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Soma and germ: an experimental approach using *Volvox*

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**SUMMARY**

The separation of soma from germ may have originated as a result of a specialization in source and sink, with somatic cells acting as sources, gathering nutrients from the external environment and germ cells as sinks, utilizing nutrients to grow and reproduce. This hypothesis can be tested in an organism, such as *Volvox*, where single germ cells can be cultured in isolation from the soma, thus serving both as source and sink, and their growth compared with that of germ cells with an intact soma where source and sink are separated into different cells. Results from such an experiment show that germ cells grown with an intact soma had greater rates of increase than those grown with disrupted soma or that were completely isolated, but the difference became greater as nutrient concentration increased, as predicted by the source-and-sink hypothesis. The advantage, however, was not sufficient to compensate fully for the initial investment in soma, especially at low nutrients, perhaps due to the energetic cost of swimming. In nature, species with segregated soma are found in nutrient-rich lakes and ponds. In experimental farm ponds, the biomass of such species increases with eutrophication more than the biomass of related species without division of labour, suggesting an advantage consistent with the source-and-sink.

**1. INTRODUCTION**

A basic feature of the design of multicellular organisms is the separation of a reproductively sterile soma from the germ line. The question of the selective advantage that has favoured this separation has been central to evolutionary biology for more than a century, ever since Weismann (1891) pointed out the problem, but the nature of the advantage has never been clarified. It has been emphasized recently that only a minority of phyla of multicellular organisms show the early segregation of soma envisaged by Weismann, and that most other phyla vary extensively in the timing and the extent of the segregation of soma from germ (Buss 1987). At least three kinds of selective advantages have been proposed: (i) advantages due to the division of labour among cells (Bonner 1965; Margulis 1981; Bell 1985, 1988; Koufopanou 1992); (ii) the control of heritable mutation rate (Whitham & Slobodchikoff 1981; Buss 1983); and (iii) perhaps, intracellular parasites (Hurst 1990). We present here two experimental tests of a theory that attributes a physiological advantage to division of labour between soma and germ.

The volvocine series of colonial green algae (Chlorophyta, Volvocales), which are thought to have evolved from a unicellular *Chlamydomonas*-like ancestor, offer a uniquely detailed example of the transition from unicellular to multicellular organisms with distinct somatic and germ tissues (reviewed by Kochert 1973; Starr 1980; Bell 1985; Koufopanou 1992). The multicellular forms range from the very simple colonies of the genus *Gonium*, with few identical *Chlamydomonas*-like cells embedded in a gelatinous matrix and arranged in a flat plate, to the most highly elaborated members of *Volvox*, with colonies of up to 50 000 cells, in which most of the cells are small and reproductively sterile but a few are much larger and specialized for reproduction. Intermediate stages in this transition are represented by forms such as *Pandorina, Eudorina* and *Pleodorina*, with a tendency towards increased size and more pronounced soma–germ differentiation. It was shown by Bell (1985) that colonial members of the Volvocales have greater maximal rates of increase than unicellular algae of comparable size, suggesting a physiological advantage to multicellularity and division of labour under optimal conditions of growth. Bell hypothesized that the critical factor is the inhibition of nutrient uptake by previously acquired resources: with Michaelis–Menten kinetics, rate of uptake is an increasing function of the concentration gradient between the internal and external environments (Rhee 1979). The possession of a germ line acting as a sink, to which resources can be translocated by a soma acting as a source, allows cells of multicellular organisms to increase their rate of uptake by reducing the concentration of products of synthesis which would otherwise accumulate and interfere with the reactions contributing to their synthesis.

The source-and-sink hypothesis can be tested in an organism where germ cells can be cultured outside the body, thus acting as both source and sink, and comparing their growth with that of intact individuals where source and sink are separated among different cells, across a series of external nutrient concentrations.

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We then predict that: (i) the rate of increase of germ plasm will be greater when germ cells are growing associated with a soma; and (ii) the difference between germ cells growing with and without a soma will increase with nutrient concentration, as it is at high nutrients that the effect of inhibition by previously acquired resources will be greatest. We report here an experiment which confirms these predictions. We used the species V. carteri (f. nagarinesis, strain HK10), which represents one of the simplest multicellular designs in the Volvocales with complete separation of soma from the germ line. In this species, as few as three genes are thought to be involved in differentiating the wild-type form from one entirely lacking such a division of labour (Kirk & Harper 1986). Cleavage of the gonidium (asexual germ cell) eventually gives rise to a hollow sphere of cells, with small somatic cells at the surface and larger germ cells in the interior. It has been known for some time that uncleaved gonidia isolated from the parental spheroid are capable of normal growth to the adult state (Pocock 1938). In V. carteri, cytoplasmic connections between cells break naturally soon after the completion of morpogenesis and the formation of germ cells (Kirk & Harper 1986), so that cells are connected to each other only by an extracellular glycoprotein matrix (Kirk et al. 1986), and can be isolated without causing any apparent damage. Another prediction from the source-and-sink hypothesis is that the occurrence of colonial forms in natural systems should increase with the degree of eutrophic, because of their greater rate of increase at high nutrient conditions, and that the biomass of increasingly complex forms with soma–germ separation should respond more to increases in nutrient levels. Data from the literature on the biomass of colonial Volvocales in fertilized farm ponds, supporting these two predictions, are also presented.

2. REMOVING THE SOMA

(a) Methods

Mechanical isolation and culture of gonidia was done following the methods described in Kirk & Kirk (1983) and D. L. Kirk (personal communication). Newly released daughter spheroids bearing young, uncleaved gonidia were disrupted as gently as possible by using a Dounce tissue homogenizer, resulting in two classes of gonidia: completely isolated cells, and ‘semi-isolated’ cells still associated with more-or-less extensive sheets of somatic tissue. These two categories were then separated under a stereoscope: non-motile, isolated gonidia sank to the bottom of the cavity, whereas those still attached on motile somatic cell sheets remained floating. Both categories of gonidia survived well and underwent normal cleavage and development. Intact colonies were collected at the same time from the same culture.

All three categories of gonidia (growing ‘with intact soma’, ‘with disrupted soma’ and as ‘isolated’ gonidia) were transferred immediately to sterile Standard Volvvox Medium (SVM) (Kirk & Kirk 1983) and cultured at four levels of dilution \(\times 10^0, \times 10^{-1}, \times 10^{-2}, \times 10^{-3}\), at 28±1 °C, under continuous, saturating, fluorescent light (150 mE m\(^{-2}\) s\(^{-1}\)) to allow maximal growth. Two replicates of each combination of state and medium concentration were set up. The volumes of gonidia were estimated from cell diameter measured at 400 × with a Zeiss compound microscope at 0 h, 14.5 h and 21 h after the beginning of the experiment, by which time the gonidia at the highest concentration were beginning to cleave; 50 gonidia per state were measured at 0 h, and 25 gonidia per replicate at 14.5 h and 21 h. To prevent the cells from being flattened by the coverslip, a fixed quantity of standard pre-swollen Sephadex beads somewhat larger than the gonidia was added to the samples before measurement. To measure the age and size at first cleavage of gonidia at all states and medium concentrations, additional samples of 25 cells were obtained every 7–10 h for up to 160 h from the beginning of the experiment.

The volume and number of somatic cells were also measured in the intact spheroids to estimate the total amount of tissue responsible for germ plasm production. The number of somatic cells at 0 h was estimated by measuring the distance between adjacent somatic cells and the diameter of the spheroid, and then applying the formula for the surface of a sphere. A mean number of 2385 somatic cells per spheroid was found, within the range previously observed for the HK10 strain (Green & Kirk 1981). The volumes of somatic cells were estimated at 0 h and 21 h from measurements of 10 cells on each of 10 individuals per replicate. There was a mean of 9.3 gonidia per spheroid.

![Figure 1. Growth of gonidia (intact soma (open circles), disrupted soma (filled squares) and isolated (filled circles)) (mean and two standard errors; means of two replicate cultures) and of somatic equivalents (open triangles) per gonidium. (a) At the highest nutrient concentration (undiluted SVM), all states had significantly greater volumes 14.5 h after the beginning of the experiment (t-tests, \(p < 0.001\)). (b) At the lowest concentration (1000 × dilution), all states were significantly larger by 21 h (t-tests, \(p < 0.001\)). The question mark represents an estimated point.](image-url)
(b) Results and discussion

(i) The advantage of soma. Figure 1 shows that gonidia increased significantly in volume at all states and medium concentrations. The growth of somatic tissue equivalents per gonidium (calculated as number of somatic cells per spheroid multiplied by the mean volume at that concentration and divided by the number of gonidia per spheroid) is also shown for comparison. Growth is exponential and, as there is a small but significant difference in initial size among states (< 6% difference between the largest and the smallest groups), due to the method of separation of the isolated and semi-isolated groups, the most appropriate way to analyse the effects of treatments is by comparing the relative growth rates of cells. This is given by the specific rate of increase, r, calculated from the model of exponential growth:

\[ G_t = G_0 e^{rt}, \quad \text{hence} \quad r = \left( \frac{1}{t} \right) \ln \left( \frac{G_t}{G_0} \right), \quad (1) \]

where \( G_0 \) and \( G_t \) are the volumes of gonidia at 0 h and \( t \) h after the beginning of the experiment.

The rates of increase of gonidia grown at different states and concentrations are shown in figure 2 and analysed in table 1. Note that, for the sake of convenience and ease of visual interpretation, all the figures and the analysis are presented using the common logarithms instead of the natural logarithms required by the exponential model. Growth increased substantially with concentration of medium, both in the unmanipulated and the manipulated groups, fulfilling the necessary condition for the hypothesis to be tested. There is no significant difference in growth between gonidia growing with disrupted soma and as isolated, confirming that the mere presence of disorganized somatic tissue has little effect (table 1).

The growth of gonidia with intact soma is greater at all concentrations, suggesting that there is transfer of nutrients from the somatic to the germ cells, but the difference between states increases with concentration. Analysis of covariance shows that there is a significant effect of both state and concentration, and that their interaction is also significant (state \( \times \) concentration: \( p = 0.04 \) at 14.5 h, and \( p = 0.01 \) for 21 h; table 1). The two predictions made by the hypothesis are therefore supported by our results.

Both soma and germ contribute to the growth of gonidia with an intact soma; growth of an isolated gonidium is attributable to the germ cell alone. The efficiency of division of labour can be further tested by comparing the amount of germ production relative to the total initial mass contributing to its growth. For an intact spheroid with gonidial and somatic masses \( G_0 \) and \( S_0 \) respectively, we can calculate the rate of increase it would have achieved had it had all its mass, \( G_0 + S_0 \), composed of germ. Division of labour will be advantageous if the rate of increase of intact-spheroid gonidia meets the following condition:

\[ G_0 e^{r(t_{\text{final}})} > (G_0 + S_0) e^{r(t_{\text{final}})}, \]

\[ r(t_{\text{initial}}) + t \ln G_0 > r(t_{\text{final}}) + (1/t) \ln (1 + S_0/G_0), \quad (2) \]

where \( r(t_{\text{final}}) \) and \( r(t_{\text{initial}}) \) are the specific rates of increase of gonidia growing with an intact soma and isolated, and \( t \) is the time they were allowed to grow. Equation (2) makes the intuitive suggestion that the condition for division of labour to be advantageous becomes more stringent as the investment in soma increases. Note also that the disadvantage due to the initial investment in sterile tissue fades out as gonidia are allowed to grow, and hence benefit from it, for longer periods of time. We have calculated the condition described by equation (2) by using the ratio of somatic to germ tissue (\( S_0/G_0 = 1.95 \)) observed at the beginning of the experiment (figure 2). This probably represents an overestimate of the condition, as equation (2) assumes a constant \( S/G \) ratio throughout the growing period of the gonidium, whereas figure 1 suggests that this ratio changes from a value of about 2 to a value of less than 1 (at high concentration, \( S/G = 1.95 \) at \( t = 0 \) h; \( S/G = 1.0 \) at \( t = 14.5 \) h, and \( S/G = 0.87 \) at \( t = 21 \) h).

We also calculated the same condition using the value of 1.1 as an approximation of the average of that ratio from 0 h to 21 h.

The interval between 0 h and 21 h covers the period between early development of gonidia, i.e. soon after the two types of cells differentiate and begin to grow, and beginning of cleavage, and thus coincides with the time period the soma normally contributes to the developing gonidium. In this species all growth of gonidia occurs before the beginning of cleavage (Kirk & Harper 1986). However, soma may also contribute during the period of post-embryonic growth, while embryos are retained within the maternal cavity. The rate of increase of gonidia in intact spheroids falls

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**Figure 2.** Gonidia associated with an intact soma (open circles) grew faster than either isolated (filled circles) or gonidia with disrupted soma (filled squares), the difference increasing with nutrient concentration. The specific rate of increase, \( r \), was calculated from equation (1). The two thick lines describe the condition for division of labour to be advantageous, for \( S_0/G_0 = 1.95 \) (top line), and for \( S_0/G_0 = 1.1 \) (bottom line, see text). The broken lines are regression lines (\( y = 0.0068(0.0006)x + 0.0458(0.0010) \), \( r^2 = 0.96 \), \( p < 0.001 \), for gonidia with intact soma; and \( y = 0.0030(0.0012)x + 0.0287(0.0022) \), \( r^2 = 0.31 \), \( p < 0.03 \), for isolated and with disrupted soma pooled; standard errors are in parentheses.)
Table 1. Covariance analysis of rates of increase of germ cells growing with an intact soma, with disrupted soma and isolated (ms, type III mean squares × 1000; n.s., not significant.)

<table>
<thead>
<tr>
<th>source</th>
<th>$t = 14.5$ h</th>
<th></th>
<th></th>
<th>$t = 21$ h</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>ms</td>
<td>p</td>
<td>d.f.</td>
<td>ms</td>
<td>p</td>
</tr>
<tr>
<td>all states</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>state</td>
<td>2</td>
<td>0.311</td>
<td>0.004</td>
<td>2</td>
<td>0.278</td>
<td>0.001</td>
</tr>
<tr>
<td>concentration</td>
<td>1</td>
<td>0.845</td>
<td>0.002</td>
<td>1</td>
<td>0.533</td>
<td>0.001</td>
</tr>
<tr>
<td>state × concentration</td>
<td>2</td>
<td>0.111</td>
<td>0.04</td>
<td>2</td>
<td>0.071</td>
<td>0.01</td>
</tr>
<tr>
<td>error</td>
<td>15</td>
<td>0.037</td>
<td>—</td>
<td>18</td>
<td>0.014</td>
<td>—</td>
</tr>
<tr>
<td>disrupted soma and isolated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>only state</td>
<td>1</td>
<td>0.026</td>
<td>n.s.</td>
<td>1</td>
<td>0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td>concentration</td>
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<td>0.257</td>
<td>0.04</td>
<td>1</td>
<td>0.174</td>
<td>0.01</td>
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<tr>
<td>state × concentration</td>
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<td>0.036</td>
<td>n.s.</td>
<td>1</td>
<td>0.045</td>
<td>n.s.</td>
</tr>
<tr>
<td>error</td>
<td>9</td>
<td>0.043</td>
<td>—</td>
<td>12</td>
<td>0.020</td>
<td>—</td>
</tr>
</tbody>
</table>

Figure 3. Mean volume at cleavage plotted against time when 50% of cells were cleaving for gonidia grown with intact soma (open circles) and isolated (filled circles). The numbers shown are logs of nutrient concentration (SVM). Lines are regression lines: intact soma, $y = -0.10x + 6.69$, $r^2 = 0.96$; isolated, $y = -1.11x + 6.20$, $r^2 = 0.60$.

below the lines describing the condition of equation (2), but approaches it at high concentrations, suggesting that the initial investment in soma is nearly compensated for at high nutrients, but at low nutrients there is a high cost (figure 2). That the observed rates of increase of gonidia with intact soma should lie below the expected line is hardly surprising, however, because these have the additional energetic cost of swimming, not entailed by isolated gonidia, because, in Volvox, differentiated gonidia do not bear flagella. The cost of swimming can be measured by comparing the growth of the wild type to that of flagella-less mutants (Huskey et al. 1979), which do not spend energy in swimming even though they do invest in soma.

(ii) The cost of soma. The cost of soma may be elucidated by comparing the growth of soma and germ in intact spheroids. Figure 1 shows that, although soma grew much slower than germ at high nutrients, it grew almost as fast at low nutrients, attaining 74% of its maximal volume, whereas germ attained only 36% of the maximal volume. This suggests that soma requires a steady input of energy, irrespective of nutrient availability, whereas germ depends on surplus energy.

Among higher organisms, reproductive effort (the ratio of reproductive to somatic tissue) also increases with food availability (for example, plants, reviewed by Harper (1977); fish, reviewed by Wootton (1979)). Because soma functions to increase the reproductive success of germ, giving higher priority to the maintenance of soma when conditions are rough may be of adaptive value, as the germ can be protected by the soma, or be carried to a more favourable environment. The effect of nutrient limitation on the allocation between the two tissues will also depend on the ontogenetic stage at which nutrient limitation is most severe. In this experiment, treatments were applied after all the cells of the two tissues had been formed, so that scarcity of nutrients could only affect the size and not the number of cells in each tissue.

The high energetic cost of a motile soma could also be responsible for the delayed cleavage of germ cells growing with an intact soma, compared with the much earlier cleavage of similarly sized isolated cells at low nutrients (figure 3). Cell division is known to have very high energetic requirements (Kirk & Harper 1986), and diversion of nutrients to somatic tissue may prevent the accumulation of enough energy to meet the threshold for entering the division cycle. Both size and time at cleavage vary with nutrient concentration, cells at low nutrients dividing later and at smaller size, in agreement with previous reports in Chlamydomonas on the effect of the environment on initiation of cell division (Donnan & John 1983). However, gonidia growing isolated follow a different strategy, dividing both earlier and at smaller size than those with intact soma.

Isolated cells of Pleodorina californica and V. obversis also divided earlier and at a smaller size than cells with an intact soma (Kikuchi 1978; Ransick 1993). Moreover, the earlier the isolation during growth, the smaller was the size at cleavage, implying that there is little growth of cells after isolation. Our study showed that isolated cells of V. carteri increased significantly in volume before initiating cell division (at high nutrients, isolated gonidia reached twice and four times their initial size at 14.5 h and 21 h, respectively, compared with four and nine times in gonidia with intact soma; no gonidia were cleaving at 14.5 h, and only those at

the highest nutrient concentrations were cleaving by 21 h), and that growth and cell division responded to environmental conditions in a predictable manner.

(iii) **The effect of experimental manipulation.** Manipulative experiments are open to the criticism that some factor other than the one directly manipulated was also altered and is responsible for the observed responses. In our experiment, it is possible that the mechanical separation of the germ cells from the surrounding soma caused the differential response to medium concentration. Although gonidia are surrounded by a thick zone of extracellular matrix (Kirk *et al.* 1986), their capacity for uptake may have been reduced by damage to membrane receptors. Because more receptors would be needed to exploit fully the richest medium, an accentuation of the difference between states at high nutrients might be expected as a result. We have rejected this explanation on three grounds. First, both manipulated groups of gonidia grew, developed and responded normally with respect to several characters; rather than behaving in a disorganized manner, their development showed a distinct strategy, different from that of gonidia with an intact soma (figure 3). Secondly, if cells were damaged during the manipulation, some would receive more damage than others, increasing the variance within treatments. If the cells were very sensitive to damage, we would expect their frequency distribution to be bimodal. We did not find any significant heterogeneity among states in the error variance (i.e. among replicates within states) in the growth rate analysis (Bartlett’s or $E_{max}$ test (Sokal & Rohlf 1981) showed that the residual $m$ from analyses of variance after the effect of nutrient concentration had been removed were not heterogeneous among different states; $p > 0.05$). Neither were there significant differences among states in the variance of the distributions of the original volume measurements within each replicate culture (Bartlett’s test for heterogeneity of variances among different states, or covariance analysis of within-replicate standard deviations, using the mean of each distribution as a covariate; $p > 0.05$), or any appreciable bimodality or difference in the shape of these distributions. Finally, the growth of isolated gonidia can be compared to that of the ‘dissolver’ mutants of *V. carteri* (Huskey *et al.* 1979), which are defective in their extracellular matrix which normally holds the spheroid together, and fall apart shortly after cleavage, their gonidia growing and developing as isolated single cells similar to those in our experiment. Results from a preliminary experiment showed that isolated gonidia grew as well as or better than gonidia of dissolver mutants (data not shown). We conclude that damage caused by manipulation did not contribute appreciably to our results.

3. **EXPERIMENTAL EUTROPHICATION OF FARM PONDS**

We have found several references in the literature to the increase of colonial forms in more eutrophic conditions (Reynolds 1983, 1984; Happen-Wood 1988), but quantitative data are scarce. The predictions from the source-and-sink hypothesis are best tested by using data from studies where series of small farm ponds have been treated with mineral or organic fertilizers (Janusczko 1970, 1971; O’Brien 1970; DeNoyelles 1971). We find that the biomass of unicellular Chlamydomonadaceae scarcely responded to an increase in eutrophication, as measured by the total biomass of phytoplankton, whereas that of colonial Volvocaceae, and particularly *Volvox*, was greatly increased. We used data from Janusczko (1971) for regression analysis of log (mean biomass, g m$^{-3}$) of a group of species against log (mean total biomass, g m$^{-3}$) of all phytoplankton species (total of 58 species). The slope (+ standard error) for unicells (species of Chlamydomonadaceae) was $b = 0.056 \pm 0.179$, (d.f. = 10, $p = 0.50$), whereas the slope for colonial Volvocaceae (Pandorina morum, Eudorina elegans, Volvox aureus) was $b = 0.669 \pm 0.226$ (d.f. = 18, $p < 0.01$). Similar data from DeNoyelles (1971) analysed by regression of log (biomass, mg ml$^{-1}$) (calculated from maximal seasonal abundance) of a group of species against log (total chlorophyll a, mg m$^{-3}$) (mean seasonal value) present in the pond (total of 81 species) gave a very similar result. The slope for unicells (species of Chlamydomonas) was $b = 0.38 \pm 0.31$ (d.f. = 29, $p = 0.22$), whereas the slope for colonial

![Figure 4. Slope of the response of log (max seasonal biomass, mg ml$^{-1}$) to increasing eutrophication (log (mean total chlorophyll a, mg m$^{-3}$)) (squares) or log (mean reactive phosphorus, mg l$^{-1}$) (triangles) of four groups of Volvocales classified in progressive steps of increasing division of labour (Kochert 1973). 0, unicells (sp. of Chlamydomonas); 1, flat multicells with no differentiation between cells (Gonium sociale, G. pectorale); 2, spheroids with various degrees of differentiation between cells (Pandorina morum, Eudorina elegans, Pleodorina californica); 3, spheroids with terminally differentiated somatic and germ cells (Volvox aureus, V. globator). Bars represent the standard errors of each slope; numbers give the sample size of each regression; Spearman rank correlations for chlorophyll or reactive phosphorus against category of division of labour, $p < 0.05$; data from DeNoyelles (1971).)
Volvocaceae (Gonium sociale, G. pectorale, Pandorina morum, Eudorina elegans, Pleodorina californica, Volvox aureus, V. globator) was $b = 1.18 \pm 0.23$ (d.f. = 45, $p < 0.01$). Figure 4 shows the response of the biomass of four groups of species in the Volvocales, classified in order of progressive steps towards the soma-germ division of labour, according to Kochert (1973), to increasing levels of eutrophication as measured by either total chlorophyll $a$ or reactive phosphorus. The scope of the response becomes progressively steeper going from unicellular forms to the most complex multicellular forms with complete division of labour.

It is a common observation that larger phytoplankton species are more abundant in eutrophic conditions (Malone 1980; Reynolds 1984; Happey-Wood 1988), and this may partly explain the results obtained in the fertilization ponds, as the colonies with the most pronounced division of labour are also the largest. Both multicellularity and division of labour are correlated with size in the Volvocales (Bell 1985; Koufopanou 1992), however, and it is difficult to separate the two effects in comparative studies. Moreover, even though the total protoplast volume increases from Chlamydomonas to Volvox, there is no simple increasing trend in the component cells of the colonies. The mean cell volumes of species in the four categories of figure 4 are 17.75 pm$^3$ for Chlamydomonas, 510 pm$^3$ for Gonium, 1532 pm$^3$ for Pandorina, Eudorina and Pleodorina, but only 40 pm$^3$ for the somatic cells of Volvox. Although body size correlates with a variety of physiological and ecological variables (Peters 1983), the mechanism of action and the extent to which the observed trends are direct or indirect consequences of body size are not well understood.

4. CONCLUSIONS

The initial separation of soma from germ in the Volvocales may be associated with an advantage due to division of labour between source and sink, as proposed by Bell (1985). This interpretation is supported by our experiment, and is consistent with physiological and ecological correlates. This is probably not the only force favouring the evolution of soma in this group, as there may also be an advantage due to a constraint in flagellation during cell division: cells of Volvox and related genera do not maintain functional flagella during embryonic cleavage, apparently because their basal bodies move to the mitotic poles and away from the flagellar bases (Koufopanou 1992). Sterile tissue may have evolved to provide functional flagella during embryogenesis. The relative importance of these two hypotheses in nature can be tested by using the ‘regenerator’ mutants of Volvox, in which the soma is regenerated into germ early on during development, and individuals spend most of their life without a soma (Kirk & Harper 1986). The source-and-sink hypothesis predicts an advantage of soma only in high-nutrient environments, whereas the flagellation-constraint hypothesis predicts an advantage regardless of nutrients.

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REFERENCES


Huskey, R. J., Griffin, B. E., Cecil, P. O. & Callahan, A. M. 1979 A preliminary genetic investigation of Volvox carteri. Genetics 91, 229–244.


O’Brien, W. J. 1970 The effects of nutrient enrichment on

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