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Polarized negative interference in the *paba1* region of *Aspergillus nidulans*

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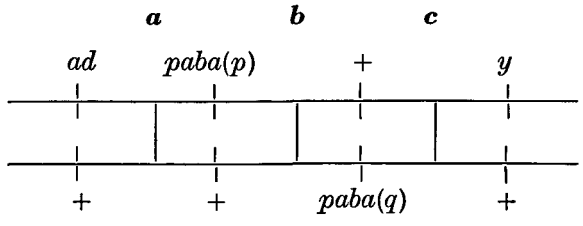
1. INTRODUCTION

The fine genetic structure of the *paba1* region has been described in an earlier publication (Siddiqi, 1962). The present paper deals with some features of localised negative interference encountered in the crosses involving allelic *paba* mutants. The term negative interference describes the excess of multiple exchanges in short intervals, so often found in crosses between closely linked markers. It has been interpreted to mean that, in any one zygote, recombination events are confined to small regions of the chromosomes which are said to be 'effectively paired'. In a population of zygotes where a particular region is 'effectively paired' only in some zygotes, a statistical consequence of such a discontinuous distribution of recombination events is the observed positive correlation (or negative interference) between exchanges in short regions. These short regions in which recombination events are assumed to occur have been termed 'effective pairing segments' (Pritchard, 1955, 1960) or 'switch areas' (Doermann, 1958).

One of the objects of the present work was to compare the intensity of negative interference on either side of the interval within which an exchange was selected. This can be done by comparing the frequencies of the two types of *paba*$^+$ recombinants, those requiring an additional exchange in the adjacent interval on the proximal side, relative to the centromere, and those which require an additional exchange in the distal interval. Consider, for example, the following type of cross:



Among *paba*$^+$ recombinants, all those which are *ad* require an additional exchange in the interval ***a*** while those which are y^+ require an additional exchange in ***c***. The excess of *ad* and y^+ recombinants over what is expected on the basis of the map lengths of the intervals ***a*** and ***c***, provides a measure of the negative interference on

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the two sides of ***b***. If negative interference is localized and extends only to a few tenths of a map unit (see Pritchard, 1960), the comparison should be relatively unaffected by any difference in the size of the two intervals. This last assumption can also be verified experimentally.

2. MATERIAL AND METHOD

For a detailed account of methods and the results of crosses between *paba* mutants, reference may be made to Siddiqi (1962). The fine structure of the *paba1* region and the linkage relationships of other markers used are shown in Fig. 1.

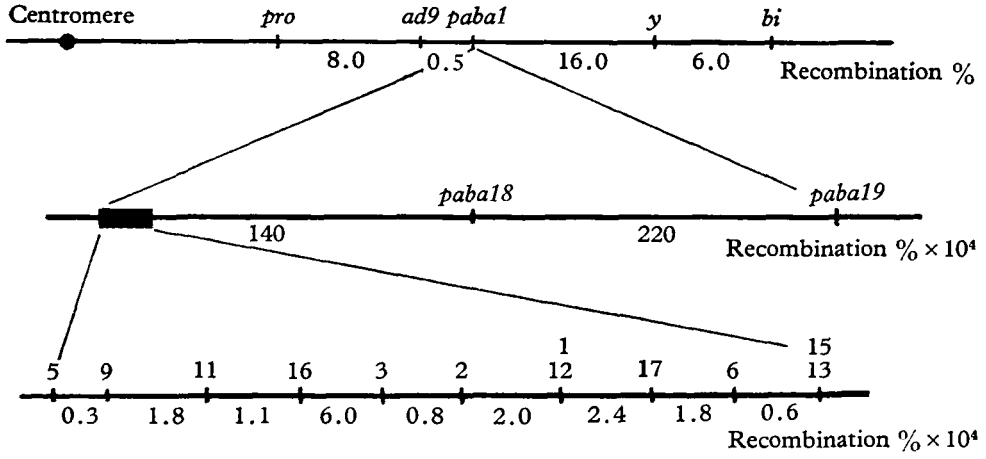


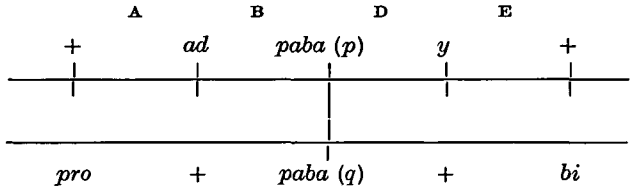
FIG. 1. Map of the right arm of chromosome I (after Käfer, 1958) showing the relationships of the markers referred to in the text. Nutritional requirements are: *pro*, proline; *ad*, adenine; *paba*, para-aminobenzoic acid; *bi*, biotin; *y* denotes yellow conidia. The *paba* cistron has been successively magnified to show the relative positions of the mutational sites. Numbers above the lines refer to *paba* markers.

3. RESULTS

Localization of negative interference

In order to test the assumption that negative interference does not extend appreciably beyond *ad9*, an additional marker *pro1*, which is 8 units proximal to *ad9*, was included in a few crosses. The results of four such crosses are summarized in Table 1, where the observed recombination percentages in the intervals involved among selected *paba*$^+$ recombinants are compared with their standard values among unselected products of meiosis. Although the results of the reciprocal crosses are not homogeneous, it is evident that the increase in the recombination fraction on the proximal side is restricted to the interval between *ad9* and *paba*. This is in agreement with the earlier observations of Pritchard (1960), which indicated that the length of effective pairing segments is no more than a few tenths of a map unit.

Table 1. *Effect of selection on recombination percentage in different intervals*



		Recombination percentage			
		A	B	D	E
Standard value:		8·0%	0·5%	16%	6%
Among *paba*$^+$ recombinants	*ad paba3 y* × *pro paba15 bi*	9·7 ± 3·5	5·6 ± 2·7*	48·6 ± 5·8*	2·8 ± 1·9
	ad paba15 y × *pro paba3 bi*	2·5 ± 1·1	2·5 ± 1·1*	39·8 ± 3·4*	3·5 ± 1·3
	ad paba18 y × *pro paba3 y*	2·9 ± 1·6	2 ± 1·5*	40 ± 4·8*	6·8 ± 2·4
	ad paba3 bi × *pro paba18 y*	10·5 ± 2·5	2 ± 1·1*	34·2 ± 3·8*	4·6 ± 1·7

* Increase over standard value significant at the 5% level.

Asymmetrical negative interference on the two sides of the interval of selection

Table 2 presents the observed increase in the recombination fractions on the two sides of the interval of selection among *paba*$^+$ recombinants from twenty-eight different crosses involving allelic *paba* mutants. Only those crosses which gave more than thirty *paba*$^+$ recombinants have been included. Apart from making the sample statistically large, this also minimizes the danger of spurious effects due to back mutation. The crosses are arranged in an increasing order of the recombination fraction between the *paba* alleles. The increase in the two intervals has been calculated by subtracting the standard value for the interval from the observed value in the selected sample. This is not strictly legitimate. As the intervals are unequal, the more appropriate comparison would be between their cross-over values. Since the present paper is concerned with gross trends rather than the precise values of these differences, we have considered this transformation unnecessary. A constant value of 0·5 units for the *ad* – *paba* interval and 16 units for the distal interval has been used as standard. These values are based on the pooled results of several workers in this laboratory. Although it is known that the actual values of recombination fractions often show considerable heterogeneity (Pritchard, 1959), it was not practicable to obtain control values in all the crosses. The standard errors of these estimates are negligible relative to the standard errors in the selected sample. The last column in the Table shows the difference in the increase on the two sides ($\alpha - \beta$). The data are represented graphically in Fig. 2.

It will be seen that on the proximal side there is a strong negative correlation between the length of the interval in which an exchange is selected and the increase in the recombination fraction of the *ad* – *paba* interval. The correlation coefficient is $-0{\cdot}5$, which is significant at the 1% level. The decrease in negative interference with an increase in the interval of selection appears to be more rapid near the origin

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Table 2. *Effect of an increase in the distance between two* paba *mutants on the intensity of negative interference on the two sides of the interval within which exchanges giving* paba$^+$ *recombinants are selected*

Intervals:		B	D	E	
Cross:	*ad9*	*paba* (*p*)		*y*	+
	+	*paba* (*q*)		+	*bi*

paba mutants in the cross ($p \times q$)	Recombination fraction between the *paba* mutants	Net increase in proximal interval, i.e. observed recombination percentage minus 0·5 (α)	Net increase in distal interval, i.e. observed recombination percentage minus 16·0 (β)	Difference ($\alpha - \beta$)
11 × 5	$9{\cdot}2 \times 10^{-7}$	25·9 ± 7·6	10·4 ± 7·6	+15·5*
2 × 3	$1{\cdot}3 \times 10^{-6}$	15·1 ± 4·9	21·2 ± 6·8	− 6·1
12 × 2	$2{\cdot}0 \times 10^{-6}$	14·8 ± 4·8	7·0 ± 5·9	+7·8
6 × 2	$4{\cdot}0 \times 10^{-6}$	7·3 ± 3·2	20·8 ± 6·2	−13·5*
1 × 6	$4{\cdot}4 \times 10^{-6}$	—	8·0 ± 6·0	−8·0
9 × 3	$4{\cdot}8 \times 10^{-6}$	25·4 ± 6·0	15·4 ± 6·3	+10·0
3 × 16	$5{\cdot}8 \times 10^{-6}$	19·5 ± 5·0	4·0 ± 5·0	+15·5*
11 × 3	$5{\cdot}9 \times 10^{-6}$	16·7 ± 3·1	12·2 ± 3·7	+4·5
16 × 3	$6{\cdot}0 \times 10^{-6}$	14·6 ± 4·0	10·5 ± 5·0	+4·1
2 × 5	$9{\cdot}4 \times 10^{-6}$	23·0 ± 4·2	7·5 ± 4·2	+15·5*
6 × 3	$1{\cdot}0 \times 10^{-5}$	9·1 ± 4·1	12·8 ± 6·3	−3·7
16 × 2	$1{\cdot}2 \times 10^{-5}$	18·9 ± 4·9	21·5 ± 5·7	−2·6
9 × 2	$1{\cdot}8 \times 10^{-5}$	10·4 ± 3·7	3·1 ± 4·6	+7·3
6 × 16	$1{\cdot}9 \times 10^{-5}$	5·5 ± 2·9	12·3 ± 5·5	−6·8
6 × 5	$3{\cdot}5 \times 10^{-5}$	14·0 ± 2·6	16·7 ± 3·4	−2·7
6 × 11	$5{\cdot}1 \times 10^{-5}$	19·1 ± 5·6	17·3 ± 6·6	+1·8
6 × 18	$1{\cdot}4 \times 10^{-4}$	0·4 ± 0·9	12·3 ± 4·4	−11·9*
13 × 18	$1{\cdot}4 \times 10^{-4}$	0·9 ± 0·8	20·5 ± 3·4	−19·6*
1 × 18	$1{\cdot}6 \times 10^{-4}$	1·2 ± 1·2	18·4 ± 4·4	−17·2*
9 × 18	$1{\cdot}6 \times 10^{-4}$	5·1 ± 1·7	17·1 ± 3·5	−12·0*
18 × 3	$1{\cdot}9 \times 10^{-4}$	3·7 ± 2·9	16·8 ± 6·8	−13·1*
5 × 18	$1{\cdot}9 \times 10^{-4}$	3·0 ± 1·7	20·8 ± 4·5	−17·8*
18 × 19	$2{\cdot}2 \times 10^{-4}$	3·3 ± 1·9	15·7 ± 4·6	−12·4*
18 × 2	$2{\cdot}6 \times 10^{-4}$	5·0 ± 3·1	17·3 ± 6·4	−12·3*
1 × 19	$4{\cdot}0 \times 10^{-4}$	0·3 ± 0·2	12·2 ± 3·9	−11·9*
9 × 19	$4{\cdot}1 \times 10^{-4}$	2·0 ± 1·4	21·1 ± 4·4	−19·1*
6 × 19	$4{\cdot}3 \times 10^{-4}$	3·3 ± 2·7	12·8 ± 5·1	−9·5*
5 × 19	$5{\cdot}1 \times 10^{-4}$	1·8 ± 2·3	9·5 ± 5·4	−7·7*

* Significant at the 5% or less level.

than in the latter part of the curve. On the distal side, no correlation between the interval of selection and the intensity of negative interference is apparent; with increasing distance between the *paba* mutants, the increase in the recombination fraction between *paba* and *y* remains unchanged.

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The differential effect on the two intervals is even more clearly revealed when the increases in the two outside intervals in each cross are compared. In the first part of Table 2, where the recombination fractions between the *paba* alleles are small, the increase in both intervals tends to be equal. Out of the first sixteen crosses listed in the table, the difference between column α and column β is significant in only four cases. What is perhaps even more important, this difference $(\alpha-\beta)$ assumes both + and − values with about the same frequency. In the second half of the table, however, the increase in the distal interval is significantly greater in all twelve crosses. The difference in some crosses is as great as twenty-fold.

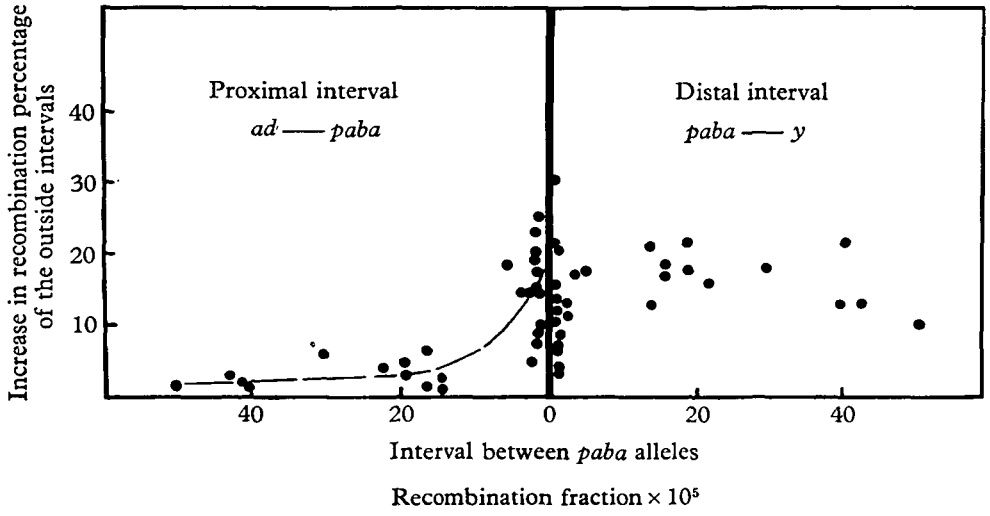

FIG. 2. Effect of increase in the distance between the two *paba* alleles on the intensity of negative interference in the adjacent intervals as measured by the net increase in the recombination percentage in the interval (a standard value of 0·5 for the distal interval and 16·0 for the proximal interval has been used).

Evidently some of the differences in column 5 of Table 2 could be artifacts and could arise either from chance fluctuation or a real deviation in the recombination fraction of the *paba* − *y* interval from the standard value of 0·16 used in our calculations. This would account for the wide scatter in the regression diagram. However, this fluctuation by itself could not produce the correlation described. The consistency and the magnitude of the effect are such that it is unlikely to be an artifact.

4. DISCUSSION

The results described above lend themselves to more than one interpretation. In the absence of more direct information on the mechanism of recombination it is not possible to choose conclusively between the alternative explanations. We therefore limit ourselves to the relevance of these observations to some of the possible explanations, without attempting to introduce specific models.

Polarization of negative interference was first noticed in the *ad8* region by Pritchard (1957) who pointed out that the increase in recombination was greater in the interval distal to the segment of selection than proximally to it. Similar asymmetry is present in the data of Calef (1957). An analogous situation is encountered in transformation experiments in *Pneumococcus* where 'crosses' between the same pair of mutants do not give identical results in reciprocal arrangements of markers between donor and recipient (Lacks & Hotchkiss, 1960).

It has been suggested by Ephrussi-Taylor (1960) that if transformation involves multiple cross-over events in the paired region, the unequal frequency of recombinants with the two parental arrangements of outside markers in *Aspergillus* and the asymmetry in reciprocal crosses in transformation may be interpreted in the

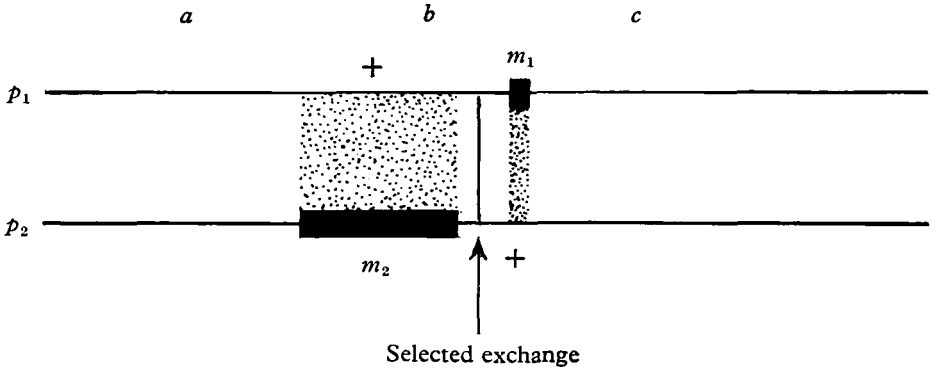

FIG. 3. Model following Ephrussi-Taylor (1960) attributing asymmetry in negative interference to different linear dimensions of the mutants. Recombinants are selected on the basis of a single exchange in the interval *b*. A second exchange within the shaded length will not be selected and, with a short length of effective pairing, the recoverable second exchange will be more often in interval *c* than in *a*. *P1* and *P2*, parental strands; *m1* and *m2*, mutants; *a*, *b* and *c*, intervals.

same manner. She has assumed that some of the mutants extend over more than one site of recombination, and that the number of sites may be different in different mutants. The consequence of this hypothesis for a cross in *Aspergillus* is represented in the diagram of Fig. 3. It will be seen that the frequency of recovery of the second exchange is reduced towards the side of the 'larger' mutant.

We consider an explanation based on Ephrussi-Taylor's model inadequate for two reasons. Applied to our results, it would imply that, in all cases where a pronounced asymmetry in the distribution of multiple exchanges is found, the proximal mutant is 'larger' than the distal one, specifically that all mutants proximal to *paba18* are larger than *paba18* and *paba18* itself is larger than *paba19*. It would also imply that all mutants proximal to *paba18* have similar dimensions as no asymmetry is encountered in crosses between them. Although this possibility cannot be rigidly excluded, it is highly unlikely. All mutants are of independent origin and four of them are known to revert to the wild type. Secondly, the hypothesis predicts that, as the interval between *paba* mutants increases, the asymmetry should become less pronounced. This is the opposite of what is found.

It has been pointed out by several authors (Calef, 1957; Chase & Doermann, 1958; Pritchard, 1960; Suyama, Munkres & Woodward, 1959) that if recombination is restricted to effectively paired regions, the intensity of negative interference in adjacent intervals should be negatively correlated with the length of the interval within which an exchange is selected. The possibility may be considered that the position of the pairing segment is fixed and in crosses where an asymmetry in negative interference is found the interval of selection lies nearer to one end of the segment. Our results are not compatible with this assumption. In crosses where the most extreme asymmetry is found, the interval of selection has been shifted towards the distal side (i.e. towards y) but the decrease in negative interference has occurred in the proximal interval.

Effective pairing may involve the simultaneous realization of more than one condition essential for recombination. It may be assumed that one of these conditions is polarized and proceeds from the distal towards the proximal end of the effectively paired region. Effective pairing could be discontinued owing to an interruption of one or more of its components, thus preventing any further recombination. This 'failure' may occur with a finite probability. If one considers a population of products of meiosis from which those arising by one exchange between two closely linked markers are selected, the possibility of failure in the distal interval is eliminated because it would also entail failure of the selected exchange. Failure could still occur before any further recombination in the proximal interval took place.

The asymmetry could also be accounted for by 'marker stimulation' as suggested by Hershey (1958), if the recombination events had a sequential distribution.

Lissouba & Rizet (1960) have studied recombination between ascospore colour mutants in *Ascobolus immersus* and have found that, in one segment of the chromosome, recombination between alleles is exclusively non-reciprocal. In all tetrads with a ratio of 3 wild type : 1 mutant, it is always the mutant on the one side which is converted to wild type, giving rise to an excess of the parental marker on the other side (majority parent). This observation has been interpreted in terms of polarized replication and the segment in question is termed polaron. It is not known how this kind of recombination affects the distribution of outside markers. In our case, in the absence of tetrad analysis, it is not possible to say to what extent, if any, non-reciprocal recombination is involved. The distribution of outside markers shows that, with the possible exception of crosses between *paba5* and *paba9* (Siddiqi, 1962), the largest number of *paba*$^+$ recombinants can be interpreted as resulting from single exchanges. Even when the interval of selection is small and the negative interference on both sides high, the proportion of recombinants between *paba* alleles which have the parental arrangements of outside markers does not exceed 50%. This is consistent with the view that all single exchanges, whether reciprocal or non-reciprocal, result in recombination between outside markers and that the *paba*$^+$ strands with parental arrangements of outside markers are produced as a result of multiple exchanges. The possibility that at least some of the 'parental type' recombinants arise by gene-conversion (i.e. 3:1 segregation) cannot be

excluded. If this conversion were polarized in such a way that the parental strand with the distal *paba* mutant was always the 'majority parent', an inequality between the two parental types such as that observed in the crosses between *paba* alleles far apart from each other would be encountered. It may, however, be pointed out that many crosses between *paba* mutants belonging to one small region of the cistron do not show any asymmetry. It is possible that the two sets of observations, one showing polarization of conversion in *Ascobolus* and the other, polarization of negative interference in *Aspergillus*, are different manifestations of the same underlying process, i.e. a polarized distribution of recombination events.

It may be argued that, as most of the crosses showing pronounced asymmetry of negative interference involve *paba18* or *paba19*, the effect is not related to the interval of selection but is a specific property of these two mutants. In so far as the possibility of allele-specific conversion rates is concerned, it has been shown elsewhere (Siddiqi, 1962) that our results do not support this hypothesis. The apparent conversion rates are related to the recombination fractions between the mutants involved and their relative positions. However, the possibility that the correlation between negative interference and the interval of selection is fortuitous and is produced by *paba18* and *paba19* cannot, at present, be entirely ruled out. As more sites become available in the *paba1* region, a confirmation of the present observations would render it more improbable.

Pritchard (1960) has examined the effect of increasing length of the interval of selection on negative interference in the *ad8* region of *A. nidulans*. He observed a decrease in negative interference on the proximal side with increasing distance between *ad* alleles. On the distal side no such decrease was discernible. The absence of a detectable decrease in negative interference on the distal side was ascribed to heterogeneity in the recombination fraction between *ad* and *y*. It is very likely that his results are of the same nature as ours. The data from the *ad9* region (Calef, 1957; Martin-Smith, personal communication) show that in this case too, negative interference on the distal side exceeds that on the proximal side. Since all the three genes *ad9*, *paba1* and *ad8* are situated on the same arm of chromosome I, it may be more than a coincidence that the direction of polarization is the same in each case. At present sufficient evidence to justify further speculation about the orientation of polarity in relation to the centromere and ends of the chromosome is not available. However, if the present observations are corroborated by results at other loci, a study of polarity in different parts of the chromosome may offer an approach to submicroscopic organization.

SUMMARY

The intensity of negative interference in 28 crosses involving 14 allelic *paba* mutants in different combinations has been measured. Among selected cross-overs between two such mutants, when the distance between the mutants is large, additional exchanges on the distal side (relative to the centromere) greatly exceed those on the proximal side. With decreasing length of the interval of selection, the inequality disappears. It is suggested that this polarity may be the outcome

of a undirectional process which imposes a time sequence on the recombination events within an effectively paired segment. Some alternative explanations of this result are discussed.

It is a pleasure to thank Prof. G. Pontecorvo, F.R.S., for much advice and stimulating criticism.

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