*31st ALTENBERG WORKSHOP IN THEORETICAL BIOLOGY*

# *The Origins and Consequences of Multicellularity*

*organized by*

*Karl Niklas and Stuart Newman*

*September 25-28, 2014*

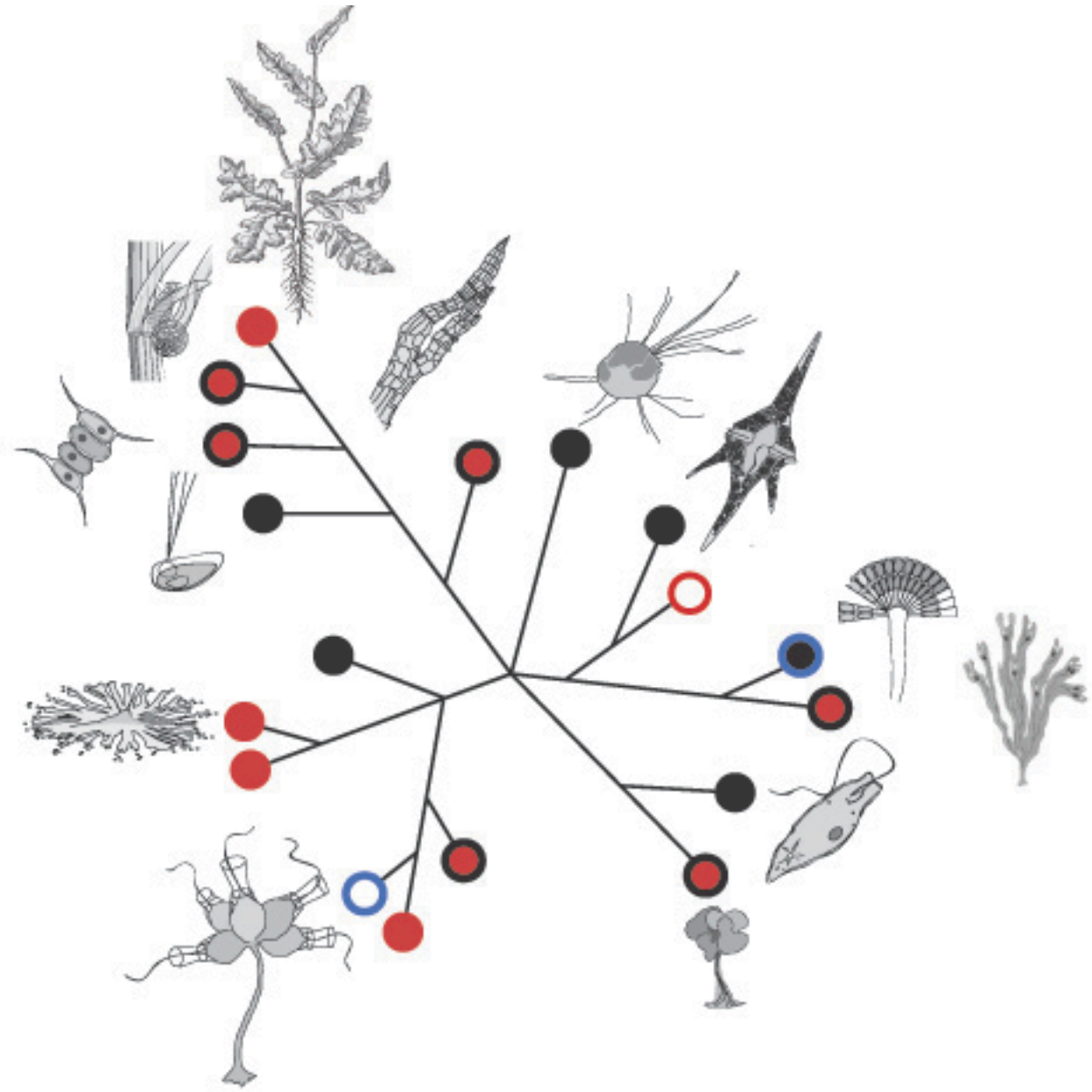
*The KLI Institute*

*Klosterneuburg, Austria*

## Welcome

to the 31th Altenberg Workshop in Theoretical Biology. The Altenberg Workshops are interdisciplinary meetings organized by the KLI Institute in Klosterneuburg, Austria. The workshop themes are selected for their potential impact on the advancement of biological theory. Leading experts in their fields are asked to invite a group of internationally recognized scientists for three days of open discussion in a relaxed atmosphere. By this procedure the KLI Institute intends to generate new conceptual advances and research initiatives in the biosciences. We are delighted that you are able to participate in this workshop, and we wish you a productive and enjoyable stay.

Gerd B. Müller
Chairman



## The topic

Multicellularity has evolved independently in ten different lineages, each of which had a unicellular ancestral condition. Its evolution involved the appearance of physiological mechanisms resulting in cell-to-cell adhesion and sustained intercellular communication among adjoining cells. A comparative approach among extant lineages shows that these two requirements have been achieved in different ways among different plant, animal, and fungal groups; e.g., cell-to-cell adhesion and communication in metazoans typically involves membrane-bound glycoproteins and tight junctions, whereas land plant cell adhesion and communication results from a pectinaceous middle lamella and plasmodesmata. Critical questions in this context are: What are the genomic and developmental commonalities (and the unique features) among multicellular lineages that permit cell-to-cell adhesion and communication? What are the selective advantages (and disadvantages) to the evolution of these defining features of multicellular organisms? What physical consequences follow from the multicellular state of life, and can these provide insight into the origins of morphological novelties?

In addition, the evolutionary transition from the unicellular to the multicellular condition is a major change in individuality since a new kind of organism emerges from the interactions and cooperation among subunits (cells). Until recently, discussions about this transition have been characterized by two divergent schools of thought, one focusing on the so-called ‘unicellular bottleneck’ between alternating generations, and another school focusing on ‘soma-germ’ specialization. More recently, the focus has become more synthetic by considering the ‘unicellular bottleneck’ in terms of the ‘alignment-of-fitness’ phase (wherein genetic similarity among cells prevents internal conflict) and ‘soma-germ’ specialization in terms of an ‘export-of-fitness’ wherein cellular components become interdependent and collaborate in reproductive effort. This perspective raises a number of important questions. For example, does the unicellular bottleneck in the life cycles of multicellular organisms assure an alignment-of-fitness? Does multicellular ‘individuality’ evolve as the result of a gain in fitness achieved by cellular specialization?

Finally, recent knowledge of the physical underpinnings of morphogenesis and pattern formation in multicellular organisms raise questions that go beyond the adaptationist framework of traditional evolutionary theory. In particular, to what extent are morphological motifs inevitabilities of a new scale of cellular life that afford opportunities to explore and create entirely novel niches? Is the evolution of multicellularity typically a “left wall”—that is, is it a generally irreversible event in the history of many, if not the majority of lineages, and if so, why?

**Format**

There will be 17 presentations, with 45 minutes allotted for each—roughly 25 minutes for each talk, followed by 20 minutes for questions on that talk and discussion. On Sunday we end with a general discussion, including publication plans.

To support discussion during the sessions, we encourage all participants to send a rough draft of their presentation and/or some materials that are relevant to their topic to the organizers in advance of the workshop, to be circulated among the participants.

**Manuscript preparation and publication**

The Altenberg Workshops in Theoretical Biology are fully sponsored by the KLI Institute. In turn, the Institute requires all participants to contribute a paper to a volume edited by the organizers. Altenberg Workshop results are usually published in the *Vienna Series in Theoretical Biology* (MIT Press). The contributors are not necessarily limited to the original participants; they may be complemented by experts on those topics that emerge as important, and may include co-authors invited at the discretion of the participants.

We expect that participants will revise their drafts as a result of our discussions at the workshop and the ensuing review process. We aim for a March 2015 date for receipt of finished manuscripts for publication. The length of the contributions should be approximately 8,000 words. The use of figures and photographs is highly encouraged. All contributions will be edited for style and content, and the figures, tables, and the like will be drafted in a common format. The editors will send specific instructions after the workshop.

Karl Niklas and Stuart Newman

# Participants

**MAJA ADAMSKA** **maja.adamska@sars.uib.no**

Sars International Centre for Marine Molecular Biology
University of Bergen
Thormøhlensgate 55
N-5008 Bergen
Norway

**ARGYRIS ARNELLOS** **argyris.arnellos@kli.ac.at**

The KLI Institute
Martinstrasse 12
3400 Klosterneuburg
Austria

**MARIANA BENÍTEZ KEINRAD** **marianabk@gmail.com**

Instituto de Ecología & Centro de Ciencias de la Complejidad
Universidad Nacional Autónoma de México
C.U., México D.F
Mexico

**DAVID R. CRAWFORD** **david.crawford@bristol.ac.uk**

Department of Philosophy
University of Bristol
31 The Praedium, Chapter Walk
Bristol BS6 6WB
UK

**LIAM DOLAN** **liam.dolan@plants.ox.ac.uk**

University of Oxford
South Parks Road
Oxford OC1 3RB
UK

**KUNIHIKO KANEKO** **kaneko@complex.c.u-tokyo.ac.jp**

Department of Basic Science
University of Tokyo
Komaba, Meguro
Tokyo 153-8902
Japan

**ANDREW H. KNOLL** **aknoll@oeb.harvard.edu**

Department of Organismic and Evolutionary Biology
Harvard University
OEB, 26 Oxford Street
Cambridge, MA 02138
USA

**MANFRED LAUBICHLER** **manfred.laubichler@asu.edu**

School of Life Sciences
Arizona State University
PO Box 4501
Tempe, AZ 85287-4501
USA

**OTTOLINE LEYSER** **OL235@cam.ac.uk**

Sainsbury Laboratory
University of Cambridge
Bateman Street
Cambridge, CB2 1LR
United Kingdom

**ALAN LOVE** **aclove@umn.edu**

Department of Philosophy and Minnesota Center for Philosophy of Science
University of Minnesota
831 Heller Hall, 271 19th Ave. S
Minneapolis, MN 55455
USA

**VIDYANAND NANJUNDIAH** **vidya@ces.iisc.ernet.in**

Centre for Human Genetics
Electronic City (Phase I)
Bangalore 560100
India

**STUART NEWMAN** **newman@nymc.edu**

Department of Cell Biology & Anatomy
New York Medical College
Basic Science Building
Valhalla, NY 10595
USA

**KARL J. NIKLAS** **kjn2@cornell.edu**

School of Integrative Plant Science
Department of Plant Biology
Cornell University
412 Mann Library
Ithaca, NY 14853-5908
USA

**WILLIAM RATCLIFF** **will.ratcliff@biology.gatech.edu**

School of Biology
Georgia Institute of Technology
311 Ferst Drive
Atlanta, GA 30332
USA

**IÑAKI RUIZ-TRILLO** **inaki.ruiz@ibe.upf-csic.es**

Institut de Biologia Evolutiva (CSIC-UPF)
Universitat de Barcelona
Passeig Marítim de la Barceloneta 37-49
08003 Barcelona
Spain

**RICARD SOLÉ** **ricard.sole@upf.edu**

ICREA- Complex Systems Lab, Institute of Evolutionary Biology
Universitat Pompeu Fabra
Dr Aiguader 88
08003 Barcelona
Spain

**PAVEL TOMANCAK** **tomancak@mpi-cbg.de**

Max Planck Institute of Molecular Cell Biology and Genetics
Pfotenhauerstrasse 108
D-01307 Dresden
Germany

# The Origins and Consequences of Multicellularity

| Thursday 25 September | Evening | |
|---|---|---|
| 6.00 pm | | Welcome reception and dinner at the KLI Institute |

| Friday 26 September | Morning | Setting the Stage: History, Paleontology, Philosophy, and Related Theory | Chair: Love |
|---|---|---|---|
| 9.00 am – 9.45 am | Laubichler | An Extended Evolution Perspective on the Origin and Consequences of Multicellularity | |
| 9.45 am – 10.30 am | Knoll | Protistan Feeding and the Origins of Complex Multicellularity | |
| 10.30 am – 11.00 am | Coffee | | |
| 11.00 am – 11:45 am | Arnellos | Integration of Constitutive and Interactive Aspects in the Transition from Unicellular to Multicellular Organisms | |
| 11.45 am – 12:30 pm | Niklas | Convergent Motifs in the Acquisition of Multicellularity | |
| 12:30 pm – 2.30 pm | Lunch | at the KLI Institute | |

| Friday<br>26 September | Afternoon | Plants and Related Theory | Chair:<br>Knoll |
|---|---|---|---|
| 2.30 pm – 3.15 pm | Dolan | Principles of Cell Development in the Evolution of Multicellularity | |
| 3.15 pm – 4.00 pm | Leyser | Auxin, Self-Organisation and the Colonial Nature of Plants | |
| 4.00 pm – 4:30 pm | Coffee | | |
| 4.30 pm – 5.15 pm | Benítez<br>Keinrad | Physicochemical Factors in the Organization of Multicellular Aggregates and Plants | |
| 6.30 pm | | Departure for Dinner | |

| Saturday<br>27 September | Morning | Amoebozoa, Fungi, and Related Theory | Chair:<br>Benítez |
|---|---|---|---|
| 9.00 am – 9.45 am | Nanjundiah | Cellular Slime Moulds and the Transition from Solitary to Social Living | |
| 9.45 am – 10.30 am | Crawford | Evolution of Multicellularity in the Fungi: Alternative Routes | |
| 10.30 am – 11.00 am | Coffee | | |
| 11.00 am – 11.45 am | Ratcliff | What Experimental Evolution in *Saccharomyces* and *Chlamydomonas* Reveal about the Transition to Multicellularity | |
| 11.45 am – 12.30 pm | Solé | Synthetic Transitions to Multicellularity | |
| 12.30 pm – 2.30 pm | Lunch | at the KLI Institute | |

| Saturday<br>27 September | Afternoon | Metazoans and<br>Related Theory I | Chair:<br>Leyser |
|---|---|---|---|
| 2.30 pm – 3.15 pm | Ruiz-Trillo | The Origin of Metazoan Multicellularity: A Genomics and Cell Biology Perspective | |
| 3.15 pm – 4.00 pm | Adamska | Sponges as the Rosetta Stone of Colonial-to-Multicellular Transition | |
| 4.00 pm – 4.30 pm | Coffee | | |
| 4.30 pm – 5.15 pm | Kaneko | Multi-level Consistency Dynamics for Multi-cellular Organisms | |
| 6.30 pm | | Departure for Dinner | |

| Sunday<br>28 September | Morning | Metazoans and<br>Related Theory II | Chair:<br>Laubichler |
|---|---|---|---|
| 9.00 am – 9.45 am | Tomancak | Developmental Hourglass and the Chicken and Egg Causality Dilemma | |
| 9.45 am – 10.30 am | Newman | The Role of the Egg in the Evolutionary Consolidation of Animal Body Plans | |
| 10.30 am – 11.00 am | Coffee | | |
| 11.00 am – 11.45 am | Love | Explaining the Origins of Multicellularity: Criteria of Adequacy and Epistemological Prerequisites | |
| 11.45 am – 12.30 pm | | General discussion and publication plans | |
| 12.30 pm – 2.15 pm | Lunch | at the KLI Institute | |
| 2.30 pm | | Departure for Danube boat trip and dinner in Schloß Dürnstein | |

# Abstracts

Manfred LAUBICHLER
Arizona State University

**An Extended Evolution Perspective on the Origin and Consequences of Multicellularity**

This paper introduces a model for the evolution of complex systems based on an integration of regulatory network and niche construction theories and applies it to the case of multicellularity.

The general model of extended evolution covers two aspects of the evolutionary process: (1) the transformation rules governing the variation of complex phenotypes and (2) the complex dynamics between evolutionary units and their niches. The model of extended evolution deals with transformations of complex networks embedded in dynamically changing environments resulting from several layers of constructed niches. Since the transformational dynamics itself depends on these niches, mutual causation is realized even at the developmental level. The transformation of complex networks includes two types of changes to the architecture of regulatory networks: (1) internalization, where elements of various niches become internalized within the network and (2) externalization, where key regulatory features become part of an external niche. This paper argues that the origin and consequences of multicellularity are captured by these two aspects of regulatory evolution.

Andrew KNOLL
Harvard University

**Protistan Feeding and the Origins of Complex Multicellularity**

Fossils indicate that eukaryotes participated in marine ecosystems as early as 1600-1800 Ma, and silicified red algae further show that crown group taxa, simple multicellularity and photosynthesis all existed within the domain by 1100-1200 Ma. Nonetheless, fossils record major eukaryotic diversification ca. 800 Ma, including testate, scale-bearing, and novel multicellular forms. By analogy with ecological hypotheses for Cambrian animal diversification, it can be argued that the evolution of eukaryvory–feeding by ingestion on other eukaryotic cells–provided an ecological driver for the observed Neoproterozoic diversification. Phylogenies and molecular clocks suggest that the last common ancestor of extant eukaryotes was a heterotroph that fed on bacteria-sized particles; principal eukaryvorous clades began to diversify later, in the Neoproterozoic Era. Multicellularity provides defense against protistan predators, helping to explain the Neoproterozoic appearance of new multicellular clades, recorded by fossils for algae and inferred from molecular clocks for animals. Phylogenetic trait mapping also suggests that major eukaryotic clades diverged along lines of feeding mode early in the history of the domain, and that feeding mode strongly constrained the subsequent probability of evolving complex multicellularity.

Argyris ARNELLOS
The KLI Institute

**Integration of Constitutive and Interactive Aspects in the Transition from Unicellular to Multicellular Organisms**

Unicellular systems exhibit an organization constituted of several functionally differentiated sub-systems and parts that are subjected to a certain type and degree of integration on whose basis the active control and regulation of the cell's interaction with the environment is organized and implemented in a functional and reciprocal relation with the underlying metabolic-constructive processes.

We argue that the transition from unicellular systems to multicellular (MC) ones raises both conceptual and ontological challenges for understanding the integration between interactive and constitutive aspects. We compare different cases of early MC systems (from bacterial aggregations and algae colonies to more integrated systems like Porifera and Cnidaria), and argue that the minimal organizational requirements for a complete integration between constitutive and interactive aspects are satisfied from a genuinely epithelial (i.e., eumetazoan) organization. An epithelial organization provides the basis both for integrated organismal actions and for the regulatory logic needed for the development of the respective differentiated and integrated MC system. More specifically, we will argue that an epithelial organization endogenously constructs its own developmental regulation, and the various operational patterns of such regulation modulate and control the generation and integration of the constitutive aspects in such a way that they are in a functional and reciprocal correspondence with its interactive aspects. We shall discuss in detail the structural and operational characteristics of this endogenously produced regulation.

We suggest that MC systems capable for organismal action exhibit a special type of integration, which in turn is directly and intimately intertwined with their developmental organization. We conclude with several implications related to contemporary biological and philosophical issues.

Karl NIKLAS
Cornell University

**Convergent Motifs in the Acquisition of Multicellularity**

Multicellularity has evolved in every major prokaryotic and eukaryotic clade. Comparative studies show that, in each case, four developmental motifs are necessary and sufficient for the acquisition of multicellularity. These motifs (1) prefigure the alignment of the plane of cell division, (2) create cell-to-cell adhesion, (3) establish and maintain intercellular communication, and (4) foster cellular polarity and differentiation. Yet, the underlying mechanisms that are employed to achieve these motifs differ, often dramatically, among multicellular organisms. Consider that intercellular adhesion in the brown algae involves phlorotannins and polymers of D–mannuronic and L–guluronic acids, whereas among the land plants (embryophytes), it is achieved by a middle lamella typically dominated by $Ca^{2+}$–rhamnogalacturonanic–rich pectins.

All of these adhesives differ chemically from the Type-1 transmembrane cadherin proteins responsible for animal cell-to-cell adhesion, or the glycoprotein-based "glues" produced by many fungi. Likewise, intercellular communication in the embryophytes involves plasmodesmata, which differ from the desmosomes and the tight or gap junctions of chordates, the plasmodesmata-like structures seen in some brown algae, or the cytoplasmic bridges in *Volvox* and colonial choanoflagellates. These and other examples of the convergent evolution are reviewed and used as evidence that natural selection typically acts on functional traits and not directly on their underlying generative mechanisms. I will also touch on alternative splicing in tandem with the formation of intrinsically disordered proteins as a variant motif for cell differentiation and tissue-type specification.

Liam DOLAN
University of Oxford

**Principles of Cell Development in the Evolution of Multicellularity**

Multicellularity evolved many times among the green plants. Evolution of multicellularity led to the development of more complex life cycles–where either the haploid, diploid or both phases of the life cycle became multicellular. The evolution of multicellularity among streptophyte algae laid the foundation for another leap in complexity–the evolution organisms with complex tissues and associated stem cells and associated dividing cells (meristems)–which is found in land plants. By comparing the mechanisms that control the development of cellular diversity and tissue formation among streptophytes (algae and land plants), the mechanistic changes that underpinned the evolution of multicellularity and complex tissue formation may be elucidated.

Ottoline LEYSER
University of Cambridge

**Auxin, Self-Organisation and the Colonial Nature of Plants**

Multicellularity is characterised by specialisation of cell types, and at a higher level of organisation, specialisation of tissues and organs. This brings with it a requirement for communication between cells, tissues and organs. In animal systems, the trend has been toward low levels of redundancy between organs and centralised co-ordination of their activities. In plants, there are usually no unique organs and co-ordination is distributed. We are interested in the role of the self-organising transport network of the plant hormone auxin in co-ordinating growth and development across the shoot system, and between the shoot and root. In particular we are analysing how competition between shoot apices for common auxin transport routes down the main stem to the root regulates shoot branching, allowing integration of local and systemic factors.

Mariana BENÍTEZ KEINRAD (joint work with Ana E. ESCALANTE)
Universidad Nacional Autónoma de México

**Physicochemical Factors in the Organization of Multicellular Aggregates and Plants**

Since the formulation of the Modern Synthesis, the causes of phenotypic variation and innovation as sources of adaptive evolution have been mainly associated with changes in the DNA sequence. However, diverse avenues of theoretical and empirical research show that our view of the possible causes and processes by which phenotypic diversity originates need to be expanded. Indeed, it has been acknowledged that ecological, social, developmental, as well as generic physicochemical processes may contribute to phenotypic variation and innovation just as the genome and changes therein do. One of the major evolutionary innovations regarding phenotypes is the appearance of multicellularity in different lineages. Multicellular organisms are not only the aggregation or incomplete separation of cells, but also involve some sort of differentiation, metabolic integration, and the appearance of new systemic properties and levels of selection. The generation of these features is now being studied with a diversity of causes in consideration. We focus on the potential role of physicochemical aspects on the formation and patterning of multicellular arrangements in lineages that, while evolutionary very distant, might exhibit suggestive commonalities. In particular, we discuss how mechanical and chemical coupling of cells and the combination of so-called Dynamical Patterning Modules may lead to the formation of recurrent patterns in plants and bacteria, and illustrate these ideas in specific model organisms. With these ideas in mind, we reconsider the notion of multicellular reproduction, as compared to multicellular recreation, and discuss some aspects of plant and microbial development that might shed light on the study of multicellularity origin, organization, and evolution.

Vidyanand NANJUNDIAH
Centre for Human Genetics, Bangalore

**Cellular Slime Moulds and the Transition from Solitary to Social Living**

The Dictyostelid or cellular slime moulds (CSMs) are soil amoebae that go through a facultative unicellular to multicellular transition in their life cycle. Thus they may offer clues to how multicellularity as such may have evolved. The proximate cause of the transition is the stress caused by starvation, which leads to the aggregation of spatially separated cells. The consequences are development and differentiation, including reproductive division of labour. When viewed from an evolutionary perspective, multicellularity is accompanied by the transfer of individual status from a single cell to a group. A variety of factors appear to have fostered the transfer in the CSMs, among them preadaptation, intercellular heterogeneity, phenotypic plasticity, and self-organisation. The outcome can be the formation of 'guilds' with a flexible membership within which tradeoffs between different fitness-related traits can maintain long-term coexistence. Genetic similarity among the members of a group may have been more a consequence of multicellularity than a cause.

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Nanjundiah V, Sathe S (2013) Social selection in the cellular slime moulds. In: Romeralo M. et al. (eds) Dictyostelids. Springer
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David CRAWFORD
University of Bristol

**Evolution of Multicellularity in the Fungi – Alternative Routes**

Hierarchical transition consists in the development or evolution in a system of a new level of selection. The standard *integration* model of hierarchical transition proposes a transition from parts to parts-in-a-whole. The classic example is the transition from unicellularity to multicellularity. In contrast, the evolution and development of multicellularity (more generally 'compartmentalization') in filamentous fungi follows an *individuation* model in which wholes transition to wholes-with-parts. The EvoDevo of filamentous fungi involves origination of multicellularity by an alternative route. The proposed individuation model highlights ontogenetic regulation of parthood, and demonstrates how transition can proceed without the fitness alignment or fitness export stages the standard model requires. The individuation model may also apply to groups outside the filamentous fungi like plasmodial slime molds or filamentous algae. I discuss the general model and explore specifics of fungal EvoDevo.

William RATCLIFF
Georgia Institute of Technology

**What Experimental Evolution in *Saccharomyces* and *Chlamydomonas* Reveal about the Transition to Multicellularity**

1) The evolutionary transition to multicellularity can occur rapidly.
   - Process. The MLS hypothesis for the origin of multicellularity posits a two-step process: a) cluster formation b) shift to among-cluster selection. We observe this directly in our yeast experiment.
   - Genes. Yeast are just one mutation away from multicellularity.
   - Outcome. We will review experimental work on the tempo and mode of multicellular adaptation in snowflake yeast.

2) Geometrically-structured clusters allow for selection to act effectively on multicellular traits.
   - The growth dynamics of snowflake yeast snowflake yeast and evolved *Chlamydomonas* result in single-cell bottlenecks.
   - Single-cell bottlenecks limit the potential for genetic conflict and increase the evolvability of cluster-level traits. For small, clonally developing clusters, the threat posed by among-cell conflict may be negligible.
   - Cluster geometry likely plays a key role in the evolution of apoptosis in yeast.

3) Single-cell traits can be co-opted for novel multicellular function.
   - The life cycles that evolved in the yeast and *Chlamydomonas* experiments are the direct result of the way that the single-celled ancestors grow.
   - The most rapid way to gain novel multicellular functionality is to exapt a trait first evolved by the unicellular ancestor.

Ricard SOLÉ
University Pompeu Fabra and Santa Fe Institute

**Synthetic Transitions to Multicellularity**

Understanding the transition towards a multicellular world might require a combined consideration of developmental, evolutionary, and ecological factors. All these components have been taken into account in previous studies, but the explicit consideration of spatial embodiment, i.e., the fact that cells, tissues, and organisms are physical entities, has not been seriously considered as a key component of the problem. Embodied models of artificial evolution of multicellular aggregates reveal unexpected paths of evolution beyond single cells that might have to do with early stages of this major transition. These models illustrate how EvoDevo and ecological transitions occur, and also provide a unique scenario for exploring the role played by constraints and history in evolving complex biological structures.

Iñaki RUIZ-TRILLO
University of Barcelona

**The Origin of Metazoan Multicellularity: A Genomics and Cell Biology Perspective**

How animals or metazoans emerged from their single-celled ancestors remains a major question in biology. Recent genome data from close unicellular relatives of Metazoa has shown that the unicellular ancestor that gave rise to Metazoa was genetically much more complex than previously thought. Thus, the unicellular ancestor of animals already had a good repertoire of genes involved in cell adhesion, cell signaling, and transcriptional regulation. This suggests that co-option and an increase in gene regulation may have played an important role in the origin of animals. All this data provides insights both into the origin of animal multicellularity and the emergence of the different animal cell types.

Maja ADAMSKA
University of Bergen

**Sponges as the Rosetta Stone of Colonial-to-Multicellular Transition**

Multicellular animals are monophyletic, and their nearest relatives are single-cell and colonial choanoflagellate protists. Sponges are traditionally viewed as the oldest surviving animal clade, and this view is supported by the majority of contemporary phylogenies. Similarities between choanocytes (the defining cell type for sponges) and choanoflagellates have long suggested an evolutionary link between them. In addition to capturing of food particles, choanocytes are the source of gametes. This mixture of somatic and germ line features bears more resemblance to protists than to specilized animal cells. Gene expression studies demonstrate similarities between choanocytes and both endodermal (e.g., gut) and stem cells of 'higher animals.' I will argue that the combination of functional morphology and gene expression signatures places choanocytes between colonial protists and the gut cells of multicellular animals. I will draw an evolutionary scenario of transitions from single-cell protists through sponges to 'true animal' body plans using only gradual steps driven by natural selection.

Kunihiko KANEKO
University of Tokyo

**Multi-level Consistency Dynamics for Multi-cellular Organisms**

Life systems generally consist of different levels with hierarchy, from molecules, cells, to organisms. States at each level change dynamically in time, while consistency between different levels is achieved for a stationary biosystem. Such consistency leads to general laws in reproduction, adaptation, development, and evolution. After surveying this general principle, I discuss consistency between growth of an ensemble of cells and of a single cell consisting of intra-cellular reaction dynamics. Cell differentiation and autonomous regulation of cell numbers of different types are generally resulted. Evolution of multicellularity is discussed as a genetic fixation of phenotypic differentiation and genetic assimilation of robustness to developmental noise, while the phenotypic origin of cancer is also mentioned.

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Pavel TOMANCAK
Max Planck Institute of Molecular Cell Biology and Genetics

**Developmental Hourglass and the Chicken and Egg Causality Dilemma**

Similarity in embryo morphology and its implications for the evolutionary process have fascinated biologists from the times of Haeckel, von Baer, and Darwin. Duboule and Raff synthesised comparative data into the developmental hourglass model that postulates that for animals of the same type, or, if one wants, a phylum, early developmental pathways tend to be quite divergent before reaching a constraint—the so-called phylotypic stage—, after which the diversification picks up again.

The evidence supporting the developmental hourglass model has been primarily morphological, and only recently has the model received strong support on the molecular level of gene expression. Using comparative transcriptomics and phylogenomics analyses the signature of the hourglass divergence pattern has appeared in many studies on diverse organisms from insects to plants. Contrasting divergence of gene expression between species at different periods of embryonic development has proved to be a useful framework to tease out interesting information from genome-wide gene expression studies. Some very recent work from modENCODE is trying to take this analysis to the next level and compare gene expression profiles across vastly divergent species such as flies and worms. I will discuss the interpretation of these studies.

What remains enigmatic is the mechanism which constrains embryo gene regulatory network and morphology at the neck of the hourglass in the middle of embryonic development. We have presented some ideas on how the nature of early development reflects the adaptation to diverse ecological niches and proposed that the phylotypic stage provides more information about the evolutionary history of the species. This lead us to speculate wildly on the origin of the animal body plans and the role of the egg stage in shaping it—following the conceptual framework of Stuart Newman.

Finally, it is clear that in order to gain insight into the mechanisms that constrain the evolution of embryo morphology and enable its early and late plasticity, we need more data. I will discuss our technological framework to study the embryonic morphology of animals and the dynamic emergence of patterns of gene expression by cutting-edge light sheet microcopy techniques.

Stuart NEWMAN
New York Medical College

**The Role of the Egg in the Evolutionary Consolidation of Animal Body Plans**

The 'egg-as-novelty' hypothesis proposes that the major animal phylum-characteristic suites of morphological motifs first emerged more than a half billion years ago in multicellular clusters that did not exhibit a germline-soma divergence. Pre-metazoan bodies were organized by "dynamical patterning modules" (DPMs), physical processes mobilized on the new multicellular scale by products of ancient conserved genes. "Proto-eggs" were enlarged cells that through cleavage, or confinement by a secreted matrix, enforced genomic and genetic homogeneity in the cell clusters arising from them. Ooplasmic segregation made the implementation of the DPMs at the multicellular stage of embryogenesis more reliable, robust, and defining of sub-phylum morphotypes. This perspective can account for the rapid diversification of biological forms at the dawn of animal evolution, the capability of most sexual reproducers to propagate vegetatively, and the "embryonic hourglass" of comparative developmental biology.

Alan LOVE
University of Minnesota

**Explaining the Origins of Multicellularity: Criteria of Adequacy and Epistemological Prerequisites**

Explaining the origins of multicellularity in a variety of lineages constitutes a complex problem agenda in evolutionary biology. The primary approach to the problem has been evolutionary theorizing about the conditions under which transitions in individuality occur and thereby make it possible for selection to operate at a higher level. Thus, multilevel selection (MLS) theory has been central to these efforts, especially the distinction between MLS1 (early stages of group formation between individual cells with group fitness deriving from the sum of component cell fitnesses—alignment of fitness) and MLS2 (later stages where stable groups exhibit individuality—export of fitness). But the specific properties that facilitate group formation (e.g., cell adhesion or cell-cell signaling), the transition between the two stages, and the specific properties that facilitate stable groups are not explained by MLS. These are distinct questions that require different answers. In addition to the issue of individuality transitions, the criteria of adequacy for the problem include elucidating the origin of developmental variation during MLS1, MLS2, and the transitional period in-between. Addressing these questions is essential to formulating generalizations about multicellular origins and accounting for key differences between them (e.g., transcriptional regulation differences), especially the differential consequences in various lineages (e.g., microbial vs. metazoan multicellularity). This implies that the epistemological prerequisites for the problem are broader than sometimes conceived, and that a more multidisciplinary approach is required, inclusive of molecular genetic and phylogenetic methodologies applied to both unicellular and multicellular models. The aim of this paper is to make some of these criteria of adequacy and epistemological prerequisites more explicit by displaying structural aspects of this complex problem in order to advance ongoing empirical and theoretical inquiry into the origins and consequences of multicellularity.