PhD PROJECT PROPOSAL

Mechanics of head eversion in Drosophila

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Abstract
Animal morphogenesis often relies on a combination of contractility, migration, and growth. These processes typically occur over long timescales, ranging from ten of minutes to hours or days, making tissue morphogenesis inherently slow. In sharp contrast, hydraulic forces can drive very fast large-scale transformations of entire tissues or organs during development. For instance, the deployment of insect wings upon emergence relies on a surge of internal pressure that forces hemolymph into the folded wings, enabling dramatic size and shape change within just a few minutes. However, the physical and biological principles of hydraulic morphogenesis remain largely unexplored. In this project, we aim to investigate the hydraulic aspects of head eversion in *Drosophila*. During eversion, the head, initially formed within the larval body, pops out on the timescale of seconds. Preliminary evidence suggests that this fast morphogenetic process is also driven by an internal pressure surge. By studying this phenomenon, we seek not only to elucidate a remarkable and understudied developmental event, but also to uncover general principles governing hydraulic morphogenesis.

Keywords
morphogenesis; biomechanics; hydraulics; *Drosophila*; live imaging; modelling; morphometrics

Scientific questions and objectives
Head eversion in *Drosophila* occurs approximately 10 hours into pupal development and consists of an inversion of tissue curvature driven by an internal pressure surge that forces the head outward. Because this event has hardly been studied, our first goal is to establish a detailed morphometric and biomechanics description of the system.

<u>Aim 1 - Morpho-dynamic description of eversion:</u> What is the 3D geometry of the tissue before, during and after eversion? On what timescales does eversion proceed and what are the key kinematic features of the process?

<u>Aim 2 - Mechanics of eversion:</u> What is the amplitude and temporal profile of the internal pressure

surge, and what factors control it? Can eversion proceed in the absence of this surge? What are the mechanical properties of the everting tissue?

Aim 3 – Biophysical modeling of eversion: Can the interplay between pressure forces and tissue mechanics characterised in Aim 2 be integrated into a quantitative biophysical model capable of reproducing the eversion morpho-dynamics measured in Aim 1?

Together, our objectives are thus to provide a first comprehensive morphometric and mechanical characterisation of head eversion in Drosophila. By combining live pressure measurements, tissue mechanical testing, and modeling, we aim to build a biophysical framework that captures the fundamental principles of governing hydraulic actuation.

Proposed approach (experimental / theoretical / computational) and research plan

Aim 1 - We will characterize the dynamics of eversion, and the associated structural changes of the tissue before, during and after eversion. Given the rapidity of the process, with movements occurring on the timescale of seconds, we will rely on complementary strategies for live imaging. High-speed brightfield imaging will be used to resolve the rapid dynamics, while live confocal/spinning-disc imaging will provide higher spatial resolution and sectioning at lower frame rates. To complement live observations, we will examine fixed samples by electron microscopy (EM) to resolve the micro-structure of the pre- and post-eversion tissue. These high-resolution data will help identify small-scale structural features that may play critically roles in the mechanics of eversion.

Aim 2 - We will directly measure internal pressure dynamics by puncturing the pupa with a capillary, using a method previously validated for wing deployment. Preliminary experiments provide proof of principle that such live measurements can be achieved during eversion. To probe causality, we will perturb muscle contractions, presumed drivers of internal pressure surge to test their influence on eversion dynamics. Following eversion, we will perform nano-indentation to probe the mechanical properties of the tissue. These measurements will quantify tissue stiffness and rheological properties, providing essential inputs for understanding how tissue mechanics regulate or respond to the pressure-driven transformation. We will use a nano-indentation set-up previously validated for *Drosophila* wing.

Aim 3 - We will develop an integrative theoretical model describing the interplay between internal pressure forces and tissue stresses during eversion. Informed by our geometric, pressure and mechanical data, this model will balance pressure forces and stress in the everting tissue to predict mechanical stability, onset conditions for eversion, and eversion dynamics. This model will serve as a first quantitative framework for understanding the mechanics of hydraulic morphogenesis.

Interdisciplinarity and implication of the two labs

This project is intrinsically interdisciplinary as it bridges developmental biology, physics of morphogenesis, and materials science. The experimental approach combines advanced live imaging with micro-manipulation and mechanical assays traditionally used in materials science. Quantitative analysis will leverage computational image processing with physical and biomechanical modeling tools. Collaboration between the two labs will ensure robust training of the recruited researcher with strong interdisciplinary training, encompassing experimental, numerical and theoretical aspects of the project.

Beyond its biological significance, this project will strengthen the growing interface between morphogenesis and soft-matter physics, offering general insights applicable into other pressure-driven shape transformations in living systems. Conversely, it will inform biomimetic strategies for deployable and reconfigurable materials actuated by hydraulic forces, an area of rapidly expanding interest in soft robotics and adaptive materials.

Specify with whom the person recruited will collaborate and on what aspects

Imaging (Aim 1) will be conducted primarily at IBDM (Raphael Clément, in collaboration with Benoit Aigouy). The morphometric aspects will benefit from the IBDM imaging platform, the EM facility, and the team's extensive expertise in live imaging.

The mechanical measurements (Aim 2) will be conducted primarily at IUSTI (Joel Marthelot). Live pressure measurements and nano-indentation set-ups have already been validated on the wing.

The modelling aspects (Aim 3) will build on the combined expertise of both supervisors in biophysics of morphogenesis (Raphael Clément) and hydraulic actuation of soft structures (Joel Marthelot). This joint effort will ensure a coherent integration of experimental results into a predictive biophysical framework.

PhD student's expected profile*

Candidates should have a strong background in biophysics, quantitative biology, or soft-matter physics. Prior experience with live imaging, image analysis, or mechanical modeling would be a plus, but is not required. Curiosity and commitment to interdisciplinarity are essential.

Is this project the continuation of an existing project or an entirely new one?
In the case of an existing project, please explain the links between the two projects (5 lines)*

The supervisors have collaborated on a previous project: the hydraulics of wing deployment. The system that we propose to study here (hydraulics of head eversion) is new to both PIs. This new direction is a way to establish a new line of research and uncover the principles of an overlooked aspect of animal development: rapid hydraulic morphogenesis.

Two to five references related to the project*

1. Pupation in drosophila melanogaster and the effect of the Lethalcryptocephal mutation
Colin G. Chadfield, Dr. John C. Sparrow
Developmental Genetics, 1984

2. Development of the morphological mutant Cryptocephal of Drosophila Melanogaster
James W Fristrom
Genetics, 1965

3. Mechanics of Drosophila wing deployment
Simon Hadjaje , Ignacio Andrade-Silva, Marie-Julie Dalbe, Raphaël Clément[corr.], Joel Marthelot[corr.]
Nature Communications, 2024

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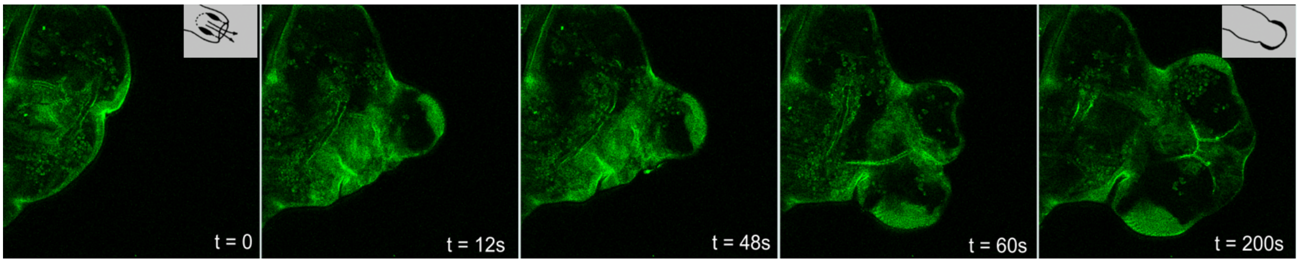
Two main publications from each PI over the last 5 years*

1. Mechanics of Drosophila wing deployment
S. Hadjaje , I Andrade-Silva, MJ Dalbe, **R. Clément**[corr.]**, J. Marthelot**[corr.]
Nature Communications, 2024

2. Fast calcium-dependent reorientation of motile cilia basal bodies in the simple metazoan, Trichoplax
M. Leria, M. Requin, M. Daroueche, R. Hill, A. Le Bivic, **R. Clément**[corr.], A. Pasini[corr.]
Biorxiv 2025

3. Bubble casting soft robotics. T.J. Jones, E. Jambon-Puillet, **J. Marthelot** and P.-T. Brun, *Nature* 2021

Project's illustrating image



Confocal imaging of head eversion. Eyes pop out consecutively, in less than 10s each (Raphael Clément & Benoît Aigouy)

*: Mandatory