ORIGINS OF TRANSLOCATIONS IN *ASPERGILLUS NIDULANS*[1]

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IT was realized early in the course of the genetic analysis of the ascomycete *Aspergillus nidulans* that several of the strains with X-ray induced mutants contain chromosomal aberrations (e.g. two cases mentioned by PONTECORVO, ROPER, HEMMONS, MACDONALD and BUFTON 1953). At that time all evidence came from meiotic analysis. In crosses heterozygous for chromosomal aberrations, crossing over appears to be reduced since certain unbalanced crossover types show low viability. In fungi, this results in an unusually high frequency of aborted and abnormal ascospores, corresponding to defective pollen in higher plants that show "semisterility" as a result of chromosome aberrations. Ascopore patterns can be used to detect visually the presence of aberrations in Neurospora, either in intact asci (McCLINTOCK 1945), or in random spores (PERKINS, GLASSEY and BLOOM 1962). The nonlinear ascus of Aspergillus is too small to make use of this method routinely even though similar patterns have been observed in asci from a cross which is now known to have been heterozygous for at least three aberrations (ELLIOTT 1960). On the other hand, the discovery of mitotic recombination in Aspergillus (ROPER 1952; PONTECORVO and ROPER 1953) has provided new genetic methods not only for the mapping of markers but also for the detection of aberrations, especially translocations (PONTECORVO and KÄFER 1958; KÄFER 1958).

Even though most X-ray mutants had been excluded from the general Glasgow stocks, several aberrations have been encountered in the course of mitotic mapping of new markers. The first of these resulted in mitotic linkage between markers of linkage groups I and VII. All available evidence supports the hypothesis that the unusual linkage is due to a translocation between the corresponding chromosomes (KÄFER 1962 and unpublished). The origin of T(I;VII) was found in a strain treated with ultraviolet light (UV) and isolated because it contained a mutant conferring a nutritional requirement. It seems likely, therefore, that in this case a translocation has been induced by UV. The spread of T(I;VII) was determined and all strains containing this translocation were eliminated from stocks. When, several years ago, it was realized that a few other translocations have spread widely in the pedigree of the commonly used stock strains, a similar project was started to trace these. The results are published at this point, because the pedigree of the translocations in the more frequently used strains has now been completely analyzed and information has been obtained on a large number of Aspergillus strains available at the Fungal Genetics Stock Center at Dartmouth College, Hanover, New Hampshire (BARRATT, JOHNSON and OGATA 1965).

The aim of the present investigation was, therefore, twofold: (1) to ascertain

whether translocations are often induced by current methods of UV-induction; (2) to trace the translocations encountered in stock strains to their origin in order to separate out translocation-free strains for future use.

MATERIALS AND METHODS

Strains: All strains studied in this investigation originated from the same haploid wild-type strain of *Aspergillus nidulans* which is used by PONTECORVO, ROPER and co-workers at Glasgow and Sheffield Universities (for details see PONTECORVO *et al.* 1953). The mutants present in the strains analyzed and their locations in the eight linkage groups are given in Figure 1, (12 of these were induced by X rays, 23 by UV, and one by nitrogen mustard; the rest arose spontaneously). The origin of most of these mutants is indicated in Figures 2, 3, 5, 6 and 7.

Two new mutants were recently identified; $p2$ = pale, a spontaneous color mutant observed in the original *ad14 bi1* strain, is allelic to *p1* found in the original *an1 bi1* strain (G. A. VAN ARKEL, personal communication). The morphological mutant *ve* = velvet (linkage group VIII) is present in almost all stock strains; it seems to have been subconsciously selected for because it increases conidiation and helps in the recognition of colour and ploidy. The wild type (*ve+*) conidiates less, but seems to produce fruiting bodies more readily. All mutants induced in *bi1* are *ve*, while the four mutants induced in the wild type or the original *y* (yellow) strain are *ve+*. Therefore, the mutation to *ve* seems to have occurred at the same time as *bi1*, which was induced by X rays in the original wild-type strain (Figure 3). To simplify strain designations,
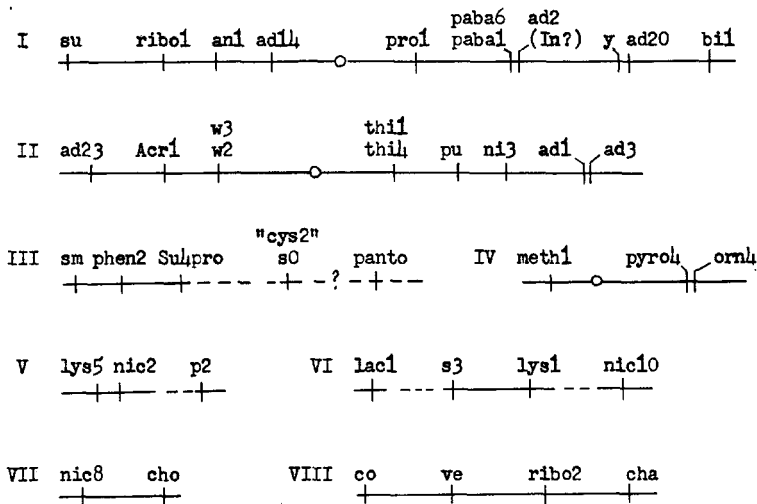

FIGURE 1.—Linkage groups I-VIII showing the mutants used. Conidial color mutants: y = yellow; $w2$ and $w3$ (allelic) = white; $p2$ = pale; *cha* = chartreuse. Morphological markers: sm = small colonies, co = compact colonies; *ve* = velvet-like conidiation. Resistance marker: $Acr1$ = increased resistance to acriflavine, semidominant. Suppressors: *su* (=$su1ad20$) = recessive suppressor of $ad20$; $Su4pro$ = dominant suppressor of *pro*. Mutant determining requirements: $ad20$ (allele of $ad8$), $ad14$, $ad2$ (allele of $ad9$), $ad23$, $ad3$ and $ad1$ = adenine; $an1$ = aneurin (=thiamine); $bi1$ = biotin; *cho* = choline; $lys1$ and $lys5$ = lysine; $meth1$ = methionine; $nic2$ and $nic10$ = nicotinic acid; $nic8$ = nicotinic acid or tryptophan; $ni3$ = nitrite; $orn4$ = ornithine; $paba1$ and $paba6$ (allelic) = p-aminobenzoic acid; *panto* = pantothenic acid; $phen2$ = phenylalanine; $pro1$ = proline; *pu* = putrescine; $pyro4$ = pyridoxine; $ribo1$ and $ribo2$ = riboflavin; $s0$ (=$s12$), "$cys2$" (allelic to $s0$) and $s3$ = sulphite; $thi1$ and $thi4$ (allelic) = thiazole. Mutant unable to use lactose as carbon source = $lac1$.

the unusual procedure of indicating the presence of (*ve+*) rather than *ve* has been adopted. The mutant *ad2* is allelic to *ad9* (PRITCHARD 1955); it appears to be associated with an intra-chromo-somal aberration, possibly an inversion, since it reduces meiotic and mitotic crossing over in the *pabal-γ* interval (PONTECORVO and ROPER 1953). For further information about all other mutants [except *cha* (KÄFER 1961) and *lac1* (ROBERTS 1963)] see PONTECORVO *et al.* (1953) and KÄFER (1958).

All multiply marked strains are descendants of crosses between the various mutant strains. The symbols and numbers given for the various crosses are those in use at McGill University, Montreal; capital letters indicate crosses carried out by PONTECORVO, ROPER and co-workers at the University of Glasgow (some published by PONTECORVO *et al.*, 1953).

Standard strains: Since the original wild-type strain cannot be used in forcing heterokaryons from which diploids might be selected, two eighth-generation backcross strains were used as the main reference strains (Fungal Genetics Stock Center Nos. 17 and 18, BARRATT *et al.*, 1965, Figure 1). It was assumed that these strains would contain the standard chromosome comple-ment of the original wild type.

Translocations-free tester strains: To obtain translocation-free strains with markers in all those linkage groups that are unmarked in the strains to be tested, a few suitably marked strains were checked against the "standard" backcross strains and intercrossed (see BARRATT *et al.*, 1965, Figure 2) to produce the majority of the tester strains (all available at the Fungal Genetics Stock Center [see Table 5 of BARRATT *et al.*, 1965] and referred to by the FGSC No. in Table 2).

Media: The minimal and complete media used here are variants of the standard media of PONTECORVO *et al.* (1953) devised to give equivalent phenotypes and growth rates for all mutants (KÄFER 1958); of the mutants used here only *panto* is inhibited on this complete medium, and it is grown on yeast extract medium instead (for details of the media see BARRATT *et al.*, 1965).

Techniques: Standard methods were used with respect to incubation temperature, plating, and meiotic analysis (PONTECORVO *et al.*, 1953). For mitotic analysis, diploids were selected from forced heterokaryons (ROPER 1952) and haploids were selected by three techniques: (1) in about 25% of the cases, they were obtained as conidial heads of mutant color within diploid colonies grown on complete medium (PONTECORVO and ROPER 1953); (2) in about 60% of the cases, as "suppressed" haploid sectors on a supplemented minimal medium without adenine, making use of the recessive suppressor *su1ad20* in diploids homozygous for *ad20* or in yellow, *γ ad20/γ ad20*, segregants from diploids originally heterozygous for *γ ad20* (PONTECORVO and KÄFER 1958; KÄFER 1958); (3) in the remaining cases, mitotic haploids were isolated as coloured sectors from colonies grown on complete medium supplemented with p-fluorophenylalanine (MORPURGO 1961; LHOAS 1961). Haploidy was determined visually by the appearance of segregants on standard complete medium, and was usually confirmed by the absence of any heterozygous markers.

EXPERIMENTS AND RESULTS

In the attempt to trace all the translocations encountered in the more important stock strains of *A. nidulans* over 150 strains have been combined with suitably marked tester strains to form diploids heterozygous for markers on all or most of the eight linkage groups. Results from 106 diploids are presented here (Figures 2 to 7 show the genotype and origin of the strains tested in these diploids). A number of haploids, most likely resulting from mitotic nondisjunction, were isolated from each test diploid and scored. Table 1 gives, as an example, the com-plete results from two test diploids containing the same tested strain. The results from test diploids containing the strains in Figure 2 are summarized in Table 2. Similar information on strains of Figures 3 to 7 is being published elsewhere (Tables 3 to 7 of KÄFER 1965; mimeographed copies are available on request). Normally such haploids show complete linkage of markers located on the

TABLE 1

Haploid and near-haploid segregants from two diploids heterozygous for
bi; w3 pu T(I;III;VIII), T(VI;VII)

Parental types for I-III-VIII

Linkage groups

I	III	VIII	II	VI	VII	IV	V	No.	Total
+*	+	+	-*	+	+	+	+	2	
						+	-	8	
						-	+	11	
						-	-	13	
			-	-		+	+	8	
						+	-	11	
						-	+	3	
						-	-	16	72
			+	+	+	+	+	5	
						+	-	5	
			-	-		+	+	1	
						+	-	2	
						-	-	3	16
-	-	-	-	-	-	+	-	2	
						-	-	1	3
								91	

Cross-overs for I-(III+VIII), or III-(I+VIII)

Linkage groups

I	III	VIII	II	VI	VII	IV	V	No.	Total
+	-	-	-	+	+	-	+	3	
				+	+	+	-	-	2	
-	+	+	-	+	+	+	-	1	
						-	-	1	
+	-	(?)	-	-	-	+	+	3	
						+	-	1	
						-	-	4	
+	-	+	+	●	+	+	-	1	
						-	-	1	17
Stable aneuploids									
+	-/+	-/+	-	+	+	+	+	1	
						-	+	3	
						+	-	1	
+	-	-/+	-	+	+	+	-	1	
	+	+	+	+	+	+	+	1	7
								24	

+ = Alleles of tester strain   ● = Most frequently selected alleles
- = Alleles of translocation strain   ? = Allele unknown because of epistasis of markers.
-/+ = Disomic linkage group (likely)

same chromosome and free recombination of markers on different chromosomes (PONTECORVO, TARR-GLOOR and FORBES 1954). However, haploids from certain diploids showed complete linkage of all markers located in two different chromosomes. This is assumed to indicate the presence of a translocation, since in translocation heterozygotes recombinant types become unbalanced, duplicated or deficient for translocated segments, and are not isolated by the methods used here. In the haploid strain, the translocations analyzed here do not affect phenotype or viability. It is, therefore, unlikely that sizable duplications (or deficiencies) are associated with these chromosomal interchanges (as found by PRITCHARD 1960). The analysis was carried out stepwise from the strain in which a translocation was first found, both back through one generation at a time to the strain of origin, and forward to all descendants from crosses segregating for the translocation. In this way it was possible to distinguish, among the stock strains, those
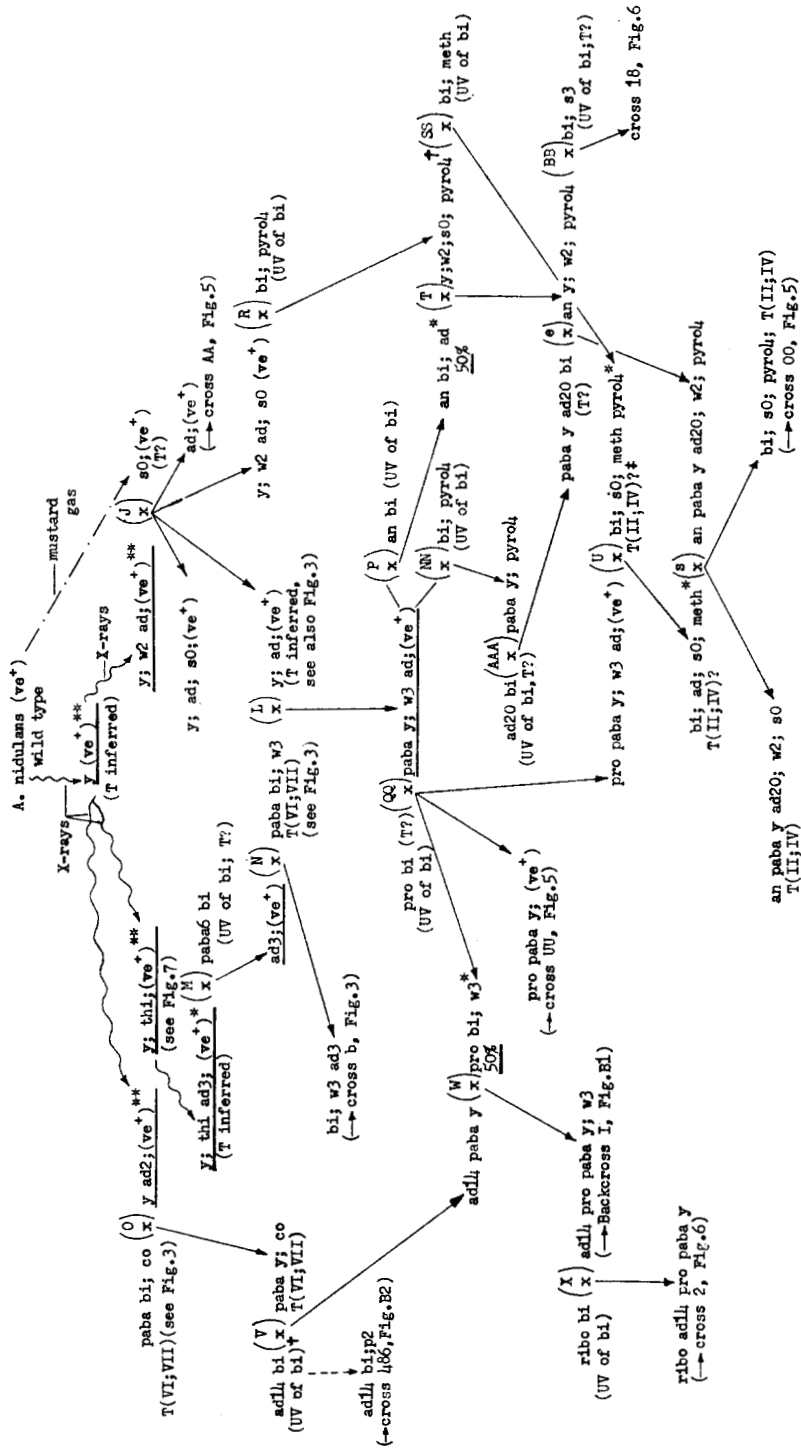
FIGURE 2.—Origin and pedigree of T(III;VIII): strains with T(III;VIII) underlined, other translocations as shown. All strains were tested except where indicated otherwise: * strain lost, untested; † strain not tested but with indirect evidence that translocation-free; "T?" other untested strains. ‡ Details in text, presence of additional translocation likely; ** details in text, presumably with translocation complex T(III;IV;VIII). For gene symbols, see Figure 1 (allele number 1 omitted in all cases). Figures 1 and 2 of BARRATT et al. (1965) are referred to as Fig, B1 and Fig, B2.

containing translocations from all others which are shown to be without trans-locations or are descendants of translocation-free strains.

Statistical analysis: x^2-tests were used to check for significant deviations from random assortment of markers in different linkage groups (28 pairwise combinations when all groups are marked in the test diploid and only one marker at a

TABLE 2

Test for deviations from random segregation of markers of different linkage groups in mitotic haploids selected from heterozygous test diploids containing strains of Figure 2 (allele number 1 omitted in all cases)

TESTED STRAIN		Tester strain FGSC no.	Unmarked linkage groups in test diploid	No. of selected haploids	Complete linkage; trans-locations (P << .01)	No. of x^2 - tests not influenced by viability or selection		
Origin	Genotype					P: > .05	.05-.01	< .01
Induced mutants								
X-ray of y (ve⁺)	y; ad2;(ve⁺)	70	-	20	T(III;IV;VIII)	23	2	-
	y; thi;(ve⁺)	105	-	29	T(III;IV;VIII)	20	-	-
	y; w2 ad;(ve⁺)	70	-	19	T(III;IV;VIII)**	21	2	1
UV of bi	ad14 bi; p2	159	-	51	no T	23	3	-
	ribo bi	155	-	25	no T	28	-	-
	bi; pyro4	171	-	50	no T	26	2	-
	bi; meth	171	-	·80	no T	26	2	-
Recombinants								
y†	ad;(ve⁺)	155	-	61	no T	21	2	-
	y; w2 ad; so;(ve⁺)	70	-	50	no T	22	3	-
	y; ad; so;(ve⁺)	44	-	49	no T	25	-	-
L	paba y; w3 ad;(ve⁺)	70	-	26	T(III;VIII)	23	1	-
M	ad3;(ve⁺)	79	-	48	T(III;VIII)	22	-	-
N	bi; w3 ad3	79	-	27	no T	26	1	-
QQ	pro paba y; w3 ad;(ve⁺)	44	-	58	no T ‡	25	3	-
	pro paba y;(ve⁺)	44	-	34	no T	26	2	-
T	an y; w2; pyro4	172	VI	64	no T	18	2	-
V	ad14 paba y	169	III, V	20	no T	13	-	2
W	ad14 pro paba y; w3	44	-	8	no T	26	2	-
X	ribo ad14 pro paba y	44	-	20	no T	26	1	1
NN	paba y; pyro4	172	II, IV	61	no T	15	-	-
e	an paba y ad20; w2; pyro4 {	179	VIII	60	no T	19	-	-
		70	IV	64		16	1	-
s	bi; so; pyro4 {	175	-	31	T(II;IV)‡	24	1	
		167	-	26		26	-	-
	an paba y ad20; w2; so	70	-	22	T(II;IV)‡	21	4	-
						561	34	4

† Cross symbol in use at McGill University, Montreal ** T(III;IV;VIII) likely, details in text
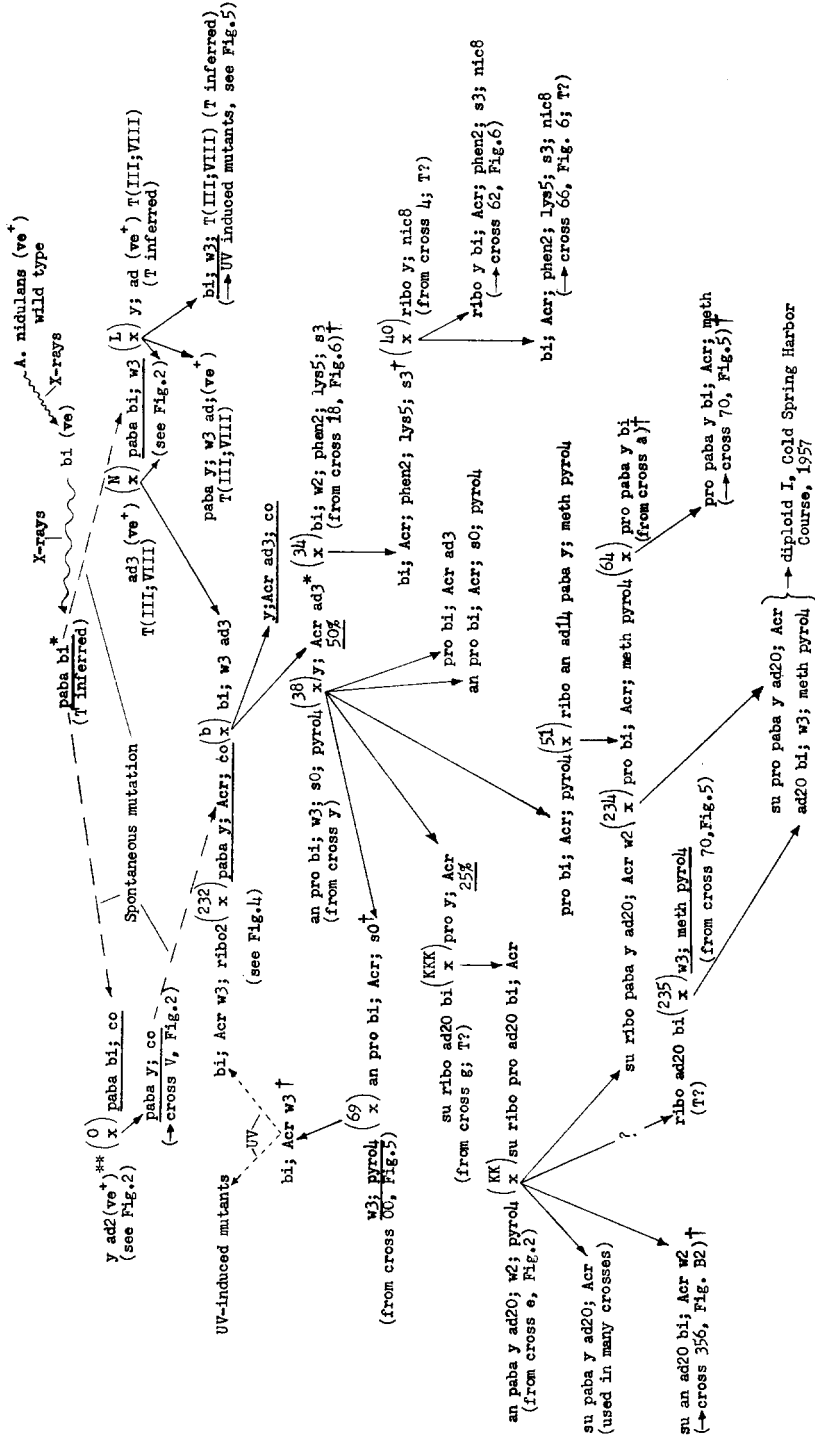
‡ Results discussed in text

FIGURE 3.—Origin and pedigree of T(VI;VII) in early crosses: strains with T(VI;VII) underlined, other translocations as indicated. For symbols see legend of Figure 2.
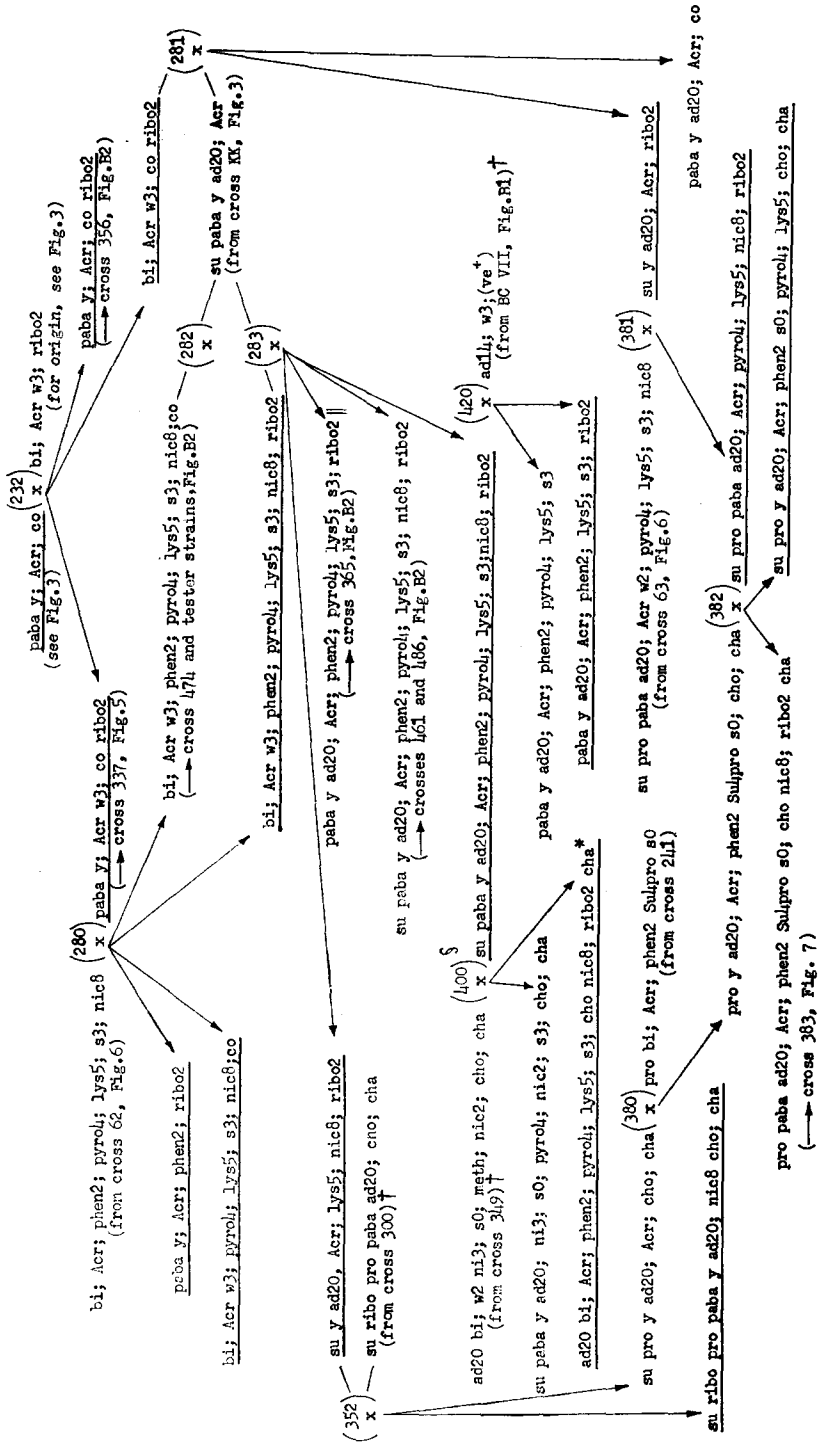
paba y; Acr; co (232) x bi; Acr w3; ribo2
(for origin, see Fig.3)

paba y; Acr; co ribo2
(see Fig.3)

(281) x   paba y ad20; Acr; co

bi; Acr w3; co ribo2

paba y; Acr; co ribo2
(⟶ cross 356, Fig.B2)

bi; Acr w3; co ribo2

su paba y ad20; Acr
(from cross KK, Fig.3)

su y ad20; Acr; ribo2

paba y ad20; Acr; co

(282) x

(283) x

ad44; w3;(ve⁺)
(from BC VII, Fig.R1)

bi; Acr; phen2; pyrol4; lys5; s3; nic8
(from cross 62, Fig.6)

bi; Acr w3; phen2; pyrol4; lys5; s3; nic8;co
(⟶ cross 474 and tester strains,Fig.B2)

paba y; Acr w3; Acr w3; ribo2

paba y; Acr w3; co ribo2
(⟶ cross 337, Fig.5)

bi; Acr w3; phen2; pyrol4; lys5; s3; nic8; ribo2

paba y ad20; Acr; phen2; pyrol4; lys5; s3; ribo2 ‖
(⟶ cross 365, Fig.B2)

paba y ad20; Acr; phen2; pyrol4; lys5; s3; nic8, ribo2

paba y; Acr; phen2; ribo2

paba y ad20; Acr; phen2; pyrol4; lys5; s3;nic8; ribo2
(⟶ crosses 461 and 486, Fig.B2)

(420) x

paba y ad20; Acr; phen2; pyrol4; lys5; s3

paba y ad20; Acr; phen2; pyrol4; lys5; s3; ribo2

bi; Acr w3; pyrol4; lys5; s3; nic8;co

su y ad20, Acr; lys5; nic8; ribo2

su ribo pro paba ad20; cno; cha
(from cross 300)

paba y ad20; Acr; phen2; pyrol4; lys5; s3; nic8
(from cross 63, Fig.6)

su pro paba ad20; Acr w2; pyrol4; lys5; s3; nic8

(381) x

su y ad20; Acr; pyrol4; lys5; nic8; ribo2

ad20 bi; w2 ni3; s0; meth; nic2; cho; cha
(from cross 349)

(400) x

su paba y ad20; Acr; phen2; pyrol4; lys5; s3;nic8; ribo2

su paba y ad20; ni3; s0; pyrol4; nic2; s3; cho; cha

ad20 bi; Acr; phen2; pyrol4; lys5; s3; cho nic8; ribo2 cha*

(382) x

su pro paba ad20; Acr; pyrol4; lys5; cho; cha

pro y ad20; Acr; phen2 Sulpro s0; cho nic8; ribo2 cha

su pro y ad20; Acr; cho; cha (380) x pro bi; Acr; phen2; Sulpro s0
(from cross 241)

pro paba ad20; Acr; phen2 Sulpro s0; cho nic8; ribo2 cha
(⟶ cross 383, Fig. 7)

su ribo pro paba y ad20; nic8 cho; cha

(352) x

FIGURE 4.—Pedigree of T(VI;VII) in later, multiply marked crosses: strains with T(VI;VII) underlined. § Cross 400 involves strains of diploid "R" (KÄFER 1961); ‖ analyzed by TECTOR (1961). For other symbols see legend of Figure 2.
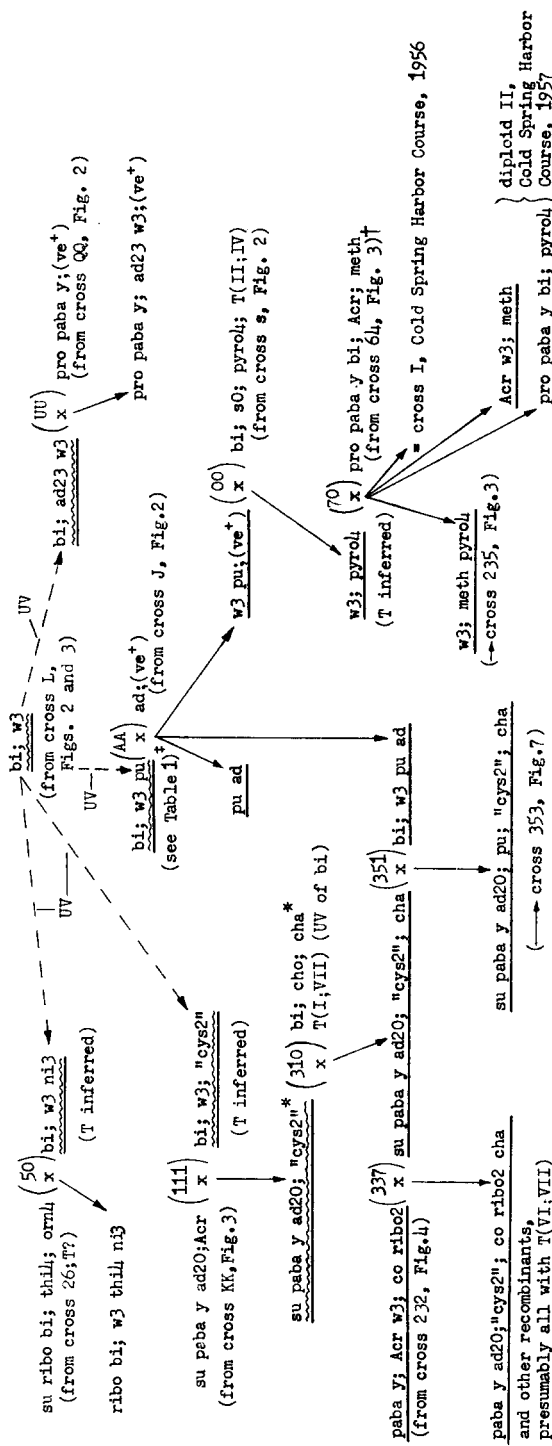
FIGURE 5.—Pedigree of T(VI;VII) and T(III;VIII) in crosses of mutants induced in *bi1*; *w3*: strains with translocations underlined; single line = T(VI;VII), double line = T(VI;VII) *and* T(III;VII). For symbols see legend of Figure 2.
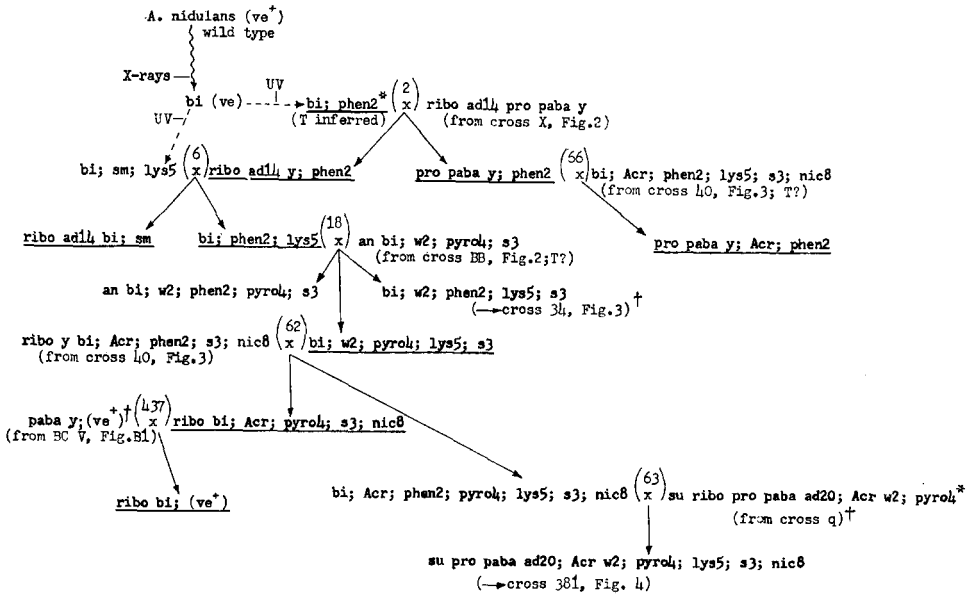
FIGURE 6.—Origin and pedigree of T(I;VIII): strains with T(I;VIII) underlined. For symbols see legend of Figure 2. The strain from cross 437 (lower left) should read *ribo bi*; *Acr*; (*ve+*). *Acr* was omitted in preparing the figure.

time is used for selection). Initially the data were broken down according to the three different methods used for the selection of mitotic haploids, since they did not appear to produce equally uniform and reliable results. However, the differences between the three methods were not statistically significant, each of them
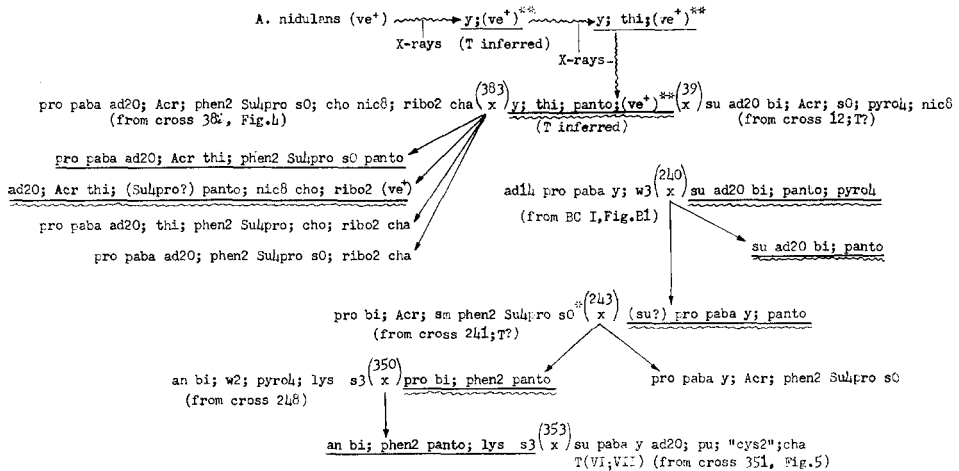
FIGURE 7.—Origin and pedigree of T(III;VIII) and T(III;VII;VIII) in *panto* strains: strains with translocations underlined; single line = T(III;VIII), double line = T(III;VII;VIII). For symbols see legend of Figure 2.

producing a few cases of aberrant results (some of these are discussed below). Data from the different methods of selection have, therefore, been combined for each tested strain (see Table 2, corresponding to Figure 2 and KÄFER 1965, Tables 3 to 7, corresponding to Figures 3 to 7).

Whenever almost complete linkage between two linkage groups was found, the presence of a translocation in one of the strains forming the test diploid was postulated (column 6 of Table 2). The x^2-value was always larger than ten, $P \ll 0.01$, for these results being chance deviations from free recombination. Rare haploids of recombinant type were found and are expected, since occasionally mitotic crossing over may precede the formation of a haploid. (Similar exceptional crossovers are found for markers located on the same chromosome.) Significant deviations are expected and were found, not only in the case of the heterozygous translocations, but also when two markers were used simultaneously for selection or when a mutant showed drastically reduced viability under the experimental conditions employed.

All other x^2-values have been classified into three groups: (1) $x^2 < 3.5$ corresponding to a value of $P > 0.05$; (2) $x^2 = 3.5-6.6$ corresponding to $P = 0.05-0.01$ and; (3) $x^2 > 6.6$ for $P < 0.01$. If all deviations are due to chance, it is expected that the first group will contain 95% of all cases, the second 4%, the last 1%. In all tables there is a total of 2204 x^2-values from 105 analyzed diploids not influenced by translocations, selection or viability. Of these, 2082 correspond to $P > 0.05$, 100 to P between 0.05 and 0.01, and 22 to $P < 0.01$. This agrees well with the expected distribution of 2094: 88:22 ($x^2 = 1.6$, $P > 0.05$) and confirms earlier observations that all chromosomes show random segregation when haploids are produced by the process of mitotic nondisjunction.

Pedigree and origin of the specific translocations: As far as possible, the pedigrees of the different translocations are presented in separate figures (Figures 2 to 7), but for the widespread ones there is some overlap, and certain of the early crosses were found to have been heterozygous for more than one translocation. Generally, all strains shown in a pedigree have been tested and the details of analysis are presented in the corresponding table. For untested or lost strains it is indicated whether they have a certain probability of containing a translocation or are probably free of any translocation. The UV-treated strains of *bi1* which have not been tested are labelled "T?", since it is possible that a few contain UV-induced translocations even though no translocations have been discovered among their descendants, and direct tests did not therefore, seem worth while. The translocations found here are designated by linkage groups only. Following the rules of mutant nomenclature of Aspergillus, all these translocations will be assigned isolation number 1 when further translocations involving the same pair of linkage groups are identified.

1. *T(III;VIII):* This translocation appears to have been induced by X rays in the same strain as the original γ mutant, since three mutants induced in this strain (*ad2, thi1* and *ad1*) contain T(III;VIII) (as well as ve^+, indicating that the $\gamma;ve$ prototroph in stock is unlikely to be the γ strain listed in these pedigrees; see Figures 2, 7). T(III;VIII) was found to segregate in six of the early crosses but was passed on to only a few offspring. Among these, the only one widely used was strain *bi1;w3* (from cross L) which also contains T(VI;VII) (see Figures 2,

5). In all cases it was T(VI;VII) rather than T(III;VIII) which was passed on to descendants used for further crosses. Perhaps T(III;VIII) may slightly reduce viability and conidiation, so that strains with this translocation were subconsciously selected against. T(III;VIII) was first encountered in certain "*cys2*"-strains (Figure 5) leading to a tentative localization of the mutant "*cys2*" in linkage group VIII (KÄFER 1958); however, it is now evident that "*cys2*" is an allele of *s0* in linkage group III.

2. *T(III;IV;VIII)*: One further aberration may have arisen at the same time as T(III;VIII), since two of the three original mutant strains [*y;thi*1 (*ve*$^+$) and *y ad2* (*ve*$^+$), Figure 2] showed not only linkage of III and VIII, but also linkage of these to IV. The same results are then expected for the strain *y; w2 ad1* (*ve*$^+$), but in this case several exceptions were found to the linkage between IV and either III or VIII as well as to the linkage between III and VIII. In addition, while T(III;VIII) was discovered among several descendants of these three strains, in no case was an aberration involving linkage group IV identified. Therefore, while an additional translocation involving linkage group IV and either III or VIII probably occurred, it is not indicated in Figures 2 and 7.

3. *T(III;VII;VIII)*: Another translocation complex linking VII to T(III;VIII) is present in many *panto* strains (Figure 7, Table 7). The mutant *panto* was induced by X rays in *y; thi*1 (*ve*$^+$) T(III;IV;VIII) and excluded from general stock. However, since no other *panto* mutant is available, several crosses were carried out (some shown at the right side of Figure 7) to separate *panto* from translocations so that it could be mapped and used as a marker. It was found (Figure 7) that in all cases *panto* was associated with T(III;VIII) but, in addition, most *panto* strains carried a further translocation involving VII as well as III or VIII. In the last cross (353) the only case of *meiotic* linkage between markers of different linkage groups was observed, namely linkage of *panto* with *cha* in group VIII (3 recombinants out of 85) as well as with "*cys2*", an allele of *s0* in group III (4 recombinants out of 21 tested). Cross (383) tested linkage of the interchange points with these and other markers of linkage groups III, VII and VIII (14 descendants analyzed, 4 of which are shown in Figure 7). Results of this extremely small sample confirmed the complete linkage of T(III;VIII) with *panto* and the close linkage of T(III;VIII) with *cha* (2 recombinants out of 14), while no linkage with *s0* was detectable (6 recombinants out of 14). No close linkage of either translocation to any other marker was found. The *panto* mutant seems to have been induced extremely close to one of the interchange points of T(III;VIII) and may belong to linkage groups III or VIII (prematurely placed in linkage group III by KÄFER 1958). The aberration involving linkage group VII was not recovered separately from T(III;VIII) and may, therefore, involve VII and either III or VIII.

4. *T(II;IV)*: Two strains were found which consistently showed complete linkage of markers belonging to linkage groups II and IV in large numbers of haploids from three test diploids (see Figure 2 and Table 2, strains *bi1*; *s0*; *pyro4* and *an1 paba1 y ad20*; *s0* from cross s). The available evidence suggests that T(II;IV) arose spontaneously, but the exact point of origin cannot be determined since several strains in the direct lineage are not available for analysis.

5. *T(VI;VII)*: By far the most wide-spread translocation is T(VI;VII), which segregates in many crosses (6 in pedigree of Figure 3, 9 in Figure 4, 7 in Figure 5). Two crosses (337 and 351, Figure 5) even turned out to be homozygous for T(VI;VII). Similarly, several of the early test diploids synthesized before "certified" tester strains were available are homozygous for T(VI;VII) (KÄFER 1965, Table 4). T(VI;VII) appears to have originated in the original *pabal bil* strain which has been lost. It was probably induced by X rays at the same time as *pabal*, since the two available strains, containing the spontaneous mutants *w3* and *co* which arose in *pabal bil*, both have T(VI;VII) (see Figure 3).

6. *T(I;VIII)*: This translocation was first found in a diploid designed to map the widespread mutant *ve*, using a strain *ribol bil* (*ve⁺*) from cross 437 to produce the heterozygous test diploid (see Figure 6, bottom). All haploids from this diploid showed complete linkage of I and VIII, and in addition no y/y segregants were recovered, presumably owing to extremely poor viability. T(I; VIII) may well be an unidirectional (insertional) translocation from VIII to the right arm of linkage group I, since, in contrast to the above case where it was in repulsion to y, diploids containing T(I;VIII) in coupling with y, produce many y/y segregants with almost normal viability (presumably carrying duplications). T(I;VIII) was traced back through five generations to the strain *bil*; *phen2*. It seems likely that it was UV-induced in *bil* at the same time as *phen2* (this original strain is lost; see Figure 6).

Experimental features complicating analysis: a. *Exceptional segregants,* that is, haploids showing crossing over between markers of the same linkage group, are usually found with low frequency (average about 1%), as expected from the rate of occurrence of mitotic crossing over. Exceptions to the complete linkage of markers on two linkage groups involved in a translocation are found with similar frequency. Generally, whenever more than one or two such exceptions were found, a second sample of haploids was isolated and tested or a second test diploid was analyzed.

Usually, clear-cut results were obtained in this way. However, it was found in a few cases that in all samples from certain diploids the exceptional types had a fairly consistent and relatively high frequency, up to 10%. Details of such a case are given in Table 1. The analyzed strain *bil; w3 pu* obviously contains T(VI; VII) (no exceptions) and, in addition, not only T(III;VIII) but also some aberration involving I as well as III or VIII. With respect to the groups I, III and VIII the linkage in mitotic haploids is evident, but not complete; haploid as well as stable aneuploid exceptional types were found. In addition, about ten test diploids with segregation of markers in more than one linkage group were recovered. These are expected to become relatively more frequent when in diploids with several aberrations the frequency of balanced haploid segregants decreases. They were excluded after careful checking for heterozygous markers and measuring of conidial diameters. This high frequency of exceptional segregants of various types obviously complicates the interpretation of the results. The formation of stable aneuploid or near-haploid exceptional types is likely to depend on the size and number of translocated segments and on their viability effects when duplicated. Their frequency is, therefore, expected to differ for different translocations, as was found in the case above.

b. *Loss of markers* owing to mitotic recombination in test diploids occurs occasionally (e.g. PONTECORVO *et al.*, 1954). If the mutant used for selection is in repulsion to such a marker in the original diploid, all selected haploids will simulate linkage between the two corresponding linkage groups.

This may be the explanation for one set of inconsistent results obtained in this investigation in the testing of the strain *ribo1 bi1*; *Acr1*; (*ve*+) (from cross 437, bottom of Figure 6; marker *Acr1* was omitted from the figure by mistake). In a first diploid all haploids, selected as yellow heads, carried the markers *y*, *phen2*, and *co* showing linkage of group I to III and VIII. A translocation T(I;III;VIII) was postulated (as indicated in Table 6). However, further analysis of the strain *ribo1 bi1*; *Acr1*; (*ve*+) as well as its tester strain, by combining each with two further standard or tester strains, did not confirm this result. The new results indicated that only a T(I;VIII) was present in *ribo1 bi1*; *Acr1*; (*ve*+), and that the tester strain was translocation-free. The apparent linkage to III may have been due to homozygosis for *phen2* in a large part of the analyzed colonies from the first test diploid.

c. *Viability effects* leading to unexpected results were found most frequently with the marker *lys5*, especially on supplemented minimal medium without adenine, and most of the large x^2s that are likely to be due to viability effects involve this marker.

When *lys5* was in repulsion to the main selected marker, an excess of *lys5*+ haploids led to a reduction of the recombinant types and, therefore, to the assumption of a translocation; e.g. a T(I;V) appeared to be present when the selective marker was on linkage group I. This happened in two cases: a *panto* strain and the strain *bi1*; *sm*; *lys5* used in cross 6 (Figure 6). In a first analysis both showed complete linkage of I and V, and a T(I;V) was tentatively postulated (KÄFER 1963). However, a second sample from medium containing more lysine showed random segregation for the markers on linkage groups I and V, indicating that no such translocation exists. Similar misleading results were also obtained occasionally for other markers. For example, in a first sample of 43 selected *y*;*co* haploids from the diploid testing the strain *pro1 paba1 ad20*; *thi1*; *phen2 Su4pro*; *cho*; *ribo2 cha* (cross 383, Figure 7) only the *cho*+ allele was recovered, which led to the assumption that this strain contained a T(VII;VIII) which would explain the III-VII-VIII linkage in its parent. However, a second sample of 53 haploids from the same diploid showed free recombination between *cho* (VII) and *co* (VIII).

Another group of puzzling results was obtained when haploids from three diploids heterozygous for the morphological marker *co* (compact, linkage group VIII) were selected on complete medium supplemented with p-fluorophenylalanine (PFP). The diploids were synthesized to test the strains *pro1 paba1 y*; *w3 ad1*; (*ve*+) (from cross QQ, Figure 2), *pro1 paba1 y*; *ad23 w3*; (*ve*+) (from cross UU, Figure 5) and *y*; *Acr1 ad3*; *co* (from cross b, Figure 3), and the same *co*-carrying tester strain (FGSC No. 44) was used in the first two. In all three cases the marker *co* was recovered only in very few of the many selected haploids, and a T(III;VIII) was postulated, since on the PFP-containing medium all haploids are *phen2*+ (III). However, haploids isolated on complete medium from these diploids showed no such linkage. Possibly *co* is selected against on the PFP containing medium; but results from a further test diploid containing the same tester strain did not confirm this hypothesis.

DISCUSSION

The data presented here show that in *A. nidulans* it is possible to detect translocations by genetic analysis based on the segregation of markers in haploids produced by mitotic recombination from heterozygous diploids. Since these mitotic haploids are the result of segregation of whole chromosomes it is possible to detect any chromosomal interchanges, but other aberrations, e.g. inversions, are not recognized. In addition, no information is obtained about interchange points of the detected translocations within the linkage groups involved. However, in a few cases these were located on a chromosome arm containing a selective marker. Such a marker permits isolation of unbalanced diploid recom-

binants produced by mitotic crossing over in the structurally heterozygous chromosome arm. The position of the break-point can thus be mapped as to chromosome arm, and some information may be obtained about the type of aberration present (e.g. T(I;VIII) likely involves an insertional translocation in IR). Generally the number of strains tested which could give information on the *meiotic* linkage of translocations to markers on the involved chromosomes is small, so that only very close meiotic linkage could have been detected. Such linkage was found for one translocation only: in agreement with earlier results (KÄFER 1962), T(III;VIII) showed meiotic linkage to *cha* of linkage group VIII and could not be separated from *panto*. This is parallel to the finding that in *A. nidulans* mutants mapped in the same linkage group by mitotic recombination very often show no meiotic linkage.

Making use of the genetic method for detection of chromosomal aberrations outlined above, the three widespread translocations T(III;VIII), T(VI;VII) and T(I;VIII), which had been encountered in multiply marked stock strains, have been traced to their origin (as described previously for T(I;VII), KÄFER 1962). In addition, six further aberrations were encountered—four chromosomal interchanges and two intrachromosomal aberrations, possibly inversions. Most of these have not spread into the general pedigree (except one of the inversions, which is being investigated). A total of eight translocations have, therefore, so far been found and traced to their origin. Four of these appear to have been induced by X rays, three by ultraviolet light, and one apparently arose spontaneously. The pedigrees include ten strains treated with X rays and 22 with UV, at radiation doses which gave similar survival values (PONTECORVO *et al.*, 1953). It can be concluded, therefore, that, as in higher organisms, X-irradiation produces in this fungus many more aberrations than does UV. This is not unexpected, and agrees with recent findings comparing effects of γ-rays and UV on diploid conidia at doses that give similar survival values (TECTOR 1961; KÄFER and CHEN 1964).

The present investigation, therefore, confirms the findings in Neurospora (PERKINS *et al.*, 1962) which indicate that it is not safe to assume that UV treated strains are free of chromosome aberrations even though, generally, the frequency of aberrations is lower than after treatment with ionizing radiation.

SUMMARY

The process of mitotic recombination that produces haploids from diploids in *Aspergillus nidulans* has been exploited to detect and trace translocations encountered in stock strains. Recombinants from an eighth-generation backcross were adopted as standard reference strains, since it is not possible to use the original prototrophic wild type for the synthesis of marked test diploids. Three

translocations were shown to segregate in many crosses (in addition to the one analyzed previously). Four further translocations were discovered in a few strains only, two of them in irradiated original mutant strains. All translocations were traced to their origin. This made it possible to exclude from general stock strains with translocations, or descendants from crosses heterozygous for trans- locations. One of the eight analyzed translocations seems to be of spontaneous origin, while all others were traced back to mutagenic treatments, four with X rays, three with ultraviolet light (UV). Since the analyzed pedigree includes descendants from nine strains irradiated with X rays and 22 with UV, it appears that translocations are induced with fairly low frequency by UV, and with extremely high frequencies by the commonly used doses of ionizing radiation.

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