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Partitioning of symbiotic *Chlorella* at host cell telophase in the green hydra symbiosis

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SUMMARY

Although there is much evidence that green hydra digestive cells control cell division of their *Chlorella* symbionts, so that the symbionts divide only at host cell division, it is not clear how the population size of symbionts (numbers per cell) is regulated. In constant culture conditions the mean number of symbionts per cell also remains constant, but with a very large variance about the mean. The way in which symbionts are partitioned at host cell division appears to account for that variation. By counting numbers of *Chlorella* in daughter cells at late telophase it was found that partitioning of *Chlorella* symbionts was not symmetrical, but at random, closely following that predicted by the binomial distribution if it is assumed that each symbiont had an equal probability of entering either host daughter cell. A better fit to the predicted distribution was obtained from observations of partition in digestive cells from excised regenerating peduncles than in those from recently fed gastric regions, possibly because in the former, algae have completed their division before the host cell divides, while in the latter algal and host cell division takes place at the same time. There was only a small effect of differences in daughter cell volume on numbers of symbionts received, but comparison of variance and coefficient of variation of numbers of algae in mother (post-algal division, pre-partition) and daughter telophase digestive cells (pre-division, post-partition) suggested that algal division at host mitosis was density dependent. Random partitioning of algae at host cell telophase would account for the wide variation in numbers of algae per cell, and compensatory density-dependent algal division at the next host cell mitosis would ensure stability of the mean algal population.

1. INTRODUCTION

Green hydra has proven to be an ideal experimental organism for the study of regulation of symbiont populations in algal–invertebrate symbioses. Hydra is easily dissociated into suspensions of intact single cells by a simple maceration process (David 1973), and population levels in the green hydra symbiosis may be rapidly determined by counting numbers of algae in host cells. The symbiotic *Chlorella* live within the endodermal digestive cells of green hydra, and each cell contains on average between 10 and 25 algae, depending upon culture conditions, the strain of the green hydra used, and the position of the digestive cell in the body column (Pardy 1974; McAuley 1980, 1981, 1985*a*; Douglas & Smith 1984).

Although it is well known that changes in growth conditions of hydra elicit predictable changes in the mean number of algae per digestive cell, it is not known what determines the size of an algal population in a particular cell. Furthermore, although the mean number of algae per cell remains constant when culture conditions are also constant, there is considerable variation about that mean (Pardy 1974; McAuley 1981), so that within a population of digestive cells containing a mean of 22 algae per cell, individual digestive cells may be observed to contain as few as one and as many as 80 algae (P. J. McAuley, unpublished results). At present, the reason for this variation in population levels of algae within individual digestive cells remains unknown. Although some workers have shown that there is a correlation between digestive cell size and the numbers of algae contained, in all cases variation in digestive cell size accounts for 30% or less of the observed variation in algal numbers (Douglas & Smith 1984; McAuley 1986).

Division of the symbiotic algae is entrained to that of the host digestive cells, so that the algae are able to divide only when the host cell enters mitosis (for review, see McAuley (1985*b*)). Measurement of variance and coefficient of variation of algal populations of mother and daughter digestive cells before and after telophase has shown that values were in agreement with those predicted by a density-dependent model of regulation, which was first proposed to explain control of division of chloroplasts in a unicellular alga (see Hennis & Birky 1984; McAuley 1986). It suggested that in dividing digestive cells with few algae, all algae divided; in those with many algae there was no algal division; and in those with intermediate numbers of algae, a proportion of the algae divided, approximately doubling the number of algae per cell.

If this model is correct, partition of the algal population between host daughter cells may have important consequences for the way in which the regulatory mechanism is expressed in terms of numbers

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of algae per cell. As algae divide only at host cell division, the way in which algae are partitioned between host daughter cells would determine population levels until the daughter cells complete their cell cycle and the next round of algal replication is permitted.

In this paper, partitioning of symbionts was determined simply by counting numbers of algae in daughter cells at late telophase. Unlike organelles such as chloroplasts and mitochondria, which are partitioned roughly equally at cell division (Hennis & Birky 1984), partitioning of each symbiont appeared to be at random. Partition of the symbiont population could be predicted by the binomial distribution assuming that every symbiont had an equal probability of entering either host daughter cell.

2. MATERIALS AND METHODS

Green hydra (*Hydra viridissima*, Pallas) of the European strain were cultured in 'M' solution (Muscatine & Lenhoff 1965) at 18 °C in continuous light (60 μmol photons m^{-1} s^{-1}). Cultures were fed each Monday, Wednesday and Friday with freshly hatched nauplii of *Artemia salina*. All animals used in experiments were taken from cultures that had been fed the previous day, and each bore a single bud.

To produce suspensions of cells suitable for microscopical examination, five gastric regions from hydra that had been fed 24 h previously or five regenerating peduncles 48 h after excision were isolated in a drop of macerating fluid (David 1973) containing 5 μg ml^{-1} of the DNA-specific fluorochrome 4,6-diamidino-2-phenylindole (DAPI). After 10 min, pieces of hydra could be teased apart into individual cells, and the preparation was examined by using Normarski interference contrast and epifluorescence microscopy. Cell volumes were estimated from plan areas after preparations had been stored overnight at 4 °C (McAuley 1986).

3. RESULTS

Distribution of symbiotic algae between daughter digestive cells at mitosis was examined in gastric regions of hydra fed 24 h previously, and in regenerating peduncles 48 h after excision. In the first case, algal and digestive mitosis take place at the same time; in the second, algal mitosis precedes that of the digestive cells and is virtually complete by the time digestive cell mitosis begins, 36 h after excision (McAuley 1982, 1986). Partition of algae between daughter cells was measured by identifying telophase cells in which separation had proceeded far enough to distinguish and count the two algal populations.

In both fed gastric and regenerating peduncle digestive cells, there was a large variation in numbers of algae in telophase mother and daughter cells (figure 1). In regenerating peduncles, telophase digestive cells contained a mean of 21.5 algae, range 5–43, and in gastric region of fed hydra a mean of 20.5 algae, range 8–48. The resulting daughter cells contained a mean of 10.8 algae, range 3–25 in peduncles, and 10.2 algae, range 1–26 in gastric regions. Numbers of algae dividing at host cell division appeared to be controlled by some form of density dependence, as shown by comparing variance and coefficient of variation of algal populations in mother cell (post-replication, pre-partition) and daughter (pre-replication, post-partition) cells (figure 1). Coefficient of variation in mother and daughter cells was similar, whereas variance in mother cells was more than three times that

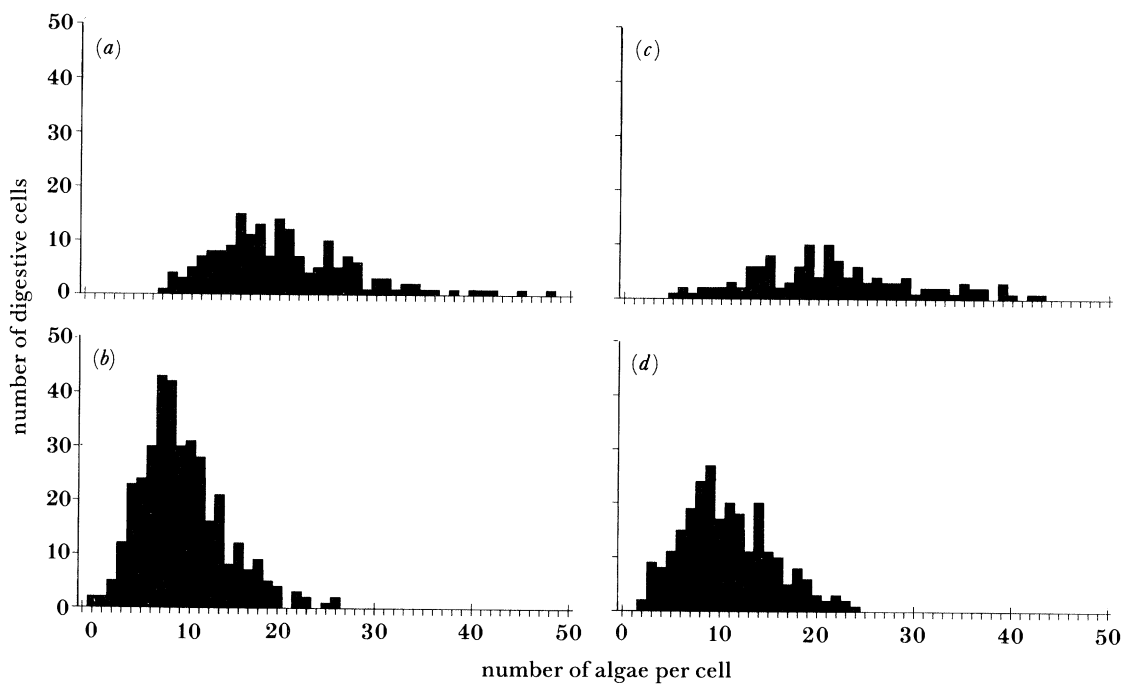

Figure 1. Numbers of algae per cell in telophase mother and daughter cells. (*a*) Mother cells ($n = 181$, $\bar{x} = 20.4 \pm 7.3$, *c.v.* = 35.6, $s^2 = 52.9$), and (*b*) daughter cells from gastric regions 24 h after feeding; ($n = 362$, $\bar{x} = 10.2 \pm 4.4$, *c.v.* = 42.8, $s^2 = 19.1$), (*c*) mother cells ($n = 126$, $\bar{x} = 21.5 \pm 8.4$, *c.v.* = 39.2, $s^2 = 71.2$) and (*d*) daughter cells from regenerating peduncles 48 h after excision ($n = 252$, $\bar{x} = 10.8 \pm 4.7$, *c.v.* = 43.2, $s^2 = 21.6$).

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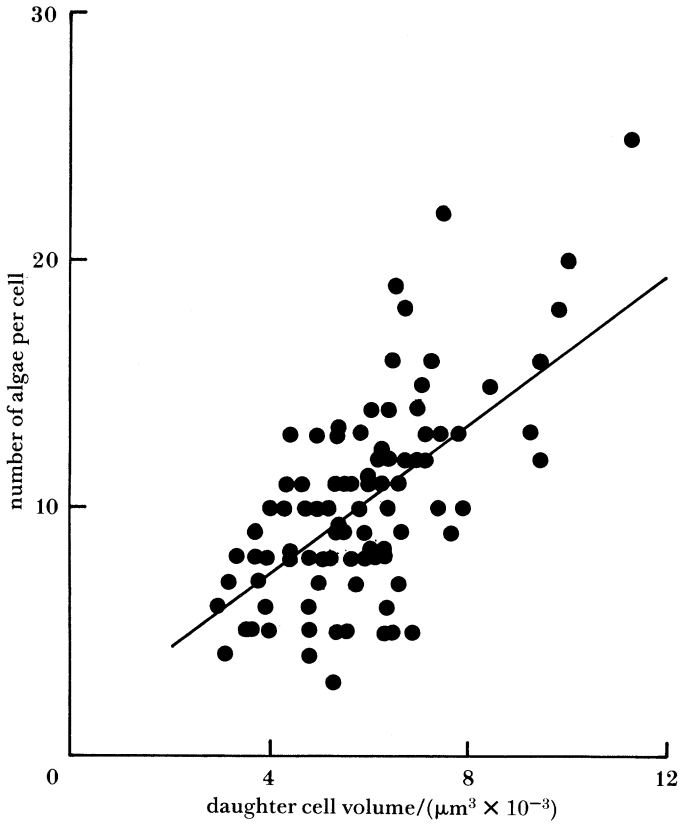

Figure 2. Linear regression of number of algae per telophase daughter digestive cell against cell volume. ($y = 1.39 + 0.0015x$; $r = 0.60$). The measurements were taken from 50 late telophase cells in which the daughter cell pairs had separated sufficiently to be allowed measurement of volumes and to count numbers of algae in each cell.

in daughter cells. This agrees with previous results (McAuley 1986), and corresponds to the predicted effects of a density-dependent control of replication of chloroplasts in the unicellular alga *Olisthodiscus*, in which all chloroplasts divided in cells containing fewer than a lower limit, no chloroplasts divided in cells containing more than an upper limit, and chloroplast number roughly doubled in cells with intermediate populations (Hennis & Birky 1984).

As algal division in digestive cells appears to be density dependent, it is possible that division is limited by the amount of space available for algal colonization; that is, by digestive cell size. However, regression of the number of algae against cell volume (estimated from plan area) of 50 pairs of daughter cells of telophase digestive cells from fed gastric regions showed that although there was a significant relation between cell volume and algal number, difference in cell volume accounted for only 36% of variation in numbers of algae (figure 2). This confirms previous work, which found only a poor correlation between the size of a cell and the number of algae that it contains (Douglas & Smith 1984; McAuley 1986).

Unlike chloroplasts and mitochondria, in which partition, although stochastic, tends towards equality (Hennis & Birky 1984), partition of algae between daughter digestive cells showed no tendency towards equality (tables 1 and 2). Instead, partition more closely resembled that predicted by the binomial distribution, assuming each algal cell had an equal but random chance of entering either daughter cell. Thus if a mother cell contains n algae, the chance that a daughter cell is produced containing x algae is equal to

$$n!/x!\,(n-x)!\,0.5^x\,0.5^{n-x}.$$

In telophase digestive cells from regenerating peduncles, the number in which partition was equal ($x = n/2$ in those digestive cells with even numbers of algae, $x = 1 + (n/2)$ in those with odd numbers), was not significantly different from that predicted by the binomial distribution (observed = 37.00, expected = 33.87; $\chi = 0.29$). However, in digestive cells from gastric regions of fed hydra, significantly fewer equal partitions were observed than were predicted by the binomial distribution (observed = 28.00, expected = 47.18; $\chi = 6.48$).

The data were further tested by comparing observed numbers in all daughter cells to those predicted by the binomial distribution. Because in no case were there sufficient mother cells with the same value of n on which to perform a χ^2 test, the data were transformed by using z, the cumulative distribution function of a standardized random variable where $z = (2x-n)/n$ (Hennis & Birky 1984). If the frequency of x, $n-x$ depended on the binomial function of n, 0.5, then the distribution of the transformed observations would resemble the normal distribution. Thus the cumulative normal frequency distribution of z (Snedcor & Cochran 1967) was divided into 10 classes of equal size, z was calculated for each observed value of x, n, and the number of transformed observations falling into each class was compared with the expected frequency, $n/10$,

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Table 1. *Distribution of algae between telophase daughter cells from regenerating peduncles*

For each telophase digestive cell that was observed, the total number of algae (n) and the number of algae in the daughter cells (x and $n-x$) were counted. The expected frequency was calculated according to the binomial distribution, which assuming each alga had an equal chance of entering either digestive cell, is given by:

$n!/x!\,(n-x)!\,0.5^{x}\,0.5^{n-x}$.

number of algae	x	$n-x$	observed N	expected N
5	3	2	1	0.31
6	3	3	1	0.50
	4	2	1	0.75
7	4	3	1	0.27
8	5	3	2	0.44
9	5	4	2	0.49
10	6	4	2	0.81
11	6	5	1	0.68
	8	3	2	0.48
12	8	4	1	0.48
	9	3	1	0.22
13	7	6	4	2.52
	8	5	2	1.84
14	7	7	2	1.25
	8	6	1	2.20
	9	5	3	1.48
15	8	7	4	3.14
	9	6	3	2.40
	11	4	1	0.66
16	8	8	1	0.39
	9	7	1	0.69
17	9	8	3	1.11
18	10	8	3	2.00
	11	7	2	1.46
	12	6	1	0.85
19	10	9	3	3.53
	11	8	1	2.88
	12	7	3	1.93
	13	6	2	1.04
	14	5	1	0.44
20	10	10	1	0.44
	11	9	3	0.75
21	11	10	3	3.37
	12	9	5	2.52
	13	8	1	1.75
	14	7	1	1.00
22	11	11	1	1.18
	12	10	3	2.10
	13	9	1	1.66
	14	8	1	1.07
	16	6	1	0.25
23	12	11	1	2.56
	14	9	2	0.80
	15	8	1	0.47
24	13	11	4	2.10
	14	10	2	1.63
	15	9	1	1.10
25	14	11	1	0.80
	16	9	1	0.36
	17	8	1	0.20
26	14	12	2	1.12
	15	11	2	0.96
27	15	12	3	0.78
28	14	14	1	0.45
	15	13	1	0.86
	20	8	1	0.08
29	15	14	3	1.12
	16	13	1	0.12
30	16	14	1	0.27
31	17	14	1	0.50
	18	13	1	0.38
32	18	14	2	0.44
33	17	16	1	0.54
	23	10	1	0.11
34	18	16	1	0.25
35	19	16	2	0.69
	21	14	1	0.07
36	19	17	1	0.50
	20	16	1	0.46
37	19	18	2	0.50
39	20	19	1	0.75
	22	17	1	0.66
	23	16	1	0.54
40	22	18	1	0.20
42	24	18	1	0.16
43	22	21	1	0.24

by using a χ^2 test with nine degrees of freedom (tables 3 and 4). This test showed that partition of algae between daughter cells of dividing digestive cells in regenerating peduncles followed that predicted by the binomial distribution ($\chi = 14.57$, $p < 0.05$). However, partition in dividing digestive cells of fed gastric regions, while similar to that expected from the binomial distribution, fell outside the limits of the test ($\chi = 21.15$, $p > 0.05$).

Measurement of the size of daughter cell pairs of telophase digestive cells from fed hydra showed that there was a tendency for one daughter cell to be somewhat larger than the other. On average, the larger of the daughter cells constituted $54.340 \pm 0.051\,\%$ of the total volume, significantly different from the mean of 50.0 % expected if partition had been equal ($p < 0.001$). That one daughter cell tended to be larger than the other may explain the difference between observed partition of algae and that predicted by the binomial distribution when for each algal cell the probability of entering either daughter cell was 0.5. If algae tend to be scattered evenly throughout the cytoplasm at telophase, as predicted by the binomial distribution, then inequality in host cell division would bias partition of algae such that the larger daughter cell would be expected to receive proportionally more algae. To test this, the expected number of daughter cell pairs receiving equal numbers of algae was calculated according to the binomial distribution when p, $q = 0.55$, 0.45 instead of p, $q = 0.5$, 0.5 as shown in table 3. In this case, expected numbers of equal partitions were 43.8 out of 181 cases, which although smaller than the value calculated for p, $q = 0.5$, was still significantly different from the observed number of equal partitions, 28 out of 181 cases ($\chi = 4.35$, $p > 0.05$). Thus the small difference in size of daughter cells was not sufficient to account for the difference in observed partition of algae in telophase cells from fed hydra from that predicted by the binomial distribution and that observed in telophase cells from regenerating peduncles.

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Table 2. *Distribution of algae between telophase daughter cells from gastric regions of recently fed hydra*

number of algae	x	$n-x$	observed N	expected N
8	4	4	1	0.27
9	5	4	2	1.97
	6	3	1	1.31
	8	1	1	0.14
10	5	5	1	0.74
	6	4	2	1.23
11	6	5	1	1.13
	7	4	2	1.62
	8	3	1	0.80
	9	2	1	0.26
12	6	6	1	1.38
	7	5	3	2.70
	8	4	1	1.70
	9	3	2	0.76
13	7	6	1	3.38
	8	5	5	2.45
	9	4	1	1.40
	12	1	1	0.03
14	8	6	3	2.93
	9	5	3	1.96
	11	3	1	0.36
	12	2	1	0.09
15	8	7	3	3.49
	9	6	3	2.70
	10	5	2	1.65
	11	4	1	0.75
16	8	8	3	3.00
	9	7	6	5.14
	10	6	5	3.64
	11	5	1	1.00
17	9	8	4	4.13
	10	7	4	3.30
	11	6	1	2.08
	12	5	2	1.04
18	9	9	2	2.38
	10	8	6	4.34
	11	7	3	3.16
	13	5	1	0.86
	14	4	1	0.30
19	10	9	3	2.45
	11	8	1	2.01
	12	7	1	1.35
	13	6	2	0.73
20	11	9	6	3.52
	12	8	6	3.36
	14	6	2	1.04
21	11	10	1	4.04
	12	9	5	3.36
	13	8	2	2.33
	14	7	3	1.33
	16	5	1	0.23
22	11	11	1	1.18
	12	10	3	2.10
	13	9	2	1.66
	14	8	1	1.07
23	13	10	1	1.12
	14	9	1	0.78
	16	7	2	0.23
24	12	12	1	0.81
	13	11	1	1.50
	14	10	2	1.17
	18	6	1	0.08
25	13	12	2	3.00
	14	11	3	2.66
	15	10	1	1.95
	16	9	1	1.22
	17	8	3	0.65
26	14	12	3	1.40
	15	11	1	1.20
	19	7	1	0.10
27	15	12	1	1.81
	16	11	4	1.36
	17	10	1	0.88
	20	7	1	0.09
28	14	14	1	0.90
	15	13	2	1.70
	17	11	2	0.96
	18	10	1	0.59
29	16	13	1	0.25
30	16	14	1	0.80
	18	12	1	0.47
	19	11	1	0.30
31	18	13	1	0.57
	20	11	2	0.23
32	18	14	1	0.22
33	18	15	2	0.47
34	18	16	1	0.50
	19	15	1	0.42
35	22	13	1	0.11
36	22	14	1	0.11
38	22	16	1	0.16
40	23	17	1	0.16
41	23	18	1	0.17
42	26	16	1	0.07
45	25	20	1	0.18
48	26	22	1	0.19

4. DISCUSSION

In the green hydra symbiosis, the wide variation about a strictly regulated mean number of algae per digestive cell may be because of separation of two regulatory processes: the first being the point at which control of number of algae dividing after initiation of host cell division is expressed; and the second the way in which the algal population is partitioned between daughter digestive cells at telophase. In fed hydra, algal and digestive cell mitoses are initiated at roughly the same time; in regenerating peduncles, division of the algae is completed before the host cells begin to divide (McAuley 1982, 1986). In both cases, algal division, and presumably expression of the density dependent control mechanism, is initiated before digestive cell telophase and subsequent partition of the algal population.

Results in this paper showed that when a digestive cell divides, the algae were apportioned between each daughter digestive cell in a manner that can be predicted by the binomial distribution. In dividing digestive cells from regenerating peduncles, each algal symbiont had an equal chance of entering either daughter host cell. The binomial distribution was found to apply less well to partition of algae at division of digestive cells from fed hydra; indeed, fewer instances of equal partitioning were observed than the

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Table 3. *Z statistics for partition of algae in digestive telophase cells of regenerating peduncles*

The value of Z was calculated for each pair of daughter cells from table 1 and the observed distribution within ten classes Z was compared with the expected distribution of equal numbers in each class, assuming that the algae were partitioned according to the binomial distribution and $p, q = 0.5$, so that the binomial distribution approximated to the normal distribution.

Z $((2x-n)/n)$	observed (o)	expected (e)	$(o-e)^2/e$
0.13	7	12.6	2.489
0.25	18	12.6	2.314
0.39	16	12.6	0.918
0.53	19	12.6	3.251
0.67	16	12.6	0.918
0.84	16	12.6	0.918
1.04	7	12.6	2.489
1.28	12	12.6	0.029
1.65	8	12.6	1.679
3.90	7	12.6	2.489
total	126	126	$\chi = 14.568$

Table 4. *Z statistics for partition of algae in digestive telophase cells of gastric regions of recently fed hydra*

The value of Z was calculated for each pair of daughter cells from table 2 and the observed distribution within ten classes Z was compared with the expected distribution of equal numbers in each class as described in table 3.

Z $((2x-n)/n)$	observed (o)	expected (e)	$(o-e)^2/e$
0.13	11	18.1	2.785
0.25	10	18.1	3.625
0.39	11	18.1	2.785
0.53	27	18.1	4.376
0.67	20	18.1	0.199
0.84	20	18.1	0.199
1.04	27	18.1	4.376
1.28	13	18.1	1.437
1.65	19	18.1	0.045
3.90	23	18.1	1.326
total	181	181	$\chi = 21.153$

binomial distribution predicted. That partition of algae in digestive cells from regenerating peduncles follows the binomial distribution more closely than partition in cells of fed hydra may be explained by differences in timing of algal and host cell division. In regenerating peduncles, algal division is complete by the time the host cell divides, but in fed hydra algal division continues through host mitosis, as shown by the increase in numbers of algae as mitosis proceeds (McAuley 1986). Algae that have completed mitosis at host telophase, but which have not separated into daughter cells, will be distributed as single cells, but may later be counted as four, causing the deviation towards inequality observed here.

Although the binomial distribution favours equal partition, it does not rule out asymmetric partition, particularly in those cases where numbers of algae are large. Thus application of the binomial distribution as the rule governing partition of the symbiotic algae will by itself lead to considerable variation about the mean. After host cell division, over- or under-population of host cells caused by partition would not be corrected until the next round of host and algal mitosis. Density-dependent algal division, implied by differences in variance and coefficient of variation in mother and daughter telophase digestive cells, would then compensate for inequality at partition, and regulate the algal population in the mother cell towards the mean, optimal level.

It is well established that variation of number of chloroplasts per cell is strongly correlated with increase in size of immature spores of the moss, *Polytrichum* (Paolillo & Kass 1977), and with increase in mesophyll cell size during the development of leaves of higher plants (Boasson *et al.* 1972; Kameya 1972; Tsuji *et al.* 1979; Scott & Possingham 1980; Whatley 1980; Wild & Wolf 1980; Olszewska *et al.* 1983). It has been suggested that growth and division of chloroplasts in mesophyll cells is regulated so that they cover a proportion of the cell surface area that is constant for a given species, even in cells of different levels of ploidy (Honda *et al.* 1971; Ellis & Leech 1985; Pyke & Leech 1987).

Although green hydra digestive cells exhibit a wide range in cell size, this is only poorly correlated with the numbers of algae that they contain. In previous studies, Douglas & Smith (1984) and McAuley (1986) found that variation in cell size of interphase and mitotic digestive cells accounted for less than 50% of variation observed in either numbers or total volume of their populations of symbiotic algae. In this paper, numbers of algae in daughter telophase digestive cells was also shown to be poorly correlated with host cell size. This suggests that the space available to the symbiotic algae plays only a small role in controlling algal cell growth and division either during or after host cell mitosis, and has little effect on partition of algae when the host cell divides. Instead, density-dependent division may be consistent with the suggestion that algal division is dependent upon supply of a 'division factor', as yet not identified (McAuley 1985*a*). In digestive cells with large numbers of algae, competition for division factor would be more limiting with regard to algal mitosis than in cells with few algae.

Partition of symbiotic algae according to binomial probability suggests that they were scattered fairly evenly through the host cell at telophase. This is in contrast with partition of chloroplasts in the marine unicellular alga *Olisthodiscus* (Hennis & Birky 1984), where in most cases the chloroplast population is divided evenly between the daughter cells, suggesting the chloroplasts were confined to a small volume of the algal cell, perhaps in a shell of cytoplasm beneath the cell wall. This difference may reflect a more primitive degree of integration between symbiotic algae and host cell architecture than that found between chloroplasts and plant cells. Study of the way in which the perialgal vacuoles interact with host cell cytoskeletal elements during and after cell division may help resolve this problem.

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