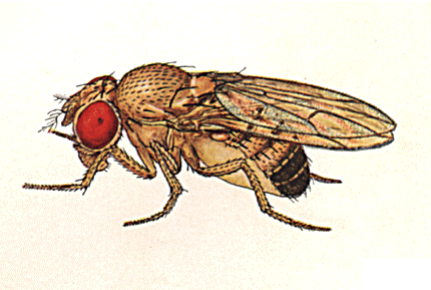
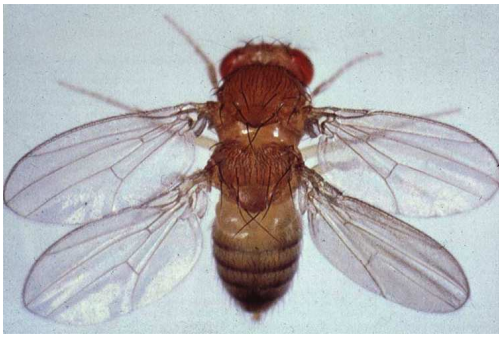
Evolution of Transcription factor function: Homeotic (Hox) proteins

- Hox proteins regulate morphology in cellular zones on the anterior-posterior axis of embryos via the activation/repression of unknown numbers of downstream effector genes.

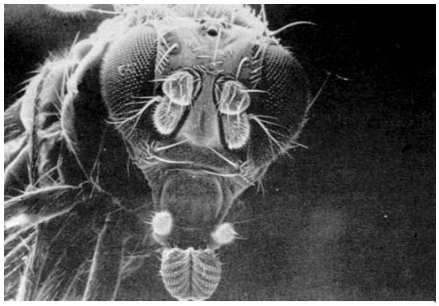


Recessive mutations in the Hox gene *Ultrabithorax* (*Ubx*)

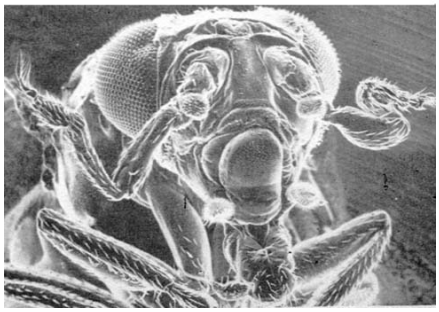
two ⟹ four-winged flies



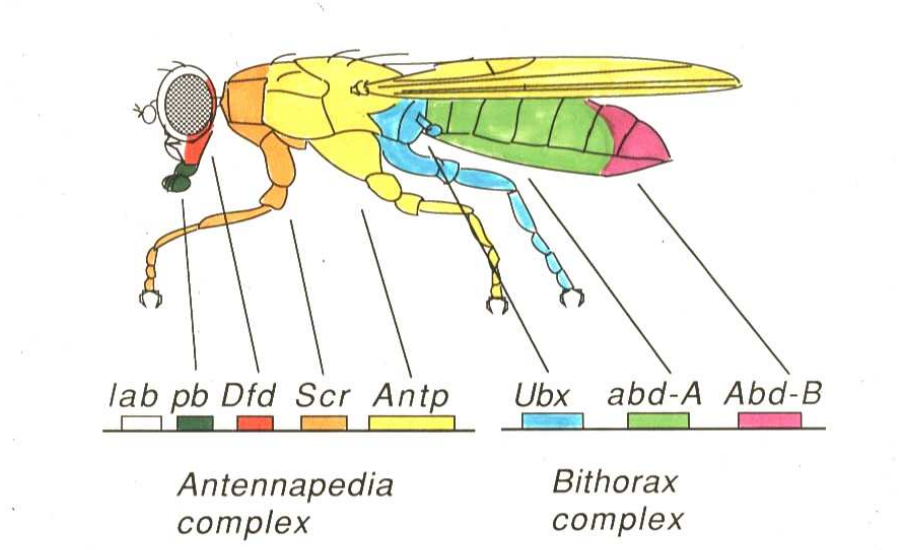
Dominant mutations in the *Antennapedia* (*Antp*) gene



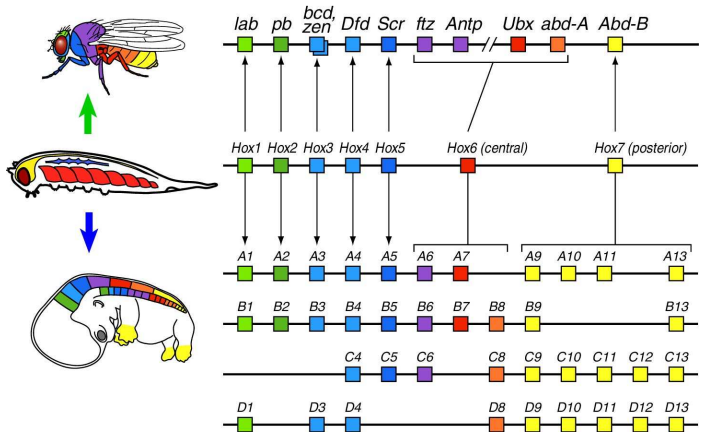
Homeotic transformations of antenna to leg



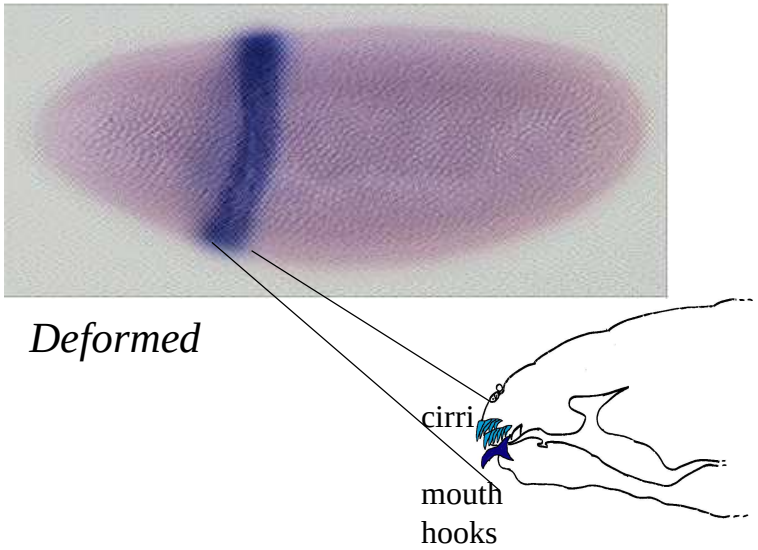
***Drosophila* homeotic (Hox) genes**

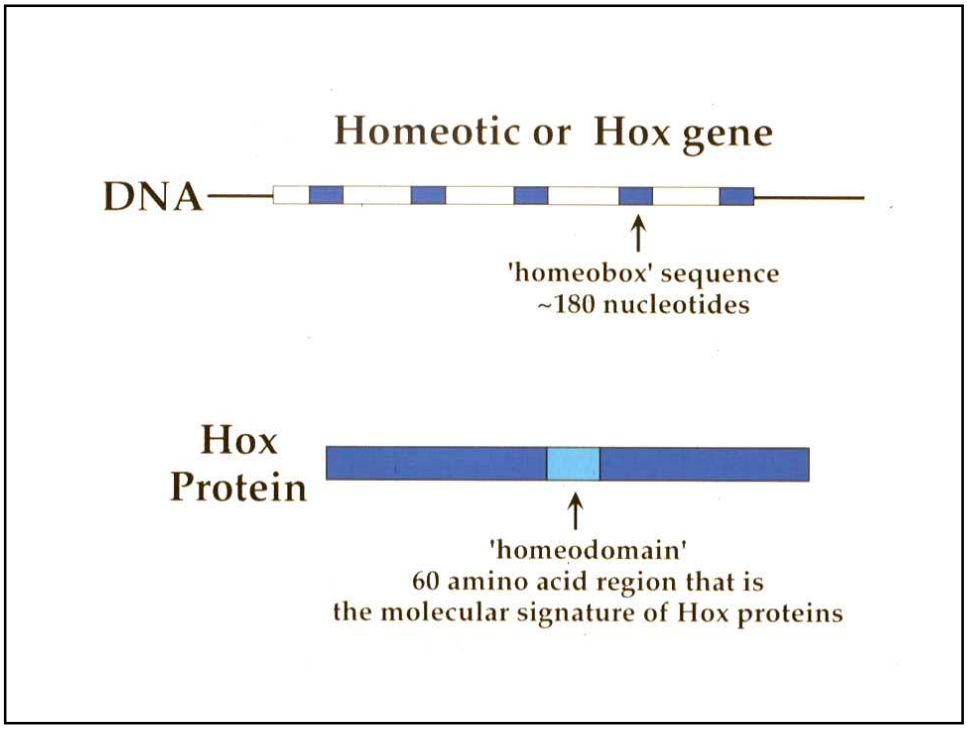


Homeotic (Hox) gene complexes in vertebrates and invertebrates



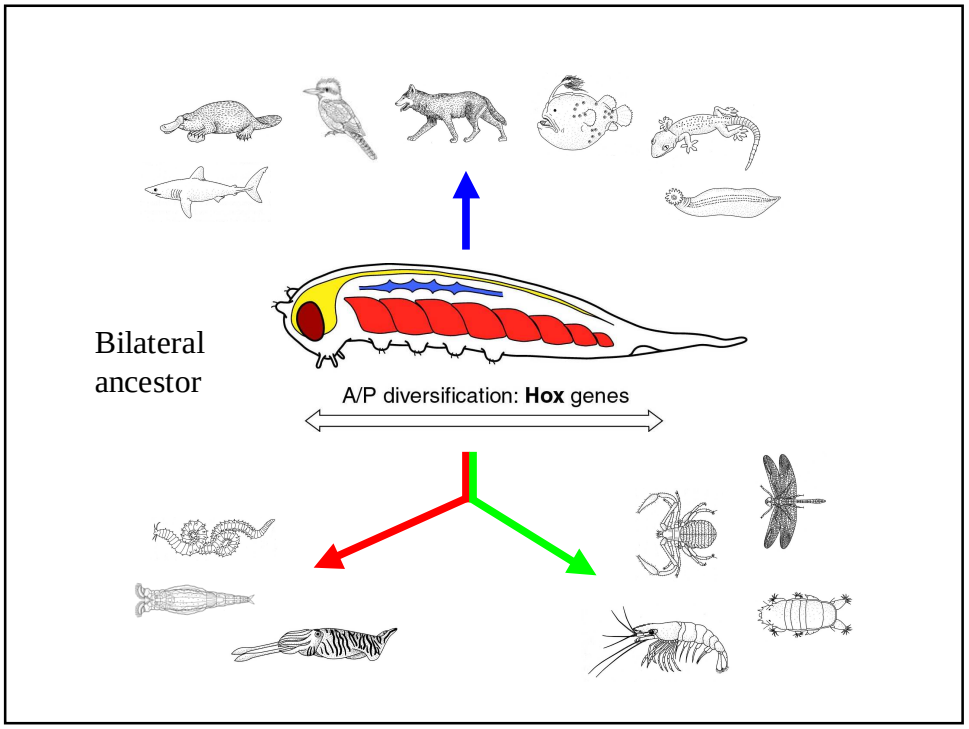
Hox genes are expressed in stripes of cells on the head-tail axis of embryos

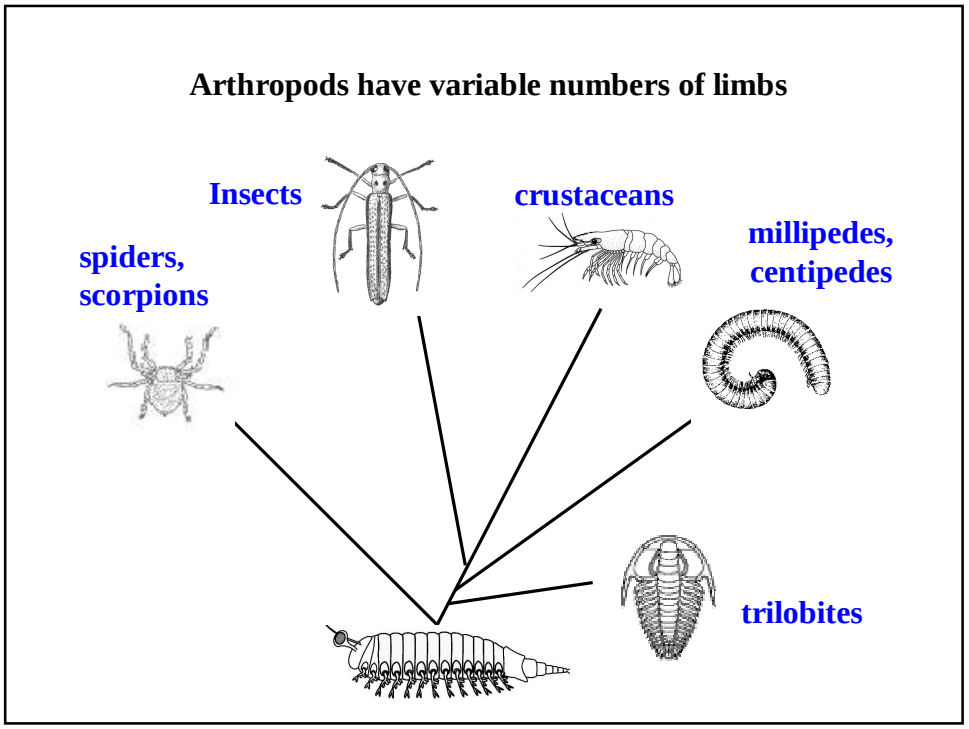




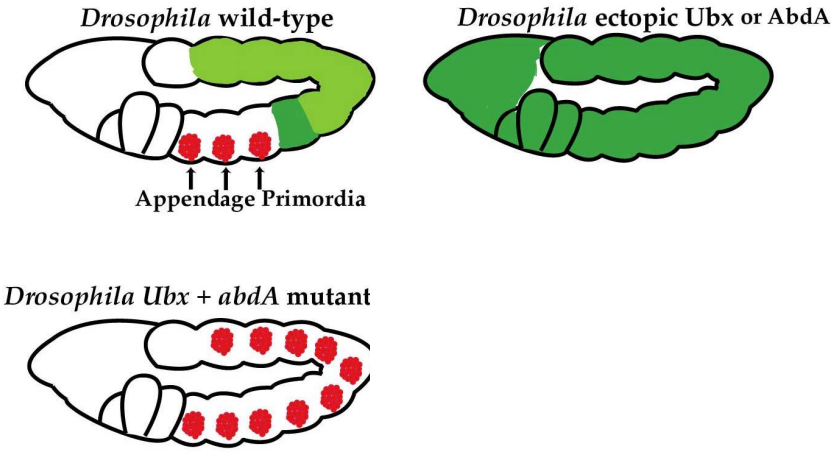
Hox genes are required to diversify morphology during animal development.

Has mutation of Hox genes diversified morphology during animal evolution?

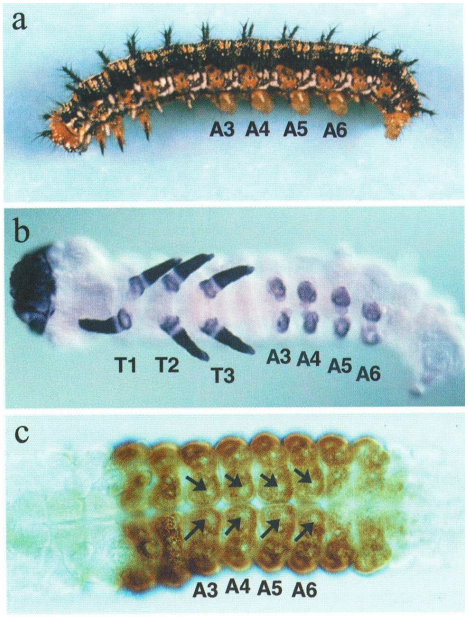

Bilateral
ancestor
A/P diversification: **Hox** genes


Arthropods have variable numbers of limbs
Insects
crustaceans
millipedes,
centipedes
spiders,
scorpions
trilobites

Distalless promotes limbs in fly embryos,
Ubx and AbdA Hox proteins repress limbs



Mutations of Hox cis-regulatory elements and morphological evolution

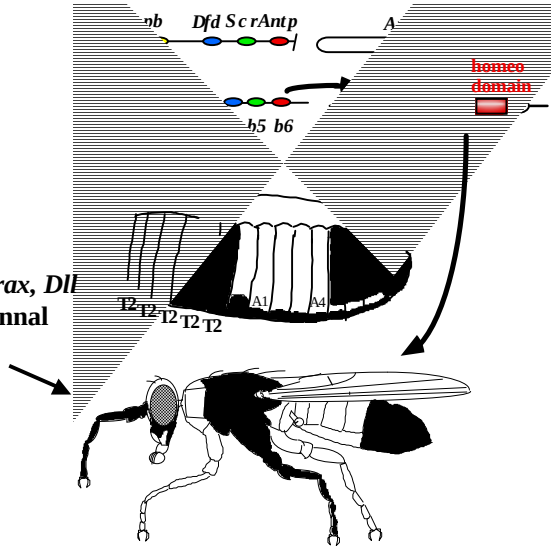


Warren et al. (1994)
Nature 372,458

When evolving different morphologies, is cis-regulatory mutation/selection where the action is?

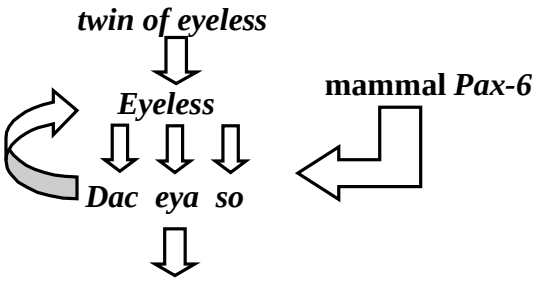
- **Much indirect evidence for Cis-reg mutation ⇨ morph. change.**
- **Cis-reg mutation has been hypothesized to be "easier" on a developmental system and organism, since it would involve changes in the expression pattern of only one gene at a time (Of course if that gene encodes a regulatory protein it isn't easier).**
- **Because of binding site "redundancy" in enhancers, Cis-reg mutations might be extremely gradual (micro-micro mutations), and therefore also beneficial from the viewpoint of organismal survival**
  Although in the case of micro-micro changes, what is there to be selected?

Evolutionarily conserved roles of transcription factors - How conserved are they? Ectopic Hox-6 in *Drosophila*.



Repression of *homothorax*, *Dll* and/or *spineless* in antennal primordia will suffice

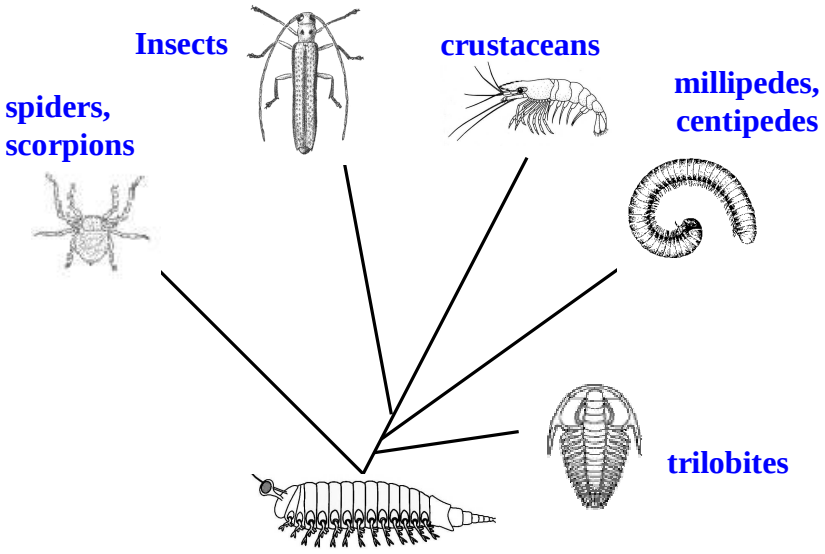
Ectopic Pax-6 protein can mimic the "master regulator" Eyeless
Is this good evidence for detailed functional conservation?



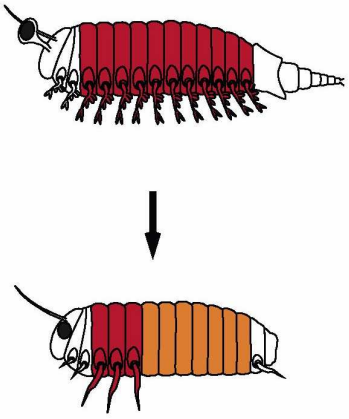
Compound eyes in *Drosophila* legs, etc

The evidence for <u>stringent</u> functional conservation
of distantly-related homologs is skimpy to non-existent.

Hexapod insects evolved from an
ancestral crustacean with many limbs



What kinds of mutations could contribute to limb loss?



1. Loss of upstream limb activator gene expression (wg, dpp).

2. Gain of Hox repressor binding sites in *Distal-less*.

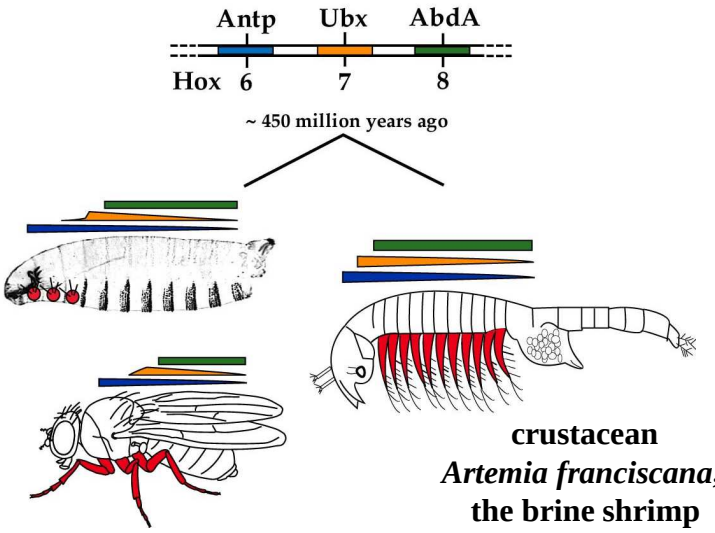
3. More anterior expression pattern of a pre-existing Hox repressor.

4. Mutation of abdominal Hox proteins to generate appendage repressor.

5. Mutation in Hox cofactor function or expression pattern.

Artemia franciscana

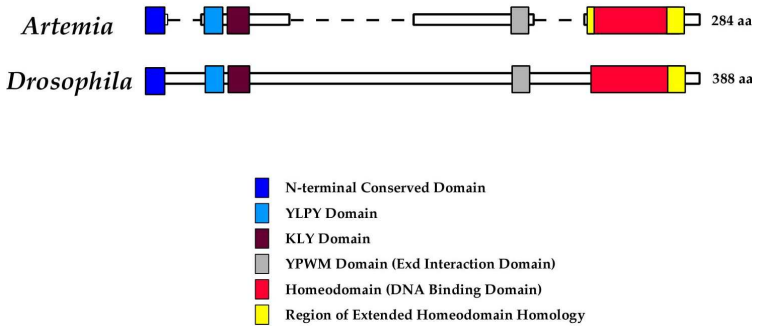
Different expression patterns between insect and crustacean "abdominal" Hox proteins fail to explain limb number evolution



crustacean
Artemia franciscana,
the brine shrimp

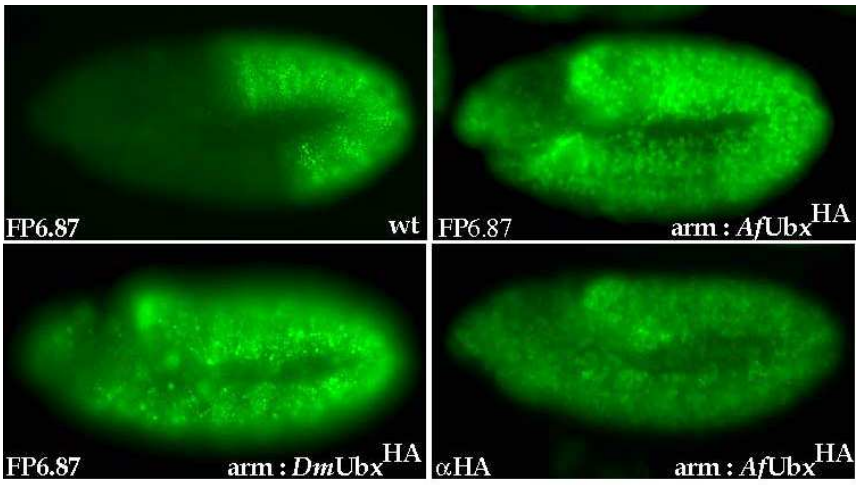
The structure of Ubx proteins from *Artemia* and *Drosophila* are highly diverged, except in the homeodomain

Ultrabithorax proteins

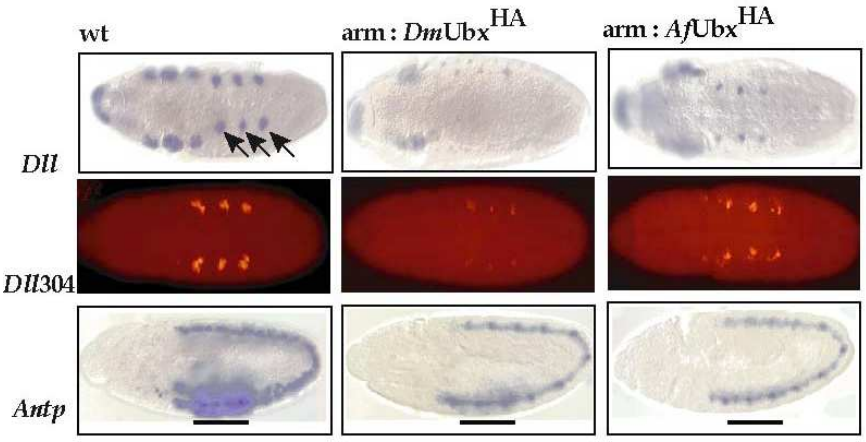


Overall match 123/388 = 32%

Expression of *Artemia* or *Drosophila* Ubx proteins in the thorax of *Drosophila* embryos. You have to quantitate.

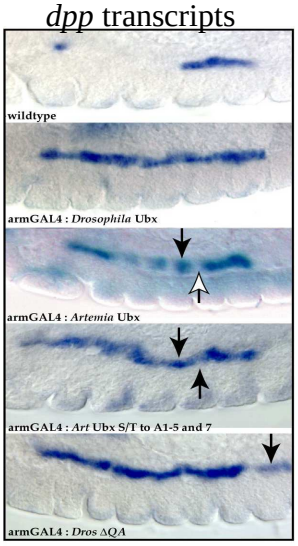


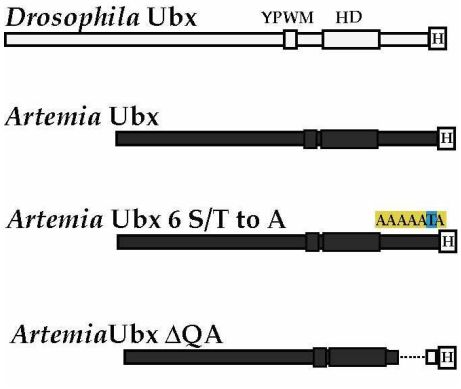
In contrast to *Dros.* Ubx, *Artemia* Ubx doesn't repress limbs or *Distalless*, but it does regulate other genes, even in the limb field.



Artemia Ubx can activate _dpp_ in a striped pattern, the stripes dependent on the C-terminal Ser/Thr aa residues

dpp transcripts





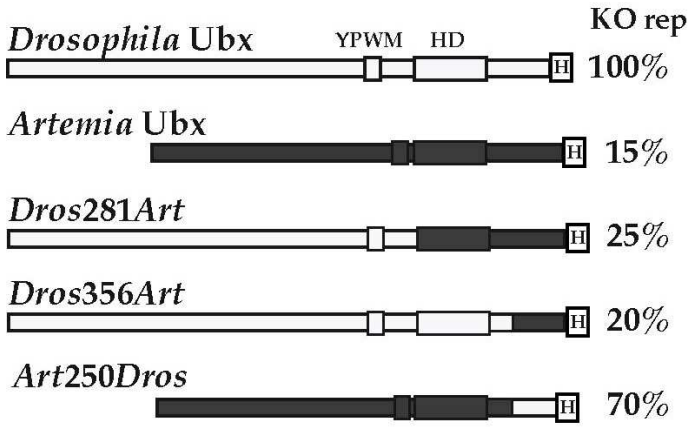
Like _Drosophila_ Ubx, _Artemia_ Ubx represses wing development

Wing driver alone

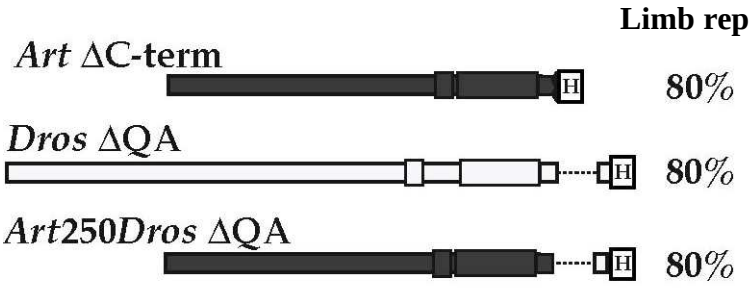
Artemia Ubx

Drosophila Ubx

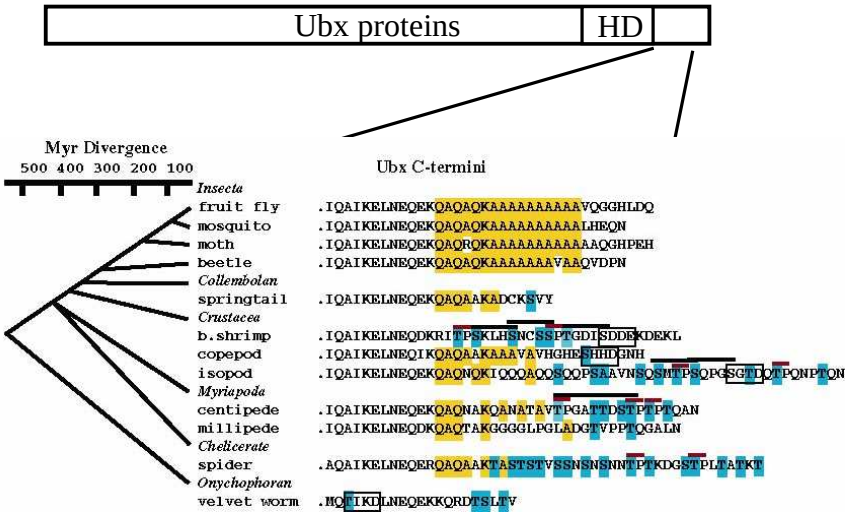
Differences in C-terminal residues between fly and crustacean Ubx control whether limbs are repressed.



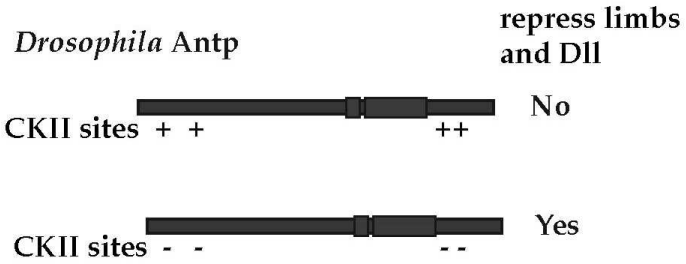
The C-terminus of *Artemia* Ubx inhibits, in cis, a covert limb repression function, the C-terminus of *Drosophila* Ubx is largely permissive for limb repression



Ubx C-termini from multilimbed arthropods have multiple Ser/Thr resides, Ubx from insects have none

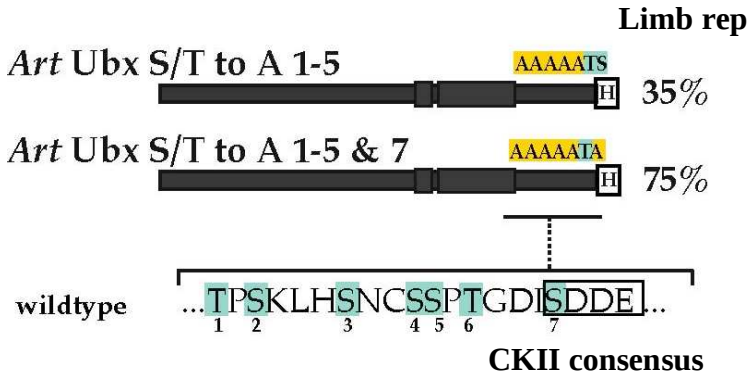


The importance of CKII sites in regulating Hox protein ability to repress limbs

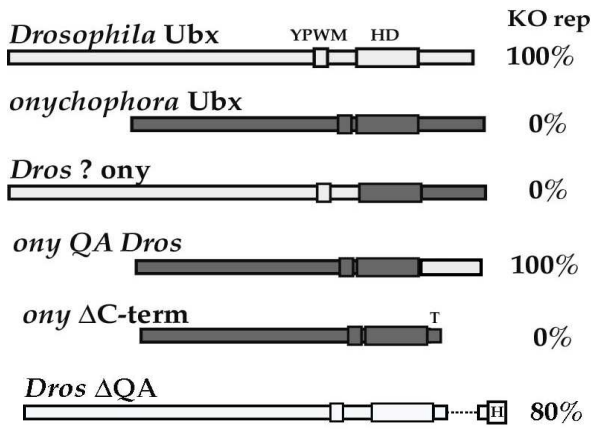


Jaffe, Ryoo, and Mann, Genes Dev. 1997

Ser/Thr residues, including one in a CKII consensus site, inhibit the limb repression function of *Artemia* Ubx

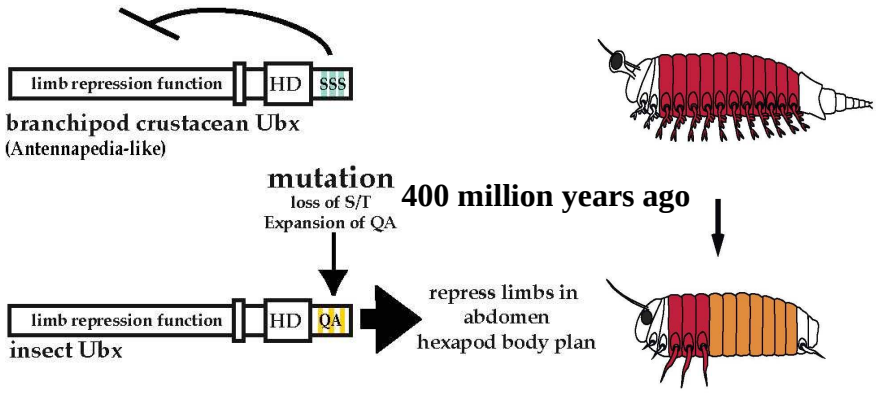


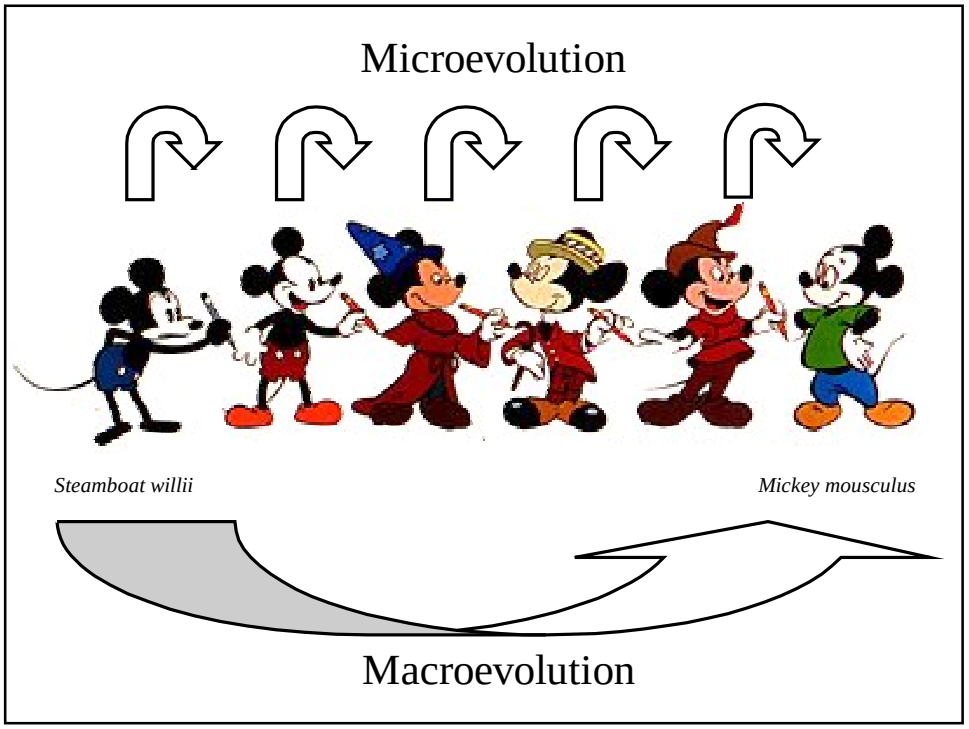
Another study on Ubx protein evolution suggested *Dros*. Ubx acquired a repression function not in onychophoran Ubx



Galant and Carroll, Nature, 2002

A serine to alanine mutagenesis scheme contributed to the evolution of the hexapoda

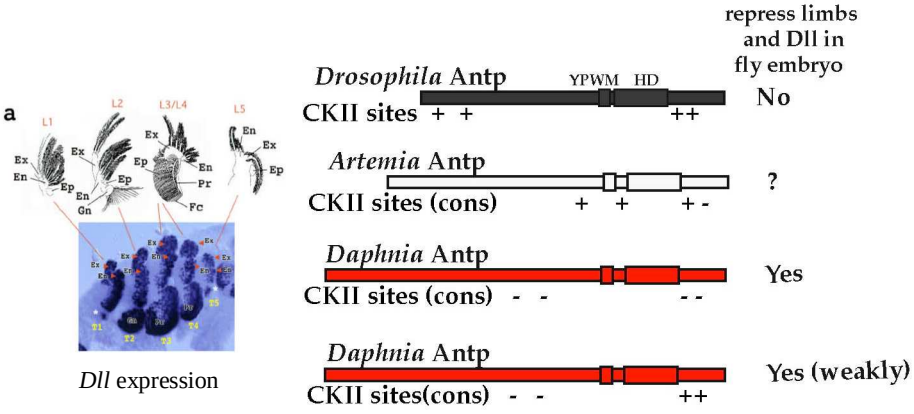




Macroevolution, and Ser/Thr-mediated regulation of Hox transcriptional repression

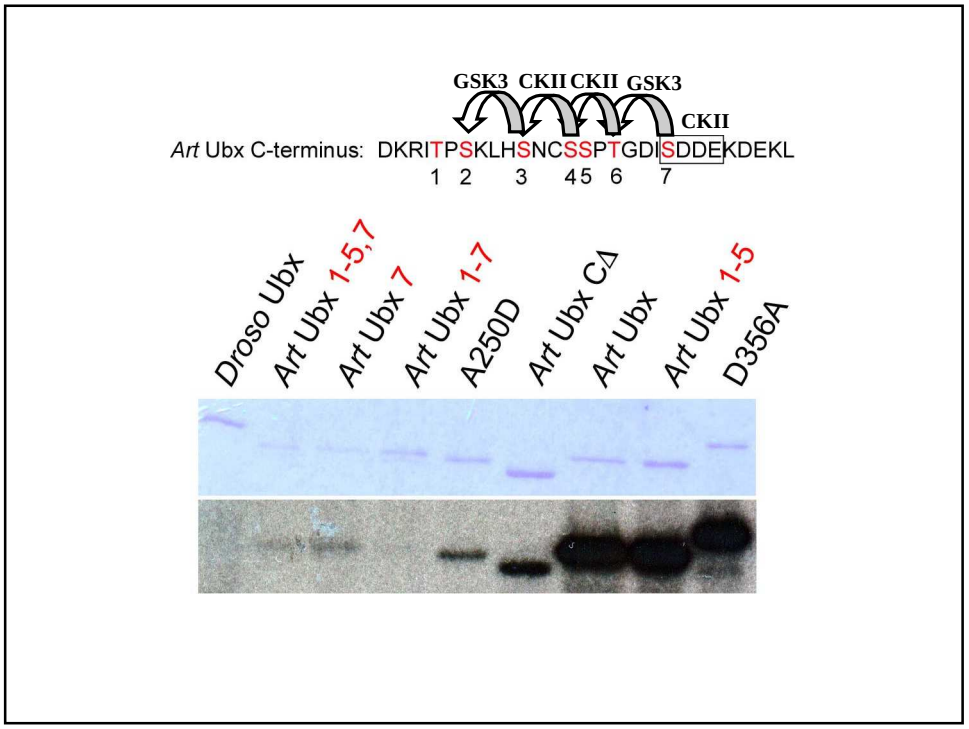
- **Ser-mutant phenotypes are dominant, no need to fix recessive alleles.**
- **Variation in number of Ser/Thr residues may allow microevolutionary steps toward a macroevolutionary event (hopeful monster) such as loss of appendages from the segments of a viable animal.**
- **The novel evolutionary variation can be regulated by cell-cell signaling pathways**

Parallel evolution? CKII site loss in Daphnia Antp protein correlates with gain of limb repression function

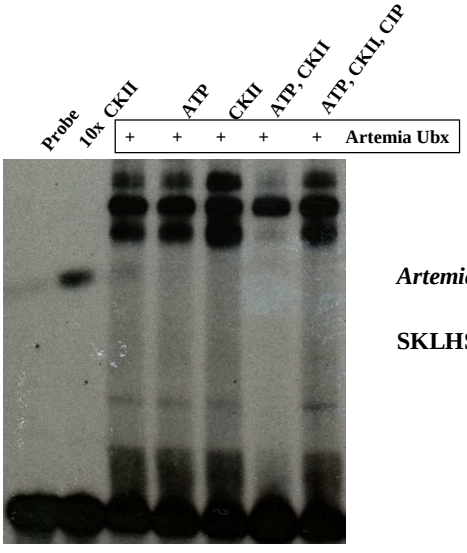


Shiga et al. Development, 2002

What biochemical properties and interactions of *Artemia* Ubx are altered by C-terminus Ser/Thr residues?



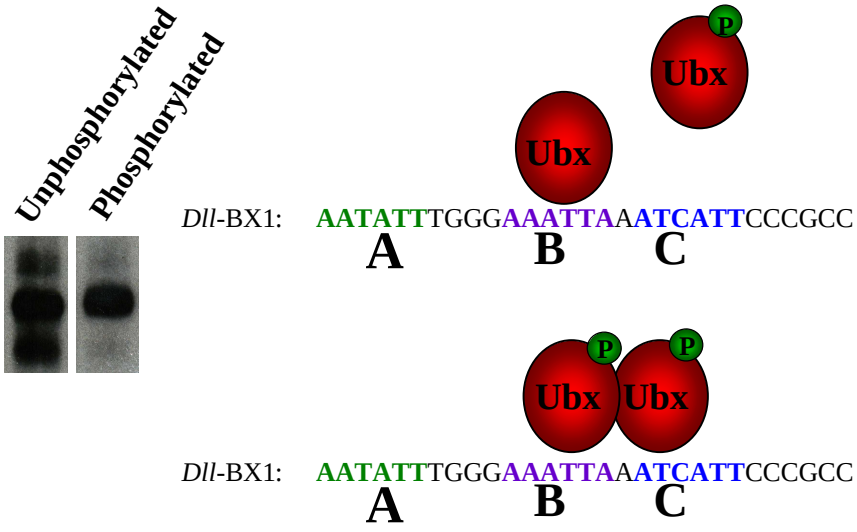
Phosphorylation of *Artemia* Ubx protein by CKII affects *in vitro* DNA binding to the *Dll*-BX1 element



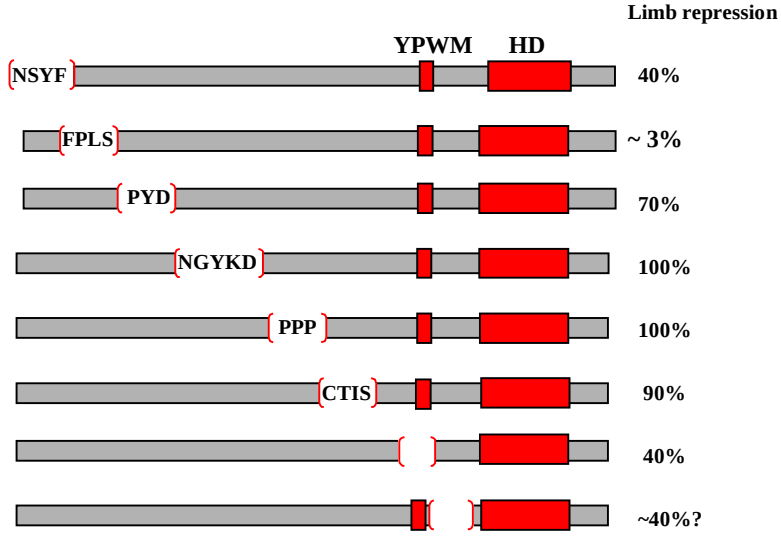
***Artemia franciscana* UBX C-Terminus:**

SKLHSNCSSPTGDISDDEKDKEKNL

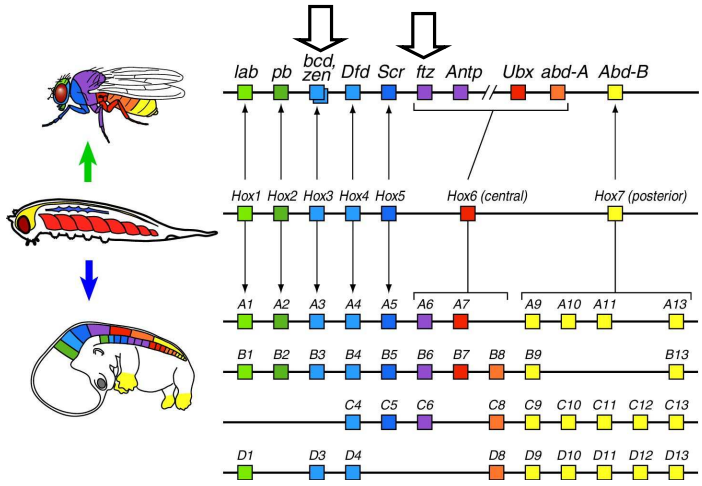
Model of *Artemia* Ubx protein binding to *Dll*-BX1 element



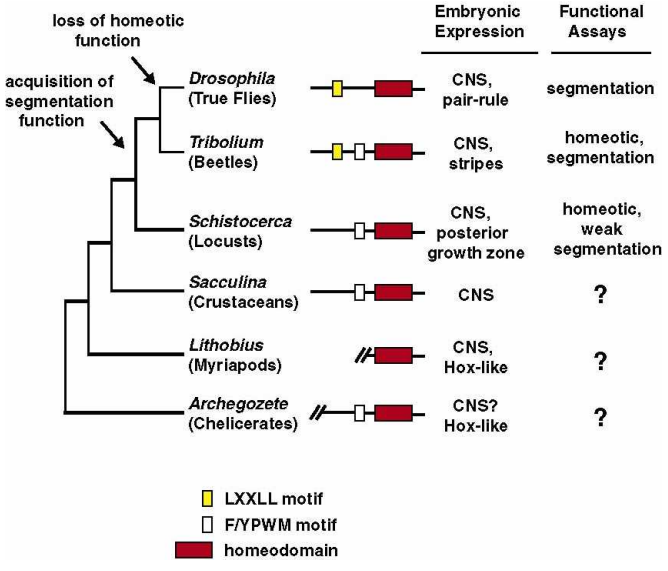
A domain required for limb repression in *Drosophila* Ubx maps between aa 20 and 61.

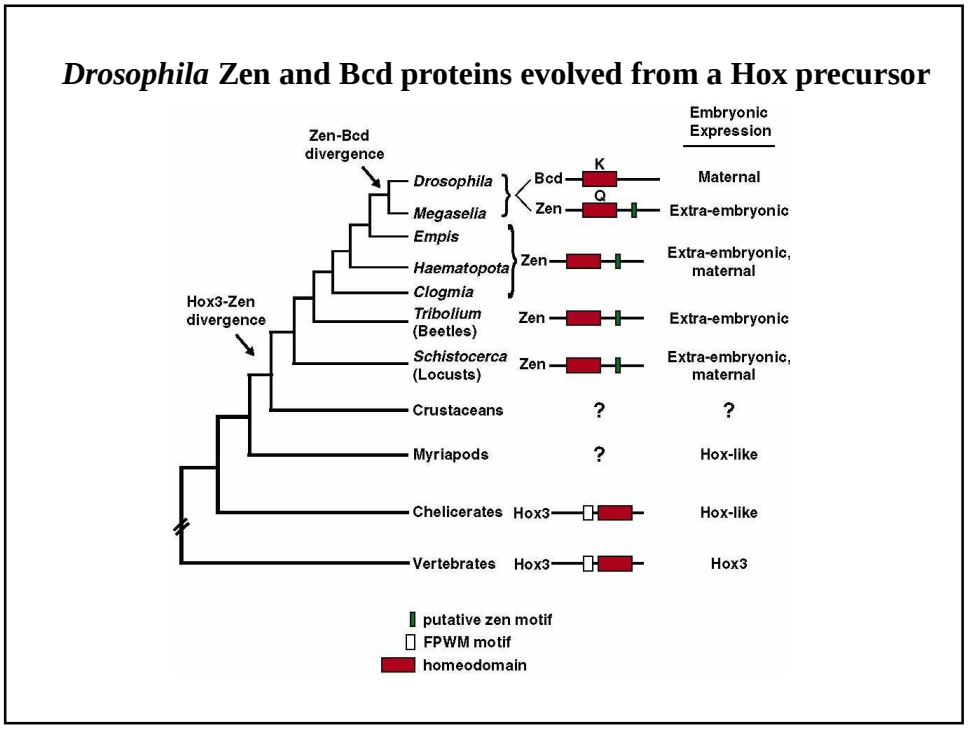


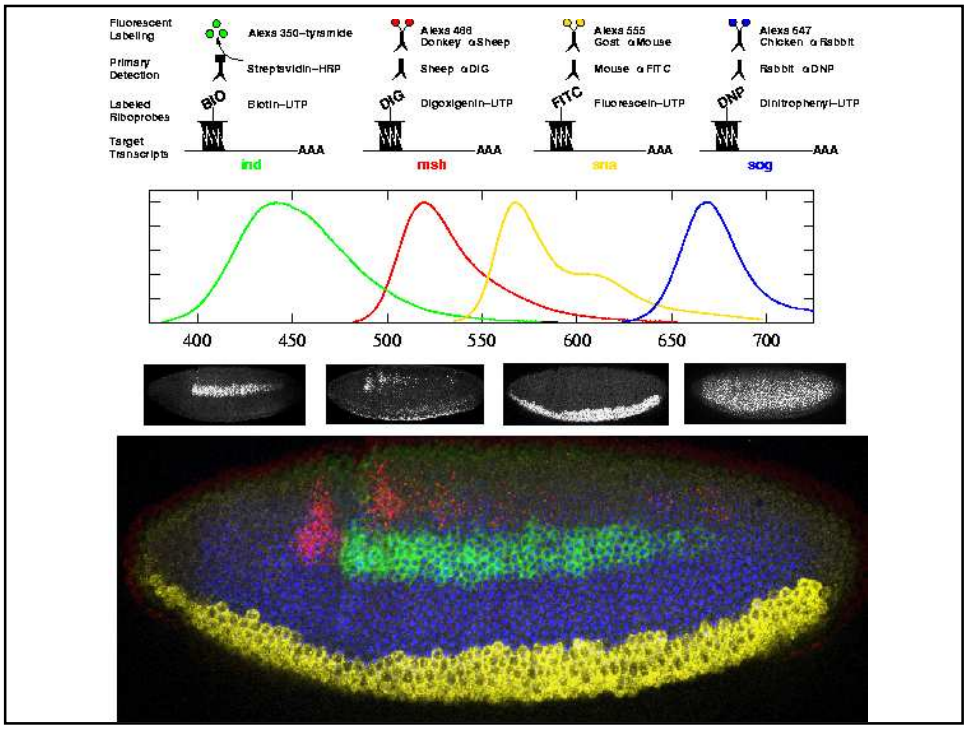
Some of the Drosophila genes in the Hox complex have lost their segment identity functions

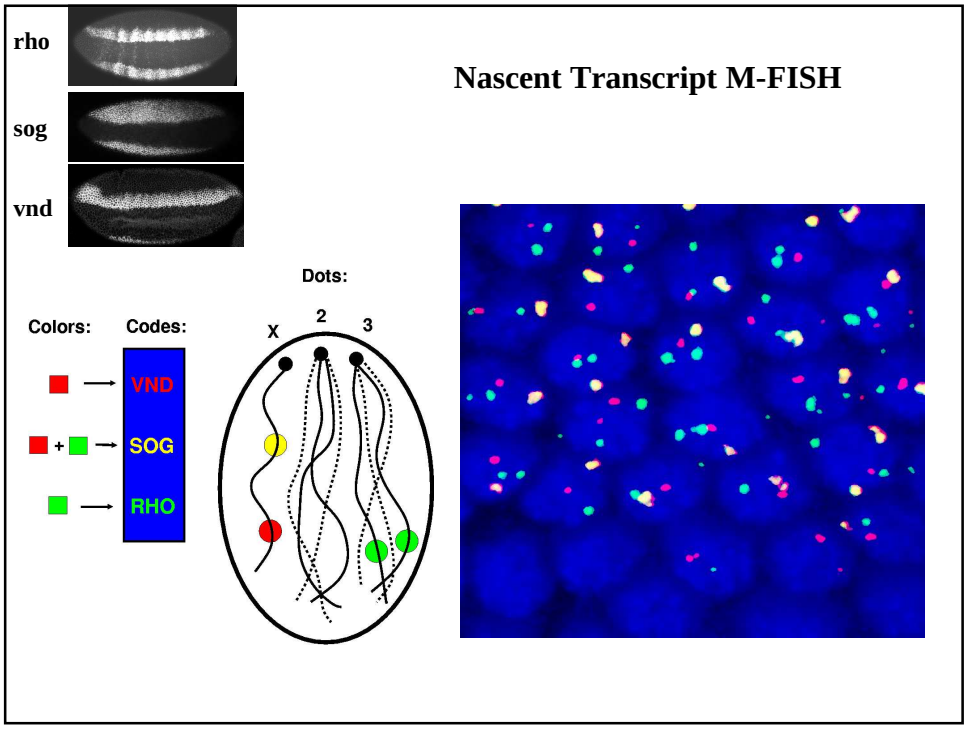


***Drosophila* Ftz protein evolved from a Hox precursor**




Drosophila Zen and Bcd proteins evolved from a Hox precursor
Embryonic Expression
Zen-Bcd divergence
Drosophila
Megaselia
Empis
Haematopota
Clogmia
Tribolium (Beetles)
Schistocerca (Locusts)
Crustaceans
Myriapods
Chelicerates
Vertebrates
Hox3-Zen divergence
Bcd
Zen
K
Q
Maternal
Extra-embryonic
Extra-embryonic, maternal
Hox3
Hox-like
?
putative zen motif
FPWM motif
homeodomain


Fluorescent Labeling
Primary Detection
Labeled Riboprobes
Target Transcripts
Alexa 350–tyramide
Streptavidin–HRP
Biotin–UTP
Alexa 488 Donkey αSheep
Sheep αDIG
Digoxigenin–UTP
Alexa 555 Goat αMouse
Mouse αFITC
Fluorescein–UTP
Alexa 647 Chicken αRabbit
Rabbit αDNP
Dinitrophenyl–UTP
BIO
DIG
FITC
DNP
AAA
ind
msh
sna
sog
400
450
500
550
600
650
700



Nascent Transcript Multiplex FISH
"3D nuclear nanoarrays"

3 probe colors - 7 genes

AntpP1	Alexa 488	Alexa 555	Alexa 647
Alexa 488	Dll	Spineless	Exd
Alexa 555		Btd	Hth
Alexa 647			wg

4 probe colors - 15 genes
5 probe colors - 31 genes
6 probe colors - 63 genes