

Volvox *barberi* likely use weak forces to aggregate into optimally packed two-dimensional flocks

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Abstract:

Volvox barberi are multicellular protists forming colonies of 10000-50000 cells. I established that typical colonies have diameters varying ten-fold from ~50 to ~500 microns, drawn from a log-normal distribution. I measured swimming speeds up to 600 microns/second, making this one of the fastest swimming *Volvox* species. I showed that *barberi* aggregates actively into “flocks” of 2 to more than 100 colonies, which move and rotate collectively at high speeds. The *Volvox* centers in flocks form a packed, irregular lattice. I hypothesized that the *Volvox* were dynamically finding the optimal packing for their size distribution. To test this, I built molecular dynamics simulations of spherical particles with a log-normal diameter distribution (matching the *Volvox*), and a weak long-range attractive force with strong local repulsion (to model mutual exclusion of colonies). Such “soft-spheres” are known to form random close-packed configurations that pack nearly optimally. I found that the lattice angle distribution in these close-packed configurations was identical to that of *Volvox* flocks. This suggests that the *Volvox* achieve random optimal packing by exerting weak, long-range attractive forces on one another. Using a dye tracer, I show that the *Volvox* create water currents as the colonies rotate by beating their flagella, and that these currents can both attract and repulse the fluid over distances ranging over many *Volvox* diameters. This provides a likely source for the forces leading to flocking.

The adaptive role of *RLS1* in *Chlamydomonas*: a direct test using genomic and RNAi *RLS1* mutants

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RLS1 is part of a volvocine-specific gene family that includes *regA* – the gene responsible for somatic cell differentiation in *Volvox carteri*. In *Chlamydomonas reinhardtii*, *RLS1* is induced in response to various environmental changes (including dark and nutrient deprivation) when the temporary down-regulation of reproduction is beneficial in terms of survival. Based on its expression pattern, we have previously suggested that *RLS1* acts as an environmentally-induced life-history trade-off gene that promotes survival at a cost to immediate reproduction. However, a direct test of this hypothesis has not been performed. Here, we used genomic and RNAi *RLS1* mutants to directly investigate the effect of *RLS1* loss on several fitness parameters, including reproduction, survival and resistance to stress. Mutant and wild-type strains were grown in various growth conditions (autotrophic and mixotrophic media; continuous light, continuous dark, and 12 h light:12 h dark cycle) and under various stresses (light stress; nutrient deprivation; antibiotics) and their growth rates and survival potentials were compared. This study provides direct evidence for the adaptive role of *RLS1* in *C. reinhardtii* and supports the suggestion that the evolution of somatic cell differentiation in *V. carteri* involved the co-option of an *RLS1*-like gene whose expression in the unicellular ancestor was conditioned on an environmental cue through shifting its expression from a temporal into a spatial (developmental) context.

Genetic Basis Underlying *De Novo* Origins of Multicellularity in Response to Predation

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Abstract:

The evolution of multicellularity is a Major Transition that sets the stage for subsequent increases in biological complexity. However, the genetic mechanisms underlying this major transition remain poorly understood. The volvocine algae serve as a key model system to study such evolutionary transitions, and comparative approaches using the three sequenced genomes of unicellular *Chlamydomonas reinhardtii*, colonial *Gonium pectorale* and fully differentiated *Volvox carteri* have revealed important genetic pathways for the evolution of multicellularity. Nevertheless, this retrospective approach limits insights into the tempo and mode of genetic changes and the possible paths a unicellular organism can explore for the transition to multicellularity. Experimental evolution in the laboratory allows direct observations of such transitions in real time.

We used the unicellular *C. reinhardtii* to generate *de novo* origins of multicellularity under predation. Outcrossed populations of *C. reinhardtii* were subjected to selection by the filter-feeding predator *Paramecium tetraurelia*. After 50 weekly transfers, two of five experimental populations evolved multicellular structures not observed in any of the three unselected control populations. To uncover genetics underlying multicellularity, we isolated DNA from 24 isolates from the two experimental populations and one control population (8 from each population) and performed Illumina whole-genome sequencing to identify mutations occurred in those evolved isolates. To investigate epistatic interactions among evolved mutations or with the ancestral variants, we conducted bulked segregant analysis (BSA). Evolved isolates were crossed with unicellular wild types, and the resulting recombinant F2 progeny were sorted into multicellular and unicellular pools. DNA from each pool was sequenced to detect differences in allele frequency, and over-represented alleles in the multicellular pool are likely interacting alleles that contribute to the multicellular phenotypes.

The multicellular isolates from the two experimental populations exhibit distinct genetic signatures from each other. The isolates from different populations were derived from different recombinant cells in the starting outcrossed population. Within each population, the multicellular isolates share a number of mutations together, but not with the multicellular isolates from the other experimental population, or with the unicellular isolates from the control population. Each isolate also accumulated mutations not found in other isolates. In addition, the results from BSA suggest instances of epistatic interactions among ancestral variants and derived mutations, and we are working on elucidating such interactions underlying the origins of multicellularity. This is the first step towards understanding the dynamics and mechanistic basis of the evolution of complexity.

Understanding the ecology of the volvocine green algae

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Abstract:

The ecology of the volvocine green algae is central to better understanding the evolution of multicellularity in this clade. However, relatively little is known about the ecology of these cosmopolitan, freshwater algae. Here, we present the methods and preliminary results from an ongoing project that aims to better understand volvocine algae ecology by systematically collecting samples at two sites in southern Arizona, Reid Park and Peña Blanca Lake. Both of our sites are known to harbor at least one species of volvocine algae. We examine several abiotic factors that may affect changes in the abundance of volvocine algae species, including temperature, rainfall, pH, nitrate, phosphate and dissolved oxygen content of the water. We also use high-throughput imaging instrument to examine biotic factors, including the abundance of algal predators and potential competitors. We welcome input, data sharing and potential collaborations.

Changes in phenotypic variability during an evolutionary transition

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Abstract:

Phenotypic variation is central to evolution, and understanding the causes and consequences of differences in the propensity to vary (variability) is considered one of the central problems in biology. While variability has been observed across hierarchical levels of organization, studies have only begun to understand how patterns of variability change across these levels during a major evolutionary transition. We use the volvocine green algae to examine how variability changes during the evolution of complex multicellularity, from simple, undifferentiated species composed of a less than a dozen cells to larger species with thousands of cells and two fully differentiated cell types. We counted reproductive cells in individual colonies and quantified cell number variability in seven species and one cellular differentiation mutant in standard growing conditions and under nutrient deprivation. We find that fully differentiated multicellular species exhibit less phenotypic variability than their smaller, undifferentiated counterparts. This indicates that the production and regulation of variability has likely changed in the volvocine green algae during a major transition in evolution.

Exploring plastid function in free-living nonphotosynthetic algae

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Abstract:

Photosynthesis is an ancient (emerged 3.2-3.5 BYA) autotrophic pathway (Blankenship, 2010) - which likely evolved first in bacteria - capable of harnessing energy from sunlight and converting it into chemical energy (Arnon, 1971). The diverse versions of photosynthesis are the main source of food on planet Earth (Blankenship, 2010). Particularly, oxygen-producing (i.e., oxygenic) photosynthesis evolved circa 2.5 BYA in the ancestors of modern cyanobacteria (Blankenship, 2010). The rise of photosynthesis dramatically altered conditions on Earth by oxygenating the atmosphere during the “Great Oxygenation Event”, which occurred about 2.4 BYA (Cardona *et al.*, 2015). However, despite the ecological relevance of photosynthesis, lineages from every domain of the Eukaryote group, including a diverse variety of land plants and algae, have lost the ability to photosynthesize numerous times independently (Wicke *et al.*, 2013). Nonphotosynthetic algae typically retain colourless plastids with highly reduced genomes. Most studies about the loss of photosynthesis have focused parasitic lineages that were once photoautotrophic (Figueroa-Martinez *et al.*, 2015). However, not all lineages that have lost photosynthesis are parasites or pathogens (Figueroa-Martinez *et al.*, 2015). Diverse free-living colourless algae presumably evolved from mixotrophic ancestors to nonphotosynthetic habits without parasitic/pathogenic stages (Troost *et al.*, 2005; de Castro *et al.*, 2009). My work aims to gain a better understanding of the physiological roles of the colourless plastids in free-living chlamydomonadalean algae. Using transcriptomic approaches, I investigated the metabolic functions of the nonphotosynthetic plastid of *Polytoma uvella*. Similarity and intracellular targeting analyses of the *P. uvella* transcriptomic data provided a detailed catalog of the functions retained in the colourless plastid after the adoption of a nonphotosynthetic lifestyle. Reconstruction of complete metabolic pathways indicate that the *P. uvella* plastid hosts multiple key metabolic functions, such as the metabolism and biosynthesis of a number of amino acids, purine and pyrimidine metabolism, terpenoid biosynthesis, fatty acid biosynthesis, starch and sucrose metabolism, fatty acid biosynthesis, glycerolipid metabolism, carbon fixation, biosynthesis of porphyrin and chlorophyll, carotenoid biosynthesis, and mismatch repair. The TOC-TIC complex for plastid protein import also seems to be fully functional. Overall, my results provide important insights into the metabolic roles of the plastids in free-living nonphotosynthetic algae.

What Ever Happened to Sex Inducer?

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Abstract:

While unicellular *Chlamydomonas reinhardtii* and many of the smaller volvocine colonials are sexually induced by nitrogen deficiency, many *Volvox* species become sexual after exposure to a pheromone called sex inducer. The diffusible pheromone is released (usually) by male sperm packets, and acts on vegetative gonidia to induce differentiation of sexual female colonies and sometimes additional sexual males. Inducer was first described by Darden in 1966 [1] in *V. aureus*, and has since been described in *V. rousseletii*, *V. dissipatrix*, *V. gigas*, *V. obversus* and, of course, *V. carteri*. All three *Volvox* clades are represented; however, only in *V. carteri* has the inducer glycoprotein been characterized both biochemically and by gene sequencing.[2] The *V. carteri* inducer protein is comprised of a single pherophorin domain and presumably evolved from this family of ECM proteins. Whether the inducers from the other *Volvox* species are orthologous proteins is unknown.

V. carteri is an obvious choice for investigating sex inducer evolution as incipient speciation leads to reproductive isolation. All *V. carteri* strains are divided into three parts/forma/subspecies called f. *nagariensis*, f. *kawasakiensis* and f. *weismannia*. The f. *weismannia* isolates come from three different locations: North America, Australia and India. Data (sometimes inconsistent) show that the inducers from some of these strains can cross induce others. Unfortunately, less information is available about the ability of the subspecies to cross-fertilize and produce viable zygotes. The reproductive isolation between f. *kawasakiensis* and f. *nagariensis* has been best described.[3]

Since sex inducer has only been sequenced from the Eve strain, a simple place to start is to clone and sequence inducers from the other strains. Screening genomic libraries is preferable, because the inducer genes are generally multi-copy which could lead to complications using gPCR. Inducer from *V. obversus* and *V. carteri* f. *weismannia* Nebraska were cloned using cosmids. Due to time pressure, cloning from f. *kawasakiensis*, f. *weismannia* Australia and f. *weismannia* India are being attempted with gPCR. The current state of our endeavours will be described.

The spermatogenic potential of the volvocine algal sex determining gene *MID* evolved prior to dimorphic sexes

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Abstract

Volvocine algae comprise a unique comparative model for investigating the evolution of distinct male and female sexes (anisogamy/oogamy) from an isogamous ancestral state with mating types. In *Chlamydomonas reinhardtii* a single *minus* mating (*MT*-) type gene called *CrMID* (minus dominance) determines mating-type. *Mid* is putative RWP-RK family transcription factor and orthologs of *Mid* are present throughout the volvocine algal lineage in either the *MT*- or male mating locus of each species. In *Chlamydomonas* ectopic expression of *CrMID* in a *MT*+ strain (*MT*+::*CrMID*-*T*) causes sexual differentiation with a *minus* phenotype, while in the multicellular species *Volvox carteri* ectopic expression of the male *MID* gene (*VcMID*) in females (*Eve*::*VcMID*-*T*) is sufficient to induce spermatogenesis (Geng S. et al 2014); but the *VcMID* and *CrMID* genes could not induce ectopic sexual differentiation when expressed heterologously in *Chlamydomonas* (*MT*+::*VcMID*-*T*) or *Volvox* (*Eve*::*CrMID*-*T*) respectively. Thus, the function of the *Mid* protein and/or its associated network in sexual differentiation has evolved in the volvocine lineage. We used ectopic cross-species expression experiments with *MID* genes from different colonial volvocine genera to identify when it acquired the ability to induce sperm development. Reciprocally, we also tested whether ectopically expressed *MID* genes from colonial Volvocine species could function in *C. reinhardtii* to control *minus* mating type differentiation. We expressed epitope-tagged *MID* genes from isogamous (*C. reinhardtii*, *G. pectorale*) or oogamous (*P. starrii*, *V. carteri*) volvocine species in *V. carteri* female strain *Eve* and tested for their ability to induce spermatogenesis. Transgenic female *V. carteri* expressing *PsMID* produced functional sperm packets during sexual development, which is similar to *Eve*::*VcMID*-*T*, but with slightly lower efficiency (95% versus 100% sperm packets). The sperm packets from *Eve*::*PsMID*-*T* strains also had hatching defects that were more severe than in *Eve*::*VcMID*-*T* strains. Transgenic female *V. carteri* expressing *GpMID* had a more complex phenotype, with smaller vegetative spheroids and a disorganized pattern of somatic cells compared with controls. Remarkably, when sexually induced, *Eve*::*GpMID*-*T* strains produced self-fertile hermaphrodites with mixtures of sperm packets and eggs within a single parental spheroid. It is somewhat paradoxical that the spermatogenic potential of *Mid* evolved in the isogamous species *Gonium pectorale* prior to the evolution of oogamy. This finding suggests that changes in the cis-regulatory networks controlled by *Mid* proteins rather than changes in *Mid* sequence were responsible for innovations leading to the emergence of anisogam/oogamy.

Different paralogs may control somatic cell development in *Volvox powersii* and *Volvox carteri*

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Abstract:

The evolution of somatic cell differentiation represents a critical step in the major evolutionary transition from unicellular to multicellular life. The volvocine green algae provide an excellent model system for studying the evolution of cellular differentiation because member species span a range of complexity from single-celled, to multicellular with two specialized cell types. Previous research¹ revealed that the *regA* gene, which encodes a putative transcription factor, controls somatic cell differentiation in the multicellular volvocine alga *Volvox carteri*. A mutation leading to loss of a functional *regA* gene product results in *V. carteri* colonies developing a characteristic regenerator phenotype where somatic cells become de-differentiated. The *regA* gene is a member of a tandem duplication of paralogs known as the *regA* cluster which arose early in the evolution of multicellularity in the volvocine green algae and is present in all Volvocacean species which differ in cell number, complexity, and developmental program². However, the functional role of the *regA* gene and related *regA* cluster genes in species other than *V. carteri* is not known. I will describe the identification of a putatively causal mutation in the *regA* cluster gene, *rlsB*, of a regenerator-like mutant of *Volvox powersii*³. The developmental program of *V. powersii* is thought to be more ancestral than *V. carteri*. In *V. carteri*, germ cells and somatic cells are specified by an asymmetric division during embryogenesis that creates large germ-progenitor cells and small soma-progenitor cells. This asymmetric division is a derived developmental character only seen in *V. carteri* and its closest relatives, but is absent in *V. powersii* and most other volvocine species. Thus, understanding the genetic basis of somatic cell development in *V. powersii* will improve our understanding of the evolution of somatic cell development in the volvocine green algae. Furthermore, the finding that *rlsB* may control soma in *V. powersii* indicates a possible history of subfunctionalization or independent co-options for *regA* cluster genes during the evolution of soma.

Anisogamy evolved with the reduced sex-determining region

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Abstract:

Volvocine green algae shape a model lineage for the evolutionary analysis in emergence of anisogamy and oogamy [1-3]. They diverged from a single-celled, isogamous *Chlamydomonas*-like ancestor 2 MYA to give rise to a wide variety of organisms including *Chlamydomonas reinhardtii*, 8- or 16-celled isogamous *Gonium*, 32-celled isogamous *Yamagishiella*, 32-celled anisogamous *Eudorina*, and >500-celled oogamous *Volvox*, enabling experimental comparisons [3]. Charlesworth [4] theoretically predicted close linkage between gamete size-determining genes and the mating type locus (*MT*, sex-determining chromosomal region) for the emergence of anisogamy. However, the molecular basis for the origin of anisogamy has been still unknown even based on the recent comparative genome analyses of *MT* of the isogamous and oogamous volvocine algae (*Chlamydomonas*, *Gonium* and *Volvox*) [5,6]. To approach the molecular basis of the evolution of anisogamy, we here focus on two closely related volvocine algae that phylogenetically link isogamy to anisogamy: advanced isogamous *Yamagishiella* and ancestral anisogamous *Eudorina*. We generated *de novo* nuclear genome assemblies of both sexes of *Yamagishiella* and *Eudorina*. The resultant assemblies contained sex dimorphic haplotype regions that corresponded to their *MT*. In contrast to the large and complex *Volvox carteri* *MT* which exceeds 1 Mb in size [5], *Yamagishiella unicocca* and *Eudorina* sp. *MT* were small and simple with only two sex-specific genes, *minus* dominance gene (*MID*) and gamete recognition gene (*FUS1*) that were localized in *minus*/male and *plus*/female *MT*, respectively. There were no other sex specifically-predicted gene models. *Eudorina* sp. female and male *MT* haplotypes were highly reduced, measuring 90 and 7 kb in size, respectively. Since sizes of female gametes of *Eudorina* and isogametes of *Yamagishiella* are almost identical to those of their vegetative cells [7], the emergence of anisogamy from isogamy in the *Yamagishiella*- or *Eudorina*-like ancestor might have been mainly based on the evolution of formation of small male gametes or sperm. While *MID* is a master gene determining the mating type *minus* in isogamous *C. reinhardtii* [9], the ortholog of oogamous *Volvox carteri* *MID* (*VcMID*) determines formation of sperm packets (bundles of sperm or male gametes) by successive divisions of reproductive cells in sexual spheroids [10]. Since both *Eudorina* and *Volvox* perform similar sperm packet formations [7], *Eudorina* sp. *MID* and *VcMID* are assumed to have essentially the same function to initiate the spermatogenesis. Thus, the changes in regulatory mechanisms under the existence of *MID* should be responsible for the emergence of spermatogenesis in the *Eudorina*-like ancestor.

Evolution of self-fertilization in the volvocine green algae

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Abstract:

Outcrossing and self-fertilization are fundamental strategies of sexual reproduction, each with different evolutionary costs and benefits. Self-fertilization is thought to be an evolutionary dead-end strategy, beneficial in the short term but costly in the long term, resulting in self-fertilization species occupying the tips of phylogenetic trees. Here we use the haploid volvocine green algae to investigate the evolution of self-fertilization. We use ancestral state reconstruction to show that self-fertilization has repeatedly evolved from outcrossing ancestors and includes multiple reversals from selfing to outcrossing. We use three phylogenetic metrics to show that self-fertilization is not restricted to the tips of the phylogenetic tree, inconsistent with the view of self-fertilization as a dead-end strategy in the volvocine green algae. We demonstrate that recombination of previously sex-restricted loci into the same genotype occurs with the evolution of self-fertilization, suggesting a genetic mechanism of recombination partially underlies the evolution of homothallic self-fertilization.

Multicellularity drives the evolution of sexual traits

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Abstract:

From the male peacock's tail plumage to the floral displays of flowering plants, traits related to sexual reproduction are complex and exaggerated. But why has sexual reproduction become so complicated? Why have such exaggerated sexual traits evolved? Early work posited a connection of multicellularity with sexual traits such as anisogamy (i.e., the evolution of sperm and eggs), which in turn drives the evolution of other forms of sexual dimorphism. Yet, the relationship between multicellularity and the evolution of sexual traits has not been empirically tested. The volvocine green algae offer a tractable system for understanding the interrelationship of multicellular complexity and sex, including anisogamy and other forms of sexual dimorphism. Here we reconstruct the evolutionary history of six sexual traits, demonstrating a complex evolutionary history, multiple gains and/or losses, in every trait. Our results demonstrate that anisogamy repeatedly evolved from isogamous multicellular ancestors and that anisogamous species are larger and produce larger zygotes than isogamous species. We show that species with higher metrics of multicellular complexity have significantly more derived sexual traits, including anisogamy and exaggerated sexual dimorphism. In the volvocine algae, the evolution of multicellularity likely drives the evolution of anisogamy, and anisogamy subsequently drives exaggerated sexual dimorphism, suggesting that multicellularity sets the stage for the overall diversity of sexual complexity.

Evolution of multicellularity in *Chlamydomonas reinhardtii* in response to predation

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Abstract:

The transition from unicellular to multicellular life was one of a few major events in the history of life that created new opportunities for more complex biological systems to evolve. Thus far, studying the proximate and ultimate causes of the resulting increases in complexity has been a major challenge in evolutionary biology. Traditionally, questions related to the emergence of multicellularity have been addressed retrospectively, through comparative studies of extant unicellular and multicellular lineages. Experimental microbial evolution allows for prospective studies that observe evolution in real time. In this study, we report the *de novo* origin of simple multicellularity in response to predation. We subjected outcrossed populations of the unicellular green alga *Chlamydomonas reinhardtii* to selection by the filter-feeding predator *Paramecium tetraurelia*. Two of five experimental populations evolved multicellular structures not observed in any of the three unselected control populations. Colonies consist of 4-16 cells enclosed within the cell wall of the maternal cell, and cells are encased within a transparent extracellular matrix. The highly structured, spheroidal colonies that evolved in this experiment are reminiscent of volvocine algae such as *Eudorina elegans*. These algae represent a completely novel origin of multicellularity, as *C. reinhardtii* has never had multicellular ancestors.^{1,2}

Evolution and variability of morphogenesis in *Volvox*

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Abstract:

All *Volvox* embryos undergo the process of inversion during which they turn themselves inside-out in order to expose their flagella. So far, inversion processes in different species have been divided into two distinct types: in type A inversion four lips at the anterior embryo pole curl backwards while type B inversion starts with a circular invagination at the equator. However, our 3D *in vivo* studies using light sheet fluorescence microscopy show that the embryos of some species including *V. dissipatrix* and *V. tertius* show features of both inversion types. This finding stresses the question how different inversion tactics evolved.

In addition to variations between species there is also a certain variability between inversions of individual embryos. How stereotypic morphogenetic processes have to be in order to be completed successfully is an important but understudied question in developmental biology. To perform three dimensional deformations, such as invagination or involution, cell sheets need to overcome geometrical bottlenecks. The local regions that act to overcome these bottlenecks need to be more closely regulated and are likely to show less variation. In order to identify such crucial areas and stages we have quantified the variability of inversion of *V. globator* embryos. Our findings are consistent with previously observed cell shape changes and with the predictions of our mathematical model of type B inversion.

Identification and Characterization of Gamete Adhesion Factor *FUS1* Orthologs in Isogamous *Yamagishiella* and Anisogamous *Eudorina*

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Abstract:

Anisogamy, which exhibits the fusion of a large female gamete and a small male gamete (sperm), has evolved from isogamous ancestors several times in independent eukaryotic lineages. To understand the mechanistic change of gamete fusion event associated with the isogamy-anisogamy transition, we focus on the two closely related volvocine algae, isogamous *Yamagishiella unicocca* and anisogamous *Eudorina* sp. Based on our whole-genome sequencing project of these algae, we identified orthologous genes of *FUS1*, an isogametic adhesion factor gene previously reported in *Chlamydomonas reinhardtii* (Ferris et al. 1996; Misamore et al. 2003) and *Gonium pectorale* (Hamaji et al. 2016). The *FUS1* orthologs of *Y. unicocca* (*YuFUS1*) and *Eudorina FUS1* (*EuFUS1*) were exclusively encoded in the *plus* and female mating-type locus, respectively. The deduced protein sequences of *YuFUS1* and *EuFUS1* contain a signal peptide, immunoglobulin-like sequences, and a transmembrane domain, in common with the other isogamous *FUS1* proteins. In *Y. unicocca*, the *YuFUS1* gene was specifically expressed in the *plus* gametes. In *Eudorina* sp., the *EuFUS1* expression was significantly up-regulated after mixing with sperm, indicating that enhancement of the *EuFUS1* expression may require some interaction to the male derived factor(s), comparable to the *FUS1* enrichment through the gamete activation treatment in isogamous species (Ning et al. 2013; Hamaji et al. 2016). These results suggest that isogamy and anisogamy in volvocine algae share the *FUS1*-mediated gamete adhesion mechanism on *plus*/female gametes. In oogamous *Volvox carteri*, no *FUS1* ortholog was found (Ferris et al. 2010). It seems that a substantial mechanistic shift in gamete fusion/fertilization might have occurred in the ancestor of *V. carteri*.

Investigations into the mechanistic role of *Chlamydomonas* RLS1 in photosynthetic acclimation using an *RLS1* genomic mutant and RNAi knockdown

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Abstract:

Photoacclimation is a universal response in photosynthetic organisms where light harvesting capacity and downstream energy utilizing pathways are adjusted in response to light intensity (and other environmental changes) in an attempt to maintain a balance between these dependent processes. This ultimately reduces photo-oxidative damage and/or limits growth and enhances survival. Long-term photoacclimation inevitably involves changes in the abundance and activity of chloroplast-localized proteins, which often requires changes in gene expression. The expression of nucleus-encoded, photosynthetic genes in response to light intensity is well described, though the specific mechanism initiating these changes as a result of the energy imbalance is unknown. *RLS1* is the closest homologue of *regA* (the gene responsible for somatic cell differentiation in *Volvox carteri*) in *Chlamydomonas reinhardtii*. While the function of RLS1 is unknown, the gene is expressed in conditions known to induce acclimation responses, such as an extended dark incubation, and both phosphate and sulphur deprivation. Since all of these conditions lead to a down-regulation of photosynthesis, we hypothesized that RLS1 could have a role in photoacclimation. Here we use an *RLS1* genomic mutant and RNAi knockdown to test whether RLS1 is a central regulator of photoacclimation in *Chlamydomonas*. The mutant/knockdown and wild-type strains were grown under different light intensities to determine if a lack of RLS1 activity affects growth rate, chlorophyll content, photosynthetic activity, and general photoacclimation capacity. These studies contribute to our understanding of the acclimation mechanism in photosynthetic organisms as well as the evolution of somatic cell differentiation in volvocine green algae.

Taxonomy of a new snow-inhabiting species of *Chloromonas* based on the use of cultured and field-collected materials

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Abstract:

Description of ‘colored snow’ dates back to the work of Aristotle. This phenomenon is principally caused by blooms of psychrophilic microalgae. Within green, reddish or brown snow, cold-adapted species of the unicellular biflagellate genus *Chloromonas* belonging to the Volvocales, are generally dominant (1).

Taxonomic studies of snow-inhabiting *Chloromonas* were primarily carried out based solely on the light microscopy of field-collected materials. Partial life cycles (from vegetative cells to zygotes) of several snow *Chloromonas* species were observed on the basis of continuous observations of field-collected materials from North America (e.g. 2). Among the species, *C. nivalis* was considered cosmopolitan because the cysts morphologically identical to the zygotes of this species from North America are distributed worldwide (2,3). Currently, *C. nivalis* is generally identified based solely on zygote or cyst morphology (e.g. 4), since germination of field-collected cysts of snow-inhabiting *Chloromonas* species has never been successfully induced (5). However, molecular data suggested the polyphyly of field-collected cysts identified as *C. nivalis* (6). Recently, Matsuzaki et al. (7) demonstrated that one Japanese lineage of such *C. nivalis* zygotes is actually conspecific with *C. miwae*, on the basis of molecular data of multiple DNA regions obtained from culture strains and field-collected cysts. Therefore, further taxonomic studies are required to reveal the accurate species identification and correct diversity of snow-inhabiting *Chloromonas* using combined molecular analyses of culture strains and field-collected cysts.

Here, we delineated a new snow-inhabiting species of *Chloromonas* based on the use of cultured and field-collected materials from Japan. The vegetative morphology of this species differed from those of all previously described snow *Chloromonas* species. Some field-collected cysts from Mt. Hakkoda, Aomori, Japan and Mt. Tateyama, Toyama, Japan could be identified as this new species on the basis of molecular data whereas they were morphologically similar to the zygotes of *C. nivalis* under light microscopy. Multigene phylogeny showed that the new species was sister to *C. miwae*, which also has *C. nivalis*-like cysts (7). However, the present field-emission scanning electron microscopy demonstrated that the cysts of the new species could be clearly distinguished from those of *C. miwae* by the differences of the flanges’ form developing on the cell wall.

Transcriptomic analysis of *Volvox carteri* cell types yields insights into the evolutionary origins of germ and somatic differentiation programs

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Abstract:

Germ-soma differentiation is a hallmark of complex multicellular organisms, yet its origins are not well understood. *Volvox carteri* is a simple multicellular green alga that has recently evolved a simple germ-soma dichotomy with only two cell types: large germ cells called gonidia and small terminally differentiated somatic cells. We conducted a comprehensive characterization of the gonidial and somatic transcriptomes of *Volvox* to uncover fundamental differences between the molecular and metabolic programming of these cell types. We found extensive transcriptome differentiation between the gonidial and somatic cells of *Volvox* with somatic cells expressing a more specialized program overrepresented in younger, lineage-specific genes, and gonidial cells expressing a more generalist program overrepresented in more ancient genes that shared striking functional overlap with a gene set from core metazoan pluripotent stem cells. Directed analyses of metabolic pathways revealed a strong dichotomy between cell types with gonidial cells expressing growth-related genes and somatic cells expressing an altruistic metabolic program geared towards the assembly of flagella, which support organismal motility, and conversion of storage carbon to sugars, which act as donors of extracellular matrix glycoproteins whose secretion enables massive organismal expansion. *Volvox* orthologs of *Chlamydomonas* diurnally regulated genes were analyzed for cell-type distribution and found to be strongly partitioned, with expression of dark-phase genes overrepresented in somatic cells and light-phase genes overrepresented in gonidial cells, a result that is consistent with cell type programs in *Volvox* arising by cooption of temporal regulons in a unicellular ancestor. Together our findings reveal fundamental molecular, metabolic, and evolutionary mechanisms that underlie the origins of germ-soma differentiation in *Volvox* and provide a template for understanding the acquisition of germ-soma differentiation in other multicellular lineages.

CRISPR/Cas9-directed mutagenesis in *Volvox carteri*

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Abstract:

Cas9 can be used to make targeted indels by non-homologous end-joining (NHEJ) or precise changes in the genome by homology-directed recombination (HDR), resulting in knockout mutations and precise genome edits, respectively. To expedite genetic analysis in *V. carteri*, we set out to develop a transgene-based CRISPR/Cas9 system that can be used to mutate candidate genes and to make precise edits. We replaced the plant-specific regulatory sequences present in an Arabidopsis/rice Cas9 vector with regulatory sequences from *V. carteri* genes and targeted test genes with known mutant phenotypes, including *glsA* and *regA*. The *V. carteri*-specific Cas9 gene is carried on one plasmid and the guide RNA gene on a second plasmid containing a hygromycin resistance marker. Biolistic co-transformation of the Cas9 plasmid and a guide RNA gene plasmid targeting *glsA* into Reg mutant 153-68 generated hygromycin-resistant transformants that produced Glc mutant progeny, and co-bombardment of the Cas9 vector with a guide RNA gene plasmid targeting *regA* into wild type strain EVE generated hygromycin-resistant Reg transformants. Mutation rates varied from <0.1% to 100%. We cloned and sequenced the targeted *glsA* and *regA* regions in those mutants and found they contained frameshift-causing indel mutations, indicating that these were Cas9-generated NHEJ mutations. Efforts are underway to test HDR via Cas9 and to mutate genes of unknown function in *V. carteri*.

Functional analysis of regA paralogs rlsA-rlsD

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Abstract:

regA encodes a somatic-cell-specific nuclear protein (RegA) that represses growth and reproduction and is essential for maintenance of the somatic cell fate in *Volvox carteri*. RegA paralogs share with RegA a single conserved region, the ~110-aa Volvocine Algal RegA-like (VARL) domain that is related to the DNA-binding SAND domain present in several plant and animal proteins. Information about the functions of these paralogs is lacking, but could shed light on cell differentiation and how it evolved in *V. carteri*. Of the four closest paralogs of *regA*, three (*rlsA-rlsC*) have transcript accumulation patterns that roughly parallel those of *regA*, while *rlsD* transcripts are not developmentally regulated during the vegetative life cycle. Here, we report results from several approaches to investigate the functions of these RegA paralogs. In addition to other experiments, we used RNA hairpin-expressing transgenes to attempt gene knockdowns, and we tested the ability of chimeric versions of RegA with paralog VARL domains to rescue a *regA* mutant. RNA hairpins targeting *rlsA*, *rlsB*, or *rlsC* have not yielded detectable phenotypes, suggesting that *rlsA*, *rlsB*, and *rlsC* individually might not be essential for somatic cell differentiation. On the other hand, an RNA hairpin targeting *rlsD* leads to severe defects in growth and reproduction, suggesting that in stark contrast to *regA*, *rlsD* promotes growth and reproduction. Meanwhile, none of the chimeric RegA proteins with paralog RegA VARL domains rescued a *regA* mutant, suggesting that the RegA VARL domain has a different function than the paralog VARL domains. Considered together, our results indicate that RegA is functionally different from its close paralogs, and that the VARL domain is key to this difference.

Two cellular events for inversion in *Volvox carteri*, cell shape changes and migration: which drives the morphogenetic movement?

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Abstract:

Volvox and their close relatives serve as a good model for investigating molecular and cellular bases of morphogenetic processes and how such processes arise during the evolution of multicellular organisms from their unicellular ancestors. The key morphogenetic process in *Volvox* development is called inversion, in which the spherical embryo turns inside out. The pre-inversion embryo is a cellular monolayer in which neighboring cells are linked to one another at the mid-level of their cell bodies by cytoplasmic bridges. The phialopore where such linkages are missing is the opening where the inversion movement will start. First, the cells become flask shaped by extending long, thin stalks from their outermost ends. Next, cells near the phialopore migrate relative to their cytoplasmic bridges until they are linked to their neighbors only at the outermost tips of their stalks, which causes the cell sheet to turn outward. In the previous work, a series of morphological mutants in *V. carteri* that failed inversion was isolated and it has been shown that these two cellular events, cell elongation and cell migration, are genetically independent. In a mutant called *InvA*, cells in the inversion stage change their shape to be elongated normally but they cannot migrate relative to the cytoplasmic bridges and the inversion arrests halfway. A similar defect of embryo morphology is also seen for another type of mutant strains, in which cell migration seems to occur normally but cells cannot elongate. Thus, it is assumed that both cellular events are complement and working together for the *V. carteri* embryo to complete the inversion process. When we examined the two cellular processes in smaller colonial species such as *Eudorina* and *Pandorina*, we noticed that the cells elongated much shorter than that in *Volvox* and inhibiting cell migration using an antisense construct of *P. morum InvA* gene was enough to prevent *P. morum* from getting its spherical shape and kept it to be a flat colony, as somehow similar to a *Gonium* colony. These results proposed that reconsideration of the inhibition mechanism in the *V. carteri* mutants without cell shape changes might be required. Video microscopy indicated that when inversion stopped for such mutants, the embryo contacted with the vesicles surrounding itself and TEM also suggested that the vesicle might interfere with embryo inversion. I would like to discuss about why cell shape changes are required for the embryo of large *Volvox* to solve such issue.

***Volvox carteri* from Taiwan**

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5. Biodiversity Research Center, Academia Sinica, Taiwan

Abstract:

The genus *Volvox* represents the most advanced member of volvocine green algae, especially in multicellularity and sex (Kirk 1998, Hiraide et al. 2013). Recently, *Volvox carteri* f. *nagariensis* has been studied extensively by cellular and molecular data (e.g. Kirk et al. 1999, Ferris et al. 2010). Although this taxon or forma was originally described based on natural sample collected in Nagari, India (Iyengar 1933), most of the recent studies used only Japanese strains such as Eve and Adam (e.g. Kirk et al. 1999, Ferris et al. 2010). In addition, the most distinctive morphological attribute of *V. carteri* f. *nagariensis* is the 1:1 ratio of sperm packets (androgonidia) to somatic cells in sexual male spheroids (Nozaki 1988). However, this ratio has been examined in only Japanese strains irrespective of the presence of Indian strains from Poona (Adams et al. 1990).

V. carteri f. *nagariensis* has heterothallic sexuality with male and female genders determined by the mating type locus where gender-limited genes are present (Ferris et al. 2010). Thus, it seems very easy to determine the gender in natural populations of *V. carteri* f. *nagariensis* based on the presence or absence of the gender-limited genes. However, no identification of natural populations of this species has been performed by using molecular identification. Furthermore, the mating type locus of *V. carteri* f. *nagariensis* is composed of ca. 1 Mbp linear chromosome where recombination is suggested to be repressed (Ferris et al. 2010). However, actual recombination of the mating type locus genes has not been examined in natural population of *V. carteri*.

During a recent field collection of the freshwater green algae in Taiwan, we fortunately encountered natural populations of *V. carteri* in rice paddies. Based on the internal transcribed spacer regions of nuclear ribosomal DNA, it was clearly identified to *V. carteri* f. *nagariensis*. However, male sexual spheroids of the Taiwan strains do not exhibit 1:1 ratio of sperm packets to somatic cells in male spheroids. Furthermore, molecular identifications of genders of natural populations of these Taiwanese strains were carried out in the present study.

Sphingolipid characterization of Volvocine algae reveals presence of glucosylceramides with α -hydroxylated 18:2 fatty acid and a possible role for sphingolipids in *Volvox carteri* embryo development

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Abstract:

Glucosylceramides (GlcCers) are sphingolipids composed of a long chain base (LCB), a fatty acid (FA), and a glucose head group. Previous studies in other model organisms have suggested GlcCers play an important role in multicellularity and cellular differentiation. In this study, we used the Volvocine algae, a collection of algae with unicellular and a variety of colony forming morphologies from the *Volvocales* order, to characterize GlcCers and other neutral sphingolipids in relation to colony morphology using HPLC-MS/MS.

We found Volvocine algal sphingolipids contain only saturated LCBs and synthesize GlcCers with a novel α -hydroxylated 18:2^{A9,12} fatty acid containing ceramide. Total levels of GlcCers tended to increase in a slight trend with increasing complexity among the Volvocine algae with exceptions for *Pandorina morum* and *Volvox carteri*. Separation of gonidia and somatic cells from *Volvox* colonies followed by sphingolipid profiling revealed gonidia to have higher levels of ceramides and GlcCers, but lower hydroxyceramides relative to somatic cells. Additionally, gonidia sphingolipids appeared to have a higher proportion of long chain FA (16-18C) containing ceramides and hydroxyceramides whereas somatic cells had a higher proportion of very long chain FA (>18C) containing ceramides and hydroxyceramides.

We identified candidates for glucosylceramide synthase (GCS) DNA sequences from both *Chlamydomonas reinhardtii* and *Volvox carteri* genomes. The GCS sequence from *Chlamydomonas* contained two insertions of repeats, one in the predicted exon 4 and the other in the intronic region between exons 4 and 5 and did not appear to be expressed. However, the *Volvox* GCS was isolated from cDNA with a coding sequence of 1551 bp and complemented a *Agcs1 Yarrowia lipolytica* strain when heterologously expressed.

Treatment of Volvocine cultures with the GCS inhibitor d,l-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) increased levels of ceramides in *Pandorina morum*, *Eudorina elegans*, *Pleodorina californica*, and *Volvox carteri*, and hydroxyceramides in *Gonium pectorale*, *Pandorina morum*, *Eudorina elegans*, and *Volvox carteri*. Only *Gonium* and *Pleodorina* showed significant decreases in GlcCers. The treatment had lethal effects for *Gonium* and *Volvox*, and repressed growth in *Pandorina* and *Eudorina*. *Chlamydomonas* exhibited no discernible changes in sphingolipid content or morphological changes under PDMP treatment. PDMP treatment of *Volvox* cultures resulted in embryos exhibiting a hollow, malformed shape as viewed under light microscopy. Together these results may suggest a role for sphingolipid metabolism in *Volvox* embryo development previously not explored.

Unravelling organelle genome transcription using publicly available RNA-sequencing data

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Abstract:

Background: Organelle genomes are the most sequenced type of chromosome, given their relative compactness and importance on molecular research. Additionally, next generation sequencing (NGS) has generated unprecedented amounts of genomic and transcriptomic data that can be used for organelle genome evolution studies. Indeed, NGS has been influencing the organelle genomics field by being a major facilitator in the current sequencing efforts. However, most of the NGS data, particularly the RNA sequencing (RNA-seq) data, is still used only for the study of cell nucleus, being the organelle reads even discarded in such investigations. Here, we used this untapped data source to investigate the transcription of publicly available organelle genomes in plastid-bearing protists. We mapped raw transcripts over genomes of 116 protist species to verify the utility of RNA-seq data in unraveling the organelle genome transcription architecture.

Results: 77 species had their organelle genomes nearly entirely (> 85%) mapped by transcripts despite of the amount of non-coding DNA (intergenic regions and introns) they had. These genomes come from various protist groups – including the volvocine algae – that exhibit diverse genome structure and content. Yet, all of them appear to go full transcription.

Conclusions: Publically available (and unused) RNA-seq data can be employed to investigate organelle genome transcription. Even though organelle genomes can exhibit significant portions of non-coding DNA, these regions are still actively transcribed. That allowed us to show that nearly entire (when not entire) organelle genomes can be recovered from RNA-seq data. This represents a valuable alternative to determine organelle genomes. Beyond that, organelles seem to be producing a plethora of non-coding RNAs that can regulate fundamental processes such as organelle differentiation in land plants and trophic mode choice in mixotrophs.

Evolution of the extracellular matrix for multicellularity

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Abstract:

The evolution of multicellularity is a major transition in the morphological organization of organisms, however, the molecular mechanisms important for this transition in any taxa are currently not well understood. In most taxa, the molecular signature of the transition to multicellularity is obscured by nearly a billion years of divergence. Multicellularity evolved recently in the volvocine algae, thereby preserving the molecular signature of this transition. The volvocine algae include members that span the range of morphological complexity from unicellular (e.g. *Chlamydomonas*) to undifferentiated multicellular (e.g. *Gonium*), to species with differentiated tissues (e.g. *Volvox*). Importantly, the genomes of *Chlamydomonas* and *Volvox* have shown to be remarkably similar, suggesting the transition to multicellularity only requires the evolution of a few genes. To find genes important for multicellularity in undifferentiated multicellular *Gonium pectorale*, we performed a genetic screen for unicellular mutants. From this we identified a mutant, *uc-1C7*, that is 99.6% unicellular. Resequencing its genome revealed that the causative mutation is in an ortholog of *GDT1*, which is conserved across all eukaryotes. *GDT1* is localized to the trans-Golgi, where it plays a role in the proper glycosylation of proteins destined for the extracellular matrix. We found that the *uc-1C7* mutant is sensitive to detergent lysis consistent with defects in extracellular matrix assembly. When the *GDT1* ortholog from *Gonium* is expressed in unicellular *Chlamydomonas*, it causes a multicellular gain-of-function phenotype. These results suggest that *GDT1* is important for multicellularity by regulating the maturation of proteins destined for the extracellular matrix to promote cell-cell adhesion.

The volvocales genomes project

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Abstract:

The transition from unicellular to multicellular organisms is a major step in the evolution of life on earth. This transition is known to have happened independently in different organismal groups. However, the molecular basis of this transition has remained largely elusive. The volvocine algae consist of several species representing increasing multicellular complexity and thus provide an excellent model for the study of the evolution of multicellularity. We are studying the genomic basis of multicellular evolution by sequencing the genomes of volvocine species representing progressively increasing complexity, in evolutionary terms. The genomes of *Chlamydomonas reinhardtii* (unicellular), *Gonium pectorale* (undifferentiated multicellular) and *Volvox carteri* (differentiated multicellular) have been sequenced. Previously, by sequencing the genome of *Gonium pectorale*, we showed that the co-option of cell cycle regulatory genes plays a key role in the evolution of multicellularity. Subsequently, we have sequenced the genomes and transcriptomes of *Yamagishiella unicocca*, *Eudorina elegans*, *Pandorina morum*, *Pleodorina starii*, and *Volvox ferissi*. To understand how multicellularity and cell type specification evolve within the Volvocales, we are comparing the evolutionary history of these genome sequences. In particular, we are focusing on how the cell cycle regulated gene expression has evolved for organismal developmental plans. An update on our progress on assembly and annotation of the Volvocales genomes will be presented along with analysis of the cell cycle transcriptomes of several key species.

Anterior–posterior gradient in Ca²⁺ sensitivity of axoneme studied using detergent-extracted *Volvox* “model”

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Abstract

Volvox rousseletii, a multicellular spheroidal green alga containing ~5,000 cells, shows remarkable photobehavior without any known intercellular communication. Its ~10,000 flagella beat with an asymmetrical/ciliary-type waveform toward the posterior pole with a slight tilt from the anterior–posterior axis of the spheroid (Mast, 1926). The direction of flagellar beating changes upon light perception from posteriorward to anteriorward while retaining the ciliary waveform (Ueki et al., 2010). This flagellar response is called “ciliary reversal”. Importantly, the sensitivity or the magnitude of this response has a gradient along the anterior–posterior axis, in that the response is more conspicuous near the anterior pole of the spheroid and rarely observed near the posterior pole (Ueki et al., 2010). In this study, to examine its flagellar properties in vitro, we developed a method for detergent-extraction of *V. rousseletii* and reactivation of its flagellar motility, while its spheroidal shape is retained. Upon addition of ATP, the demembrated flagella (axonemes) in the spheroids actively beat and the whole spheroids swam as if they were alive. Furthermore, we examined effects of Ca²⁺ on the motility of reactivated axonemes. Under Ca²⁺-free conditions, the axonemes showed planar and asymmetrical waveforms beating toward the posterior pole, as in live spheroids swimming without light stimulation. In contrast, in the presence of 10⁻⁶ M Ca²⁺, they beat somewhat three-dimensionally and toward the anterior pole, like the flagellar beating in photo-stimulated live spheroids. Intriguingly, this Ca²⁺-dependent change in flagellar beating direction was more conspicuous near the anterior pole of the spheroid and not observed near the posterior pole. We propose that the anterior–posterior gradient of flagellar Ca²⁺ sensitivity underlies the gradient of photosensitivity of flagellar response in *V. rousseletii*.

Mating type locus-like regions in the homothallic species *Volvox africanus*.

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Abstract:

In the oogamous genus *Volvox*, there are two types of sex determination, heterothallism and homothallism. The former type has genetically determined sexes and each sex produces male or female sexual spheroids (dioecism), whereas the latter has both sexes in a single strain producing monoecious sexual spheroids (containing both eggs and sperm packets) or sometimes dioecious sexual spheroids. Sexual differentiation in heterothallic species of the unicellular and colonial/multicellular volvocine algae is controlled by a sex-determining or mating type locus (*MT*) containing *MID* in the minus or male strain (Ferris and Goodenough 1997; Ferris et al 2010; Hamaji et al 2016). Comparative analyses of *MT* loci in volvocine algae are important to elucidate the molecular and genomic basis of evolution of sexual differentiation.

We recently reported that a *MID* ortholog is present in the homothallic species *V. africanus* which produces both male and monoecious sexual spheroids in a single strain (Yamamoto et al. 2017). In *V. africanus*, monoecious spheroid-specific down regulation of gene expression of the *MID* ortholog was demonstrated (Yamamoto et al 2017). Furthermore, androgonidia (male reproductive cells) in the male strain of heterothallic *V. carteri* develop to be eggs by experimental suppression of *VcMID* expression (Geng et al. 2014). Therefore, we hypothesized that the homothallic species *V. africanus* might have evolved directly from a male strain of the heterothallic ancestor by modification of the regulation system of *MID* expressions in sexual spheroids (Yamamoto et al. 2017).

In order to test our hypothesis of the evolution of homothallic *V. africanus* (Yamamoto et al. 2017), we here generated *de novo* nuclear genome assemblies of a strain of *V. africanus* and male and female strains of its closely related heterothallic species *V. reticuliferus* (Nozaki et al. 2015). Our comparative analyses of these three genomes revealed male and female haplotypes of *V. reticuliferus MT*, and *MT*-like regions in the *V. africanus* genome. Male and female haplotypes in *MT* of *V. reticuliferus* measured approximately 1 Mb long and contained a male-specific *MID* ortholog and a female-specific *FUS1*-like sequence. In *V. africanus*, an *MT*-like region measured approximately 1.2 Mb long and harbored a *FUS1*-like sequence. However, *MID* ortholog of *V. africanus* was found in another scaffold, in which five tandemly repeated *MID* sequences and homologs of two gametologs in *V. carteri* (*SPS1* and *MTF1436*) constituted a very small genome region (ca. 50 kb).

Cell Biological Analysis of the Embryogenesis in *Gonium* (Volvocales, Chlorophyta)

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Abstract:

Volvocine algae include various species from unicellular *Chlamydomonas reinhardtii* to multicellular *Volvox* with germ-soma division of labor and are thought to be the unique model for research of the evolution of multicellularity. Within the volvocine lineage, the evolution from flattened colony to spheroidal colony might have occurred in two independent lineages: Volvocaceae and genus *Astrephomene* (Goniaceae). After successive cell divisions during embryogenesis, volvocacean species undergo inversion, turning the cell layer of an embryo inside out to orient flagellar positions of the daughter protoplasts toward the outside, to form a spheroidal colony. The inversion is accompanied by the change in shape of daughter protoplasts and the movement of protoplasts relative to cytoplasmic bridges, which produce the driving force of cell sheet folding (Viamontes & Kirk 1977, Green et al. 1981). On the other hand, *Astrephomene* undergoes the rotation of daughter protoplasts during successive cell divisions to form a spheroidal cell layer, instead of inversion (Yamashita et al. 2016). However, details of the evolution of these cellular events involved in the two types of spheroidal colony formation (shape change, movement and rotation) are unclear based on previous studies on more ancestral volvocine algae with flattened colony such as *Gonium* (e.g. Hallmann 2006, Iida et al. 2013). Here, with an active strain newly established from a rice field in Japan, we observed in detail the cell biological event during the embryogenesis of *Gonium pectorale* using light microscopy time-lapse imaging as well as immunofluorescence microscopy of basal bodies with anti-CrSAS-6 antibody. Rotation of daughter protoplasts was not observed during successive cell divisions. After the successive cell divisions, the concave cell sheet of an embryo expanded gradually without significant cell shape change, which might be the phenomenon called ‘partial inversion’ in previous studies (e.g. Hallmann 2006, Iida et al. 2013). The present results suggest that the two different ancestors of Volvocaceae and *Astrephomene* might have newly invented the cellular mechanisms to form a spheroidal colony after the divergence from the ancestors of Goniaceae and *Gonium*, respectively.