THE 3rd INTERNATIONAL VOLVOX CONFERENCE

August 19th – 22nd, 2015

Centre for Mathematical Sciences, University of Cambridge
Cambridge, United Kingdom

SPONSORSHIP AND SUPPORT
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SYNOPSIS

This is the 3rd of what we hope to be a long series of *Volvox* meetings to be held every other year, alternating with the Chlamydomonas meeting. We are delighted to be hosting the 3rd international *Volvox* meeting in the beautiful and historic city of Cambridge, UK. The city centre is tightly packed with exquisite architecture and a multitude of parks, the College Backs and the beautiful river Cam add to its appealing atmosphere.

The aim of this meeting is to bring together international scientists working with *Volvox* and its relatives (aka Volvocales or volvocine algae). We cordially invite experimentalists and theorists interested in these fascinating organisms. The Volvocine algae have become an important model system for the evolution of multicellularity, development and cellular differentiation, and have yielded important results in fields as diverse as genomics, biological physics, hydrodynamics, and social evolution. We hope that these meetings will foster exchange of ideas and expertise, and will initiate new collaborations to expand and strengthen the Volvocales community.

WEBSITE

http://www.damtp.cam.ac.uk/user/ah659/volvoxindex.html

CONFERENCE ORGANIZERS

Aurelia Honerkamp-Smith, University of Cambridge, UK
Stephanie Höhn, University of Cambridge, UK
Raymond E. Goldstein, University of Cambridge, UK
Aurora M. Nedelcu, University of New Brunswick, Canada
James Umen, Donald Danforth Plant Science Center, USA
Matthew Herron, University of Montana, USA
Hisayoshi Nozaki, University of Tokyo, Japan
Erik R. Hanschen, University of Arizona, USA
David Smith, Western University, Canada

PROGRAM AT A GLANCE

Day	Time	Activity	Location
Wednesday, August 19th			
	7:15 – 8:15	Breakfast	Wychfield Site, Trinity Hall
	12:00 – 15:00	Algae Collection Walk	
	5:00 – 9:00	Registration	
	6:00	Welcome Reception	CMS, Central Hall
Thursday, August 20th			
	7:15 – 8:15	Breakfast	Wychfield Site, Trinity Hall
	8:30 – 10:20	Morning Session 1: Life Cycle	CMS, Manns Room
	10:20 – 10:40	Break	CMS, Central Hall
	10:40 – 12:05	Morning Session 2: Cell Differentiation	CMS, Manns Room
	12:05 – 1:30	Lunch	CMS, Central Hall
	1:30 – 2:55	Afternoon Session 1: Development	CMS, Manns Room
	2:55 – 3:15	Break	CMS, Central Hall
	3:15 – 4:40	Afternoon Session 2: Evolution	CMS, Manns Room
	4:40 – 5:30	Guess Speaker: Pauline Schaap	CMS, Manns Room
	5:30 – 6:30	Poster Session	CMS, Central Hall
	6:30	Paella dinner	CMS Garden
Friday, August 21st			
	7:15 – 8:15	Breakfast	Wychfield Site, Trinity Hall
	8:30 – 10:20	Morning Session 1: Biophysics	CMS, Manns Room
	10:20 – 10:40	Break	CMS, Central Hall
	10:40 – 12:05	Morning Session 2: Taxonomy, Phylogeny, & Ecology	CMS, Manns Room
	12:05 – 1:30	Lunch	CMS, Central Hall
	1:30 – 2:55	Afternoon Session 1: Genetics Workshop	CMS, Manns Room
	2:55 – 3:15	Break	CMS, Central Hall
	3:15 – 5:30	Afternoon Session 2: Genomics Workshop	CMS, Manns Room
	5:30 – 6:30	Image, Video, Arts Competition	CMS, Manns Room
	6:30 – 6:50	Walk to Magdalene College	
	7:00	Award Ceremony & Banquet	Magdalene College, Hall
Saturday, August 22nd			
	7:15 – 8:15	Breakfast	Wychfield Site, Trinity Hall
	9:15 – 10:15	Round Table	
	10:45 – 12:10	View City Centre, Market Place	
	12:10 – 12:30	Walk to Scudamore Punting Station	
	12:30 – 1:30	Punting	The Mill
	1:50	Afternoon Tea	Double Tree on Hilton

SCIENTIFIC PROGRAM

THURSDAY, AUGUST 20, 2015

MORNING SESSION 1: LIFE CYCLE (Chair: James Umen)

8:30 – 8:40 **Introduction**

8:40 – 9:05 **Hiroko Kawai-Toyooka** (University of Tokyo)
Isolation and characterization of the plus and minus tubular mating structures from the isogamous volvocine alga *Gonium pectorale*

9:05 – 9:30 **Takashi Hamaji** (Danforth Plant Science Center)
Transcriptome analysis of sexual and vegetative development in *Volvox carteri*

9:30 – 9:55 **Sa Geng** (Danforth Plant Science Center)
Cross-species complementation experiments reveal a complex evolutionary history of the MID gene and its regulatory networks in Volvocine algae

9:55 – 10:20 **Dinah Davison** (University of Arizona)
Phenotypic stability and flexibility in the Volvocaceae

MORNING SESSION 2: CELL DIFFERENTIATION (Chair: Erik Hanschen)

10:40 – 10:50 **Introduction**

10:50 – 11:15 **Zach Grochau-Wright** (University of Arizona)
Genetic Basis for Cellular Differentiation is Present in Undifferentiated Volvocine Green Algae

11:15 – 11:40 **Arash Kianianmomeni** (Bielefeld University)
Cell-type specific light-mediated transcript regulation in *Volvox*

11:40 – 12:05 **Gavriel Matt** (Washington University)
Transcriptomic analysis of cell types in *Volvox carteri* yields new insights into the molecular-genetic basis of germ-soma differentiation

AFTERNOON SESSION 1: DEVELOPMENT (Chair: Matthew Herron)

1:30 – 1:40 **Introduction**

1:40 – 2:05 **Shota Yamashita** (University of Tokyo)
Another evolution for spheroidal colony formation: developmental analysis of *Astrephomene* (Volvocales, Chlorophyta)

2:05 – 2:30 **Pierre Haas/Stephanie Höhn** (Cambridge University)
Mechanics of a *Volvox* Embryo Turning Itself Inside Out

2:30 – 2:55 **Alexey Desnitskiy** (Saint Petersburg State University)
Geographical distribution of the species of *Volvox* and the light/dark control of gonidial divisions

AFTERNOON SESSION 2: EVOLUTION (Chair: Aurora Nedelcu)

3:15 – 3:25 **Introduction**

3:25 – 3:50 **Matt Herron** (University of Montana)
Experimental evolution of multicellularity in *Chlamydomonas reinhardtii*

3:50 – 4:15 **Margrethe Boyd** (University of Montana)
Motility in Multicellular *Chlamydomonas reinhardtii*

4:15 – 4:40 **Erik Hanschen** (University of Arizona)
Cell cycle regulation and the evolution of multicellularity

4:40 – 5:30 **Guest Speaker: Pauline Schaap** (University of Dundee)
Evolution of developmental signalling and cell-type specialization in the Dictyostelia

EVENING SESSION: POSTERS

P1: Benjamin Klein (Bielefeld University)
The inducible *nit*A promoter provides a powerful molecular switch for transgene expression in *Volvox carteri*

P2: Arash Kianianmomeni (University of Bielefeld)
Genome-wide analysis of alternative splicing in *Volvox carteri*

P3: Thomas Pröschold (University of Göttingen and University of Vienna)
Molecular phylogeny and genetic variability within *Chlamydomonas* revealed by multiple gene analyses and AFLP technique

P4: Erik Hanschen (University of Arizona)
Eudorina illinoisensis and the origin of somatic cells

P5: Sarah Cossey (Kansas State University)
Predator induced aggregation of *Chlamydomonas reinhardtii* by a diffusible signal

P6: Yoko Arakaki (University of Tokyo)
Unveiling the initial stage of multicellularity within the Volvocales

P7: Linh Bui (Danforth Plant Science Center)
Developing genetic markers and a method for rapid identification of mutations in Volvox carteri through whole genome resequencing and SNP identification in polymorphic strains

P8: Jillian Walker (Georgia Institute of Technology)
The novel multicellular life cycle of *C. reinhardtii* in predator co-culture

P9: Kyriacos Leptos (University of Cambridge)
The flagellar photoresponse is optimised for high-fidelity phototaxis in *Chlamydomonas*

P10: Jose Ortega-Escalante (University of Maryland Baltimore County)
Resistance to antibiotics hygromycin and blasticidin as new selectable markers for *Volvox carteri*

FRIDAY, AUGUST 21, 2015

MORNING SESSION 1: BIOPHYSICS (Chair: Ray Goldstein)

8:30 – 8:40 **Introduction**

8:40 – 9:05 **Doug Brumley** (Massachusetts Institute of Technology)
Sync and swim: flagellar dynamics in *Volvox carteri*

9:05 – 9:30 **Tim Pedley** (University of Cambridge)
Squirmers with swirl: a model for *Volvox* swimming

9:30 – 9:55 **Kristy Wan** (University of Cambridge)
Evolutionary Roots of Multiflagellation

9:55 – 10:20 **Noriko Ueki** (Tokyo Institute of Technology)
Eyespot overrides the cellular lens effect and is important for determination of correct phototactic direction in Volvocales

MORNING SESSION 2: TAXONOMY, PHYLOGENY, & ECOLOGY (Chair: Hisayoshi Nozaki)

10:40 – 10:50 **Introduction**

10:50 – 11:15 **Hisayoshi Nozaki** (University of Tokyo)
Taxonomic re-examination of two sexual types of "*Volvox africanus*" by Starr (1971), based on the use of new strains from Lake Biwa, Japan

11:15 – 11:40 **Thomas Pröschold** (University of Arizona)
New insights on the phylogenetic position of unicells among the Volvocales

11:40 – 12:05 **Cristian Solari** (University of Buenos Aires)
Predicting abundance as a response to temperature: A chemostat approach using the volvocine green algae

AFTERNOON SESSION 1: GENETICS WORKSHOP (Chair: David Smith)

1:30 – 1:40 **Introduction**

1:40 – 2:05 **Kayoko Yamamoto** (University of Tokyo)
Identification and characterization of the MID orthologs from two homothallic species of *Volvox*

2:05 – 2:30 **Aurelia Honerkamp-Smith** (University of Cambridge)
Geometry of volvocalean somatic cells

2:30 – 2:55 **Tara Marriage** (Kansas State University)
The genetic basis for multicellularity by evolutionary transcriptomics

AFTERNOON SESSION 2: GENOMICS WORKSHOP (Chair: Bradley Olson)

3:15 – 3:25 **Introduction**

3:25 – 3:50 **Matheus Sanita Lima** (University of Western Ontario)
Volvocine chloroplast genomes: what do they hide?

3:50 – 4:15 **Jonathan Featherston** (University of the Witwatersrand)
An Update on the Genome Sequence of *Tetrabaena socialis* NIES-571

4:15 – 4:40 **Aurora Nedelcu** (University of New Brunswick)
Gene co-option, antagonistic pleiotropy, and the evolution of somatic cell differentiation in *Volvox carteri*

4:40 – 5:30 **Bradley Olson** (Kansas State University)
The Volvocales Genome Project: Update and Methodology

EVENING SESSION

5:30 – 6:30 **Image, Video, and Arts Competition**

SATURDAY, AUGUST 22, 2015

9:15 – 10:15 **ROUNDTABLE**

ABSTRACTS

Abstracts published in this volume or posted on the website should be treated as Personal Communications and only cited with the consent of the authors.

TALKS (BY SESSION)

Life Cycle (Chair: James Umen)

Isolation and characterization of the *plus* and *minus* tubular mating structures from the isogamous volvocine alga *Gonium pectorale*

Hiroko Kawai-Toyooka[1], Toshiyuki Mori[1], Shiori Nakazawa[2], Lixy Yamada[2], Masahiro Suzuki[1], Yuko Mogi[1], Takashi Hamaji[3], Shin-ya Miyagishima[4], Hitoshi Sawada[2], and Hisayoshi Nozaki[1]

1. Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan
2. Sugashima Marine Biological Laboratory, Graduate School of Science, Nagoya University, Mie, Japan
3. Donald Danforth Plant Science Center, St. Louis, MO, USA
4. Department of Cell Genetics, National Institute of Genetics, Shizuoka, Japan

Gamete fusion is an essential process of sexual reproduction, although its precise molecular mechanism still remains to be elucidated. The 8- or 16-celled colonial volvocine alga, *Gonium pectorale*, undergoes sexual reproduction by the fusion of equal-sized gametes (isogamy), similar to that in the model unicellular alga, *Chlamydomonas reinhardtii*. Only the *plus* gametes of *C. reinhardtii* possess actin-filled cellular protrusions called tubular mating structures (TMSs), whereas both the *plus* and *minus* gametes of *G. pectorale* possess TMSs (bilateral TMS). The bilateral TMS of *G. pectorale* provides a unique opportunity to focus on the sex-based differences of the fusion organelle. Recent studies have shown the presence of the *G. pectorale* TMS proteins that exclusively localize and function in one sex: a *minus*-specific gamete fusion protein, GpGCS1 (Kawai-Toyooka et al. 2014), and a *plus*-specific gamete attachment protein, GpFUS1 (Hamaji et al. 2013, the 2nd International Volvox Conference). Toward further identifying novel sex-specific TMS proteins that potentially play important roles in gamete fusion, we isolated the TMSs from both the sex gametes of *G. pectorale* by using a modified version of a previously published TMS isolation method for *C. reinhardtii plus* gametes (Wilson et al. 1997) to compare the protein profiles of the *plus* and *minus* TMSs. Activated *G. pectorale* gametes with TMSs obtained by cyclic AMP analog treatment (Mogi et al. 2012) were homogenized and fractionated using sucrose density gradient centrifugation. The fractions with relatively high concentration of TMSs were selected by visualizing the TMSs using fluorescently labeled phalloidin staining. Western blot analysis performed using anti-actin and anti-GpGCS1 antibodies indicated that the TMSs were indeed concentrated in these fractions. In addition, we confirmed

that the TMSs isolated from the cell bodies attached to those of activated gametes of the opposite sex, indicating that they retained the essential factor(s) for attachment. We are in the process of performing/analyzing comparative proteomics of the *plus* and *minus* TMSs by liquid chromatography-tandem-mass spectrometry (LC-MS/MS) on condensed fractions of both TMSs using our on-going nuclear genome sequencing project-based database of *G. pectorale.*

References

Kawai-Toyooka, H., Mori, T., Hamaji, T., Suzuki, M., Olson, B.J.S.C., Uemura, T., Ueda, T., Nakano, A., Toyoda, A., Fujiyama, A., and Nozaki, H. (2014). *Eukaryot. Cell* 13:648-656.

Mogi, Y., Hamaji, T., Suzuki, M., Ferris, P., Mori, T., Kabeya, Y., Miyagishima, S., and Nozaki, H. (2012). *J. Phycol.* 48:670-674.

Wilson, N.F., Foglesong, M.J. and Snell, W.J. (1997). *J. Cell Biol.* 137:1537-1553.

Transcriptome analysis of sexual and vegetative development in *Volvox carteri*

Takashi Hamaji[1], Sa Geng[1], Ayano Miyagi[1], Gavriel Matt[1], Ezra Umen[1], James Umen[1]
1. Donald Danforth Plant Science Center, St. Louis, USA.

Genome-enabled approaches to understanding life cycle transitions in *Volvox* have great promise for elucidating regulatory networks that control growth and development. Deep transcriptome sequencing (RNA-seq) allows comprehensive interrogation and quantitation of gene expression patterns and is an ideal tool to use for identifying gene regulatory networks (GRNs). We performed RNA-seq analyses from samples of synchronous *Volvox carteri* cultures from each sex at two or three hour intervals across all stages of sexual and vegetative development to generate 64 transcriptomes. We have begun to mine these data by identifying genes that are expressed specifically during the sexual cycle of males and females. We identified three classes of sex-regulated genes that were expressed only during sexual development: male specific, female specific and non-gender specific. Among each group were genes expressed early in development as well as those that were expressed in terminally differentiated germ cells. Interestingly, male specific genes were the most numerous class among the three supporting the idea that spermatogenesis entails a more complex developmental and cell-type specialization program than oogenesis. The male specific genes included cell cycle/cell division/embryogenesis-related ones such as cyclin D, DP1, inversion kinesin InvA and regA-like protein RlsC to produce small and abundant sperm cells in sperm packets. In those groups, we also recognized homologs of several known volvocine sex-related genes such as *GSP1*, *GSM1*, and *GCS1*/*HAP2*.

Cross-species complementation experiments reveal a complex evolutionary history of the MID gene and its regulatory networks in Volvocine algae

Sa Geng[1], Ayano Miyagi[1], James G. Umen[1]
1. Donald Danforth Plant Science Center, St. Louis, USA.

Volvocine algae comprise a unique comparative model for investigating the evolution of sexes from an isogamous ancestral state with mating types. In *Chlamydomonas* a single *MT-* gene called *CrMid* (minus dominance) determines mating-type. Mid orthologs are present throughout the Volvocine lineage in either *MT-* or male strains of each species, and in *V. carteri Mid (VcMid)* expression in females is sufficient to induce spermatogenesis. We used ectopic cross-species *Mid* expression experiments to identify when *Mid* from different Volvocine genera 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. When we expressed epitope-tagged *CrMid*, *Gonium pectorale Mid* (*GpMid)* or *VcMid* under the control of a strong promoter in a *C. reinhardtii* mutant *mid-2*, only *CrMid* expression resulted in complementation. In all three cases transgenic mRNA was detected, but only in the case of *CrMid* could Mid protein be detected on Western blots suggesting that GpMid and VcMid were turned over rapidly in *C. reinhardtii*. In contrast, we also 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. *Eve::VcMid-T, Eve::CrMid-T, Eve::GpMid-T* and *Eve::PsMid-T* transgenic strains all constitutively expressed *Mid* mRNAs and tagged Mid proteins meaning that, unlike the case in *C. reinhardtii,* ectopically-expressed Mid proteins from other species are not rapidly turned over in *V. carteri*. *Eve::CrMid-T* lines had no phenotype as reported previously (Geng et al, 2014). *Eve::PsMid-T* lines appeared normal when grown vegetatively, and when sexually induced had a pseudo-male phenotype similar to *Eve::VcMid-T,* but with slightly lower penetrance (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. *Eve::GpMid-T* strains 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 prior to the trait oogamy. This finding suggests that changes in the cis-regulatory networks controlled by Mid proteins rather than changes in Mid were responsible for major innovations leading to the emergence of oogamy.

References:
Geng et al. (2014) PLoS Biol 12:e1001904

Phenotypic stability and flexibility in the Volvocaceae

Dinah R. Davison[1], Richard E. Michod[1]
1. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA

Phenotypic plasticity is the capacity of a given genotype to produce multiple phenotypes. While many traits are known to be plastic, phenotypic plasticity in life history traits may be particularly important in determining organismal fitness in variable environments. Previous work has posited that developmental and morphological complexity may be important determinants of the degree of plasticity particular traits exhibit. Organismal complexity may therefore affect the extent to which differential investment in survival and reproduction varies across environments and is predicted to constrain phenotypic flexibility. This study will use four Volvocaceae species of varying levels of morphological complexity, defined here as the presence or absence of differentiated germ and somatic cells, to test the hypothesis that complexity constrains adaptive phenotypic plasticity in life history traits in response to stress. *Volvox carteri*, *Volvox gigas*, *Pleodorina starrii* and *Eudorina elegans* UTEX 1205 will be exposed to heat shock, cold shock and nitrogen deprivation and monitored for four generations. Data on the mean of and variability in number of germ and somatic cells, colony roundness and colony size at hatching will be collected daily. Results will be presented in full. This study will address the long-standing question regarding the relationship between complexity and phenotypic stability. Moreover, this work will elucidate the ways in which increased complexity may constrain the ability to respond via plasticity to environmental stress.

Cell Differentiation (Chair: Erik Hanschen)

Genetic Basis for Cellular Differentiation is Present in Undifferentiated Volvocine Green Algae

Zachariah I. Grochau-Wright[1], Erik R. Hanschen[1], Patrick J. Ferris[1], Bradley J. S. C. Olson[2], Richard E. Michod[1]
1. Department of Ecology and Evolutionary Biology, University of Arizona. Tucson, USA.
2. Division of Biology, Kansas State University. Manhattan, USA.

The evolution of multicellular individuality from unicellular ancestors represents a major increase in biological complexity. Reproductive division of labor such as germ-soma differentiation plays a critical role in this transition. However, the evolution of the genetic toolkit for germ-soma differentiation remains poorly understood. The volvocine green algae provide an excellent model system to study how the genetic basis for multicellularity and germ-soma differentiation evolves because they range from unicellular *Chlamydomonas* species to *Volvox* species with thousands of cells and germ-soma differentiation. In *Volvox carteri* somatic cell differentiation is controlled by the *regA* gene which is part of a tandem duplication of putative transcription factors known as the *reg* cluster. While previous work has found the *reg* cluster in disparate *Volvox* species, the origin and distribution of the *reg* cluster in the volvocine algae remains unresolved. We used cosmid cloning and sequencing to search for the *reg* cluster from diverse volvocine algae species both with and without somatic cells. We found that the *reg* cluster is present in species lacking specialized somatic cells suggesting a more complicated evolutionary history than previously thought. The genetic basis for cellular differentiation arose prior to the evolution of specialized cell types in this group and was later coopted to produce somatic cells. We propose that the evolution of cellular differentiation in the volvocine algae arose through a change in the genotype-phenotype map, rather than the acquisition of new transcription factors.

Cell-type specific light-mediated transcript regulation in *Volvox*

Arash Kianianmomeni[1]
1. Department of Cellular and Developmental Biology of Plants, University of Bielefeld. Bielefeld, Germany.

The multicellular green alga *Volvox carteri* makes use of none less than 13 photoreceptors, which are mostly expressed in a cell-type specific manner. This gives reason to believe that transcriptome pattern of each cell type could change differentially in response to environmental light. Here, we report that transcript accumulation of genes encoding proteins involved in chlorophyll and carotenoid biosynthesis, light-harvesting complexes, circadian clock and cell cycle control changes differentially between cell types in response to different light qualities. Namely, blue light tends to be effective to accumulate transcripts in the somatic cells; while red light leads to accumulate transcripts predominantly in the reproductive cells. Blue light also induced marked accumulation of two components of circadian rhythms only in the somatic cells, indicating that these clock-relevant components are affected by blue light in a cell-type specific manner. Further, we show that photosynthetic associated genes are regulated distinctly among cell types by different light qualities. These results demonstrate that *Volvox* uses different cell-type specific light signalling pathways to modulate gene expression in a cell-type specific manner. This sophisticated gene expression system has been potentially assured through cell type specific expression of photoreceptors and allows differential regulation of genes involved in various cellular and metabolic pathways in response to environmental light. The existence of cell-type specific light signalling pathways in multicellular organism like *Volvox* reflects an early development of cell-type specific signalling pathways during evolution to ensure maintenance of differentiation.

References:
Kianianmomeni (2015) Plant Signaling & Behavior 10:e1010935.
Kianianmomeni & Hallmann (2015) Current Genetics 61:3.
Kianianmomeni (2014) BMC Genomics 15:764.
Kianianmomeni & Hallmann (2014) Planta 2391:1.

Transcriptomic analysis of cell types in *Volvox carteri* yields new insights into the molecular-genetic basis of germ-soma differentiation

Gavriel Matt[1,2], James Umen[2]
1. Washington University in St. Louis, Molecular Genetics and Genomics. Saint Louis, USA.
2. Donald Danforth Plant Science Center. Saint Louis, USA.

The distinction between a reproductive germline and a sterile soma is foundational to the division of labor in multicellular organisms. *Volvox carteri* provides an excellent model system to study the developmental control of germ-soma differentiation as it is composed of only two cell types: germ cells, called gonidia, and sterile somatic cells. To gain a deeper understanding of the molecular-genetic and metabolic differences underlying germ-soma differentiation in multicellular organisms, we have performed a transcriptomic analysis of *Volvox* gonidial and somatic cells, using high-throughput RNA sequencing. Contrary to a previous report of only 31 cell-type-specific transcripts in *Volvox* (1), we have found over 6000 genes to be significantly differentially expressed between gonidial and somatic cells, demonstrating that germ-soma differentiation in *Volvox* is under much greater transcriptional control than previously thought. *Volvox* germ cells express a broader range of cellular and metabolic functions than do somatic cells and somatically expressed genes are more biased in their expression patterns than are gonidially expressed genes, suggesting that somatic cells are a more specialized cell type. Additionally, somatic-upregulated genes are enriched for genes specific to the Volvocine algae, whereas gonidial-upregulated genes are deeply conserved across the tree of life, demonstrating that differentiation of somatic cells requires the innovation of evolutionarily novel gene sets. Genes involved in photosynthesis and chlorophyll biogenesis are biased towards gonidia, but are still expressed at appreciable levels in somatic cells. This finding challenges the prevailing hypothesis that a central mechanism for germ-soma differentiation in *Volvox* is the global suppression of the chloroplast machinery in somatic cells. Analysis of the cell-type expression patterns of central carbon metabolism genes has revealed that carbon anabolism and catabolism are allocated to gonidial and somatic cells, respectively. In addition, the glyoxylate and gluconeogenesis pathways are significantly upregulated in somatic cells, suggesting that non-photosynthetic mechanisms of sugar biosynthesis play a central role in somatic cell metabolism. These findings in *Volvox* have provided a deeper understanding of the molecular and metabolic mechanisms that evolved during the evolution of multicellularity to specify germ and somatic cell lineages.

References:
1. Tam & Kirk (1991) Developmental Biology 145:51.

Development (Chair: Matthew Herron)

Another evolution for spheroidal colony formation: developmental analysis of *Astrephomene* (Volvocales, Chlorophyta)

Shota Yamashita[1], Yoko Arakaki[1], Hiroko Kawai-Toyooka[1], Hisayoshi Nozaki[1]
1. Department of Biological Sciences, University of Tokyo. Tokyo, Japan.

The volvocine algae constitute a monophyletic group including from unicellular *Chlamydomonas reinhardtii* to multicellular *Volvox* with germ-soma division of labor through various intermediate organisms. Thus, this group is thought to be the unique model for research of the evolutionary pathway from unicellular to multicellular organisms (1). Within the volvocine lineage, there may be an evolutionary tendency from flattened to spheroidal colonies in relation to the increase in colony cell number. Recent phylogenetic studies suggested that the spheroidal colony might have evolved in two independent lineages within the volvocine algae: the Volvocaceae and the genus *Astrephomene* (Goniaceae) (2,3,4).

Astrephomene has 32- or 64-celled spheroidal colonies resembling those of the volvocacean genera *Eudorina* and *Pleodorina*, but it is different from the volvocacean species in having posterior somatic cells in the colony (5). The most distinguishing feature of *Astrephomene* from the Volvocaceae is lack of inversion during colonial development or embryogenesis (5); the embryo of the Volvocaceae undergoes inversion, turning its cell layer inside out to orient flagellar positions of the embryo protoplasts toward the outside. In the model organism, *Volvox carteri*, the shape change and the movement of protoplasts in the embryo might be the fundamental factors of inversion (6,7). However, embryogenesis of *Astrephomene* has not been studied in detail.

Here, we conducted a detailed analysis for the colonial development of *Astrephomene*. As the strains in culture collection are old and do not show normal morphology, we newly established strains of two species of *Astrephomene* from soil samples of rice fields in Japan. Using light microscopy time-lapse imaging, we observed details of divisions and movements of protoplasts during embryogenesis of *Astrephomene*. During 3^{rd}-6^{th} cell divisions, gradual rotation of longitudinal axes of daughter protoplasts occurred to form radially arranged protoplasts. In addition, daughter protoplasts became slender tentatively soon after the last cell division. The latter morphological change resembles that of *Eudorina* and *Volvox* during inversion. The present results will lead to further research in the colonial development of *Astrephomene* using cell biological and molecular data to unveil the genetic bases of parallel evolution of the spheroidal colony formation within the volvocine algae.

References:
1. Kirk (2005) *BioEssays* 27:310.
2. Nozaki & Ito (1994) *J. Phycol.* 30:353.
3. Nozaki et al. (2000) *Mol. Phylogenet. Evol.* 17:256.
4. Herron et al. (2009) *PNAS* 106:3254.
5. Pocock (1954) *Trans. Roy. Soc. S. Afr.* 34:103.
6. Viamontes & Kirk (1977) *J. Cell Biol.* 75:719.
7. Green et al. (1981) *J. Cell Biol.* 91:756.

Mechanics of a *Volvox* Embryo Turning Itself Inside Out

Pierre A. Haas[1], Stephanie Höhn[1], Aurelia R. Honerkamp-Smith[1], P. Khuc Trong[1], and Raymond E. Goldstein[1]
1. Department of Applied Mathematics and Theoretical Physics, University of Cambridge. Cambridge, UK.

The deformations of cell sheets pervade early animal development, but they are driven by an intricate interplay of cell shape changes, cell migration, cell intercalation and cell division. The elucidation of the mechanics and dynamics of these processes has therefore proven difficult. By contrast, the inversion process in *Volvox* (during which the spherical embryos turn themselves inside out to expose the flagellar cell poles) arises from cell shape changes alone, and is therefore more amenable to a physical description. We have recently acquired the first four-dimensional visualisations of inversion in *V. globator* using selective plane illumination microscopy (SPIM) enabling first quantitative analyses of the occurring cell sheet deformations (1). A theoretical model, in which cell shape changes correspond to local variations of the intrinsic bending, contraction and stretching of an elastic shell, reproduces the *in vivo* shapes of "type-B" inversion (2) in *V. globator* (Fig. 1). Our results suggest that active contraction and stretching complements active bending to enable inversion.

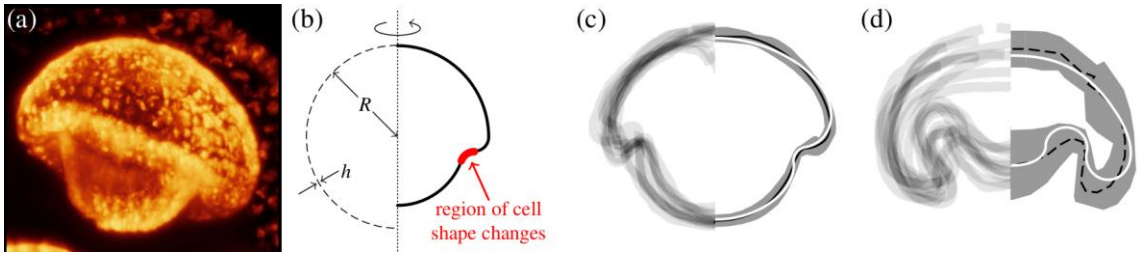

Figure 1. "Type-B" inversion in *Volvox globator*. (a) Embryo of *V. globator* during the early stages of inversion (SPIM). (b) Schematic of elastic model: an elastic sphere of radius *R* and thickness *h* deforms under variations of its intrinsic curvature and stretches. (c,d) Experimental cross-sections of *N* = 10 *Volvox* embryos at two stages during invagination (left) and corresponding fit of the elastic model (right; black line: average of experimental data; grey area: standard deviation thereof; white solid line: shape from elastic model).

Of particular interest is the first step of this "type-B" inversion, the formation of a circular invagination (a generic cell sheet deformation shared with developmental processes in higher organisms). Asymptotic and numerical analyses (3) illustrate how local deformations of the cell sheet overcome global elastic and geometric constraints to stably invaginate the alga, and invert the posterior hemisphere. This adds to our previous work by rationalising the timecourse of the observed cell shape changes and, in particular, an initially narrow invagination that widens as its curvature relaxes.

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Geographical distribution of the species of *Volvox* and the light/dark control of gonidial divisions

Alexey G. Desnitskiy[1]
1. Department of Embryology, St Petersburg State University. St Petersburg, Russia.

In the cultures of *Volvox carteri*, *V. spermatosphaera* and several other species of *Volvox* (as well as in more primitive colonial Volvocaceae) a long period of light-dependent gonidial growth is followed by a palintomic series of rapid consecutive divisions, which may occur in darkness. This type of light/dark control is ancestral for the family Volvocaceae. By contrast, in *V. aureus, V. globator, V. tertius* and several other *Volvox* species with reduced forms of palintomy, a period of light-dependent growth is followed by a series of slow and light-dependent divisions (Herron et al. 2010). This type of light/dark control is derived.

Data on the geographical distribution of all members of the genus *Volvox* have been summarized. Both cosmopolitan species (*V. aureus*) and species with local distribution (e.g., *V. gigas, V. powersii, V. spermatosphaera*) have been detected (Desnitskiy 2008a). An attempt was made to trace a correlation of their latitudinal distribution with the type of light/dark control during asexual development. In high latitudes of the Northern hemisphere (northward of 50—57° north) only *V. aureus, V. globator,* and *V. tertius* occur, in which slow gonidial divisions start in the morning and are temporarily blocked during the night. These features may have adaptive significance under the conditions of long summer day and might have been important for the formation of modern (Holocene) flora of volvocine algae in high latitudes of the Northern hemisphere. Interestingly, in the Southern hemisphere southward of 35—36° south no palintomic *Volvox* species occur, but five nonpalintomic species (*V. aureus, V. barberi, V. globator, V. perglobator*, and *V. tertius*) with light-dependent gonidial divisions have been found there.

The family Volvocaceae originated at least 200 million years ago (Herron et al. 2009). Therefore, *Volvox* evolution predominantly occurred under the warm climate conditions of Jurassic, Cretaceous, and considerable part of Cenozoic. Even during winter the temperature in high latitudes of both Northern and Southern hemispheres might have been favorable for *Volvox* vegetation. However, our experiments show that under the diurnal 8 h light/16 h dark regime (instead of the routine 16/8 regime) development in cultures of *V. carteri* is blocked, whereas *V. aureus* is able to complete the asexual life cycle (Desnitskiy 2008b). These data imply that evolutionary reorganizations of *Volvox* development, which are primarily connected with changes in the rate and light/dark control of gonidial divisions, might have occurred as an adaptation to warm and short winter day in high latitudes in the deep past.

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Evolution (Chair: Aurora Nedelcu)

Experimental evolution of multicellularity in *Chlamydomonas reinhardtii*

Matthew D. Herron[1], William C. Ratcliff[2], Jacob Boswell[1], Michael Travisano[3], Frank Rosenzweig[1]
1. University of Montana, 32 Campus Dr., Missoula, Montana, USA.
2. Georgia Institute of Technology, 310 Ferst Dr., Atlanta, Georgia, USA.
3. University of Minnesota, 100 Church St. S.E., Minneapolis, Minnesota, USA.

The transition 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. Indeed, multicellularity is a prerequisite for the evolution of large, complex organisms such as plants and animals. We have generated *de novo* origins of simple (undifferentiated) multicellularity in two separate experiments in the green alga *Chlamydomonas reinhardtii*, a species that has not had multicellular ancestors. In the first experiment, simple multicellular structures evolved in response to selection for increased size by low-speed centrifugation. In the second experiment, colonial forms reminiscent of *Pandorina* or *Volvulina* evolved in response to predation by the ciliate *Paramecium tetraurelia*. The form of multicellularity observed differs substantially between experiments, suggesting that the particulars of the transition to multicellular life depend not only on the nature of the unicellular ancestor, but on the specific selective pressures driving the transition as well. To understand the genetic changes underlying the transition to multicellular life, we used a combination of whole-genome sequencing, genome-wide expression analysis, and bulked segregant analysis.

Motility in Multicellular *Chlamydomonas reinhardtii*

Margrethe Boyd[1], Frank Rosenzweig[1], Matthew Herron[1]
1. Division of Biological Sciences, University of Montana. Missoula, USA.

The advent of multicellularity was a watershed event in the history of life, yet the transition from unicellularity to multicellularity is not well understood. Multicellularity opens the door to innovations in intercellular communication, cooperation, and specialization, which can provide selective advantages under certain ecological conditions. *Chlamydomonas reinhardtii,* a photosynthetic green alga in the Chlamydomonadaceae, never had a multicellular ancestor yet it is closely related to the volvocine algae, some of which express multicellularity in colonies of up to 50,000 cells. In response to predation and settling rate-based selection, Ratcliff, Herron and colleagues have observed the evolution of simple multicellular structures in several *Chlamydomonas reinhardtii* populations (1). Predation is thought to favor multicellularity by causing an increased survival rate of clusters too large to be ingested, generating a selective advantage in multicellular groups of algae (2). Structures formed in response to predation consisted of individual cells held within a shared transparent extracellular matrix. Importantly, these cells form multicellular clusters obligately in culture conditions where their wild type ancestors do not. Because *C. reinhardtii* is a photosynthetic organism, it possesses an eyespot and two flagella with which it moves towards or away from light in order to secure an optimal input of radiant energy. Motility contributes to *C. reinhardtii* fitness because it allows cells or colonies to achieve this optimum. Utilizing phototaxis as a method to assay motility, I have observed that newly-evolved multicellular strains do not exhibit significant directional movement. Because motility impacts their growth and survival it is an important determinant of fitness for all motile algae, including *C. reinhardtii.* I will continue to compare the phototactic responses of multicellular and unicellular strains in order to better understand the potential trade-offs that may result when unicellular *C. reinhardtii* undergo the transition to multicellularity.

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Cell cycle regulation and the evolution of multicellularity

Erik R. Hanschen[1]
1. Department of Ecology and Evolutionary Biology, University of Arizona. Tucson, USA.

The volvocine green algae have long been viewed as an important model system for the evolution of multicellularity. Recently, the *Chlamydomonas* and *Volvox* genomes revealed strikingly similar genomes suggesting few genetic changes are necessary to evolve multicellularity; however, these data were not able to determine when these genetic changes occur. Sequencing the genome of *Gonium pectorale*, we have demonstrated the early evolution of cell cycle regulation in a multicellular context, through co-option of the retinoblastoma cell cycle regulatory pathway. Significantly, expression of the *Gonium* retinoblastoma cell cycle regulator in unicellular *Chlamydomonas* causes it to become multicellular. The genes which underlie large body size (pherophorins and metalloproteases) and cellular differentiation (VARL genes) are not expanded in *Gonium*, though additional research suggests the *regA* gene cluster evolved shortly after the speciation of *Gonium* and *Volvox* lineages. We propose a novel, three-stage framework for the evolution of multicellularity which and may apply more generally to evolutions of multicellularity. The presence of these changes in undifferentiated *Gonium* highlights the importance of cell cycle regulation during the evolution of multicellularity and indicates extensive group level adaptation during the initial step in the evolution of multicellularity.

Evolution of developmental signalling and cell-type specialization in the Dictyostelia

Pauline Schaap[1]
1. College of Life Sciences, University of Dundee. Dundee, UK

The macroscopic world is dominated by life forms such as animals, plants and fungi that arise through repeated divisions from a cellular spore or zygote. However, in both prokaryotes and eukaryotes, multicellularity evolved many times by aggregation and colony formation of single cells. Currently, aggregative multicellularity has reached the highest level of complexity in the Dictyostelia, members of the eukaryote supergroup Amoebozoa. Up to a million dictyostelid amoebas can move together to form a multicellular organism, that displays light-oriented migration and construction of an architecturally complex fruiting structure. This structures consist of spores that propagate the species and up to four somatic cell types, which have specialized roles in supporting the spore mass and anchoring the structure to the substratum. We performed extensive comparative phenotypic analysis to retrace the evolution of phenotype in Dictyostelia and participated in projects to sequence genomes of unicellular Amoebozoa and Dictyostelium that span the breadth of genetic diversity in Dictyostelia. We use comparative analysis of gene expression patterns and gene modification in clade-representative species to retrace how the function and expression of developmental control genes changed during Dictyostelid evolution. I will discuss how developmental signalling in Dictyostelia evolved from a stress response in the unicellular ancestor and outline how both positional mechanisms and cell sorting contributed to the emergence of somatic cell-type diversification during Dictyostelid evolution.

Biophysics (Chair: Ray Goldstein)

Sync and swim: flagellar dynamics in *Volvox carteri*

Douglas R. Brumley[1], Marco Polin[2], Kirsty Y. Wan[3], Timothy J. Pedley[3], Raymond E. Goldstein[3]
1. Massachusetts Institute of Technology. Cambridge, USA.
2. University of Warwick, UK.
3. University of Cambridge, UK.

Groups of eukaryotic cilia and flagella are capable of coordinating their beating over large scales, routinely exhibiting collective dynamics in the form of metachronal waves (MWs). Despite the ubiquity of such systems, the mechanisms behind this striking coordination remain unclear, in large part due to a lack of quantitative experimental studies.

The colonial alga *Volvox carteri* is an ideal model organism for studying the collective dynamics of eukaryotic flagella, owing to its large size and ease of visualisation. We first present experiments in which two micropipette-held somatic cells, isolated from *Volvox* and with distinct intrinsic beating frequencies, are studied by high-speed imaging as a function of their separation and orientation (1). Analysis of time series shows that the interflagellar coupling, constrained by lack of connections between cells to be hydrodynamical, exhibits a spatial dependence consistent with theory. At close spacings it produces robust synchrony for thousands of beats, while at increasing separations synchrony is degraded by stochastic processes. This study proves unequivocally that flagella coupled solely through a fluid can achieve robust synchrony despite differences in their intrinsic properties.

We also report experiments in which the global flagellar dynamics on the surface of *Volvox* are studied *in situ*. Using particle image velocimetry, we measure the time-dependent fluid velocity around 60 different *Volvox* colonies from 30 s long movies (~1000 beats in each), and use these results to characterize in detail the flagellar coordination for each colony (2). These systems exhibit robust symplectic MWs, in which the wave direction coincides with the direction of the flagellar power stroke. Our studies also reveal for the first time that the average metachronal coordination is punctuated by periodic phase defects during which synchrony is partial and limited to specific groups of cells. A minimal model of hydrodynamically coupled oscillators – inspired by the dynamics of individual flagella (1) – can reproduce semi-quantitatively the characteristics of the average metachronal dynamics, and the emergence of defects (3).

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Squirmers with swirl: a model for *Volvox* swimming

T. J. Pedley[1], D. R. Brumley[2]
1. University of Cambridge, Cambridge, UK
2. Massachusetts Institute of Technology. Cambridge, USA.

A *Volvox* colony takes the form of a perfect sphere that swims because each cell on its surface has a pair of beating flagella. The flagella of the different cells are coordinated, almost certainly hydrodynamically (1), to beat approximately in a meridional plane, with axis of symmetry in the swimming direction, but with a roughly 20 degree azimuthal offset which means that the colonies rotate about their axes as they swim. Experiments on colonies held stationary on a micropipette show that the beating pattern takes the form of a symplectic metachronal wave (1). Here we extend the Lighthill/Blake axisymmetric, Stokes-flow model of a free-swimming spherical squirmer to include azimuthal swirl. The kinematics of the metachronal wave are used to calculate the coefficients in the eigenfunction expansion and hence calculate the swimming speed and rotation rate (proportional to the square of the beating amplitude); measuring these provides a simple means of assessment of the flagellar beating parameters of individual colonies. Extension of the model to include colony interactions, with each other and a plane boundary, leads to simulations of Volvox ‘dancing’: the observed bound states of ref (2).

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Evolutionary Roots of Multiflagellation

Kirsty Y. Wan[1], Raymond E. Goldstein[1]
1. DAMTP, University of Cambridge. Cambridge, United Kingdom.

The large colonial alga *Volvox* - the proverbial “spherical cow”, swims using thousands of flagella which are attached to surface-embedded somatic cells. In the absence of a brain or central nervous system the vigorous, incessant rowing motions of these arrays of tiny flagella are considered “coxless”, and yet waves of highly coordinated activity akin to metachronal waves in a football stadium can extend across the organism. Recent work has demonstrated that hydrodynamic interactions through the immersing fluid can be sufficient to couple each flagellum to its neighbour, leading to synchronization. However, for microorganisms which possess multiple cilia or flagella just how generic is this mechanism?

In the phylogeny of the green algae and in particular its myriad unicellular forms (many of which are ancestral with respect to *Volvox*) we find numerous instances where this naive perspective is found lacking. Here we identify and present ingenious strategies for flagellar coordination adopted by species bearing 2^n flagella (where n is a small integer), reconciling these motility phenomena with the ultrastructural complexity of their flagellar apparatuses.

Eyespot overrides the cellular lens effect and is important for determination of correct phototactic direction in Volvocales

Noriko Ueki[1,2], Shota Mochiji[3], Ken-ichi Wakabayashi[1]
1. Chemical Resources Laboratory, Tokyo Institute of Technology. Meguro, Japan.
2. Department of Biological Sciences, Chuo University. Hachioji, Japan.
3. Department of Biological Sciences, University of Tokyo. Tokyo, Japan.

To move toward photo-environments suitable for survival, motile green algae change their swimming directions by switching between positive and negative phototaxis, i.e., swimming toward or away from the light source. In the unicellular green alga *Chlamydomonas*, cellular reduction-oxidation (redox) poise plays a key role: cells exhibit positive phototaxis after treatment with reactive oxygen species (ROS) while they exhibit negative phototaxis after treatment with ROS quenchers (Wakabayashi et al., 2011). We isolated a *Chlamydomonas* mutant, *ips1* (inverse phototactic sign 1), that exhibits redox-dependent switching of the sign of phototaxis in a manner opposite to the switching in the wild type: negative phototaxis after ROS treatment and positive after ROS-quencher treatment. The *ips1* mutation causes a single amino-acid substitution in the catalytic domain of phytoene synthase (PSY), an enzyme involved in an early step of carotenoid biosynthesis in plants and algae. The *ips1* cells contain carotenoids in only about half the amount in wild type, and about 70% of the cells lack the eyespot. To examine the link between the phototactic sign and the eyespot existence, phototaxis was assayed in known *eyeless* mutants. Surprisingly, like *ips1*, all of the three *eyeless* mutants examined also exhibited an inverse redox-dependent change in the phototactic sign. These observations can be explained by the properties of the *Chlamydomonas* cell body to act as a convex lens. In a wild type cell swimming with bodily rotation around its long axis, the light-shielding effect of the eyespot produces periodically modulated light signal at the photoreceptor site. In eyespot-less mutants, the cellular lens effect causes stronger illumination on the backside of the photoreceptor than on the front side, also leading to periodic stimulation in a swimming and rotating cell. In this case, however, the phase of light signal modulation perceived by the photoreceptor is opposite to that in the wild type cell. The inverted relative light intensity eventually results in inverse sign of phototaxis. We confirmed the presence of the lens effect by direct observation. The importance of eyespot for correct phototactic behavior of multicellular species of Volvocales will also be discussed.

Taxonomy, Phylogeny, & Ecology (Chair: Hisayoshi Nozaki)

Taxonomic re-examination of two sexual types of "*Volvox africanus*" by Starr (1971), based on the use of new strains from Lake Biwa, Japan

Hisayoshi Nozaki[1], Ryo Matsuzaki[1], Kayoko Yamamoto[1], Fumio Takahashi[2]
1. Department of Biological Sciences, University of Tokyo. Tokyo, Japan.
2. Ritsumeikan University, College of Life Sciences. Shiga, Japan.

Volvox is a fantastic alga that was described as a genus by Linnaeus (1758). Smith (1944) recognized ca. 20 species that were subdivided into four sections, based on the morphological difference in spheroids. *Volvox* sect. *Merrillosphaera* is characterized by lacking cytoplasmic bridges between adult cells, and contains about eight species (Nozaki and Coleman 2011).

The vovocine algae (including *Chlamydomonas reinhardtii* and *Volvox*) are an excellent model lineage to study evolution of female and male genders based on comparative analyses of related species (Nozaki et al. 2006, Ferris et al. 2010), in which *V.* (sect. *Merrillosphaera*) *carteri* has been generally studied as a model species of the most advanced members (e.g. Ferris et al. 2010, Prochnik et al. 2010). However, *V. carteri* may represent a non-typical attribute as the most advanced volvocine member such as *MAT3* genes (Hiraide et al. 2013). Thus, information of other species of *Volvox*, especially sect. *Merrillosphaera*, is needed to understand main evolutionary tendency in the volvocine lineage.

Starr (1971) reported four types of sexual reproduction in several strains identified as "*V*. (sect. *Merrillosphaera*) *africanus"* originating from Australia, South Africa, USA and India. However, further taxonomic studies of these strains have not been carried out. Unfortunately, only one [Dara 4 (UTEX 1890; http://www.utex.org/default.asp)] of them is available, but phylogenetic analyses of their sequences of internal transcribed spacer-2 (ITS-2) of nuclear ribosomal DNA (rDNA) were carried out (Coleman 1999).

Here, we studied morphology, sexual reproduction and taxonomy of two species of *V. africanus*-like strains isolated recently from Lake Biwa, Japan. These two species are very similar to two sexual types described by Starr (1971); one with dioecious sexual spheroids and the other producing both male and monoecious spheroids. The former species produced zygotes with a reticulate cell wall whereas a smooth zygote wall was observed in the latter species as in *V. africanus* reported previously from various localities of the world (West 1918, Shaw 1923, Iyengar 1933). Phylogenetic analyses demonstrated that these two species are very closely related to each other. However, presence of a compensatory base change in the nuclear rDNA ITS2 secondary structure and morphological difference in the extracellular matrix of the spheroid supported the separation of the two species, *V. africanus* with a smooth zygote wall and a new species of *Volvox* with a reticulate zygote wall. Although Starr (1971) did not report zygotes of "*V. africanus*," his strains can be considered to include the present two species of *Volvox* based on the ITS2 sequences deposited to NCBI.

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New insights on the phylogenetic position of unicells among the Volvocales

Thomas Pröschold[1,2]
1. Experimental Phycology and Culture Collection of Algae, University of Göttingen. Göttingen, Germany
2. Department of Limnology and Biooceanography, University of Vienna. Vienna, Austria.

It has been considered that the colonial Volvocales evolved among the line *Chlamydomonas- Tetrabaena/Basichlamys- Gonium- Pandorina/Volvulina- Yamagishiella- Astrephomene- Eudorina- Platydorina/Colemaniosphaera- Pleodorina- Volvox*. However, phylogenetic analyses have revealed that unicellular genera such as *Chlamydomonas* and *Vitreochlamys* are distributed among the colonial Volvocales. For example, *Chlamydomonas reinhardtii*, *C. incerta*, and *C. debaryana* are closely related to strains of *Gonium pectorale*.

We studied several species of *Chlamydomonas* and *Vitreochlamys* using an integrative approach (morphology, life cycle, sporangium autolysin, and SSU, ITS and rbcL sequences). The results confirmed that *C. reinhardtii* and its close relatives form together with *Gonium* a monophyletic lineage. Species of *Vitreochlamys* are closely related to *Tetrabaena/Basichlamys*. In addition, several taxa previously designated to different species of *Chlamydomonas* and *Vitreochlamys* represent the sister group of the Volvocales. The phylogenetic reconstruction using complex molecular bioinformatic tools revealed that unicellular taxa derived several times from different lineages of volvocine species. Therefore, the comparative study of uni- and multicellular taxa can provide new insights of the evolution to multicellular organisms.

Predicting abundance as a response to temperature: A chemostat approach using the volvocine green algae

Christian Solari[1]
1. CONICET, Laboratorio de Biología Comparada de Protistas, Departamento de Biodiversidad y Biología Experimental (FCEN), Universidad de Buenos Aires, Buenos Aires, Argentina

Physiologists have a well-developed theory for how the intrinsic rate of increase (*r*) changes with temperature (*T*). Yet, it is not clear how predictions about *r* translate into predictions about the average abundance across a stochastic equilibrium, *N**. To increase our knowledge on how abundance responds to temperature we have analyzed the chemostat model to first understand how a population at equilibrium (*N**) relates to *r* in the most simplified system possible. The chemostat dynamics shows that *N** depends not only depends on *r,* but also on the saturation constant of a limiting nutrient (*K*) and on the flow rate (*w*), which controls the recycling of nutrients and the mortality rate. The model shows that the power of prediction of *r* over *N** highly depends on the values of *K* and *w*, and the two variables might also correlate with temperature. We measured *r* and *K* (using phosphorous as the limiting nutrient) as a function of temperatures in different species of the volvocine green algae to analyze how the response to temperature changes as size and complexity increases, and if those measured values could be used to predict *N**. Our results shows that *r* has the typical asymmetrical humped-shaped response in all species, with a smooth gradual decrease from the optimal to the lower temperatures, and a steep decline to high temperatures, but with very different temperature tolerance. In contrast, the *K* response to temperature varied considerably between species, multicellular *Volvox* species having much higher *K* values than unicellular or small colonial species. Moreover, *K* gradually increased with temperature in *Chlamydomonas*, whereas in *Volvox K* showed a similar response to *r*. Our analysis shows that *r* might not be a good proxy to predict the change in abundance of a population in response to temperature.

Genetics Workshop (Chair: David Smith)

Identification and characterization of the *MID* orthologs from two homothallic species of *Volvox*

Kayoko Yamamoto[1], Toshiyuki Mori[1], Hiroko Kawai-Toyooka[1], Hisayoshi Nozaki[1]
1. Department of Biological Sciences, University of Tokyo. Tokyo, Japan.

In the oogamous genus *Volvox*, there are two types of sexual differentiation, 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. *MID* gene, discovered as a *minus* sex determination gene of *Chlamydomonas reinhardtii* (Ferris & Goodenough, 1997), has been known as a factor to control the sexual differentiation of volvocine algae. *MID* gene ortholog of heterothallic *Volvox carteri* (*VcMID*) is similarly present in its male genome (Ferris et al. 2010). Recently, Geng et al (2014) demonstrated that *VcMID* controls the production of sperm packets. They also reported that transgenic male *V. carteri* with a partial knockdown of *VcMID* produces monoecious species-like sexual spheroids with both eggs and sperm packets. This indicates that the *MID* orthologs in the genus *Volvox* may have some role in determining whether the sexual spheroids are dioecious or monoecious. However, *MID* orthologs have not previously been reported in homothallic or monoecious species of *Volvox*.

Here, we examined *MID* orthologs in two homothallic species of *Volvox*, *V. africanus* (belonging to section *Merrillosphaera* with *V. carteri*) and *V. ferrisii* (section *Volvox*). The former species originating from Lake Biwa, Japan is very similar to one of the four sexual types reported by Starr (1971), producing both male and monoecious spheroids. To obtain information from other homothallic species in other sections of the genus *Volvox*, we focus on a monoecious species of section *Volvox*, *V. ferrisii,* that is phylogenetically separated from *V. carteri* and *V. africanus*. We determined the entire sequences of *MID* homologs from these two homothallic species. These sequences were not significantly different from those of heterothallic species of *Gonium*, *Pleodorina* and *Volvox* in synonymous/nonsynonymous substitution or functional constraint. Our results suggest that *MID* gene in homothallic species has the same mode of action as that of heterothallic species.

In *V. carteri,* the *VcMID* gene is constitutively expressed in both asexual and sexual spheroids of male strain, and post-transcriptional, rather than transcriptional, controls have been shown to restrict the *VcMID* function to androgonidia (sexual male germ cells) (Ferris et al. 2010; Geng et al. 2014). We here measured *MID* expression level in asexual and sexual spheroids of *V. africanus* by semi-quantitative RT-PCR. Unlike *VcMID*, expression of the *MID* gene in *V. africanus* was significantly higher in male spheroids than that in monoecious and asexual spheroids, suggesting that the *MID* gene in *V. africanus* is regulated by some transcriptional mechanisms to produce sperm in this organism.

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Geometry of volvocalean somatic cells

Aurelia Honerkamp-Smith[1], Hugo Wioland[2], Stephanie Höhn[1], Raymond E. Goldstein[1]
1. Department of Applied Mathematics and Theoretical Physics, University of Cambridge. Cambridge, United Kingdom.
2. Institut Jacques Monod. Paris, France.

The hexagonal pattern of somatic cells forming the spheroidal body of *Volvox carteri* and other species is familiar to anyone who has viewed them. While hexagonal packing is the most efficient way to pattern a flat surface, defects in the pattern are required to achieve a spherical surface. Here we investigate the extent to which somatic cell placement is consistent with the purely geometric constraint of evenly covering the surface of a sphere. Are there deviations from the optimal distribution that correspond to biological or developmental constraints? We use light sheet microscopy to obtain the location in three dimensions of every cell of small volvox colonies and quantify their pattern. We then compare this pattern to theoretical and experimental idealized distributions.

The genetic basis of multicellularity by evolutionary transcriptomics

Tara N. Marriage[1], Bradley J.S.C. Olson[1]
1. Division of Biology, Kansas State University. Manhattan, USA.

Multicellularity has independently evolved at least 25 times throughout the history of life on earth. Multiple hypotheses have been proposed to explain how multicellularity evolved, yet no definitive answer exists for its origin. The Volvocine algae are a model system to study the evolution of multicellularity because they consist of a recently evolved (~200 mya) monophyletic group of organisms that morphologically span from unicellular (*Chlamydomonas reinhardtii*) to colonial multicellular (*Gonium pectorale*) to multicellular with germ and soma differentiation (*Volvox carteri*). *C. reinhardtii* and *G. pectorale* grow and divide similarly; however when *G. pectorale* cells under go multiple fission (multiple rounds of mitotic divisions), the daughter cells remain attached to each other, whereas in *C. reinhardtii*, the daughter cells separate. This, along with other experimental evidence, suggests that the colonial multicellular phenotype of *G. pectorale* is under cell-cycle regulation. In this experiment, we used RNA-Seq analysis to investigate changes in gene expression across the 24-hour cell cycle of *G. pectorale*. Strong candidate *G. pectorale* multicellularity genes were identified through a two-step process. First genes were filtered by mitotic expression profiles, and secondarily filtered by whether these differentially mitotically expressed genes showed evidence of purifying selection. This two-step filtering process revealed five strong candidate genes, one of which is a fasciclin-like gene, that when transformed into the unicellular *Chlamydomonas,* results in a multicellular phenotype with cytoplasmic connections.

Genomics Workshop (Chair: Bradley Olson)

Volvocine chloroplast genomes: what do they hide?

Matheus Sanitá Lima[1], David Roy Smith[1]
1. Department of Biology, University of Western Ontario. London, Canada.

Chloroplasts, the photosynthetic organelles, originated from an endosymbiosis between the ancestral eukaryotic cell and cyanobacteria. Since then, they have been spread throughout the eukaryotic tree of life by multiple later endosymbiotic events, always maintaining their own genomes (1). We first described these chloroplast (cp) genomes as circular DNA molecules ranging between 110-190 kb (2). However, after 30 years of organelle genomics, we have found cp genomes ranging from ≥ 1Mb (in *Acetabularia acetabulum*) to ~30 kb (in *Symbiodinium* sp) and exhibiting linear and circular structures with a wide variety of content (3).

Within this genomic myriad, the Volvocales occupy a special place. *Volvox carteri* and *Dunaliella* spp. have expanded cp genomes (~525kb and at least 370kb, respectively) with a large amount of noncoding DNA and very low genetic diversity (4,5). On the other hand, *Polytomella* spp. seem to have a plastid without any genetic material (note that *Polytomella* spp. are a non photosynthetic lineage, thereat they do not have a chloroplast, but a plastid) (6). Why *Polytomella* spp lack their plastid genome is still under investigation, while *V. carteri*'s bloated genome seems to be well explained by the mutational hazard theory (7,8).

However, the mutational hazard theory is only one in many theories trying to explain why we have this wide variation in genome size (the so called "C-value enigma") (8). Therefore in this talk, we would like to address some of the main theories about genome size variation, emphasizing the role of the Volvocales in the development and corroboration of these theories. Even though the Volvocales have very famous model organisms (e.g. *Chlamydomonas reinhardtii* and *V. carteri*), this group of green algae, as a whole, is still not well known (9). The future prospects rely on the genomics of the group, so we also call the Volvocales Genome Project. Who knows what is hidden in the chloroplast genomes of the several volvocine species? Maybe the explanation for the diversity of genome size, structure and content.

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An Update on the Genome Sequence of *Tetrabaena socialis* NIES-571

Jonathan Featherston[1,2] and the Volvocales Genome Project Consortium
1. Department of Molecular Medicine and Sydney Brenner Institute for Molecular Biosciences, University of the Witwatersrand. Johannesburg, South Africa.
2. Agricultural Research Council, Biotechnology Platform. Pretoria, South Africa.

The Tetrabaenaceae family are basal to the Goniaceae and Volvocaceae families and are of great interest for early developments required for the evolution of multicellularity such as the evolution of an extracellular matrix and the genetic modulation of cell number. Here we describe the genome sequencing initiative of the four-celled archetypal member of this family; *Tetrabaena socialis,* described as the “simplest integrated multicellular organism”. *T. socialis* strain NIES-571 was sequenced using Illumina sequencing chemistry on a combination of Hiseq and Miseq platforms. The draft genome assembly was performed predominantly using AllpathsLG but was improved by combining the assembly using Metassembler with a SPADES assembly. The assembly was further improved using GapFiller and Pilon. The final (version 1) draft assembly yielded a total assembly size of 135,7MB in 5856 scaffolds. The current L50 of the assembly is 263 and scaffold N50 is 145,9KB (contig N50 of 7,4KB). At ~65% GC the genome was found to be similar in GC content to *Chlamydomonas reinhardtii* and *Gonium pectorale*. When compared to other lineages (such as Human versus Chicken) the analysis of synteny between *C. reinhardtii* and *Volvox carteri* revealed a high-degree of structural rearrangement. Similar levels of syntenic rearrangements were identified in *T. socialis* (scaffolds ≥ to 100KB). To date, using only protein homology as evidence a total 7740 genes have been modeled using the MAKER genome annotation pipeline, of which over 65% have domains present. Transcriptome sequencing is currently being performed and will be used for gene modeling. Functional annotation will be completed using the Volvocales Genome Project annotation pipeline.

Gene co-option, antagonistic pleiotropy, and the evolution of somatic cell differentiation in *Volvox carteri*

Aurora M. Nedelcu[1] and Stephan G. Koenig[1]
1. Department of Biology, University of New Brunswick, Fredericton, Canada.

The evolution of a non-reproductive cell lineage (i.e., soma) was instrumental to the increase in complexity observed in many multicellular lineages. Although the presence of somatic cells can be explained in terms of the selective advantages of specialization and division of labor, the mechanistic basis for the evolution of somatic cell differentiation is less understood. We have proposed that the evolution of soma involved the co-option of ancestral life-history genes whose expression was conditioned on environmental cues (as an adaptive strategy to enhance survival at an immediate cost to reproduction), through shifting their expression from a temporal/environmentally-induced into a spatial/developmental context. In the multicellular green alga *Volvox carteri*, a single gene (*regA*) is necessary and sufficient to establish (by an unknown mechanism) the terminally differentiated somatic cell fate. We have reported that *regA*'s closest homolog (*rls1*) in a unicellular relative of *V. carteri* (*Chlamydomonas reinhardtii*) is expressed in environmental settings in which reproduction is temporary arrested. More recently, using a new *regA*$^{-}$/gonidialess double mutant strain we showed for the first time that in addition to its developmental expression, *regA* can be induced by environmental stimuli, and this induction is dependent on cell size. Furthermore, in mutants expressing a non-functional RegA protein, the conditions that trigger *regA* expression also induce programmed cell death, which points towards a dual function for *regA* in cell fitness: to decrease cell reproduction (by repressing cell growth) and to increase cell survival (by conferring resistance to stress). Genes with antagonistic pleiotropic effects on fitness have been proposed to stabilize cooperation, and *regA* is the first such example in multicellular organisms with unitary development (i.e., developed from a single cell). This work provides new insights into our understanding of somatic cell differentiation, from both a mechanistic and an evolutionary perspective.

The Volvocales Genome Project: Update and Methodology

Bradley J.S.C. Olson[1], Erik R. Hanschen[2], Tara N Marriage[1], Takashi Hamaji[3], Pierre Durand[2,4], Hisayoshi Nozak[i3], Patrick J. Ferris[2] Jonathan Featherston[4], Masahiro Suzuki[3], Hiroko Kawai-Toyooka[3], Asao Fujiyama[5], Atsushi Toyoda[5], and Richard E. Michod[2]

1. Division of Biology, Kansas State University. Manhattan, USA.
2. Department of Ecology and Evolutionary Biology, University of Arizona. Tucson, USA
3. The Danforth Plant Science Center, St. Louis, USA.
4. Department of Molecular Medicine and Sydney Brenner Institute for Molecular Biosciences, University of the Witwatersrand. Johannesburg, South Africa.
5. National Institute of Genetics, Department of Genetics, Shizuoka, Japan

The Volvocales and their unicellular relatives have long been viewed as an important model system for the evolution of multicellularity and cellular differentiation. *Chlamydomonas* and to a lesser degree *Volvox* are well developed molecular-genetic model systems, but other Volvocacean species representing important evolutionary steps toward multicellularity, have not been as well developed. The completion and publication of the genomes of *Chlamydomonas* and *Volvox* has demonstrated that even though these organisms differ markedly in the morphology, their genomes are surprisingly similar. This suggests that the evolutionary path to multicellularity and cellular differentiation requires only a few genetic changes. The upcoming publication of the genome of *Gonium pectorale* is consistent with this interpretation, though additionally reveals the early evolution of much of this genetic basis. With the availability of next-generation sequencing technology, the Volvocales Genome Project seeks to focus on sequencing the genomes of *Basichlamys sacculifera*, *Tetrabaena socialis*, *Astrephomene gubernaculifera*, *Pandorina morum*, *Pleodorina starrii*, and *Volvox ferrisii.* The project is emphasizing quality data sets including genome sequence, annotation and life-cycle transcriptomes.

POSTERS

P1

The inducible *nit*A promoter provides a powerful molecular switch for transgene expression in *Volvox carteri*

Benjamin Klein[1], Eva Laura von der Heyde[1], Lars Abram, Armin Hallmann[1]
1. Department for Developmental and Cell Biology of Plants, Bielefeld University. Bielefeld, Germany.

Functional and molecular analyses of various aspects of multicellularity in *Volvox carteri* require a wide array of molecular tools for genetic engineering. So far there are only a limited number of these tools available in *Volvox*. We showed that the promoter of the *V. carteri* nitrate reductase gene (*nit*A) is a powerful molecular switch for induction of transgene expression. Strong expression is triggered by simply changing the nitrogen source from ammonium to nitrate. We also showed that the luciferase (*g-luc*) gene from the marine copepod *Gaussia princeps*, which previously was engineered to match the codon usage of the unicellular alga *Chlamydomonas reinhardtii*, is a suitable reporter gene in *V. carteri*. Emitted light of the chemiluminescent reaction can be easily detected and quantified with a luminometer. Long-term stability of inducible expression of the chimeric *nit*A/*g-luc* transgenes after stable nuclear transformation was demonstrated by transcription analysis and bioluminescence assays.

P2

Genome-wide analysis of alternative splicing in *Volvox carteri*

Arash Kianianmomeni[1], Cheng Soon Ong[2], Gunnar Rätsch[3], Armin Hallmann[1]
1. Department of Cellular and Developmental Biology of Plants, University of Bielefeld. Bielefeld, Germany.
2. Machine Learning Group, NICTA Canberra Research Laboratory. Canberra, Australia.
3. Biomedical Data Science Group, Memorial Sloan-Kettering Cancer Center. New York City, USA.

Alternative splicing is an essential mechanism for increasing transcriptome and proteome diversity in eukaryotes. Particularly in multicellular eukaryotes, this mechanism is involved in the regulation of developmental and physiological processes like growth, differentiation and signal transduction. Here we report the genome-wide analysis of alternative splicing in the multicellular green alga *Volvox carteri*. The bioinformatic analysis of 132,038 expressed sequence tags (ESTs) identified 580 alternative splicing events in a total of 426 genes. The predominant type of alternative splicing in *Volvox* is intron retention (46.5%) followed by alternative 5′ (17.9%) and 3′ (21.9%) splice sites and exon skipping (9.5%). %). Our analyses show that at least ~2.9% of intron-containing genes in *Volvox* are alternatively spliced. Considering the number of analyzed ESTs, it is very likely that *Volvox* genome possesses more favorable conditions, e.g., changes in the length and GC content of introns, for the occurrence of alternative splicing than those of the closely related *Chlamydomonas*. On the other hand, an analysis of the alternative-splicing status of homologous genes from the closely related alga *Chlamydomonas* could show that a fraction of the genes that are alternatively spliced in *Volvox* are not alternatively spliced in *Chlamydomonas*. Concurrently with our study, Urrutia and colleagues examined how alternative splicing was related to organismal complexity by analyzing alternative splicing in 47 eukaryotic species. They found that alternative splicing has steadily increased over eukaryotic evolution and is strongly associated with organismal complexity and cell-type number. Therefore, it might be conceivable that alternative splicing acts as a key regulatory factor to facilitate the evolution of multicellularity in volvocine algae. To confirm the alternative splicing events identified by bioinformatic analysis, several genes with different types of alternatively splicing have been selected followed by experimental verification of the predicted splice variants by RT-PCR. The results show that our approach for prediction of alternative splicing events in *Volvox* was accurate and reliable. Moreover, quantitative real-time RT-PCR appears to be useful in *Volvox* for analyses of relationships between the appearance of specific alternative splicing variants and different kinds of physiological, metabolic and developmental processes as well as responses to environmental changes.

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P3

Molecular phylogeny and genetic variability within Chlamydomonas revealed by multiple gene analyses and AFLP technique

Thomas Pröschold[1,2], Tatyana Darienko[1]

1. Experimental Phycology and Culture Collection of Algae, University of Göttingen. Göttingen, Germany.

2. Department of Limnology and Biooceanography, University of Vienna. Vienna, Austria.

Phylogenetic analyses have revealed that the genus Chlamydomonas (in traditional sense) is polyphyletic. Taxonomically revised the genus contains only four species, C. reinhardtii, C. incerta, C. debaryana, and a new species, which is not described yet. We studied all available strains using an integrative approach (morphology, life cycle, sporangium autolysin, and SSU, ITS and rbcL sequences). To detect the genetic variability within the species, we analyzed the AFLP patterns (Amplified Fragment Length Polymorphism) using two pairs of restriction enzymes (EcoRI+MseI and EcoRI+PstI).

All Chlamydomonas species showed similar morphology and life cycle. However, the strains could be clearly differentiated to four species by phylogenetic analyses of SSU, ITS and rbcL sequences. The new species, the closest relative to C. reinhardtii, differs by mucilage surrounding of vegetative cells and compensatory base changes in the ITS sequences to the other species of Chlamydomonas. Using synchronized cultures the sporangium autolysins of each species could be harvested and tested in crossing experiments. The autolysins of C. reinhardtii, C. incerta, and the new species react on fixed sporangia of these species in both directions, but do not lyse the sporangia cell walls of C. debaryana. In contrast, the autolysin of C. debaryana react on sporangia of all species. The AFLP technique showed characteristic pattern for each species.

P4

Eudorina illinoisensis and the origin of somatic cells

Erik R. Hanschen[1], Baltazar Chavez-Diaz[1], Matthew Herron[2], Richard E. Michod[1]
1. Department of Ecology and Evolutionary Biology, University of Arizona. Tucson, USA.
2. Division of Biological Sciences, University of Montana. Missoula, USA.

The volvocine green algae have long been viewed as an important model system for the evolution of multicellularity. Extensive research has been performed on the evolution of somatic cellular differentiation including genetic, empirical, phylogenetic, and mathematical analysis. One remaining question is the evolutionary origin of somatic cells, and understanding how original function of somatic cells relates to current function in existing species. We hypothesize that the number of somatic cells at the origin of cellular differentiation was low and beneficial functions may have increased as the number of somatic cells increase. To test this hypothesis, we investigate the likely ancestral state of soma in the volvocine algae. Using empirical approaches, we investigate somatic cells in *Eudorina illinoisensis*, which is reported to sometimes have a few anterior somatic cells. Using *E. illinoisensis* as a model for the possible origin of soma, we demonstrate photosynthetic and flagellar function. After investigating the possible function of these somatic cells in nutrient deplete conditions, we suggest possible future directions.

P5

Predator induced aggregation of *Chlamydomonas reinhardtii* by a diffusible signal

Sarah Cossey[1], Christopher Berger[1], Bradley J. S. C. Olson[1]
1. Division of Biology, Kansas State University. Manhattan, USA.

The evolution of multicellularity is a major evolutionary transition, occurring at least twenty five independent times across eukaryotic lineages. However, the ecological pressures that stimulate this transition are poorly understood. *Chlamydomonas* and its multicellular relatives show a linear progression of morphological complexity, from unicellular *Chlamydomonas reinhardtii* to colonial multicellular *Gonium pectorale* to *Volvox carteri* which is multicellular with differentiated tissues. It has been hypothesized that multicellularity evolved to increase organismal size thus, allowing it to evade predation. In the presence of predators such as *Daphnia*, we found that *C. reinhardtii* will rapidly form aggregates. However, in nutrient limited media we did not see aggregation. We hypothesize that the aggregation response seen in the predated *C. reinhardtii* has become genetically permanent in multicellular Volvocine algae species. To test this hypothesis, we are characterizing the predator response in *C. reinhardtii* and exploring how it evolved in its multicellular relatives. First, bacteria can induce aggregation of *C. reinhardtii* cells, so we developed a method to prepare axenic *Daphnia* (removal of bacterial and fugal contaminants) such that their sole food source is *C. reinhardtii* cells. The axenic *Daphnia* then feed on the *C. reinhardtii* cells and a rapid aggregation response is observed. When media from the predator treated *C. reinhardtii* cells is added to untreated *C. reinhardtii* an aggregation response is also observed, suggesting that there is a mobile signal released into the media, causing this response. Interestingly, *G. pectorale*, a colonial multicellular relative of *C. reinhardtii* does not respond to predation by aggregation, suggesting that the aggregation response may be genetically permanent in this species. Here we will report the characterization of the signal that is responsible for this aggregation response. In summary, our data indicates that predation may have driven the evolution of multicellularity in the Volvocine algae.

P6

Unveiling the initial stage of multicellularity within the Volvocales

Yoko Arakaki[1], Mizuho Sugasawa[1], Ryo Matsuzaki[1], Hiroko Kawai-Toyooka[1], Hisayoshi Nozaki[1]
1. Department of Biological Sciences, University of Tokyo. Tokyo, Japan.

Multicellularity is one of the general evolutionary events in eukaryotic lineages (1), although it may represent one of the most mysterious biological issues. Most eukaryotic lineages lack transitional forms from unicellular to multicellular; thus how single cells were combined, integrated, and differentiated into a multicellular organism has remained almost obscure. However, volvocine algae, which encompass both unicellular *Chlamydomonas reinhardtii* and various multicellular species, may be a powerful tool for studying multicellularity.

Colonial volvocine algae constitute a robust monophyletic group composed of Tetrabaenaceae, Goniaceae, and Volvocaceae. Both morphological and molecular data suggest that the most basal group is Tetrabaenaceae (2, 3). Thus, the four-celled tetrabaenacean species *Tetrabaena socialis* is very important to discuss the initial stage of multicellularity. Until recently, however, morphological data were not sufficient to demonstrate whether *T. socialis* has multicellular morphological traits or not. We established synchronous cultures of *T. socialis* (4) and carried out immunofluorescence microscopic and ultrastructural observations that demonstrated that *T. socialis* has two important multicellular features proposed by Kirk (5): “rotational asymmetry of cells” to let the cells become components of the individual and “cytoplasmic bridges between protoplasts in developing embryos” to maintain the species-specific form of the multicellular individual. These two features are also found in more advanced members of colonial or multicellular volvocine algae such as *Gonium* and *Volvox*. Therefore they might have evolved in the common ancestor of the extant colonial volvocine algae. *T. socialis* is the simplest integrated multicellular “volvocine” alga (4).

Pascherina tetras is another member of the four-celled volvocalean algae (6, 7). *P. tetras* belongs to the family Spondylomoraceae because it lacks a gelatinous matrix encompassing the colony. Nakada et al. (8) performed phylogenetic analysis of a strain labeled “*P. tetras*”; however this strain forms only unicellular cells and lacks evidence for species identification. Thus, phylogenetic position of *P. tetras* had been still unclear until we very recently established new strains of four-celled colonies of *P. tetras* from Japan (9). Our multigene phylogeny demonstrated that the four-celled alga *P. tetras* is separated from other colonial volvocalean algae that constitute three lineages. This indicated that multicellularity of *P. tetras* have evolved in a different origin from other colonial Volvocales and *P. tetras* may represent an ongoing initial stage of multicellularity. Further studies using *P. tetras* and *T. socialis* may unveil the mystery of multicellular evolution.

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P7

Developing genetic markers and a method for rapid identification of mutations in *Volvox carteri* through whole genome resequencing and SNP identification in polymorphic strains

Linh T. Bui[1], James G. Umen[1]
1. Donald Danforth Plant Science Center. Saint Louis, USA.

Forward genetics is an important and well-established approach for identifying developmental regulators in model organisms, and has uncovered many loci in *Volvox carteri* with interesting and informative phenotypes. Historically, a rate-limiting step in forward genetic analysis has been the time and effort required for meiotic mapping and molecular cloning. Whole genome sequencing (WGS) relieves this bottleneck by providing a fast and effective tool for identifying causative mutations. We are developing a hybrid approach to "cloning by re-sequencing" that will involve low-resolution meiotic mapping followed by identification of linked mutations through genome re-sequencing. To do so we are sequencing seven *V. carteri* f. *nagariensis* isolates to identify SNP markers that will be used to catalog genetic variation, build a unified physical and genetic map, and generate meiotic mapping markers. Markers will be identified for each major contig in the reference genome assembly and a set of F1 mapping progeny will be generated from suitable parental strains and used to establish genetic linkage between *Volvox* genomic contigs. These markers will be used in future studies to map mutations in developmental regulators that can subsequently be identified rapidly by re-sequencing. Progress in SNP identification and molecular genetic mapping will be presented.

P8

The novel multicellular life cycle of *C. reinhardtii* in predator co-culture

Jillian Walker[1], Matthew Herron[2], Frank Rosenzweig[2], William Ratcliff[1]
1. School of Biology, Georgia Institute of Technology, Atlanta, Georgia, USA
2. Division of Biological Sciences, University of Montana, Missoula, Montana, USA

The transition to multicellularity allowed for the evolution of large organisms with complex traits and structures, but the early steps in this shift remain poorly understood. To explore the origins of multicellularity, we co-cultured *Chlamydomonas reinhardtii* with a unicellular predator (*Paramecium*). Over approximately 750 generations, multicellular isolates evolved in three separate lines. Here, we examine their novel multicellular life cycles and find that they are quite regulated, with consistent temporal patterns that persist even in the absence of predators. This suggests that there may be some genetic control over cell number and dispersal, a key step in the evolution of multicellularity in the volvocine algae.

P9

The flagellar photoresponse is optimised for high-fidelity phototaxis in *Chlamydomonas*

Kyriacos C. Leptos[1], Raymond E. Goldstein[1]
1. Department of Applied Mathematics and Theoretical Physics, University of Cambridge. Cambridge, United Kingdom.

Motile photosynthetic algae have the ability to change their direction of motion towards light in order to harvest it for their photosynthetic requirements, a process called *phototaxis*. In this study, we investigate how the flagellar photoresponse in the biflagellate alga *Chlamydomonas* is optimised to efficiently reorient its direction of swimming towards light. In particular, to steer towards light the cell has to differentially change the front amplitudes of its two flagella in a timely and alternating fashion, as it rotates while swimming. This coordinated response takes place while the cell's eyespot is experiencing light of oscillating intensity. Using high-speed video microscopy on light-stimulated immobilised single cells, we measure the two time scales involved in the photoresponse, a fast (reaction) one and a slow (adaptation) one. Moreover, using these measured values along with a mathematical model for light adaptation, we theoretically predict that an optimal response towards an oscillating light stimulus would take place at a frequency that is about the cell body's rotation frequency. Additional preliminary experimental data corroborate the theoretical prediction.

P10

Resistance to antibiotics hygromycin and blasticidin as new selectable markers for *Volvox carteri*

Jose A. Ortega-Escalante[1], Owen Kwok[1], Stephen Miller[1]
1. University of Maryland Baltimore County. Maryland, USA.

Volvox carteri is an excellent model system for investigating evolution of multicellularity and cell differentiation. Several selectable markers for nuclear transformation of *V. carteri* have been developed, including the *nitA* gene (encoding nitrate reductase) and genes for resistance to the antibiotics zeocin and paromomycin, but there are disadvantages to using each of these markers. For instance, *nitA* can only be used to transform Nit mutants, and transformants resistant to the antibiotic resistance markers can be difficult to select and/or are sometimes unstable. To improve nuclear transformation of *V. carteri*, we developed vectors that provide stable, easily selectable resistance to the antibiotics hygromycin and blasticidin. Our lethal-dose experiments indicate that *V. carteri* is sensitive to both antibiotics at a concentration of 10 ug/mL. We generated vectors with *Volvox*-specific regulatory sequences (*hsp70A*/*rbcS3* promoter and *rbcS2* 3' UTR) and codon-optimized hygromycin and blasticidin resistance genes from *Escherichia coli* and *Bacillus cereus*, respectively, that contain the first intron of the small subunit of the *rbcS2* gene included into the coding region of these markers. We are also testing additional 5' and 3' regulatory sequences, including cassettes from the β-tubulin, ferredoxin and ribosomal protein L14 genes. Here we report results from transformation and co-transformation experiments with these vectors, including transformation rates, transgene insertion, transgene expression and stability of resistance and describe their utility as new selectable marker genes for *V. carteri*.

LIST OF PARTICIPANTS

First Name	Surname	Email	Institution	Page
Yoko	Arakaki	yk_arakaki@bs.s.u-tokyo.ac.jp	University of Tokyo, Japan	**43**
Margrethe	Boyd	margrethe.boyd@umontana.edu	University of Montana, USA	**21**
Douglas	Brumley	brumley@mit.edu	Massachusetts Institute of Technology, USA	**24**
Linh	Bui	lbui@danforthcenter.org	Danforth Plant Science Center, USA	**44**
Sarah	Cossey	scossey@ksu.edu	Kansas State University, USA	**42**
Ottavio	Croze	oac24@cam.ac.uk	Cambridge University, UK	**--**
Dinah	Davison	dinahdavison@email.arizona.edu	University of Arizona, USA	**13**
Alexey	Desnitskiy	adesnitskiy@mail.ru	St. Petersburg State University, Russia	**19**
Jonathan	Featherston	featherstonj@arc.agric.za	University of the Witwatersrand, South Africa	**35**
Sa	Geng	sgeng@danforthcenter.org	Danforth Plant Science Center, USA	**12**
Ray	Goldstein	R.E.Goldstein@damtp.cam.ac.uk	Cambridge University, UK	**--**
Zachariah	Grochau-Wright	zig@email.arizona.edu	University of Arizona, USA	**14**
Pierre	Haas	pah59@cam.ac.uk	Cambridge University, UK	**18**
Takashi	Hamaji	thamaji@danforthcenter.org	Danforth Plant Science Center, USA	**11**
Erik	Hanschen	hanschen@email.arizona.edu	University of Arizona, USA	**22**
Matthew	Herron	matthew.herron@mso.umt.edu	University of Montana, USA	**20**
Stephanie	Höhn	S.hoehn@damtp.cam.ac.uk	Cambridge University, UK	**18**
Aurelia	Honerkamp-Smith	ah659@cam.ac.uk	Cambridge University, UK	**32**
Hiroko	Kawai-Toyooka	hiro_toyo@bs.s.u-tokyo.ac.jp	University of Tokyo, Japan	**9**
Arash	Kianianmomeni	kianian@uni-bielefeld.de	Bielefeld University, Germany	**15**
Benjamin	Klein	bklein@uni-bielefeld.de	Bielefeld University, Germany	**38**
Kyriacos	Leptos	K.Leptos@damtp.cam.ac.uk	Cambridge University, UK	**46**
Tara	Marriage	tmarria@ksu.edu	Kansas State University, USA	**33**
Gavriel	Matt	gavrielmatt@wustl.edu	Washington University, USA	**16**
Haydee	Montoya T	haydmon@yahoo.com	Natural History Museum, Peru	**--**
Aurora	Nedelcu	anedelcu@unb.ca	University of New Brunswick, Canada	**36**
Hisayoshi	Nozaki	nozaki@bs.s.u-tokyo.ac.jp	University of Tokyo, Japan	**28**
Brad	Olson	bjsco@k-state.edu	Kansas State University, USA	**37**
Jose	Ortega	ortegaj1@umbc.edu	University of Maryland, USA	**47**
Tim	Pedley	T.J.Pedley@damtp.cam.ac.uk	University of Cambridge, UK	**25**
Thomas	Pröschold	Thomas.Proeschold@me.com	Goettingen University, Germany	**29, 40**
Matheus	Sanita Lima	matheus.sanita@unb.ca	University of Western Ontario, Canada	**34**
Pauline	Schaap	p.schaap@dundee.ac.uk	University of Dundee, Scotland	**23**
David	Smith	dsmit242@uwo.ca	University of Western Ontario, Canada	**--**
Cristian	Solari	cristiansolari@yahoo.com	University of Buenos Aires, Argentina	**30**
Noriko	Ueki	volvoxchlamy@mac.com	Tokyo Institute of Technology, Japan	**27**
James	Umen	jumen@danforthcenter.org	Danforth Plant Science Center, USA	
Eva Laura	Von der Heyde	evdheyde@uni-bielefeld.de	Bielefeld University, Germany	**38**
Jillian	Walker	will.ratcliff@biology.gatech.edu	Georgia Institute of Technology, USA	**45**
Kirsty	Wan	yw265@cam.ac.uk	University of Cambridge, UK	**26**
Kayoko	Yamamoto	haihailotuslotus@gmail.com	University of Tokyo, Japan	**31**
Shota	Yamashita	yamashii@bs.s.u-tokyo.ac.jp	University of Tokyo, Japan	**17**