these results do not preclude direct effects of temperature at meiosis.

The present results confirm and extend those obtained in *N. crassa* by McNelly-Ingle *et al.* (1966). By comparing conidiation and crozier-transfers, the present results are more specific than those obtained by McNelly-Ingle *et al.* by a double-

transfer technique, in excluding the early ascogenous hyphal phase from possible sensitive stages for temperature effects on recombination. Mitchell (1957) found that heat-shock of *N. crassa* protoperithecia reduced conversion sevenfold; he also stated that crossing-over was unaffected although the data show a small (7%) reduction in recombination compared with controls. This reduction could be due to thermolabile effectors in protoperithecia.

(ii) *The relative importance of meiotic and pre-meiotic controls of recombination frequencies*

By comparing results from transferred crosses with ones from constant-temperature crosses made at the pre- and post-transfer temperatures, one obtains an indication of the relative importance of recombination controls at pre- and post-transfer developmental stages. From Fig. 1 it can be seen that for both the 18 → 29 °C transfer results, recombination values were closer to those from constant incubation at the post-transfer temperature, 29 °C, than to those from incubation at the pre-transfer temperature, 18 °C. The 29 → 18° crozier-transfer results were closer to those from incubation at the pre-transfer temperature.

The relative importance of meiotic and pre-meiotic controls is therefore partly dependent on temperature. In the 18 → 29 °C crosses, controls operating after conidiation and the crozier stage had more effect than those operating before these stages, especially in the + ♀ crosses. Since crozier-transfers were made when croziers were first visible in perithecia (that is, when most future asci were still at pre-crozier stages), possible controls operating after such a transfer include some pre-meiotic, as well as meiotic, ones. Crozier-transfers therefore give only maximum estimates of meiotic controls: true values would be less if any pre-meiotic controls operate during the period from immediately pre-crozier stages to the beginning of meiosis. The 29 → 18 °C crozier-transfer results therefore suggest that temperature effects acting directly at meiosis may in some cases be considerably less important than those acting well before meiosis.

The premeiotic effects studied here, operating at several-to-many nuclear divisions before meiosis, are clearly not comparable with ones observed immediately before meiosis, such as the preleptotene effects of radiation in *Tradescantia* described by Lawrence (1961). Chauhan & Abel (1968), using *Impatiens* and *Salvia*, reported that homologous chromocenters remained associated in pairs at least during the last premeiotic interphase, and were loosely aligned in other tissues. Even if such associations occurred and led to some recombination before meiosis, this would not show as the premeiotic controls of recombination found here because the ordered-tetrad scoring system detects recombination only during meiosis.

(iii) *The effects on recombination of the method of making a cross and of protoperithecial age at conidiation*

The data are shown in Table 2 and Fig. 2. At 18 °C both mixed-inoculation crosses gave higher second-division segregation frequencies than any of the crosses made by conidiation. At 25 °C the mixed-inoculation crosses gave values

as high as the highest ones from conidiated crosses. Although for convenience in Table 2 and Fig. 2 the mixed inoculations are shown as the equivalent of conidiation at day zero, they are not completely comparable with crosses conidiated later: in the latter, one strain develops vegetatively and may produce protoperithecia before being conidiated, whereas in the former crosses, sexual, and possibly vegetative development is by heterokaryons between the two strains.

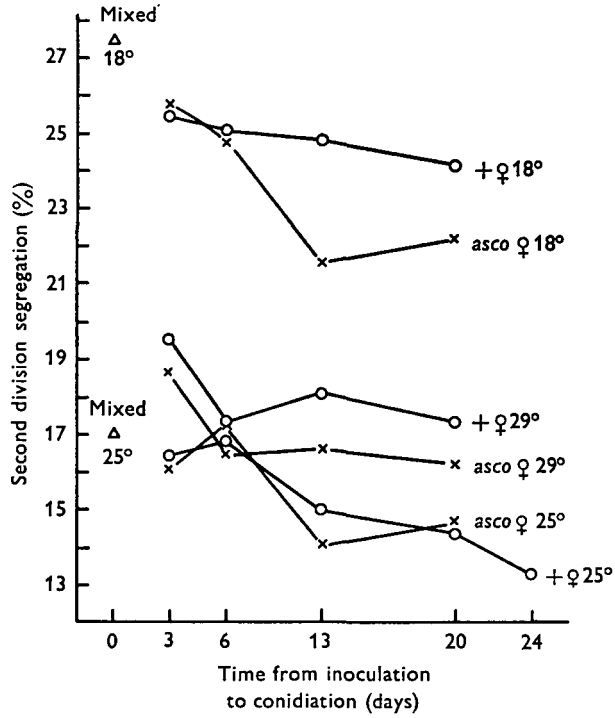

Fig. 2. Effect of the method of crossing and the age of protoperithecial cultures at conidiation on second-division segregation frequencies. △, Value from mixed-inoculation cross (mean of values from crosses with and without lysine supplementation); ○, value from $+a$ ♀ × *asco A* ♂; ×, value from *asco A* ♀ × $+a$ ♂.

Differences between conidiated and mixed-inoculation crosses presumably arise from events before ascogenous hyphal development because stages from then on should be genetically identical in both kinds of cross. The highest recombination values occurred in the mixed-inoculation crosses, where vegetative and sexual development were presumably by heterokaryons of *asco A* and $+a$, instead of one or other homokaryon as in conidiated crosses. This suggests that some kind of complementation or gene-product interaction occurred between the two components of the heterokaryon, which implies a difference between the strains in genes controlling premeiotic effector production. Such a difference is quite likely because Abbott and Lindegren wild-type strains (from which $+a$ and *asco A*, respectively, are largely derived) are known to differ in recombination properties (Frost, 1961).

Complementation in mixed-inoculation crosses increased recombination, but Griffiths & Threlkeld (1966) and Griffiths (1968) reported a complementation of recombination-*inhibiting* factors in crosses where heterokaryons were used as protoperithecial or conidial parents in *Neurospora*. Their data from protoperithecial heterokaryons are best explained in terms of pre-meiotic effectors produced before or during protoperithecial development, but their conidial data, like those of Mitchell (1957), are not easily explained.

Table 2. *The effect on recombination of the method of crossing and the age of protoperithecial cultures at conidiation*

		Conidiated crosses*				Mixed-inoculation crosses*			
		+ *a* ♀		*asco A* ♀		Without lysine		With lysine in medium	
Temperature (°C)	Age of protoperithecial culture (days)	Total asci	% 2nd div. asci	Total asci	% 2nd div. asci	Total asci	% 2nd div. asci	Total asci	% 2nd div. asci
18	0	—	—	—	—	5267	27·0	6305	28·0†
	3	7950	25·5	5915	25·8†				
	6	9559	25·1	6377	24·8				
	13	3184	24·8	3540	21·6				
	20	5624	24·2	5439	22·2				
25	0	—	—	—	—	4276	16·8	3398	17·2
	3	6291	16·4	4266	16·1				
	6	4025	16·8	6525	17·2				
	13	3870	15·0	5005	14·1				
	20	2000	14·4	2135	14·7				
	24	1547	13·3						
29	0	—	—	—	—	Not fertile			
	3	8503	19·5	4823	18·7				
	6	9917	17·3	9307	16·5				
	13	4791	18·1	4473	16·6				
	20	4240	17·3	2266	16·2				

* See Methods and Terminology for details.
† Repeats or replicates heterogeneous at $P = 0{\cdot}05$.

Amongst the present conidiated crosses, there was a clear general tendency for crosses conidiated youngest (3 or 6 days after inoculation) to have higher second-division segregation frequencies than those conidiated later (13–24 days). Although there was a general decrease in second-division segregation frequencies with increasing age at conidiation, the decrease was sometimes small or irregular (Fig. 2). There are several possible explanations for such decreases, such as an accumulation of recombination-inhibitors with age, but they cannot be distinguished from the present data. One general point arising from the *asco* ♀ values at 25 and 29 °C (Fig. 2) is that, exceptionally, the temperature giving minimal recombination may depend on the age of the protoperithecial culture at conidiation.

(iv) *Recombination differences between reciprocal crosses*

From Tables and Figs. 1 and 2 it can be seen that second-division segregation frequencies for $+ a$ ♀ crosses were higher than those for corresponding *asco A* ♀ crosses in 13 cases, lower in 4 cases and equal in 1 case. A few of these differences are statistically significant at the $P = 0{\cdot}05$ level or lower; for example, amongst crosses made at 18 °C and conidiated after 13 days, the $+ a$ ♀ value is significantly higher at the $P = 0{\cdot}01$ level than the *asco A* ♀ one. Differences between reciprocal crosses depended on temperature and age at conidiation of ♀ cultures. For example, at 29 °C, the $+ a$ ♀ values were all higher than corresponding *asco A* ♀ ones but at 25 °C they were approximately equal; if this temperature-dependence of reciprocal cross differences applies to other *Neurospora* crosses, it would explain why such differences have not been widely reported, since crosses are usually made at 25 °C.

Table 3. *The effect of lysine concentration on recombination results*

Cross	$+ a$ ♀		*asco A* ♀		Mixed-inoculation	
Lysine conc.*	Total asci	% 2nd div. asci	Total asci	% 2nd div. asci	Total asci	% 2nd div. asci
0	9559	25·1	No growth		5267	27·0
× ·1	—	—	5670	24·2	—	—
× ·25	—	—	2357	26·4	—	—
× 1·0	4104	25·9	6377	24·8	6305	28·0†
× 4·0	2719	24·3	—	—	—	—

* × 1·0 concentration = 0·5 g/l lysine.

† Replicates heterogeneous at $P = 0{\cdot}05$.

All crosses made at 18 °C. Crosses conidiated 6 days after inoculation except for mixed-inoculation crosses.

Differences between reciprocal crosses in recombination have been known in higher organisms for many years (references in Phillips, 1969) but there the separate processes of micro- and mega-sporogenesis differ in many ways. In *Neurospora*, however, instead of separate male and female meioses, meiosis takes place on presumably identical dikaryons in each reciprocal cross. Because the ascogenous hyphae are genetically identical in both reciprocal crosses, the resulting recombination differences imply a difference in effector production before ascogenous hyphae are formed. The differences in recombination between reciprocal crosses are therefore consistent with genetic differences between the strains in premeiotic effector production, as suggested from different evidence in section (iii) above. In the present experiments, $+ a$ ♀ × *asco*33 *A* ♂ crosses generally gave higher recombination values than the reciprocal cross. In previous experiments (McNelly-Ingle *et al.* 1966) a different re-isolate of *asco* (37402), *asco* 35*A*, gave a similar difference which was small yet consistent over a wide range of temperatures, with the $+ a$ ♀ crosses giving the higher values. One vegetative subculture of *asco* 33*A* gave higher recombination values in the *asco A* ♀ cross than in the reciprocal cross (Lamb, unpublished), suggesting that neither the *asco* nor mating-type loci were responsible themselves for reciprocal cross differences.

Differences between reciprocal crosses were not due to lysine-supplementation of only the *asco* ♀ crosses: data in Table 3 show no significant effects of lysine concentration in the medium on recombination results. Comparing results from crosses made at different lysine concentrations, homogeneity χ^2 tests gave values of 2·2, D.F. = 2, $P = 0{\cdot}3$–$0{\cdot}5$ for $+a$ ♀ crosses, 4·0, D.F. = 2, $P = 0{\cdot}1$–$0{\cdot}2$ for *asco* *A* ♀, and 1·4, D.F. = 1, $P = 0{\cdot}2$–$0{\cdot}3$ for mixed-inoculation crosses.

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