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# Regulation of numbers of symbiotic *Chlorella* by density-dependent division

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## SUMMARY

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. However, it is not clear how the population size of the symbionts is determined, although repeated measurements show that in constant culture conditions the mean number of symbionts per cell also remains constant. In this paper, simple density-dependent compensatory models were tested by simulating large numbers of host cell divisions by using computer modelling techniques. Stability of the mean number of algae per cell was achieved over a wide range of values simply by altering the value of the boundary between division of all symbionts in a cell and density-dependent division. Changes in the boundary between density-dependent division and total inhibition of symbiont division had little effect on the mean number of symbionts per cell, but instead altered variance and the shape of the distribution. Correlation between operation of the mathematical models and possible regulatory mechanisms operating within the symbiosis are discussed.

## INTRODUCTION

Regulation of growth and population size of symbionts by host cells is essential for the stability and persistence of any intracellular symbiosis. If symbionts grow more slowly than host cells, they will become diluted so that more and more host cells without symbionts appear; if symbionts grow more quickly than the host, excess numbers must be ejected (in those symbioses between autotrophs and heterotrophs, this would result in decreased primary productivity), or the host cells would be destroyed.

There is considerable evidence that digestive cells of green hydra control the cell division cycle of their *Chlorella* symbionts so that the symbionts divide only at host cell division (for review see McAuley (1985*a*)). However, although it is well known than changes in growth conditions of hydra elicit predictable changes in the mean number of symbionts per cell (Pardy & Muscatine 1973; Pardy 1974; Pool 1976; McAuley 1980, 1981*a*; Muscatine & Necklemann 1981; Necklemann & Muscatine 1983; Blank & Muscatine 1987), and that the mean number varies between different strains of hydra (McAuley 1984), the same strain of hydra infected with different strains of algae (Douglas & Smith 1984), and even between different regions of the body column of the same hydra (Pardy 1974; McAuley 1981*b*), it is not known what mechanism determines the mean population size, or the size of an algal population in any particular cell. Furthermore, the *Chlorella* symbionts each produce four autospores at cell division to the host digestive cell's two daughter cells. Therefore, at each round of host cell division, only a proportion of algal symbionts can be allowed to divide so as to maintain the stable population level.

Numbers in digestive cells could be controlled at either or both of two stages during the cell division cycle of host and symbionts. First, the proportion of the algal population which can divide before host cell partition will influence the number of algae in subsequent daughter cells. Second, when the host cell divides, any asymmetry in the distribution of algae within the mother cell or in the hydra division process could lead to a correspondingly asymmetric distribution of algae into the daughter cells. The former process offers the opportunity for the animal to regulate the symbiont within its cells while the latter process will tend to confound regulation, especially if high degrees of asymmetry were experienced.

These hypothetical rules, which may control the proportion of symbiotic algae dividing in a digestive cell and determine how algae are distributed between daughter cells when the host cell divides, may be tested by using computer modelling techniques. The green hydra symbiosis is particularly suited to this type of analysis, as it consists of large numbers of discrete population units (that is, digestive cells each containing a certain number of algae), and it may be rapidly and easily disassociated into its component cells so that actual populations may be compared with populations derived from computer simulations. In a preliminary report (McAuley & Darrah 1990), a single somewhat empirically derived model was shown to generate a population of digestive cells whose distribution according to the numbers of algae they contained was similar to that of an actual population of digestive cells. Here,

a number of mathematical models of the way in which numbers of *Chlorella* may be controlled were constructed with variations on the simple hypothetical rules described above. To evaluate the validity of each model, the stability of the mean number of algae per cell and distribution of numbers of algae per cell after many division cycles in a large number of cells were tested against the distribution observed in actual digestive cells.

## MATERIALS AND METHODS

### (*a*) *Organisms*

Green hydra (*Hydra viridissima*, Pallas) of the European strain were grown as previously described (McAuley 1990*a*). To determine distribution of *Chlorella* symbionts in digestive cells, the gastric regions (Pardy & Muscatine 1973) of five standard hydra (each bearing a single bud) were excised and isolated in a single drop of macerating fluid (David 1973). After ten minutes, the pieces of hydra could be teased apart into individual cells, and the preparation was examined with Normarski interference contrast microscopy. Numbers of algae were counted in 100 cells in each of ten preparations.

### (*b*) *The mathematical model*

In formulating the mathematical model, rules had to be devised for each process, giving rise to a series of models.

(i) *Algal division rules*

Two rule systems were evaluated, both based on density dependent regulation rules: the step model; and the linear model.

The step model was based on that previously suggested for density dependent division of *Chlorella* symbionts (McAuley 1986*a*, 1990*a*), and consisted of three rules:

(i) below a certain algal density (LL), all algae within the hydra mother cell divide, giving a quadrupling of the population (since division of a single *Chlorella* cell usually gives rise to four autospores (McAuley & Muscatine 1986));

(ii) above a certain algal density (UL), none of the algae within the mother cell divide, giving an unchanged population;

(iii) between the lower and upper limits, one third of the algae divide, giving an approximate doubling of the population.

The linear model also consists of three rules, the first two being identical to the step model rules given above. The third rule is:

(iii) between the lower and upper limits, an inverse linear density rule is applied to predict the proportion of the algal population that divide. Hence in a hydra cell containing only a few more algae than LL, most of the population would divide while for an algal population close to UL, relatively few algae divide.

The linear model is hence a true continuous density-dependent model whereas the step model is only an approximation to density-dependent regulation.

(ii) *Hydra division rules*

Two rule systems were also evaluated for the way in which *Chlorella* cells are partitioned at host cell division.

(i) The random partition model assumed that the partition of algae between daughter cells is essentially random, giving equal weighting to the chances of a population of 10 algal cells partitioning 10:0 or 5:5. Thus the random rule was applied by generating a random number in the range 0–1 representing the fraction of the algal population which partitions into one of the daughter cells.

(ii) The binomial partition rule assumed that algal partition occurred according to a binomial distribution (McAuley 1990*a*), hence giving a much higher weight to the probability of a 5:5 partition than to a 10:0 partition. The rule is applied by constructing a weighted partition table based on the binomial distribution, assuming each *Chlorella* cell had an equal, but random chance of entering either daughter digestive cell. Thus if a mother cell contained $n$ algae, the chance that a daughter cell would receive $x$ algae is equal to:

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

The construction and use of this table is best shown with an example. For a population of just three algae, the population can be partitioned between the two daughter cells in four ways (0:3, 1:2, 2:1 and 3:0), and the binomial distribution assigns these partition ratios different weights of 0.125, 0.375, 0.375 and 0.125, respectively. To construct the entry for this number of algae in the weighted probability table the interval 0–1 is divided into four fractional increments of 0–0.125, 0.125–0.5, 0.5–0.875 and 0.875–1.0. This process is repeated for each algal number up to the maximum number of algae anticipated. To use the table, a random number is generated in the interval 0–1, the appropriate level in the table is selected, and the partition ratio determined by finding which fractional increment the random number falls into.

It would be possible to construct four mathematical models from the rule combinations given above, but in practice only three were evaluated: model 1, step rules for algal division, random algal partition (SR); model 2, step rules for algal division, binomial algal partition (SB); model 3, linear rules for algal division, binomial algal partition (LB).

To run the models, the rules were applied to a large digestive cell population, normally 1000 cells. As a population of hydra takes about 10 days to double in number (P. J. McAuley, unpublished data), 10% of the model population of digestive cells were assumed to be capable of division in each division cycle (that is, each division cycle roughly equals one day). Initially, each digestive cell was assumed to contain 22 algae. The dividing cells were sampled at random, algal division and partition simulated, and the population size restored to its initial size by discarding 10% of the total population at random; this is equivalent to loss of cells due to production of buds, and to a lesser extent, sloughing and cell death. This process was repeated over many division cycles (normally 1000).




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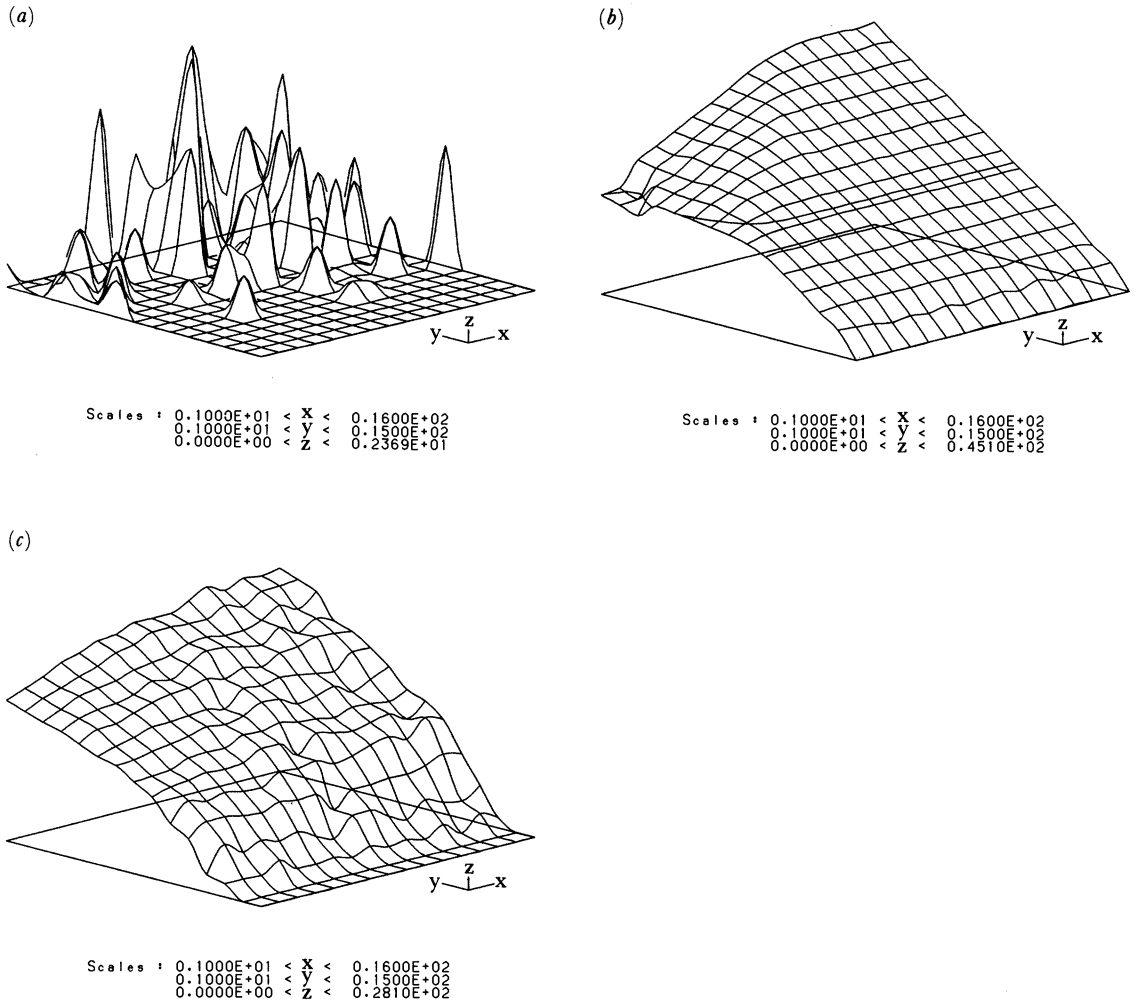


Figure. 1. Predicted mean algal numbers per hydra cell after 1000 division cycles as a function of the lower (LL) and upper (UL) density dependent limits for the three models. (*a*) SR model; (*b*) LB model, and (*c*) SB model, see text for details. The *x*-axis scales correspond to UL values of 25–70 while the *y*-axis scales correspond to LL values of 1–15. The *z* axis shows the predicted mean algal numbers for each combination of LL and UL.

## RESULTS

One of the most important features of the natural system, which mathematical models must be capable of simulating, is the relative stability of mean algal numbers per hydra cell that is exhibited over many division cycles in constant growth conditions (Pardy 1974; Pool 1976; McAuley 1980, 1981*a*). Accordingly, each model was run for a range of combinations of LL and UL to assess the stability of the mean. The results are presented in the form of three-dimensional graphs with LL and UL values on the independent axes and the mean number of hydra per cell after 1000 division cycles on the dependent axis (figure 1).

There were dramatic differences between the stability of the three models. The random partition model (SR) was unconditionally unstable for all the density-dependent limit values imposed, with the mean dropping to very low levels or to zero for all combinations.

In contrast, the linear-binomial model (LB) exhibited excellent stability with complete elimination of algae from digestive cells occurring only at very low values of LL. The three-dimensional plot (figure 1*b*) showed that almost any value for the mean algae per cell can be obtained by the choice of appropriate values for LL and UL. It also showed that different combinations of LL and UL did not produce a unique solution: the contour line, shown as the enhanced line on the plot, denotes those paired values of LL and UL, which maintain the initial mean value over the 1000 division cycles. The plot also showed the differential effect changes to LL or UL have on the mean value: changes in LL having a pronounced effect, especially at low UL values while changes in UL having no effect for a range of low LL values and only a very moderate effect at higher values of LL.

The step-binomial model (SB) showed an intermediate stability with total elimination of algae from digestive cells again occurring only at very low values of LL. The general predictions were similar to those of the LB model although it was noticeable that changes in the upper density-dependent limit had rather more effect on the mean over the whole range of LL examined. The figure was also more 'noisy', indicating a looser control over algal division than that in the LB model.

On the basis of the stability plots, it was possible to

Table 1. *Comparison of the predicted mean, range, skewness and kurtosis of an experimentally observed distribution of algal numbers in hydra cells with those predicted by the* LB *and* SB *models after 1000 division cycles for various combination of the upper and density-dependent parameters. Tests for skewness and kurtosis were either significant at the 1% level* (**) *or non-significant at the 5% level* (*n.s.*).

| model | limits LL | limits UL | mean | min. values | max. values | skewness | kurtosis |
|---|---|---|---|---|---|---|---|
| experimental data ... | | | 22.10 | 1 | 76 | 1.491** | 4.476** |
| LB | 7 | 25 | 19.69 | 6 | 32 | −0.147 n.s. | −0.125 n.s. |
| LB | 7 | 40 | 21.02 | 9 | 34 | 0.043 n.s. | −0.124 n.s. |
| LB | 7 | 55 | 21.02 | 9 | 34 | 0.043 n.s. | −0.124 n.s. |
| LB | 7 | 70 | 21.02 | 9 | 34 | 0.043 n.s. | −0.124 n.s. |
| LB | 3 | 40 | 9.02 | 2 | 17 | 0.110 n.s. | −0.220 n.s. |
| LB | 5 | 40 | 14.99 | 7 | 26 | 0.086 n.s. | −0.256 n.s. |
| LB | 7 | 40 | 21.02 | 9 | 34 | 0.043 n.s. | −0.124 n.s. |
| LB | 9 | 40 | 26.98 | 12 | 41 | 0.017 n.s. | −0.103 n.s. |
| LB | 11 | 40 | 31.66 | 14 | 48 | −0.408** | 0.410** |
| SB | 11 | 25 | 16.51 | 6 | 34 | 0.320** | −0.215 n.s. |
| SB | 11 | 40 | 18.88 | 5 | 40 | 0.760** | 0.450** |
| SB | 11 | 55 | 20.61 | 6 | 56 | 1.180** | 1.513** |
| SB | 11 | 70 | 21.47 | 6 | 68 | 1.624** | 3.215** |
| SB | 11 | 85 | 21.99 | 6 | 82 | 1.951** | 5.027** |
| SB | 11 | 100 | 22.54 | 6 | 102 | 2.317** | 7.345** |
| SB | 7 | 40 | 13.17 | 2 | 40 | 1.307** | 2.261** |
| SB | 9 | 40 | 15.88 | 3 | 46 | 1.166** | 1.861** |
| SB | 11 | 40 | 18.88 | 5 | 40 | 0.760** | 0.450** |
| SB | 13 | 40 | 22.07 | 7 | 46 | 0.495** | −0.073 n.s. |
| SB | 15 | 40 | 23.92 | 10 | 46 | 0.415** | −0.115 n.s. |

eliminate the SR model, but no meaningful distinctions could be made between the LB and SB models. To establish which of the two remaining models gave the better simulation of algal regulation, it was necessary to compare the different distributions of algal numbers in the hydra cell population predicted by the models with an experimentally determined distribution.

The experimental distribution shown in figure 2 was similar to previous determinations (Pardy 1974; McAuley 1981*b*). Although approximating to the normal distribution, it exhibited properties of skewness (i.e. with the mean value differing from the median) and kurtosis (i.e. with an excess number of values close to the mean and far from it, and with a corresponding reduction in the number of intermediate values). Calculation of the $g_1$ and $g_2$ statistics (Snedecor & Cochran 1967) for these data shows that the distributions are significantly different from a Normal distribution at the 1% level for skewness and kurtosis (table 1).

The statistics were also calculated for the distributions predicted by the two models and these results are also shown in table 1. The data showed significant differences in the distributions predicted by the two models. The LB model predicted the distribution of algae within the hydra cell population was Normally distributed; the one exception to this displayed a significant skew towards the lower end of the distribution, i.e. in the opposite direction to the skew exhibited by the experimental data. In contrast, all the distributions predicted by the SB model were significantly and positively skewed and the majority also exhibited significant positive kurtosis. On this basis, the SB model appeared to provide a better fit to the experimental data.

Inspection of the minimum and maximum values for algae per cell for the various simulations also revealed interesting differences. For the LB model, changes in UL for a constant LL value had almost no effect on the extreme values of the distribution; in contrast, changing LL at constant UL affected both ends of the distribution causing both an upwards displacement of the distribution and a broadening of the range with increasing LL. For the SB model, the effect of changing UL while maintaining LL constant was to increase the spread of the distribution to higher values while not affecting the lower limit. The reverse was true when LL was varied at constant UL: when the lower limit was changed, there was little effect on the upper limit value. Figure 2 shows the distributions found for several values of LL and UL selected from table 1 and graphically shows the appearance of skew and kurtosis in the distribution.

The models can also be used to stimulate another important aspect of algal regulation: the decline to a new stable mean number of algae per cell, which occurs when the hydra are transferred from light to continuous darkness (Pardy 1974; McAuley 1981*a*). Transfer of green hydra to continuous darkness initiates a rapid decline in mean numbers of algae per cell to a new stable level, reached after 14–28 days, that is much lower than that in the light.

To simulate this behaviour, the SB and LB models were run over 2000 division cycles, with a simulated transfer from light to dark or vice versa every 500 division cycles. At each transfer point the upper and




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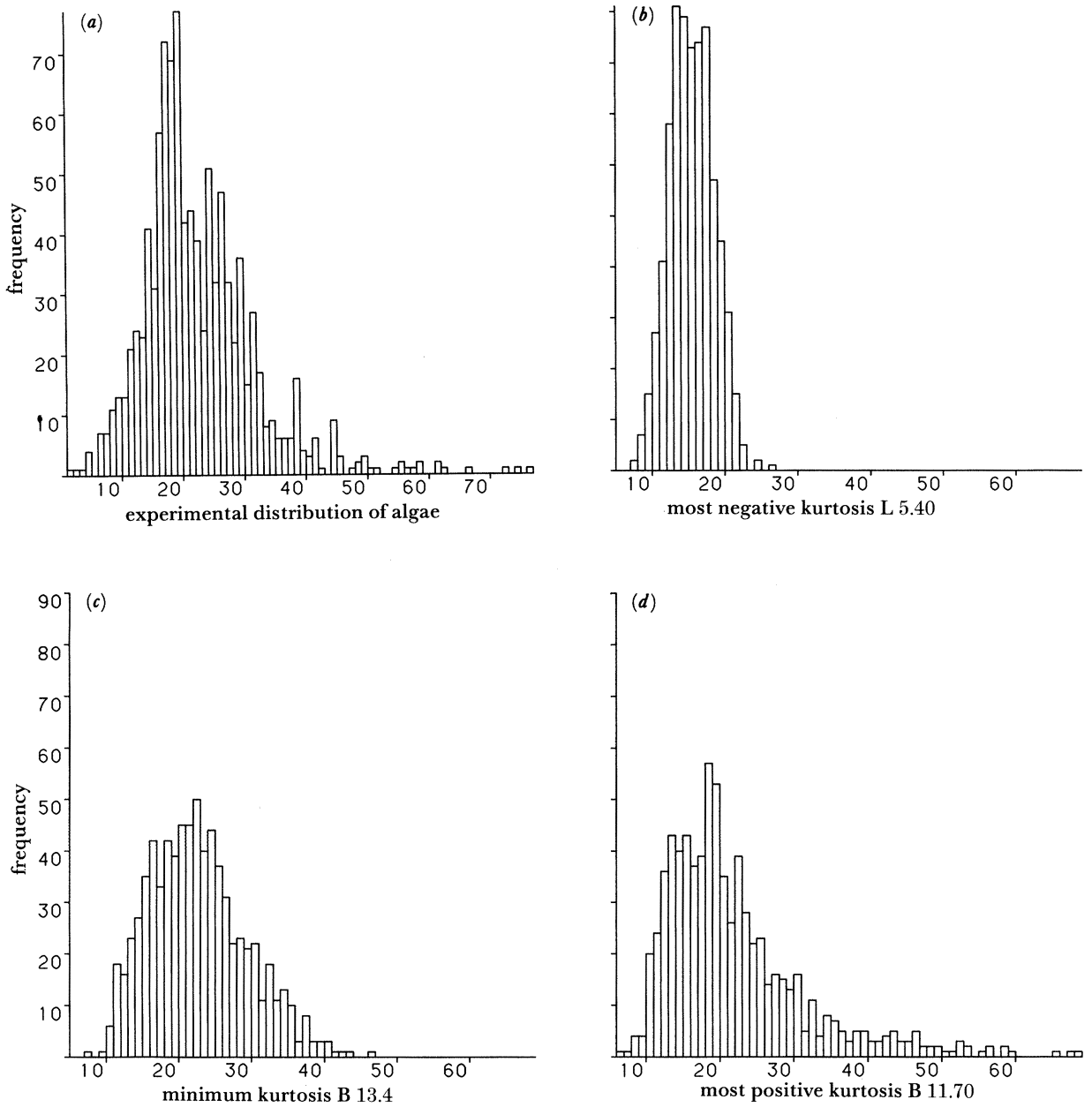


Figure 2. The distribution of algae in hydra digestive cells. (*a*) Experimentally observed distribution according to numbers of algae per cell of 1000 digestive cells from the gastric regions of 20 European green hydra. The other figures show predicted distributions and show some of the extremes of kurtosis and skewness found in the analysis of the models summarised in table 1. (*b*) LB model with LL = 5, UL = 40; (*c*) SB model with LL = 13, UL = 40, and (*d*) SB model with LL = 11, UL = 70.

lower density-dependent limits were adjusted to new values. The effect of the changes to the mean number of algae per cell are shown in figure 3. The two models showed markedly different behaviour; although both seemed capable of simulating the change in algal density resulting from a change in growth condition, the kinetics of the change were very different. The SB model reacted very sluggishly to a change from light to dark and dark to light with a new steady-state mean value not being achieved within 500 division cycles (because green hydra and its symbiont population doubles approximately once every ten days (P. J. McAuley, unpublished data) and in these models 10% of the digestive cells divide at each division cycle, one model division cycle approximates one day of actual hydra growth). In contrast, the LB model reacted very smoothly to the imposed change, with a new steady state value being reached within approximately 50 division cycles which, although slower than observed changes, is more realistic than that achieved with the SB model. An intermediate model, using the SB rules in the light and the LB rules in the dark showed intermediate behaviour.

The results highlight one potential deficiency in the models' formulation. The models assume that each digestive cell had a 1 in 10 probability of division at each division irrespective of the antecedent division history of the cell: in reality it is more likely that most of the digestive cells in the population will divide within 10 division cycles. The models therefore have an

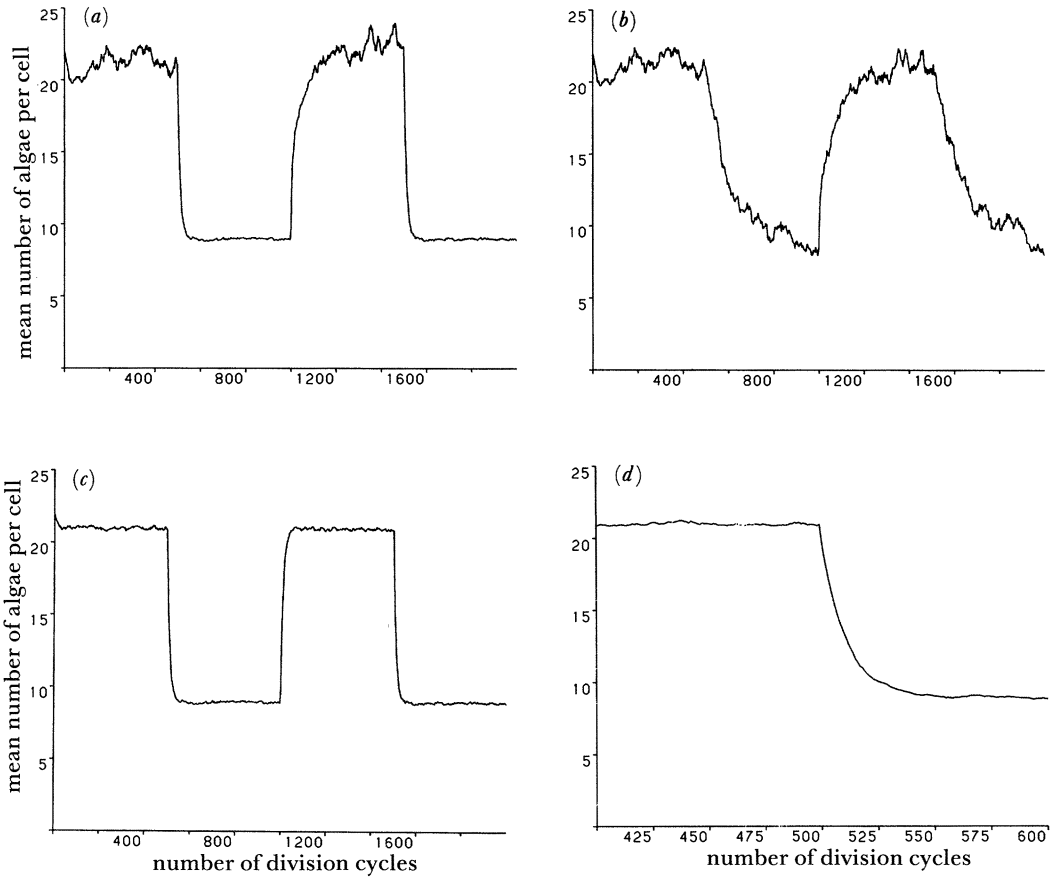


Figure 3. Simulated effects of alternate light–dark treatments on the mean number of algal cells per hydra. (*a*) SB model in light (LL = 11, UL = 80), LB model in dark (LL = 3, UL = 80); (*b*) SB model in light (LL = 11, UL = 80), SB model in dark (LL = 5, UL = 80); (*c*) LB model in light (LL = 7, UL = 80), LB model in dark (LL = 5, UL = 80); (*d*) shows expansion of part of (*c*). The upper and lower density-dependent limits were selected from figure 1 to give comparable algal densities for each model combination.

inbuilt predisposition to perform sluggishly after an environmental change leading to a change in algal density. Although it would be possible to correct this deficiency, further experimental data would be required before such transition phenomena could be accurately predicted.

## DISCUSSION

### (*a*) *Validity of models*

In this paper, simple rules of density-dependent symbiont division, applied at each round of host cell division, could adequately compensate for random partition of the symbionts at host cell division in two related mathematical models, the LB and SB models. The models were based on three assumptions supported by previous research:

(i) the symbionts divide only at host cell division (McAuley 1981*b*, 1982, 1985*a*, *b*);

(ii) symbiont division is density dependent (McAuley 1985*b*, 1986*b*);

(iii) when the host cell divides, symbionts are partitioned at random between daughter cells, in a way that can be described by the binomial distribution, assuming $p$, $q = 0.5$ (McAuley 1990*a*).

To simplify the model, it was assumed that symbiont division was completed before that of the host cell commenced. Although this is true for the case of digestive cells in excised regenerating peduncles, in digestive cells of fed hydra symbionts and host cells divide at the same time (McAuley 1982, 1986*b*). This will have a slight but significant effect on the way in which the symbionts are partitioned, as symbionts which have not completed cytokinesis when the host cell divides would behave as a single cell at partition, but would be counted as four cells afterwards, tending to increase inequality of partition and hence variation in numbers of symbionts per host cell.

In both models described in this paper, stability of the average number of symbionts per cell was achieved over a wide range of values simply by altering the lower limit (LL), the boundary between division of all symbionts and density-dependent division. Changes in the upper limit (UL), the boundary between density dependent and total inhibition of symbiont division, had relatively little effect on the mean number of symbionts per cell, but instead altered variance and the shape of the distribution. Interaction between LL and UL was surprisingly complex. A single stable value for mean number of symbionts per cell could be maintained by very many pairs of different LL and UL values, and there appeared to be no empirical method for determining the mean number of symbionts per cell that a given pair of LL and UL values would generate.

There is no immediately obvious reason for preferring the SB model of regulation over the LB model,

except that the former generates distributions of digestive cells against symbiont number that more closely resemble those of actual digestive cell populations. The extent to which this is fortuitous is uncertain, and further experimental work on the mechanisms underlying the regulation process is required before the model can be validated. However, both models show that very simple rules of division and partition can generate richly complex distributions and changes in populations of intracellular symbionts. This may have relevance not only on the regulation of symbionts, but also to control of replication of organelles. For instance, it is widely assumed that replication of chloroplasts in higher plant cells is density dependent (Boasson *et al.* 1972; Honda *et al.* 1972; Kameya 1972; Paolillo & Kass 1977; Tsuji *et al.* 1979; Scott & Possingham 1980; Whatley 1980; Wild & Wolf 1980; Olszewska *et al.* 1983; Ellis & Leech 1985; Pyke & Leech 1987).

During preparation of this paper, Taylor *et al.* (1989) published an account of a computer model of regulation of numbers of algae per cell in green hydra based on assumptions that were radically different from those used here. The chief difference between the Taylor model and the models described in this paper is that the Taylor model used estimates of mitotic indices and duration of cell mitosis to calculate limits for the probability of a particular alga or digestive cell dividing at any given time. This does not take into account the large body of evidence that algal division is entrained to that of the digestive cells (McAuley 1981*b*, 1982, 1985*a*, *b*, 1986*a*), but assumes that algal and digestive cell division are independent of each other. Furthermore, while the Taylor model assumed that algal division was density dependent, no reason was given for the choice of a single set of values of upper and lower limits for minimum and maximum mitotic indices; and it was also assumed that digestive cell mitosis was influenced by numbers of algae per cell (so that digestive cells with many algae were more likely to divide than those with few algae), although no evidence was presented for this form of density dependence. The Taylor model also assumed that at digestive cell division the algae were partitioned equally between daughter cells, an assumption since invalidated (McAuley 1990*a*). Finally, although the Taylor model produced a stable mean population of algae, and a distribution of algae per cell resembling that of an actual distribution, it was necessary during the running of the model to eliminate digestive cells which were occasionally produced with very large numbers of algae. The limit at which digestive cells were eliminated was set at 55 algae per cell, although observations given in this paper show that in European hydra, cells in natural populations may contain up to 76 algae.

The necessity to remove cells with large numbers of algae shows that control in the Taylor model is less precise than in the models described here. However, the Taylor model provides an interesting, alternative regulatory mechanism in which algal division is density-dependent, but is not directly coupled to host-cell division. It may be more applicable to marine alga and invertebrate symbioses, especially as the relatively large symbiotic zooxanthellae algae occupy a much higher proportion of the host cell in marine symbioses than do symbiotic *Chlorella* algae in green hydra. This would favour a more equal distribution of symbionts at host-cell division than that observed in green hydra digestive cells.

### (*b*) *Density dependence and 'division factor'*

The ultimate aim of mathematical modelling should be to replace the current, essentially empirical model by a mechanistic model of symbiont regulation. In this section, an attempt is made to relate the SB and LB models to previous theories of symbiont regulation based on experimental observations.

Density-dependent division implies that the symbionts of a population inside a single digestive cell compete with each other for some factor required for completion of their cell cycle. Some workers have suggested that this limiting factor may simply be the amount of space available inside the host cells (Muscatine & Pool 1979; Douglas & Smith 1984), but there is only a poor correlation between the size of a digestive cell and the number of symbionts it contains (Douglas & Smith 1984; McAuley 1986*a*, 1990*a*). An alternative explanation is that the symbionts require an external supply of metabolite or metabolites to complete their cell division cycle (McAuley 1985*b*). This 'division factor', derived from host food, would be supplied via host metabolic pools only at host cell division (McAuley 1985*b*). There is as yet no evidence for the identity of the putative 'division factor', although it has been established that the symbionts show some characteristics of nitrogen limited growth, and may be dependent upon host supply of amino acids (McAuley 1986*b*, 1987, 1990*b*; Rees *et al.* 1989). Other workers have suggested that supply of inorganic nutrients may control symbiont division (Muscatine & Necklemann 1981; Necklemann & Muscatine 1983; Blank & Muscatine 1987; Rees 1986, 1989). Again, supply would be density dependent.

The critical amount of 'division factor' needed for symbiont division may vary according to growth conditions of the symbiosis, thus causing differences in the mean number of symbionts per cell that have been measured in hydra grown under different light intensities, feeding regimes or photoperiods (Pardy & Muscatine 1973; Pardy 1974; McAuley 1981*a*). For instance, increasing the diurnal period of illumination may reduce dependence upon 'division factor' and hence reduce the LL and increase the UL, resulting in more symbionts per cell.

Availability of 'division factor' is closely related to the density-dependence rules used in the SB and LB models. Thus in digestive cells with small numbers of symbionts, the digestive cell's internal pool of 'division factor' may be sufficient to allow all the symbionts to divide without addition from digestion of food. In digestive cells with many symbionts, limits on the amount of food an individual digestive cell could process, and competition for 'division factor' between symbionts, would reduce the amount that each symbiont received to a level below that necessary to initiate cell division. At intermediate population sizes,




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intermediate numbers would be able to divide, based on the ability of digestive cells to phagocytose food particles during extracellular digestion of captured prey.

This complex interaction between a fixed and variable source of 'division factor', and a variable number of symbionts, may explain why the SB model more closely approximates the actual distribution of symbionts per digestive cell, rather than the LB model, although the smooth change in rate at which number of symbionts dividing increases with numbers of symbionts per cell in the latter might be thought to reflect competition for 'division factor' more accurately. However, modelling the rapid fall in numbers of symbionts per cell when hydra are transferred to continuous darkness suggested that while the SB model of regulation may apply in the light, the LB model may apply in continous darkness. Simply lowering the LL of the SB model resulted in only a very sluggish decline in numbers of symbionts per cell, while lowering the LL and switching to the LB model caused a rapid decline more closely approximating that observed in experimental situations (Pardy 1974; McAuley 1981*a*). Possibly, this represents a switch in dependence of the symbiont population upon a two-phase supply of 'division factor' in the light, to a single-phase supply of organic carbon, because in darkness the symbionts must depend entirely upon host supply of fixed carbon (McAuley 1986*c*).

To test the models further for the dark–light simulation, experimental data are required on the timecourse of change in mean algal numbers per cell resulting from a change in environmental growth conditions. Additionally, data are required on changes in the distribution of algae within the hydra population at various stages during the transfer period, as the two models predict substantially different distributions.

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