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Spatial patterns in the fruiting bodies of the cellular slime mold *Polysphondylium pallidum*

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Abstract. During morphogenesis in the slime mold *Polysphondylium pallidum* cell masses are periodically pinched off from the base of the developing sorogen. These masses round up and differentiate into secondary sorogens, which become radially ordered arrays of secondary fruiting bodies called whorls. Here we describe the morphogenesis of *P. pallidum* and characterize the spacing of whorls along the central stalk of the fruiting body and the spacing of sorocarps within whorls. We find both are highly regular. We propose that the linear spacing of whorls can be accounted for satisfactorily by a model that views the periodic release of cell masses from the base of the developing sorogen as the consequence of an imbalance between forces that orient amoebae toward the tip of the culminating sorogen, and cohesive forces between randomly moving cells in the basal region of the sorogen, which act as a retarding force. The orderly arrangement of fruiting bodies within whorls can be explained most easily by models that employ short-range activation and lateral inhibition.

Introduction

The cellular slime molds *Dictyostelium discoideum* and *Polysphondylium pallidum* have been widely used to study the origins of biological form. When a scattered population of slime mold amoebae starves, they aggregate to form a pseudoplasmodium that differentiates into a well-organized fruiting body whose cells are accurately partitioned between spores and stalks. There is little if any cell division following aggregation, and thus morphogenesis is entirely the consequence of cell-cell interaction and cell movement. Because of this relative simplicity one can hope to understand the cellular and molecular basis of morphogenesis in *D. discoideum* and *P. pallidum* in considerable detail.

P. pallidum, and other members of the genus, form secondary (2°) fruiting bodies arranged as whorls about the primary (1°) stalk. The whorls are more or less equally spaced [2]. An example of such a mature fruiting body is shown in Fig. 1 a.

Whorls first form as spheroidal masses of cells, which separate from the base of the culminating sorogen and then break up into small 2° sorogens, each of which culminates at approximately right angles to the 1° stalk. There they form whorls of 2° sorocarps (Fig. 1 b, c).

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Although *Polysphondylium* species have been described by several authors (see a recent and comprehensive review by Raper [18]), there has not been a systematic study of such basic questions as the morphology of developing whorls, the relationship between the number of cells in a sorogen and the number of sorocarps, the accuracy of the linear spacing of whorls and the radial spacing of sorocarps, and the degree to which morphogenetic detail is reproducible from fruiting body to fruiting body and from day to day. Thus one goal of this paper is to describe and quantify the major temporal and spatial morphogenetic features of this organism. We also discuss models that help explain the regular spacing of whorls along the central stalk, and the spacing of sorocarps within whorls.

Methods

Strains and culture media. P. pallidum PN500 [8] was used throughout this study. LP agar plates contained, per liter of water: 1 g lactose, 1 g peptone, and 20 g agar. GYP agar plates contained, per liter: 1 g glucose, 2 g peptone, 0.25 g yeast extract, 4.2 g KH_2PO_4, 5.1 g $Na_2HPO_4 \cdot 7H_2O$, and 25 g agar. The 2% agar plates used contained 20 g agar per liter water. Saline contained 8.5 g NaCl per liter water. Agar, peptone, and yeast extract were from Difco (Detroit, USA). Other chemicals were of reagent grade.

Growth conditions and morphological measurements. Cultures were propagated on *Escherichia coli* B/r growing on LP agar at 22° C. For morphological measurements, small volumes of spores or amoebae were transferred with an inoculating loop onto *E. coli* streaked on LP or 2% agar plates. Plates were then incubated at 22° C either with or without a layer of charcoal spread over the lid of the inverted plates [9]. Two to three days later, fruiting bodies were photographed directly or flattened under coverslips for observation with a microscope. Fruiting bodies propagated in this way are well separated from each other along the streak. The size of the sorogen can be controlled by streaking variable amounts of *E. coli* on 2% agar so that there is little or no bacterial growth. For the analysis of interwhorl distance, sorocarp length, and cell numbers, tracings were made with a drawing tube and digitizing tablet. For the measurement of sorocarp angles, untouched whorls oriented in the focal plane were measured. Angles taken from tracings gathered with a drawing-tube attachment were measured with a protractor. Spore counts were made as previously described [13].

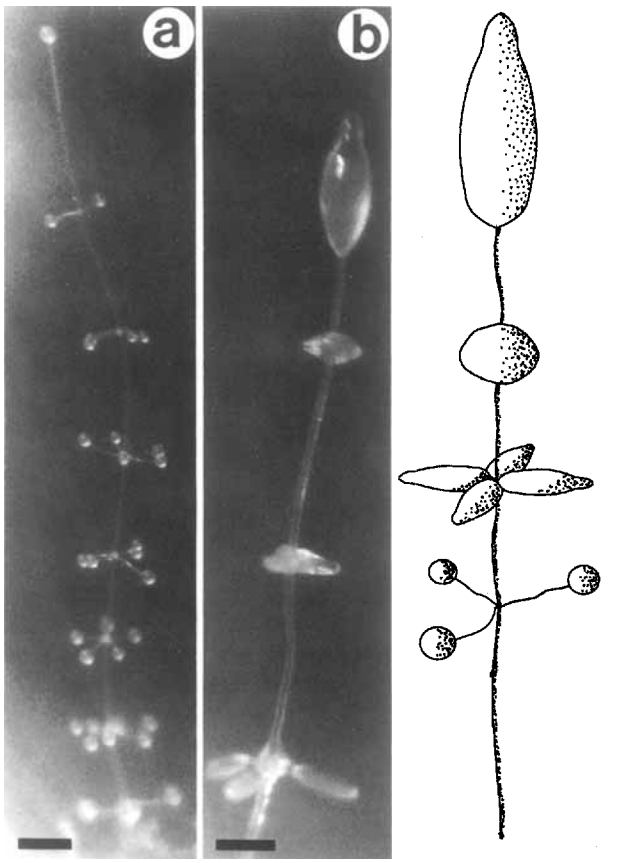

Fig. 1a–c. *Polysphondylium pallidum* morphology. **a** Mature fruiting body with regularly spaced whorls of sorocarps; *scale bar*, 200 μm. **b** Culminating sorogen that has deposited three cell masses in various stages of whorl maturation, the basal whorl being the oldest; *scale bar*, 100 μm. **c** Descriptive terms used in the text. The mature fruiting body has in the past been called a sorocarp [18]. Here we use this term to refer to the arms terminated by balls of spores, be they in a whorl or at the top of the fruiting body. Where there is a possibility for confusion, sorus, sorogen, sorocarp, and stalk are refered to as primary (1°) or secondary (2°), depending on whether or not they are part of a whorl

Scanning electron microscopy. Log-phase amoebae were harvested and plated on a membrane filter (Millipore, 0.45-μm pore size, type HA, Medford, USA) at a density of approximately 5×10^4 cells/mm^2. The filter was placed on 2% agar plates and incubated at 22° C. When numerous sorogens and fruiting bodies appeared, the filters were exposed to osmium tetroxide vapors at room temperature for 20–30 min, frozen to liquid N_2 temperature on a copper block, and then the cold block was placed in a vacuum evaporator overnight at 10^{-4} Torr. The freeze-dried specimens were mounted on stubs, coated with gold-palladium, and viewed with a JEOL SEM35 microscope operated at an accelerating voltage of 20 kV.

Sorogen elongation rates. Small agar strips containing aggregating centers were cut from 2% agar plates and mounted on the surface of a 2% agar plate. The plate was sealed and mounted under a dissecting microscope so that the sorogen culminated downward. Plates were incubated at 22° C. This procedure allowed us to follow very large sorogens without the fruiting body bending out of the plane of focus. A video camera and tape recorder were used to prepare time-lapse records.

Antibody staining. Sorogens were fixed and stained with primary antibody and rhodamine-conjugated secondary antibody as described by Byrne and Cox [3].

Results

Morphology. A fruiting body of *P. pallidum* begins to develop as the sorogen rises off the substratum (Fig. 2a). This process starts as the sorogen first takes shape at the late aggregate stage, and is not complete until the last whorl is formed. Under our growth conditions the sorogen does not migrate across the substratum.

At a time that depends on culture conditions and sorogen size, a bulge destined to become the first whorl appears at the basal end of the culminating sorogen (Fig. 2b, c). The bulge increases in diameter and decreases in length, while a constriction forms to separate the presumptive whorl from the main body of the sorogen (Fig. 2c). The bulge continues to move up the stalk with the sorogen until the two are completely separated; thereafter it remains in a fixed position (Fig. 1b). Just before, during, or shortly after separation, small tips appear around the circumference of the presumptive whorl (Fig. 2d, e). Some of the cells in the whorl become associated with each tip (Fig. 2f), and a ring of small, cylindrical 2° sorogens develops, each having approximately the diameter of the tip behind which it forms (Fig. 2g). At this stage the stalk of each sorocarp has achieved its mature length and is surrounded by a single layer of wedge-shaped cells aligned perpendicular to it, resulting in the cylindrical shape of the sorogen. These outer cells then move to the tip of the stalk (Fig. 2h) to form a spherical sorus of spores (Fig. 2i).

The process of whorl deposition is repeated at intervals until the 1° sorogen changes from an elongated pear-shape to a cylinder having the diameter of the tip region. Then the remaining active cells of the 1° sorogen move to the tip of the central stalk, and encyst to form a spherical sorus of spores (Fig. 2h).

We next measured the two major patterns illustrated in Figs. 1 and 2, the one-dimensional pattern of whorls

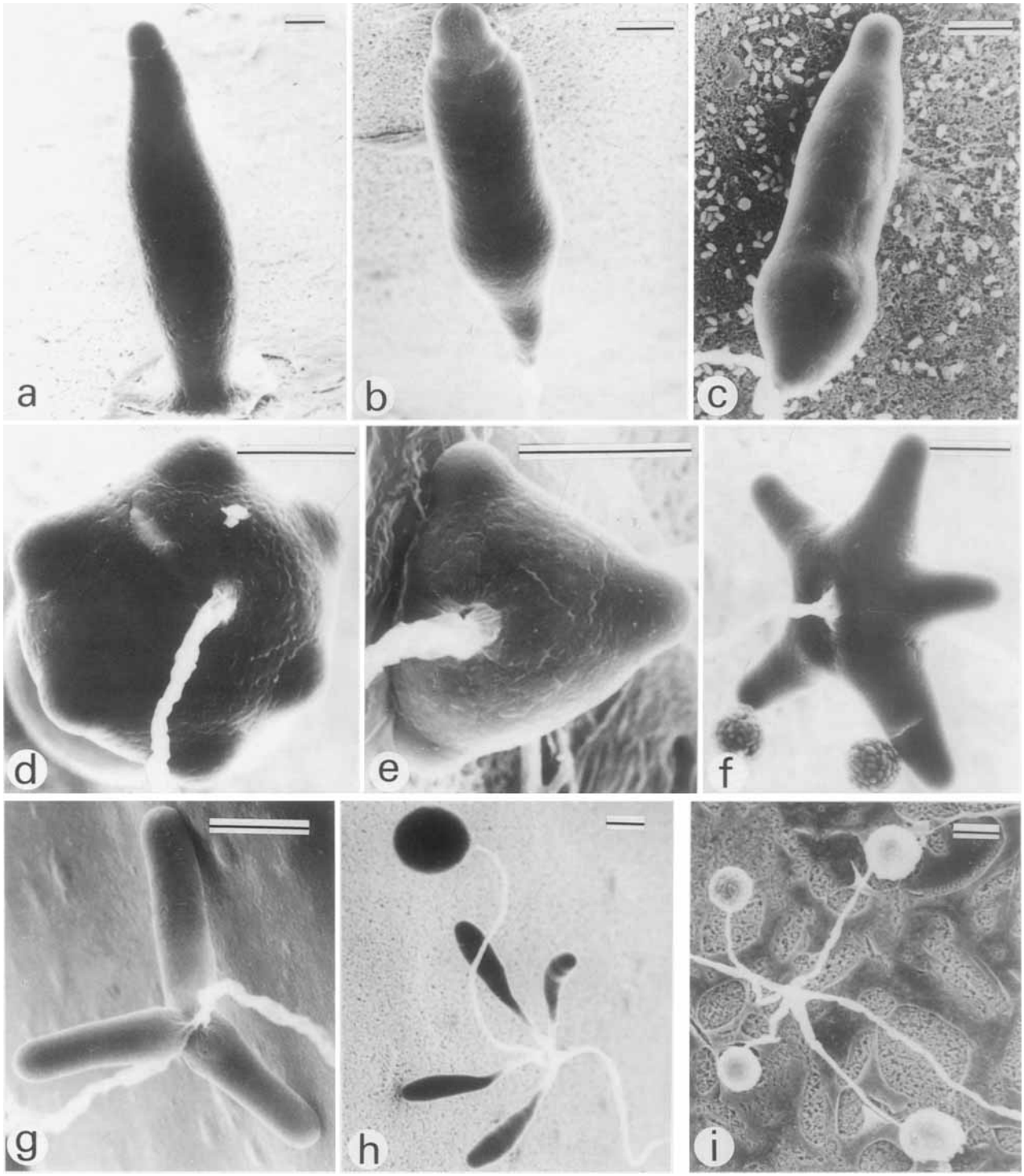

Fig. 2a–i. Scanning electron micrographs of sequantial stages of whorl development. **a** Newly formed sorogen beginning to rise off the substrate. **b** Sorogen with slight basal bulge, the presumptive whorl. **c** Sorogen with more pronounced bulge than **b** and a constriction beginning to form between the bulge and the apical portion of the sorogen. **d** Recently separated presumptive whorl in which six tips have appeared around the equator. **e** Presumptive whorl with three well-defined tips. **f** Presumptive whorl partially divided into five 2° sorogens. **g** Presumptive whorl completely divided into three 2° sorogens. **h** Presumptive whorl in which amoeboid cells of 2° sorogens are migrating to the ends of the 2° stalks. Note nearly mature terminal sorus at the apex of the 1° stalk. **i** Mature whorl; *scale bars*, 30 μm

along the stalk, and the two-dimensional radial pattern of sorocarps within a whorl.

Interwhorl distance. The spacing between whorls is highly regular, with a mean interval length of 344 μm (Fig. 3a). The mean spacing between whorls as a function of position for the data summarized in Fig. 3a is recorded in Fig. 3b, which includes the number of cases in each category as well as the average spacing between intervals, together with the 95% confidence intervals for interwhorl distances.

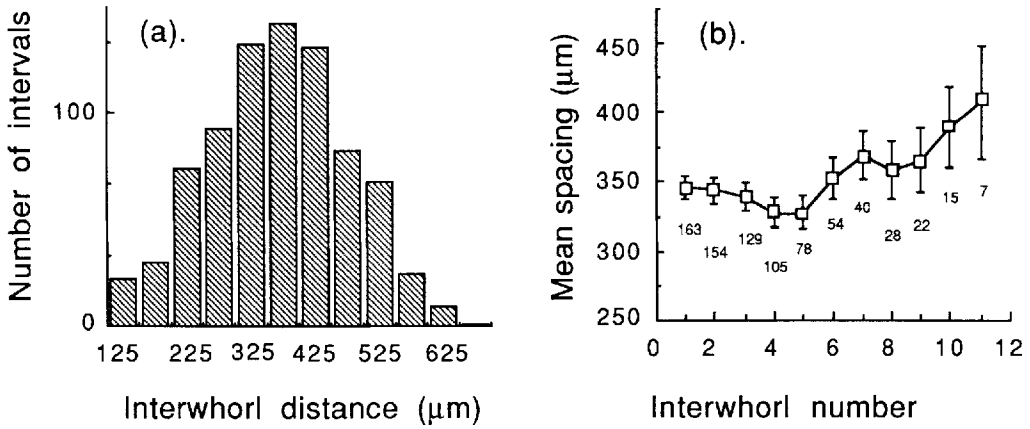

Fig. 3. a Frequency distribution of 807 interwhorl intervals from 163 fruiting bodies. The mean interval is 344 μm. The standard deviation and standard errors of the mean are 109 and 3.8 μm, respectively. 34 fruiting bodies in this data set were grown over charcoal. Their mean spacing did not differ significantly from the population mean. The data are normally distributed. **b** The mean spacing of whorls as a function of position on the fruiting body. The means ±95% confidence level are given. The number of intervals measured is recorded at the *base of each error bar*

These data can be analyzed to see if there is any tendency within a population of fruiting bodies for the distance between adjacent whorls either to increase or decrease from the base to the apex. This comparison was done by aligning fruiting bodies in such a way that all first intervals (most basal) were compared to each other, all second to each other, all third, and so on. The fruiting bodies were also aligned in the reverse order. The results were essentially the same. The 95% confidence intervals overlap (Fig. 3b), and an analysis of variance on the entire data set shows that all intervals are chosen from the same distribution (P, 0.419 for the null hypothesis that they are not, 13 degrees of freedom).

A useful and size-independent measure of the "evenness" of spatial patterning is the extent to which data depart from a random expectation [6]. This measure is the ratio of the observed nearest-neighbor distances between whorls to the Poisson expectation (the R value). In one dimension R is 2.0 for perfectly equal intervals between points. For random spacing R is 1.0. For the data in Fig. 3, R is 1.74, surprisingly close to the values measured for heterocyst spacing in the blue-green alga *Anabaena* [22]. Thus, although interwhorl spacing is not crystalline in its regularity, it deviates significantly from randomness. Analysis of subsets of data chosen from Fig. 3 and gathered on different days also shows that the mean spacing is constant. However, when individual fruiting bodies are measured, interwhorl distances seem on average to be more uniform than population averages (data not shown). Thus we conclude that some fraction of the variance in Fig. 3 is caused by "noise" in the culture conditions, since even though our culture conditions are reasonably well controlled there are nonetheless unavoidable inhomogeneities in each petri dish.

The basal and terminal intervals. The distance to the first whorl (the basal interval) and the distance between the last whorl and the terminal sorus (the terminal interval) are more variable than other interwhorl distances as Fig. 4 shows.

The basal interval is rarely shorter than the average interwhorl distance, and can be quite large under our culture conditions. It is also related to the size of the sorogen, as measured by the final length of the mature fruiting body – the larger the fruiting body, the longer the basal interval (Fig. 4a). Under the conditions used in these studies, culmination begins as aggregation ends. This leads us to believe that the deposition of the first whorl depends upon the regulation of a gradient along the length of the ascending sorogen (see Discussion).

The terminal interval may be quite short compared to other intervals or it may be up to twice the average interval length of the fruiting body. It is very rare for the terminal interval to exceed twice the average interval length of 344 μm (Fig. 4b).

Spore number per whorl. Does the number of cells per 2° sorocarp bear any relationship to whorl location? Since under our culture conditions and with the strain used here, approximately 95% of the cells in a 2° sorocarp are spores [13] we counted spores per sorocarp as a function of location on the 1° stalk. On average, the initial and final sorocarps contain fewer cells than their nearest neighbors. There is also a clear tendency for the number of spores per sorocarp to increase, then decrease with distance from the basal whorl (Table 1).

The number of sorocarps per whorl. A second measure of the relation between position and whorl size is the number of sorocarps per whorl as a function of distance along the stalk. The first and last whorls have fewer sorocarps than their neighbors (Fig. 5).

An analysis of the inidividual sorocarps summarized in Fig. 5 shows that this relationship is not an obligatory one for the most-basal whorl. For example, individuals with three sorocarps in the first whorl can have two in the second. Nonetheless, the trend is clear – basal whorls are on average smaller than central ones. When we examine the data for the terminal and penultimate interval, however we find that the number of sorocarps in the last whorl is always less than or equal to the one before it (data not shown).

These results, taken together with those bearing on the number of cells per whorl, suggest that the mechanism partitioning cells into sorocarps is not rigidly dependent on position, although clearly the largest whorls are on average nearer the middle of the mature fruiting body.

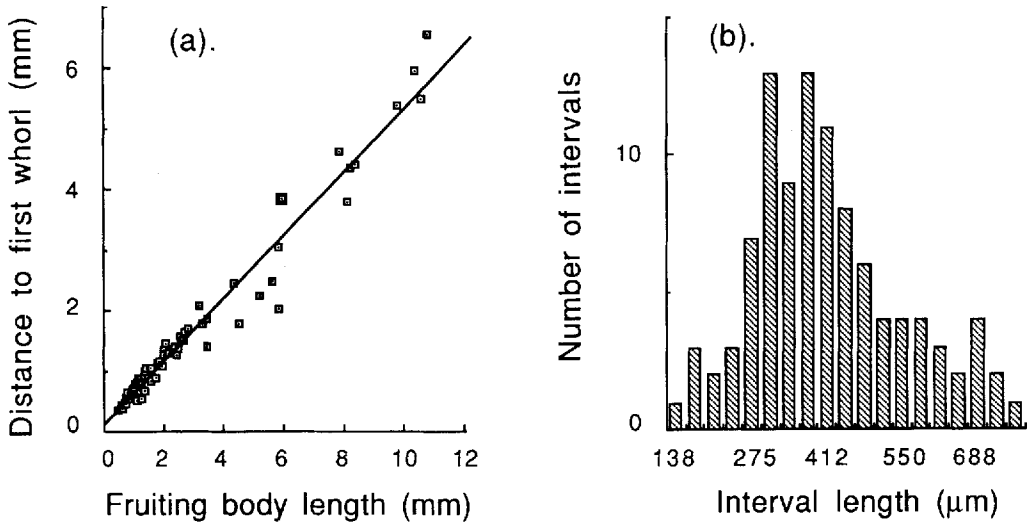

Fig. 4. **a** The distance between the first basal stalk cell and the first whorl as a function of fruiting body length. This sample contains 23 fruiting bodies with one whorl, 18 with two, 9 with three and 9 with four or more. **b** The distance between the last whorl and the terminal sorus; 106 intervals were measured. The mean spacing is 416 μm

Table 1. Spores per sorocarp, base to apex

Whorl position	Spores/sorocarp (±S.E.)	*n*
1	208 (26)	24
2	290 (26)	24
3	357 (21)	24
4	351 (22)	22
5	389 (29)	21
6	375 (25)	15
7	353 (25)	11
8	315 (46)	9
9	360 (51)	5
10	302 (83)	4
11	254 (82)	3
t[a]	557 (49)	24

[a] The terminal sorocarp

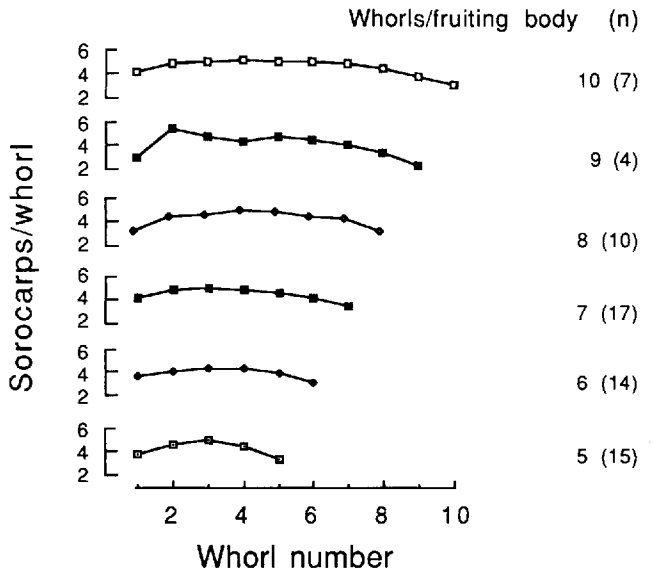

Fig. 5. The number of sorocarps per whorl for fruiting bodies of various sizes as a function of whorl number

Stenhouse and Williams [20] have studied the cell/stalk ratio in *P. pallidum* PP28S using data gathered from small and large fruiting bodies. They concluded that there is a linear relationship between the number of cells in a sorogen and the number of 2° sorocarps it eventually forms, which is consistent with the results presented here. The ratio of spore/stalk cells in their study, ten, was about half the number we have consistently observed (results not shown), possibly attributable to strain differences.

The relationship between successive whorls. Does the patterning in one whorl influence its nearest neighbors, as do, for example, successive leaves and branches in many plants? We have studied this question in two ways, by asking if there is a rotational relationship between successive whorls, and if the number of sorocarps in a whorl is related in any way to the number above and below it. As a general rule, sorocarps within successive whorls on a fruiting body bear no rotational relationship to each other (data not shown); and few if any nearest-neighbor relationships are forbidden (Table 2).

The results summarized in Table 2 reveal several interesting relationships. First, approximately 80% of the whorls are randomly distributed with respect to their nearest neighbors. Second, many of the pairs that deviate from the expected distribution are found near the base or the apex of the fruiting body (data not shown). For example, all of the one-armed whorls were found apically or basally. This effect is also seen in the excess pairs containing twos and threes, and this is undoubtedly because the shape of the sorogen influences the average number of sorocarps per whorl (Table 1; Fig. 5). Third, the deviation from randomness for several of the pairs containing sevens is explained by the fact that many of these pairs come from a single unusually large fruiting body containing five whorls with seven sorocarps each. The only nearest-neighbor pair that is not simply explained is the five/six pair. Finally, with the exception of nearest neighbors that are statistically improbable given the size of the data set (e.g. the neighbors one and eight, there being relatively few ones and only four eights; and so on), any number of sorocarps/whorl can be observed to precede or follow any other. Whilst

Table 2. Whorl nearest neighbors

		Number of nearest neighbor pairs with n sorocarps[a]								
		n	1	2	3	4	5	6	7	8	
											n
			0	**8**	**14**	14	10	2	0	0	1
	m			4	**22**	**54**	**10**	4	0	0	2
Calculated	1		1		22	104	72	**6**	**0**	0	3
nearest-	2		2	3		108	216	48	**10**	2	4
neighbor	3		6	13	17		138	**98**	18	0	5
pairs m[b]	4		15	33	84	107		14	**16**	2	6
	5		16	35	89	235	119		10	0	7
	6		5	10	26	66	69	10		2	8
	7		1	3	8	21	22	6	1		
	8		0	1	0	4	4	1	0	0	
		m	1	2	3	4	5	6	7	8	

[a] This table summarizes the data gathered from 90 fruiting bodies with 1028 nearest-neighbor pairs. Of 603 whorls, 316 contained odd numbers of 2° sorocarps, 287 even. Observed values that differ significantly from the random expectation ($P \leq 0.05$) are in bold type. The observed and calculated values were compared by a χ^2 analysis

[b] Calculated on the assumption that nearest neighbors were randomly distributed. Because of round-off errors, the total number equals 1033. For the same reason, not all entries are even

there is a clear tendency for large whorls to be preceded or followed by large whorls, especially in the middle of the fruiting body, Table 2 emphasizes that this is not an obligatory relationship.

Symmetry within whorls. Under growth conditions in which fruiting bodies are well-separated, most sorocarps within a whorl are radially symmetric in a plane at right angles to the axis of the central stalk. Sorocarps within a whorl are also of similar length. In many whorls with six or more sorocarps, the planar arrangement of the sorocarps breaks down, and they lose their simple rotational symmetry. In extreme cases a large cylindrical mass of cells can separate from the rear of a sorogen and break up to give rise to many sorocarps, giving that portion of the fruiting body a "bottle-brush" appearance [5].

The number of spores per sorocarp is a convenient measure of symmetry within a whorl, since any mechanism that divides the whorl mass into roughly equal proportions should produce sorocarps containing approximately the same number of spores. That this expectation is the case is shown in Fig. 6, where we have reported data for whorls containing two, three and four sorocarps per whorl.

Figure 6 summarizes our results in the following way. Each point contains information on a single whorl. For example, the variance in spore number of a four-branched whorl with 67, 106, 117, and 142 spores (the largest variance for four-branched whorls in this data set), calculated according to the figure caption, is 0.0052, which, when normalized by the random expectation of 0.05, is 0.1044 (asterisk, Fig. 6a insert). Inspection of the equation used to calculate normalized variance (legend, Fig. 6) will show that perfectly partitioned spore counts assume values of 0.0. Random distributions plotted this way have mean values of 1.0, clumped distributions of greater than 1.0. Very clearly, spores are partitioned among sorocarps with a high degree of nonrandomness.

This plot obscures the fact that the number of cells in a whorl is only weakly related to the number of sorocarps

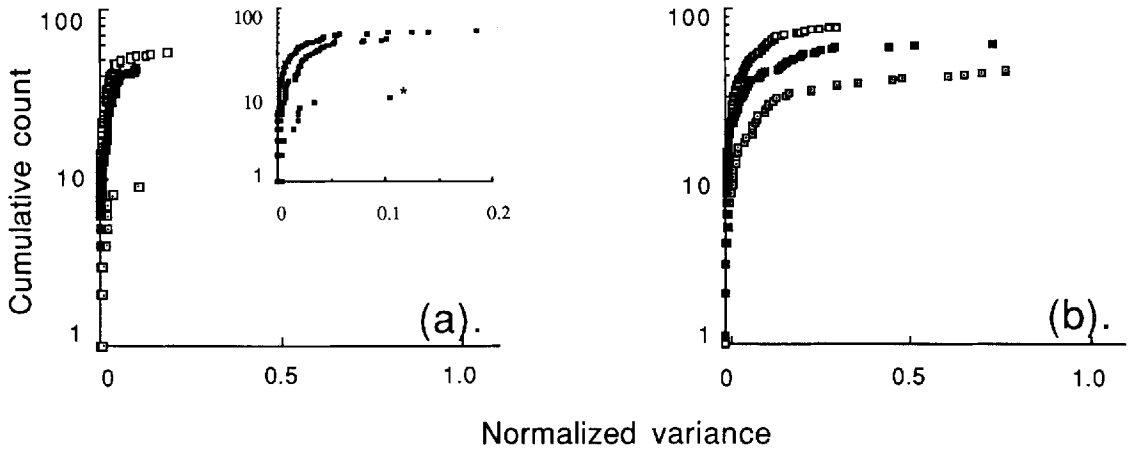

Fig. 6a, b. The variance in the number of spores per sorocarp within a whorl **a** and the variance in the angles between 2° sorocarps **b**. The *insert* in **b** is the spore data on a larger scale. In this representation perfect partitioning of spores among 2° sorogens, and perfect spacing of the angles between sorocarps within a whorl, give values of 0.0; random spacing, 1.0; and clumped distributions > 1.0. The variance within each whorl was calculated from the expression $\sum_{i=1}^{n} \frac{(x_i - 1/n)^2}{n-1}$, where n, no. of sorocarps per whorl, and x is the fractional no. of spores/sorocarp **a** or the fractional no. of degrees between a sorocarp and its neighbor **b**. The random expectation for whorls with two, three, and four sorocarps can be shown to be 1/6, 1/12, and 1/20, respectively [7], and hence the observed variance was normalized by dividing the experimental result by the random expectation. The no. of samples counted in panel **a** for whorls with two, three, and four sorocarps was 56, 44, and 9, respectively; for **b**, the no. of whorls measured was 44, 61, and 78; 90% of the spore data lie below 0.057, 0.079, and 0.033, respectively, for two, three, and four branched whorls; for sorocarp angles, the values are 0.62, 0.25, and 0.16. Thus the departure from randomness is much greater for the spore data. Four-branched whorls, ⊡; three-branched whorls, ■; two-branched whorls, □

that will form. Thus a whorl mass containing one thousand cells can give rise to a whorl containing three, four or even five sorocarps [13]. This unexpected result appears to have its origins in how the initial symmetry in the whorl is broken to give the organizing tips visible in Fig. 2d, and is discussed in more detail in the Discussion.

A second measure of symmetry within a whorl is the angular separation of whorl sorocarps. We examined 261 whorls with two to eight sorocarps on well-separated erect fruiting bodies grown on plain agar. Measurement of the angular separation of the sorocarps for the most-abundant classes, two through four sorocarps per whorl, shows that they exhibit radial symmetry (Fig. 6b), and that the angular separation between sorocarps varies significantly from a random pattern. The spacing is not, however, as uniform as it is for spore partitioning, possibly because as sorocarps develop, there is considerable sorocarp movement caused by stalk cell differentiation and swelling.

The radial symmetry is most pronounced in two- to five-branched whorls and becomes less regular in those with more sorocarps. This could be the result of the relatively small sample sizes for whorls with greater than six sorocarps, or because large numbers of sorocarps place physical constraints on regular spacing in a planar arrangement. Perhaps also initial symmetry-breaking events controlling the transition of the spheroidal mass of cells to the radially distributed sorocarps within whorls is sensitive to the size and symmetry of the initial cell mass (and see Discussion).

Culmination velocity. Culmination velocity is independent of sorogen size and constant until the last whorl has been deposited. These results are summarized in Table 3 and Fig. 7, where we present data chosen from small and large fruiting bodies observed separately and in the same microscopic field.

The velocity of culmination can be quite variable. For example, in 11 different experiments at constant temperature and humidity, we observed velocities of 3.5–7.3 μm/min. This range of velocities was light-dependent, varying from day to day under the experimental conditions used. The fruiting bodies studied had from zero to ten whorls. There was no relationship between sorogen size and culmination rate, unlike the results obtained by Bonner and Eldredge [1] with *D. discoideum*. A detailed analysis of each time-lapse record gave results very similar to Fig. 7, and thus we can conclude that over a large size-range culmination velocity is independent of sorogen size under constant culture conditions.

These results can be compared to those of O'Day and Durston [15]. They did not examine the relationship between culmination rate and sorogen size, the number of examples was small, and the sorogen size was not recorded. However, it is clear from their data that culmination rate is constant for at least the first two whorls. They also reported an average sorocarp number per whorl (3.77) and noted that there is a gradient of whorl size, with the largest ones most basal. Neither result is in agreement with the data presented here. Perhaps the differences are due to culture conditions, since, as we have noted here, and as Harper [11] noted many years ago, light intensity strongly influences branching, and their published data (Fig. 5) shows highly irregular patterning of a kind we find is induced by strong light and heat.

Table 3. Sorogen culmination velocity[a]

Experiment[b]	No. of whorls	Whorl order[c]	Velocity (μm/min)
1	2	[6, 4]	7.0
2	3	[7, 7, 7]	5.7
3	5	[5, 5, 6, 4]	5.8
4	5	[5, 5, 6, 4, 4, 1]	7.3
5	10	[5, 6, 3, 5, 5, 3, 3, 5, 2, 2]	6.8
6	1	[5]	4.1
	2	[5, 2]	3.9
	3	[5, 4, 4]	4.2
	3	[5, 2, 5]	4.6
7	1	[4]	3.9
8	1	[2]	2.8
	3	[6, 6, 2]	3.5
	4	[6, 6, 5, 5]	4.2
9	1	[5]	6.9
	3	[5, 7, 4]	6.2
10	2	[5, 3]	5.8
11	3	[5, 6, 6]	3.9

[a] Measured as described in Methods

[b] Sorogens in experiments 6, 8, and 9 are from experiments on different days. Within each of these experiments, the sorogens come from the same microscopic field

[c] The number of sorocarps per whorl is listed from the most basal to the most apical whorl

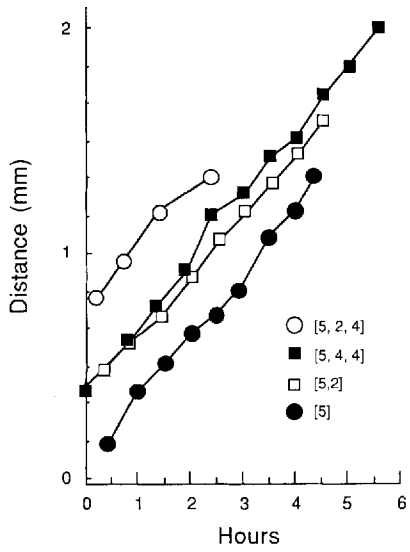

Fig. 7. Sorogen elongation velocities for sorogens of different sizes. These results were recorded from a single field (Experiment 6 of Table 3). The *numbers in square brackets* are the numbers of sorocarps per whorl ascending from the most basal whorl to the most apical. Not all fruiting bodies in the field could be followed from the beginning to the end of culmination. [5, 2] means five sorocarps in the first whorl, two in the second

The gradient of an antigen under developmental control is size-regulated. AntiPg101, a monoclonal antibody directed against a tip-specific antigen, has been used to study the origins of tip prepattern in *P. pallidum* [3, 4]. *AntiPg101* detects a gradient of Pg101 in the 1° sorogen that is size-regulated (Fig. 8). The small sorogen in Fig. 8 is caught at a developmental stage where a whorl mass has just formed. There is a gradient of Pg101 extending from the

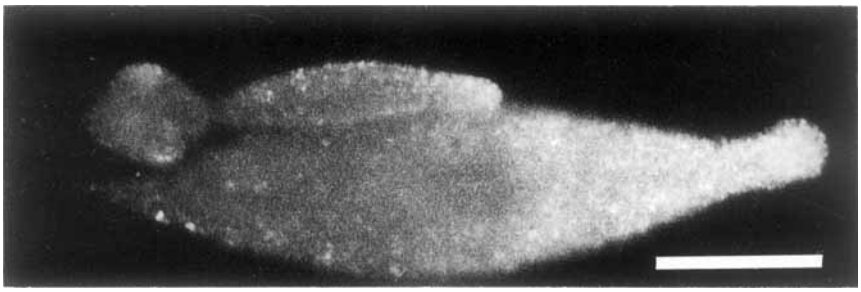

Fig. 8. Size-independent regulation of a pattern-specific antigen, Pg101. Sorogens were fixed and stained with the monoclonal antibody *antiPg101* as described in Methods; *scale bar*, 100 μm

sorogen apex to the base. Pg101 is also present in patches about the equator of the whorl mass (How these patches arise is described by Byrne and Cox [4]). The larger of the two sorogens, while it has not yet formed a whorl mass, also exhibits a clear gradient of this antigen.

Discussion

Spacing between whorls. Our results suggest a qualitative model that helps explain spacing of whorls along the stalk. Since we have shown that the culmination velocity of the ascending sorogen is constant and independent of sorogen size, a mechanism that allows for the deposition of whorl masses at constant time intervals will account for whorl spacing independent of fruiting-body length. How might this happen? We believe this is most easily understood as the outcome of two opposing phenomena, the tendency of cells to chemotax towards the tip of the sorogen, on the one hand, and the tendency of cells to cohere to each other, and hence round up, on the other.

Clearly the net movement of cells toward the sorogen tip must be due to the net forward velocity of individual amoebae, arguably the sum of chemotactic and chemokinetic forces [16]. If either one decreases below a threshold value at the base of the sorogen, then amoebae will begin to move randomly. We suggest that the regulated gradient underlying Pg101 expression mirrors a graded chemotactic or chemokinetic activity. Thus as the basal concentration of the gradient continuosly falls during sorogen culmination, basal amoebae find themselves outside the threshold value for net directed movement, and lose their way. When cohesive forces between these cells and between the cells and the central stalk exceeds the motive force directing them towards the sorogen tip, a whorl mass is left behind on the stalk. Thus the continuous culmination of the sorogen is translated into the discontinuous deposition of whorls.

One prediction of this proposal is that cells within an incipient whorl should be seen to move randomly before the incipient whorl pinches off from the sorogen. Moreover, randomly moving cells should first appear basally, and the size of the region containing them should grow apically until the whorl pinches off. The interval between the earliest stages of culmination and the appearance of randomly moving basal cells should correspond to sorogen size, since, as noted below, we believe the explanation for the relationship between sorogen size and distance to the first whorl is most readily accounted for by assuming larger sorogens take longer to regulate their chemoattractant or chemokinetic signal. For this reason, we might expect the gradient of Pg101 to be established faster in smaller sorogens if the two are related.

The distance between the substratum and the first whorl is directly proportional to fruiting-body length (Fig. 4a). Since culmination velocity is not a function of sorogen size, longer sorogens must therefore require more time than shorter sorogens to pinch off the first whorl mass. This also suggests the existence of a regulated chemotactic or chemokinetic signal whose initial state is above the response threshold everywhere. The regulation of such a gradient would be length-dependent. Because second and subsequent whorls are released at regular intervals, this further suggests that once the gradient is established it is regulated independently of sorogen length. Indirect evidence for a regulated gradient is clearly demonstrated by *antiPg101* staining patterns (Fig. 8; see Byrne and Cox [4]). Possible ways to accomplish this in the slime molds have been reviewed by MacWilliams and Bonner [12].

When a 1° sorogen tip is excised, the remaining cells quickly round up [5], and thus the forces postulated to explain the shape and segregation of an incipient whorl exist, and appear to be under the control of the 1° tip. The observation that diploid and haploid strains have the same interwhorl spacing [19] is also consistent with this proposal. Although diploid stalks contain, per unit length, only half as many cells as a haploid, the establishment of a chemotactic or chemokinetic gradient, as noted above, should be length-dependent, not cell-number-dependent [14].

The first and last whorls contain fewer cells and thus form fewer sorocarps (Fig. 5). We can think of two possible explanations for why this is so. One is that the sorogen may change shape during culmination in such a way that its basal region is narrower when the first and last whorls segregate. The second possiblity is that the young sorogen might already be divided up into more or less equal length segments corresponding to the whorl masses that will form during culmination. If so, then the first and last whorls would be smaller, since the cross-sectional area of a sorogen increases towards the center of the sorogen, remains approximately cylindrical for a distance proportional to sorogen length (Fig. 1b), and then begins to taper at the tip. The difficulty with this explanation is that it implies that the majority of the cells in the sorogen are committed to become a whorl mass of a certain size soon after the sorogen forms. While this seems possible to us, there is considerable cell movement and mixing during culmination in the slime molds (reviewed by Odell and Bonner [16]), suggesting that it would be difficult for masses of cells grouped early on to maintain their identity throughout the patterning process.

Why do the first and last whorls have fewer spores per sorocarp than intermediate whorls (Table 1)? McNally et al. [13] showed that the number of sorocarps per whorl

is proportional to the surface area of the whorl mass, not the volume or number of cells. Consequently larger whorls will have more spores per sorocarp than smaller whorls, since the surface-to-volume ratio in large whorls is smaller, and more prespore cells must therefore be partitioned to each sorocarp. This does not of course explain why the first and last whorl masses are on average smaller, but it does explain the relationship apparent in Table 1, given the fact that they are.

Spacing within whorls. The results summarized in Fig. 6 reveal that the whorl mass is partitioned into roughly equal portions, whether measured by the angles between sorocarps or by the numbers of spores. This is also clearly shown in Fig. 2, particularly panels d and e. Although the number of spores per sorocarp in a particular whorl is relatively constant, the total number of cells in a whorl mass is only a weak predictor of the number of sorocarps that will eventually form. In Fig. 6, for example, the number of spores per single-branched whorl was 60–460, and for two branched whorls, 165–1172 (and see McNally et al. [13]). As we show elsewhere [4], the spacing between visible tips, and the relationship between the number of cells in the whorl and the number of sorocarps formed can be simulated accurately by models first described by Turing [21], Gierer and Meinhardt [10], and Oster et al. [17]. Several aspects of these models lead to variability in the number of sorocarps per whorl for whorls with identical numbers of cells. The reason is that various periodic components compete for domination of the final pattern. Thus the number of pattern elements formed is not rigidly determined, but depends on which periodicities finally emerge. In particular, simulation of whorl patterning by a reaction-diffusion mechanism [14] reveals that the number of sorocarps in a whorl depends strongly on the variance in the initial morphogen concentration ([4]; and unpublished observations). Thus we explain the variance in sorocarps per whorl for a particular whorl mass by noting that the initial distribution and amplitude of morphogens (or mechanical instabilities) contributes to the outcome for a particular whorl. This is an inherently noisy process, and consequently the outcome for a whorl mass of a particular size class is expected to show some variance. Exactly how much depends strongly on the initial amplitude of the instabilities that drive the patterning (Byrne and Cox, unpublished observations).

These models also produce variability in the number of spores per sorocarp. Again, the final pattern predicted theoretically usually consists of several overlapping periodic components. The superposition of these components results in imperfect spacing of pattern elements. In a whorl, this corresponds to an imperfect positioning of tips. Because tips recruit cells, irregularities in tip spacing lead to unequal partitioning of cells to each tip, and so to variability in the number of spores per sorocarp.

Finally, our nearest-neighbor analysis shows that the number of sorocarps in a whorl is not rigidly determined by the number above or below it. This clearly demonstrates that the radial positioning of sorocarps within whorls is independent of events preceeding and succeeding it, and further justifies the modeling of the radial prepattern as the growth of instabilities within a statistically uniform initial state [4].

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