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Genetic analysis of an unequal chromosomal translocation in *Aspergillus nidulans*

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SUMMARY

Translocation T(III–VIII) in *Aspergillus nidulans* has been analysed by the detection of meiotic linkage between markers previously located separately on linkage groups III and VIII. The breakage points have been mapped by the detection of linkage between the crinkled type and genetic markers in the region of the break. A segment from linkage group III, approximately 43 units long and including the markers *moC96*, *sC12*, *sA1* and *cnxH3*, has been translocated into linkage group VIII. The breakage point is between *su6proA* and *moC96* and the attachment point is close to *cha* in linkage group VIII. It seems probable that the segment has been inserted into linkage group VIII.

1. INTRODUCTION

Previous work on a chromosomal translocation involving linkage groups three (III) and eight (VIII) had suggested that this translocation was unidirectional or that it involved the interchange of two very unequal segments (Bainbridge & Roper, 1966). Evidence for this came from the regular segregation at meiosis of an aneuploid type. This was shown to be a duplication type which could revert to a more normal type by loss of chromosomal material. The detailed mechanism of this reversion has attracted considerable attention (Nga & Roper, 1968, 1969).

The work reported in this paper was undertaken to define the structure of this translocation in more detail. A preliminary report of this work has appeared (Bainbridge, 1968).

2. METHODS

(*a*) *Media.* The standard minimal medium for *Aspergillus nidulans* was prepared without the carbon source. Glucose or galactose was prepared separately and autoclaved at 5 lb./109° for 10 min. They were added aseptically as required to media at 45° immediately before use to give a final concentration of 1% (w/v). Minimal medium (MM) contained 1·5% Davis agar. Complete medium (CM) was identical to the medium used by MacKintosh & Pritchard (1963) except that again glucose was autoclaved separately. Methionine, phenylalanine and arginine were added as a supplement of 0·01% (w/v) as CM is deficient in these amino-acids.

(*b*) *Strains.* Strains were obtained from stocks held in the Genetics Departments at Glasgow, Sheffield, Cambridge and Warsaw Universities. Additional

strains were obtained from the Fungal Genetics Stock Centre, Dartmouth College, U.S.A. Other strains were selected from crosses during the course of the work. The strains are numbered as in the Queen Elizabeth College collection by the prefix BWB.

(*c*) *Genetic markers.* Most of the markers used in this work have been described previously (Käfer, 1958; Barrett, Johnson & Ogata, 1965; Dorn, 1967). The following were the markers of major interest: *adD3*, *adE20*, *argA1*, *argB2*, *bi-1*, *cnxH3* (Pateman, Rever & Cove, 1967), *cys2* = *sO* = *sCl2* (Käfer, 1965), *lysB5*, *methG2*, *pabaA1*, *pabaA9*, *phenA2*, *proA1*, *proA6*, *pyro-12*, *riboB2*, *sA1* and *sC12;* growth requirements respectively for adenine, arginine, biotin, nitrite, cystine, lysine, methionine, *p*-aminobenzoic acid, phenylalanine, proline, pyridoxine and thiosulphate. *cha*-chartereuse conidia, *y* yellow conidia, *w-1*, *-2*, *-3* white conidia. The following were morphological mutants: *aba-6* (A. J. Clutterbuck, unpublished), *moB9*, *moB50*, *moC96* (Bainbridge, 1966). *Su1proA; Su4proA* (E. Forbes, unpublished, Käfer, 1958) and *su6proA* (Weglenski, 1966) were suppressors of the proline mutant *proA*. $AcrA^R$ and Act^R resistance respectively to acriflavin and actidione (Roper & Käfer, 1957; Warr & Roper, 1965). *gal1*, a strain unable to utilize galactose.

(*d*) *Genetic analysis.* The general techniques of analysis were those already published (Pontecorvo *et al.* 1963; Pontecorvo & Käfer, 1958) except that the following modifications were made.

The growth requirements of the duplication type, crinkled, were tested using a loopful of a dilute spore suspension in 0·1 % Tween 80 (v/v) containing approximately 10^5 spores/ml. This was to minimize the possibility of reversion as this has been shown to affect particular genetic markers (Bainbridge & Roper, 1966).

Certain crosses were made using the arginine crossing technique (Bainbridge, 1965). Translocations were detected by the haploidization technique using *p*-fluorophenylalanine (Käfer, 1965).

Mitotic analysis of diploids heterozygous for Actidione resistance (Warr & Roper, 1966) was made by a modification of the acriflavin technique (Roper & Käfer, 1957). Diploids were inoculated on to CM containing 20 mg/ml of Actidione. Actidione resistance is semi-dominant and fully resistant sectors were selected.

3. RESULTS

(*a*) *Genetic analysis of linkage groups III and VIII.* A genetic map of part of linkage group III is shown in Fig. 1. This is based on extensive data, a selection of which can be seen in Table 1. The order of the markers *moC96-sC12-sA1* is based on selective platings not shown in this table.

The location of the centromere between *Act1* and *phen2* is based on the analysis of two diploids. Diploid I had the following genotype:

$$\begin{array}{ll} \text{BWB 177} & \dfrac{+\quad +\quad Act^R\ phenA2\ sC12}{} \\ \text{BWB 149} & adE20\ bi\text{-}1\quad +\quad +\quad + \end{array}.$$

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Sixteen diploid sectors obtained from this diploid on CM plus actidione were all *phen*$^+$ *s*$^+$. Diploid 2 had the following genotype:

$$\begin{array}{l} \text{BWB 305 } \underline{adE20\ bi\text{-}1} \\ \text{BWB 139 } \underline{+\ y\ +} \end{array} \quad \begin{array}{l} \underline{methG2\ argG2\ Act^R\ phenA2} \\ \underline{+\quad +\quad +\quad +} \end{array}.$$

Exposure of this diploid to Actidione gave eight diploid sectors which were all *meth arg Act*^{R} *phen*$^+$. The most likely position for the centromere is therefore between *Act* and *phenA2*.

The linkage data for linkage group VIII is shown in Table 1. It should be noted that *moB9* prevents the accurate classification of *cha* and *aba*. The two crosses shown here were based on analysis of *moB9*$^+$ colonies only (crosses E and F).

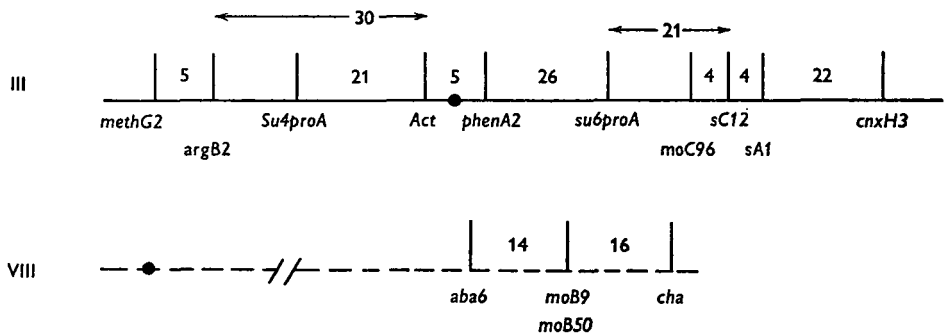

Fig. 1. Genetic maps of parts of linkage groups III and VIII.

(*b*) *Detection of T(III–VIII)*. Haploidization analysis of diploids heterozygous for the translocation T(III–VIII) showed that there was complete linkage between genetic markers previously located separately to linkage groups III and VIII. This confirmed work already published (Käfer, 1965). This method also revealed the presence of T(III–VIII) in strain BWB 366 *proA6 pabaA9 bi-1; su6proA* obtained from Dr P. Weglenski. Haploid sectors were analysed from a diploid of the form:

$$\begin{array}{l} \underline{su6proA\quad +} \\ \underline{+\qquad gal\ 1} \end{array} \quad \begin{array}{l} \underline{\quad +} \\ \underline{riboB2} \end{array}$$

gal 1 is located on linkage group III and *riboB2* is located on linkage group VIII. Only two genotypes relevant to these markers were obtained, *su6pro gal*$^+$ *ribo*$^+$ and *su6pro*$^+$ *gal 1 ribo*. Markers in the remaining six linkage groups showed independent assortment. A cross between BWB 366 and BWB 171 *bi, w, cys2 moC50* T(III–VIII) gave rise to morphologically normal progeny (Cross I). It would appear from this that the translocations in these two strains are identical or at least very similar. For this reason data from this strain are included in the analysis. *su6pro* has not been separated from the translocation and is presumed to be located very close to the breakage point.

(*c*) *Meiotic linkage between markers in linkage groups III and VIII*. Linkage between genetic markers previously located separately on linkage groups III and VIII were detected in crosses homozygous or heterozygous for the translocation.

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Table 1. *Linkage data for genetic markers in linkage groups III and VIII*

Cross	Strain no. (BWB)	Genotypes and relevant intervals	Single cross-over in region's			Double cross-over in regions			Triple cross-over	No. of colonies tested
			1	2	3	1–2	1–3	2–3		
A	181	*proA1 pabaA1 y* + + + *phenA2 cys2*	16	4	44	1	15	1	1	156
	327	*proA1* + + *bi* *Su4pro Act*^{R} + + (21, 5, 39)								
B	172	*y* + *adD3* *moC96 sA1* +	4	18	—	2	—	—	—	92
	359	+ *bi* + + + *cn* × H_3 (7, 22)								
C	181	*proA1 pabaA1 y* + *phenA2* + *cys*$_2$	11	9	—	1	—	—	—	47
	366	**T proA6 pabaA9* + *bi* + *su6proA* + (26, 21)								
D	236	*adE20 bi* + + *Act*^{R} *phenA2*	6	61	5	5	1	4	—	237
	285	+ *bi* *meth2 argB2* + + (5, 30, 4)								
E	472	*proA1y* + + *cha*	50†	42	—	†	—	—	—	348‡
	481	+ *y* *aba6* *moB9* + (14, 16)								
F	414	+ *bi* *phenA2* + + *cha*	34	67	—	11	—	—	—	228‡
	482	*y* *bi* + *aba-6* *moB9* + (20, 34)								

* *su6* has not been separated from T(III–VIII).

† Double crossover class indistinguishable from single crossover in position I.

‡ *moB9*$^+$ colonies only classified for *aba* and *cha*.

Table 2. *Linkage between markers previously located in linkage groups III and VIII*

(In crosses segregating crinkled types only normal colonies were analysed.)

Cross	Strains (BWB)	Genotypes	Translocation state of cross	Interval	Recombinant	Total colonies	Recombination units	*P* (1:1 ratio)
G	200	*adE20* + *bi* / + *y* +	Homozygous	*moB9-sC12*	29	52	Unlinked	> 0·3
	207	*w*$_3$ *AcrA*^{R} + + / *sC12* + + / + *argA1* *moB9*	*unT*					
H	137	*T* *y* / *y* + *adD3* / *w* +	Homozygous	*moB9-cys2*	73	178	41 ± 3·7	< 0·02
	174	*T* + + + + / *sA1* *cys2* *pyro12* *moB9*	*T*					
I	171	*T* + + *bi* / *proA1* *pabaA9* *bi*	Homozygous	*moB50-cys2*	10	36	27·8 ± 7·5	< 0·02
	366	*T* *w*$_3$ / + + *cys2* *moB50* / *su6pro* + +	*T*					
J	J (G0187)	*T* *bi* / *bi* *w*$_3$ / + + / *cys2* *pyro12* / + + / *lysB5* + / *cha*	Heterozygous	*cha-cys2*	37	100	37 ± 4·8	< 0·01
	124		*T*					
K	209	*T* *y* / *y* *w*$_3$ / + *adD3* / + + / *phenA2* *cys2* *moB9* + / + *cha*	Heterozygous	*phenA2-moB9*	37	101	36·5 ± 4·7	< 0·01
	417		*T*	*phenA2-cha*	33	101	32·7 ± 4·7	< 0·01
				cha-cys2	25	101	24·8 ± 4·2	< 0·01

* *moB9*$^+$ colonies only classified in this cross.

G. Glasgow stock number.

In this analysis normal colonies only were classified for genetic markers. A summary of this linkage data is shown in Table 2. No linkage was detected in homozygous untranslocated crosses.

(*d*) *Location of the interchange* (*breakage*) *point.* Initially, location of the breakage point was attempted by analysis of recombinant strains from crosses heterozygous for the translocation. These crosses were of the general type *moB9 T* × *moB9*$^+$. Six *moB9* recombinant normal strains were backcrossed to the *moB9*$^+$ parental strain. Five of these strains gave crinkled progeny while the sixth strain did not. This suggested that *moB9* was approximately 17 recombination units from breakage point.

Table 3. *Data from crosses heterozygous for T(III–VIII)*

Cross L BWB 140 *y; argA1*
BWB 170 *bi*; w_3 *adD3 : moB9T*
Cross M BWB 140 *y; argA1*
BWB 117 *bi : Act*^{R} *cys2/T*

	Cross L		Cross M	
	moB9	*moB9*$^+$	*cys2*	*cys2*$^+$
Normal	60	59	33	37
Crinkled	70	11	6	64

This method of analysis was, however, laborious and a second method was devised to map the position of the breakage point. Essentially this method consisted of treating the crinkled type as a genetic marker. Linkage between the crinkled type and a given genetic marker gave an estimate of the position of the breakage point. For example, in the cross already mentioned linkage of *moB9* to the breakage point was reflected in an excess of crinkled *moB9* colonies (cross L, Table 3). This indicated linkage of about 14 %. It should be noted that this is a direct estimate of the linkage between *moB9* and the breakage point, as *moB9* is not located in the translocated segment. Also shown in Table 3 are the results from cross M, in which an excess of *cys*$^+$ were found. *cys2* is located in the duplicated segment, and consequently *cys2* crinkled segregants are genotypically *cys2/cys2.* The reciprocal recombinant *cys2*$^+$*/cys2*$^+$ cannot be distinguished from *cys2*$^+$*/cys2* by nutritional analysis. It is therefore necessary to multiply the recombinant class by two to obtain a true estimate of the linkage between *cys2* and the breakage point. A collection of more extensive data is summarized in Table 4. From this data and from the data already presented it is possible to construct a map of the translocation which is shown in Fig. 2(*a*) and (*b*). Also shown is a T-shaped map showing the linkage relationship observed.

The position of *su6pro* just outside the translocated region is based on the analysis of crinkled reversion in cross C. *su6* is a recessive suppressor of proline requirement and consequently *su6*$^+$*/su6* crinkled types require proline. Loss of the *su6*$^+$ allele by crinkled reversion should result in a revertant sector which is now independent of proline. An intensive study of revertants from 6 proline requiring

Table 4. *Location of the breakage point* (T_B)

Cross	Strains (BWB)	Genotypes	Recombinant crinkled	Total crinkled	Interval	Linkage relationship (s.e.)
M	140	$\frac{y\ +\ +\ \ \ +\ \ argA1}{+\ bi\ \ AcuR\ \ cys2\ +}\ \ T$	6 (×2)	70	$cys2T_B$	17·2 ± 4·5
	117					
L	140	$\frac{y\ +\ +\ +\ \ argA1\ \ +}{+\ bi\ \ w_3\ \ adD3\ \ +\ \ moB9}\ \ T$	11	81	$moB9\text{-}T_B$	13·6 ± 3·8
	170					
N	149 (G07)	$\frac{+\ \ adE20\ bi\ \ +\ \ +}{y\ \ +\ \ +\ \ argA1\ \ moB9}\ \ T$	20	113	$moB9\text{-}T_B$	17·7 ± 3·6
	217					
O	140	$\frac{y\ +\ +\ +\ \ argA1\ \ +}{+\ bi\ \ w_3\ \ cys2\ \ +\ \ moB50}\ \ T$	5	37	$moB50\text{-}T_B$	13·5 ± 5·6
	171					
C	181	$\frac{proA1\ pabaA1y+\ \ phenA2+cys_2}{proA6\ pabaA9+bi\ \ +su_6proA+}\ \ T$	9	38	$phenA2\text{-}T_B$	23·7 ± 6·9
	366		2	38	$su6proA\text{-}T_B$	5·3 ± 3·6

G. Glasgow stock number.

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crinkled colonies failed to reveal any evidence of *pro*$^+$ reversion. It is therefore concluded that *su6* is located just outside the translocated segment.

(*e*) *Analysis of* su1 pro *strains.* *Su$_1$pro* has already been located to linkage group IIIR by previous workers (E. Forbes, unpublished; Käfer, 1958). Attempts have been made to relate this marker to the genetic maps presented here. Results were complex and an intrachromosomal arrangement appeared to be the best way to

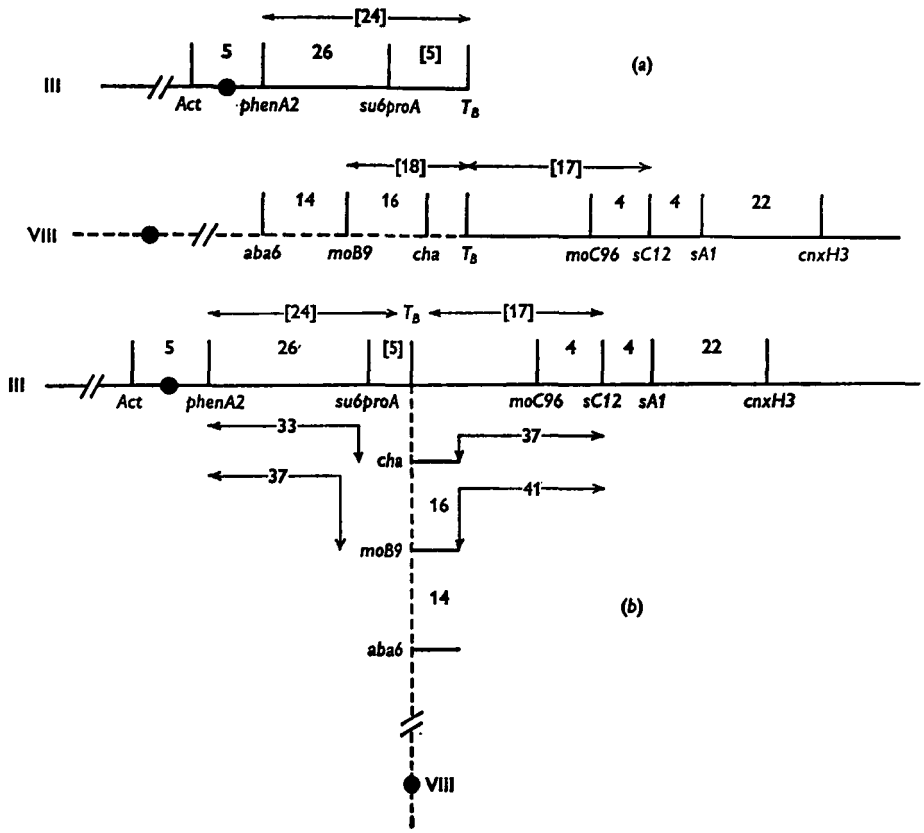

Fig. 2. Genetic maps of the translocated chromosomes. (*a*) The translocated chromosomes in strains carrying T(III–VIII); (*b*) map to show complex inter-chromosomal linkages. [17]linkage data obtained by crinkled analysis as described in the text.

explain these results. Crosses between *Su1pro* strains, e.g. BWB 233 *proA1, bi1; Su1proA* and BWB 172 *y; adD3, moC96 sA1* (lacking the T(III–VIII)), gave rise to a low frequency of aneuploid types similar to crinkled. However, crosses between BWB 233 and strains known to have T(III–VIII) yielded a further aneuploid type. Attempts to isolate relevant diploids to check the translocation status of BWB 233 have so far failed. For the above reasons all crosses involving *Su1pro* have been omitted.

4. DISCUSSION

The results presented above show that T(III–VIII) involves the transfer of a chromosomal segment at least 43 units long from the right arm of linkage group III to the right arm of linkage group VIII. The breakage point in linkage group III is between *su6proA* and *moC96* and the attachment point is close to *cha* in linkage group VIII. There are two possible structures for the translocation. It may be reciprocal, involving a short dispensable tip from linkage group VIII and a longer region of linkage group III. Alternatively the translocation may result from the insertion of a segment of linkage group III into linkage group VIII. An example of the second type of translocation has been reported in *Neurospora* (Perkins, 1966), and there are close similarities between the two translocations. However, no evidence in T(III–VIII) has been found for genetic markers located distally to the translocated segment. A choice between these two alternatives must await the availability of more genetic markers on the right tip of linkage group VIII. The most likely alternative however would appear to be that T(III–VIII) is an insertional translocation.

A recent study has been made in *Aspergillus nidulans* of the occurrence of aneuploid types in meiotic products (Pollard, Käfer & Johnston, 1968). Aneuploids occurred at a frequency of between 0·3 and 4·2% in crosses involving translocations. It is possible that aneuploids of the type n + 1 for linkage groups III or VIII have occurred in this work. However, crinkled colonies account for 33% of all meiotic products, so that it seems unlikely that the occurrence of a second aneuploid type such as n + 1 will have seriously affected the data presented in this paper.

It has been suggested that the results obtained from strains carrying the T(III–VIII) could be explained by an intra-chromosomal inversion (Millington-Ward, 1967). Although there are a number of similarities between the results obtained from T(III–VIII) and a pericentric inversion in *Neurospora* (Newmeyer & Taylor, 1967) it seems unlikely that the results presented above can be explained by a chromosomal rearrangement involving only one linkage group.

The detailed structure of this translocation is crucial to our understanding of the mechanism of crinkled reversion. A detailed analysis of crinkled reversion has been made of a related translocation in *Aspergillus*, T(I–II) (Nga & Roper, 1968; 1969). Reversion of the duplication type appears to occur both by interstitial loss and by loss of most or all of the duplicated segment. If, however, both of these translocations are insertional, then the duplication types will have a section of linkage groups VIII or II located distally to the translocated segment. If this segment is essential for growth then all loss from this chromosome will be interstitial. Loss from the other chromosome could also occur by complete loss of the tip of linkage groups III or I, respectively.

A detailed understanding of these translocations and the mechanism of crinkled reversion must await the availability of relevant genetic markers. It will also be necessary to subject the translocated strains, crinkled colonies and revertants to a detailed analysis by ascus analysis as well as by haploidization.

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