12. G. SERMONTI. — **Genetics of Penicillium Chrysogenum. — II. Segregation and recombination from a heterozygous diploid.**

Summary. — From two strains of *Penicillium chrysogenum* with different nutritional requirements and different colours of conidia (parent strains), a balanced heterokaryon (22y13 + 7w16) was obtained. From this a heterozygous diploid strain was isolated, which had all the properties of the common ancestor (Q 176 Wis) of the parent strains (22y13/7w16) (Pontecorvo & Sermonti, 1954).

The present study concerns the segregants of the above-mentioned diploid (new symbol: 55 me hy/66 ad cy w).

After plating about a thousand conidia of the diploid on CM (complete medium) it was possible to distinguish and separate from the great number of colonies which retained the diploid appearance, a few dozen colonies which gave the colour (yellow or white) of one of the parents (termed segregants selected for colour). When plating was carried out on MM (minimal medium) to which the substances required by the parent strains were added in limiting quantities, there could be observed a few colonies with very stunted growth among the normally-developing colonies which constituted the large majority. When transferred on to CM and MM to which no growth factors had been added, a fair number of these were shown to have nutritional requirements (termed segregants selected for nutritional requirements).

All the selected segregants were tested for all the markers of the parent strains, and were classified as regards colour and nutritional requirements.

All the segregants selected directly from the diploid (first order segregants) showed (isolated or in various combinations) the recessive properties of one or the other parent strain. No strain showed recombination of recessive properties deriving from both parent strains.

Further selection of segregants from four first order segregants (second order segregants) gave rise to 16 strains, 10 of which showed recombination of recessive properties from both parent strains.

This represents a complete confirmation of the process of « parasexual » recombination in the fungus imperfectus, *Penicillium chrysogenum* Thom.

Riassunto. — Da due ceppi di *Penicillium chrysogenum* con diverse richieste nutrizionali e differente colore dei conidi (ceppi genitori) è stato ottenuto un eterocarion bilanciato (22y13 + 7w16). Da questo è stato

isolato un ceppo diploide eterozigote, con tutte le proprietà dell'ascendente comune (Q176 Wis) dei ceppi genitori (22y13/7w16) (Pontecorvo & Sermonti, 1954).

Il presente lavoro riguarda lo studio dei segreganti dal diploide suddetto (nuovo simbolo: 55 me hy y / 66 ad cy w).

In seguito a piastramento di un migliaio di conidi del diploide su CM (terreno completo) si possono individuare e prelevare tra la massa delle colonie che conservano l'aspetto del diploide, alcune decine di colonie che presentano il colore (giallo o bianco) di uno dei genitori (segreganti selezionati per il colore).

Se il piastramento è eseguito su MM (terreno minimo) cui le sostanze richieste dai ceppi genitori sono aggiunte in quantità limitanti, tra la massa delle colonie che si sviluppano normalmente, se ne presentano alcune a crescita molto stentata. Trasferite su CM e MM buona parte di queste risultano avere richieste nutrizionali (segreganti selezionati per le richieste nutrizionali).

Tutti i segreganti selezionati sono stati saggiati per tutte le proprietà dei ceppi parentali e classificati in riguardo al colore e alle richieste nutrizionali.

I segreganti selezionati direttamente dal diploide (segreganti di primo ordine) presentano, isolate o in varie combinazioni, proprietà recessive dell'uno o dell'altro ceppo genitore. Nessun ceppo presenta però la ricombinazione di proprietà recessive provenienti dai due ceppi genitori.

L'ulteriore selezione di segreganti da quattro segreganti di primo ordine (segreganti di secondo ordine) ha dato luogo a 16 ceppi, 10 dei quali presentavano la ricombinazione di proprietà recessive dei due ceppi genitori.

Ciò rappresenta un'esauriente conferma del processo di ricombinazione parasessuale nel fungo imperfetto *Penicillium chrysogenum* Thom.

Résumé. — En partant de deux souches de *Penicillium chrysogenum* qui avaient des exigences nutritives différentes et dont les conidies étaient de couleurs différentes (souches parentales) on a obtenu un hétérokaryon balancé (22y13-7w16). A partir de celui-ci, on a isolé une souche hétérozygote diploïde, présentant toutes les propriétés de l'ancêtre commun (Q 176 Wis) des souches parentales (22y13/7w16) (Pontecorvo et Sermonti, 1954).

L'étude actuelle concerne les ségrégants des diploïdes susdits (nouveau symbôle: 55 me hy / 66 ad cy w).

Après « plating » d'un millier de conidies du diploïde sur CM (milieu complet) il a été possible de distinguer et de séparer parmi le grand

nombre de colonies qui conservaient l'apparence diploïde, quelques douzaines de colonies ayant la couleur jaune et blanche) d'une des souches parentales (appelées ségrégants choisis par la couleur). Lors du « plating » sur MM (milieu minimum) auquel les substances nécessaires aux souches parentales ont été additionnées en quantités sub-optimales, on a pu observer une croissance très médiocre de quelques colonies, parmi celles qui se développaient normalement et qui constituaient la grande majorité. En les transportant sur CM et MM, un bon nombre de cellesci avaient des besoins nutritifs (appelées ségrégants choisir par les besoins nutritifs).

Tous les ségrégants sélectionnés furent examinés pour toutes les caractéristiques des souches parentales et furent classés par rapport à leur couleur et leurs exigences nutritives.

Tous les ségrégants sélectionnés directement du diploïde (ségrégants de premier ordre) on montré (isolés ou différemment combinés) des propriétés de l'une ou de l'autre des souches parentales. Aucune souche n'a montré une recombinaison des propriétés récessives dérivant des deux souches parentales.

Une sélection ultérieure de ségrégants, en partant de quatre ségrégants de premier ordre, (ségrégants de second ordre) donna naissance à 16 souches, dont 10 montraient la récombinaison de propriétés récessives des deux souches parentales.

Ceci est une confirmation complète du processus de recombinaison « parasexuelle » dans le fungus imperfectus, *Penicillium chrysogenum* Thom.

Zusammenfassung. — Aus zwei Stämmen von *Penicillium chrysogenum*, die verschiedene Ernährungsbedürfnisse und verschiedene Konidienfarbe besassen (Elternstämme), wurde ein balanziertes Heterocaryon (22y13 + 7w16) erhalten. Aus diesem wurde eine heterozygoter, diploider Stamm isoliert, der alle Eigenschaften des Stammes (Q 176 Wis) hatte, aus dem die beiden Eltern (22y13/7w16) hervorgegangen waren (Pontecorvo und Sermonti, 1954).

Die gegenwärtigen Untersuchungen behandeln die Aufspaltungen des oben erwähnten Diplonten (neues Zeichen: 55 me hy/ 66 ad cy w).

Nach Aussaat von ungefähr tausend Konidien des Diplonten auf CM (vollständigem Medium) war es möglich unter der grossen Anzahl der diploiden Kolonien einige Dutzend Kolonien zu isolieren, die die Konidienfarbe der Elternstämme (gelb oder weiss) besassen. (Auswahl der Aufspaltungen hinsichtlich der Farbe!). Nach einer Aussaat auf einem MM (Minimalmedium), dem in begrenztem Masse die von den Eltern benö-

tigten Stoffe zugesetzt wurden, konnte man unterden sich in der Mehrzahl normal entwickelnden Kolonien einige mit kümmerlichen Wachstum beobachten. Bei Übertragung dieser Kolonien auf ein CM und ein MM, denen keine Wachstumsfaktoren zugesetzt worden waren, zeigte sich, dass eine grosse Anzahl Ernährungsdefekte beass. (Aufspaltung hinsichtlich der Ernährungsbedürfnisse!).

Alle diese Aufspaltungen wurden auf alle Eigenschaften der Elternstämme getestet und klassifiziert hinsichtlich ihrer Farbe und ihrer Ernährungsbedürfnisse.

Alle Aufspaltungen, die unmittelbar aus dem Diplonten isoliert wurden (Auspaltungen erster Art), zeigten (allein oder in verschiedenen Kombinationen) die rezessiven Eigenschaften des einen oder anderen Elternteils. Kein Stamm zeigte Neukombination der rezessiven Eigenschaften der Elternstämme.

Die weitere Selection von Aufspaltungen aus vier Aufspaltungen erster Art ergab 16 Stämme (Aufspaltungen zweiter Art). 10 dieser Stämme zeigten Neucombinationen der rezessiven Eigenschaften der Eltern.

Dies ist die vollständige Bestätigung des Vorganges der « parasexuellen » Neukombination bei dem imperfekten Pilz *Penicillium chrysogenum* Thom.

Recombination between characters of different strains, outside or in the absence of a sexual cycle, was recently obtained in three species of filamentous fungi, viz., *Aspergillus nidulans* (Roper, 1952; Pontecorvo & Roper, 1952; 1953), *Aspergillus niger* (Pontecorvo, 1952; Pontecorvo, Roper & Forbes, 1953) and *Penicillium chrysogenum* (Pontecorvo & Sermonti, 1953; 1954).

The procedure followed in all three cases consisted of the following three steps:

(1) synthesis of balanced heterokaryons between pairs of mutant strains differing in nutritional requirements and in other properties;

(2) isolation from the balanced heterokaryons of strains carrying heterozygous diploid nuclei in their hyphae;

(3) selection from the heterozygous diploid strains of segregants showing the properties of the original strains in new combinations.

The processes leading to recombination of characters otherwise than *via* sexual reproduction are termed « parasexual » (Pontecorvo, 1953).

In the case of *Penicillium chrysogenum*, the greatest difficulties in

the introduction of the parasexual cycle were encountered in the first step i.e. the formation of balanced heterokaryons (Pontecorvo & Sermonti, 1954). Facile techniques for the synthesis of heterokaryons in *P. Chrysogenum* were developed only subsequently (Sermonti, 1954).

The isolation of heterozygous diploids from balanced heterokaryons was, however, as easy as in the case of *A. niger* (Pontecorvo, Roper & Forbes, 1953). From two balanced heterokaryons two heterozygous diploids were obtained, respectively designated by the symbols: 22y DW1/7w DW4 and 22y13/7w16.

From the diploid 22y DW1/7w DW4, first and second order segregants were isolated (Pontecorvo & Sermonti, 1953; 1954). Among the latter, one strain with white conidia (like the parent 7w DW4) and requiring hypoxanthine (like the parent 22y DW1) showed recessive properties of the original strains in a new combination.

The subject of this paper is the isolation of segregants and recombinants from the heterozygous diploid 22y13/7w16.

The paper aims at presenting a fuller and more exhaustive documentation of recombination in *P. chrysogenum*.

(1) Synthesis of heterozygous diploid 55/66 (= 22y13/7w16).

The procedure by means of which synthesis of the diploid 22y13/7w16 was obtained, has been described in detail in an earlier paper (Pontecorvo & Sermonti, 1954) and will here be referred to only summarily.

The first stage of the work consisted in the formation of two strains, each differing from the original strain (Q 176) in three pairs of alternative characters, and one differing from the other in six pairs. The new characters were obtained by means of successive ultra-violet treatments. The strains obtained were given the code numbers 22y13 and 7w16. In this laboratory collection these strains now carry the respective code numbers *55 me hy y* and *66 ad cy w*, and will henceforth be referred to by these symbols. The number indicates the place of collection, and the letters refer to the properties induced, in inverse order of induction (see table 1):

me = requires methionine for normal growth on MM.
hy = requires either hypoxanthine or adenine.
y = yellow colour of conidia.
ad = requires adenine.
cy = requires either cysteine or methionine.
w = white colour of conidia.

TAB. 1. — Properties of heterozygous diploid 55/66 compared with those of the two haploid component strains, and of the original haploid wild type Q 176 Wis

Strain	*Growth factors requirement*			*Colour of conidia*
	On MM + adenine (a)	*On MM + methionine (b)*	*On MM*	
Q 176 Wis	—	—	—	green
55 me hy y	methionine	adenine/hypoxanthine*	a + b	yellow
66 ad cy w	methionine/cysteine *	adenine	a + b	white
55/66	—	—	—	green

* The symbol / indicates alternative requirements.

From the two strains a balanced heterokaryon was synthesized, that is to say, a new strain carrying nuclei from both parent strains in its hyphae. The heterokaryon was established by isolating small fragments of a more vigorously growing mycelium from the zones of contact between colonies of two types growing on complete medium agar.

The heterokaryon developed on minimal medium (i.e. on a medium in which the growth factors required by the parent strains were absent) and gave a yellowish sporing surface. This could be transferred by means of mycelial fragments and single hyphae, but when transferred by conidia it segregated into the two parent types.

The conidia of *P. chrysogenum* contain a single nucleus (Tonolo & Urbani, 1953) and are therefore not able to maintain the heterokaryotic state.

8 million conidia of the heterokaryon were plated in Petri dishes on minimal medium. Only a few colonies developed (about 100 for every 10^6 conidia plated): the majority of these were new heterokaryons, presumably developed from hyphal fragments accidentally plated together with the conidia. Three colonies grew more than the others and gave a green sporing surface. When isolated and purified by isolation of a single conidium, they were shown to be heterozygous diploids. Similar heterozygous diploids were also obtained as sectors of the new heterokaryotic colonies. A diagram of the « parasexual » process in *P. chrysogenum* is given in fig. 1.

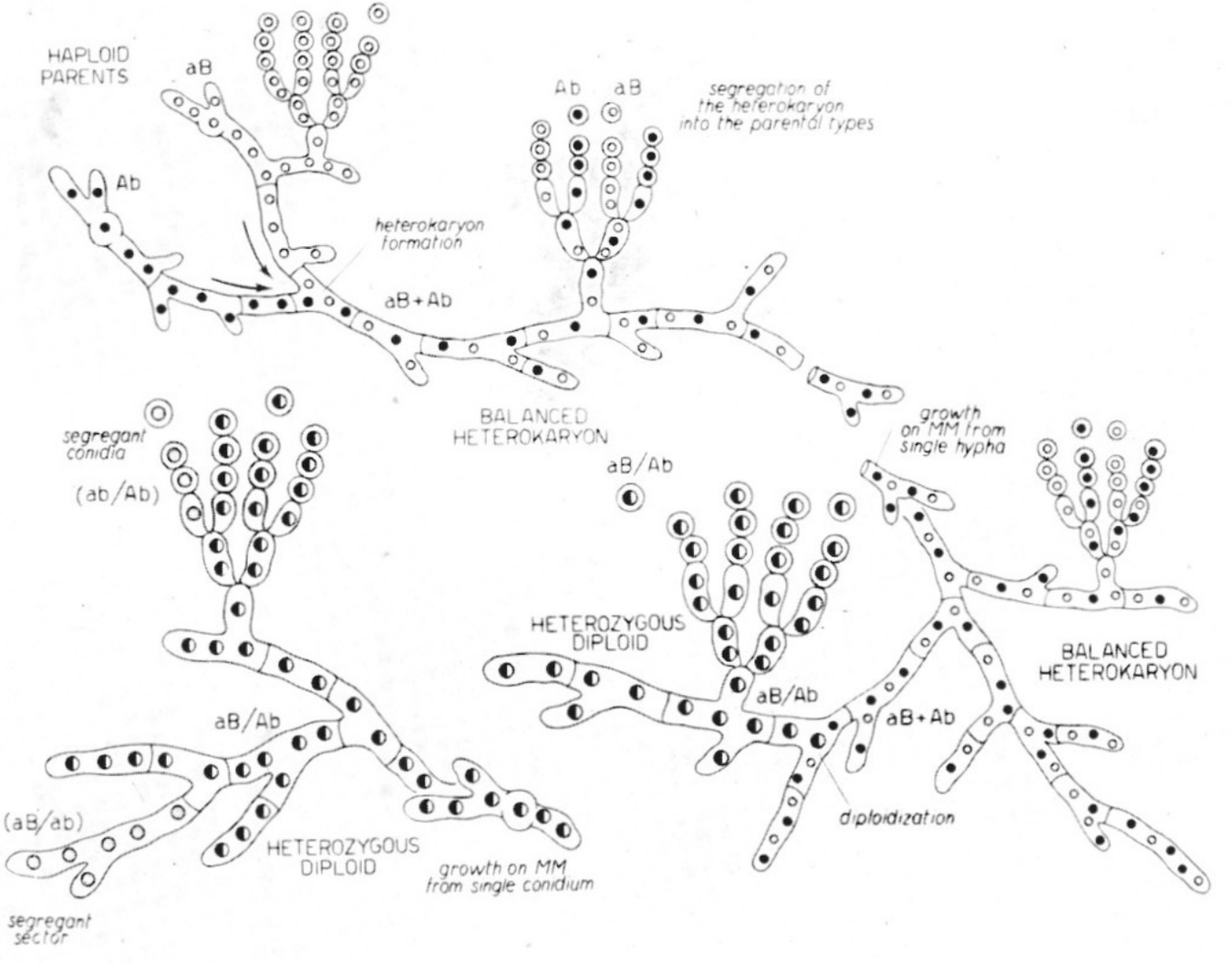

Fig. 1. - Scheme of the " parasexual " process in *Penicillium chrysogenum* Thom. First step: formation of the heterokaryon from two haploid strains; second step: formation of heterozygous diploid nuclei within the heterokaryon; third step: recombination in the diploid nuclei. Formation of haploid nuclei from diploid, though not yet observed, may reasonably be assumed to occur.

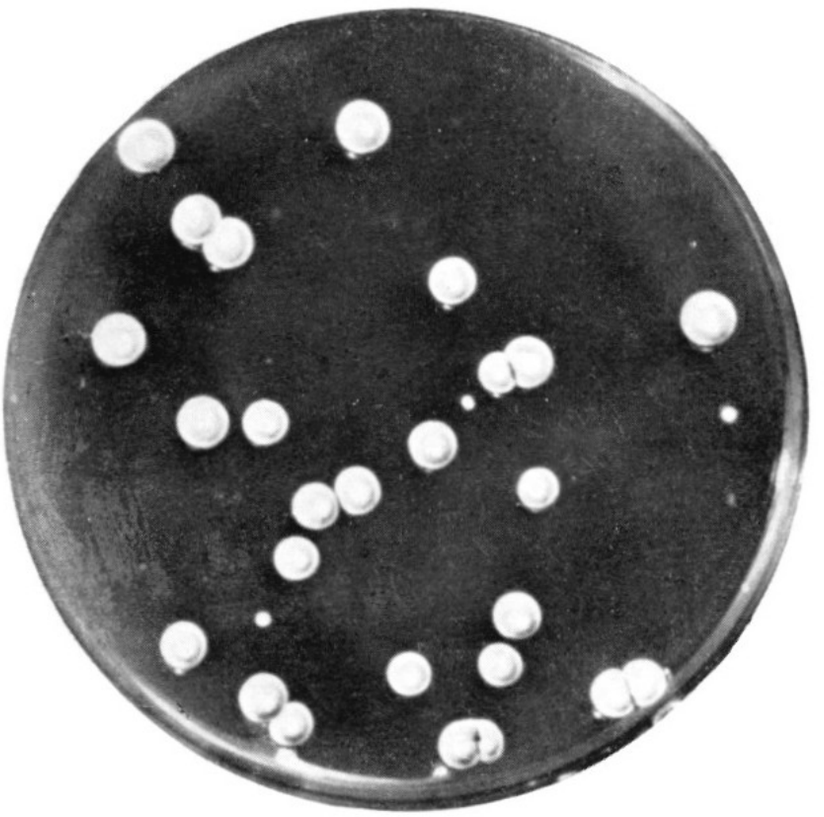

Fig. 2. - Selection of segregants for nutritional requirements on limiting medium. The three small colonies will be picked up and tested.

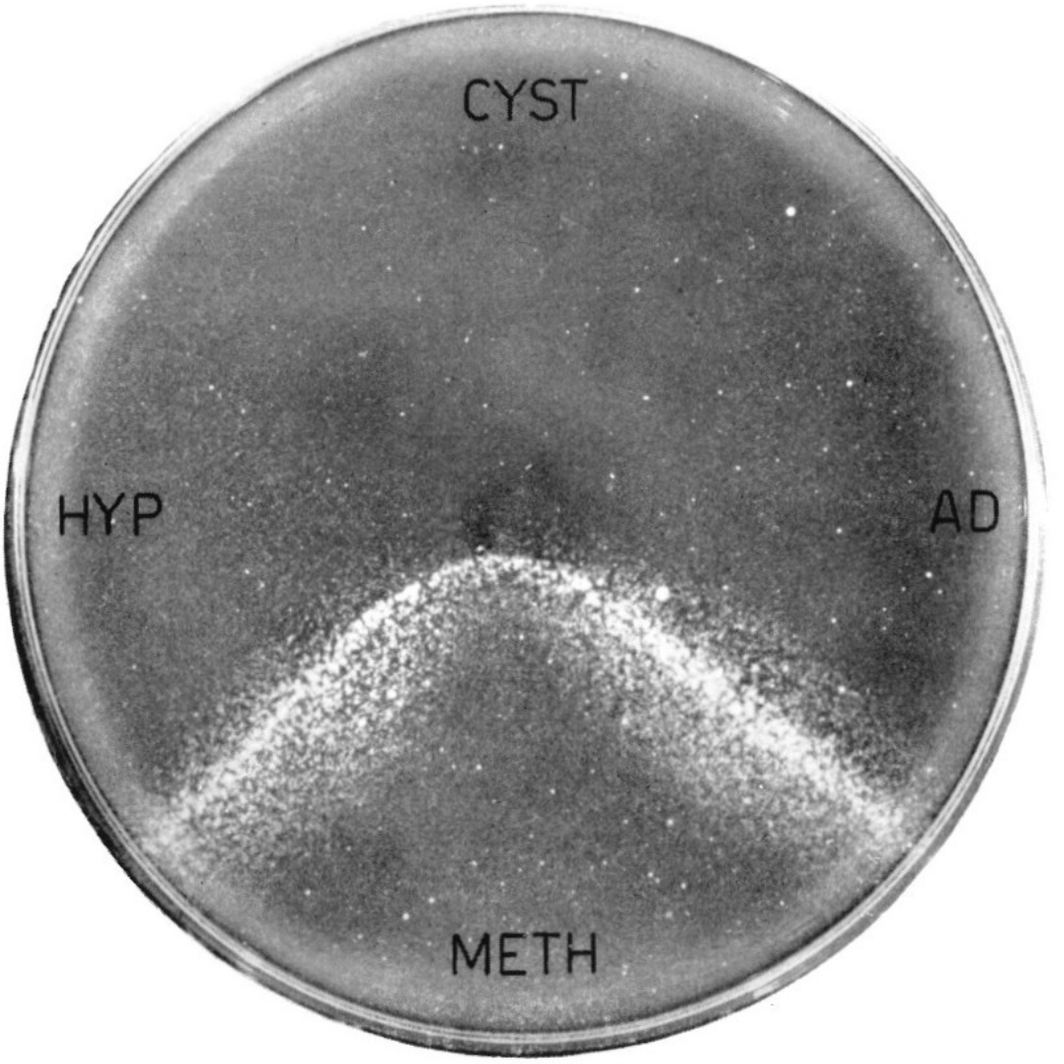

Fig. 3. - Auxanographic test: strain *55 me hy y* (requires methionine + adenine/hypoxanthine). Growth occurs in the zones where both requirements are available.

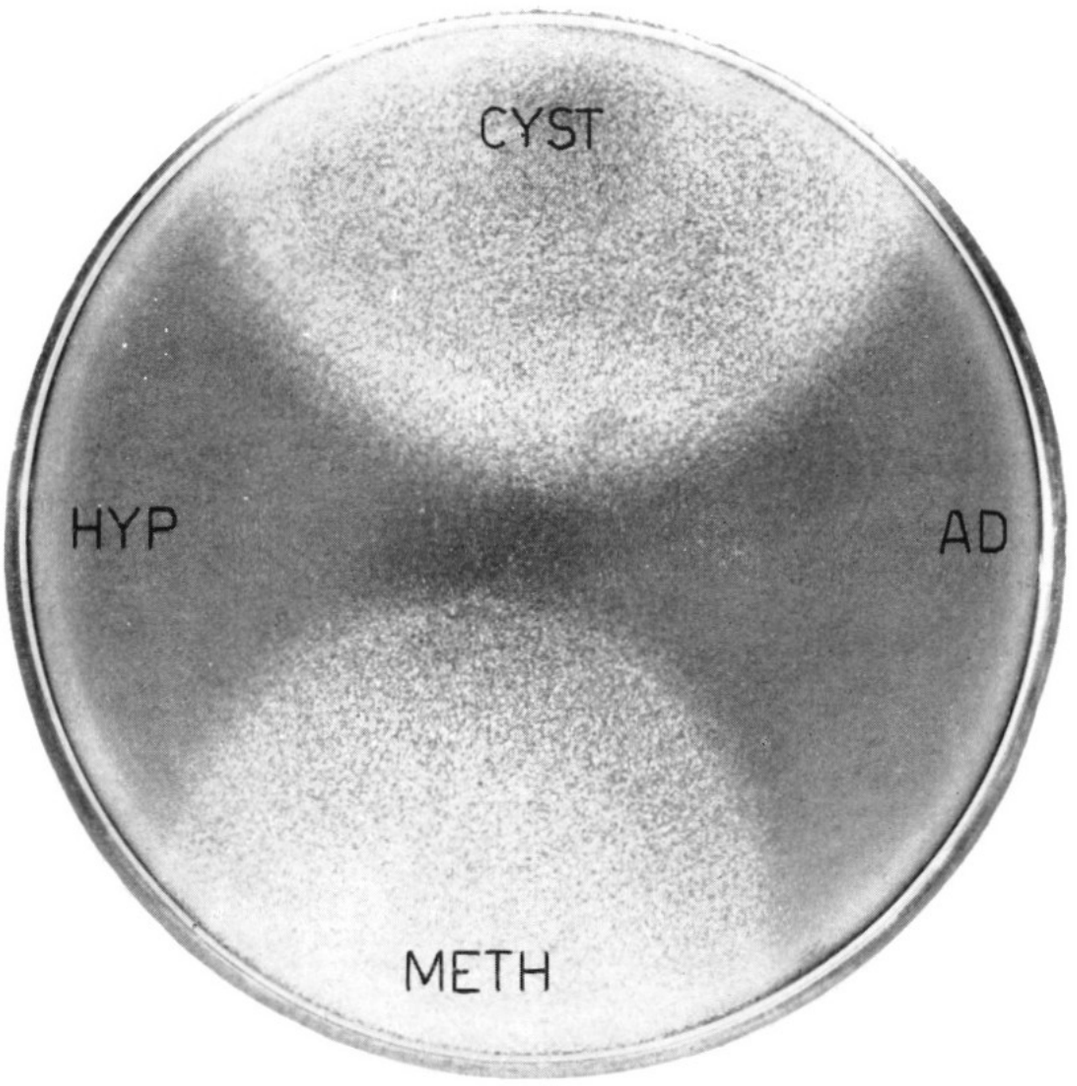

Fig. 4. - Auxanographic test: first order segregants from diploid 55 me hy y/66 ad cy w, cystine-less (requires methionine/cystine). Growth occurs where either requirement is available.

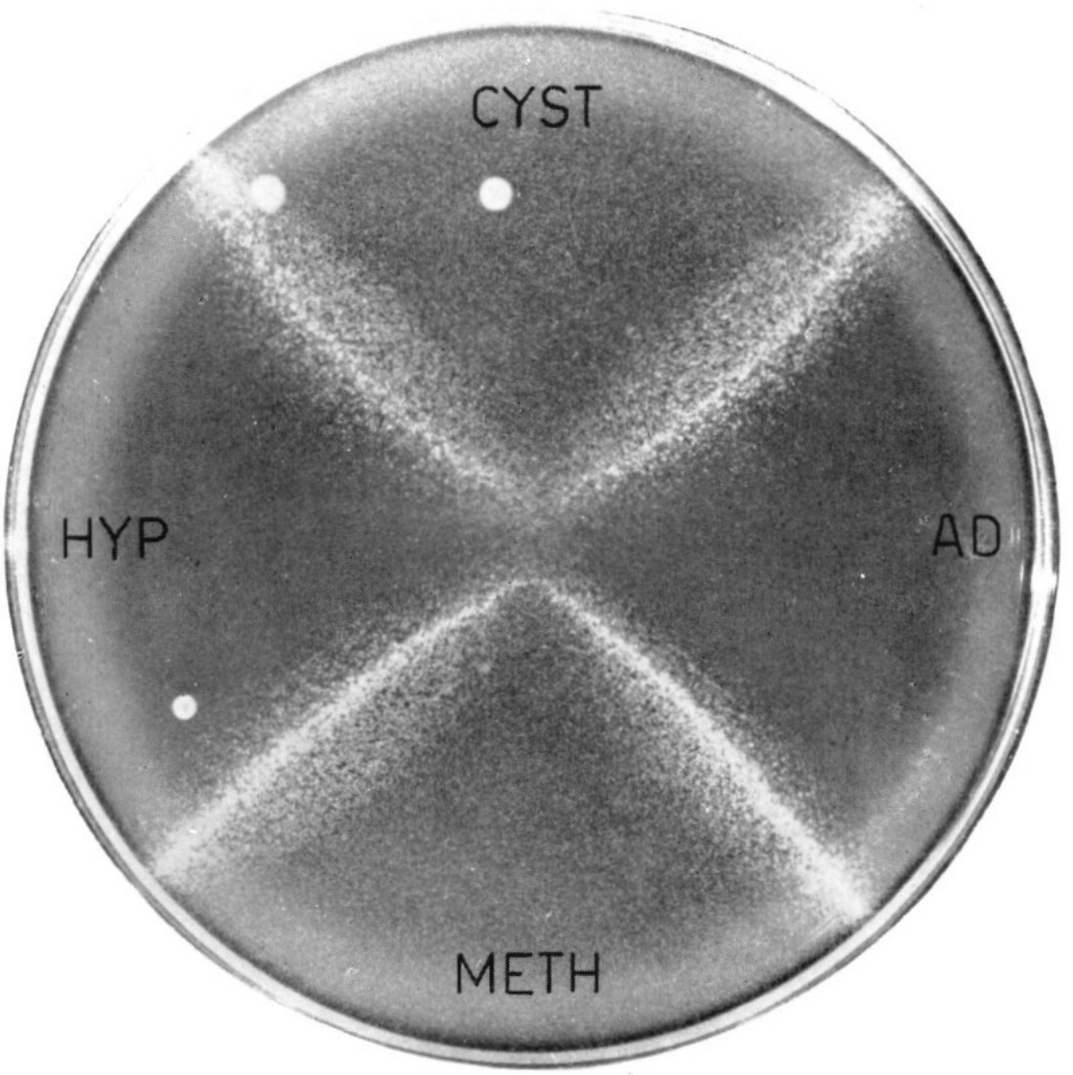

Fig. 5. - Auxanographic test: second order segregant from diploid 55 me hy y/66 ad cy w (from a hypoxanthine-less first order segregant), white, cystine-less, hypoxanthine-less (requires methionine/cystine + adenine/hypoxanthine). Growth occurs in the zones where two complementary requirements are available.

Fig. 6. - Colonies of heterozygous diploid 55 me hy y/66 ad cy w. A yellow segregant is sectoring out from one of them.

(2) Properties of heterozygous diploid 55/66.

The heterozygous diploid 22y13/7w16 will henceforth be indicated by the code number 55/66, or more fully:

$$\frac{55 \text{ me hy y}}{66 \text{ ad cy w}}$$

This grows normally on minimal medium, giving rise to regular colonies which continue to grow when transferred by a single conidium. It gives green conidia which microscopically are of greater dimensions than those of the haploid parents of their common ancestor.

It thus does not show any of the properties induced by mutation in the strain *55 me hy y* and *66 ad cy w* (table 1). (By mutation is meant the sudden appearance of a clone which differs from the original clone in respect of any property, after treatment with a mutagenic agent). This means that each of the properties induced by mutation in one of the parent strains is recessive in respect of the original state of such property, retained in the other strain.

In genetic terms this means that the six mutations induced occurred in different loci, even if, as in the case of *y* and *w*, they concern the expression of the same character. As it generally happens, the mutant alleles are recessive relative to the original alleles.

The properties induced by mutation in the parent strains will henceforth be termed *recessive properties*, and will be designated by small letters. The strains will be designated by the symbols of the recessive properties that they show phenotypically. Only the full designation of the heterozygous diploid contains the symbols of latent recessive properties, as an indication of its genotype.

(3) Materials and techniques.

The techniques utilized in this work, where not explicitly stated, are the same as those described in the first paper of this series (Sermonti, 1954).

The media adopted were:

a *minimal medium* (MM): modified Czapek-Dox;

a *complete medium* (CM), « Costantino », casein hydrolysate, 1.5 g; « Costantino » peptone 1.5 g; « Costantino » autolysed yeast, 1.5 g; corn-steep liquor, 10 g; hydrolysed yeast nucleic acid, 3 ml;

vitamin solution, 1 ml; glucose, 20 g; agar, 20 g; water, to 1 litre. For details see the quoted paper.

(4) Selection of first order segregants.

The heterozygous diploids synthesized in *Aspergillus* (Pontecorvo, 1953) and in *Penicillium chrysogenum* (Pontecorvo & Sermonti, 1953; 1954) are not completely stable, and show a tendency to segregation of separate clones, in which recessive properties of the original haploid strains reappear in the original combinations or in new combinations.

Segregation from the heterozygous diploid strain of clones carrying the recessive properties of the parent strains is a proof of the heterozygous, and therefore diploid, state of the strain being tested.

Identification of the segregant strains is easy when these have the conidial colour of one of the parent strains, yellow or white, which contrasts with the green colour of the heterozygous diploid. Yellow and white segregants can be detected as yellow or white sectors or spots, (fig. 6) which occasionally appear from heterozygous diploid colonies, or as occasional single colonies appearing among the many colonies which have retained the green colour of the heterozygous diploid. after plating its conidia.

It is more difficult to detect the segregants that differ from the diploid in nutritional requirements alone. In this case individual colonies had to be tested, arising after plating conidia of the diploid on CM.

A method for rough visual selection of auxotrophic segregants (i.e. with nutritional requirements), which was utilized in the course of the present study, is described later.

The diploid 55/66, before being studied in relatior. to segregation, was purified by isolation of a single conidium with the micro-manipulator (Pontecorvo & Sermonti, 1954).

The segregants deriving directly from the purified heterozygous diploid are termed first order segregants.

Selection for colour of first order segregants from the diploid 55/66 was carried out as follows:

Conidial suspension of the heterozygous diploid 55/66 were spread on agar CM, in order to obtain, in different series of Petri dishes, 5, 30 and 150 colonies per dish. After one week it was possible to observe yellow and white colonies among the green colonies of the heterozygous diploid that had appeared; they were well isolated in the dishes containing a small number of colonies, but were closely intermingled with the green colonies in the other dishes. The percentage of segregants for

colour which could be observed in the colonies that appeared, diminished markedly as the density of the colonies per dish increased. Altogether, out of 1330 colonies counted, 97 segregants were selected for colour. These were purified by streaking on CM and were then re-isolated, checked once more for colour and then tested for nutritional requirements.

Of 81 colour segregants subjected to further tests, 72 were yellow and 9 white. These were spot-transferred on to different dishes of MM, supplied with substances required by the original strains, in different combinations, so that their nutritional requirements could be ascertained, according to the criteria listed in table 2.

TAB. 2. — Media for testing § the nutritional requirements of segregants from heterozygous disploid $\frac{55\ me\ hy\ y}{66\ ad\ cy\ w}$

Possible segregants *	*Supplements to MM* **					
	None	*Meth., Aden.*	*Meth. Hypox.*	*Methion.*	*Aden., Cyst.*	*Adenine*
cy hy	—	+	+	—	+	—
cy ad	—	+	—	—	+	—
cy	—	+	+	+	+	—
me hy	—	+	+	—	—	—
me ad	—	+	—	—	—	—
me	—	+	+	+	—	—
hy	—	+	+	—	+	+
ad	—	+	—	—	+	+
prototroph	+	+	+	+	+	+

§ +: growth; —: no growth.

* No other kind of segregant is detectable, due to the epistasis of *me* to *cy*, and of *ad* to *hy*.

** Methionine satisfies a requirement of cysteine (not vice-versa), adenine satisfies a requirement of hypoxanthine (not vice-versa).

69 strains out of 81, all with yellow conidia, showed the double requirement of methionine + hypoxanthine. Two strains had single requirements, ten were prototrophic (tab. 3A). The nutritional requirements of 14 of the auxotrophic strains, among them the two with single requirements, were checked by the auxanographic test (Pontecorvo, 1949) (fig. 3, 4, 5).

The details of the characteristics of the first order segregants for colour are given in table 3A.

TAB. 3. — First order segregants from diploid $\frac{55\ me\ hy\ y}{66\ ad\ cy\ w}$

Colour of colonies selected	A. - *Selected for colour*							
	Proto-troph	*hy*	*me*	*cy*	*ad*	*hy me*	*ad cy*	*Tot*
yellow	2	0	1	0	0	69	0	72
white	8	0	0	1	0	0	0	9
	10	0	1	1	0	69	0	81
	B. - *Selected for requirements*							
green	—	2	0	3	0	0	0	5
yellow	—	0	1	0	0	4	0	5
white	—	0	0	0	0	0	1	1
	—	2	1	3	0	4	1	11

Selection for nutritional requirements of the first order segregants of the diploid 55/66 was obtained as follows:

A minimal medium was prepared, supplied with methionine and adenine (i.e., with substances able to restore normal growth of any possible segregant) in limiting concentrations. By limiting concentration of a substance is meant the concentration which will only allow stunted development of a colony requiring that substance for its growth (Sermonti, 1954). Methionine was added to MM in concentration in agar of 0.02 mg/ml, adenine in a concentration of 0.001 mg/ml.

A conidial suspension of the diploid was spread over a number of dishes of such a medium, in order to get from 30 to 100 colonies per dish. The colonies which showed markedly reduced growth six days after plating were spot transferred on to dishes of CM and MM (fig. 2). A similar method was devised by Davis (1949) for selection of auxotrophs in bacteria. From 1101 colonies that appeared on 20 dishes, 47 colonies with reduced growth were collected. Of these 11 showed nutritional deficiency.

All eleven colonies were tested by the auxanographic method for nutritional requirements and were classified by colour. The details of this experiment are given in table 3B.

From the results in tables 3A and 3B it is possible to infer that, when the conidia of heterozygous diploids are plated, segregants appear showing the recessive properties of the original strains either isolated or in combination. It is also seen that of the 92 first order segregants observed, none showed recessive properties derived from different parents.

(5) Selection of second order segregants.

Four of the first order segregants were used for detection of further segregants (second order segregants), viz.:

one prototrophic white strain (*w*),
one prototrophic yellow strain (*y*),
one green hypoxanthine-less strain (*hy*),
one green cysteine-less strain (*cy*) (fig. 4).

From the first three strains the second order segregants were selected for nutritional requirements; from the fourth, for colour.

The procedures adopted were the same as those adopted for the first order segregants. In the case of the strain *hy*, hypoxanthine in optimal concentration was added to the minimal medium with methionine and adenine in limiting quantities.

All the nutritional requirements were tested by the auxanographic technique.

Altogether 16 segregants were isolated. Among these, 10 showed recessive properties from the two parents in new combinations, and were thus true recombinants.

Tab. 4. — Second order segregants from diploid *55 me hy y/66 ad cy w*

First order segregants phenotypes	*Observed colonies*	*Selection of segregants for*	*Selected colonies*	*Second order segregants*			
				Phenotypes *	*Number*	*Tot.*	*% among selected*
w	215	requirements	15	*w me hy*	3	3	20
hy	357	requirements	9	y me hy *w cy hy*	5 2	7	78
y	967	requirements	22	y me hy *y cy*	1 2	3	17
cy	688	colour	5	*y (cy) me hy*	3	3	60

* among the phenotypes of the second order segregants the recombinants are in italics.

The second order segregants are listed in table 4; the recombinants are given in italics. They belong to four different groups:

(1) *w me hy*: white conidia (from strain 66), methionine and hypoxanthine requirement (from strain 55).

(2) *w cy hy*: white conidia and cysteine requirement (66), hypoxanthine requirement (55) (fig. 5).

(3) *y cyst*: yellow conidia (55) and cysteine requirement (66)

(4) *y me (cy) hy*: yellow conidia and methionine + hypoxanthine requirement (55), presumed cysteine requirement (66). The cysteine requirement, presumed since the strain is a segregant from a cysteine-less first order segregant, could not be verified in the presence of methionine requirement.

We have thus exhaustively confirmed recombination between recessive properties of two different strains of *Penicillium chrysogenum*.

(6) Discussion.

The results of this work confirm the occurrence of the phenomenon of « parasexual » recombination in *Penicillium chrysogenum* Thom, as observed by Pontecorvo & Sermonti (1953; 1954)

The data relating to the relative and absolute frequency of segregants with regard to different properties cannot be considered as an expression of the rate of the processes of segregation, since no effort was made to avoid the effect of clonal distribution of the segregant nuclei, and in any case it is possible that these multiply differentially.

In *Aspergillus nidulans* (Pontecorvo, 1953a), segregation of recessive properties of the parent strains from heterozygous diploids seems to occur by means of two different processes: somatic crossing-over and somatic reduction.

In the first case, the segregants retain their diploid state, carrying one or more (originally heterozygous) genes in the recessive homozygous state, the remainder being in the primitive heterozygous state. In the second case, the segregants carry haploid nuclei which may contain chromosomes deriving from two different parents.

After somatic crossing-over, recombination between recessive properties of the two parents can take place only by means of two successive segregation processes (except in the unusual case where two somatic

crossing-over are produced simultaneously). The segregants, still heterozygous for some genes, can give rise to further segregation.

The haploid segregants derived from a process of somatic reduction can show recombination of recessive properties of the two parent strains in a first segregation, and do not give rise to further segregation.

Among the first order segregants of the diploid studied, none showed recombination of recessive properties of the two parents, and, on the other hand, all four segregants utilized for research on further segregation, produced second order segregants. This suggests that the segregation process occurs in *P. chrysogenum* chiefly by means of somatic crossing-over (Stern, 1936), as in *Aspergillus nidulans* (Pontecorvo, 1953a).

The demonstration of « parasexual » recombination in *P. chrysogenum* makes possible the application of the methods of biochemical genetics to the study of biosynthetic mechanisms in this species.

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