BUGS AND BABIES

Endosymbiotic Embryogenesis in Carpenter Ants

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Science Circle

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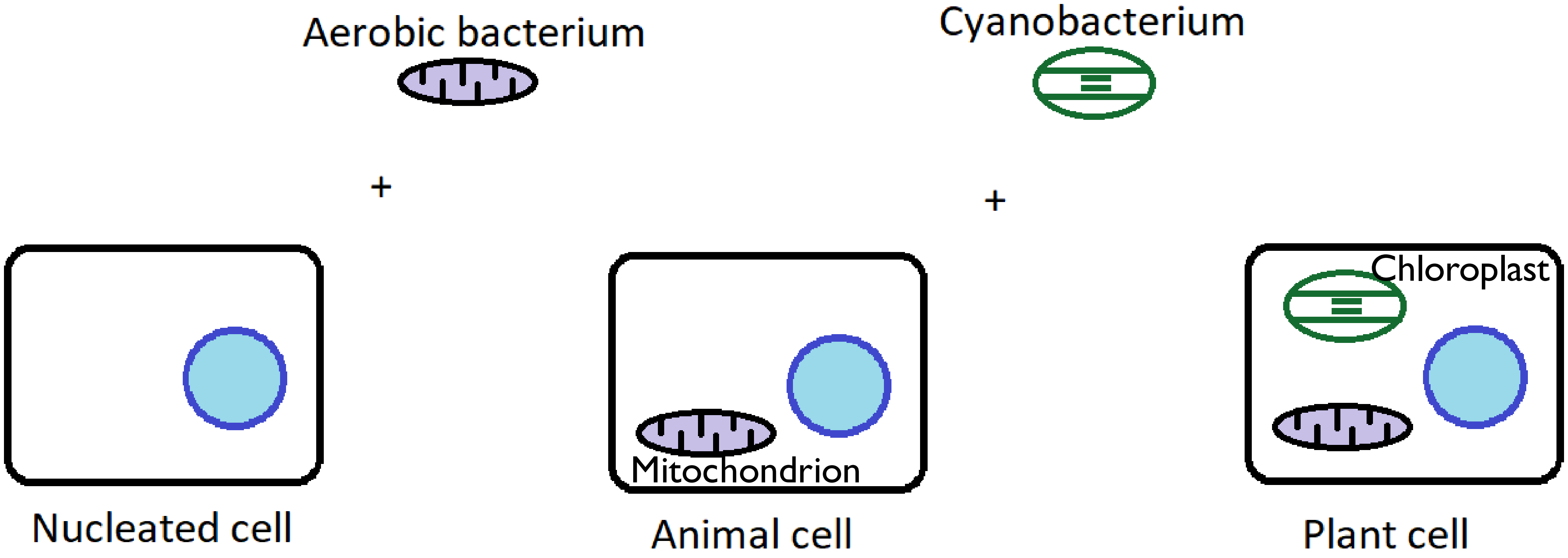
SYMBIOSIS AND ANIMALS

- Symbiosis is an association between two different species that is beneficial to both.
- Examples:
  - Animals do not have genes for making or breaking down cellulose. But many herbivorous animals can digest cellulose by hosting bacteria or protists that know how to break down cellulose in their digestive tracts.
  - One species of squid (*Euprymna scolopes*) produces a molecule (diketopiperazine) in its light organ that attracts light-producing *Vibrio fischeri* from ocean water. The light organ is attached to the ink sac and reflects the microbial light using proteins encoded by the squid's eye genes.

SYMBIOSIS AND ENDOSYMBIOSIS

- Symbiosis just involves two species living together. They may be so dependent on each other that they can only survive together, but each species retains its individual identity.
- In endosymbiosis one species, usually a prokaryote – some kind of bacterium, is actually carried inside the cells of the host species and becomes an essential part of the host.

ENDOSYMBIOSIS AND EUKARYOTES



INSECT ENDOSYMBIONTS

- Drosophila: *Wolbachia spp*.
- Aphids: *Buchnera aphidicola*
- Psyllid: *Carsonella rudii*
- Carpenter Ants: *Blochmannia spp*.

MANY OF THESE ENDOSYMBIOTIC RELATIONSHIPS ARE NOT FULLY UNDERSTOOD

- *Wolbachia* is a terrifically successful endosymbiont in many insects and other arthropods. It was first discovered in mosquitoes in 1924, and in Drosophila in 1965. *Wolbachia* may protect Drosophila from other bacterial infections, or contribute to longevity, but it also can lead to sterility or the death of male progeny. Cool factoids: *Wolbachia* is an alphaproteobacterium related to the ancestors of mitochondria. In one species of Drosophila (*D. ananassae*), at least one full copy of the 1.4 Mb *Wolbachia* genome has been inserted into the genome of its host and is transcribed.
- In aphids, *Buchnera* provides essential amino acids that the aphid doesn’t get from its sappy diet. *Buchnera* is related to enterobacteria like *E. coli*, but its genome is only 641K.
- The *Carsonella* endosymbiont provides a similar service for its psyllid host, also a sap-feeder. *Carsonella* has the smallest known prokaryotic genome, 174 K.
- The relationship between *Blochmannia* and its carpenter ant hosts is what we will be looking at today. Cool factoid: *Blochmannia* is related to *Buchnera* and may originally have been acquired by lateral transfer of a similar endosymbiont from mealybugs.

CARPENTER ANTS

Carpenter ant worker (*C. floridanus*) by Bob Peterson from North Palm Beach, Florida, Planet Earth!, CC BY-SA 2.0 https://creativecommons.org/licenses/by-sa/2.0, via Wikimedia Commons

Tree sawdust by NaCl58 - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=69995487

- 12,000 species of ants.
- 1000 species of carpenter ants in the genus *Camponotus*.
- Carpenter ants tunnel in wood, but don't eat the sawdust.
- All carpenter ants contain obligate endosymbionts, which produce some essential amino acids.
- The ants provide shelter for the bacteria and transmit them to their offspring.

BLOCHMANNIA
AND CARPENTER ANTS

- So far, *Blochmannia* is a typical endosymbiont. But wait, there's more!
- *Blochmannia* was one of the first insect endosymbionts to be described (1882). Blochmann, its first observer, identified it as a fungus, but it was later found to be bacterial.
- Recently, researchers at McGill University, were studying the relationship between *Blochmannia* and its ant host by looking at ant embryos.
- Their findings were published in 2020 in Nature (Abdul Matteen Rafiqi, Arjuna Rajakumar & Ehab Abouheif. Origin and elaboration of a major evolutionary transition in individuality. Nature 585: 239-244.)

HOW DOES BLOCHMANNIA INTERACT WITH CARPENTER ANTS?

The *Blochmannia* – Ant interaction involves all of the following:

- The ant embryo germ line
- Maternal genes of the ant
- Hox genes of the ant

THE EMBRYONIC GERM LINE

- Animal embryos develop from single cells: fertilized eggs.
- The "germ line" cells which will produce eggs and sperm for the next generation are partitioned off very early in development of all animals.
- The gonads – testes and ovaries – actually develop after the germ cells, which then migrate into these structures to produce sperm cells and eggs.

THE INSECT GERM LINE

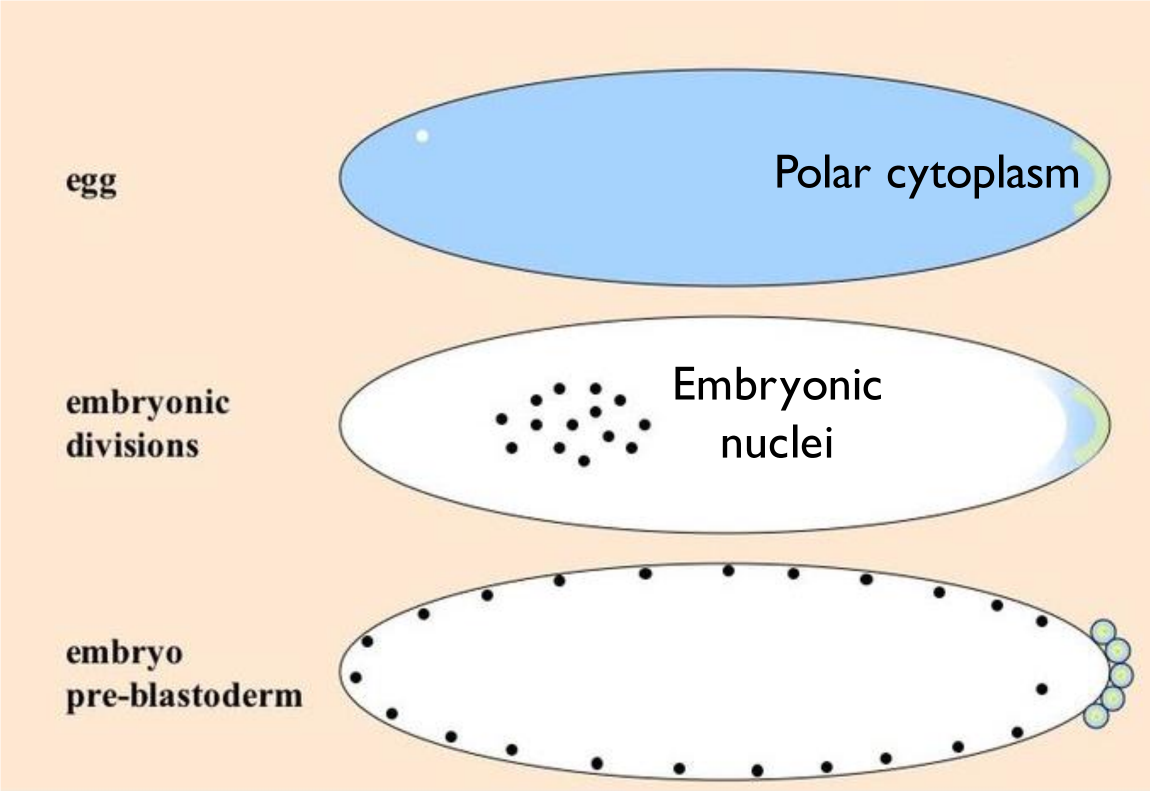


Image modified from: https://www.researchgate.net/figure/Preformation-model-of-germ-cell-formation-in-Drosophila-In-the-germarium-germ-line-stem_fig2_235225764

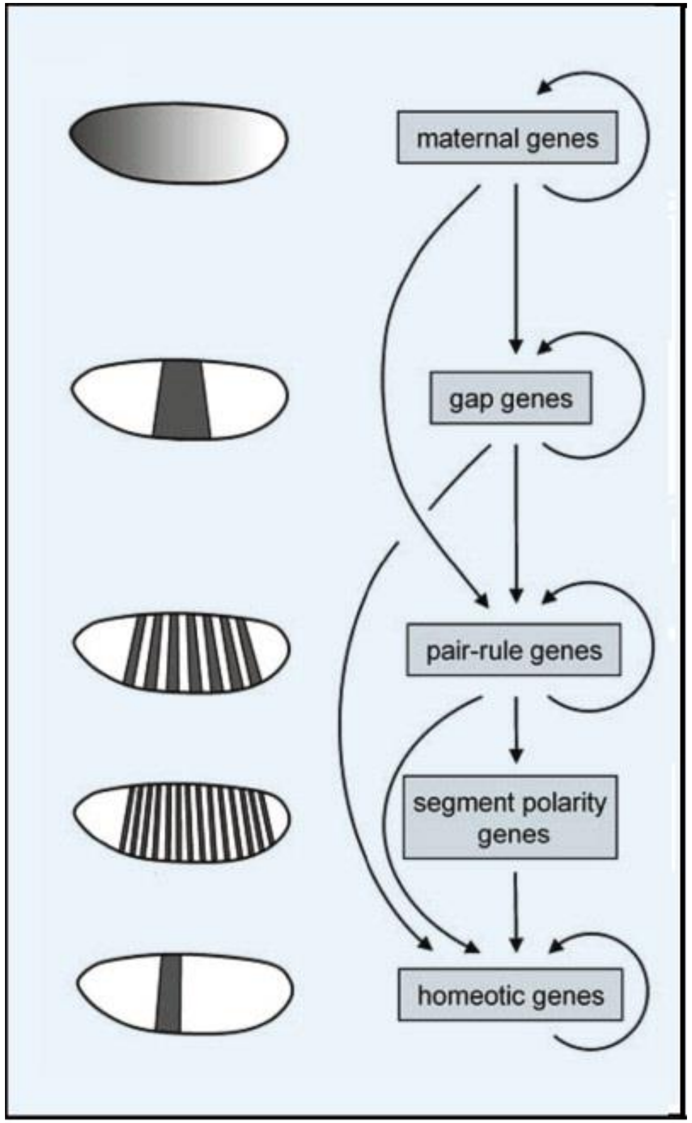
Cytoplasm is on the surface of the egg, surrounding the yolk.

Polar cytoplasm (green) laid down maternally.

Nuclei divide and migrate to surface cytoplasm.

Nuclei migrating into the polar cytoplasm are cellularized before other nuclei and become the primordial germ cells

EMBRYONIC DEVELOPMENTAL SEQUENCE

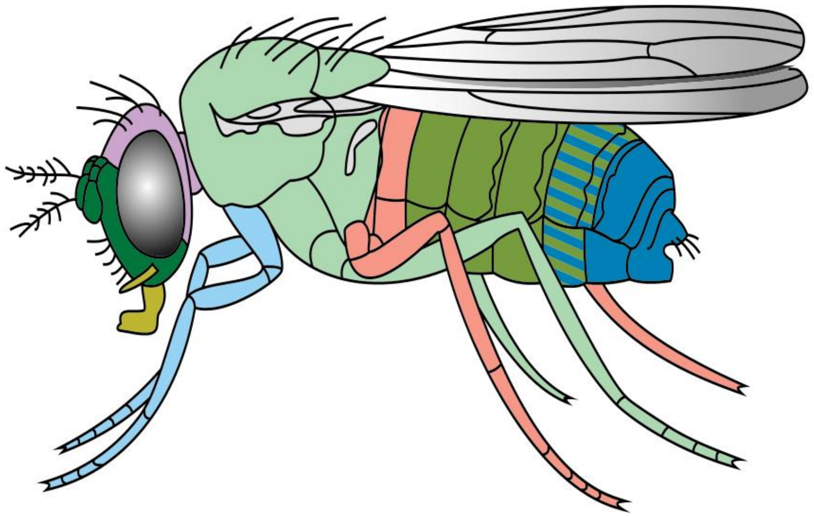


- Maternal effect genes: set up the anterior-posterior axis.
- Gap genes: subdivide the embryo into several major sections along the anterior-posterior axis.
- Pair-rule and segment polarity genes: Subdivide the embryo further with each set of pair-rule genes defining a given embryonic segment.
- Hox genes: Assign specific structures to an embryonic segment. Hox genes are a specific subset of homeotic genes that all include a 180-base homeobox sequence, encoding a specific protein domain (probably DNA-binding).

Image: modified from https://www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/gap-gene

INSECT HOX GENES
(DROSOPHILA)

Hox genes encode developmental regulators that determine the location of specific body structures in the embryos of all bilaterally symmetrical animals.



Hox genes are ordered on the chromosome in the same relative positions as the body regions they determine, and in their activation order.

Humans have four sets each on a different chromosome (7, 17, 12, 2 for sets A-D).

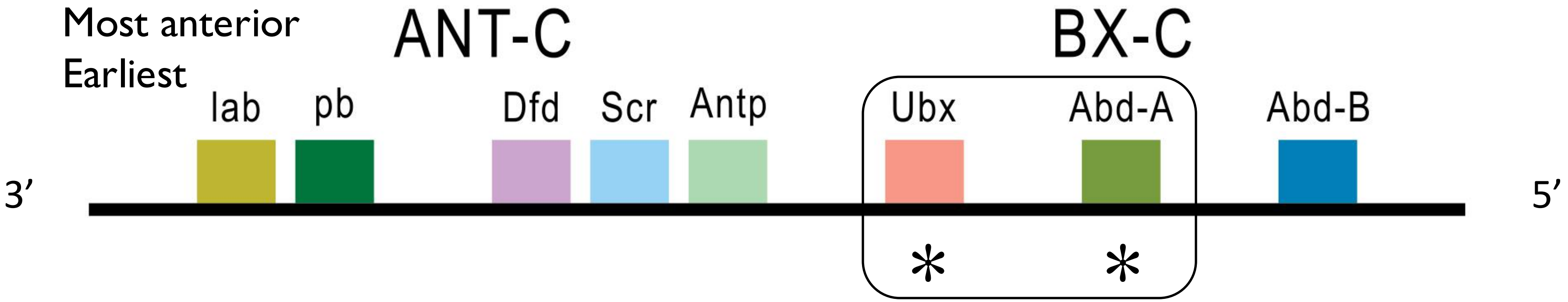
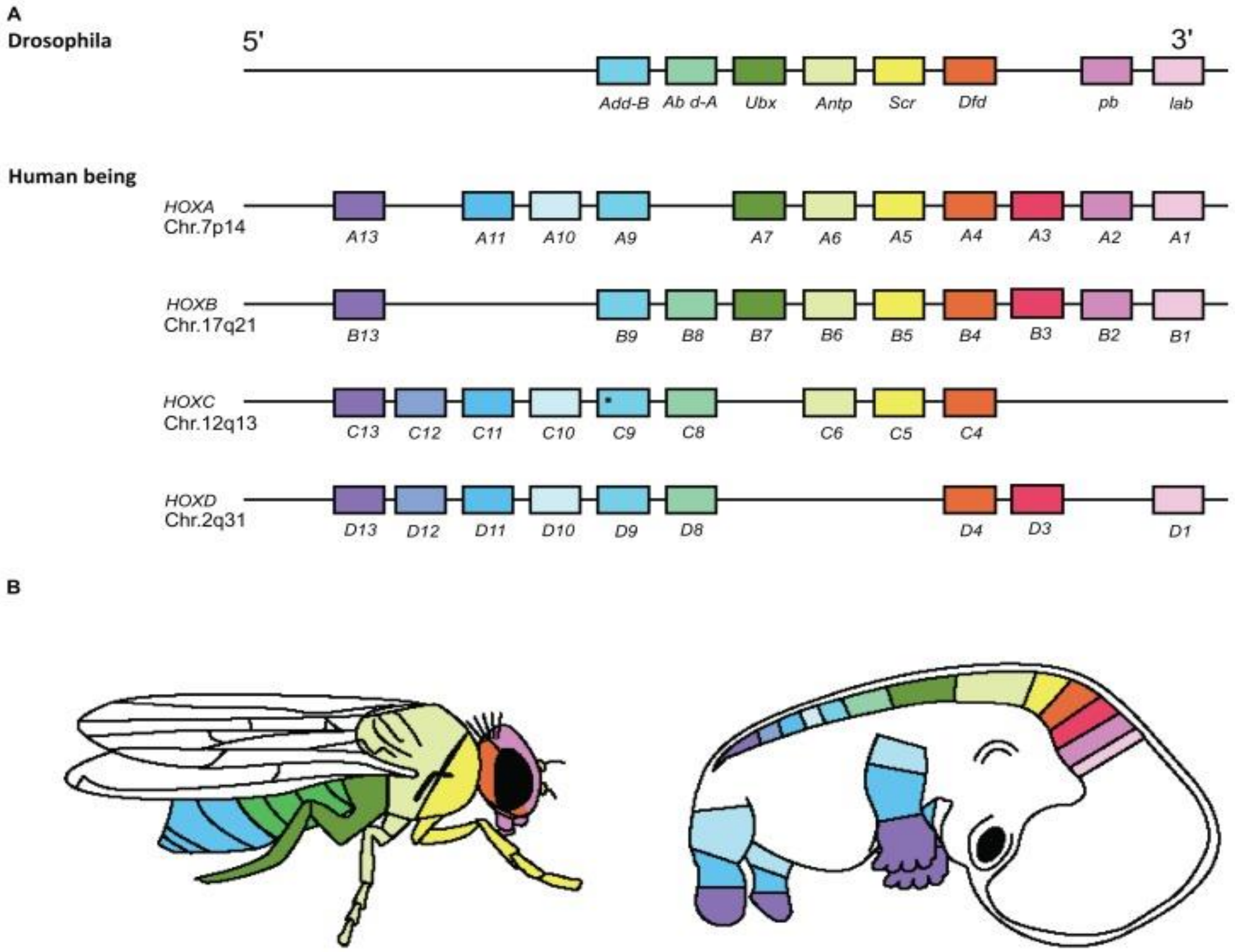


Image: Hox genes by PhiLiP - self-made, based on Hoxgenesoffruitfly.png, Public Domain, https://commons.wikimedia.org/w/index.php?curid=2118712

COMPARISON OF HOX GENES

The Hox genes are named for their mutant effects in Drosophila, but similar genes are found in all bilaterally symmetrical animals.

- lb: labial
- pb: proboscipedia
- Dfd: Deformed
- Sc: Sex comb reduced
- Antp: Antennapedia
- Ubx: Ultrabithorax
- AbdA: Abdominal A
- AbdB: Abdominal B



REVIEW OF EMBRYONIC DEVELOPMENTAL SEQUENCE

- Maternal effect genes: set up the anterior-posterior axis.
- Gap genes: subdivide the embryo into several major sections along the anterior-posterior axis.
- Pair-rule genes and segment polarity genes: Subdivide the embryo further with each set of pair-rule genes defining a given embryonic segment.
- Hox genes: Assign specific structures to an embryonic segment. Hox genes are a specific subset of genes that include a 180-base homeobox sequence, encoding a specific protein domain (probably DNA-binding).

FIRST WEIRDNESS: HOX GENES

- In their study of carpenter ant embryos, Rafiqi, Rajakumar and Abouheif noted some very odd things:
- Two Hox genes were being expressed very early – Ubx and Abd-A, which usually act to define the last thoracic and first abdominal segments in the ant embryo.
- These two genes were being expressed maternally, from mRNAs in the germ line cytoplasm, not from the embryonic genome.
- The two genes had been modified to interact with the germ line genes.

SECOND WEIRDNESS: EMBRYONIC REARRANGEMENT

- The posterior germ cell region wasn’t used to produce gametes.
- Most of the endosymbionts collected in the regular germ cell regions and were used to produce bacteriocytes, which were carried to the developing gut.
- The embryo region was displaced toward the anterior of the egg.
- A different germ cell region was used to produce gametes.
- Two more germ cell regions served other functions.

BACTERIOCYTES

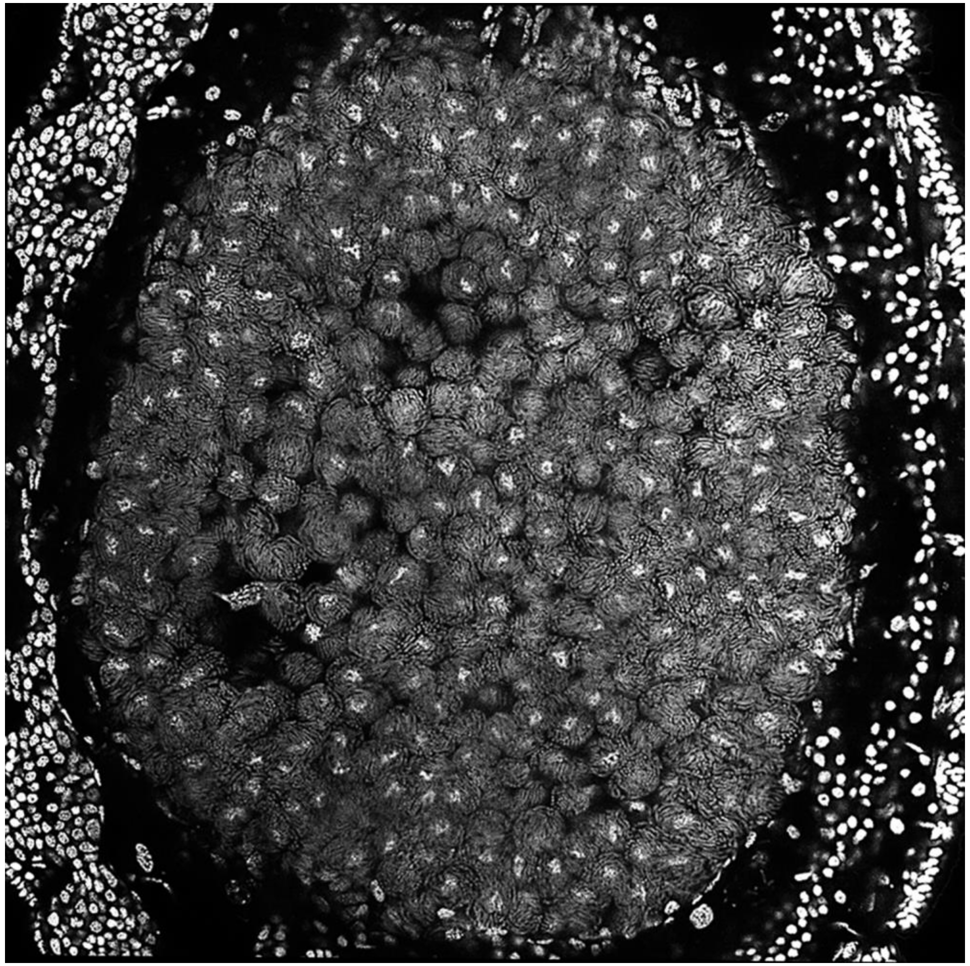


Image: Abouheif Lab, McGill University

- Bacteriocytes are *Blochmannia* cells incorporated into cells from the original germ line zone.

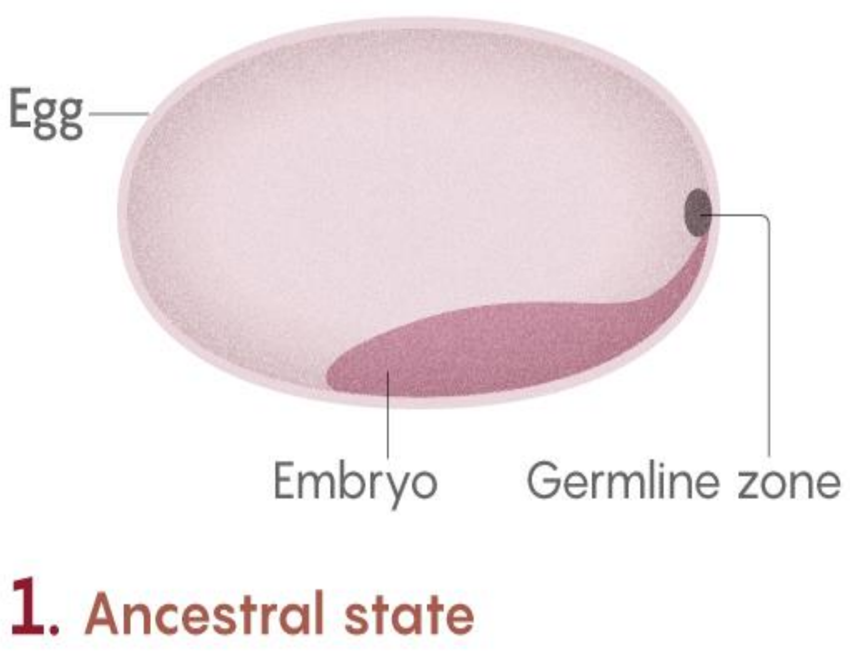
- The cells carry the endosymbiont to the gut, where they produce essential amino acids.

COMPARISON OF CAMPONOTUS WITH RELATED INSECT SPECIES

To figure out how all of this happened, the investigators compared the embryos of carpenter ants to those of a lot of other related species and asked several questions:

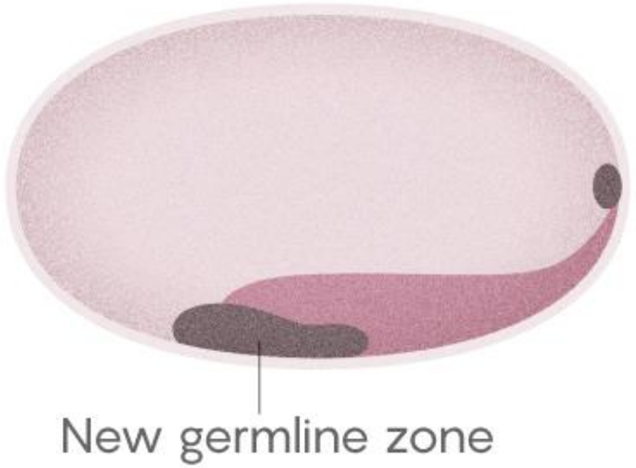
- Where is the germ line located?
- Where are maternal hox genes active?
- Do they have obligate endosymbionts and if so what kind?
- Where is the embryo located?
- Which of the germ lines produces gametes and what do the others do?

HISTORY OF DEVELOPMENTAL MODIFICATIONS



Normally the cytoplasm that marks the germ cells is concentrated at the posterior end of the insect egg. The embryo body develops nearby. The germ cells are then carried into the body and snuggle up in the developing gonad. This arrangement is typical of most insects.

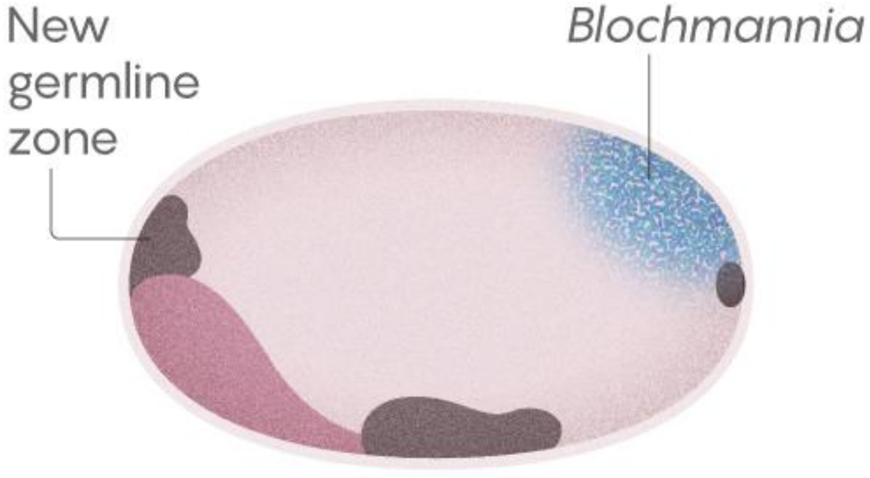
HISTORY OF DEVELOPMENTAL MODIFICATIONS



2. New germline expression

Relatives of carpenter ants were examined to determine when the extra germ lines might have appeared. The first seems to have predated the acquisition of the *Blochmannia* endosymbiont, although a some of the nearest relatives had different endosymbionts and others had none. In these species the maternal Abd-A and Ubx were expressed in both germline zones. What was the second germ line for?

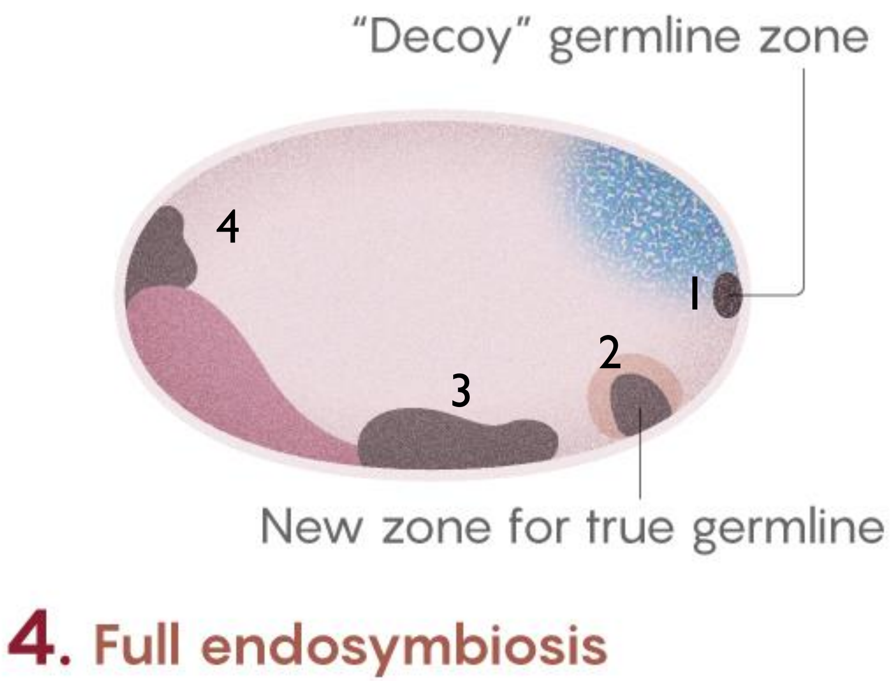
HISTORY OF DEVELOPMENTAL MODIFICATIONS



3. Initial endosymbiosis

After the acquisition of the *Blochmannia* endosymbiont, about 51 mya, the embryo region of the egg moved forward, and a second new germline region appeared. The *Blochmannia* concentrated in the original germline cells, which prevents competition with the reproductive cells. This combination is seen in some carpenter ant species.

HISTORY OF DEVELOPMENTAL MODIFICATIONS



In most *Camponotus* species, the original germ cell region (1) now produces the bacteriocytes that carry the endosymbionts into the developing gut, where they will assist with nutrition. A fourth germ line region (2) now serves as the real germ line, and also picks up a few endosymbionts for transmission to the next generation of ants. Zone 3 seems to pattern the embryonic midline and zone 4 may assist with the migration of the bacteriocytes into the gut.

PROGRESSION OF BLOCHMANNIA ENDOSYMBIOSIS

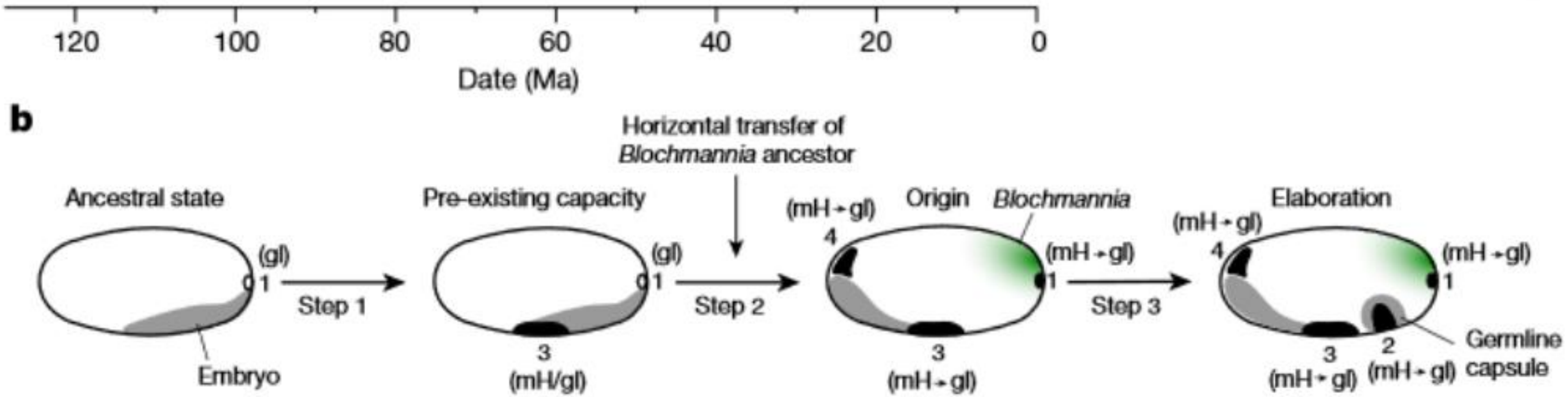


Image: Rafiqi et al.,
doi: 10.1038/s41586-020-2653-6

THE HOX GENES AND THE GERM LINES

- What are the maternal Hox genes Ubx and Abd-A doing?
- Abd-A mRNAs localize in germ line zones 1 and 3.
- Ubx mRNAs localize in all four germ line zones.
- Blocking the activity of the Ubx and Abd-A mRNAs leads to abnormal embryos.
  - Blocking Abd-A leads to misexpression of the germline genes. *Blochmannia* is not incorporated and the bacteriocytes do not form.
  - Blocking Ubx leads to misplacement of the zone 2 germ cells, which do not enter the embryo.
  - Embryonic posterior sections do not develop due to blocking of embryonic Hox genes.

WHAT DO THE ENDOSYMBIONTS DO FOR THE ANTS?

- Endosymbionts were eliminated from embryos using antibiotics.
- Half of the embryos failed to develop.
- Embryos that hatched had defective germ cells.

SO

- The endosymbionts seem to activate genes in the embryo.
- The Hox genes in the ants have been modified to activate the ant germ line to incorporate the bacteria, some as bacteriocytes and others to carry the endosymbiont into the gametes.

WOLBACHIA VS BLOCHMANNIA

- *Wolbachia* is one of the most successful of the insect endosymbionts, with representatives in about 2/3 of all insect species. However, it is not essential to the survival of many of its host species.
- *Wolbachia* is also found in some nematodes, and has become essential to those species it infects. The smaller genome size in the nematode endosymbionts (1.2 vs 1.6 Mb) suggest that it may have started there and transferred to insects later.
- *Blochmannia* is essential to the survival of all carpenter ants and has effectively become part of this entire group of insects.

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