

UNIVERSITY OF TARTU
FACULTY OF SCIENCE AND TECHNOLOGY
INSTITUTE OF MOLECULAR AND CELL BIOLOGY

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Mechanisms of head capsule development in *Drosophila melanogaster*

Bachelor's Thesis

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Ivan Uljanov

Supervisor

Professor Osamu Shimmi PhD

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Mechanisms of head capsule development in *Drosophila melanogaster*

Live imaging of *Drosophila* larval eye-antennal imaginal discs and *ex vivo* culture methods allow for clear observation of head development. Our goals for this work were to map the development, underlying molecular mechanisms and signalling during fly head morphogenesis. In this work, a previously developed *ex vivo* culturing technique is utilized and further refined for extended time-lapse and detailed imaging. Using this approach, five distinct developmental stages, as well as specific morphogenetic movements of peripodial epithelium (PE) and disc proper (DE), were captured, analysed and described in detail. In addition, the imaging of disc fusion and head cuticle formation was improved. The intricate movements of the previously overlooked disc proper (DP) were analysed, providing a detailed description of its complex structural changes, fusion and spatial reorganization. Additionally, caspase signalling was mapped during fusion, confirming its presence and importance as one of the main factors in imaginal discs development.

Key words: *Drosophila*, eye-antennal imaginal discs, head morphogenesis, disc proper (DP), peripodial epithelium (PE).

CERCS: B350 Development biology, growth (animal), ontogeny, embryology

***Drosophila melanogaster* peakapsli arengu mehhanismid**

Äädikakärbse (*Drosophila melanogaster*) vastse silma-tundla imaginaaldiskide reaalajas jälgimine *ex vivo* kultiveerimismeetoditega võimaldavad dokumenteerida pea struktuuride arengumehhanisme. Antud bakalaureusetöös kasutati varem väljatöötatud *ex vivo* koekultuuri tehnikat, mida täiustati, et võimaldada pikajalist ja detailset reaalajas biokuvamist. Selle käigus jäädvustati, analüüsiti ja kirjeldati üksikasjalikult viite erinevat arenguetappi ning peripodiaalse epiteeli (PE) ja imaginaaldiski põhiosa spetsiifilisi morfogeneetilisi liikumisi. Töös analüüsiti silma-tundla imaginaaldiskide dünaamikat, neis toimuvaid keerulisi struktuurimuutusi, sulandumisprotsessi ja kudedes asukoha morfoloogilisi muutusi. Lisaks iseloomustati kudedes sulandumisprotsessi ajal toimuvat kaspaside signalisatsiooni, mis kinnitas selle olulisust imaginaaldiskide arengus.

Märksõnad: Äädikakärbes, silma-tundla imaginaaldiskid, pea morfogenees, peripodiaalne epiteel (PE), imaginaaldiski põhiosa.

CERCS: B350 Arengubioloogia, loomade kasv, ontogenees, embrüoloogia

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ABBREVIATIONS AND ACRONYMS

DP – Disc Proper

ECM – Extracellular Matrix

EMT – Epithelial–Mesenchymal Transition

Gal4 – Yeast transcriptional Activator in GAL4/UAS System

GAL80ts – Yeast Temperature Sensitive GAL4 Repressor

GFP – Green Fluorescent Protein

L1 – First Instar Larva

L3 – Third Instar Larva

M – Marginal Zone

MET – Mesenchymal–Epithelial Transition

PE – Peripodial Epithelium

RFP – Red Fluorescent Protein

RNAi – RNA Interference

UAS – Upstream Activation Sequence in GAL4/UAS System

INTRODUCTION

Morphogenesis of the eye-antennal discs of the fruit fly *Drosophila melanogaster* (hereinafter referred to as *Drosophila*) is a very complex process that involves the fusion of imaginal discs of the fly larva into the organs of an adult fly. This process involves numerous molecular and cellular interactions that have not yet been fully understood with sufficient precision. The difficulties arise first from the fact that tissue morphogenesis, during which mostly flat disc pairs move and fuse together, is very difficult to observe *in vivo*.

This bachelor's thesis project aims to investigate the specific processes that occur during the morphogenesis and capture in detail fusion of primordial tissues into three-dimensional head structures, using the *ex vivo* observation method developed in our lab and described in the work of Robin Sarv 2024. This method allows observation of the development of the specimen in time, space and at different wavelengths. By utilizing this *ex vivo* method this project seeks to achieve a detailed understanding of the transition mechanism from third instar larval eye-antennal imaginal discs to the pupal head eversion stage. Creating a map of the occurring movements as well as investigating the cell fate during disc fusion. Also exploring some molecular mechanisms necessary for fly head formation and morphogenesis.

During our observations we were able to accurately map the fusion process in several variations, allowing us to reliably describe the movements occurring during development. We also analysed the movements of the disc proper (DP) and its structural changes throughout the process, which turned out to be much more complex than previously thought.

We also confirmed the importance of apoptosis during imaginal disc and specifically peripodial epithelium (PE) fusion. Caspase signalling was mapped throughout all developmental stages using multiple expression patterns. Using fly stocks with inhibited apoptosis we were able to observe different abnormal head phenotypes as well as increased mortality rates for all strains used.

Overall, this work aims to improve the understanding of *Drosophila* head morphogenesis combining long-term *ex vivo* imaging and fluorescent visualization of developing tissues. By analysing tissue fusion, structural rearrangements, and apoptosis-related signalling pathways during eye-antennal disc development, this study aims to provide a more detailed description of the processes driving head formation in *Drosophila*.

1. LITERATURE REVIEW

1.1. *Drosophila melanogaster* as a model organism

Drosophila is one of the most established model organisms in genetic and developmental biology research for over a century.

The use of *Drosophila* as a model organism began in the early 20-th century with the pioneering work of Thomas Hunt Morgan, who demonstrated using *Drosophila* that genes are located on chromosomes. Several biological and technological advantages make *Drosophila* especially well-suited for developmental research. The fly has a short life cycle of around 10 days at a temperature of 25 °C and it produces a lot of offspring, which allows for rapid genetic manipulation and subsequent analysis across multiple generations. In addition, the genome of *Drosophila melanogaster* is relatively small and well annotated. Many developmental pathways found in flies are highly conserved in vertebrates, including humans. Key signalling pathways such as Wnt/Wingless, Hedgehog and Notch were firstly characterised in *Drosophila* and later it was found that they regulate analogous developmental processes in other animals (Bier, 2005) *Drosophila melanogaster* has a unique embryological anatomy which allows for a more simple and cost-effective model for studying complex developmental processes that are otherwise hard to observe in higher organisms. In particular imaginal discs provide an ideal model for organogenesis. For these reasons *Drosophila melanogaster* remains one of the most widely used model systems in developmental biology.

1.2. Imaginal discs: structure and function

In the *Drosophila* imaginal discs such as leg, wing and eye-antennal discs, are undifferentiated tissues that develop into adult body structures during later stages of morphogenesis. The eye-antennal imaginal discs are precursors to the fly's head bristles and antennae as well as most of the head capsule and eyes (K. Kenyon et al., 2003).

In *Drosophila* at the first larval stage (L1), the disc is a small, uniform epithelial group of cells with minimal regional differentiation. Intensive proliferation during larval development gradually transforms the initially simple epithelia into a highly organized structure (B. P. Weasner and Kumar, 2022; A. Younossi-Hartenstein, 1993). This rapid proliferation occurs during the second and third larval stages (L2–L3), expanding the eye-antennal disc from the initial small population of cells into a structure containing thousands of cells. Simultaneously, the disc subdivides into two main

morphogenetic fields: the eye field and the antennal field. These regions further differentiate into primordia, ultimately resulting in the formation of two primordia: one for eye including cuticle, and ocelli, and second for the antennal field that includes primordium for maxillary palps, and cuticle (Figure 1) (B. P. Weasner and Kumar, 2022; K. Kenyon et al., 2003).

During the third larval instar (L3), retinal differentiation initiates at the posterior margin of the eye field induced by Hedgehog signalling (Domínguez and Hafen, 1997). Differentiation then progresses anteriorly as a wave-like epithelial indentation named the morphogenetic furrow (MF) (Tsai et al., 2007). During that BMP Decapentaplegic (Dpp) signalling contributes to coordinated cell-cycle arrest anteriorly to the furrow (Domínguez and Hafen, 1997). Ahead of the morphogenetic furrow, proliferating cells express transcription factors such as *homothorax* (*hth*), *eyeless* (*ey*), and *teashirt* (*tsh*), which maintain cells in an undifferentiated proliferative state (Hummel et al., 2002; Singh et al., 2002). Repression of *hth* expression by Dpp signalling allows cells to exit the cell cycle and initiate retinal differentiation (Lee and Treisman, 2001).

The eye-antennal disc functions as a dynamic epithelial structure composed of the disc proper (DP) and the overlying peripodial epithelium (PE). Although initially described as a passive epithelial covering, the PE is now recognized as an important signalling and morphogenetic regulator (Cho et al., 2000; Gibson and Schubiger, 2000). Signalling molecules Hedgehog (Hh), Decapentaplegic (Dpp), and Wingless (Wg) are expressed throughout first and second instar in the PE and regulating proliferation and patterning of the underlying disc proper (Gibson and Schubiger, 2000; Atkins and Mardon, 2009). Cytoplasmic extensions between the PE and DP may also facilitate communication between both epithelial layers (Cho et al., 2000).

Multiple lineage-tracing studies also reveal that the PE contributes significantly to external cuticular structures in the adult fly (Chouinard and Kaufman, 1991; Stulz et al., 2006; Lee et al., 2007). These structures include middle of the head cuticle, the vibrissae, and ventral head structures including the maxillary palps. As well as providing the force for the movement and fusion of the discs (Milner et al., 1983).

Furthermore, the PE generates mechanical forces necessary for epithelial movement, tissue rearrangement, disc fusion and alignment during morphogenesis (McClure and Schubiger, 2008). Coordinated interactions between the PE, marginal cells, and the disc proper are therefore crucial for the correct formation of adult head structures, including the compound eyes and antennae. (McClure and Schubiger, 2008; Andrew, 1987).

Although the molecular and signalling mechanisms underlying the differentiation and formation of the eye-antennal disc are partially understood, the large-scale morphogenetic events occurring during head formation and especially head eversion remain poorly understood due to limitations in long-term imaging techniques.

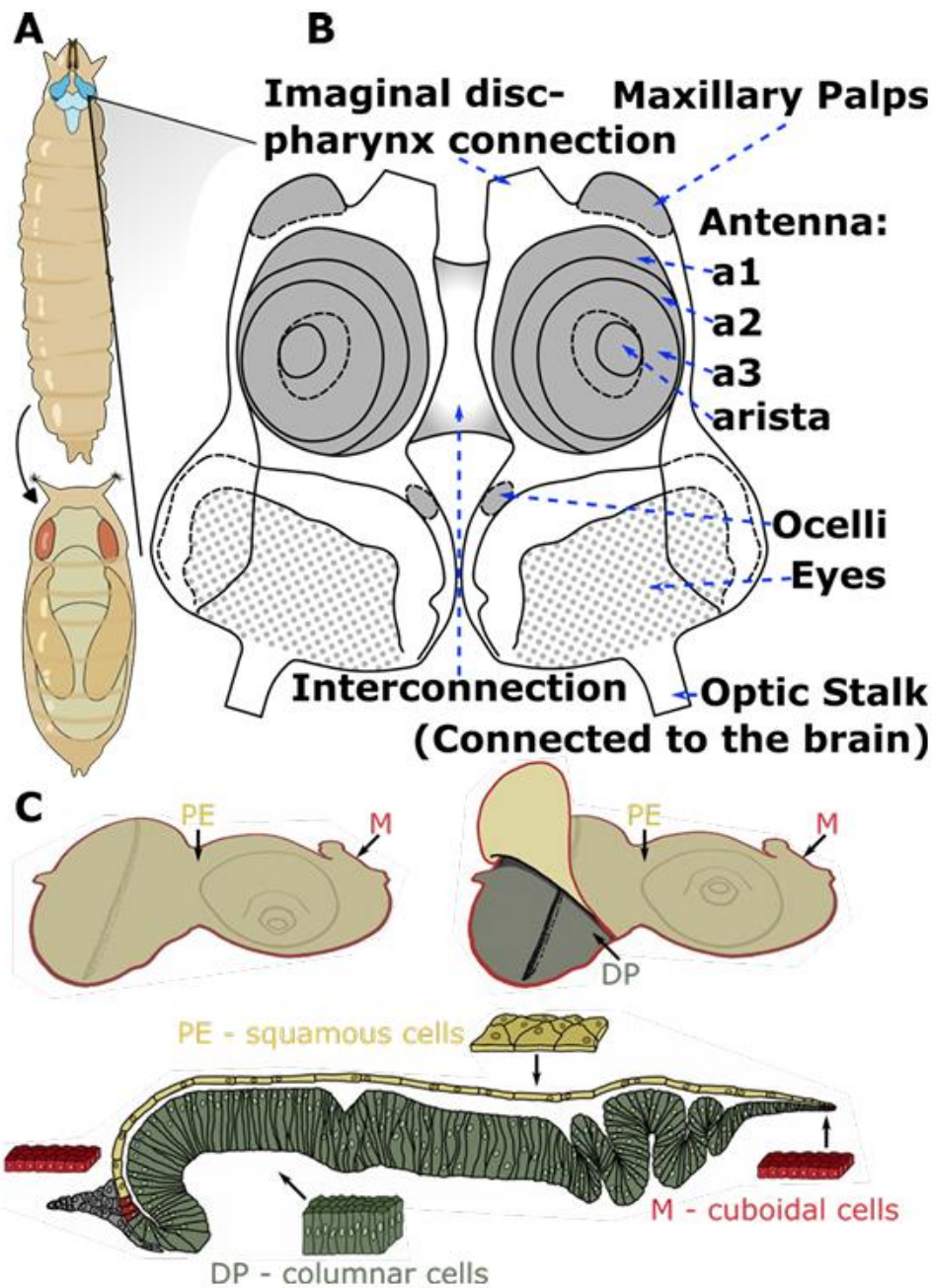


Figure 1. Anatomy of the *Drosophila melanogaster* eye-antennal imaginal disc. (A) Schematic representation showing the positioning of the eye-antennal imaginal discs within the larva (created with BioRender). (B) Microscopic view of pair of dissected eye-antennal discs. Two discs are interconnected by a thin epithelial sheet of cells. Two distinct regions are observable the antennal region, consisting of segments a1, a2, a3 (first, second, and third antennal segments), the arista, and the eye region. Ocelli and maxillary palps have their own primordia inside eye-antennal disc structure. They are not visible as a separate structure but are confirmed by using the antibody staining and fate maps (Kenyon et al., 2003). (C) Structural composition of the eye-antennal disc, consisting of two distinct cell layers and three cell types: the disc proper (DP), a pseudo-stratified epithelial layer of columnar cells, and the covering peripodial epithelium (PE) composed of squamous cells. These cell layers are connected along their edges via a set of cuboidal cells known as margin cells (M) (modified from Weasner & Kumar, 2022).

1.3. Morphogenesis of imaginal discs

The morphogenesis of the eye-antennal imaginal discs has been described by very few old studies. However, these provide preliminary descriptions of its movements and morphogenesis.

In *ex vivo* culture the two discs respond to the presence of hydroxyecdysone in the medium, which activates disc movement. The peripodial membrane plays a key role in generating tension during movement. The peripodial membrane decreases in area and bends ventrally, bringing the disc proper with it. This is confirmed by cutting the peripodial membrane during this bending. After this, the bending stops and the disc returns to its original position (Milner et al., 1983). Ventral bending of the peripodial membrane continues, bending the disc proper laterally into a more convex shape. This causes lateral rotation of both discs to fuse the two edges of the peripodial membrane into a single sac containing the antennal portion of the imaginal disc as well as the disc proper (Milner et al., 1984). During the fusion of the two peripodial epithelia opposing cells create a multitude of filopodia and pseudopodia as well, as exhibit some degree of cell migration in order to perfectly align the two tissue layers (Milner et al., 1984).

During the final stages of fly head morphogenesis, head eversion takes place where haemolymph pressure caused by the contraction of remaining larval muscles pushes the head capsule out of the larvae body, marking the beginning of the pupal stage (Bainbridge and Bownes 1981; Arranz et al., 2014).

1.4. Adult head structures

The head of the fly is located at the anterior end of the fly's body. Head is divided into an acron (not considered as a segment) and 6 other distinct segments. The 6 segments contain primary sensory organs of the fly, including the compound eyes, the antennae, and the clypeolabrum, which is built by the labrum and the clypeus (Figure 2). The labrum is a movable plate connected to the border of the clypeus, which is a frontal piece of the head. The fourth to sixth segments comprise three pairs of mouth appendages: the mandibles, the maxillae with maxillary palps and lobes, and the labium (Figure 2). These structures develop from the clypeolabral and labial imaginal discs. In flies, the maxillae form an elongate sucking tube, called the proboscis. The distal part of the labium is modified into a pair of prominent porous lobes, the labial palps. The most prominent features of the fly's head are the large compound eyes. They are located laterally on the fly's head and consist of 700-750 ommatidia, or facets with one sensory bristle between each facet (Figure 2) (Jürgens et al., 2024).

At fly's head medial-dorsal side the head bears small light-sensing organs called the ocelli (Figure 2). In flies and other flying insects, there is always a triad of ocelli, arrange in a triangular shape with a median one facing the anterior and a pair of ocelli facing the posterior parts of the head. The ocelli are situated in a distinct area called the interocellar cuticle, which also harbours a specific set of sensory bristles, which are located between the ocelli. With the exception of the proboscis and the eyes the entire head is densely covered by tiny bristles that are used for mechanical sensation. The bristles vary in size and occur isolated or in groups (Jürgens et al., 2024).

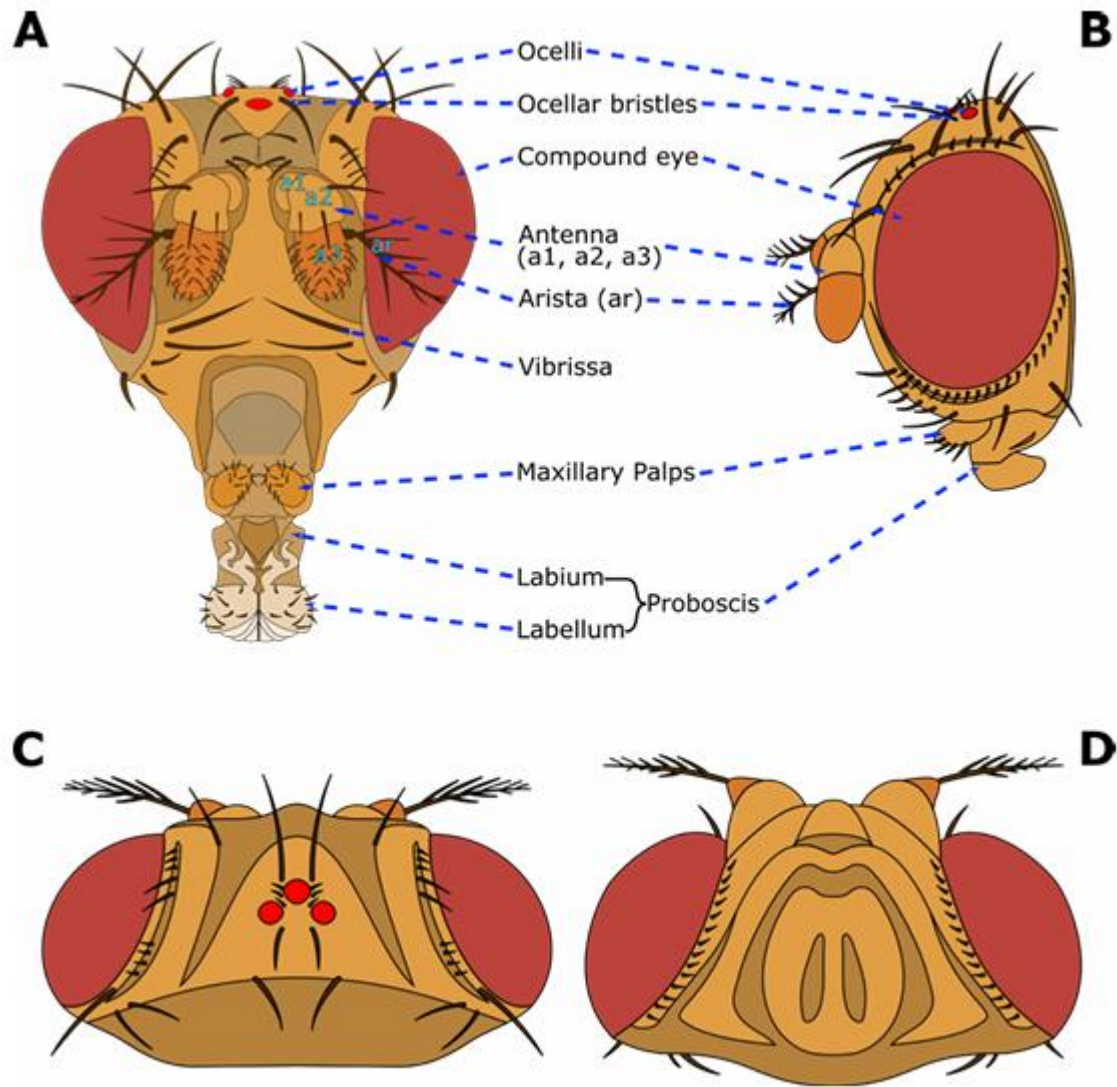


Figure 2. Adult head anatomy of *Drosophila melanogaster*. (A) Anterior view of the head. Individual antennal segments (a1, a2, a3) and the arista (ar) are indicated. Reporter analysis suggests that the cuticle beneath the eye, the second antennal segment (a2), the vibrissae, ocelli and the maxillary palps contain cells derived from the peripodial epithelium (Atkins & Mardon, 2009). The labium and labellum are derived from the clypeo-labral disc and labial disc, respectively. Adult head morphology shown in (B) lateral, (C) dorsal, and (D) ventral views.

1.5. Apoptosis signalling during tissue development

Apoptosis is a crucial morphogenetic mechanism during craniofacial development of head bearing animals and is present overall during embryonic development of all animals. Where its precise activation and temporal control is needed to shape the structures of the developing organism.

During the early stages of mammalian embryogenesis, the frontonasal, maxillary and mandibular processes must fuse along specific epithelial seams forming a unified face. For example, in mouse embryo epithelial apoptosis regulates the fusion of facial prominences at specific seams, and this process is under the transcriptional control of proteins with homeodomain Pbx. They regulate epithelial tissue remodelling through coordinated regulation of apoptosis and epithelial to mesenchymal transition (EMT) (Losa et al., 2018). Therefore, apoptosis does not play a passive role of tissue clearing mechanism, but it acts as an active force of change and regulation of epithelial architecture at the precise sites of the developmental boundaries.

The functional necessity of apoptosis in this context is demonstrated most clearly by studies in which core apoptotic machinery has been genetically disrupted. In gene-targeted *Apaf1* mice where APAF1 is a central mediator of the mitochondrial apoptosis pathway. Animals displayed striking craniofacial abnormalities alongside a marked reduction in developmental apoptosis, indicating that APAF1-dependent programmed cell death is critical for normal head formation (Yoshida et al., 1998). Similar principles also apply to human head morphogenesis. For instance, disruptions in apoptotic signalling are known to cause severe craniofacial abnormalities, as seen in Megalocephaly and Lissencephaly Syndrome, Richieri Costa–Pereira Syndrome (Favaro et al., 2014; Kim et al., 2017). Additionally, studies on *Apf1* mutants have demonstrated the critical role of programmed cell death in head development (Moreno et al., 2002). Although these diseases are caused by overexpression of apoptosis and or broken apoptotic regulation where one region receives too much signalling and other too little.

Although structurally distinct, fly and mammalian head morphogenesis share highly conserved genetic pathways and cellular processes, including apoptosis. Caspases are the main executioners of the apoptotic process in both cases (Kumar, 2007). Apoptotic signalling is present during all stages of the fly's development starting from early embryonic stage such as dorsal closure to late pupal stages (Toyama et al., 2008). During metamorphosis, apoptosis begins to play an increasingly important role as during pupal stage fly undergoes extensive tissue remodelling, where all of the

larval organs and structures are dissolved and rearranged, while imaginal discs are retained after which they move and fuse to form adult body structures e.g. wings, legs, head and eyes.

Similarly to mammalian face region fusion fly head formation involves the fusion of imaginal discs. Although in this case it is more simplistic with just one pair of imaginal discs. During fusion it was noticed that apoptotic signalling was present (Sarv, 2024) as well as simply in non-developing eye-antennal imaginal discs (Zhu et al., 2016). Although the specific locations of the signals in eye-antennal imaginal disc tissues were not specified. Mutants that have apoptosis signalling inhibited or hindered in any way exhibit central nervous system defects as well as head formation abnormalities as missing eyes or strange eye shape (Ge et al., 2017; Sarv, 2024).

1.6. Genetic and molecular tools used in *Drosophila*

Drosophila melanogaster has become one of the most powerful model organisms for studying developmental processes, including head morphogenesis. A wide range of genetic tools enables precise spatial and temporal control of gene expression and functional manipulation of specific cell populations. Among these, the GAL4-UAS system is one of the most used, allowing targeted gene activation in defined tissues such as the eye–antennal imaginal disc. In combination with complementary systems, these tools provide a versatile framework for studying the genetic and cellular mechanisms of morphogenesis (Yamada et al., 2020).

The GAL4/UAS system is a binary gene expression system. Consisting of the GAL4 yeast derived transcriptional activator and an upstream activating sequence (UAS) to which GAL4 binds. GAL4 expression can also be spatially restricted through the use of tissue-specific enhancers. In the GAL4 fly lines, expression is placed under the control of a tissue- or cell-specific enhancer, creating a driver line. In a second fly line, the gene of interest is positioned under the control of UAS. When these two lines are crossed, GAL4 binds to the UAS sequences and in the progeny activates transcription of the target gene specifically in cells or tissues specified by the GAL4 driver line. Common reporter genes used in this system include for example non-fluorescent Chloramphenicol acetyltransferase (CAT) and lacZ to monitor transcriptional activity inside the cells as well as numerous fluorescent reporter genes such as GFP, and RFP, that are employed to visualize cellular expression, cell shape, location and movement (Li et al., 2018; Duffy, 2002).

For temporal control of the GAL4 system, a temperature sensitive variant of the GAL80 repressor was developed. Because most promoters and enhancers that control GAL4 expression are active through multiple stages of development, thus temporal control is limited. GAL80 is also yeast-derived inhibitory protein. GAL80 inhibits GAL4 activation by blocking its activation domain, preventing interaction between GAL4 and the transcriptional machinery (Nogi and Fukasawa, 1989).

GAL80ts is a thermosensitive GAL80 variant. Where at room temperature 20°C, GAL80ts is active, and able to bind to GAL4-repressing its expression, and at the higher temperature of 29°C, GAL80ts is inactive and repression does not occur allowing for normal GAL4 gene expression and activation of subsequent gene of interest (Barwell et al., 2017).

Alternative temporal control possibility for GAL4 system is use of chemically induced GAL4 lines. Where a normal GAL4 transcription factor is modified with an addition of ligand-binding domain

derived from steroid hormone receptor. This type of modified GAL4 remain stable and does not activate transcription at the UAS. In order to activate the transcription a chemical inducer must be added e.g. progesterone analogue mifepristone (RU-486). It will bind to the modified GAL4 transcription factor restoring its normal expression (Osterwalder et al., 2001).

Although this method can achieve temporal control of the expression it also requires creation of new GAL4 lines, therefore not efficient and are rarely used (Kogenaru et al., 2024).

1.7. *Ex vivo* culture methods of imaginal discs

The imaginal discs of *Drosophila melanogaster* are an excellent model for studying developmental processes. However, their study is often hampered by their deep location within the animal. To address this, experiments with *ex vivo* methods began as early as the 1960s (Hanly et al., 1967).

Ex vivo methods allow for simplification of the complex organ systems, enabling focused analysis of individual tissues. The resulting images are of much higher quality and easier to manipulate. *Ex vivo* systems for *Drosophila* require different conditions than mammalian cell cultures. Current methods allow for maintaining *Drosophila* tissues *ex vivo* cultures for a couple of days. For the optic disc studied in this study, a culture life of several days is sufficient. Key movements and fusions occur early in development. The detailed components required for longer-term observations are not yet known, but currently, insulin and 20 hydroxyecdysone are required components of the culture. This hormone is involved in the initiation of pupalization and the onset of imaginal disc differentiation in *Drosophila* (Riddiford, 1993).

Using this hormone in combination with insulin stabilizes the proliferation and differentiation of imaginal disc cells. Without it, *ex vivo* cultures are unable to develop.

Ex vivo studies are often performed on neural tissue from the brain, as well as wing, leg, and eye-antennal discs, but rarely on eye-antennal discs as paired structures (Kumar, 2018; Rabinovich et al., 2015). This is primarily due to the difficulty of dissection and the inconsistency of results from such *ex vivo* cultures.

1.8. Current research gaps and method limitations

Live imaging of *Drosophila* development remains technically challenging despite major advances that have enabled visualization of key morphogenetic processes such as dorsal closure, wound healing, organogenesis and tissue patterning (Nienhaus et al., 2012; Wood and Jacinto, 2005). However, many aspects of head morphogenesis remain insufficiently characterized due to imaging limitations.

Imaginal discs in general provide excellent models for studying organogenesis and offer valuable insights into molecular processes, yet they are inherently difficult to image. Their small size, thin and transparent epithelial organization, and deep localization within larval tissues complicate long-term observation. In addition, surrounding organs and extracellular tissues obstruct optical access and reduce imaging stability during development.

In the case of the eye-antennal imaginal disc, the complexity is increased as an isolated structure as wing or leg discs. It is tightly associated with the larval nervous system particularly the brain hemispheres. While recent studies achieved some success in neural tissue live imaging, simultaneous eye-antennal and brain live imaging is difficult because of the media composition as it must suit both structures (Boto and Tomchik, 2024).

Established *ex vivo* techniques are present for the eye-antennal imaginal discs. It consists of larvae preparation, complex dissection and preparation of specific growing medium. After which we can observe the head capsule, eyes and sometimes maxillary palp morphogenesis. The inability to sustain active morphogenesis for more than 18-20 hours. This temporal limitation prevents continuous observation of later developmental events that occur during pupal stages and restricts our understanding of final organ differentiation and organogenesis (Sui and Dahmann, 2025).

Consequently, significant gaps still remain in our understanding of *Drosophila* head morphogenesis on the cellular and molecular levels during the eye-antennal disc development (Atkins and Mardon, 2009). Large scale morphogenetic movements forming future adult head remain relatively poorly understood. In particular, the mechanisms underlying head eversion, coordinated tissue remodelling and integration of developing eye-antennal disc with brain structures remain poorly understood (Arranz et al., 2014).

These limitations are closely associated with technical difficulties of long imaging during in vivo development. It is because most of the processes that interest us happened during the pupal stage,

which is limited by modern *ex vivo* methods. Our current understanding of head formation remains fragmented.

Thus, developing better techniques of visualizations ability to sustain physiological development including both nervous and epithelial tissues will have a great impact on understanding of how head morphogenesis is coordinated into the final architecture of the head.

2. EXPERIMENTAL PART

2.1. Aims of the Thesis

The aims of this thesis are:

- 1) To map the morphogenetic processes that occur during head formation.
- 2) Investigate and describe the molecular and cellular mechanisms of the observed processes.
- 3) Elucidate the cell fate during face formation by capturing it in a detailed time-lapse movie.
- 4) Test and map the specific apoptotic signalling pathways crucial for head formation.
- 5) Optimize and develop the current *ex vivo* live-imaging methodology.

2.2. Materials and methods

2.2.1. Used fly stocks

The fly stocks used in this study were obtained from the following sources:

Bloomington Drosophila Stock Centre (BDSC).

Table 1. List of fly lines used

Stock	Purpose	Source	Identifier
sd[12]P{ry[+t7.2]=neoFRT}19A/FM6;P{w[+mW.hs]=GawB}mirr[DE]/TM6B, Tb[1]	DE-Gal4, which is expressed in the dorsal part of the eye-antennal disc	BDSC	78371
y[1]; P{w[+mW.hs]=GawB}c311	C311-Gal4 which is expressed in eye -antennal disc peripodial epithelia	BDSC	5937
mutX/FutB;t/cyo; DE-GaL4(III), C311-Gal4(III)/ Tm6 Tb	DE-Gal4 and C311-Gal4 recombinant combining both expression patterns	N/A	N/A

w[1118]; P{y[+t7.7] w[+mC]=20XUAS-IVS-mCD8::mStayGold}su(Hw)attP5/CyO	Expresses membrane bound GFP under the control of UAS	BDSC	606314
W;skh,mch/Cyo; UASGC3Ai/Tm6TBSb	Combines specific fluorescent reporter for apoptosis and mCherry red fluorescence background	N/A	N/A
W; Gal80ts/Cyo; DE-Gal4/Tm6B	Temperature sensitive DE-Gal4	N/A	N/A
w[*]; P{w[+mC]=UAS-p35.H}BH1	Expresses the baculovirus p35 cell death inhibitor under UAS control	BDS	5072
w[*]; Kr[If-1]/CyO; P{w[+mC]=UAS-GC3Ai}3	Expresses a GFP-based apoptosis sensor under the control UAS	BDS	84343
sqh:sqh-mCherry (II)	Live fluorescent marker for Non-muscle Myosin II	Obtained from Xiaobo Wang	N/A
w[*]; P{w[+mC]=tubP-GAL80[ts]}20; TM2/TM6B, Tb[1]	Temperature-sensitive GAL80 repressor line used primarily for temporal control of gene expression in the GAL4/UAS system	BDSC	7019

2.2.2. Culture media composition

In this study, Shields and Sang M3 insect medium (Sigma-Aldrich, S8398) was used as the basis for both dissection and growth media. It was prepared by dissolving 0.5 grams of KHCO_3 and M3 powder in 1 litre of distilled sterile water. This solution was used as the dissection medium. Growth media was prepared from this M3 solution. 100 millilitres of IMS (10x insect medium supplement, (Sigma-Aldrich, I7267) and 10 millilitres of penicillin-streptomycin (final concentration 100U/mL penicillin, 0.1 mg/mL streptomycin) were added to 900 millilitres of M3 solution (Sarv, 2024). This solution is referred to hereafter as M3/IMS growth media. Both dissection and growth media were stored under sterile conditions at 4 °C. Before the experiment, the M3/IMS solution was aliquoted into 50-ml test tubes, to which 63 μl of insulin and 25 μl of 20-Hydroxyecdysone (Sigma-Aldrich, H5142) were added, to reach a final volume of 50 ml. This solution was prepared before the experiments, and used within 1 week, after which fresh solution was made for subsequent experiments.

2.2.3. Dissection and Imaging

The experiments were performed on third instar *Drosophila* larvae that had begun to exhibit wandering behaviour and were located away from the food source. After collecting the larvae of the desired stage, they were washed in sterile PBS, disinfected with 72% alcohol and then transferred to dissection medium (Sui and Dahmann, 2025).

The Dissection protocol was developed by adapting methods described by Sui and Dahmann (2025) and improved by Robin S. (bachelor's thesis 2024). Dissection was performed using tweezers and microscalpel on a silicone disc. The cleaned larva is completely submerged into a drop of dissection media on the silicone plate. To isolate the discs, the larva's facial region, which includes the brain, mandibles, and eye discs, was isolated by pulling on the mandibles with one pair of tweezers and holding the body using the other pair (Figure 3A). All unnecessary fat, leg discs, and trachea were then removed from the pulled-out facial region using tweezers. The brain was then cut in half with a scalpel to make room for the next stage (Figure 3A). After the brain was cut, the nerve connections between the eye part of the eye-antennal discs and the brain hemispheres were cut with a scalpel. After the brain was removed, it is necessary to remove the remains of the mandibles and the connecting tissues between them and the eye-antennal discs (Figure 3A). If necessary, the discs were further cleaned of residual tissues. Using tweezers, the prepared discs were carefully transferred from the silicone disc to a dish containing the growth media (Figure 3B).

After collecting a sufficient number of discs for observation, an initial observation was performed. During this stage, the condition of the discs was analysed, and the healthiest disc was selected for detailed observation to improve the image quality. The subsequent observation was performed using a fluorescent microscope for 18–20 hours.

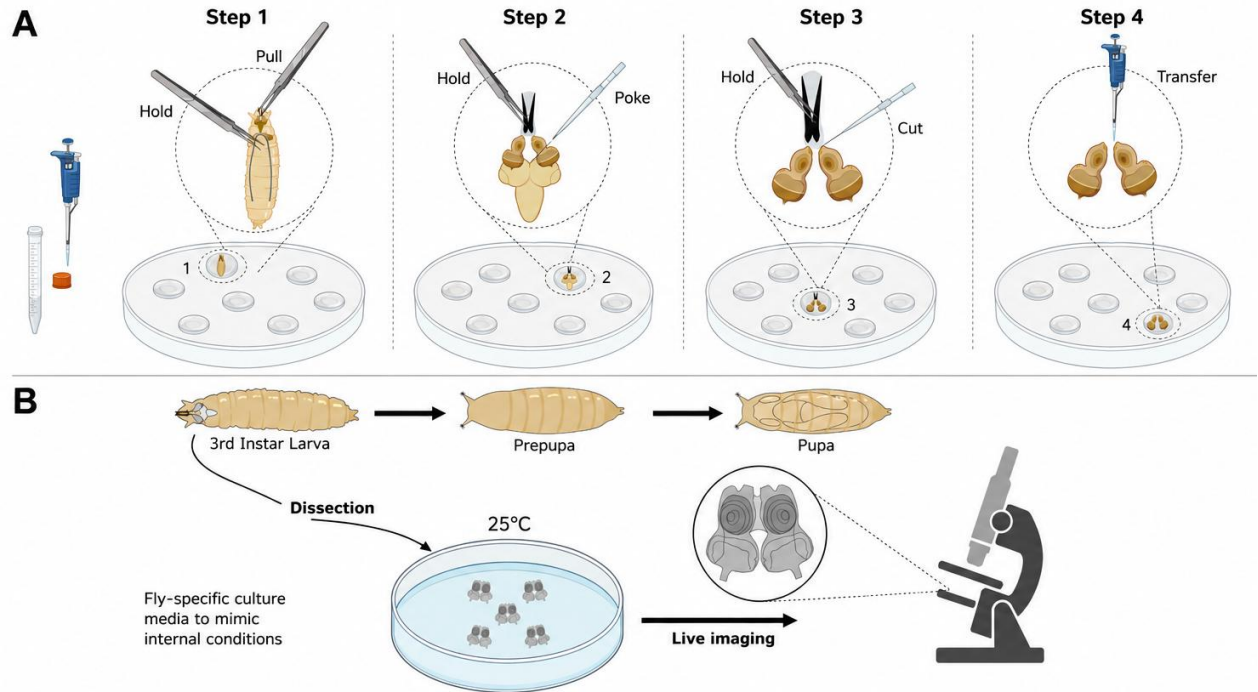


Figure 3. Schematic representation of the dissection and *ex vivo* culturing method. (A) Larval dissection showing isolation of the head region from the larval body, followed by removal of the brain and separation of the mouth hooks. (B) Schematic of the *ex vivo* method, in which isolated eye-antennal imaginal discs are transferred to culture medium supplemented with Insulin and the steroid hormone Ecdysone for time-lapse imaging.

2.2.4. Microscopy

For this work, EVOS M7000 Imaging System, an inverted fluorescent microscope for thick tissue, as well as an Olympus IX81 Fluorescent Microscope, an inverted DIC microscope used for live imaging DIC and fluorescent experiments, were used. The EVOS was used to capture non fluorescent or strong fluorescent time-lapse imaging. While the Olympus microscope was used for more detailed imaging.

Exact settings differ depending on the experiment disc anatomy and microscope. Z stack parameters were typically in the range of 100-150 micrometres. Z stack acquisition and live imaging snapshots were recorded at 10-minute intervals. Analysis of the data was performed using Imaris.

2.3. RESULTS

2.3.1. Eye-antennal imaginal disc morphogenesis.

By further refining the dissection technique and *ex vivo* imaging in this work we managed to consistently observe disk morphogenesis and capture in detail all movements occurring during this process. Because of the improvements, the overall duration of the time-lapses imaging was increased to around 18-20 hours.

As previously demonstrated in the work of Robin Sarv in his “Live Imaging of *Drosophila melanogaster* Eye-Antennal Imaginal Disc Ex vivo Culture: Craniofacial Morphogenesis”, the most significant events occur between 0 to 4 hours (Sarv, 2024). During this period, key processes including alignment and Folding of the disc proper under the tension generated by the peripodial membrane, bringing the antennal part closer to the eye field (Figure 4, 0-4 hours). After the initial movement, a period of rapid morphogenetic change takes place, during which the disc transitions from a flat tissue into a more voluminous structure. Between 4-7 hours, the discs begin to rotate to align with respect to future forming structures. Future antennae and ocelli are placed opposing each other and the whole shape of the discs is changing into a rectangle with rounded shorter sides. Between hours 7 to 11 hours, disc fusion emerges as the crucial even in disc development (Figure 4). During this stage PE, which covers the whole disc, fuses firstly at the anterior part of the disc (Figure 4), followed by fusion at the posterior part of the disc (Figure 4). At this point, major morphogenetic movements stop and further structural changes are primarily limited to structural refinement, particularly in the eye regions located laterally (Figure 4, Figure 6B). In the final stages of fusion at 11 hours, the formation of the head cuticle becomes visible, and. the PE forms a continuous layer covering the entire eye antennal disc.

After finalizing the epithelial fusion, the structure remains relatively stable between 12 and 15 hours. After 15 hours, the anterior and posterior fusion regions begin to bend outward of the structure (Figure 4, Figure 6B) and the structure as a whole undergoes gradual contraction. Between 16 –20 hours, the structure changes from rectangle shape with rounded edges into a more compact, oval structure with small outward bends of the PE at the places of fusion.

This final process is most likely a part of the normal development rather than tissue degeneration. Even at 20,5 hours, the disc continues to exhibit clear movements, internal structures within the forming head capsule remain well defined (Figure 4).

When the disc is dying or dead, no movement is observed and rapid tissue deterioration occurs; under the microscope, this appears as a dark cell mass with no clear boundaries, which eventually covers the entire imaginal disc and makes it impossible to see anything further.

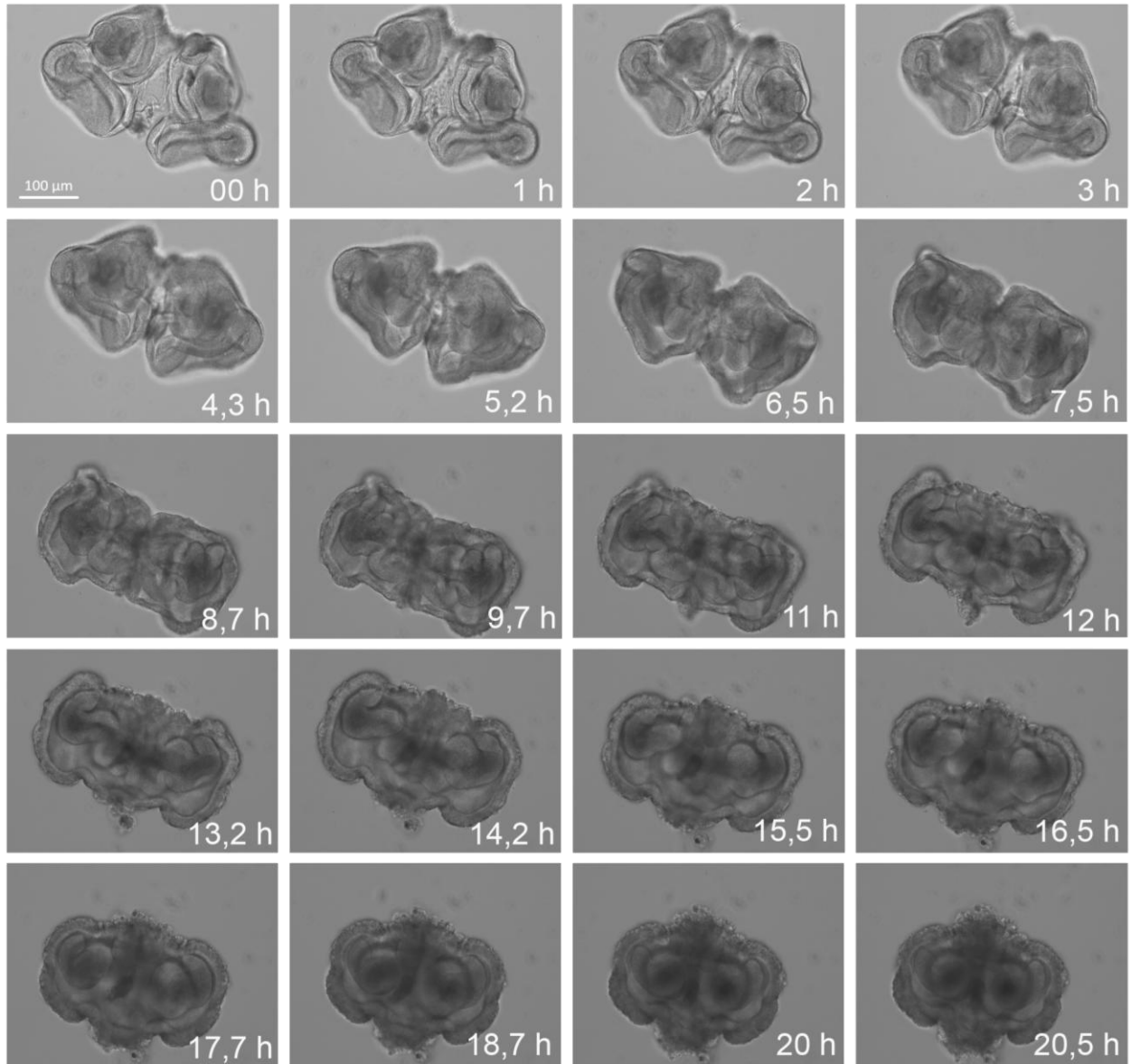


Figure 4. *Ex vivo* time-lapse imaging of the eye-antennal imaginal disc. Set of DIC images showing all major morphogenetic movements from 0 to 20 hours.

2.3.1.1. Disc proper morphogenetic movements during imaginal disc formation

Thanks to the development of our imaging and dissection techniques, we were also able to capture the intricate movements of the discs proper within the developing head capsule. The fine movement can be divided into five distinct stages, extending the previously described four by R. Sarv (Sarv, 2024 bachelor's thesis).

During **stage 1**, the eye region of the eye-antennal disc folds and shortens moving posteriorly toward to the antennal region of the imaginal disc, which itself moves anteriorly, toward the eye field (Figure 5A and B). Also, during this stage two discs move closer to one another and interconnective membrane becomes visibly smaller (Figure 5B).

Stage 2 begins with the start of the eye inversion, where folded DP turns inside out to cover the lateral arc of the eye shape (Figure B). Part of the antennal DP also folds and separates from the main primordia (Figure 5B). It remains connected to the main primordia but at the same time it elongates and migrates toward the corresponding structure in the opposite disc. Partial fusion of the cuticle near the antennal primordia, r aligning the two epithelia and bringing the two discs closer together.

Stage 3 marks the beginning of the eye field rotation. During this process two discs, previously located at a sharp angle relative to each other, rotate into a more perpendicular position (Figure 5 C). In the fusion regions, V-shaped tissue folds begin to take shape. They will later smooth out as fusion progresses. During this stage, the antennal primordia (Figure 5C) move toward the lateral sides of the disc near the developing eye arcs, allowing the a1 and a2 segment regions to bulge up and form a fold adjacent to the main antennal primordia (Figure 5C). Main antennal primordia and a1 and a2 folds are still connected by a DP extension, forming together an elongated S-shaped structure. Similar structure forms in the opposing imaginal disc. Main antennal primordia remains relatively round (Figure 5 C). And two discs are still distinguishable from one another.

Stage 4 is characterized by complete fusion of the two discs, forming a continuous head structure. Firstly, fusion occurs at the posterior fold, followed by the anterior fold (Figure 4). The previously formed V-shaped folds flatten into a smoother epithelial surface with minor indurations remaining (Figure 5D). The eyes fields take on semicircular shape c and assume their final lateral position. During this final cuticular fusion DP fusion also occurs. The a1 and a2 regions, which migrated toward the disc centre near the fusion region, fold again and fuse with their counterpart from the opposite disc, forming a line with four folds (Figure 5D). The main antennal primordia, previously

rounded, stretch and fold into a curved furrow located parallel to the frontal plane of the animal head (Figure 5D). Connective part of the DP between a1, a2 and main antennal regions is pushed toward the site of the first fusion at the anterior part of the disc.

In **stage 5**, the two main fusion regions, previously smoothed, continue their movement and begin to evert. During this eversion, the entire structure begins to contract, decreasing in size and volume. The Eyes shift toward the centre of the forming head. Internally, the head a1 and a2 regions of the DP move ventrally and anteriorly, hiding from the field of view under the main antennal primordia (Figure 5E). The main antennal primordia also decrease in size, forming an oval with a small lateral extension. This oval then also begins to move towards the centre and then front of the structure almost touching the opposing antennal primordia (Figure 5E). This anterior location is almost exactly where antennae are located on the adult head. This final movement of the main antennal primordia are more closely visible in Figure A.1. in the appendix.

Final shape of the head at 20 hours is a smaller, more compact oval shape. Symmetrical protrusions are present along its lateral sides, and the internal structures have already shifted toward the front part of the head, corresponding to their final place in the adult head.

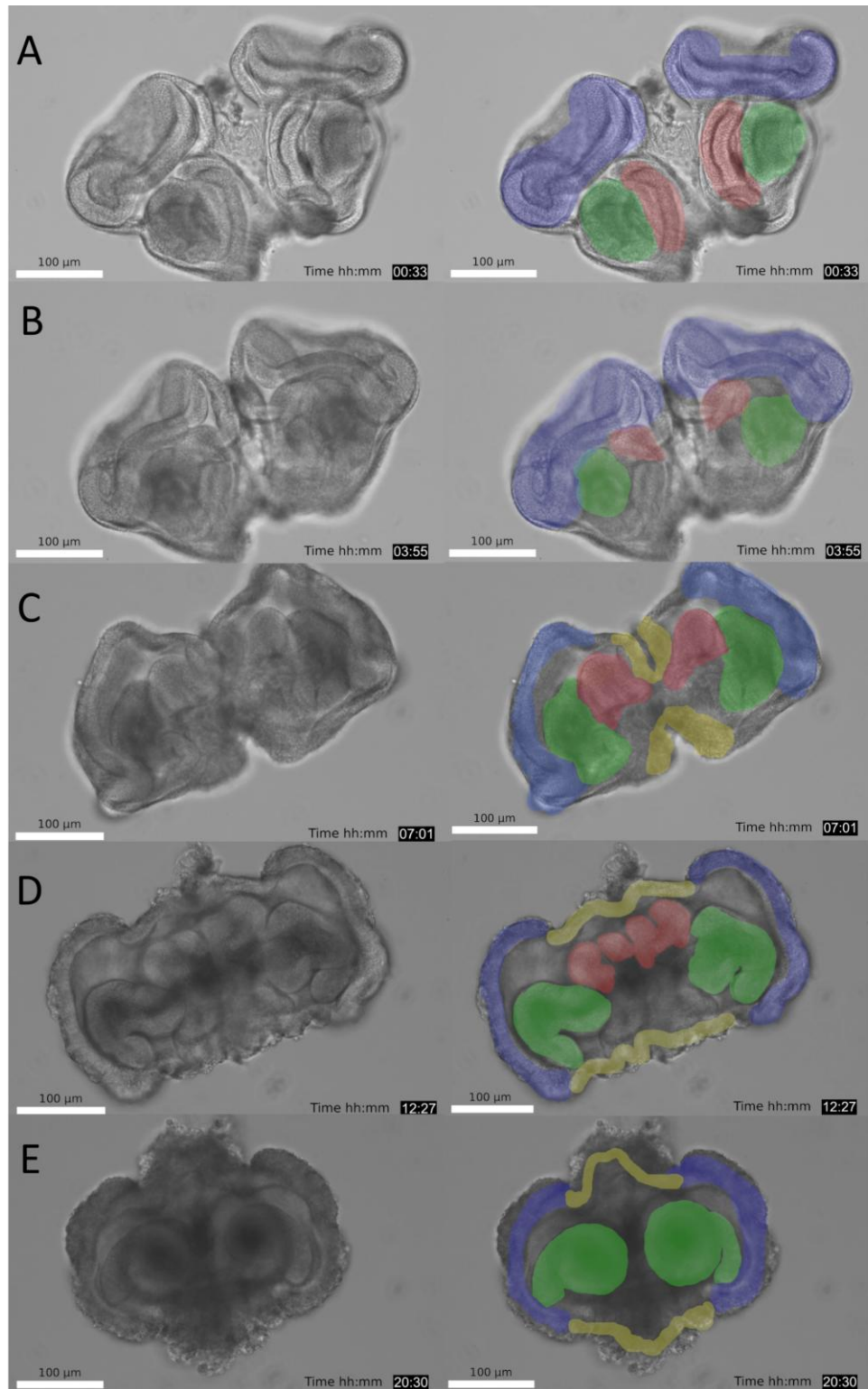


Figure 5. The five major developmental stages of eye-antennal imaginal disc, with internal structures indicated. Description of the five main developmental stages: (A) stage 1 (0-4 hours), (B) stage 2 (4-7 hours), (C) stage 3 (7-11 hours), (D) stage 4 (11-15 hours), (E) stage 5 (15-20 hours). Indicated structures: the eye region of the DP (blue), the two major fusion regions of the PE (yellow), the main antennal primordia (green), and the a1 and a2 regions of the DP (red). Scale bar: 100 μm.

2.3.1.2. Peripodial membrane role in final structure formation

By extending the imaging window past 16 hours through methodological improvements, we successfully observed the complete progression of disc development up to its final stages at 20,5 hours.

During fusion and after it in stage 4, brightfield imaging revealed few visible changes in PE shape, aside from minor eye shape finalization and overall alignment (Figure 6). Using GFP expression driven by the C311-GAL4 peripodial membrane-specific driver, in which fluorescence is restricted to PE cells, we visualized PE dynamics during the final stages of development (Figure 9). We observed that during these final stages of disc fusion, the peripodial membrane actively moved posteriorly along the curve of the disc's outer surface, while simultaneously reducing its surface area (Figure 7). These observations suggest that PE movement contributes to Stage 4 shape correction and later stage 5 overall head compaction. Due to technical difficulties, we were not able to continue observation after the 11-hour mark in the case of Figure 5.

These observations were further supported using another peripodial membrane specific DE-gal4 driver. In this case, expression is limited to a dorsal part of the eye antennal disc (i.e. peripodial membrane), forming a line near the fusion regions (Figure 8). This driver produces much brighter fluorescence, facilitating more precise observations of the cellular movements. Following fusion, the fluorescent signals form an almost complete circle.

Observing stage 4 from 8 to 12 hours we saw a subtle shift in the dorsal part of the fluorescent signal (Figure 6). This corresponded to similar dynamics observed using the C311 driver at comparable time points, although captured from a different focal plane and using a different microscope (Figure 9). Continuous observations into stage 5 and onwards revealed that during the rounding and compaction of the structure, the peripodial membrane location shifted further anteriorly relative to its initial position. During this movement, the anterior and lateral sides of the fluorescent signal moved closer together, indicating a shrinkage of the PE area (Figure 6). We also, observed that the posterior part of the fluorescent PE signal exhibited minimal displacement but instead it becomes brighter over time, likely due to adjacent fluorescent PE cell accumulation.

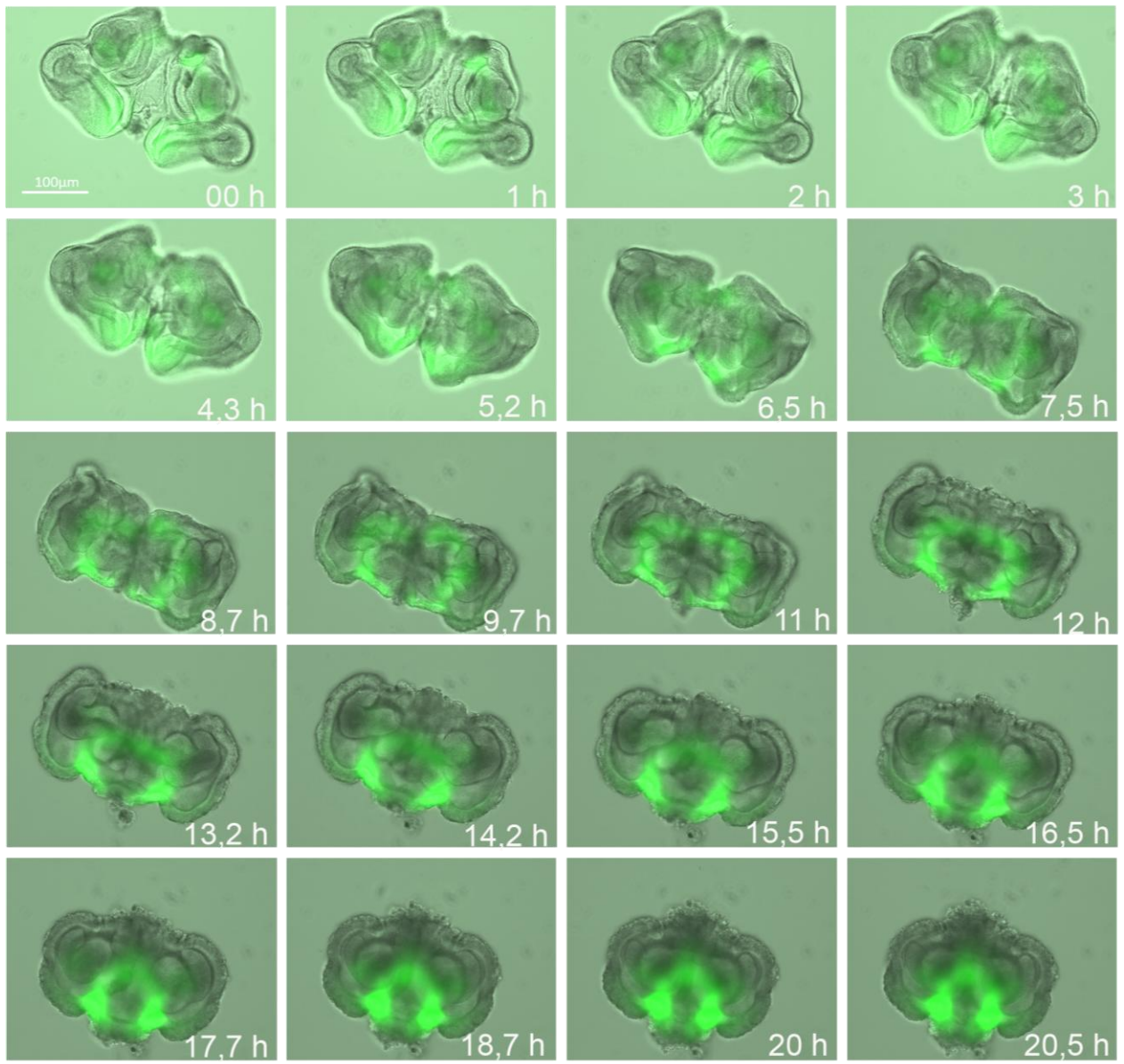


Figure 6. *Ex vivo* eye-antennal imaginal disc time-lapse with DE-gal4 expression visualising PE movement. A series of GFP and DIC images depicting all major morphogenetic movements of the peripodial epithelia from 0 to 20 hours.

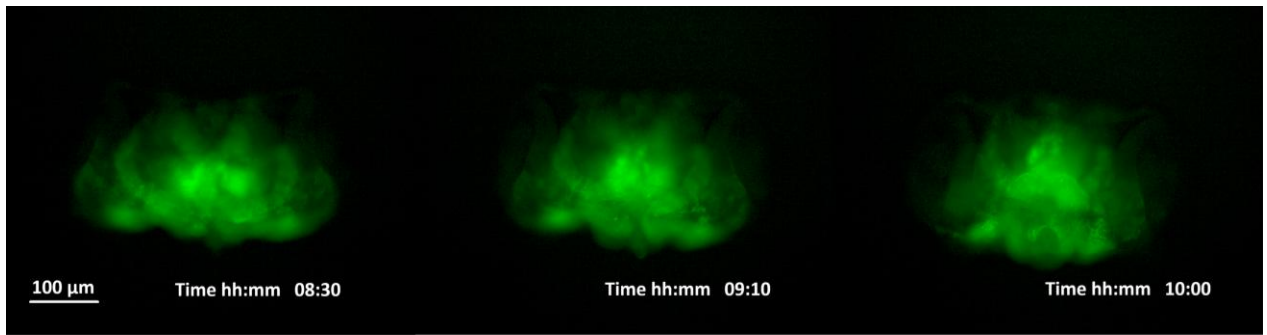


Figure 7. Live imaging of peripodial membrane movements during disc fusion. The Peripodial membrane is visualised using C311-gal driver induced GFP expression, showing its posterior movement and subsequent contraction in surface area.

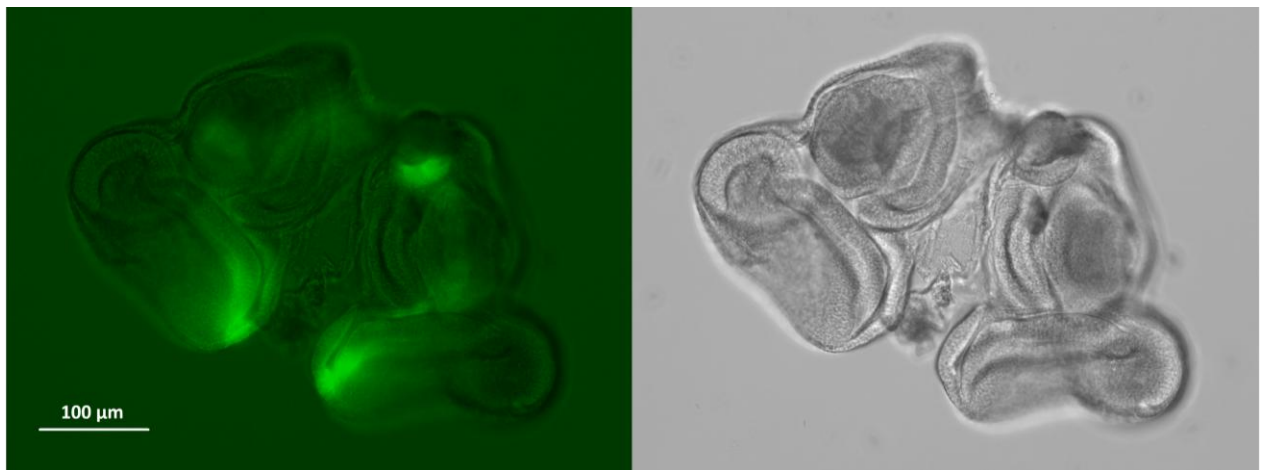


Figure 8. DE-Gla4 expression pattern. Membrane bound DE-mStayGold expression in the eye-antennal imaginal disc. Representative images show the green, fluorescent signal (GFP) restricted to the nuclei alongside the corresponding brightfield channel to illustrate overall disc morphology. Expression is localized in the dorsal part of the disc in the medial part of the peripodial membrane. Scale bar: 100 μm.

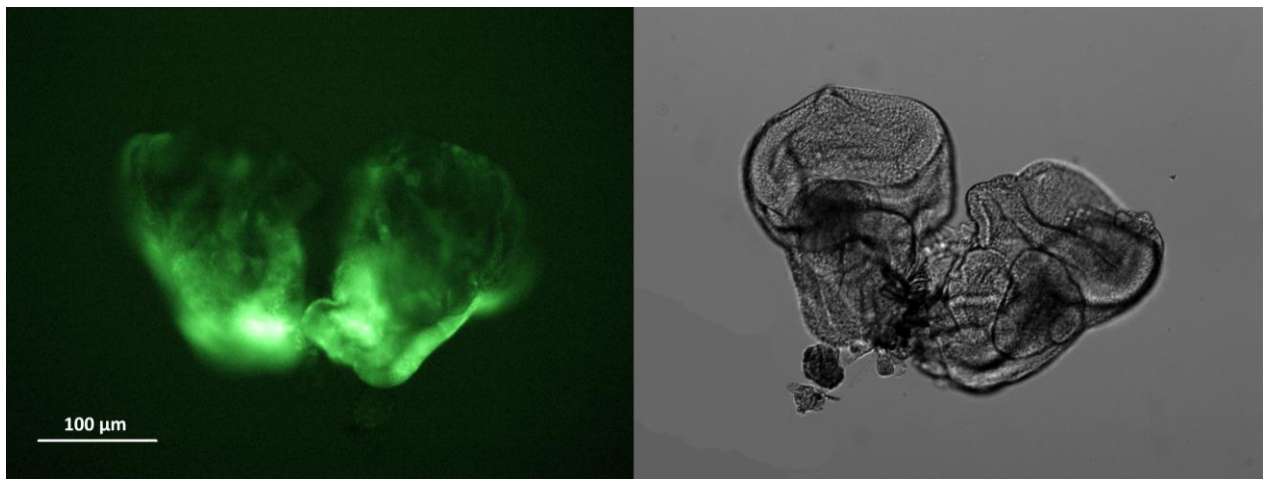


Figure 9. C311-Gal4 expression pattern. Membrane-bound mStayGold expression induced by C311-Gal4 in the eye antennal disc, in both GFP and DIC to illustrate overall disc morphology. Expression is concentrated inside the thin layer of cell of the peripodial membrane, with brighter regions near the disc interconnection. Scale bar: 100 μm.

2.3.2. Apoptotic signalling during morphogenesis

As previously established, apoptosis plays a vital role in animal organ development. To investigate the spatiotemporal dynamics of apoptotic signalling during *Drosophila* head development and to map its activity, we utilized the GC3Ai reporter. GC3Ai consists of a modified GFP that remain in a non-fluorescent conformation due to an inserted caspase-cleavable sequence. When effector caspases activate, the linker is cleaved which in turn allows GFP to fold into its active fluorescent conformation. Because fluorescence is directly activated by presence of the effector caspase this tracker provides a relatively rapid readout of caspase activation compared to transcription-based systems. In UAS-GC3Ai system, the reporter is expressed under the control of Gal4 drivers, allowing for tissue specific caspase activity to be detected. While the GC3Ai successfully confirms exposure to caspase-3 signals, it does not distinguish between lethal, sub-lethal, or non-lethal apoptotic outcomes.

During our observations we tested two different Gal4 drivers to compare the expression pattern as well as its intensity. In the case of C311-Gal4 driver, caspase activity increased progressively as development continued (Figure 10). During the start of the first fusion at the 7-hour mark, caspase activity was clearly localized to the V-shaped folds outlining the fusion regions. In the final stages, we observed a more uniform, punctate expression throughout the central fusion regions. This suggest that the morphological processes occurring stages 4 and 5 may also depend on caspase signalling for their development. It's worth noting that the C311 expression pattern definitely extends to the eye part of the imaginal disc, but in this case, we did not see any obvious caspase signals from these regions (Figure 10).

DE-Gal4 driver showed a caspase activity in limited regions in the beginning of the live imaging and increased substantially over time. Similarly at the start of the disc fusion around hour 7 expression was localised to the posterior and anterior parts of the disc (Figure 10), where it continued to intensify during later stages. We do see a similarly patterned expression of the middle regions of the antennal disc, as with the C311 driver (Figure 10), although it was weaker and more spatially restricted. This is likely due to the DE specific expression pattern, in which fluorescent expression is extremely potent and highly concentrated in its designated regions. Therefore, by the 6-hour mark, individual cells within most expressing regions become indistinguishable, confining the detectable signal strictly to the DE pattern.

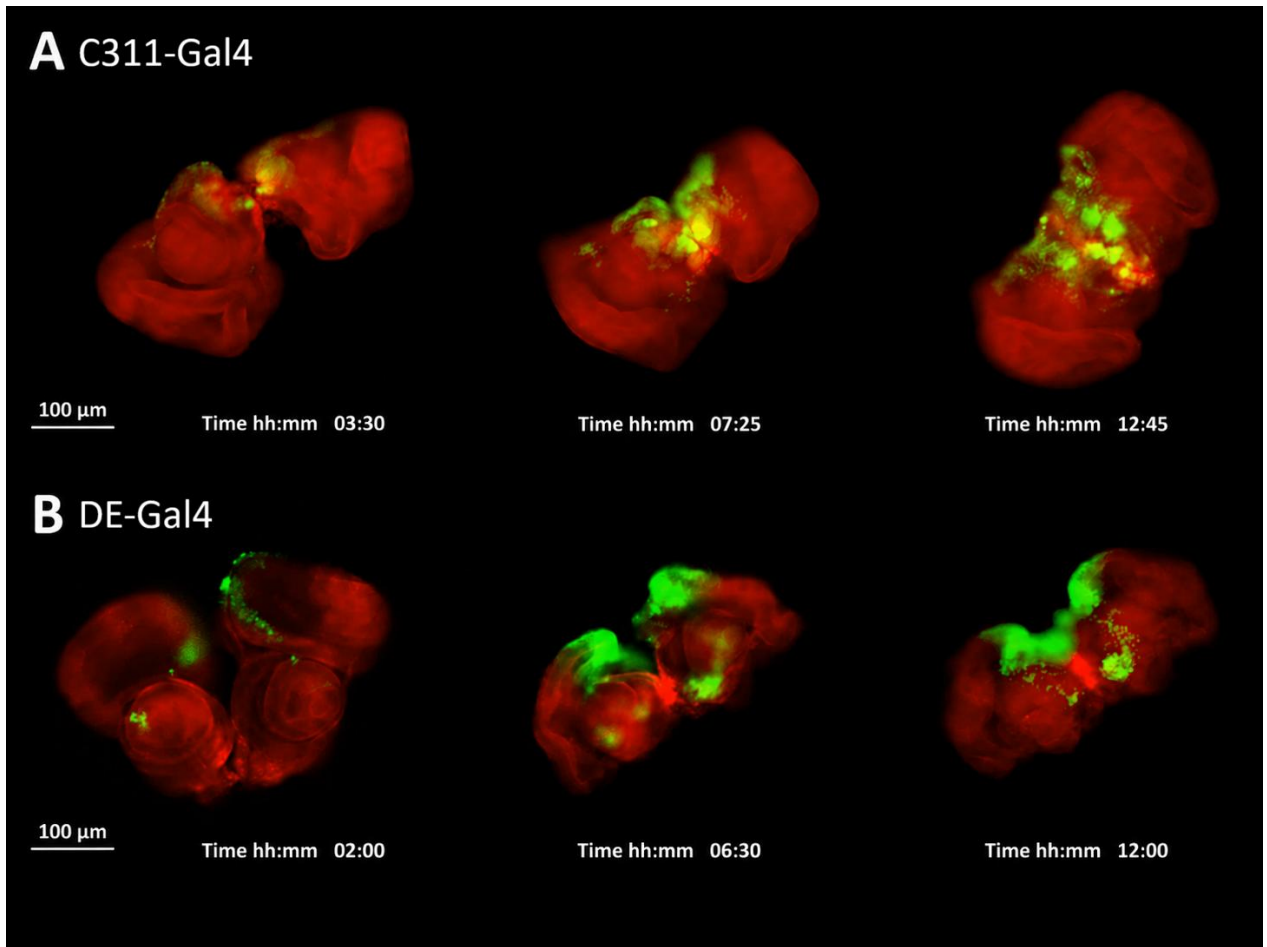


Figure 10. C311-Gal4 and DE-Gal4 crossed with caspase sensor time-lapse. Representative frames illustrate the major stages of eye-antennal disc development, with caspase tracing indicating the intensity and spatial distribution of apoptosis. (A) Apoptotic signal increases throughout the stages of the development, concentrating at the fusion sites as well as uniformly across the medial region of the structure. (B) Apoptosis signal is similarly increasing over time, with enrichment near the fusion regions but comparatively lower expression in the middle of the two discs, where only isolated apoptotic puncta are visible. Squash:mCherry was used to capture the tissue structure. Scale bar: 100 μ m.

To verify the functional significance of apoptosis, phenotypic data and mortality rates were evaluated for both expression patterns crossed with an apoptotic inhibitor strain.

Each laboratory grown stock exhibits a natural mortality rate, meaning that not that not all flies will complete their development. In this study, embryonic and larval mortality could not be observed, therefore the data acquired consists of flies who died at pupal stage. C311 and p35 stocks showed relatively low natural mortality rates of 4% and 8% respectfully (Figure 11). The DE stock showed a higher natural mortality rate of approximately 26% (Figure 11).

When these flies were used for crossing with a caspase inhibitor p35, increased pupal mortality was observed (Figure 12). C311 when crossed with p35, mortality increased to approximately 60%. Because dead pupae could not be genotyped, the recorded mortality includes all progeny types. Examining the dead pupa phenotypes revealed severe defects in head development. In most cases, head region seemed underdeveloped, sometimes missing completely inside pupal case (Figure 13). The head itself seem to form initially but then it quickly degrades and loses its structure after which the animal dies (Figure 9). The few flies that survived to the adult stage exhibit strong head capsule abnormalities with small round or underdeveloped eyes (Figure 13).

DE cross with apoptotic inhibitor shows increased mortality relative to the parental stock mortality rate, reaching approximately 40% (Figure12). Similarly, all dead pupae recorded include all progeny genotypes. In contrast to the C311 cross a larger number of flies survive to the adult stage. Abnormalities in this case include small misshapen eyes, fragmented eyes and sometimes missing eyes and parts of the head (Figure 14). Normal progeny phenotypes were also recorded. This shows that apoptosis during imaginal disc fusion and later differentiation is crucial for normal eye and head formation.

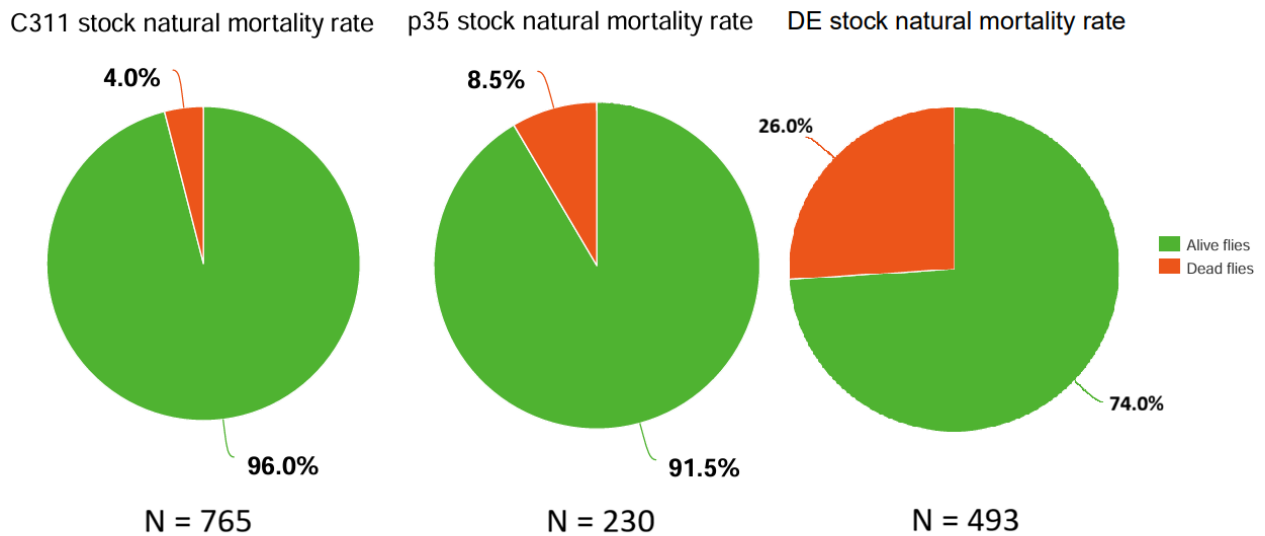


Figure 11. Natural mortality rates of used fly stock. In case of lab grown fly stock it is expected that not all individuals will reach maturity for one reason or another. The mortality rate represents the percentage of individuals that failed to complete development and died prior to adult emergence (eclosion).

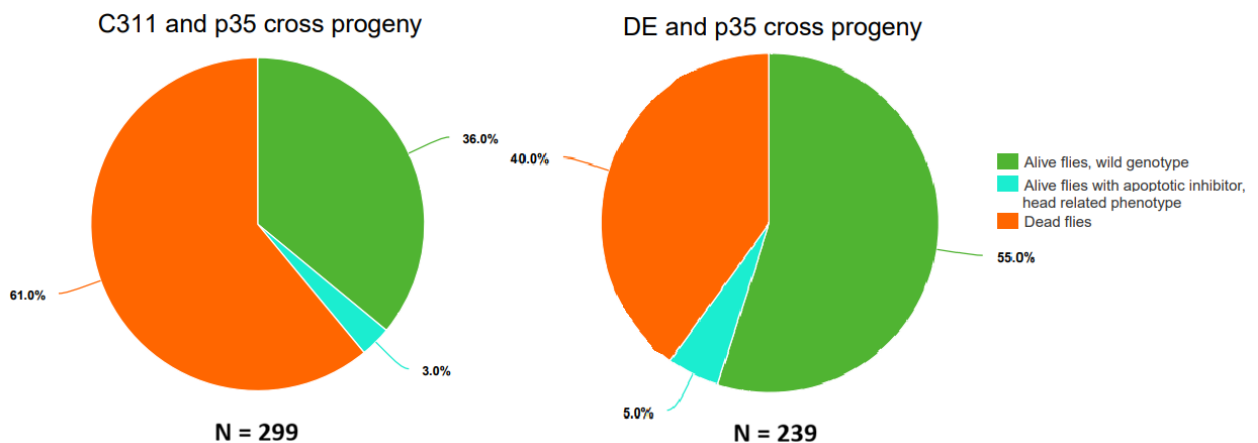


Figure 12. C311 and DE cross with p35 progeny statistics. Quantitative analysis demonstrates a significant increase in mortality among individuals expressing the apoptotic inhibitor. In both cases almost half of the progeny dies and very few that have apoptotic inhibitor genotype live to the adult stage. Among the rare surviving adults possessing apoptotic inhibitor genotype, the vast majority present with severe head capsule deformations or entirely absent eyes.

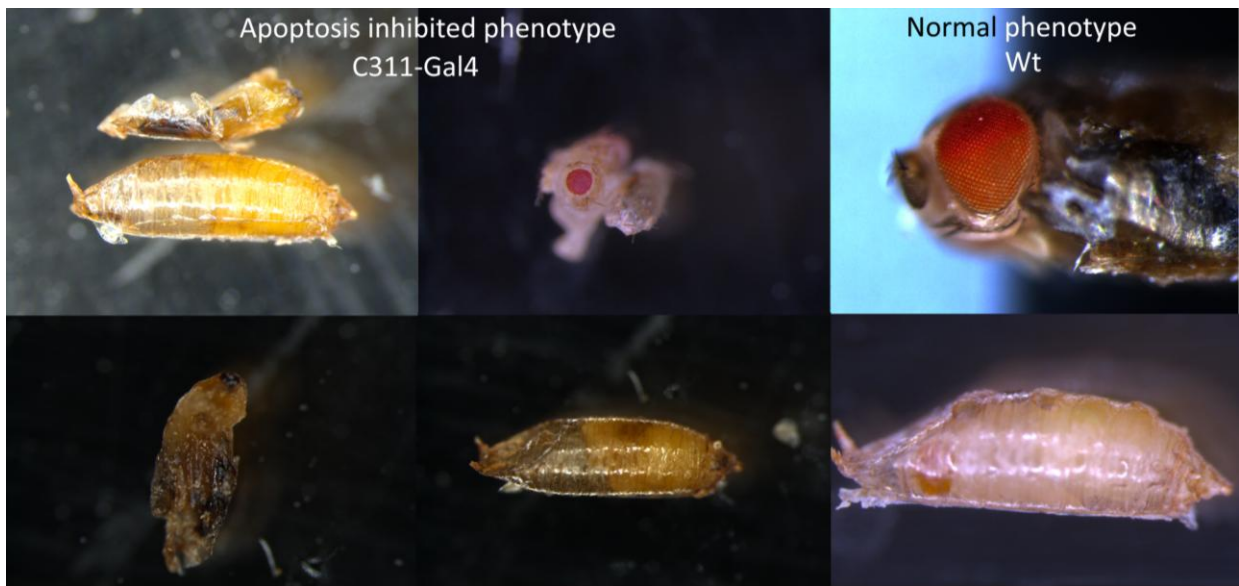


Figure 13. Phenotypic defects resulting from the C311 and p35 cross. Expression of the apoptotic inhibitor p35 induces aberrant phenotypes during pupal development. High pupal lethality is observed, with pupae exhibiting severe head deformities and developmental delay. The rare individuals that survive to the adult (imago) stage display small, underdeveloped rounded eyes.

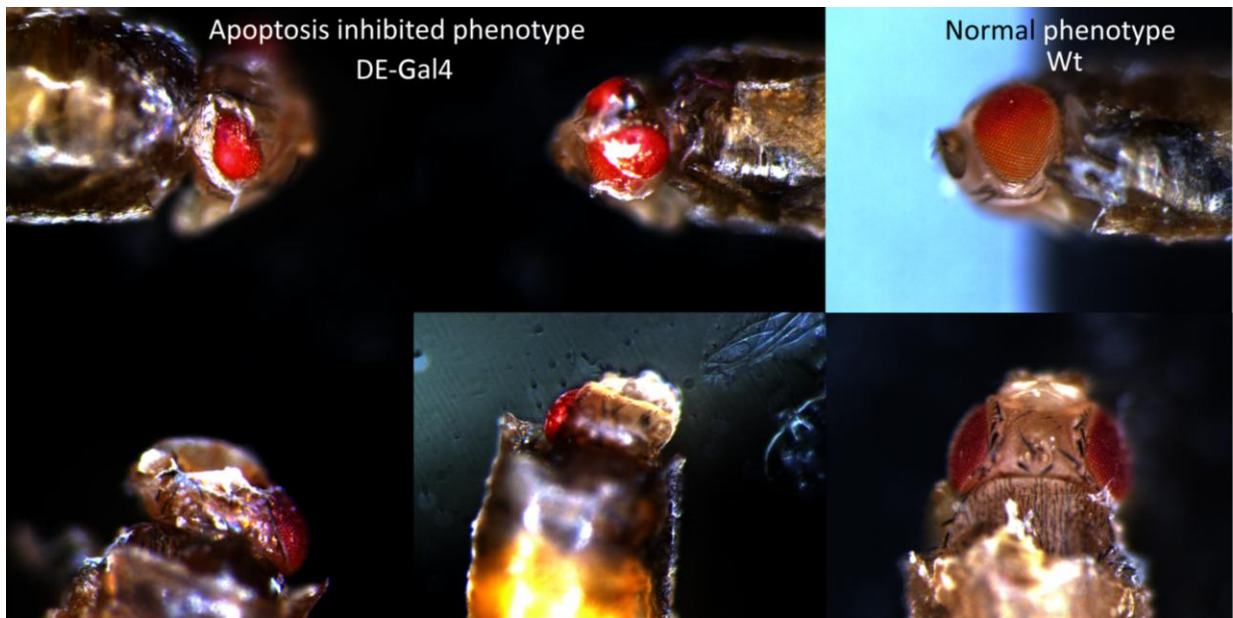


Figure 14. Phenotyping defects resulting from the DE and p35 cross. This genotype exhibits lower pupal lethality compared to the C311 cross with p35, with a substantially higher proportion of individuals surviving to the adult (imago) stage. Surviving adults exhibit severe head capsule deformations, resulting in misshapen, reduced or entirely missing eyes.

DISCUSSION

In this study, using an improved *ex vivo* culture method, we were able for the first time to continuously observe the morphogenesis of the complete eye-antennal disc pair of *Drosophila melanogaster* for more than 20 hours and identify five distinct developmental stages. This is a significant advancement compared to previous studies (Sarv, 2024; Sui and Dahmann, 2025), in which the maximum observation duration did not exceed 16 hours, and the description was limited to four stages. Our observations are consistent with earlier studies of eye-antennal disc morphogenesis, which showed that the PE generates mechanical tension required for disc movement and fusion (Milner et al. 1983, 1984). We observed that the PE reduces its area and bends, pulling the disc. Importantly, we showed that the PE continues to actively move after fusion during stages 4 and 5, shifting posteriorly and further compacting the head structure. This movement is likely key to the onset of stage 5, so the current protocol should be improved to capture the entire movement process in detail.

An important result is the detailed characterization of disc proper movements, which remained undescribed until this study. We described how the antennal portion of the DP separates from the main primordium, forms an S-shaped structure, and then rearranges itself and migrates toward the anterior part of the head. This suggests that the DP not only serves as a passive substrate but also actively participates in the spatial organization of future antennal structures and its various components.

Using two different Gal4 drivers C311 for the entire PE and DE for the dorsal PE, we mapped caspase signalling throughout most stages of head development. Both drivers showed a gradual increase in apoptotic activity over time, but with distinct spatial distributions: in the C311, the signal was more uniform in the central fusion zone, while with DE, it was more spatially restricted. The most compelling evidence for the importance of apoptosis came from experiments involving p35 mediated caspase inhibition. Inhibition of caspase activity throughout the PE in the case of C311 resulted in 60% lethality rate and severe head capsule deformities, including absent eyes, and deformed or underdeveloped eyes. With partial expression in the dorsal part of the disc, as is in the case of DE cross with p35, lethality was somewhat lower around 40%, but the phenotypes included asymmetric deformities. This directly demonstrates that apoptotic signal is required for proper

morphogenesis during the pupal stage, similar to the observations in mammalian systems, such as Pbx-dependent EMT process (Losa et al., 2018).

Despite its success, the method has limitations. First, the maximum disc lifespan in vitro remains approximately 20–22 hours, preventing observation of the latest events of the pupal period, if any such occur. Second, GC3Ai reports effector caspase activation almost in real time but it does not distinguish between lethal and non-lethal caspase signalling. Third, due to optical limitations, it was not possible to simultaneously visualize the deep regions of the DP and the superficial PE with the same resolution as well as acquired images lack depth of field in late stages.

Thus, our work not only clarified the chronology of morphogenesis but also demonstrated for the first time that the DP undergoes five complex remodelling stages, the PE actively moves even after fusion, and apoptosis is involved in fusion with spatiotemporal precision.

Further experiments should utilize caspase sensors to more accurately determine the type of caspase signalling involved. To capture late processes of PE movement after stage 4 during head shape correction and subsequent compaction a further improved protocol is needed. As well as we need to improve the quality of imaging to a more detailed cellular level. In order to fully assemble all the details of morphogenesis into a single whole

The proposed approach can be applied to the study of other paired structures and for modelling human craniofacial disorders.

CONCLUSION

In this thesis we utilized long-term *ex vivo* culture and live imaging of *Drosophila* eye-antennal imaginal discs to study developmental stages of head morphogenesis.

Under enhanced imaging conditions, developmental processes were observed continuously for over 20 hours, allowing for a detailed characterization of five morphogenetic stages, expanding on previous descriptions. The results demonstrate that the peripodial epithelium undergoes active positional rearrangements during and after tissue fusion and likely contributes to the final head shape formation, including compaction and the positioning of antennal primordia. Furthermore, the disc itself exhibited complex structural movements and positional changes visible across the five stages, suggesting a more important role in morphogenesis than previously assumed. Disc proper undergoes a substantial shape change, during which antennal part of the disc separates into a continuous, elongated structure that reorganizes and positions itself at the front of the head, where future adult structure will form.

Fluorescence imaging using PE specific drivers confirmed the presence of apoptotic signalling in the peripodial membrane during late stages of development. Caspase signalling was identified in fusion-related processes, at two major fusion locations as well as later across the region of contact between two discs. Flies expressing caspase inhibitor showed that hindering apoptosis in any way brings severe damage to animals' development. With a correlation corresponding to amount of peripodial membrane affected. Inhibition across the entire peripodial membrane resulted in marked higher lethality.

Overall, this work expands our understanding of craniofacial morphogenesis in *Drosophila* and highlights the role of caspase signalling in this process. It also demonstrates the value of long-term *ex vivo* imaging methods for studying developmental processes under conditions resembling those *in vivo*.

***Drosophila melanogaster* peakapsli arengu mehhanismid**

Ivan Uljanov

RESÜMEE

Käesoleva bakalaureusetöö eesmärk oli kaardistada ja iseloomustada äädikakärbse (*Drosophila melanogaster*) silma-tundla imaginaaldiskide morfogeneesi, kasutades täiustatud *ex vivo* kultiveerimismeetodeid ja reaajas pildistamist. Kuigi varasemad uuringud on kirjeldanud üldiseid arenguetappe, on suuremahulised morfogeneetilised liikumised, täpsemalt imaginaaldiski põhiosa reorganiseerumine ja peripoodiaalse epiteeli (PE) roll äädikakärbse pea lõpliku kuju kujundamisel, jäänud halvasti mõistetavaks. Määravaks on saanud peamiselt pikaajalise pildistamise tehnilised piirangud. Samuti on küllaltki vähe teada apoptootiliste signaalide ajalis-ruumilise jaotuse kohta äädikakärbse pea morfogeneesis.

Selles uuringus kasutati varem väljatöötatud *ex vivo* kultiveerimisprotokolli, mida täiustati, et võimaldada pikemaid pildistamisperioode (kuni 20,5 tundi). Kolmanda arengustaadiumi (L3) vastsete silma-tundla imaginaaldiske kultiveeriti M3/IMS söötmes, millele oli lisatud insuliini ja 20-hüdroksüekdüsooni. Pildistamiseks kasutati inverteeritud fluorestsentsmikroskoobe (EVOS M7000, Olympus IX81) 10-minutilise vahemikuga pildistamisega. PE dünaamika ja kaspaasi aktiivsuse visualiseerimiseks kasutati mitmeid spetsiifilisi GAL4 draiver liine (C311-Gal4 PE-spetsiifiline, DE-Gal4 dorsaalne PE) ja reportervalke (mStayGold, CaspaseTracker GC3Ai, p35 apoptoosi inhibiitor). Täiustatud pildistamistingimustes jälgiti arengut üle 20 tunni, mis võimaldas tuvastada viis selgelt määratletud morfogeneetilist etappi (0–4 h, 4–7 h, 7–11 h, 11–15 h, 15–20+ h). Esmakordselt kirjeldati detailselt DP keerulisi liikumisi: tundlapiirkonna eraldumine, S-kujulise struktuuri moodustumine, silmaväljade pööramine, lõplik sulandumine ja tundlate imaginaaldiskide liikumine pea esiossa. PE aktiivset liikumist ja selle pindala vähenemist visualiseeriti ka 4. ja 5. etapis, mis näitab PE otsest rolli pea lõplikus kompakteerimises. Apoptootilise signaaliülekanne kaardistamine näitas, et kaspaasi aktiivsus suureneb arengu käigus lineaarselt, muutudes erit kontsentreerituks V-kujulistes sulanduspiirkondades ja seejärel ühtlasemalt kahe imaginaaldiski vahelises kontaktpiirkonnas. Apoptoosi inhibeerimisega genotüübid näitasid oluliselt suuremat suremust ja ellujäänutel esinesid peakapsli deformatsioonid

(väikesed või puuduvad silmad, ebakorrapärane pea kuju). Sõltuvus oli seda tugevam, mida suurem oli PE piirkond, kus apoptoos oli blokeeritud.

Täiustatud *ex vivo* meetod võimaldab usaldusväärselt uurida hiliseid morfogeneetilisi protsesse, mida pole varem kirjeldatud. Üldiselt on see uuring andnud olulise panuse meie arusaamisele äädikakärbse pea morfogeneesist, eriti DP ja PE dünaamika ning apoptoosi rolli osas. See näitab ka pikaajalise *ex vivo* pildistamise potentsiaali arengubioloogia uurimisel.

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APPENDIX

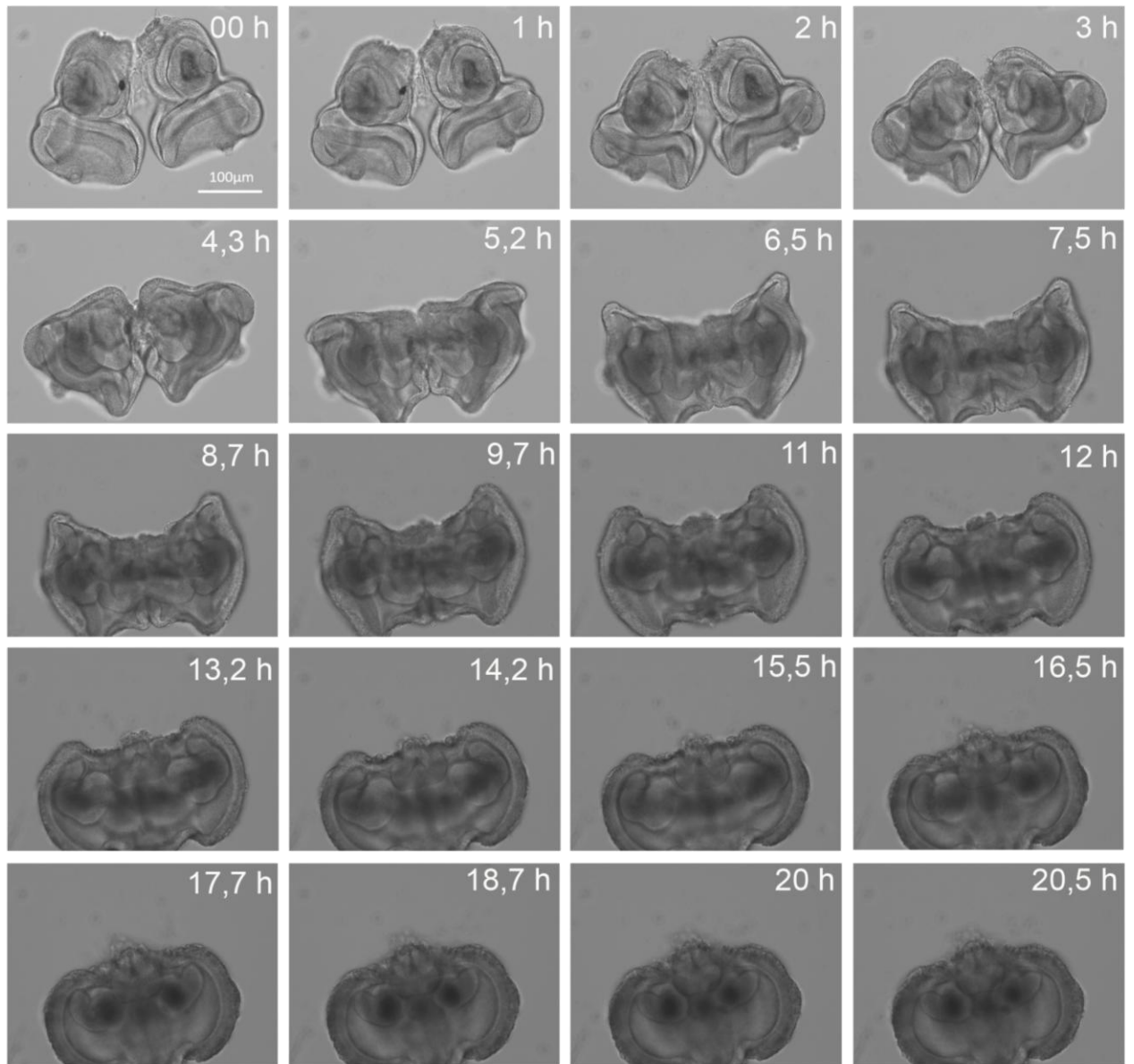


Figure A.1. Live imaging of the eye-antennal imaginal discs. Brightfield images of an *ex vivo* culture captured from 0 to 20 hours, illustrating morphogenetic movements.

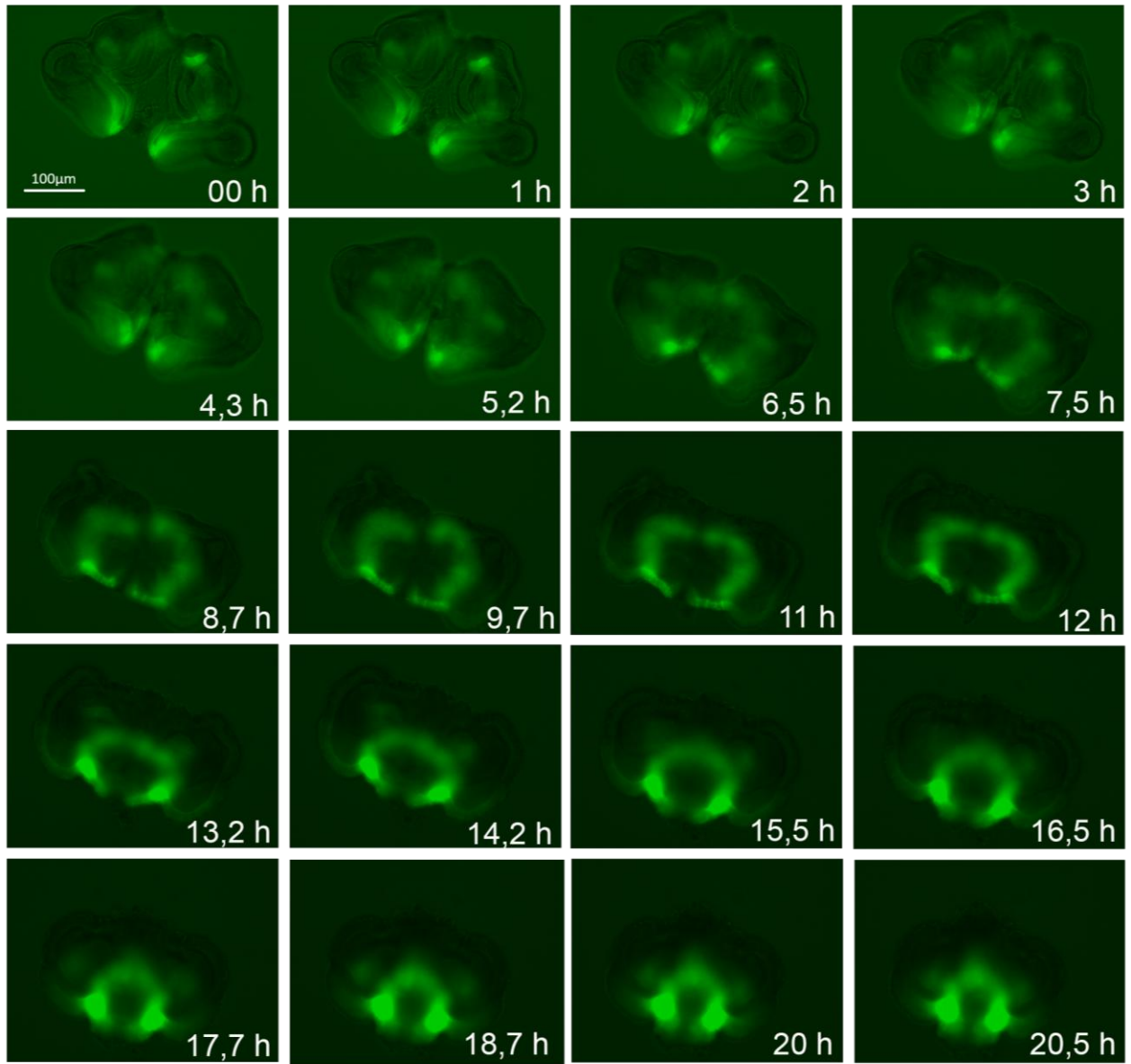


Figure A.2. Live imaging of the DE-Gal4 expression pattern in the eye-antennal imaginal discs. The same time-lapse sequence as in Figure 5, displayed without brightfield channel to show only GFP channel.

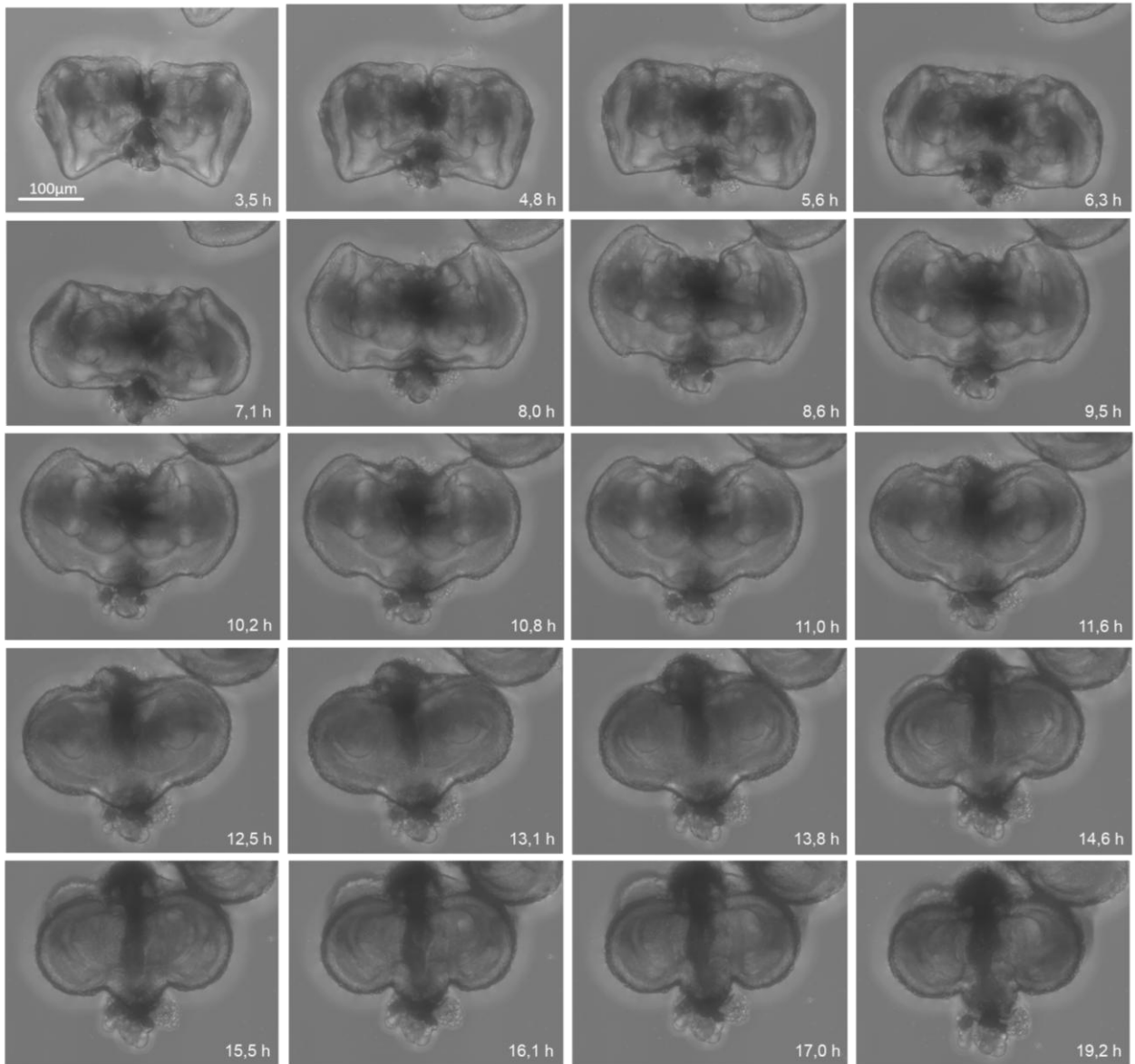


Figure A.3. Live imaging of the eye-antennal imaginal discs. Time-lapse sequencing illustrating the downward movement of the antennal primordia toward the outer edge of the disc during the final stage, becoming fully excluded from the field of view. This movement is observable from the 14.5-hour time point until the end of the experiment. This specific phenomenon was not observed in other replicates.

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