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The genetic control of protoperithecial production in *Sordaria brevicollis*

BY D. J. BOND AND D. J. MACDONALD
Institute of Animal Genetics, King's Buildings, West Mains Road, Edinburgh, Scotland

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SUMMARY

Mutations have been detected which affect the production of protoperithecia in *Sordaria brevicollis*. These mutations have been called *perithecial-1* and *perithecial-2*. Ascus analysis revealed that the former was a centromere unlinked gene, whilst the latter was very close to the mating-type locus. The *per* 1^- *per* 2^- genotype was female sterile. Recovery of fertility by a sterile culture occurred and perithecial analysis revealed that this recovery was due to the accumulation of revertant *per* 1^+ nuclei in the mycelium. *Per* 1^- *per* 2^+ colonies were fertile and gave a cross of characteristic appearance.

1. INTRODUCTION

The production of the female reproductive structures, protoperithecia, in Ascomycete fungi has been subjected to genetic analysis by many workers using a variety of different organisms.

Early work established that the development of protoperithecia was under genetic control (Aronescu, 1933; Dodge, 1946). Subsequently several workers have reported female sterile mutants in which the perithecial development was completely blocked (Westergaard & Hirsch, 1954; Horowitz *et al.* 1960; McNelly-Ingle & Frost, 1965; Tan & Ho, 1970). Probably the most complete analysis was carried out by Esser & Straub (1958) using *Sordaria macrospora*. They found a variety of mutants blocked at different stages in development. Carr & Olive (1959) used heterokaryon formation to study two non-allelic sterile mutants of *Sordaria fimicola*. They discovered that the two mutants complemented each other in newly established heterokaryotic mycelium, but heterokaryons formed by migration of one mutant into mycelium of the other remained sterile. Thus the phenotype of the already established mycelium was fixed and was not modifiable by migration of a complementary nucleus.

Genetic studies have also been carried out using mutants which had effects on fruiting other than complete sterility. In *Glomerella*, Wheeler & McGahen (1952) reported mutants in which the distribution of perithecia was under genetic control. One locus determined whether sexual or asexual structures (perithecia or conidia) were produced whilst another locus determined whether the fruiting structures were scattered or clumped together.

From studies such as these it is apparent that fungal morphogenesis is very

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amenable to genetic analysis. This work in *Sordaria brevicollis* was initiated in an attempt to develop a system in which the interaction of genetic and environmental influences on development could be studied. This paper reports on genetic segregations which affect the distribution of protoperithecia.

2. MATERIALS AND METHODS

(i) *Strains*

The cultures of *Sordaria* used in this paper were originally obtained from Professor L. S. Olive via Miss June Shaw. Some cultures are deposited with the American Type Culture Collection.

The mutants *perithecial-1* and *perithecial-2* reported in this paper were isolated from two reciprocal crosses (*B136* and *B137*). Neither parent had been subjected to mutagenic treatment, although both were descendants from crosses in which some mutagenic treatment had been involved. One parent carried a morphological mutant (*mo-1*) and a pyridoxine requirement. The morphological mutant is characterized by a sharper colony outline and slower growth rate than wild type. Both these mutants were isolated separately after UV treatment, *mo-1* by H. M. V. Cooray, and both were extensively backcrossed to wild-type isolates. The double mutant was made by recombination. In the results presented in this paper the pyridoxine requirement has been ignored, since it segregated independently of all other markers and had no effect on crossing phenotype. In later crosses the pyridoxine requirement was removed. The other parent was apparently wild type and was isolated from a cross of a spore colour mutant to wild type. The spore colour mutant was induced by UV several generations earlier.

(ii) *Culture media*

Crosses were routinely carried out on corn-meal agar of composition 1·7% corn-meal agar, 0·3% sucrose, 0·2% glucose and 0·1% yeast extract. Cultures were grown in tubes containing Vogel's (1956) N medium to which 1·5% Difco agar and 2% glucose were added. Germination of ascospores was stimulated by the addition of 0·7% sodium acetate.

(iii) *Crossing method*

Preliminary experiments established that any isolate gave a reproducible crossing phenotype if a standardized crossing procedure was followed. Crossing phenotype is defined as the distribution of protoperithecia on the mycelium. The male and female cultures were inoculated separately into plastic Petri dishes and incubated for 4 days at 25 °C. A standard volume (20 ml) of medium was used. Microconidia were harvested from the male parent in sterile distilled water by scraping the colony surface with a glass rod and the resulting suspension was filtered through sterile muslin to remove mycelial fragments and protoperithecia. Two ml of microconidial suspension was pipetted over the female culture and the distribution of perithecia determined after further incubation at 25 °C.

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Control experiments established that for the cultures used in these experiments there was no differential fertilization. The distribution of perithecia was an accurate reflexion of the underlying distribution of the smaller protoperithecia. Cultures were fertilized merely to facilitate determination of crossing phenotype.

3. RESULTS

(i) *The detection of* perithecial-1 (per-1)

Table 1 shows the results obtained by ascus dissection, from two reciprocal crosses, *B136* and *B137*. In both crosses it was apparent that female sterility was segregating, but it was also obvious that sterility was not caused by a single gene. Several asci were found in which sterility was absent, whilst in the majority of asci a single spore pair exhibited sterility. The data suggested that sterility was caused by two unlinked genes.

Table 1. *Segregation of female sterility from cross* B136, ♀ mo-1⁻ per 1⁻a × mo-1⁺ per 1⁺A, *and cross* B137, ♀ mo-1⁺ per 1⁺A × mo-1⁻ per 1⁻a

Cross no.	No. of asci with: 0 female steriles	2 female steriles	4 female steriles
B136	5	15	2
B137	0	8	2
Total and frequency	5 (0·16 ± 0·065)	23 (0·72 ± 0·079)	4 (0·12 ± 0·057)

From both crosses the female sterile cultures were always *A* mating type, which suggested that mating type (or a gene closely linked to it) was one of the genes involved. The other component was a previously undetected gene which we have called *perithecial-1* (*per-1*) (Bond & MacDonald, 1975).

The hypotheses that *per 1⁻* existed, and that *per 1⁻A* was female sterile, were confirmed by intercrossing isolates from two tetratype asci. The results of these intercrosses are presented in Table 2. From each tetratype, female sterility was recovered from one of the fertile × fertile crosses. These crosses identified the *per 1⁻a* isolates. As expected, crosses of these isolates to a female sterile gave 50 % female sterile progeny in every ascus.

(ii) *The crossing phenotype of fertile* per 1⁻ *cultures*

In several asci a spore pair was obtained which gave a cross with a characteristic appearance. Plate 1 shows the crossing phenotype of the three fertile genotypes from cross *B136*. In one of these (Plate 1*c*) perithecia were located in a concentrated ring just in from the edge of the Petri dish. This 'crossing away from the edge' (c.a.f.e.) phenotype was always *a* mating type and on analysis proved to be *per 1⁻a*.

The c.a.f.e. phenotype was not found in every tetratype ascus and this was

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shown to be due to the modifying effect of a morphological mutant (*mo-1*). *Per 1⁻a* cultures were c.a.f.e. phenotype provided that they were also mo^+; *mo-1⁻ per 1⁻a* genotypes were fertile but with a reduced number of perithecia located mainly at the plate margin (see Plate 2*c*).

Table 2. *Detailed analysis of asci numbers 11 and 14 from cross* B136

Ascus 11					Ascus 14				
Isolate no.	*mo-1*	m.t.	Crossing phenotype	Proposed *per 1* genotype	Isolate no.	*mo-1*	m.t.	Crossing phenotype	Proposed *per 1* genotype
11.1	−	a	reduced*	−	14.1	−	A	sterile	−
11.3	+	A	sterile	−	14.3	+	a	fertile	+
11.5	−	a	fertile	+	14.5	−	A	fertile	+
11.7	+	A	fertile	+	14.7	+	a	c.a.f.e.*	−

	No. of asci with:				No. of asci with:		
Isolates crossed	0 female steriles	2 female steriles	4 female steriles	Isolates crossed	0 female steriles	2 female steriles	4 female steriles
11.1 × 11.3	0	0	9	14.7 × 14.1	0	0	16
11.1 × 11.7	1	5	3	14.3 × 14.1	0	8	3
11.5 × 11.3	1	2	1	14.7 × 14.5	0	3	1
11.5 × 11.7	4	0	0	14.3 × 14.5	3	0	0

* For explanation see text.

This hypothesis that mo^+ *per 1⁻a* had a c.a.f.e. phenotype was confirmed by two tests. It was shown that c.a.f.e. could be recovered from a reduced fertile *per 1⁻a* culture if crossed to wild type and in crosses in which the morphological mutant was not segregating all *per 1⁻a* isolates were c.a.f.e.

(iii) *The detection of* perithecial-2

Although *per 1⁻* apparently interacted with *A* mating type to give female sterility, it was always possible the interaction was really with another locus closely linked to *A* mating type in the original cross. Evidence to support this was obtained from the analysis of an exceptional ascus which contained a female sterile *a* spore pair. A *per 1⁻A* culture which was not sterile was also found in the same ascus. The following genotype was proposed for this ascus:

spore pair no. 1 *per 1⁻* *per 2⁻A*,
spore pair no. 2 *per 1⁻* *per 2⁺A*,
spore pair no. 3 *per 1⁻* *per 2⁻a*,
spore pair no. 4 *per 1⁻* *per 2⁺a*.

The proposed recombinant spore pairs were crossed and the results contrasted with a cross of 1 × 4. The results supported the recombination hypothesis. Female sterile cultures were predominantly *a* in cross 2 × 3 and *A* in cross 1 × 4.

For reasons which are raised in the discussion it was not possible to estimate the frequency of recombination between *per 2* and mating type.

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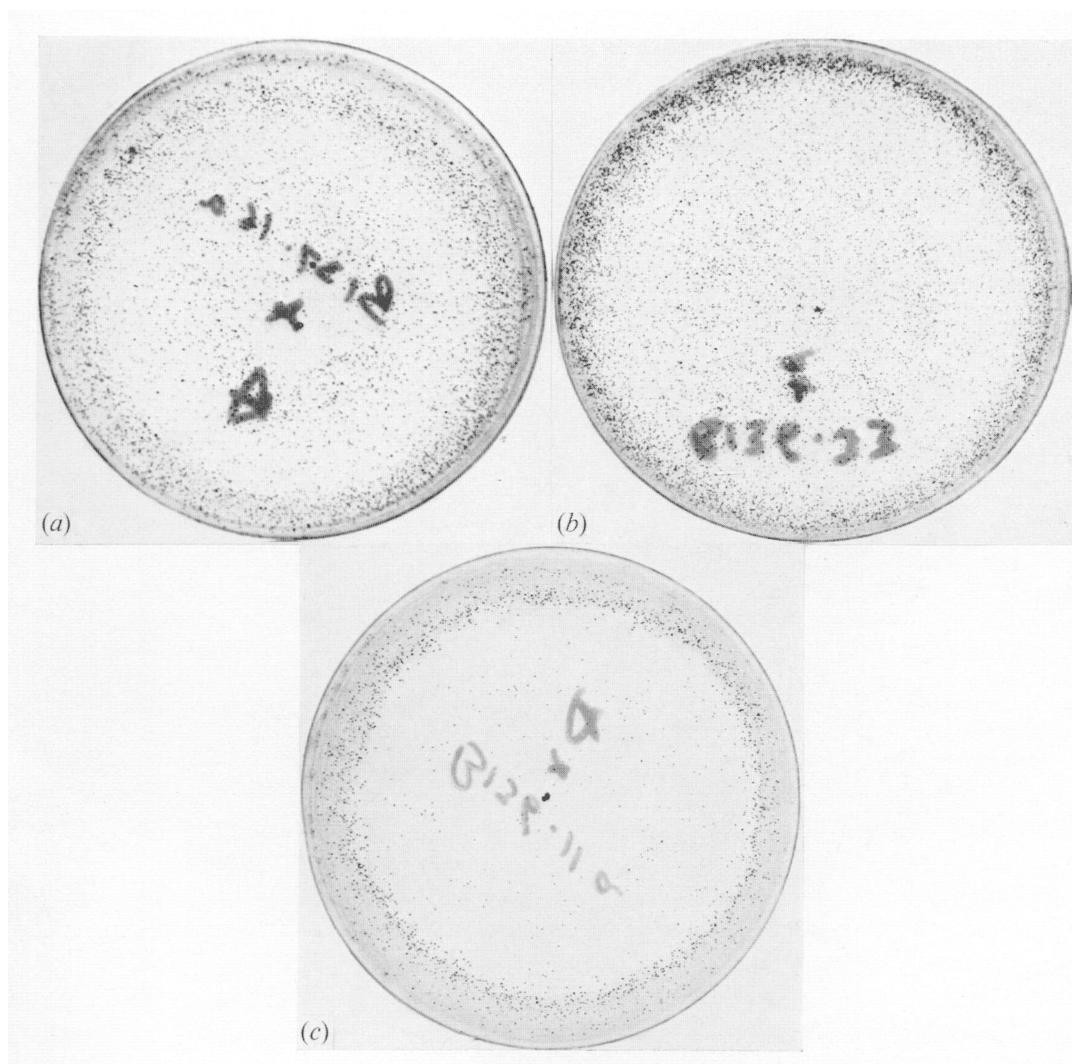

Crossing phenotype of the three fertile genotypes from cross *B136* and *B137*. (*a*) *mo*$^{+}$ *per 1*$^{+}$ *per 2*$^{+}$, (*b*) *mo*$^{+}$ *per 1*$^{+}$ *per 2*$^{-}$, normal phenotypes. (*c*) *mo*$^{+}$ *per 1*$^{-}$ *per 2*$^{+}$, c.a.fe. phenotype.

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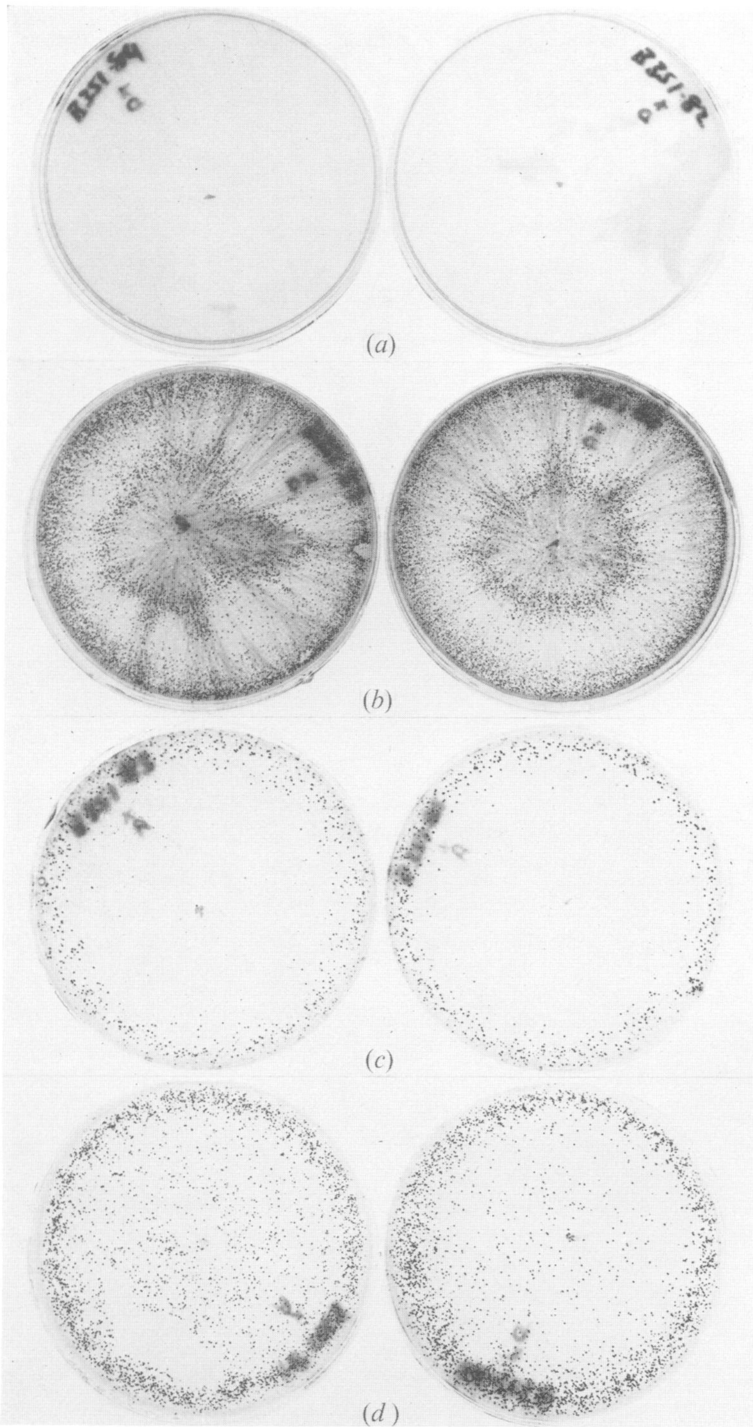

(a)

(b)

(c)

(d)

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Further tests of this hypothesis were made by crossing these recombinant genotypes with the original non-recombinants. The following crosses were analysed:

(*a*) *Per 1⁻ per 2⁻A* × *per 1⁺ per 2⁻a*

This cross segregated for *per 1* only and, as expected, female sterility was inherited independently of mating type. Fifteen asci were analysed; all asci contained 4 female sterile and 4 fertile cultures; 9 of the asci were tetratypes for mating type and female sterility, with 5 non-parental ditypes and 1 parental ditype.

(*b*) *Per 1⁺ per 2⁺A* × *per 1⁻ per 2⁺a*

This cross was segregating for *per 1*, as shown by the c.a.f.e. phenotype of the *a* parent and of the progeny, but, as expected, did not give female sterile cultures.

Table 3. *Results of crosses using the same isolates before and after recovery of female sterile culture*

(Proposed genotype: before recovery – *per 1⁻ per 2⁺a* × *per 1⁻ per 2⁻A*; after recovery – *per 1⁻ per 2⁺a* × *per 1⁻ per 2⁻ A* : *per 1⁺ per 2⁻A*.)

Before recovery				After recovery			
Cross no.	No. of asci with			Cross no.	No. of asci with		
	0 f.s.*	2 f.s.*	4 f.s.*		0 f.s.*	2 f.s.*	4 f.s.*
B190	0	0	9	*B351*	2	5	14
B195	0	0	16	*B347*	2	6	3
B283	0	0	17	*B316*	3	7	3

* f.s. = female sterile.

(iv) *Recovery of fertility by female sterile cultures*

Female sterile cultures were invariably completely devoid of protoperithecia when first tested. With time almost all isolates recovered fertility. Genetic analysis of recovered female sterile strains clearly established that they had become heterokaryotic with revertant nuclei. Table 3 contrasts the results obtained from crosses of female sterile cultures before and after recovery. In these crosses the *per 1⁻ per 2⁻A* isolates were used as a male both before and after recovery. These

PLATE 2

Crossing phenotype of a typical tetratype ascus from a cross: *mo-1 per 1⁻ per 2⁺a* × *mo⁺ per 1⁺ per 2⁻A*. Genotype of spore pairs, (*a*) *mo⁺ per 1⁻ per 2⁻A*, female sterile; (*b*) *mo⁺ per 1⁺ per 2⁻A*; (*c*) *mo⁻1 per 1⁻ per 2⁺a*, 'reduced' phenotype; (*d*) *mo⁻1 per 1⁺ per 2⁺a*. In addition the cross segregated for a gene conferring a characteristic radial striped appearance to half the fertile cultures. In this ascus spore pair (*b*) shows the phenotype, which is masked by the sterility in spore pair (*a*). The wild-type phenotype of spore pair (*d*) is slightly modified by *mo-1*, resulting in larger perithecia.

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results are understandable if it is proposed that the male parent after recovery is heterokaryotic, so that the fertilizing microconidia are of two sorts.

Some recovered sterile crosses were subjected to perithecial analysis and the results supplied clear confirmation of these ideas. For two crosses (*B351* and *B282*) two types of perithecium were found, depending on whether the fertilizing microconidium was revertant or not. In the third case analysed (cross *B314*) the recovered sterile culture was used as a female. From this cross single asci from each of 16 perithecia were dissected in the first instance. In five cases the initial ascus dissected gave four female sterile cultures, and the perithecia from which these asci came were subjected to further analysis. This method was adopted in order to maximize the possibility of detecting two types of perithecia. The results again clearly indicated that two types were present. Type 1 (perithecia nos. 1, 2 and 4), resulting from the cross ♀ $per\ 1^{+}\ per\ 2^{-}A \times per\ 1^{-}\ per\ 2^{+}a$ and type 2 (perithecium 3 and probably 5) from the cross ♀ $per\ 1^{-}\ per\ 2^{-}A \times per\ 1^{-}\ per\ 2^{+}a$. Additional crosses carried out with the $per\ 1^{-}\ per\ 2^{+}a$ male parent, to a variety of different females, gave no evidence for heterokaryosis of the male parent of cross *B314*.

(v) *Detection of other genetic segregations*

The ascus dissection method, which was adopted in order to detect mutations affecting the morphogenesis of protoperithecia, proved to be an extremely powerful tool. In the absence of post-meiotic segregation and mutation a spore pair is genetically identical. In every ascus the difference between members of a spore pair is therefore a measure of the environmental variation in crossing. Plate 2 is an example of an ascus in which *mo-1*, *per 1* and *per 2* were segregating. It can be seen that another genetic marker was also segregating in this cross, which gave characteristic radial stripes to some of the fertile cultures. In the cross as a whole half the fertile cultures exhibited this phenotype. Thus the four spore pairs in Plate 2 are clearly different from each other. Segregations of this type proved to be commonplace. Although ascus dissection enabled very subtle changes in crossing phenotype to be detected, in practice many of these mutants proved difficult to work with. The mutants were themselves subject to modification by genetic background which made subsequent analysis difficult. Small changes in crossing phenotype of wild-type cultures were therefore ignored. Analysis was concentrated on changes such as c.a.f.e. which were less easily modifiable.

4. DISCUSSION

(i) *Location of* per 1 and per 2

The equality of parental ditype and non-parental ditype asci in crosses *B136* and *B137* is clear evidence that the two components of sterility are unlinked to each other. The frequency of tetratype asci ($72 \pm 7{\cdot}9\%$) indicates that at least one of these genes is not centromere linked. *Per-2* is closely linked to the mating type locus, which is itself close to the centromere of linkage group I (Chen, 1965),

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and this indicates that *per-1* is the centromere unlinked gene in this system. *Per 1* has not been mapped further.

It is not possible, for the following reason, to estimate the frequency of recombination between *per 2* and mating type. From crosses in which *per* 2^- was in coupling with *A* mating type, 10 exceptional asci out of a total of 143 were observed. Exceptional asci were identified by the presence of a female sterile *a* spore pair and a fertile *per* 1^-A pair. Not all of these exceptional asci proved to be recombinants. Two out of three which have been analysed by intercrossing proved to be non-recombinant, in that female steriles from them were not predominantly *a* mating type. Investigations are continuing into these asci. At present it is not possible to estimate the proportion of exceptional asci which are genuine recombinants and the proportion arising from other events.

Despite the difficulties which the non-recombinant exceptional asci present, there is no doubt that recombination can occur between mating type and the genetic element which we have called *per-2*.

Table 4. *Perithecial analysis of three crosses using recovered female sterile cultures*

(Proposed genotype: *B351* and *B282*, ♀ *per* 1^- *per* 2^+a × ♂ *per* 1^+ *per* 2^-A : *per* 1^- *per* 2^-A; *B314*, ♀ *per* 1^+ *per* 2^-A : *per* 1^- *per* 2^-A × ♂ *per* 1^- *per* 2^+a.)

Cross	Perithecium	No. of asci with 0 f.s.*	2 f.s.*	4 f.s.*
B351	1	0	0	8
	2	0	7	5
	3	0	0	8
	4	2	5	1
	5	0	0	8
B282	1	2	4	3
	2	0	0	9
	3	0	0	9
	4	0	0	9
	5	1	6	2
	6	3	2	4
	7	1	4	4
	8	2	5	2
B314	1	2	5	2
	2	1	3	3
	3	0	0	5
	4	1	3	4
	5	1??	0	7

* f.s. = female sterile.

(ii) *The reversion of* per 1 *and* per 2

In this paper the term reversion of course includes both back mutation and suppression as possible genetic means of recovery of fertility. A rigorous analysis of recovered sterile cultures has not yet been carried out, but in some cases, for example, cross *B351* (Table 4), revertant nuclei did not give sterile progeny when

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crossed to *per 1*$^+$, indicating that back mutation, rather than suppression, had occurred in these cases.

No case has yet been discovered of reversion of the *per 1* locus in *per 1*$^-$ *per 2*$^+$ cultures but the possibility of such cultures becoming heterokaryotic cannot be excluded in all instances.

No example of recovery by back mutation of the *per 2* locus has been discovered. Recovered cultures of this sort would be heterokaryotic for *per 1*$^-$ *per 2*$^+$ and *per 1*$^-$ *per 2*$^-$ nuclei. Hence when crossed to *per 1*$^-$ *per 2*$^+$ cultures perithecia of two sorts would be obtained. Type one with no asci containing female steriles (from $1^-2^+ \times 1^-2^+$ crosses), and type two with every ascus containing 4 sterile cultures (from $1^-2^- \times 1^-2^+$ crosses).

When a *per 1*$^-$ *per 2*$^-$ culture reaches the edge of the Petri dish in which it is growing the levels, in the mycelium, of various metabolites such as glucose-6-phosphate and ATP sharply declines (manuscript in preparation). Evidence has been obtained which is consistent with the idea that this decline is a consequence of the onset of premature dormancy. When such a dormant culture is subcultured we believe that revertant nuclei have a selective advantage and consequently increase in the mycelium. When revertants increase above a threshold value the mycelium as a whole becomes fertile. This hypothesis would explain why fresh isolates are completely sterile because there would have been no selective pressure for revertant nuclei to accumulate and the threshold would not have been exceeded. Some evidence to support these ideas has been obtained (unpublished) and experiments to test the threshold hypothesis are continuing.

(iii) *The interaction of* per 1 *and* per 2

The analysis of heterokaryotic stocks provides clear evidence that *per 1*$^+$ is dominant and non-autonomous. In the presence of a sufficient proportion of *per 1*$^+$ nuclei the *per 1*$^-$ *per 2*$^-$ genotype can function as a female nucleus. *Per 2* is epistatic to the wild-type allele at the *per 1* locus, that is the *per 2* gene can only be detected in the presence of a mutant allele at the *per 1* locus.

No information has been obtained on whether the *per 1* and *per 2* effects on protoperithecial production are direct or indirect. Without information on the biochemical basis of the *per 1* and *per 2* lesions we do not wish to speculate on the nature of the interaction which results in sterility of the double mutant. Fitzgerald (1962) reported a case of female sterility in *Neurospora* which, at least superficially, has some similarities with that described here. The sterile strain which he described differed from wild type by two genes. This genotype exhibited phenotypic variation, e.g. recovery of fertility. It was claimed that this phenotypic variation was not due to gene differences but rather to 'different equilibrium states of a cellular system intervening between [the genotype] and its phenotypic effects'. Thus a serial subculturing regime resulted in phenotypic changes. No evidence for a similar epigenetic system has been obtained in *Sordaria*. In *Neurospora* several of the cultures exhibited pigmentation abnormalities with the accumulation of patches on the mycelium. Whilst tyrosinase and melanin production have been

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implicated in perithecial production in *Neurospora* (Hirsch, 1954), a fairly intensive search in *Sordaria* has produced no evidence for a similar correlation (MacDonald, unpublished).

We have now established stock crosses which are homozygous for all combinations of *per 1* and *per 2*. These crosses will be the source of material for all future genotype testing. It is hoped to extend this analysis of protoperithecial production in two main directions. Firstly, to extend the genetic analysis to other mutations affecting protoperithecial production, and secondly, to analyse environmental influences on fruiting. Ultimately we hope these approaches will enable us to understand more fully the ways in which genotype and environment interact in fruiting body production.

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