Ecological dynamics of aggregation and dispersal in the algae *Chlamydomonas*

1 Research plan

The transition from unicellular to multicellular organization occurred more than twenty times independently along the tree of life. Multicellularity provides cells with novel opportunities, such as increased resistance to predation and division of labour. However, larger group sizes are also associated to increased competition for resources and environmental toxification. Hence, while multicellularity yields benefit, the biological and physical conditions that initially led individual cells to form groups remain unclear. Interestingly, the vast majority of multicellular life forms undergo cycles that comprise a single-cell stage (a propagule, e.g. spores, gametes). Multicellular groups not only form by clonal growth, as in higher metazoans ("staying together"), but also through aggregation, as in cellular slime moulds and myxobacteria ("coming together") [1, 2]. Elucidating the mechanistic determinants of aggregative life cycles is needed to understand the emergence of primitive multicellular life cycles and the evolution of collective-level functions.

Chlamydomonas species, flagelleted green algae (Fig. 1b), are an excellent laboratory model for studying the emergence of multicellularity since they belong to the order of *Chlamydomonadales*, which is comprised of families that are either unicellular or multicellular. In natural habitats, when sensing the threat of predation *Chlamydomonas* form groups of cells that are either clonal (containing 4 to 16 genetically identical cells within one cell wall) [3, 4] or aggregative (where 10 to 1000 cells stick together thanks to the production of an extra-cellular-matrix, Fig. 1c) [5, 6]. It has been suggested that such behavior protects cells against predation, because the multicellular cluster size exceed the size of food item the predators can ingest. The aggregative process occurs on a faster time scale than cell division (group formation occurs in $\sim$10 h, half the generation time) and is also much more plastic. Notably, since aggregation depends mainly on the concentration of chemical signals perceived by the algae (Fig. 1e), multicellularity is reversed once the predation signal is removed [5]. In addition to extra-cellular-matrix production that glues cells together, the motility of algae has been shown to play a role on aggregation: fast-swimming strains tend to aggregate less [7]. However, if the main determinants of the dynamics have been identified, no precise quantification has been performed on this system to enable microscopic ecological modeling of group formation and dispersal, thus limiting ecological models to phenomenological descriptions. Importantly, the issue of dispersal has never been addressed neither theoretically nor experimentally.

In this project, we will quantify the microscopic determinants (extra-cellular-matrix secretion, chemo-attraction, flagellation) for group formation and dispersal in *Chlamydomonas reinhardtii* in order to inform an ecological model that will pinpoint the requirements for the emergence of aggregative life cycles. The project also opens interesting perspectives for the biofuel industry. Currently, the most energy-intensive process to obtain oil is the separation of biomass from the growth medium. No commercial-scale solution has yet been proposed, making the overall process non-profitable compared to fossil fuel. Current techniques involve high-cost centrifugation or flocculation by addition of often toxic chemicals followed by sedimentation. Optimizing the aggregation process represents therefore a promising route to recover the biomass with low financial and energy cost.

The PhD work will combine experiments, realized in team 1 (Nicolas Desprat and Raphaël Jeanneret, LPENS), and modelling, developed in team 2 (Silvia De Monte, IBENS). It will be divided in two parts, addressing first the ecological dynamics of aggregation/dispersal process, and then devise evolutionary models based on the relevant microscopic features of the system.

a) Macroscopic ecological quantification of aggregation/dispersal. We will make use of low magnification video microscopy and image analysis (Fig. 1a) in order to quantify the growth rate of cell clusters, their shape (i.e. fractal/compact), the aggregate size distribution, as well as the fraction of aggregated and planktonic cells. These measures aim to quantify the dynamics and steady-state of aggregating populations. The predatory threat will be produced using the culture filtrate of the natural predator of *Chlamydomonas*, *Peranema trichophorum* [5], organism already cultivated in the team (Fig.

1d). To understand the mechanisms of disaggregation upon removal of the predation signal, we will perform microfluidic experiments based on designs that integrate agar gel bridges between the main observation chamber (where the algae grow) and a flushing channel where the environmental conditions are imposed (Fig. 1f) [8]. Such setup allows to quickly modify, by diffusion, the environmental cues perceived by the algae. We will measure if clusters break up in clumps or if cells break off individually, and when cell motility is recovered. Inspired by these observations, agents-based models and simulations will be implemented in team 2 (Silvia De Monte) at IBENS in order to pinpoint the minimal microscopic ingredients (e.g. adhesiveness, motility, chemosensing) necessary to reproduce the experimental macroscopic data measured previously. This will allow exploring the phase diagram of the system by systematically varying the control parameters of the problem such as cell density and intensity of predator signal, and to make predictions on regimes that are the most relevant for measuring the microscopic parameters.

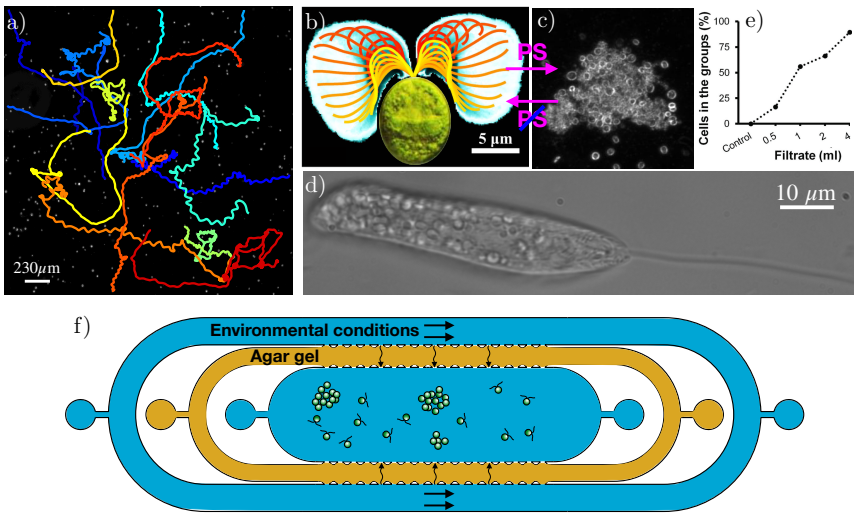

Figure 1: a) Single tracks of *Chlamydomonas* cells. b) *Chlamydomonas* cell with beating flagella superimposed. c) When sensing a predation signal (PS) from *Peranema*, cells aggregate in large clusters. d) Photograph of the predator *Peranema trichophorum*. e) The aggregative state in the population depends on the level of predatory threat. f) Schematic example of a microfluidic chip enabling to impose specific environmental conditions without disturbing the dynamics of the system [8].

b) Microscopic-based eco-evolutionary model of multicellular life cycles. We will first experimentally measure the microscopic parameters that have been identified by the ecological model described above. We anticipate that the production of extra-cellular-matrix and chemosensing will have a strong impact on group dynamics. The variability of extra-cellular-matrix production will be measured using transcriptional fluorescent reporters [9] at increasing concentrations of predation signal and cell densities. In parallel, the statistical features of the swimming behavior will be assessed by tracking individual cells at a high frame rate (Fig. 1a). Then, we will incorporate natural selection on those microscopic ingredients to design eco-evolutionary models, where different selective pressures can be imposed on cells both in the unicellular and the aggregated phases of the life cycle. We will test what selective regimes allow the maintenance of aggregative life cycles in spite of within-group conflicts. These are produced by introducing "mutant" types, characterized by different microscopic parameters (e.g. adhesiveness, motility, chemosensing), and letting them co-aggregate with a predominant resident type, in the spirit of adaptive dynamics [10].

2 Interdisciplinarity of the project

The biological relevance of the project relies on a good knowledge of the ecological scenarios for making sense of the microscopic parameters measured in the experiments. This expertise is brought by the lab of Silvia de Monte (IBENS), who is currently studying the conditions under which natural selection could

favor groups undergoing cyclic aggregation and dispersal. On the other hand, measuring the microscopic parameters of the system requires a strong expertise in video-microscopy and image analysis that will be brought by the lab of Nicolas Desprat and Raphaël Jeanneret (LPENS).

The PhD candidate will perform the experiments and run simulations. Since a novelty of our approach aims to describe the group dispersal upon removal of the predation signal, the candidate should preferentially have received training in microfluidics. Physics background will be privileged to handle both experimental and modelling aspects of the work. For modelling, the ability to code in MatLab or Python is mandatory.

3 Thesis advisors

Nicolas Desprat (Physics Department, LPENS) is a biophysicist. He will supervise microscopy and quantitative image analysis. He has worked with cells, embryos and bacteria. Since he joined the LPENS, he has developed microscopy and image analysis to quantify over long timescale the spatial dynamics within bacterial microcolonies. He has investigated siderophore secretion [11], a de-novo switch for capsule production [12] and the mechanics of microcolony morphogenesis [13].

Silvia De Monte (Institute of Biology of ENS) is a modeller interested in the eco-evolutionary dynamics of microbial collective behavior, in particular in organisms that have aggregative multicellular life cycles. She uses models for group formation in order to augment evolutionary models with more realistic descriptions of ecological interactions [14, 15, 16, 17].

Raphaël Jeanneret (Physics Department, LPENS) is a physicist interested in the biology and ecology of microorganisms. He has a strong experience in microfluidics [18] and microscopy as well as microorganismal culturing (in particular *Chlamydomonas*) for motility and active matter studies [19, 20]. He has successfully imported this model organism in the lab (Fig 1a,c,d).

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