

Cell-type allocation and variability in diverse *Volvox* species

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Motivating questions and goals

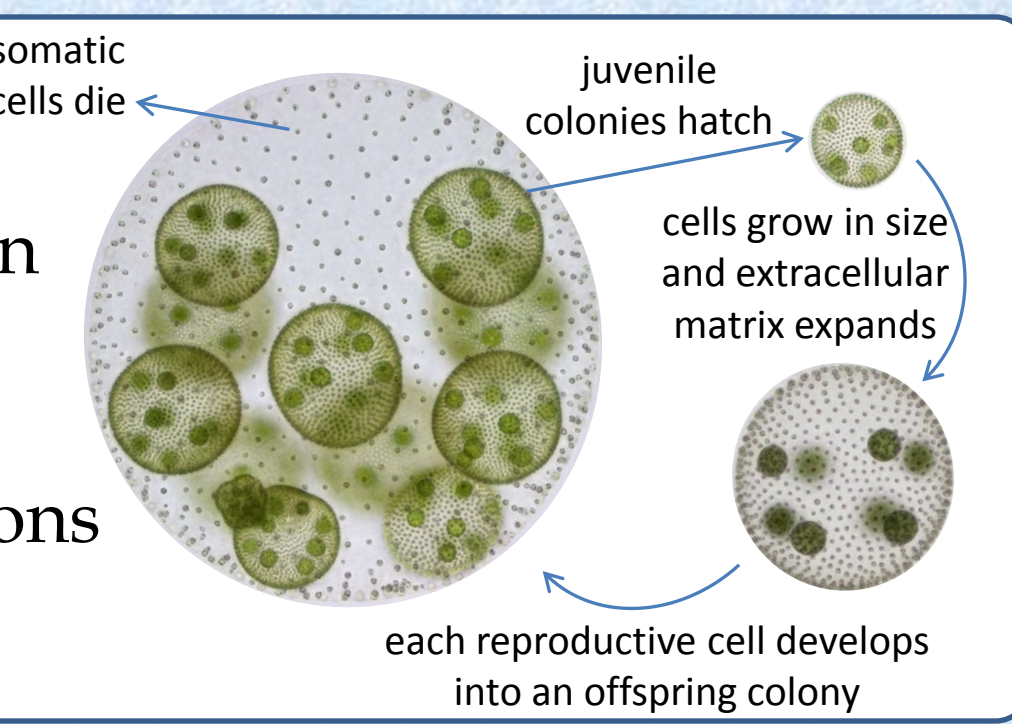
With the appearance of cellular specialization, cell-type allocation strategy and variability of cell-type number immediately arise as new fitness-affecting traits. How do developmental mechanisms affecting these traits first evolve? How do volvocine algae resolve the life history strategy dilemmas posed by these traits?

In this study our aims were to:

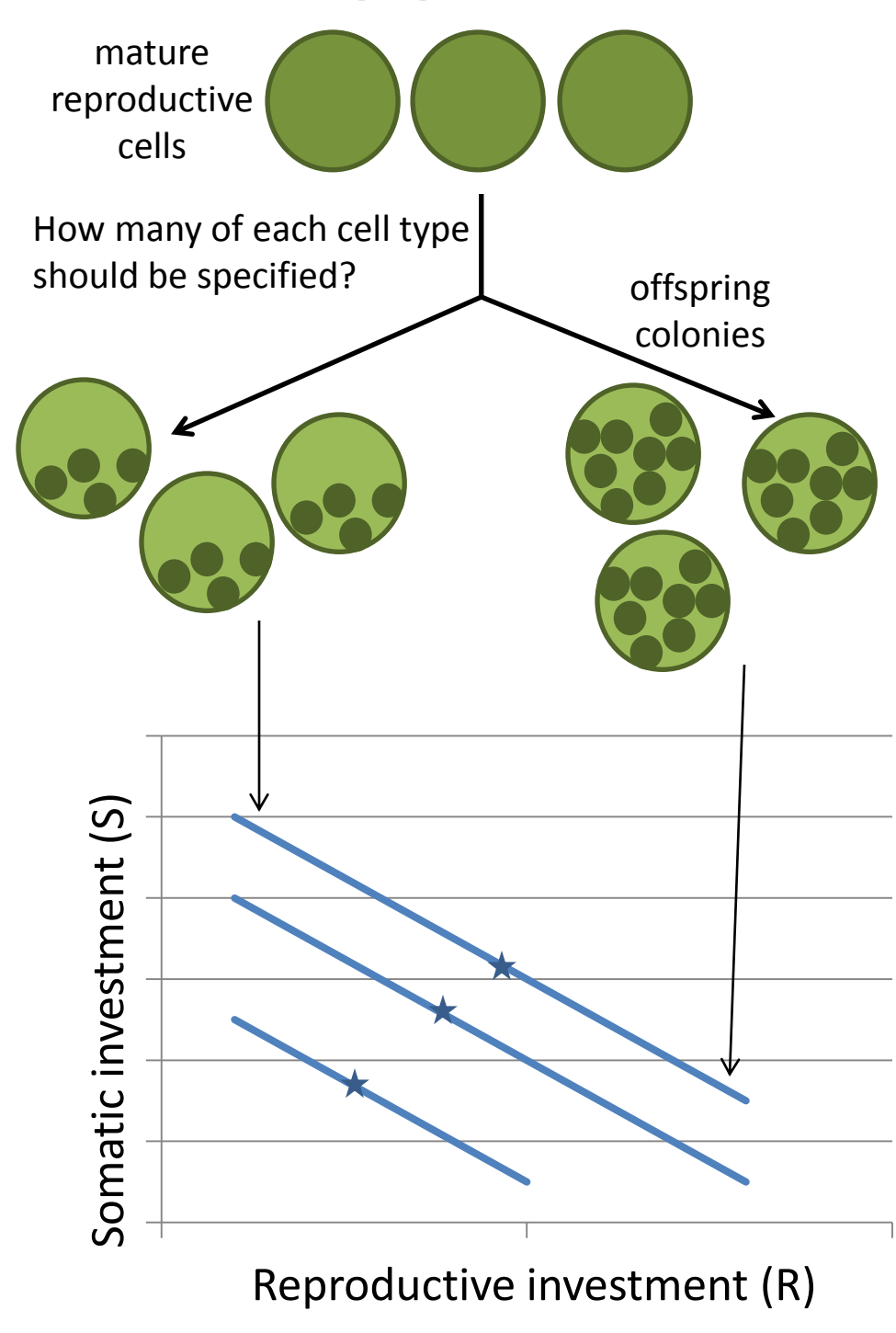
1. Confirm between-species somatic/reproductive scaling relationship (allocation strategy)
2. Look for relationship between developmental traits and within-species somatic/reproductive scaling relationship (allocation strategy)
3. Look for relationship between developmental traits and variability of cell specification

Asexual cycle

- Each reproductive cell (usually) produces an offspring colony.
- Offspring colonies hatch with a full suite of reproductive and somatic cells; no cell divisions occur outside of embryogenesis.

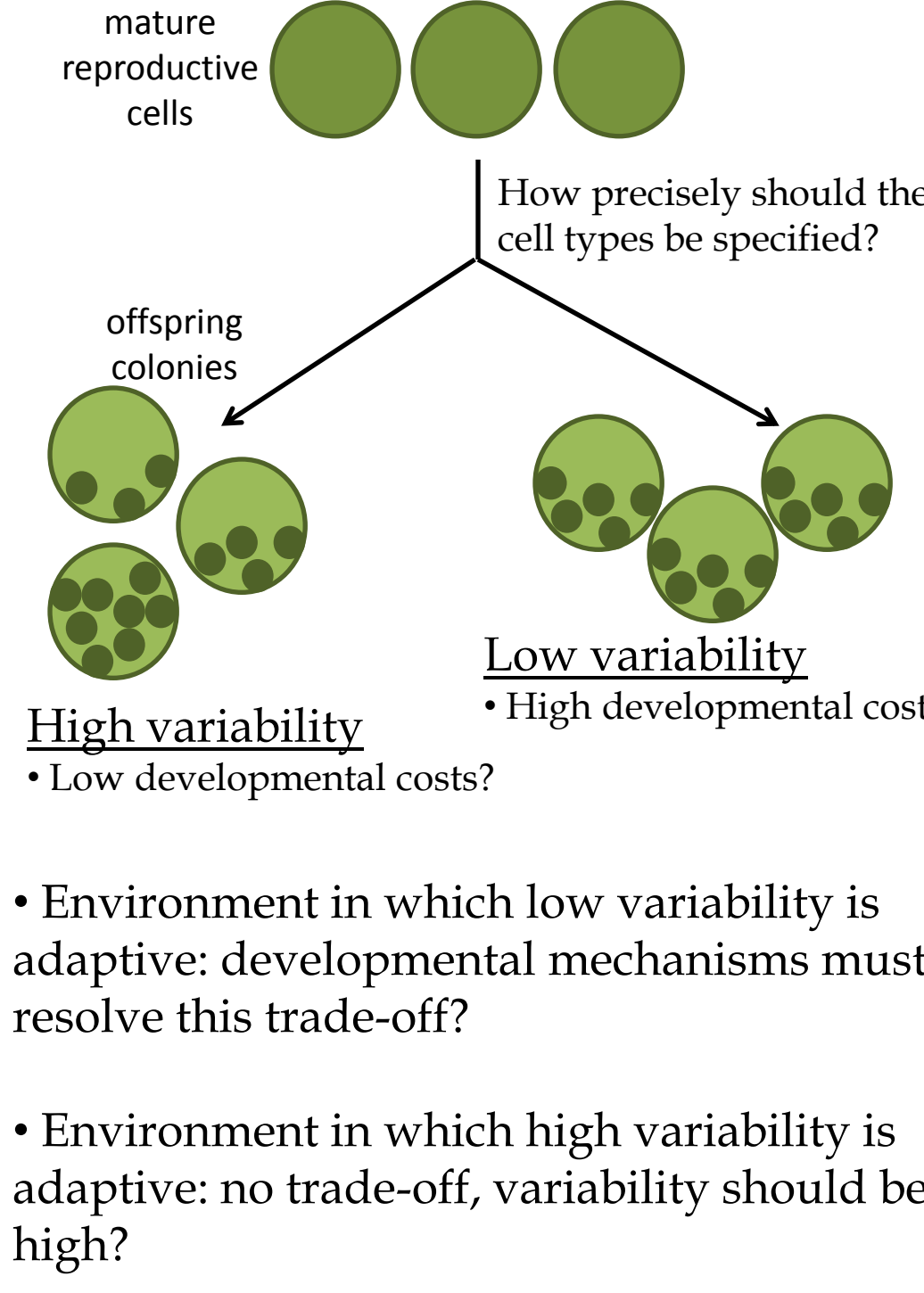


Cell-type allocation



- Developmental mechanisms determine investment in S and R and evolve in response to selective pressures for an adaptive allocation strategy.
- *Volvox* is an ideal system for addressing the evolution of the developmental mechanisms underpinning allocation strategies because they are relatively simple yet developmentally diverse.
- Although total investment in R and S trade-off for fixed levels of total resources ($R+S=T$), differences in acquisition (blue lines) can lead to positive correlations between observed R and S (blue stars) (1).

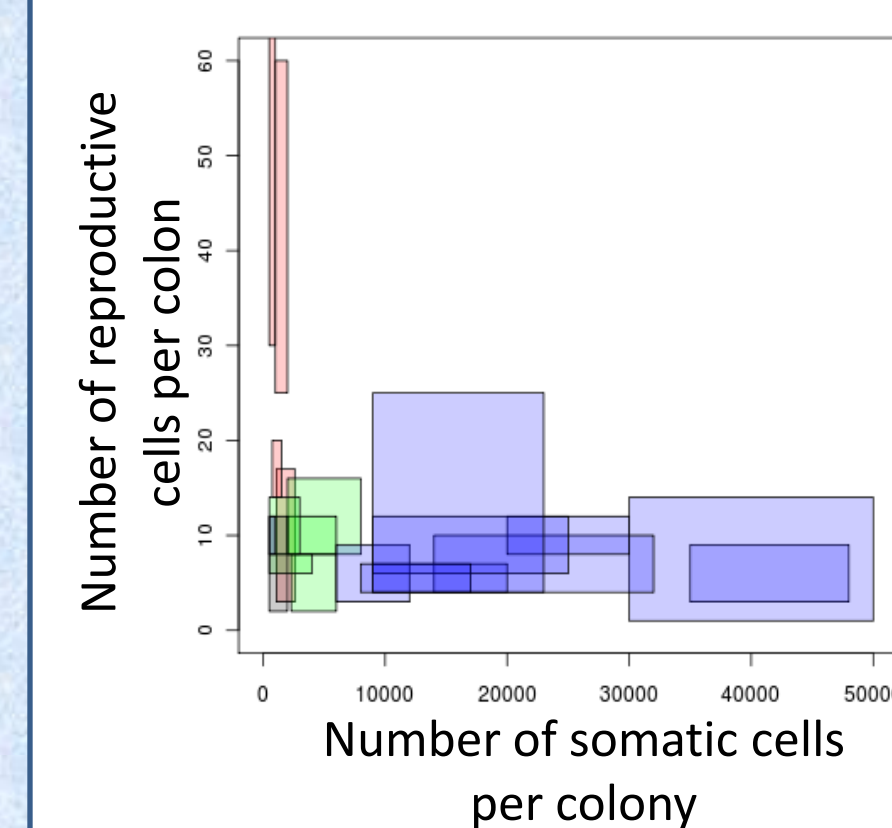
Variability of allocation



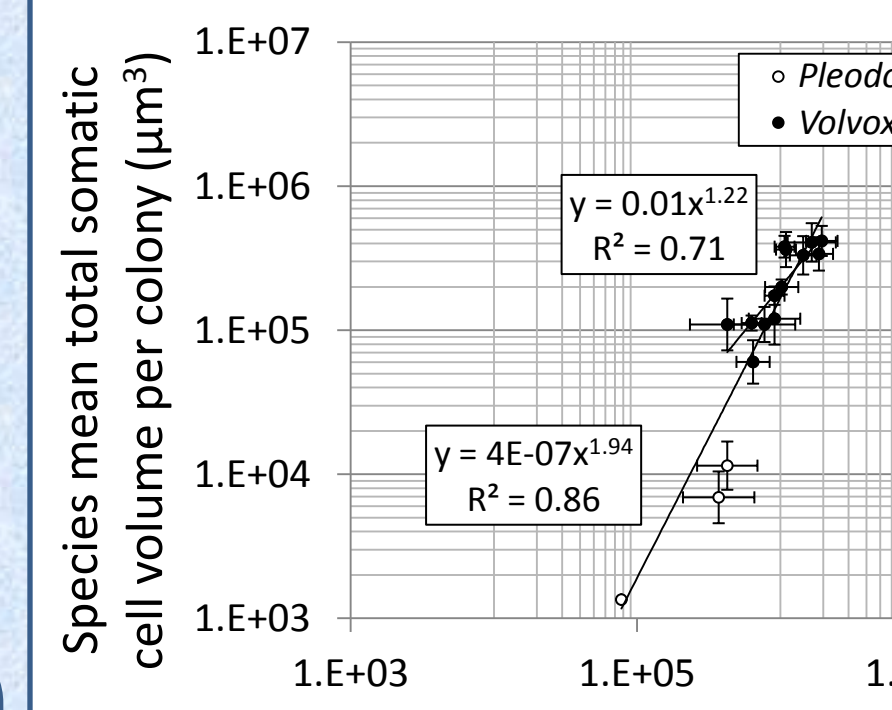
- Environment in which low variability is adaptive: developmental mechanisms must resolve this trade-off?
- Environment in which high variability is adaptive: no trade-off, variability should be high?

- Under stabilizing selection in a constant environment, less variability is generally also favored by selection.
- However, when the environment varies in an unpredictable way, high variability (bet hedging) can be a successful strategy (2).
- The developmental mechanisms that affect allocation variability are often obscured by the overall complexity of development and have not been addressed in relatively simple multicellular organisms.

Selected previous work



From Koufopanou (1994) (8):

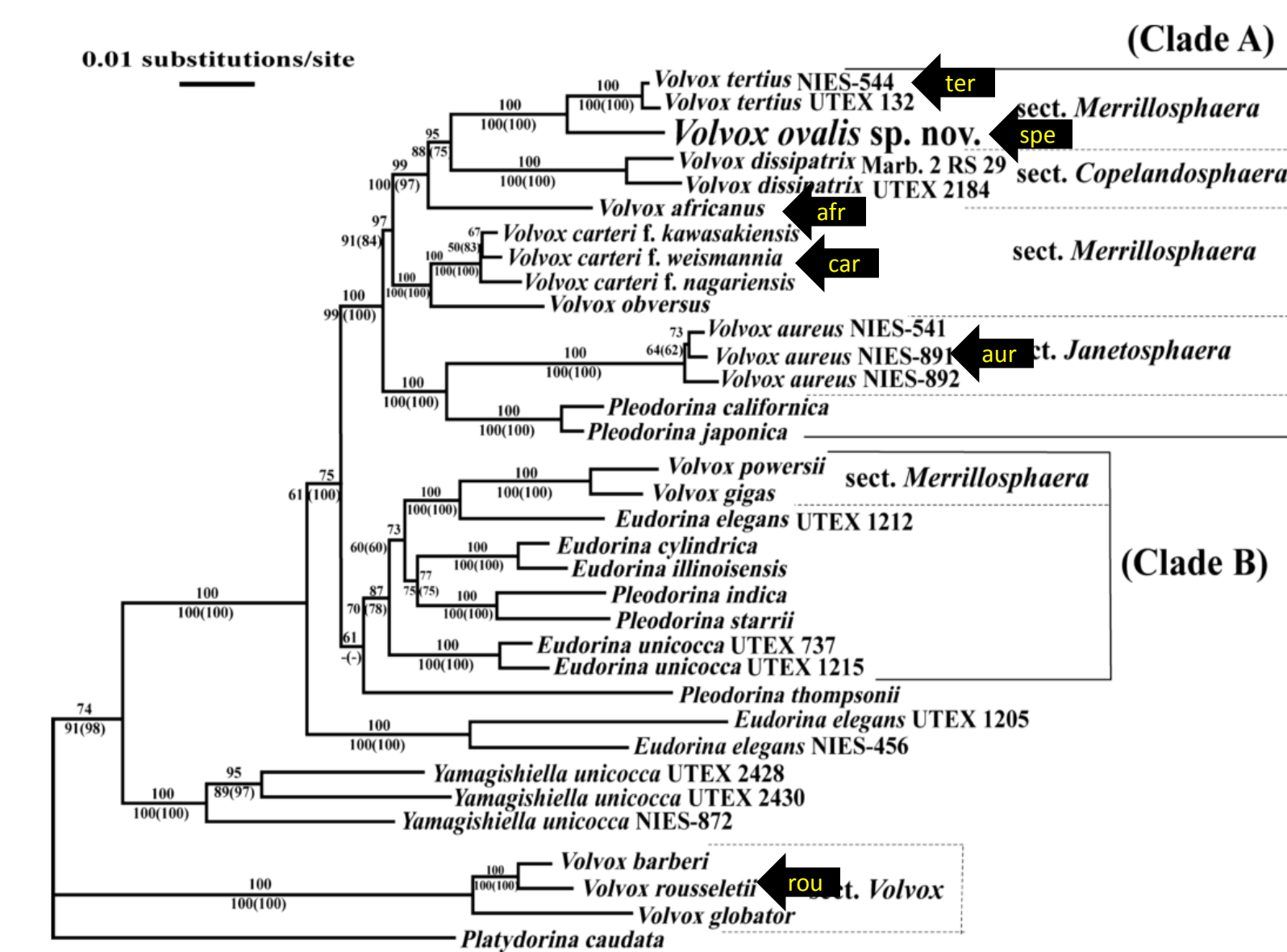


- At left, each box shows the reported range of *Volvox* asexual cell numbers for a species. Color corresponds to developmental program (3-10) (red=program 1, green=program 2, gray=program 3, blue=program 4).
- Koufopanou (8) observed that total somatic volume per colony increases about twice as fast as total reproductive volume per colony for comparisons among *Volvox* and *Pleodorina* species.
- Considering only Koufopanou's (8) *Volvox* data gives a substantially different picture (scaling exponent of 1.22 rather than 1.94; graph at left).
- Additionally, there may be a relationship between the residuals and the developmental traits of species (bottom graph), so a model that pools only developmentally similar species may describe the empirical relationship better.
- Modeling and empirical work by

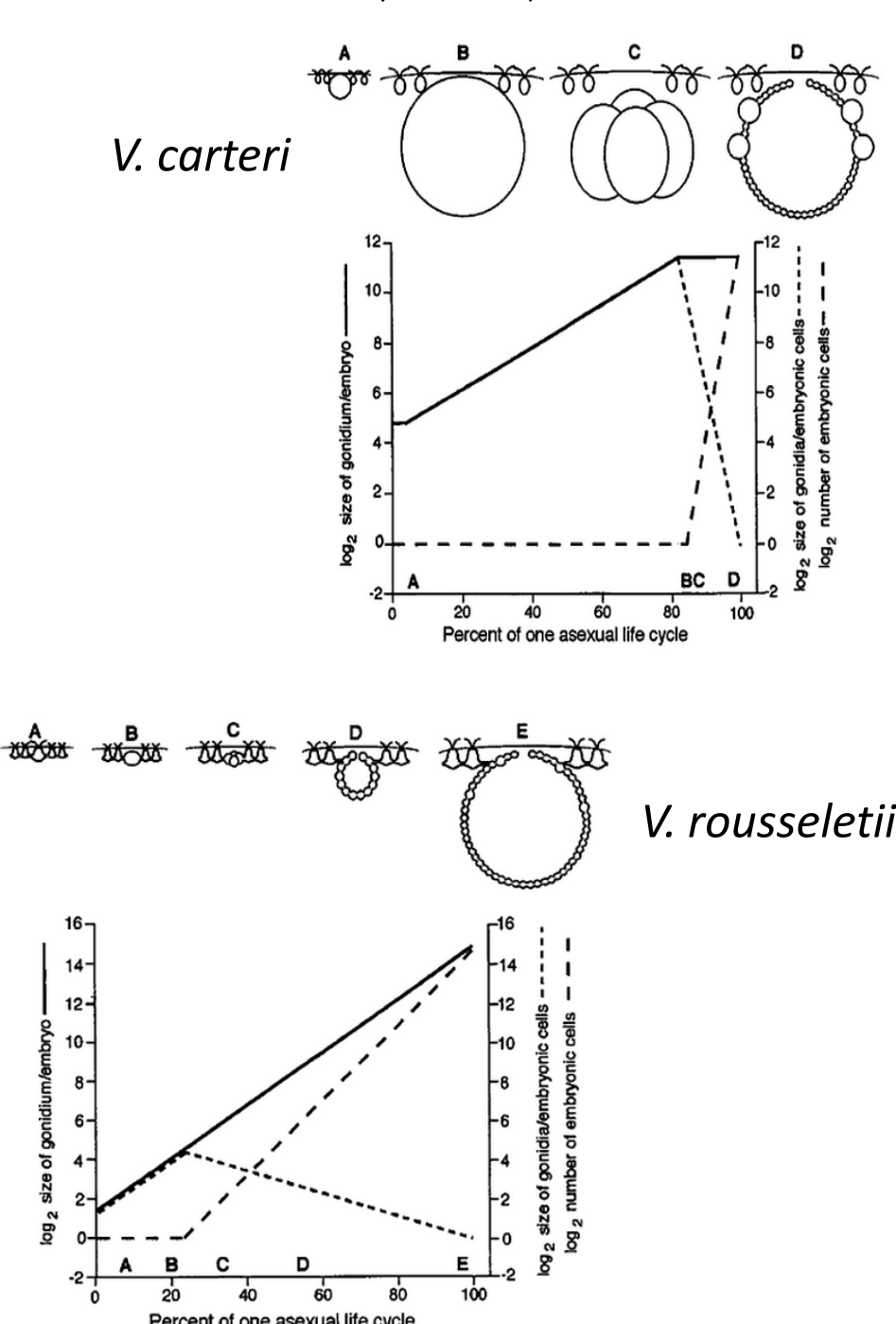
- Solari and colleagues (11) examined developmental and hydrodynamic constraints of increasing size.
- They supported a >1 scaling exponent for proportion of somatic cells on total cell number.
- However, the slope of the scaling exponent that Koufopanou (8) observed (somatic volume on reproductive volume) has not been derived from first principles or followed up on with further empirical study.

Species in this study

Nozaki and Coleman (2011) (12) phylogenetic tree based on five chloroplast genes, showing the approximate position of the species studied here.



Figures of two representative *Volvox* species from Kirk (1998) (13):

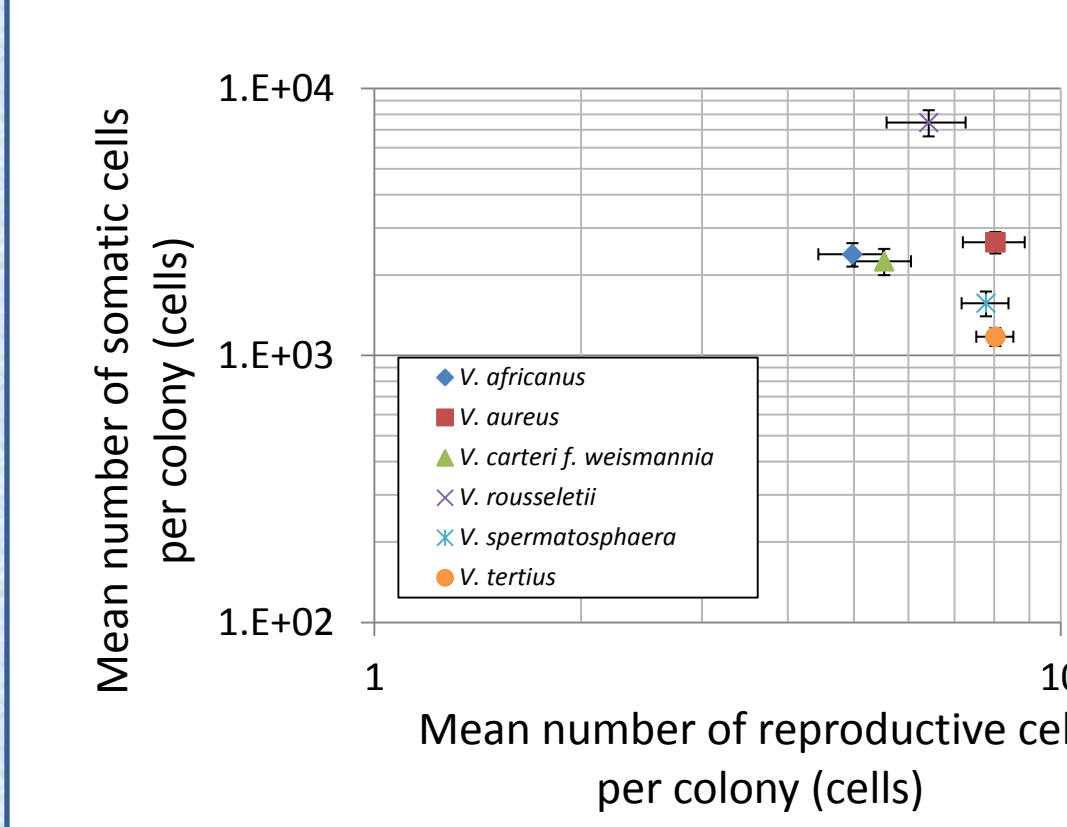


Methods

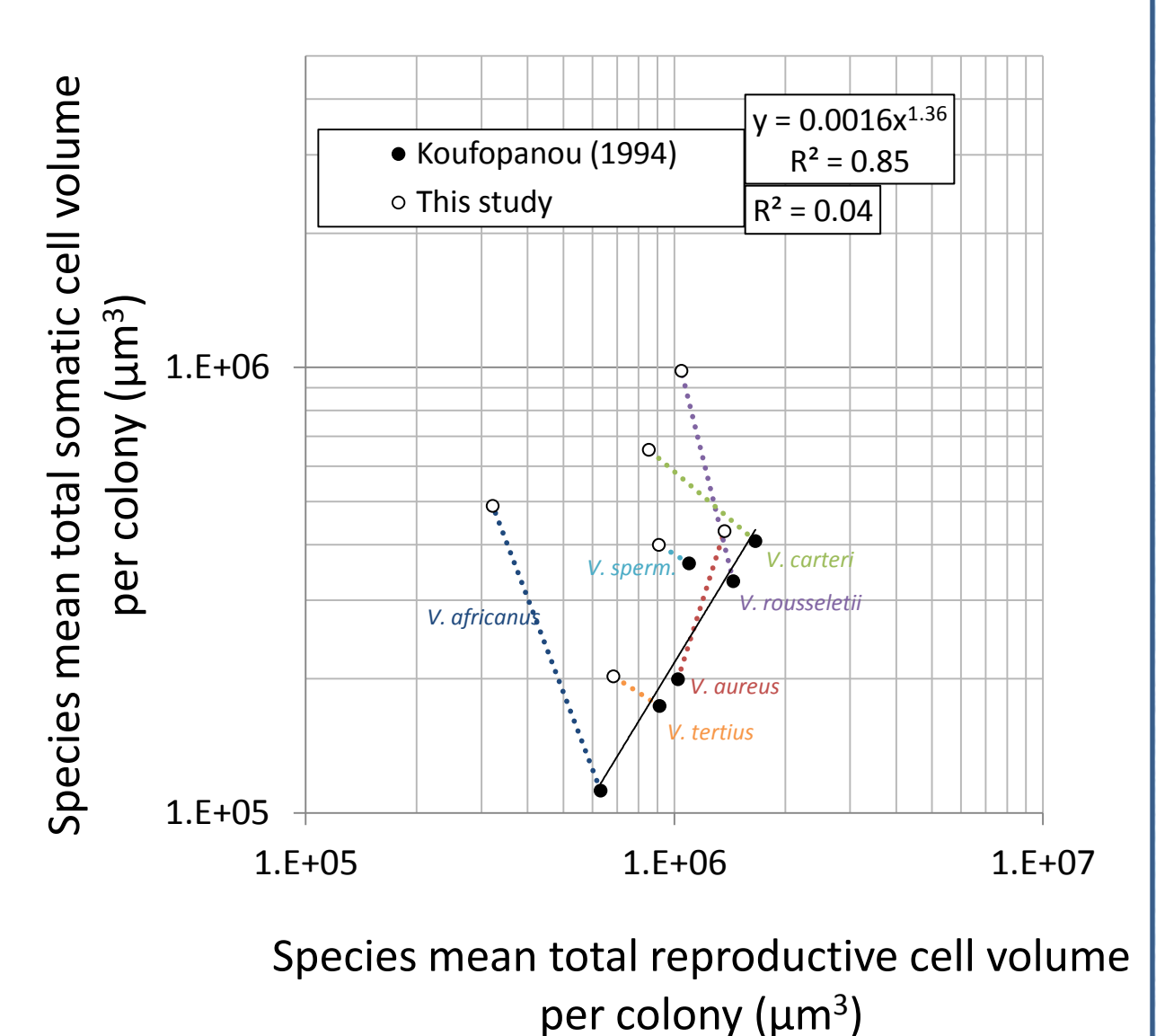
- Six species with diverse developmental features (3-4) were studied.
- From growing, asexual populations, 40 colonies of each species were sampled. Reproductive cells were counted and somatic cell number was estimated by counting the somatic cells visible in the great circle.
- Total volume of reproductive and somatic cells was estimated using the species-specific geometric means for the volume of each cell-type (standardized to a particular time in development) reported in Koufopanou (8).
- For an additional 200 colonies per species, reproductive cell number was counted.

Species (program)	Intercellular bridges in adult colony?	Slow, light-dependent divisions?	An asymmetric division?	Small gonidia, growth between divisions?
<i>V. tertius</i> (3)	No	Yes	No	No
<i>V. spermatozoa</i> (1)	No	No	No	No
<i>V. africanus</i> (2)	No	No	Yes	No
<i>V. carteri f. weismannia</i> (2)	No	No	Yes	No
<i>V. aureus</i> (4)	Yes (thin)	Yes	No	Yes
<i>V. rousseletii</i> (4)	Yes (thick)	Yes	No	Yes

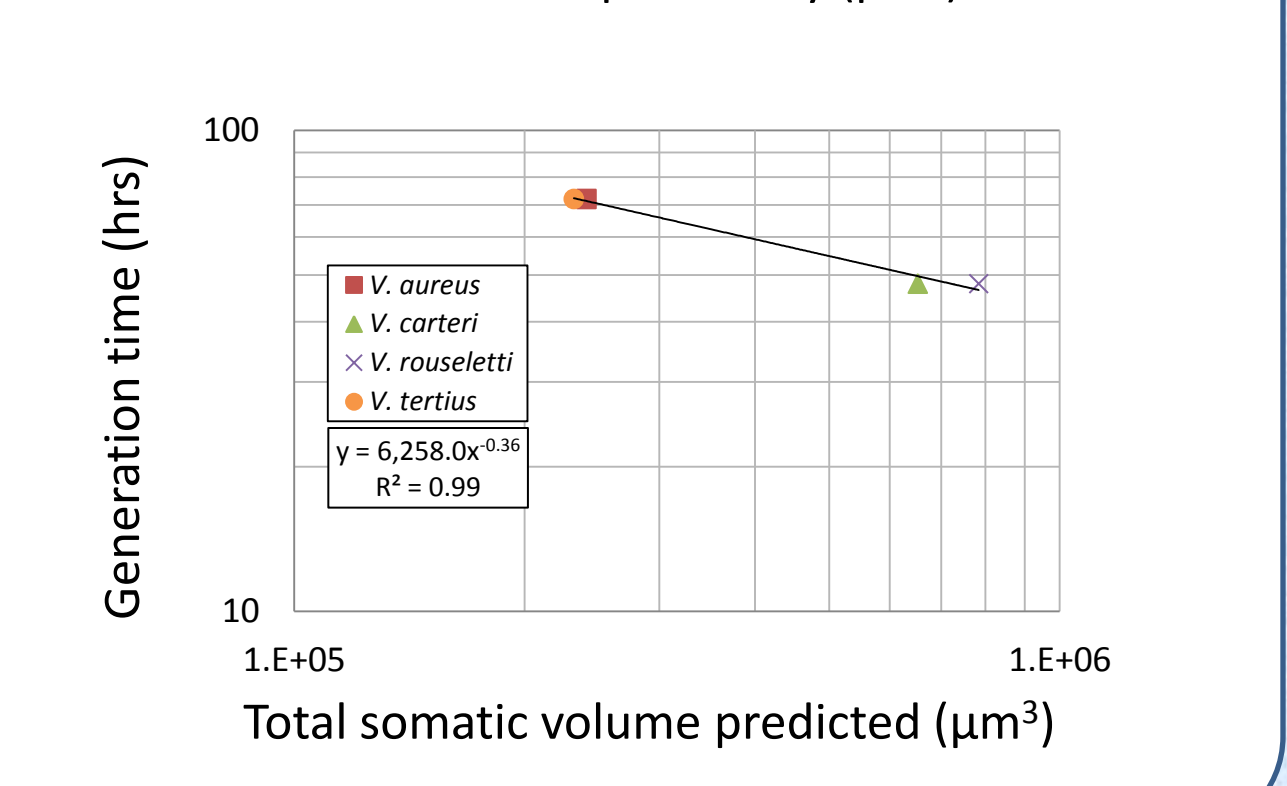
1. Between-species allocation patterns



- Between-species comparisons in cell number (shown at left) do not take into account species differences in cell sizes, and thus do not bear directly on issues of allocation of total resources. We show the patterns in our cell number data here (left graph) for descriptive purposes only.
- *V. rousseletii* had a particularly high number of somatic cells, and *V. carteri* and *V. africanus* (program 2 species) had the lowest number of reproductive cells.

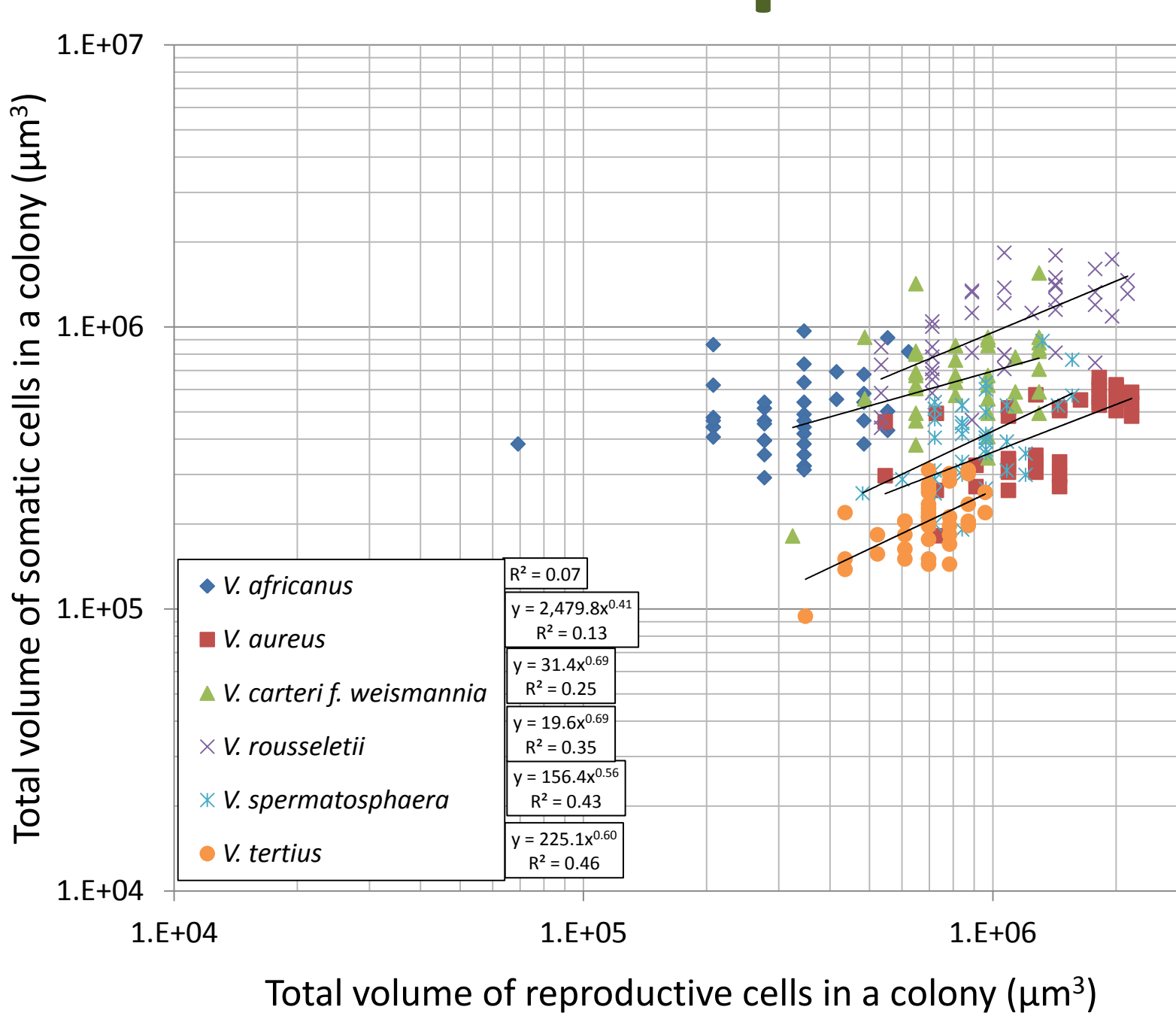


- Our data shows no correlation between species mean reproductive and somatic total volume (upper right graph).
- Our data generally show less reproductive and more somatic volume compared to Koufopanou (8), likely due to different culture conditions.
- *V. tertius* stands out with low mean somatic volume and *V. africanus* stands out with low mean reproductive volume.
- The somatic volume predicted (by the within-species analyses for $8 \times 10^5 \mu\text{m}^3$ reproductive volume) correlates with the generation times reported by Solari et al. (11). This preliminary result suggests that a benefit of investing in soma could be a shorter generation time.



2. Within-species allocation patterns

- We did ordinary least squares regressions of total estimated somatic volume on total estimated reproductive volume (with both estimates \log_{10} -transformed, shown at right as power law regressions).
- Akaike information criterion (AIC) model selection (14) indicates that species differ with respect to the intercept (proportionality constants) and that species have similar, or possibly the same, slopes (scaling exponents).
- The slope was 0.51 in the model with a common slope, indicating that somatic volume increases about half as fast as reproductive volume for species studied here. Thus, for the underlying S-R relationship, proportion somatic volume declines with increasing total volume. This contrasts with the previously-reported between-species pattern.
- We calculated the amount of somatic volume (height of the line) at a reproductive volume within the range of all species ($6 \times 10^5 \mu\text{m}^3$). In ascending order of overall somatic investment at this level of reproductive volume, the species are: *V. tertius* ($190,000 \mu\text{m}^3$), *V. aureus* ($206,000 \mu\text{m}^3$), *V. spermatozoa* ($269,000 \mu\text{m}^3$), *V. africanus* ($552,000 \mu\text{m}^3$), *V. carteri* ($580,000 \mu\text{m}^3$), *V. rousseletii* ($659,000 \mu\text{m}^3$).
- The program 4 species (*V. rousseletii* and *V. aureus*) had very different levels of somatic investment whereas the program 2 species (*V. africanus* and *V. carteri*) had similar levels of somatic investment.



3. Variability of reproductive cell number

- Species differed with respect to gonidial cell specification variability. However, there was no clear pattern of the pattern of the coefficient of variation with developmental traits.
- There may be a positive relationship between somatic investment and reproductive cell number CV, though more data would be needed to test this (graph above; jackknifing was used to estimate the standard error of the CV).
- Overall, *Volvox* appears to have higher CV for fecundity than other, more complex organisms (0.15-0.25 for comparable data) (15-17). It is an open question whether high *Volvox* variability is an adaptive strategy or a reflection of rather simple developmental control of cell-type allocation.

Conclusions

1. We did not find support for the previously-reported between-species somatic/reproductive cell volume scaling relationship. Our number of species was too small for robust between-species analyses, but do indicate that further investigation is warranted.
2. We found a positive correlation between somatic and reproductive cell number within species. The scaling exponent of this relationship was similar for all species, and indicates that investment in somatic volume increases more slowly than investment in reproductive volume. Species differed with respect to investment in somatic volume (for a given reproductive volume) but no pattern with respect to developmental traits emerged. This result could be sensitive to quality of the standardized species-specific cell volumes that we used from the literature.
3. Species differences in reproductive cell variability (for clones raised in one environment) did not correspond to known developmental differences. The overall level of variability was generally high compared to more complex organisms.

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Acknowledgements

We thank B. Enquist, B. Walsh, J. Bear, and D. Billheimer for helpful discussions and statistical advice. We are also grateful for many helpful discussions with J. Monti-Masel, A. Badyaev, D. Elliott, A. Nedelcu, M. Herron, C. Solari, V. Galzani, Q. Li, P. Ferris, E. Hanschen, and M. Leslie.