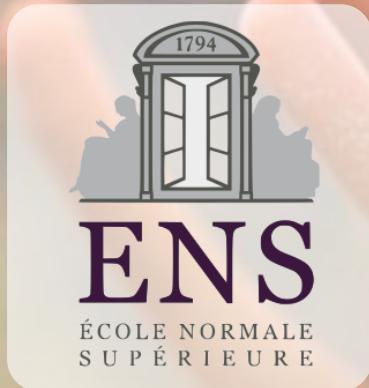
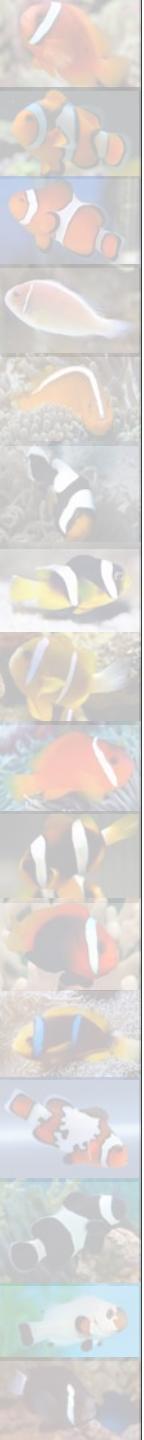


# Coloration: a great system to study genotype-phenotype relationships

M1 – ENS  
Module "Genomes and Phenotypes"  
Nov. 14<sup>th</sup> 2019

Thibault Lorin





OBSERVATOIRE  
OCÉANOLOGIQUE  
de Banyuls/Mer  
1882



Laudet's lab



(No lab)



Institut de Génomique  
Fonctionnelle de Lyon



Volf's lab

# Introduction



"Coloration" or "pigmentation"?

---



# "Structural colors"

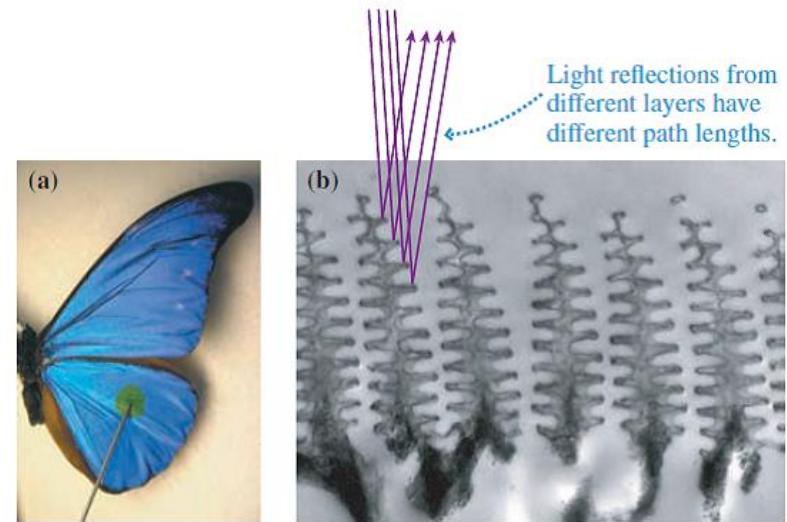


FIGURE P17.73



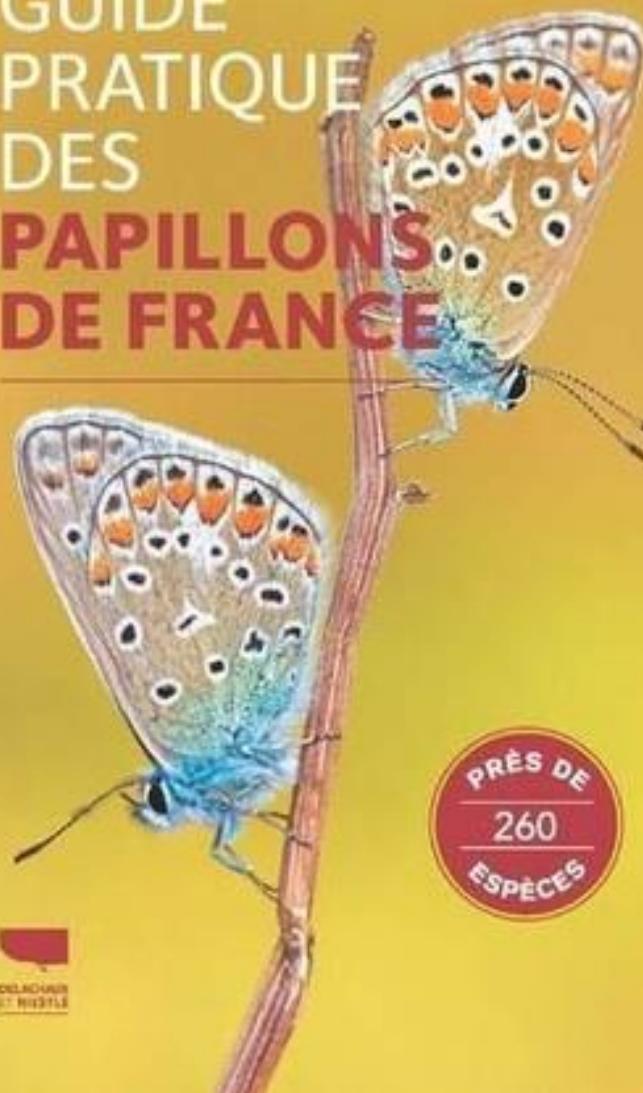
## GUIDE DELACHAUX



DELACHAUX  
ET FILS

JEAN-PIERRE MOUSSUS, THIBAULT LORIN, ALAN COOPER

# GUIDE PRATIQUE DES **PAPILLONS** **DE FRANCE**



PRÈS DE  
260  
ESPÈCES

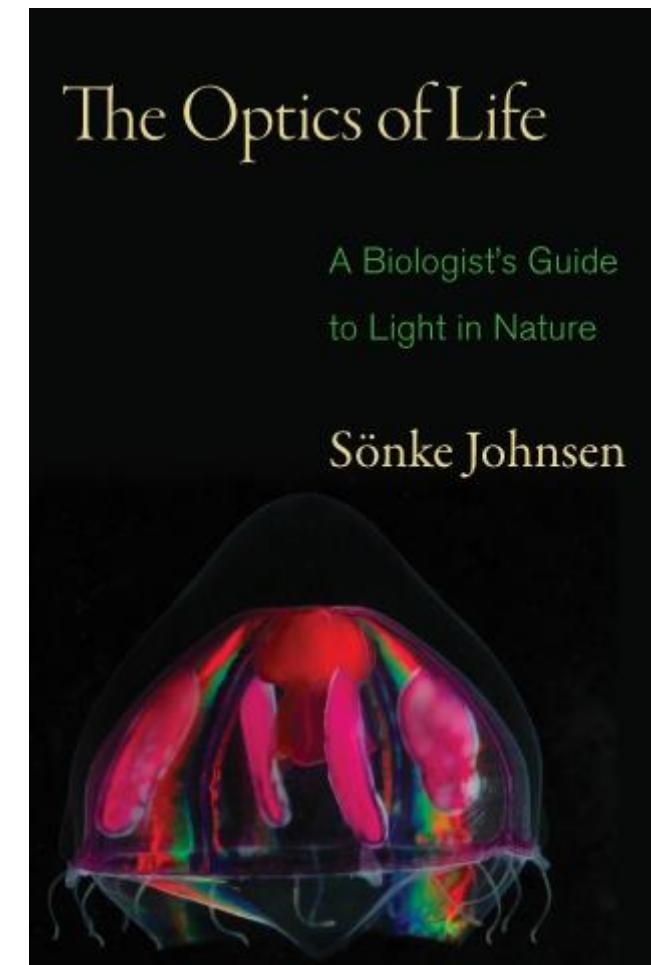


DELACHAUX  
ET FILS

# "Coloration" ≠ "pigmentation"



"Coloration" ≠ "pigmentation"



2011

# Genotype-phenotype relationships

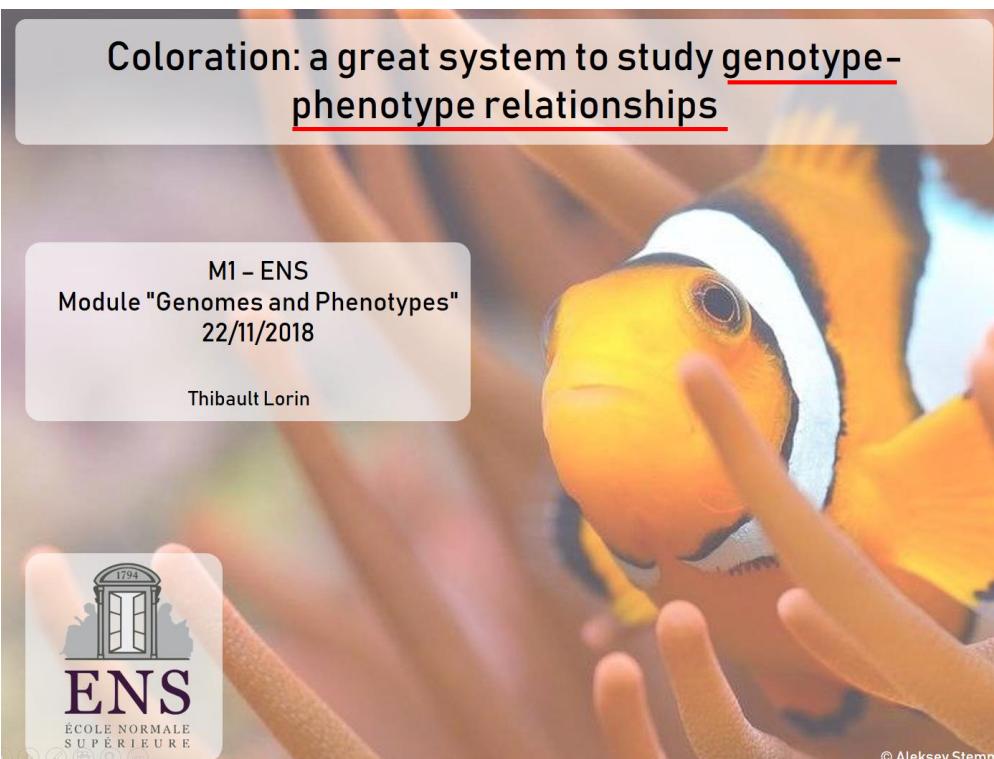
Coloration: a great system to study genotype-phenotype relationships

M1 – ENS  
Module "Genomes and Phenotypes"  
22/11/2018

Thibault Lorin



Relationships between genes and the phenotype, and their modulation by the environment



# Genotype-phenotype relationships

(more detail and info here)



HYPOTHESIS AND THEORY  
published: 19 May 2015  
doi: 10.3389/fgene.2015.00179

## The differential view of genotype–phenotype relationships

*Virginie Orgogozo<sup>1\*</sup>, Baptiste Morizot<sup>2</sup> and Arnaud Martin<sup>3</sup>*

<sup>1</sup> CNRS, UMR 7592, Institut Jacques Monod, Université Paris Diderot, Paris, France, <sup>2</sup> Aix Marseille Université, CNRS, CEPERC UMR 7304, Aix en Provence, France, <sup>3</sup> Department of Molecular Cell Biology, University of California, Berkeley, CA, USA

## Introduction

I. Coloration: a great system to study genotype-phenotype relationships

II. A case study: clownfish coloration

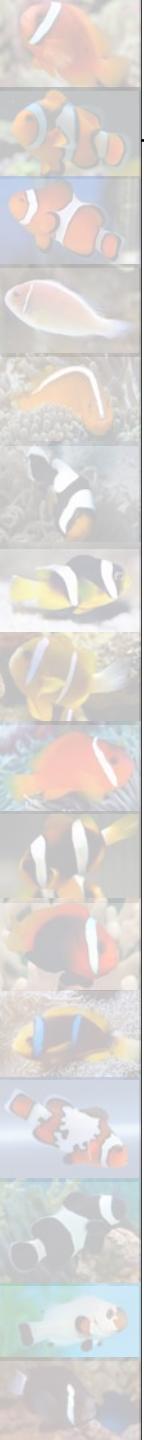


## Introduction

### I. Coloration: a great system to study genotype-phenotype relationships

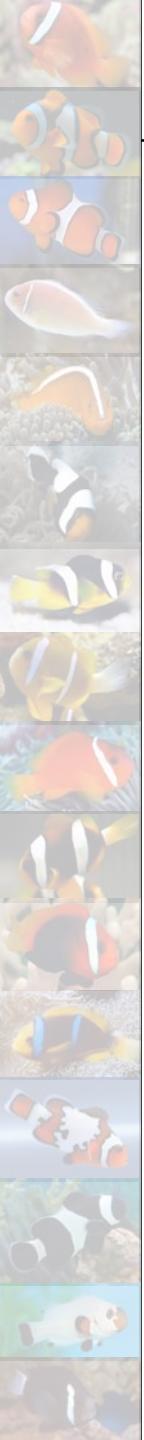
- a. Coloration in the History of Science
- b. Coloration in Vertebrates





## Why study coloration?

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## Why study coloration?

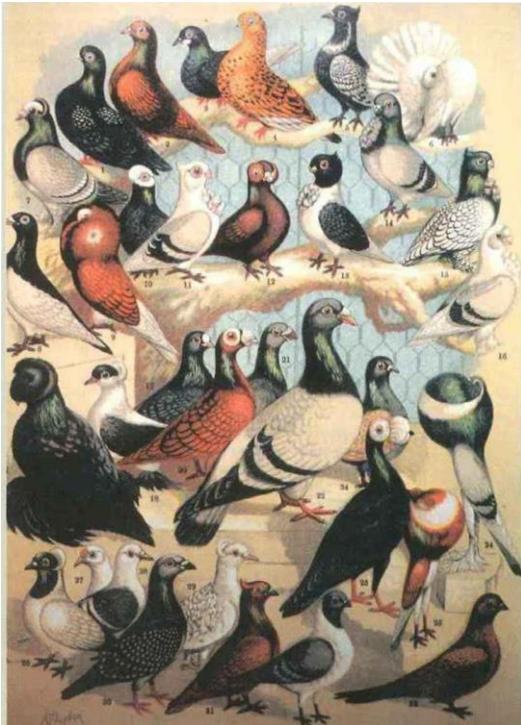
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→ Aesthetic

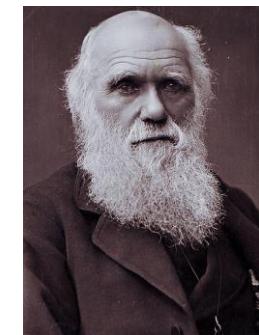
→ Profitable

→ Convenient

## Coloration and naturalists' interest



*Whitwell Elwin*



*Charles Darwin*

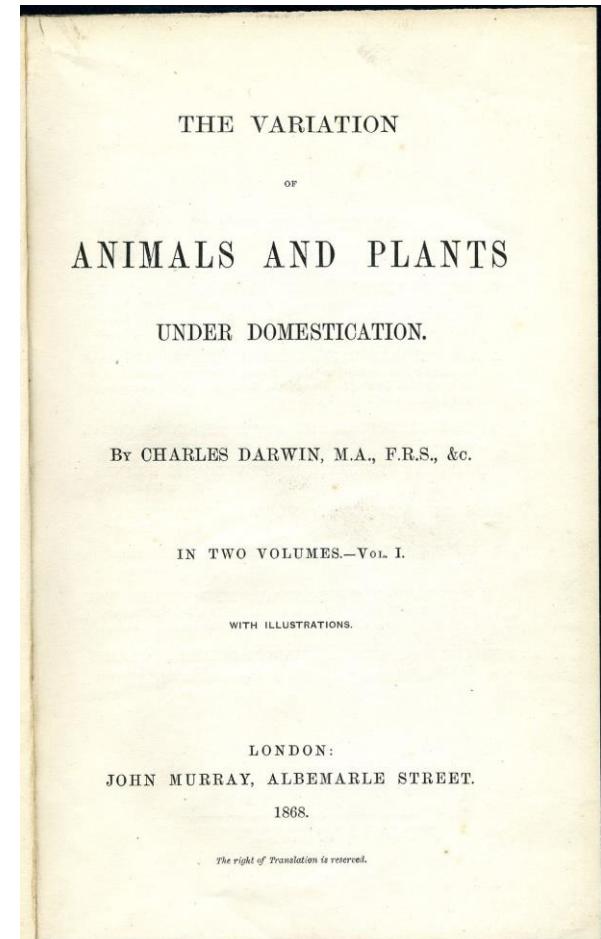
*“Everybody is interested in pigeons”.  
[A book like this would] “be reviewed  
in every journal in the kingdom and  
soon be on every table.”*

# Coloration and naturalists' interest

COLUMBA LIVIA OR ROCK-PIGEON.

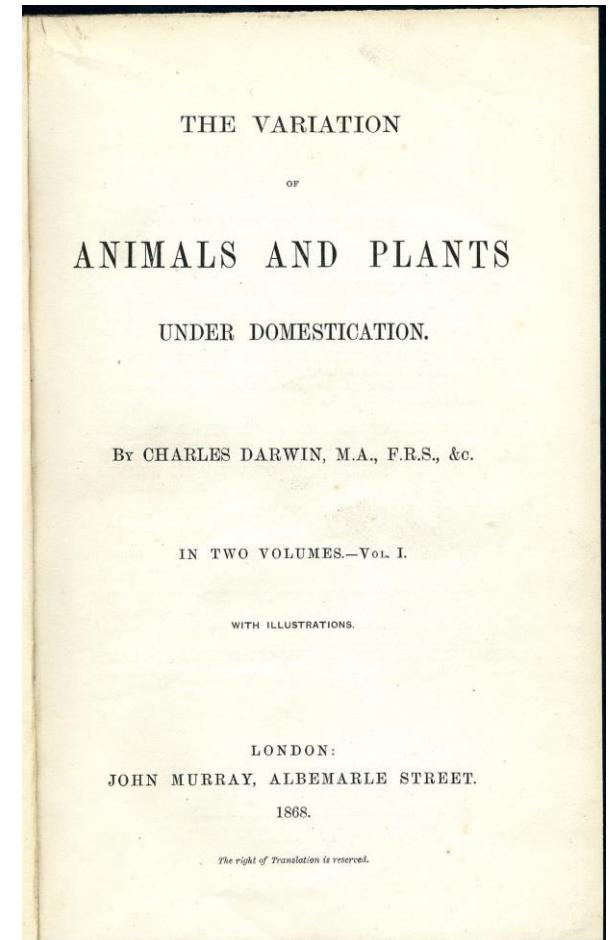
GROUP I.		GROUP II.		GROUP III.			GROUP IV.			DOMESTIC PIGEONS :	CHAR. V	
1.	SUB-GROUPS.	2.	3.	4.	5.	6.	7.	8.	9.	SUB-GROUPS.	10.	11.
Kali-Par.						Persian Tumbler				Dove and Pigeon		
Murasia.						Lotan Tumbler				Stoolion.		
Bussorah.						Common Tumbler				Spit.		
Gorman P.										Nim.		
Lille P.										English Frill-neck		
Dragon										Lapinger.		
Pigeon										Trumpeter.		
Cygne												
Dutch P.												
English Pouter.												
English Carrier.												
Raut.												
Barb.												
Fantail.												
African Owl.												
Short-Faced Tumbler.												
Indian Frill-back.												
Jacobin.												
Turbit												

*"I do not hesitate to affirm that some domestic races of the rock-pigeon differ fully as much from each other in external characters as do the most distinct natural genera."*

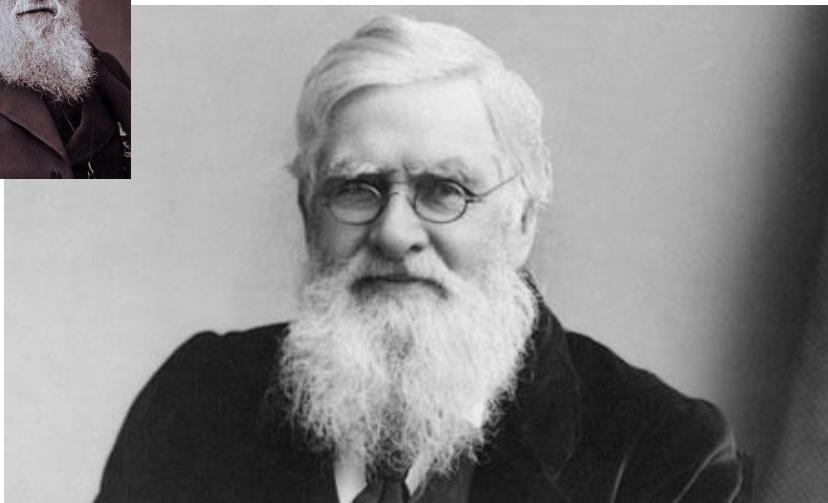
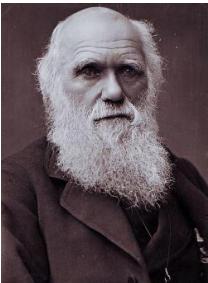


# Coloration and naturalists' interest... also in plants!

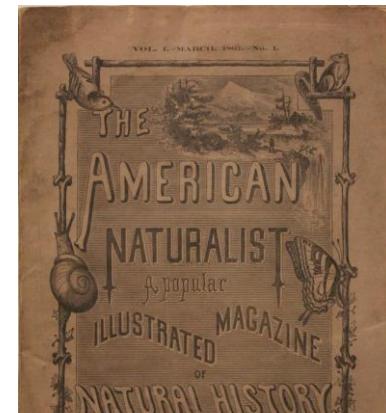
*Maize has varied in an extraordinary and conspicuous manner .... The seeds are coloured – white, pale-yellow, orange, red, violet ...*



# Wallace and "coloration"



Alfred R. Wallace (1823–1913)

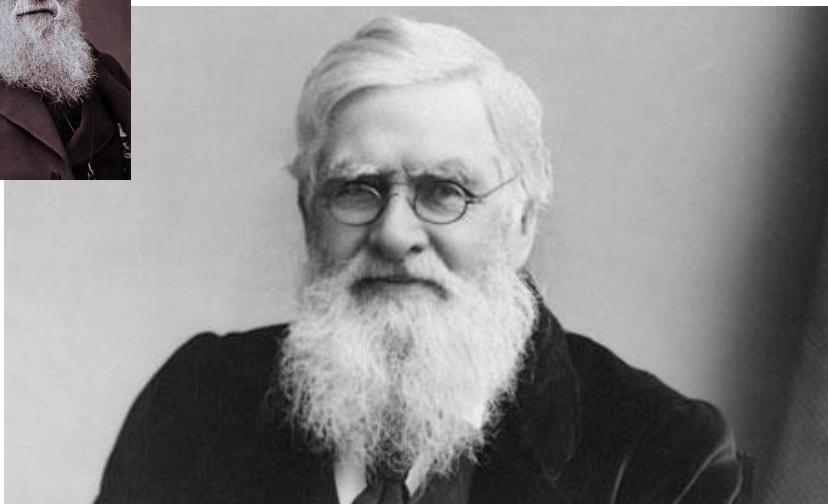
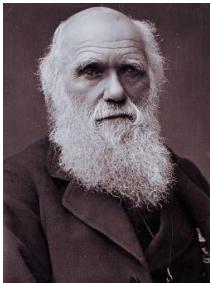


VOL. XI.—NOVEMBER, 1877.—No. 11.

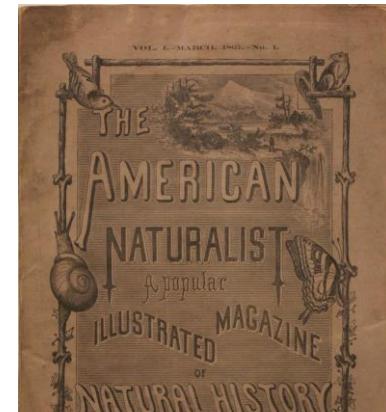
THE COLORS OF ANIMALS AND PLANTS.<sup>1</sup>

BY ALFRED RUSSEL WALLACE.

# Wallace and "coloration"



Alfred R. Wallace (1823–1913)



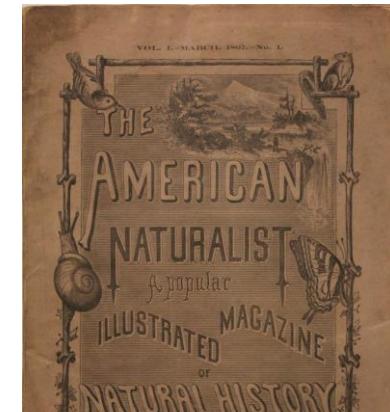
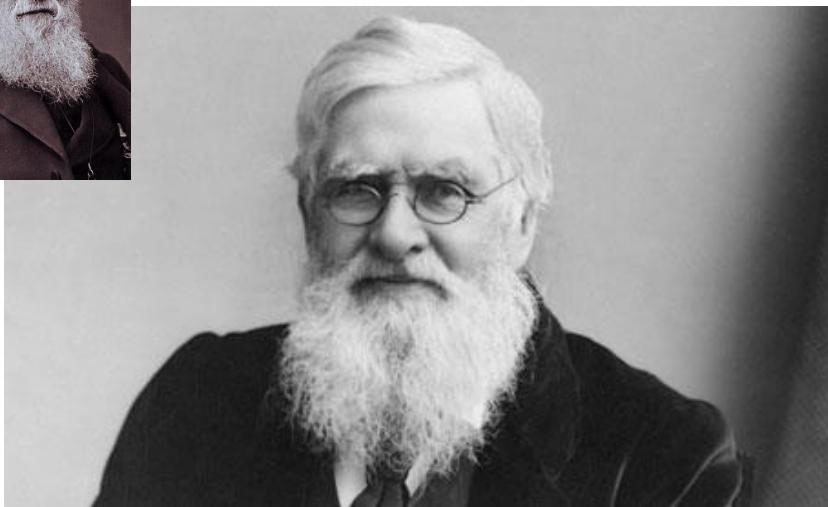
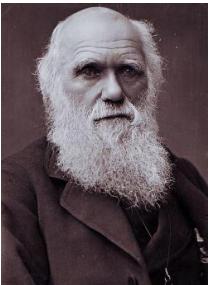
VOL. XI.—NOVEMBER, 1877.—No. 11.

THE COLORS OF ANIMALS AND PLANTS.<sup>1</sup>

BY ALFRED RUSSEL WALLACE.

- Animals. { 1. Protective colors.  
          2. Warning colors.  
          3. Sexual colors.  
          4. Typical colors.  
Plants.     5. Attractive colors

# Wallace and "coloration"



VOL. XI.—NOVEMBER, 1877.—No. 11.

THE COLORS OF ANIMALS AND PLANTS.<sup>1</sup>

BY ALFRED RUSSEL WALLACE.

Alfred R. Wallace (1823–1913)



Camouflage

Animals.

- 1. Protective colors.
- 2. Warning colors.
- 3. Sexual colors.
- 4. Typical colors.



Intra- or Interspecific  
communication colors

## Opinion

# Wallace on Coloration: Contemporary Perspective and Unresolved Insights

Tim Caro<sup>1,2,\*</sup>

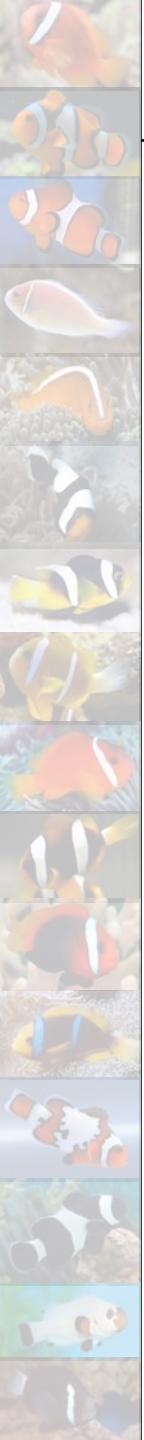
I examine Alfred Russel Wallace's six biological categories of coloration of animals and plants, and review how they have been developed over the subsequent century and a half. These categories are: protective colors; warning colors; mimicry; sexual colors; 'typical colours'; and attractive colors in flowers and fruits. Incredibly, Wallace missed little in his appraisal of the evolutionary drivers of coloration, despite being out of step with modern sexual selection theory, and his categories still characterize much of the way that this burgeoning field is organized. Even now his encyclopedic knowledge of natural history raises intriguing functional questions about coloration that still demand investigation.

### Trends

Wallace wrote extensively on animal and plant coloration and organized the field into six functional categories that are still highly relevant.

These categories are crypsis, aposematism, mimicry, sexually selected traits, coloration involved in inter- and intraspecific signaling, and floral and fruit colors.

While understanding of coloration in



# Coloration and the History of the comprehension of GP relationships

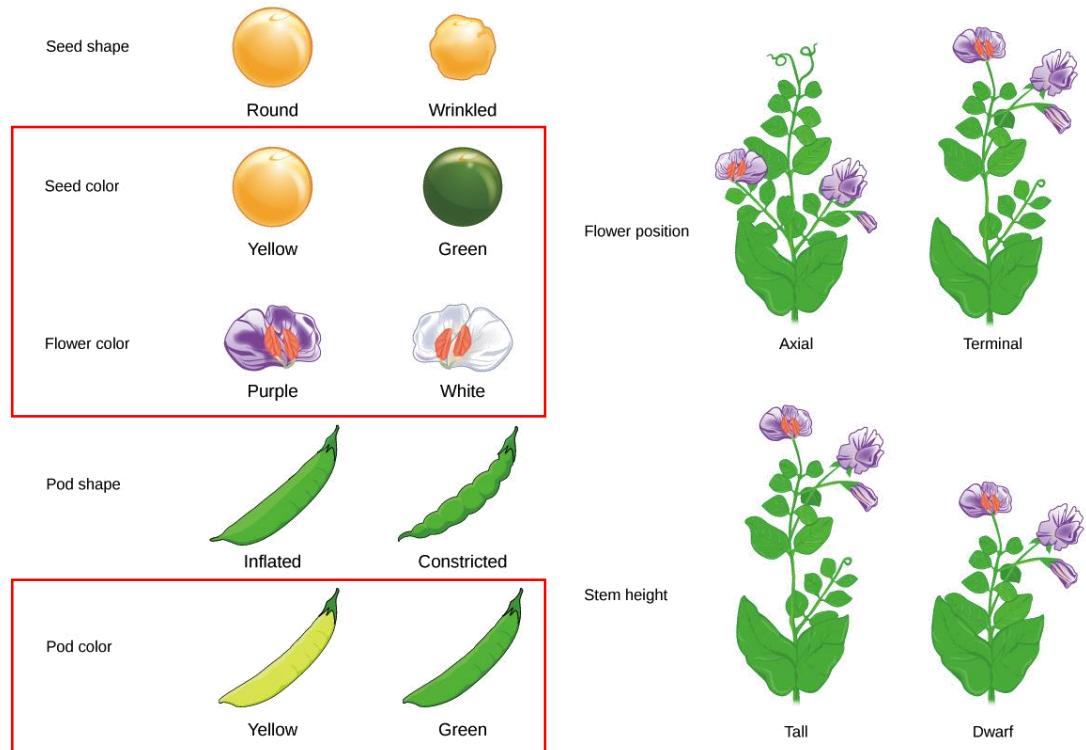
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# Mendel



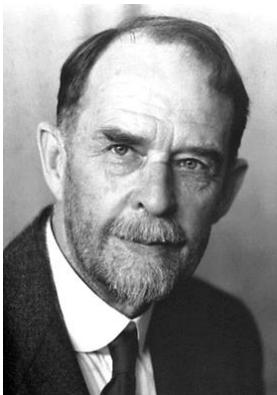
**Gregor Mendel**  
**(1822-1884)**

"Father of modern  
genetics"  
→ Discovery of "heredity  
factors"



→ Coloration at the basis of the Mendel's Laws of Inheritance:  
Law of Segregation, Law of Independent Assortment, Law of Dominance

# Morgan



Thomas Hunt  
MORGAN  
(1866-1945)



Lilian MORGAN  
(1870-1952)

1909 (Nobel 1933)

AMERICAN BREEDERS' ASSOCIATION.

365

## WHAT ARE "FACTORS" IN MENDELIAN EXPLANATIONS?

BY PROF. T. H. MORGAN.

*Columbia University, New York, N. Y.*

In the modern interpretation of Mendelism, facts are being transformed into factors at a rapid rate. If one factor will not explain the facts, then two are invoked; if two prove insufficient, three will sometimes work out. The superior jugglery sometimes necessary to account for the results may blind us, if taken too naively, to the common-place that the results are often so excellently "explained" because the explana-



*white gene*

# McClintock



*Barbara McClintock  
(1902-1992)*

**THE ORIGIN AND BEHAVIOR OF MUTABLE LOCI IN MAIZE**

BY BARBARA McCLINTOCK

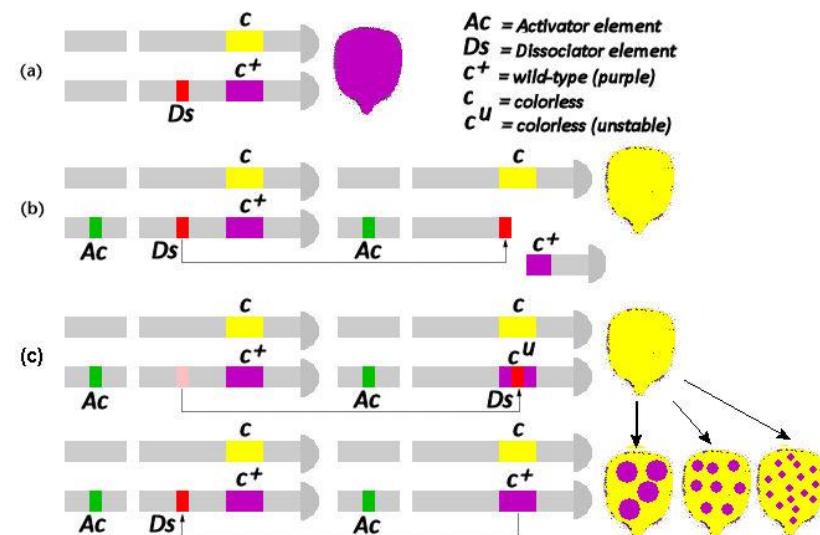
DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION, COLD SPRING HARBOR, NEW YORK

Communicated April 8, 1950

In the course of an experiment designed to reveal the genic composition of the short arm of chromosome 9, a phenomenon of rare occurrence (or recognition) in maize began to appear with remarkably high frequencies in the cultures. The terms mutable genes, unstable genes, variegation,



*Maize variegation*



# McClintock



*Barbara McClintock  
(1902-1992)*

→ Coloration at the basis of the discovery of major genome sequences, transposable elements ("controlling elements")



*Maize variegation*

## CLASS I - Retroelements (RNA intermediate)

Endogenous retrovirus (HIV, HERV)



Ty3/gypsy - BEL transposons



Ty1/copia retrotransposons



DIRS1-like retrotransposons



Non-LTR retrotransposons (L1, L2)



Penelope-like retrotransposons



Non-autonomous retrotransposons (SINEs)

(Alu, B2, B4)

## CLASS II - DNA transposons

DDE transposons (piggyBac, Mariner, Transib)



Helitrons



Polintons / Mavericks



Non-autonomous DNA transposons (MITEs)  
(MADE1)



# Coloration and the History of genetics: a summary (in Vertebrates only)

**Table 1** Timeline of some milestones and fundamental discoveries using pigmentation phenotypes

Date	Milestone	Representative reference
1700s	Establishment of laboratory mouse strains with 'fancy' coat color patterns	Morse (1978)
1902–1904	Demonstration of <u>Mendelian inheritance in vertebrates</u> using the albino locus	Castle and Allen (1903), Cuenot (1904)
1915	Establishment of genetic linkage in mammals using two pigmentation loci	Haldane <i>et al</i> (1915)
1917	Seminal papers on coat color genetics in laboratory animals	Wright (1917a, b, c, d)
1920s	Natural history studies linking vertebrate pigmentation to environmental variation	Sumner (1921, 1929a, b), Benson (1933), Dice and Blossom (1937)
1948	First mathematical treatment of clinal variation based on adaptive pigmentation traits	Haldane (1948)
1950s	First estimate of radiation-induced <u>mammalian mutation rates</u> at six coat color loci	Russell (1951), Russell and Major (1957)
1960s	Estimates of spontaneous mammalian mutation rates using coat color phenotypes	Schlager and Dickie (1966, 1969)
1986	Cloning of the first pigmentation gene	Shibahara <i>et al</i> (1986)
2000s	Linking mutations in pigmentation genes to adaptive phenotypic variation in the wild	

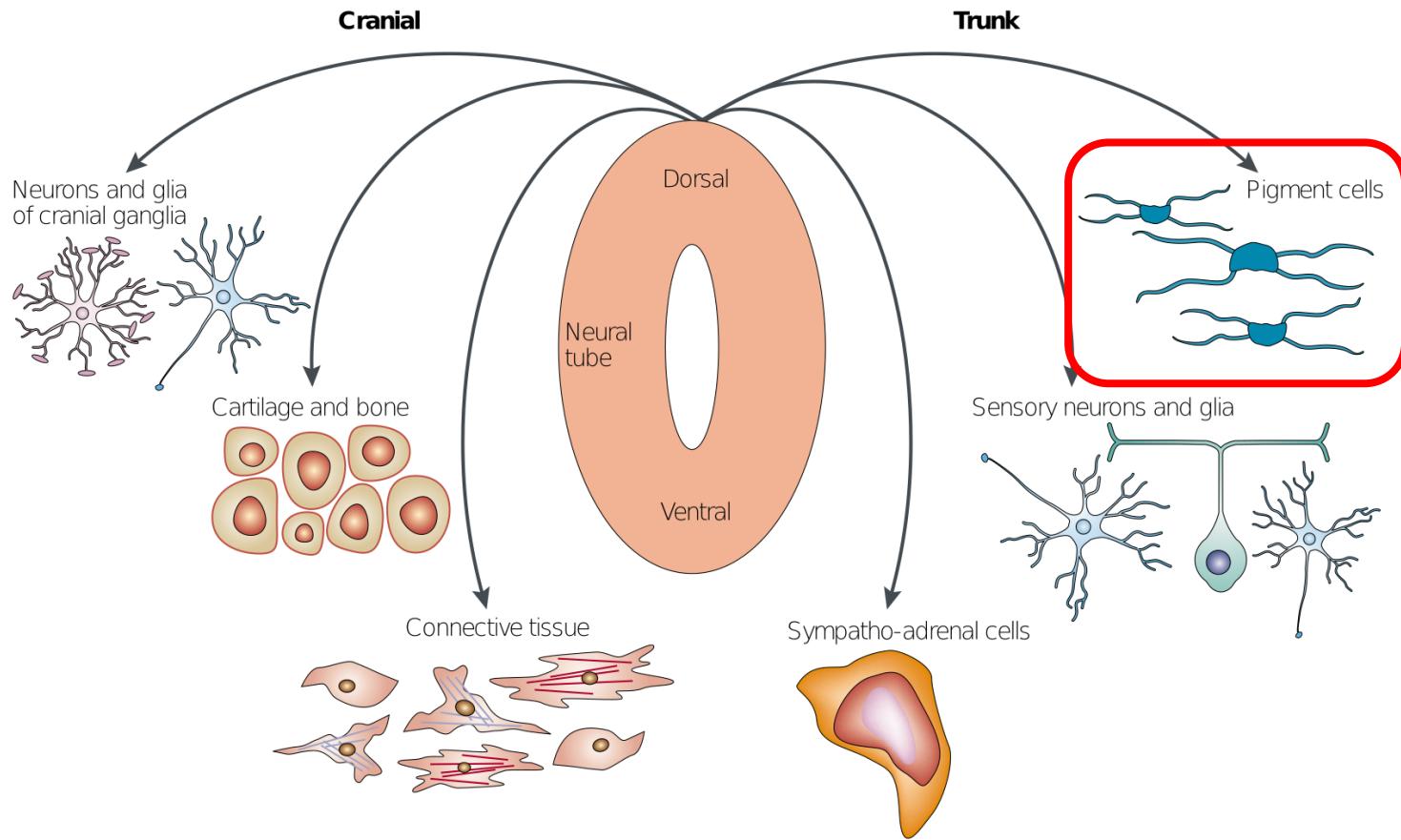
## Introduction

### I. Coloration: a great system to study genotype-phenotype relationships

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# Pigment cells in vertebrates originate from the neural crest



PLANET  
VIE

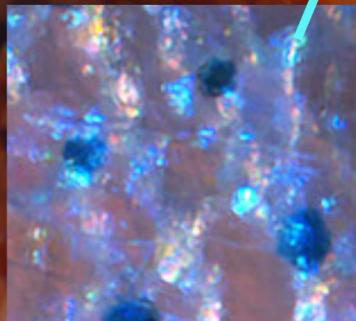
Les cellules des crêtes neurales : « la seule chose intéressante » chez les Vertébrés ?

Thibault Lorin

Xanthophore



Iridophore

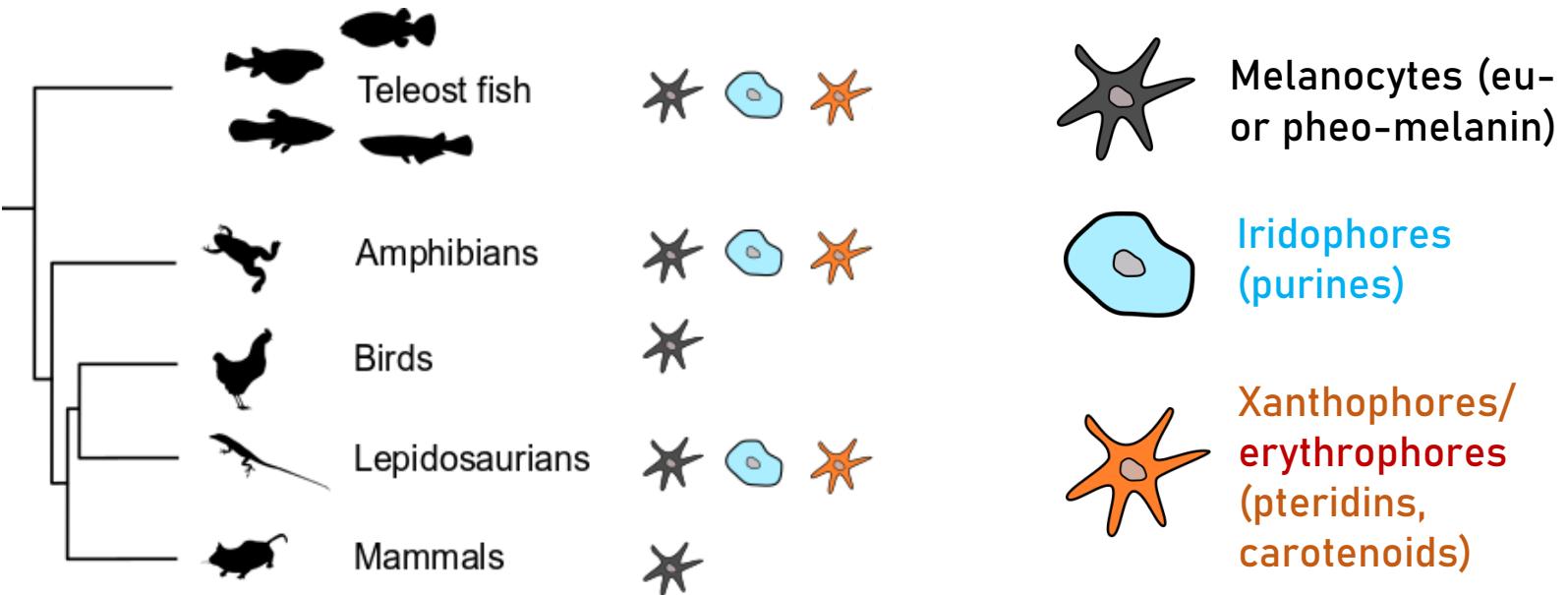


Mélanocyte

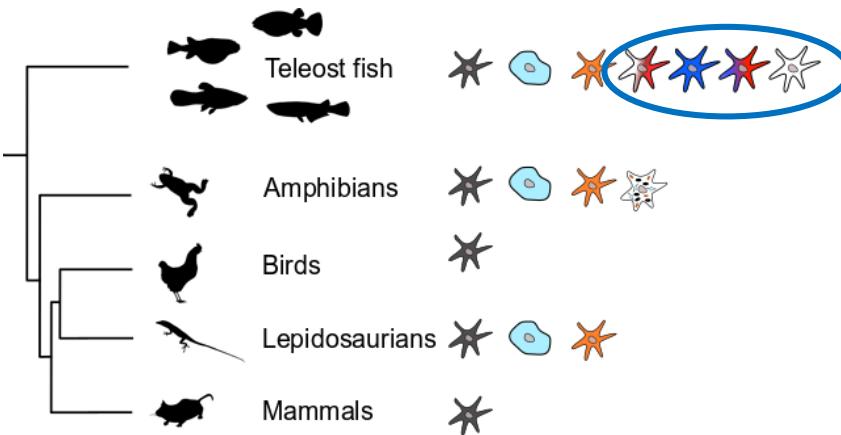
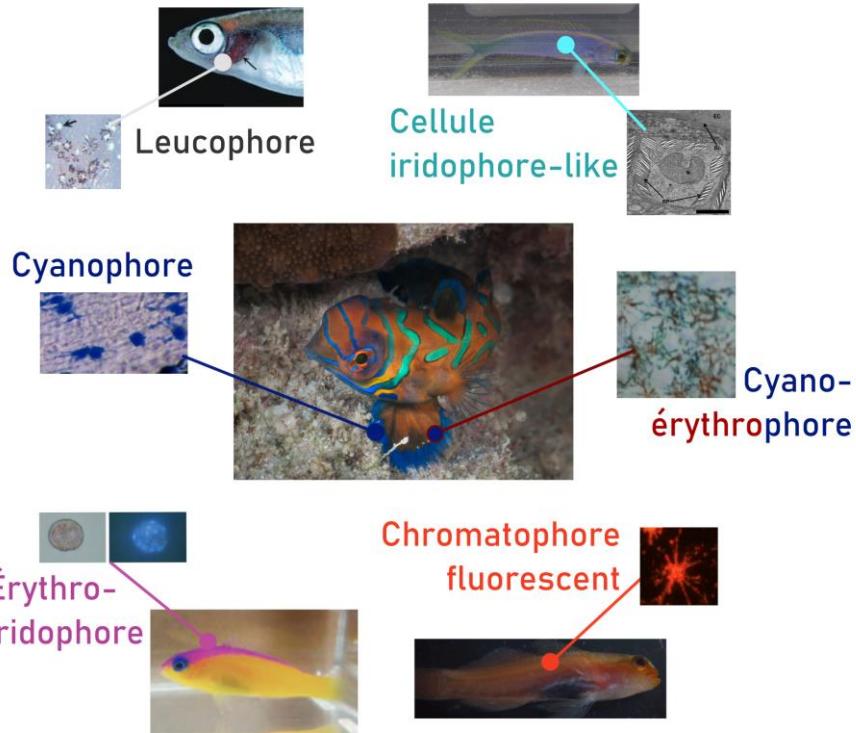
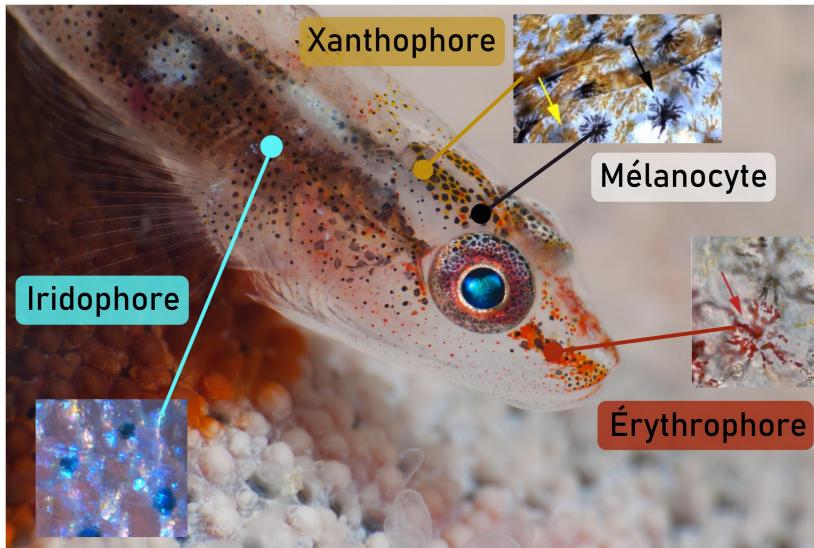
Érythrophore

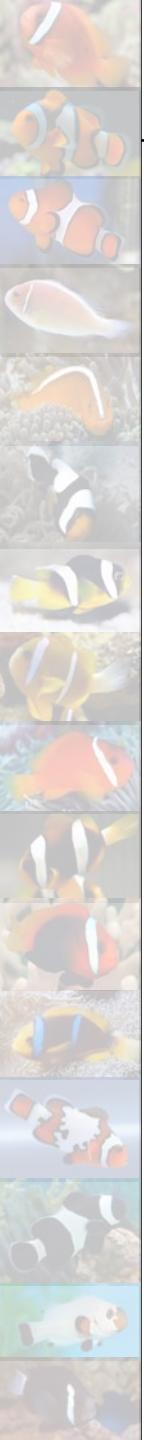


# Developmental, cellular and molecular basis of pigmentation in Vertebrates

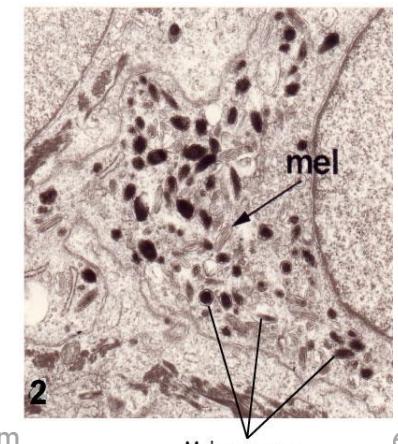
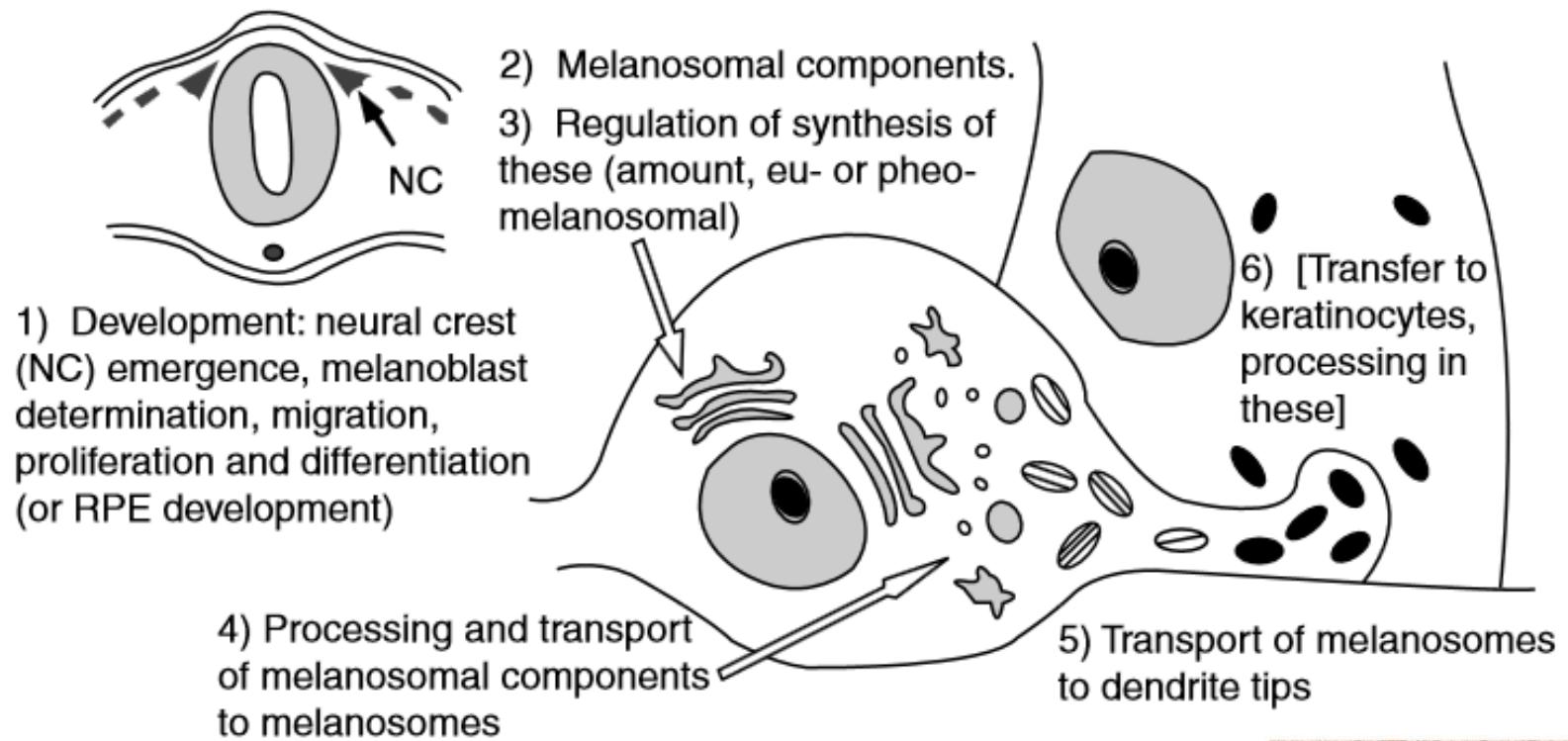


# Developmental, cellular and molecular basis of pigmentation in Vertebrates





## Coloration is a very diverse trait...

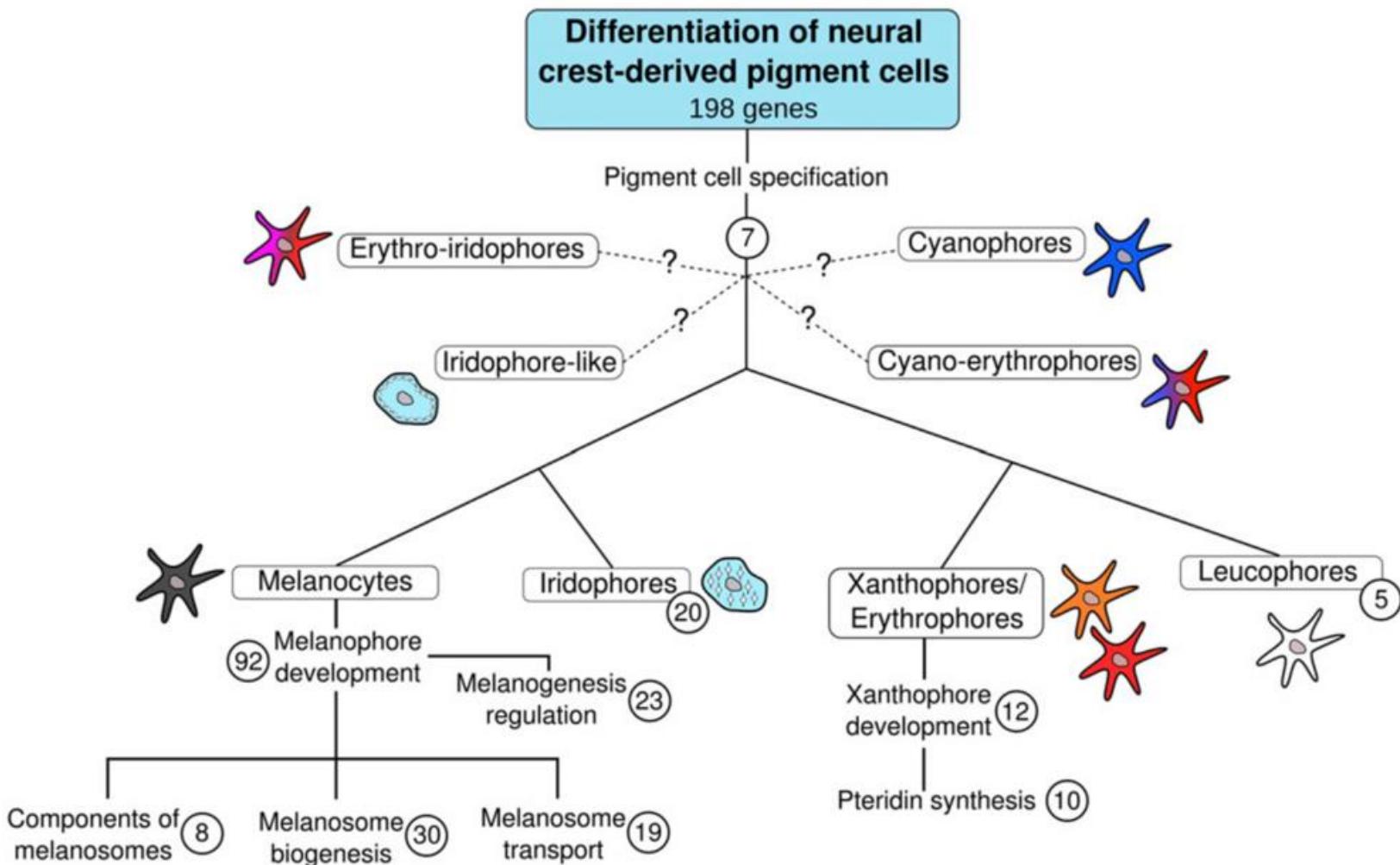


# Coloration is a very diverse trait... encoded by a plethora of genes

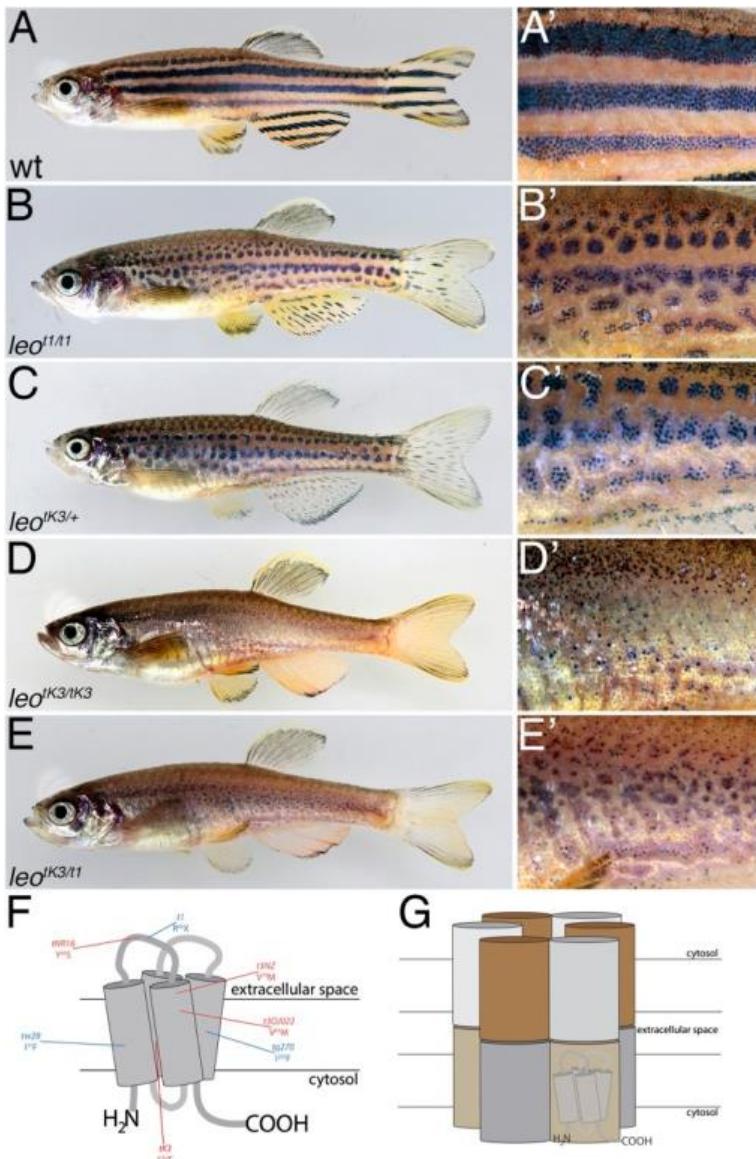
Table 1. Summary of the cloned mouse color genes

Symbol (old symbol)	Name (old name)	Chromosome	Function	Human symbol	Human chr'some	Syndrome
<b>(a) Melanocyte development</b>						
<i>Adam17</i>	A disintegrin and metalloprotease domain 17	12	Protease, processing various bioactive proteins	<i>ADAM17</i>	2p25	N
<i>Adamts20 (bt)</i>	A disintegrin and metalloprotease domain (reprolysin type) with thrombospondin type 1 motif, 20 (belted)	15	Metalloprotease. Melanoblast migration?	<i>ADAMTS20</i>	12q12	N
<i>Brcal</i>	Breast cancer 1	11	Development of various organs; tumor suppressor	<i>BRCA1</i>	17q21	BC
<i>Eda (Ta)</i>	Ectodysplasin-A (tabby)	X	Sweat gland, tooth and hair morphogenesis	<i>ED1</i>	Xq12-q13	EDA/HED
<i>Edn3 (ls)</i>	Endothelin 3 (lethal spotting)	2	Growth and differentiation factor	<i>EDN3</i>	20q13	HD, WSS
<i>Ednrb (s)</i>	Endothelin receptor type B (piebald spotting)	14	Growth factor receptor	<i>EDNRB</i>	13q22	HD, WSS
<i>Egfr (Dsk5)</i>	Epidermal growth factor receptor (dark skin 5)	11	Growth factor receptor	<i>EGFR</i>	7p12.3	N
<i>Fgf2r</i>	Fibroblast growth factor receptor 2	7	Growth factor receptor	<i>FGFR2</i>	10q26	CrS, Pfs
<i>Ikbkg</i>	Inhibitor of κB kinase, γ subunit (NEMO)	X	IκB kinase. Required for NFκB signaling	<i>IKBKG</i>	Xq28	IP, HED-ID, EDA-ID
<i>Kit (W)</i>	Kit oncogene (white spotting)	5	Growth factor receptor	<i>KIT</i>	4q11-q12	PS
<i>Kitl (Sl)</i>	Kit ligand (steel)	10	Growth and differentiation factor	<i>KITLG</i>	12q22	N
<i>Krt2-17 (Dsk2)</i>	Keratin 2-17 (dark skin 2)	15	Cytoskeleton	<i>KRT2A</i>	12q11-q13	IBS
<i>Lmx1a (dr)</i>	LIM homeobox transcription factor 1α (dreher)	1	Transcription factor	<i>LMX1A</i>	1q22-23	N
<i>Mcoln3 (Va)</i>	Mucolipin 3 (varintint-waddler)	3	Cation channel	<i>MCOLN3</i>	1p22.3	N
<i>Mitf (ml)</i>	Microphthalmia-associated transcription factor (microphthalmia)	6	Transcription factor	<i>MITF</i>	3p12-14	WS2
<i>Pax3 (Sp)</i>	Paired box gene 3 (splotch)	1	Transcription factor	<i>PAX3</i>	2q35	WS1, WS3
<i>Sfxn1 (f)</i>	Sideroflexin 1 (flexed tail)	13	Tricarboxylate carrier	<i>SFXN1</i>	5q35.3	N
<i>Snat2</i>	Snail homolog 2/Slug	16	Transcription factor	<i>SNAI2</i>	8q11	WS2
<i>Sox10 (Dom)</i>	SRY-box containing gene 10 (dominant megacolon)	15	Transcription factor	<i>SOX10</i>	22q13.1	WSS
<i>Sox18 (rg, Dcc1)</i>	SRY-box containing gene 18 (ragged, dark coat color 1)	2	Transcription factor	<i>SOX18</i>	20q13.33	N
<i>Wnt1</i>	Wingless-related MMTV integration site 1	15	Growth factor/morphogen	<i>WNT1</i>	12q13	N
<i>Wnt3a</i>	Wingless-related MMTV integration site 3A	11	Growth factor/morphogen	<i>WNT3A</i>	1q42	N
<b>(b) Components of melanosomes and their precursors</b>						
<i>Dct (slt)</i>	Dopachrome tautomerase (slt)	14	Melanosomal enzyme	<i>DCT</i>	13q31-q32	N
<i>Gpnmb</i>	Glycoprotein (transmembrane) NMB	6	Apparent melanosomal component	<i>GPNMB</i>	7p15	N
<i>Matp (uw)</i>	Membrane-associated transporter protein (underwhite)	15	Apparent transporter	<i>MATP</i>	5p	OCA4
<i>Rab38 (cht)</i>	RAB38, member RAS oncogene family (chocolate)	7	Targeting of Tyrp1	<i>RAB38</i>	11q14	N
<i>Sl (si)</i>	Silver (silver)	10	Melanosome matrix	<i>SILV</i>	12q13-q14	N
<i>Tyr (c)</i>	Tyrosinase (color, albino)	7	Melanosomal enzyme	<i>TYR</i>	11q21	OCA1
<i>Tyrp1 (b)</i>	Tyrosinase-related protein 1 (brown)	4	Melanosomal protein	<i>TYRP1</i>	9p23	OCA3
<b>(c) Melanosome construction/protein routing (HPS-related)</b>						
<i>Ap3b1 (pe)</i>	Adaptor-related protein complex AP-3, β 1 subunit (pearl)	13	Organellar protein routing	<i>AP3B1</i> [HPS2]	15q15	HPS
<i>Ap3d (mh)</i>	Adaptor-related protein complex AP-3, δ subunit (mocha)	10	Organellar protein routing	<i>AP3D1</i>	19p13.3	N
<i>Vps33a (bf)</i>	Vacuolar protein sorting 33a (buff)	5	Organellar protein routing	<i>VPS33A</i>	12q24.31	N
<i>cno (cno)</i>	Cappuccino	5	Organelle biogenesis	<i>CNO</i>	4p16-p15	N
<i>Hps1 (ep)</i>	Hermansky-Pudlak syndrome 1 homolog (pale ear)	19	Organelle biogenesis and size	<i>HPS1</i>	10q24	HPS
<i>Hps3 (coa)</i>	Hermansky-Pudlak syndrome 3 homolog (cocoa)	3	Organelle biogenesis	<i>HPS3</i>	3q24	HPS
<i>Hps4 (le)</i>	Hermansky-Pudlak syndrome 4 homolog (light ear)	5	Organelle biogenesis and size	<i>HPS4</i>	22q11-q12	HPS
<i>Hps5 (ru2)</i>	Hermansky-Pudlak syndrome 5 homolog (ruby-eye 2)	7	Organelle biogenesis	<i>HPS5</i>	11p14	HPS
<i>Hps6 (ru)</i>	Hermansky-Pudlak syndrome 6 homolog (ruby-eye)	19	Organelle biogenesis	<i>HPS6</i>	10q24.31	HPS
<i>Lyst (bg)</i>	Lysosomal trafficking regulator (beige)	13	Organelle biogenesis and size	<i>LYST</i>	1q42	CHS

# Coloration is a very diverse trait... encoded by a plethora of genes



# Changes in "coding sequences" → changes in coloration: "direct GP relationship"



*gene cx41.8 in zebrafish*

```

Cx41.8  1 MADNSLLGNFLEEVQENHSTSVCRVWLTILPFRILVLGTRAESSWGDEQEDFTCDTEQPGCENVC█IAFFIAHIRFW█ 80
GJAS  1 MGDNNSFLGNFLEEVHHRSTVVGKVNWLTVLPIFRMLVLGTAEESSWGDEQADFRCTDQPGCQNVCYDQAFFISHIRYRNL 80
cons.  M DNS LGNFLEEV HST VGVWLT LPIFR LVLGTAEESSWGDEQ DF CDT QPGC NVCYD AFFI HIR RNL

Cx41.8  81 QIVP█TFSLIYMGHAMHIVRREKK█NEKELDDDEGAQRDGE--KYPEDOKNKED--EGGC█VRLKGALLQTY█Y█ 155
GJAS  81 QIIFVSTPSLVYMGHAMHITVRNQEKRKLREAERAKEVRSGSYYEYPAEKALSCWEEGNRGIALQGTILNTVVCSELIR 160
cons.  QI FVSTPSL YMGHAMH VR EKR . . G YP - K E G GR L G LL TTV SLLIR

Cx41.8  156 TVMEVIFIIIQYLIYGVFLSALYVCKAPPCHPVNCYISRPTEKRVF█IVPMALAVAASVLLSIVELYHLANKQLRKYVHG 235
GJAS  161 TTMEVGFIVQQYPIYGIFLTTLHVCRNSPCPHPVNCYVSRFTEKRVFIVPMALAVAASVLLSIAELYHLGNWKIRQRFV- 239
cons.  T MEY FI OY IYG FL - L VC PCPHPVNCY SRPEKRVFIVPMALAAA SLLIS ELYHL KK .. R.

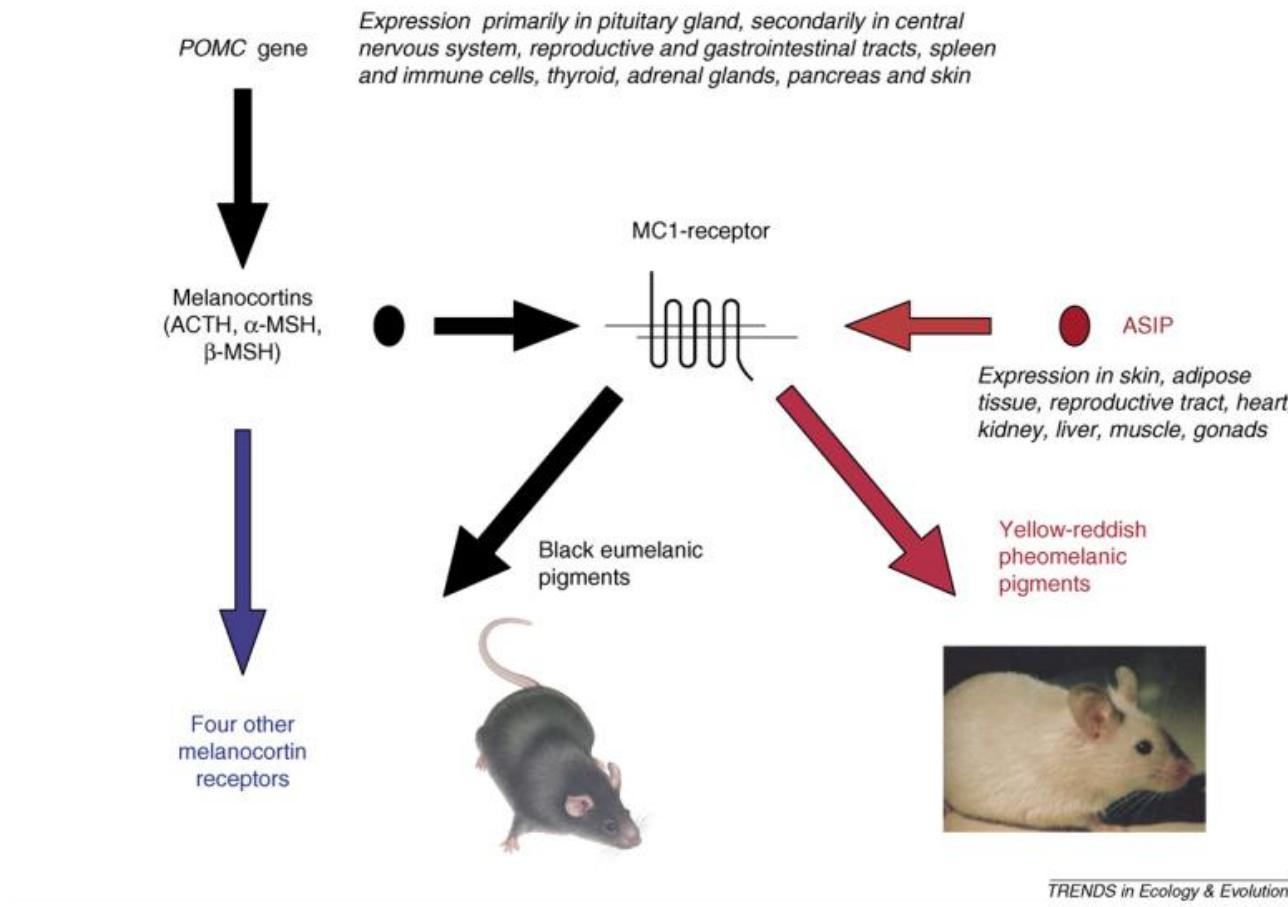
Cx41.8  236 YKASKQRPNTFPSTMPALSPNPSTPNRACTTPPDFNQCLTSPPSSPTLQTHSLLHTCPPFHDRLAHQQNSANMVTERHRG 315
GJAS  240 -----KPRQEMAKCQLSGPSVGIVQSCTPPDFNQCLENGPGKFFN-----PFSNNMASQQNTOINLVTQVRG 303
cons.  . P - LS ..CTPPPFDNQCL P . PF - A QQN N VTE RG

Cx41.8  316 QD-YLGVNPLS--FSQTPTEPNASCAPSFLGSDFE-DKRRFKSSGTSSRMRFDDLAV 370
GJAS  304 QEQTPGEGFIQVRYGGKP-EVPNGVSPGHRLPHGYRSDKRRLSKAS---SKARSDDLSV 358
cons.  Q G F . Q P E FN . L . DKRR SK S S R DDL V

```

But... The GP relationship is more complex than "one gene / one trait"

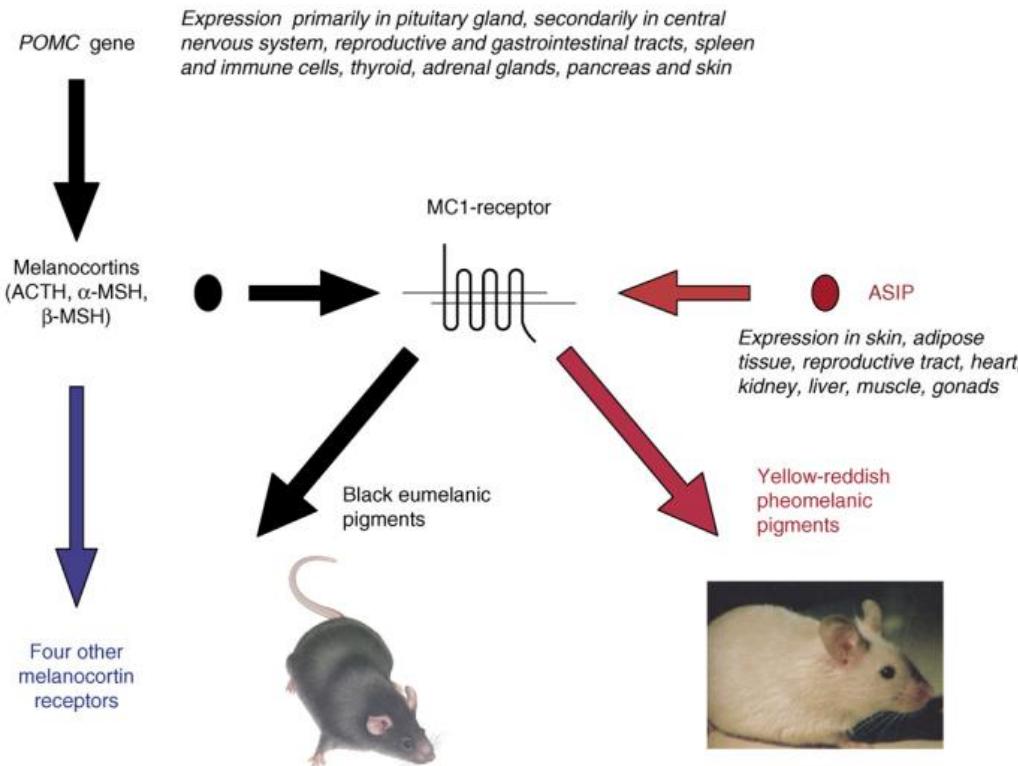
→ One gene / MULTIPLE traits: **Pleiotropy** of "color genes"



But... The GP relationship is more complex than "one gene / one trait"

→ One gene / MULTIPLE traits: Pleiotropy of "color genes"

→ MULTIPLE genes / one trait



TRENDS in Ecology & Evolution

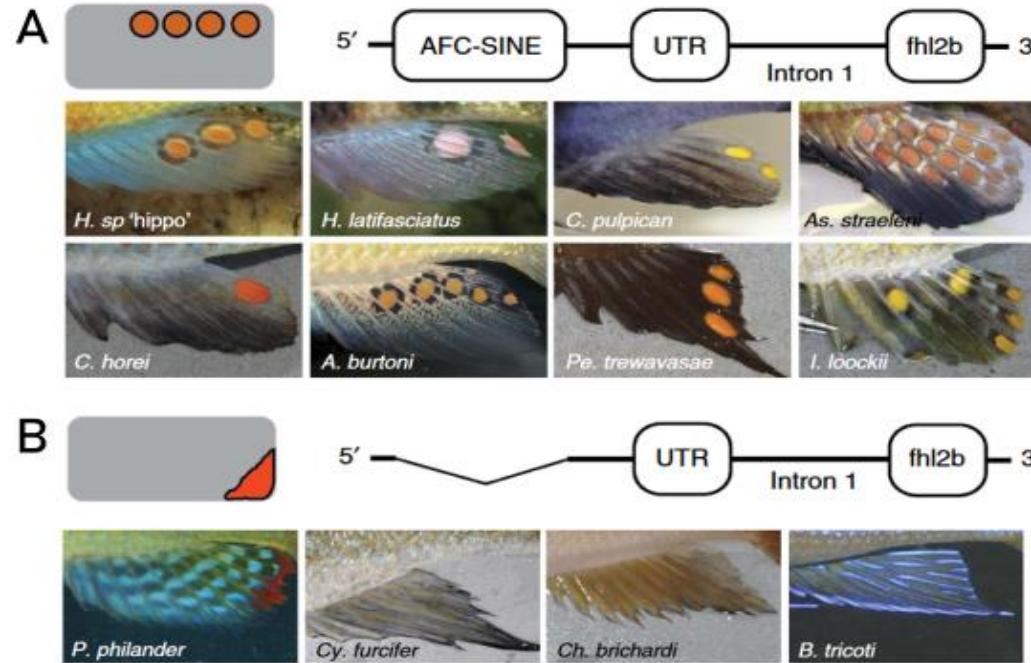
Ducrest et al. (2008) TREE

# Coloration is sometimes due to regulatory sequences rather than "genes"



Cichlid fish

## 1. *Cis*-regulatory sequences

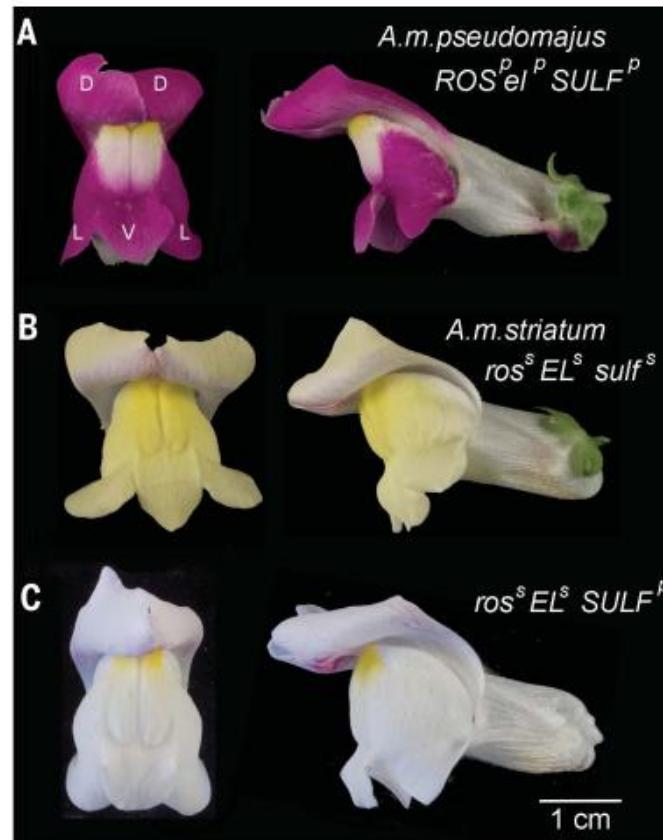


Coloration is sometimes due to regulatory sequences rather than "genes"



### 1. *Cis*-regulatory sequences

### 2. Non-coding RNAs

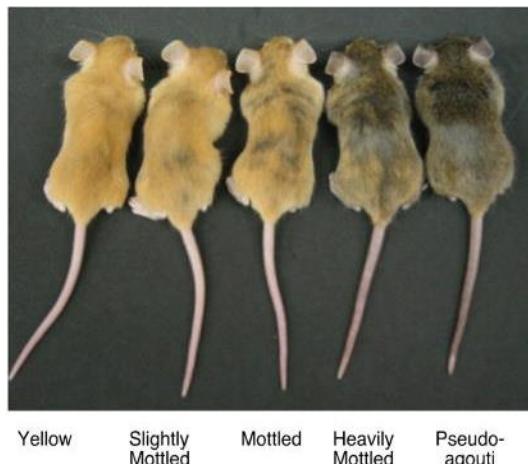


Coloration is sometimes due to regulatory sequences rather than "genes"

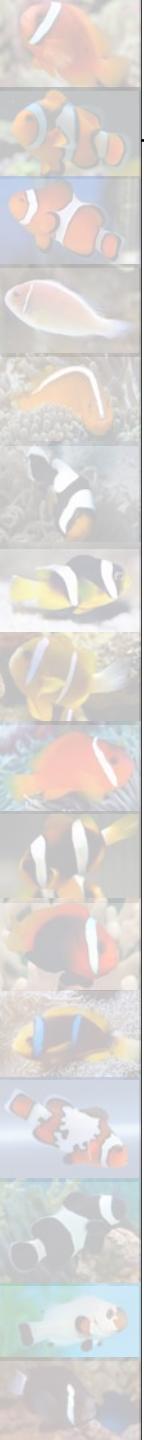
1. *Cis*-regulatory sequences

2. Non-coding RNAs

3. Epigenetics



*"Yellow mice are hypomethylated at the transposable element upstream of the Agouti gene allowing maximal ectopic expression"*



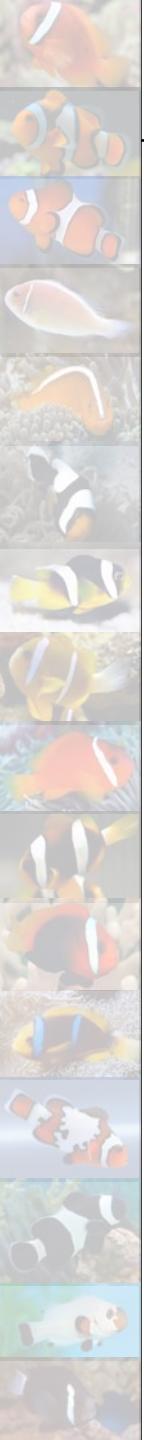
## Why study coloration?

---

→ Profitable

→ Aesthetic

→ Convenient

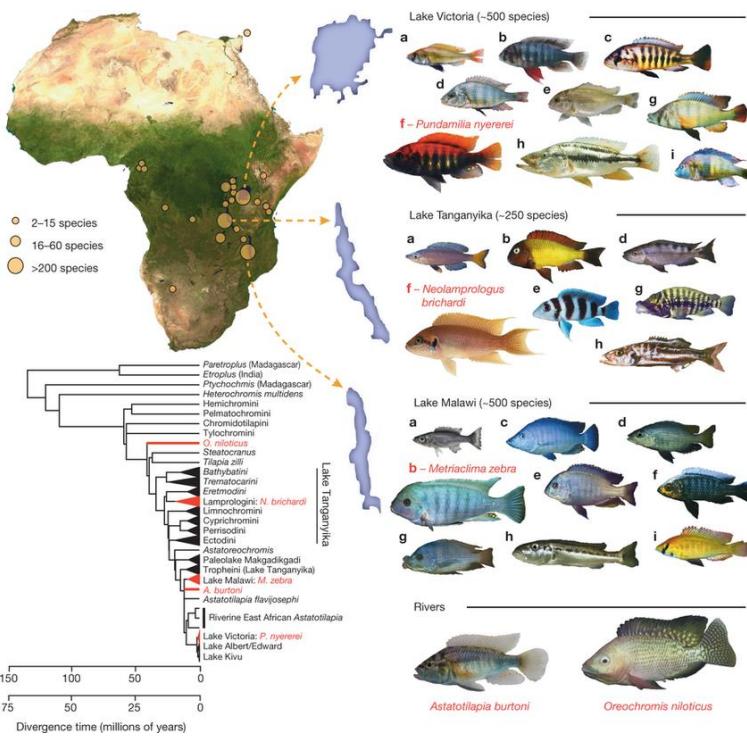


## Why study coloration?

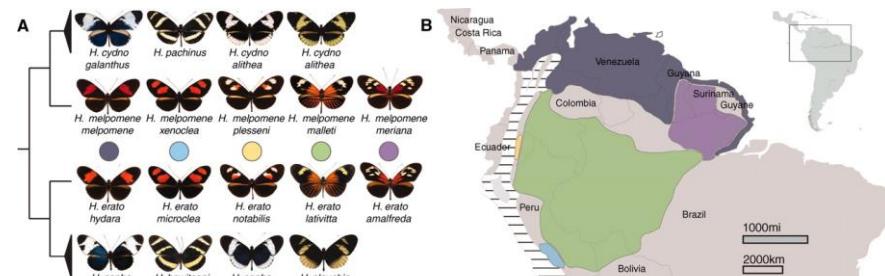
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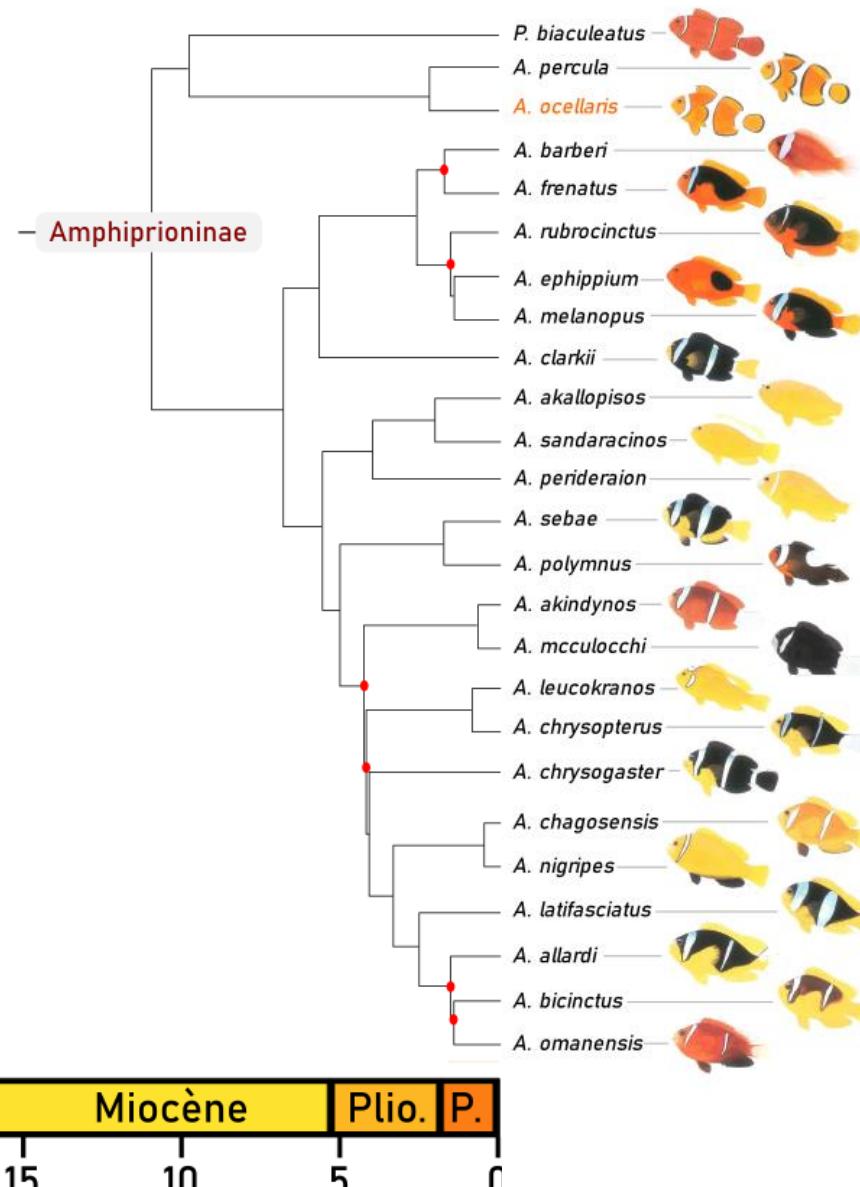
Coloration is a **fast-evolving trait**

# Coloration is a fast-evolving trait



Gautier et al. (2018) Current Biol.





Adapted from Litsios *et al.* (2012), Litsios, Pearman *et al.* (2014) and Litsios and Salamin (2014)

## Introduction

### I. Coloration: a great system to study genotype-phenotype relationships

#### a. Coloration in the History of Science

#### b. Coloration in Vertebrates

- Many cell types and genes
- Not only genes
- Fast evolution



## Introduction

### I. Coloration: a great system to study genotype-phenotype relationships

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- b. Coloration in Vertebrates

### II. A case study: clownfish coloration

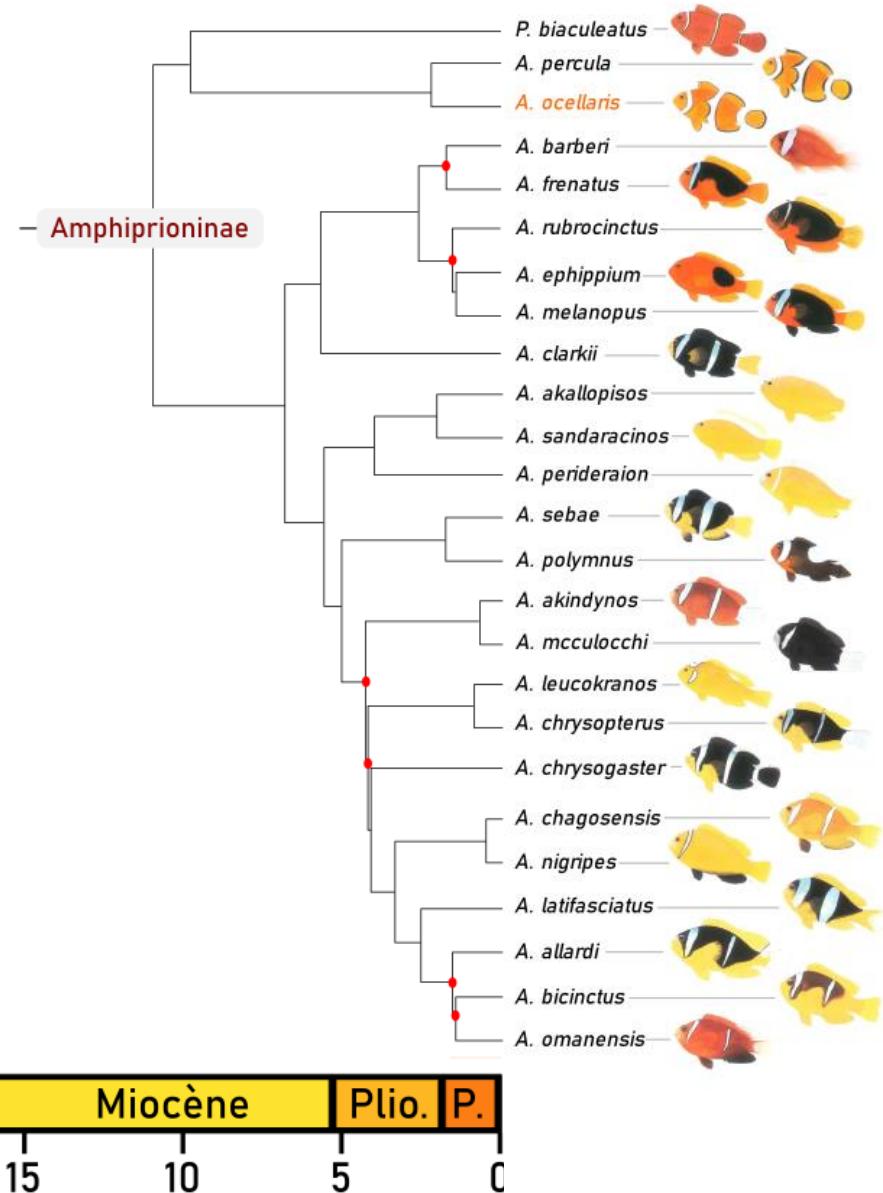
- a. Clownfish: a new model system
- b. Determinism of clownfish coloration





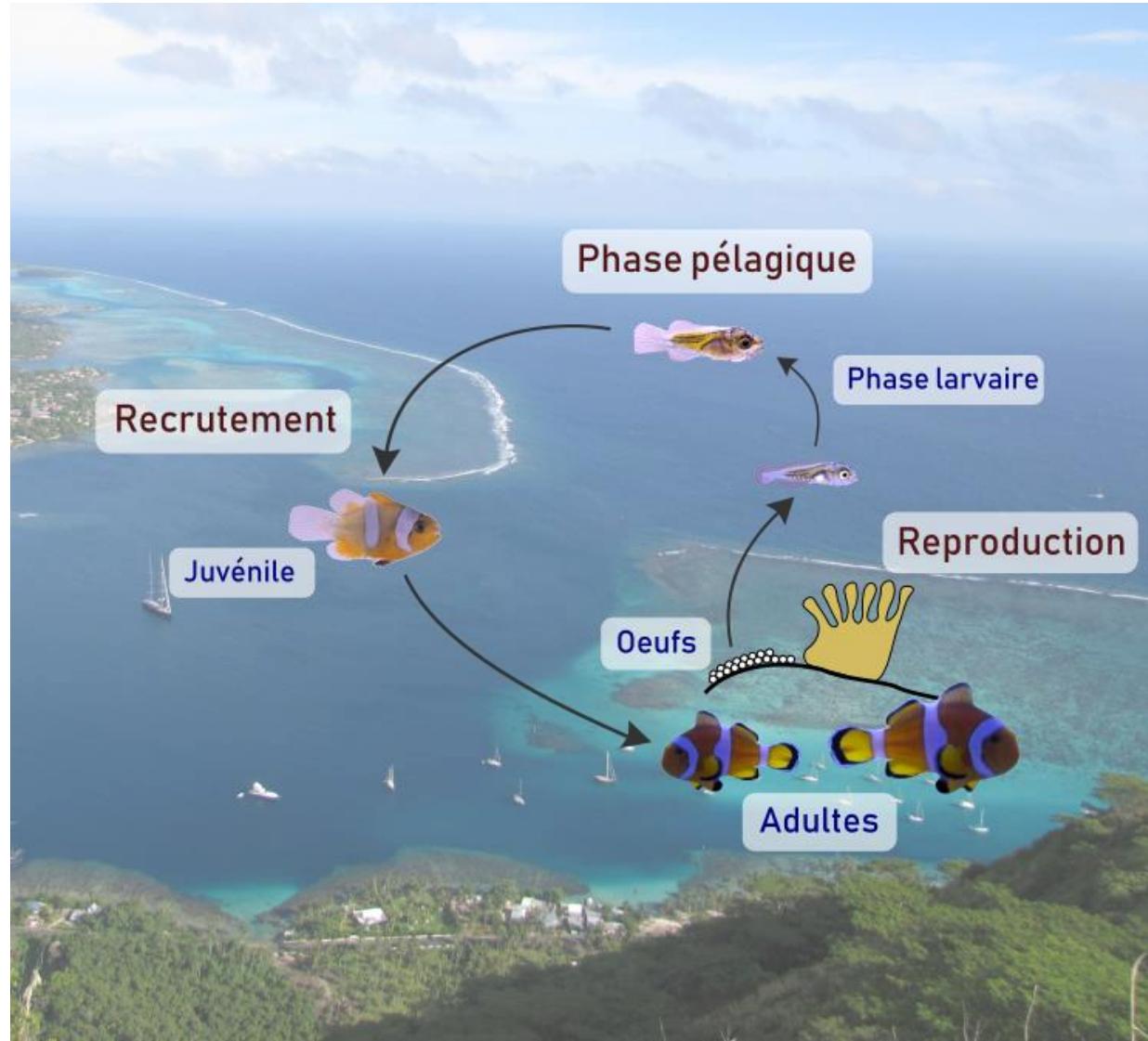
Why a new model system ?

# Ecology and evolution of clownfish *Amphiprion ocellaris*

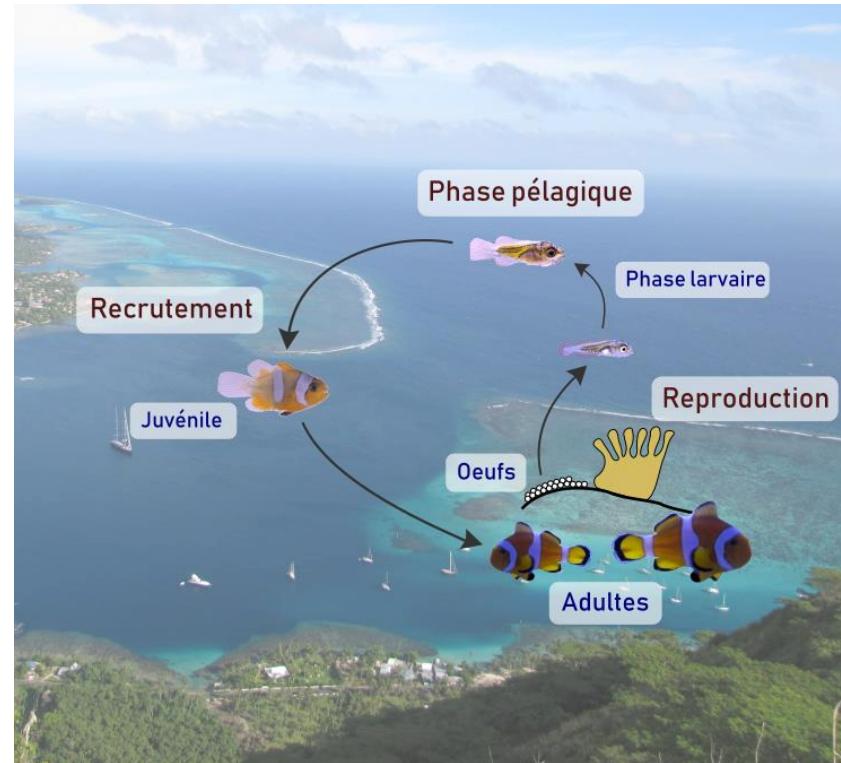
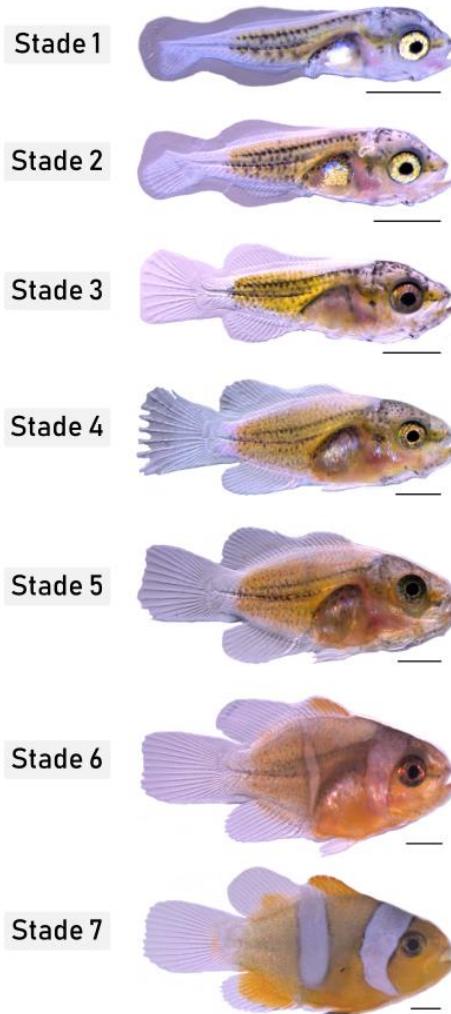


Topology adapted from Litsios *et al.* (2012), Litsios, Pearman *et al.* (2014) and Litsios and Salamin (2014)

# Lifecycle of clownfish *A. ocellaris*



# Pigmentary changes during *A. ocellaris* post-embryonic development



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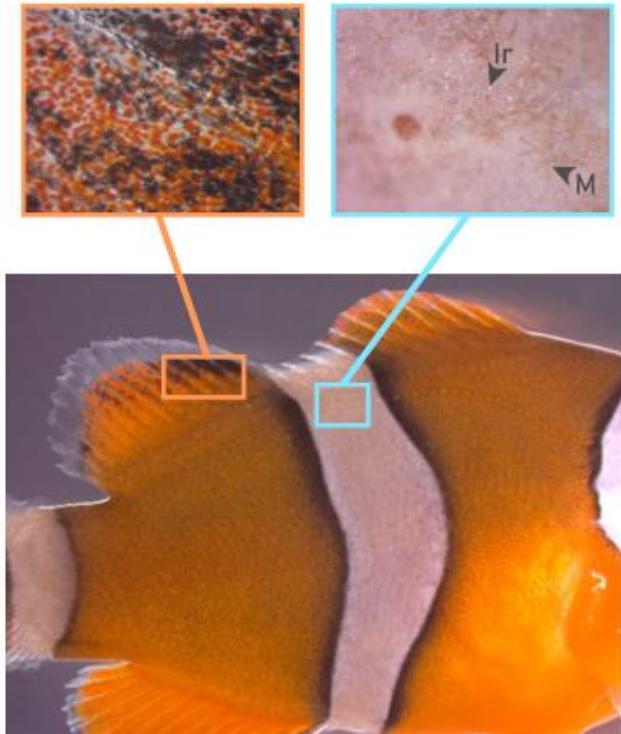
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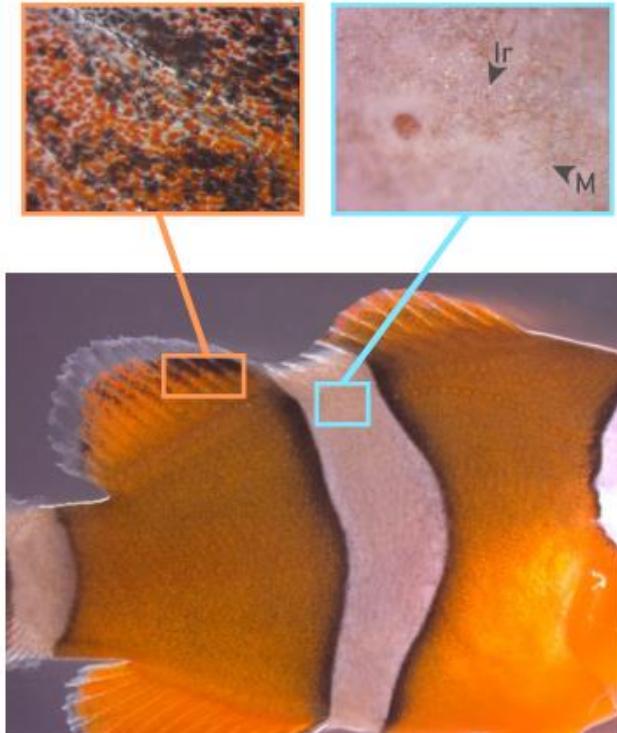


## Cells present in each band in *A. ocellaris*

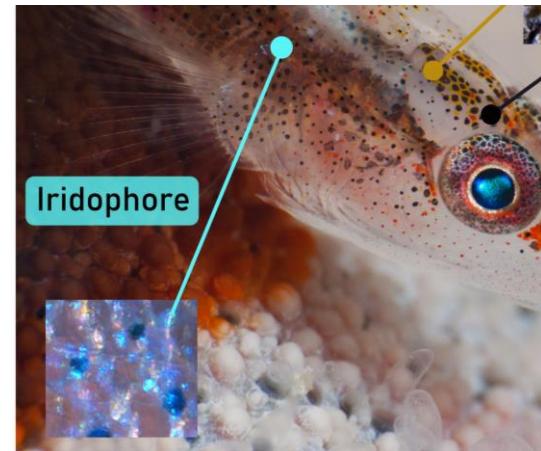


Pictures: N. Roux

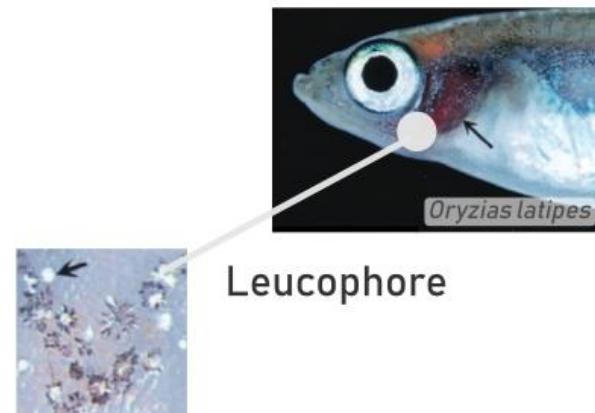
## Cells present in each band in *A. ocellaris*



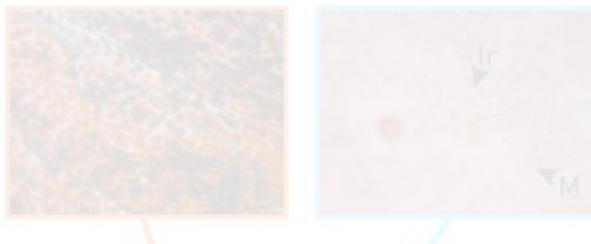
Pictures: N. Roux



?



## Cells present in each band in *A. ocellaris*



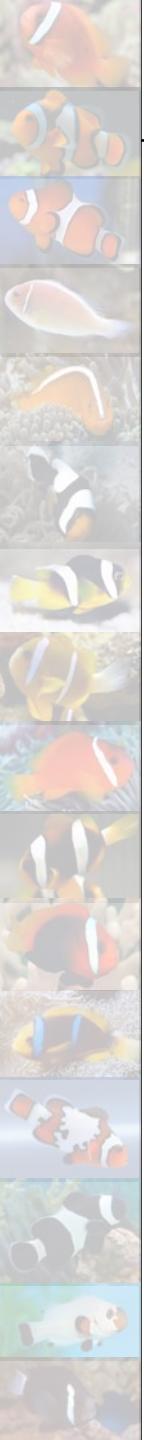
→ Genetic determinism of white band

→ Identify new candidate genes for white band development

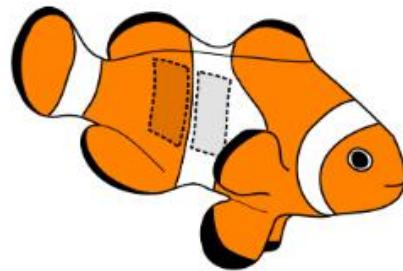
Pictures: N. Roux



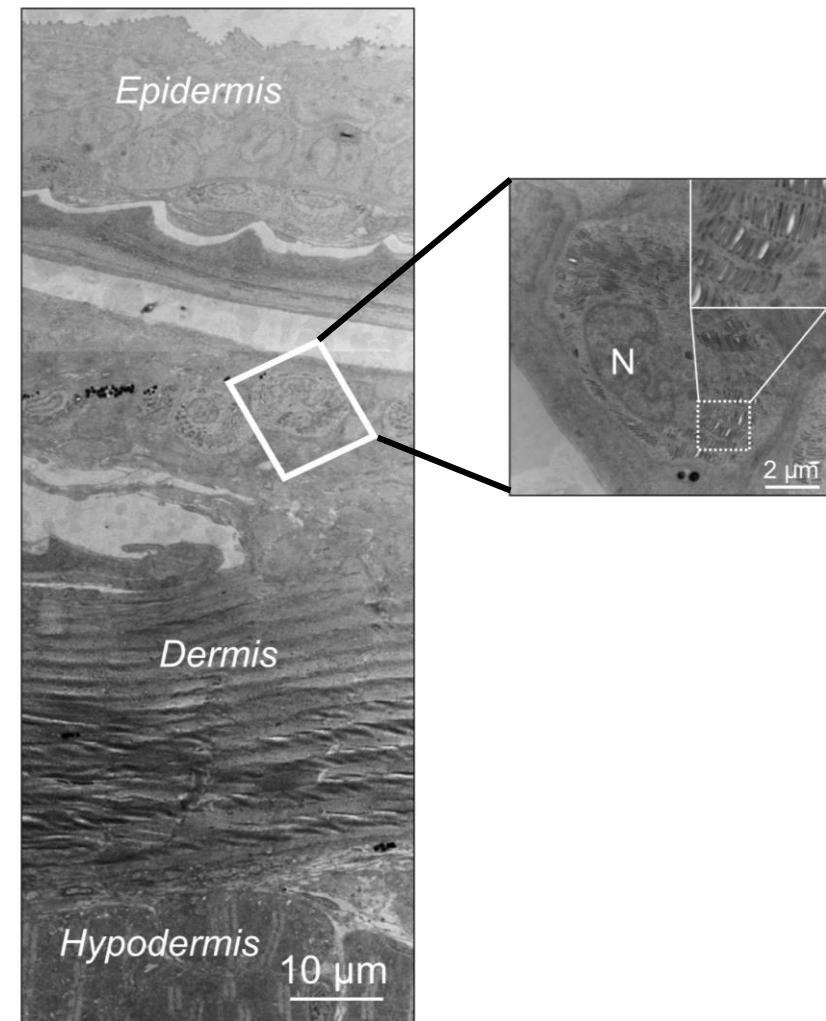
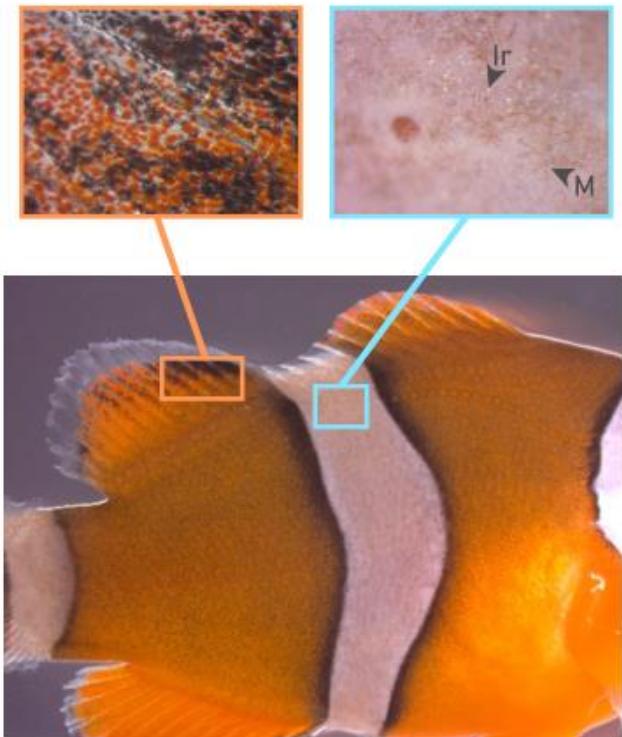
Leucophore



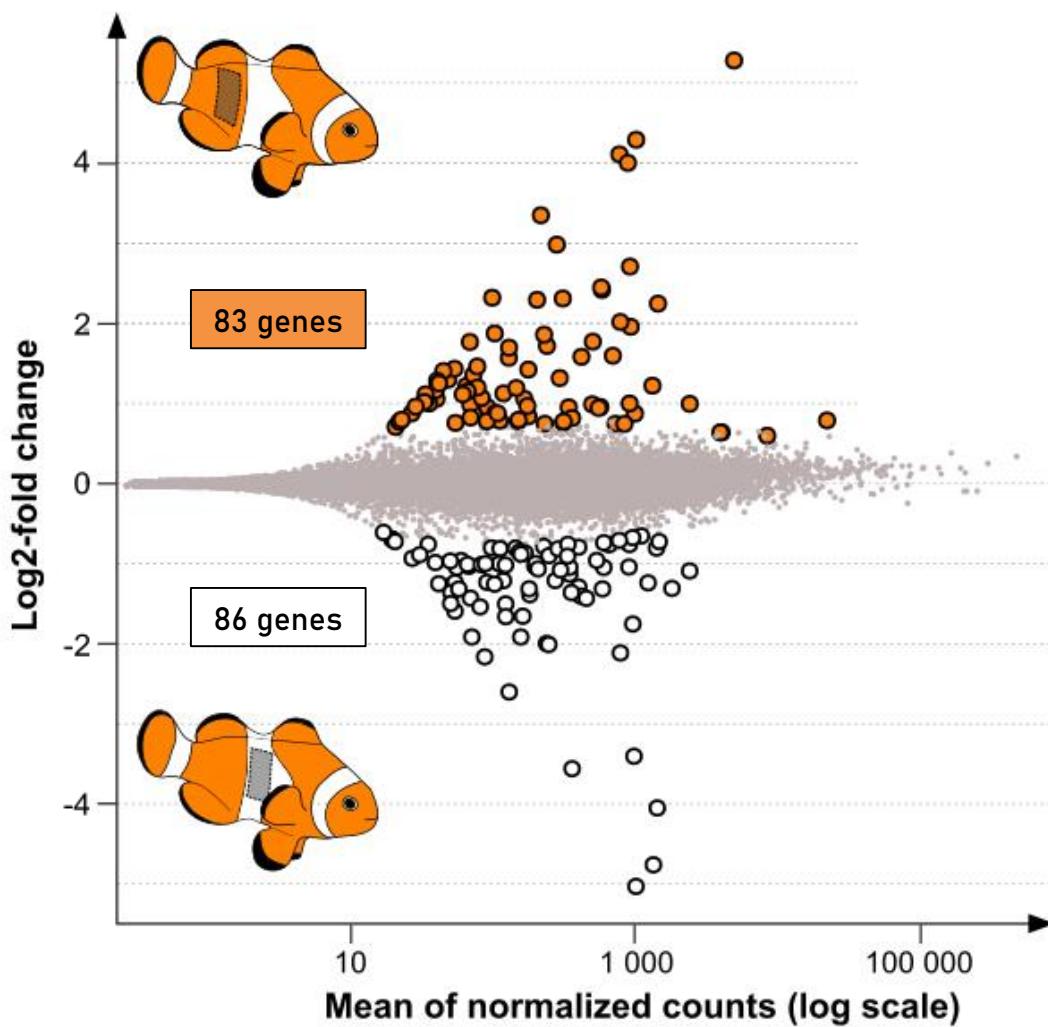
## Comparison white vs. orange skin using RNA-Seq



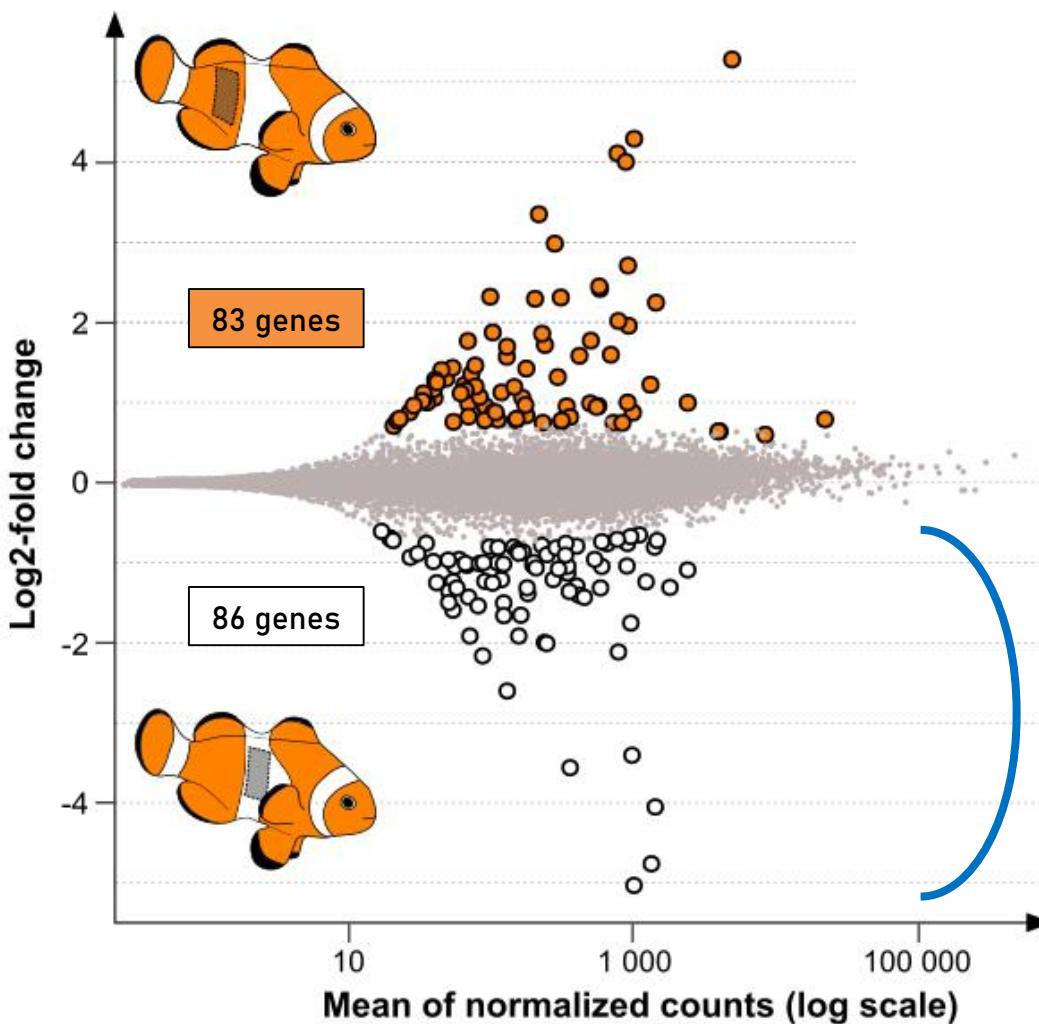
# White skin of *A. ocellaris* harbors iridophores, not leucophores



# RNA-Seq comparison of white vs. orange skin

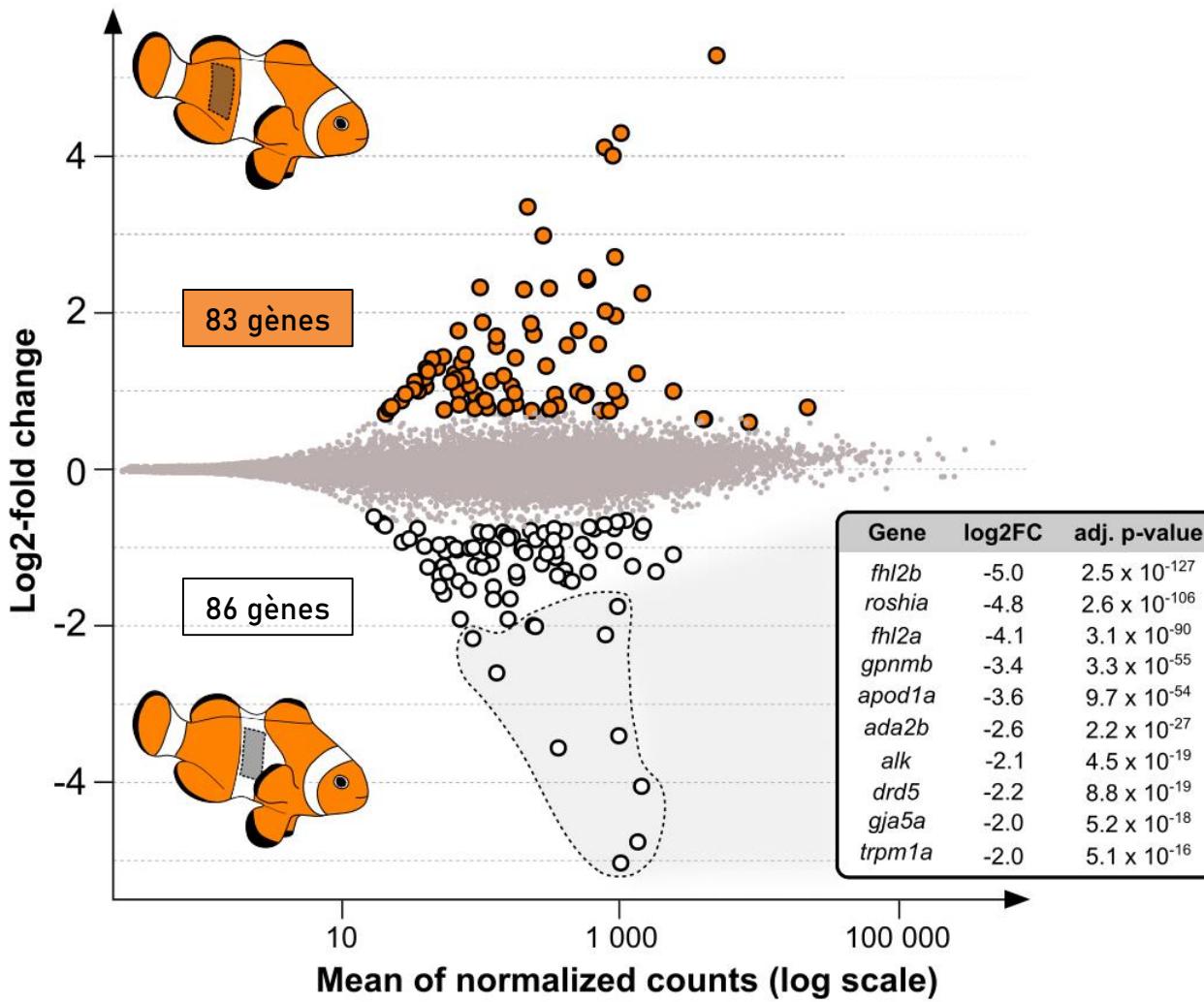


# RNA-Seq comparison of white vs. orange skin

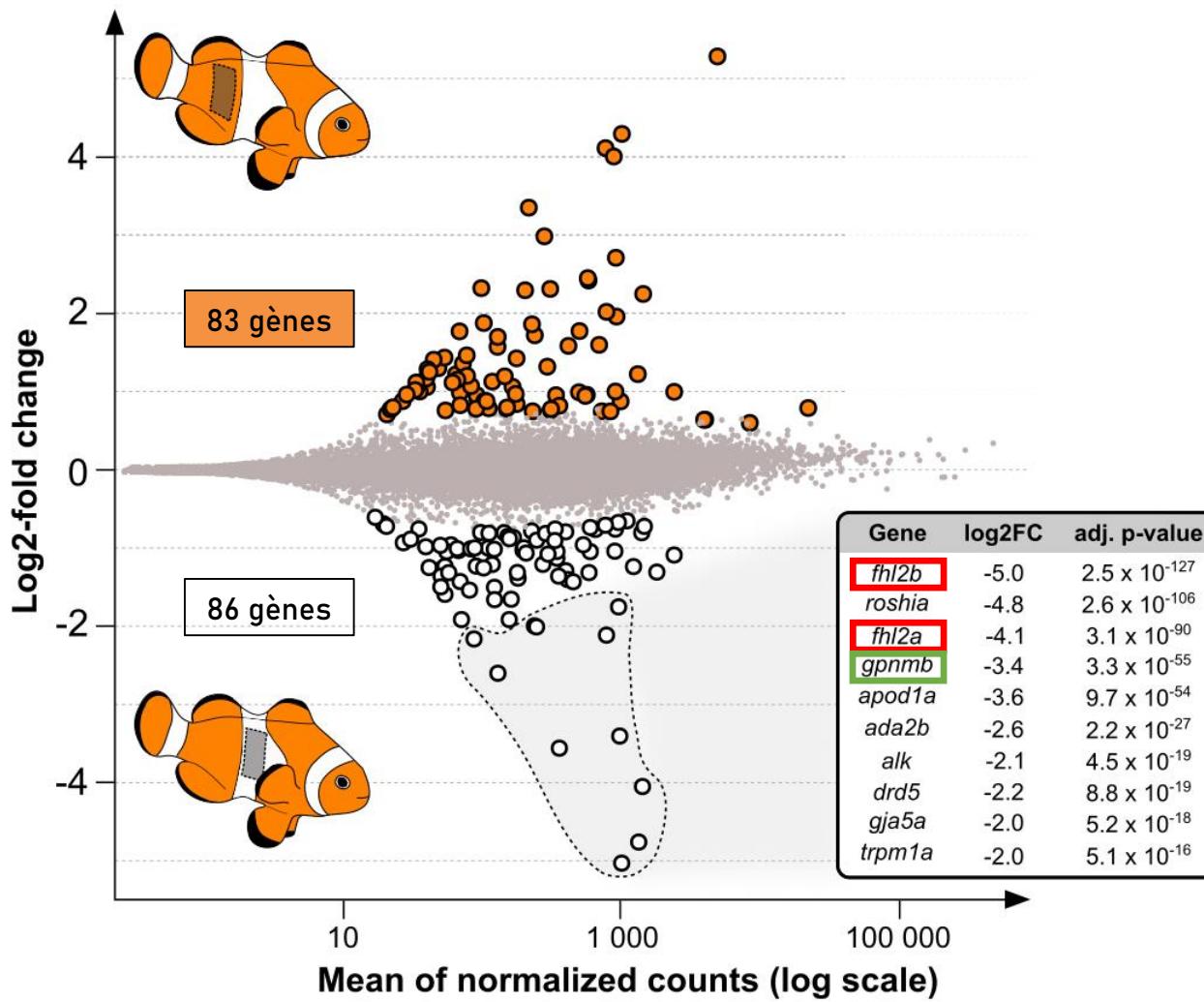


Comparable with  
zebrafish iridophores

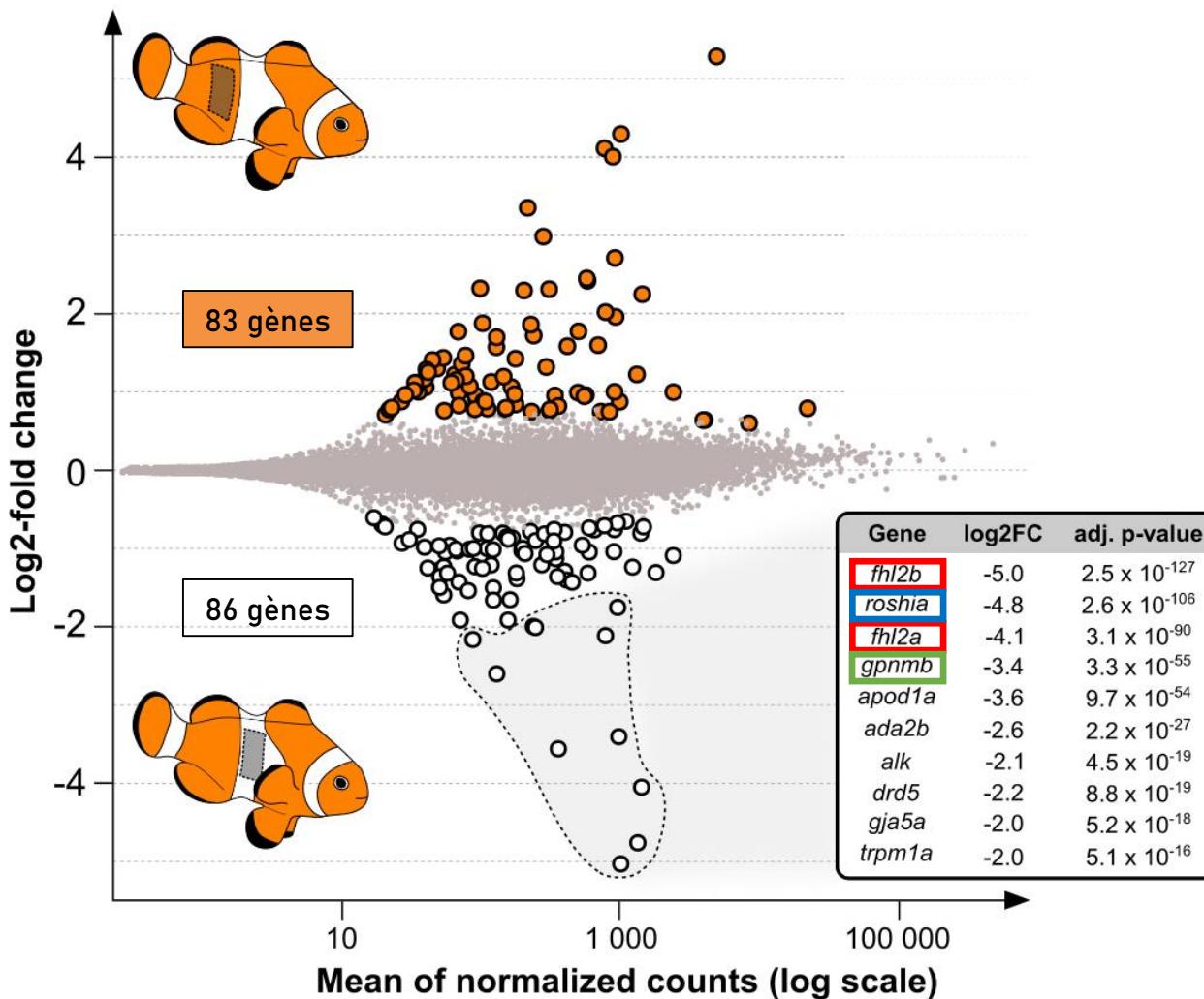
## 10 most DEGs in white skin



## 10 most DEGs in white skin



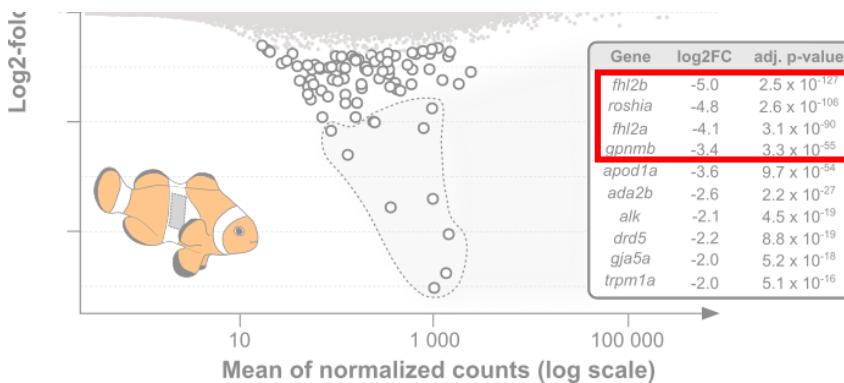
# 10 most DEGs in white skin



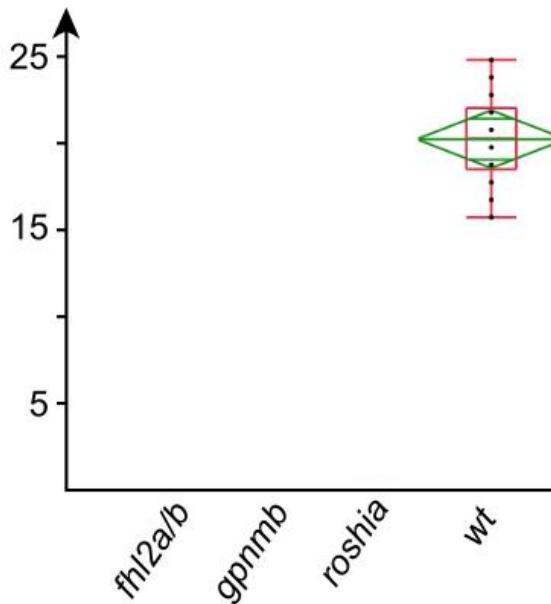
*non-annotated  
→ roshi-a*



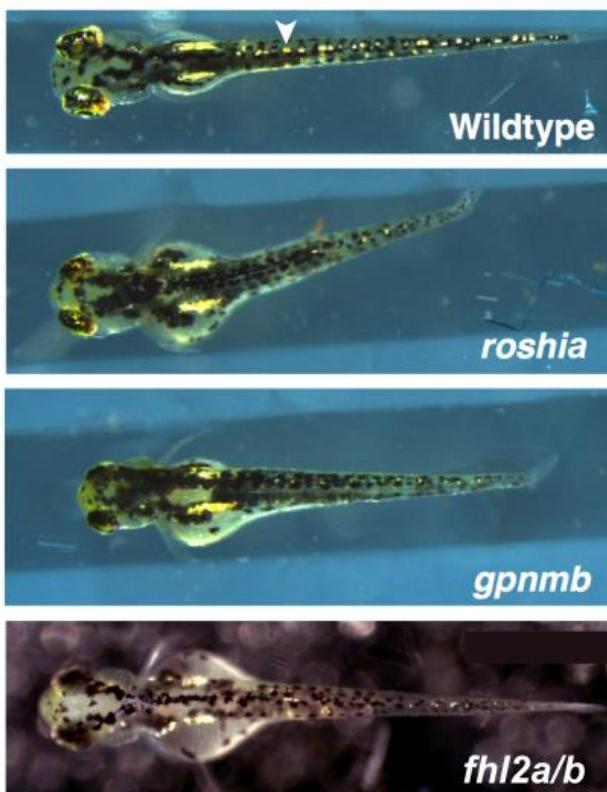
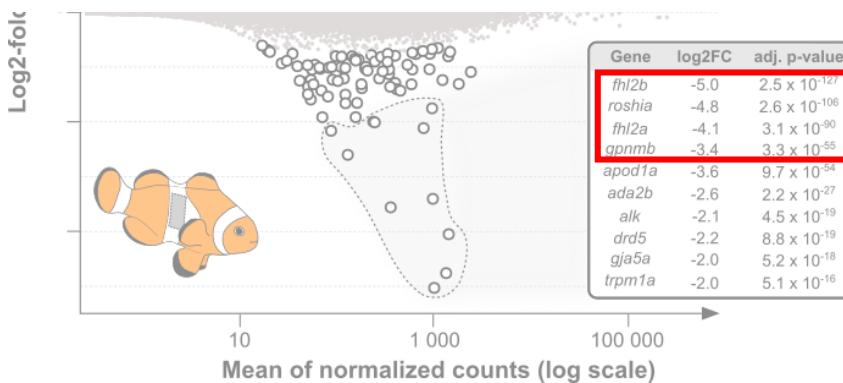
# Functional validation using CRISPR-Cas9 in *D. rerio*



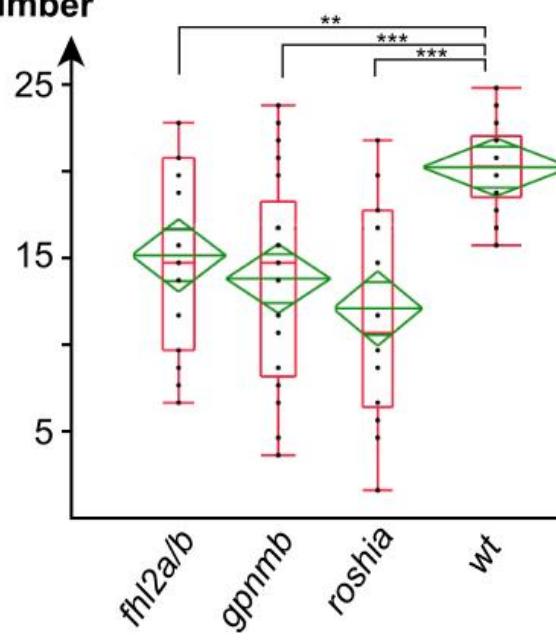
Iridophore  
number



# Functional validation using CRISPR-Cas9 in *D. rerio*



Iridophore  
number



## Introduction

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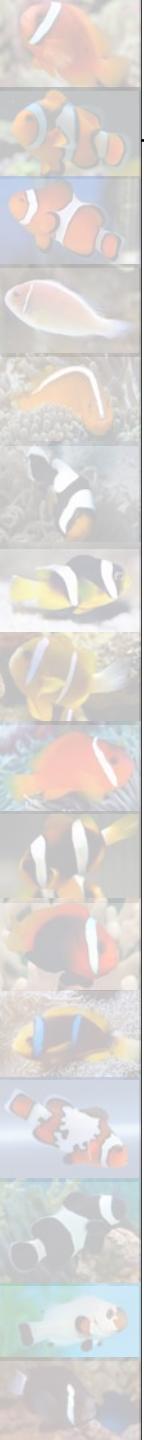
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### II. A case study: clownfish coloration

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## Conclusion





# Evolution of GP relationships

The EVOLUTION of

Coloration: a great system to study genotype-phenotype relationships

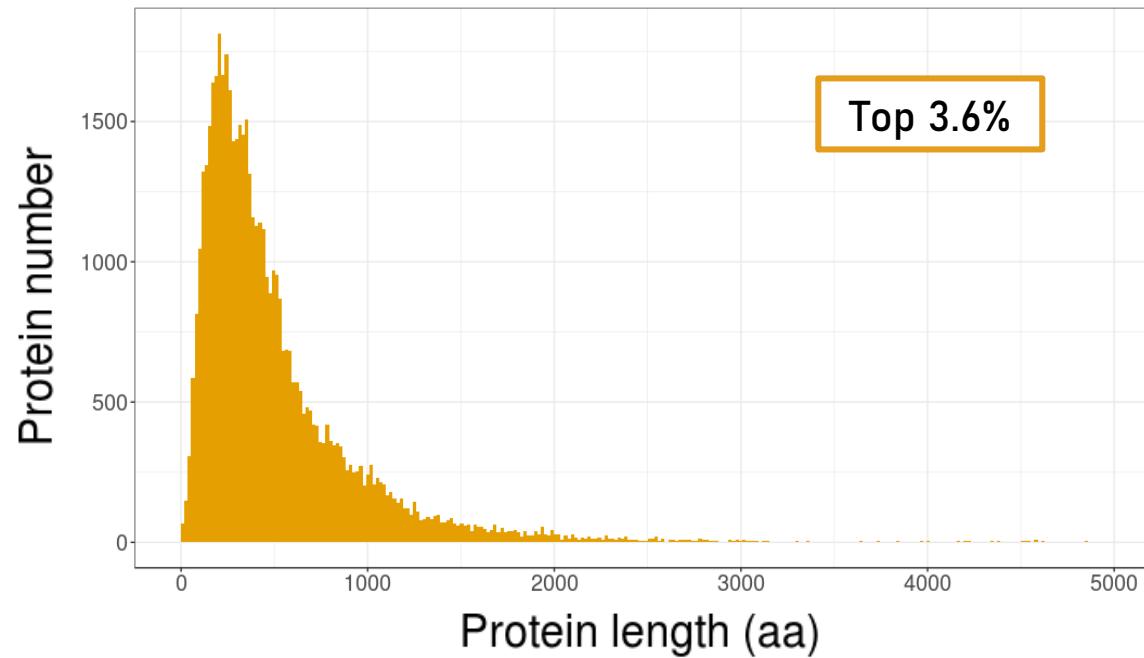
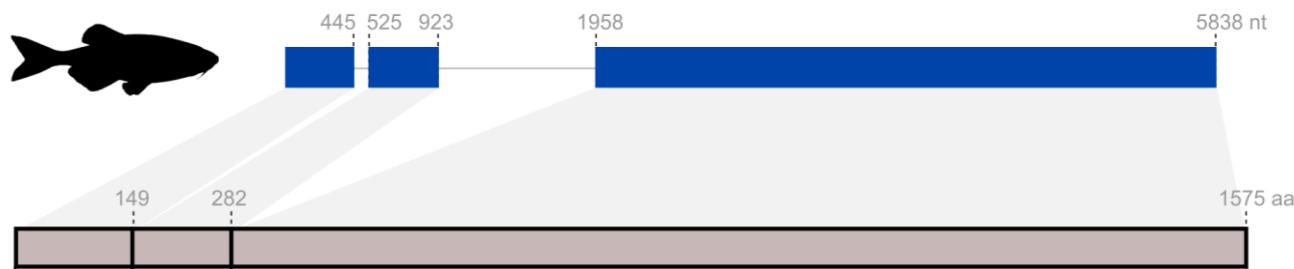
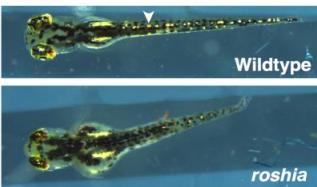
M1 – ENS  
Module "Genomes and Phenotypes"  
Nov. 14<sup>th</sup> 2019

Thibault Lorin

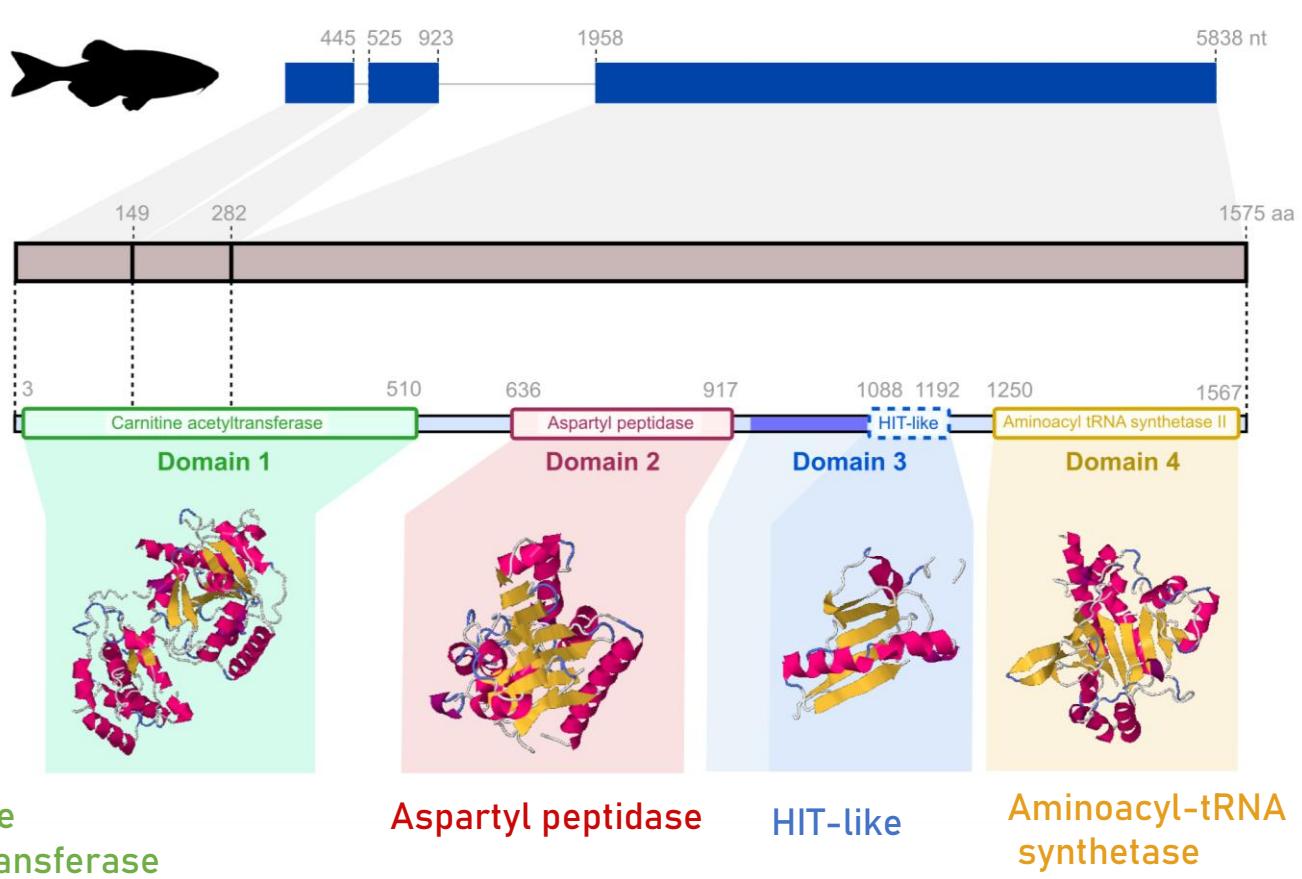
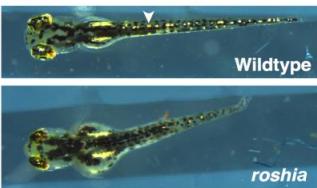


© Aleksey Stemmer

# Structure and putative function of Roshi-a



# Structure and putative function of Roshi-a



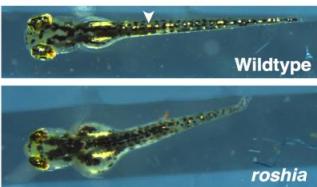
Carnitine  
acetyltransferase

Aspartyl peptidase

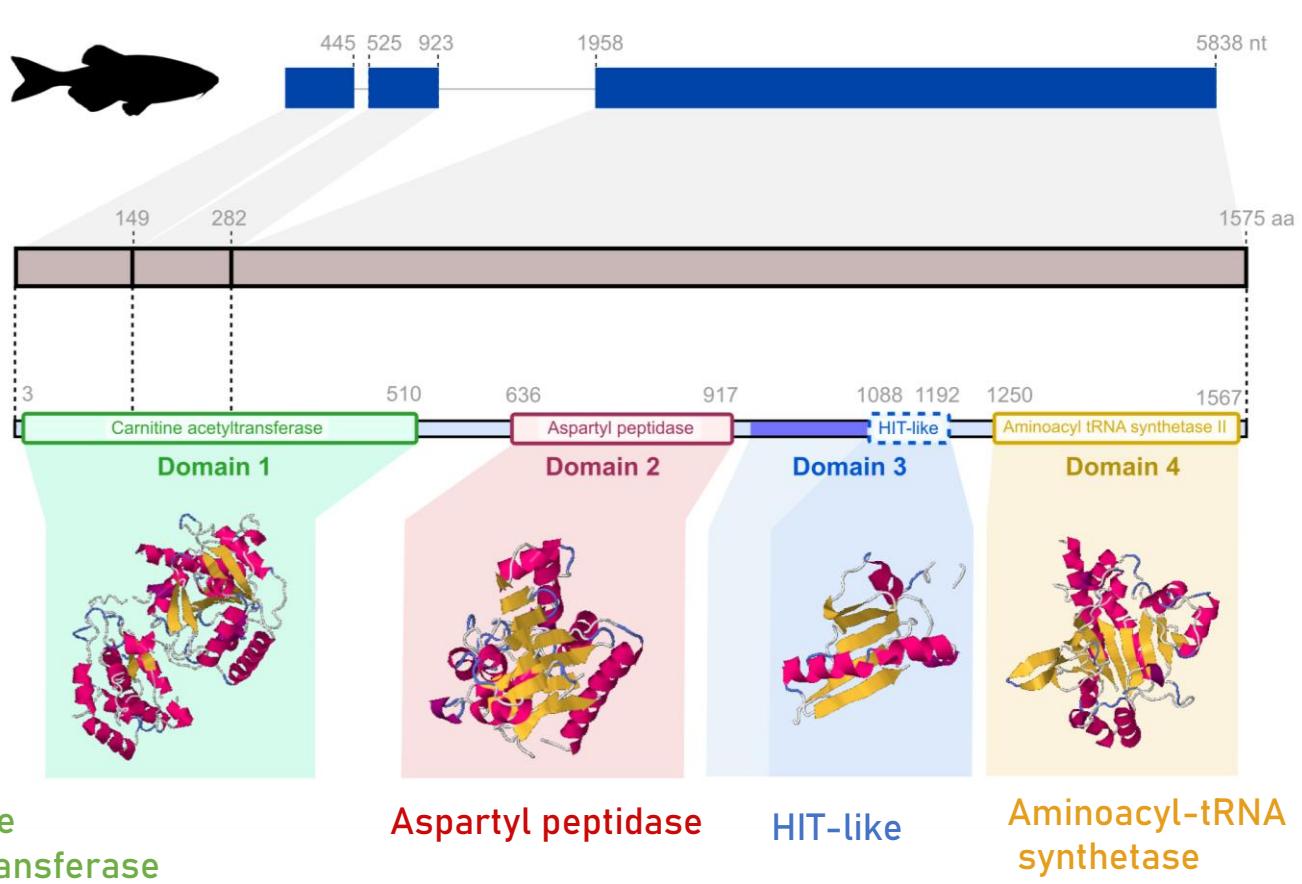
HIT-like

Aminoacyl-tRNA  
synthetase

# Structure and putative function of Roshi-a



Roshi-a is a "Long Putative Multifunctional Cytosolic Enzyme"



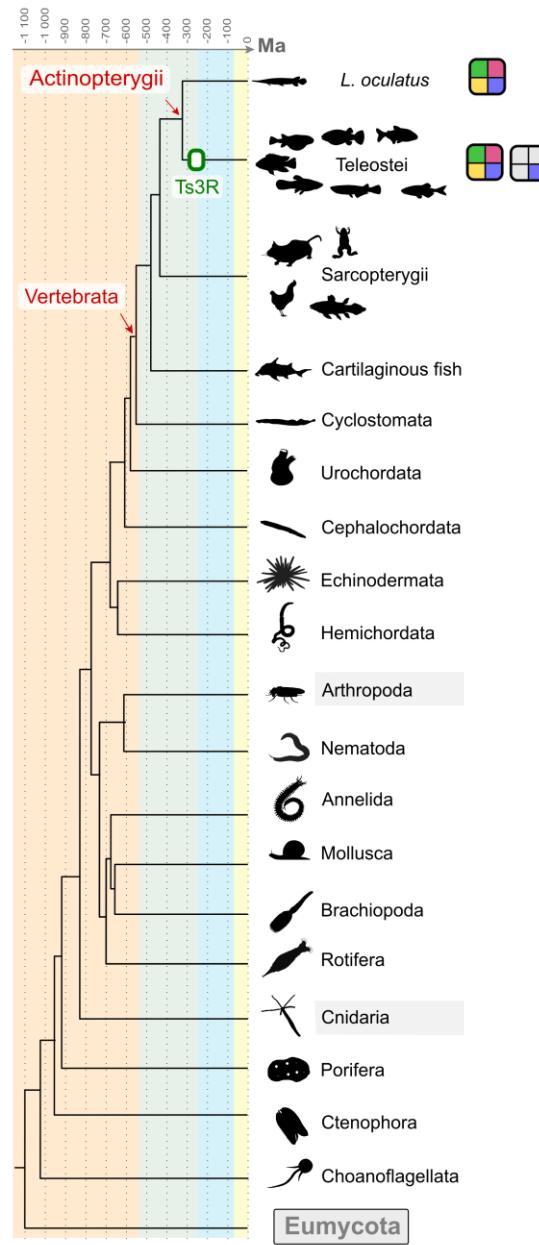
Carnitine  
acetyltransferase

Aspartyl peptidase

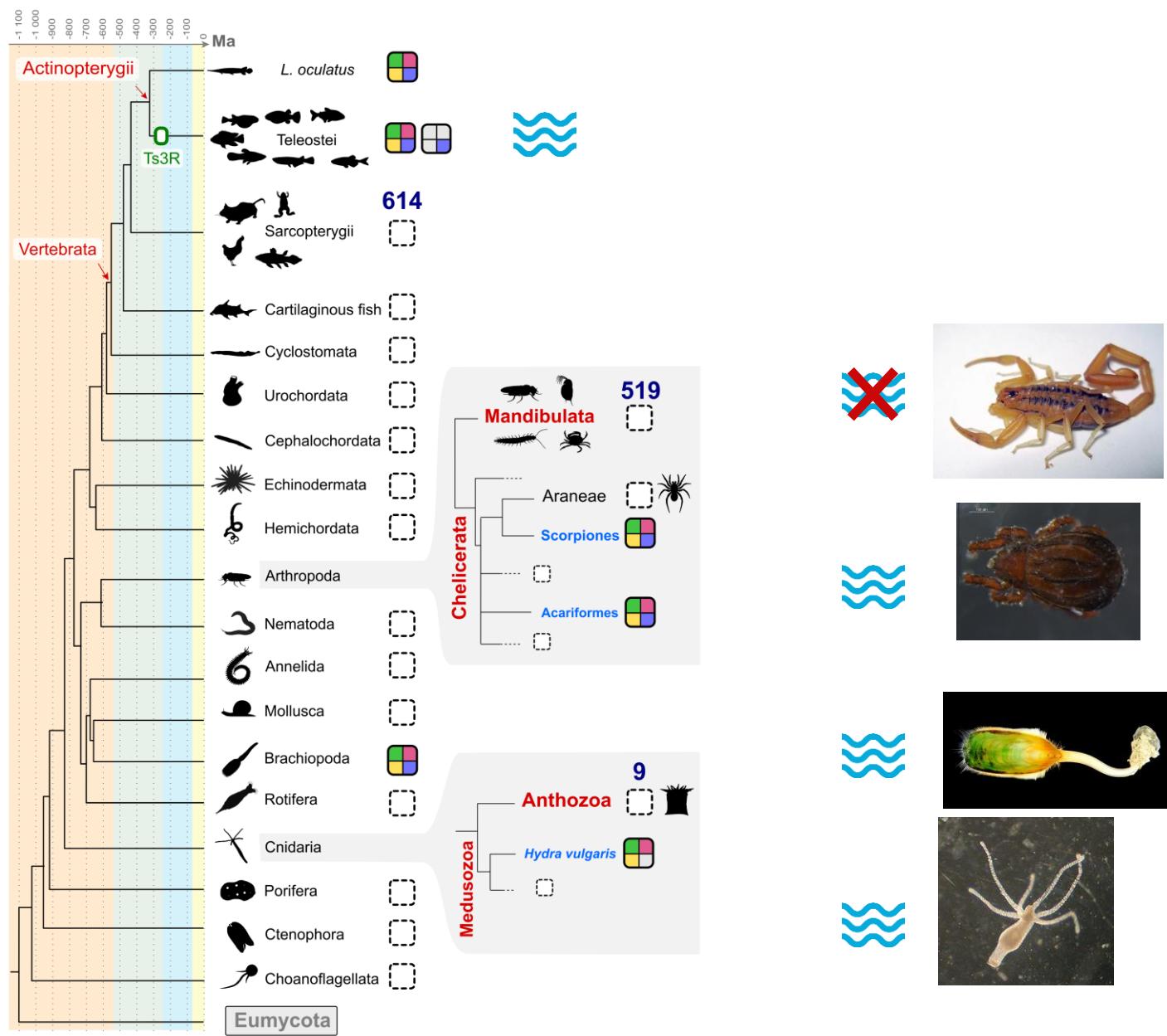
HIT-like

Aminoacyl-tRNA synthetase

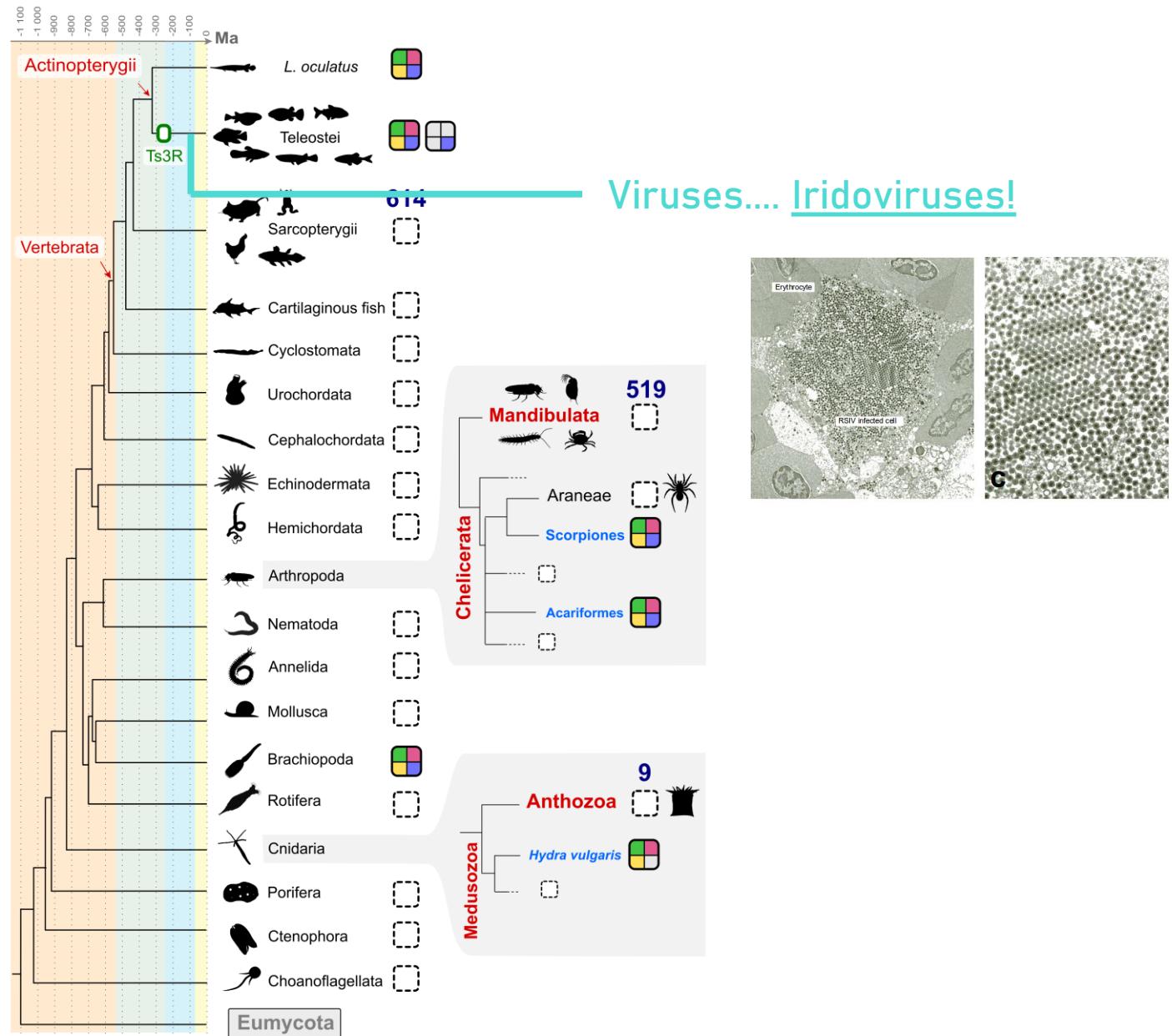
# Roshi has a patchy distribution within animals



# Roshi has a patchy distribution within animals

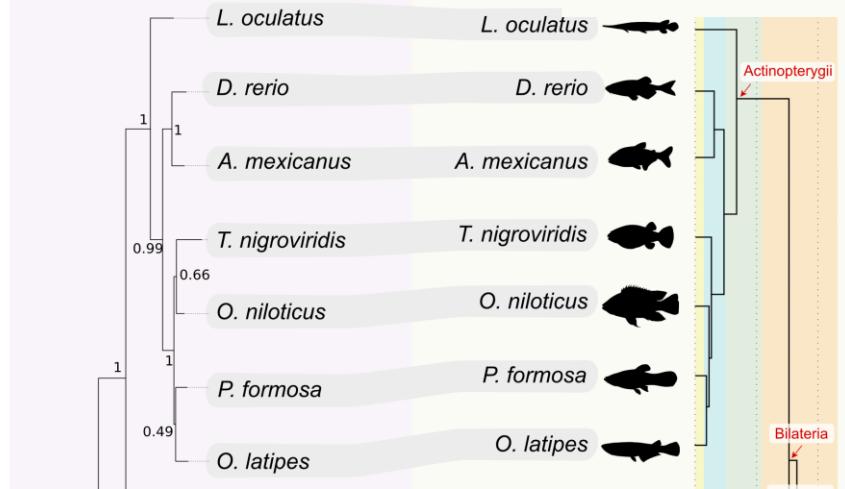


# Roshi has a patchy distribution within animals

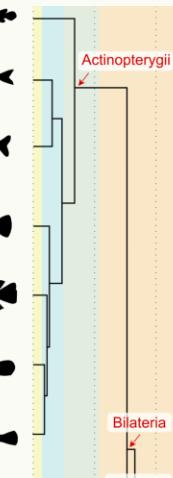


# *roshi* gene tree does not correspond to species tree

## Roshi protein phylogeny

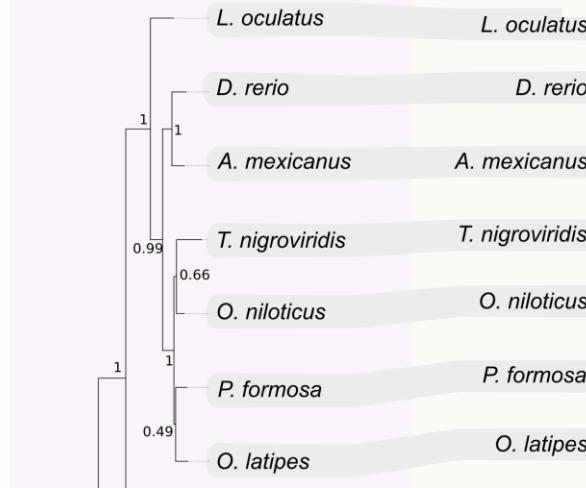


## Species phylogeny

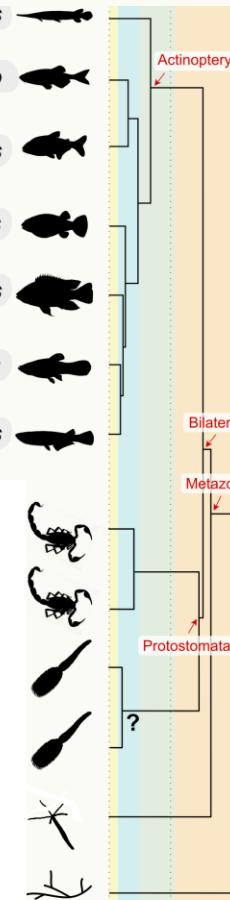


# *roshi* gene tree does not correspond to species tree

## Roshi protein phylogeny



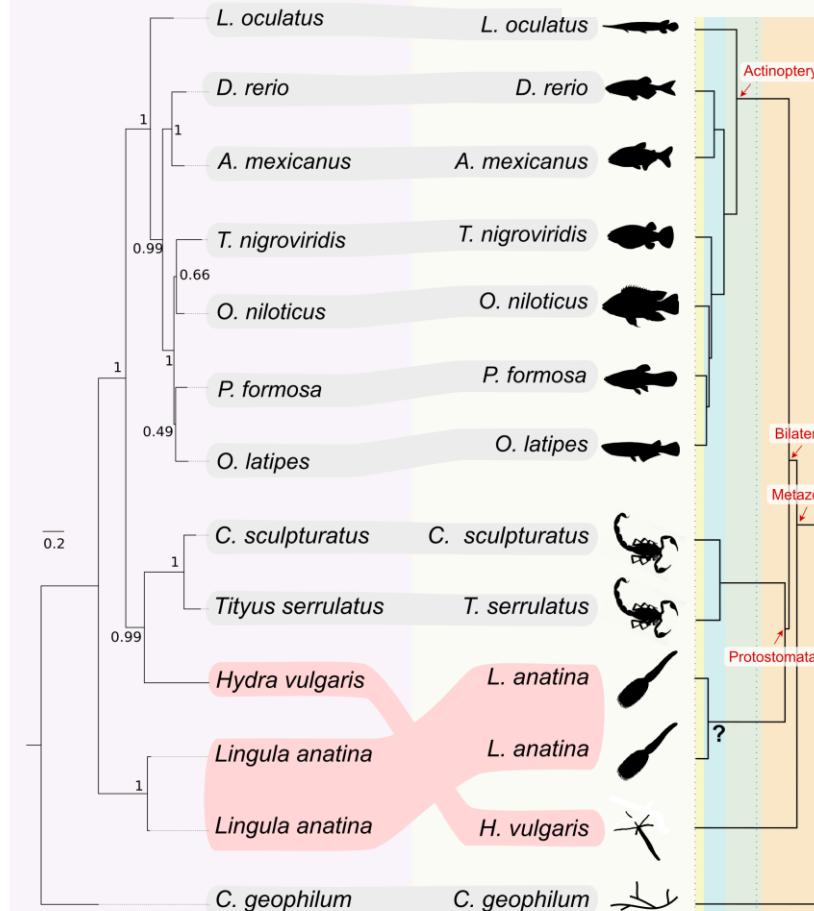
## Species phylogeny



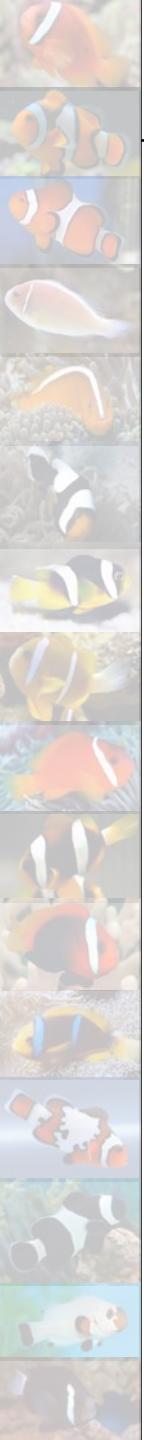
# *roshi* gene tree does not correspond to species tree

Lateral Gene Transfer ?

## Roshi protein phylogeny



## Species phylogeny

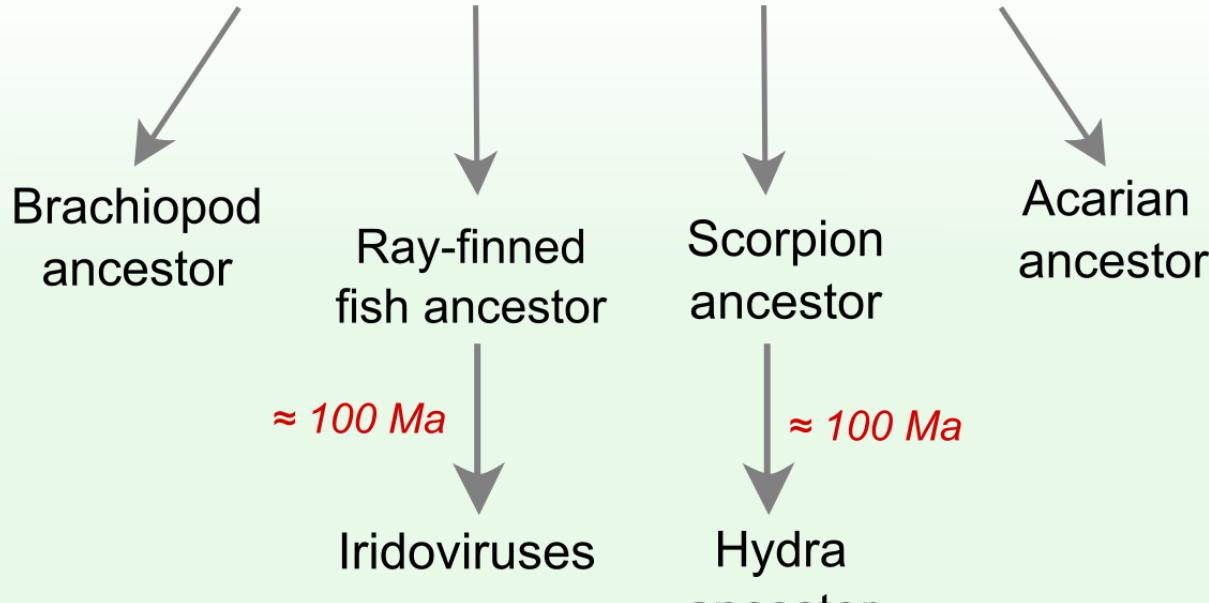


## Hypothetical scenario for *roshi* evolution

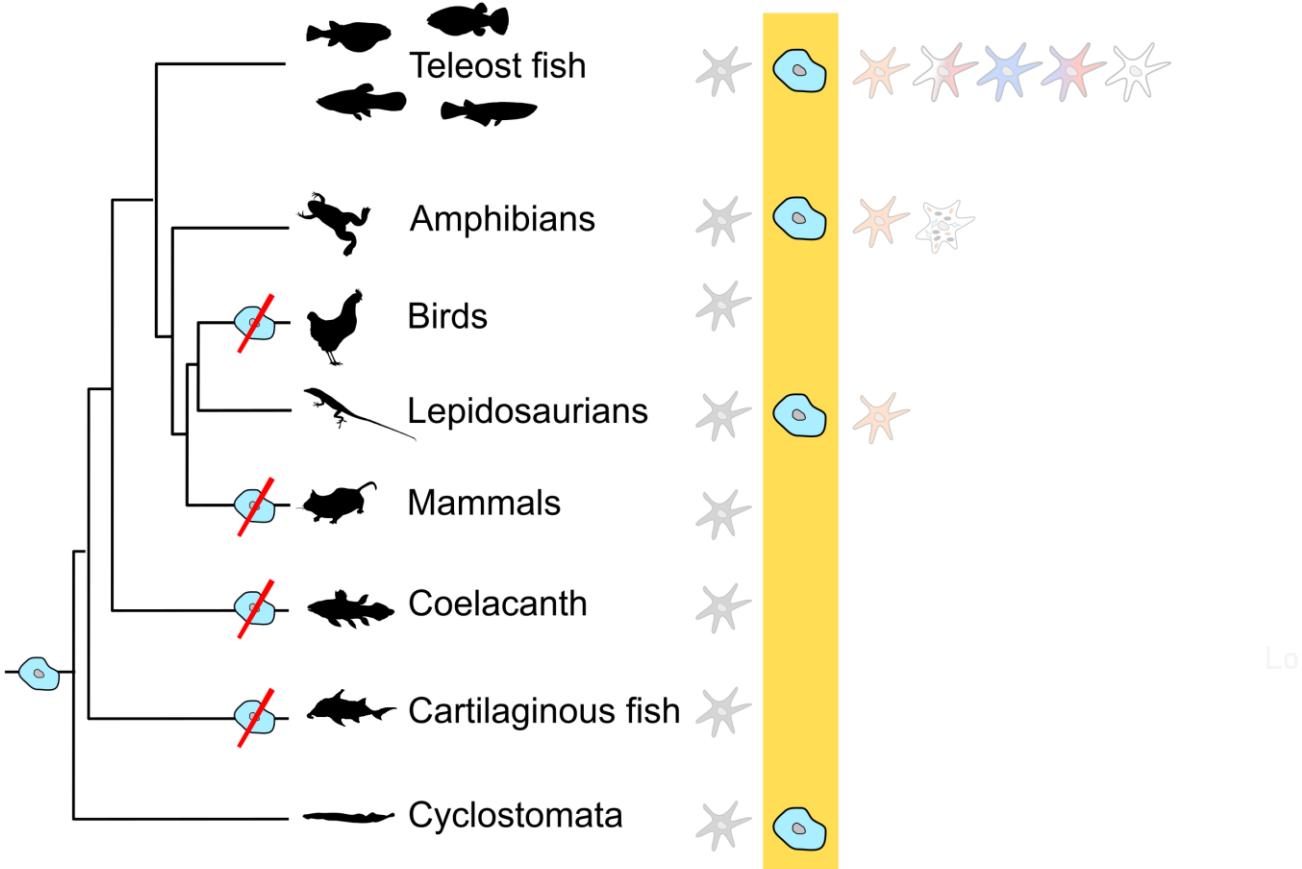
### Roshi acquisition in a fungal or in an animal ancestor



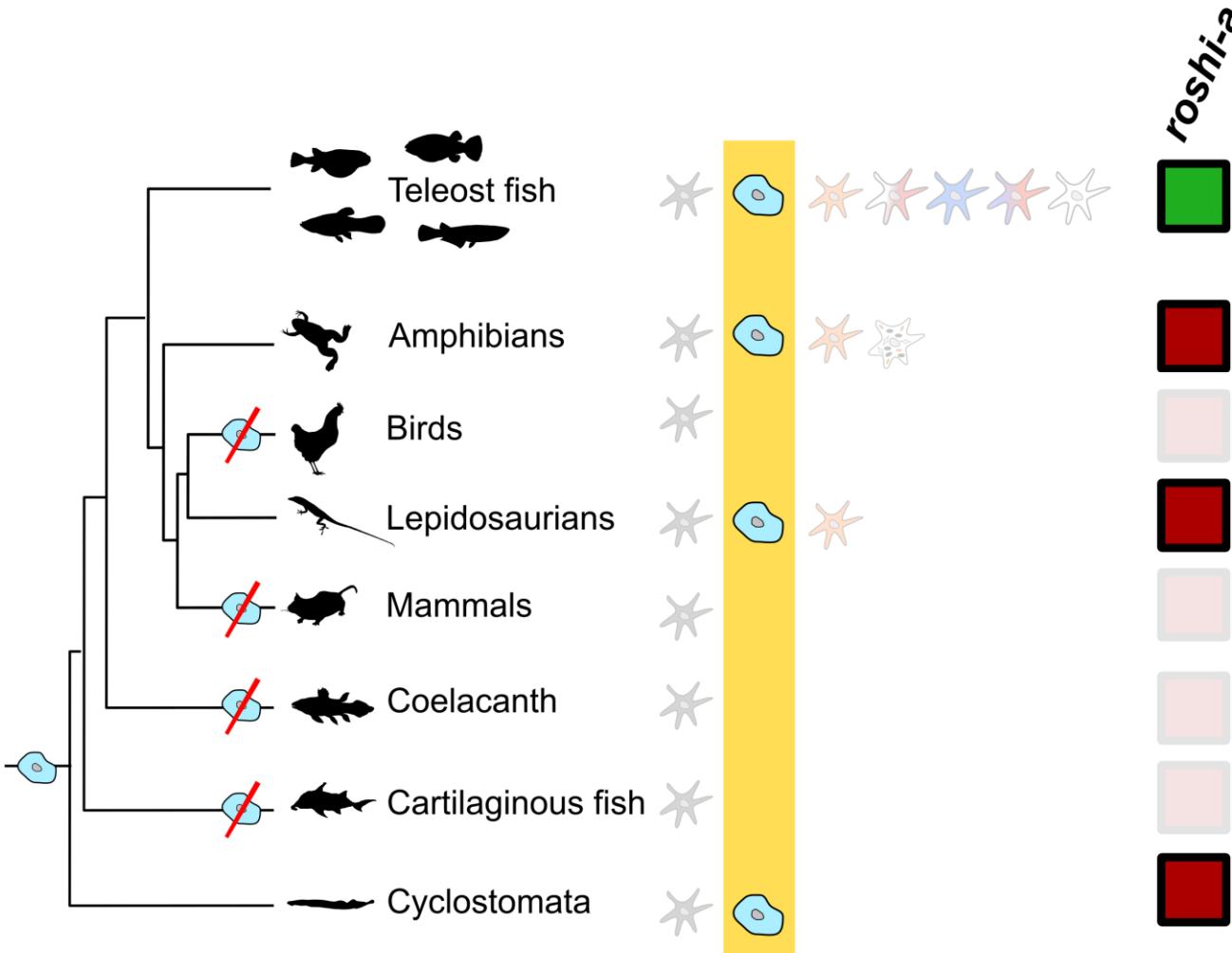
$\approx 400 \text{ Ma}$



# Iridophore evolution: monophyly within vertebrates?



# Iridophore evolution: monophyly within vertebrates?



# Evolution of GP relationships

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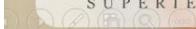
M1 – ENS  
Module "Genomes and Phenotypes"  
22/11/2018

Thibault Lorin



**ENS**

ÉCOLE NORMALE  
SUPÉRIEURE



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**Thank you for your attention**

# WHAT IF I TOLD YOU

INTERNSHIPS WERE MORE IMPORTANT THAN  
ANY GRADE, PROJECT, MIDTERM, OR FINAL

Made on imgur

Thank you for your attention

NOT SURE IF INTERN  
OR SLAVE

# WHAT IF I TOLD YOU

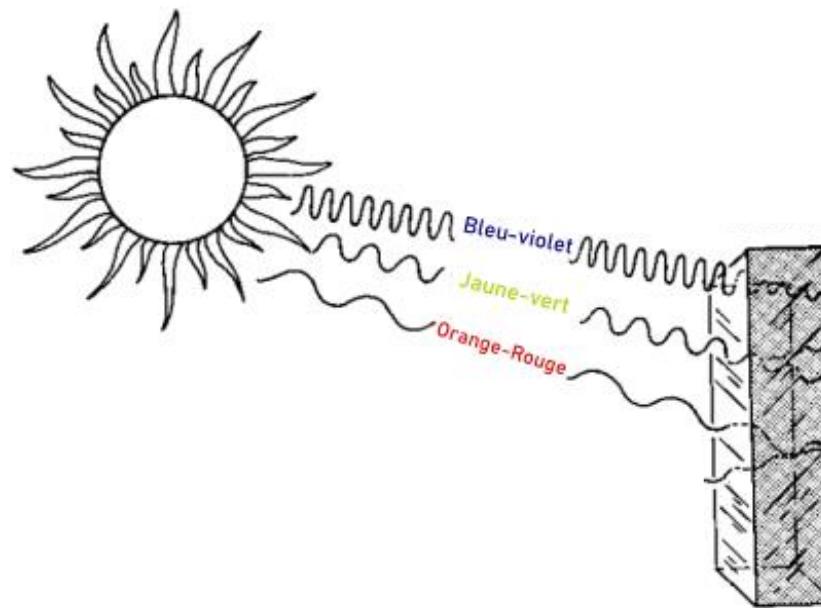
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Made on imgur

Thank you for your attention

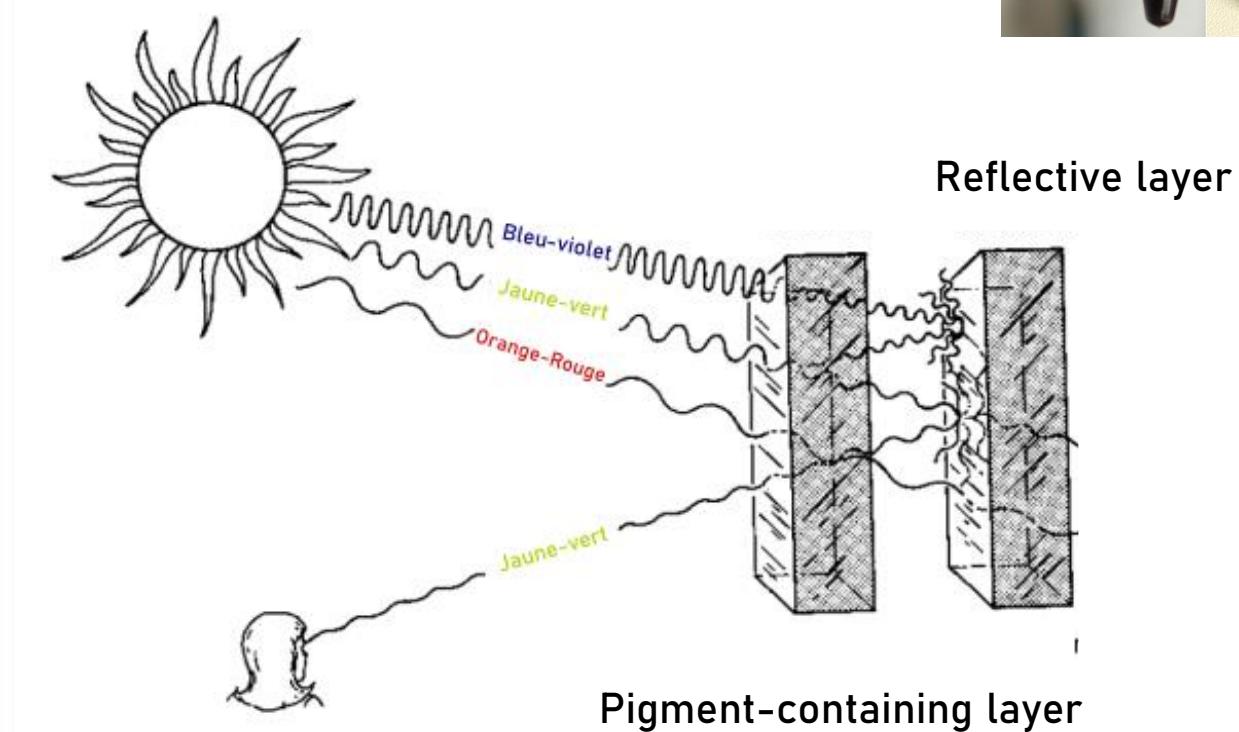
NOT SURE IF INTERN  
OR SLAVE

## "Chemical" colors...

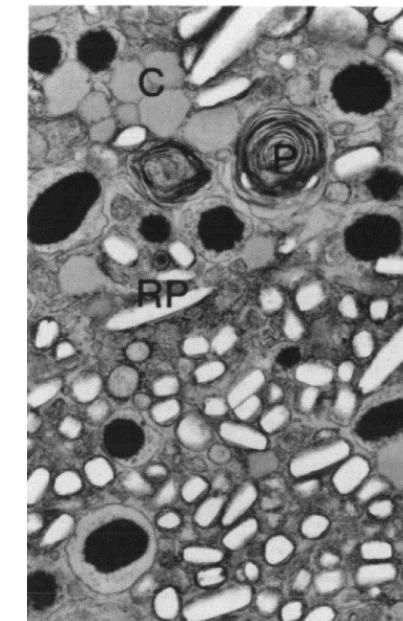
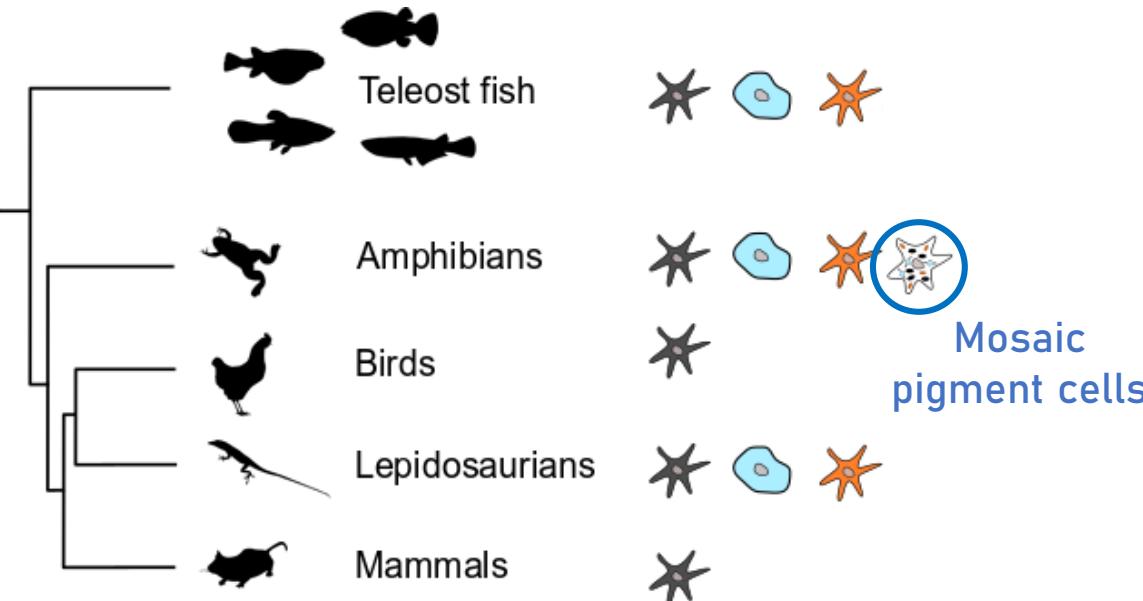


Pigment-containing layer

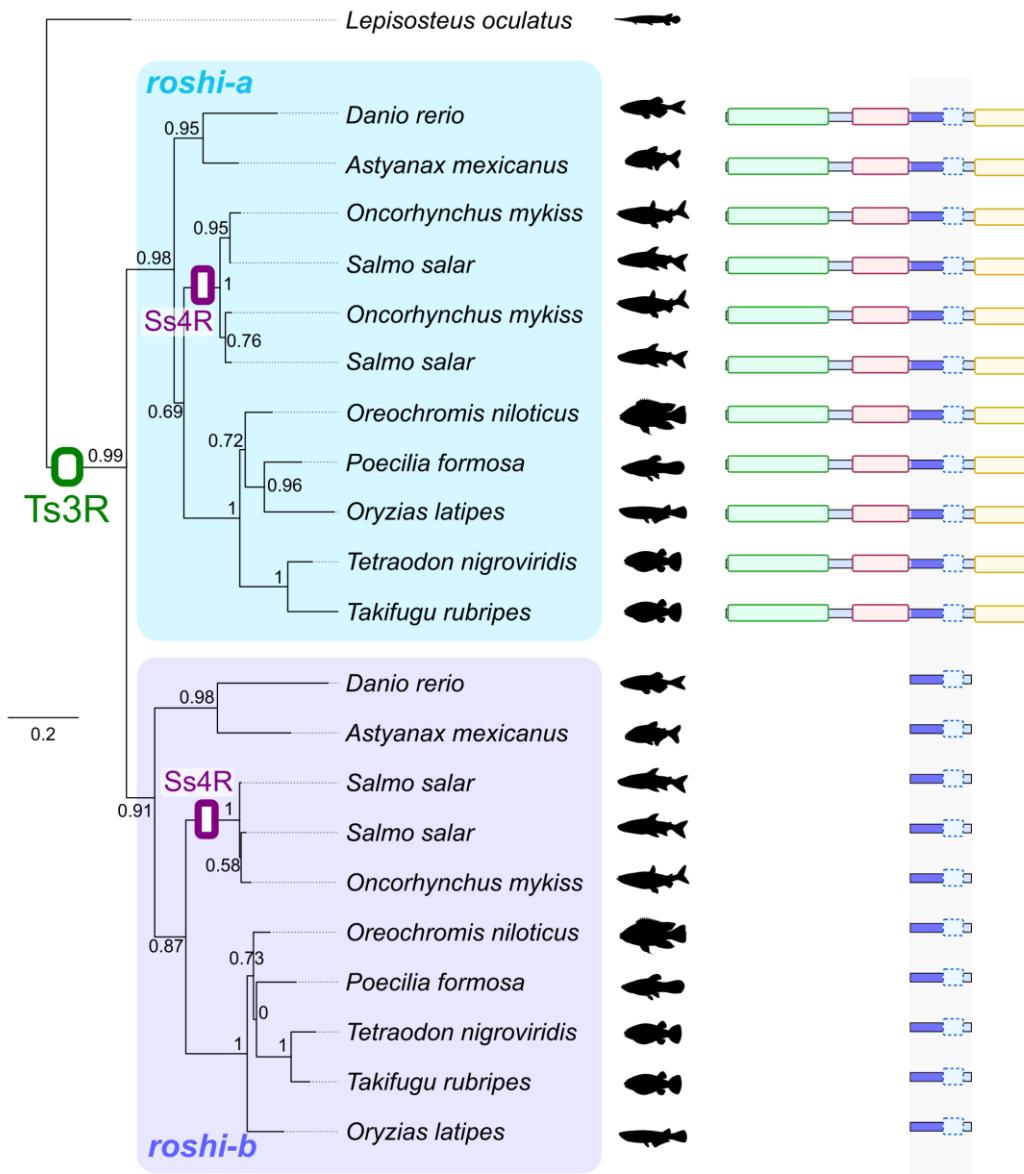
# "Chemical" colors.... Need structural layers!



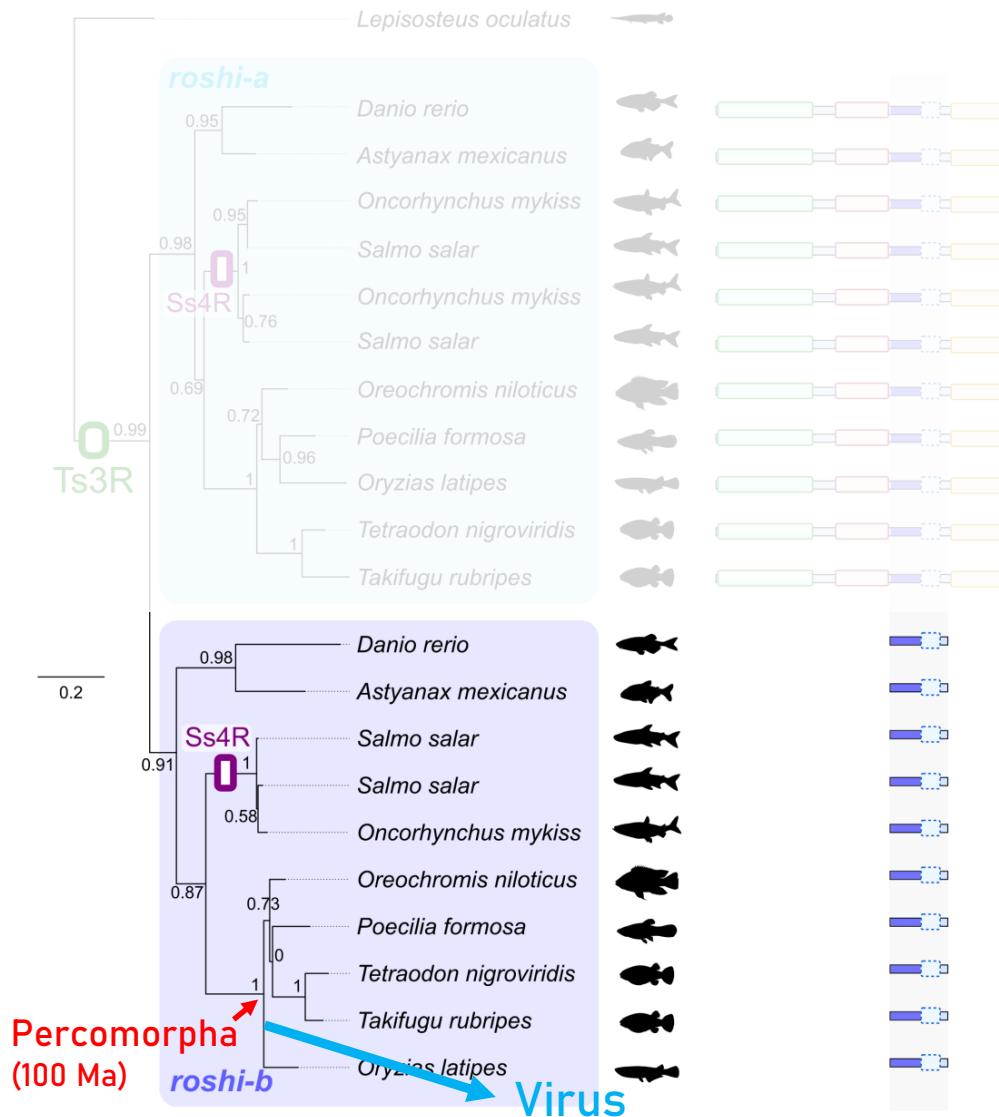
# Developmental, cellular and molecular basis of pigmentation in Vertebrates



# Roshi a été dupliqué et retenu après la Ts3R

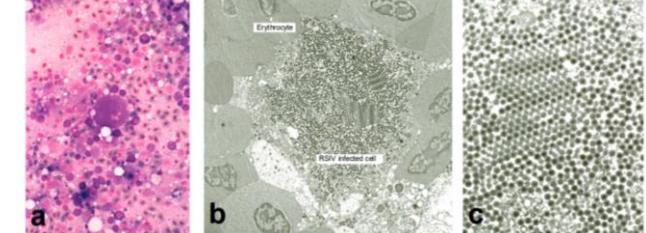


# Roshi-b a été transféré à un virus de manière horizontale

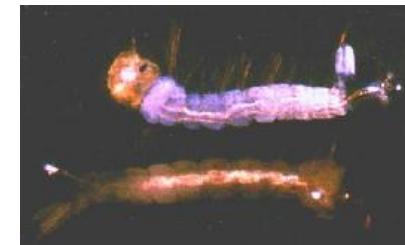


## Séquences de Mégalocytivirus...

- Anémies
- Hémorragies
- Exophtalmie
- Gonflement de la rate



... qui sont des Iridovirus !

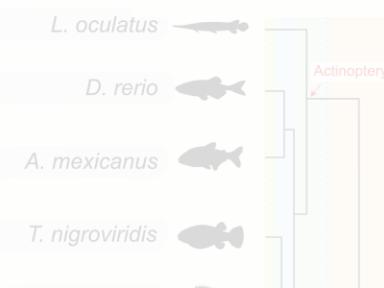


# La phylogénie de *roshi* ne correspond pas à la phylogénie des espèces

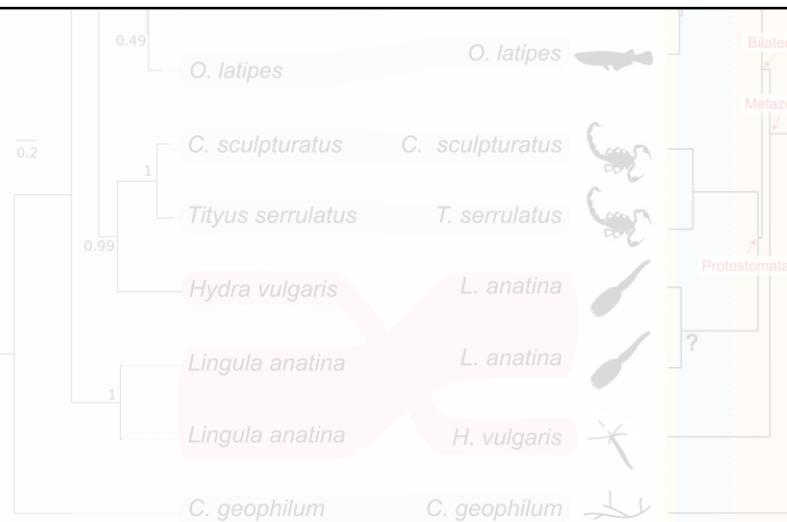
## Roshi protein phylogeny



## Species phylogeny



Patron évolutif suggérant une transmission horizontale du gène



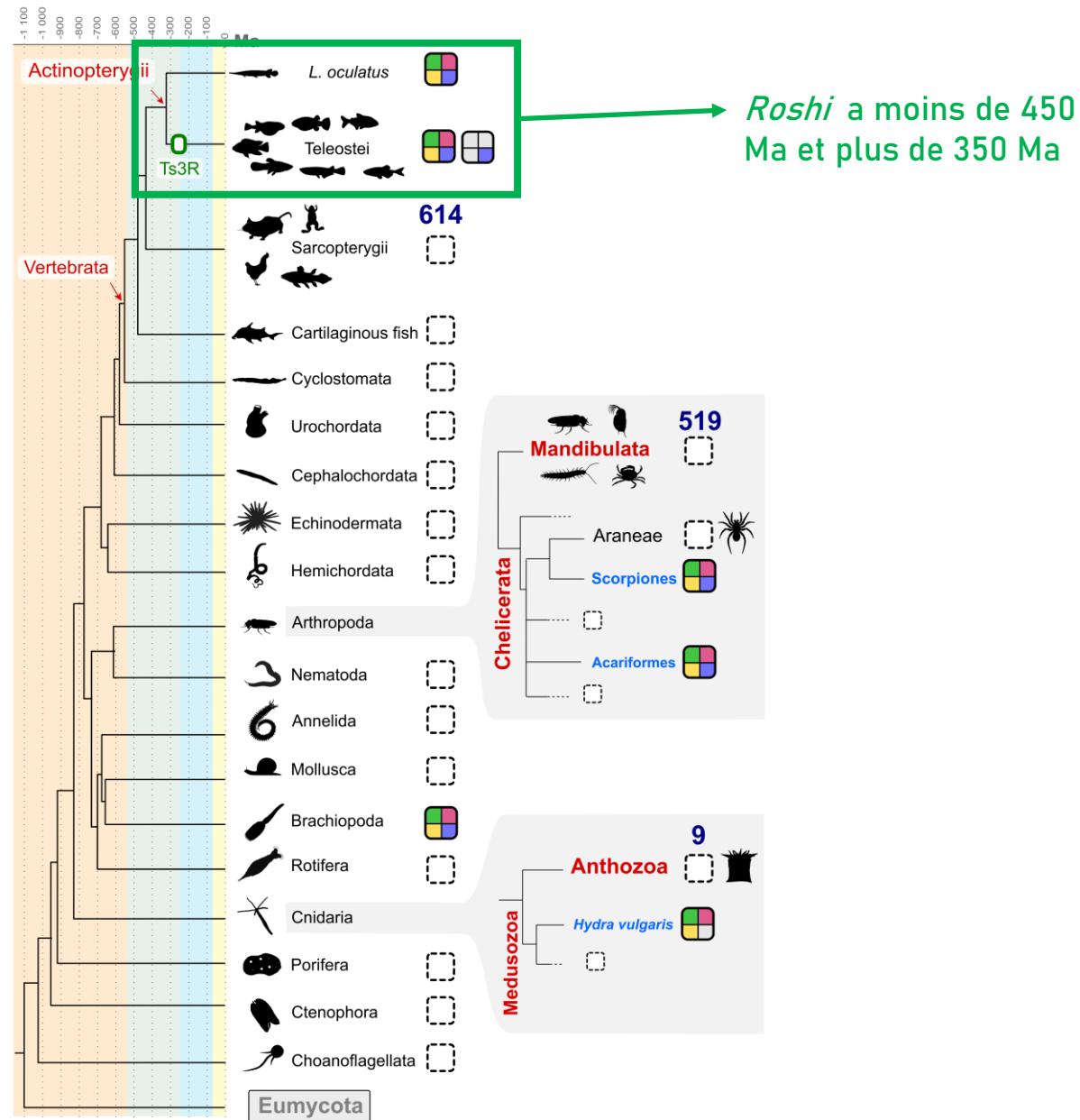
Transfert :

- de "qui" vers "qui" ?
- "quand" ?
- "comment" ?

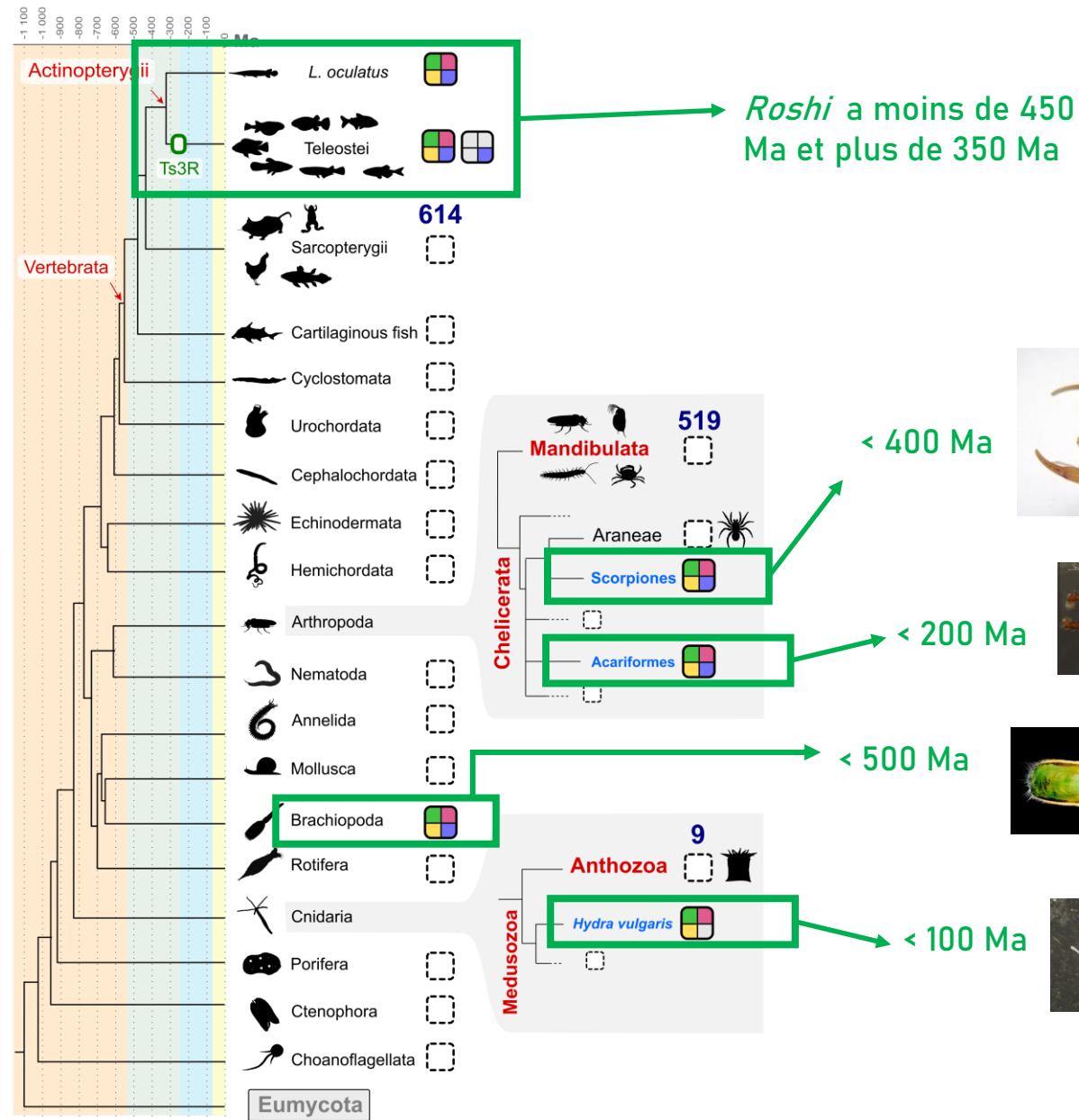


Vincent Daubin

# Estimation des âges de transfert de *roshi* chez les Animaux



# Estimation des âges de transfert de *roshi* chez les Animaux



More recently... "Color" is still a first-considered trait



Nature cover (1981)

Among all possible mutants used to describe the emergence of a new model system, guess which one was used?

*Golden*... a pigmentary mutant!

More recently... "Color" is still a first-considered trait



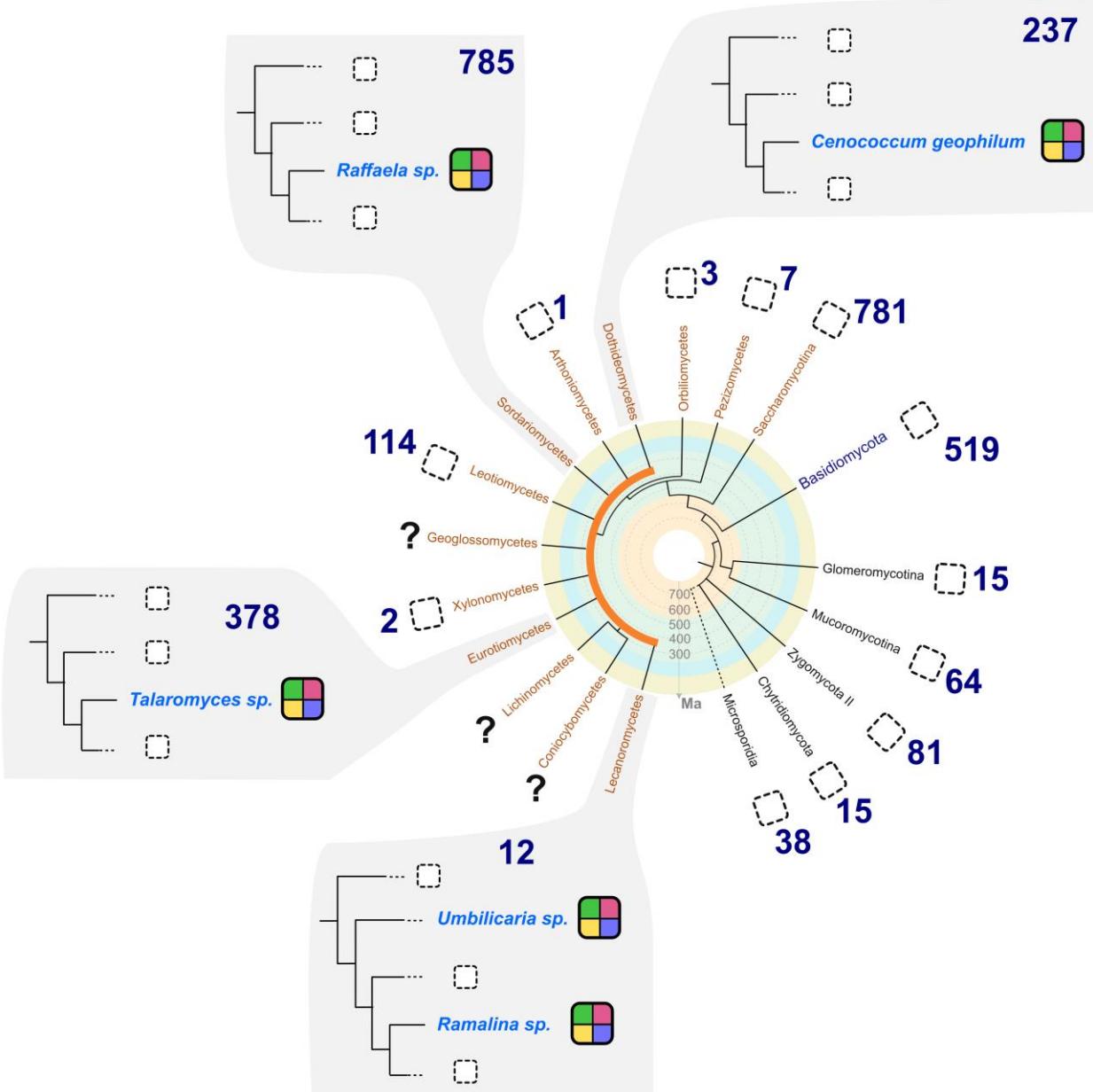
Nature cover (1981)

Among all possible mutants used to describe the emergence of a new model system, guess which one was used?

*Golden*... a pigmentary mutant!

Potential biases in the choice  
of model systems?

# Distribution de *roshi* chez les Eumycètes



# Quels sont les modes de transfert envisagés pour *roshi* ?

## 1. Les HGT sont moins fréquents mais tout de même possibles chez les Eucaryotes

Huang et al. (2010) *Science*

Sun et al. (2015) *Sci Rep*

## 2. Les HGT "inter-Animaux" sont rares

Gilbert et al. (2012) *Mol Biol Evol*

Graham et al. (2012) *BMC Evol Biol*

Peccoud et al. (2017) *PNAS*

## 3. Les mécanismes de transfert reposent probablement sur des vecteurs viraux

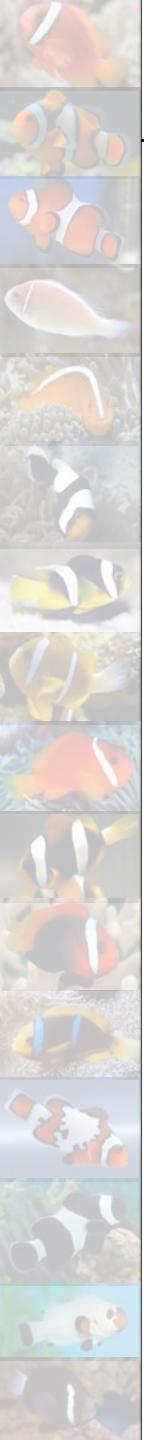
Gilbert et al. (2010) *Nature*

Gilbert and Cordaux (2017) *Curr Opin Virol*



Waddington et al. (2015)  
*Biol. Letters*

- ✓ a. *Roshi-b* transféré horizontalement à des Iridovirus
- ✓ b. *Roshi* présent chez des Acariens
- ✓ c. Ecologie compatible (aquatique)



# Quels sont les modes de transfert envisagés pour *roshi* ?

## 1. Les HGT sont moins fréquents mais tout de même possibles chez les Eucaryotes

Hughes *et al.* (2010) *Science*

Sun *et al.* (2015) *Sci Rep*

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## 3. Les mécanismes de transfert reposent probablement sur des vecteurs viraux

Gilbert *et al.* (2010) *Nature*

Gilbert and Cordaux (2017) *Curr Opin Virol*

## 4. Au sein des Champignons, les transferts horizontaux sont possibles

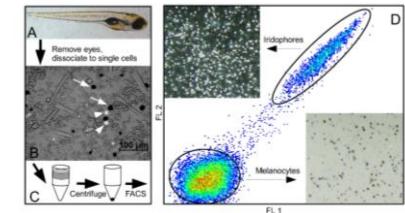
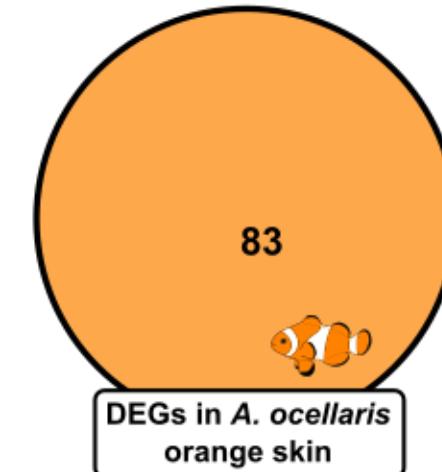
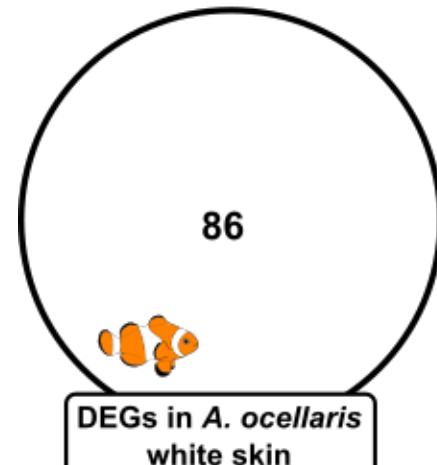
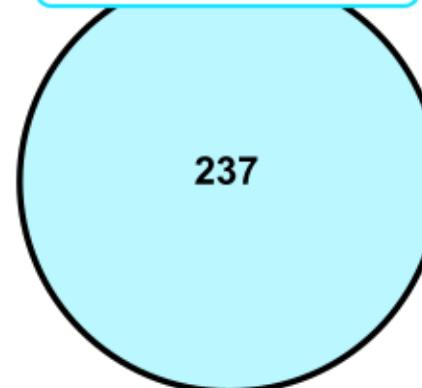
Fitzpatrick (2012) *FEMS Microbiol Lett*

Xie *et al.* (2008) *BMC Evol Biol*

# La peau blanche a une "identité d'iridophores"

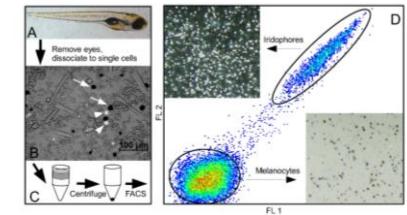
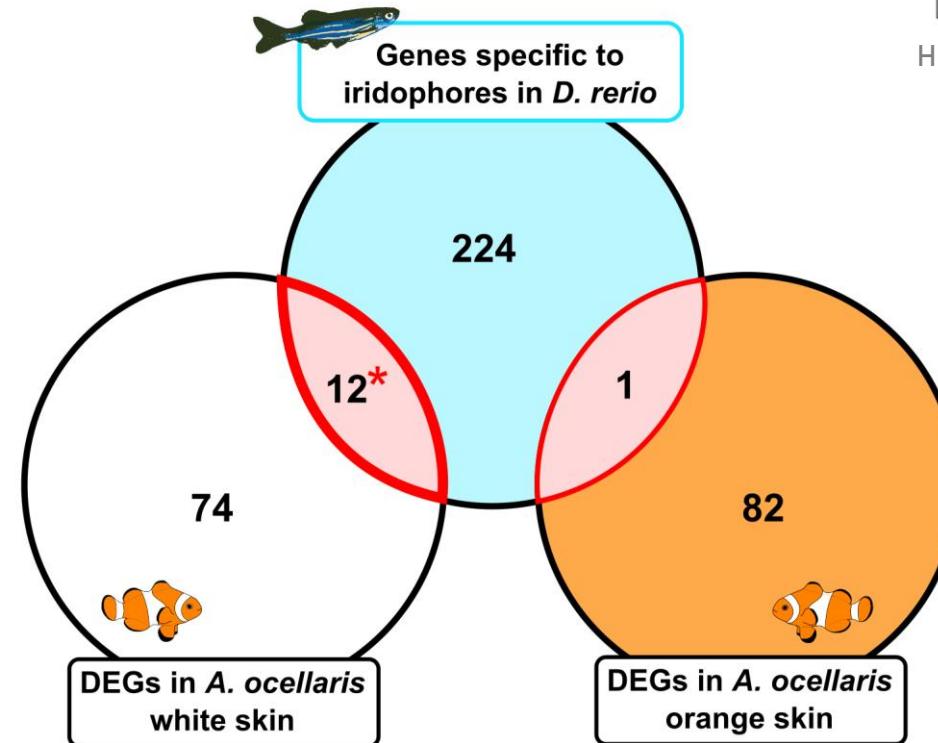


Genes specific to  
iridophores in *D. rerio*



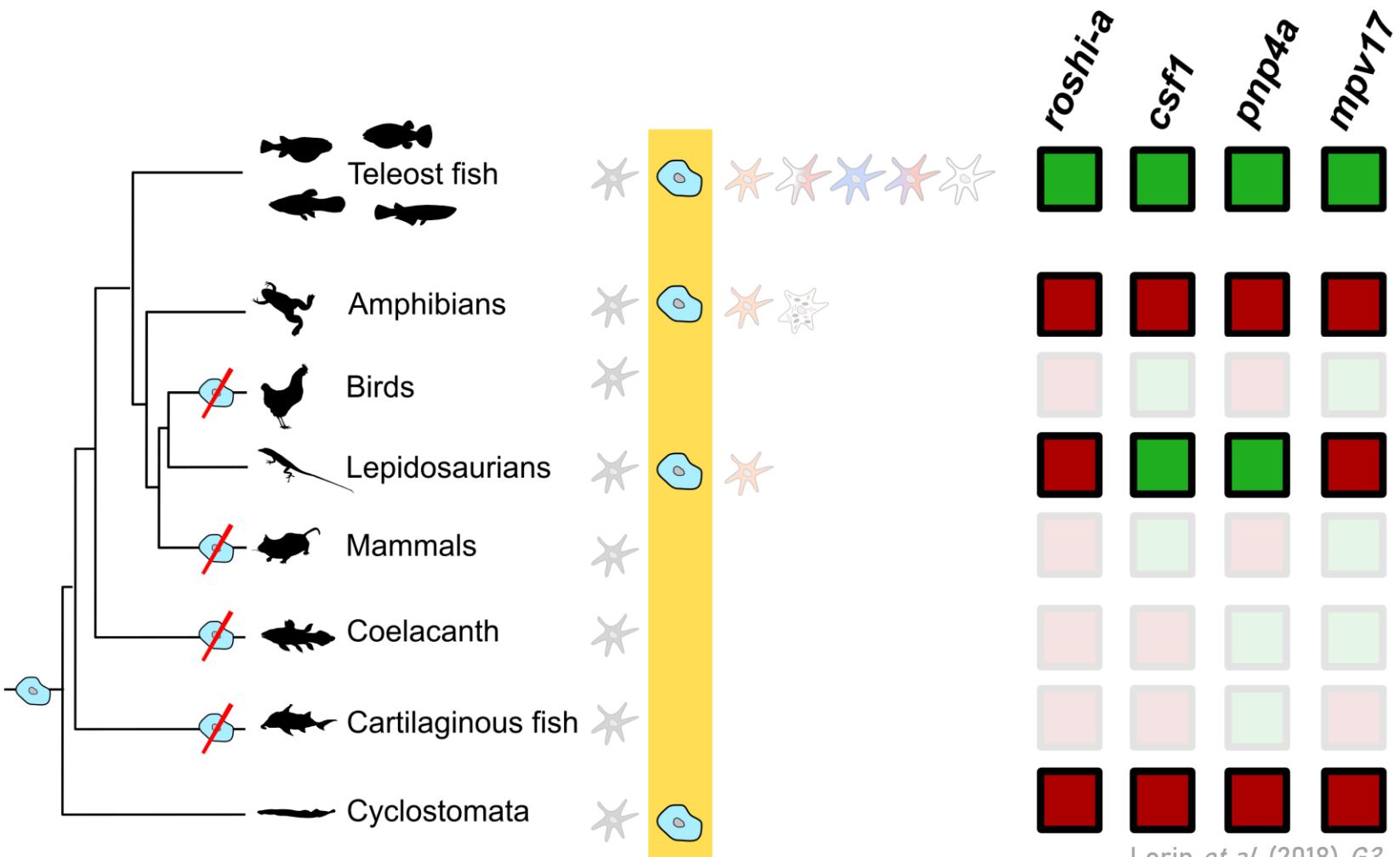
Higdon et al. (2013) Plos One

# La peau blanche a une "identité d'iridophores"



Higdon *et al.* (2013) *Plos One*

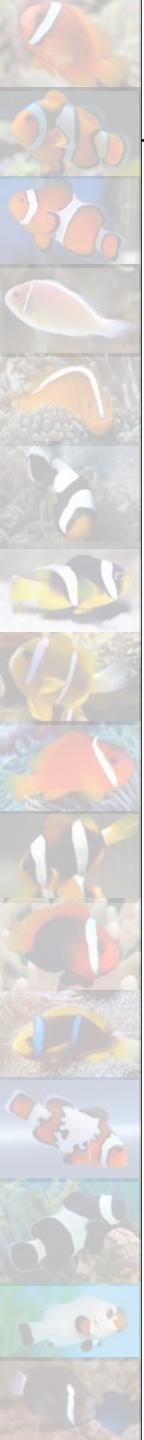
# Roshi et d'autres gènes invitent à envisager l'évolution des iridophores chez les Vertébrés sous un nouvel angle

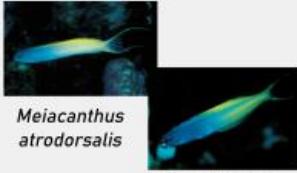
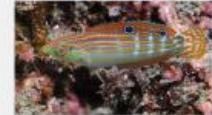


Lorin et al. (2018) G3

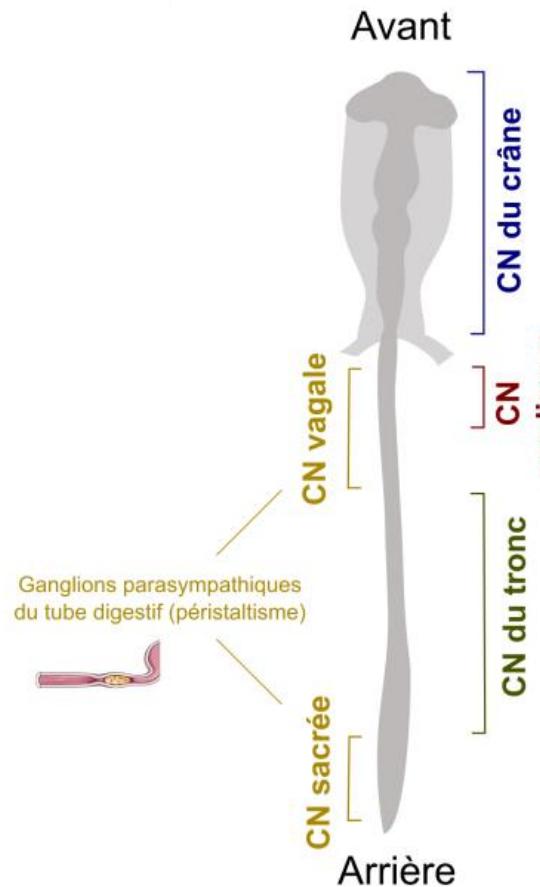
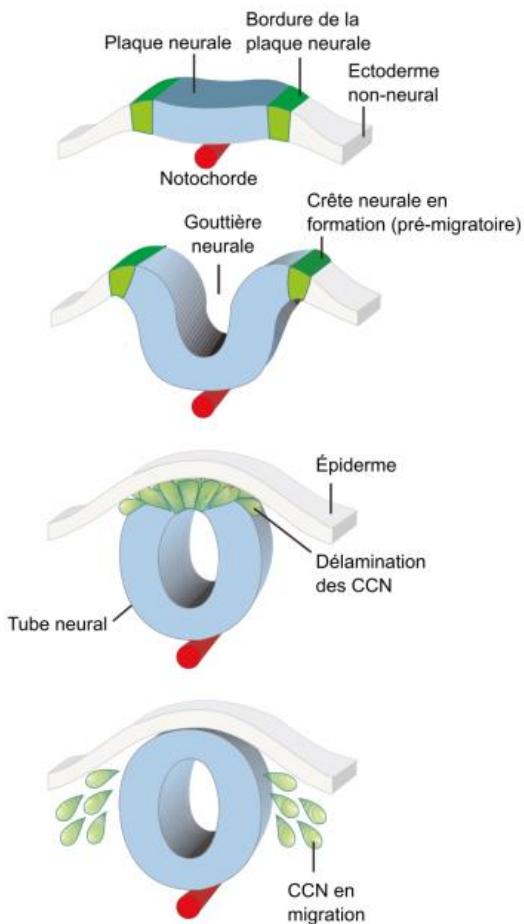
# Wallace et la "coloration animale"

## Une classification revisitée sur l'exemple des poissons coralliens



CAMOUFLAGE ("Protective colors")		COULEURS D'AVERTISSEMENT ("Warning colors")	COULEURS DE COMMUNICATION INTRA- OU INTERSPÉCIFIQUE ("Typical colors")
<b>Cryptisme</b>		<b>Organismes naturellement protégés</b>	<b>Communication intraspécifique (hors sélection sexuelle)</b>
Assortiment à l'arrière-plan		Aposématisme	Compétition intrasexuelle
Spécialisé	Généraliste	 <i>Pterois volitans</i> (Nelsen et al. 2014)	 <i>Chaetodon lunula</i> (Hamilton et al. 1971)
 <i>Hippocampus bargibanti</i> (Randall et al. 2005)	 <i>Antennarius commerson</i> (Randall et al. 2005)		
<b>Countershading</b>	<b>Mascarade</b>	<b>Mimétisme müllerien</b>	<b>Signaux de regroupement</b>
 <i>Chaetodon lunula</i> (Hamilton et al. 1971)	 <i>Juvénile de Platax orbicularis</i> (Randall et al. 2005)	 <i>Meiacanthus atrodorsalis</i> <i>Plagiotermus laudandus</i> (Randall et al. 2005)	 <i>Myripristis murjan</i> (McRobert et al. 1998)
<b>Coloration trompeuse</b>		<b>Organismes non-protégés</b>	<b>Communication interspécifique</b>
 <i>Dascyllus aruanus</i> (Phillips et al. 2017)		<b>Mimétisme batésien</b>	<b>Confusion de prédateurs</b>
<b>Transparence</b>	<b>COULEURS SEXUELLES ("Sexual colors")</b>	 <i>Meiacanthus nigrolineatus</i> (peu comestible, à gauche) et <i>Ecsenius gravieri</i> (inoffensif) (Randall et al. 2005)	 <i>Halichoeres brownfieldi</i> (Lönnstedt et al. 2013)
 <i>Larve d'Acanthurus triostegus</i> (Holzer, Besson et al. 2017)	<b>Dimorphisme sexuel</b>		
	 <i>Femelle (en haut) et mâle (en bas) de Poisson-perroquet</i>		

# Les cellules pigmentaires proviennent de la crête neurale



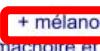
Neurones et glie des ganglions crâniens



Os et cartilage de l'avant de la tête



Tissu conjonctif de la tête



+ mélanocytes odontoblastes + os de la machoire et de l'oreille interne + conjonctif de la thyroïde, du thymus...



Conjonctif des grosses artères + mélanocytes + cartilage des arcs pharyngiaux/branchiaux



Glande médullo-surrénale



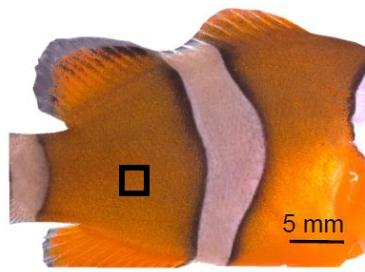
Système nerveux périphérique (cellules de Schwann, ganglions spinaux)



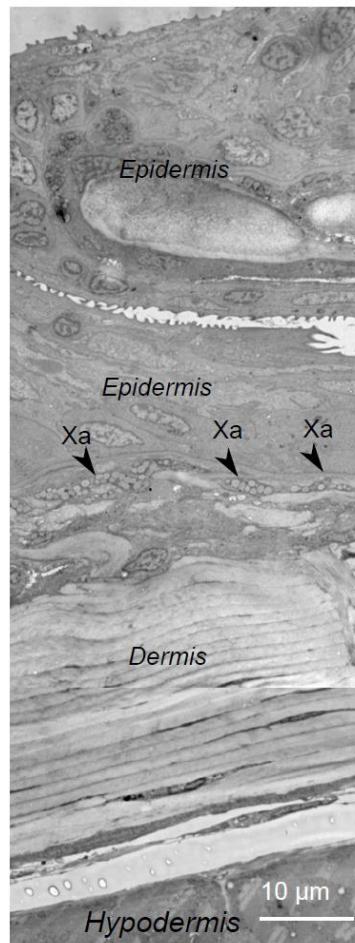
Mélanocytes chez les Mammifères, autres cellules pigmentaires chez d'autres Vertébrés

# Peau orange en microscopie

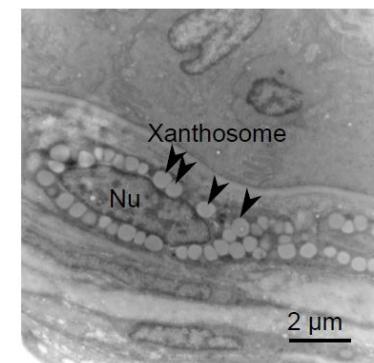
A.



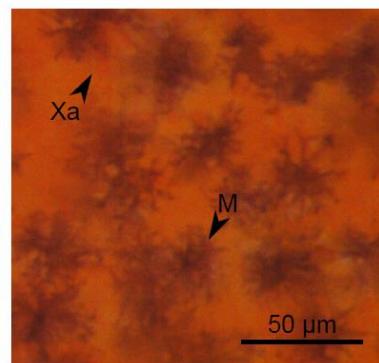
C.



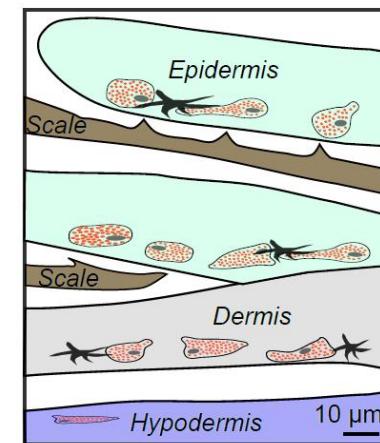
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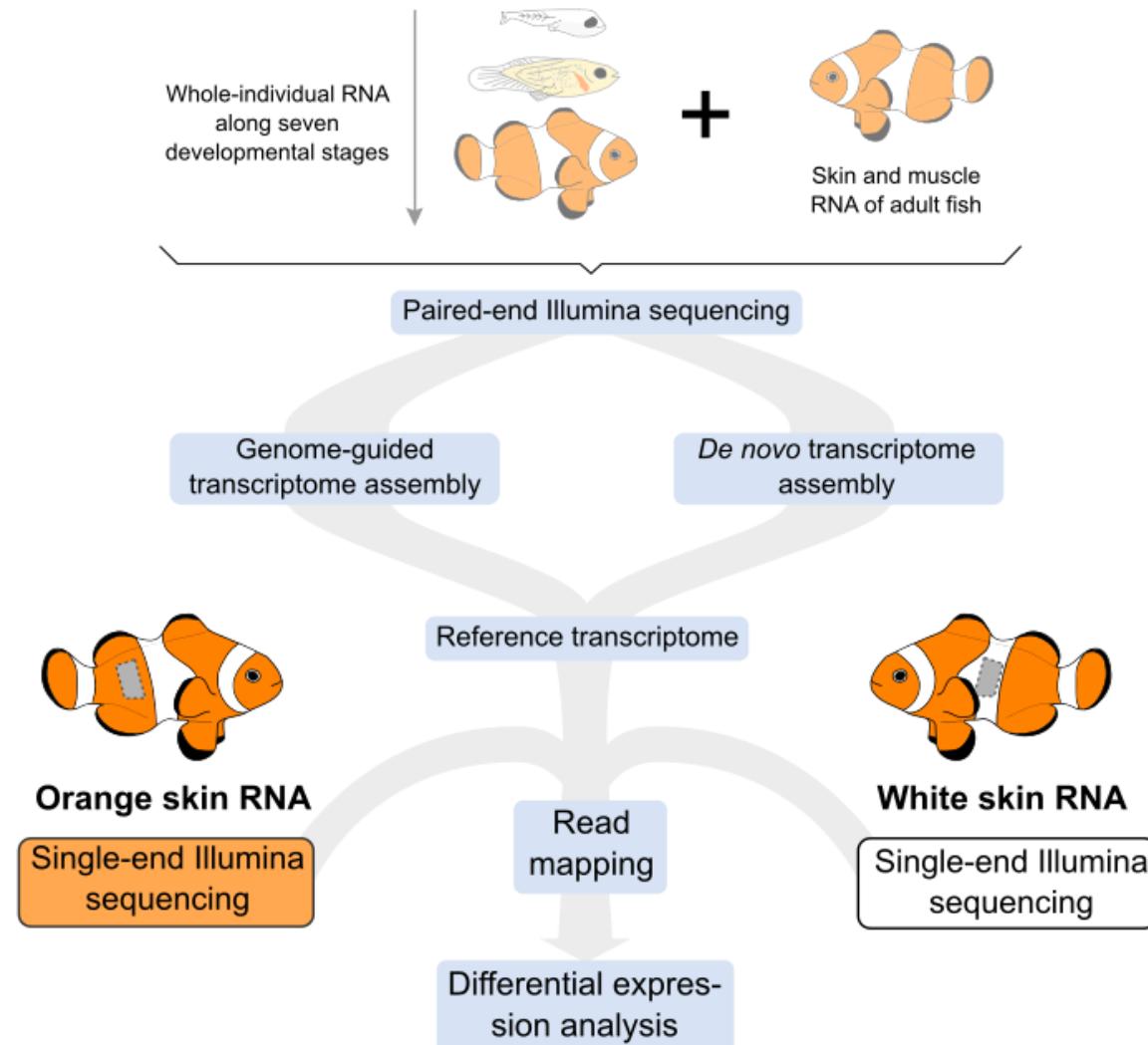
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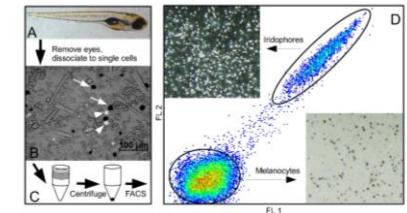
E.



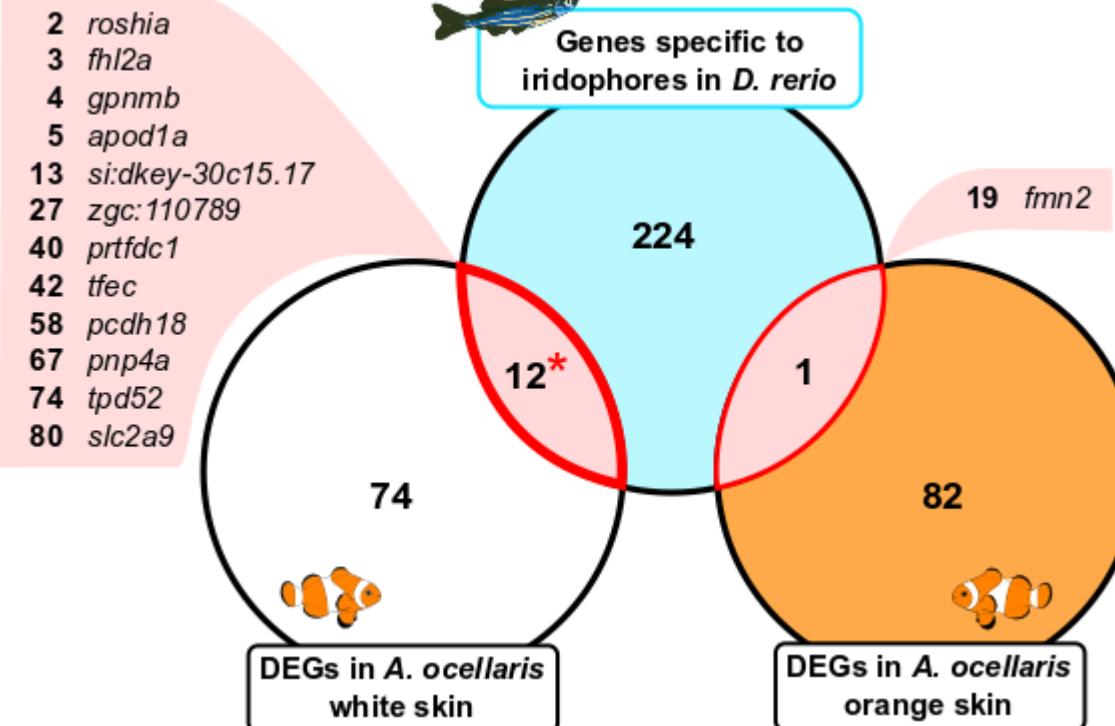
# Pipeline utilisé pour l'analyse des différences d'expression



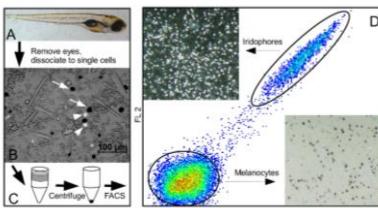
# La peau blanche a une "identité d'iridophores"



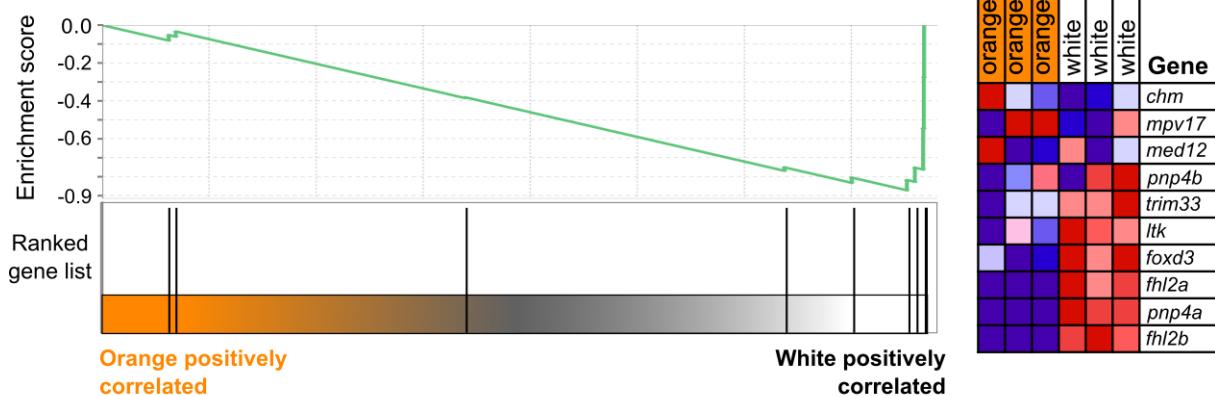
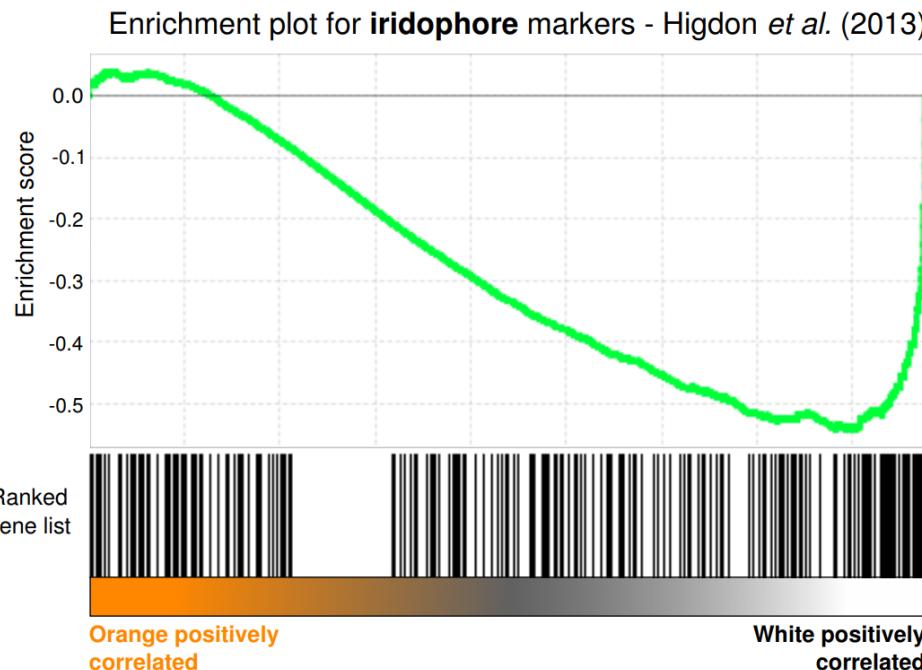
Higdon *et al.* (2013) Plos One



# La peau blanche a une "identité d'iridophores"



Higdon et al. (2013) Plos One



# Megalocytivirus (Iridoviridae)

