CONTROL OF HYPHAL ORIENTATION IN COLONIES OF *MUCOR HIEMALIS*

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This is a study of factors controlling the development of hyphae of *Mucor hiemalis* on a plane uniform agar surface, whereby a single germ tube elongating linearly gives rise to a colony enlarging symmetrically on an approximately uniform front through 360° from its centre. Most branches arise closely behind an existing elongating hyphal tip, and only a small number are formed on older parts of hyphae. The observed distances between successive points of of origin of hyphae fit a γ distribution with estimated shape parameter 1·5 and an estimated scale parameter of 0·011 μm. The observed angles at which branches diverged from their parents' axes ranged within a normal distribution (mean 56° σ 17°). The first formed hyphae extend at about 20 μm per h for about 1 h after germination. Subsequently some stop growing permanently or temporarily; variation in the rates of elongation at the remaining tips, and at those branches formed after this first hour fits a half normal curve (mean 85 μm h^{-1}).

Growing hyphal tips repel each other, the maximum distances between tips at which this interaction has been seen range from about 10 μm to about 20 μm. No appreciable re-orientation has been seen when living tips come close to dead ones, or if they approach agar sites from which another growing tip has been removed. The effects of moving growing tips close to older parts of the mycelium are less regular. Half of 68 observed cases stopped elongating, approximately one quarter (16) grew away from the older hyphae before making contact, approximately one quarter (18) crossed the older hyphae with no visible change in direction.

Analysis of patterns produced by computer simulation indicates that the circular shape is the expected result of any system having the observed variation in branching and rates of elongation. No other controlling factor is needed to explain the patterns seen. In particular there is no evidence that orientating interactions between hyphal tips have any biologically important effect on colony shape. The amount to which the observed variation could be increased or reduced without affecting the growth patterns in any biologically important way is being studied.

Most fungal colonies which grow from a single spore onto a plane surface of a uniform nutrient medium are approximately circular, unless their shape has been influenced by some asymmetric environmental factor. The peripheral hyphae lie approximately radially in such colonies, and they extend on an approximately even front. The colony starts as a linear structure, however, i.e. as a germ-tube elongating by apical growth. Usually there is a single germ-tube; lateral branches arise from this, but in the early stages the lateral diameter of the colony will be very small compared with that on the axis of the germ-tube (Fig. 1A/B). The development of the circular shape therefore involves a change from a linear system enlarging in one direction to a branching system enlarging symmetrically through 360° from its centre (Figs 1–4). This is a study of the factors which lead to this result.

A circular colony would not form if branches arose separately at equal intervals, and if all hyphal tips extended at the same constant rate at the same constant angle of divergence between their axes. For example, Figs 5–7 are shapes which would develop if branches developed regularly at uniform rate at 90°, 45° or 10° of divergence. Decrease of distance between branches, regardless of divergence, would eventually reach a limit in which all growth points are in contact, i.e. one in which the cell enlarges spherically, not linearly (Fig. 8).

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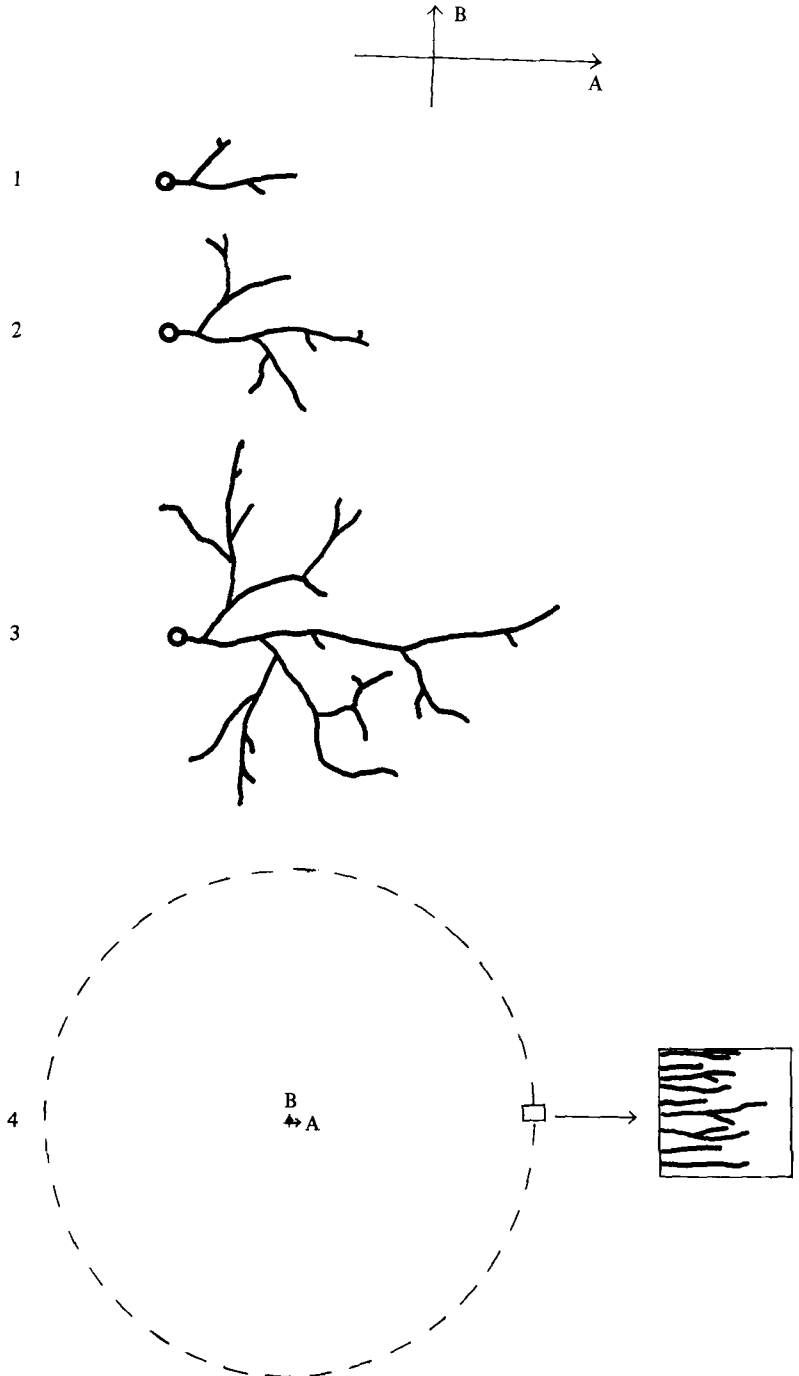
Figs 1–4. Schematic development of a circular fungal colony by apical growth
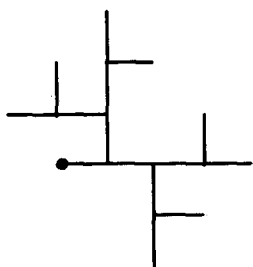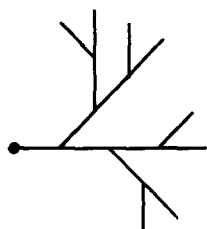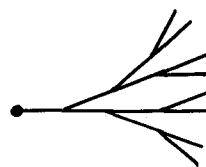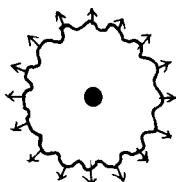and branching from a single germ-tube.

Fig. 5. Branch angle 90°.   Fig. 6. Branch angle 45°.   Fig. 7. Branch angle 20°.

Fig. 8. Negligible internode distance.

Figs. 5–8. ●, Spore on germination. Schematic results of linear branching at uniform angles and internode distance.
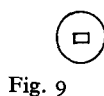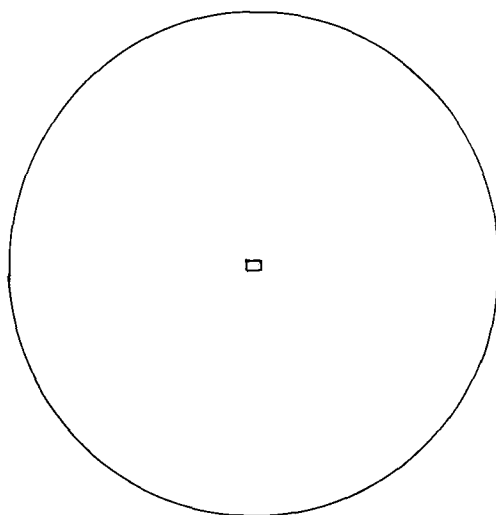

Fig. 9

Fig. 10

Figs. 9, 10. Schematic comparison of effect of shape of young colony as point of origin in relation to colony size at maturity.

Within the normal concept of 'apical growth', however, reduction in the interval between branch origins would affect the compactness of the developing colony, but not its geometric symmetry, and no such changes in a regular system could produce a perfectly circular colony. This effect of asymmetry in the early stages of growth of a colony would become less noticeable by eye in later stages. In particular the difference between any two diameters at right angles to each other would become small compared with their mean. Thus an asymmetric figure resulting from the early branching of hyphae may become in effect a 'single point' origin as a colony ages (Figs 9, 10). The possible developments become almost infinitely more varied if the angles and frequency of branching, and the growth rates, are not constant; this is the normal situation in practice. Each development is of course the product of 'controlled' metabolism; this control may be direct by

limitation of the genotype, or by phenotypic response to variables in the environment. We do not, however, know of any experimental investigation of the relative importance of such control in the development of colony shape.

The principle of the 'Random Walk' would apply to any one hyphal tip changing its direction at random intervals through random angles, and its eventual mean displacement would be zero, i.e. after any period there would be an equal chance of the tip lying at any point on the circumference of any circle around the point of origin having a radius less than or equal to the mean displacement during that period. Silvey (1977) has pointed out that where branching occurs it is plausible to seek an explanation in terms of a 'contact growth process' (Mollison, 1977). Thus a 'group' can be envisaged in which each individual has a fixed location in space. These individuals produce 'offspring' which wander off from the parents' location and take up their own location, this displacement being dependent on variables within known distributions. If the growth is on the plane we can talk of the probability that offspring will be located at a specified distance and direction from the parent. There are theoretical results (Mollison, 1977) which show that for simple arrangements of this sort the groups will eventually become circular.

For fungi we can think of 'nodes' (i.e. points of origin of branches) as the individuals, the nearest neighbours on branches in the direction of growth as offspring of that node. Typically an individual will have two offspring, though sometimes it will have only one or none. This process has the complicating factor that the locations of the two offspring are not independent of each other. Because of this built-in dependence it is not possible to appeal to proven theoretical results about eventual shape. Nevertheless, the dependence does seem weak enough to justify the conjecture that the eventual circular shape could be explained in this way. However, theoretical results of this sort seem certain to be restricted to the asymptotic shape, that is its approximate shape after a 'sufficiently long' period of time. Theoretical results concerning the time that must elapse before asymptotic formulae become suitable approximations, and theoretical studies of the pattern of growth in the early stages, seem certain to be mathematically intractable; it is, however, precisely such results which are of greatest interest in the examination of whether self-regulatory mechanisms play any important part in colony growth.

Two lines of investigation have therefore been followed here. In one the shapes of colonies after known periods of growth have been compared with the shapes of computer simulations based on measurements of the range of behaviour found in these colonies. The information so obtained is then reviewed in relation to the identified mathematical possibilities of the systems. The other line investigates visible interactions between hyphal tips by micromanipulation.

METHODS

A single authentic strain of *Mucor hiemalis* Wehm. (Commonwealth Mycological Institute, Strain 2123) was used throughout the investigation. This was maintained on agar slopes of 2 % malt agar (Difco malt extract 2 g, agar 2 g, deionized water 100 ml; autoclaved at 120° for 20 min). Hyphal growth and orientation were studied by direct observation and by measurements of changes recorded in series of time-lapse photographs of colonies growing on 10 ml of 2 % malt agar in 9 cm Petri dishes. This amount gave a thin layer of agar which was convenient for photography by transmitted light, and which ensured that most of the branching occurred on a plane close to or on the agar surface. An aqueous suspension of spores was spread over the surface at a concentration which left approximately one spore per field of view of a × 10 objective. Inoculated dishes were incubated at 23° for seven to ten h, by which time germ-tubes were visible on most of the spores. Colonies developing from selected spores were then photographed at room temperature at 30 min intervals. The photographic assembly was operated in normal laboratory light, with additional transmitted light through the microscope lenses for the time of film exposure only. If other spores were present in the field of view they were removed by micromanipulation at the beginning of the observations. The retained colonies grew over the sites from which these spores had been removed, with no visible sign of being affected by any residual matter in or loss of nutrients from the medium.

Hyphae were measured on prints of successive photographs at magnification of × 25 to × 160; the measurements were converted to give a record of extension of the hyphae in terms of μm per h.

In the literature a variety of terms are used to refer to hyphae at different stages of development of the colonies; to avoid confusion the following terms will be used:

Primary hypha. The hypha clearly recognizable as developing from the first formed hyphal tip. In the early stages this is commonly referred to by others as the 'germ-tube'. This name is, however, not a conveniently informative one for the parts of

this hypha which can still be recognized in substantial colonies in which much branching has occurred, and there seems to be no useful purpose in retaining it for some arbitrarily defined early stages of growth, e.g. the phase until a lateral branch is formed.

Primary axis. The axis which the primary hypha follows in the colony.

Parent hypha. A hypha from which branches originate.

Branch hypha. A hypha formed from a growing point which develops behind the growing point of a parent hypha.

First order, second order, third order branches. Branches formed successively on a branching system:

Node. The point of origin of a branch on a parent hypha.

The effects of moving hyphal tips into new directions of elongation by micromanipulation was examined in colonies 7 to 10 h old in the same conditions. The subsequent directions of elongation of realigned hyphae were recorded by camera lucida diagrams at 30 min intervals.

RESULTS

Measurements of parameters of growth found in colonies

Growth rates. Records of rates of elongation of primary hyphae in each of 6 colonies during the first 13 h after germination (Table 1); of primary hyphae and their first branches of first order and of
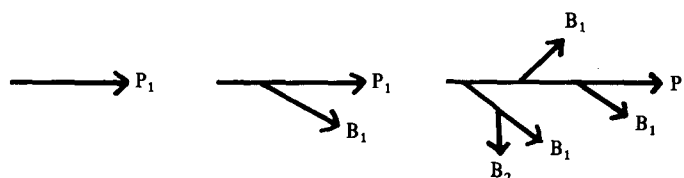
P_1, Parent; B_1, first-order branch for parent P_1; B_2, second-order branch for parent P_1.

Table 1. *Rate of growth ($\mu m/h$) of six primary hyphae in 6 separate colonies*

Time after germination (h)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 6
0·5	20	26	36	30	20	18
1	30	52	36	30	30	26
1·5	54	64	58	36	30	54
2	54	64	58	60	52	54
2·5	78	76	64	70	60	64
3	78	116	76	72	60	94
3·5	78	168	94	76	72	94
4	116	168	94	166	78	184
4·5	154	220	190	166	152	228
5	154	56	208	166	152	184
5·5	154	276	260	220	230	276
6	230	220	260	56	152	276
6·5	154	276	248	276	230	0
7	230	220	282	220	230	276
7·5	230	276	302	276	230	276
8	230	276	320	220	152	276
8·5	230	276	190	276	306	306
9	230	276	282	276	306	368
9·5	156	276	340	276	306	368
10	308	332	320	276	306	184
10·5	308	332	340	276	230	0
11	308	332	340	332	306	0
11·5	308	332	320	332	384	0
12	232	332	360	332	384	0
12·5	308	332	340	332	380	0
13	308	332	340	332	306	0

Table 2. *Rates of growth ($\mu m/h$) of six primary hyphae and of their first branch of the first order and of the first branch of the second order up to a period of 6·5 h after germination*

Time after germination (h)	Order of branch*			Order of branch			Order of branch		
	P	1	2	P	1	2	P	1	2
	Colony 1			Colony 2			Colony 3		
0·5	19	—	—	19	—	—	19	—	—
1	76	—	—	19	—	—	19	—	—
1·5	95	95	—	57	—	—	38	—	—
2	95	76	—	76	57	—	57	—	—
2·5	95	95	—	95	76	—	38	—	—
3	95	95	—	95	152	—	133	—	—
3·5	152	95	—	190	57	—	133	—	—
4	114	114	—	209	152	171	171	—	—
4·5	152	133	—	266	114	247	247	152	—
5	152	152	57	266	76	114	266	152	—
5·5	152	133	95	247	76	228	285	190	57
6	209	209	133	285	114	171	285	190	95
6·5	228	209	171	304	209	152	360	152	152
	Colony 4			Colony 5			Colony 6		
0·5	19	—	—	20	—	—	18	—	—
1	38	—	—	40	—	—	26	—	—
1·5	38	—	—	55	—	—	54	—	—
2	76	—	—	55	—	—	54	26	—
2·5	95	—	—	55	—	—	64	26	—
3	133	—	—	111	—	—	94	54	—
3·5	184	92	—	111	—	—	94	94	54
4	276	92	—	166	55	—	184	94	94
4·5	276	184	—	166	55	—	228	228	94
5	276	378	276	221	0	—	184	228	368
5·5	276	276	276	55	0	110	276	285	94
6	0	0	92	277	0	55	276	0	110
6·5	368	368	276	221	55	55	0	228	110

* P, Primary hypha; 1, first-order branch; 2, second order branch.

second order in each of six colonies during the first 6·5 h after germination (Table 2); a complete record of rates of all the branches of a colony between 4·5 and 14 h after germination (Table 3); a frequency distribution of rates in a sample which is discussed below (Table 12).

Sites of origin of branch hyphae. Records of distances between nodes on primary and first order branches of each of 6 colonies (Table 4); of numbers of branches produced by standard lengths of typical hyphae in successive circumferential zones of each of 3 colonies (Table 5); of numbers of branches formed on older parts of primary hyphae of each of three colonies (Table 6).

A frequency distribution of internodal distances in a sample which is discussed below (Table 13).

Orientation of branches. Records of angles which branch hyphae form with parent hyphae in different circumferential zones of each of three colonies (Table 7).

A frequency distribution of angles in a sample which is discussed below (Table 14).

Factors affecting orientation made by realignment experiments

Effects of the following micromanipulations are recorded: (i) bringing living hyphal tips close to each other (Table 8); (ii) bringing living hyphal tips onto agar from which another living tip has been removed (Table 9); (iii) bringing living hyphal tips close to dead ones (Table 10); (iv) bringing living hyphal tips close to older parts of the parent hyphae (Table 11).

DISCUSSION

There is a recognizable overall pattern of approximately linear increase of rate of elongation of the primary hypha from about 20–30 $\mu m/h$ in the first hour after germination to a maximum ranging

Table 3. *Rate of growth (μm/h) of individual hyphae in a colony of* Mucor hiemalis *from 4·5 h after germination up to 14 h of growth*★

Time after germination (h)

(h)	P	P	1	2	2	2	2	2	2		1	1	2	1	1	2	2	2	2	2
4·5	166	110	55																	
5	166	166	55								166									
5·5	222	0	0								110	110								
6	55	0	0	110	110						55	110								
6·5	277	166	0	55	55						0	0	277		110					
7	222	110	55	55	110						0	222	166		222					
7·5	277	0	55	0	110	55					0	0	110		277	110				
8	222	55	166	0	55	110					0	0	55		166	222				
8·5	277	0	110	0	166	55					0	0	0		166	277				
9	277	0	166	0	166	0	55	110			0	55	0		277	0	110			
9·5	277	0	110	0	110	0	55	166			0	0	55		332	0	222			
10	277	0	55	55	277	55	0	222			0	0	166		222	0	277	110	110	
10·5	277	0	166	0	222	0	0	222	110		0	0	0	166	222	0	222	0	222	
11	332	0	277	0	222	0	0	166	55		0	0	0	222	332	0	166	0	277	
11·5	332	0	110	0	222	0	0	55	0	110	0	0	110	166	277	0	277	0	332	277
12	332	0	0	222	55	0	0	222	0	222	0	0	55	222	332	0	222	0	277	332
12·5	332	0	0	0	332	0	0	277	0	222	0	0	0	55	332	0	222	0	332	332
13	332	0	0	0	332	0	0	277	110	166	0	0	0	166	277	0	277	0	332	222
13·5	332	0	0	0	332	0	0	332	222	0	0	0	0	222	277	0	110	0	332	277
14	332	0	0	0	332	0	0	388	222	0	0	0	0	0	277	0	332	0	388	322

(h)	1	2	2	2	2	2	2	2	1	1	1	2	2	2	1	2	2	2	1	1	2	1
8	166																					
8·5	277								110													
9	277	166							166	55												
9·5	277	277							110	277												
10	277	277	110						0	110	110				166							
10·5	332	166	222	222					0	110	166				332	110						
11	277	166	0	277					0	0	332	55			166	222		222				
11·5	332	0	0	332	277				0	0	277	55			222	332		277	166			
12	332	0	0	332	166	110			0	0	277	0	110	110	222	388		166	277		55	
12·5	332	0	0	277	0	332	55	55	0	55	166	0	222	222	277	332	55	166	222	110	277	
13	388	0	0	332	0	222	222	332	110	0	222	0	277	55	277	277	222	110	55	0	166	332
13·5	332	0	0	332	0	0	55	277	222	0	222	0	222	0	222	332	166	277	0	0	222	388
14	332	0	0	388	0	0	0	110	332	0	22	0	55	0	222	332	0	332	0	0	222	388

Footer labels: 1 | 2 (brace) | 1 | 1 | 1 | 2 (brace) | 1 | 2 (brace) | 1 | 1 | 2 | 1

★ Primary hypha; 1, First order branch; 2, Second order branch.

Table 4. *Distance (μm) between branch origins on primary hyphae and on*
first order branches of six colonies

	First five laterals from the primary hypha					First five second-order branches from the first branch				
Replicates	1	2	3	4	5	1	2	3	4	5
Colony 1	50	100	44	69	88	206	288	300	163	250
Colony 2	63	44	69	56	81	56	38	81	56	81
Colony 3	44	38	38	69	50	225	56	119	88	69
Colony 4	30	139	83	55	111	55	30	55	110	83
Colony 5	46	184	206	300	385	225	184	300	225	184
Colony 6	77	119	77	38	119	77	154	119	119	192

Table 5. *Number of branches produced on 500 μm lengths of typical hyphae at different distances from the spore: three hyphae were recorded from three sides of each colony*

Replicates	0–500 μm from the spore	500–1000 μm from the spore	1000–1500 μm from the spore	1500–2000 μm from the spore
Colony 1	2	2	0	1
	3	2	2	1
	3	2	1	3
Colony 2	5	5	5	4
	0	2	3	3
	3	3	4	3
Colony 3	2	4	1	0
	3	2	4	4
	2	2	4	3

between about 200 μm/h and 350 μm/h after 8–10 h of growth (Table 1). This pattern of change is similar to that reported by Trinci (1974) and earlier authors (Brown, 1923; Smith, 1924; Plomley, 1959). There are, however, very big fluctuations within this general pattern, and many

cases where the rate of elongation is substantially reduced or even halted after different periods of growth (Tables 1–3). In most colonies the primary hypha remained identifiable and continued to elongate for more than 13 h after germination. Most branches do not start with the period of slow elongation; usually they attain the rate of their parent within half an hour to an hour of formation, and in some cases they exceed the parents' rate. They show fluctuations in rates similar to those shown by primary hyphae, and some stop elongating within a short time of formation. Mostly the hyphae which stopped elongating did not start again within the period of observation, some gave a few intermittent short periods of extension, rarely a hypha continued elongating for several hours after restarting from a pause.

No correlation has been found between the times of fluctuation in different elements of each branching system; in particular the rate of elongation of parent hyphae was not apparently affected by the formation of a branch. This agrees with the observations of *Coprinus disseminatus* made by Butler (1961) but there is no indication here of a consistent difference between the extension rates of primary, first-order and second-order branches

Table 6. *Occurrence of branch hyphae in the older parts of the primary hyphae in three colonies*

Number of newly formed laterals in the older parts of the primary hypha

Replicates	3–5 h after germination	5–7 h after germination	7–9 h after germination	9–12 h after germination
Colony 1	—	1 appeared after 4 laterals had been formed	1 appeared after 5 laterals had been formed	3 appeared after 8 laterals had been formed
Colony 2	1 appeared after 2 laterals had been formed	1 appeared after 4 laterals had been formed	—	2 appeared after 10 laterals had been formed
Colony 3	1 appeared after 1 lateral had been formed	1 appeared after 2 laterals had been formed	1 appeared after 5 laterals had been formed	1 appeared after 14 laterals had been formed

Table 7. *Angles which branch hyphae form with parent hyphae at different diam from spore; eight angles at random were measured on each diam*

Replicates	0–500 μm from the spore	500–1000 μm from the spore	1000–1500 μm from the spore	1500–2000 μm from the spore
Colony 1	35°	45°	80°	35°
	55°	40°	35°	35°
	45°	35°	45°	45°
	85°	45°	50°	55°
	35°	65°	60°	55°
	45°	40°	50°	60°
	55°	55°	60°	75°
	75°	65°	45°	45°
Mean	54°	49°	53°	51°
Colony 2	95°	40°	40°	80°
	40°	70°	40°	65°
	50°	35°	45°	55°
	60°	55°	50°	50°
	65°	65°	35°	50°
	55°	40°	60°	60°
	45°	45°	55°	55°
	50°	60°	45°	45°
Mean	57°	51°	46°	57°
Colony 3	70°	55°	55°	50°
	55°	70°	50°	35°
	60°	55°	60°	50°
	65°	50°	55°	65°
	70°	70°	45°	45°
	45°	55°	75°	45°
	55°	40°	45°	70°
	45°	45°	55°	35°
Mean	58°	55°	55°	49°

Table 8. *Reactions of two growing hyphal tips when they were brought close to each other*

	Number treated		Numbers reacting		
	36	30	3	1	2
Diagrams of the treatment and reactions*					
Reaction of the growing tips after 30 min		Grew away from each other before making contact	Grew away from each other after making contact	Stopped growing	Proximity had no effect on direction

* – – – →, Original growth direction; ———→, growth of reoriented hyphal tips.

such as she observed in that fungus. The photographed records have been supplemented by many short-term direct observations of rates using an ocular micrometer. These observations were made of young colonies and of hyphae advancing at the edges of older colonies. These all fit within the ranges of the tables, and no consistent pattern of fluctuations or of mean rates has been found in any of them.

The records of sites of nodes and of initial directions of growth of branches are made from the first stages at which these are measurable on the photographs. Hence the preponderance of hyphae growing on the surface of the agar, and the

Table 9. *Reactions of the growing hyphal tips to the agar from which other growing hyphal tips had been removed*
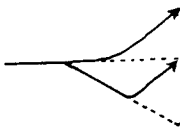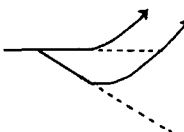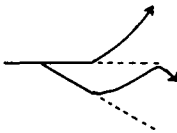
	Number treated	Numbers reacting	
	30	25	5
Diagrams of the treatment and reactions*			
Reaction of the growing tips after 30 min		Growth of reoriented laterals apparently unaffected by the agar on which parent had grown	Proximity to agar on which hypha had grown had some effect on orientation

* – – – –, Original growth direction; ——, growth of the reoriented hyphal tips.

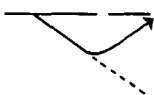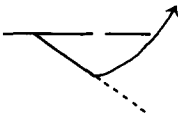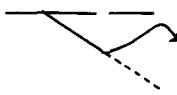Table 10. *Reactions of the growing hyphal tips to the presence of severed hyphal tips which were no longer growing*

	Number treated	Numbers reacting	
	26	24	2
Diagrams of the treatment and reactions			
Reactions of the growing tips after 30 min		Growth of reoriented lateral apparently unaffected by the presence of the severed tip	Grew away from the severed tip

* – – – –, Original growth direction; ——, growth of the reoriented hyphal tips.

Table 11. *Reactions of the growing hyphal tips when they were brought close to the older parts of the hyphae*

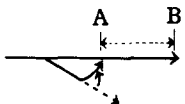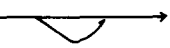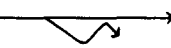|	Number tested		Numbers reacting		
	68	34	5	11	18
Diagrams of the treatment and reactions*					
Reaction of the growing tips after 30 min		Laterals did not grow after reorientation	Laterals grew away before making contact with the hypha	Laterals grew away after making contact with the hypha	Crossed over the hypha without showing any reaction

* – – – –, Original growth direction; ——, growth of reoriented hyphal tip; A⊢– – – –⊣B ranged from approximately 30 μm to approximately 75 μm in different experiments.

Table 12. *Frequency table of rate of growth for a sample of* 135 *unimpeded branches*

Growth rate ... (μm/h)	0–119	120–239	240–359	360–479
Frequency ...	41	43	37	14

A half-normal distribution HN $(0, \sigma^2)$, with probability density function

$$p(x|\sigma) = \{\sqrt{2}/(\sqrt{\pi}\sigma)\} \exp\{-x^2/(2\sigma^2)\} \quad (0 < x < \infty),$$

was fitted to the raw data and σ estimated by maximum likelihood as 107.

Factors affecting orientation obtained from realignment experiments

Most hyphae continued to extend after they had been moved, and they did so initially in whatever direction they were pointed at the end of the manipulation. The angles at which lateral branches lie in relation to parent hyphae may therefore result from some control of the direction in which the branch growing point is pointed when it forms. Small divergencies from the axis of elongation appear apparently irregularly as hyphae grow across an apparently uniform unimpeded agar surface. These may be due to small unmeasured differences of the environment at different sites on the agar, or perhaps to the geometry of the process of apical elongation (Madelin *et al.*, 1978).

The possibility that these hyphal alignments are affected by a metabolite or metabolites liberated from growing hyphae, or by nutrient exhaustion in agar near living hyphae, is examined by the manipulations recorded in Tables 8 and 9, and by the observation that the direction of growth of hyphae is not visibly affected if they grow across agar from which other germinated spores have recently been removed. The evidence does not support either hypothesis: it does not, however, discredit the alternative hypothesis that an unstable metabolite is involved, or one which is very rapidly inactivated or diffused through the agar after secretion. The five cases of reorientation on the sites from which growing hyphae had been removed (Table 9) could be due to exceptional accumulation or to slow inactivation of such a metabolite. Table 10 shows that the interaction depends on the presence of living hyphal tips; in all these cases the cyto-

'initial' direction, may not represent either the precise position of the initial growing point, or of the first axis of departure from the parent, in relation to the circumference of the hypha.

The great majority of the branches are formed in acropetal succession in racemes behind the parent tip, but a small number are also formed on older parts of the hyphae (Table 6). The inter-nodal distances mostly varied in the range 30–385 μm (Tables 4, 13). No consistent patterns have been seen in the variations within or between different circumferential zones (Table 5), and no correlations have been seen between rates of elongation and frequency of branching in any tables. The lateral distributions of branches may show a bias to one side or another for short periods, but no consistent pattern has been seen overall.

The angles in Table 7 and in the bigger random sample in Table 14 mostly lie within the range of 20°–90°, with means between 46°–59°. No consistent patterns have been seen within these limits in any zones or samples.

Table 13. *Frequency table of internodal distances* (μm) *for a sample of* 178 *branches*

Internodal distance ...	0–59	60–119	120–179	180–239	240–299	300–359	360–419	420–479	480–
Frequency	35	42	46	26	15	10	2	1	1

A gamma distribution Ga (α, θ), with probability density function

$$p(x \mid \alpha, \theta) = \theta^\alpha x^{\alpha-1} e^{-\theta x}/\Gamma(\alpha) \quad (0 < x < \infty),$$

was fitted to the raw data and (α, θ) estimated by maximum likelihood as (1·5, 0·011) respectively.

Table 14. *Frequency table of angles of branching* (degrees) *for a sample of* 202 *branches: a distinction is not made between clockwise and anti-clockwise angles*

Angle ...	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–109	110–119
Frequency ...	2	5	23	47	47	44	14	13	5	1	1

A normal distribution $N(\mu, \sigma^2)$, with probability density function

$$p(x \mid \mu, \sigma) = (\sigma\sqrt{2\pi})^{-1} \exp\{-(x-\mu)^2/(2\sigma^2)\} \quad (-\infty < x < \infty)$$

was fitted to the raw data and (μ, σ) estimated by maximum likelihood as (56°, 17°) respectively.

plasm of the severed tip became packed with vacuoles; and it did not extend further during the period of observation. The two cases in which the direction changed could be related to some difference in the rate at which the tip died, or to exceptional accumulation or slow inactivation of the postulated metabolite(s) after severance.

The interactions of growing tips with older parts of the hyphae are more irregular (Table 11). Only 7 % of the tips grew away before coming into contact, a further 16 % grew away after coming into contact, and 20 % crossed the hypha without changing direction at all. The differences in behaviour did not show any consistent relationship with the differences in distance of the site from the tip of the parent hypha. These reactions could be expected if the postulated metabolite is produced only at the hyphal tip, and if it is inactivated or diffuses to low concentration rapidly after extrusion. This hypothesis would not account, however, for the 50 % of cases in which the branches stopped elongating after realignment. This is possibly associated with the production and/or accumulation of a different metabolite or mixture of metabolites in the older parts of the colony.

The most revealing information from this series of observations is, however, that the realignment effects have been seen only when living hyphae grow very close to each other (i.e. commonly at distances of the order of 10–20 μm or less). In a developing colony most of the hyphal tips are far wider apart than this, and branch origins are usually first visible at 50 μm to 300 μm from the tip. Hence these effects might contribute to the initial orientation of the growing point as a branch forms on a hypha, but it seems unlikely that they will have much functional importance in the gross orientation of vegetative hyphae in later phases of their development.

Simulation of colony growth

Possible patterns which might result from growth randomly distributed within known parameters for the distributions of direction, internodal length and growth rates have been examined by simulation. The main aim was to simulate, and to display visually, the growth of a hyphal system exhibiting the same individual growth morphology as found in a genuine colony, without introducing any other regulating or coordinating mechanisms. Measurements of growth rates, internodal distances and branching angles for unimpeded branches at the edges of colonies of a variety of ages were therefore collected; naturally, the growth characteristics of such unimpeded branches largely determine the shape of the colony. Half-normal, gamma and normal distributions were

fitted to the resulting frequencies (see Tables 12, 13 and 14 respectively), and unknown parameters were estimated by the method of maximum likelihood.

Simulations were performed on an ICL 2976 machine at Glasgow University with output directed to a System 90 'Economist' graph plotter. Each simulation consisted of the growth of a colony in 25 'half-hourly cycles', starting from the emergence of the primary hypha, and a plot of the colony was output at every fifth cycle. With a suitable scale change, and an adjustment for the slower growth rate in the first few hours, a direct visual comparison with the time lapse photographs of genuine colonies is possible (see Fig. 11). Details of the simulation are as follows.

At the beginning of each cycle, a straight-line growth distance for each 'parent' branch is independently generated from an HN $(0, 107^2)$ distribution, using the Box & Muller (1958) transformation. The position of the next node on the parent branch is then examined, the internodal distance being independently generated from a Ga $(1\cdot5, 0\cdot011)$ distribution using the method of Whittaker (1974). If this next branching is to take place during the current cycle, a branch angle is generated independently from an N $(56, 17^2)$ distribution, clockwise or anti-clockwise with equal probability. The growth, if any, of the offspring during its first cycle is appropriately related to the distance of its node from the parental tip.

Storage requirements escalate rapidly with the number of cycles and it is not feasible to keep track of possible intersections of growing tips with old branches. The simulations therefore permit branches to cross each other without impediment. The shape of the colony is, however, determined by the tips near the periphery, which are involved in few if any intersections. The unrealistic element of continued unimpeded growth in the older parts of the colony is partially alleviated by the introduction of a pruning routine aimed at deleting some of the growing tips which would be obstructed by other hyphae.

Simulation results

Figs 11–13 show that, in essential respects, the growth of the real colony is accurately reflected in the simulation. Ten repeat simulations showed some variability in the timing, but not in the pattern, of the simulated colony growth. For example, after 12·5 h the maximum dimensions of the colonies, measured parallel and perpendicular to the alignment of the primary hypha, varied from (2·96, 2·99) mm to (3·92, 3·72) mm respectively. However, this variation is caused almost solely by the variability in the number of branches
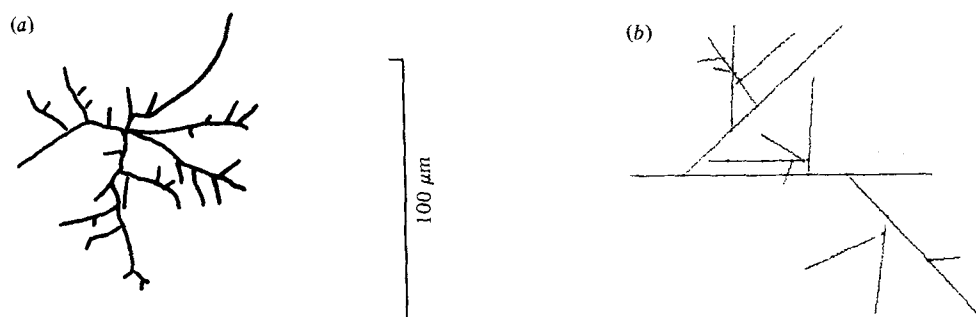
Fig. 11. (*a*) Tracing from a microphotograph of a colony of *M. hiemalis* approximately 5 h after emergence of the germ-tube. (*b*) Result of computer simulation after 5 h run.
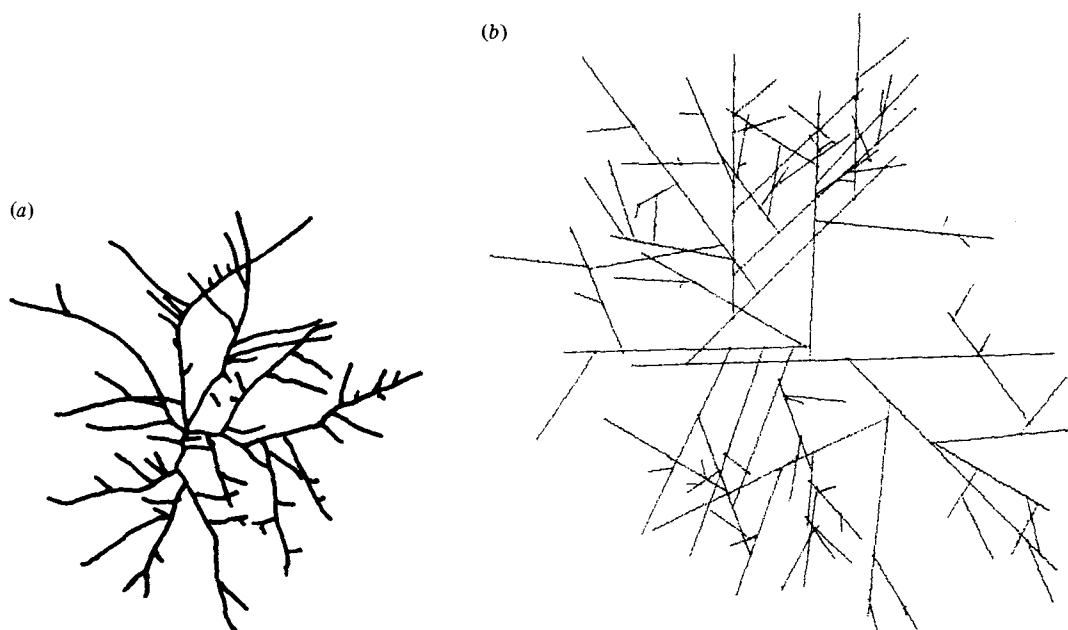

Fig. 12. (*a*) Tracing from a microphotograph of a colony of *M. hiemalis* approximately 7·5 h after emergency of the germ-tube. (*b*) Result of computer simulation after 7·5 h run.

produced during the first few cycles; with knowledge of the number of branches present after the first 2·5 h, say, the future course of the simulation is largely predictable as regards aggregate properties such as number of branches, size of colony, attainment of circular shape, etc.

The above results lead us to suggest that the shape of the colony is an expected consequence of randomly varying growth within certain parameters. These parameters are presumably under genetic control. On the basis of 'Occams Razor' there is no need to introduce any other inter-hyphal orientating factors or self-regulatory systems co-ordinating growth of parent and branch hyphae. They may have little importance even if they are present.

The question now arises of whether the particular values of the parameters of growth rates, internodal distances and angles of branching are critical. To what extent could they be altered without affecting the result in any functionally important way? This is now being examined by further simulations. Preliminary results suggest that the distributions measured in the real colonies may be optimal for rapid formation of circular colonies. This could be functionally important
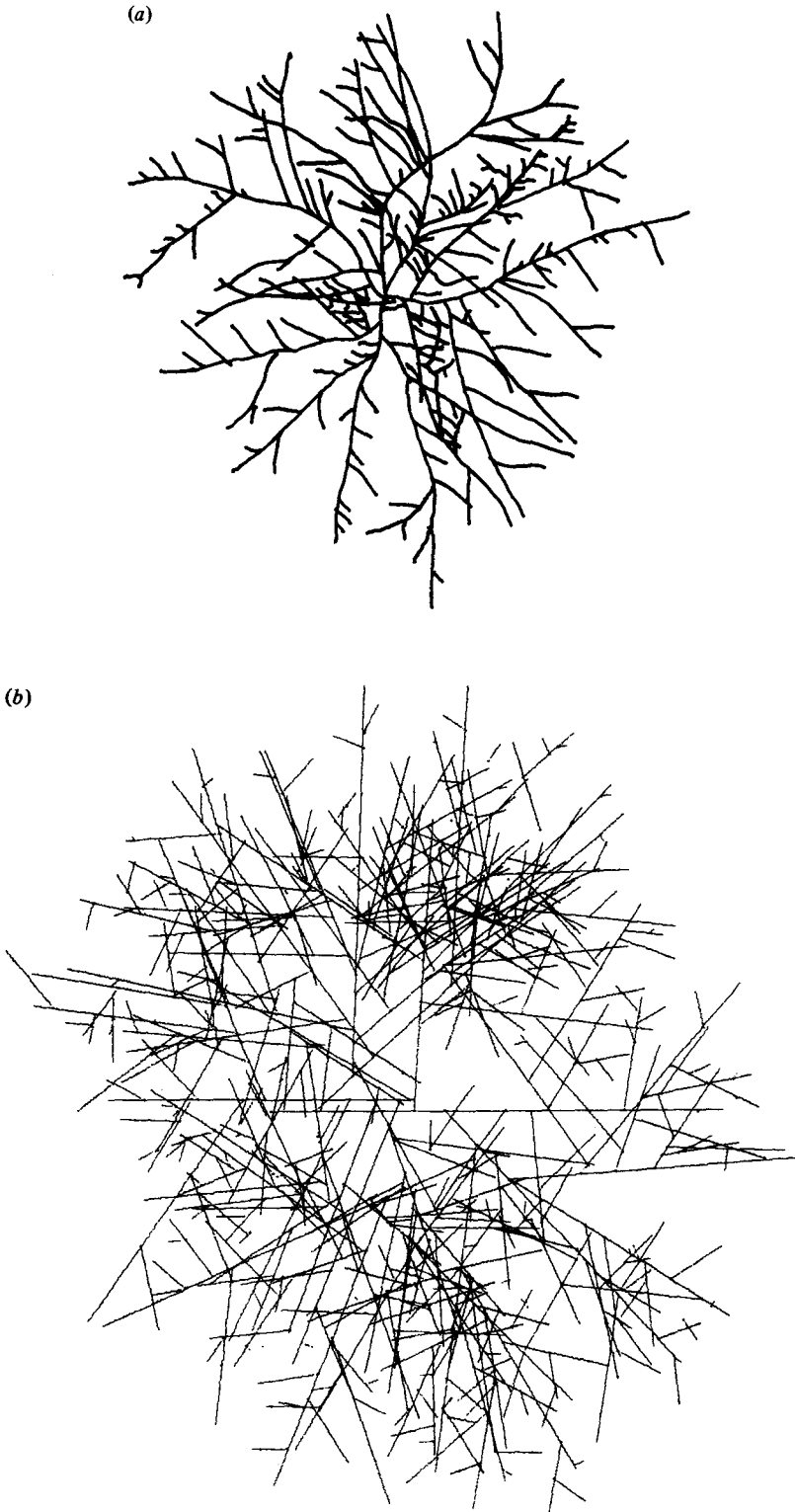
(a)

(b)

Fig. 13. (a) Tracing from a microphotograph of a colony of *M. hiemalis* approximately 10 h after the emergence of the germ tube. (b) Result of computer simulation after 10 h run.

in exploiting new areas within mixed populations.

The approximately circular shape is an expected result of random and independent variation of orientation and rate of growth of hyphae within the limits found in observed colonies. The distances between hyphal tips at which orientating interactions have been seen are too small for these interactions to affect colony shape substantially.

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