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Developmental Genetics of *Coprinus cinereus:* Genetic Evidence that Carpophores and Sclerotia Share a Common Pathway of Initiation

David Moore

Department of Botany, The University, Manchester M13 9PL, Great Britain

Summary. Five haploid monokaryons of the basidiomycete *Coprinus cinereus* were already known to be unable to form sclerotia (asexual resting structures) on the vegetative monokaryotic mycelium. Genetic analyses had shown that four distinct genes (symbolised *scl*) were represented, all being "recessive" to their sclerotium-producing alleles. In the study reported, homoallelic dikaryons were constructed and the effect of the sclerotium-negative genes on carpophore formation investigated. In the homoallelic state these defective genes prevent the formation of both sclerotia and carpophores by the dikaryon. Thereby demonstrating that these two structures share a common pathway of initiation. It is also shown that the expression of effects of *scl* genes on carpophore maturation in heteroallelic dikaryons is subject to the influence of modifying genes. Possible modes of action of modifying genes and of the *scl* genes are discussed and a carpophore developmental pathway is presented.

Key words: Developmental genetics – Coprinus – Morphogenesis – Modifiers

Introduction

Sclerotia of *Coprinus cinereus* are globose, small (c. 250 μm diam.) but multicellular, persistent, resting structures. They are formed by vegetative mycelia, both monokaryotic and dikaryotic. The mushroom carpophores are normally produced only on the dikaryon. The early stages of the pathway which leads to the formation or the carpophore primodium (Matthews and Niederpruem 1972) are strikingly similar to events described for the initiation of sclerotia (Waters et al. 1975), and for this and other circumstantial reasons it has been suggested that for the dikaryon the two structures are alternative outcomes of a single initiating pathway (Moore and Jirjis 1976).

A possible approach to a test of this proposition is offered by developmental variants which fail to form sclerotia on monokaryons. Five strains have been identified which are unable to form sclerotia (Waters et al. 1975). Genetic analyses have shown that four distinct genes are involved; these are all "recessive" although two allelic forms are known for one of the genes, one "dominant" to the other (Hereward and Moore 1979). Although complementation testing was done to establish the number of genes involved, no attempts were made to construct homoallelic dikaryons. In the present work such homoallelic dikaryons have been made and the effect of the "recessive"sclerotium-negative genes on carpophore formation investigated. It is shown that in the homoallelic state these defective genes prevent formation of both sclerotia and carpophores, and that their expression in heteroallelic dikaryons is subject to the influence of modifying genes.

Materials and Methods

The strains used are described in Table 1. Culture methods and techniques of genetical analysis were described by Moore (1967), Waters et al. (1975) and Hereward and Moore (1979).

Results

Analysis of scl-1. Two alleles of *scl-1* are known. The monokaryon BC9/6,6 carries *scl-1*^{0} and fails to produce sclerotia, while strain number H1 carries *scl-1*^{H} and produces sclerotia which have an abnormal multilayered rind and very compact medulla (Hereward and Moore 1979).

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Table 1. Origins of cultures

Code number	Mating type	Sclerotium genotype	Origin	Reference
H1	A_5B_5	*scl-1*^{H}	Hertfordshire, England	Day and Anderson 1961
H9	A_6B_6	*scl-1*^{0}	Hertfordshire, England	Day and Anderson 1961
BC9/6,6	A_6B_6	*scl-1*^{0}	Nine backcrosses, H1 x H9	D. H. Morgan (pers. comm.)
BC9/6,5	A_6B_5	*scl-1*^{0}	Nine backcrosses, H1 x H9	D. H. Morgan (pers. comm.)
BC9/5,6	A_5B_6	*scl-1*^{0}	Nine backcrosses, H1 x H9	D. H. Morgan (pers. comm.)
BC9/5,5	A_5B_5	*scl-1*^{0}	Nine backcrosses, H1 x H9	D. H. Morgan (pers. comm.)
B1	A_9B_9	*scl-2*	Hertfordshire, England	Day 1963
2H1	$A_{21}B_{21}$	*scl-3*	Hertfordshire, England	Day 1963
L1	$A_{27}B_{25}$	*scl-4*	Hertfordshire, England	Day 1963
PA1	$A_{31}B_{12}$	*scl*$^+$	Gelasna, Poland	Day 1963
ZBw601/40,40	$A_{40}B_{40}$	*scl*$^+$	Prague, Czechoslovakia	Moore and Stewart 1972
mc-1,3	not known[a]	*scl*$^+$	Connecticut, USA	P. R. Day (pers. comm.)
Okayama-4	not known[a]	*scl*$^+$	Okayama, Japan	Takemaru and Kamada 1972
Singapore-7	not known[a]	*scl*$^+$	Singapore	Chang-Ho and Yee 1977
Singapore-12	not known[a]	*scl*$^+$	Singapore	Chang-Ho and Yee 1977

[a] The exact mating type specificity of these strains has not been determined; Singapore-7 is not compatible with Singapore-12, but otherwise these strains are compatible with all the others on the list

Strains BC9/5,5, BC9/5,6 and BC9/6,5 were all derived from the same series of backcrosses as BC9/6,6 and all carry *scl-1*^{0}. Dikaryons homoallelic for *scl-1*^{0} made either by crossing BC9/6,6 with BC9/5,5 or BC9/6,5 with BC9/5,6 failed to form carpophores either on agar medium (maltose-CM medium of Stewart and Moore 1974) or on sterilised horse dung. It is evident that when *scl-1*^{0} is homoallelic carpophore initiation is prevented; the dikaryotic mycelium is featureless, with no organised structures being formed at all.

Strain H1 was crossed with ZBw601/40,40 and progeny of mating type $A_{40}B_{40}$ which formed H-type sclerotia were isolated. Dikaryons made between these progeny mycelia and H1 were therefore homoallelic for *scl-1*^{H}; they formed H-type sclerotia and fruited readily, the carpophores being normal in form and development. Evidently, the defect caused by *scl-1*^{H}, which seems to influence the later stages of sclerotium maturation (Hereward and Moore, 1979), has no effect on the processes of initiation and carpophore maturation.

Analysis of scl-2. Strain B1 (which carries *scl-2*) was crossed with Okayama-4 and progeny compatible with B1 which were unable to form monokaryotic sclerotia were isolated. Dikaryons made between these progeny and the B1 parent (i.e. *scl-2/scl-2* homoallelic dikaryons) failed to form either sclerotia or carpophores when grown on horse dung or maltose-CM medium. The homoallelic *scl-2* defect clearly prevents initiation of fruiting structures.

Dikaryons made between B1 and other wild types like BC9/6,6, Singapore-12, H1 and ZBw601/40,40 (i.e. heteroallelic *scl-2/scl*$^+$ dikaryons) were vigorous and readily produced normal carpophores. This was not true for dikaryons made with progeny of B1. Although dikaryons made between B1-progeny and ZBw601/40,40 behaved quite normally, crosses with H1 produced mature, but very pale, carpophores bearing a lesser crop of spores than normal, and dikaryons with Singapore-12 produced many primordia which aborted after developing to heights of about 12 to 25 mm; only after prolonged incubation were mature fruits formed. Dikaryons made with BC9/6,6 were weak in growth and failed to fruit.

It is clear that in the original strain, B1, the aspect of *scl-2* function which influences carpophore formation is completely "recessive". However, in the cross which was used to prepare mating type recombinants of *scl-2* it seems likely that genetic factors which modify the penetrance of *scl-2* also segregated with the result that the B1-progeny carried re-assorted complements of modifiers which in some dikaryons allowed *scl-2* to exhibit "semi-dominance" or "dominance". We shall see later that *scl-2* was not unique in being responsive to changes in the "background genome".

Analysis of scl-3. The strain which carries *scl-3* (2H1) has been used extensively to study linkage relationships (Waters et al. 1975) and is therefore known to be able to fruit normally in many crosses. In this work, however, the dikaryons 2H1 + H1 and 2H1 + ZBw601/40,40 did not form carpophores although sclerotia and carpophore initials were produced. The 2H1 + Okayama-4 dikaryon fruited normally and 2H1-progeny carrying recombinant mating type specificities were isolated. None of the selected progeny were able to make sclerotia on the monokaryon.

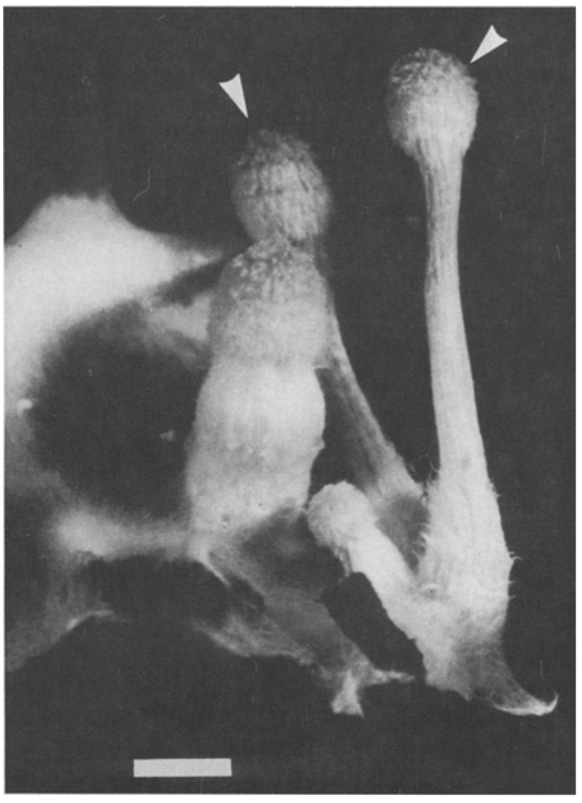

Fig. 1. Primordia produced by the dikaryon L1 + H1. Primordia in process of senescing are indicated with an arrow head. The scale bar corresponds to 4 mm

Dikaryons made between 2H1 and 2H1-progeny (*scl-3/scl-3* homoallelic dikaryons) were vigorous, but failed to form either sclerotia or carpophores.

In common with other *scl* genes, *scl-3* was always "recessive" in terms of the sclerotial phenotype. However, expression of its effect on carpophore formation showed variable "dominance". The parental 2H1 strain was unable to fruit in some dikaryon combinations and 2H1-progeny did not fruit normally in any dikaryon which was tested. For the most part these *scl-3/scl*$^+$ dikaryons produced primordia which aborted at early stages. In some combinations (notably dikaryons with PA1) carpophores did mature but even these were usually abnormal. Like *scl-2,* therefore, it seems that expression of the phenotypic effect of *scl-3* on carpophore development in the heteroallelic dikaryon is dependent on the overall complement of modifying genes contributed to the dikaryon by both parental haploid nuclei. The implication is that the modifying genes act through cytoplasmic gene products.

Analysis of scl-4. The *scl-4* allele differs from the other three sclerotium-negative genes in that the strain in which it was identified, L1, was recorded as being able to prevent fruiting.

Dikaryons involving L1 '... produced sporophore initials which successfully developed into primordia but then aborted, to be replaced by a new crop of initials which behaved similarly ...' (Waters et al. 1975). This observation was confirmed (Fig. 1). Dikaryons involving L1 also had an abnormally low frequency of formation of clamp connections (only 25–68% of cross walls being accompanied by clamp connections compared with 97–100% for control dikaryons) and often exhibited distorted and vacuolate hyphae. A further general feature of the vegetative growth of these dikaryons was that extension growth rates of colonies on complete medium were very much slower than those of normal dikaryons, and slower even than growth rates of the monokaryotic components of the dikaryons. Johnson (1978) found perithecial mutants of *Neurospora crassa* to have altered growth rates, and it may be significant that a feature of common-*A* heterokaryons in *Coprinus* is that they show lower growth rates than their monokaryotic parents (Swiezynski and Day 1960; Casselton and Lewis 1967).

Observations with the H1 + BC9/6,6 dikaryon of *C. cinereus* have shown that large quantities of glycogen are translocated from the stipe to the cap during the early and middle phases of normal carpophore development (Moore et al. 1979). Since primordia formed by L1-dikaryons seem to abort at about the stage of development that this translocation of glycogen is judged to commence, attempts were made to measure glycogen levels in both normal and aborting primordia. Analyses of carpophores produced by four normal dikaryons confirmed that this translocation of glycogen is a general characteristic of this organism and not simply part of the phenotype of the *scl-1*^{H}/*scl-1*^{0} (H1 + BC9/6,6) dikaryon (Fig. 2A). Accumulation of glycogen in the caps of normal carpophores is positively correlated with development of those caps. In contrast, little if any glycogen is accumulated beyond the basal level in carpophore caps of L1-dikaryons, and general cap development is also arrested (Fig. 2B).

Although the total quantity of glycogen contained in carpophores produced by L1-dikaryons was low in comparison with normal carpophores of comparable fresh weights, there were no indications of reproducible defects in glycogen metabolism of vegetative monokaryotic mycelia.

Whether the absence of translocation of glycogen into the cap of aborting primordia produced by L1-dikaryons is a cause of their lack of development or merely another symptom is not known. However, this analysis is important in showing that the abnormal morphology of the carpophores is accompanied by fundamental physiological abnormalities.

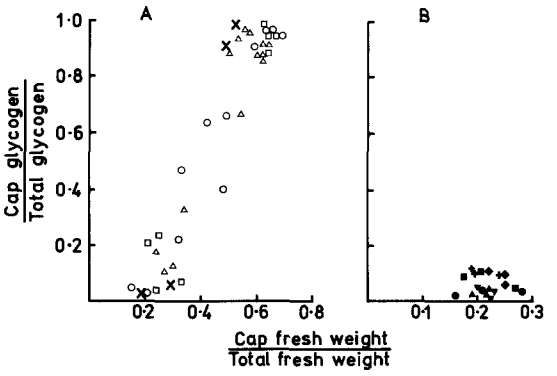

Fig. 2 A and B. Proportion of glycogen located in the cap expressed as a function of the proportion of the total fresh weight represented by the cap. Data in A were derived from normal dikaryons, and in B from dikaryons involving the *scl-4* strain, L1. Each dikaryon is represented by a different symbol: BC9/6,6 + ZBw601/40,40 (□); H1 + ZBw601/40,40 (○); mc-1,3 + Singapore-7 (△); PA1 + ZBw601/40,40 (×); L1 + BC9/6,6 (■); L1 + ZBw601/40,40 (●); L1 + mc-1,3 (▲); L1 + Singapore-7 (+); L1 + H1 (▼); L1 + PA1 (◆). Note that the scale on the abscissa differs between A and B

Table 2. Analysis of progeny from the cross L1 (*scl-4*, A_{27}, B_{25}) x ZBw601/40,40 (*scl*$^+$, A_{40}, B_{40})

447 germinated spores were isolated but 210 failed to grow further. Among the viable progeny the genotype frequencies were:

	Mating type				Totals
	$A_{40}B_{40}$	$A_{27}B_{25}$	$A_{40}B_{25}$	$A_{27}B_{40}$	
scl-4	16	32	13	19	80
scl$^+$	28	53	30	46	157
Totals	44	85	43	65	237

Table 3. Progeny genotypes expected in the cross detailed in Table 2 assuming segregation of two unlinked lethal genes and a modifier

Parental genotypes assumed	
L1	*scl-4, mod,* A_{27}, *let*
ZBw601/40,40	*scl*$^+$, +, A_{40}, +

Expected genotype	phenotype
scl-4, mod, A_{27}, *let*	carpophore defective
scl-4, mod, A_{27}, +	carpophore defective
scl-4, mod, A_{40}, *let*	postgermination lethal
scl-4, mod, A_{40}, +	carpophore defective
scl-4, +, A_{27}, let	postgermination lethal
scl-4, +, A_{27}, +	postgermination lethal
scl-4, +, A_{40}, *let*	postgermination lethal
scl-4, +, A_{40}, +	postgermination lethal
scl$^+$, *mod,* A_{27}, *let*	normal
scl$^+$, *mod,* A_{27}, +	normal
scl$^+$, *mod,* A_{40}, *let*	postgermination lethal
scl$^+$, *mod,* A_{40}, +	normal
scl$^+$, +, A_{27}, *let*	normal
scl$^+$, +, A_{27}, +	normal
scl$^+$, +, A_{40}, *let*	postgermination lethal
scl$^+$, +, A_{40}, +	normal

Although the established phenotype of L1 when crossed with other strains is that carpophore primordia abort prior to the completion of meiosis, on one occasion mature carpophores were produced.

On one culture of the dikaryon L1 + ZBw601/40,40 four carpophores arose at one place and continued normal development, producing crops of basidiospores. At other places on the same culture primordia were aborting, and cultures made from the stipes of the matured carpophores themselves produced only aborting carpophores. Evidently the occurrence of these mature fruits was an exceptional event which was not due to any permanent alteration in the genotype of the dikaryon.

Spores were plated on complete medium and germinated overnight by incubation at 37 °C. The germination frequency was 72.2%. A total of 447 germinated spores were individually isolated to fresh medium. Only 237 of these progeny grew on to form colonies; the rest died, indicating segregation of a post-germination lethal gene. Viable progeny were characterised for mating type specificities and were also crossed against PA1 and the dikaryons were cultured on dung so that the presence of *scl-4* could be scored by its ability to cause carpophore abortion. Results are listed in Table 2.

The *B* mating type factor segregates 109:128 which is not significantly different from 1:1. However, both *scl-4* and the *A* mating type factor show disturbed segregations. There is a seriously reduced number of *scl-4* progeny, and of A_{40} progeny. At the same time that these progeny were being isolated and classified, strain ZBw601/40,40 was in use in other crosses in which postgermination viabilities and mating type segregations were entirely normal. Any disturbing factors in this cross must therefore have been contributed by L1.

The results can be explained by postulating two independently segregating lethal genes. Suppose that *scl-4* itself causes postgermination death but that L1 also carries an unlinked modifier which ameliorates the phenotype to that already described – "recessive" inability to form sclerotia and "dominant" inability to form mature fruits. Then L1 would have the genotype (*scl-4, mod*) and ZBw601/40,40 the genotye (*scl*$^+$, +). Progeny genotypes and phenotypes would be as follows:

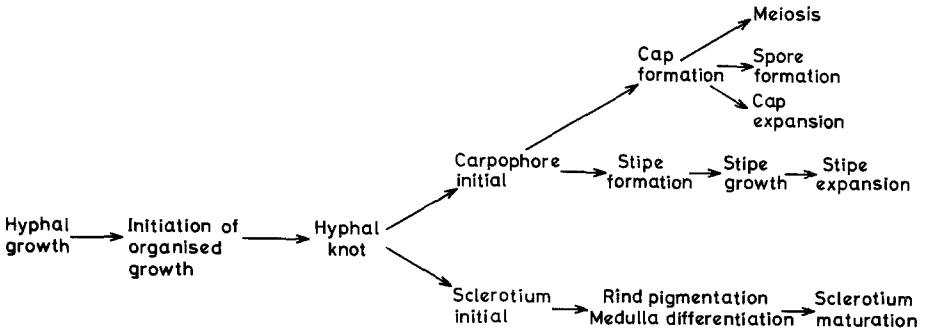

Fig. 3. Developmental pathway describing the relationship between carpophore and sclerotium development in *Coprinus cinereus*

scl-4, mod	alive, showing primordium abortion
scl-4, +	lethal
scl^+, *mod*	alive, normal primordium development
scl^+, +	alive, normal primordium development

To account for the loss of the A_{40} allele a second lethal (contributed to the dikaryon by L1) could be assumed which was only lethal in the presence of A_{40} (or some unknown factor closely linked to this allele). In this case the L1 genotype would be (A_{27}, *let*) and ZBw601/40,40 would have the genotype (A_{40}, +), giving progeny combinations as under:

A_{40}, *let*	lethal
A_{40}, +	normal
A_{27}, *let*	normal
A_{27}, +	normal

Combining these two models, and assuming there is no linkage between the four genes concerned, gives the progeny distribution shown in Table 3. The observed data do not differ significantly from the segregating pattern predicted by this interpretation ($\chi^2_4 = 3.067$, $p = 0.5$ to 0.6).

Discussion

Monokaryons carrying any of the four *scl* genes are unable to form sclerotia. This phenotype is "recessive". Dikaryons which are homoallelic for any of the four *scl* genes are unable to form either sclerotia or carpophores. These observations confirm suspicions based on comparison of microscopic observations that a common pathway of initiation is used by both carpophores and sclerotia (Fig. 3).

The broadest study of developmental mutants in this organism has been done with the Japanese strains of *C. cinereus*. It is worth emphasising that although these are called *C. macrorhizus* they are conspecific with the European isolates (Moore et al. 1979) and, in fact, Okayama-4 was isolated by the present author as a progeny spore of the 5026 + 5132 dikaryon used in the isolation of developmental mutants. Takemaru and Kamada (1972) isolated over 1,500 developmental variants following mutagen treatment of the 5026 + 5132 dikaryon.

Takemaru and Kamada (1972) isolated mutants (designated 'knotless') which were unable to differentiate. This phenotype is similar to that of homoallelic *scl*-negative dikaryons. However, since Takemaru and Kamada (1972) dealt solely with dikaryotic isolates it seems likely that their knotless mutants were "dominant" and thus different in nature from the "recessive" *scl*-genes. Penetrance of *scl*-genes in heteroallelic dikaryons depended on segregation of modifiers. The effect of these modifiers may help in understanding the very high frequency of "dominant" mutations observed by Takemaru and Kamada (1972). The assumption made by these authors was that their developmental variants arose as the result of mutations in genes controlling development. However, mutations in modifers could allow both the expression of previously "recessive" variants present in the original genome, and also make more likley the expression of newly-induced developmental gene mutations. About 15% of the survivors of mutagen treatment were found to carry "dominant" developmental variations (Takemaru and Kamada 1972), but nearly 75% of the variants were assigned (in about equal number) to just two phenotypes. It is possible that "recessive" genes for these phenotypes occurred in the parental dikaryon and that the high frequency of their occurrence in "dominant" form in the mutagen survivors was due to increased penetrance because of mutations in members of what could be a large and heterogeneous population of modifying loci, rather than to mutations in genes involved in morphogenesis.

The *scl* loci are not the only *Coprinus* genes for which "dominance" modifiers have been identified. Senathirajah and Lewis (1975) and Lewis and Vakeria (1977) have studied modifiers which cause normally "recessive" mutants which confer resistance to inhibitions caused by *p*-fluorophenylalanine to become "dominant". One of these resistance genes, pfp^r-*10*, is thought to specify a hexameric product and the modifier locus was presumed to act by keeping pfp^r-*10* gene products separate in het-

eroallelic dikaryons. One way in which the modifiers could cause this effect would be if they were involved in processing signal sequences of structural proteins in such a way that in the presence of particular modifier alleles (which cause the change in penetrance) the signal sequences are wrongly processed and either normal structural proteins fail to reach their correct intracellular destination or abnormal proteins are partially corrected so that they can reach the correct target site. There is evidence that differentiation in basidiomycetes does involve extensive protein processing (Zantinge et al. 1979; de Vries et al. 1980; Moore and Jirjis 1981).

Comparisons of monokaryotic and dikaryotic fruiting in *Polyporus ciliatus* and *Agrocybe aegerita* have led to the conclusion that the same two genes (fi^+ and fb^+) are responsible for carpophore initiation and carpophore differentiation respectively in both mycelial types (Stahl and Esser 1976; Esser and Meinhardt 1977). Although more genes were found to be involved in carpophore differentiation in *Schizophyllum commune*, a single gene was again associated with initiation (Esser et al. 1979). The latter authors suggest, as part of their general model of carpophore development in basidiomycetes, that the fi^+ gene is responsible for the 'step over the threshold from mycelial to plectenchymatic growth, whereas the other genes determine the shape of the fruit body' (Esser et al. 1979). The data given in the present paper show that this is an oversimplification. The *scl*-genes are clearly involved in the change-over from mycelial to organised tissue growth in both monokaryon and dikaryon and are consequently analogous to fi^+. But in the dikaryon they do not specify a pathway which leads inevitably to carpophores. Since in *Coprinus* commitment to carpophore formation occurs subsequent to formation and partial differentiation of the hyphal knot, other genes must be involved in determining commitment to carpophore formation as well as determining the shape of the fruit body.

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