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The parasexual cycle in *Verticillium albo-atrum*

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1. INTRODUCTION

The parasexual cycle consists of the formation of diploid nuclei by nuclear fusions in heterokaryons followed by the occurrence of mitotic crossing-over and haploidisation in this diploid nuclear lineage (Pontecorvo, 1956). These mechanisms form two classes of segregant genotypes, diploid segregant genotypes which arise following mitotic crossing-over, and haploids following haploidization. Both mitotic crossing-over and haploidization are relatively infrequent in most fungi studied, and selective techniques are usually employed to recover the rare segregant genotypes. The application of these devices to chromosome mapping in *Aspergillus nidulans* has been illustrated by Pontecorvo (1959).

Genetic recombination in the imperfect fungus *Verticillium albo-atrum* has been reported previously (Hastie, 1962). Heterokaryosis was demonstrated using auxotrophic mutants, and it was shown that heterozygous nuclei formed in these heterokaryons were very unstable and yielded two classes of novel genotypes. The first of these consisted of nuclei heterozygous at some loci but homozygous (or hemizygous) at other loci, and these were presumably diploid (or aneuploid). The second class of novel genotypes were stable and presumably haploid. The phenomena responsible for recombination in *V. albo-atrum* therefore seemed essentially similar to parasexuality, although the novel genotypes were recovered from the original heterozygous strain at a very much higher frequency than that usually found in the parasexual systems of other fungi.

These earlier investigations with *Verticillium albo-atrum* were confined to a single diploid involving strains isolated from wilted hops in Kent (Hastie, 1962). The present study was undertaken to determine whether the high frequency of recombination observed previously was common to other heterozygotes of independent origin, and to investigate the mechanism responsible for genetic recombination.

2. MATERIALS AND METHODS

A modified Prune Extract agar medium (PE) (Talboys, 1960) was used as a complete medium for routine culturing of auxotrophs. Czapek-Dox Agar medium (MM) was used as a minimal medium, and appropriately supplemented as required.

The strains used were isolated from wilted potatoes and tomatoes. Nutritional

mutants were isolated after using ultra-violet radiation as a mutagen, and the following symbols are used to indicate mutant requirements: *arg* = arginine; *gu* = guanine, *inos* = inositol, *meth* = methionine, *nic* = nicotinic acid, *paba* = *p*-aminobenzoic acid, *phen* = phenylalanine, *pyr* = pyridoxine, *thr* = threonine. Diauxotrophs were obtained by irradiating single auxotrophs, and the strain *pyr-1 meth-6 phen-1*, used as a parent of diploid 3, was obtained as a stable segregant from the diploid *pyr-1 meth-6/phen-1*.

About 99% of the conidia formed by *Verticillium albo-atrum* are uninucleate, and most nuclei in wild-type isolates are haploid (Buxton & Hastie, 1963). Conidia from eight-day-old haploid (stable) and diploid (segregating) cultures are about 6μ and 11 μ in length respectively, and these measurements provide a reliable distinction between haploid and diploid isolates if the measured conidia are taken from young cultures.

The techniques used for the selection of heterokaryons and heterozygous diploids were essentially those of Roper (1952). The genotypes of the diploids synthesized were:

Diploid 1. *pyr-1 paba-1/gu-2 inos-3*
Diploid 2. *thr-2 arg-4/nic-3 meth-14*
Diploid 3. *pyr-1 meth-6 phen-1/gu-2 inos-3*

The following procedure was adopted to obtain segregants of independent origin for the genetic analysis. Selected heterozygous strains were purified by obtaining a monoconidial culture which was shown, by taking random monoconidial isolates, to be segregating for all the markers included in the original mixed culture. These progeny (1st-order isolates) were classified by replication on appropriate test media, and by making conidial measurements. They included numerous diploid prototrophs, and fifty-two random monoconidial isolates (2nd-order isolates) were taken from each of these. The classification of these 2nd-order isolates revealed the markers for which the 1st-order prototrophs were segregating, and only progeny from 1st-order diploid prototrophs shown to be segregating for all the markers were considered in the subsequent analysis.

The 2nd-order isolates included stable and segregating progeny of a variety of phenotypes. Only one haploid and/or one diploid segregant of each phenotype was considered from any one 1st-order prototroph, and consequently only novel genotypes of independent origin were used in the analysis of segregation and recombination. The genotypes of independent 2nd-order diploids were determined by taking about fifty-two 3rd-order isolates at random from the fourteen-day-old diploid cultures. This sample was sufficiently large to be reasonably certain of detecting the segregation of any heterozygous marker.

3. RESULTS

(*i*) *Selection of prototrophic diploid conidia*

Attempts to select prototrophic conidia from heterokaryons were made after various periods of incubation (8 to 18 days). The proportion of prototrophic conidia

recovered was from 10^{-5} to 10^{-7}, and the first prototrophic conidia recovered from any mixed culture always formed colonies bearing large conidia. The genetic analysis of these selected prototrophs showed that they, or strains derived from them, consistently segregated for all the markers introduced from both parents. Because such prototrophic conidia are uninucleate it can be concluded that they contain a heterozygous diploid nucleus (Hastie, 1962).

(*ii*) *Breakdown of diploid strains*

The origin of haploid segregants was studied by spreading conidia of diploid 3 on dilute MM containing nutrients at 1/1000th of their usual concentration. Diploid mycelium sporulated poorly on this medium, but sectors of dense sporulation formed during the growth of colonies from a diploid conidium. Thirty-one of these sectors, distributed among five colonies, were examined by taking twenty-six random conidia from each. The resulting isolates were classified by replication and conidial measurements. All the isolates from twenty-eight of the sectors were haploid, and all the isolates from any one of these sectors had the same phenotype. Both diploid (or aneuploid) and haploid conidia were recovered from each of the other three sectors, and a few different phenotypes were represented among the progeny from each of these sectors.

The sectors sampled were on different radii of the respective colonies, and adjacent sectors usually gave conidia with different phenotypes. The sectors were therefore almost certainly of independent origin. Because most of the sectors yielded conidia of only one stable (haploid) phenotype it is concluded that these haploids arise by some mitotic mechanism perhaps essentially similar to haploidization as described by Kafer (1961) in *Aspergillus nidulans*.

The breakdown of heterozygous diploid strains grown on PE medium was followed by taking random samples of conidia from monoconidial segregating cultures of various ages. Samples of fifty conidia were taken from each of three replicate cultures at each sampling time. All the conidia from one-week-old cultures were diploid, but the proportion of diploids decreased rapidly until less than 5% of the conidia from three-week-old cultures were diploid.

It was also found that four-day-old cultures formed by single prototrophic diploid conidia yielded an average of 3×10^6 conidia, while similar cultures formed by haploid conidia yielded about 3×10^7 conidia. The large proportions of haploid conidia obtained from older segregating cultures is therefore at least partly accounted for by more prolific sporulation of haploid mycelium formed during the growth of segregating cultures.

In the following genetic analyses it is assumed that heterozygous segregant genotypes arise by mitotic recombination following mitotic crossing-over (Stern, 1936; Pontecorvo, Tarr-Gloor & Forbes, 1954), and some justification for this assumption is given later. The proportions of such segregants among random conidia from four-day-old cultures often exceeded 5%. Haploid segregants formed during the later growth of segregating cultures may therefore be derived from nuclei which have already undergone mitotic crossing-over, and may therefore be recombinant

with respect to linked markers. Because a variable number of nuclear generations may intervene between mitotic recombination and haploidization, the proportion of such recombinants among independent haploid segregants will be a more complex function of the genetic distance between linked markers than percentage recombination as estimated in meiotic analysis.

(*iii*) *Analysis of diploid 1* (pyr-1 paba-1/gu-2 inos-3

A young purified culture of diploid 1 yielded ten diploid prototrophs amongst the 1st-order isolates. Fifty-two random isolates were taken from each of these prototrophs when the cultures were ten days old. The classification of these 2nd-order isolates revealed that eight of the 1st-order diploid prototrophs were heterozygous for all four markers. There were sixty-three independent haploids, and thirty-two independent diploid segregants amongst the 2nd-order isolates from the prototrophs which segregated for all four markers, and these are used in the following analysis of segregation and recombination (Table 1).

Table 1. *Segregation and recombination of markers among* 63 *independent haploids derived from diploid 1* (pyr-1 paba-1/gu-2 inos-3)

Genotypes				Percentage recombinant
pyr paba / 19	*pyr* + / 16	+ *paba* / 15	+ + / 13	49·2
pyr gu / 18	*pyr* + / 17	+ *gu* / 15	+ + / 13	49·2
pyr inos / 15	*pyr* + / 20	+ *inos* / 14	+ + / 14	46·1
paba gu / 19	*paba* + / 15	+ *gu* / 14	+ + / 15	54·0
paba inos / 1	*paba* + / 33	+ *inos* / 28	+ + / 1	3·2
gu inos / 15	*gu* + / 18	+ *inos* / 14	+ + / 16	50·8

None of the allele ratios for the haploids listed in Table 1 is significantly different from a 1 : 1 expectation. When recombination between all possible pairs of markers is considered only the proportion of recombinants for *paba-1* and *inos-3* (3·2%) is very different from an expected proportion of 50% based on independent segregation. The markers *paba-1* and *inos-3* are therefore linked in repulsion in this diploid.

The genotypes of the thirty-two diploids of independent origin recovered among the 2nd-order isolates were determined by taking 3rd-order isolates from them, and these thirty-two diploids are classified according to their genotypes for all possible pairs of markers in Table 2.

In the analysis of these heterozygous segregants it is assumed that they are diploid rather than aneuploid or hemizygous with respect to markers which showed no segregation in the 3rd-order isolates, and that they are formed by mitotic recombination. If this is so loci on the same, or homologous, chromosome arms will tend to become homozygous simultaneously in the formation of these diploid segregants, and therefore the data for a pair of markers linked in this way will tend to be distributed along one of the diagonals of the appropriate section of Table 2. This distribution applies to only that section of Table 2 in which the genotypes with respect to the *paba-1* and *inos-3* loci are tabulated. All diploids homozygous at the *paba-1*

Table 2. *Classification of* 32 *independent diploid genotypes from diploid 1 with respect to all possible pairs of markers*

	paba/paba	*paba/+*	*+/+*	*pyr/pyr*	*pyr/+*	*+/+*	*gu/gu*	*gu/+*	*+/+*
inos/inos	0	0	7	1	5	1	0	5	2
inos/+	0	18	0	6	10	2	6	8	4
+/+	7	0	0	0	7	0	1	4	2
gu/gu	1	6	0	0	6	1	–	–	–
gu/+	4	8	5	4	12	1	–	–	–
+/+	2	4	2	3	4	1	–	–	–
pyr/pyr	0	6	1	–	–	–	–	–	–
pyr/+	7	10	5	–	–	–	–	–	–
+/+	0	2	1	–	–	–	–	–	–

locus are also homozygous for *inos-3*, and *vice versa*. The linkage of *paba-1* and *inos-3* indicated by the analysis of haploids is therefore confirmed by the analysis of diploids, and this latter analysis gives the additional information that the two markers are located on homologous chromosome arms.

The data supports the hypothesis that these diploid segregant nuclei are formed by mitotic recombination in that the two genotypes *paba-1* + */paba-1* + and + *inos-3*/+ *inos-3* are recovered equally frequently (Table 2). A single mitotic cross-over between the more proximal of these markers and its centromere will lead to the formation of either both, or neither of the above genotypes according to the way in which the chromatids segregate at the subsequent mitotic division. These two genotypes must therefore be expected to appear with the same frequency.

None of the above heterozygous segregants was heterozygous for one of the linked markers and homozygous for the other, and it is consequently impossible to decide which marker is proximal.

Reference to Table 2 shows that some heterozygous segregants were homozygous for pairs of markers which showed no evidence of linkage in the analysis of independent haploids (e.g. one heterozygous segregant was homozygous for both *paba-1* and *gu-2*). Heterozygous segregants homozygous for two unlinked markers were presumably formed by either simultaneous or successive crossovers, but aneuploidy cannot of course be excluded as an alternative explanation.

(*iv*) *Analysis of diploid 2* (thr-2 arg-4/nic-3 meth-14)

Fifteen prototrophic diploids were obtained among 1st-order isolates from a purified culture of diploid 2. The classification of 2nd-order random isolates showed six of these 1st-order prototrophs to be segregating for all four markers. The following analysis utilizes forty independent haploids, and twenty-four independent diploids derived from these six 1st-order prototrophs (Table 3).

Table 3. *Segregation and recombination of markers among* 40 *independent haploids derived from diploid 2* (thr-2 arg-4/nic-3 meth-14)

Genotypes				Percentage recombinant
thr arg / 4	*thr* + / 10	+ *arg* / 12	+ + / 14	55·0
thr nic / 4	*thr* + / 10	+ *nic* / 14	+ + / 12	40·0
thr meth / 7	*thr* + / 7	+ *meth* / 9	+ + / 17	60·1
arg nic / 15	*arg* + / 1	+ *nic* / 3	+ + / 21	90·0*
arg meth / 8	*arg* + / 8	+ *meth* / 8	+ + / 16	60·1
nic meth / 7	*nic* + / 9	+ *meth* / 11	+ + / 13	50·0

* In calculating this percentage of recombinants, it was assumed that these two markers were in repulsion in diploid 2, but see text.

The segregation of the threonineless marker among the haploids recorded in Table 3 is 26 ± :14 *thr-2*, and the probability of this being due to chance is 0·05. This shortage of *thr-2* haploids is probably attributable to the poor sporulation of threonineless strains. The allele ratios of none of the other markers showed a significant deviation from a 1 : 1 expectation. The proportions of recombinants are between 40% and 60% for all pairs of markers except *arg-4* and *nic-3*, and the markers *arg-4* and *nic-3* show obvious evidence of linkage. These two markers were introduced into diploid 2 from different parents and must therefore have been originally in repulsion, but 90% of the haploids recovered have *arg-4* and *nic-3* in coupling and it must be inferred that a phase change, from repulsion to coupling, occurred in the nuclear lineage from which a single conidium was taken to purify diploid 2. This would mean that *arg-4* and *nic-3* are in coupling in diploid 2, and that the 10% haploids with these markers in repulsion are recombinants. A further phase change, from coupling back to repulsion, was found among the 2nd-order diploid segregants. The probable significance of these phase changes will be discussed later.

The genotypes of the twenty-four 2nd-order diploid segregants are classified in such a way as to detect non-independent segregation of any pair of markers (Table 4). Although *arg-4* and *nic-3* were found to show linkage when independent haploid segregants were considered, there was no tendency for them to segregate simul-

Table 4. *Classification of* 24 *independent diploid genotypes from diploid 2 with respect to all possible pairs of markers*

	meth/meth	*meth/+*	*+/+*	*nic/nic*	*nic/+*	*+/+*	*thr/thr*	*thr/+*	*+/+*
arg/arg	4	4	2	4	3	3	1	7	2
arg/+	6	3	0	2	6	1	4	2	3
+/+	2	3	0	5	0	0	1	1	3
thr/thr	2	3	1	2	2	2	–	–	–
thr/+	4	5	1	6	4	0	–	–	–
+/+	6	2	0	3	3	2	–	–	–
nic/nic	5	6	0	–	–	–	–	–	–
nic/+	6	2	1	–	–	–	–	–	–
+/+	1	2	1	–	–	–	–	–	–

Table 5. *Segregation and recombination of markers among* 89 *independent haploids derived from diploid 3* (pyr-1 meth-6 phen-1/gu-2 inos-3)

Genotypes				Percentage recombinant
inos phen / 15	*+ phen* / 23	*inos +* / 24	*+ +* / 27	47·2
inos meth / 16	*+ meth* / 24	*inos +* / 23	*+ +* / 26	47·2
inos pyr / 23	*+ pyr* / 22	*inos +* / 16	*+ +* / 28	57·3
inos gu / 22	*+ gu* / 27	*inos +* / 17	*+ +* / 23	49·4
meth pyr / 20	*+ pyr* / 25	*meth +* / 20	*+ +* / 24	50·6
meth phen / 14	*+ phen* / 24	*meth +* / 26	*+ +* / 25	56·2
meth gu / 7	*+ gu* / 42	*meth +* / 33	*+ +* / 7	16·7
pyr phen / 18	*+ phen* / 20	*pyr +* / 27	*+ +* / 24	52·8
pyr gu / 24	*+ gu* / 25	*pyr +* / 21	*+ +* / 19	48·3
phen gu / 16	*+ gu* / 23	*phen +* / 22	*+ +* / 28	60·7

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Table 6. *Classification of 24 independent diploid genotypes from diploid 3 with respect to all possible pairs of markers.*

	meth/meth	*meth/+*	*+/+*	*pyr/pyr*	*pyr/+*	*+/+*	*phen/phen*	*phen/+*	*+/+*	*inos/inos*	*inos/+*	*+/+*
gu/gu	0	0	8	2	4	2	1	7	0	1	5	2
gu/+	0	9	2	1	6	4	1	8	2	3	6	2
+/+	5	0	0	1	3	1	0	3	2	1	3	1
inos/inos	1	2	2	0	4	1	0	5	0	–	–	–
inos/+	3	5	6	2	8	4	2	8	4	–	–	–
+/+	1	2	2	2	1	2	0	5	0	–	–	–
phen/phen	0	1	1	0	2	0	–	–	–	–	–	–
phen/+	3	6	9	4	8	6	–	–	–	–	–	–
+/+	2	2	0	0	3	1	–	–	–	–	–	–
pyr/pyr	1	1	2	–	–	–	–	–	–	–	–	–
pyr/+	3	5	5	–	–	–	–	–	–	–	–	–
+/+	1	3	3	–	–	–	–	–	–	–	–	–

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taneously in the formation of diploid segregants. If these diploid segregant genotypes are formed by mitotic recombination, this is accounted for if their loci are on opposite sides of their centromere.

(v) *Analysis of diploid 3* (pyr-1 meth-6 phen-1/gu-2 inos-3)

The 2nd-order isolates derived from a purified culture of diploid 3 included eighty-nine independent haploids and twenty-four independent diploids. The haploids obtained comprised twenty-six of the thirty-two possible genotypes, and the segregation and recombination of the markers is tabulated in Table 5. None of the allele ratios are significantly different from a 1 : 1 expectation, and it is also shown that only the markers *meth-6* and *gu-2* yield a proportion of recombinants (16·7%) which deviates widely from the 50% expected (Table 5). This indicates that *meth-6* and *gu-2* are linked.

The independent diploid genotypes are classified with respect to all possible pairs of markers in Table 6. Only the markers *meth-6* and *gu-2* show any tendency to segregate simultaneously. These two markers segregate as if they are located on homologous chromosome arms, and the detection of two diploid segregants heterozygous at the *gu-2* locus but homozygous at the *meth-6* locus indicates that *gu-2* lies between *meth-6* and the respective centromere.

4. DISCUSSION

Heterozygous prototrophs of *Verticillium albo-atrum* have always been recovered before haploid (stable) prototrophs from mixed cultures of complementary auxotrophs. This indicates that the detected genetic recombination takes place only after the formation of an unstable heterozygote, and this is presumed to be diploid because all recessive markers introduced can always be recovered from it. Stable haploid genotypes usually appear singly from this heterozygote, and these haploids may arise following mitotic non-disjunction as demonstrated by Kafer (1961) in *Aspergillus nidulans*. The mechanism by which haploids are formed in *V. albo-atrum* cannot be fully analysed until all the chromosomes in a diploid can be labelled.

The proportions of haploid segregants recovered among random asexual progeny of diploid strains of *Verticillium albo-atrum* are always of the same order of magnitude as indicated in this paper. This proportion varies greatly with culture age, and from three-week-old cultures grown from a single diploid conidium it is about 95%. Of the numerous fungi in which somatic segregation has been reported, only diploids of *Cephalosporium mycophilum* have spontaneously yielded similarly high proportions of segregants among random isolates (Tuveson & Coy, 1961). The comparable figure for *Aspergillus nidulans* is about 0·1% (Pontecorvo, 1959). This near thousandfold difference in the proportions of haploid segregants recovered amongst random conidia of *Aspergillus* and *Verticillium* may reflect a difference in the frequency with which haploids are formed, but it is also at least partly explained by the more prolific sporulation of haploid as opposed to diploid mycelium of *V. albo-atrum*. There seems to be no recorded difference in the sporulation of haploid and diploid mycelium of *A. nidulans*.

About 5% of the heterozygous conidia from young segregating cultures of *Verticillium albo-atrum* are auxotrophic segregants, and they appeared before haploid conidia were detected from these cultures. In the genetic analysis it is assumed that such heterozygous segregants are diploid rather than aneuploid or hemizygous, and that they are formed from the original heterozygous diploid genotype following mitotic crossing-over. The segregation patterns for all markers observed among independent heterozygous segregants agree with this hypothesis, although they do not exclude all other possible mechanisms (e.g. terminal deletions). The assumed mechanism also requires that changes in the phase of linked markers should be detectable because of the two ways in which the chromatids may segregate following mitotic crossing-over (Roper & Pritchard, 1955; Kafer, 1961). The occurrence of phase changes with respect to linked markers, as shown for diploid 2 and for a previously analysed diploid (Hastie, 1961), justifies the assumption that the mechanism involved is mitotic crossing-over. Given that phase changes occur in this way, it is certain that at least some of the heterozygous segregant genotypes detected must arise following mitotic crossing-over.

Genetic recombination in *Verticillium albo-atrum* is therefore accomplished through a parasexual system involving haploidisation and mitotic recombination like that described by Pontecorvo (1956). However the phenomena responsible for the production of novel genotypes may occur at a much higher frequency in *V. albo-atrum* than has been reported in diploid strains of most other fungi. The estimation of the frequency of genotypic changes in filamentous fungi will usually involve the assumption that the population of nuclei in the conidia truly reflects the nuclear population in the mycelium. This assumption is perhaps rarely justifiable, and it seems likely that it may be avoided in estimating the frequency of mitotic recombination and haploidisation in *V. albo-atrum*.

Conidia of *Verticillium albo-atrum* are borne on verticillately branched conidiophores, and unpublished work suggests there is a single nucleus in each conidiophore branch. Each branch bears ten to forty uninucleate conidia which accumulate in a drop of water at its tip, and these droplets can be conveniently isolated using a micro-dissector. It may therefore be possible to study the type and frequency of genotype changes occurring during a relatively small number of nuclear divisions by using the populations of conidia from single conidiophore branches.

SUMMARY

Genetic recombination through the parasexual cycle, or some very similar system, was demonstrated. Diploid strains were very unstable, yielding about 95% haploid conidia from three-week-old cultures. This high frequency of haploid segregants was at least partly attributable to greater sporulation of haploid mycelium, but may also reflect a higher frequency of haploidization than that found in *Aspergillus nidulans*.

Mitotic crossing-over also occurred frequently, and gave segregants homozygous for some markers but heterozygous for others. It was also detectable by changes in

the phase of linked markers occurring during vegetative growth. Some heterozygous segregants were either aneuploids, or were formed by double mitotic crossovers.

Haploid segregants, derived from nuclei which had previously undergone mitotic crossing-over, were often recovered. This coincidence of mitotic crossing-over and haploidization in one nuclear lineage, together with the probable occurrence of double mitotic crossovers, makes mitotic analysis less clear cut than in *Aspergillus nidulans*.

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REFERENCES

Buxton, E. W. & Hastie, A. C. (1962). Spontaneous and ultra-violet irradiation-induced mutants of *Verticillium albo-atrum*. *J. gen. Microbiol.* **28**, 625–632.

Hastie, A. C. (1961). Cultural and pathogenic variability in the hop-wilt fungus *Verticillium albo-atrum*. Ph.D. Thesis, University of London.

Hastie, A. C. (1962). Genetic recombination in the hop-wilt fungus *Verticillium albo-atrum*. *J. gen. Microbiol.* **27**, 373–382.

Kafer, Etta (1961). The processes of spontaneous recombination in the vegetative nuclei of *Aspergillus nidulans*. *Genetics*, **46**, 1581–1609.

Pontecorvo, G. (1956). The parasexual cycle in fungi. *Ann. Rev. Microbiol.* **10**, 393–400.

Pontecorvo, G. (1959). *Trends in Genetic Analysis*. London: Oxford University Press.

Pontecorvo, G., Tarr-Gloor, E. & Forbes, E. (1954). Analysis of mitotic recombination in *Aspergillus nidulans*. *J. Genet.* **52**, 226–237.

Roper, J. A. (1952). Production of heterozygous diploids in filamentous fungi. Experimentia, **8**, 14–15.

Roper, J. A. & Pritchard, R. H. (1955). The recovery of the complementary products of mitotic crossing over. *Nature, Lond.*, **175**, 639.

Stern, C. (1936). Somatic crossing over and segregation in *Drosophila melanogaster*. *Genetics*, **21**, 625–730.

Talboys, P. W. (1960). A culture medium aiding the identification of *Verticillium albo-atrum* and *V. dahliae*. *Plant Path.* **9**, 57.

Tuveson, R. W. & Coy, D. O. (1961). Heterokaryosis and somatic recombination in *Cephalosporium mycophilum*. *Mycologia*, **53**, 244–253.