Coprinus cinereus: an ideal organism for studies of genetics and developmental biology

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Introduction

The group of fungi known as basidiomycetes includes the mushrooms and toadstools. Many of these fungi can be found in fields and woods, forming striking and interesting members of the ecological communities. Some of these organisms are extremely difficult to grow in pure culture, but there are a few which can be grown easily and one such organism, *Coprinus cinereus* (one of the ink-cap mushrooms) is described here. Basidiomycetes which can be grown readily in culture provide us with ideal material for study of a wide range of biological problems. The organisms are of interest in themselves, but they also offer the chance to study cellular biochemistry, genetics, and differentiation. Another reason for doing research with an organism like *Coprinus* is that other members of the group are of considerable commercial importance. About 60 000 tonnes of commercially grown mushrooms (*Agaricus bisporus*) are consumed in the United Kingdom annually; at retail prices this crop is worth more than £100M, and the world-wide crop value of *A. bisporus* (total production being about 1 million tonnes) is in the region of $3000M. So the economic value of this species is very evident. Ironically, the basidiomycetes which have commercial value are not the easiest to use for research in the laboratory. While some research must, obviously, be done with these species (Chang and Hayes, 1978), rather more has been done with others which are better candidates as experimental organisms while still being representative of the less tractable basidiomycetes. Among these, *Schizophyllum commune* and some *Coprinus* species stand out as having been the subjects of the most extensive and most wide ranging researches.

Coprinus cinereus is a widespread and very cosmopolitan species. Collections have been made from Europe, the USA, and the Far East. The wild population can be sampled using semi-selective isolation techniques; North (1980) has described a very simple dilution-plating method which is effective. It uses the usual *Coprinus* minimal medium (see Appendix I) supplemented with 25 mg cm^{-3} streptomycin and 7.5 mg cm^{-3} chloramphenicol to discourage bacterial growth (both antibiotics are obtainable from Sigma Chemical Company Ltd—see Appendix II). Samples of horse manure or samples from old compost heaps were suspended in water and the supernatant plated at various dilutions after the mix had been allowed to settle. Incubation of the plates at 37 °C discouraged growth of contaminating fungi.

Such methods allow the isolation of dikaryotic mycelia (having binucleate cells, see below) which can be fruited in culture for progeny monokaryons to be germinated from basidiospores. In this paper the biology of *Coprinus cinereus* is described, and its use in illustrating the principles of genetics and developmental biology is shown.

Abstract

All stages of the life cycle of the basidiomycete fungus Coprinus cinereus *are readily cultured, and the organism is easy and safe to manipulate. It is representative of many other filamentous microorganisms and provides opportunity to demonstrate microbiological techniques with an amenable organism which can also be used to illustrate gene segregations, and cytological, biochemical, and morphological aspects of the morphogenesis of the highly differentiated mushroom fruit bodies.*

Life cycle of *Coprinus cinereus*

The life cycle is depicted in figure 1; the caption explains the various technical terms used in the subsequent text.

The mushroom fruit bodies are the most conspicuous aspect of the life cycle (figure 2), but as they represent the culmination of the sexual process, and in many cases the culmination of particular experiments, it is easier to begin the description with the haploid, monokaryotic mycelium. This is the mycelium which is formed by germination of a single isolated basidiospore and is, in most cases, the type of mycelium which would be obtained from culture collections like the Commonwealth Mycological Institute and commercial suppliers like Philip Harris Biological Ltd.

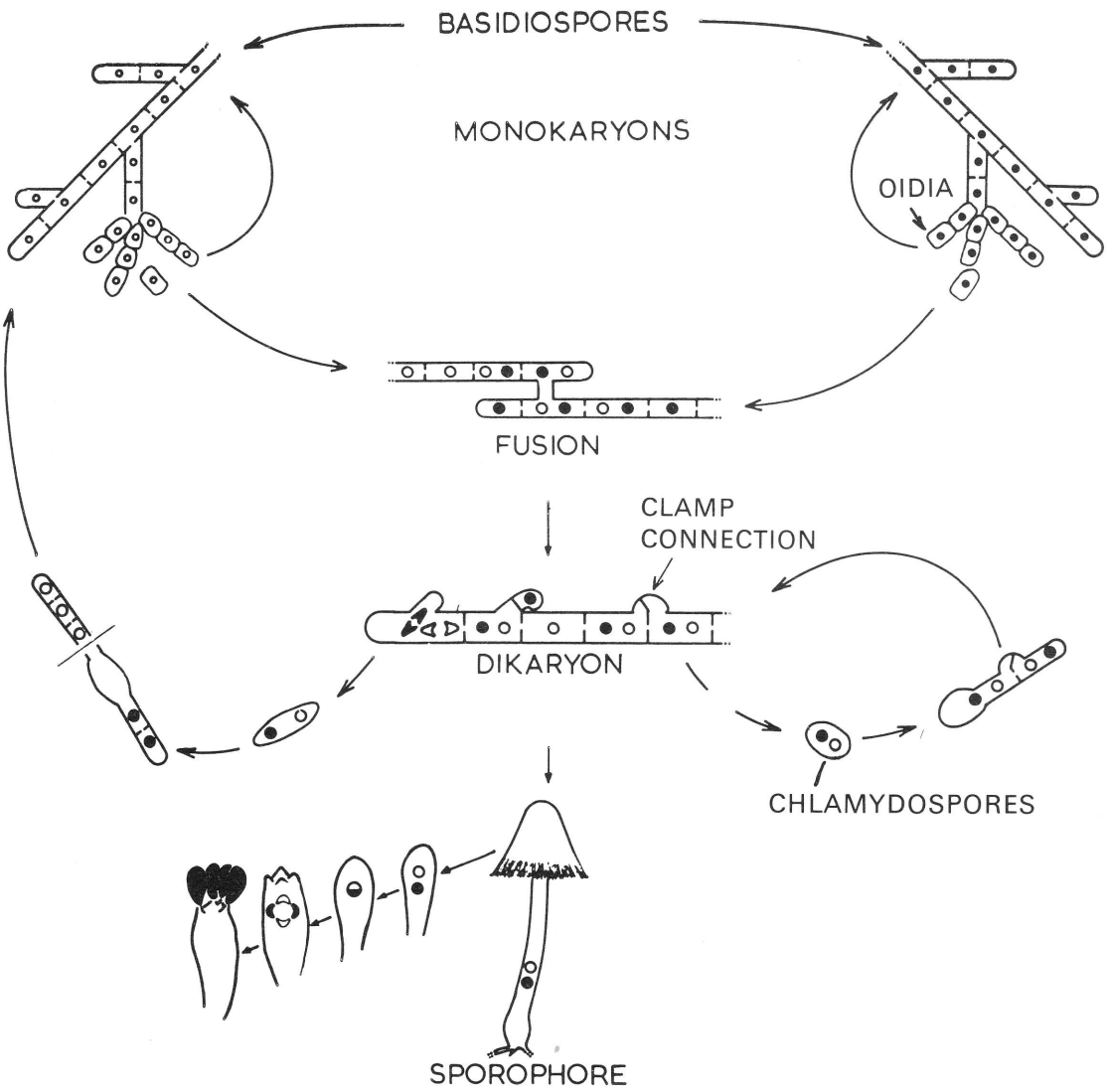

Figure 1 Life cycle of *Coprinus cinereus*. Basidiospores germinate to form mycelium which has cells containing a single haploid nucleus. These *monokaryons* differ in mating type. Hyphae from monokaryons of compatible mating type anastomose (fuse) to form a *dikaryon* which has two nuclei (one of each mating type) in each cell. Growth of this mycelium involves formation of a *clamp connection* at each newly formed septum; one of the two nuclei divides in this specialized branch while the other divides in the main body of the cell. Monokaryons produce *oidia*—uninucleate, unicellular asexual spores—which can germinate to reform the monokaryotic mycelium. Dikaryons can form dikaryotic *chlamydospores* which may germinate to form either a dikaryotic germ tube or two monokaryotic branches. Under the influence of light and at lower temperatures than are optimal for mycelial growth, the dikaryon gives rise to the mushroom fruiting body or *sporophore*. Meiosis occurs in basidia which cover the gill plates of the fruit body cap. Subsequently each basidium forms four *basidiospores* and one meiotic daughter nucleus migrates into each.

(a) Monokaryotic mycelium

The mycelium formed by germination of basidiospores has a single haploid nucleus in each cell and is therefore described as monokaryotic. The monokaryon will continue to grow as long as it is supplied with nutrients (see Appendix I for media recipes), and can be vegetatively cultured indefinitely by serial transfer from Petri dish to Petri dish. The monokaryon is a mycelium of fungal hyphae which grow within the substrate (submerged mycelium), on the substrate (surface hyphae), and into the air above the substrate (aerial mycelium).

Aerial mycelium can differentiate to form asexual spores called oidia which will themselves germinate

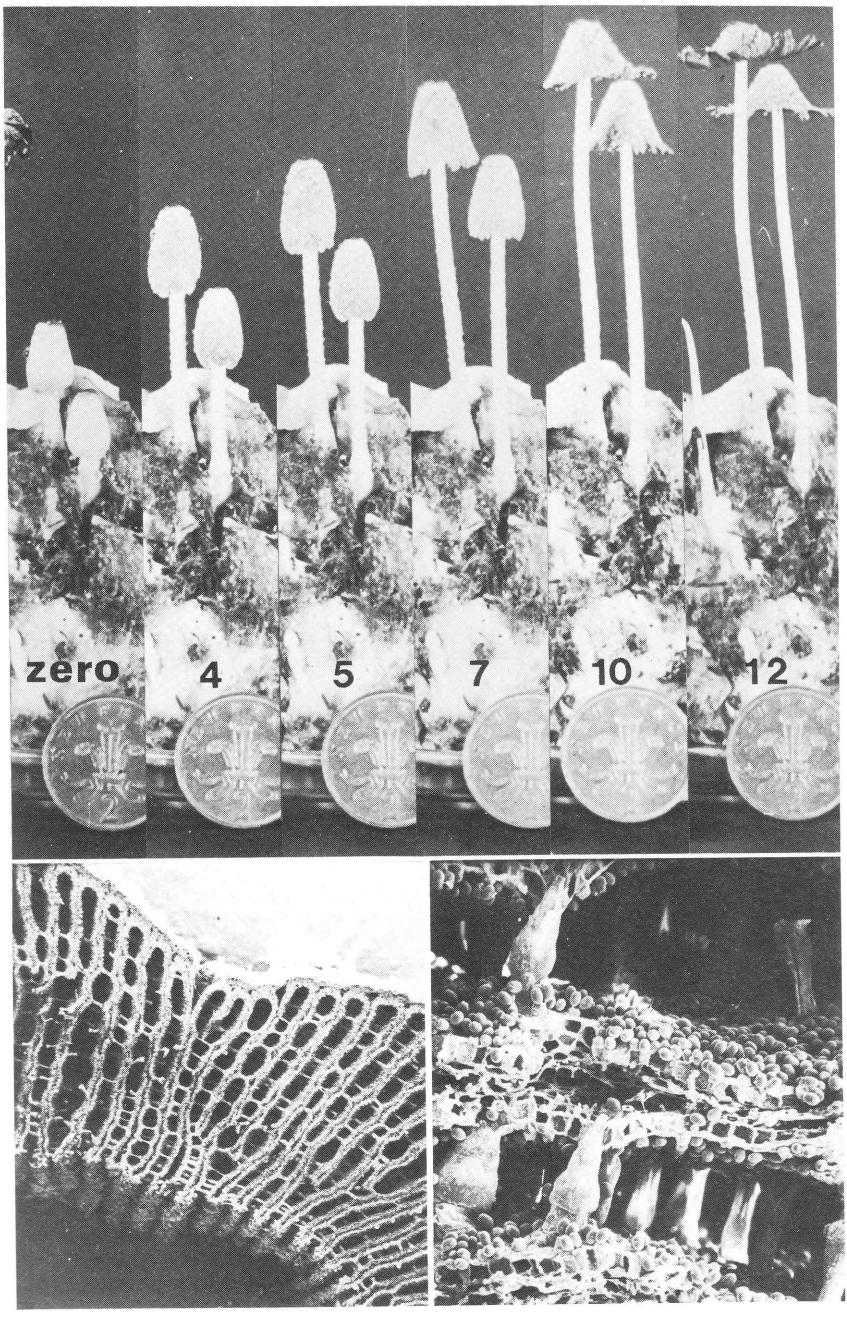

Figure 2 (*Top*) The final 12 hours in the developmental sequence of the *Coprinus cinereus* sporophore. The compost-grown culture was photographed at room temperature. Numbers show the elapsed time in hours since the start of the sequence at time zero. The coin is 25 mm in diameter. Note that the extremely rapid extension of the stipe or stalk of the fruit body is accompanied by expansion and elevation of the cap. (*Bottom left*) General view of the cut surface of a transverse slice of the cap of a fruit body at about the five-hour stage of the above sequence. Note the closely packed gill plates which are kept apart by the large cystidial cells. (*Bottom right*) Closer view of the gills showing the basidiospores covering their surface.

on a suitable medium to produce a progeny monokaryon and which therefore provide a valuable source of inoculum. Oidia are uninucleate, about $5 \times 2\,\mu m$, and colourless, being little more than fragmented hyphae. Oidial suspensions can be prepared by flooding Petri dishes (or slant-culture tubes) bearing confluent monokaryotic growth with sterile water. Oidiospores are scraped into suspension with a flame-sterilized spatula, the hyphal debris being removed by filtration through a very thin layer of well-teased cotton wool. Oidial suspensions can be counted, diluted, plated, or stored in silica gel in the same way as described for basidiospore suspensions below. The functional difference, of course, is that the oidial suspension is a clone of identical genotype, while the basidiospore suspension is a population of meiotic products of different genotypes. Oidial suspensions are ideal for mutagen treatments or hunts

for spontaneous mutants, because the treated cells are so uniform. They can also be used as inocula for plates, slopes, or liquid cultures. However, for this latter use it is pointless to filter-off hyphal debris, and in fact mycelium homogenized in a small amount of water either in a sterile glass homogenizer, blender, or mortar and pestle is entirely satisfactory. A very simple but effective technique is to crush a piece of mycelium (about 1 cm^2 would be enough for most purposes) into fragments which are small enough to be sucked up into a sterile plastic 1 cm^3 syringe (without a needle); then fit a needle (18 to 20 gauge) and force the suspension out of the syringe. Shear forces generated in the needle are sufficient to break up the hyphal fragments. The germination of oidia is erratic and viability is frequently less than 20 per cent.

As well as oidia, most monokaryons are able to form sclerotia (Waters *et al.*, 1975a and b; Hereward and Moore, 1979). These are small (up to 1 mm diameter), globose perennating structures. They have an outer layer of cells, called the rind, with thick walls impregnated with a dark brown melanic pigment. Sclerotia are formed in both the aerial and submerged parts of the colony. They are capable of withstanding adverse environments and when conditions improve will germinate to re-establish a mycelium like the parent.

The radial growth rates of monokaryons vary from strain to strain and also, but to a lesser extent, from medium to medium. The normal range is from about 200 to about 400 $\mu m\ h^{-1}$. At such rates of growth a single inoculum in the centre of a 9 cm diameter Petri dish can grow to fill the plate in about five days incubation at 37 °C. The manner of growing the monokaryon can be manipulated to suit a wide range of experimental needs.

Nutritional tests can be carried out either in liquid medium or on agar-solidified medium. Except for special cases the latter is to be preferred because of the ease of handling. Nutritional tests broadly divide into those which are simply intended to show whether a particular isolate will grow or not, and those in which the response must be quantified in some way.

All-or-none tests are carried out by placing freshly-cut inocula on to the surface of agar-solidified test media (remove as much of the old medium as possible from the inoculum). Each test must be accompanied by an appropriate control. After suitable incubation growth/no growth can be scored by eye. This sort of test is applicable to analyses of auxotrophs in which case the test media would be minimal medium and minimal supplemented with the suspected growth factors, the control medium being complete medium (see Appendix I). It is also applicable to tests for sensitivity to inhibitors, the test medium containing the suspect inhibitor and the control medium lacking it. Examples of such test plates are shown in figure 3. In these cases, the more inocula that can be put on each plate the better, and a 9 cm plate can accommodate up to about 40 or 50 individuals. Of course, incubation time is limited to about 48 h, as after this the colonies will certainly overgrow. It is also necessary to use small, reasonably regular inocula. These are best cut from the colony to be tested using an inoculating needle made of tungsten wire, which because of its tensile strength is far easier to use than the platinum or nichrome wires usually employed. A suitable wire is black-drawn tungsten wire, 0.012″ (0.3 mm) diameter (Tungsten Manufacturing Company Ltd). A 3–4 cm length can be mounted in the chuck of a commercially-available handle or crimped into the flame-softened end of a length of glass tubing. Bending the terminal 4–5 mm at right angles and sharpening the tip in molten $NaNO_2$ produces an implement which is ideal for isolating individual germinated spores and cutting and transferring all sorts of mycelial inocula.

Where the response to the medium needs to be quantified it is necessary to consider carefully the type of information required. There are two forms of the test; in one the response is expected to become more positive with increasing dosage (for example, determination of the response of an auxotroph to increasing concentrations of its required growth factor), while in the other the response is expected to become more negative with increasing dose (for example, response to increasing concentrations of an inhibitor). Both of these cases can be accommodated by growth tests done in liquid medium from which the mass of the mycelial harvest is determined. However, because of the way in which the filamentous fungal colony grows, only the latter type of investigation (quantifying reduction in growth) can be reliably done by measuring the rate at which hyphae extend across the surface of agar media as described below. This is an important point. Measurement of the hyphal growth rate is technically very attractive. It is an easy experiment and the same colony can be measured over a long period of incubation. Determination of dry mass of mycelia is technically more demanding, mainly because it is a destructive test and sufficient replicates have to be made so that mycelia can be removed and dried successively during the incubation period. The reason that hyphal extension growth rate analysis is not universally applicable rests on the ability of most fungi to maintain a 'normal' growth rate even in nutritionally deficient conditions. The growth rate of *Coprinus disseminatus* on water agar was very little different from the rate on nutrient agar (Butler, 1961). Of course, very few hyphae are formed in these circumstances, but those which are formed grow at the normal rate; as conditions improve, more hyphae and hyphal branches are formed. Thus when the test is for a positive (increasing growth) response dry mass measurements must be used. On the other hand full use can be made of the much simpler growth rate measurements when the additions to the medium are expected to inhibit growth; but even here it must be remembered that the connection between linear

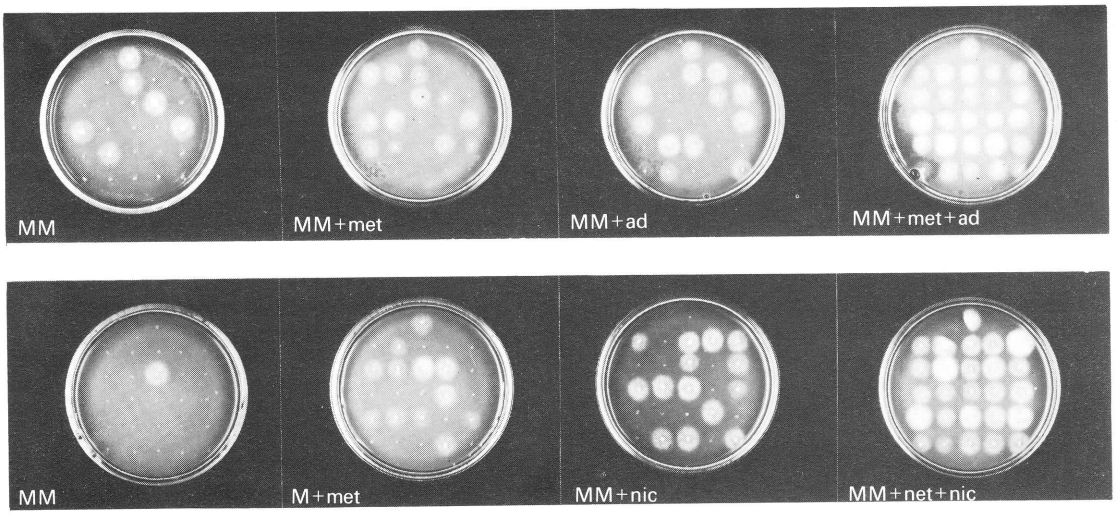

Figure 3 Examples of some test plates scoring crosses between auxotrophic mutants of *Coprinus cinereus*. The first example, in the four plates across the top of the display, shows some progeny from a cross between a strain requiring methionine for growth and one requiring adenine. The progeny are tested on minimal medium (MM—on which only prototrophs can grow), on minimal + methionine (on which prototrophs and methionine-requirers can grow), on minimal + adenine (on which prototrophs and adenine-requirers can grow), and lastly on minimal + methionine + adenine (on which all progeny can grow, but this is the only medium on which doubly-mutant recombinant progeny will grow). In inoculating these plates the progeny were laid out in a standard pattern and the position of each individual progeny in that pattern was maintained. Comparison of the growth of each inoculum of the 26 progeny tested on each medium shows that seven require methionine, seven require adenine, six are double mutants requiring both supplements, and the remaining six are prototrophs requiring neither supplement. Thus there are about equal numbers of parental and recombinant progeny types, so these two genes must have segregated independently at meiosis. The second cross in the lower set of photographs involves the same methionine-requirer as the first but now it has been crossed with a strain that requires the vitamin nicotinic acid. The tests show that this sample of spores contained eleven methionine-requiring progeny, thirteen nicotinate-requirers, one prototroph and one which required both methionine and nicotinate. In this cross, then, recombinants represented only about 8 per cent of the total so these two genes must be linked together on the same chromosome.

growth rate of the colony margin and 'cubical' growth of the whole colony is a complex one (Bull and Trinci, 1977; Trinci, 1978, 1979).

Extension growth tests are done by inoculating the centre of Petri dishes which contain agar-solidified medium and measuring the diameter of the colony at regular intervals during incubation. To maintain sterility the measurements are usually carried out by viewing the colony through the base of the dish with the lid in place. Thus it is essential to use a clear medium and, preferably, plastic Petri dishes too. Measurement can be done with a millimetre scale rule made of clear plastic. This is quite adequate providing it is remembered that the data are accurate only to about 1 to 2 mm. More exact measurements can be made with the aid of a microscope but this is necessary only where behaviour of individual hyphae is of interest. Colonies are generally best measured to the nearest 0.5 mm across two diameters at right angles. Some investigators have inscribed the diameters to be measured on the bottom of the plate before inoculation. However, inhibitors sometimes cause the usually circular colony to lose its circularity. To ensure that representative growth rates are still obtained it is best to measure the colony first across a diameter chosen at random and then across a second at right angles to the first. Tests should always be replicated; five replicates are best, but three will suffice. It is important that the inocula be standardized as far as possible. They are standardized in size by being cut with a punch which gives circles 2–5 mm in diameter (an old cork borer which can be flame-sterilized is fine), and in vigour by being cut from a region about 2 mm behind the margin of a vigorous stock plate (about four days old). Remove the old medium from the inoculum before planting it on to the fresh medium. Colony diameter varies linearly with time after about 24 hours and until the colony begins to reach the edges of the dish (at about 120 hours). Thus an adequate estimate of growth rate can be obtained from four measurements taken at 24, 48, 72, and 96 hours (figure 4). Further measurements will improve the estimate and where a medium supplement greatly inhibits growth it may be necessary to continue beyond 120 hours.

The preferred technique is to grow in liquid medium. Any of the media listed in Appendix I can be used in this way, simply by omitting agar. Inocula can

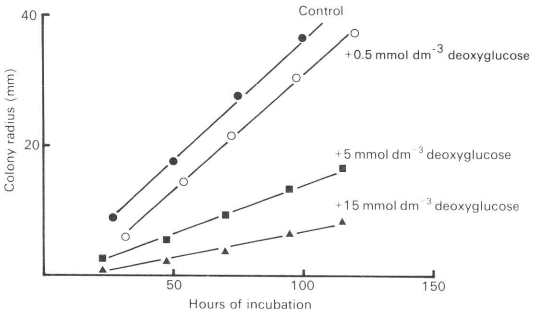

Figure 4 Use of measurements of colony radius to monitor growth inhibitions. Plot shows the effect of adding the glucose analogue 2-deoxy-D-glucose to medium containing 5 mmol dm^{-3} glucose as sole source of carbon.

be suspensions of spores or hyphal fragments and the mycelium can be grown as a surface culture (without agitation) or as a submerged culture on a shaker. An effective small-scale technique is to inoculate into a film of 10–15 cm^3 of liquid medium contained in a 9 cm diameter plastic Petri dish. Depending upon the size of the inoculum, one to three days incubation will fill the plate with a hyphal mat which can be separated readily from its culture medium. This technique can be scaled up by increasing the volume *pro rata* with increase in area of the container. The volume of medium can be increased if evaporation proves to be a problem in particular incubators.

However grown, harvested mycelium should be washed with distilled water, blotted dry with paper towels, and the mass measured. This gives a fresh mass which depends more on the amount of blotting than anything else. However, either the whole mycelial pad or a portion of it can be dried to constant mass at 90 °C to obtain a truly representative dry mass. Mycelium which is not used immediately for biochemical experiments can be preserved in the freezer at −20 °C or lower. It should be wrapped in aluminium foil or polythene before being frozen.

(b) Dikaryotic mycelium

The dikaryon (two nuclei per cell) is the fertile mycelium; it is formed by mating two (parental) monokaryons. The mating response in *Coprinus cinereus* is controlled by an outbreeding mechanism which depends on the operation of two incompatibility genes called A and B. Like the incompatibility genes of higher plants, A and B exist in a wide range of different (allelic) forms; these are distinguished by numeral subscripts—A_1, A_6, B_2, B_5, and so on. For an attempted mating to form a dikaryon, the two parental monokaryons must carry different alleles at both A and B; such a pairing is said to be compatible. So $A_1B_2 \times A_2B_1$ or $A_1B_1 \times A_2B_2$ are compatible matings which will form dikaryons, whereas $A_1B_2 \times A_2B_2$ is an incompatible mating which will not give rise to a dikaryon. The A and B genes are located on different chromosomes so they segregate at random during meiosis.

When small pieces of compatible monokaryons are put side by side on the surface of an agar medium and incubated, growing hyphae intermingle, fuse, and dikaryotization occurs by nuclear migration (Giesey and Day, 1965). The dikaryotic growth which occurs within 24 to 48 hours is readily distinguished by its clamp connections, acute angled branching habit and rapid growth rate (Lewis, 1961; Casselton, 1978). Normally, both parents are dikaryotized as a result of nuclear exchange and migration; in this case either of the parental (originally monokaryotic) mycelia can serve as a source of dikaryon inoculum. However, only nuclei migrate, so these two mycelia are reciprocally constituted as far as their cytoplasms are concerned.

An interesting way to demonstrate segregation of the mating type genes is to isolate about 20 to 40 basidiospores (see section 2(e)) from a fruit body and make attempted matings in all combinations. The progeny sample (providing it is truly representative) will fall into four classes. For example, assume that we have a fruit body from a dikaryon of mating type AxBx × AyBy; then the table of results from such an experiment would be of the form:

	AxBx	AyBy	AxBy	AyBx
AxBx	−	+	−	−
AyBy	+	−	−	−
AxBy	−	−	−	+
AyBx	−	−	+	−

(+ = no dikaryon formed; − = no dikaryon)

Of course, this does not tell us the identity of 'x' and 'y' but merely illustrates their segregation. However, the mating type genes can provide valuable additional information from experimental crosses so their routine scoring can be useful. The best way to do this is to carry out crosses with monokaryotic strains with just two different A alleles and two B alleles, and to have at hand a set of test strains of known mating type which can be used to determine the mating type of any progeny monokaryon. For example, progeny from any combination of strains which carry A_5/A_6 and B_5/B_6 can be scored for dikaryosis with testers of mating type A_5B_5, A_5B_6, A_6B_5, and A_6B_6. This mating type series is available from Philip Harris Biological Ltd.

Tester stocks are best grown in Petri-dish culture and when used for testing cut into small blocks of 1–2 mm^2. The blocks of the tester mycelium are spaced out on a dish of medium (up to 16 per 9 cm diameter dish) and inocula of the unknowns placed alongside to touch. After 36 hours incubation at 37 °C, or 48 hours at 27 °C, the matings can be scored. Scoring can be done by eye, but confirmation needs microscopic examination of the fringe of hyphae lying on the agar surface on the tester side to ensure that they have clamp connections. Clamps are more difficult to see on aerial and submerged hyphae.

The dikaryon mycelium can be grown in vegetative culture in the same way as can the monokaryon, it readily forms sclerotia in both the aerial and submerged parts of the colony but does not form oidia.

(c) Fruiting bodies

The most important pathway of dikaryon differentiation, of course, is the fruit body or carpophore (Moore *et al.*, 1979). Containers of fruiting medium (see Appendix I) inoculated with some large pieces of a dikaryon, incubated in the dark for three days at 37 °C, and then placed in an illuminated incubator at 26–28 °C, will produce fruiting bodies in 7 to 10 days (Moore and Ewaze, 1976). Fruiting will not normally take place at temperatures above 30 °C, nor in continuous darkness. In natural daylight conditions fruit bodies frequently begin discharging spores in the late evening. This is the criterion for fruit body maturity. Soon after maturation the cap undergoes the autolysis which is characteristic of the 'inkcap' fungi; as it matures the fruit body cap self-digests so as to remove spent gill tissue from the path of the forcibly ejected spores, but some spores become trapped in the liquid autolysate which thus becomes black and ink-like. Autolysis will be virtually complete by dawn. Use can be made of the evening-to-dawn fruiting synchrony in order to arrange for the supply of fruit bodies in defined stages at convenient times by the use of incubators with lights controlled by time switches. A cycle of 16 h light : 8 h dark is most convenient and if it is arranged that the lights are switched on at about 0900 h freshly autolysed fruits will be available during the working day. Advancing the time of this artificial 'dawn' enables fruit bodies to be harvested at any desired stage of development. This procedure has been refined by Lu (1974), who reports that if the light cycle is begun at 1600 h then karyogamy occurs and initiates meiosis at 0900–1000 h on the fifth day after transfer to the illuminated incubator. Although illuminated incubators allow for experimental sophistication of this sort, cultures will fruit perfectly well at room temperature on window-sills. Light is required for initiation of fruiting, but only very low light levels are needed; a North-facing window is quite adequate; a single 60 W tungsten light-bulb will do.

(d) Cytology

Meiosis occurs in a remarkably synchronous manner in *Coprinus*, and can be easily visualized using simple staining techniques (Lu and Raju, 1970). A small segment of the developing cap can be removed with a razor blade without impairing subsequent development of the rest of the fruit. The tissue is fixed in a mixture of 9 parts absolute ethanol, 6 parts propionic acid, and 2 parts 10 per cent chromic acid (10 g chromium trioxide in 100 cm^3 water), and can be stored in this solution for long periods at −20 °C.

To monitor karyogamy a small piece of gill tissue is crushed (using a brass rod) in 3 per cent ferric ammonium sulphate dissolved in 50 per cent propionic acid, and a drop of 2 per cent haematoxylin is added. After squashing, the preparation can be viewed immediately and the proportion of nuclei which contain two nucleoli (= pre-karyogamy) or one nucleolus (= post-karyogamy) can be determined. However, the chromosomes are not visualized unless the fixed gill segments are subjected to acid hydrolysis. To do this transfer the fixed material to hydrolysis solution (1 part concentrated HCl + 1 part absolute ethanol) which has been prewarmed to 70 °C. Hydrolyse for one minute at this temperature and then a further two minutes at room temperature. Chill on ice and transfer the gill segments to 70 per cent ethanol. Transfer through three changes of 70 per cent ethanol before staining and squashing as above. This treatment produces material in which the cytoplasm is little stained, though nucleoli still stain intensely and chromosomes are visible. If the nuclei are squashed out of the basidia they swell, and gentle tapping on the coverglass will separate the chromosomes. Contrast can be enhanced by heating the slide over a small flame before squashing, or by sealing the cover with wax and allowing the stain to ripen overnight.

Basidia can also be examined without the extensive disruption of gill structure caused by crushing and squashing. After fixation a single layer of basidia can be stripped away from the gill using watchmakers' forceps, hydrolysed, and stained. After a brief rinse in 70 per cent ethanol, the 'half gill' segment can be mounted in glycerol for examination. Such preparations provide a striking opportunity for demonstration of the synchronous meiotic process in *Coprinus* (Pukkila, Yashar, and Binninger, 1984).

(e) Basidiospore isolation

The basidiospores are fairly large (10 × 6 μm) and black and are consequently easy to see with moderate magnification. Basidiospores are meiospores, formed immediately following the meiotic division, and are therefore haploid. Basidiospores are borne on basidia which carpet the gill plates (figure 2); spore suspensions are most easily made by removing a small piece of the gill (less than a cubic millimetre) and shaking it in 5 to 10 cm^3 of sterile water. The process should be done aseptically if it is intended to germinate the spores. Very large numbers of spores are produced on the gills so very dense spore suspensions can be prepared. Suspension densities can be estimated using a haemocytometer counting chamber, and aseptic dilutions made with sterile water to make the desired inoculation density.

Basidiospores are not dormant; they will germinate as soon as they are furnished with appropriate conditions. In fact, when fruit bodies are allowed to collapse into an autodigested spore mass, the spores will germinate *in situ*. This can be prevented by

refrigerating the culture. Providing the moisture layer around such deposited spores does not freeze they can be kept undamaged for many weeks in the refrigerator. However, contamination may be a problem, so if a spore suspension is to be kept for a long period it is best to mix it with an equal volume of glycerol (as an anti-freeze) and store at −20 °C. Basidiospores from freshly-autolysed fruit bodies can be stored dry in small screw-capped vials of silica gel. Chromatography grade silica gel is best (60–120 mesh, BDH Chemicals. Product number 15049). About 1 to 2 cm^3 of the powder is added to the vial which is then sterilized in a hot-air oven at 160 °C for three hours. After being allowed to cool, pieces of fruit body gill are thoroughly mixed into the silica gel with a sterile glass rod. The preparation is suitable for long-term storage. Much the same technique can be used for storage of the asexual spores, oidia, obtained from the monokaryotic mycelium. Here, though, a water suspension of oidia of at least 1×10^6 per cm^3 is mixed with an equal volume of sterile non-fat milk (e.g. Skim Milk Powder, Oxoid Ltd. Product number L31. Do NOT use domestic dried milk preparations). Approximately 0.25 cm^3 of the mixture is added to the silica gel. Since the reaction of water with silica gel is exothermic the preparation should be kept cool during the initial period of water absorption by being plunged into an ice bucket. The silica gel tubes can be stored refrigerated (best for oidia) or at room temperature (basidiospores). Viable spores have been recovered from silica gel preparations over eight years old.

Basidiospores are germinated experimentally by being spread on to the surface of an agar-solidified medium (see Appendix I for recipes). The spreading can be done by pipetting 0.1 to 0.25 cm^3 of suspension on to the agar and then distributing the liquid over the whole surface with the aid of a spreader made by bending a piece of glass rod into an 'L' shape. Sterilize the glass rod by dipping into alcohol and flaming; allow to cool, but spread the spore suspension as soon as possible after pipetting on to the medium. The spore suspension should be adjusted by dilution of the original preparation so that a total of about 400 spores will be plated on to a standard 9 cm diameter Petri dish. This is about optimum for microscope observation of germinating spores and their subsequent isolation to other plates. If the spread plates are to be used to observe colonies visible to the naked eye fewer spores (100 to 200) should be plated and/or a growth inhibitor (like sorbose, see Appendix I) should be included in the medium, otherwise the colonies will overgrow one another. Incubation at 37 °C for 18 to 24 hours is sufficient to allow good germination, and fair-sized colonies will be evident after 36 to 48 hours. Basidiospore germination is generally quite good. It is unusual for viability to fall below 50 per cent, and 70 to 90 per cent viability is not uncommon.

Study of morphogenesis and development

Although *Coprinus* is a 'simple' eukaryote, the fruit body is differentiated into a number of tissues, and shows a variety of growth responses which can form the basis of interesting experiments. Fruit body formation requires light and temperatures lower than about 30 °C. Thus there is scope for experimentation on the effects of higher temperatures (fruit bodies are usually malformed and frequently have fewer spores than usual), and the effects of continued culture in darkness (fruit body primordia may be formed but they do not mature), or after various light exposures. The effective wavelengths are in the blue region of the spectrum and even coloured light-filters can be used to show this. Fruit body growth is negatively geotropic and this again can be simply demonstrated by changing the orientation of cultures which are producing fruits. Since basidiospores are distributed by air currents and must therefore fall clear of the cap from the gills on which they arise, this response to the gravity vector is extremely important in the biology of the species.

Probably the most striking aspect of fruit body development is the extension of the stipe or stalk. In the final stages this extends by about 8 cm in as many hours. The extension process is endotrophic (relying on internal nutritional reserves) so this structure also lends itself to interesting experimentation; extension continues in fruits, and even isolated stipes, removed from the parent mycelium. Gooday (1974) has reported a series of experiments on such an *in vitro* system which could form the basis for demonstration of the physiological effects of desiccation, humidity, and growth inhibitors.

The biochemistry of fruit body development has been extensively studied (for review see Moore, Elhiti, and Butler, 1979) and if photometric facilities are available numerous enzyme systems could be examined. Assay methods have been described by Moore and Ewaze (1976, mainly enzymes of carbohydrate and Krebs cycle metabolism), Ewaze, Moore, and Stewart (1978, mainly enzymes of nitrogen metabolism), North (1977, phosphatase enzymes), and Al-Gharawi and Moore (1974, 1977, glutamate dehydrogenase enzymes). The NADP-linked glutamate dehydrogenase is of particular interest as it is developmentally regulated, being found in the fruit body cap but not in the stipe (Stewart and Moore, 1974; Moore, 1981).

Enzymes involved in mobilizing nutrients in the substrate are particularly useful as many are easy to assay, yet they can be used to illustrate aspects of regulation which are important in the general biology of the organism. Good examples are the use of the chromogenic *p*-nitrophenyl glucosides to assay glucosidases in culture filtrates and mycelium of cultures grown on different carbon sources (Wilson, 1967), and assay of extracellular proteases using the milk-agar clearing technique (Cohen, 1981).

Genetic analysis

Most of the genetic analysis done so far has made use of conventional nutritional mutants which have normally been produced by treating oidia with ultraviolet light or with chemical mutagens. An ordinary germicidal UV lamp can be used quite effectively to mutate populations of oidia suspended in sterile water. Use about 7 cm^3 of the suspension in a standard Petri dish (so that there is a uniform thin layer of suspension over the base of the dish) and control the time of treatment by removing and replacing a glass cover as appropriate. Treatment times depend on the power of the lamp and the distance between the lamp and the dish. In a pilot experiment, determine the amount of kill for a small range of exposure times by plating untreated and treated samples, after dilution, on to complete medium. Identify the treatment which gives closest to 99 per cent kill and either use that as a routine treatment or prepare a more detailed kill-curve (which can be used to study mutation kinetics).

The range of mutants which have been dealt with in *C. cinereus* has been listed by Lewis and North (1974), and they also give a linkage map. Anderson (1971) describes the basic genetics of the organism and the sorts of experiments which can be used to demonstrate simple genetic principles. Anderson's book can be obtained from Philip Harris Biological Ltd (Product number A90010/2) and the content applies to *Coprinus cinereus*.

Mutants with altered colony morphology are often encountered in mutation runs, but the only one which has been used much is *den* (dendroid) which causes stunted growth due to excessive hyphal branching. This phenotype has been used as a means of selectively identifying recombinants in crosses intended for the study of recombination processes (Lu, 1969, 1974).

It is a great deal easier to use a selective technique to isolate mutants, as when one is interested in mutants resistant to some growth-inhibiting supplement, than to search for nutritionally defective mutants. Very large numbers of spores can be plated in selection experiments because the bulk of them are not expected to grow. For example oidia were plated at about 10^5 per plate during experiments to isolate mutants resistant to inhibition by sugar analogues (Moore and Stewart, 1971; Moore, 1973). With such large numbers of spores it is best to use 'pour plates'; instead of being spread over the agar surface the spores are mixed with the molten medium (cooled to about 50 °C) before the plates are poured.

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We wish to thank Mr Douglas Adams and the Sirius Cybernetics Corporation for the invocation to share and enjoy.

Appendix I: Media recipes

Coprinus cinereus is a very tolerant organism and can be grown on a number of the standard mycological media, like malt extract agar and potato-dextrose agar. Fruit bodies will be produced on a fruiting medium consisting of 4 g yeast extract + 10 g malt extract + 4 g glucose + 10 g agar per dm^3. The basic minimal medium contains 20 g glucose, 2 g asparagine, and 30 cm^3 of the salt solution described below, per litre (agar can be added to a concentration of 10 to 15 g dm^{-3}). Generally, tap water can be used to make up media. If distilled water is used one of the standard mycological trace element solutions should be added. The salt solution used in minimal medium is made up as a 600 cm^3 stock containing 10 g ammonium tartrate, 29 g disodium hydrogen phosphate, 27 g potassium dihydrogen orthophosphate, 6 g sodium sulphate (all of these salts should be anhydrous), and 20 mg thiamin hydrochloride. Minimal medium may be supplemented with individual requirements of auxotrophic mutants by adding specific amino acids (100 mg dm^{-3}), purines and pyrimidines (50 mg dm^{-3}) or vitamins (2 mg dm^{-3}). For complete medium the minimal recipe is supplemented with 30 cm^3 dm^{-3} of a nutrients solution containing (in 500 cm^3), 12 g hydrolysed casein, 12 g yeast extract, 16 g malt extract, and 25 cm^3 of a nucleic acids solution. The last is prepared as follows: 1 g each of yeast nucleic acid and thymus nucleic acid (both obtainable from BDH Chemicals Ltd) is mixed with 15 cm^3 of 1 mol dm^{-3} NaOH, and 1 g of each is mixed with 15 cm^3 1 mol dm^{-3} HCl. The two mixtures are autoclaved at 103.5 kPa for ten minutes, then mixed, adjusted to pH 6, filtered while hot, and made up to 40 cm^3 with water.

After autoclaving (10 to 15 minutes at 103.5 kPa) the media should have a pH in the region of 6.8.

The simplest basal solution which can form the basis of media in which sources of carbon and nitrogen are controlled consists of 1.45 g disodium hydrogen phosphate, 1.35 g potassium dihydrogen orthophosphate, 0.29 g sodium sulphate (all salts anhydrous), 0.12 g magnesium sulphate $7H_2O$, and 1 mg thiamin hydrochloride per litre. Carbon and nitrogen sources can be added as desired.

Sorbose can be added as a growth inhibitor to restrict mycelial growth. Its concentration depends on the level of the normal carbon source also included in the medium. If glucose is used as carbon source then five to ten times as much sorbose must be used; if either fructose or acetate are used as carbon sources then an equal or less than equal amount of sorbose will effectively restrict colony growth.

Appendix II: Suppliers

BDH Chemicals, Poole, Dorset BH12 4NN.
Commonwealth Mycological Institute, Ferry Lane, Kew, Richmond, Surrey TW9 3AF.
Oxoid Ltd, Basingstoke, Hants RG24 0PW.
Philip Harris Biological Ltd, Oldmixon, Weston-super-Mare, Avon BS24 9BJ.
Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset BH17 7NH.
Tungsten Manufacturing Company Ltd, Fishergate Works, Portslade, Brighton, Sussex BN4 1PY.

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