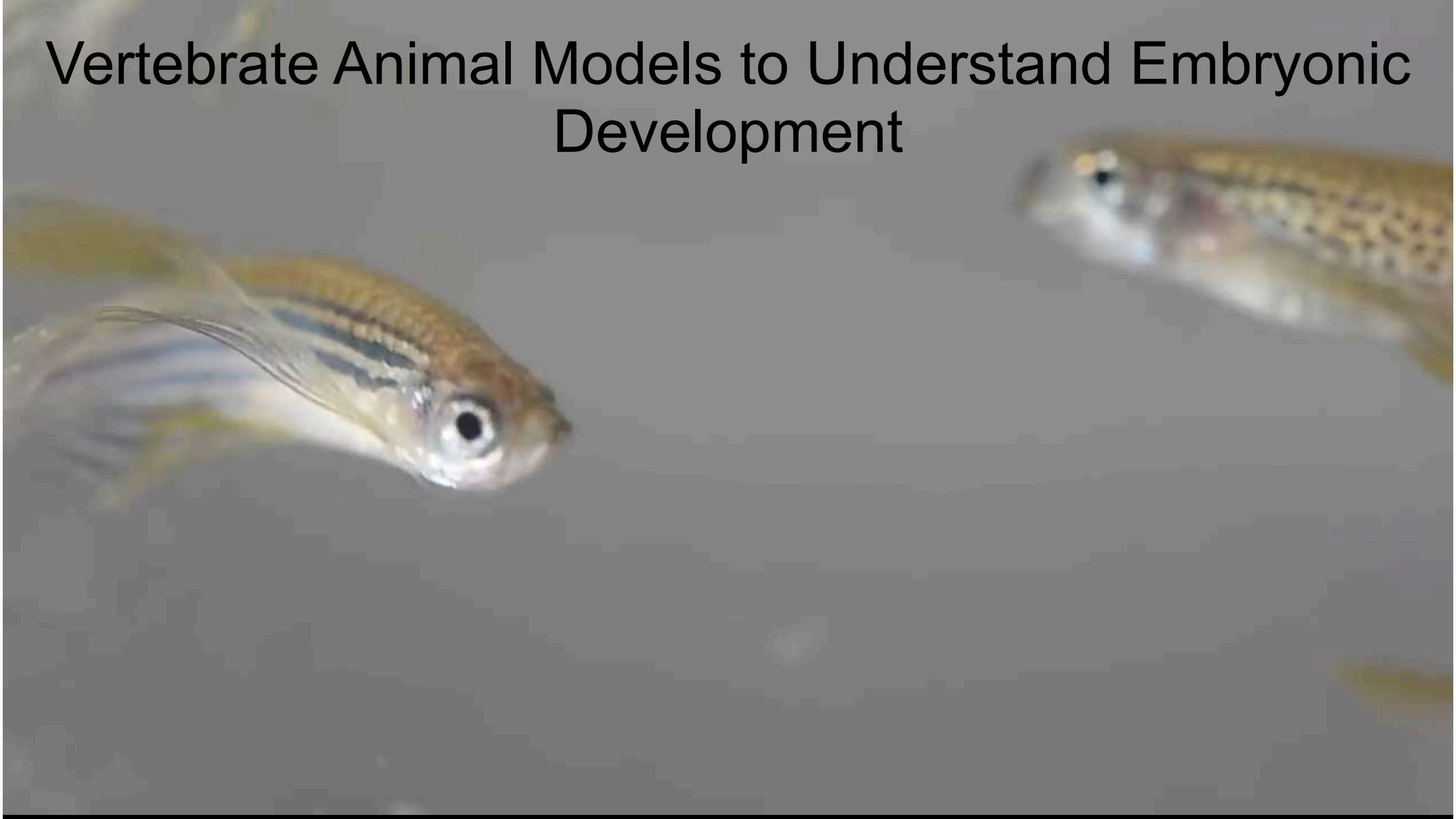
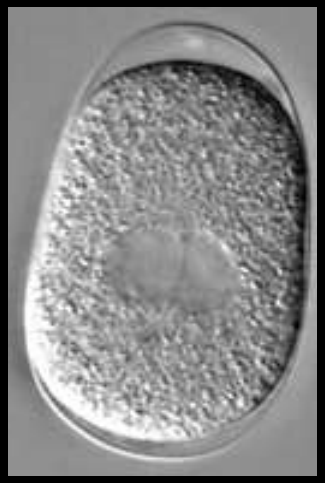
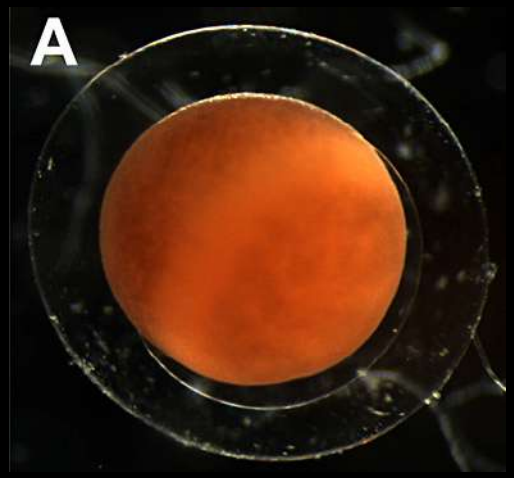
Vertebrate Animal Models to Understand Embryonic Development



Sreelaja Nair
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Bombay, India

Everything complex is simple to begin with
Developmental biology is an attempt to understand how complexity evolves






Entry into science (and space)

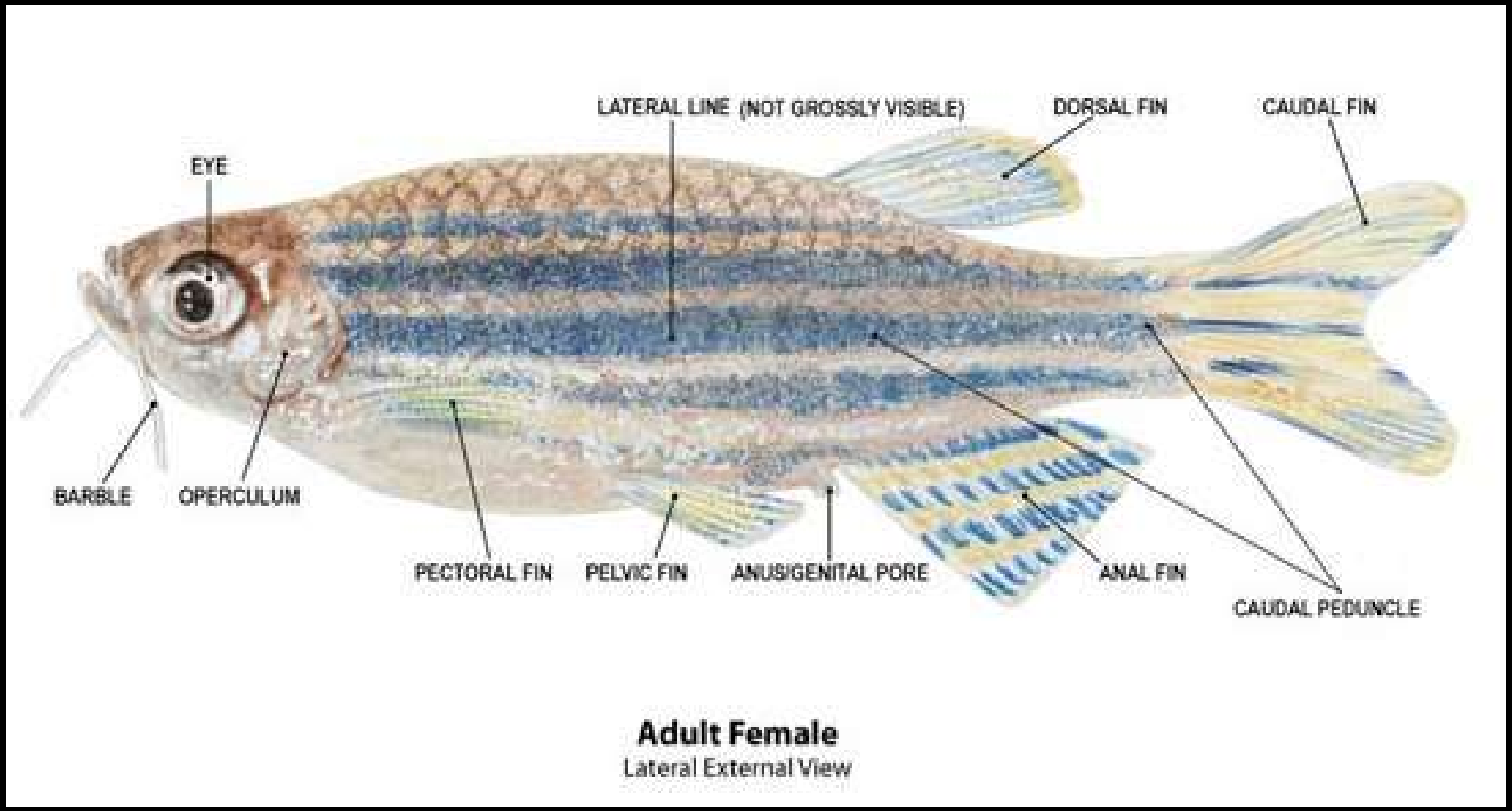
- *Danio rerio* or zebrafish are native to the Gangetic plains in East India and Myanmar
- Typically found in slow moving waters (gentle streams, paddy fields)
- Aquarium shops sell zebrafish as “hard to kill” pets

- First used as a model organism by George Streisinger at the University of Oregon in the late 1960s
- Streisinger was looking for a vertebrate model organism, in addition to the then popular models which were either invertebrates (flies, worms) or mammals (mice)
- By 1976 the USSR had sent zebrafish into space on the Salyut 5 space mission

- Chuck Kimmel, Streisinger`s colleague at Oregon continued Streisinger`s work and used zebrafish to study the nervous system and craniofacial cartilage development

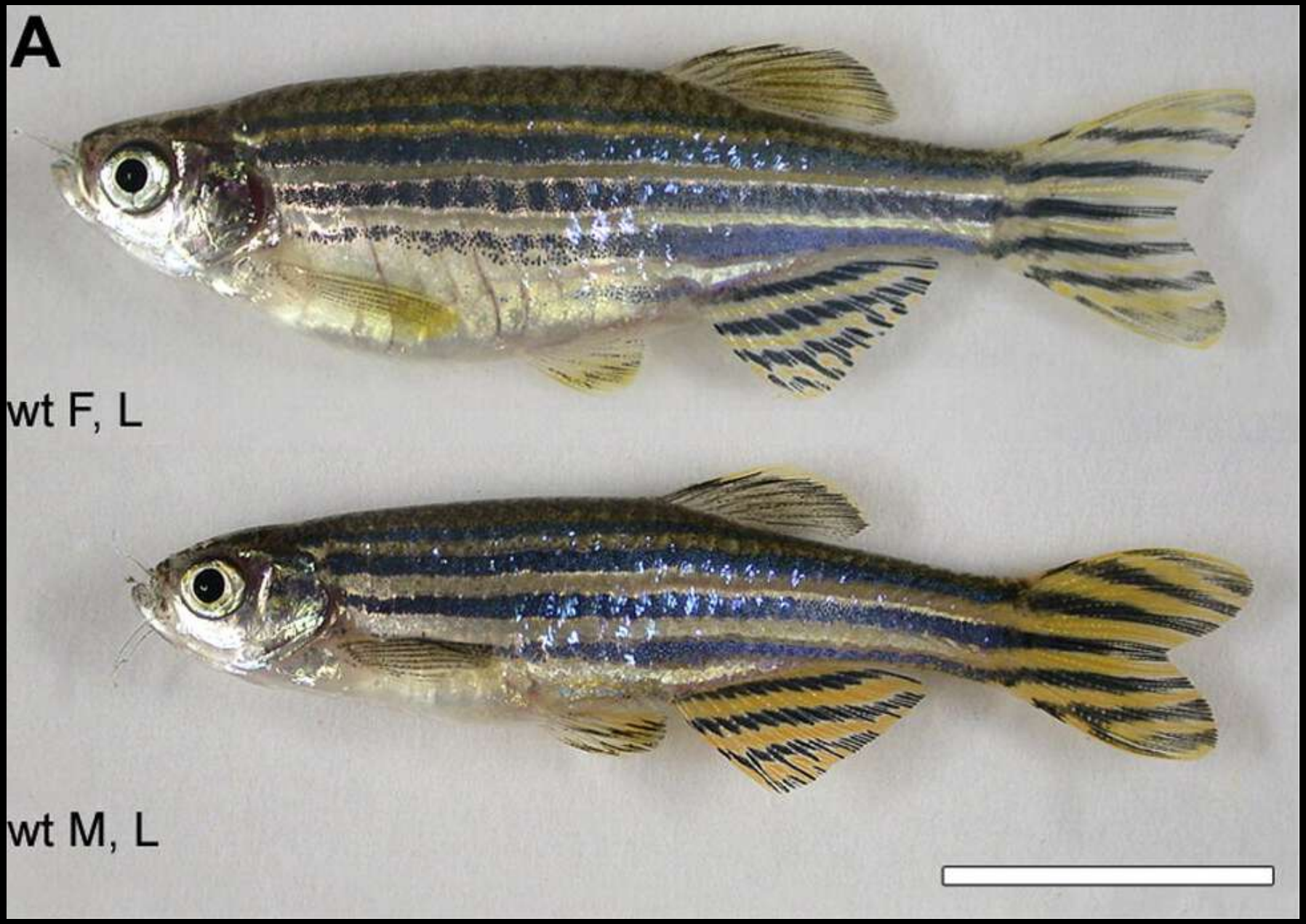
Entry into mainstream science

- In the 1900s two research groups, one in Germany and one in the USA began large scale genetic screens in zebrafish
- The german group was led by Christiane Nusslein-Volhard at the Max Plank Tubingen
- The american group was led by Wolfgang Driever at Harvard Medical School
- The ~4000 mutants that were isolated as part of both the screens was published in a giant issue of the journal Development in December 1996
- In 2001 the zebrafish community initiated the sequencing of the zebrafish genome at the Wellcome Trust Sanger Institute in UK, which is mostly complete

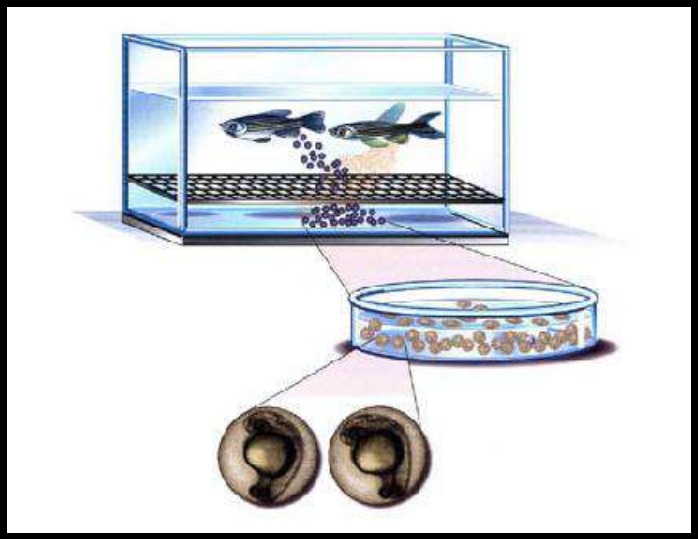


Adult Female

Lateral External View


A
wt F, L
wt M, L

The zebrafish aquatic facility

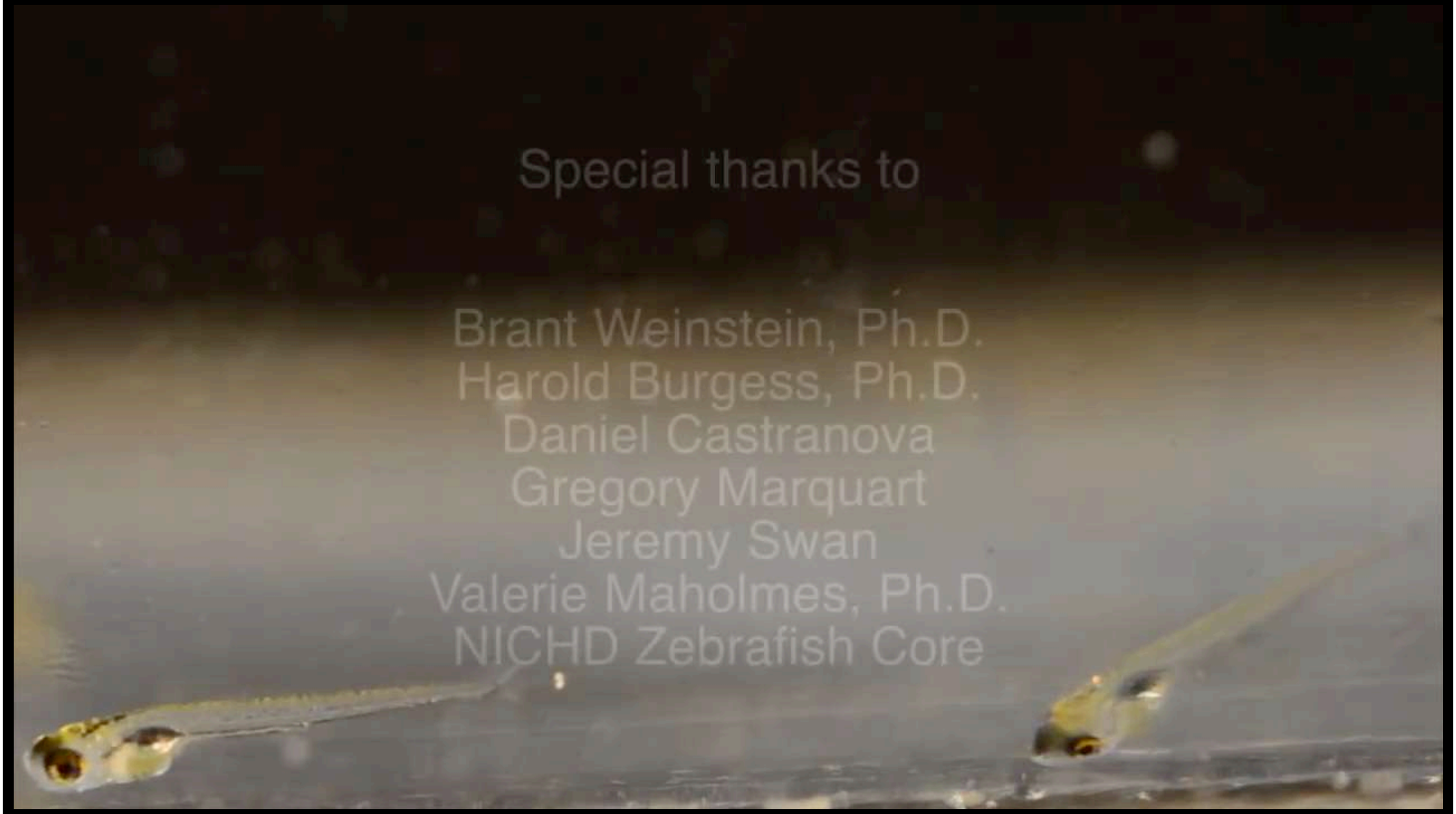


Aquaria are maintained on a 14 hours light and 10 hours dark cycle
Paired up fish in mating tanks spawn in the morning
A single pair can produce ~1000 embryos

zebrafish spawn just after lights come on in the morning

Youtube video by Dr. Tessa Montague

Embryonic development is rapid



A typical strategy to isolate mutants in zebrafish

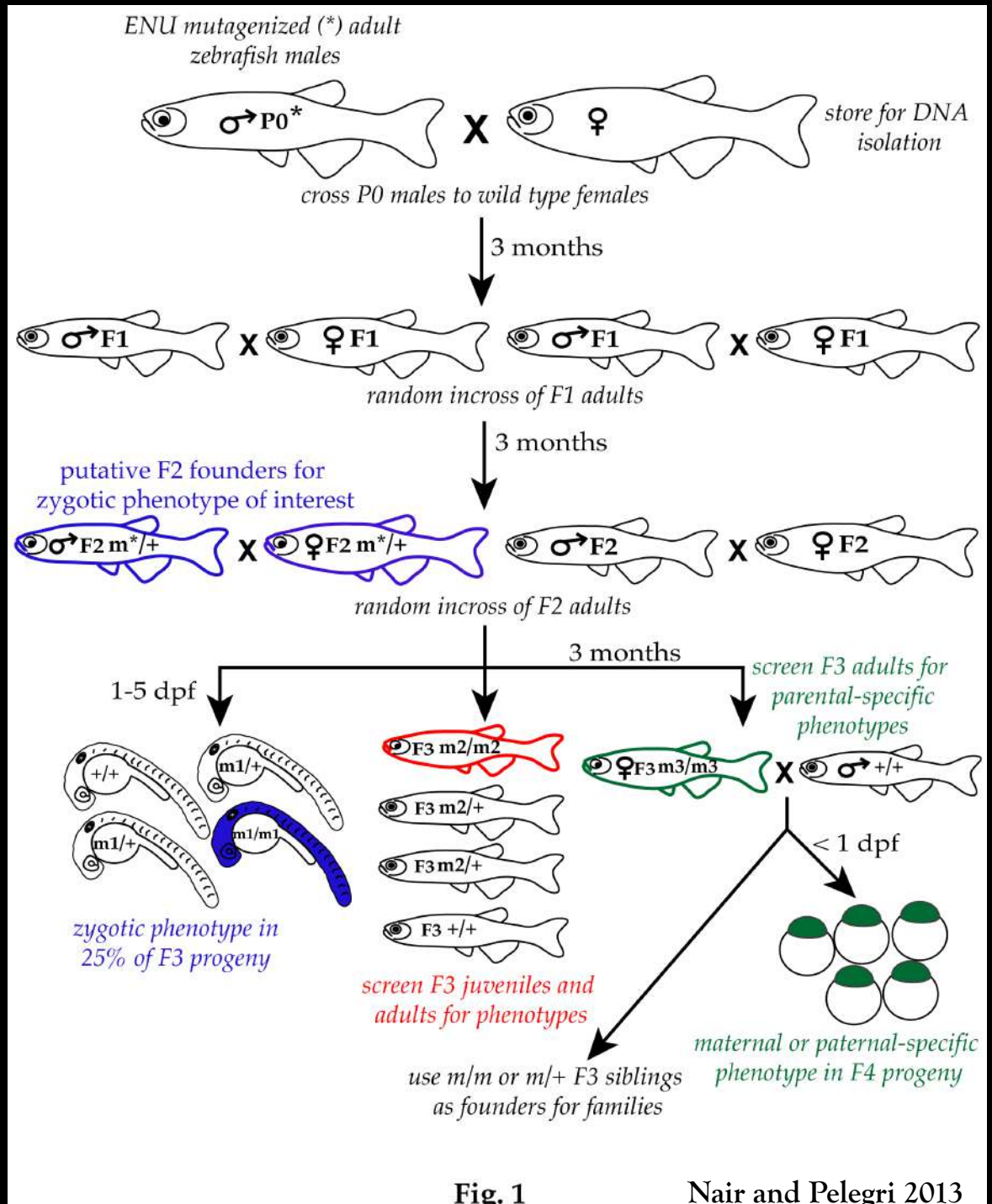


Fig. 1 Nair and Pelegri 2013

An expedited strategy to isolate mutants in zebrafish

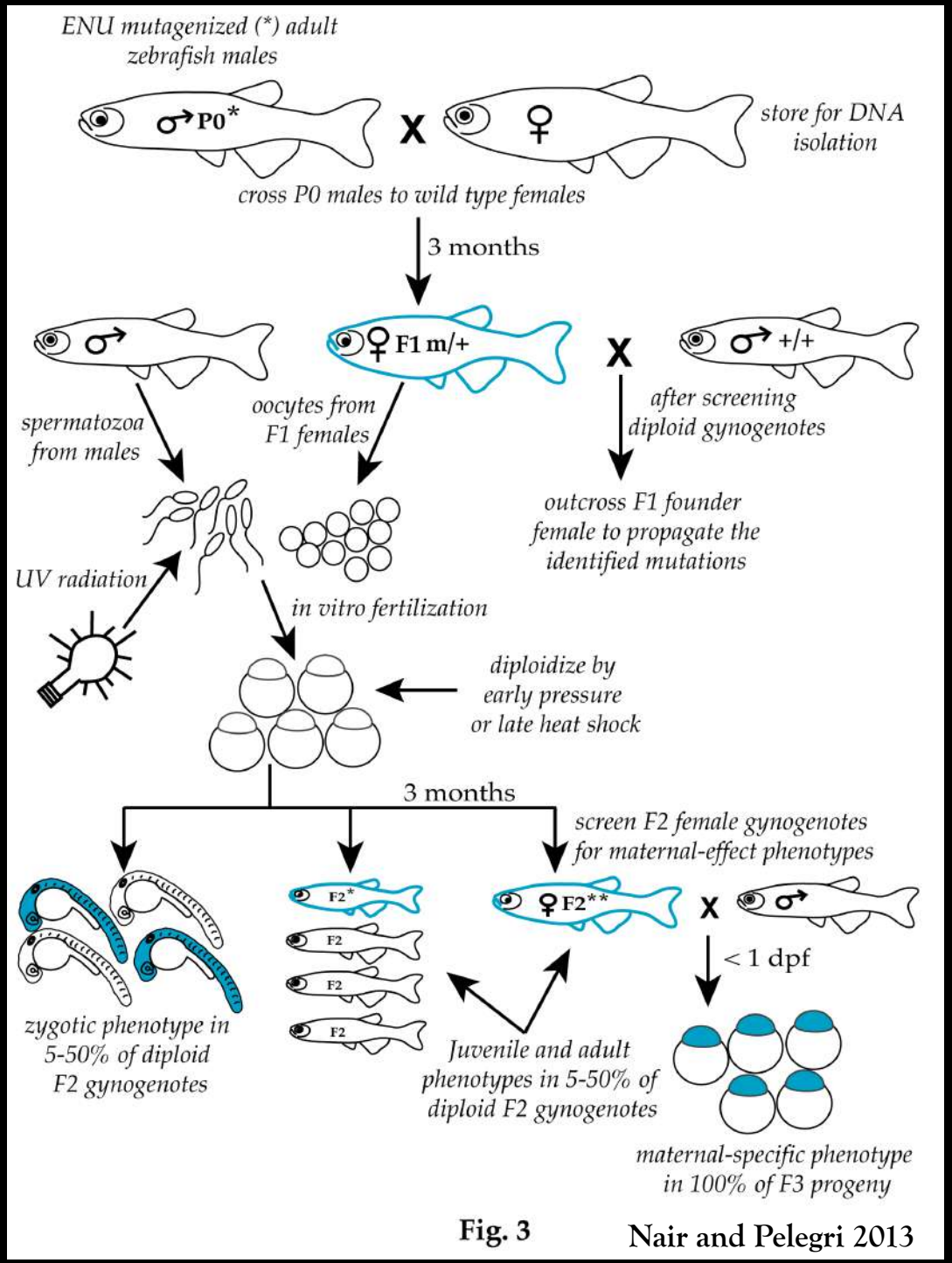
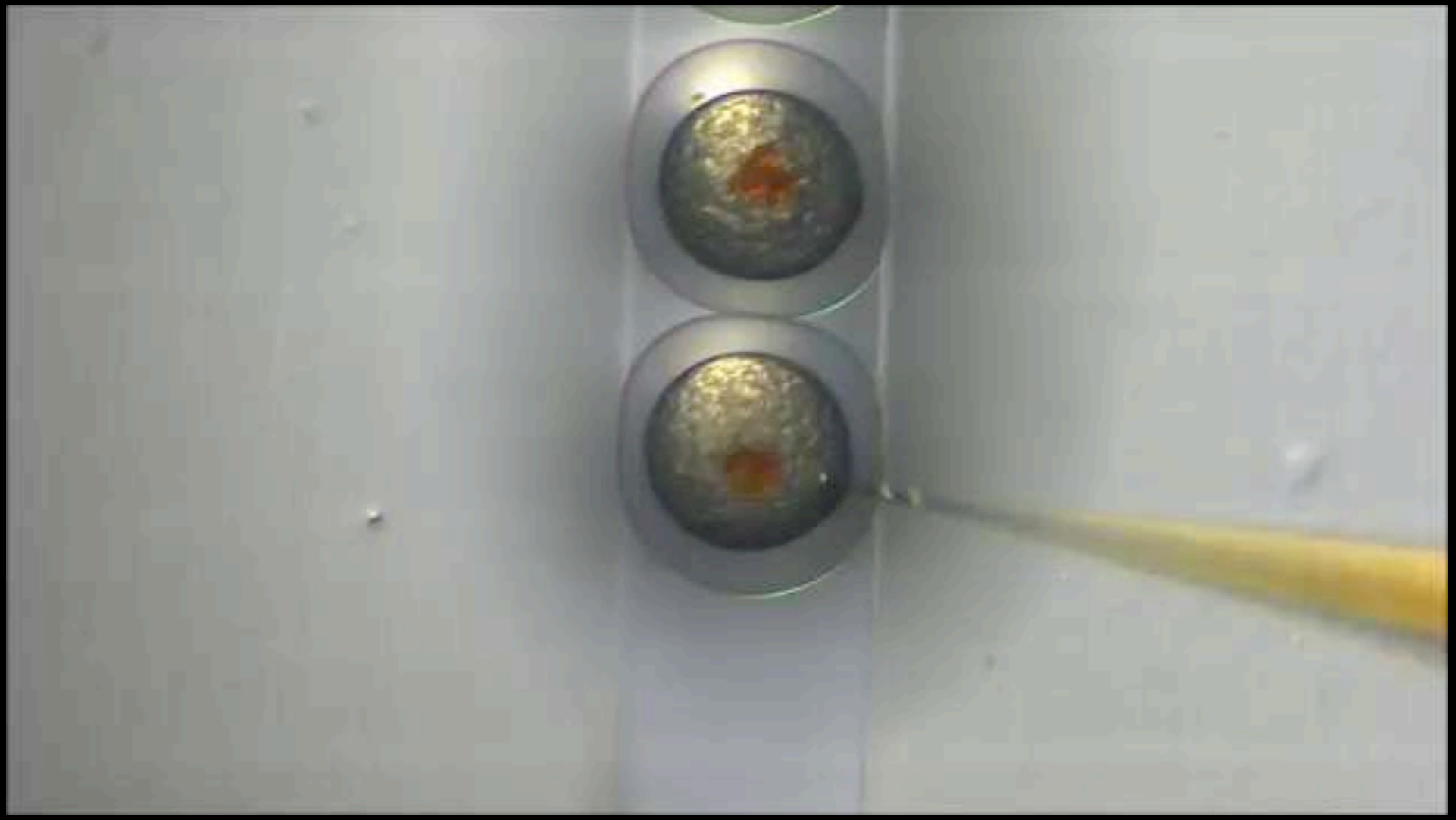
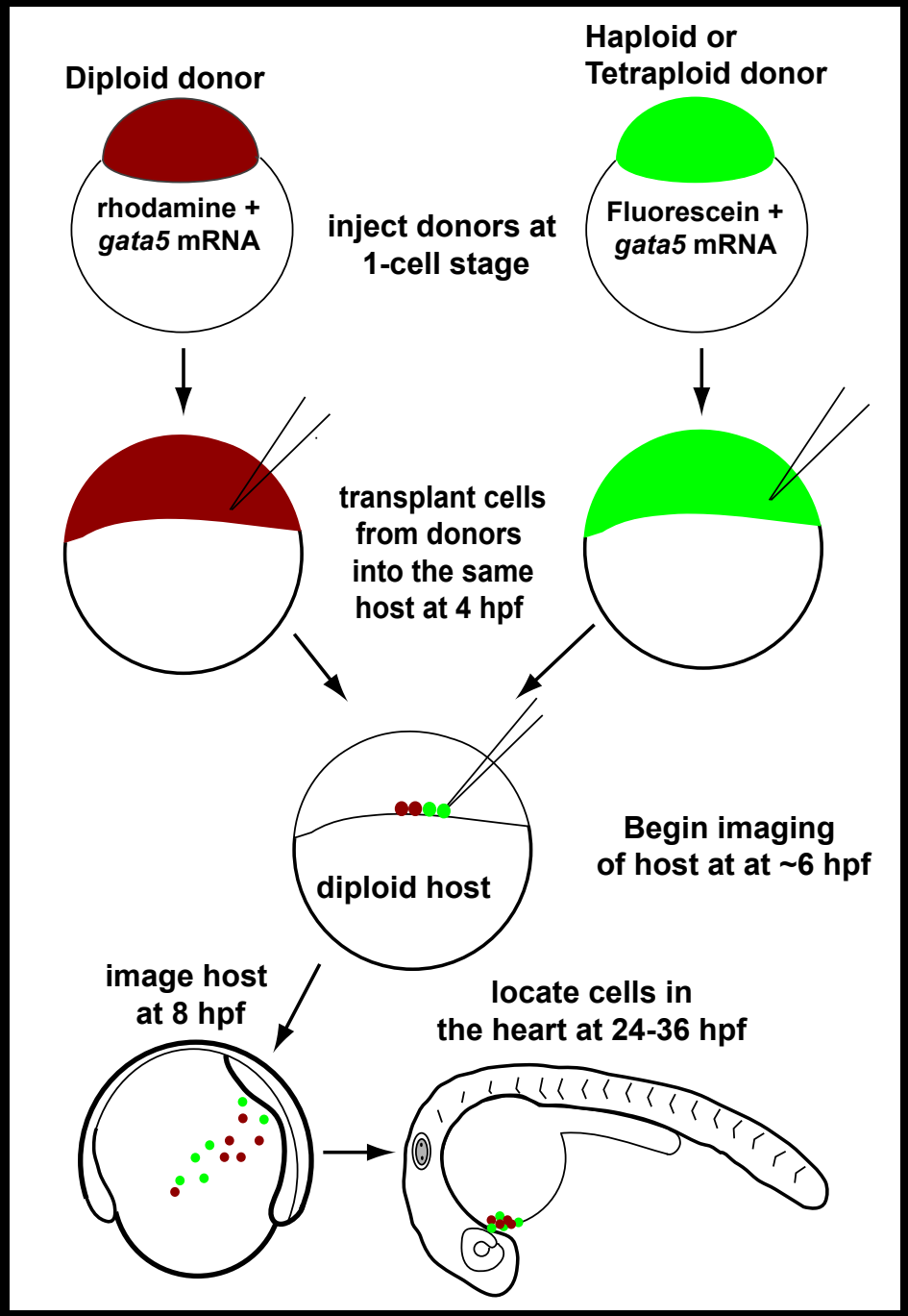


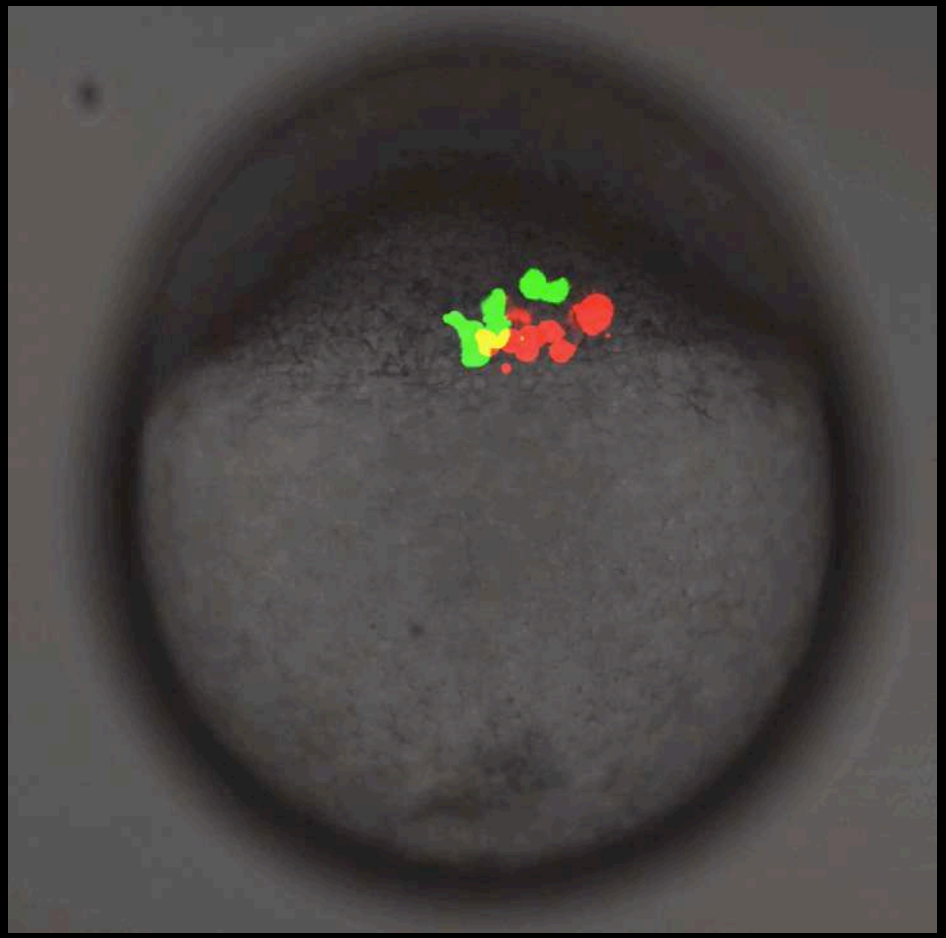
Fig. 3 Nair and Pelegri 2013

Zebrafish embryos are easy to manipulate for reverse genetics as well

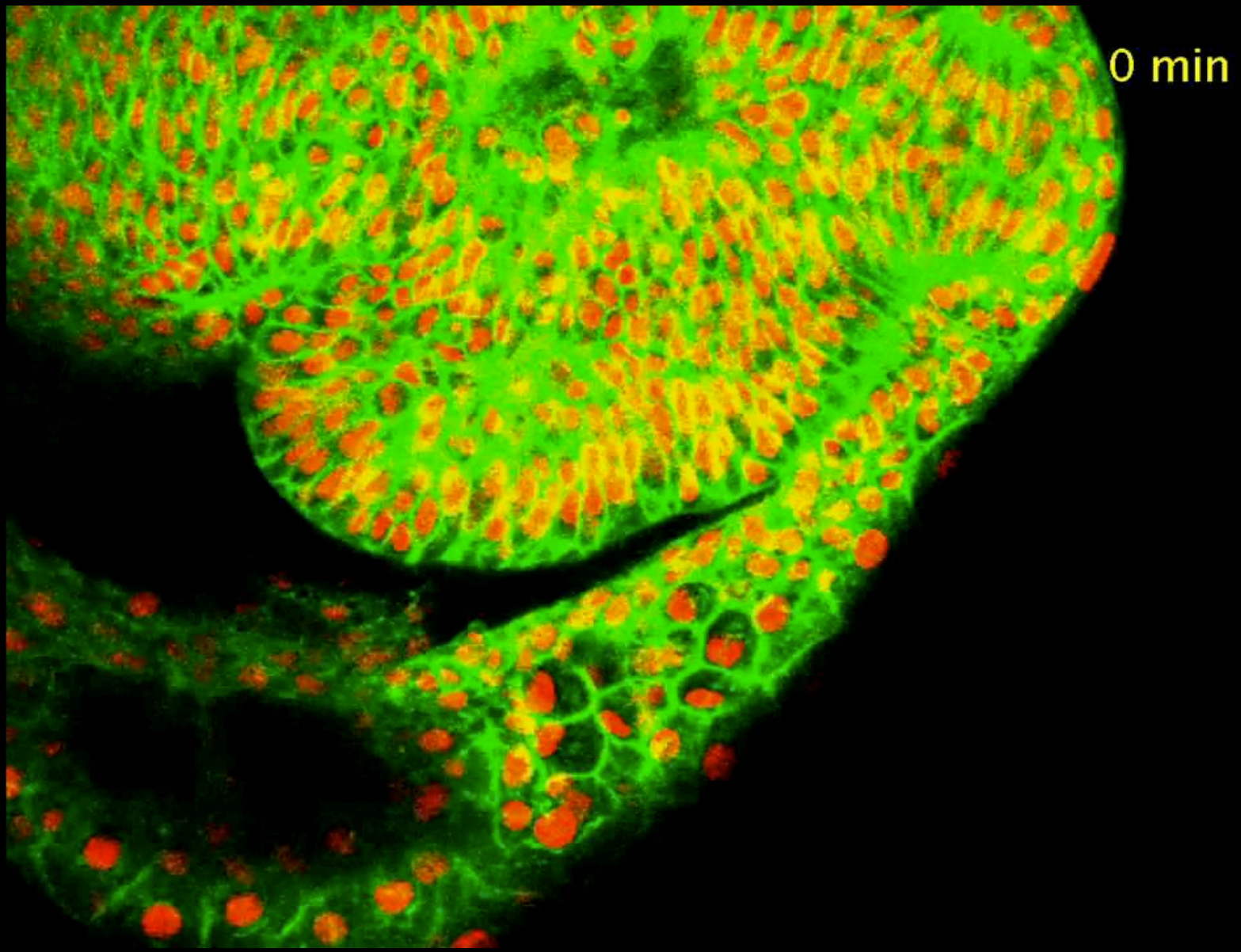


Mosaic zebrafish embryos are easy to generate





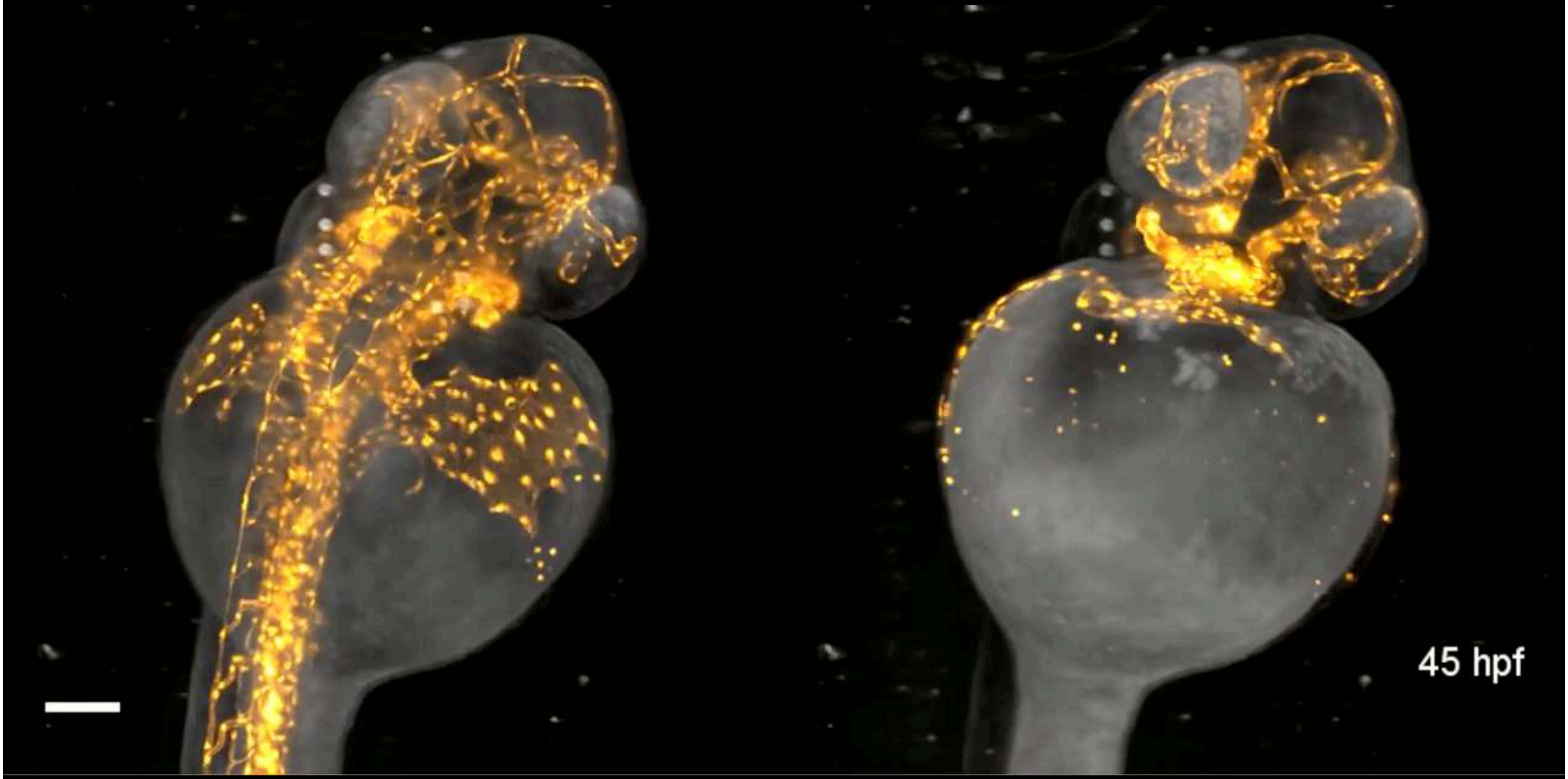
Long term live imaging is a big advantage, particularly in a vertebrate model



Development of organs can be studied in real time, in live larvae

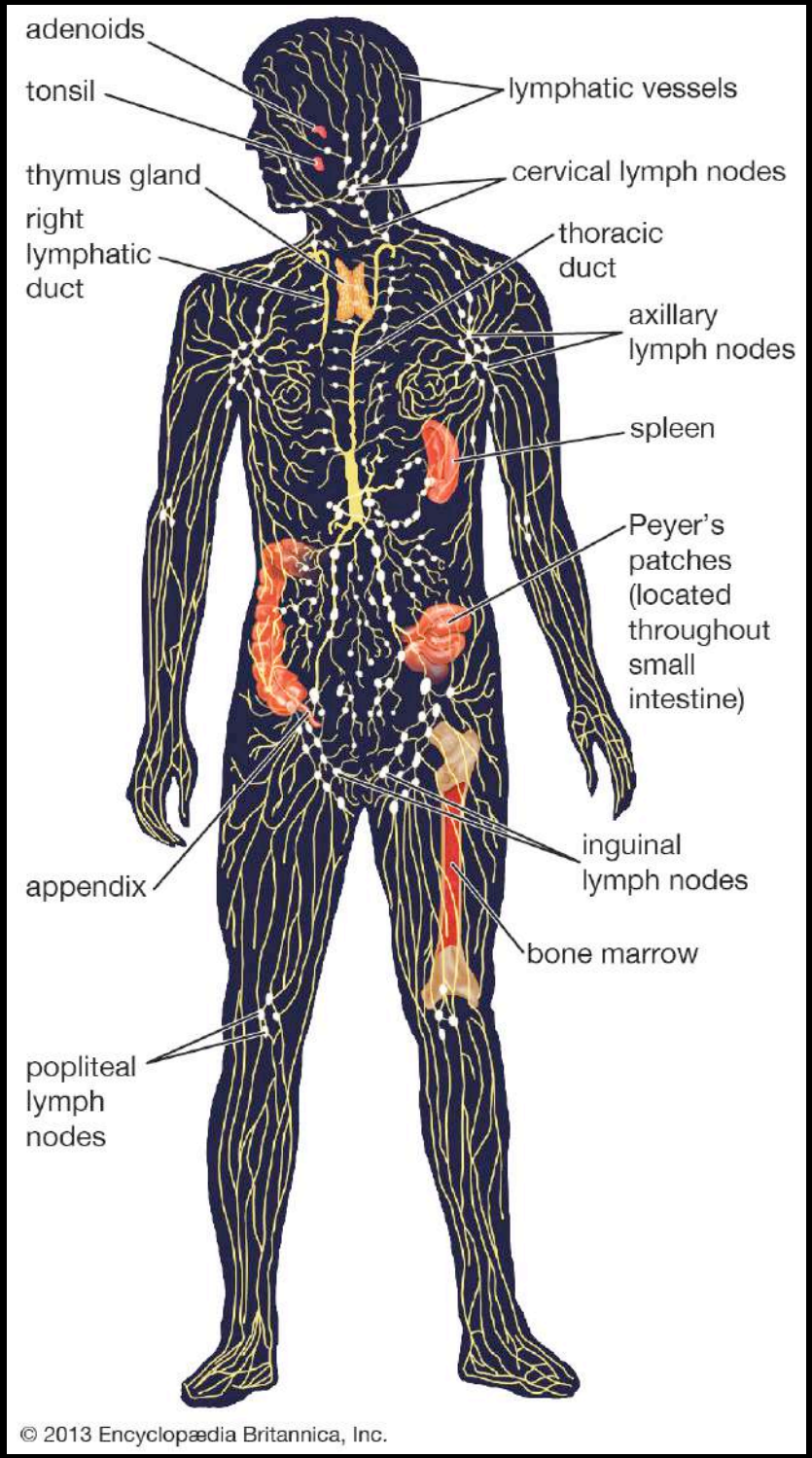
Zebrafish heart regenerate after injury
A model system to study organ regeneration

regeneration, response to drugs, evolution of diseases can be monitored live



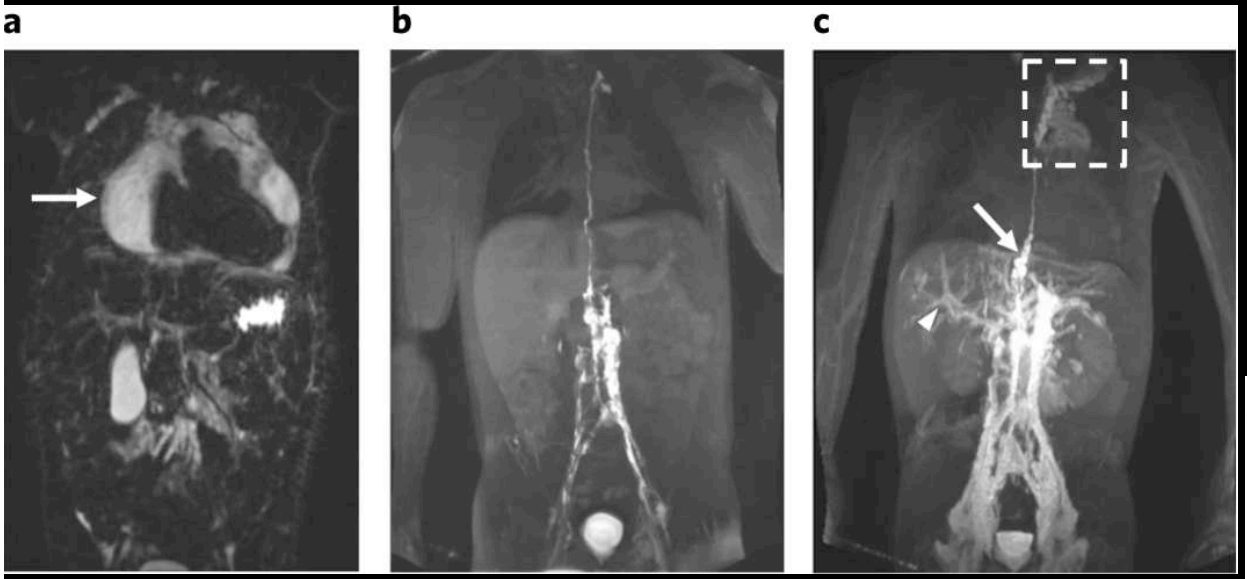
fli1:eGFP transgenic line labelling vasculature

adult fish injected with human tumor cells are a valuable model to study drug responsiveness against cancer - an aspect of personalised medicine

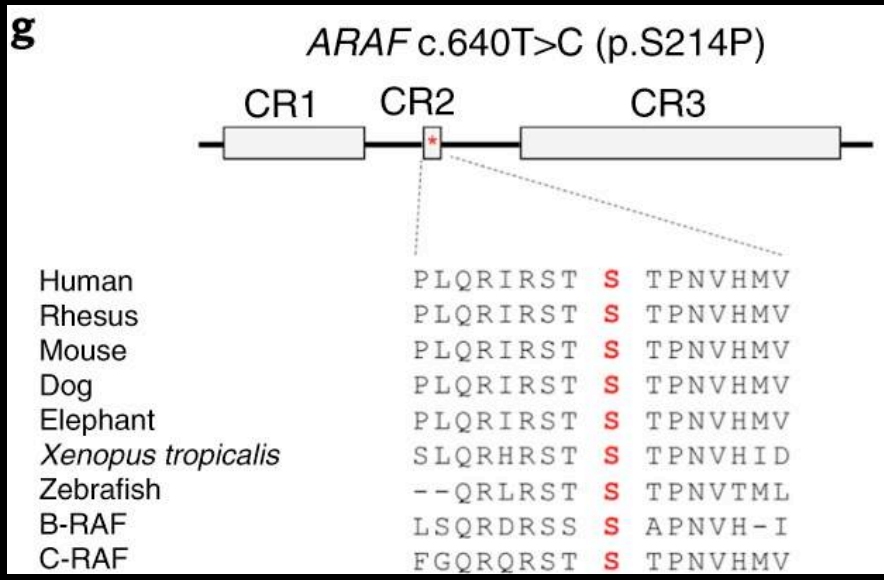


The Lymphatic System

CANCER RESEARCH UK

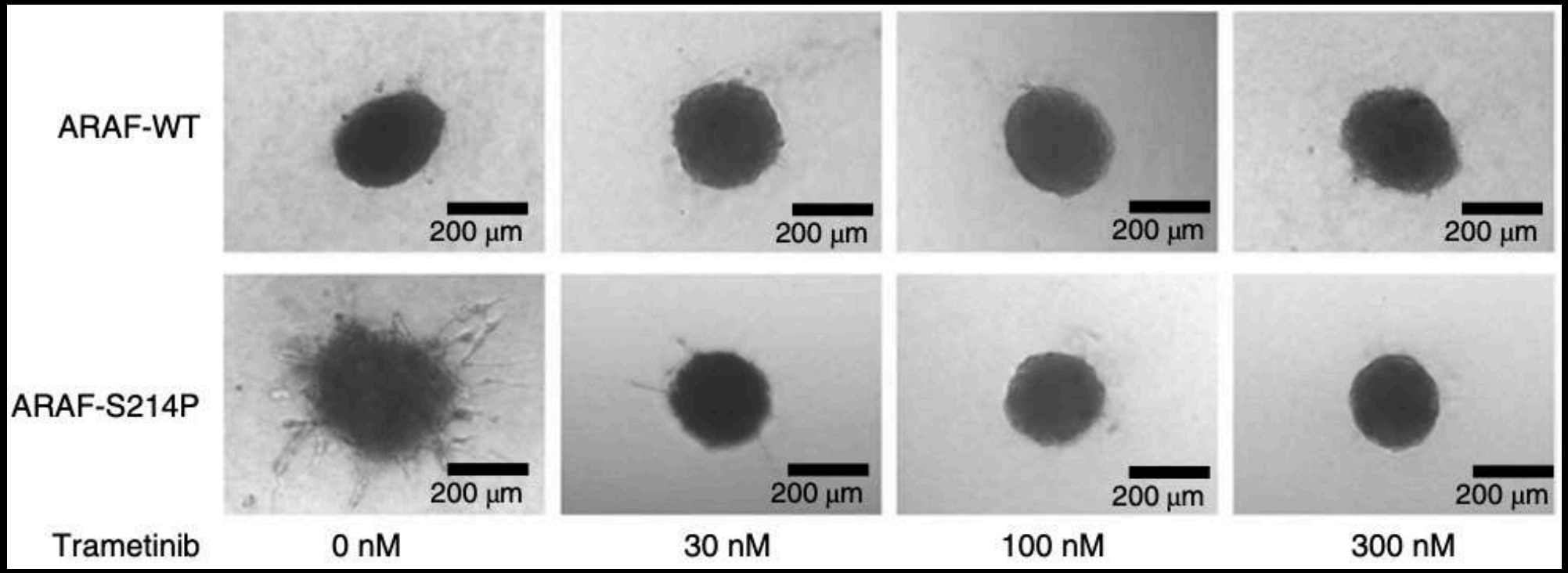


- Patient was not responding to Sirolimus (Rapamycin)
- Sirolimus inhibits vascular endothelial growth factor (VEGF)-C
- Suppresses cell division and migration of lymphatic endothelial cells



The Ser 214 residue is highly conserved across vertebrate species and all RAF isoforms

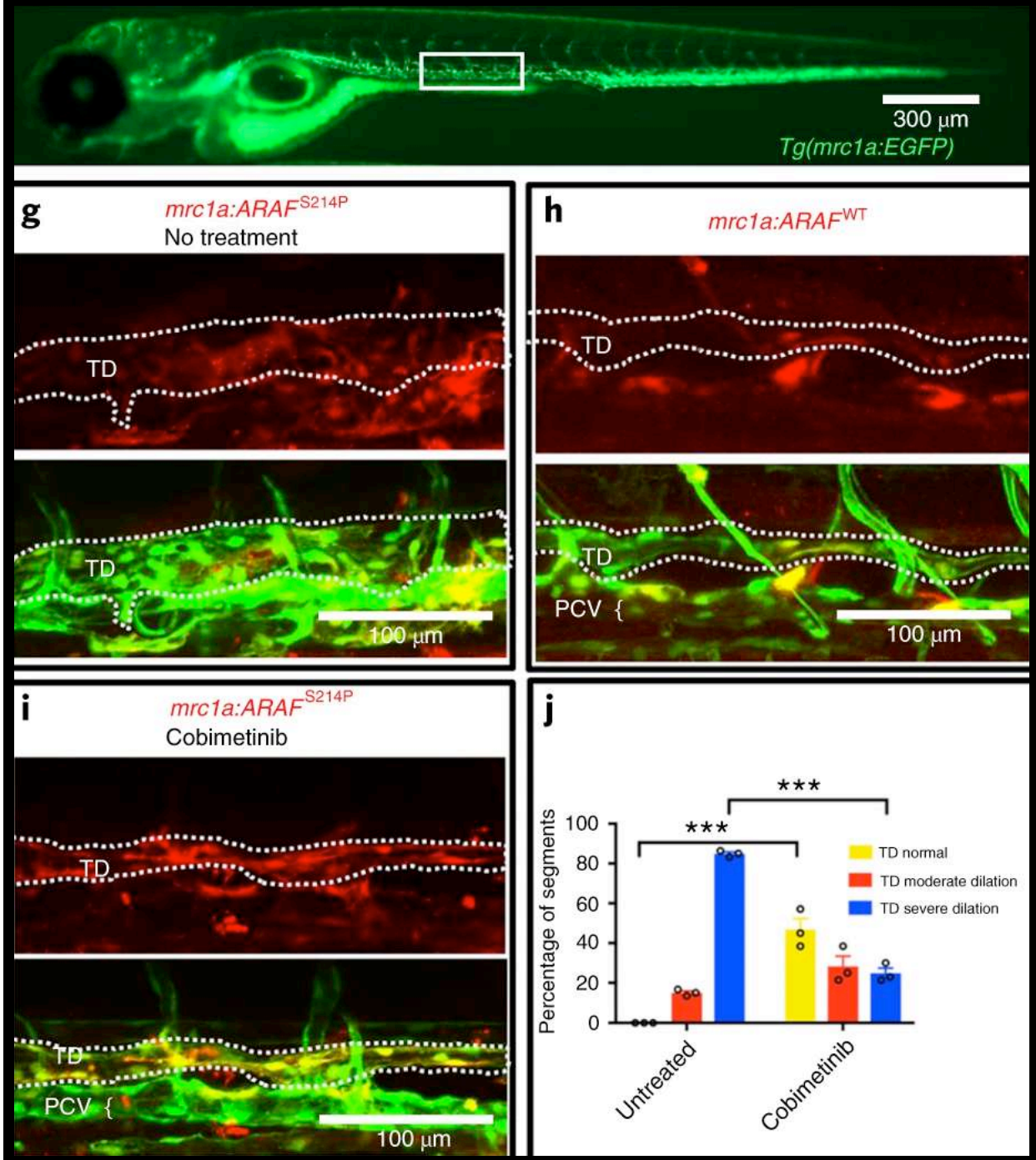
- A Serine to Proline mutation was found in ARAF
- This Serine is phosphorylated normally and the ARAF function is inhibited
- RAF proteins interact with 14-3-3 proteins at this Serine
- Binding of 14-3-3 to RAF at the phosphorylated Serine prevents membrane erecruitment of RAS Mutation of the S to P causes a gain of function of ARAF



- Three-dimensional lymphatic spheroid sprouting assay showed elevated sprouting activity in HDLECs (Human Dermal Lymphatic Endothelial Cells) expressing ARAF-S214P compared with ARAF-WT

- Spheroids were also cultured in increasing concentrations of Trametinib, which significantly reduced both the number of sprouts and sprout length

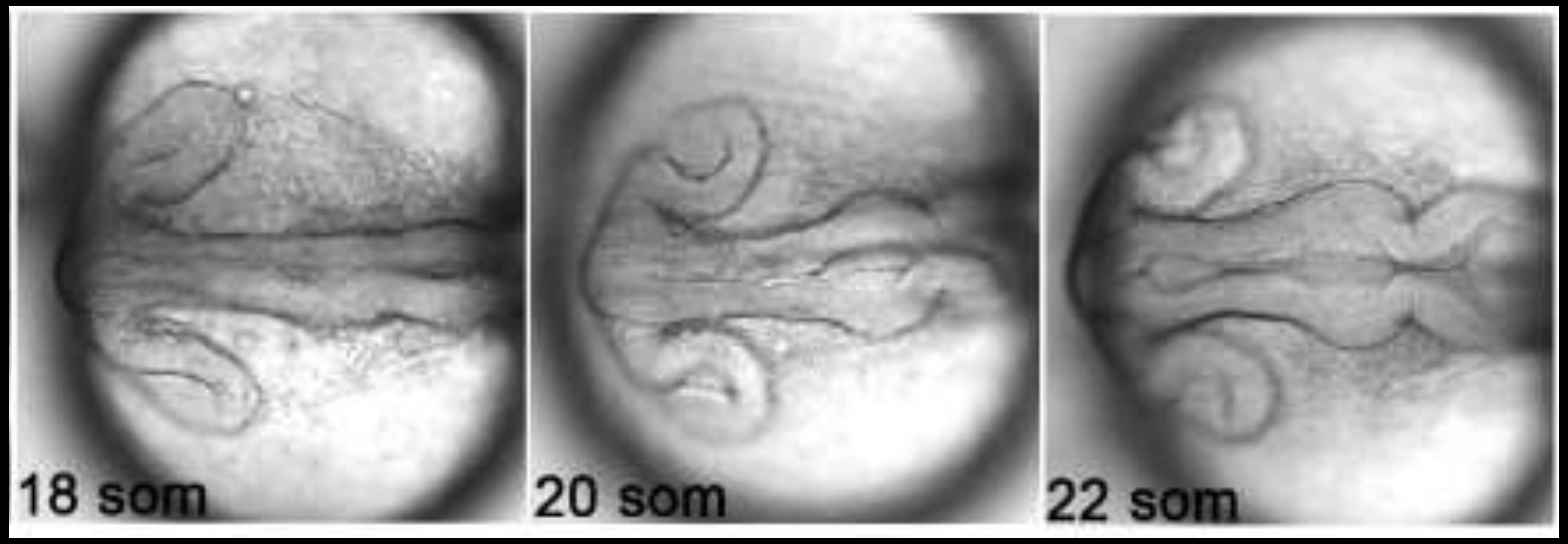
- Trametinib is a kinase inhibitor that inhibits MEK1 and MEK2 (Mitogen-Activated Extracellular Kinase)

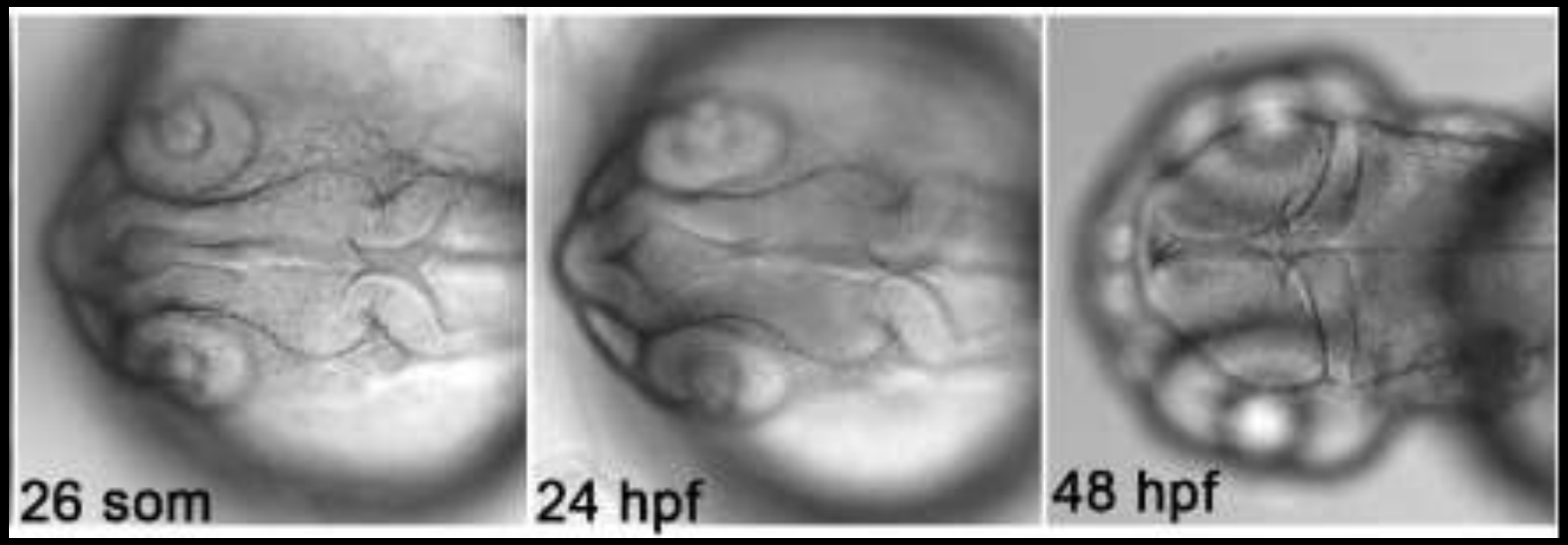


- Transgenic zebrafish in which the lymphatic system is fluorescently labeled with GFP
- Express ARAF-mCherry under the control of a lymphatic vessel promoter
- Express either wild type ARAF or Serine mutant
- Serine mutant ARAF has dilated thoracic duct
- Cobimetinib reduced the dilation of thoracic duct seen in ARAF Serine mutant expression

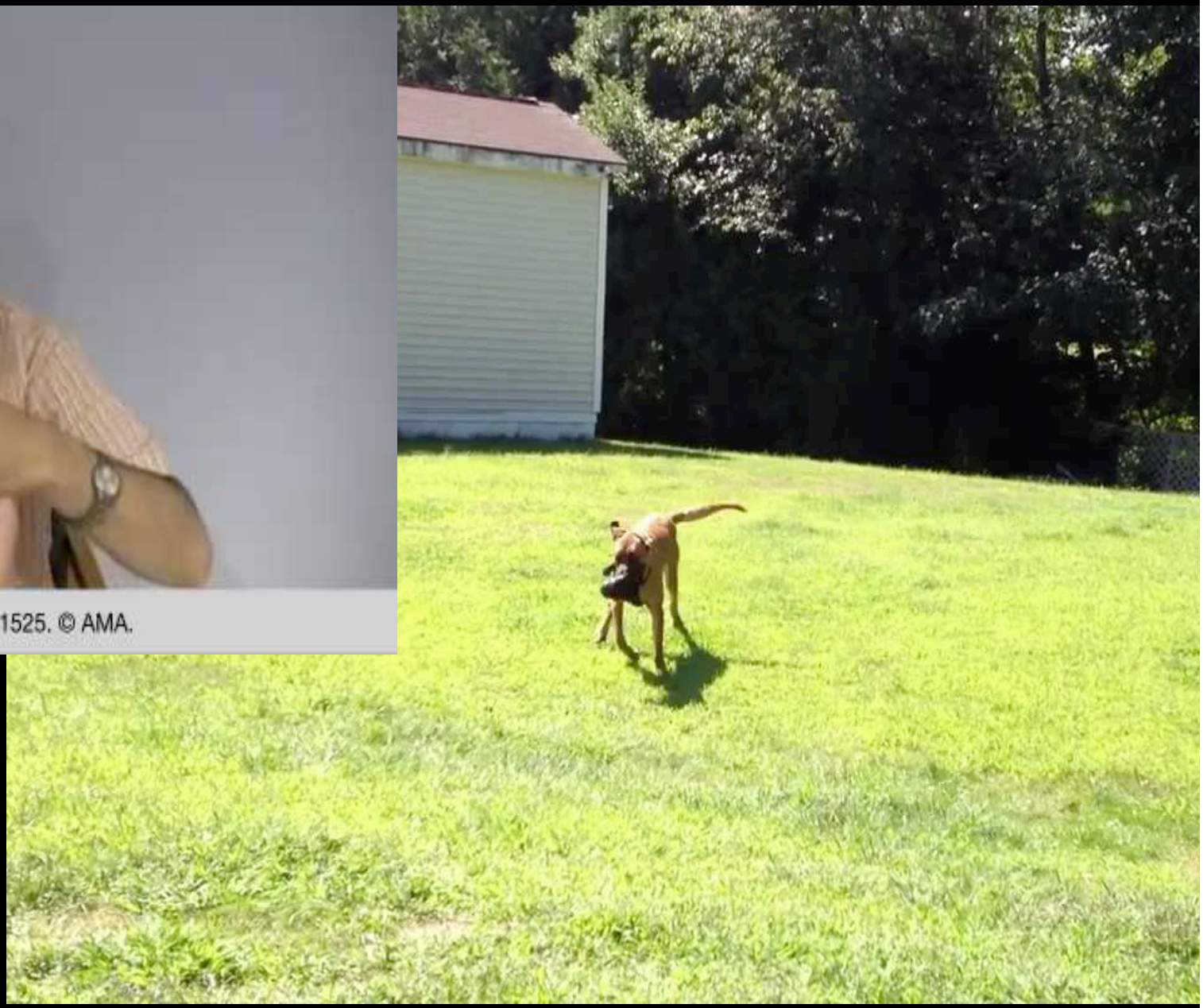
- Patient treated with Trametinib based on genetic and model organism evidence
- Was not responsive to Sirolimus
- Recovered and lymphatic system showed remodelling in response to recovery

Central nervous system is visually accessible at all stages of development



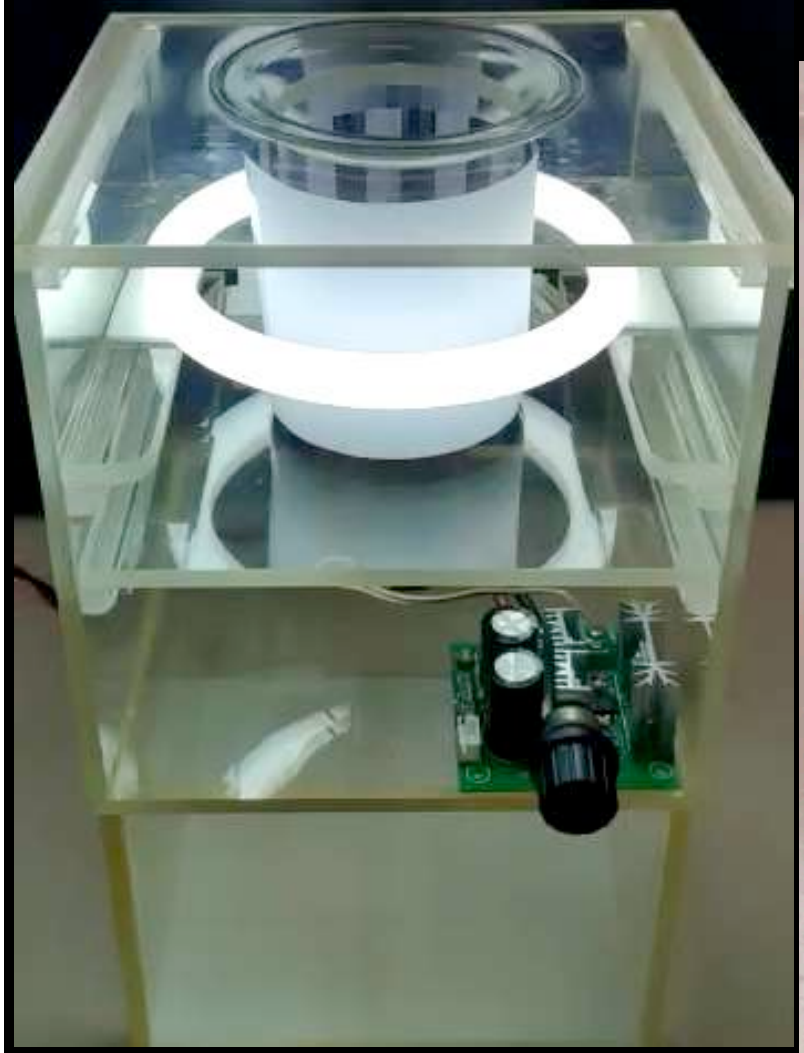


Supplement to *Arch Neurol.* 2012;69(11):1524-1525. © AMA.



Jose Fidel Baizabal, 2012
https://www.youtube.com/watch?v=VXzjKPOb1f4

We build our own small devices to study zebrafish



zebrafish optokinetic response

Zebrafish Optokinetic response

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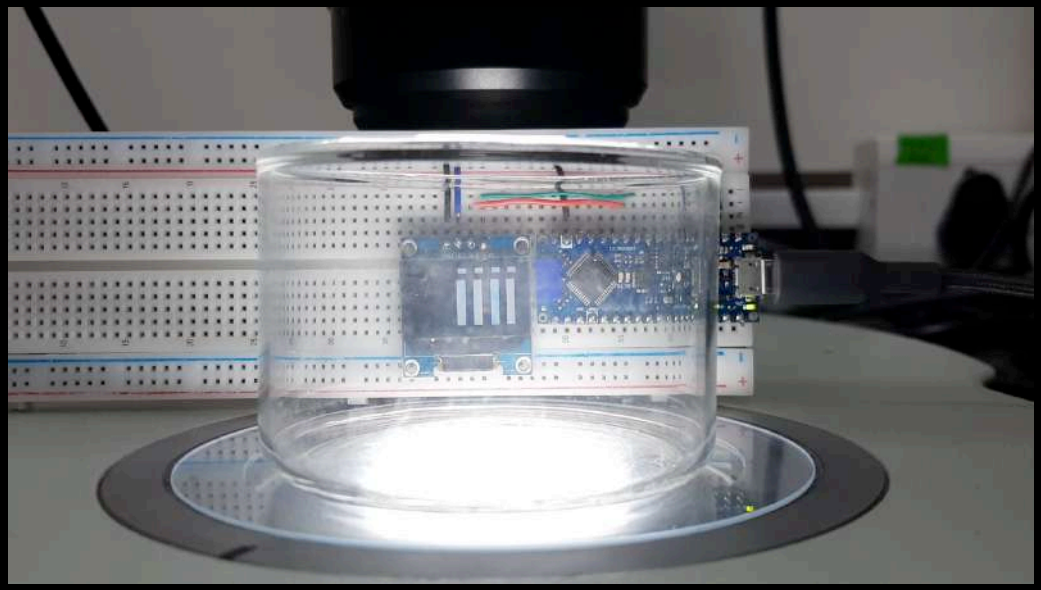
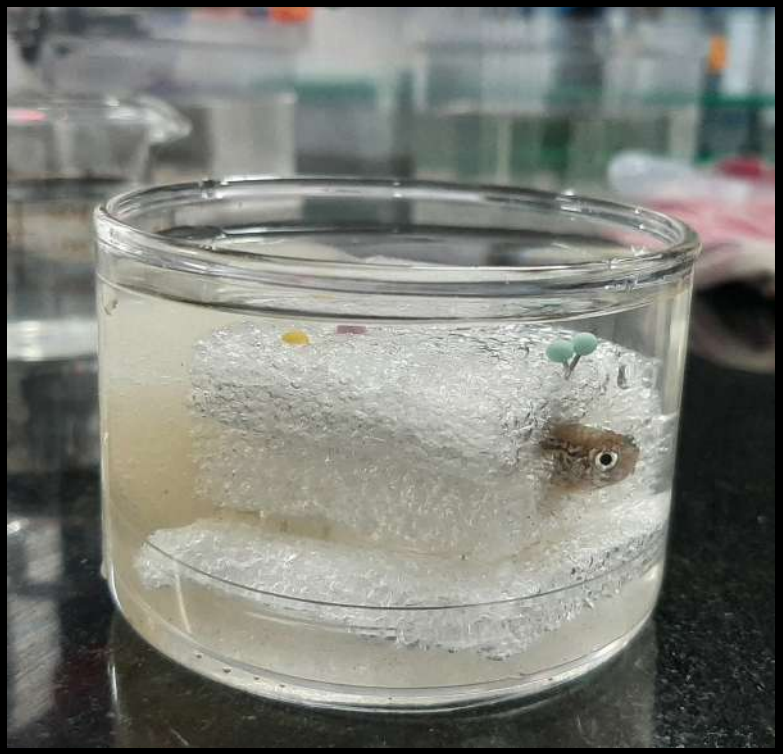
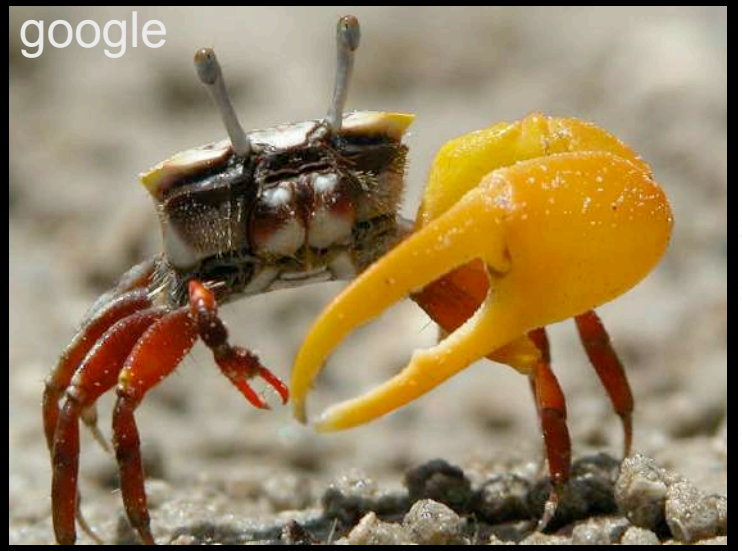




Scaling in biological systems

female fiddler crab

male fiddler crab

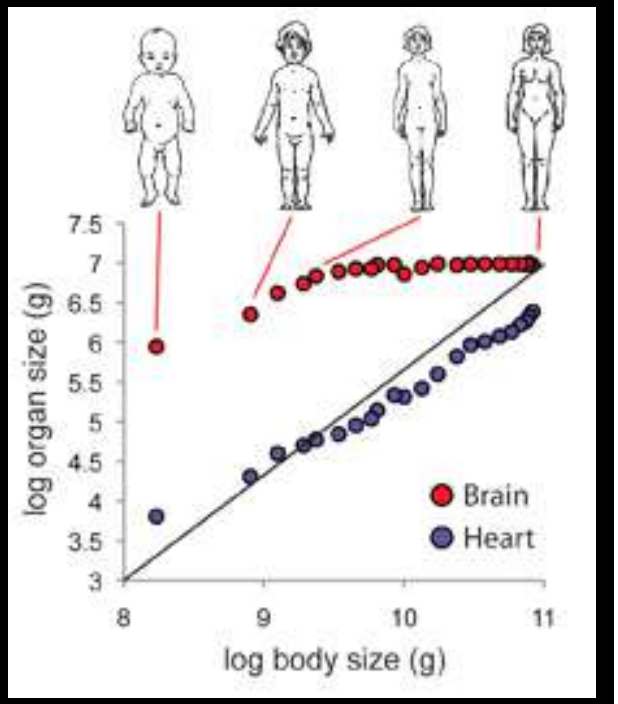


isometric scaling

organ or trait has same growth rate as the rest of the animal

allometric scaling

organ or trait has differential growth rate as the rest of the animal



Shingleton, A. (2010)
Adapted from Moore 1983;
Data from Thompson 1917

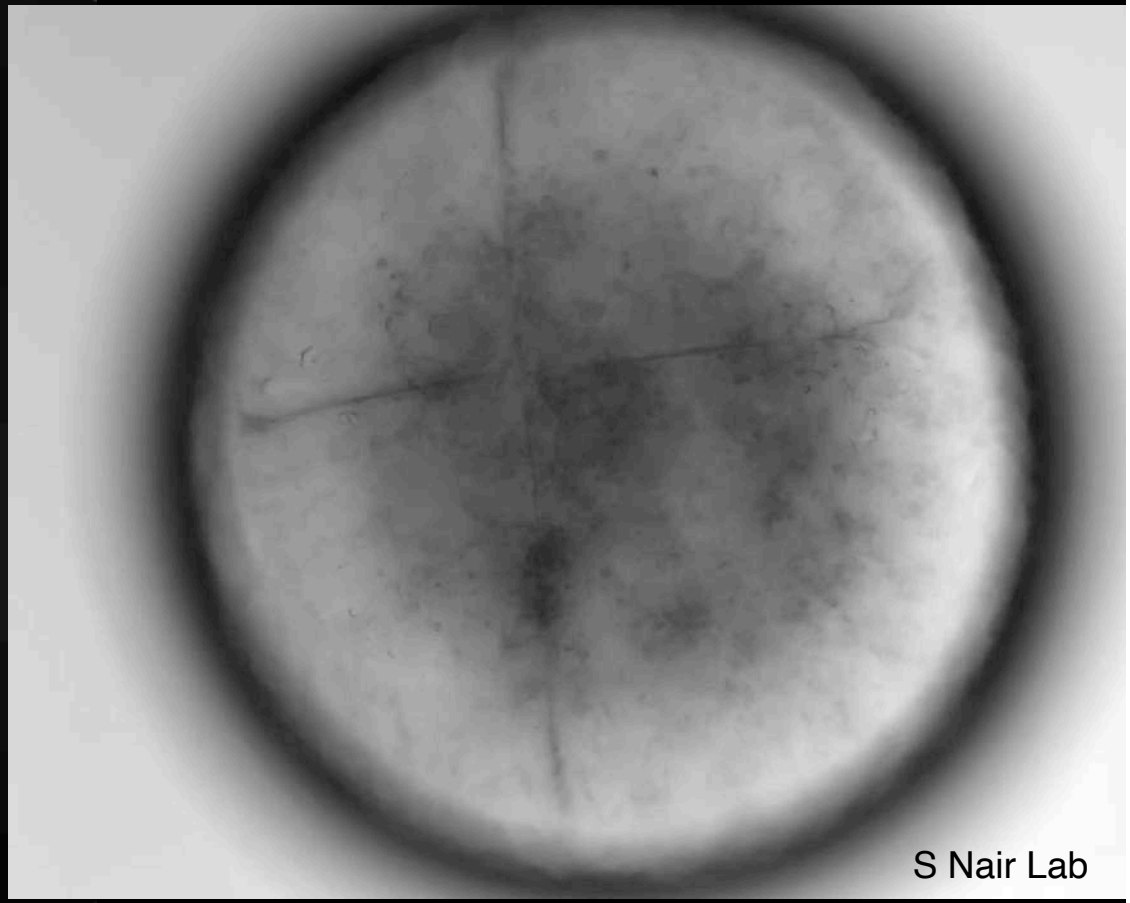
Movement is an inherently complex phenomenon

- Self co-ordination
- Co-ordination with neighbours
- Awareness of external environment
- Back-up plans
- Influence of external forces

Movement in an embryological context always gives rise to form

- Self co-ordination?
- Co-ordination with neighbours?
- Awareness of environment??
- Back-up plans???

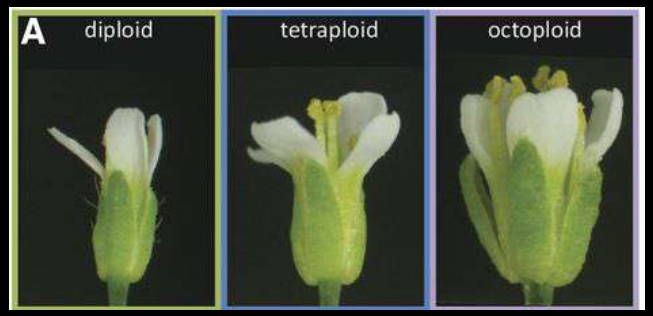
Karlstrom and Kane 1996

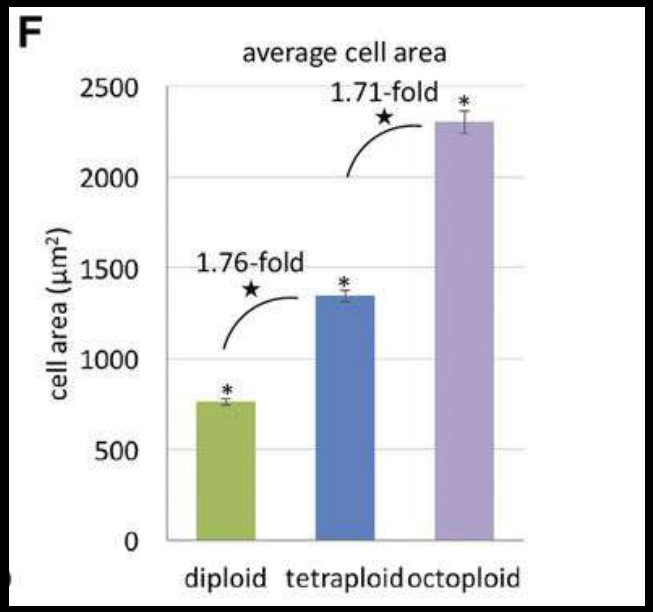


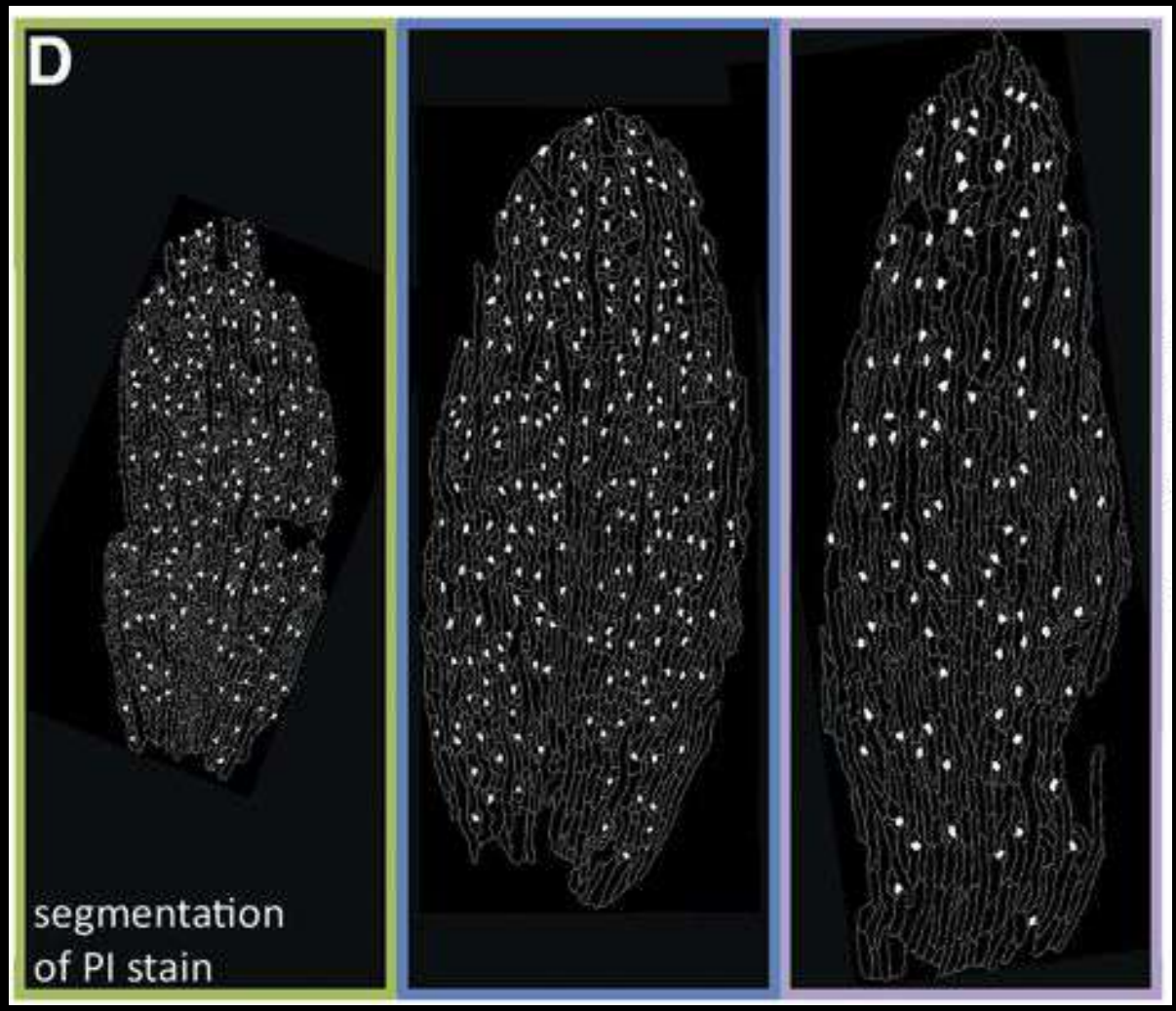
S Nair Lab

How does one change cell size when one does not know what regulates cell size?

Cell size scales with ploidy (DNA content in a nucleus)







Robinson et al Plant Cell 2018

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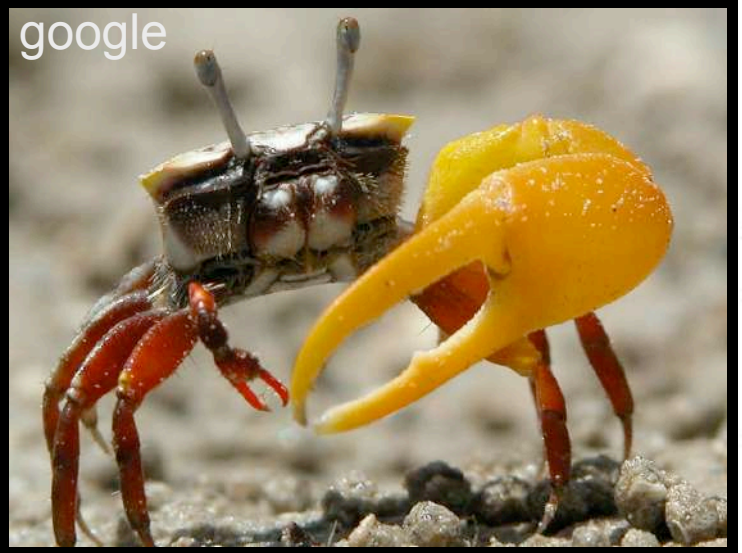

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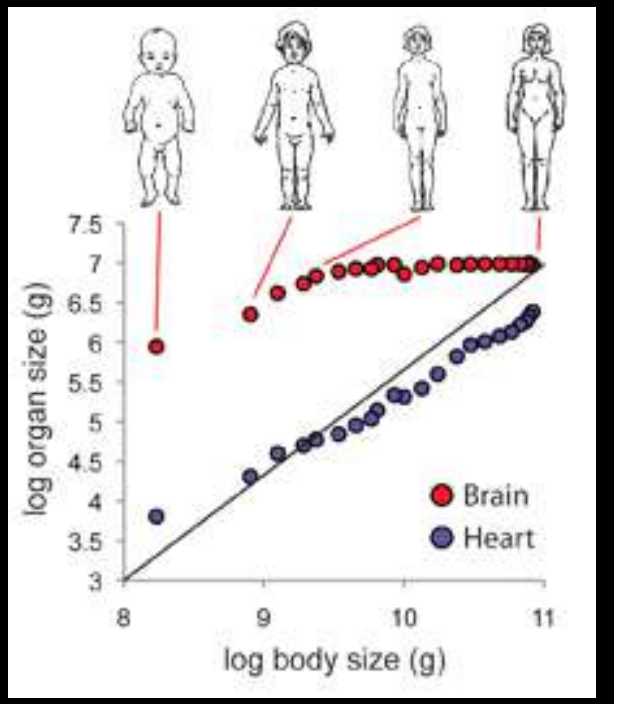

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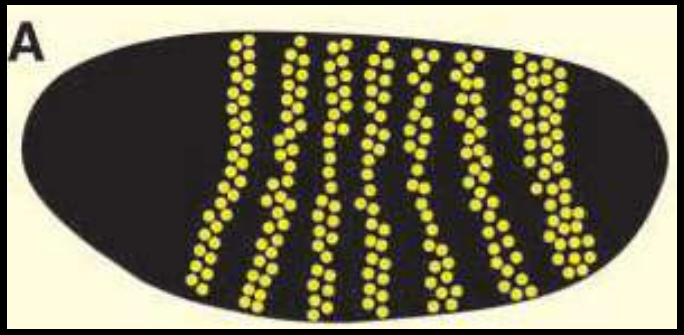
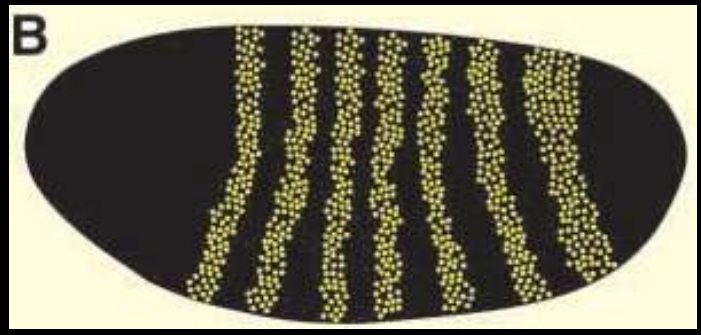


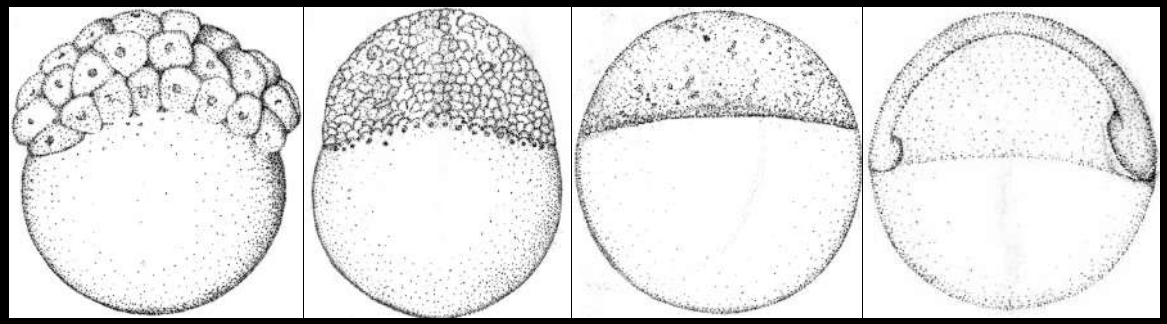


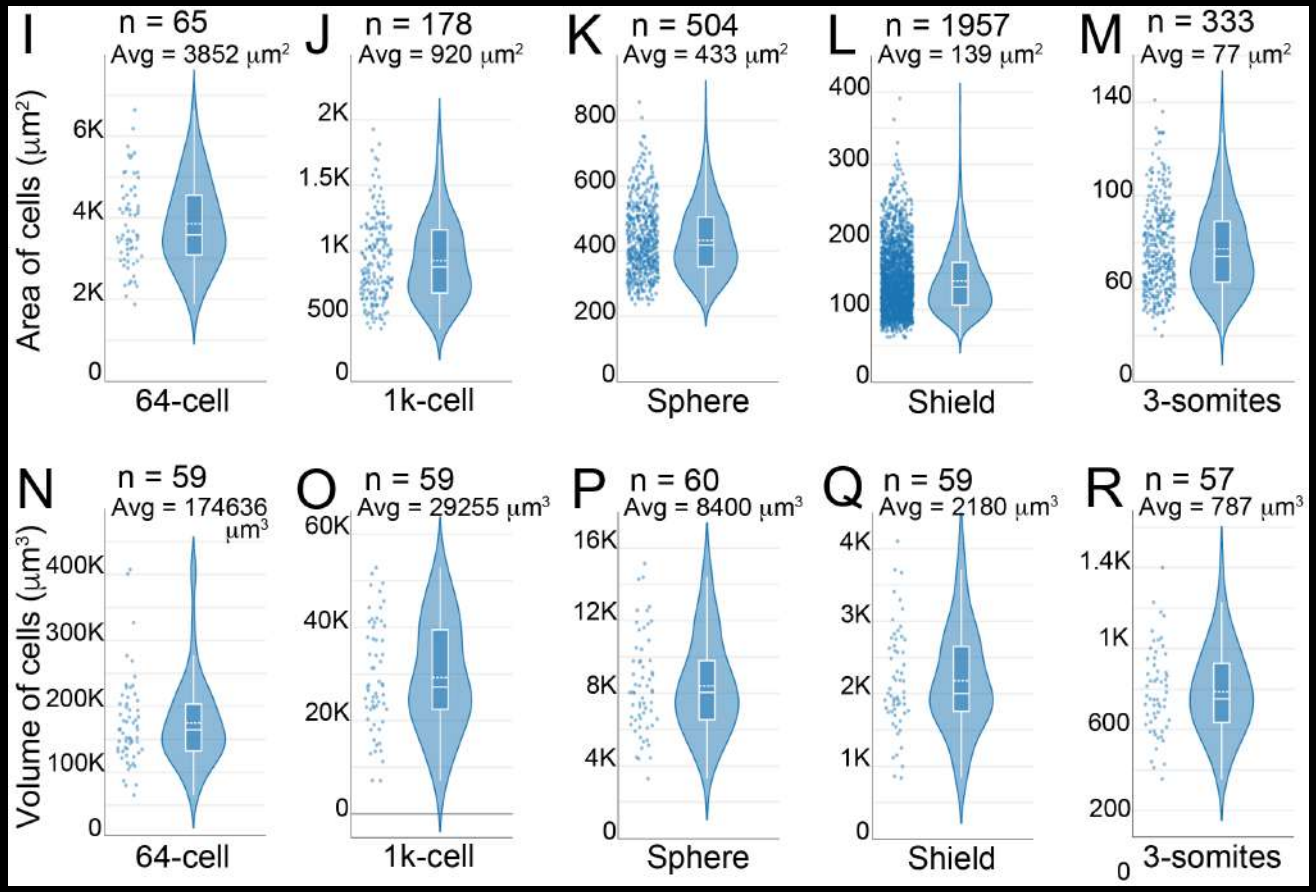
cell size scales with ploidy and “tissue” size is maintained by adjusting cell numbers

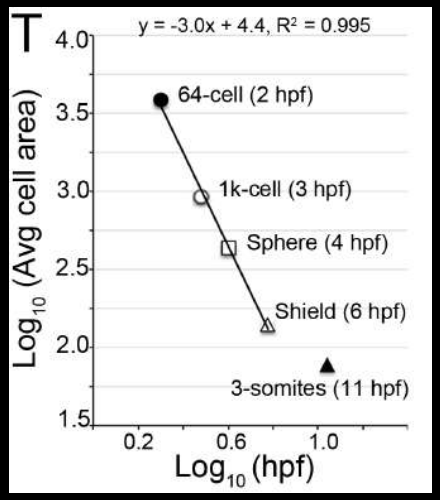
Day and Lawrence Development 2000

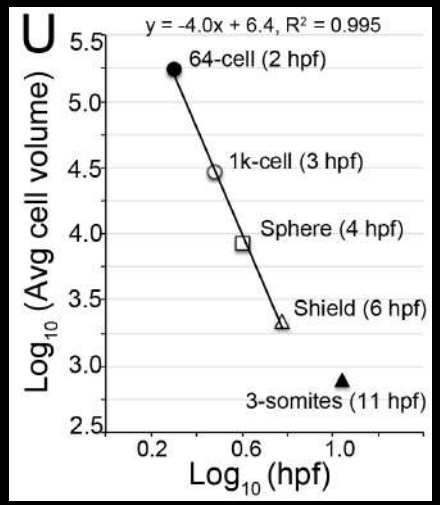
Cell size reduction follows power law during early stages of cell divisions in zebrafish

i.e. as the embryo develops, a proportional reduction in cell sizes occur
what is the embryo sensing to decide the magnitude of this proportion?

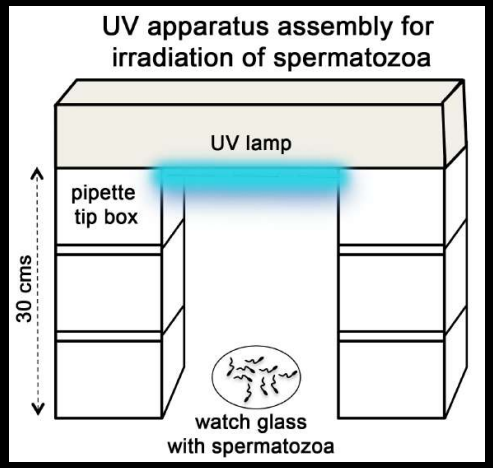


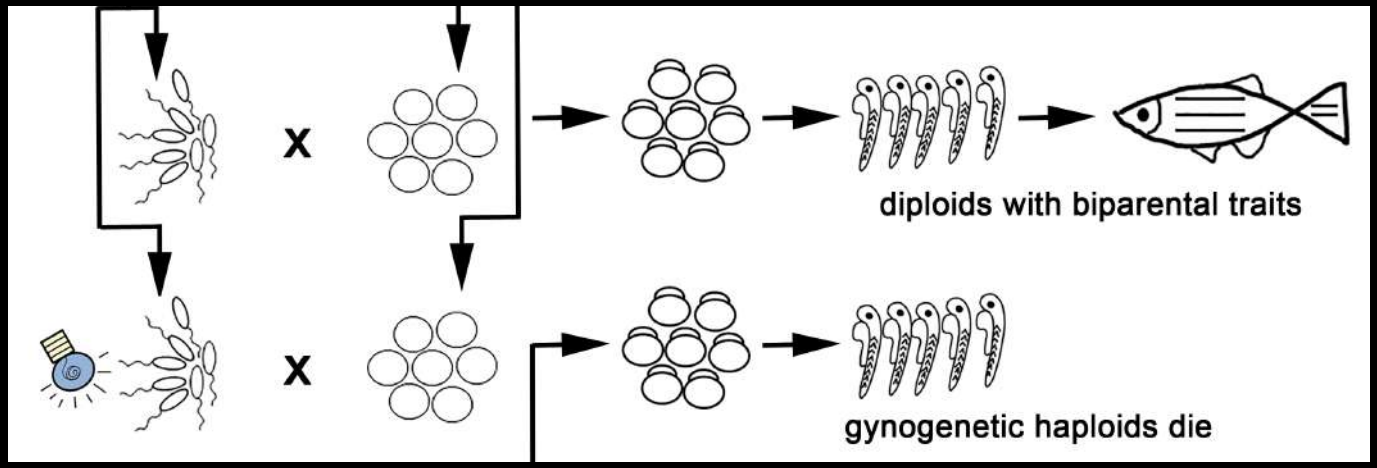


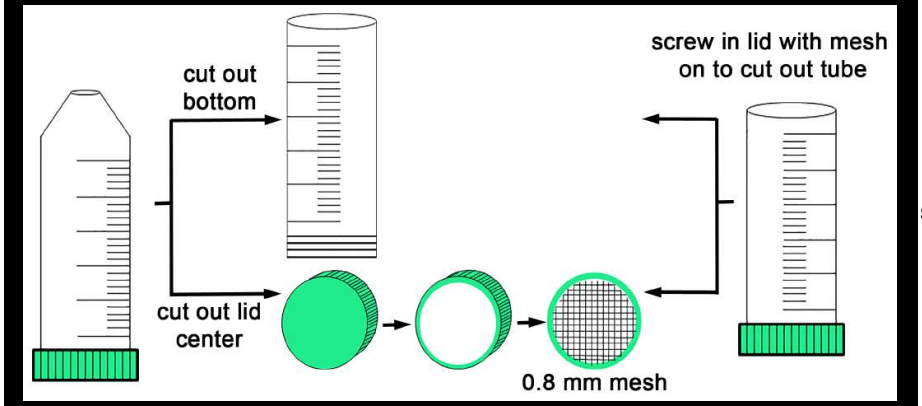


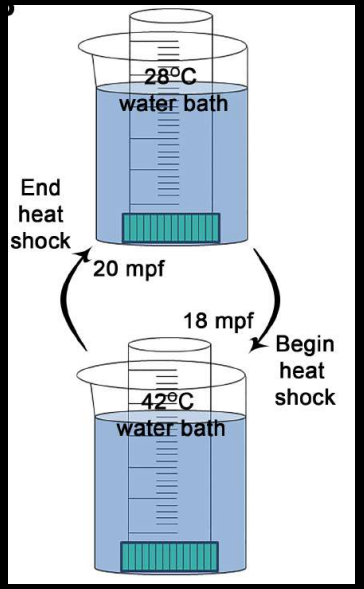


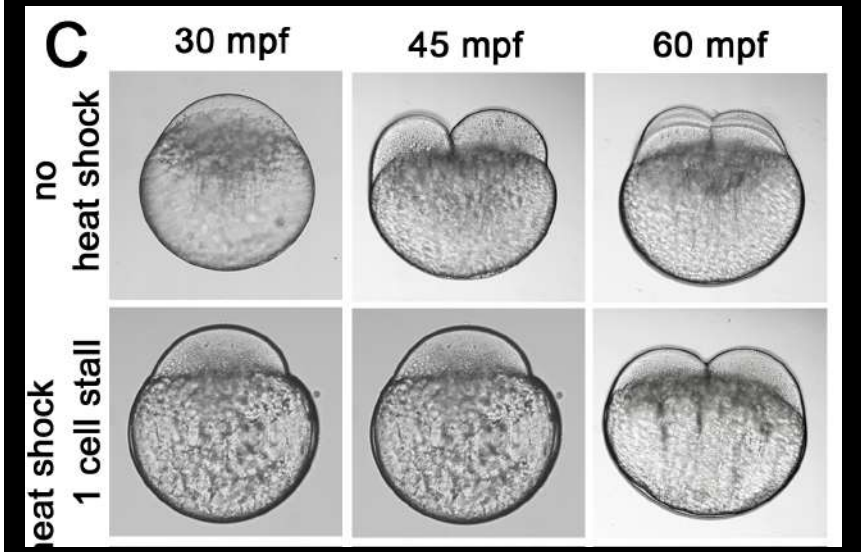
Generated zebrafish haploids and tetraploids to create embryos made of altered cell sizes





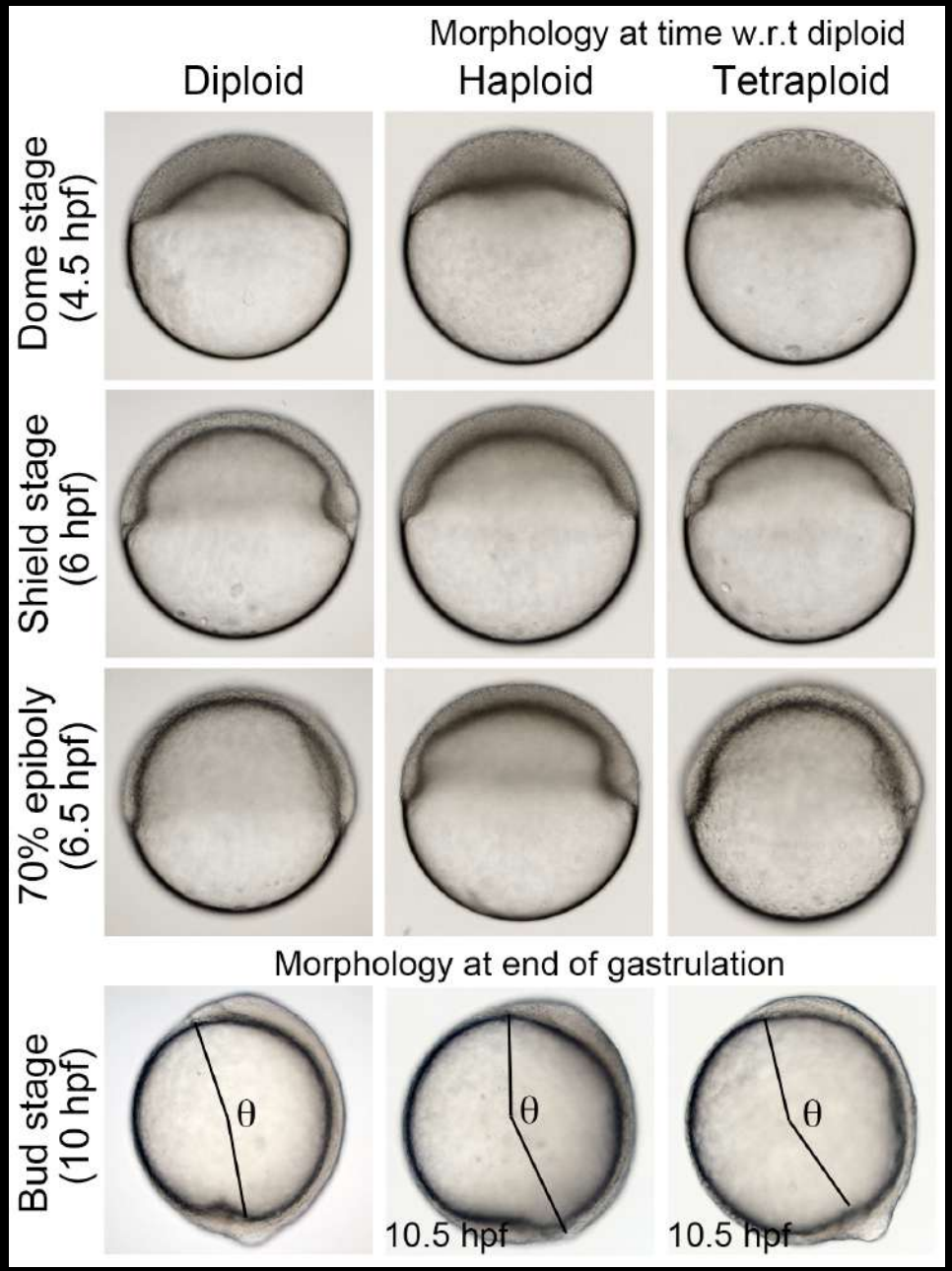


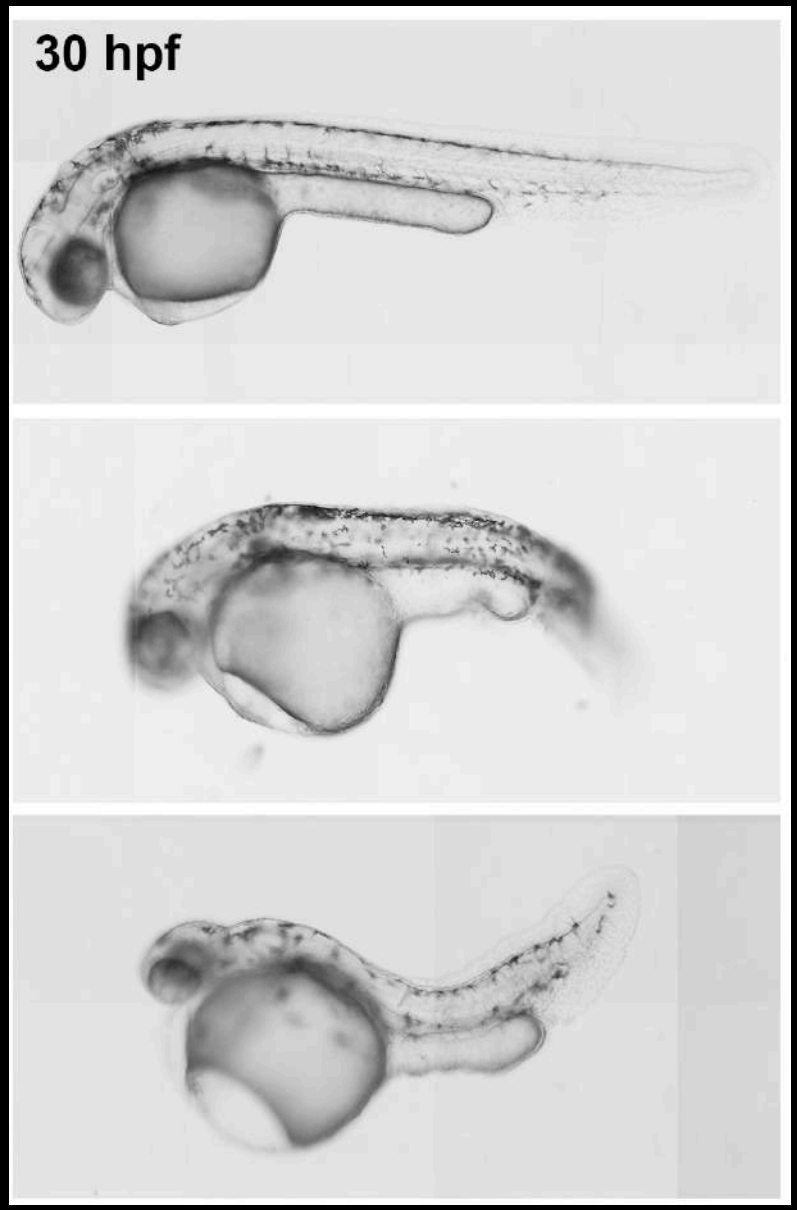




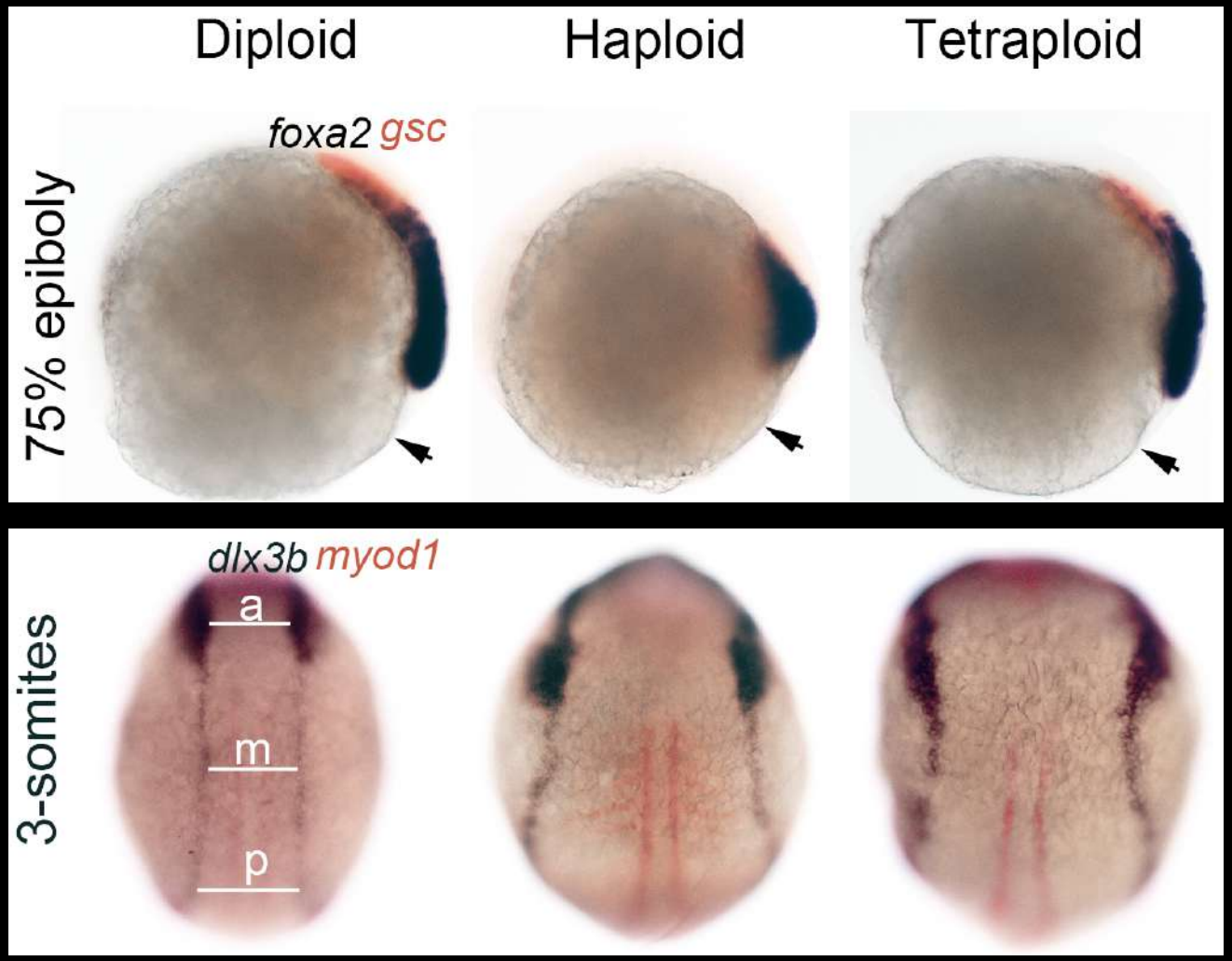
Menon and Nair Dev Dyn 2018
Menon and Nair MiMB 2019

Embryos made of smaller or larger than normal cells are delayed in epiboly initiation, progression and gastrulate abnormally





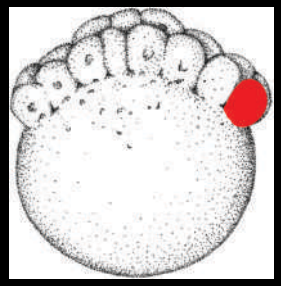
Embryos made of smaller or larger than normal cells have defects in anterior migration, midline convergence and midline extension of cells during gastrulation

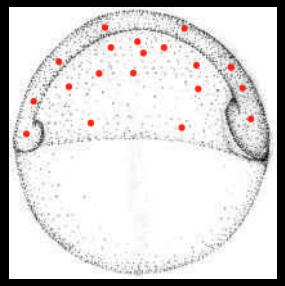


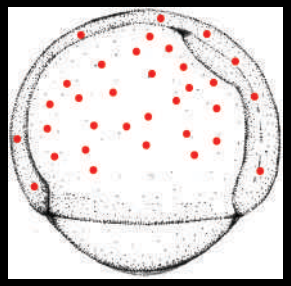
Karlstrom and Kane 1996

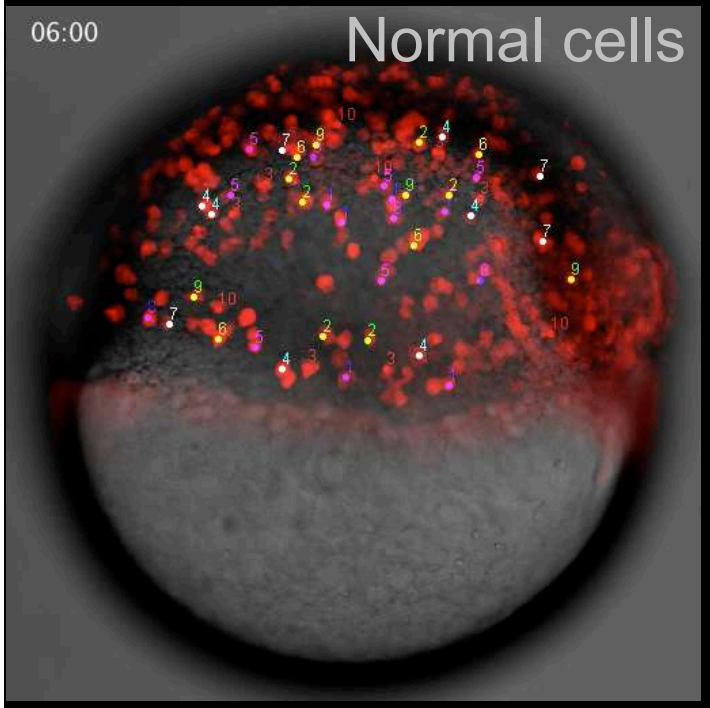
Do sub-optimally sized cells migrate aberrantly during gastrulation?

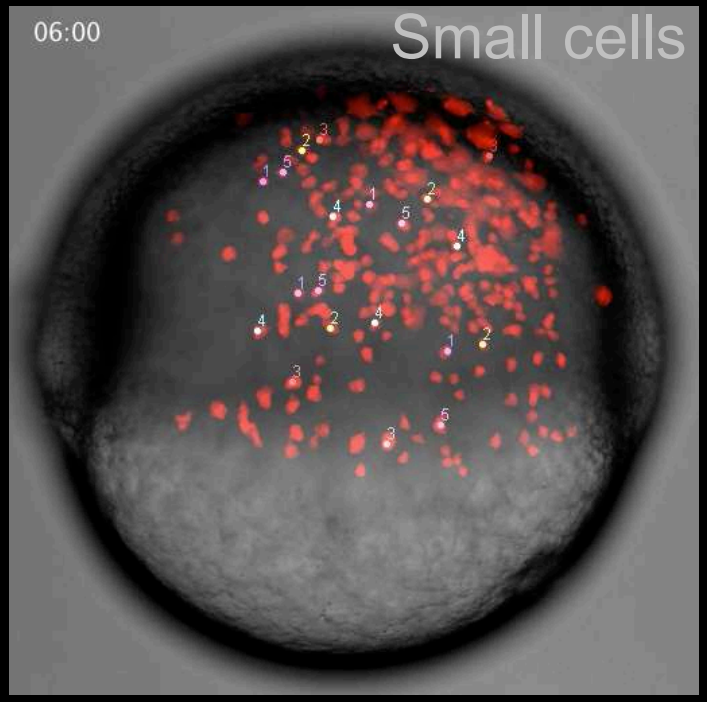
Analysing cells in an embryo by clonal labelling

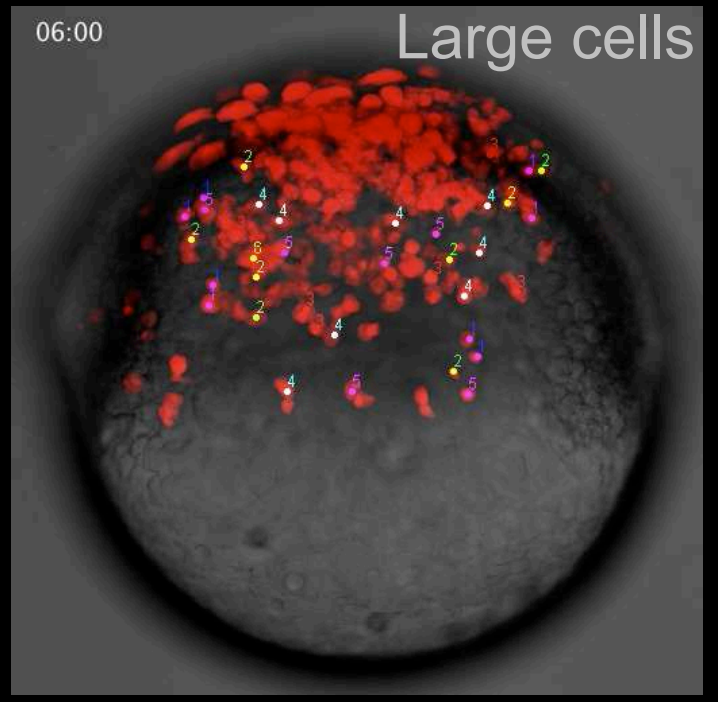




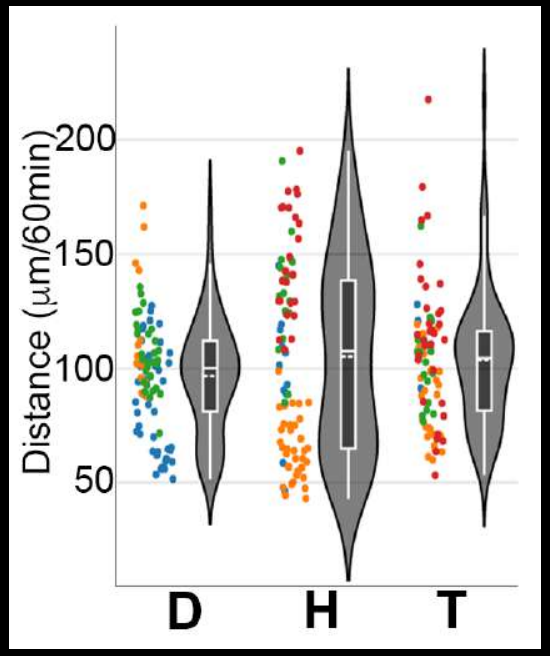


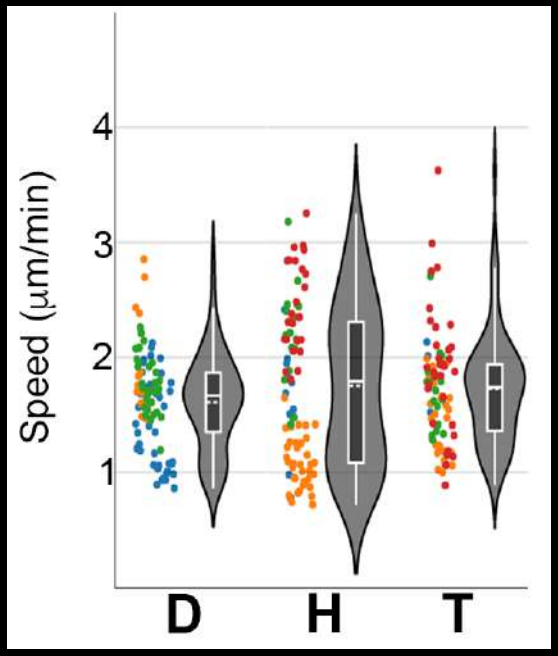




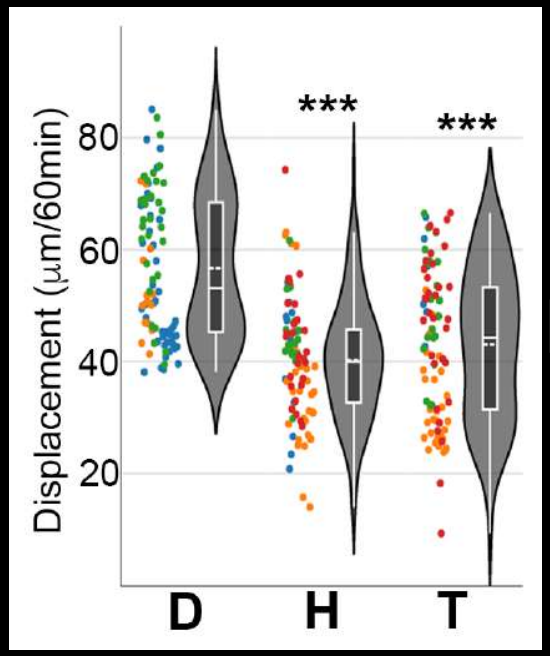


The migratory behaviour of cells in a suboptimal sized cell collective is altered

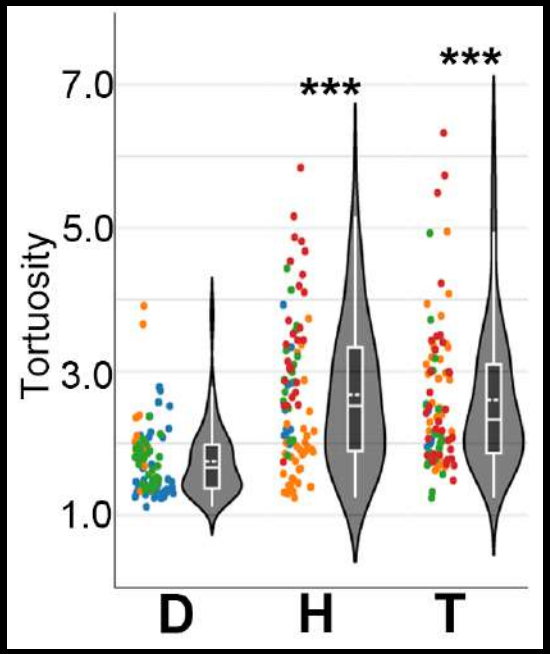




cells travel the same distance at comparable speeds



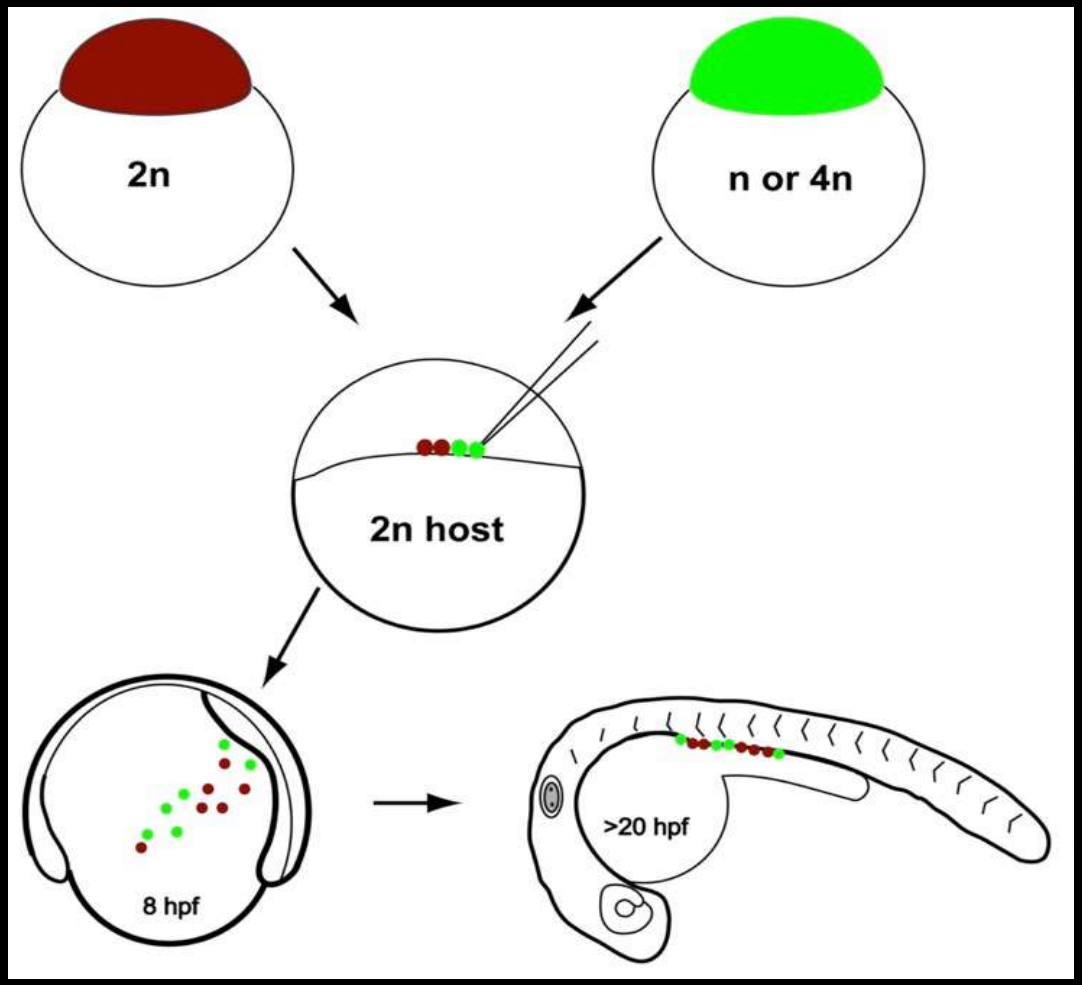
but are displaced less



because they wander more

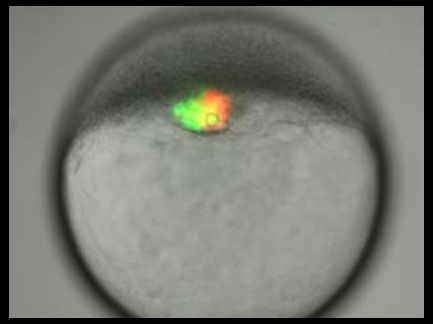
Do sub-optimally sized cells migrate aberrantly during gastrulation?

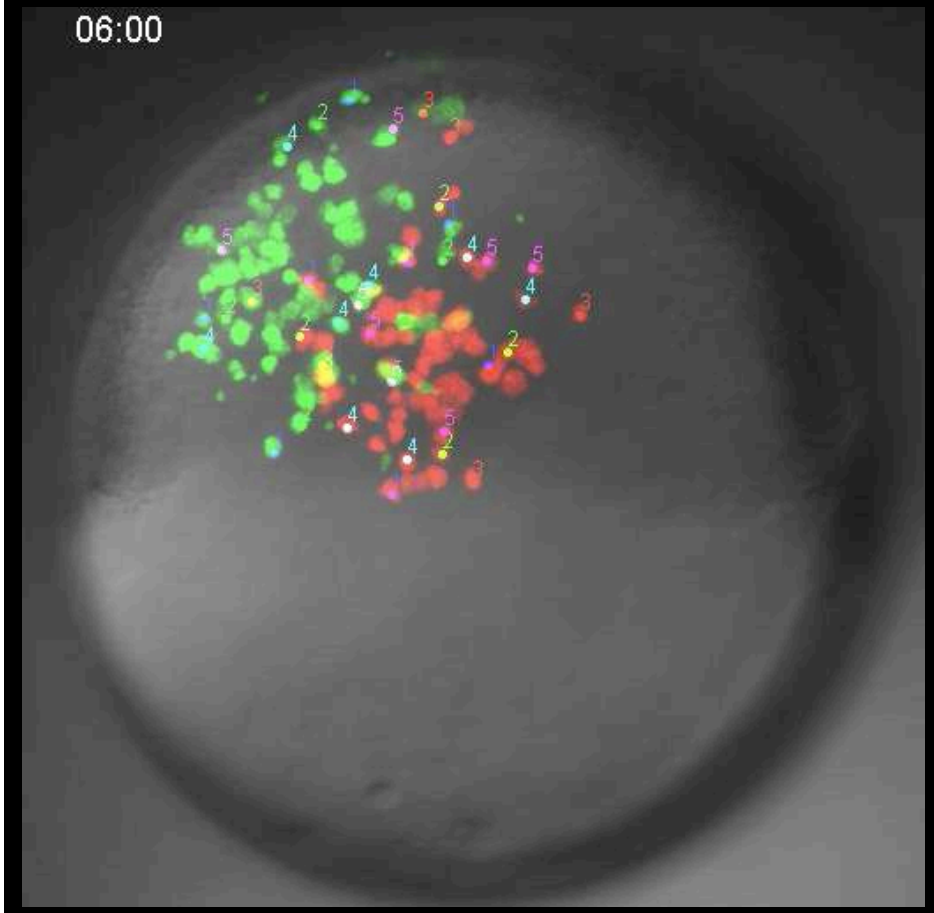
Analysing cells in an embryo by generating mosaic embryos
Tests cell autonomous vs non-autonomous nature of a phenomenon



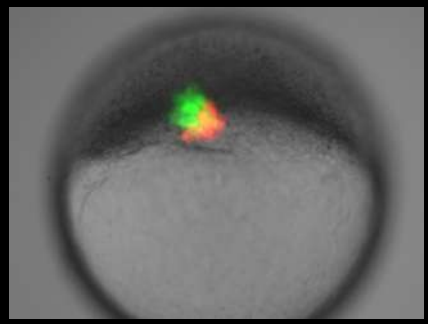
Smaller or larger cells continue to migrate aberrantly during gastrulation, even when placed in a normal environment

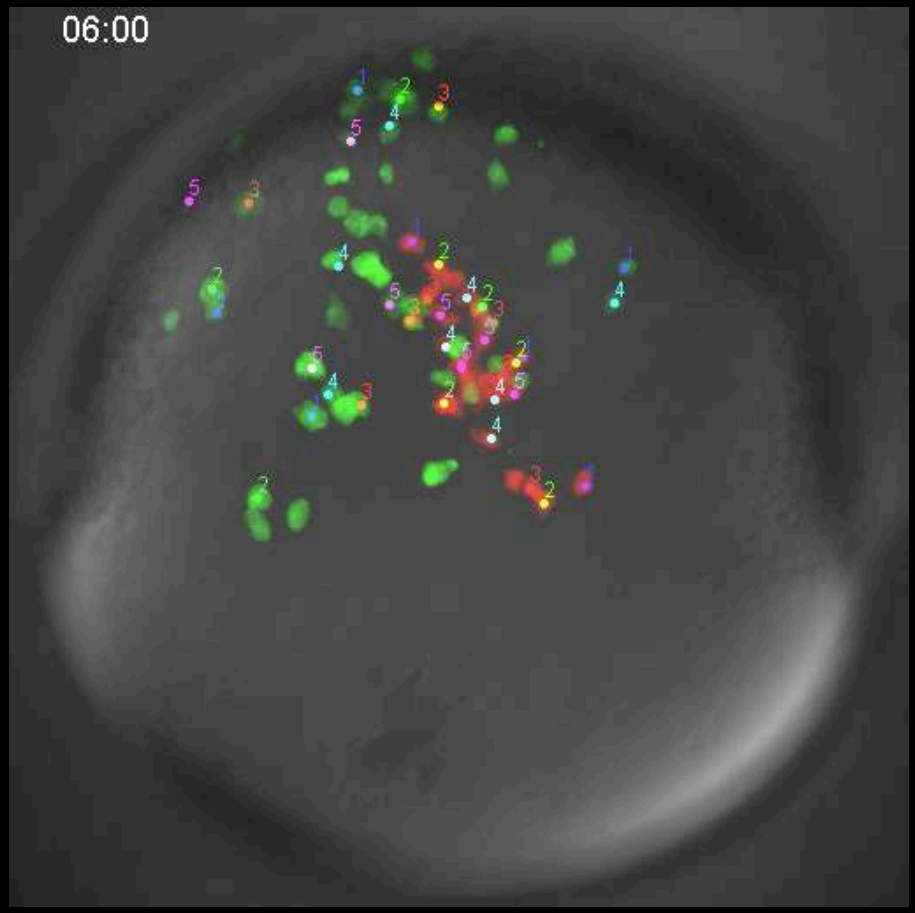
Normal + Small cells into Normal hosts



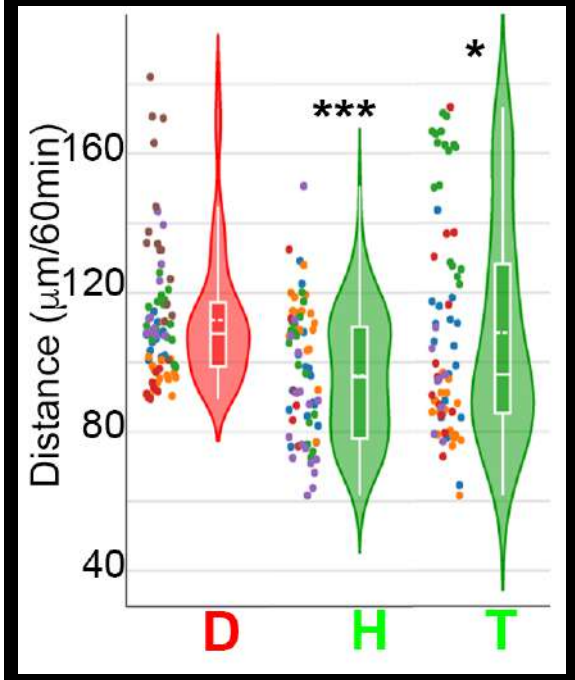


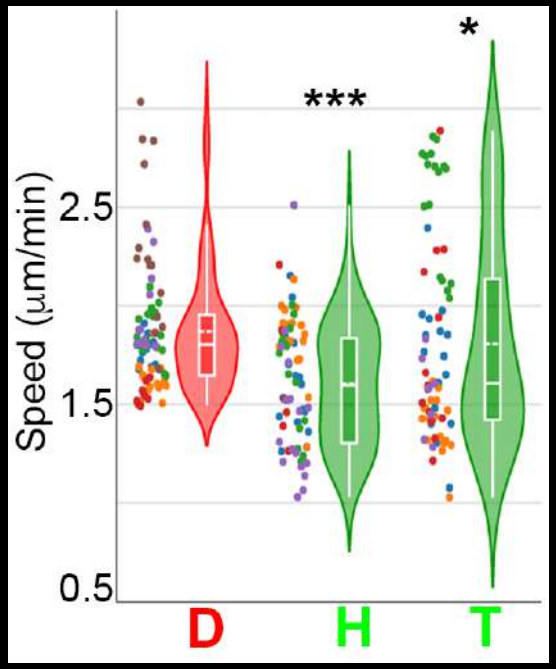
Normal + Large cells into Normal hosts



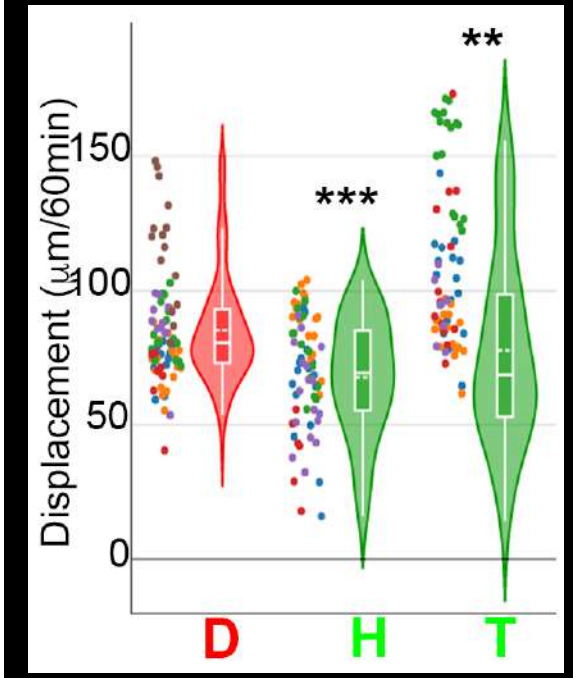


Migration is influenced by environment of the cell collective

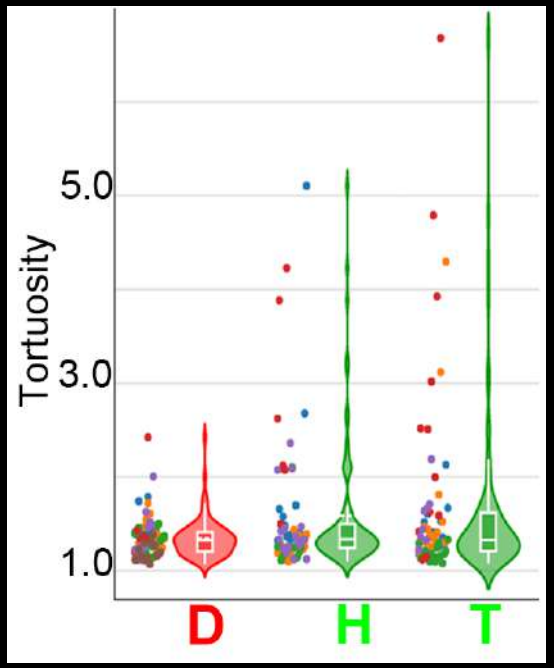




cells travel less distance
and are relatively slow



are displaced less



but cells do not wander

Vertebrate Embryogenesis Group and our Funders

- DST-Science and Engineering Research Board
- Indian Institute of Technology Bombay
- Wadhwani Research Centre for Bioengineering
- India Alliance DBT/Wellcome Intermediate fellowship
- Japan Society for the Promotion of Science
- Department of Atomic Energy
- Tata Institute of Fundamental Research

and Prakashmani Kumar