Genet. Res., Camb. (1970), **16**, *pp.* 303–316
With 3 *text-figures*
Printed in Great Britain

A variegated position effect in *Aspergillus nidulans*

By A. J. CLUTTERBUCK
Department of Genetics, University of Glasgow, Glasgow, Scotland

(*Received* 23 *June* 1970)

SUMMARY

In mutants at the '*bristle*' locus of *Aspergillus nidulans* the conidiophore remains as a stiff hypha rather than developing a vesicle, sterigmata and conidia. The *brlA*12 allele of this locus has a variegated phenotype, and genetic analysis has shown that this is associated with a translocation which has a breakpoint in the map interval adjacent to the *bristle* locus.

The mutant phenotype is partially repaired on high-salt medium at low pH, and can also be repaired by suppressors, one of which has been mapped at a locus unlinked to *brlA*12.

The mutant provides proof that variegation is due to instability of gene expression and not to mutability since *brlA*12 is genetically stable and can be propagated from either conidia or sterile conidiophores, the structures formed at the two extremes of variegation, and the resulting colonies in both cases are identical to the original strain.

It has been shown by mitotic recombination that the translocation associated with the variegated mutant is a 'simple translocation' in which the distal half of linkage group VIII is attached to the end of linkage group III. This terminal attachment site does not appear to be damaged in any genetically detectable way.

1. INTRODUCTION

In the course of a search for mutants of *Aspergillus nidulans* defective in conidial development (Clutterbuck, 1969*a*) one mutant was obtained which was unusual in having a variegated phenotype. It was considered possible that this might be an instance of position effect variegation, and in order to test this the genetic analysis described here was undertaken. The results confirm this suggestion by showing that the mutant is associated with a translocation which has a breakpoint close to the variegating locus.

Variegated position effects (reviewed by Lewis, 1950; Baker, 1968) have been described in *Drosophila*, in *Oenothera* (Catcheside, 1947) and in the mouse (Russell & Bangham, 1961). This form of variegation is of interest since it appears to be an instance of aberrant control of gene expression acting, at a fairly gross level, on the chromosome itself. The occurrence of such a phenomenon in a fungus is of interest firstly in showing that fungi share a complexity of chromosome organization in common with higher eukaryotes, and secondly for the possibilities that the mutant may provide for the investigation of nutritional and other environmental influences on variegation.

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2. MATERIALS AND METHODS

General techniques are those of Pontecorvo *et al.* (1953). Strains were all from the Glasgow stocks of *Aspergillus nidulans* (Clutterbuck, 1969*c*). Minimal medium (MM) is as described by Pontecorvo *et al.* (1953), the complete medium in current use is described by Clutterbuck (1969*a*).

The use of master strains for the location of markers on linkage groups is described by McCully & Forbes (1965), and genetic analysis by mitotic recombination by Pontecorvo & Käfer (1958). In genetic analysis *brlA*12 segregants were classified for nutritional markers by replication from CM + 1 M-NaH_2PO_4.

The linkage map of *A. nidulans* is given by Dorn (1967) with additions due to Sinha (1969), D. J. Cove (personal communication) and Clutterbuck (1969*a*). Genetic markers mentioned in this work are as follows: *bi, ribo, arg, meth, phen, s, cnx, orn, nia, paba, nir, thi* (requirements for biotin, riboflavin, arginine, methionine, phenylalanine, sulphite, nitrite, ornithine, nitrite, *p*-aminobenzoic acid, ammonium and thiazole respectively); *gal* and *fac* determine inability to utilize galactose and acetate respectively, *fw* (fawn), *y* (yellow), *cha* (chartreuse), *w* (white) and *drk* (dark) are conidial colour markers, and *aba* is an aconidial mutant (Clutterbuck, 1969*a*). *Acr* determines resistance to acriflavine and *fpa* to fluorophenylalanine. Gene symbols are used in accordance with the proposals of Clutterbuck (1968, 1969*b*).

3. RESULTS

(i) *Isolation and properties of the variegated mutant*

The variegated mutant was found among survivors of ultraviolet irradiation of the *biA*1 strain (Glasgow collection 051) carried out by U. Sinha. Survival was approximately 10%.

The mutant had the superficial appearance of a '*bristle*' mutant (Clutterbuck, 1969*a*) and was given the provisional symbol *brl*-12. *Bristle* mutants are characterized by the presence of colourless conidiophores that elongate into stiff hyphae more than ten times the normal conidiophore length, but uniformly fail to develop a vesicle or any subsequent structures of the conidial apparatus (see Fig. 1). All *bristle* mutants so far found are allelic. Leaky *bristle* mutants have a characteristic non-variegated phenotype distinct from both the wild-type and non-leaky mutants (Fig. 1). In contrast to these, the variegated mutant *brl*-12 has bristles of the non-leaky morphology amongst which there are heads of almost wild-type appearance.

Wild-type heads were most frequent on somewhat dried-out medium, e.g. at the top of an agar slope, and further investigation showed that high glucose concentrations, or better KCl, stimulated their production. NaH_2PO_4 was more effective than Na_2HPO_4, but pH differences alone had little effect. Low temperature was also ineffective.

The mutant *brl*-12 is genetically stable and has shown no tendency to produce spontaneous variants. Subculture by means of hyphal tips, bristles or conidia gives, in all cases, colonies indistinguishable from the original mutant.

Variegation of *brl*-12 was unaffected by incorporation in a heterokaryon, and

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like other *bristle* mutants, expression of the mutant in heterokaryons was autonomous. In a diploid with master strain F (see below) *brl*-12 was recessive. A diploid between *biA*1; *brl*-12 and *ornB*7 *brlA*42, on the other hand, was mutant in phenotype, but showed the peculiar properties of both parents in that, like *brl*-12, it had a few normal conidial heads at 37 °C and, like *brlA*42, it was temperature-sensitive and conidiated well at 22 °C. It is concluded from this that *brl*-12 behaves as an allele of *brlA*42 (and therefore of all other *bristle* mutants) and is henceforth designated *brlA*12.

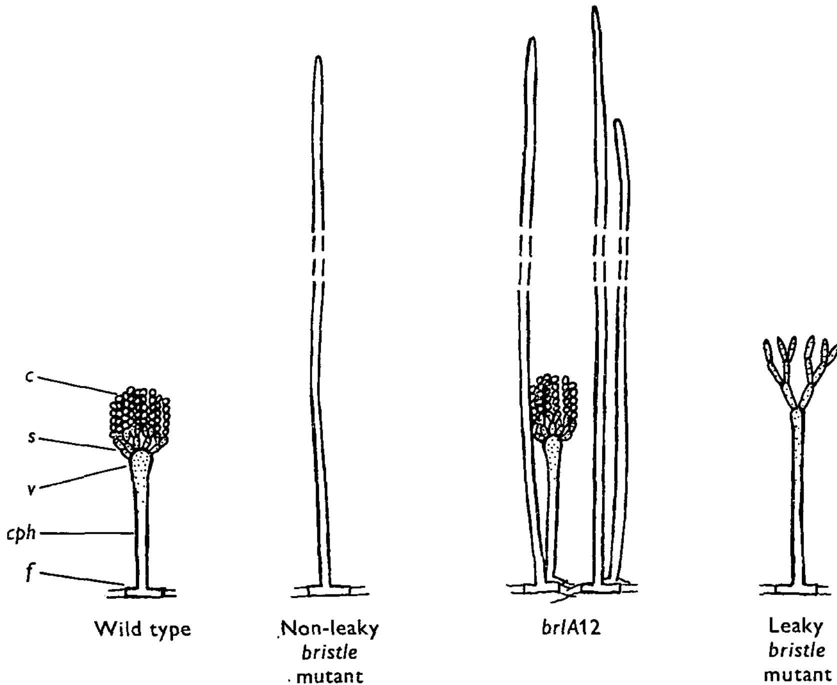

Fig. 1. Conidial heads of wild-type, leaky and non-leaky *bristle* mutants of *Aspergillus nidulans*. *c*, Conidium; *s*, sterigmata; *v*, vesicle; *cph* conidiophore; *f*, foot. The stippling indicates brown pigmentation.

Table 1. *Haploids obtained from the diploid* biA*1*;brlA*12*+*master strain F*

Markers	Linkage group of second marker	Haploids obtained			
		+ +	+ −	− +	− −
	First haploidization				
*brlA*12-*AcrA*	II	13	*0*	*1*	10
*brlA*12-*galA*	III	*0*	13	11	*0*
*brlA*12-*riboB*	VIII	*0*	13	11	*0*
	Second haploidization				
*brlA*12-*AcrA*	II	22	*5*	*9*	1
*brlA*12-*galA*	III	*0*	27	10	*0*
*brlA*12-*riboB*	VIII	*0*	27	10	*0*

Italicized numbers are recombinants.

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(ii) *Mapping by haploidization*

A diploid between *biA*1;*brlA*12 and the master strain MSF (McCully & Forbes, 1965) was obtained and haploidized on *p*-fluorophenylalanine. The relevant results are summarized in Table 1; markers not given here (with the exception of *biA*1 and *yA*2 which are both in linkage group I) all segregated independently. The *bristle* locus is in linkage group VIII and *brlA*12, as expected, shows complete linkage to *riboB* in the same linkage group; *brlA*12 also shows complete linkage with *galA* (linkage group III) which is indicative of the presence of a III–VIII translocation (Käfer, 1965). In the first haploidization there was also a suggestion that linkage group II was involved in the translocation, but in a second experiment using the same diploid the linkage group II marker segregated freely from the III–VIII complex.

(iii) *Meiotic analysis*

(*a*) *Slow-growing progeny*

In all crosses between *brlA*12 and *brl*+ strains, a conspicuous feature was the occurrence of slow-growing colonies among the progeny. These slow growers were present in frequencies up to one-third of the total progeny, but were not usually counted since only in very dilute platings could one be sure that they were not overgrown by other segregants. Moreover, they were unstable (see below), which made accurate assessment even more difficult. Similar slow-growing segregants from crosses involving a translocation have been interpreted by Bainbridge & Roper (1966) as a sign that the translocation is non-reciprocal. In such a translocation a piece of one chromosome becomes added to a non-homologous chromosome, so that in crosses with an untranslocated strain, segregation of the first, deficient chromosome with a normal homologue of the second will give deficient, inviable progeny, while the second, recipient chromosome, in combination with a normal homologue of the first, will give offspring carrying a duplication. If the *brlA*12 translocation is non-reciprocal this duplication class, which should comprise one-third of the viable offspring, may be the slow-growing segregants in the crosses described here. The slow growers were not included in the classification of progeny for mapping purposes, but evidence indicating that they are the expected duplication class is presented below (section iv).

(*b*) *Linkage of* brlA*12 to linkage group III markers*

Since the haploidization data indicate that the *biA*1;*brlA*12 mutant contains a III–VIII translocation, linkage was sought between *brlA*12 and the following linkage group III markers: *methH*2, *argB*2, *galA*1, *phenA*2, *sC*12 (see Fig. 2). In each cross, over 100 progeny were analysed but only in the case of *sC*12 was any linkage to *brlA*12 detected. The recombination fraction between these markers was $20 \pm 2{\cdot}8\,\%$. A *yA*2; *sC*12; *brlA*12 segregant from this cross was then crossed with *biA*1;*cnxH*4 (Glasgow strain 063), confirming this linkage and showing that *brlA*12 maps $9 \pm 3{\cdot}5$ units beyond *cnxH*, at the end of the linkage group (Fig. 2). The map distance between *sC* and *cnxH* is reduced in this cross as compared with translocation-free crosses (Fig. 2).

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(*c*) *The map position of* brlA*12 in linkage group VIII*

Fig. 2 gives the linkage maps obtained with *brlA*1 and *brlA*42 alongside that obtained with *brlA*12. It can be seen that although *brlA*12 maps in the same place as the other *bristle* mutants, the map distances to the left of *brlA*12 are reduced. The most pronounced reduction is between *brlA*12 and the next marker, *niaD*, but there are similar reductions in the distances between other markers to its left. Recombination in this region exhibits negative interference: among recombinants for the *brlA* to *fwA* region, recombination between *fwA* and *ornB* was 31 ± 5·4 % (i.e. similar to the normal figure), while among the progeny in general it was only 16 ± 2·6 %.

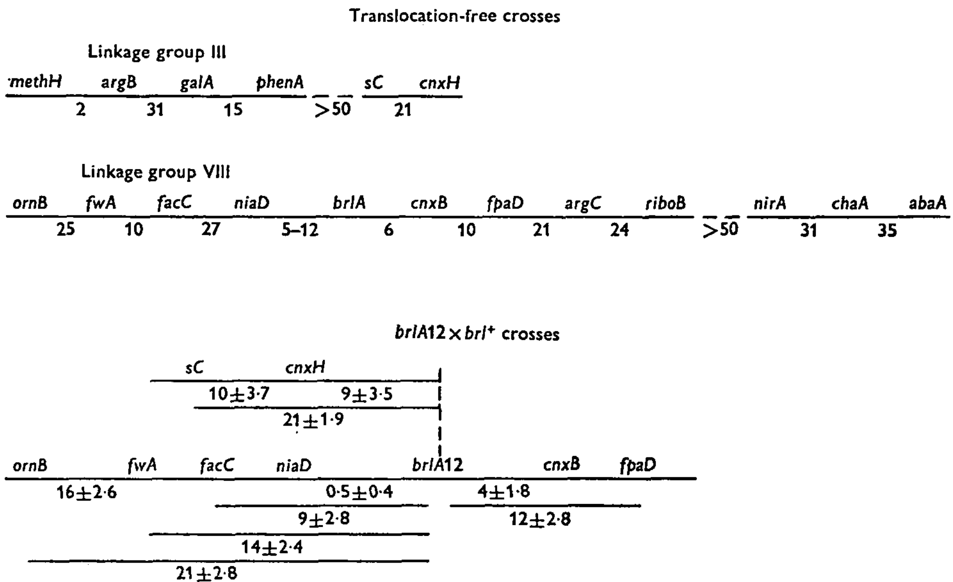

Fig. 2. Maps of linkage groups III and VIII in translocation-free crosses compared with linkages found in crosses heterozygous for *brlA*12. Linkages in the translocation-free crosses are as found in Dorn (1967) with modifications and additions by D. J. Cove (personal communication), Sinha (1969) and Clutterbuck (1969*a*).

To the right of *brlA*12 reduction in map distance to the two nearest markers (*cnxB* and *fpaD*) was not significant. Two crosses involving *argC* did give reduced linkages; however, these crosses also gave very unequal allele ratios and a considerable proportion of the progeny were of abnormal appearance so it seems likely that the *argC* strains contain an additional chromosome abnormality and these crosses will be ignored. In crosses involving markers towards the right-hand end of linkage group VIII map distances were normal and no linkage of *brlA*12 to *palB*, *chaA* or *abaA* was detected.

(*d*) *Crosses of* brlA*12 to other* bristle *mutants*

Two leaky *bristle* mutants with distinctive phenotypes were chosen for crossing to *brlA*12 so that hybrid cleistothecia could readily be distinguished and also

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because leaky mutants are likely to be due to point mutation rather than deletion. In both crosses the frequency of *brl*+ recombinants was calculated (Table 2) and in the second cross, with *brlA*42, the *brl*+ recombinants were also classified for the outside markers *niaD*15 and *fpaD*43 (Table 3).

Table 2. *Numbers of* brl+ *colonies obtained in crosses of* brlA*12 to* brlA*7 and* brlA*42*

Cleistothecium	*brl* Large colonies	*brl* Small colonies	*brl*+ Large colonies	*brl*+ Small colonies	*brl*+ frequency Large	*brl*+ frequency Small
Hybrid *brlA*12 × *brlA*7	18464	1693	18	2	9.7×10^{-4}	1.2×10^{-3}
Selfed *brlA*12	6090	0	0	0	—	—
Selfed *brlA*7	12028	0	0	0	—	—
Hybrid *brlA*12 × *brlA*42	6026	Not counted	12	0	2.0×10^{-3}	—

From Table 2 it can be seen that selfed cleistothecia of both the *brlA*12 and the *brlA*7 parents produced no *brl*+ progeny, which indicates that back mutation is unlikely to account for any significant proportion of the *brl*+ progeny from the hybrid cleistothecia. The recombination frequency between *brlA*12 and the two other *bristle* alleles is obtained by doubling the *brl*+ frequency from the hybrid cleistothecia in order to include the double mutant recombinant class. The results from the two crosses are not significantly different, so they can be pooled, giving a recombination frequency of 2.5×10^{-3}. This is not outside the limits for intragenic recombination in fungi (Fincham & Day, 1963) although it is relatively high. On the other hand, if *brlA*12 is a position effect mutant, the effective site of the mutant will be the translocation breakpoint. Recombination very close to this breakpoint is likely to be inhibited, so that map distances in this region may be underestimated.

In these crosses platings were made at high density (200–1000 colonies per dish) on medium containing sodium deoxycholate (Mackintosh & Pritchard, 1963) so that slow-growing colonies were likely to be overgrown in many cases. However, where slow growers were counted, *brl*+ recombinants were as frequent as among the normal segregants.

The analysis of outside markers shown in Table 3 clearly indicates that *brlA*12 maps to the left of *brlA*42, i.e. towards *niaD*. Interpretation of the *brlA*12 site as a breakpoint of a non-reciprocal translocation between linkage groups III and VIII means that either a piece of linkage group III is inserted into linkage group VIII to the left of the *bristle* locus, or that the piece of linkage group VIII including the *bristle* locus and regions distal to it, i.e. to its right, is translocated to linkage group III.

The existence of the postulated translocation in the vicinity of the *bristle* locus was tested with a cross homozygous for *brlA*12 and including the same outside markers as before. In such a cross, if a piece of linkage group III is inserted in this

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region, the map distances should be increased accordingly, while if part of linkage group VIII is translocated to linkage group III, markers in the two parts of VIII should no longer be linked at all. In the event, 87/180 (48 %) recombinants were obtained between *niaD* and *fpaD* instead of the untranslocated expectation of *c.* 22 %. This confirms the presence of a translocation in this region and supports particularly the second of the above hypotheses. Further support for this interpretation comes from the genetic analysis of the slow-growing segregants (section iv).

Table 3. *Outside marker classification of* brl+ *segregants from the cross* biA*1*; brlA*12* × yA*2*; niaD*15* brlA*42* fpaD*43*

Genotype	Number
niaD fpaD	2
niaD fpaD+	9
niaD+ *fpaD*	0*
niaD+ *fpaD*+	2

* One colony of this genotype had an appearance intermediate between *brlA*12 and *brl*+. It is considered most likely to be a partial revertant rather than a recombinant.

(iv) *Further analysis of slow-growing recombinants*

Bainbridge & Roper (1966) showed that slow-growing colonies among the progeny of crosses heterozygous for a non-reciprocal translocation resulted from the duplication of the translocated fragment. The reciprocal product, deficient for the same region, was inviable. The extent of the duplication could be tested in these segregants since some were heterozygous for markers on this part of the chromosome. Spontaneous loss of one or other copy of the region (further studied by Nga & Roper, 1968, 1969) gave rise to sectors with increased growth rate and more normal morphology which could be tested for the segregation of markers heterozygous in the original slow-growing segregant.

Similar faster growing sectors arose from the slow-growing segregants obtained in crosses between *brlA*12 and *brl*+ strains. Classification of these demonstrated that the slow-growing colonies were heterozygous for the following loci: *cnxB*, *fpaD*, *argC*, *riboB*, *nirA*, *chaA*, and *abaA*. In each of these cases slow-growing colonies were found with the phenotype determined by the dominant allele of the locus concerned, but whose sectors included some with the phenotype of the recessive allele. No such heterozygosity was found for markers to the left of the *bristle* locus or for markers in linkage group III.

It is therefore concluded that in *brlA*12 strains the whole of the mapped length of linkage group VIII distal to the breakpoint at the left of the *bristle* locus is translocated to linkage group III. It is the segregation of this translocation compound of III + VIII with the normal homologue of VIII in a cross which gives rise to slow-growing colonies with the translocated region in duplicate.

The majority of sectors from the slow-growing colonies were more or less wild-type in their conidiation, but a minority had *bristle* morphology. This ability to segregate for the *bristle* phenotype indicates that the slow-growers are heterozygous

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for the *bristle* locus itself and therefore that this locus is in the translocated section. The *bristle* sectors obtained in this way differed from the original *brlA*12 in that they failed to conidiate even on medium containing 1 M-NaH_2PO_4; however, recombinants of one of these derivatives with a suppressor of *brlA*12 (see below) could be induced to conidiate.

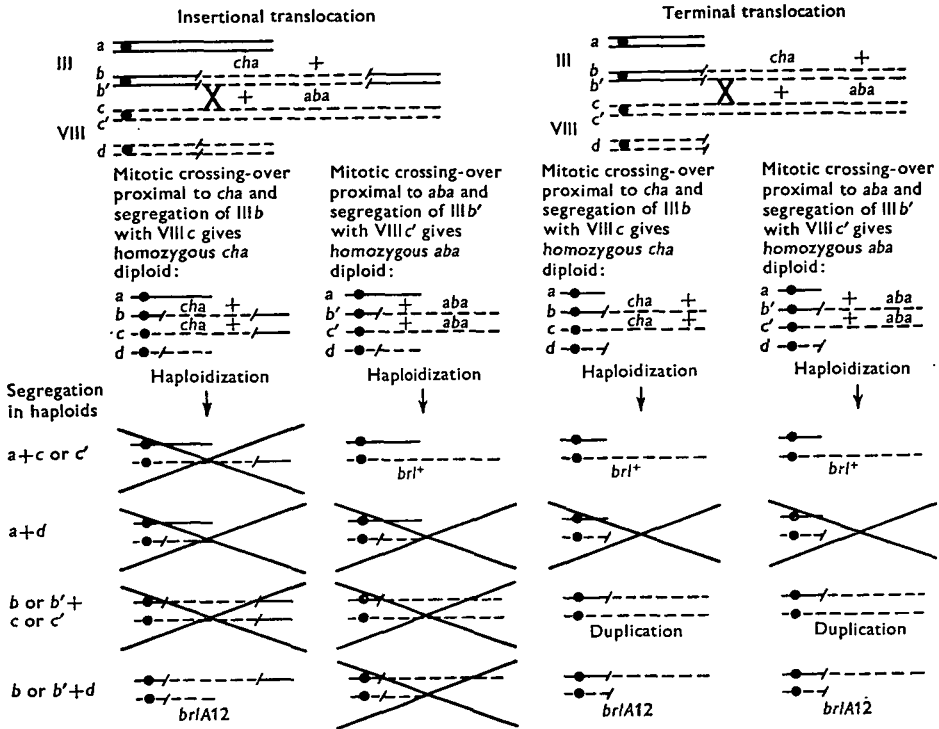

Fig. 3. Mitotic recombination and haploidization in insertional and terminal translocation models of a diploid heterozygous for *brlA*12. –●——— = linkage group III, ·●----- = linkage group VIII, —/— = breakpoint. Haploids which lack the full chromosome complement are assumed lethal and are scored out.

(v) *Determination of the nature of the translocation by mitotic recombination*

A non-reciprocal translocation is normally expected to consist of the insertion of a segment of one chromosome into another (Muller, 1940; Swanson, 1957). However, such an interstitial translocation should result in disturbances in mapping at both ends of the inserted fragment, and in fact, the map of this should be in the form of a loop. Since no disturbances in the mapping of the distal end of linkage group VIII were found, this may mean that the linkage group extends well beyond the portion at present mapped, or that the translocation is terminal rather than insertional, i.e. in *brlA*12 strains the distal half of linkage group VIII is attached to the end of linkage group III.

This possibility has been tested by mitotic recombination. Recombination during mitosis is a rare event and double crossovers are therefore unusual (Pontecorvo & Käfer, 1958). Fig. 3 shows that single crossovers within an insertional

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translocation should lead to inviable duplication-deficient progeny, whereas all the products of crossing over in a terminal translocation should be viable.

A diploid was synthesized which would be heterozygous for *brlA*12 and also for *chaA*3 and *abaA*14 marking the two homologues of the distal part of linkage group III. Visual selection was made for mitotic recombinants homozygous for each of the latter two markers and the resulting diploid recombinants were then haploidized to see if haploids containing each type of recombinant chromosome would be viable (see Fig. 3). The results, given in Table 4, show that normal

Table 4. *Haploids obtained from mitotic recombinants of a diploid heterozygous for* brlA*12*

Diploid	Diploid constitution	Haploids	
		*brlA*12	*brl*⁺
Parent diploid (control)	Heterozygous $\frac{chaA\ +}{+\ abaA}$	7	9
Mitotic recombinant 1	Homozygous *abaA*	13	8
Mitotic recombinant 2	Homozygous *chaA*	5	5
Mitotic recombinant 3	Homozygous *chaA*	7	4
Mitotic recombinant 4	Homozygous *chaA*	4	6
Mitotic recombinant 5	Homozygous *chaA*	4	2
Mitotic recombinant 6	Homozygous *chaA*	3	7
Mitotic recombinant 7	Homozygous *chaA*	7	4

haploids of both *brlA*12 and brl^+ genotypes were readily obtained from all recombinant diploids. Homozygous *cha* diploids were more conspicuous than homozygous *aba* recombinants, and the latter were also difficult to purify, so that only one has been tested. The results, however, show that neither recombinant chromosome gives inviable haploids and this is compatible only with the simple translocation model in which the distal portion of linkage group VIII is attached to the end of linkage group III. The only alternative (but improbable) explanation would be that all the mitotic recombinants recovered were in fact the result of double crossing over.

(vi) *Suppressors of* brlA*12*

An attempt was made to obtain back mutants resulting from the retranslocation of the chromosome VIII fragment to a location which did not result in inactivation of the *bristle* locus.

Twenty-eight mutants conidiating better than the parent strain were picked up after ultraviolet irradiation of *biA*1;*brlA*12. These mutants were classified into three classes according to the degree of reversion: class 'A' differed only slightly from wild type, class 'B' were intermediate and class 'C' had only slightly more conidia than *brlA*12. Seven revertants were analysed by haploidization of diploids with master-strain E; all retained the III–VIII translocation and none had any additional translocation. Revertants 2 and 8 (both class B) failed to give any haploids with the original *brlA*12 phenotype, indicating that the reversion is inseparable from the III–VIII complex and may be due to an independent

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suppressor in one of these linkage groups or to an event in the region determining the *brlA*12 mutant itself. Revertant 7 (class C) proved to be due to a suppressor in linkage group VII and four revertants of class A all contained a suppressor in linkage group II. Outcrossing showed that the C 7 suppressor had no phenotypic effect on its own, but the linkage group II suppressors all had conidia with darker than normal pigmentation and a tendency to lose an outer layer. Both these features are characteristic of 'dark' (*drk*) mutants (Clutterbuck, 1969*a*), the only mapped member of which, however (*drkA*1), is in linkage group VII.

Three previously isolated dark mutants; *drkA*1, *drk*-2 and *drk*-3, were therefore crossed to a *brlA*12 strain, but there was no evidence among the *brlA*12 progeny of any distinction between *drk* and drk^+ segregants other than colour of conidia.

One of the A group suppressors was designated *drkB*5 and this mutant was further mapped 13 units to the right of *riboE*, but freely recombining with the next nearest marker; *thiA* (see Dorn, 1967). This suppressor was also crossed to a leaky *bristle* mutant: *brlA*9, to see if it had any effect on other *bristle* mutants. *brlA*9 shows a response to high salt concentrations (Clutterbuck, 1969*a*) so that it could also be used to test whether the action of *drkB*5 mimics the action of high salt in respects other than the suppression of *brlA*12. The cross progeny, however, included only one type of *brlA*9 segregant, comprising half the total progeny, indicating that no effect of *drkB*5 was distinguishable in this mutant.

A cross between *drkB*5 and a *bristle* sector from a slow-growing duplication colony has already been mentioned in section (iv). This cross gave two types of *bristle* colonies: roughly half were like the parent *bristle* sector, bearing no conidia even on 1 M-NaH_2PO_4 medium, while the other half had brown forked bristles and did conidiate on 1 M-NaH_2PO_4. Brown-coloured forked bristles are characteristic of leaky *bristle* mutants (Clutterbuck, 1969*a*) and are assumed to indicate partial activity of the *bristle* locus. This situation contrasts, therefore, with the behaviour of the original *bristle* mutant, which appeared to switch in individual conidiophores between the fully mutant phenotype and more or less fully wild type.

It was noticed during the process of mapping *drkB*5 that colonies of *drkB*5; *brlA*12 adjacent to morphologically wild-type colonies produced a line of bristles along the junction. This shows that *drkB*5 is recessive and that its wild-type allele can override it by action across a colony boundary. This observation afforded a test for other suppressors: all eleven revertants of the highly suppressed A class were streaked alongside a morphologically wild-type strain, and all showed the same phenomenon. The less highly suppressed revertants could not be tested in this way since they produced too many bristles on their own; the A class revertants can, however, all be considered to carry recessive suppressors.

4. DISCUSSION

The *brlA*12 mutant described here was first recognized as a possible instance of position effect variegation on account of its inconstant phenotype and, on the basis of this, a translocation close to the *bristle* locus was predicted. The finding of such

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a translocation within the map interval *niaD-brlA* is therefore seen as strong evidence in favour of this hypothesis.

Variegated position effects have been studied most intensively in various species of *Drosophila* (Baker, 1968) and have also been found in *Oenothera* (Catcheside, 1947) and, in a specialized form, in the mouse (Russell & Bangham, 1961). A variegated position effect has also been postulated by Ball (1967) as an explanation for a genetic instability in *Aspergillus nidulans*. The apparent absence of more clear-cut examples in fungi may be due to the relatively slight attention previously given to visible mutants at loci with localized action. In addition it is likely that many position effects would be lethal in a haploid organism: the existence of a haploid gametophyte stage has been used to explain the relative rarity of any phenotypic effects of homozygotes for translocations in maize as opposed to *Drosophila* (Burnham, 1956). Käfer (1965) has analysed eight translocations in *Aspergillus nidulans*, none of which has a detectable phenotypic effect, nor have 45 translocations analysed by Nga (1968). It is worth noting that since position effects involving extensive chromosome inactivation are likely to be lethal in a haploid organism, it is not surprising that the map position of *brlA*12 (postulated to be the translocation breakpoint) is very close to the *bristle* locus.

Variegated position effects in *Drosophila* are characteristically due to translocations between eu- and heterochromatin, but there is at present little chance of distinguishing these components in the minute chromosomes of *Aspergillus nidulans* (Elliott, 1960; Robinow & Caten, 1969). Little is in fact known about the composition of fungal chromosomes—for instance, the presence of histones, which might well be of importance in position effects, is debatable (e.g. Dwivedi, Dutta & Bloch, 1969). On the other hand, *Aspergillus* is well suited to a study of environmental effects on variegation and it is to be hoped that a further study of these, in conjunction with the suppressor mutants, may yield some evidence on the nature of the variegation process. At present it is only evident that high salt concentrations at low pH increase wild-type activity in the *brlA*12 mutant, but this is a reaction similar to that found in many leaky mutants in which the osmotic effect almost certainly occurs at the protein level (Lacy, Mellen & Pomerance, 1968), whereas in *brlA*12 an effect on the chromosome itself is most likely.

The pattern of variegation in *brlA*12 suggests that the gene inactivation event occurs at, or before the start of conidiophore formation, since whole conidiophores tend to take either the wild-type or the mutant form. The *bristle* locus activity is required for the formation of both the vesicle and the sterigmata (Clutterbuck, 1969*a*), which means that in heads of wild-type morphology, at least the majority of sterigmata must receive nuclei in which the *bristle* locus is active. A clone of nuclei must therefore retain a state of activation determined at the start of conidiophore formation. There is no evidence here, however, of a long interval between the determinative event and the time when gene activity is apparent as there is in variegated eye-colour mutants of *Drosophila* (Baker, 1968), nor is there evidence in this system of clones of activated or inactivated nuclei of the dimensions of a whole colony as postulated by Ball (1967).

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The action of *brlA*12 suppressors on the original *brlA*12 mutant appears to increase the proportion of wild-type to mutant conidiophores rather than, for instance, increasing the level of *bristle* locus activity to an intermediate level in nuclei previously largely inactivated. On the other hand, the leaky *bristle* phenotype of segregants from the cross of *drkB*5 to the otherwise aconidial sector from a duplication strain does indicate intermediate levels of *bristle* locus activity. These observations are not readily interpreted without further investigation, but they may depend on suppressor action both at an early stage controlling the activation or inactivation of clones of nuclei, and secondly at a later stage when the level of activity in each nucleus is determined. A similar hypothesis has been put forward by Spofford (1969) for the action of a suppressor of position effect variegation in *Drosophila*.

The *brlA*12 mutant provides a unique proof that variegation is due to instability in gene expression rather than to a mutable gene. It is possible to propagate the fungus from structures derived from both states of variegation: conidia and bristles. Since both structures give rise to colonies identical to the parent type, it is apparent that mutation is not involved. This agrees with Baker's conclusion (1968) derived from features of a wide variety of position effects, and does not support Roper & Nga's (1969) suggestion that variegation may be due to chromosomal deletions.

An unusual feature of the translocation described in this paper is that it appears to be a 'simple translocation', i.e. chromosome VIII is broken at one point only, and the fragment is attached to the unbroken end of chromosome III. Nga & Roper (1968) have similarly concluded that a I–II translocation of *A. nidulans* is a simple translocation, although their data was from meiotic recombination only and could be explained if the linkage groups were much longer than was apparent so that crossovers were common between the last marker and the breakpoint. It may be, therefore, that in *Aspergillus*, unlike other organisms studied (Swanson, 1957), terminal attachment to unbroken ends is possible, or that genetically undetectable damage to the end of a chromosome may provide a 'sticky end' for attachment of another fragment, i.e. that the chromosome end is either genetically inert or redundant. The latter hypothesis would fit with the idea that the end of linkage group III is heterochromatic and it is the association of this heterochromatin with the euchromatic piece of linkage group VIII that causes the variegation at the *bristle* locus.

In crosses heterozygous for the translocation, reduction of linkage distances has been found in both linkage groups III and VIII in the regions between the centromere and the breakpoint, but not in the translocated arm of VIII. This type of result has been explained by Burnham (1956) as due to the predominance of alternate, rather than adjacent segregation of homologues from the translocation complex at meiosis, so that crossover products go preferentially into the cells with unbalanced chromosome constitutions.

An alternative explanation is that meiotic synapsis starts at the ends of the chromosomes and works towards the centromere as in *Coprinus* (Lu & Raju,

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1970). This would be likely to cause failure of pairing proximal to a breakpoint and hence a reduction in recombination.

I am grateful to Mrs June Baxendale for doing most of the hard work.

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