ANALYSIS OF VEGETATIVE SEGREGATION AND RECOMBINATION IN PENICILLIUM CHRYSOGENUM

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On the basis of an analysis of vegetative segregation in heterozygous diploids of Aspergillus nidulans, Pontecorvo et al. (Pontecorvo 1953; Pontecorvo, Tarr Gloor and Forbes 1954) concluded that the segregant nuclei arise as a consequence of two independent processes. The first is mitotic crossing over (Stern 1936), giving rise to diploid nuclei which are homozygous for some of the markers. The other is irregular distribution of whole chromosomes at mitosis; this produces haploid nuclei in a proportion of cases. Roper and Pritchard's (1955) recovery of complementary products of mitotic crossing over provided conclusive evidence for the first of these processes. Pontecorvo and Käfer (1956) have shown with Aspergillus nidulans the practicability of using mitotic crossing over and haploidization for mapping chromosomes.

The two types of vegetative segregant, haploid and diploid, have also recently been identified in Penicillium chrysogenum (Sermonti 1956), so that it is a reasonable assumption that vegetative segregation takes place by means of the same processes in this species. In the same paper the author shows that the spore size of a segregant is diagnostic for its ploidy: diploid spores are about twice the volume of haploid spores.

Observations of spore diameter have not been carried out in the present experiments, and the distinction of strains into haploid and diploid has been based on the phenotype of the segregants and on second-order segregation data.

MATERIALS AND METHODS

Heterozygous diploids. An analysis was made of the three following heterozygous diploids of Penicillium chrysogenum:

IV: 63 cy p y / 51 thi pr
VII: 63 cy p y / 46 me w
XII: 86 y thi pr / 46 me w

The Roman numeral is the code number of the diploid. The symbol is made up of the symbols of the two constituent haploids separated by an oblique. Each strain is symbolized by an Arabic number (the strain number) followed by the symbols of the mutations in inverse order of induction. All the strains trace back to the strain Wis. 47.1564; the method of their production and the meanings of the mutation symbols have been described elsewhere (Sermonti 1954a).1 The two y markers of strains 63

1 cy, cysteine or methionine requirement; me, methionine requirement; pr, proline requirement; thi, thiamine requirement; y, yellow conidia; w, white conidia; p, absence of penicillin production.
and 86 are regarded as alleles, because diploid 86 y thi pr / 39 arg y has yellow conidia, and strains 39 and 63 are both descended from the same mutant, 15 y. Strain 86 is derived from strain 51.

The heterozygous diploids IV and VII were obtained by selection on minimal media from conidia of the corresponding heterokaryons, and diploid XXI as a sector from a heterokaryon colony. All three diploids have green spores and grow on minimal medium, like the wild type strain Wis. 47.1564. The heterozygous diploidy of the three strains is asserted on the evidence of the present work.

All three diploids were purified by isolation of a single conidium with a micro-manipulator (Sermonti 1954a), and kept on agar slants of complete medium. The cultures were renewed by washing the whole sporing surface and using a sample for transfer. These precautions were devised to reduce to a minimum any bias due to sampling errors in the nuclear population under examination.

Culture media. The following media were employed: a minimal medium (MM), Czapek-Dox as modified by Clutterbuck, Lovel and Raistrick (1932); a complete medium (CM) with corn steep base as described in an earlier paper (Sermonti 1954a). A modified form of the latter was also used in the second part of the experiment. Its composition was as follows: KH₂PO₄, 1 gm; MgSO₄, 0.5 gm; KCl, 0.5 gm; FeSO₄, 0.01 gm; sucrose, 30 gm; corn steep liquor, 10 gm; nucleic acid hydrolysate (Pontercorvo 1953), 3 ml; sodium phenylacetate, 1 gm; dl-methionine, 0.05 gm.

Segregant isolation. 1) Colour segregants: Conidia of each diploid are plated on CM; the colonies which grow are observed for colour; all white or yellow colonies are transferred to fresh CM agar for further testing. 2) Nutritional deficiency segregants: Conidia of each diploid are plated on limiting medium (Sermonti 1954a); colonies which do develop but show only poor growth are collected and subjected to further testing. 3) Segregants for absence of capacity to produce penicillin were selected after total isolation of the colonies obtained by plating conidia of diploid VII on CM. The culture and the titration techniques are described in an earlier paper (Caglioti and Sermonti 1956).

Characterization of segregants. Colour segregants were checked for uniformity of colour (and purified in cases of nonuniformity) and then classified for nutritional requirements (Sermonti 1954b). Deficiency segregants were first classified for their specific deficiencies and then transferred to CM agar and examined for their colour, which was uniform in every case.

Strains whose response was open to doubt were transferred to CM agar slants and subjected to more accurate tests. Strains which were to be investigated further were also kept in this way. In all cases in which some doubt arose as to the nutritional requirements of a strain it was subjected to an auxanographic test (Pontercorvo 1949), and the same test was applied to all the strains of critical importance in the present work.

RESULTS

1. Selection of first-order segregants. Table 1 shows the rate at which colour segregants were obtained from the three diploids studied and from diploid XIII (Ca-
TABLE 1

Rate of occurrence of segregants selected for colour from some heterozygous diploids

<table>
<thead>
<tr>
<th>Heterozygous diploid</th>
<th>Colonies observed no.</th>
<th>Yellow segregants</th>
<th>White segregants</th>
<th>Ratio of yellow to white segregants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>IV</td>
<td>30,009</td>
<td>76</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>VII</td>
<td>14,875</td>
<td>80</td>
<td>0.54</td>
<td>179</td>
</tr>
<tr>
<td>XIII*</td>
<td>15,130</td>
<td>16</td>
<td>0.11</td>
<td>24</td>
</tr>
<tr>
<td>XXIa</td>
<td>1,648</td>
<td>146</td>
<td>8.86</td>
<td>201</td>
</tr>
<tr>
<td>XXIb</td>
<td>3,788</td>
<td>1</td>
<td>0.026</td>
<td>2</td>
</tr>
<tr>
<td>Totals†</td>
<td>35,441</td>
<td>243</td>
<td></td>
<td>406</td>
</tr>
<tr>
<td>Averages†</td>
<td></td>
<td>2.38‡</td>
<td>±2.16</td>
<td>3.40</td>
</tr>
</tbody>
</table>

* CAGLIOTI and SERMONTI (1956).
† Exclusive of IV.
‡ The standard error is shown as well as the average.

Under diploid XXI the segregation rates observed in two different clones isolated from the same sector of a balanced heterokaryon are reported. The first of these, XXIa, was pale green; the second, XXIb, a more brilliant green. The very wide variability in the rate of occurrence of the colour segregants appears from table 1. The difference in segregation rate between the two clones (a) and (b) of diploid XXI is particularly striking (21.06 percent in the first and 0.076 percent in the second), although all the markers of the diploid segregated from each clone (see table 2) and the two clones were of about the same age. As is shown by the last column of table 1, the ratio of yellow to white segregants is comparatively much less variable.

Table 2 shows the types of segregant selected from the three diploids IV, VII and XXI (a and b), with the frequencies of each, subdivided according to the selection method by which they were detected. The figures given for deficiency segregants from diploid VII include five segregants selected for absence of capacity to produce penicillin.

All the markers carried by the parents of each diploid reappear in its segregants. The appearance of the expression of a marker (or mutant gene) in a segregant will be referred to hereinafter as "segregation" of that particular marker (or mutant gene). Caution is required in the evaluation of segregation data, as is suggested by a comparison between the colour segregants and the deficiency segregants from diploid XXIa; it will be observed that the rates of types (such as \( w \) me \( thi \) and \( y \) thi \( pr \)) which are detectable either as colour segregants or as deficiency segregants stand in widely different relations in the second and third columns of table 2. This indicates that at least one of the two selection methods is not very efficient. Since the deficiency method requires an elective enrichment the results obtained by this procedure are more likely to be biased.

This last observation, and the presumable occurrence of differences in fitness
### Table 2

**Segregants from heterozygous diploids**

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Colour selected</th>
<th>Deficiency selected</th>
</tr>
</thead>
</table>
| (A) Diploid IV: 63 y p cy/51 thi pr  
   (Green prototrophic)  
   y p cy thi | 1 | 1,335 |
| y p cy | 75 | 0 |
| thi pr | n.d.* | 8 |
| thi | (1) | 2 |
| (B) Diploid VII: 63 y p cy/46 me w  
   (Green prototrophic)  
   y p cy | 49 | 125 |
| y p | 31 | 5 |
| p | n.d. | (5)† |
| w me | 159 | n.d. |
| w | 20 | |
| (C) Diploid XXI: 86 y thi pr/46 me w  
   (clone a)  
   (Green prototrophic)  
   y thi pr | 9 | 289 |
| y thi | 1 | 7 |
| y pr | 129 | 0 |
| y | 7 | n.d. |
| w me thi | 131 | 1 |
| w me | 67 | 6 |
| w | 3 | n.d. |
| thi | n.d. | 8 |
| (clone b)  
   w me thi | 2 | |
| y pr | 1 | |

* The phenotype is indicated by the symbol of the expressed markers.
  Deficiency segregants were selected on limiting media.
* n.d. = not detectable.
† Selected for nonpenicillin production out of 476 colonies tested.

between different segregants, make it preferable to neglect (in principle) the figures concerning the frequencies of the segregants in working up the segregation data, and consider only the actual types obtained.

2. **Isolation of second-order segregants.** Genetic analysis of some of the segregants clones was carried out by study of further segregants from them. The selection of second-order segregants was carried out by the same methods as were adopted for first-order segregants. Table 3 shows the phenotypes of some first-order segregants and data of their second-order segregation. The second-order segregants are classified according to the selection method by means of which they were detected.

3. **Ploidy of segregants: inferences based on phenotype.** Ploidy determination of some of the segregants can be carried out on the basis of their phenotypes, on the
TABLE 3

Second-order segregants from heterozygous diploids

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No.</th>
<th>Phenotype</th>
<th>Colour selected</th>
<th>Deficiency selected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Total observed</td>
</tr>
<tr>
<td>(A) Diploid IV:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63 y p cy/51 thi pr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thi</td>
<td>1</td>
<td>thi cy y p</td>
<td>5</td>
<td>908</td>
</tr>
<tr>
<td>thi</td>
<td>2</td>
<td>thi pr</td>
<td>—</td>
<td>2,359</td>
</tr>
<tr>
<td>thi pr</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>3,448</td>
</tr>
<tr>
<td>cy y p</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>thi cy y p</td>
<td>2*</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(B) Diploid VII:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63 y p cy/46 me w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y p</td>
<td>1</td>
<td>y p me</td>
<td>(1)</td>
<td>1,068</td>
</tr>
<tr>
<td>y p</td>
<td>2</td>
<td>y p cy</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>y p me</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>cy y p</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p</td>
<td>2</td>
<td>p y cy</td>
<td>6</td>
<td>1,786</td>
</tr>
<tr>
<td>p w me</td>
<td>2</td>
<td>p w</td>
<td>2</td>
<td>3,448</td>
</tr>
<tr>
<td>p w</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>w</td>
<td>2</td>
<td>w me</td>
<td>4</td>
<td>2,025</td>
</tr>
<tr>
<td>(C) Diploid XXI:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 me w/36 y thi pr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thi</td>
<td>3</td>
<td>thi w me</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>thi w</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>1,385</td>
</tr>
<tr>
<td>thi y pr</td>
<td>45</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>thi y</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The allele symbols denote phenotypes. Data concerning segregants giving qualitatively similar second-order segregations are pooled together.

Deficiency segregants were selected on limiting media supplemented by the substances required by the first-order segregants in optimal concentrations.

* One was a second-order segregant.
† From diploid XXIb.

model of vegetative segregation in Aspergillus nidulans (Pontercorvo, Tarr Gloor and Forbes 1954; Pontercorvo and Käfer 1956). Any statement made in this paper henceforth on possible patterns of gene behaviour in vegetative segregation from diploids of Penicillium chrysogenum should be understood in terms of this model.

Following the random segregation of whole chromosomes which leads to haploidization, haploid nuclei with recombinant chromosomes may be formed. This can give rise to phenotypes with recombinant characters only in the case in which genes in repulsion are carried on different chromosomes. Mitotic crossing over (see below) cannot possibly give rise to phenotypically recombinant clones among the first-order
segregants. A phenotype carrying recombinant recessive characters therefore supplies the following information: (a) it is haploid; (b) the recombinant genes are on different chromosomes; (c) if, of two genes in coupling, only one has segregated, the other must be on a different chromosome.

Since mitotic crossing over occurs at the four-strand stage and is followed by equational segregation of the chromatids, a crossing over chromatid segregates either together with the chromatid with which it has exchanged a part, or together with the non crossing over chromatid, the partner of the latter. In the first case, the resultant nucleus remains heterozygous for all genes which were originally heterozygous; in the second case it will become homozygous for only those genes which are on the exchanged portion. This process may lead to partial or total segregation of the genes which are in coupling on the same chromosome arm. Since the rate of mitotic crossing over is so low, the possibility of the occurrence of more than one mitotic crossing over in the same nucleus may be practically ruled out. Once the study of haploid segregants has made it possible to establish linkage groups, the recovery of a phenotype in which segregation takes place of only a part of the genes in coupling belonging to the same linkage group supplies the following information: (d) it is diploid; (e) the nonsegregant genes are not distal to the segregant genes.

4. Haploid segregants. Only two types of recombinants between recessive alleles have been identified in the first-order segregants (table 2), thi cy y p from diploid IV and thi me w from diploid XXI. There is only one example of the first type, but the frequency of the second is high. On the basis of point (a) of section 3, these segregants must be haploid, and the gene thi/THI must be on a chromosome (I) different from the chromosome or chromosomes carrying the genes cy/CY, p/P, y/Y, me/ME and w/W. It must also be on a different chromosome from the one carrying pr/PR, on account of point (c) of section 3. If pr, which is in coupling with thi, were on the same chromosome as this, it ought always to be present in the above mentioned haploid recombinants. Since no other recombinant phenotype has been found in the first-order segregants of the three diploids examined, all the genes except thi/THI must probably lie on the same chromosome (II). Other haploid segregants include all those which carry the two markers thi and pr; since these lie on different chromosomes (see above), they can only segregate together in haploids. These segregants are thi pr from diploid IV and thi pr y from diploid XXI. The following linkage situation may thus be inferred for the three diploids examined (each line indicates a chromosome; the indicated sequence of the genes on the chromosomes is not significant).

\[(\text{IV }) \quad \text{thi} \quad \frac{pr}{(cy\ y\ p)} \quad (\text{cy}\ y\ p) \quad \text{VII} \quad \frac{(cy\ y\ p)}{(me\ w)} \quad \text{XXI} \quad \frac{thi}{(pr\ y)} \quad \frac{(pr\ y)}{(me\ w)}\]

From this diagram it is easy to visualize the possible types of haploid segregants, assuming that haploidization follows a process of irregular distribution of whole
5. Segregants of uncertain ploidy. In each heterozygous diploid, chromosome pair II (see diagram above) is always marked on both the component chromosomes, so that each haploid must show all the markers of linkage group II of one or other of the parent strains. Apart from this, the marker thi should theoretically be present in half the haploid segregants from diploid IV and XXI (see section 4); in cases in which it is not present there is no phenotypic evidence for the occurrence of haploidization. Strains which belong to this second category, of putative haploids, are the cy y p strains segregating from diploids IV and VII, the me w strains segregating from diploids VII and XXI and the pr y strains segregating from diploid XXI. These strains occur with relatively very high frequencies. Similar segregants could also arise from mitotic crossing over near the centromere of chromosome II if the alleles of each of the above mentioned groups all lie on the same chromosome arm (see above).

6. Diploid segregants. These include those in which the thiamine requirement (from diploids IV and XXI) is present alone, for the reasons given at the beginning of section 5, and also include those in which a part only of the markers of linkage group II carried by one parent or the other have segregated (see section 3, point c). To this group belong the y p, p and w segregants from diploid VII, and the y and w segregants from diploid XXI. Diploid segregants probably originate, as already suggested, from a mitotic crossing over process.

7. Use of diploid segregants in locating genes relative to the centromere. A segregating gene following mitotic crossing over is always accompanied by all the genes in coupling with it which lie on the same chromosome arm distal to it relative to the centromere. This assumption makes it an easy matter to fix the sequence of the various markers in coupling relative to the centromere when the phenotypes of the segregant diploids are known.

The three genes cy, y and p appeared in segregant diploids with phenotypes p and y p and also in the phenotype cy y p, whose ploidy is doubtful. Thus the centromere must be located outside the three genes on the cy side, they being themselves in the order cy y p.

Of the two genes w and me, only w appeared in segregants which are certainly diploid, alongside me w phenotypes whose ploidy is doubtful. Thus the centromere must be outside them on the me side and the two genes must be on the same chromosome arm.

Of the two genes y and pr, only y appeared in segregants which are certainly diploid, alongside pr y phenotypes whose ploidy is doubtful. Thus the centromere must be outside them on the pr side, and the two genes must be on the same chromosome arm, i.e. the same one which also carries the genes cy and p, seeing that both these are also on the same arm as the gene y.

The gene groups discussed must therefore have the following locations relative to the centromere:

\[
\begin{align*}
\circ & \text{cy y p} & \circ & \text{me w} & \circ & \text{pr y}
\end{align*}
\]
8. Information derived from study of second-order segregants. A strain from which recessive markers segregate is by definition heterozygous for these markers, and thus diploid. All the first-order segregants treated as diploid in section 6 and so far analysed have given second-order segregants (see table 3). In particular, they have been proved to be heterozygous for all the genes between the first-order segregant markers and the centromere of the chromosome to which they belong according to the gene map at the end of section 7, and also to be heterozygous for all genes located on a different chromosome from the one on which the first-order segregant markers lie. This is precisely what might be expected if the segregant diploids are assumed to originate from mitotic crossing over.

Second-order segregants may supply information on the relative position of markers in repulsion lying on the same chromosomes. The \( p \) segregants from diploid VII give second-order segregants for \( w \) and \( me \), while \( y \ p \) segregants give \( me \) segregants but no \( w \) segregants. This is compatible with the hypothesis that the gene \( w/W \) is between the genes \( Y/y \) and \( P/p \), and the gene \( me/ME \) between \( Y/y \) and the centromere:

\[
\begin{align*}
\begin{array}{c}
\text{me} \\
\text{ME} \\
\end{array}
\begin{array}{c}
Y \\
W \\
\end{array}
\begin{array}{c}
p \\
p \\
\end{array}
\end{align*}
\]

A homozygous \( p/p \) segregant might remain heterozygous for \( w/W \), but a homozygous \( y/p/y \ p \) segregant must be also \( W/W \) homozygous, though it might still be \( me/ME \) heterozygous. These points are made on the assumption that, as in *Aspergillus nidulans* (Pontecorvo 1953), \( w \) is epistatic to \( y \) and not vice versa.

9. Genotypes of some segregant diploids. The genotypes of some of the segregant diploids can be completely deduced from the second-order segregation data (see also section 8). This applies to the \( p \) and \( y \ p \) segregants from diploid VII, whose genotypes must be as follows:

\[
\begin{align*}
\begin{array}{c}
\text{(CY me)} \\
\text{(cy ME)} \\
\end{array}
\begin{array}{c}
Y \\
W \\
\end{array}
\begin{array}{c}
x \\
x \\
\end{array}
\begin{array}{c}
p \\
p \\
\end{array}
\end{align*}
\]

and also to the \( thi \) segregants from diploid XXI, whose genotype must be:

\[
\begin{align*}
\begin{array}{c}
\text{(PR me)} \\
\text{(pr ME)} \\
\end{array}
\begin{array}{c}
Y \\
W \\
\end{array}
\begin{array}{c}
x \\
x \\
\end{array}
\end{align*}
\]

The segregants mentioned must be derived from mitotic crossing over processes in correspondence with the points marked in the three diagrams. The markers shown as in the homozygous state are all phenotypically expressed in the first-order segregants, the markers shown as in the heterozygous state all emerge in the second-order segregants and the markers whose wild type allele is present in the homozygous state do not appear in the phenotype of either first or second-order segregants.

10. Proposed map for seven genes. No information has been gained on the position
relative to one another of the genes \( pr, cy \) and \( me \), which however must all be between gene \( y \) and the centromere of chromosome II—the first two on the basis of the first-order segregation results (section 7), and the third on the basis of the argument in section 8. The order of the genes \( y, w \) and \( p \) has also been fixed in section 8. They are distal to the genes already mentioned on the same arm of chromosome II, in the order given. Gene \( thi \) is on a different chromosome (section 4). These findings may be summed up in the following diagram:

\[
\begin{array}{c}
\text{O } \text{thi} \\
\text{O } (cy, me, pr) y w p
\end{array}
\]

11. Coupling and repulsion. The markers \( thi \) and \( pr \) are in coupling with \( y \) in diploid XXI, and in repulsion with it in diploid IV. Since \( thi \) is on a different chromosome from \( y \), and \( pr \) is on the same arm as \( y \), the situation particularly favours a check of the assumptions which have been made. The situation may be illustrated diagrammatically as follows:

IV

\[
\begin{array}{c}
\text{O } \text{thi} \\
\text{O } \text{pr} + \\
\text{O } + y
\end{array}
\]

XXI

\[
\begin{array}{c}
\text{O } \text{thi} \\
\text{O } \text{pr} y \\
\text{O } + +
\end{array}
\]

The data concerning segregation from the two diploids are precisely what would be expected (table 2): \( y \) segregates sometimes with and sometimes without \( thi \) in the two diploids IV and XXI (and the same is true of \( thi \) in its relation to \( y \)); on the other hand, \( y \) never segregates with \( pr \) in diploid IV, and in diploid XXI it generally segregates with \( pr \) but sometimes without it (mitotic crossing over between \( pr \) and \( y \)).

DISCUSSION

This paper is based on the model of vegetative segregation in \textit{Aspergillus nidulans}, as formulated by Pontecorvo, Tarr Gloor and Forbes (1954). Since the data might not in fact have suited the model, they supply some information on the applicability of this model to the species. Two types of evidence are particularly unequivocal; in the first place, all the strains which were analysed on the assumption (based on the phenotype) that they were diploids turn out to be diploids on the evidence of their second-order segregations, and in the second place, expectations concerning the behaviour of some genes which are in coupling in one diploid and in repulsion in another are fully satisfied. The experiments carried out have given fairly reliable results on the location of some genes, but on the other hand labour under serious difficulties. The chief of these is the necessity of obtaining an adequate estimate of the frequency of the segregation process. Such an estimate is presumably biased by at least three sources of selective pressure on the material: 1) differences in fitness in the segregant clones; 2) the clonal distribution of the segregant nuclei; and 3) variations in the practical detectability of the segregant strains.

The only way to overcome the first of these difficulties would be the application of methods which allowed immediate isolation of nuclei deriving from segregation processes. Correction of the bias by means of reconstruction experiments intended to estimate the fitness of various clones is laborious and doubtful, since the selection
may act both between nuclei on the same heterokaryotic hypha and between different hyphae.

The second difficulty could be obviated by selecting not more than one segregant of each type from a large number of heterozygous diploid colonies of monoconidic origin (PONTECORVO, TARR GLOOR and FORBES 1954). A precaution such as this would vastly increase the laboriousness of the experiments and would be of comparatively little effect unless the first source of error was reduced. In any case, segregant isolation from small samples taken from large numbers of conidia carefully mixed, as used in the present experiments, makes it unlikely that the same segregant clone would be isolated more than once.

The third difficulty crops up when segregant isolation is obtained after enrichment of the segregants by some kind of artificial selection method. The difficulty could be obviated by total isolation of the population in which the segregants are looked for, but this expedient again would increase the laboriousness of the work to an excessive degree.

Further work on gene location in *Penicillium chrysogenum* by study of vegetative segregation from heterozygous diploids will not make appreciable progress until markers are available allowing for very efficient segregant detection; generally speaking, the type of marker required is one which confers on haploid or homozygote strains a very definite advantage over such strains which are heterozygous for this marker. Markers such as recessive suppressor of adenine requirement and incompletely dominant resistance to acriflavine (PONTECORVO and KÄFER 1956) are of this type.

The gradually increasing body of knowledge of the genetic map of Penicillium will make it continually easier to design new experiments for this purpose, since reference points for the location of new genes will become available and criteria will be set up for the distinction of strains into haploid and diploid on the basis of phenotype.

**SUMMARY**

An analysis of vegetative segregation has been carried out in three heterozygous diploid strains of *Penicillium chrysogenum*, using as model the vegetative segregation of *Aspergillus nidulans* (PONTECORVO, TARR GLOOR and FORBES 1954). The results do not contradict the model in any case. On the basis of the segregation data of seven markers it has been possible to identify two (mitotic) linkage groups and the sequence of a number of genes.

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**LITERATURE CITED**


1953  The genetics of *Aspergillus nidulans*. Advances in Genet. 6: 141–238.


