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The effects of temperature on aberrant ascus frequencies at the *Buff* locus in *Sordaria brevicollis*

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SUMMARY

Single-point crosses using five allelic spore colour mutants at the *buff* locus were carried out at different temperatures. The data suggest (i) that fixed or preferred opening points in the DNA, required for initiation of recombination events, are available more often at higher than at lower temperatures, (ii) opening points at or beyond both proximal and distal ends of the *buff* locus respond similarly to variations in temperature, and (iii) the correction pattern seems to be independent of temperature at the *buff* locus in *S. brevicollis*.

1. INTRODUCTION

Hybrid DNA models of recombination (Hastings & Whitehouse, 1964; Holliday, 1964) predict fixed opening points in the DNA where events leading to recombinant formation are initiated. The initial single strand breaks are presumed to occur in identical positions on strands of either opposite chemical polarity (Whitehouse, 1963) or identical chemical polarity (Holliday, 1964) in the two DNA molecules involved in the recombinational event. This is followed by uncoiling of single strands of DNA from each parent and necessitates a small amount of DNA synthesis according to the scheme proposed by Whitehouse whereas no DNA synthesis is expected on Holliday's model. The 'free' or 'aggressive' single strands find their complementary partner of the opposite parent, giving rise to stretches of hybrid DNA. These hybrid DNA segments would be mismatched in regions where parent molecules differ.

Mismatches in the DNA are thought to induce repair enzymes which recognize heterozygous structures and convert them to either mutant or to wild type. If the hybrid DNA regions are reciprocal, one would observe 5+ : 3m, 3+ : 5m, 6+ : 2m and 2+ : 6m aberrant ascus types, depending upon correction taking place in one or both DNA molecules. In the absence of correction aberrant 4+ : 4m segregations would be expected. For the sake of simplicity correction which restores the 4+ : 4m (normal) segregation and the consequences of non-reciprocal hybrid DNA will not be discussed. Perhaps it is necessary to add that ignoring hybrid DNA with non-

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reciprocal distribution does not alter materially the arguments developed later in this paper.

The current hybrid DNA models suggest that stretches of hybrid DNA, although initiated at fixed opening points, can be variable in length (the end-point not being fixed). No specific reason for such length variation has been put forward; however, this variability provides a neat explanation of polarity patterns observed fairly regularly in two-point crosses in fungal systems (for references see Whitehouse, 1973).

In single-point crosses (mutant × wild type) the total frequency of aberrant asci would depend upon (i) the frequency with which initial incision of single strands of DNA at gene end/s take place; (ii) the distance of the mutant site from the initial opening point/s. The relative frequencies of the different aberrant ascus types would depend upon the efficiency with which mismatches are recognized and repaired.

A suitable system to test at least some aspects of the models outlined above is available in the ascomycete *Sordaria brevicollis*. This system consists of a series of allelic spore colour mutants at the *buff* locus, whose map position has been established (Bond, 1969). The approach used was to determine the basic conversion and post-meiotic segregation frequencies of mutants at the *buff* locus and to look for the influence of temperature on (i) the total aberrant ascus frequency at the *buff* locus, (ii) total aberrant ascus frequencies of the individual *buff* alleles, and (iii) the relative frequencies of the four classes of aberrant ascus types which can be scored in this system (6+ : 2m, 2+ : 6m, 5+ : 3m and 3+ : 5m). Temperature effects have been used fairly extensively to obtain some idea about the underlying processes involved in recombination (see McNelly-Ingle, Lamb & Frost, 1966; Stamberg & Simchen, 1970). Relatively less attention has been devoted to investigating the influence of temperature on gene conversion (Boucharenc, Mousseau & Rossignol, 1966; Lamb, 1968, 1969). The experiments reported here were intended to provide a detailed analysis of aberrant ascus frequencies in single-point crosses in order to test some of the predictions of hybrid DNA models of recombination.

2. MATERIALS AND METHOD

The *buff* locus in *S. brevicollis* is located on linkage group II (Fig. 1), 1·5 map units distal to the morphological marker *mo-1* (Cooray, unpublished results) and 1·1 map units proximal to the nicotinamide-requiring marker *nic-1* (Bond, 1969). The five *buff* ascospore colour mutants used in these experiments originated as follows: *S180* from D. J. Bond and *S156*, *S44*, *S41*, and *S6* from M. H. V. Cooray. These mutants were obtained by UV-treatment of microconidia before fertilization.

The optimum temperature for crosses in *S. brevicollis* is 25 °C. Asci take 11–13 days to reach maturity and about 200 intact asci can be obtained from a single perithecium. At 30 °C mature 2 days earlier compared to 25 °C, in addition the percentage of aberrant asci is also increased. These advantages, however, are lost because of a substantial increase in the frequency of infertile crosses (40–50 % of the crosses are infertile at 30 °C compared to < 5 % at 25 °C).

Mutant × wild-type crosses were carried out (at 30 and 25 °C) by inoculating strains of opposite mating type, designated *A* and *a*, about 4–5 cm apart on malt agar Petri plates (Westergaard salt solution × 10, 100 ml; sucrose 10 g; malt extract, 20 mg; Difco agar 15 g; total volume brought up to 1 l). Perithecia are formed along the meeting point of the two strains.

At lower temperatures the process of hyphal growth, fertilization and ascal maturity is considerably retarded. At 20 °C crosses take 18–21 days to reach maturity and the number of asci/perithecium is slightly reduced (100–150 mature asci/perithecium). At 15 °C crosses take approximately 30–35 days and the number of mature asci in individual perithecia is greatly reduced, generally varying from 30 to 60 mature asci/perithecium. To avoid the delay caused by slow hyphal growth crosses at 20 and 15 °C were done in the following way. The two parents were grown separately and incubated at 25 °C for 4 days, the protoperithecial parent on malt agar plates and the microconidial parent on yeast extract plates (0·3 % yeast extract, 1·5 % Difco agar). Microconidial suspensions were prepared with distilled water (after 4 days incubation at 25 °C) and a thin layer of this suspension was spread evenly over the malt agar plate having the protoperithecial parent. The plates were then incubated at the required temperature. All crosses were routinely carried out using reciprocal combinations of the mating type. No significant maternal effect on the frequency of aberrant asci was observed, that is, buff ♀ × +♂ gave similar aberrant ascus frequencies as buff ♂ × +♀.

To obtain counts of asci a few perithecia (5–10) were transferred to a drop of 5 % sucrose solution on a glass slide. Under a dissecting microscope contents of these perithecia were squeezed out and the ascal mass was separated into small clumps of 20–30 asci. After removal of perithecial wall and other debris a coverglass was carefully dropped on to the slide. All scanning was done with a Reichert neopan microscope (100 × magnification). This magnification is adequate for distinguishing the various ascus types; however, all aberrant asci were confirmed visually, using four times the magnification mentioned above.

To check for the possible contribution of mis-scoring due to phenocopies, a few intact aberrant asci were dissected and individual spores back-crossed to the two parents. It was established that the genotypes of the spores corresponded to their phenotype. This relationship may not be absolute (as the number of asci tested was small) but it can be said with a reasonable degree of certainty that mis-scoring error due to phenocopies is negligible.

Another factor which can be the cause of mis-scoring is immature wild-type spores being scored as mutants. The development of pigment in *S. brevicollis* ascospores follows the pattern, white (transparent) → yellow → grey → black (M. H. V. Cooray, unpublished). The *buff* spore colour seems to be an offshoot and does not appear in the regular pathway of ascosphore pigment development. As a result confusion between immature wild-type spores and *buff* spores in an ascus does not pose too big a problem.

3. RESULTS AND DISCUSSION

(i) *The* buff *gene map*

The five *buff* spore colour mutations described here (*S180*, *S156*, *S44*, *S41*, and *S6*) are assumed to be point mutations. The map of the *buff* locus (Fig. 1) was derived by Bond (1969) on the basis of the difference in frequency of the two classes of recombinant spores with nonparental outside markers.

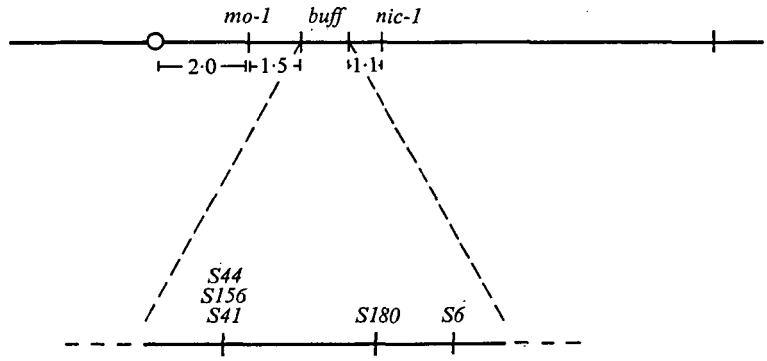

Fig. 1. A map of linkage group II of *S. brevicollis* with a fine structure map of the *buff* locus (Cooray, unpublished data; Bond, 1969).

Bond's data from interallelic crosses at the *buff* locus show that *S44*, *S41* and *S156* are mutations which do not recombine with each other and are probably homoalleles. The fact that these mutants show different aberrant ascus frequencies, which are not observed in multiple copies of crosses using a single mutant, suggests that either they are homoalleles that give rise to structurally different heterozygosities in hybrid DNA (e.g. transition *versus* transversion) or they are heteroalleles but map very close to each other. Where it was necessary to show the alleles *S44*, *S41* and *S156* as separate points (e.g. Fig. 2), the order given is arbitrary. The other two *buff* mutants, *S180* and *S6*, map distal to this cluster of mutants.

(ii) *Relationship between temperature and aberrant ascus frequencies at the* buff *locus*

The data from mutant × wild-type crosses at the *buff* locus is summarized in Table 1. A complete regression analysis of these data would appear as follows:

	Degrees of freedom	Mean square	F
Average regression on temperature	1	89	165**
Differences between crosses			
In mean frequency	4	6·1	11·3**
In regression on temperature	4	0·74	1·37
Error	10	0·54	

**$P < 0{\cdot}001$

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Table 1. *Effects of temperature on the frequency of total aberrant asci and aberrant ascus types at the* buff *locus*

Cross	Temp. (° C)	Total asci	Aberrant asci: Total	Aberrant asci: /$10^3 \pm$ S.E.	3+ : 5m	5+ : 3m	6+ : 2m	2+ : 6m	Total PMS 3+ : 5m	Total PMS 5+ : 3m	Total conv. 6+ : 2m	Total conv. 2+ : 6m	Corr. to + 6+ : 2m	Corr. to + 5+ : 3m	Corr. to m 2+ : 6m	Corr. to m 3+ : 5m
S44 × WT	30	4902	57	11·6 ± 1·0	24	6**	16	11	30		27		22		35	
	25	8641	91	10·5 ± 1·1	34	18*	24	15	52		39		42		49	
	20	5172	45	8·7 ± 0·8	12	13	16	4	28		20		39		16	
	15	5413	28	5·2 ± 0·5	8	4	9	7	12		16		13		15	
S41 × WT	30	6321	71	11·2 ± 1·0	29	21	8	13	50		21**		29		42	
	25	16814	145	8·6 ± 1·0	41	31	47	26*	72		73		78		67	
	20	8711	63	7·2 ± 0·7	21	18	14	10	39		24		32		31	
	15	3709	16	4·3 ± 0·3	4	2	7	3	6		10		9		7	
S156 × WT	30	3867	47	8·6 ± 0·7	12	5	10	7	17		17		15		19	
	25	14309	112	7·8 ± 0·9	29	26	37	20*	55		57		63		49	
	20	4154	28	6·7 ± 0·5	6	9	6	7	15		13		15		13	
	15	5051	21	4·2 ± 0·4	9	3	5	4	12		9		8		13	
S180 × WT	30	5436	47	8·6 ± 0·8	17	11	13	6	28		19		24		23	
	25	11512	75	6·5 ± 0·7	11	12	26	18	31		44		38		37	
	20	3078	19	6·1 ± 0·5	5	4	7	3	9		10		11		8	
	15	4107	15	3·7 ± 0·3	5	4	4	2	9		6		8		7	
S6 × WT	30	3178	28	8·8 ± 0·7	9	6	10	3	15		13		16		12	
	25	9549	71	7·4 ± 0·8	18	16	25	12*	34		37		41		30	
	20	6135	37	6·0 ± 0·6	13	10	9	5	23		14		19		18	
	15	6471	13	2·0 ± 0·2	4	3	2	4	6		7		5		8	

* $P = < 0{\cdot}05$. ** $P = < 0{\cdot}01$.

This regression analysis shows that (*a*) there is a significant difference between the five crosses in the mean frequency of aberrants, (*b*) that the regression slopes for individual *buff* alleles do not vary significantly and (*c*) that all crosses show a significant and similar regression of aberrant ascus frequency on temperature.

The finding that all the *buff* mutants looked at show a similar response to increase in temperature suggests that the observed increase in total aberrant ascus frequencies is not a mutant-dependent phenomenon but a general feature of the *buff* locus. The data of Bond (1973) from mutant × mutant crosses at the *buff* locus suggests that hybrid DNA formation is initiated at or beyond both ends of the *buff* gene. The observation that the proximal cluster of mutants (*S44*, *S41*, *S156*) and also the distal alleles (*S180* and *S6*) show a similar response to temperature seems to suggest that events of proximal as well as distal origin are influenced in the same way.

Hybrid DNA models suggest that the frequency with which endonucleolytic cuts are made in the DNA (at fixed or preferred opening points) determines the frequency of hybrid DNA formation for that region of the chromosome. Aberrant ascus frequencies from single point crosses provide a method of looking at the frequency of hybrid DNA formation. It is conceivable that variations in temperature influence the initial reaction of substrate (preferred opening points in the DNA) to enzyme (endonuclease) and this may well be happening at opening points located at or beyond the ends of the *buff* locus.

The assumption that either proximal or distal or both proximal and distal opening points are available more often at 30 °C than at 25, 20 or 15 °C would be sufficient to explain the behaviour of total aberrant ascus frequencies at the *buff* locus.

As aberrant 4+ : 4m asci cannot be detected in this system it is conceivable that temperature is merely affecting the proportion of aberrant 4+ : 4m asci relative to the other four types of aberrants. If one assumes that the frequency of aberrant 4+ : 4m asci increases with decrease in temperature (although total aberrant ascus frequency stays constant) one could also explain the observed effects of temperature on total aberrant ascus frequency. For reasons that will be discussed later (in section (iv)) the assumption outlined above though conceivable is considered unlikely.

Alternatively one could argue that the hybrid DNA segments are shorter at the lower temperatures and often fail to reach the mutant sites, as a result one would expect aberrant asci to occur less frequently at lower temperatures. This possibility seems to be an unlikely one and is discussed in detail in section (iv).

(iii) *Comparison of relative frequencies of aberrant ascus types*

The frequencies of the different aberrant ascus types in the case of the five *buff* alleles looked at are higher at 30 °C than at 15 °C (Table 1). Within these two extremes the distribution of the different classes of aberrant asci (6+ : 2m, 2+ : 6m, 5+ : 3m and 3+ : 5m) in general show a gradual increase with rise in temperature, similar to the trend in total aberrant ascus frequency. This finding in *S. brevicollis*

is more or less in agreement with the results of similar experiments in *Sordaria fimicola* (Lamb, 1969). In *S. fimicola* the different classes of aberrant asci followed the pattern shown by total aberrant asci, for both *hyaline* and *grey* spore colour loci.

The data presented in Table 1 have been classified so as to emphasize three possible ways in which the influence of temperature could be detected: (*a*) Changes in the relative frequencies of the two post-meiotic segregation classes (5+ : 3m *versus* 3+ : 5m) compared to the relationship between the two conversion type asci (6+ : 2m *versus* 2+ : 6m). (*b*) Influence on the relationship between the sum of the two post-meiotic segregation classes and the two conversion classes put together (5+ : 3m and 3+ : 5m *versus* 6+ : 2m and 2+ : 6m). (*c*) Alteration in the ascus classes with majority wild-type spores relative to asci with majority mutant spores (5+ : 3m and 6+ : 2m *versus* 3+ : 5m and 2+ : 6m).

If temperature changes influence the correction enzyme/s to a 'meaningful' extent, it should be possible to detect this effect when the data are classified as shown in Table 1. One can see that the distribution of aberrant ascus types in general is not influenced significantly by temperature changes. There are a few exceptions but the general conclusion from these data is that the repair mechanism is independent of temperature at the *buff* locus in *S. brevicollis*. Lamb (1968) reported a similar lack of response to variations in temperature at the *hyaline* and *grey* loci in *S. fimicola*.

(iv) *Hybrid DNA formation at the* buff *locus*

Assuming that aberrant asci are the result of hybrid DNA formation and the subsequent events related to it, the results obtained from mutant × wild type crosses at 25 °C (optimum temperature) suggest a heterogeneity in the formation of hybrid DNA at the *buff* locus (Table 1, Fig. 2). It is possible that this heterogeneity, which is significant ($\chi^2 = 11{\cdot}07$, $P = 0{\cdot}05$–$0{\cdot}01$ for 4 D.F.), reflects the frequency with which hybrid DNA crosses the *buff* alleles *S44*, *S41*, *S156*, *S180* or *S6*. This frequency would depend upon the position of the mutant site in relation to the opening point for hybrid DNA formation.

At temperatures other than 25 °C the differences between the *buff* mutants in the total aberrant ascus frequency are not significant ($\chi^2 = 4{\cdot}6$, $P = 0{\cdot}5$–$0{\cdot}25$ for 4 D.F. at 30 °C; $\chi^2 = 3{\cdot}32$, $P = 0{\cdot}75$–$0{\cdot}5$ for 4 D.F. at 20 °C; $\chi^2 = 8{\cdot}78$, $P = 0{\cdot}10$–$0{\cdot}05$ for 4 D F. at 15 °C). One reason for the absence of significant heterogeneity (at 30, 20 and 15 °C) could be a trivial one – that is, the smaller sample size at these temperatures compared to 25 °C. An alternative possibility is that hybrid DNA regions are longer at higher (30 °C) as well as at lower temperatures (20 and 15 °C). This would also lead to a loss of significant heterogeneity in the data. This type of change in lengths of hybrid DNA segments would be accompanied by an overall increase in hybrid DNA formation at 30 °C and an overall decrease in hybrid DNA formation at 20 and 15 °C as discussed earlier in this section.

If the hybrid DNA segments were shorter at lower temperatures and often failed

to reach the mutant sites (a possibility mentioned earlier) one would expect the heterogeneity in the data at least to persist if not enhanced at lower temperatures.

One drawback of this system in *S. brevicollis* is that the frequency of aberrant 4+:4m cannot be scored due to a high degree of spindle overlap at the post-meiotic mitosis. It is possible that hybrid DNA formation is constant in frequency at the *buff* locus but at 25 °C some of the hybrid DNA is left uncorrected in both chromatids and hence undetected. If this non-correction is mutant-dependent it could give rise to a significant heterogeneity. There is no clear-cut evidence to rule out this possibility giving rise to the significant heterogeneity observed in the

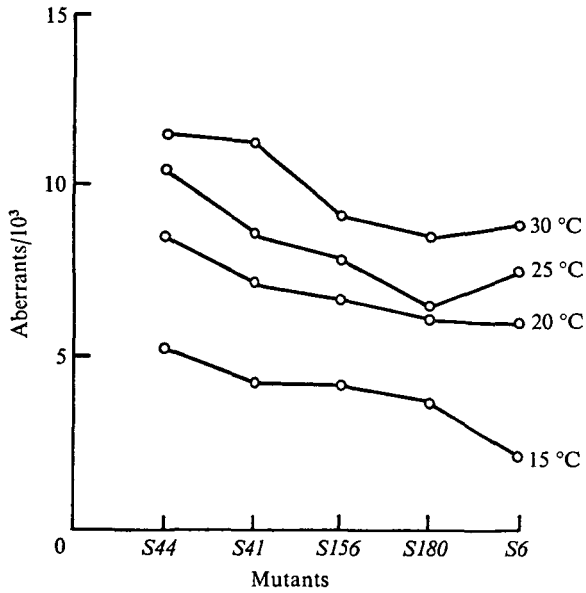

Fig. 2. Frequencies of aberrant asci at the *buff* locus at different temperatures.

single-point data at 25 °C. However, it is perhaps important to note that the relationship between total postmeiotic segregation 5+:3m, plus 3+:5m and total conversion 6+:2m plus 2+:6m is the same for all the *buff* mutations at 30, 25, 20 and 15 °C (with one exception *S41* × wild type at 30 °C). It is hard to reconcile this observation with the explanation of heterogeneity on the basis of a marker dependent contribution of aberrant 4+:4m asci which cannot be scored in this system: one would expect an increase in aberrant 4+:4m asci to be associated with an increase in 5+:3m and 3+:5m asci. This expectation is supported by the direct correlation between the frequency of aberrant 4+:4m asci and the frequencies of the two post-meiotic segregation classes 5+:3m and 3+:5m observed by Leblon & Rossignol (1973) in *Ascobolus immersus*.

(v) *Conclusions*

From crosses of mutant × wild type at the *buff* locus in *S. brevicollis* the following general conclusions can be drawn. (*a*) The fixed or preferred opening points in the DNA for initiation of recombination events appear to be available more often at higher than at lower temperatures, over the temperature range 15–30 °C. Opening points at or beyond both proximal and distal ends of the *buff* locus respond similarly to variations in temperature. (*b*) The correction pattern seems to be independent of temperature at the *buff* locus in *S. brevicollis*, a finding which is in agreement with the single-point data of Lamb from two loci (*hyaline* and *grey*) in *S. fimicola*. (*c*) The average length of hybrid DNA segment seems to be influenced by temperature changes.

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