Alternative model organisms:
New tools, new insights into the evolution of development

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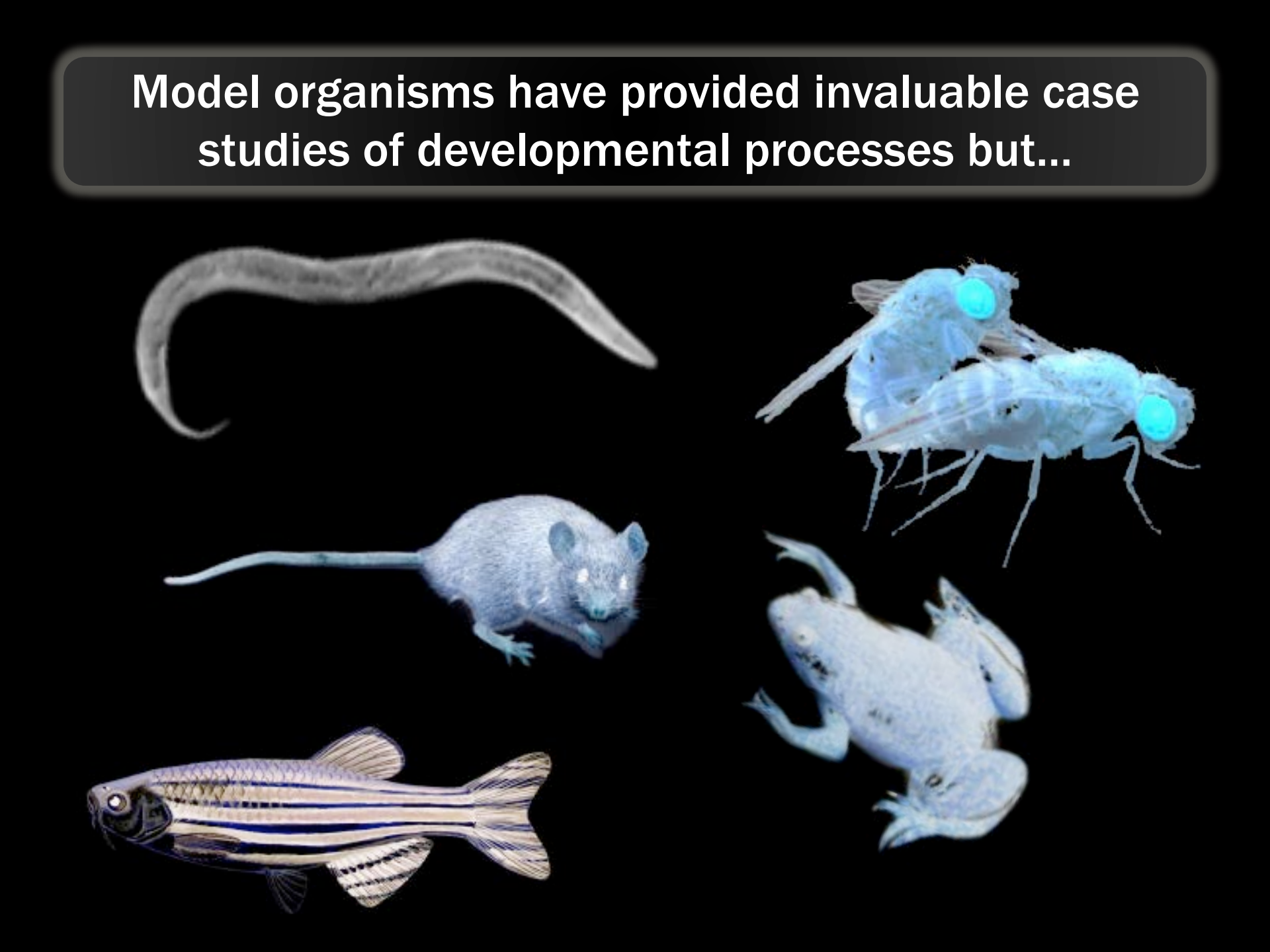
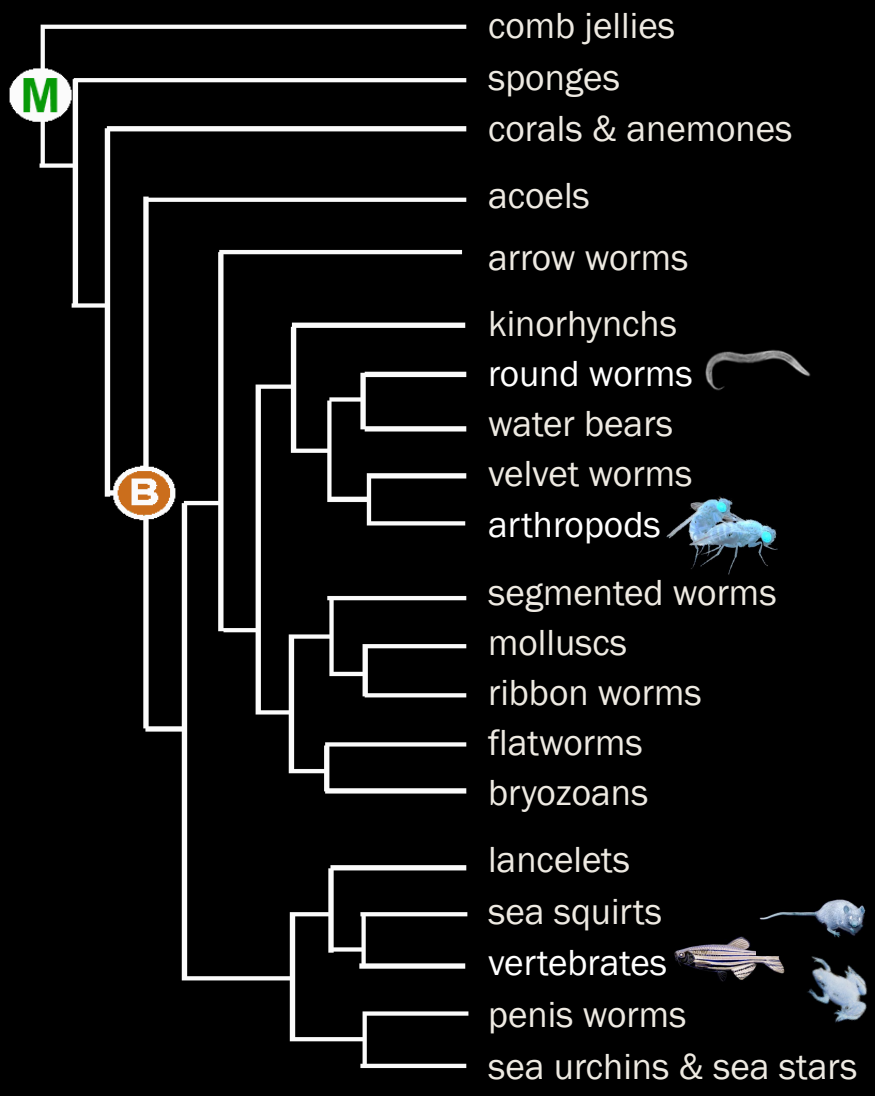
Model organisms have provided invaluable case studies of developmental processes but...



...traditional model organisms represent very little of animal diversity



1. How do we choose organisms for study?

2. Case study: a basally branching model offers insight into the evolution of germ line specification.

3. Tool building: quantitative analysis of putative “ancestral” insect development.

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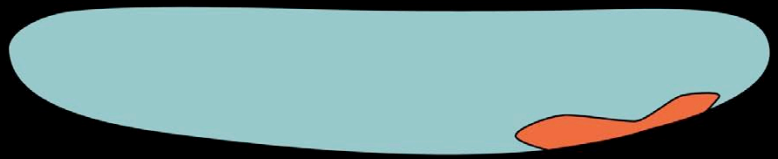
Traditional model organisms are derived relative to basally branching members of their clades

most body plan elements
established maternally
(earlier in development)

Lab models tend to be selected because of their:
fast development
rapid sexual maturation
large population sizes
high tolerance for environmental variability

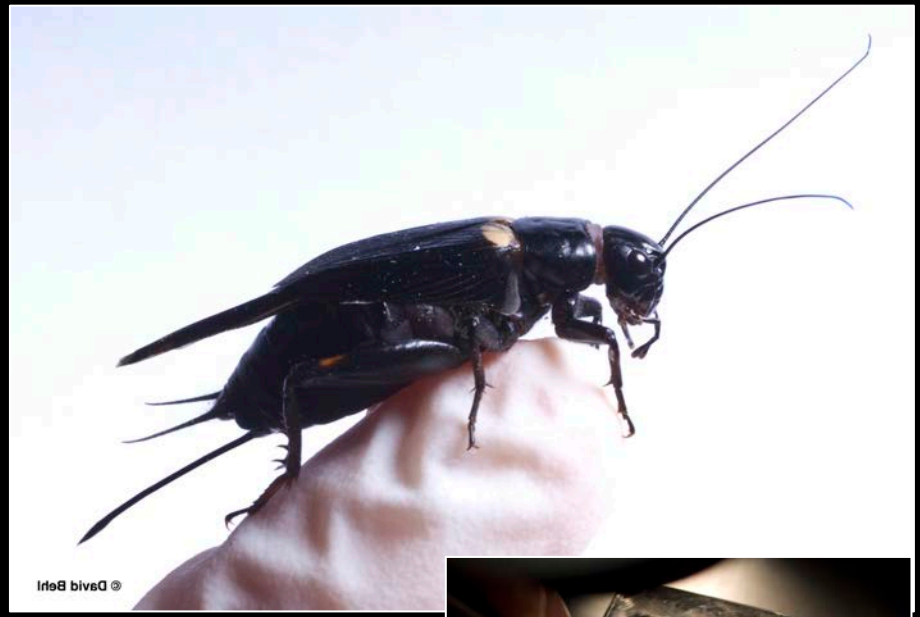


most body plan elements
established zygotically
(later in development)

Drosophila melanogaster

- Easy culture
- Community
- Complete genome
- Embryos all year
- 24 hour embryogenesis
- 10 day life cycle
- Powerful genetics

Gryllus bimaculatus



cricket embryo dissection

- Easy culture
- Transcriptome
- Draft genome
- Embryos all year
- 10 day embryogenesis
- 6 week life cycle
- RNA interference, CRISPR/Cas9

Drosophila melanogaster

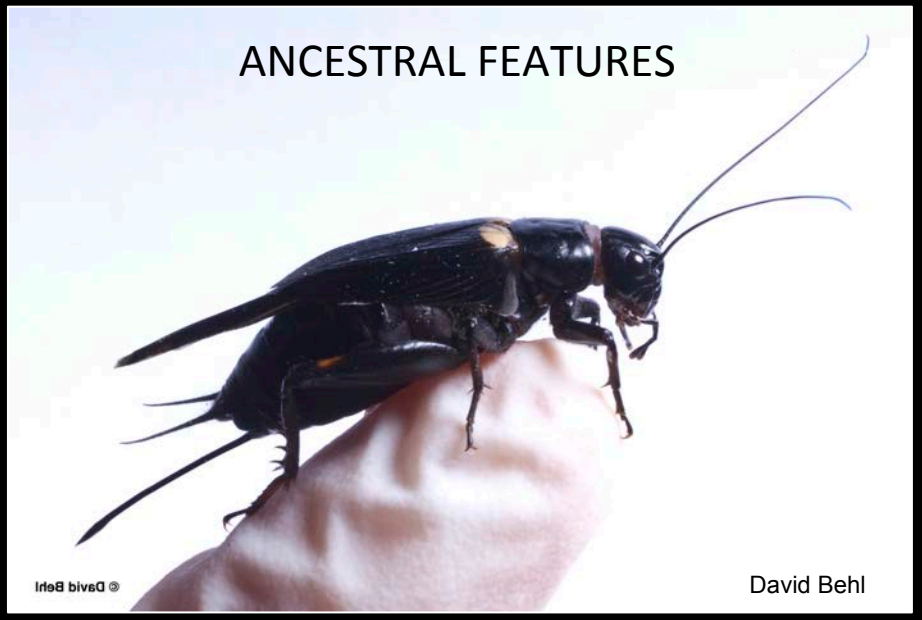
DERIVED FEATURES

Kuntzei lab

- Holometabola (metamorphosis)
- Long germ development
- Simultaneous segmentation
- Meroistic ovaries (nurse cells)
- Reduced extra embryonic membrane
- Large embryonic rudiment
- No blastokinesis

Gryllus bimaculatus

ANCESTRAL FEATURES



David Behl

- Hemimetabola (direct development)
- Short germ development
- Sequential segmentation
- Panoistic ovaries (no nurse cells)
- 2 Large extra embryonic membranes
- Small embryonic rudiment
- Blastokinesis

Embryonic development in *Gryllus bimaculatus*

Donoughe and Extavour, 2016 Dev Biol; Nakamura et al, 2010 Curr Biol

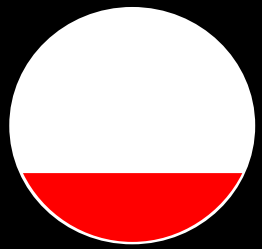
1. How do we choose organisms for study?
   - The most convenient laboratory organisms may provide limited evolutionary inference.
   - Phylogenetic position and experimental tools should correspond to the questions being asked.
   - Basally branching insect and arthropod models can help place findings from *Drosophila melanogaster* in an appropriate evolutionary context.
   - *Gryllus bimaculatus* is a promising model to understand the evolution of arthropod development.

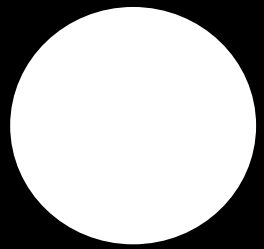
1. How do we choose organisms for study?

2. Case study: a basally branching model offers insight into the evolution of germ line specification.

3. Tool building: quantitative analysis of putative “ancestral” insect development.

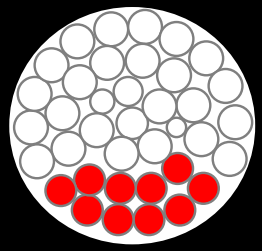
Animals have two main ways of making primordial germ cells (PGCs)

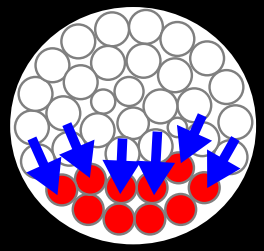




Inheritance: cytoplasmic determinants (germ plasm)

Induction: cell-cell signals may be ancestral in animal PGC formation.





Inheritance **Induction**

germ cells somatic cells

Extavour & Akam *Development* 2003

Zygotic mechanisms rather than maternal determinants make germ cells in basally branching arthropods

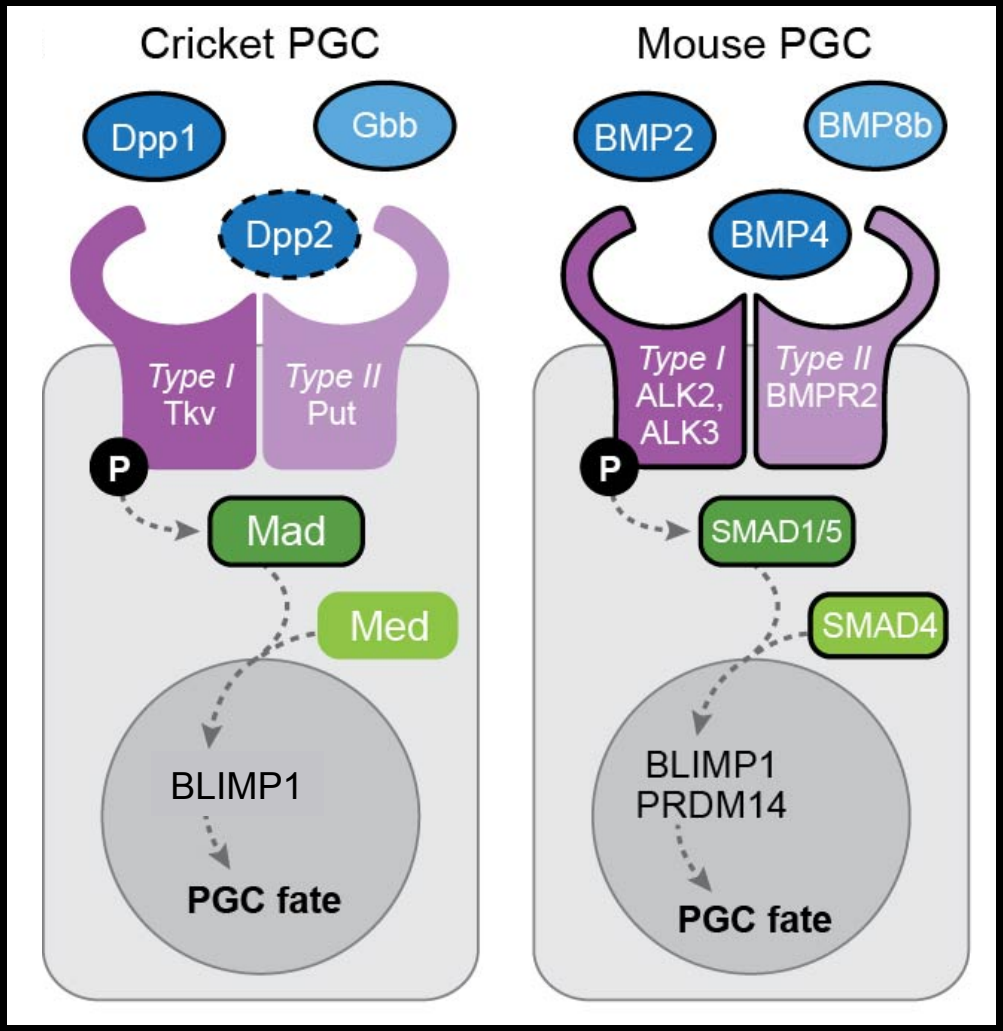
Oncopeltus fasciatus

Gryllus bimaculatus

Parasteatoda tepidariorum

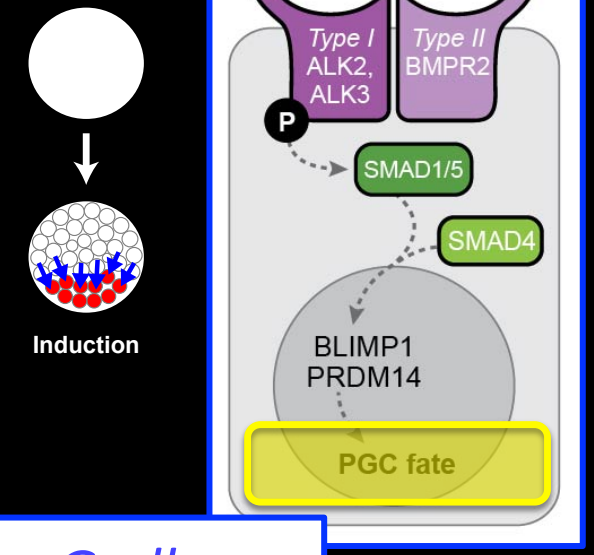
Ewen-Campen et al. ***BMC Genomics*** 2011
Zeng et al. ***BMC Genomics*** 2011
Zeng & Extavour ***Database*** 2012
Zeng et al. ***PLoS ONE*** 2013
Ewen-Campen et al. ***Current Biology*** 2013
Ewen-Campen et al. ***BiO*** 2013
Posnien et al. ***PLoS ONE*** 2014
Schwager et al. ***Developmental Biology*** 2015

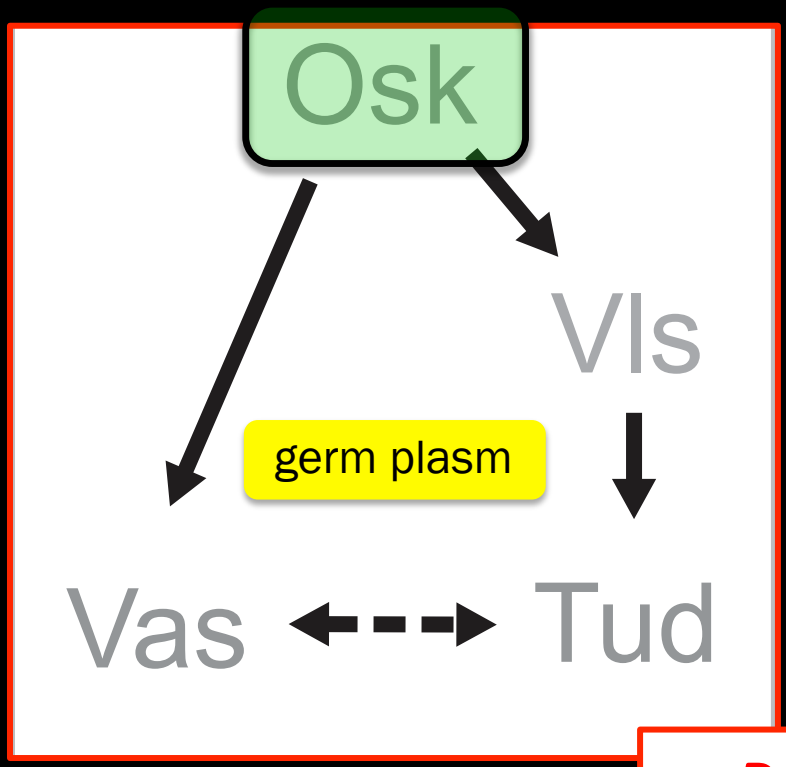
Mouse and cricket PGCs are generated by BMP signalling upstream of Blimp-1

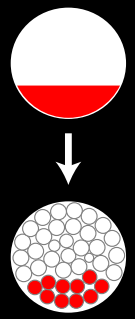


Donoughe et al. *PNAS* 2014
Nakamura & Extavour, *Development*, 2016

How did germ plasm assembly evolve?







Mus, Gryllus:
BMP
Induction

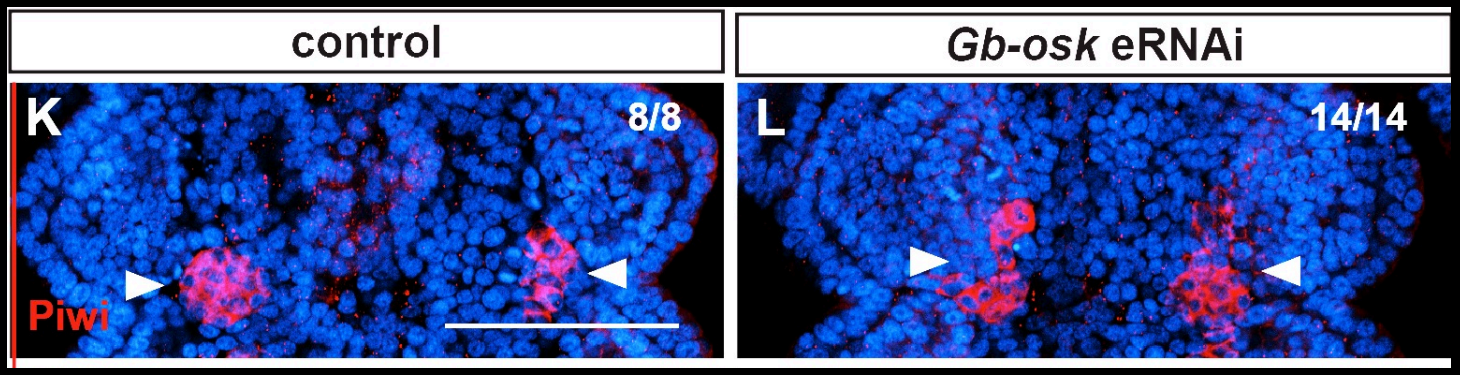
There is a conserved molecular signature of germ cell fate, independent of the upstream mechanisms

Drosophila:
Oskar
inheritance

Saitou *Cold Spring Harbor Perspectives in Biology* 2012; Anne *PLoS ONE* 2010

Gryllus oskar does not play "canonical" *oskar* roles

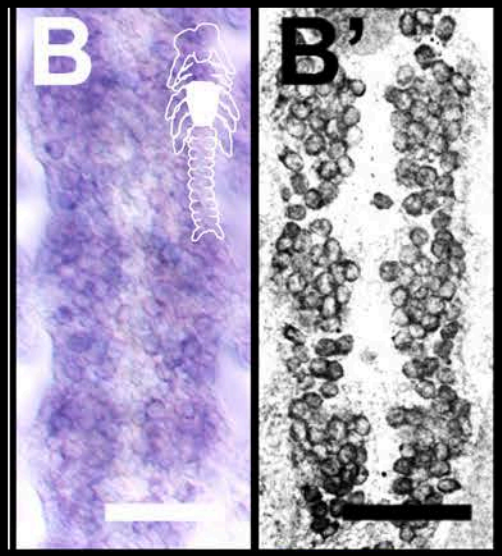
DNA
Germ cells



Drosophila oskar:

- Asymmetric localization in oocyte or early embryo
- PGC formation
- Oogenesis
- Posterior determinant localization or patterning

NO



Gb-osk **transcript** and **protein** are expressed in cricket neuroblasts.

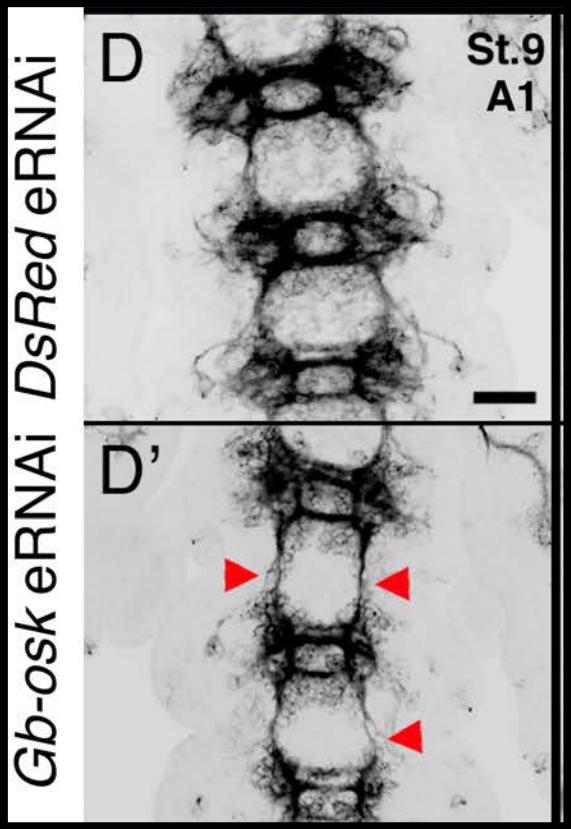
Ewen-Campen et al. *Current Biology* 2012

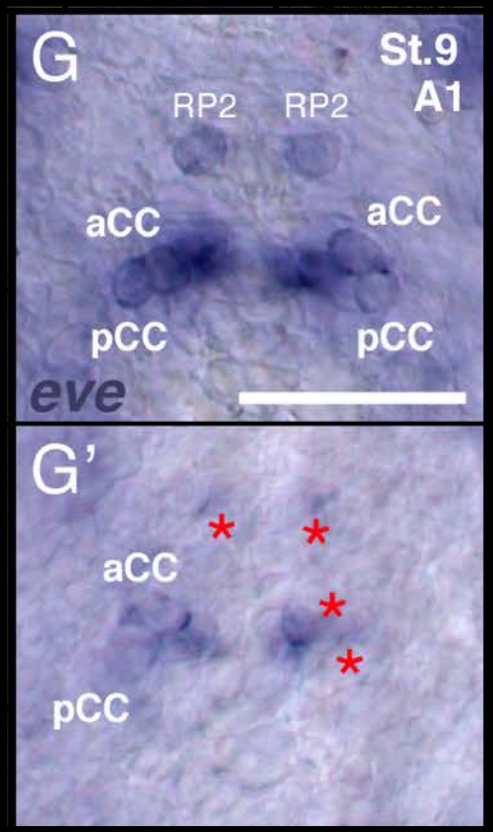
Gryllus oskar knockdown causes defects in embryonic axonal morphogenesis and neuronal patterning

Longitudinal connective defects

Anterior & posterior commissure defects

Missing RP2, aCC or pCC neurons





Ewen-Campen et al., *Current Biology* 2012

2. Case study: a basally branching model offers insight into the evolution of germ line specification.
   - Germ plasm is a derived mode of germ line specification in insects (and likely in animals).
   - Cell-cell signaling (BMP/Blimp-1 based?) likely ancestral.
   - *oskar* may have played an ancestral neural role in Hemimetabola, and then been co-opted to a germ line role in Holometabola, facilitating the evolution of germ plasm.

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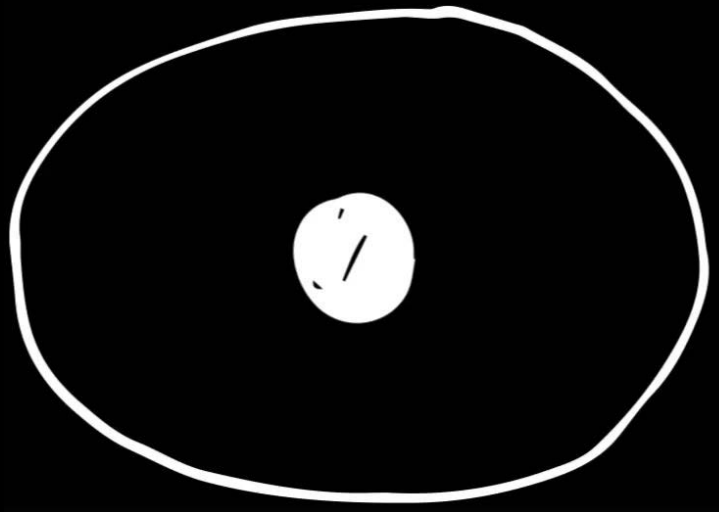
Animals have one of two types of embryonic cleavage

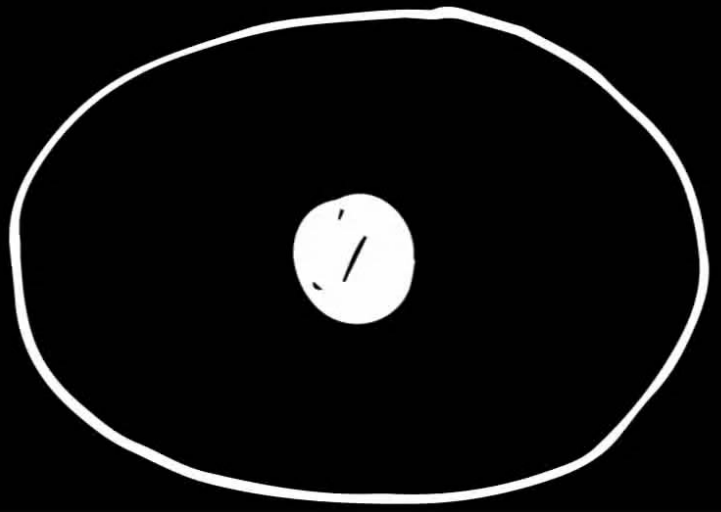
Holoblastic

Nuclear division
with cell cleavage

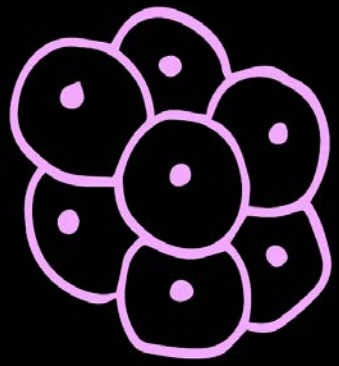


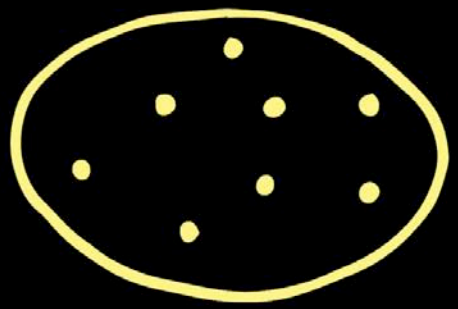
Syncytial

Nuclear division
without cleavage



Nuclear divisions *with* cell cleavages





Nuclear divisions *without* cell cleavages

Anemones

Vertebrates

Nematodes

Crustaceans


springtail


dragonfly


cricket


milkweed bug

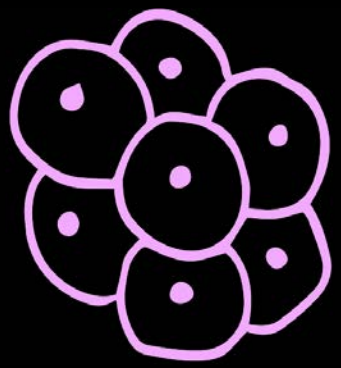

beetle

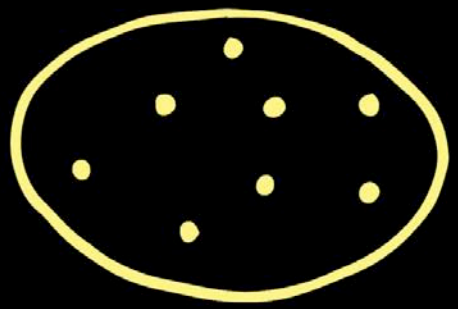

moth


flea


fruit fly

Nuclear divisions *with* cell cleavages





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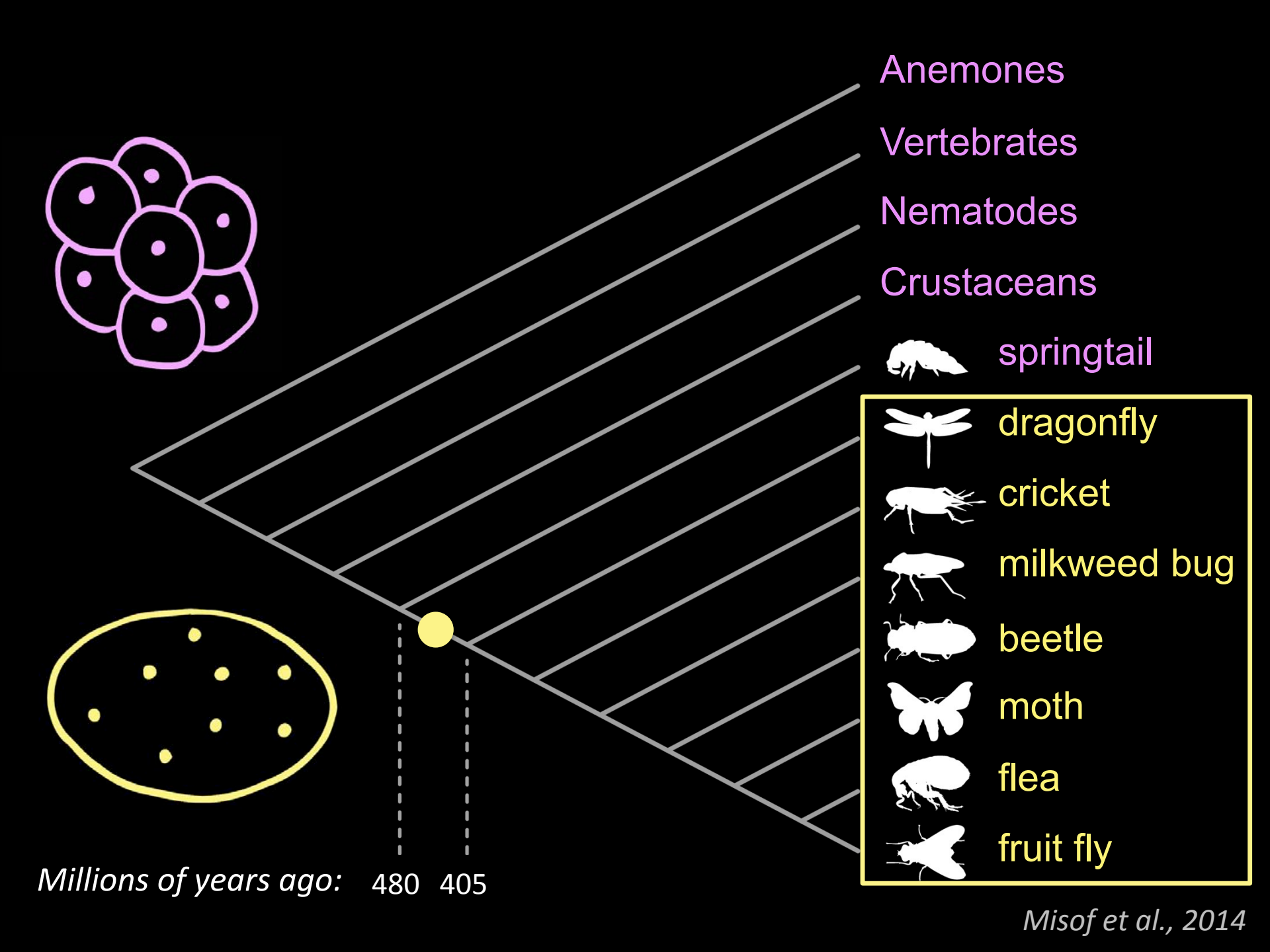


Misof et al., 2014

Syncitial cleavage is not uniform across insects

Gryllus bimaculatus

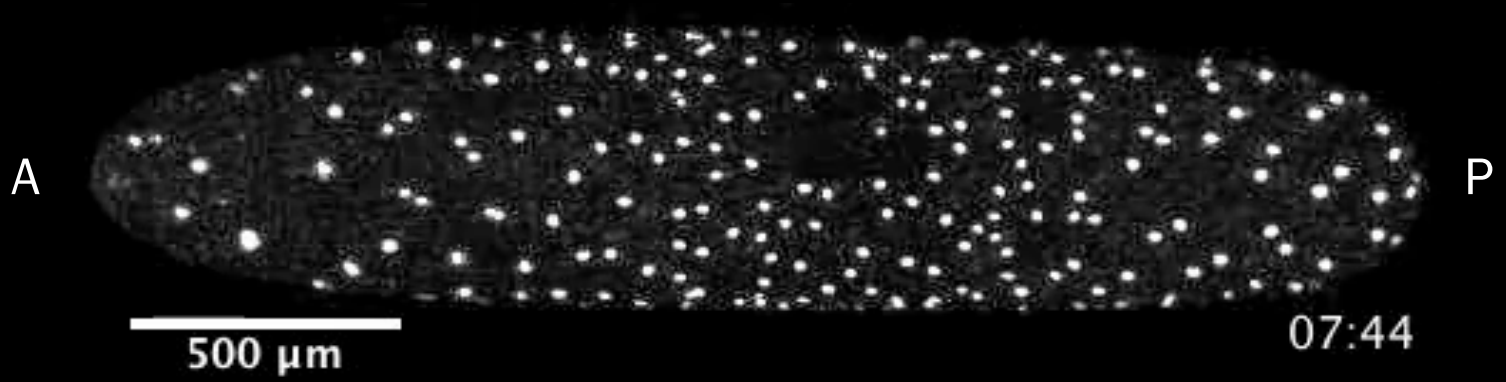
Nakamura et. al., 2010

Drosophila melanogaster

Tomer et al. 2012

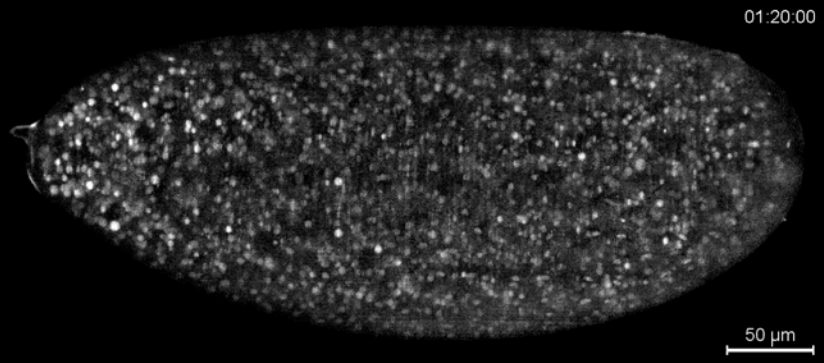
Blastoderm stages, embryonic and extra-embryonic establishment are not uniform across insects

Gryllus bimaculatus

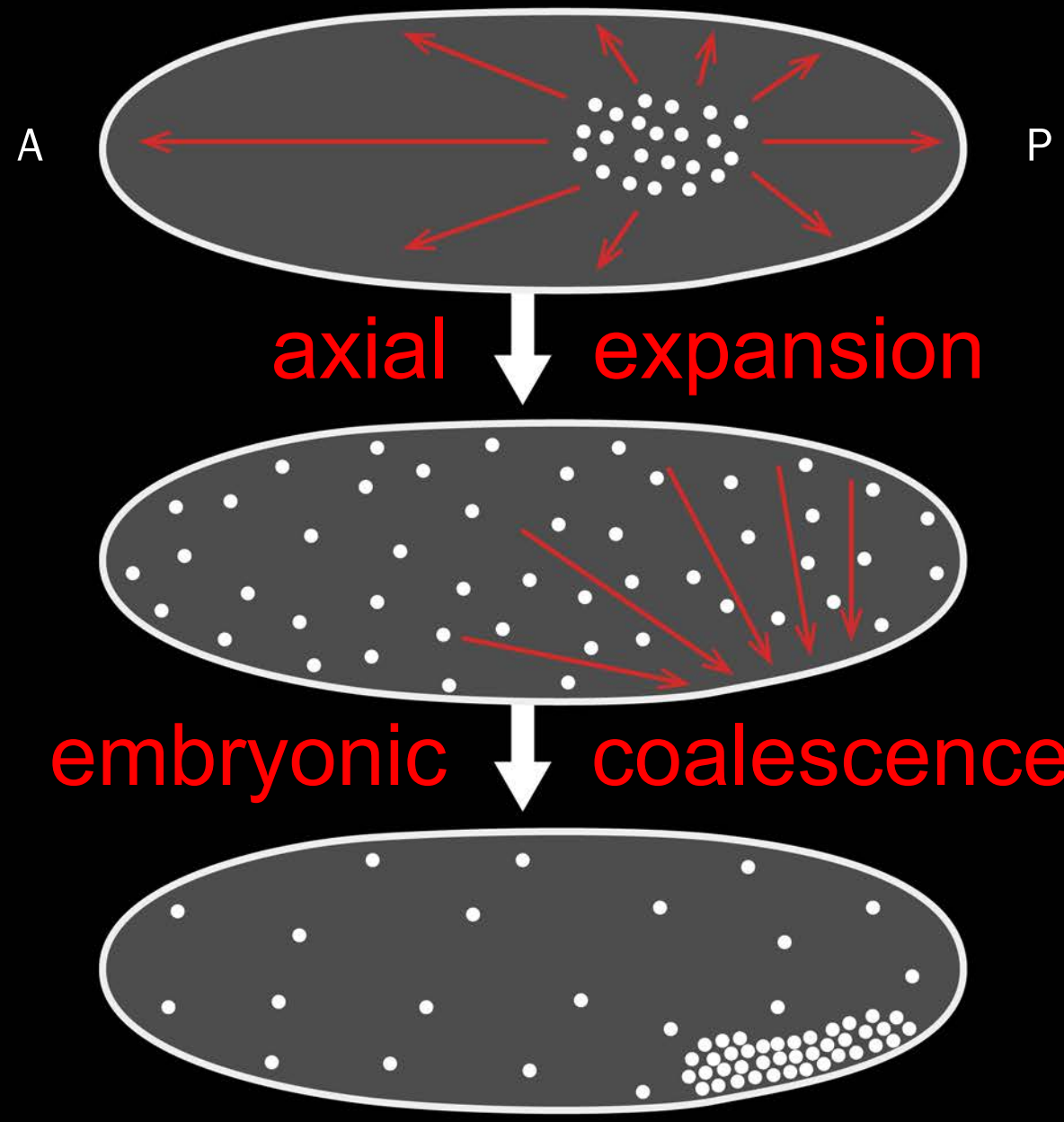


Nakamura et. al., 2010

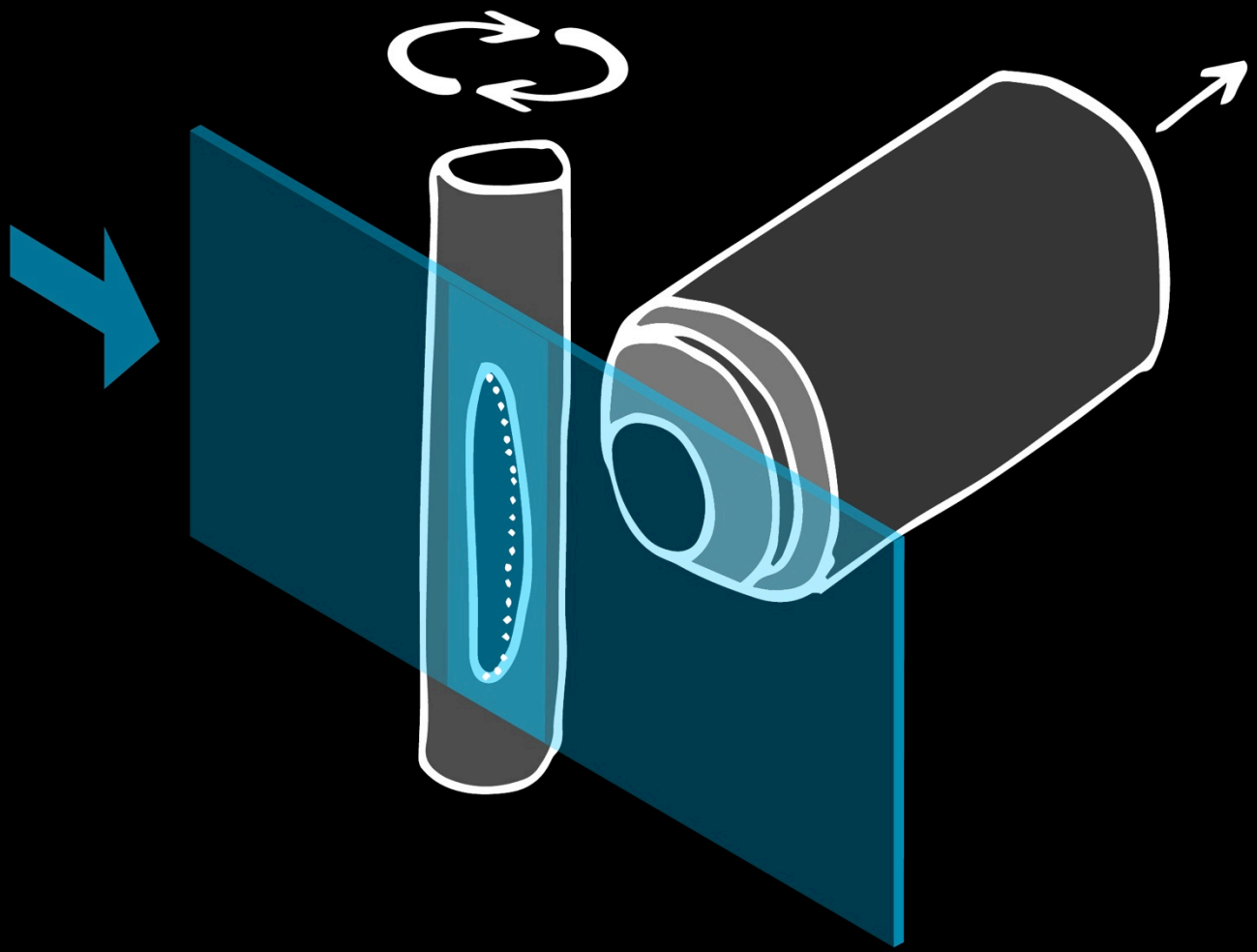
Drosophila melanogaster

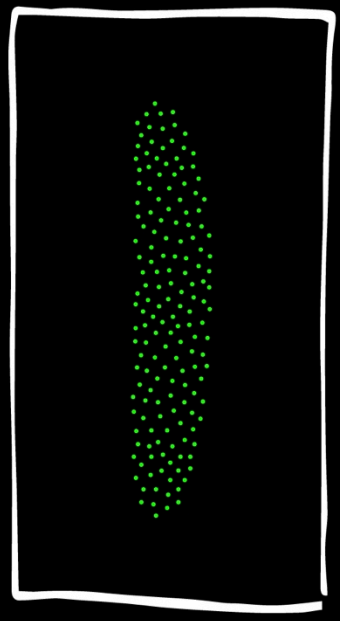


Tomer et al. 2012


A
P
axial expansion
embryonic coalescence

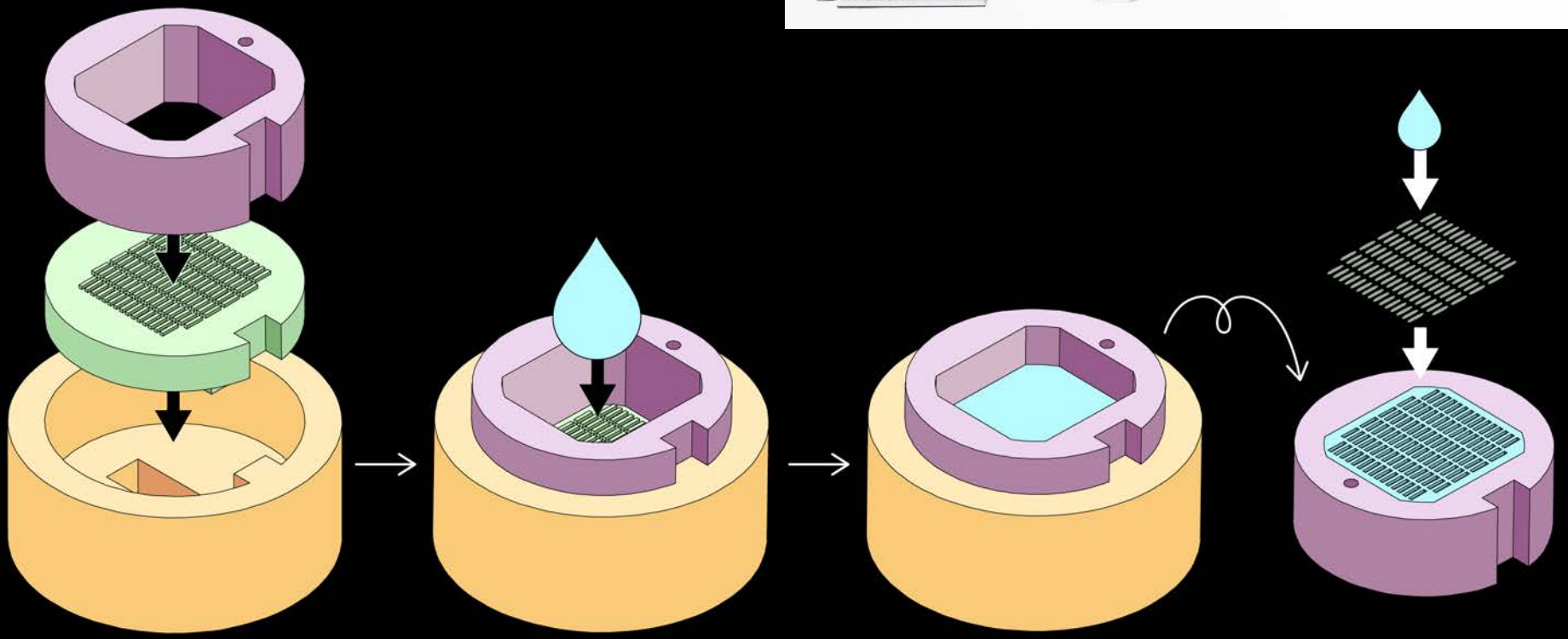
Method 1:
Lightsheet Z.1





Captured image

Method 2:
Cell Observer



Donoughe, Kim & Extavour *in prep.*

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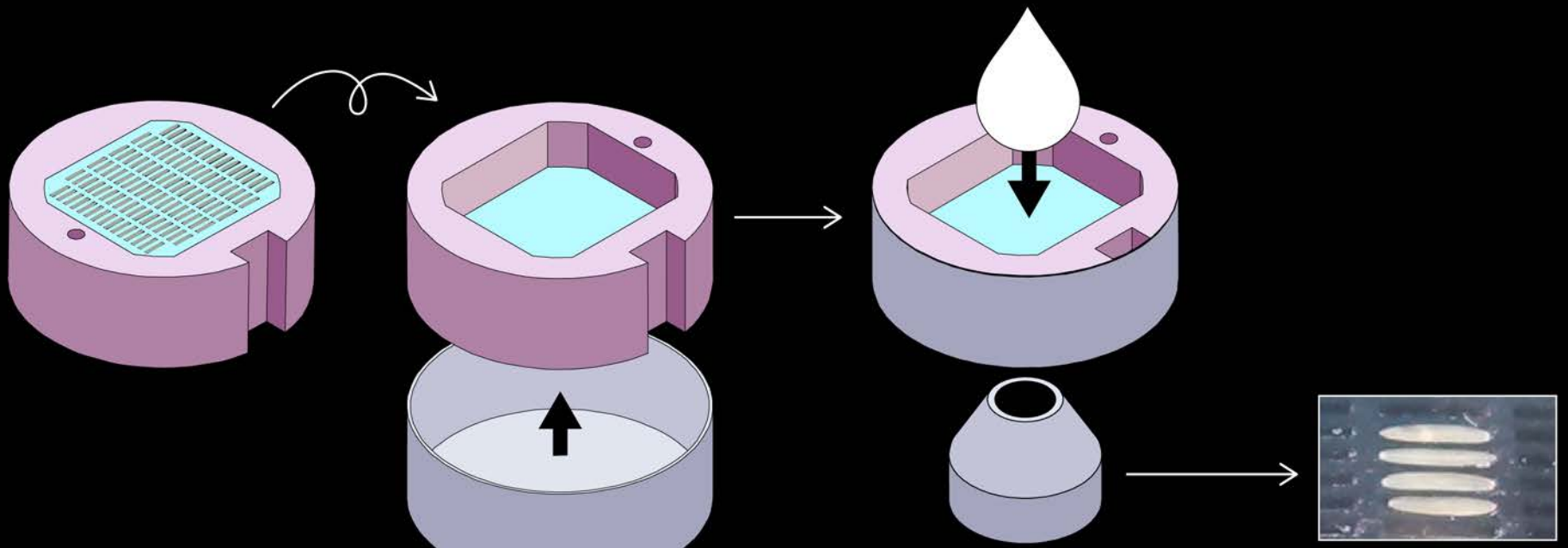
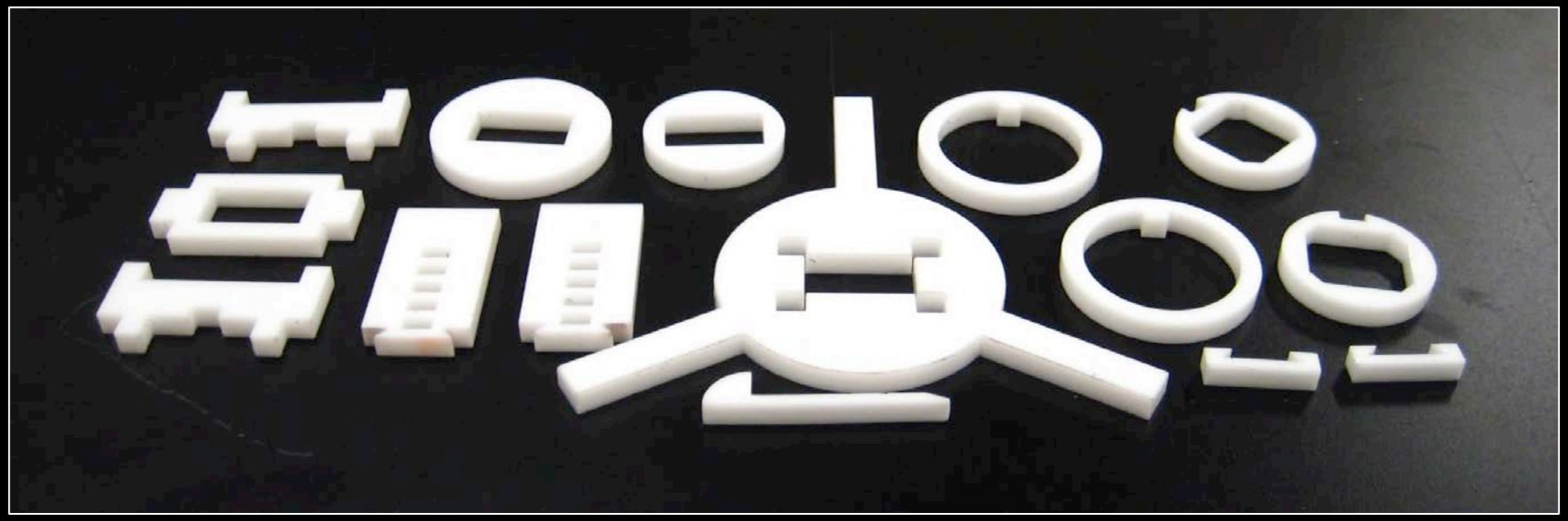
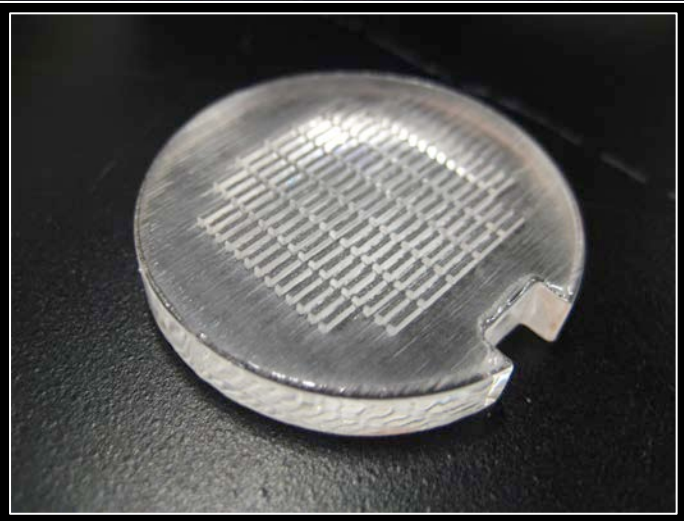


Donoughe, Kim & Extavour *in prep.*

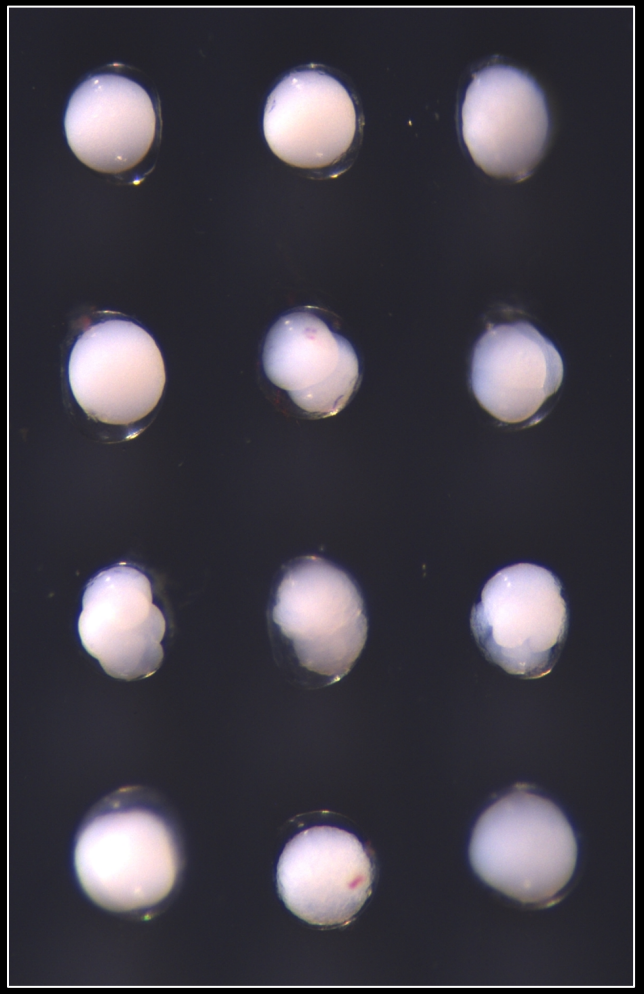
Modular device, easy to make with a laser cutter





Easy to design inserts for different species

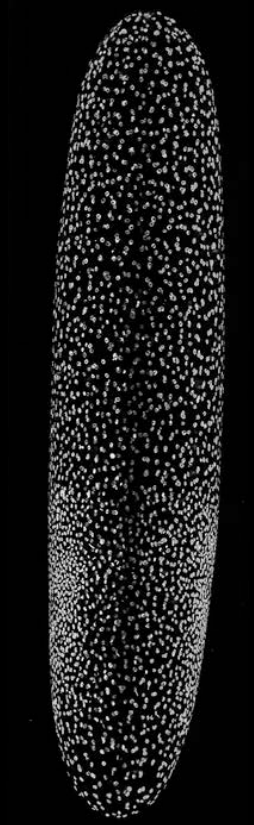
Eleutherodactylus coqui (frog)



Hofstenia miamia (acoel)

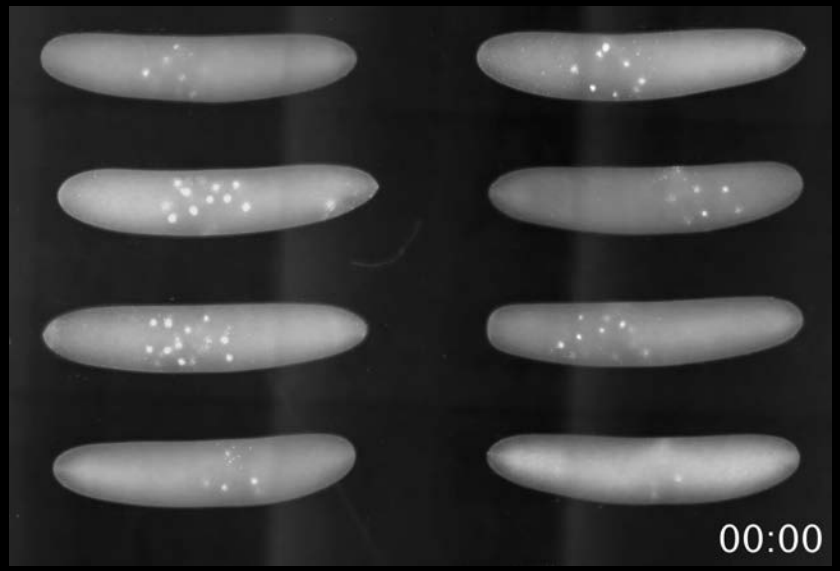
Lightsheet Z.1

- 3 dimensional nucleus position information
- 1 Embryo at a time
- 4 angles, ~500 time points

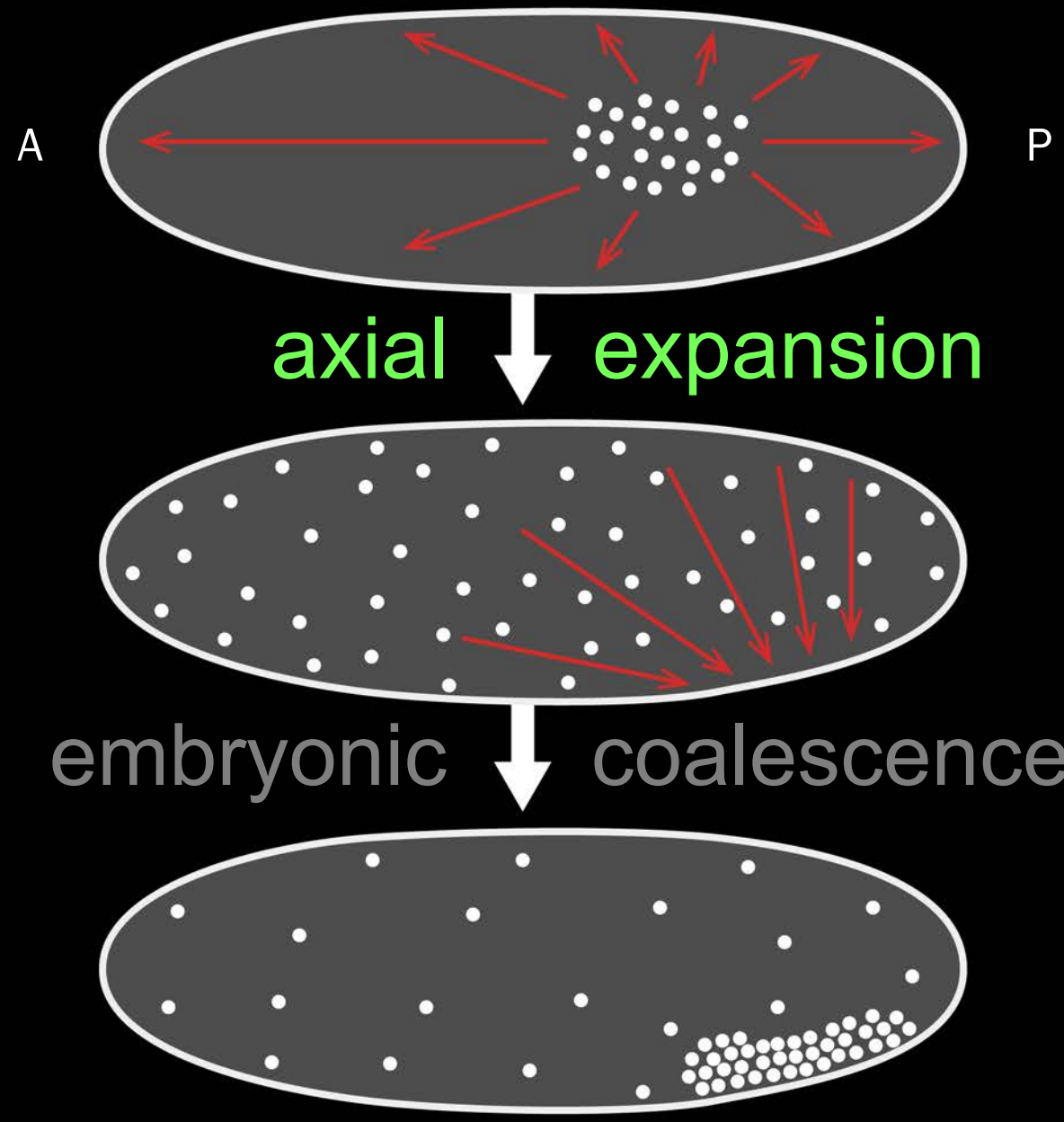


Cell Observer

- 2 dimensional nucleus position information
- ~20 embryos at a time
- 1 angle, ~500 time points



Donoughe, Hoffmann, Rycroft & Extavour ***unpublished***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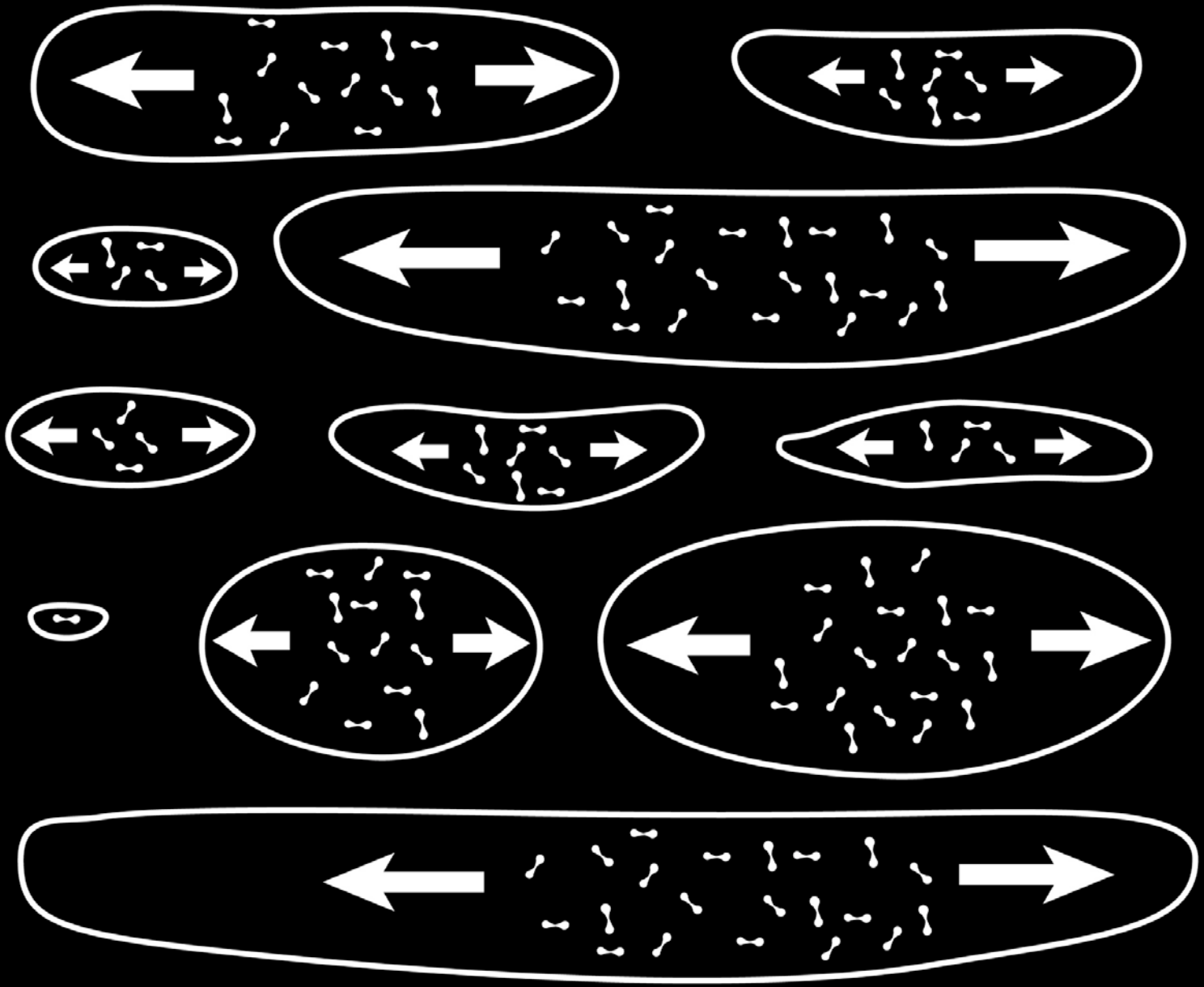

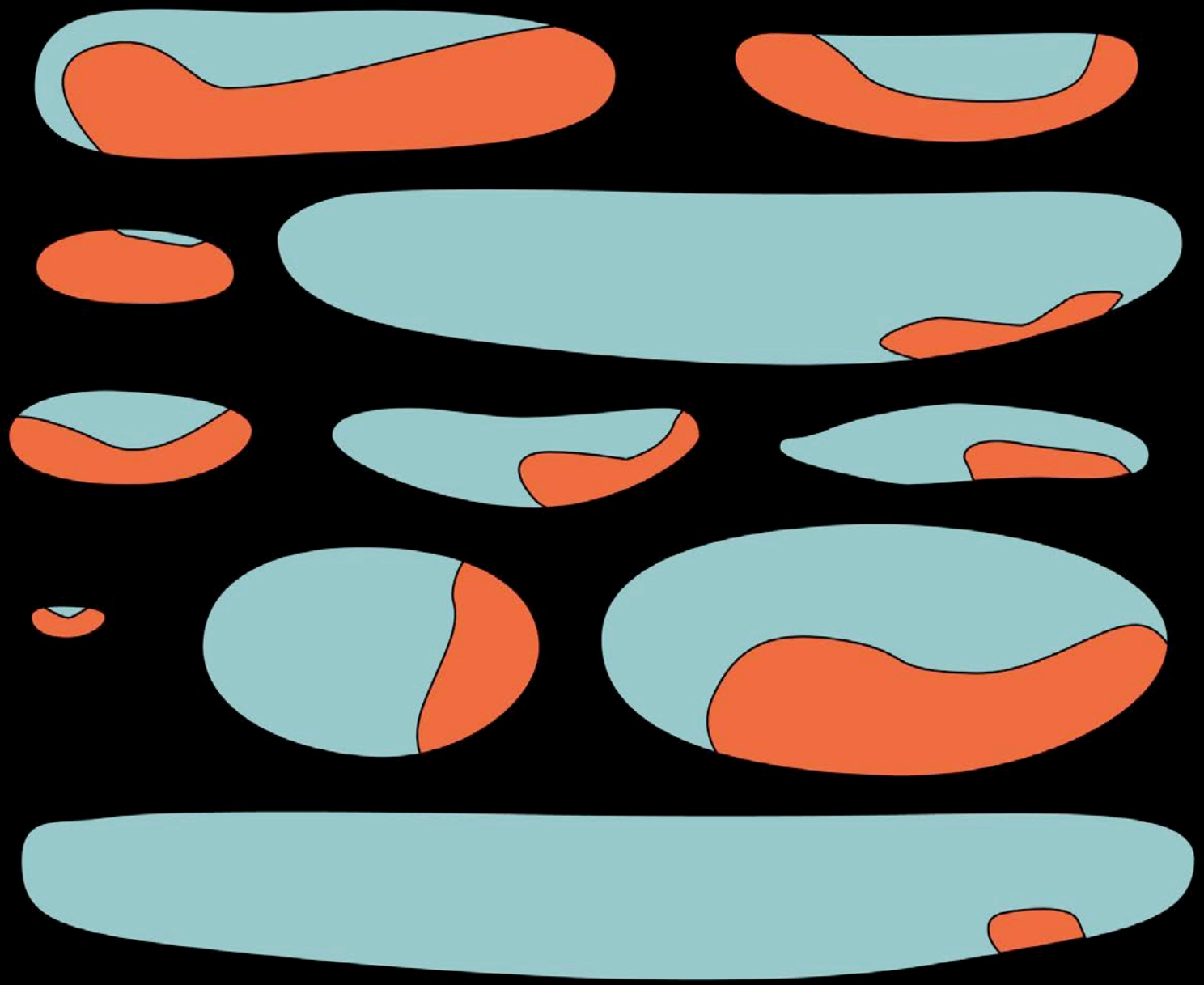
extra-embryonic tissue
embryonic rudiment