

Life History of Volvocine Algae

By

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Abstract

The evolution of somatic cells is a key aspect in the evolution of single to multicellular life forms. Our model is based on an empirically tested model of the growth of unicellular *Chlamydomonas* algae and a suggestion by Koufopanou (1994) that the basic mechanism underlying *Chlamydomonas* growth and reproduction may better explain the diversity of more complex volvocine algae morphologies. Six strains of *Pleodorina starrii* investigated in terms of the parameters of the model, yielded data for the proportion somatic (p) at various cell number sizes.

Introduction

The advancement of life from its earliest to most recent forms has followed an analogous progression in complexity of body organization. This evolution started with associated organic compounds turning into self-reproducing RNA molecules, into genomes enclosed in membranes, into prokaryotic and eukaryotic cells, and finally into multicellular organisms. In each step of this evolution, previously distinct individuals have incorporated into a higher-level entity with the transition from single to multicellular organisms being a key event in the evolution of life as seen on Earth today.

Multicellularity may be defined as being comprised of more than one cell, but in the context of complex cellular differentiation, the specialization of cells to a certain function, is also important. The fundamental distinction of differentiation is between somatic and germ cells: somatic cells form the body and its tissues, while germ cells bear the reproductive burden of carrying on the germ line. The establishment of the germ-soma (G-S) distinction is an important threshold in the evolution of individuality (Michod, 2006). Somatic cells give rise to the complex body plans characteristic of macroscopic organisms; however, from an evolutionary biology aspect, the evolution of soma is problematic, as somatic cells forfeit reproduction and become an evolutionary dead-end.

Volvocine green algae are a model system for the evolution of multicellularity and cellular differentiation. Volvocine algae express a variety of body types and sizes including single and multicellular (colonial) forms. Volvocine algae are photosynthetic eukaryotes expressing an array of cell numbers in colonial forms, though all consist of a total of a power of 2 cells (4, 8, 16..) up to ~50,000 in the largest forms. The larger spherical colonies are characterized by large germ cells in the interior with somatic cells spaced evenly on the exterior of the colony. Germ cells reproduce through palintomy, in which the germ cell grows to several times its initial size and then undergoes several rounds of division with little to no growth between divisions. Somatic cells are flagellated to provide the colony with motility and aid in nutrient uptake (Solari 2006). The species of colonial volvocine algae I have used in testing the model is *Pleodorina starrii*. *P. starrii* are characteristic of colonial green algae as briefly described above, (see Nozaki et al, 2006 for further description).

In organisms that have transitioned to multicellularity, understanding how development produces multicellular body forms is a major challenge; however, in the case of *Volvox*, simpler relatives in which phyletic transitions are represented (Kirk, 1998), may provide a useful model for exploring these concepts in more complex multicellular algae. *Chlamydomonas reinhardtii* has traditionally been considered the closest unicellular relative of the colonial volvocine algae (e.g. Kirk 1998), and has a well-studied cell cycle (Koufopanou, 1994). Based on a suggestion by Koufopanou (1994) that the basic mechanism underlying *Chlamydomonas* growth and reproduction may explain the diversity of volvocine algae morphologies, Matt Herron, a recent PhD graduate from the Ecology and Evolutionary Biology department of the University of Arizona, and I have developed a mathematical model that will provide a framework for understanding the morphological changes caused by selection in multicellular volvocine algae. My research has entailed testing the assumptions of the mathematical model we have developed using experimental manipulations of colonial volvocine algae. More specifically, my emphasis has focused on the germ-soma differentiation traits of *P. starrii* and what those results illuminate about the model.

The Model

The model includes six phenotypes, which are assumed to be quantitative traits, each of which can be described by a single numerical value): the checkpoint size (c), the time between the checkpoint and the beginning of mitosis (b), the threshold size (m), the proportion of energy allocated to ECM (e), the proportion of somatic cells (p), and the relative growth rate of somatic cells (s).

Trait	Symbol	Description
Checkpoint size	c	size of commitment to divide at least once
Timer	b	time from commitment to the start of mitosis
Threshold size	m	minimum size required for a cell to undergo division
Allocation strategy	e	proportion of energy allocated to ECM rather than cell

		growth
Proportion somatic	p	proportion of cells differentiated as soma
Somatic growth rate	s	growth rate of somatic cells relative to reproductive cells

The first trait I tested was the proportion somatic (p), the proportion of somatic cells to germ cells in a colony.

Methods

Laboratory Techniques

Six strains of *Pleodorina starrii*, NIES 1361-1366, were grown in 6-well tissue culture plates in 5 mL standard *Volvox* medium (SVM; Kirk & Kirk, 1983). Plates were grown at 25°C on a 16 hour light, 8 hour dark cycle and placed 10 cm away from 24" fluorescent lights (17W, 4100K). Plates were re-inoculated every seven days to ensure minimal environmental effects.

Eight hundred μL of colonies growing at low density were pipetted into a nine-well glass spot plate (Corning, Inc., Corning, NY) and stained with 2 μL iodine. After settling for ~1 hour, allowing dead colonies to slide to the bottom of well, all or nearly all colonies could be aspirated into 18 μL , which was transferred to a glass microscope slide. Slides were photographed on an 8 _ 12 or 12 _ 12 grid pattern at 150x magnification at a resolution of 3840 _ 3072 pixels on a Nikon DS-Ri1 digital camera mounted on a Nikon Eclipse Ti inverted microscope. Morphological measurements were made using Nikon NIS-Elements AR Imaging Software version 3.00 (Build 550), (see Herron, 2009 for details on methods).

Analyses

Colony diameter was estimated as the average of the longest axis and the longest perpendicular to the longest axis. Cell size was measured as an area, and cell diameter was the equivalent diameter for the measured area, that being the measured cell area divided by π . Only mature colonies, those in which cell types could be distinguished, but which had not begun to reproduce, were measured for colony diameter.

Proportions of somatic cells were estimated by a combination of methods. For mature (but non-reproductive) colonies, equivalent diameters of cells were sorted into two categories using K-means clustering with the Hartigan-Wong algorithm (Hartigan & Wong, 1979) in R version 2.9.2 (R Development Core Team). In addition, the ratio of diameters of the smallest germ cell to the largest somatic cell was required to be the largest ratio of two consecutively ranked cells (colonies that did not meet this requirement were not counted). For reproductive colonies, somatic cells and embryos were counted manually.

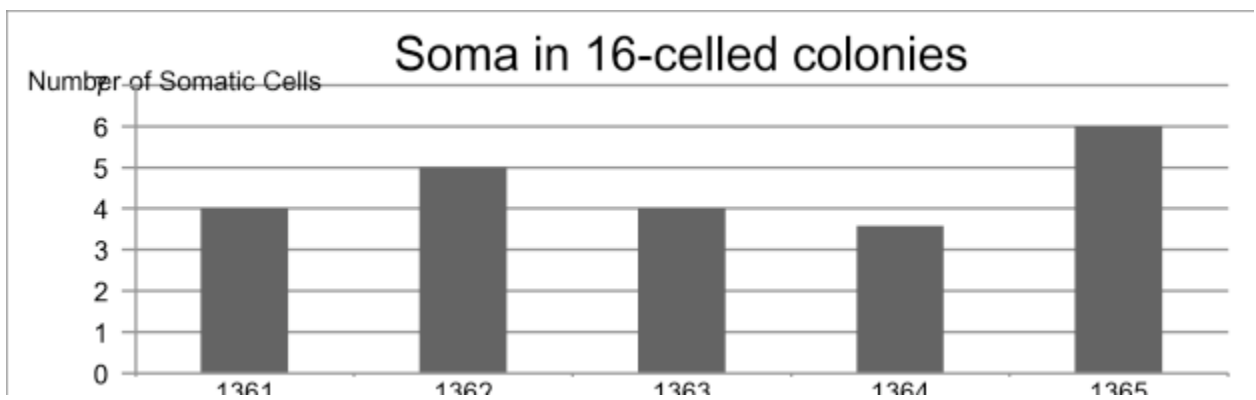
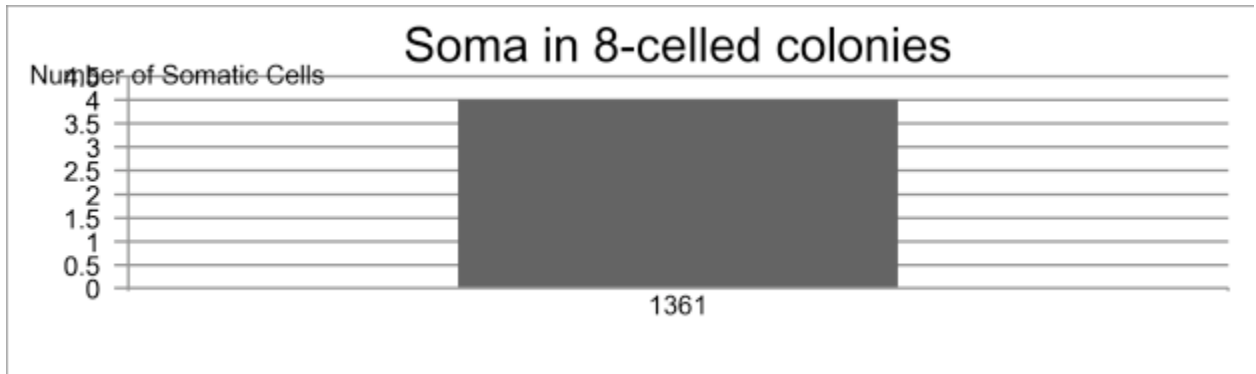
The distribution of cell numbers in each isolate strain was estimated by tabulating 200 colonies (in a few cases, fewer than 200 colonies were photographed; in these cases all photographed colonies were counted). Weighted averages of colony diameter, cell number, and proportion of soma were estimated by summing the product of the mean value for colonies of a given cell number and the proportion of colonies with that cell number (i.e. $\sum \{\text{mean trait value of n-celled colonies} \times \text{proportion of n-celled colonies}\}$). The average value of a trait within an isolate line was considered the genetic value.

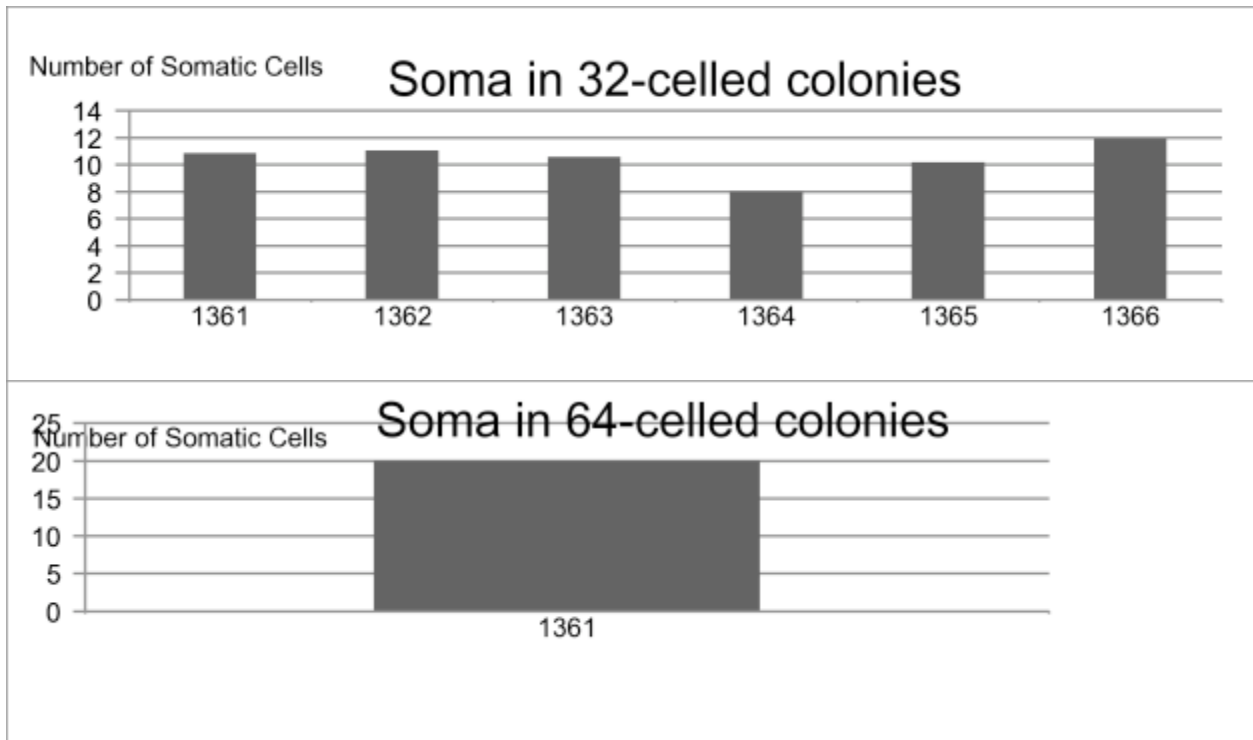
Results

In the six parental strains, when growing at low density in fresh medium, most strains included colonies of two or more different cell numbers (e.g. 32- and 64-celled colonies). Colonies with 4, 8, 16, 32, and 64 colonies were observed across the parental strains, however due to the criteria mentioned in the analyses section, several cell number sizes were too rare to be considered in the results.

The mean number of somatic cells was 4 somatic cells in 8-celled colonies, 4 somatic cells in 16-celled colonies, 11 somatic cells in 32-celled colonies, and 20 somatic cells in 64-celled colonies. A small amount of variation occurred in the number of somatic cells in the different colony sizes. Figure 1 shows the number of somatic cells for a given cell number size across the six parental strains.

Fig. 1 A-D. Number of somatic cells in varying colony sizes across six parental strains. Error Bars represent one standard deviation.





Below is a summary of the mean (\pm standard deviation) number of somatic cells in the cell number sizes observed across the six parental strains (Table 1), along with the resulting proportion somatic (p) for those cell number sizes (Table 2).

Table 1 Mean Number of Soma

Strain	4 Cell Colonies	8 Cell Colonies	16 Cell Colonies	32 Cell Colonies	64 Cell Colonies
NIES 1361		4.0 \pm 0.0	4.0 \pm 0.0	10.9 \pm 1.3	
NIES 1362			5.0 \pm 0.0	11.1 \pm 1.1	20.0 \pm 1.4
NIES 1363			4.0 \pm 0.0	10.6 \pm 1.6	
NIES 1364			3.6 \pm 1.1	8.0 \pm 4.2	
NIES 1365				10.2 \pm 1.7	
NIES 1366			6 \pm 2.8	11.9 \pm 0.4	

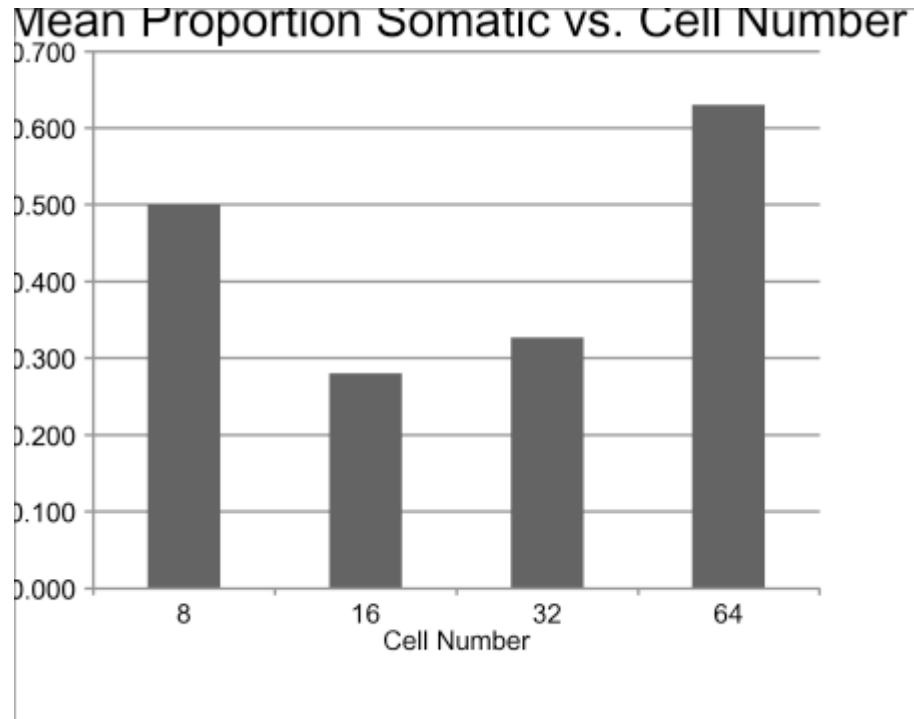
Table 2 Proportion Somatic (p)

Strain	4 Cell Colonies	8 Cell Colonies	16 Cell Colonies	32 Cell Colonies	64 Cell Colonies
NIES 1361		0.5 \pm 0.0	0.25 \pm 0.00	0.34 \pm 0.04	
NIES			0.31 \pm 0.00	0.35 \pm 0.03	0.63 \pm 0.02

1362		
NIES	0.25 ± 0.00	0.33 ± 0.05
1363		
NIES	0.22 ± 0.00	0.25 ± 0.26
1364		
NIES		0.32 ± 0.05
1365		
NIES	0.38 ± 0.18	0.37 ± 0.01
1366		

Across the six strains, mean proportion of soma was highest in the 16- and 64-celled colonies (Fig. 5 below).

Fig. 5 Mean proportion somatic vs. cell number



Distributions of cell numbers differed among the six strains (Table 1). In only NIES 1361, was there a high enough occurrence of 8-celled colonies for it to be counted. The

proportion somatic for those 8-celled colonies was always found to be 0.5 (4 somatic cells). 16-celled colonies were found at large enough occurrences in all six of the parental strains except for NIES 1365. In those 16-celled colonies, the range of proportion somatic was between 0.22 and 0.38 with an average of 0.28. 32-celled colonies are the most commonly found cell number size and were observed across all six parental strains. The average proportion somatic for a 32-celled colony was 0.32 across the six strains with the range of proportion somatic was between 0.25 and 0.37, a range that is thrown off by much larger deviations found in only one of the strains. NIES 1364. NIES 1364 exhibits a much smaller proportion somatic compared to the other five strains (see discussion). Only NIES 1362 exhibited 64-celled colonies at a high enough occurrence to be counted, with a proportion somatic of 0.63.

An interesting parabolic pattern can be observed across the different cell number sizes with the smallest (8-celled) and largest (64-celled) cell numbers expressing a larger proportion somatic compared to the middle cell number sizes (16 and 32-celled).

Discussion

Generally, it may be expected in any multicellular organism that the larger the organism is, the more somatic cells it will have. More somatic tissue is needed to support larger body plans, and this can be observed in the 3 larger cell number sizes (16, 32, and 64-celled) as they have an increasing proportion somatic with increasing numbers of cells. However, the smallest cell number size (8-celled), does not fit this pattern. Rather, as mentioned above, a curvilinear relationship was found between the different cell number sizes across the six parental strains, where the mean proportion somatic of the smallest and largest cell number sizes was larger in comparison to the medium cell number sizes. This relationship echoes previous findings by Herron in his selection experiments (Herron, 2009), where the proportions of soma were highest in colonies with the largest and smallest cell numbers. As for a possible explanation as to why smaller colonies have such a large proportion of somatic cells, especially when considering they are sacrificing half of their reproductive potential, one explanation may be the alignment of cells within the colonies. Herron (2009) suggests that this curvilinear relationship is the result of cells in *P. starrii* colonies arranging in tiers and as a result of responding to a chemical gradient, entire tiers of cells are differentiated as soma or germ cells. I have observed many *P. starrii* colonies of all cell number sizes exhibiting this tiered colonial development and would support Herron's suggestion regarding an explanation to this curvilinear relationship; that being that small colonies do not exhibit optimal proportion somatic ratios because of the tiered organizational constraint.



As mentioned above, the strain NIES 1364 produced a proportion somatic unlike the other five parental strains. As can be seen in the Figure 6, in NIES 1364 colonies, the difference in size between germ and soma was much less pronounced in comparison to the other five strains with an example of a NIES 1362 colony in Figure 7. Not only did this make measuring the differentiation harder in N1364 colonies, but it also contributed to a great deal of the variation in the proportion somatic seen in Figures 2 and 3 and Tables 1 and 2 in N1364 colonies. This disparity in N1364 colonies was so pronounced that several questions were raised pertaining to if N1364 was even exhibiting differentiation in germ and soma, whether N1364 employed the same developmental program as the other five parental strains, and furthermore, if N1364 was even related to the other 5 strains that it had been grouped with since its discovery or if it was even *P. starrii* at all.

In order to determine what was different about N1364, I sequenced several DNA fragments from N1364 samples and compared them to known gene sequences on GenBank. The DNA was sequenced at the Laboratory of Molecular Systematics and Evolution at the University of Arizona. The resulting contigs were assembled, with help from Dr. Adam Bjork from the Ecology and Evolutionary Biology Department at the University of Arizona. Four DNA contigs were found and when compared to known gene sequences on GenBank, all four ranged from 98-100% similarity to known sequences of *P. starrii*. From this it is concluded that the N1364 parental strain is in fact *P. starrii*, though its Germ-Soma differentiation is different or at least considerably harder to detect than the other five parental strains.

The results of my research have yielded the proportion somatic (p) for various cell number sizes of the six parental strains NIES 1361- NIES 1366 of *Pleodorina starrii*. This is one of the parameters of the model discussed above with more research needed to finish investigating the validity of the model and its further implications in explaining the diversity of volvocine algae morphologies.

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