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# The use of *p*-fluorophenylalanine with 'master strains' of *Aspergillus nidulans* for assigning genes to linkage groups

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## 1. INTRODUCTION

Haploidization in diploid strains (Pontecorvo, Tarr Gloor & Forbes, 1954; Pontecorvo, 1956; Pontecorvo & Käfer, 1956, 1958) has been used for assigning markers of unknown location (Forbes, 1959) to one of the eight chromosomes of *Aspergillus nidulans*. In Forbes' technique a heterozygous diploid between a strain carrying an unlocated marker and a 'master' strain marked on each or several of the chromosomes is synthesized. Haploid segregants show free recombination between the unlocated marker and all the markers of the master strain except those on the chromosome carrying the wild-type allele of the unlocated marker. Since coincidence of mitotic crossing-over and haploidization in one nuclear lineage is rare (Pontecorvo *et al.*, 1954; Pontecorvo & Käfer, 1958; Käfer, 1961), all the markers on one chromosome almost always segregate together and independently of the markers on other non-homologous chromosomes. From a multiply-heterozygous diploid, therefore, four classes—two parental and two recombinant—are recovered, $++$, $--$, $-+$, and $+-$, for any two markers on different chromosome pairs, but only the two parental classes for two markers on the same chromosome pair. Such complete linkage identifies the chromosome pair on which the new marker is located.

Originally, haploid segregants were obtained from heterozygous diploid strains by double selection, i.e. of two recessive markers on different chromosome pairs (Käfer, 1958; Forbes, 1959). Because of the rarity of coincidence in the same nuclear lineage of mitotic crossing-over, or of non-disjunction, in two chromosome pairs, or of crossing-over in one pair and non-disjunction in the other, such segregants are almost always haploid or monosomic for the two selected chromosomes: the proportion of diploid 'double' segregants is negligible. Double selection methods have the disadvantage that only the *selected* chromosomes of the relevant two chromosome pairs are recovered. Thus, if the unlocated marker should be on either of these pairs, one cannot determine on which of the two it is located.

A more convenient and more general method for selecting haploids became possible following Morpurgo's discovery (see Lhoas, 1961*a*) that *para*-fluorophenylalanine induces segregation in *Aspergillus nidulans* diploids. This discovery,

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adapted first to *Aspergillus niger* diploids by Lhoas (1961) and later by various workers to *Aspergillus nidulans* diploids, led to the further recognition that growth on *p*-fluorophenylalanine leads in time to haploidization of nearly all the hyphae of a diploid culture. Lhoas found (1961*b*) that the growth rate of haploids is greater than that of diploids on medium containing *p*-fluorophenylalanine. It is possible that *p*-fluorophenylalanine produces haploidy via successive chromosome losses, and its selective action perhaps depends on the advantage that haploid nuclei have over sub-diploid and diploid nuclei. Certain other chemical treatments and radiations seem to act in this way, according to Käfer (1963).

We wish to report on the use of four 'master' strains of *Aspergillus nidulans* which permit the rapid assignment of markers of unknown location to any one of the eight chromosomes, when used in conjunction with haploidization by *p*-fluorophenylalanine. The four master strains differ in conidial colour markers so that a new mutant can be allocated to its chromosome, regardless of the conidial colour of the strain in which the mutant was obtained.

The present paper gives one example of the use of each of these master strains for location of markers.

Another use of the master strains, not exemplified here, is to allow rapid detection of reciprocal translocations, which are known to be common in *Aspergillus nidulans* strains (Käfer, 1962). If a *reciprocal* translocation is present in a strain used to synthesize a diploid with a master strain, two markers of the master strain known to be on different chromosome pairs show complete linkage in *cis* among the haploids. This is simply the consequence of the well-known fact that *both* recombinant classes have a deficiency of a chromosomal segment and a duplication of another and are therefore *both* usually non-viable. On the other hand, of the recombinant classes resulting from a diploid heterozygous for an *insertional* translocation, one is deficient for a chromosome segment and the other has it duplicated. The latter class is usually viable though not necessarily fully so. Insertional translocations, therefore, give segregation ratios which, without further analysis, may be equivocal, especially when other kinds of viability effects distort the segregation ratios (see, e.g. Tables 2 and 4).

## 2. MATERIALS AND METHODS

The general methods of *Aspergillus nidulans* genetic analysis and the terminology of mutant strains are described in Pontecorvo, Roper, Hemmons, MacDonald & Bufton, 1953; Pontecorvo & Käfer, 1958; Käfer, 1958. Diploid strains were isolated according to the method of Roper (1952).

Haploidization of diploid strains was accomplished by point inoculation of conidia on the complete medium containing approximately 7 mg. of DL-*p*-fluorophenylalanine per 100 ml. A few strains require somewhat more *p*-fluorophenylalanine for efficient haploidization. After 4–7 days' incubation at 37°C., haploid sectors are easily identified by their more vigorous growth and better conidiation than the parent diploid colony. The haploid sectors are identified under the

dissecting microscope, touched lightly with a sterile probe, and inoculated directly on to a master dish of complete medium. Usually 50–75% of the haploid sectors can be isolated free of diploid conidia by this method. Green diploid colonies and green haploid colonies are readily distinguished on the dish by the difference in the shade of green, since the haploids are somewhat darker.

Four master strains, MSE with white conidia, MSD and MSF with yellow conidia, and MSG with green conidia, were isolated from appropriate crosses, and the markers on the various chromosomes are indicated in Table 1. The alleles *ad20*, *phen2*, *pyro4*, *lys5*, *s3*, *nic8* and *ribo2* indicate requirements for adenine, phenylalanine, pyridoxine, lysine, reduced sulphur, nicotinic acid and riboflavin, respectively. *Acr1* indicates resistance to acriflavin; *ap2*, resistance to aminopterin; *gal1*, inability to grow on galactose as sole carbon source; *facA303*, inability to grow on acetate as sole carbon source (as well as resistance to fluoroacetate; Apirion, 1962); *su1ad20*, suppressor of *ad20*; *y*, yellow conidia; $y^+$, green conidia and *w3*, colourless conidia (white). As *w3* is epistatic to *y* and $y^+$, strains marked

Table 1. *Genotypes of four 'master' strains of* Aspergillus nidulans

| Master strain | Chromosome I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| MSD | *su1ad20 y ad20* | *Acr1* | *phen2* | *pyro4* | *lys5* | *s3* | *nic8* | *ribo2* |
| MSE | *su1ad20 y ad20* | *w3* | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |
| MSF | *su1ad20 y ad20* | *Acr1* | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |
| MSG | *y* + * | — | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |

* As MSG was obtained from crosses including *su1ad2o*, it may well carry this suppressor.

with *w3* may be carrying *y* or $y^+$, i.e. phenotypically white strains may have the genotype *w3*(*y*) or *w3*($y^+$). Since it is desirable to have different conidial colours segregating in the diploid, the MSE master strain (white) is convenient for combinations involving yellow or green strains and MSF or MSD (yellow), for those involving white or green strains. MSG (green) has markers on neither chromosome I nor II and is useful for strains already marked on chromosomes I and II by *y* and *w*, respectively. MSD was the first of the four master strains to be used, but it was found to be less useful in general because of the inviability of haploids carrying *phen2*, when haploidizing on *p*-fluorophenylalanine. Also, haploids carrying *lys5* showed very poor viability.

## 3. RESULTS AND DISCUSSION

Heterozygous diploids were synthesized between the master strains and the *bi1* strain or mutant strains obtained spontaneously (*ap2 bi1*) or after ultra-violet irradiation of the *bi1* strain (*bi1*; *w4* and *ad42 bi1*). The *bi1* strain was chosen because no translocations have been detected in extensive use of this strain. The results of haploidization of the four diploid strains, *ad42 bi1*/MSE, *bi1*/MSF, *bi1*; *w4*/MSG and *ap2 bi1*/MSD are presented in Tables 2, 3, 4 and 5, respectively.

In Table 2 segregation of all possible pairs of markers is shown in full, so that the

effect of poor viability can be demonstrated and the detection of translocations is made possible. It also shows that only by the complete absence of both recombinant classes can a new marker be located and that the absence of one parental class in no way affects this location. In fact, if both a parental and a recombinant class were missing, this again would not interfere with the new marker's location.

In Tables 3, 4 and 5 the data are grouped for shortness.

No translocations were found in any of these experiments.

MSE. Among 236 haploids (Table 2) *ad42* and *bi1* recombine with every marker of strain MSE, except *y*, at about 50% frequency. No recombinants were obtained between *ad42*, *y* or *bi1*. Hence *ad42*, *y* and *bi1* are all on the same chromosome (I). Free recombination between all markers on different chromosomes excludes translocations in either the *ad42 bi1* or MSE strain. This evidence, of course, does not exclude the possibility that the *ad42 bi1*/MSE diploid could be heterozygous

Table 2. *Segregation of markers in 236 haploid strains isolated from diploid:*

| MSE: | *su* | + | *y* | *ad20* | + | *w3* | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | *ad42* | + | + | *bi1* | + | + | + | + | + | + | + |

| Joint segregation of markers (*cis*)* | Parental | | Recombinant | | Recombination fraction** |
|---|---|---|---|---|---|
| | − − | + + | + − | − + | |
| *y* & *gal1* | 45 | 5 | 7 | 58 | 65/115 |
| *y* & *pyro4* | 56 | 5 | 7 | 47 | 54/115 |
| *y* & *facA303* | 51 | 0 | 12 | 52 | 64/115 |
| *y* & *s3* | 39 | 2 | 10 | 64 | 74/115 |
| *y* & *nic8* | 49 | 7 | 5 | 54 | 59/115 |
| *y* & *ribo2* | 40 | 10 | 2 | 54 | 56/115 |
| *w3* & *gal1* | 61 | 63 | 52 | 60 | 112/236 |
| *w3* & *pyro4* | 57 | 57 | 63 | 64 | 112/236 |
| *w3* & *facA303* | 70 | 52 | 63 | 51 | 114/236 |
| *w3* & *s3* | 58 | 66 | 49 | 63 | 112/236 |
| *w3* & *nic8* | 67 | 61 | 54 | 54 | 108/236 |
| *w3* & *ribo2* | 51 | 63 | 52 | 70 | 122/236 |
| *gal1* & *pyro4* | 56 | 59 | 64 | 57 | 121/236 |
| *gal1* & *facA303* | 63 | 53 | 70 | 50 | 120/236 |
| *gal1* & *s3* | 59 | 75 | 48 | 54 | 102/236 |
| *gal1* & *nic8* | 58 | 60 | 63 | 55 | 118/236 |
| *gal1* & *ribo2* | 53 | 73 | 50 | 60 | 110/236 |
| *pyro4* & *facA303* | 72 | 55 | 61 | 48 | 109/236 |
| *pyro4* & *s3* | 55 | 64 | 52 | 65 | 117/236 |
| *pyro4* & *nic8* | 66 | 61 | 55 | 54 | 109/236 |
| *pyro4* & *ribo2* | 48 | 61 | 55 | 72 | 127/236 |
| *facA303* & *s3* | 65 | 61 | 42 | 68 | 110/236 |
| *facA303* & *nic8* | 73 | 55 | 48 | 60 | 108/236 |
| *facA303* & *ribo2* | 59 | 59 | 44 | 74 | 118/236 |
| *s3* & *nic8* | 61 | 69 | 60 | 46 | 106/236 |
| *s3* & *ribo2* | 46 | 72 | 57 | 61 | 118/236 |
| *nic8* & *ribo2* | 59 | 71 | 44 | 62 | 106/236 |
| *ad42* & *bi1* | 16 | 220 | 0 | 0 | 0/236 |

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*Table 2—continued*

| Joint segregation of markers (*trans*)* | Parental | | Recombinant | | Recombination fraction |
|---|---|---|---|---|---|
| | + − | − + | − − | + + | |
| *bi1* & *y* | 103 | 12 | 0 | 0 | 0/115 |
| *bi1* & *w3* | 117 | 12 | 4 | 103 | 107/236 |
| *bi1* & *gal1* | 103 | 6 | 10 | 117 | 127/236 |
| *bi1* & *pyro4* | 111 | 7 | 9 | 109 | 118/236 |
| *bi1* & *facA303* | 117 | 0 | 16 | 103 | 119/236 |
| *bi1* & *s3* | 94 | 3 | 13 | 126 | 139/236 |
| *bi1* & *nic8* | 114 | 9 | 7 | 106 | 113/236 |
| *bi1* & *ribo2* | 100 | 13 | 3 | 120 | 123/236 |
| *ad42* & *y* | 103 | 12 | 0 | 0 | 0/115 |
| *ad42* & *w3* | 117 | 12 | 4 | 103 | 107/236 |
| *ad42* & *gal1* | 103 | 6 | 10 | 117 | 127/236 |
| *ad42* & *pryo4* | 111 | 7 | 9 | 109 | 118/236 |
| *ad42* & *facA303* | 117 | 0 | 16 | 103 | 119/236 |
| *ad42* & *s3* | 94 | 3 | 13 | 126 | 139/236 |
| *ad42* & *nic8* | 114 | 9 | 7 | 106 | 113/236 |
| *ad42* & *ribo2* | 100 | 13 | 3 | 120 | 123/236 |

* The terms '*cis*' and '*trans*' in relation to two mutants are used, for short, to indicate parental origins; *cis* = both mutants from the same haploid strain; *trans* = one mutant from each of the haploid strains.

Allele ratios:

$$\frac{bi1}{bi1^+}\ \frac{16}{220} \qquad \frac{ad42}{ad42^+}\ \frac{16}{220} \qquad \frac{w3\left\{\begin{matrix} y & - \\ y^+ & - \end{matrix}\right\}}{w3^+\left\{\begin{matrix} y & 103 \\ y^+ & 12 \end{matrix}\right\}}\ \frac{121}{115} \qquad \frac{gal1}{gal1^+}\ \frac{113}{123}$$

$$\frac{pyro4}{pyro4^+}\ \frac{120}{116} \qquad \frac{facA303}{facA303^+}\ \frac{133}{103} \qquad \frac{s3}{s3^+}\ \frac{107}{129} \qquad \frac{nic8}{nic8^+}\ \frac{121}{115}$$

$$\frac{ribo2}{ribo2^+}\ \frac{133}{103}$$

236 haploid strains were isolated from the *ad42 bi1*/MSE diploid as described in Materials and Methods. The number of parental and recombinant strains for each pair of markers in the experiment is presented. Pairs of markers showing zero recombination fraction belong to the same chromosome pair.

** The total number classifiable in respect of the two alleles *y* (yellow) and $y^+$ (green) is 115 i.e. the segregants carrying $w3^+$, since *w3* (colourless) is epistatic to *y* and $y^+$.

for other types of chromosome rearrangements (e.g. inversions), or homozygous for the same translocation. If the latter were the case, either strain would show a translocation when haploidized with a strain not carrying this translocation.

MSF. The data in Table 3 show that among 82 haploids *bi1* recombines at about 50% frequency with every marker of strain MSF except *y*, indicating—as already known from meiotic mapping—that *bi1* is on chromosome I.

MSG. The data in Table 4 show that among 112 haploids *bi1* and *w4* recombine freely with each other and with every marker of the MSG strain. MSG is marked

on only six chromosomes, and since *bi1* is known to be on chromosome I, *w4* must be on the other unmarked chromosome, i.e., chromosome II.

MSD. The data in Table 5 show that among 31 haploids *ap2* and *bi1* recombine freely with every marker of the MSD strain except *y*. Again, *bi1* is known to be on chromosome I and *ap2* turns out to be on the same chromosome.

Table 3. *Segregation of markers in 82 haploid strains isolated from diploid:*

| MSF: | *su* | *y* | *ad20* | + | *Acr1* | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | + | + | *bi1* | + | + | + | + | + | + | + |

| Segregation of markers | Recombination fraction |
|---|---|
| *bi1* & *y* | 0/82 |
| *y*; *bi1*; *Acr1*; *gal1*; *facA303*; *s3*; *nic8* & *ribo2*, in all possible pairs except the one above | 25 to 55/82 |

Allele ratios

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *y* | 55 | *bi1* | 27 | *gal1* | 29 | *pyro4* | 35 |
| *y*+ | 27 | *bi1*+ | 55 | *gal1*+ | 53 | *pyro4*+ | 47 |
| *facA303* | 53 | *s3* | 18 | *nic8* | 33 | *ribo2* | 31 |
| *facA303*+ | 49 | *s3*+ | 64 | *nic8*+ | 49 | *ribo2*+ | 51 |

For the purpose of location one needs merely a few haploid strains recovered from a diploid to show that a marker of unknown location recombines with every marker of the master strain except those of one linkage group, and shows complete linkage in *trans* with the latter. With the MSD strain the recovery of *phen2* and *lys5* was very poor (Table 5). On the other hand, the recovery of the markers of the MSE,

Table 4. *Segregation of markers in 112 haploid strains isolated from diploid:*

| MSG: | + | *w4*+ | *gal1* | *pyro4* | *facA303* | *s3* | *nic8* | *ribo2* |
|---|---|---|---|---|---|---|---|---|
| | *bi1* | + | + | + | + | + | + | + |

| Segregation of markers | Recombination fractions |
|---|---|
| *bi1*; *w4*; *gal1*; *pyro4*; *facA303*; *s3*; *nic8* & *ribo2* in all possible pairs | 46 to 71/112 |

Allele ratios

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *bi1* | 52 | *w4* | 81 | *gal1* | 49 | *pyro4* | 59 |
| *bi1*+ | 60 | *w4*+ | 31 | *gal1*+ | 63 | *pyro4*+ | 53 |
| *facA303* | 56 | *s3* | 56 | *nic8* | 84 | *ribo2* | 43 |
| *facA303*+ | 56 | *s3*+ | 56 | *nic8*+ | 28 | *ribo2*+ | 69 |

MSF and MSG strains has been close to 50% with the exception of the *ad42 bi1* chromosome (Table 2) which shows poor recovery for unknown reasons.

Resistance to *p*-fluorophenylalanine has not been found among the haploid strains isolated from haploidization on *p*-fluorophenylalanine. In fact, one recessive *p*-fluorophenylalanine-resistant mutant has been located to a chromosome by this technique (McCully, unpublished).

Table 5. *Segregation of markers in 31 haploid strains isolated from diploid:*

| MSD: | *su* | + | *y* | *ad20* | + | *Acr1* | *phen2* | *pyro4* | *lys5* | *s3* | *nic8* | *ribo2* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | *ap2* | + | + | *bi1* | + | + | + | + | + | + | + |

| Segregation of markers | Recombination fraction |
|---|---|
| *ap2* & *bi1* | 0/31 |
| *bi1* & *y* | 0/31 |
| *ap2* & *y* | 0/31 |
| *phen2* & *lys5* | 1/31* |
| *ap2*; *y*; *bi1*; *Acr1*; *phen2*; *lys5*; *s3*; *nic8* & *ribo2*, in all possible pairs except the above | 7 to 21/31 |

Allele ratios

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ap2* / *ap2*+ | 10 / 21 | *y* / *y*+ | 21 / 10 | *bi1* / *bi1*+ | 10 / 21 | *Acr1* / *Acr1*+ | 17 / 14 | *phen2* / *phen2*+ | 0 / 31 | *pyro4* / *pyro4*+ | 17 / 14 |
| *lys5* / *lys5*+ | 2 / 29 | *s3* / *s3*+ | 7 / 24 | *nic8* / *nic8*+ | 15 / 16 | *ribo2* / *ribo2* | 11 / 20 | | | | |

* The four classes were:

*Parental:* *phen lys*: 0: *phen*+ *lys*+: 30

*Recombinant:* *phen*+ *lys*−: 1: *phen*− *lys*+: 0

These abnormal segregations are due to poor viability of *phen2* on *p*-fluorophenylalanine and of *lys5*.

No crossover events have been observed in the 349 haploids where they could have been detected (Tables 2, 3 and 5).

## SUMMARY

Four 'master' strains of *Aspergillus nidulans*, three of which are marked on each of the eight chromosomes, are used for assigning genes of unknown location to linkage groups. In this technique a heterozygous diploid is synthesized between one 'master' strain and a strain carrying an unlocated marker. This diploid is haploidized by growth on *p*-fluorophenylalanine, and the haploids thus obtained are classified for recombination between the unlocated marker and each of the markers of the 'master' strains. The marker or markers of the 'master' strain which show no recombination with the unlocated marker identify the chromosome pair on which the latter is located. One example is presented for each of the four 'master' strains.

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