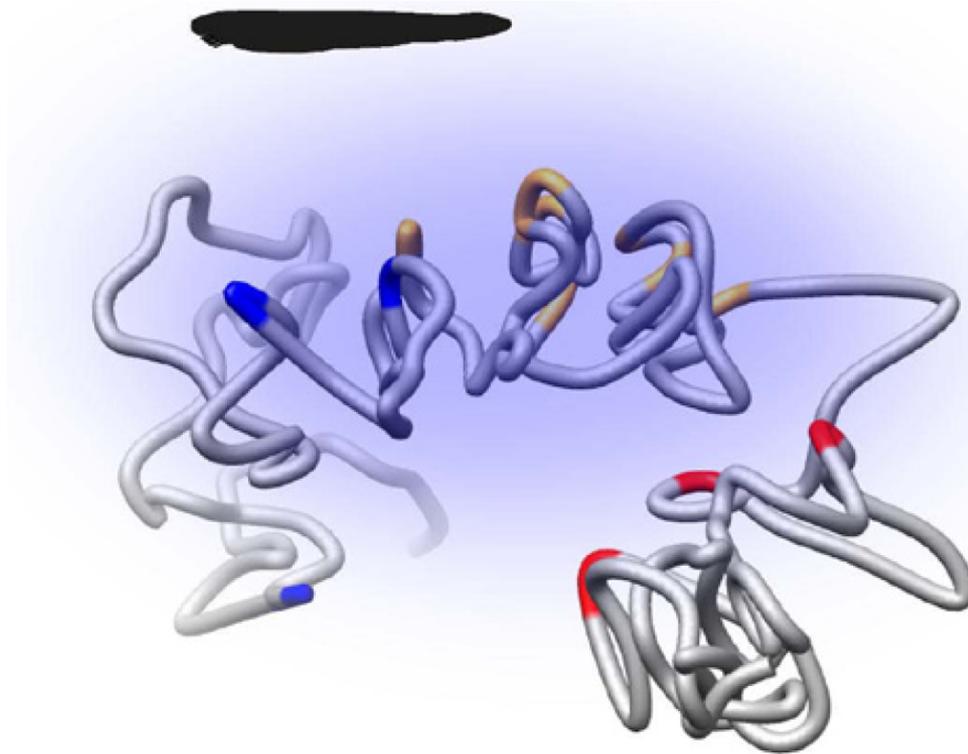
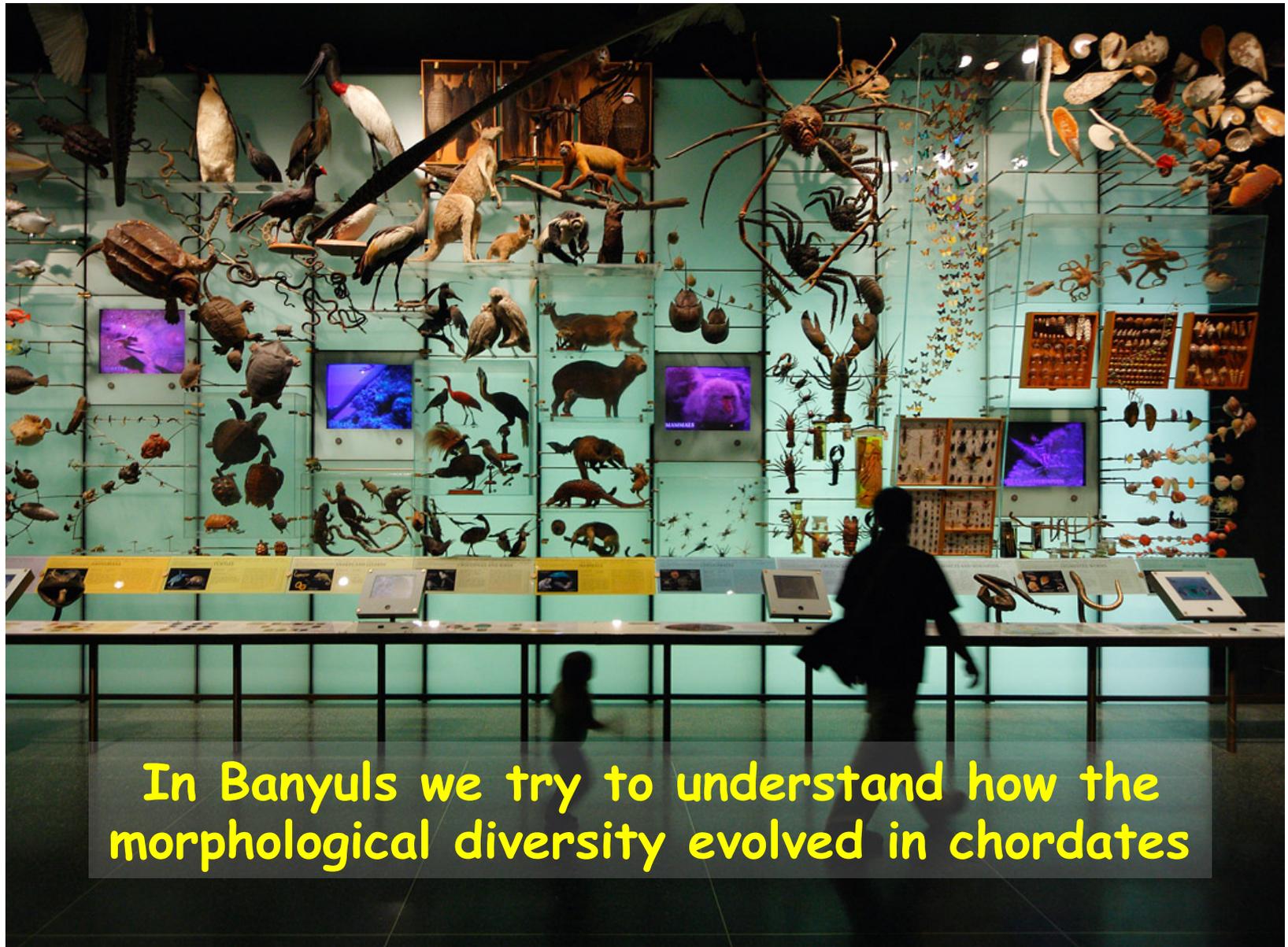


A single three-dimensional chromatin compartment in amphioxus indicates a stepwise evolution of vertebrate Hox bimodal regulation



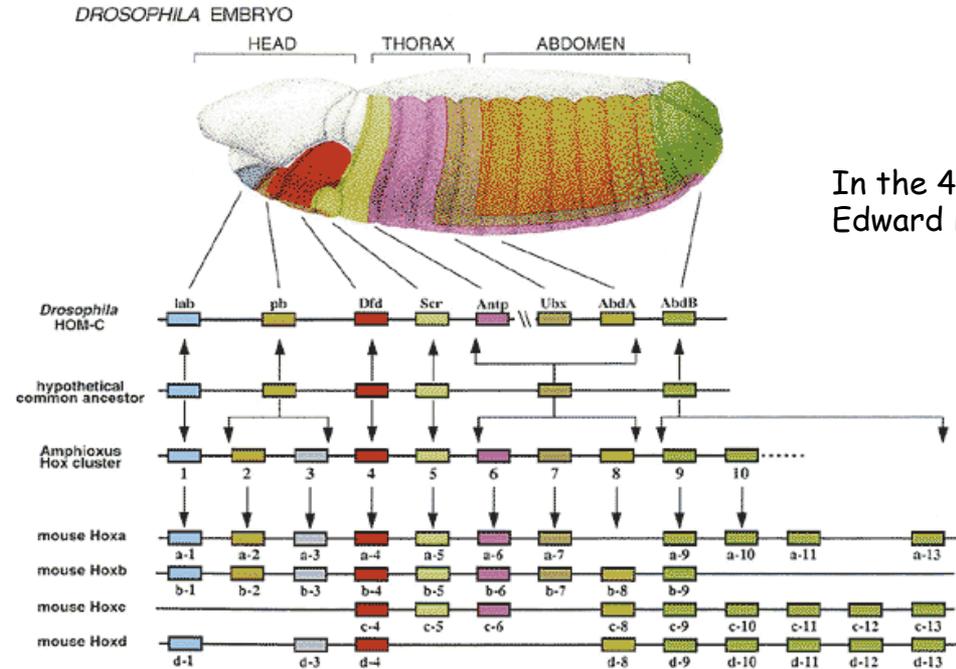
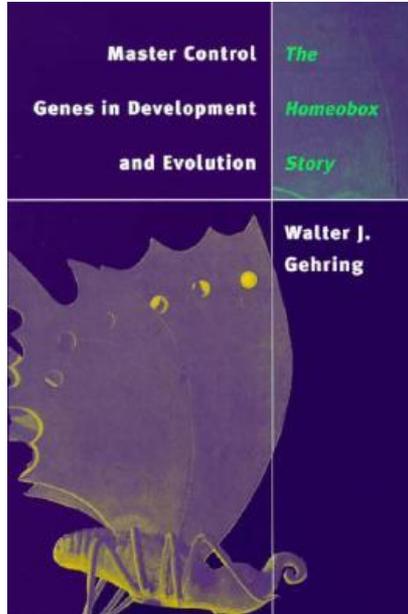
Hector Escriva
Equipe "Evolution et développement des chordés"
Laboratoire Arago
Banyuls sur Mer



In Banyuls we try to understand how the morphological diversity evolved in chordates

HOX genes are conserved in all Metazoans

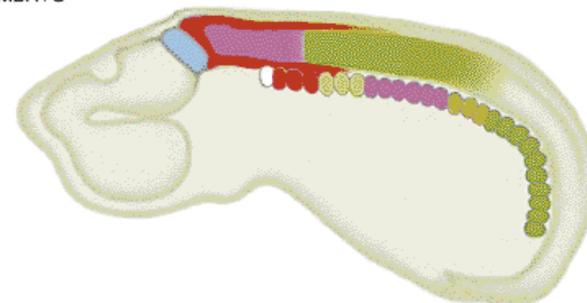
This discovery is considered the birth of EvoDevo (1984)



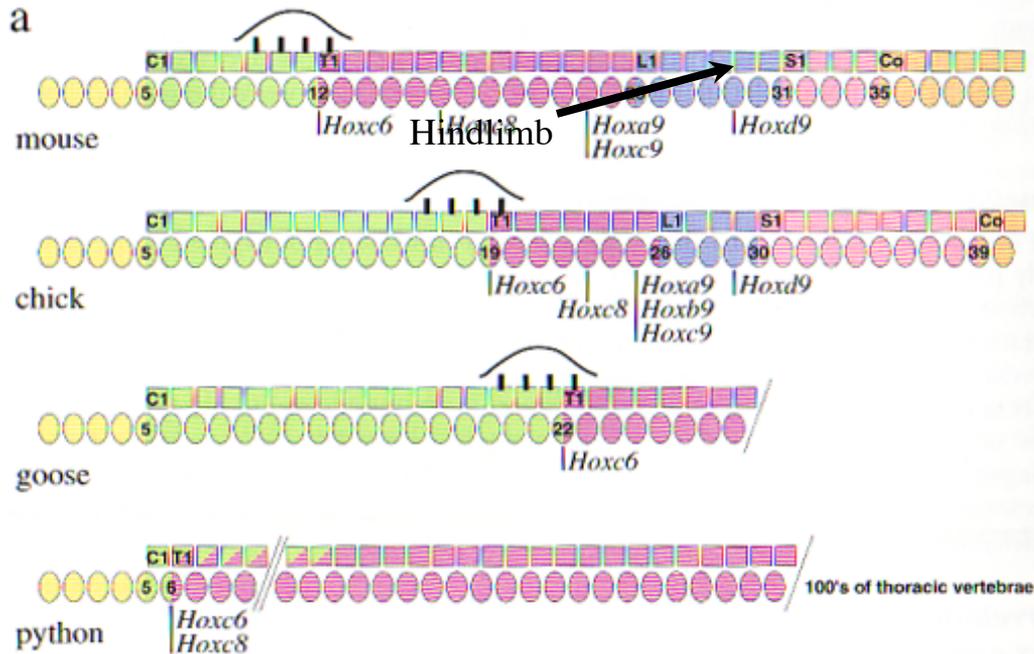
In the 40's
Edward B. Lewis



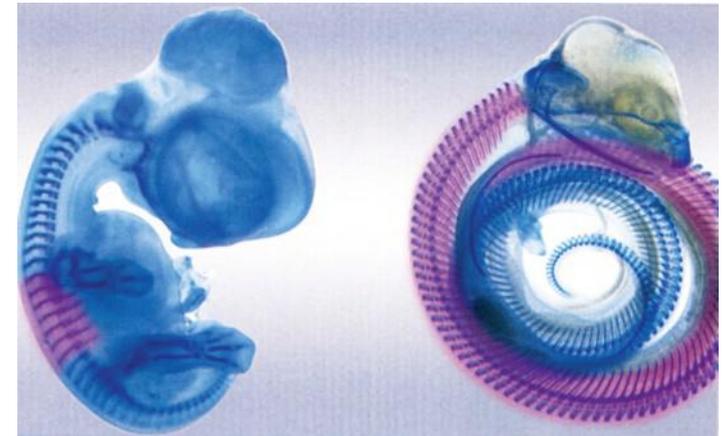
MOUSE EMBRYO



HoxC6 and the cervical/ thoracic boundary



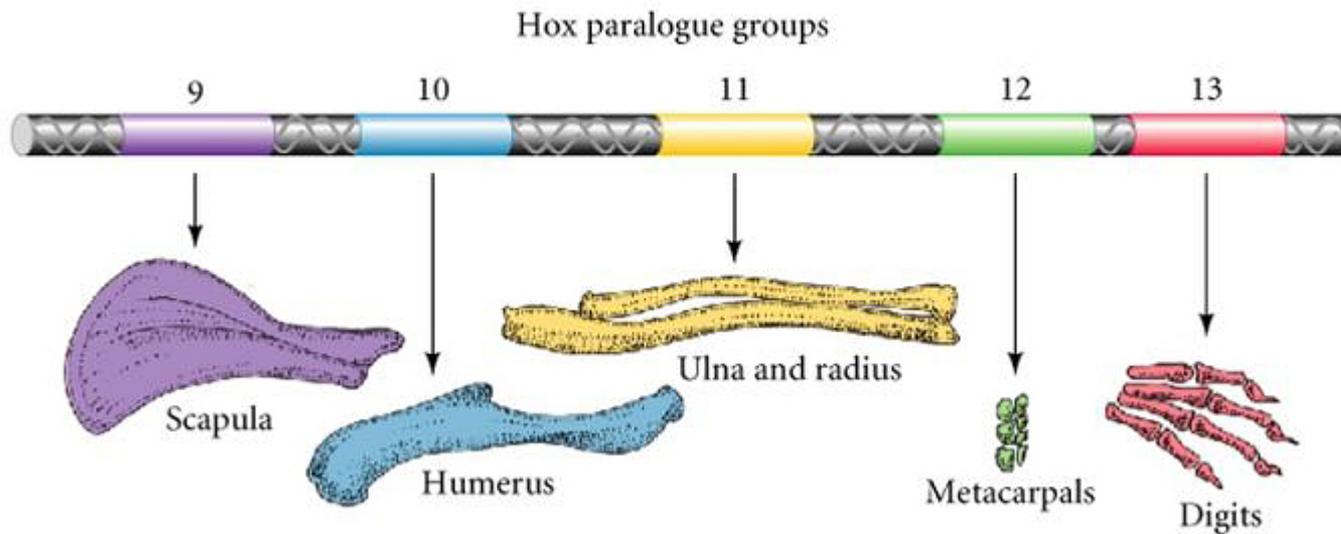
Adapted from Burke et al (1995)



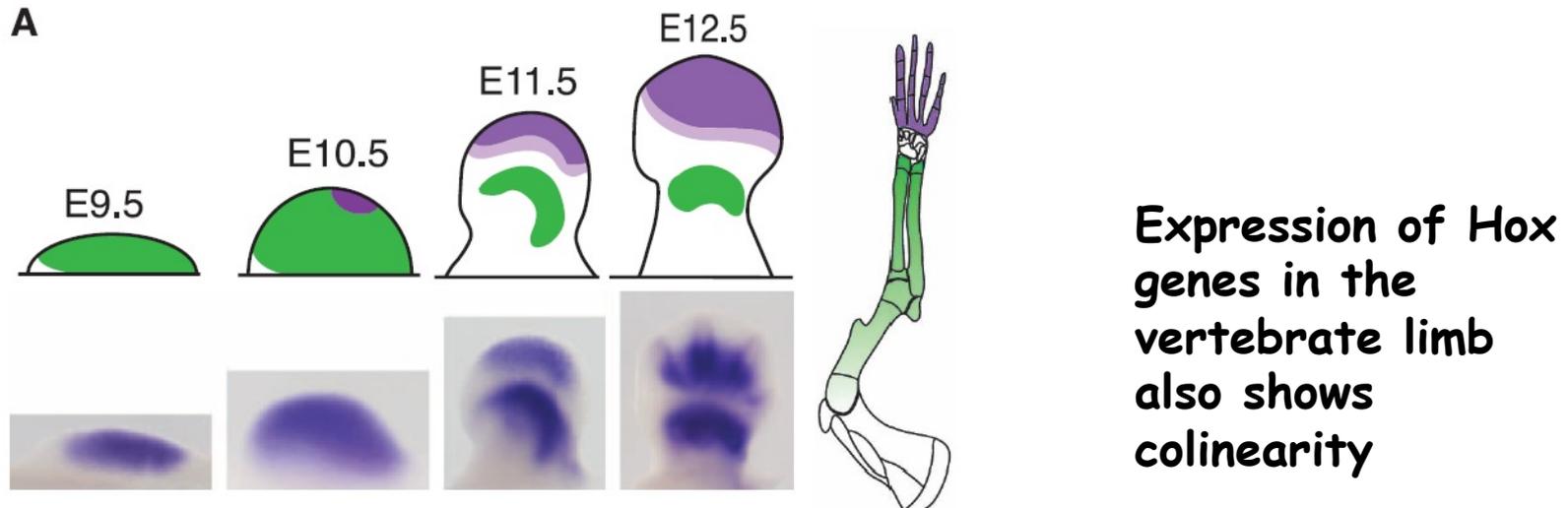
The number of cervical metameres is different, but the Hoxc6 always marks the cervical/ thoracic boundary

EvoDevo needs comparative approaches

In addition to their function in AP patterning
**HOX genes also have an important function in
patterning vertebrate limbs**



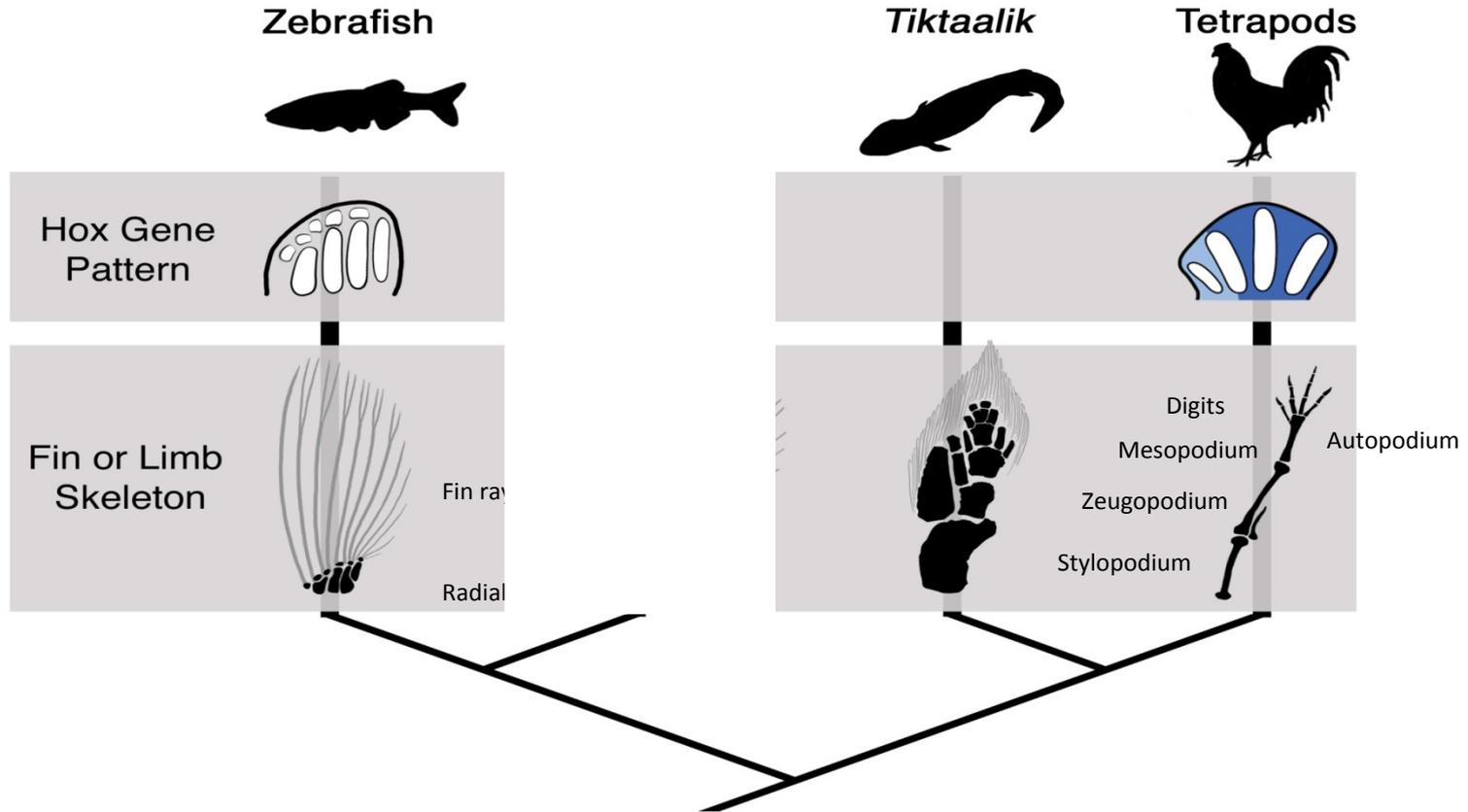
HOX genes also have an important function in patterning vertebrate limbs



A Switch Between Topological Domains Underlies HoxD Genes Collinearity in Mouse Limbs
Guillaume Andrey *et al.*
Science **340**, (2013);

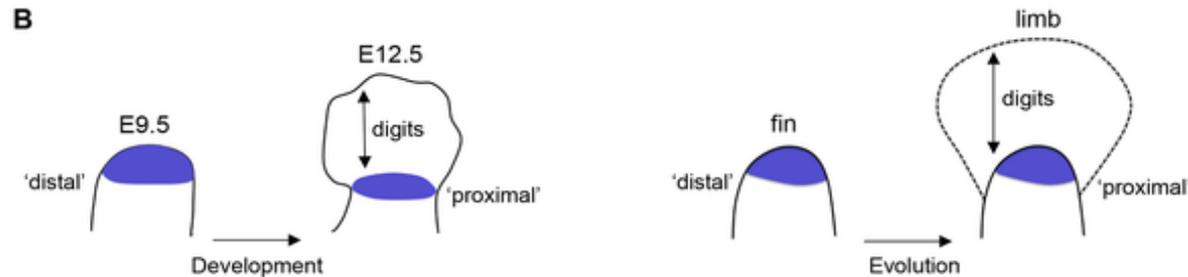
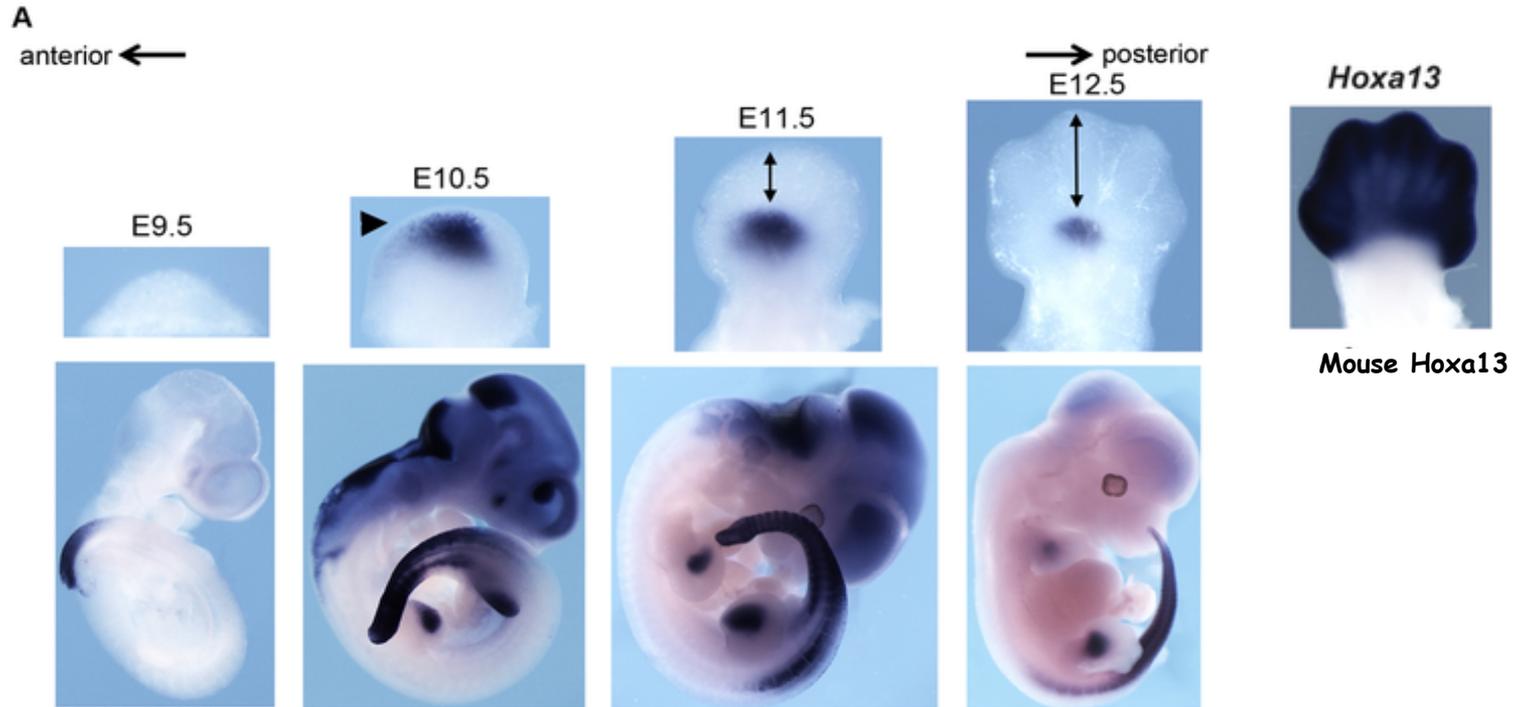
Hox function in the limbs was thought to be tetrapod-specific because Hox gene expression was not conserved in fins of classical chondrichthyan animal models

HOX genes function during fin/limb development is conserved in vertebrates



Hox gene expression is conserved, but...

The Tetraodon *Hoxa13b* expression domain in mice: from “distal” to “proximal.”

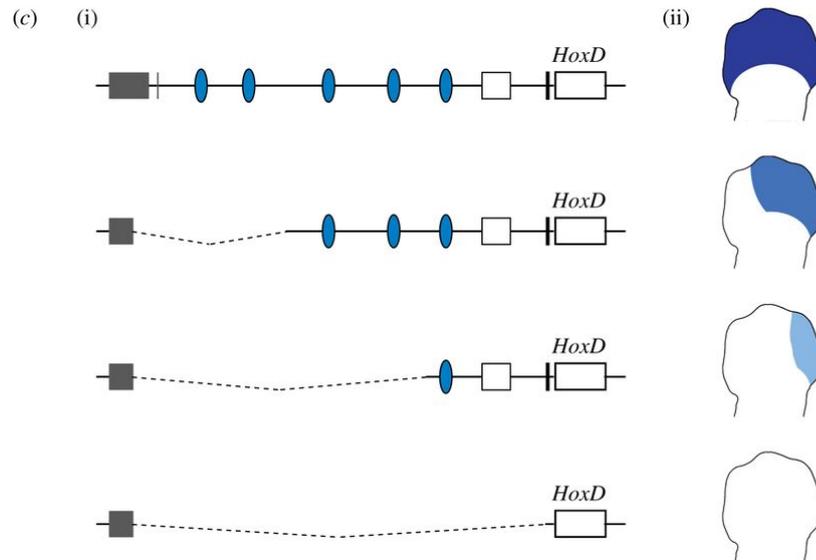
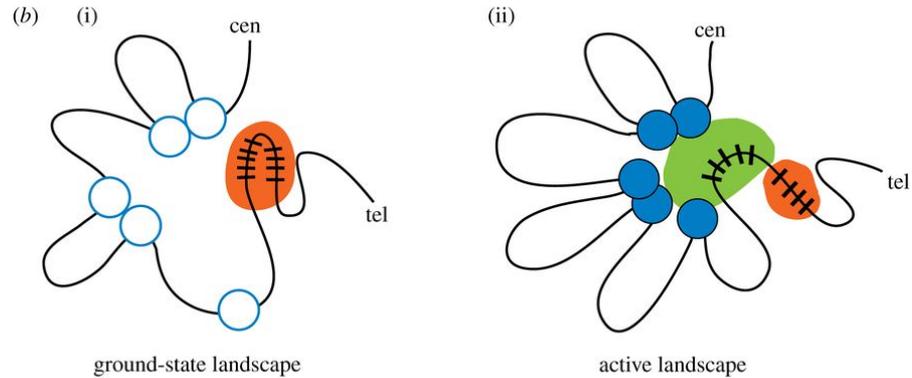
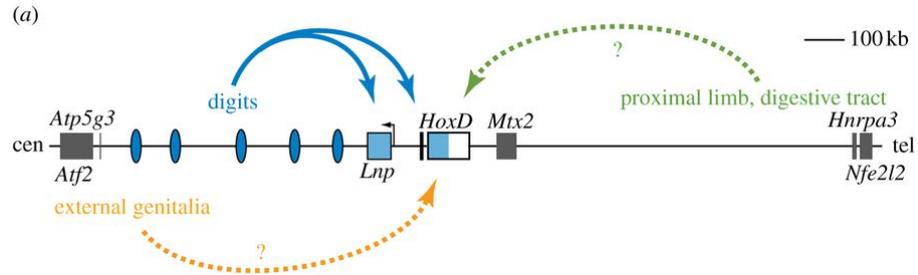


Woltering JM, Noordermeer D, Leleu M, Duboule D (2014) PLoS Biol

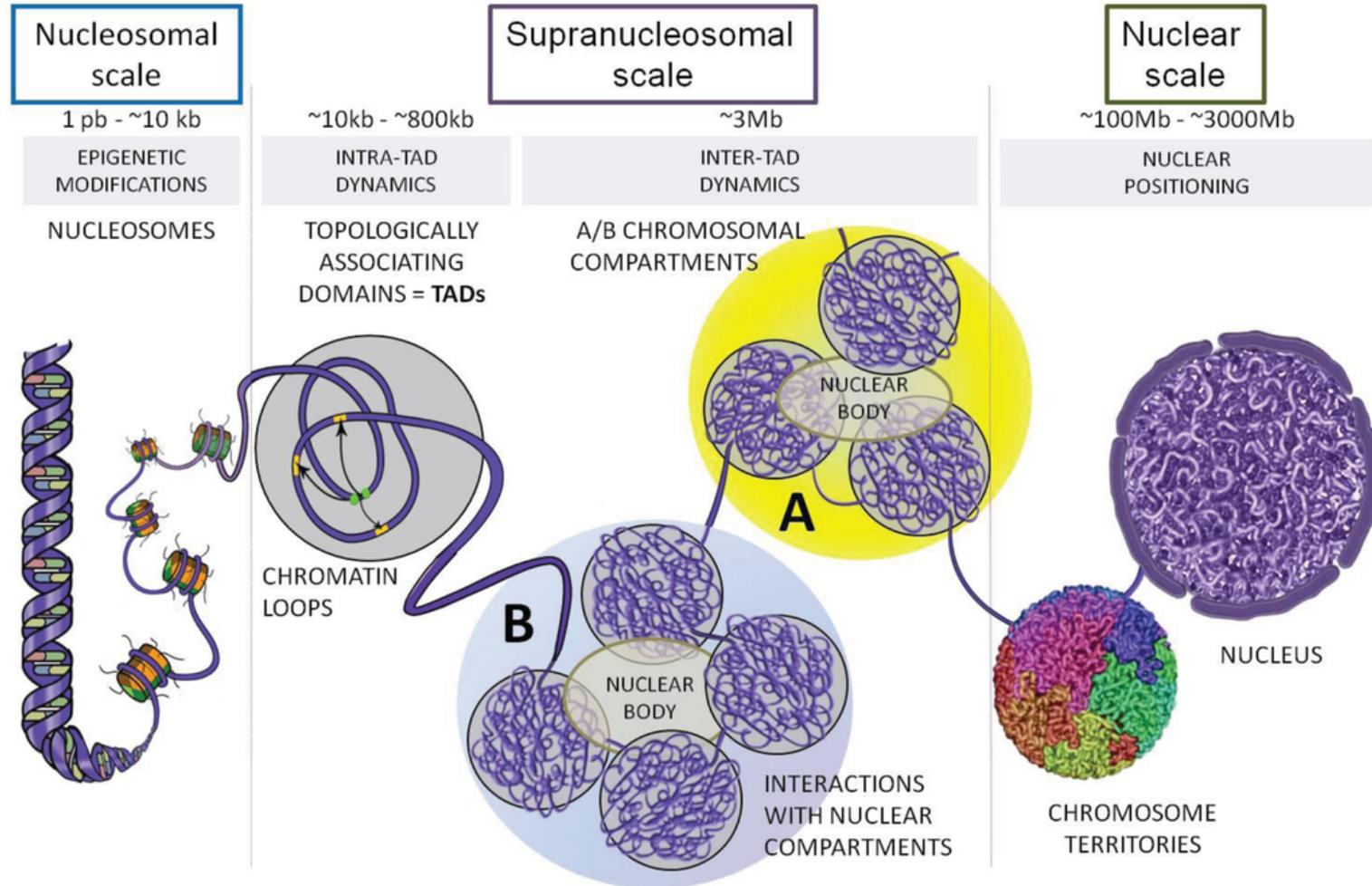
How Hox gene expression is controlled?

Expression of HoxD genes in the fins/limbs is controlled by distant enhancers

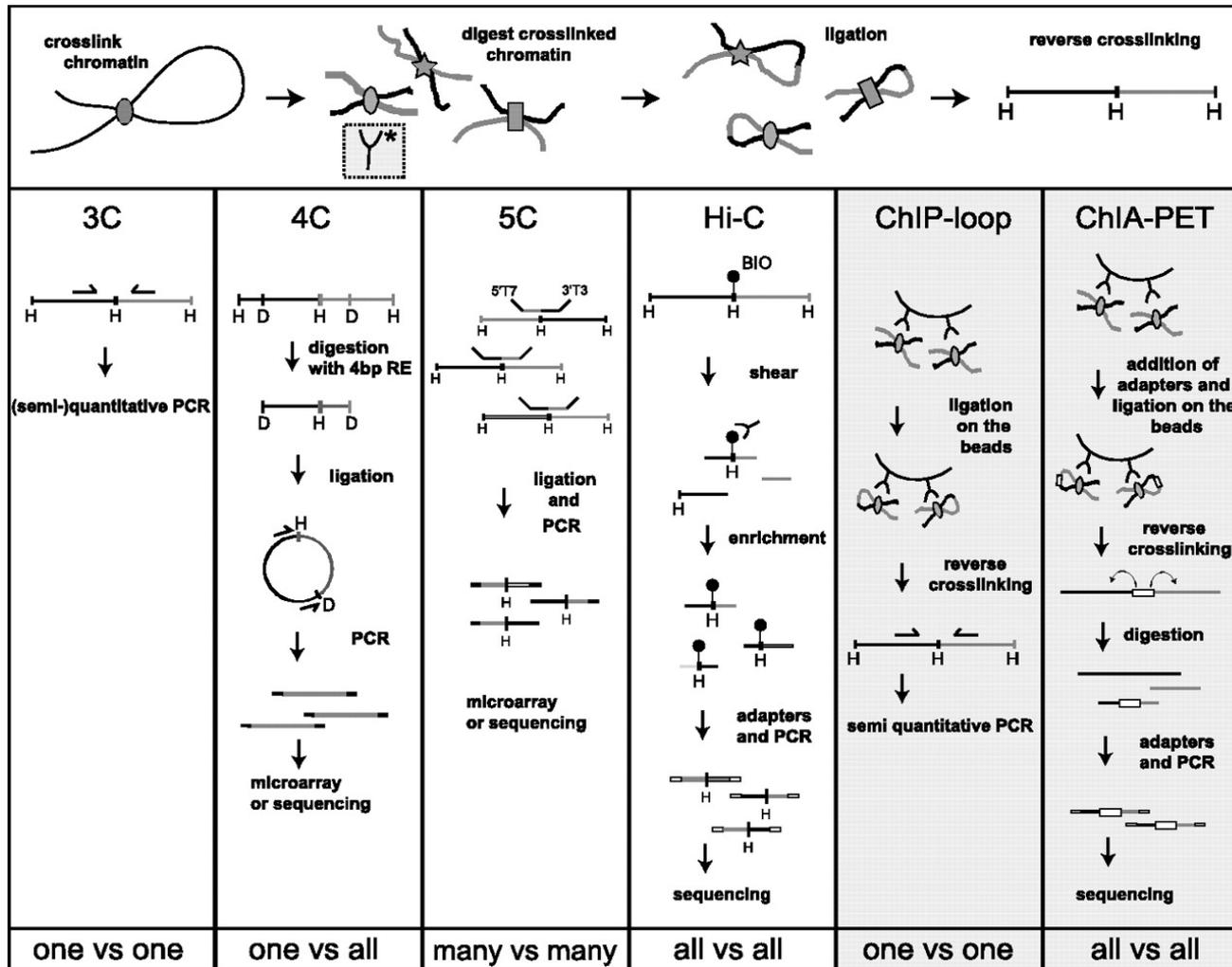
How distant enhancers may control gene expression?



Chromatin is compartmentalized into supranucleosomal structures called TADs



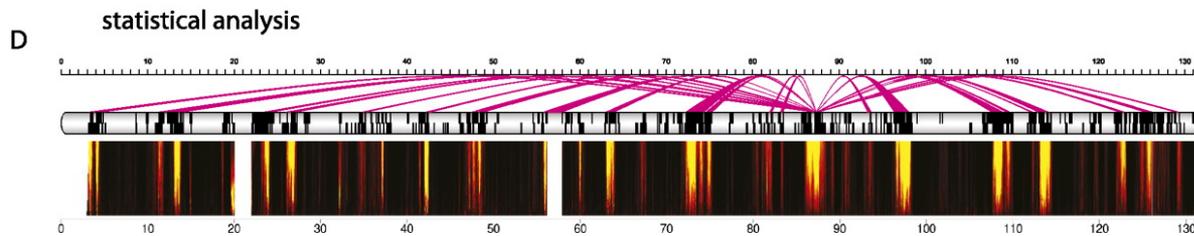
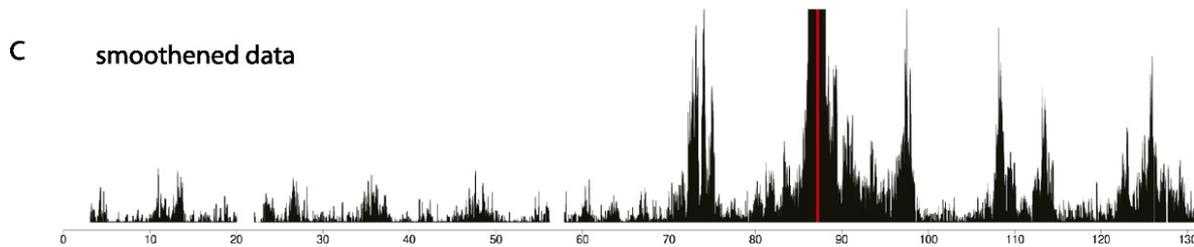
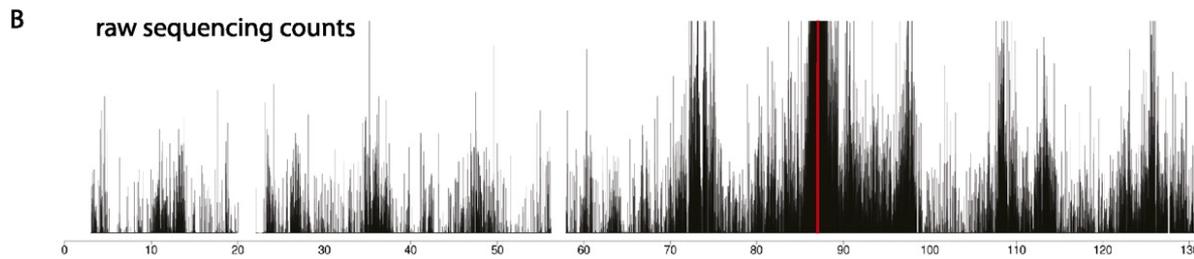
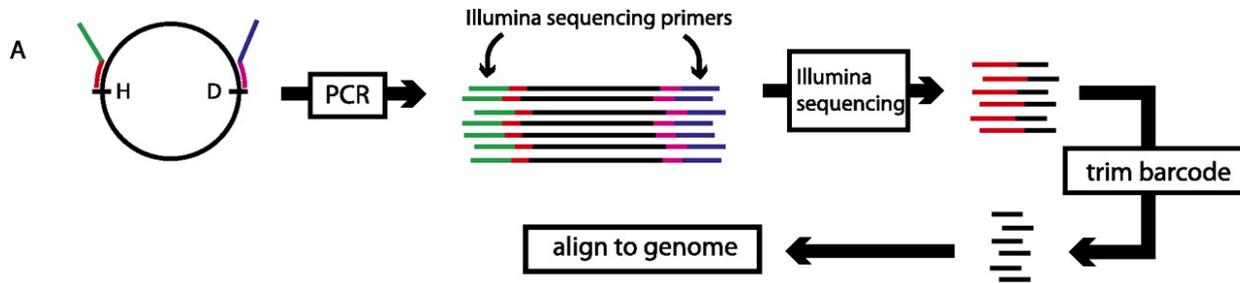
Overview of Chromosome Conformation Capture (3C)-derived methods



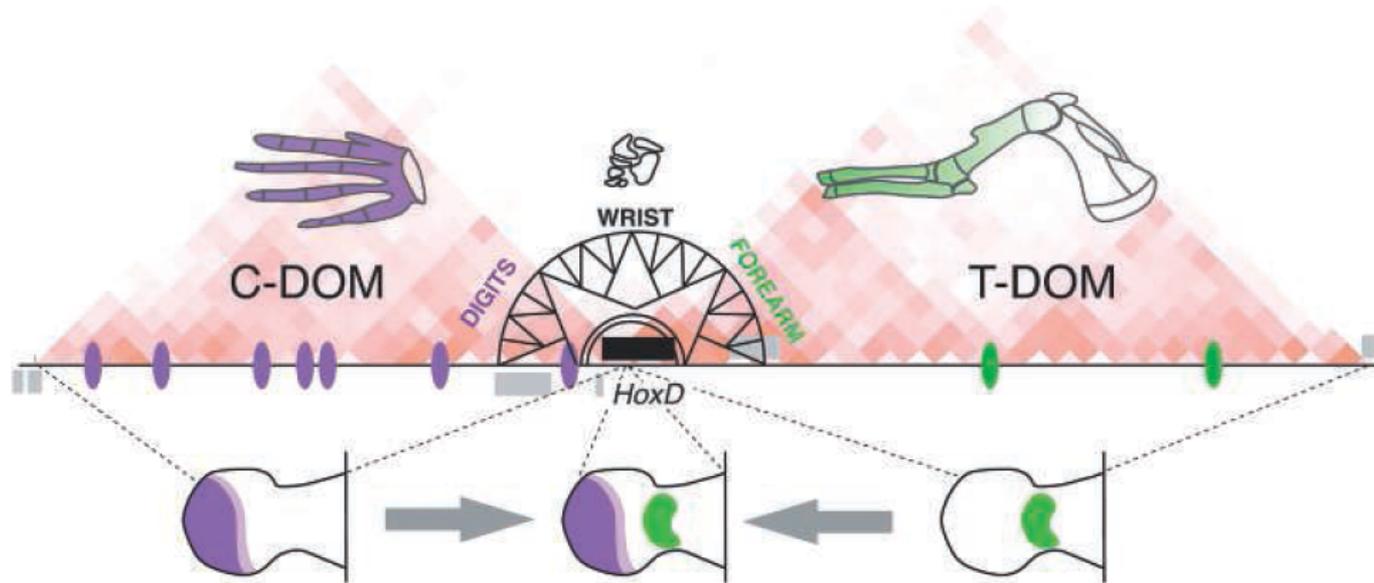
Elzo de Wit, and Wouter de Laat *Genes Dev.* 2012;26:11-24



4C-seq

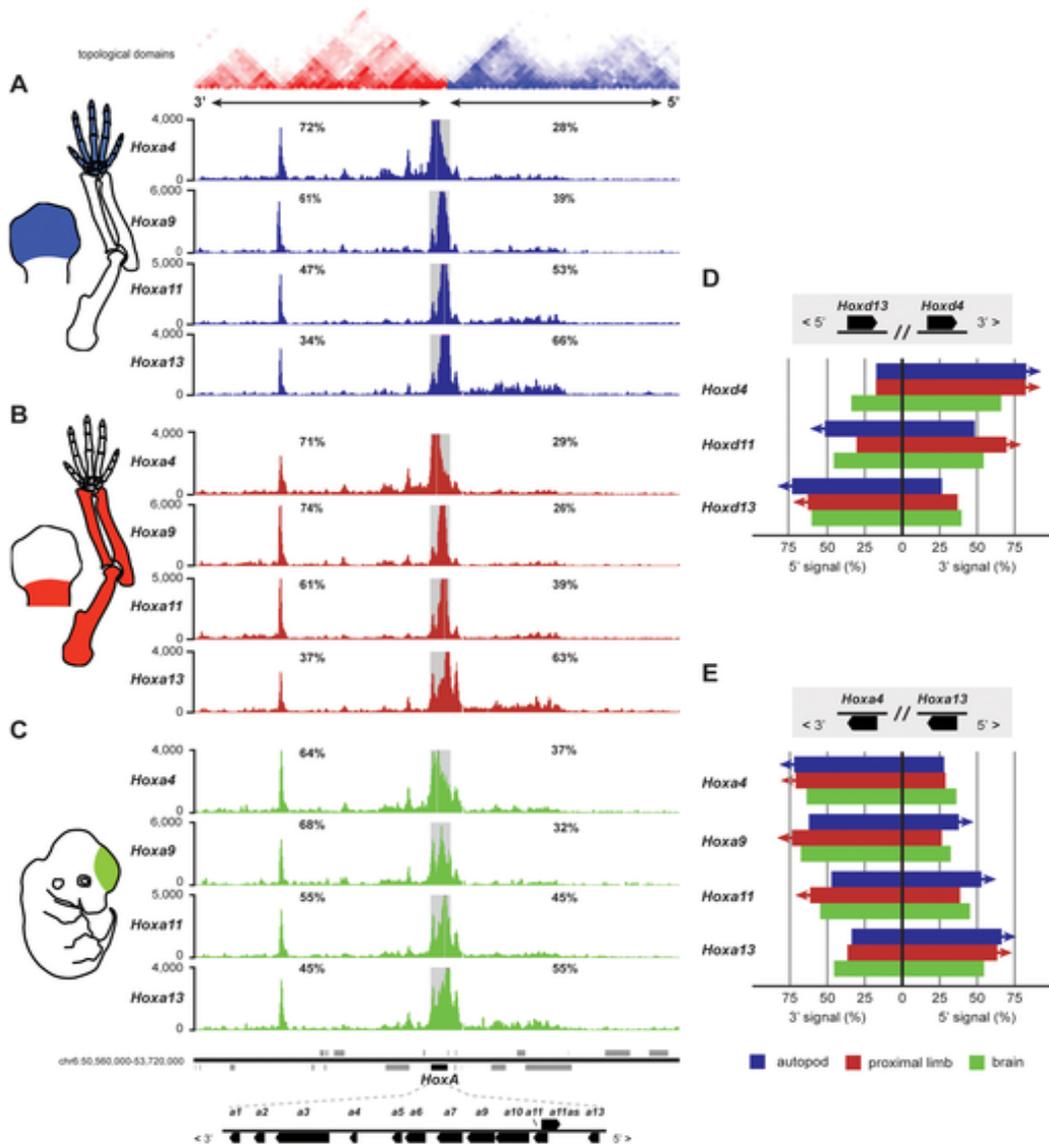


Proximal and distal HoxD gene expression is regulated from distant elements placed at 3' and 5' of the Hox cluster



A Switch Between Topological Domains Underlies *HoxD* Genes Collinearity in Mouse Limbs

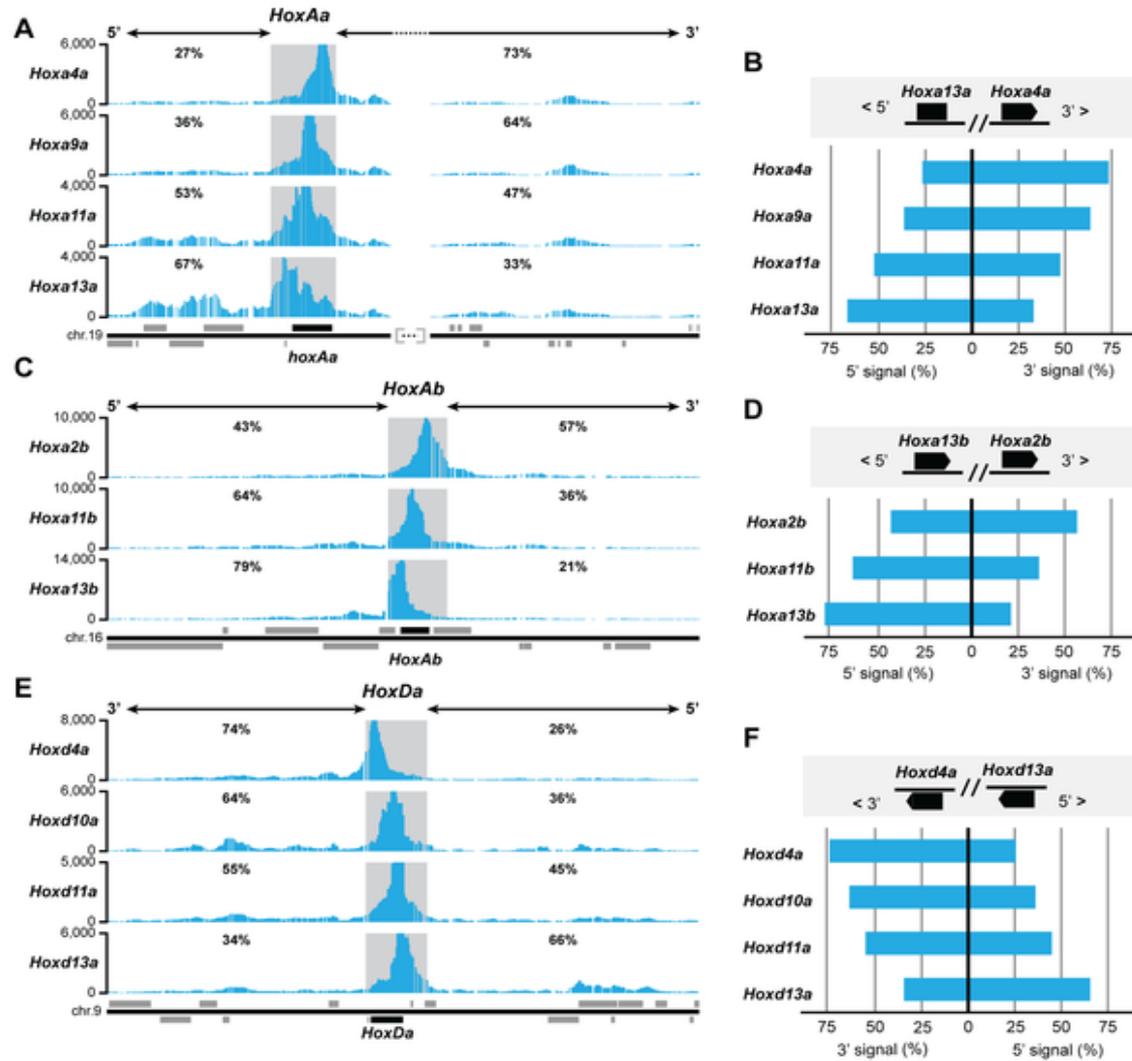
Cite this article as G. Andrey *et al.*, *Science* **340**, 1234167 (2013). DOI: 10.1126/science.1234167



HoxA genes are controlled in a similar way as HoxD

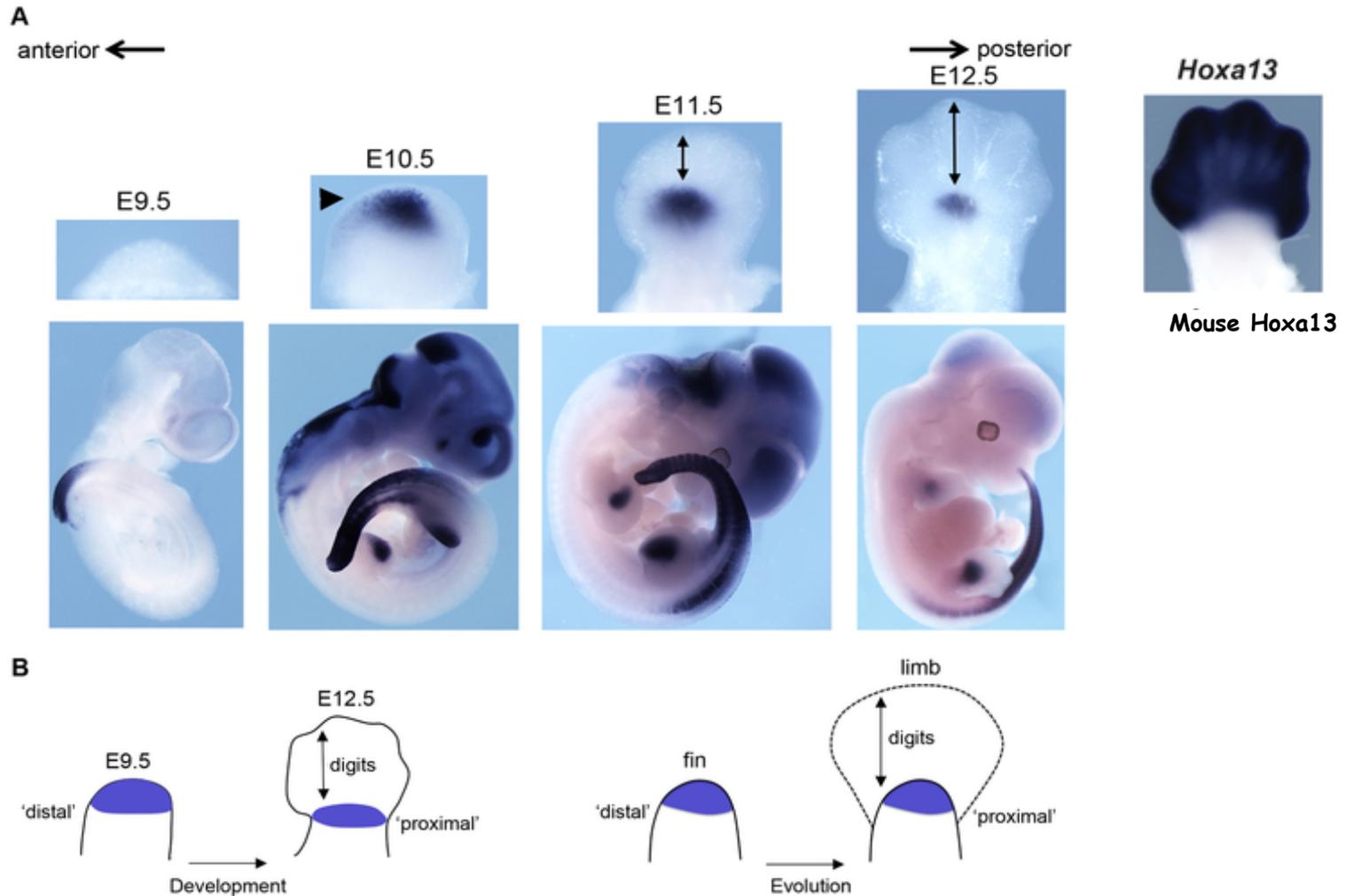
The same chromatin structure is observed in autopod, stylopod and the brain

Zebrafish Hox clusters are partitioned into 3' and 5' interaction domains



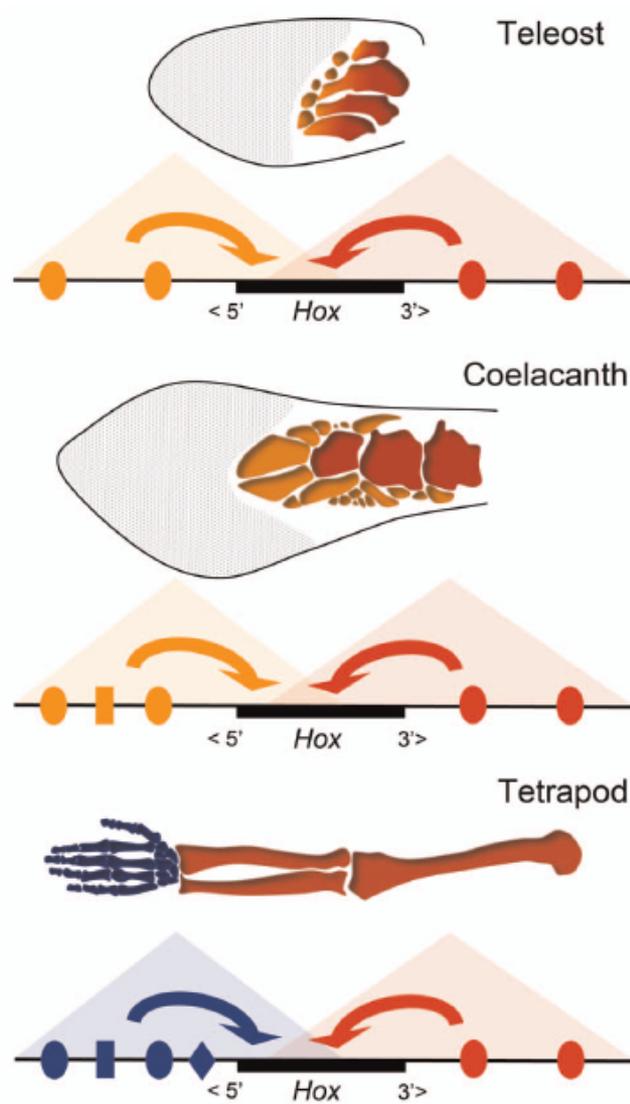
Woltering JM, Noordermeer D, Leleu M, Duboule D (2014) Conservation and Divergence of Regulatory Strategies at Hox Loci and the Origin of Tetrapod Digits. PLoS Biol 12(1)

The Tetraodon *Hoxa13b* expression domain in mice: from “distal” to “proximal.”



Woltering JM, Noordermeer D, Leleu M, Duboule D (2014) Conservation and Divergence of Regulatory Strategies at Hox Loci and the Origin of Tetrapod Digits. PLoS Biol 12(1): e1001773. doi:10.1371/journal.pbio.1001773

Vertebrate fin to limb evolution



These results suggest that limbs evolved from a bimodal "proximal-proximal" patterning system in fins to a bimodal "proximal-distal" system postulates the transformation of a regulatory landscape from a "proximal" to a "distal" specificity

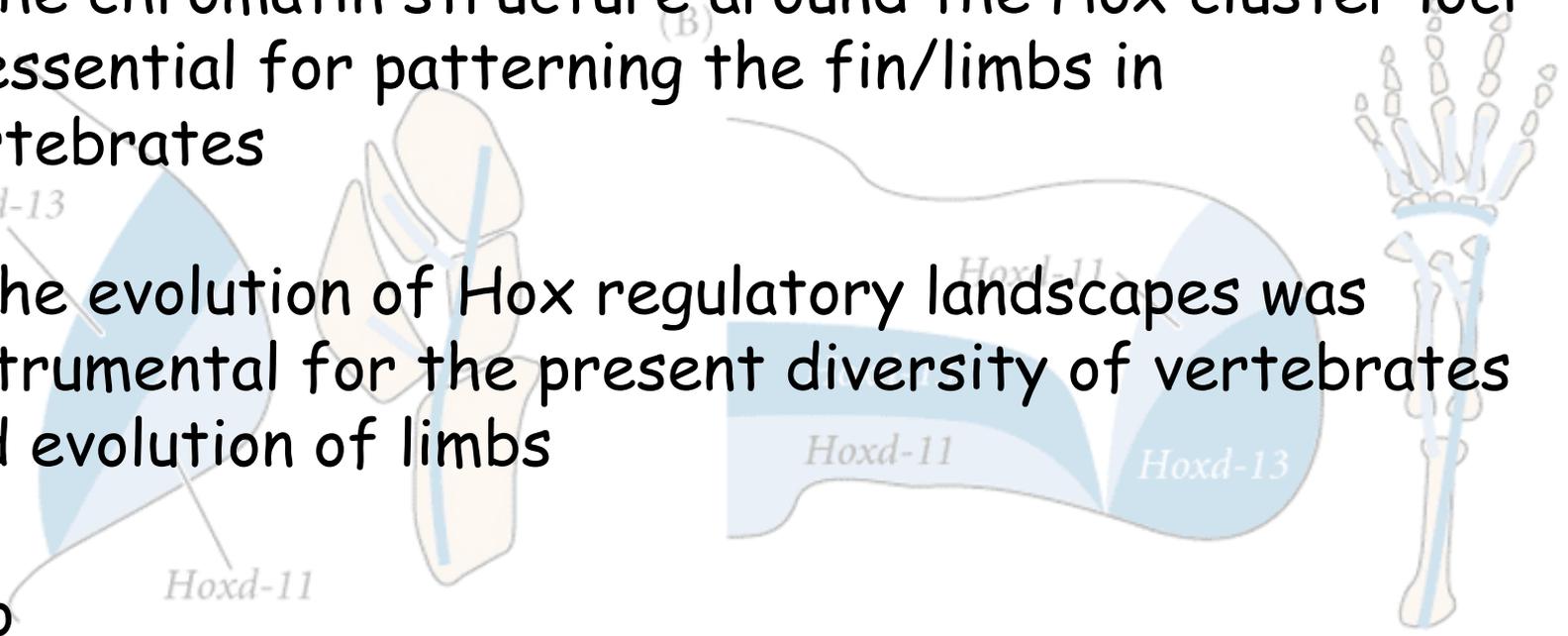
This implies the transformation of a regulatory landscape from a "proximal" to a "distal" specificity

These results show:

(A) - The chromatin structure around the Hox cluster loci is essential for patterning the fin/limbs in vertebrates

(B) - The evolution of Hox regulatory landscapes was instrumental for the present diversity of vertebrates and evolution of limbs

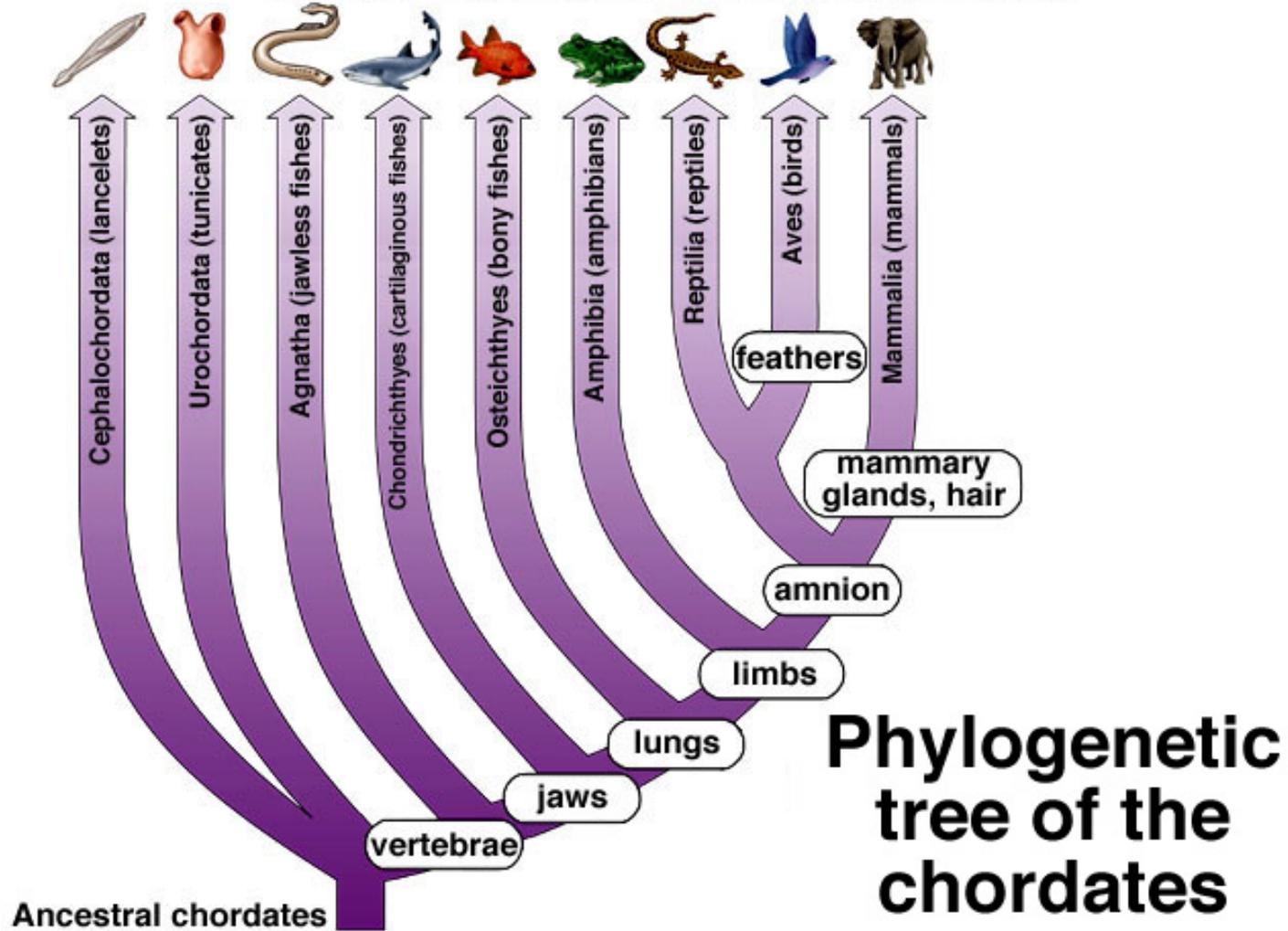
So



How and when the bimodal regulation of Hox gene expression appeared during evolution?

How and when the bimodal regulation of Hox gene expression appeared during evolution?

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.



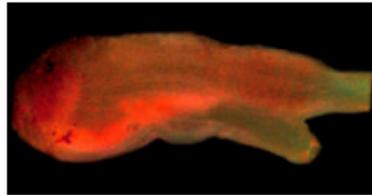
A "non-duplicated" genome with a maximum gene retention and no derived features

Subphylum

Cephalochordates



Urochordates



Vertebrates

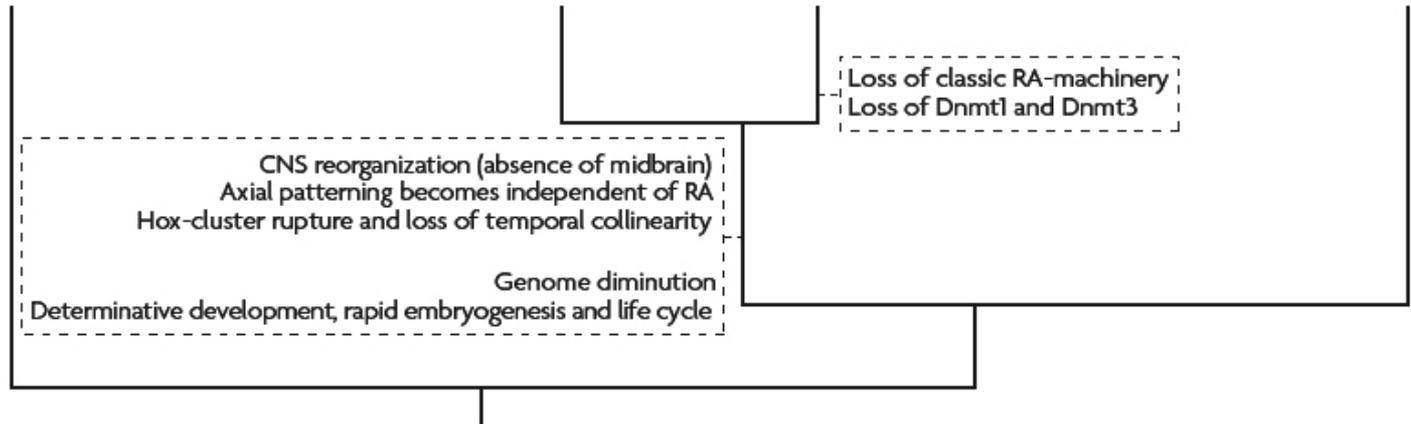
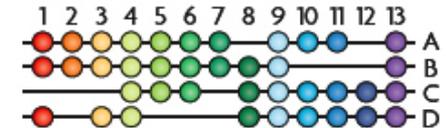
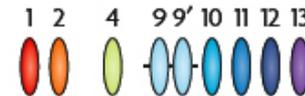
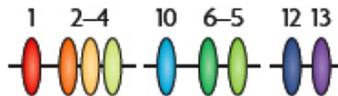
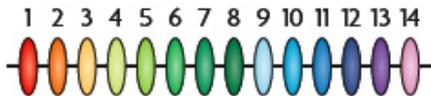


Class

Ascidians

Larvaceans

Hox clusters



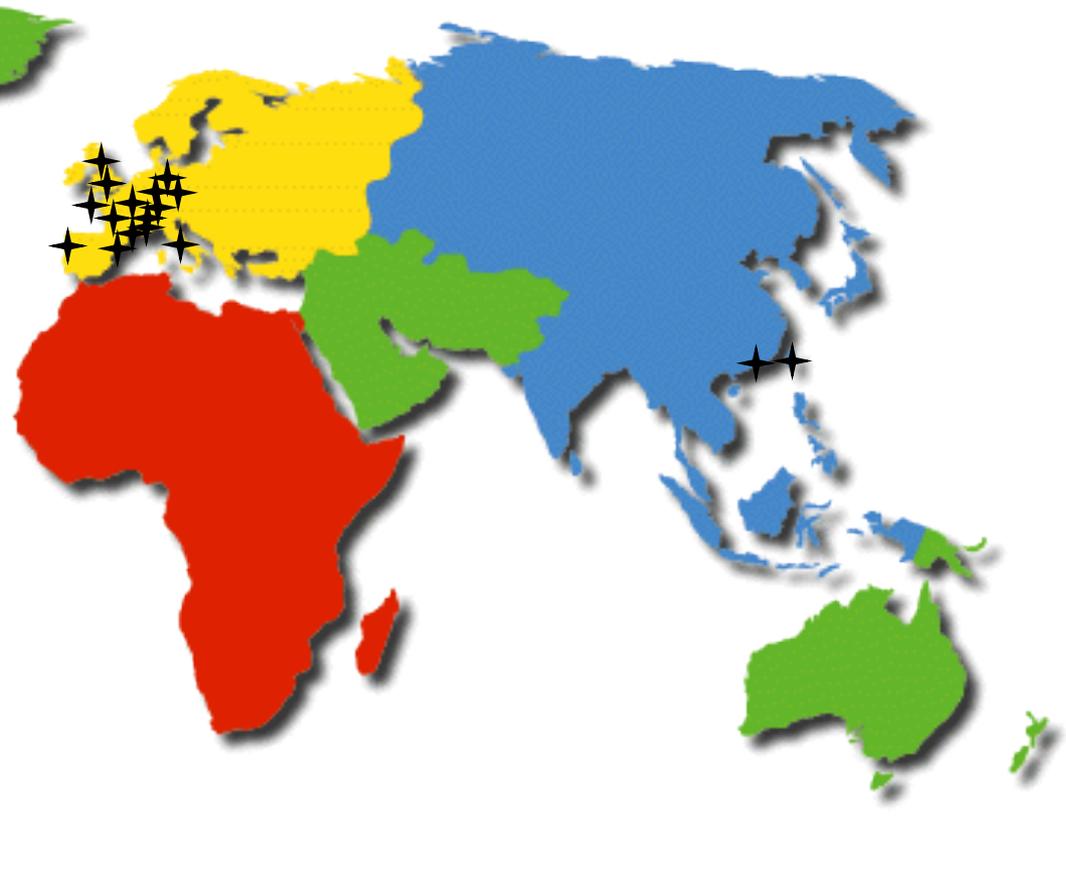
Amphioxus, *Branchiostoma lanceolatum*, genome project



From the
National
Geographic Society

Branchiostoma lanceolatum genome consortium

Ricard Albalat/Cristian Canestro/Josep Francesc Abril
Detlev Arendt/Elia Benito
Salvatore Daniello
Filipe Castro
Hector Escriva
Dave Ferrier
Jordi Garcia Fernandez
Ildiko Somorjai
Estelle Hirsinger
Peter Holland
Olivier Mirabeau
Zbynek Kozmik
Vincent Laudet
Patrick Lemaire
Julia Morales
Nadine Peyri eras
Pierre Pontarotti/Benedicte Wirth
Marc Robinson-Rechavi
Hugues Roest-Crollius
Herve Seitz
Michael Schubert
Tokiharu Takahashi
Herve Tostivint
Boris Lenhard
Jose Luis Gomez Skarmeta/Nacho Maeso
Manuel Irimia



46,573

COLUMBIA UNIVERSITY BIOLOGICAL SERIES. II.

AMPHIOXUS AND THE ANCESTRY
OF THE VERTEBRATES

BY

ARTHUR WILLEY, B.Sc.

TUTOR IN BIOLOGY, COLUMBIA COLLEGE; HALFOUR STUDENT OF THE
UNIVERSITY OF CAMBRIDGE

WITH A PREFACE

BY

HENRY FAIRFIELD OSBORN

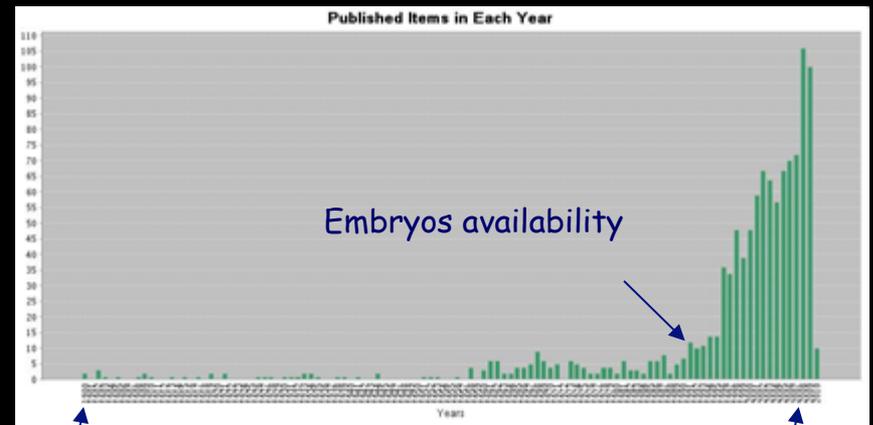


New York
MACMILLAN AND CO.
AND LONDON
1894

All rights reserved

Amphioxus:
Phylum: Chordata
Subphylum: Cephalochordata

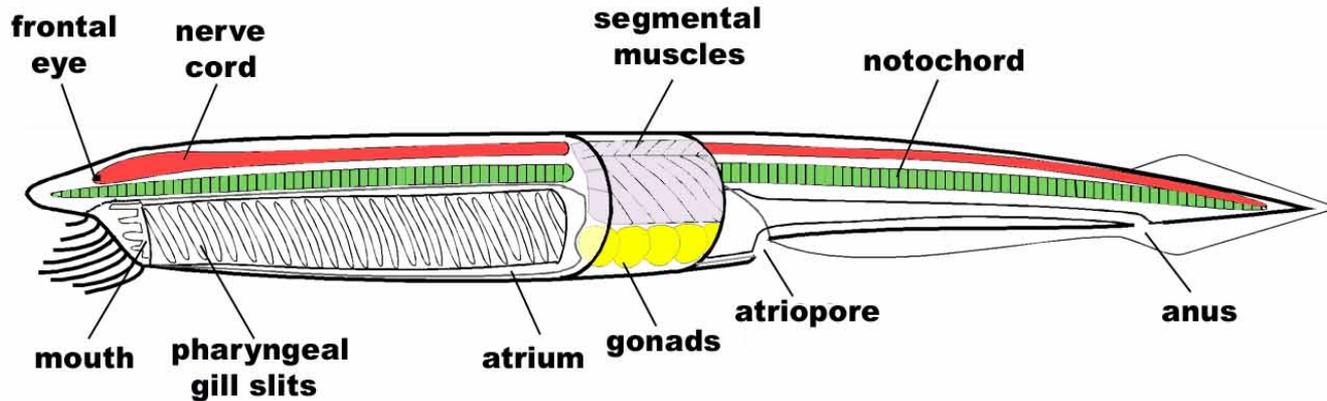
- Genus Branchiostoma, ~30 species
- Genus Epigonichthys, 1 specie
- Genus Asymmetron, 2 species
- Widely distributed in tropical and temperate seas
- Adults in sandy and shell-sand habitats
- 0,5-40? m deep
- Sexual reproduction,
- Separate sexes



1900

Genome sequence

Amphioxus Is vertebrate-like but simpler



Amphioxus has

- segmental muscles
- segmental gonads
- pharyngeal gill slits
- dorsal hollow nerve cord
- notochord
- pronephric kidney
- pituitary and thyroid homologs
- a prototypical vertebrate genome

Amphioxus lacks

- paired, image-forming eyes
- ears
- limbs
- neural crest
- the extensive gene duplications characteristic of vertebrates

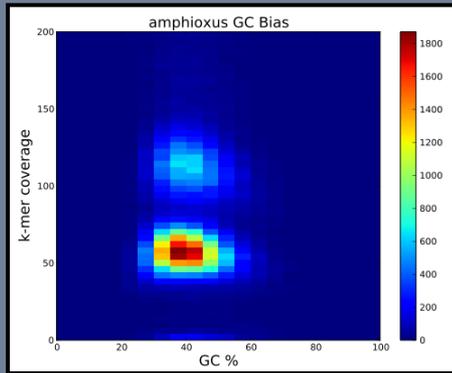
Amphioxus embryonic development is very fast and metamorphosis occurs 1-3 months after hatching



B. lanceolatum genome



DATA



Paired-end lib. 2
Mate-end lib. 6
Total sequence 85 Gb
Haploid coverage 150x
Est. genome size 570Mb
High polymorphism

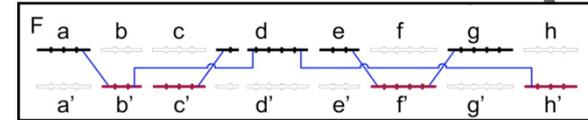
SOAPdenovo



Diploid assembly

48,019 scaff
1185Mb
N50: 529kp
3% NNNs

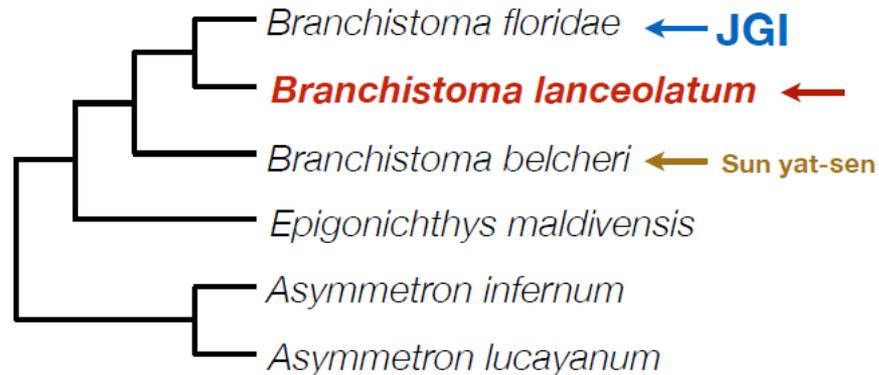
Haplomerger



Haploid assembly

10,247 scaff
495Mb size
N50 1.29Mb / L50 78
5% NNNs
90% cegma compl.

Cephalochordate genomics

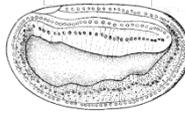


Species	Year	Institute	Technique	Coverage	Size	N50	L50
<i>B. floridae</i>	2006	JGI	Sanger	11.5x	522Mb	2.6Mb	62
<i>B. belcheri</i>	paper submitted	Sun yat-sen university	454/Illumina	23x / 50x	426Mb	2.4Mb	51
<i>B. lanceolatum</i>	en route	Génoscope	Illumina	150x	527Mb	1.13Mb	91
<i>A. lucayanum</i>	en route	L. Holland/ N. Putnam	454	2x (?)	-	-	-

A genome-wide regulatory map of the European amphioxus: Overview of the datasets.



Early gastrula (8h)



Early neurula (15h)



Premouth larva (36h)

ChIP-seq Histone
Modifications
(Skarmeta lab)

H3K4me3

H3K27ac

H3K27me3

Open chromatin
profiling
(Skarmeta lab)

ATAC-seq

Transcriptome
profiling

RNA-seq
(Irimia lab,
Garcia Fernandez
And others)

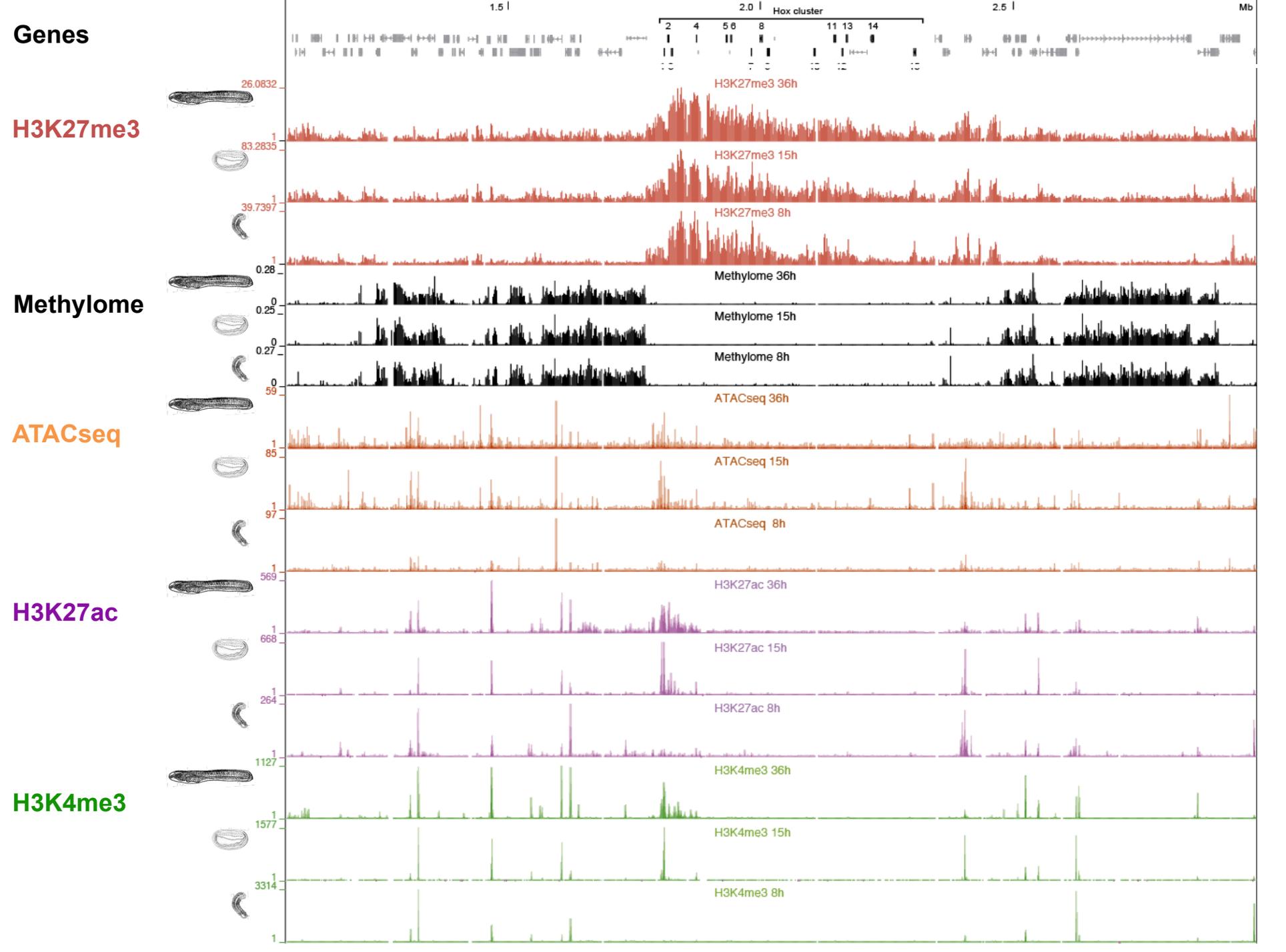
CAGE-seq
(Boris Lenhard lab)

Methylation
(Ryan Lyster lab)

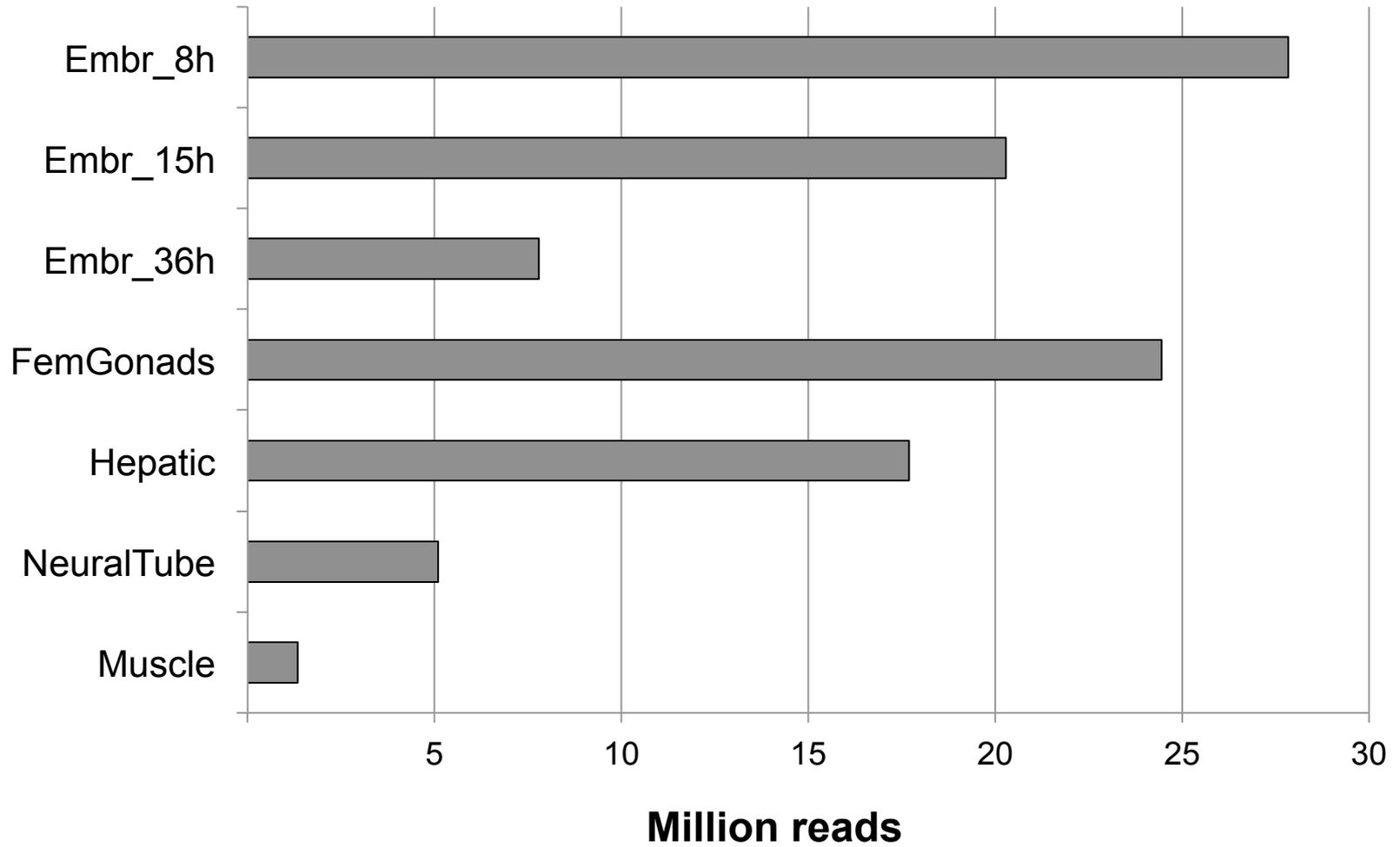
Single base
resolution
Methylome

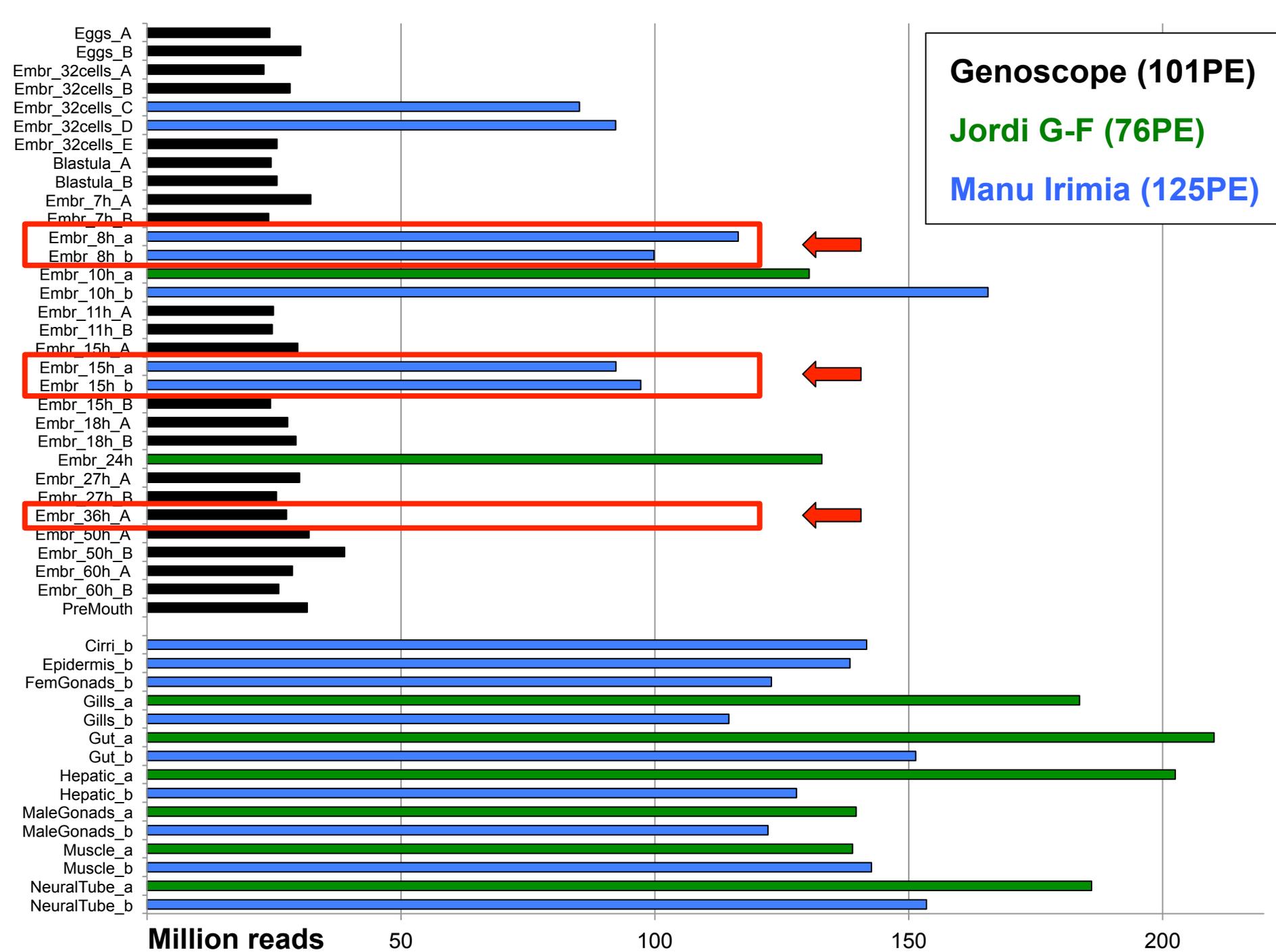
3D organization
of the chromatin
(Skarmeta lab)

4C-seq of
developmental
genes



CAGE-seq





Already implemented in different databases (still not public)

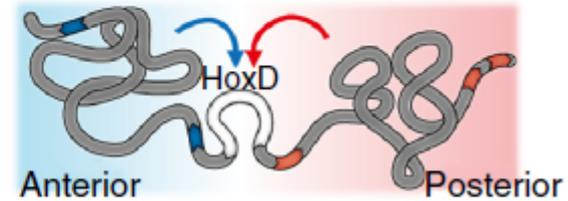
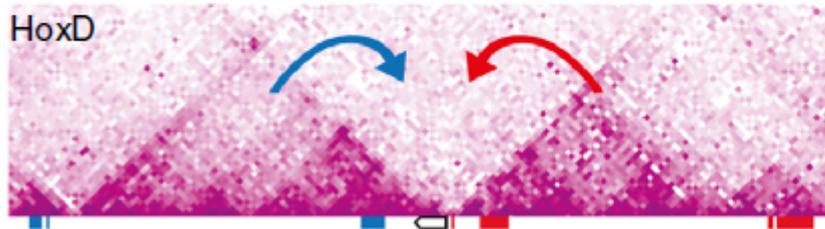
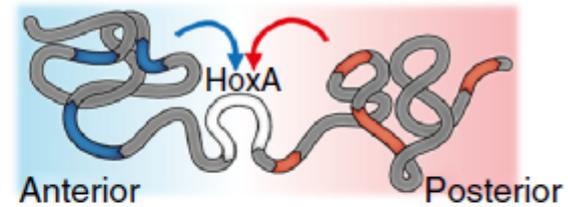
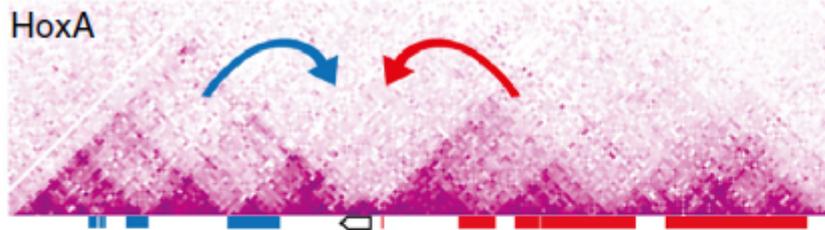
Collaboration with P. Lemaire

Collaboration with H. Roest Crolius

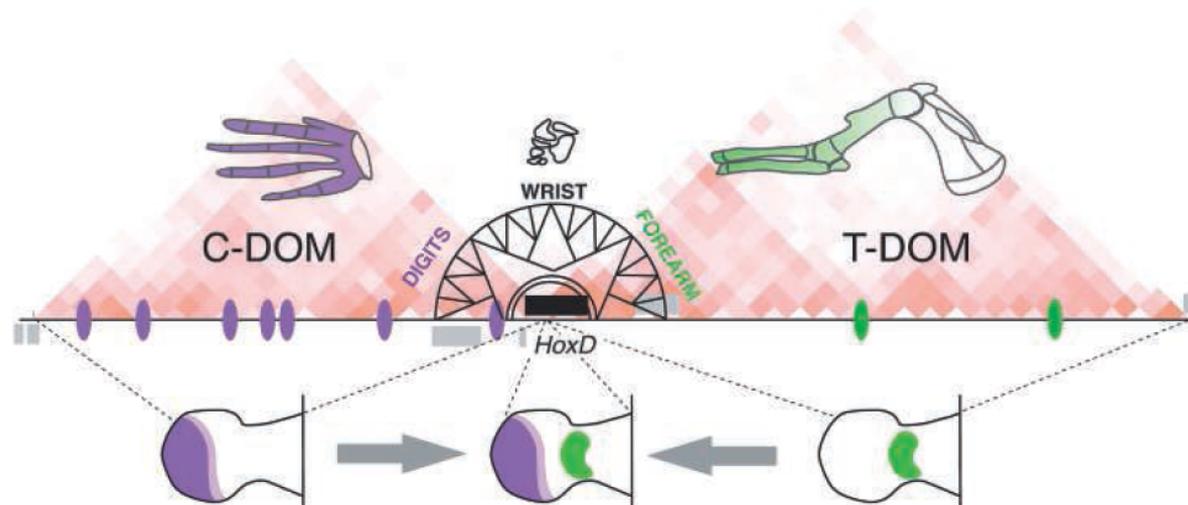
Collaboration with EBI

Vertebrate Hox clusters are organized into TADs

What about amphioxus Hox locus?



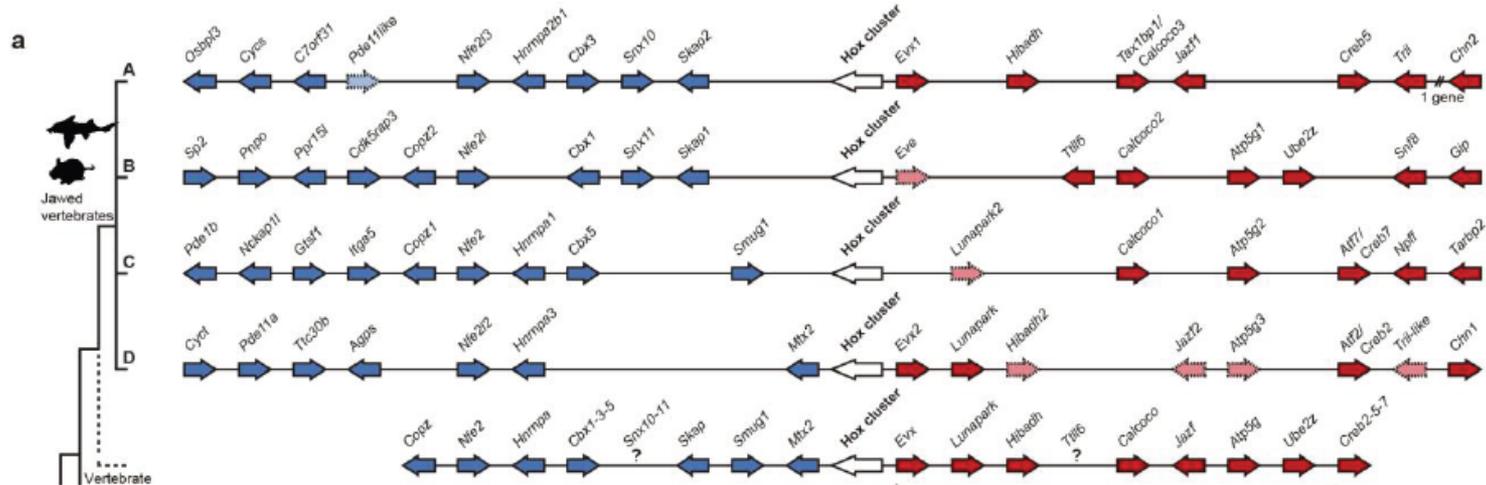
Acemel et al Nat Gen (2016)



Andrey et al Science (2013)

How the bimodal regulation of Hox genes evolved in vertebrates?

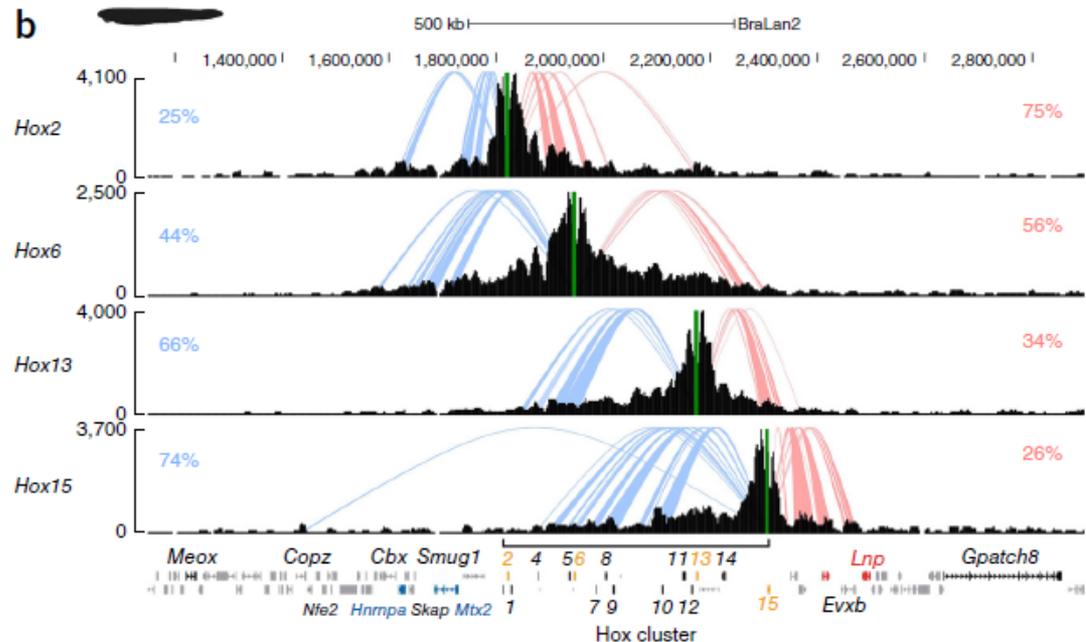
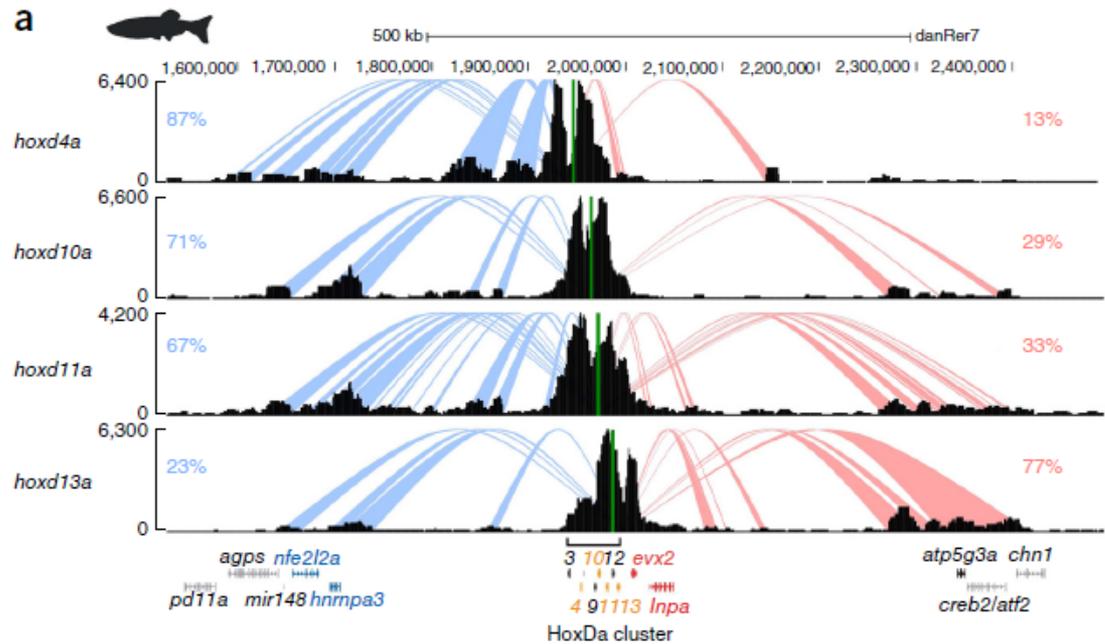
Acemel et al Nat Gen (2016)



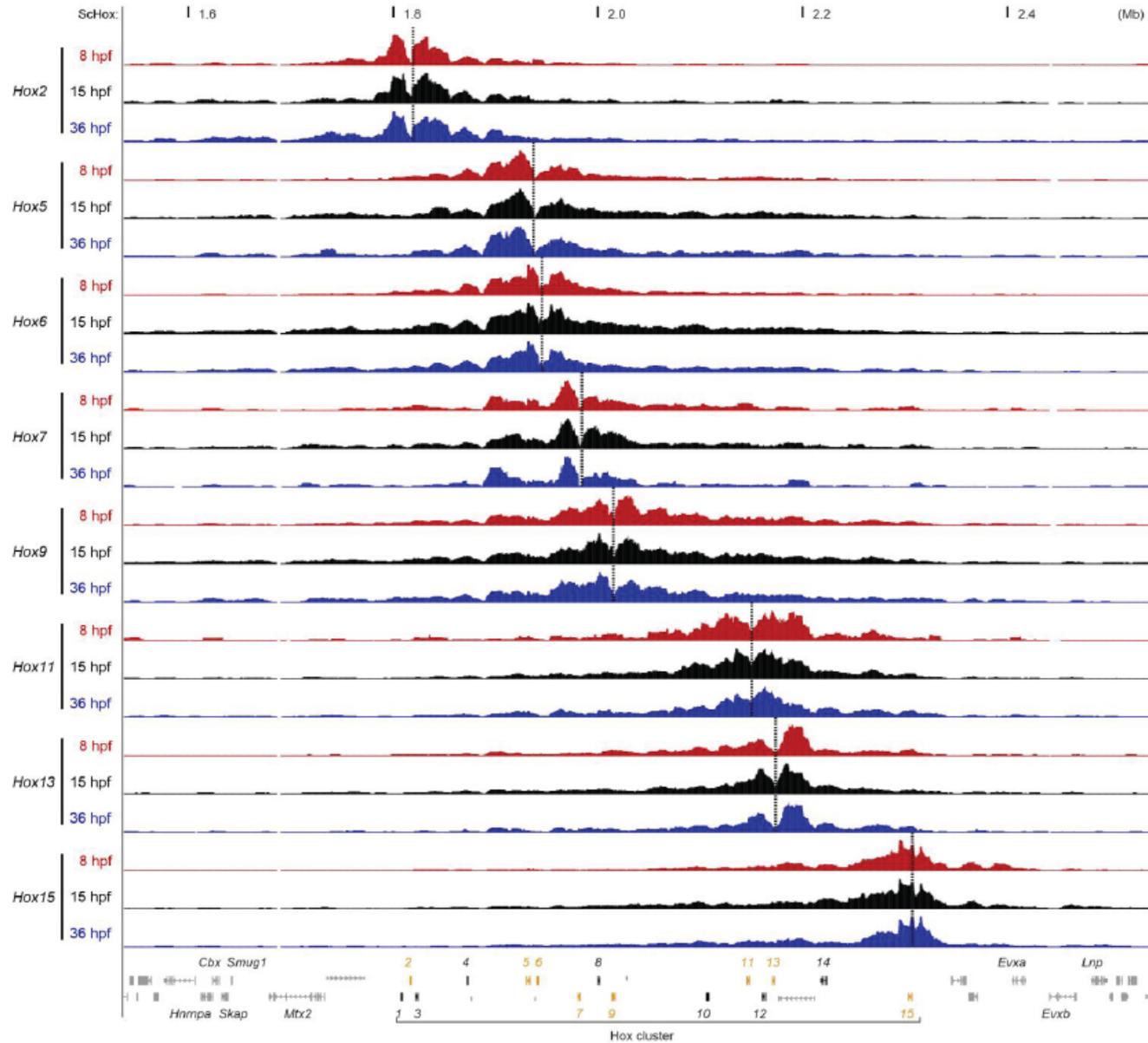
We tested conservation of the 3D structure of the Hox locus using 4-C seq

- 14 viewpoints (8 Hox genes and 6 neighboring genes) in three developmental stages in amphioxus
- 9 viewpoints (4 Hox genes and 5 neighboring genes) in zebrafish
- a total of 73 4C-seq were generated
- Chromatin contacts were quantified

While zebrafish interactions show the bimodal regulation characteristic of vertebrates, most of the interactions in amphioxus were within the Hox locus



Amphioxus 4C-seq profiles were similar irrespectively of their position in the cluster and developmentally stable

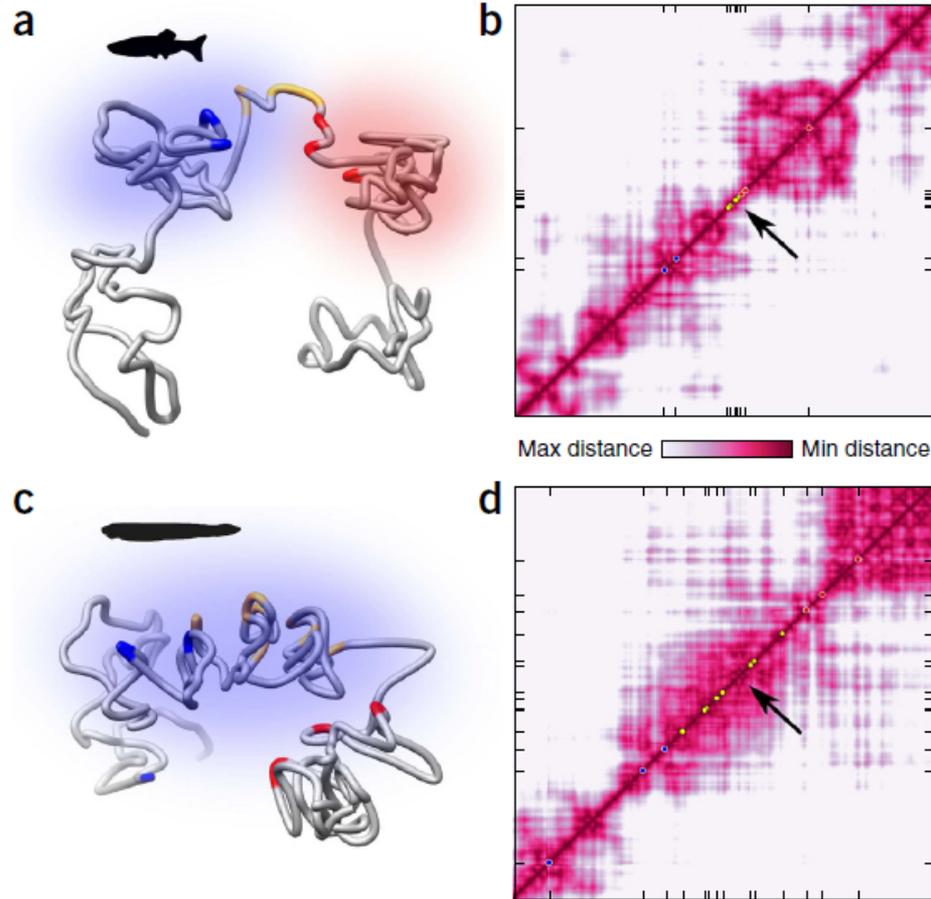


Although most contacts are within the Hox cluster, anterior and posterior sides behave differently with more contacts anteriorly

This is in agreement with the synteny conservation in the anterior region of the cluster

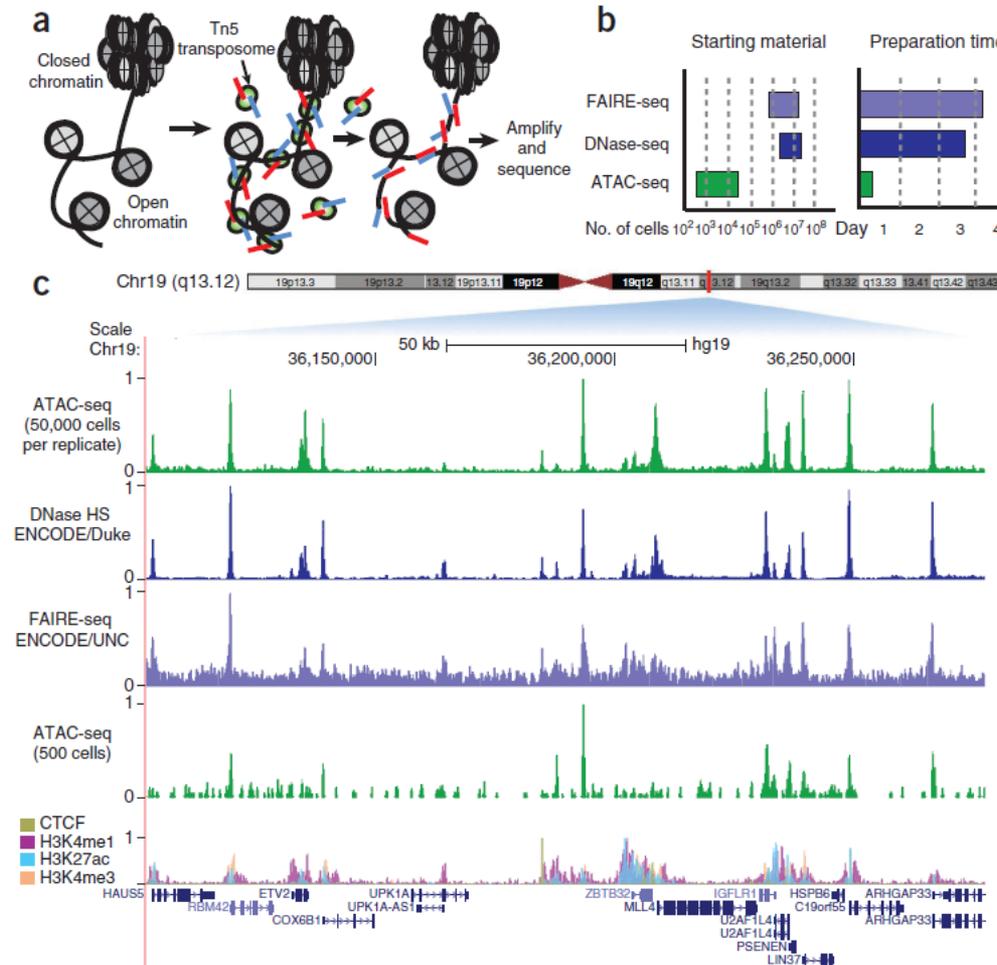
Viewpoints	Left	Right	Anterior	Cluster	Posterior
<i>Meox</i>	44.2%	55.8%	100.0%	0.0%	0.0%
<i>Hnrpa</i>	38.5%	61.5%	71.7%	25.6%	2.7%
<i>Mtx2</i>	30.9%	69.1%	41.2%	42.7%	16.1%
<i>Hox2</i>	24.8%	75.2%	24.8%	75.2%	0.0%
<i>Hox5</i>	38.5%	61.5%	18.8%	80.2%	1.0%
<i>Hox6</i>	44.1%	55.9%	24.7%	71.3%	4.0%
<i>Hox7</i>	51.6%	48.4%	21.6%	74.1%	4.3%
<i>Hox9</i>	46.9%	53.1%	14.1%	83.2%	2.6%
<i>Hox11</i>	62.1%	37.9%	0.0%	99.5%	0.5%
<i>Hox13</i>	65.7%	34.3%	1.7%	88.7%	9.5%
<i>Hox15</i>	74.2%	25.8%	5.3%	68.9%	25.8%
<i>Evxa</i>	66.1%	33.9%	11.6%	45.6%	42.8%
<i>Lnp</i>	73.0%	27.0%	6.4%	35.5%	58.1%
<i>Gpatch8</i>	50.6%	49.4%	12.0%	3.5%	84.5%

Virtual Hi-C shows that amphioxus Hox locus is included in a single domain including the anterior region, while zebrafish contains two domains



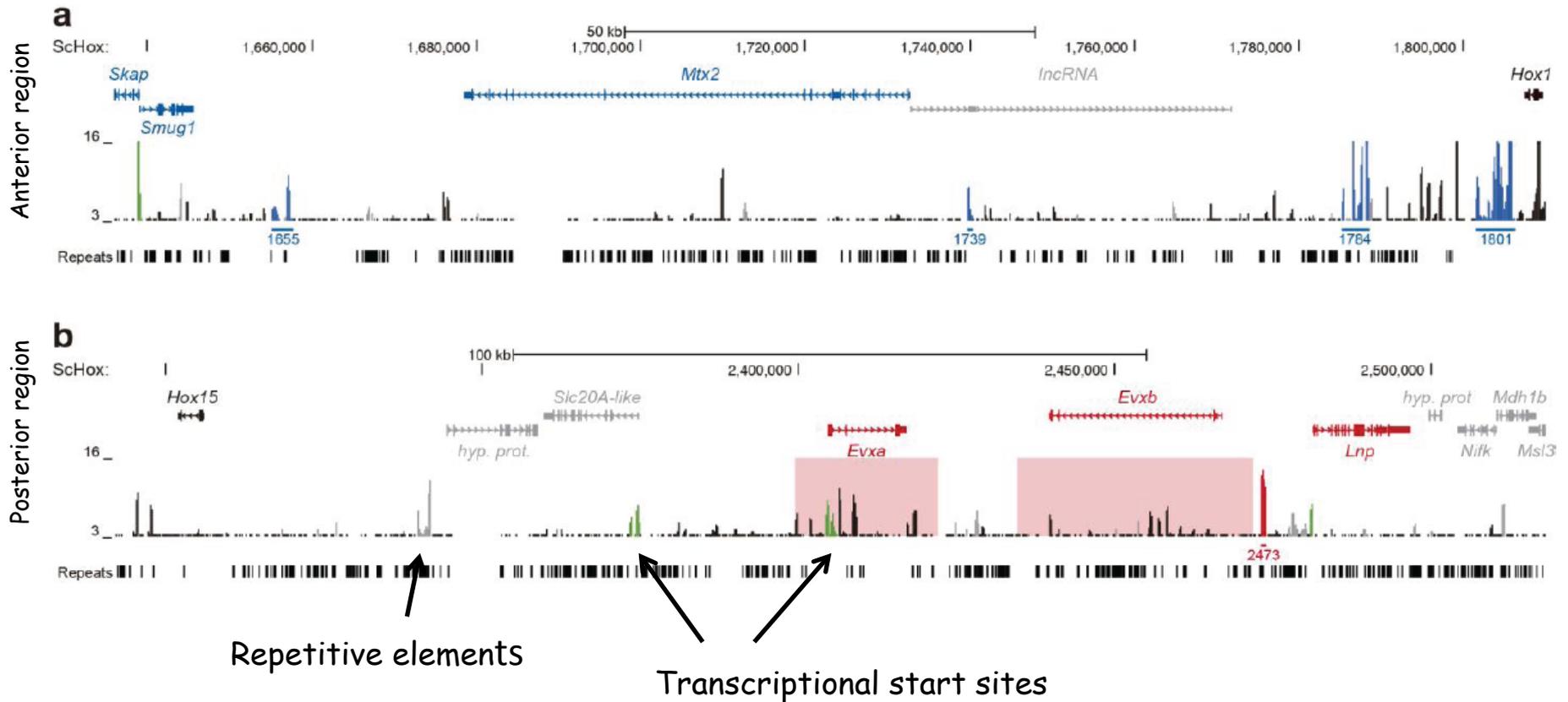
If this is true, we should find regulatory elements anteriorly but not posteriorly

ATAC-seq (assay for transposase-accessible chromatin using sequencing)

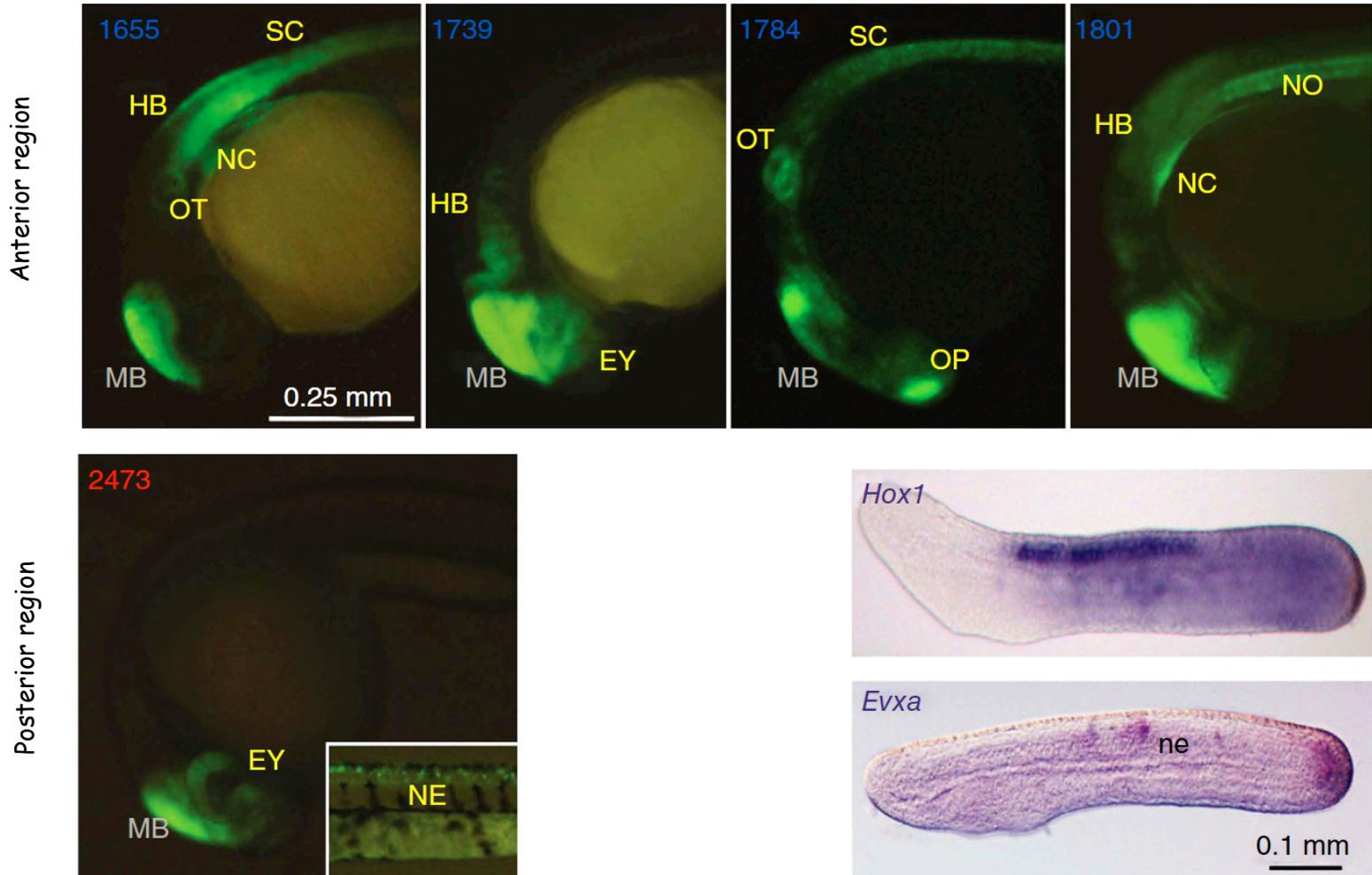


From Buenrostro et al 2013, Nat Met

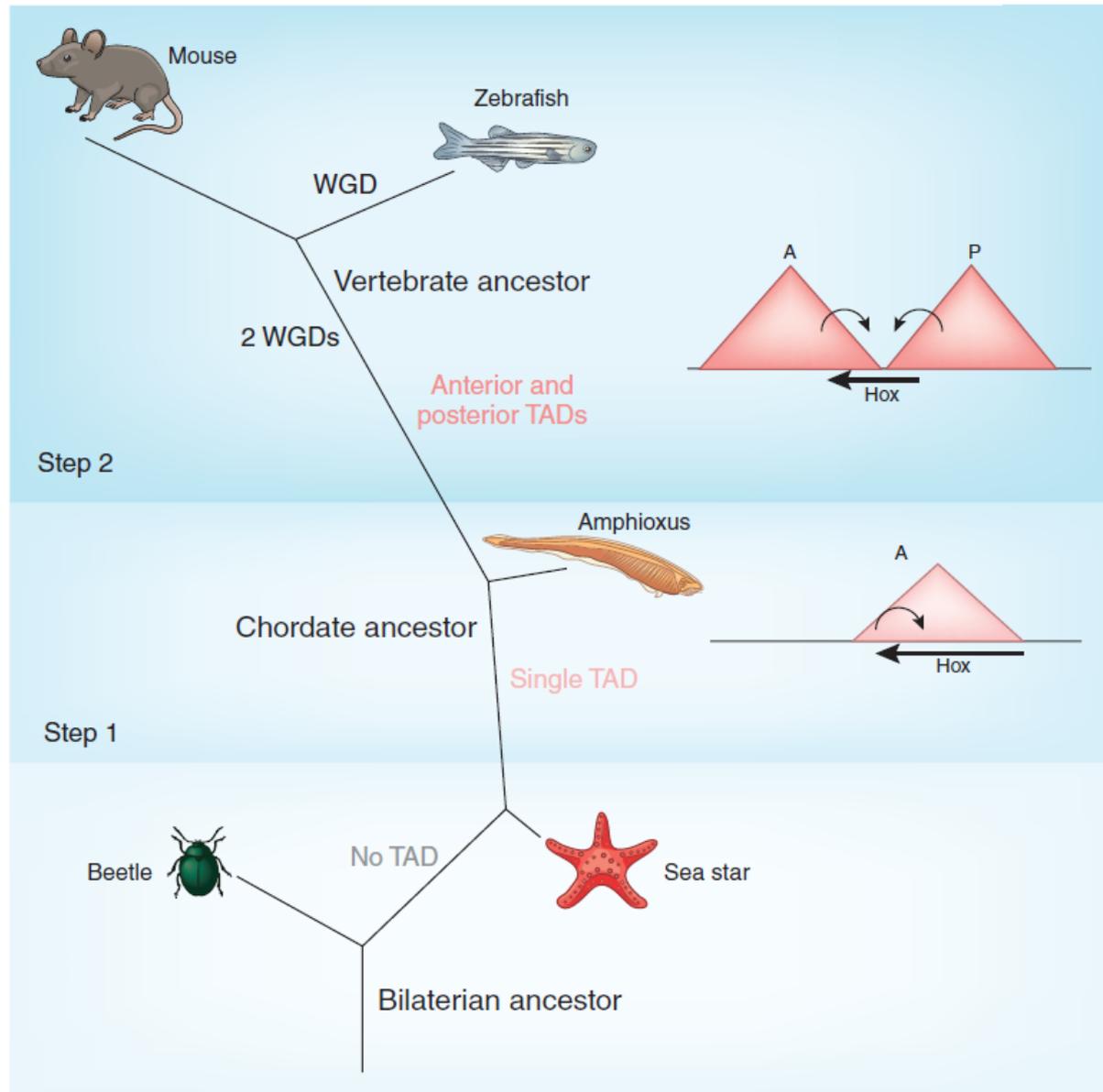
Accessible chromatin regions obtained by ATAC-seq analyses are only present in anterior region



Accessible chromatin regions obtained by ATAC-seq analyses are only present the in anterior region



Stepwise evolution of the bimodal regulatory machineries of HoxA and HoxD clusters in vertebrates



Thanks to:



Centro Andaluz de Biología del Desarrollo
Sevilla, Spain

Rafael D Acemel
Juan J Tena
Ibai Irastorza-Azcarate
Carlos Gómez-Marín
Elisa de la Calle-Mustienes
Sergio G Diaz
Damien P Devos
Ignacio Maeso
José Luis Gómez-Skarmeta



Ferdinand Marlétaz
Peter W H Holland



Jean-Marc Aury
Sophie Mangenot



Stéphanie Bertrand
Daniel Aldea
Lucie Subirana
Anthony Leon

