GENETICS OF PHYTOPATHOGENIC FUNGI. XIV. THE PARASEXUAL CYCLE IN *PENICILLIUM EXPANSUM*

E. D. GARBER AND L. BERAHA

*Department of Botany, University of Chicago, and Agricultural Research Service,
Market Quality Research Division, Chicago, Illinois*

Received February 16, 1965

THE parasexual cycle first demonstrated in *Aspergillus nidulans* (PONTECORVO, ROPER, HEMMONS, MACDONALD and BUFTON 1953) has provided a means for genetic investigations in other species of filamentous fungi, particularly those lacking a perfect stage. In the genus *Penicillium*, the parasexual cycle has been demonstrated in *P. chrysogenum* (PONTECORVO and SERMONTI 1954), *P. expansum* (BARRON 1962; BERAHA and GARBER 1965) and *P. italicum* (STRØMNAES, GARBER and BERAHA 1964) but not in *P. digitatum* (STRØMNAES *et al.* 1964; BERAHA, unpublished data). Two linkage groups were reported for *P. chrysogenum* (SERMONTI 1957) and *P. expansum* (BARRON 1962; BERAHA and GARBER 1965). The evidence for two linkage groups in *P. expansum* suggested that this species might be developed into a valuable tool for fungal genetics.

This paper presents a genetic study of eight heterozygous diploid strains involving 14 markers in *P. expansum* by means of the parasexual cycle. Although the data support the preliminary evidence for two linkage groups, 13 markers were assigned to one linkage group and one marker to a second group.

MATERIALS AND METHODS

The composition of the complex ("complete") and defined ("minimal") media, cultural conditions and procedures for inducing and characterizing mutants with nutritional requirements have been reported (STRØMNAES and GARBER 1963). Supplements were added in concentrations given by TUVESON and GARBER (1959). Acriflavine (30 mg/l) or para-fluorophenylalanine, PFPA, (500 mg/l) was added to the complex medium to screen for mutants resistant to these inhibitory compounds or to score the segregant conidia for resistance.

Heterocaryons were prepared by the frontier or mat method (BERG and GARBER 1962). Although heterozygous diploid sectors were occasionally detected in heterocaryotic colonies, heterozygous diploid colonies were usually obtained by plating approximately 10^5 conidia from heterocaryotic colonies on defined medium.

Crowding decreased the frequency of diploid colonies exhibiting segregant color sectors on complex medium. This problem was resolved by adding only two very small diploid colonies to each plate. The plates were then incubated for 5 days at 30°C and left at room temperature until obviously segregant color sectors could be seen. Conidia were taken from only one sector with each color in each colony to insure a random sample. Although the size of conidia may be used to distinguish haploid from diploid segregants, the segregant genotypes have proved to be an equally reliable criterion of ploidy. In doubtful cases, the size of conidia was determined.

The sporulating colonies of *P. expansum* are green and prototrophic. The following markers have been used to synthesize diploids: *wh-1*, white conidia; *be-1*, beige conidia; *go-1*, gold conidia; *arg-1,2*, arginine; *met-1*, methionine; *ade-2*, -3, adenine; *leu-1*, leucine; *his-1*, histidine; *nic-1*,

nicotinic acid; *pab-1*, para-aminobenzoic acid; *ACR-1*, acriflavine-resistance; *fpa-1*, PFPA-resistance.

Conidia from segregant color sectors were streaked onto supplemented complex medium to detect contaminating heterozygous diploid conidia. The homogeneous streaks provided conidia to form grids of 26 colonies, each representing a different segregant sector. The genotype of each colony was determined by replicating the colonies to complex and defined media, complex medium with an inhibitory compound, and defined medium supplemented with the appropriate nutrients. In a series of experiments, conidia from apparently nonsectoring heterozygous diploid colonies were plated on PFPA-complex medium. The resulting colonies gave conidia which were handled in the same manner as those from segregant color sectors to determine their genotype.

RESULTS

Mutant strains: Mutant strains with the same terminal deficiency were included in hereocaryons which exhibited good growth on the defined medium: *arg-1*, -2, and *ade-2*, -3. Eight heterozygous diploids were obtained using mutant strains with different color and nutritional markers and in certain diploids, resistance to acriflavine or PFPA (Table 1). The diploids were green and prototrophic, indicating that the color and nutritional markers were recessive. The diploids heterozygous for resistance to acriflavine and PFPA were resistant to acriflavine and sensitive to PFPA, indicating dominance for acriflavine-resistance and recessiveness for PFPA-resistance.

Diploid strains: The color sectors in the diploids colonies were usually small, infrequent and peripheral in location. Nonconidiating sectors were occasionally observed but they were generally not segregants. Occasionally, green sectors were diploid segregants homozygous for a nutritional or a resistance marker. The ploidy, frequency, and type of conidia from segregant color sectors in colonies of eight diploid strains are summarized in Table 1.

The frequency of sectors with the color of one or the other haploid component strains depended on the genotype of the diploid. For example, approximately 80% of the sectors from diploid D but approximately 2% from diploid E were *go-1*. The relative frequency of haploid or diploid sectors also seemed to be determined by the genotype of the diploid. Only diploid segregants were obtained from diploid H (Table 2), mostly diploid segregants from diploids J, K and L and, with one exception, mostly haploid segregants from Diploids D, E, F and G.

Diploid segregants: Mitotic crossing over yields diploid segregants homozygous for some markers but heterozygous for others. The following markers occurred in homozygous diploid segregants: *fpa-1*, *wh-1*, *be-1*, *go-1*, *ade-2*, *ade-3* and *nic-1*. Diploid segregants from diploid K were either *fpa-1* green or *fpa-1 wh-1*.

Haploid segregants: The number of haploid segregants with different genotypes ranged from zero to four, depending on the genotype of the diploid. For example, no haploid segregants were obtained from diploid H (Table 2) and haploid segregants of only one genotype were isolated from diploids G, J, K and L (Table 1). Since haploid segregants provided the necessary data to detect linkages, the absence of all detectable haploid segregant sectors from a diploid posed a problem that required special techniques to surmount.

Selection experiments: Colonies of diploid H were grown on acriflavine

TABLE 1

Ploidy, frequency, type and presumed origin of segregant sectors from diploid strains

Strain	Diploids Genotype	Segregant sectors		
		Ploidy	Number	Type*
D	<i>be-1 ade-2/go-1 arg-1</i>	2n	1	gr ade
		n	5	be ade
		n	22	go arg
E	<i>wh-1 met-1 nic-1/ go-1 arg-1</i>	2n	2	wh +
		n	2	wh met†
		n	138	wh met nic
		n	1	go arg
		n	2	go arg nic‡
E†		2n	1	wh +
		2n	1	go +
		n	4	wh met‡
		n	58	wh met nic
F	<i>wh-1 met-1 nic-1/ be-1 ade-2</i>	2n	7	wh +
		2n	6	be +
		2n	2	gr nic
		2n	3	be nic‡
		n	18	wh met nic
		n	1	wh met ade‡
F†		2n	1	be +
		n	24	wh met nic
		n	3	wh met ade‡
G	<i>fpa-1 wh-1 met-1/ be-1 ACR-1 nic-1</i>	2n	3	be + ACR
		n	62	fpa wh met
H	<i>fpa-1 wh-1 met-1 ade-3/ be-1 leu-1 ACR-1 nic-1</i>	2n	128	be + ACR
J	<i>fpa-1 wh-1 met-1 ade-3/ be-1 his-1 ACR-1 nic-1</i>	2n	8	fpa gr + ACR
		2n	1	gr ade ACR
		2n	21	fpa be + ACR‡
		n	1	fpa wh met ade
K	<i>fpa1 wh-1 met-1 ade-3/ be-1 pab-1 ACR-1 nic-1</i>	2n	12	fpa gr + ACR
		2n	1	fpa wh + ACR
		2n	17	fpa be + ACR‡
		2n	5	gr ade ACR
		2n	1	gr nic ACR
		2n	1	be ade ACR‡
		n	2	fpa wh met ade
L	<i>fpa-1 wh-1 met-1 ade-3/ be-1 arg-2 ACR-1 nic-1</i>	2n	4	fpa gr + ACR
		2n	1	gr ade ACR
		2n	8	fpa be + ACR‡
		n	2	fpa wh met ade

* gr—green conidia, indicating heterozygosity for color markers; + —prototrophic, indicating heterozygosity for nutritional markers; for meaning of other symbols see MATERIALS AND METHODS.

† Diploid colonies grown on complex medium without nutritional supplements required by component haploid strains.

‡ Secondary segregant.

medium. Although numerous sectors occurred early in the growth of these colonies when compared with those grown on complex medium, conidia from these sectors had the same phenotype as those from colonies on complex medium

TABLE 2

Ploidy, frequency, and type of segregant sectors or conidia from colonies of Diploid H grown on complex or acriflavine-complex medium

Medium	Sectors			Conidia on PFPA medium		
	Ploidy	Number	Type	Ploidy	Number	Type*
Complex	2n	128	be + ACR	2n	27	fpa wh + ACR
				n	15	fpa wh met ade
Acriflavine	2n	104	be + ACR	2n	1	fpa gr + ACR
				n	39	fpa wh met ade
				n	21	fpa wh met ade ACR

* See Table 1 footnote.

(Table 2). Approximately 10^4 conidia from one to three nonsectoring diploid colonies grown on complex or acriflavine medium were added to plates containing PFPA medium. The resulting colonies were either diploid or haploid segregants which were PFPA-resistant (Table 2). Although the frequency of segregants with different phenotypes was determined, conidia of the same type may have originated either as isolated conidia or as members of the same segregant patch. These observations indicate that segregant nuclei may enter conidia but not be able to form detectable sectors. Furthermore, the presence of acriflavine in the medium did not result in the formation of sectors with a phenotype other than that found when the medium lacked this inhibitory compound.

Approximately 10^4 conidia from nonsectoring colonies of diploids J, K and L were added to PFPA medium. The resulting colonies were either diploid or haploid segregants resistant to PFPA (Table 3). As in diploid H, segregant types not

TABLE 3

Ploidy, frequency, type and presumed origin of segregant conidia from diploid colonies plated on PFPA medium

Diploids		Segregant sectors		
Strain	Genotype	Ploidy	Number	Type*
J	<i>fpa-1 wh-1 met-1 ade-3/ be-1 his-1 ACR-1 nic-1</i>	2n	1	fpa gr + ACR
		2n	1	fpa wh + ACR
		2n	1	fpa wh ade ACR†
		n	26	fpa wh met ade
		n	43	fpa wh met ade ACR
K	<i>fpa-1 wh-1 met-1 ade-3/ be-1 pab-1 ACR-1 nic-1</i>	n	1	fpa be his nic ACR†
		2n	3	fpa gr + ACR
		n	30	fpa wh met ade
L	<i>fpa-1 wh-1 met-1 ade-3/ be-1 arg-2 ACR-1 nic-1</i>	n	13	fpa wh met ade ACR
		2n	2	fpa gr + ACR
		2n	1	fpa wh + ACR
		n	20	fpa wh met ade
		n	11	fpa wh met ade ACR

* See Table 1 footnote.

† Secondary segregant.

detected in the sectors of colonies grown on complex medium were obtained in the colonies on PFFA.

DISCUSSION

First-order segregant sectors in heterozygous diploid colonies generally result from haploidization or mitotic crossing over. Markers on the same chromosome are completely linked and those on different chromosomes show independent segregation in a random sample of haploid segregants. A mitotic crossing over between a locus and the centromere is usually responsible for diploid segregants and indicates that the locus is relatively distal from the centromere. Furthermore, mitotic crossing over between genes on the same chromosome may yield diploid segregants which furnish the necessary information to place these genes in a linear arrangement. A second-order haploid or diploid segregant may be obtained from a first-order diploid colony. Second-order segregants are rarely obtained directly from a heterozygous diploid colony of *A. nidulans* (PONTECORVO 1958) but are recovered in *Verticillium albo-atrum* (HASTIE 1964).

BARRON (1962) using seven markers in a diploid strain of *P. expansum* assigned four markers to one linkage group and three markers to a second group. BERAHA and GARBER (1965) analyzed three diploid strains of this species involving six markers and placed four markers in one linkage group and two markers in a second group. Although BARRON's data were compatible with two linkage groups, those of BERAHA and GARBER could have indicated only one linkage group.

Two assumptions were needed to place the 14 markers involved in eight diploid strains of *P. expansum* used in our work in two linkage groups: the relatively high frequency of certain haploid segregants indicated first-order segregants, and the absence of expected first-order segregants indicated a selection. The sequence of the relatively distal markers (underlined) in the first linkage group is tentative. The groups are: 1. *fpa-1 wh-1 met-1 arg-1 arge-2 leu-1 his-1 pab-1 ade-2 go-1 be-1 nic-1 ade-3*; 2. *ACR-1*.

A number of haploid segregants could be explained by assuming that *wh-1* is epistatic to *be-1* and that certain haploid or diploid segregant are second-order segregants obtained directly from the heterozygous diploid colonies.

Two of the six markers used by BERAHA and GARBER (1965) were not involved in the eight diploid strains. Since the two markers could be assigned to the first linkage group, this group would include 15 markers and the second group one marker. Furthermore, second-order segregants in each of two diploid strains analyzed by BERAHA and GARBER (1965) were almost as frequent as first-order segregants.

Although the two linkage groups proposed for *P. expansum* satisfactorily explain the results obtained by the parasexual cycle, the number of markers in each linkage group was markedly different. Since these markers were obtained by ultraviolet light, reciprocal translocations may have been induced in the course of preparing multigenic haploid strains (KÄFER and CHEN 1964) so that pseudo-linkage might have yielded a linkage group with many markers.

This investigation was supported by a grant, GB-2249, from the National Science Foundation and, in part, by a grant from the DR. WALLACE C. and CLARA A. ABBOTT Memorial Fund, University of Chicago.

SUMMARY

Eight diploid strains involving 14 markers were analyzed by means of the parasexual cycle. The results could be explained by placing 13 markers in one linkage group and one marker in a second group. Certain haploid or diploid segregants were assumed to be second-order segregants obtained directly from heterozygous diploid colonies.

LITERATURE CITED

- BARRON, G. L., 1962 The parasexual cycle and linkage relationships in the storage rot fungus *Penicillium expansum*. Can. J. Botany **40**: 1603-1613.
- BERAHA, L., and E. D. GARBER, 1965 Genetics of phytopathogenic fungi. XI. A genetic study of avirulence due to auxotrophy in *Penicillium expansum* by means of the parasexual cycle. Am. J. Botany **52**: 117-119.
- BERG, C. M., and E. D. GARBER, 1962 A genetic analysis of color mutants of *Aspergillus fumigatus*. Genetics **47**: 1139-1146.
- HASTIE, A. C., 1964 The parasexual cycle in *Verticillium albo-atrum*. Genet. Res. **5**: 303-315.
- KÄFER, E., and T. L. CHEN, 1964 Translocations and recessive lethals induced in *Aspergillus* by ultra-violet light and gamma-rays. Can. J. Genet. Cytol. **6**: 249-254.
- PONTECORVO, G., 1958 *Trends in Genetic Analysis*. Columbia University Press, New York.
- PONTECORVO, G., and G. SERMONTI, 1954 Parasexual recombination in *Penicillium chrysogenum*. J. Gen. Microbiol. **11**: 94-104.
- PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD, and A. W. J. BUFTON, 1953 The genetics of *Aspergillus nidulans*. Advan. Genet. **5**: 141-238.
- SERMONTI, G., 1957 Analysis of vegetative segregation and recombination in *Penicillium chrysogenum*. Genetics **42**: 433-443.
- STRØMNAES, Ø., and E. D. GARBER, 1963 Heterocaryosis and the parasexual cycle in *Aspergillus fumigatus*. Genetics **48**: 653-662.
- STRØMNAES, Ø., E. D. GARBER, and L. BERAHA, 1964 Genetics of phytopathogenic fungi. IX. Heterocaryosis and the parasexual cycle in *Penicillium italicum* and *Penicillium digitatum*. Can. J. Botany **42**: 423-427.
- TUVESON, R. W., and E. D. GARBER, 1959 Genetics of phytopathogenic fungi. II. The parasexual cycle in *Fusarium oxysporum* f. *pisi*. Botan. Gaz. **121**: 74-80.