Fish genomics

Dr. Balázs Kovács

Genetics background of fish

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

- Fishes are the most diverse group of vertebrates
- 30,000 different species.
- Tree evolutionary different groups
  - Agnatha (hagfish, lamprey)
  - Chondrichthyes (cartilaginous fishes – sharks, rays)
  - Osteichthyes (Teleost fishes - carp, zebrafish)

Genome structure 1.

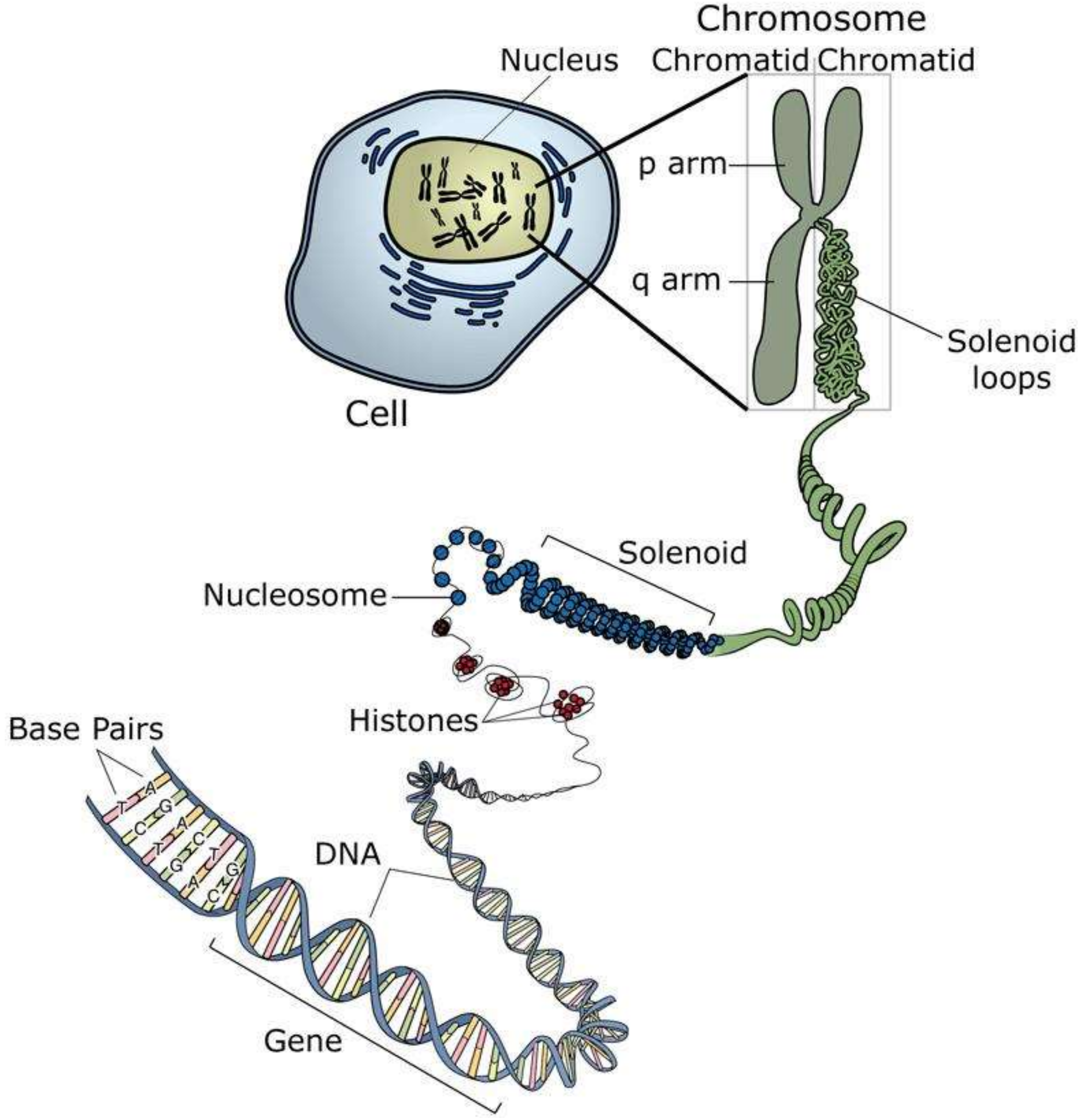


Image adapted from: National Human Genome Research Institute.

Genome structure 3.

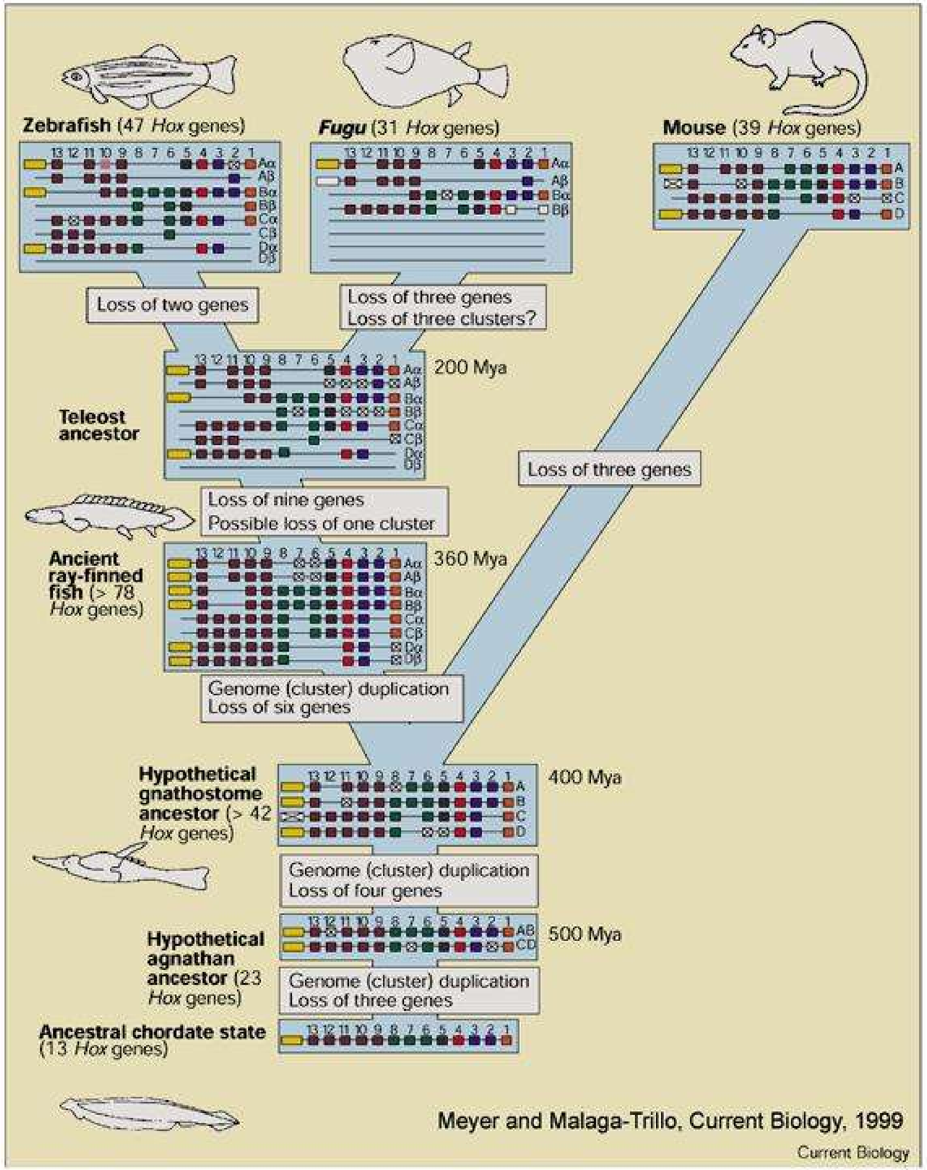
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

- One nucleus per cell
- Two chromosome set per cell
- Replication with cell reproduction
- Mendelian inheritance
- recombination
- Big size / milliards bp
- ~30 thousand genes
- ~1-3% coding region
- ~1-2% regulating region

Genetics background of fish

More genes in fish?

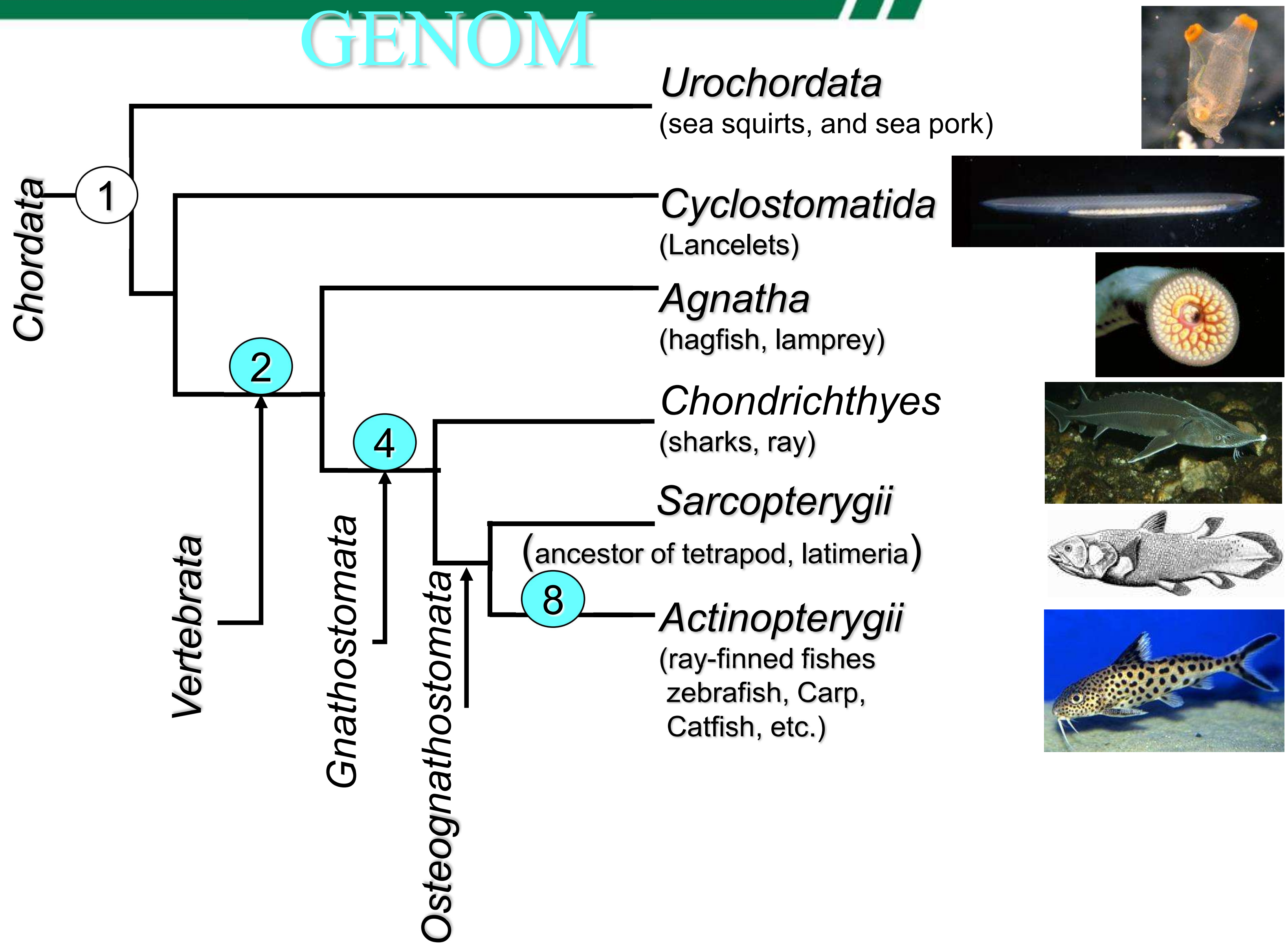
Changes of Hox gene clusters and gene numbers
During the evolution.



Genetics background of fish

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

GENOM

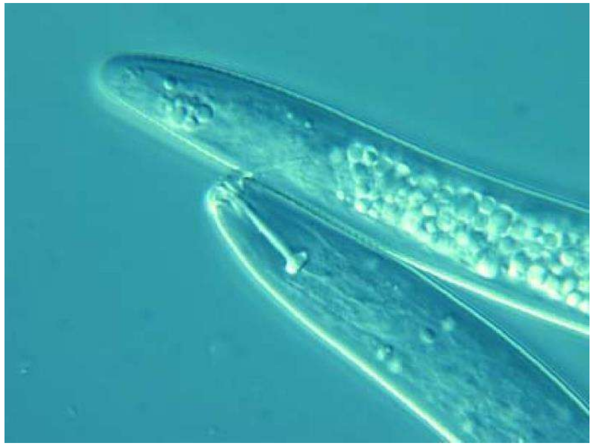


Genome size 1.

Smallest animal genome size:0.02pg

Plant-parasitic nematode

(*Pratylenchus coffeae*),



Human genome size: 3.5pg

(*Homo sapiens*)

Genome size 2.

Smallest vertebrate and fish genome size: 0.35pg

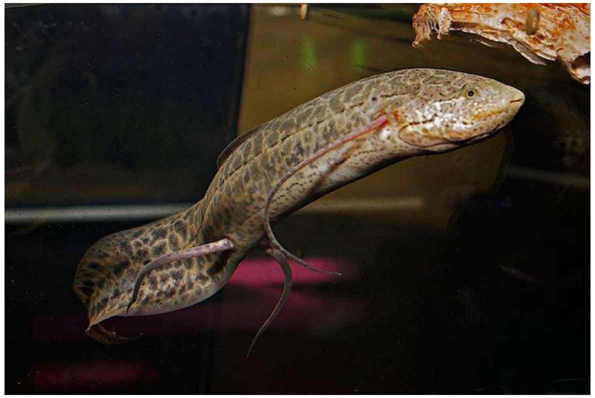
Green puffer fish

(*Tetraodon fluviatilis*)

Largest fish and animal genome size:132.83pg

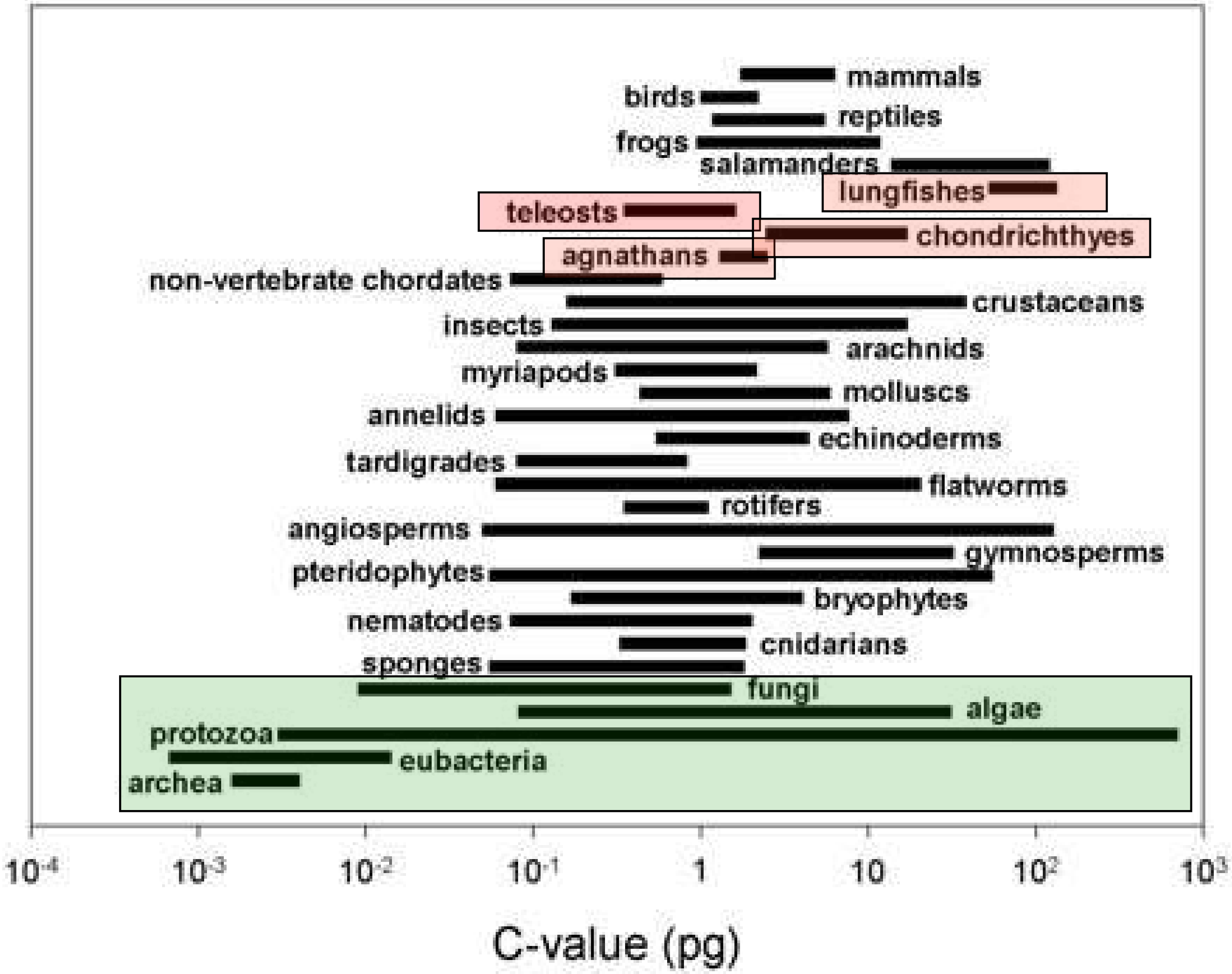
Marbled lungfish

(*Protopterus aethiopicus*)

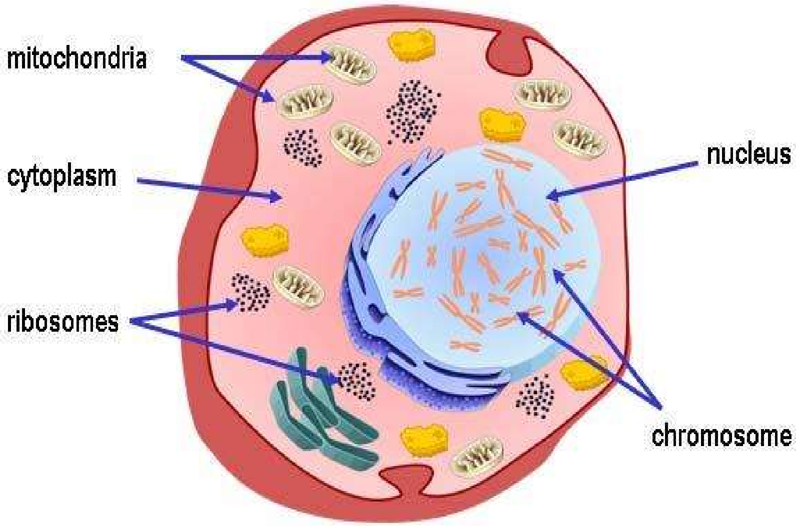


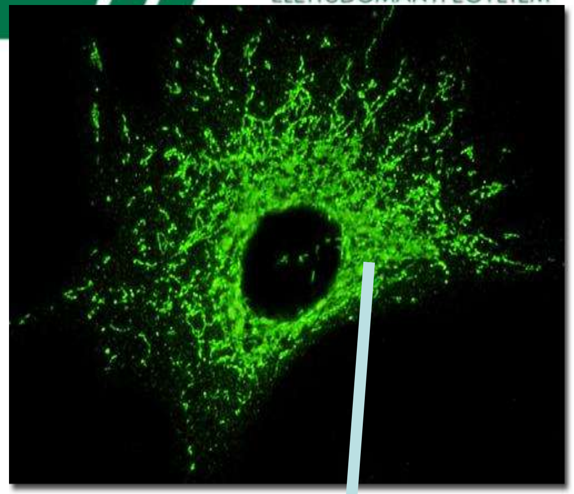
Genome size 3.

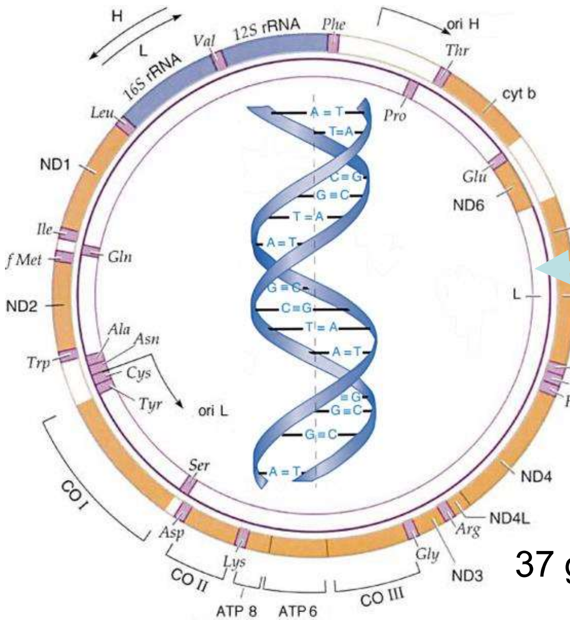
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM



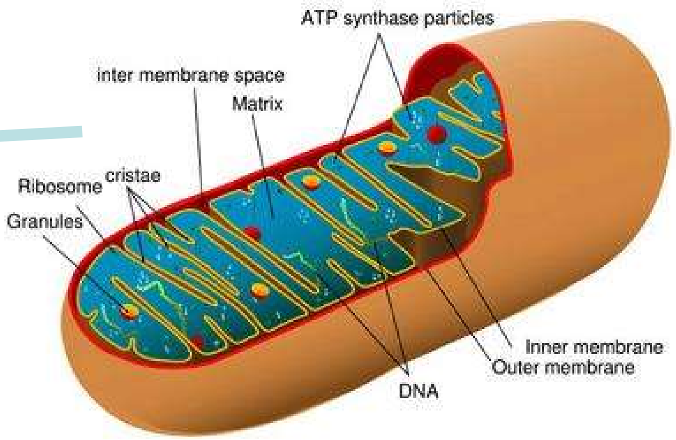
Mitochondrial DNA 1.







37 genes

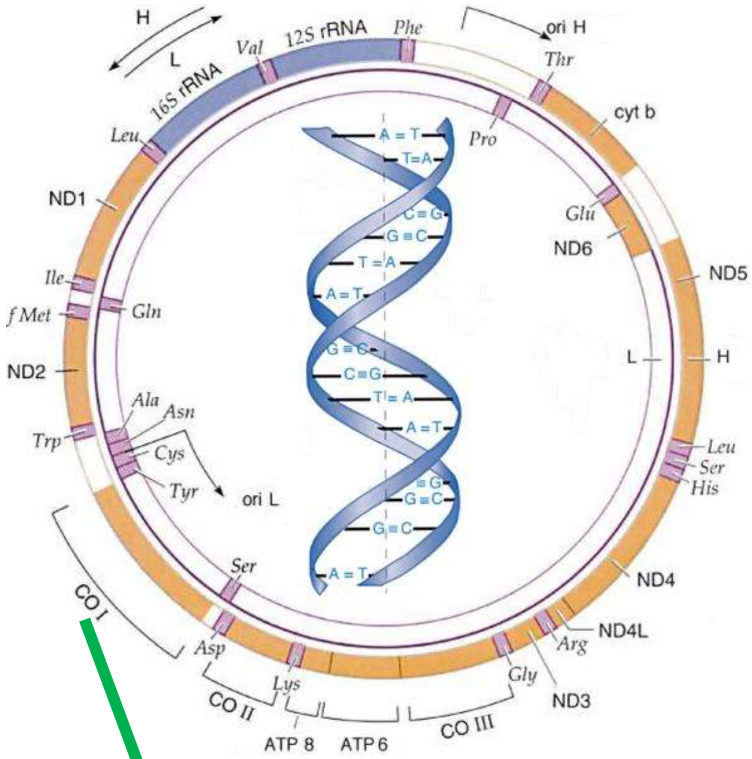


Mitochondrial DNA 2.

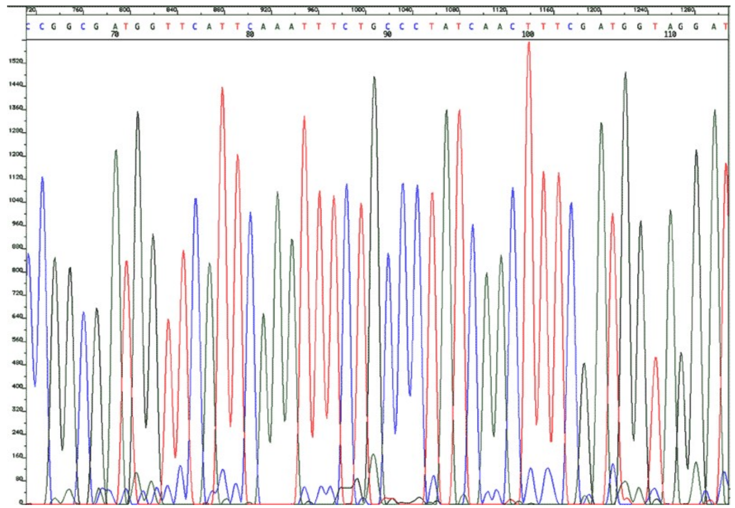
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

- More thousand mitochondria per cell
- Independent replication from the nucleus
- Circular molecule
- Maternal inheritance
- No recombination
- Small size/16-17 thousand bp (1% of a bacterial genome)
- 37 gene

Barcoding 1.



sequencing



Species identification

Cytochrome oxidase I

Barcoding 2.

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

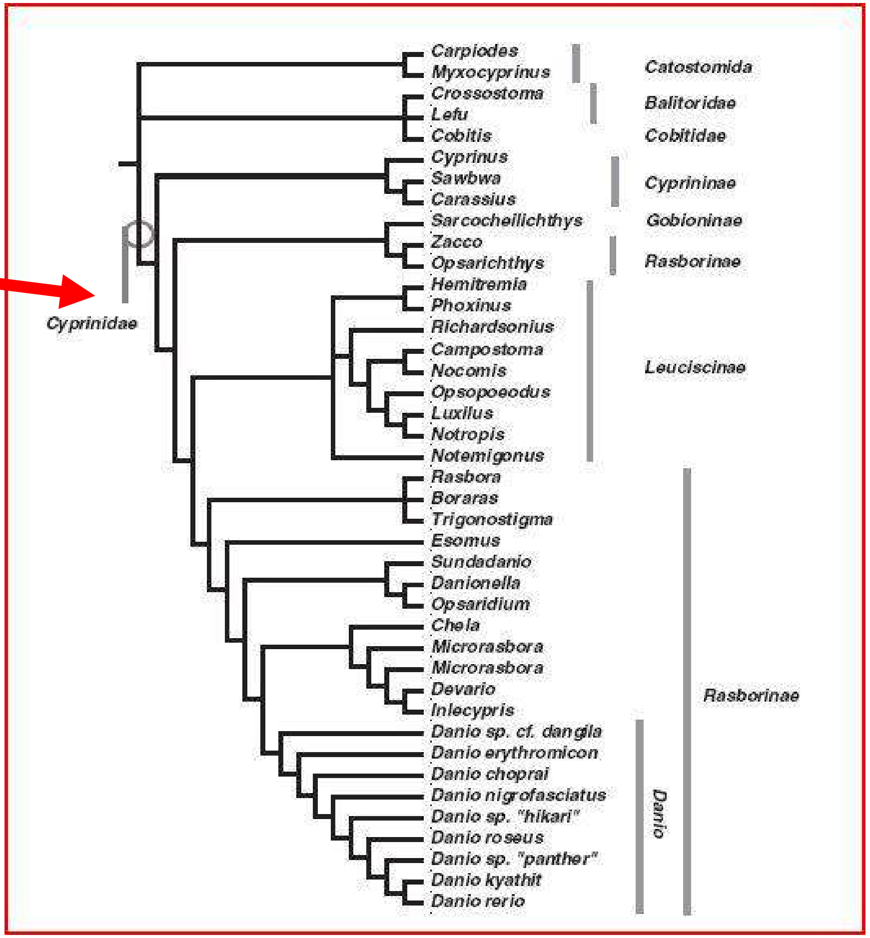
Species identification

BOLDSYSTEMS

BARCODE OF LIFE DATA SYSTEM

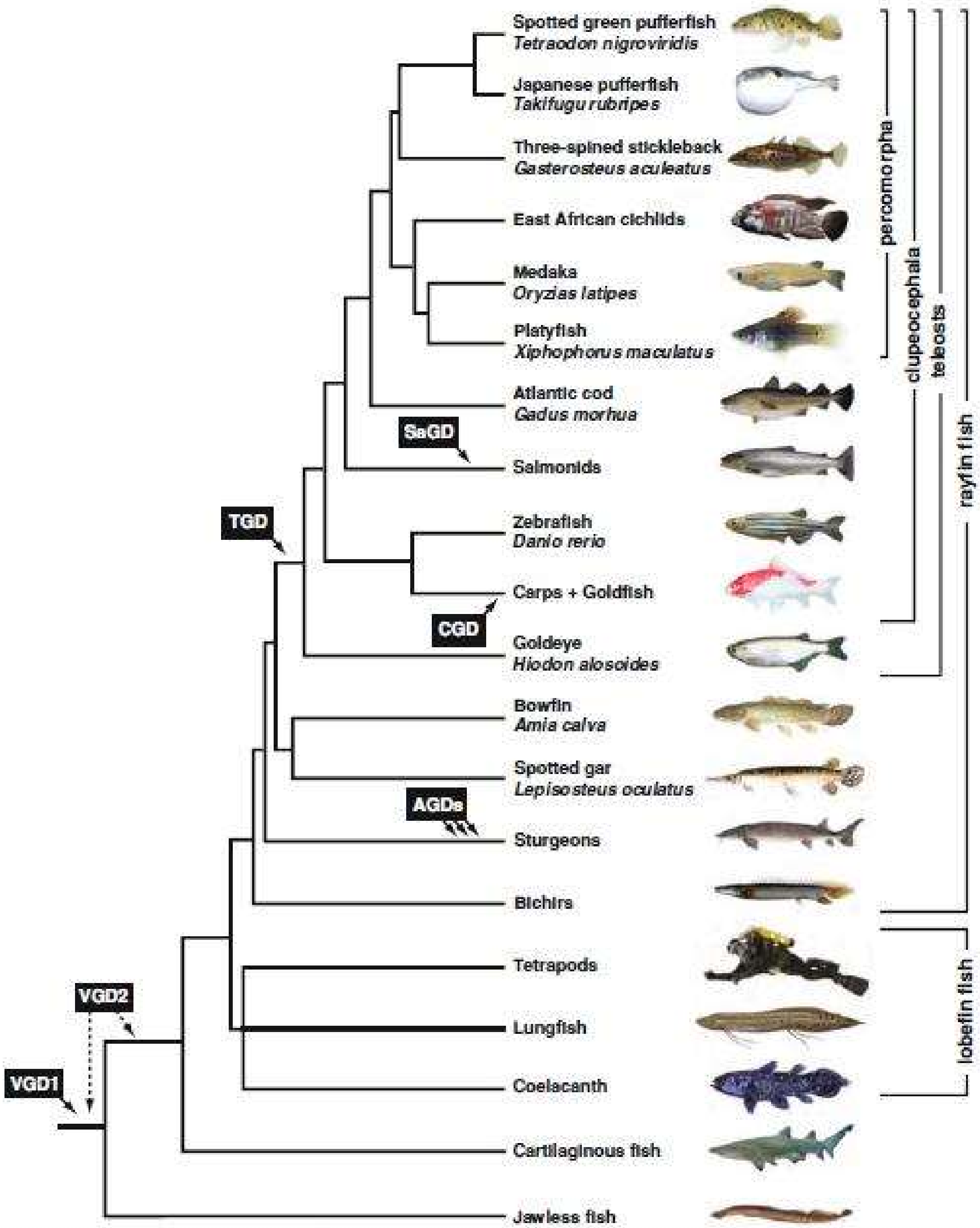
http://www.boldsystems.org/

More than 22,607 fish species



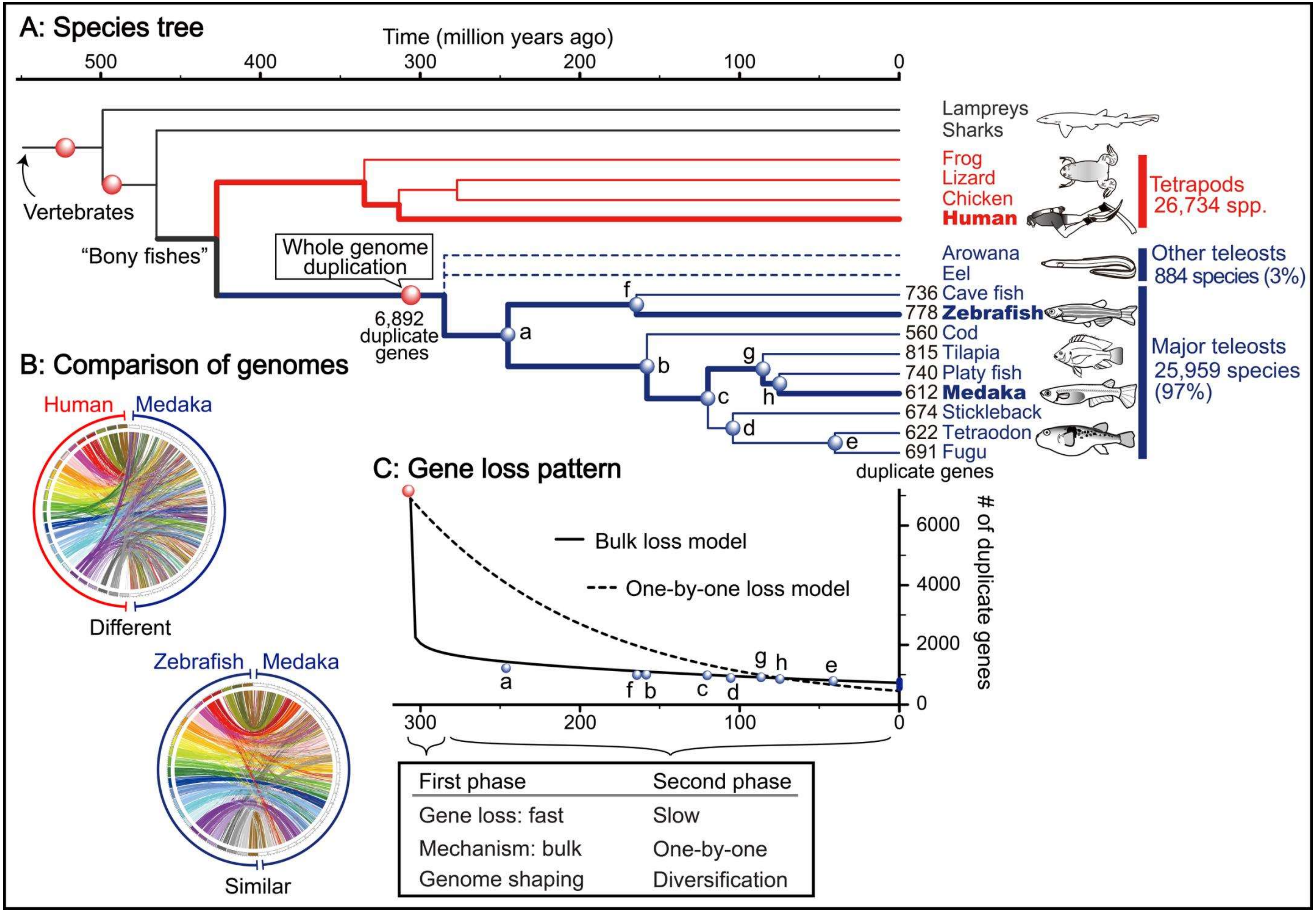
Taxonomy

http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=77



MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

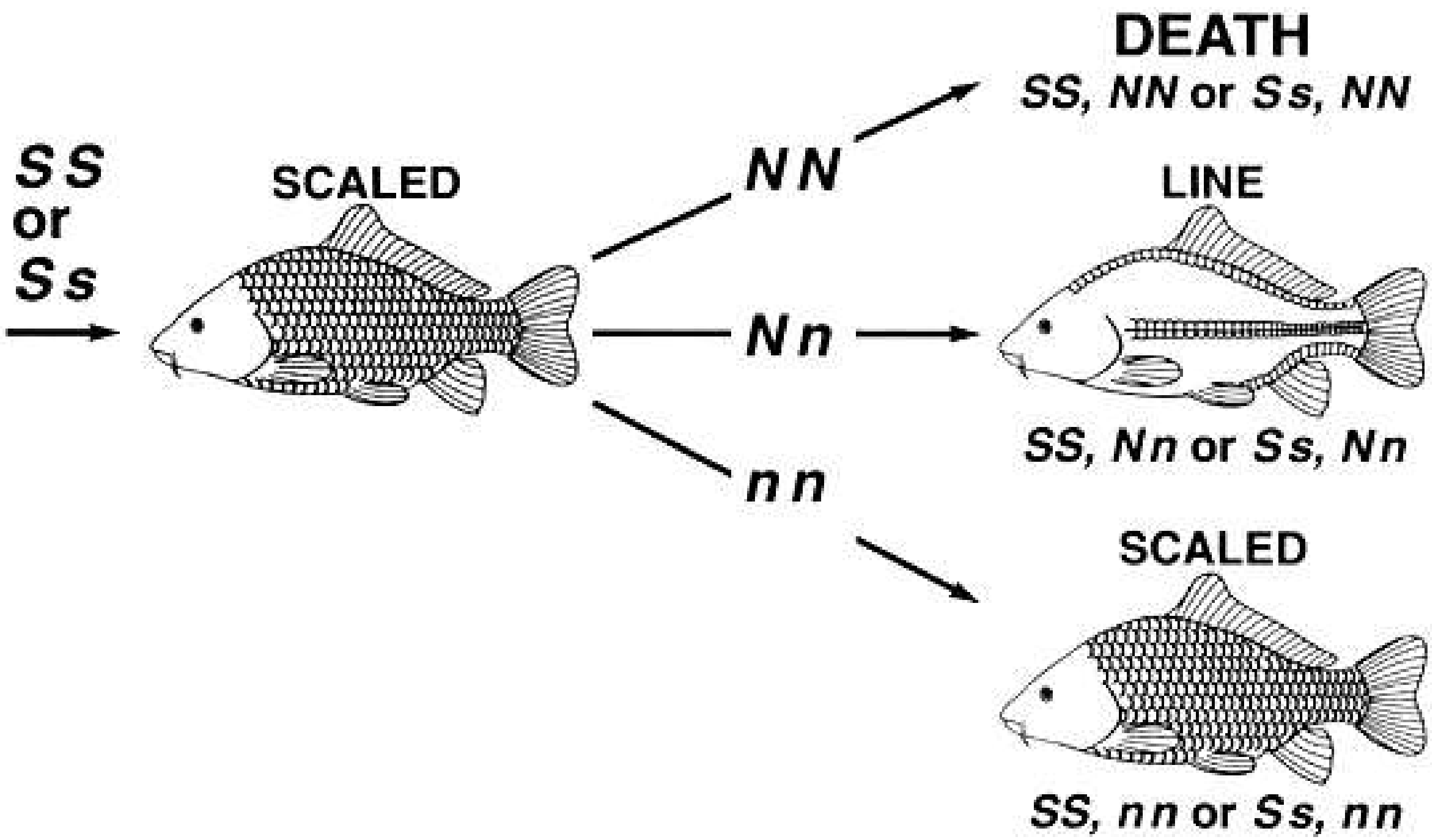
Mank and Avise 2006,Proc.R.Soc.Lond.B.Bio.Sci.273(1582):33–38.



A: Species tree
Time (million years ago)
500
400
300
200
100
0
Lampreys
Sharks
Frog
Lizard
Chicken
Human
Tetrapods
26,734 spp.
Vertebrates
"Bony fishes"
Whole genome duplication
6,892 duplicate genes
Arowana
Eel
Other teleosts
884 species (3%)
736 Cave fish
778 Zebrafish
560 Cod
815 Tilapia
740 Platy fish
612 Medaka
674 Stickleback
622 Tetraodon
691 Fugu
duplicate genes
Major teleosts
25,959 species
(97%)
a
b
c
d
e
f
g
h
B: Comparison of genomes
Human
Medaka
Different
Zebrafish
Medaka
Similar
C: Gene loss pattern
Bulk loss model
One-by-one loss model
# of duplicate genes
6000
4000
2000
0
300
200
100
0
First phase
Second phase
Gene loss: fast
Slow
Mechanism: bulk
One-by-one
Genome shaping
Diversification

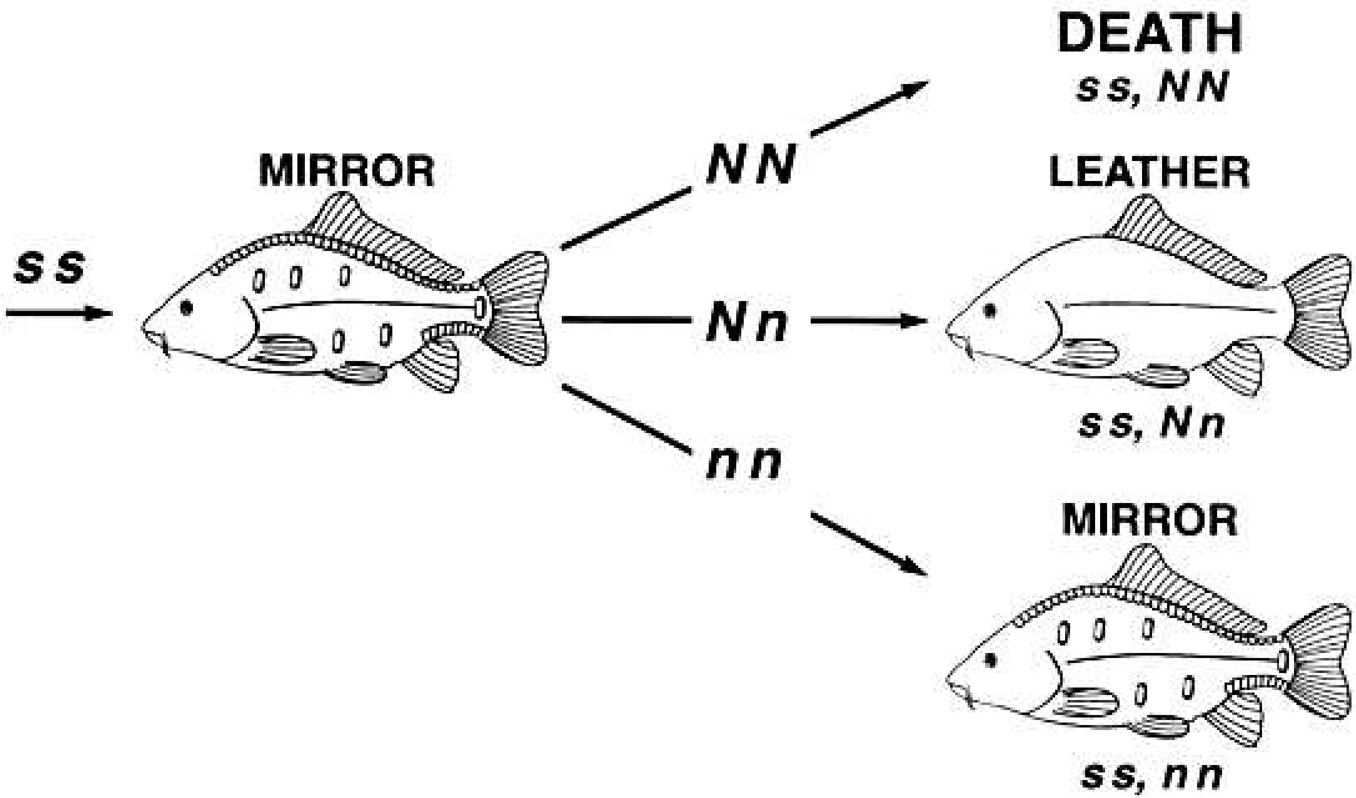
Cumulateive, Epistaticc, Pleiotropic gene effekt

Scale patter in carp 1



Cumulateive, Epistaticc, Pleiotropic gene effekt

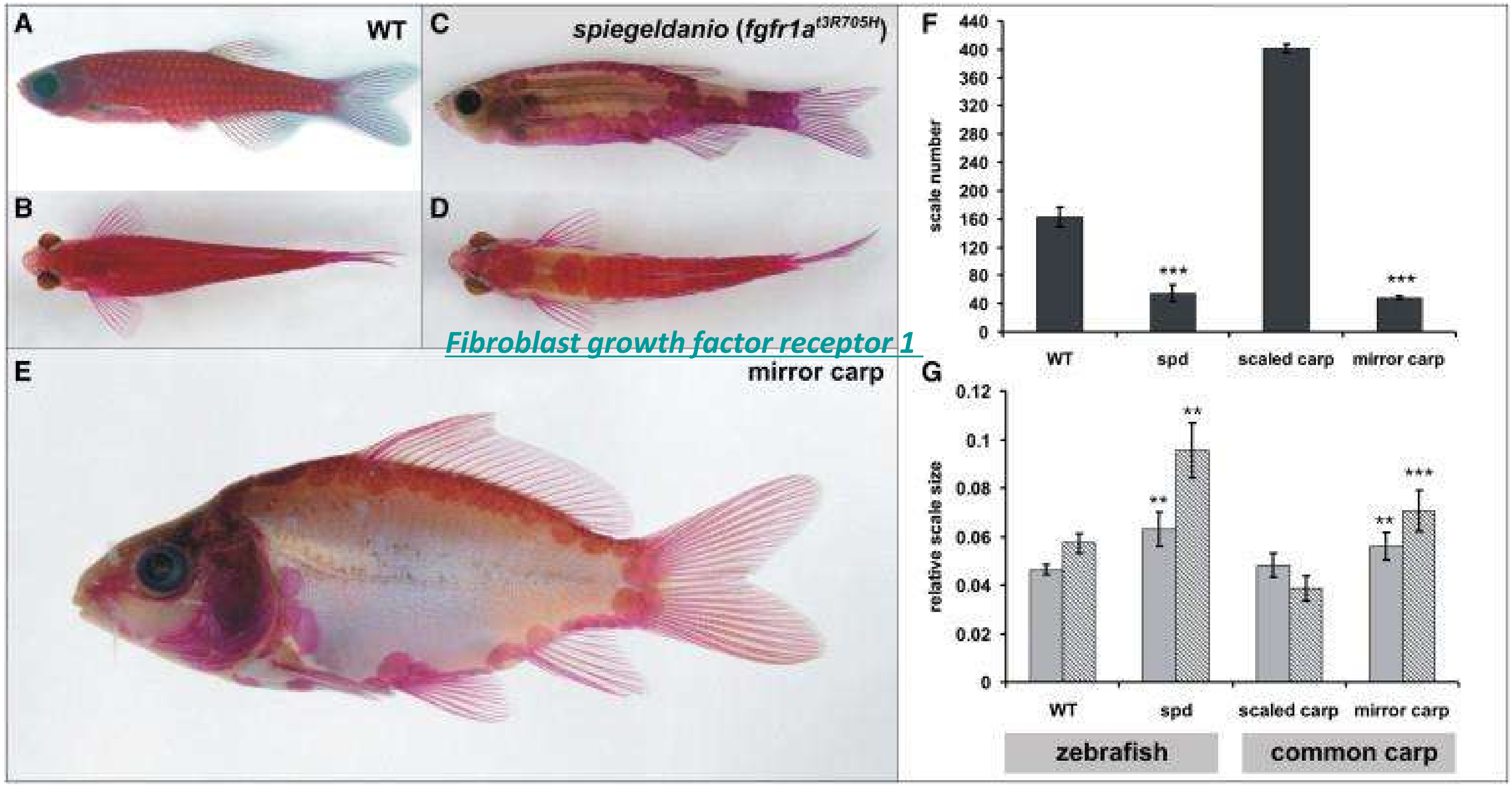
Scale patter in carp 2



Duplication of fgfr1 Permits Fgf Signaling to Serve as a Target for Selection during Domestication

Nicolas Rohner, Miklós Bercsényi, László Orbán,

Maria E. Kolanczyk, Dirk Linke, Michael Brand, Christiane Nüsslein-Volhard,



Fibroblast growth factor receptor 1

Current Biology Volume 19, Issue 19, 2009, Pages 1642–1647

Duplication of fgfr1 Permits Fgf Signaling to Serve as a Target for Selection during Domestication

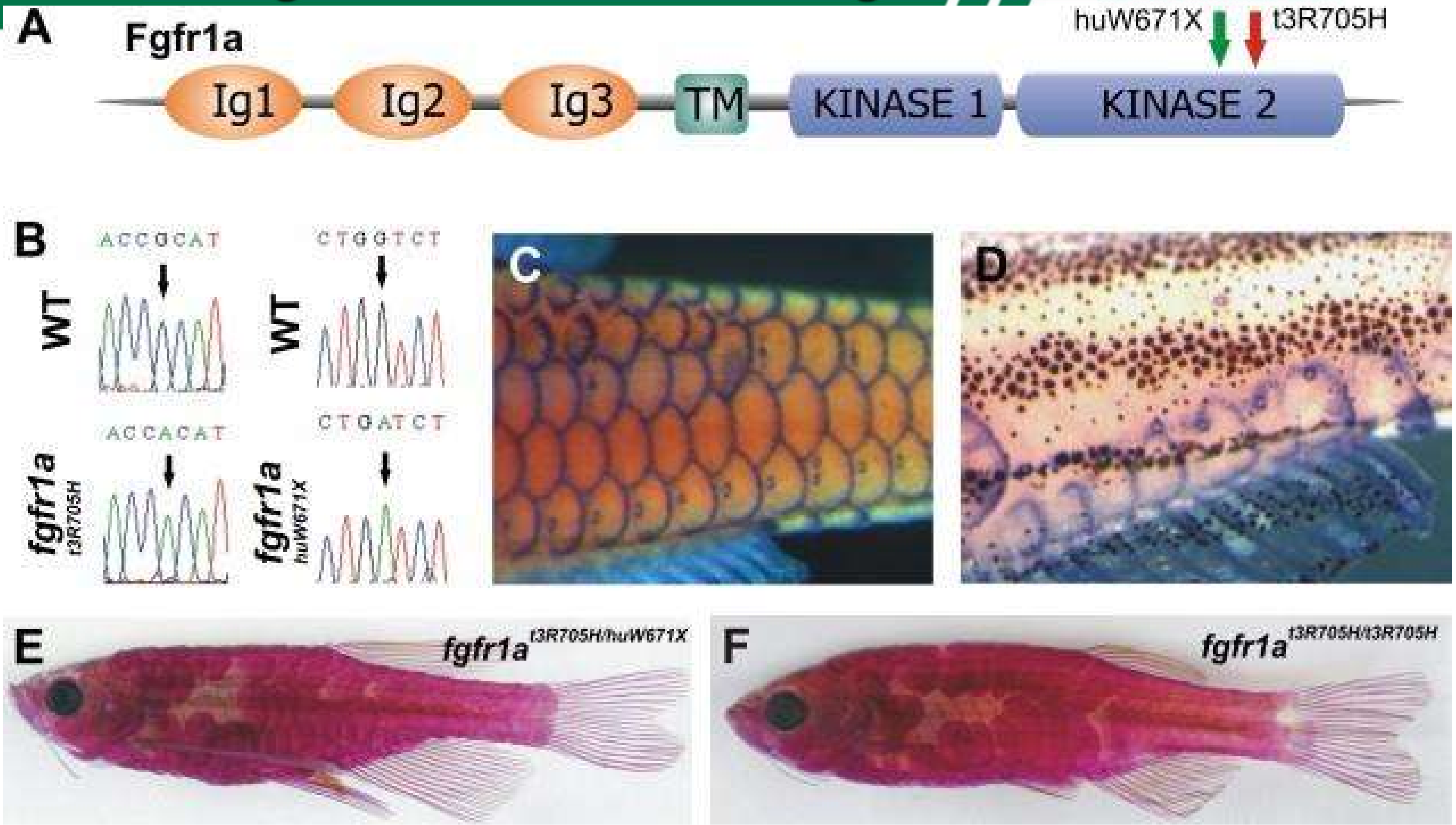
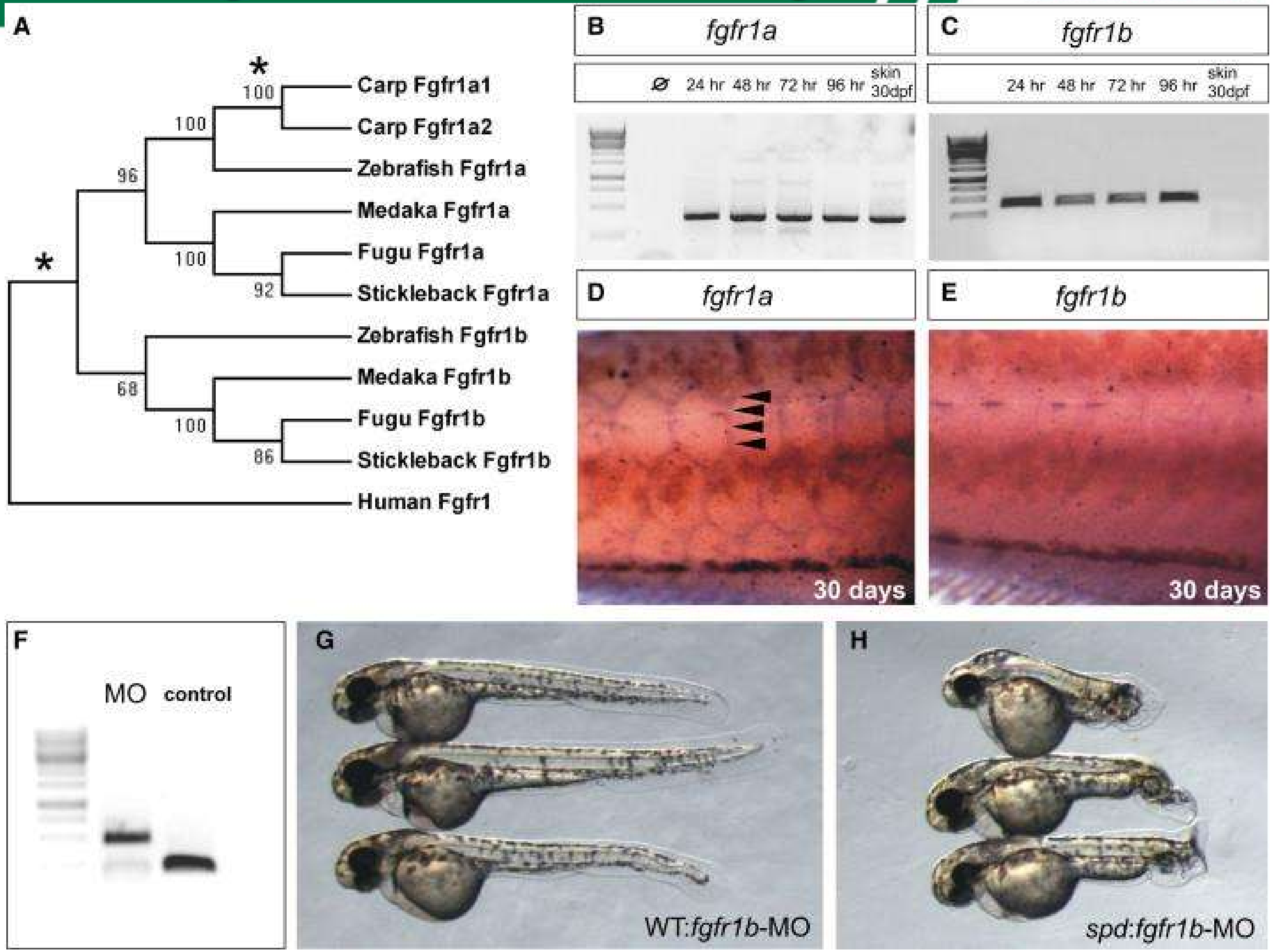


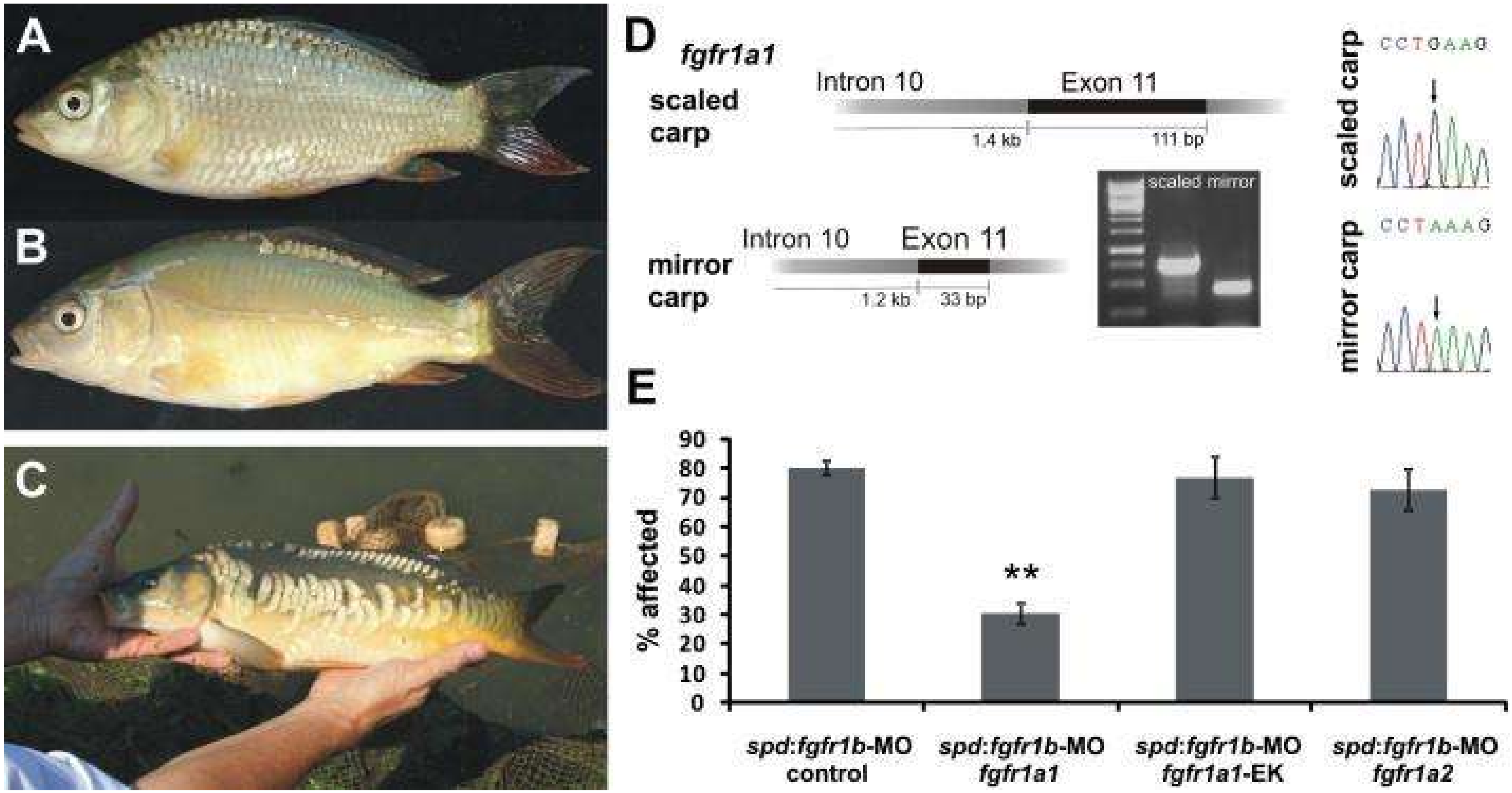
Figure 2. A Mutation in *fgfr1a* Causes the *spd*Phenotype(A) Schematic view of predicted *fgfr1a* structure and site of zebrafish mutations.(B) Nucleotide alteration in *fgfr1a*^{t3R705H} and *fgfr1a*^{huW671X}.(C and D) *fgfr1a* expression in the developing scales and lateral-line organs in wild-type fish (C) and in remaining scales of *spd* fish (D) (both 10 mm standard length).(E and F) Phenotype of transheterozygous (*fgfr1a*^{t3R705H}/*fgfr1a*^{huW671X}) fish (E) showing scale loss similar to *spd* homozygotes in different genetic backgrounds (F).

Duplication of fgfr1 Permits Fgf Signaling to Serve as a Target for Selection during Domestication



20% of zebrafish genes that exist as a single copy in mammals

Duplication of fgfr1 Permits Fgf Signaling to Serve as a Target for Selection during Domestication



Sex determination of fish 1.

Hermaphroditizm

Protandrous

Having the male sex organs maturing before the female,

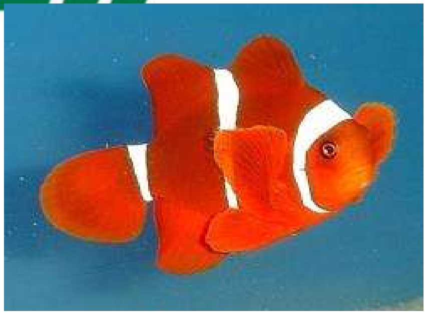
Protogynous

Having the female sex organs maturing before the male,

Synchronous

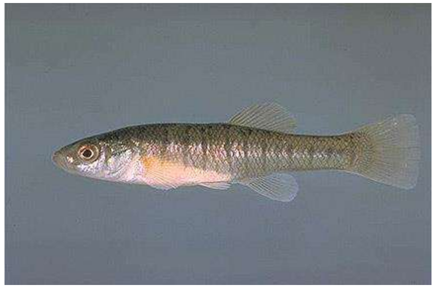
Both sex organs are present at same time

Sequential hermaphrodites



Premnas biaculeatus

Centropyge loriculus



Fundulus diaphanus

Sex determination of fish 2.

Gonochorism

The sex of an individual does not change throughout its lifetime.

Deep sea Anglerfish

Sex determining factor

Social factors

- Connection with the dominant sex

Environmental factors

-Temperature (High temperature More Male)

-PH (low PH more Male)

Genetics factors (most of the fish species)

Sex determination of fish 3.

Gonochorism with Genetics sex determining factors

Most of the species

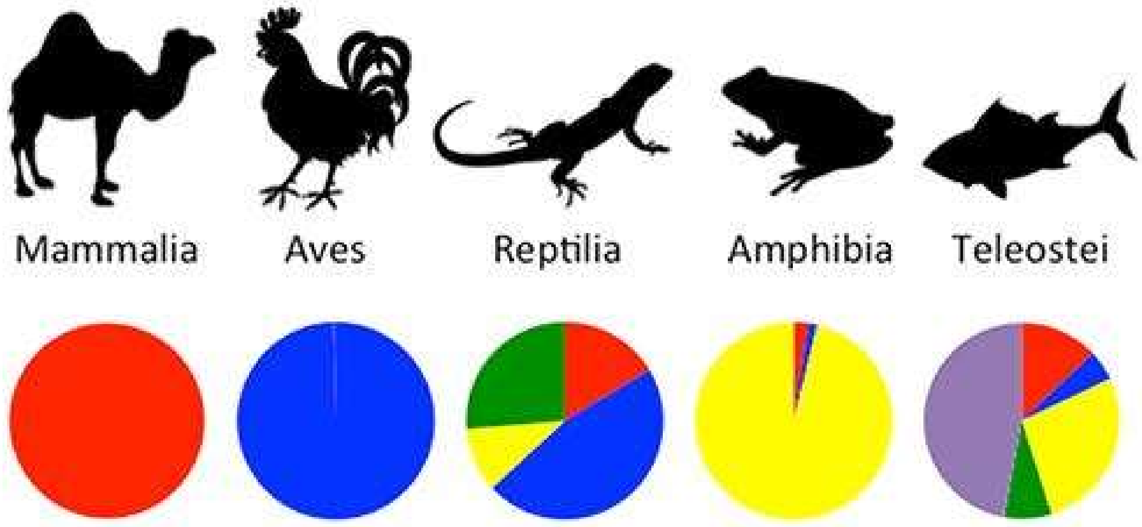
There are NO sex chromosomes

More than 1700 species

There are sex chromosomes

176 species

•(Devlin és Nagahama, 2002)

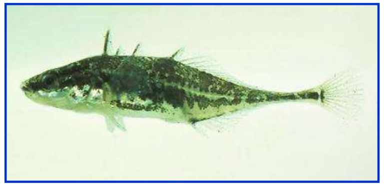
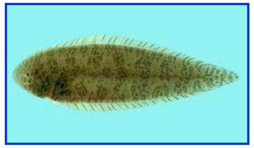


The diversity of sex chromosome systems varies among different types of animals. Mammals all use an XY system (red) and birds all use a ZW system (blue). But reptiles and fish employ a variety of different systems. Some choose sex based on temperature (green), others are hermaphrodites (purple), and some have sex chromosomes that look the same (yellow)

Sex determination of fish 4.

(Devlin és Nagahama, 2002)

Sex chromosomes types

- XX/XY  *Clarias gariepinus*
- ZW/ZZ  Apeltes quadracus
- ZZ/Z0 Dose dependent  *Cynoglossus puncticeps*
- XX/X0 Dose dependent  *Colisia lalia*
- ZW/ZW '/ZZ  *Scardinius erythrophthalmus*
- X,Z,Y  *Xiphoporus maculatus*

Transgenic Fish

Transgenic Fish--- Differences in terms?

The transgenic creature contain foreign DNA (genes).

The transgenic creature contain, chromosomally integrated , artificially engineered and inserted gene construction. That is expressing protein and cause phenotypic change

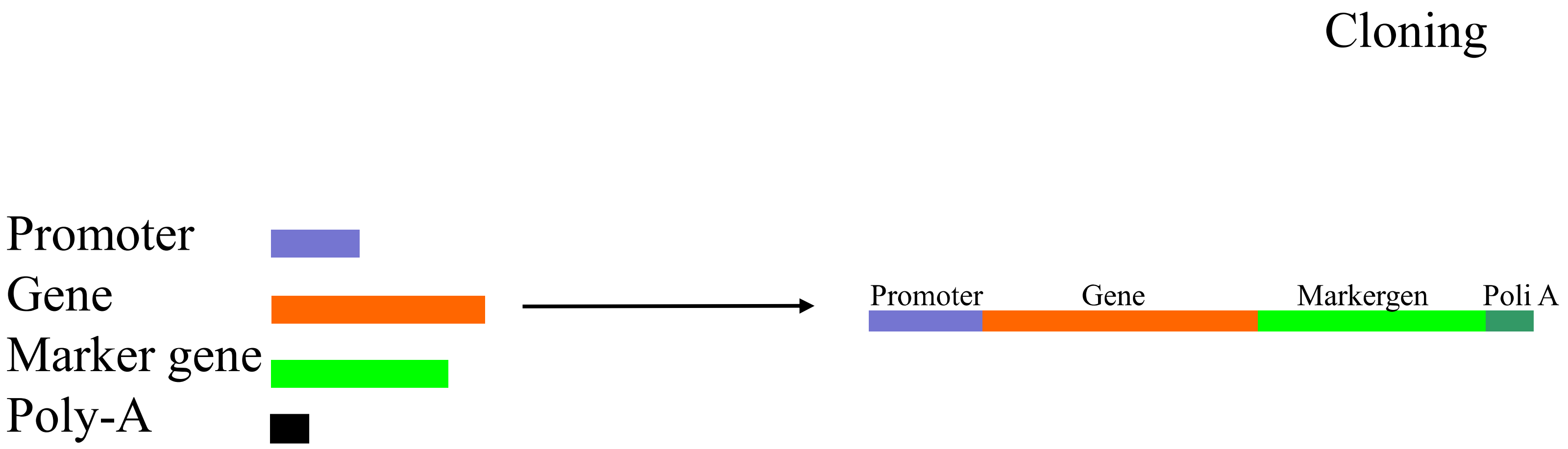
The transgenic creature contain artificially engineered gene construction.

Steps for making transgenic Fish

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Step 1. Decide Gene/Protein to Add.

Step 2. Prepare Gene Construction.



Gene Construction

Promoters: regulator element

Mammalian / avian viruses

strong, constitutive, all-tissue

RSV (Rous sarcoma virus)

TK (thymidine kinase)

SV40 (simian virus)

Tissue specific

Developement stage specific

Other regulators

- Enhancers
- Silencer

Reporter genes: detection

Genes:

Comes from any species (bacteria, plant, animal)

Reporter genes: detection method

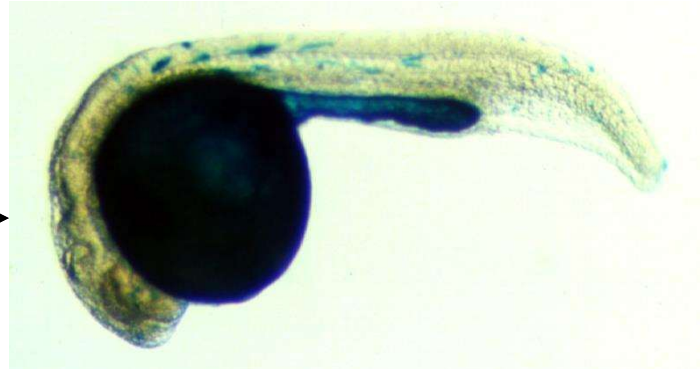
Luciferase:	luminiscence assay (firefly)
CAT:	radioactive assay
LacZ:	colorimetric assay/staining
GFP:	fluorescence microscopy
RFP:	fluorescence microscopy
YFP:	fluorescence microscopy
BFP:	fluorescence microscopy

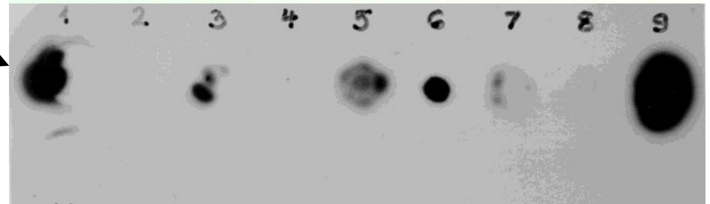
Poly-A signal:

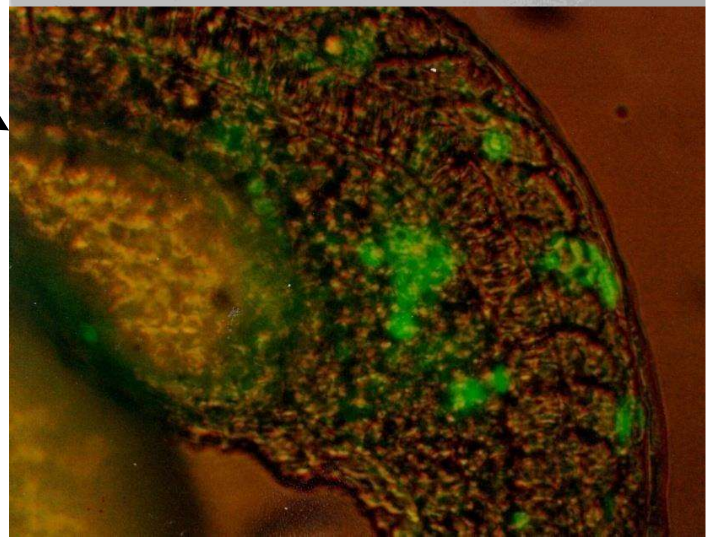
Comes from any species (bacteria, plant, animal)

Reporter genes: detection

- lacZ (E. coli)
- luc (P. pyralis)
- lux (V. harveyi)
- gfp (A. victoria-jellyfish)





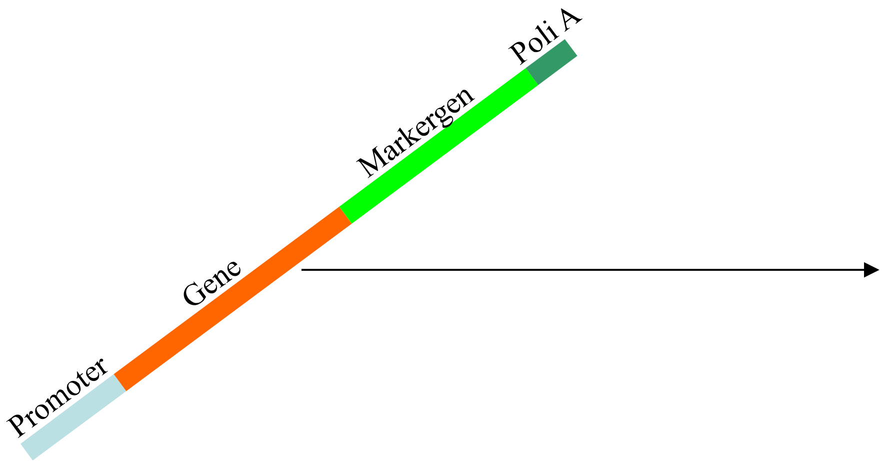


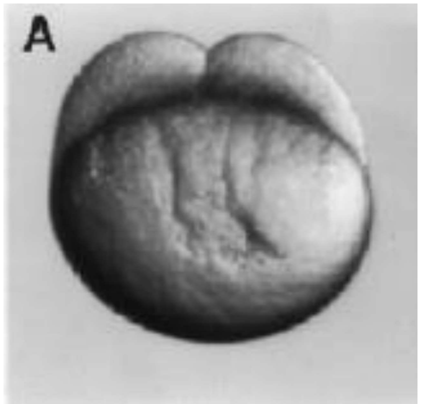
Gene insertion methods

- Microinjection
  - to the first cells
  - To cytoplasm
- Sperm mediated
- Embryo electroporation
- Sperm electroporation
- Liposome mediated
- Gene Gun
- Retrovirus mediated
- Transposon mediated

Steps for Making Transgenic Fish

Step 6. Insert Gene constructions into Newly Fertilized Egg.





Fate of Gene constructions

- Replication
- Elimination
- Circulation
- Concatamerisation
- Integration

- Genetic mosaics
  - Exrachromosomal transgene
  - transients expression

Steps for Making Transgenic Fish

- Nursing and raise the larvaes

- Selection of positive individuals among survivals

- Examination of transgene expression and phenotype

- Examination of Inheritance.

Tissue specific expression

Transient inheritance

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

	brain	whiskers	skin	tail	muscle	gill	liver	heart	kidny	gonad	offsprings
F0 19 egyed	44%	36%	47%	31%	6%	14%	7%	31%	14%	29%	76%
F1 34 egyed	50%	47%	37%	30%	6%	28%	29%	68%	23%	52%	
F2 8 egyed	75%	50%	25%	13%	25%	25%	0%	75%	63%	75%	100%

HA2837/1	non tested
HA2837/2	45,5%
HA2837/3	22,7%
HA2837/4	non tested
HA2837/5	non tested
HA2837/6	11,4%
HA2837/7	non tested
HA2837/8	9,7%

Transgenic lines

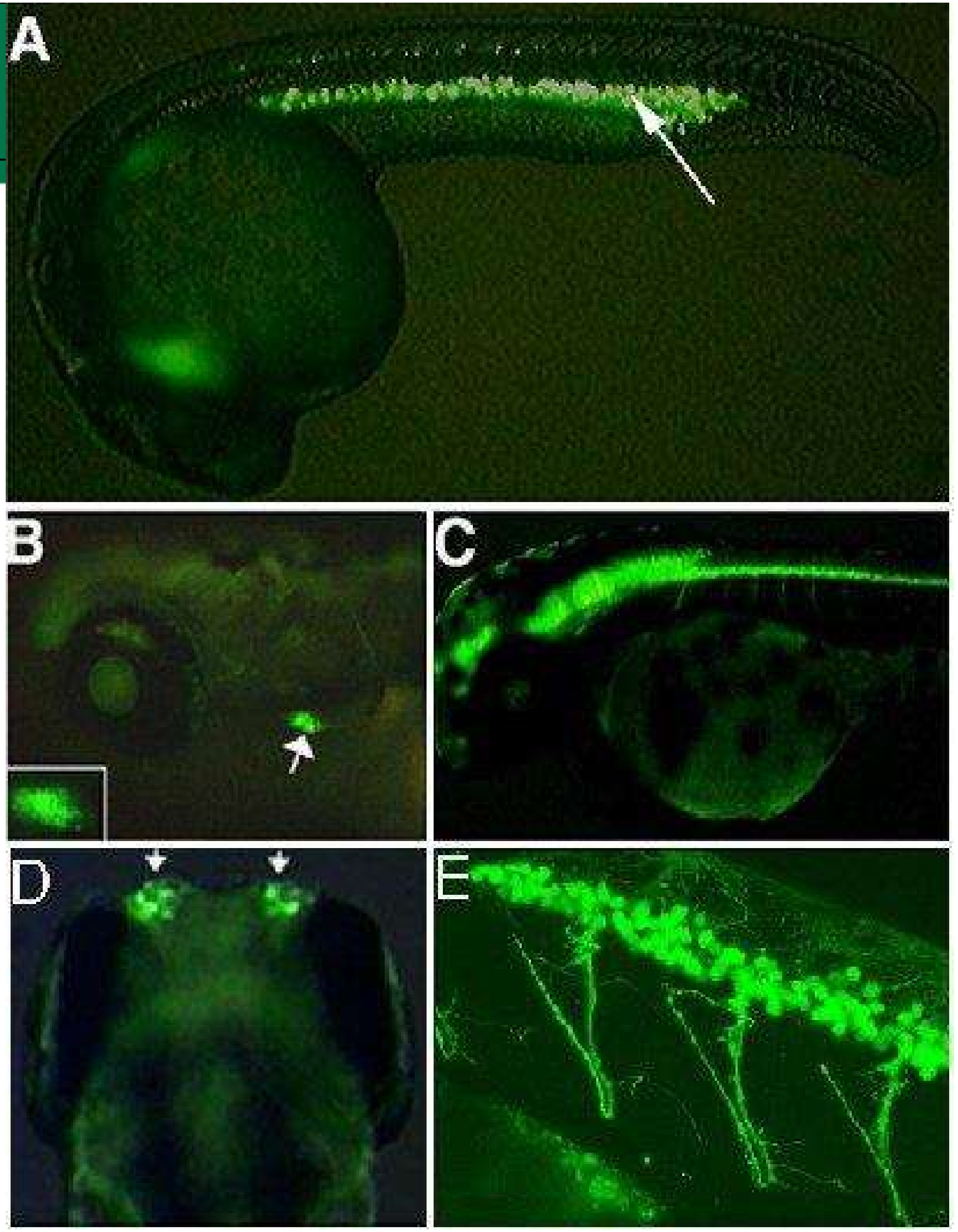
A. Blood

B. Thymus

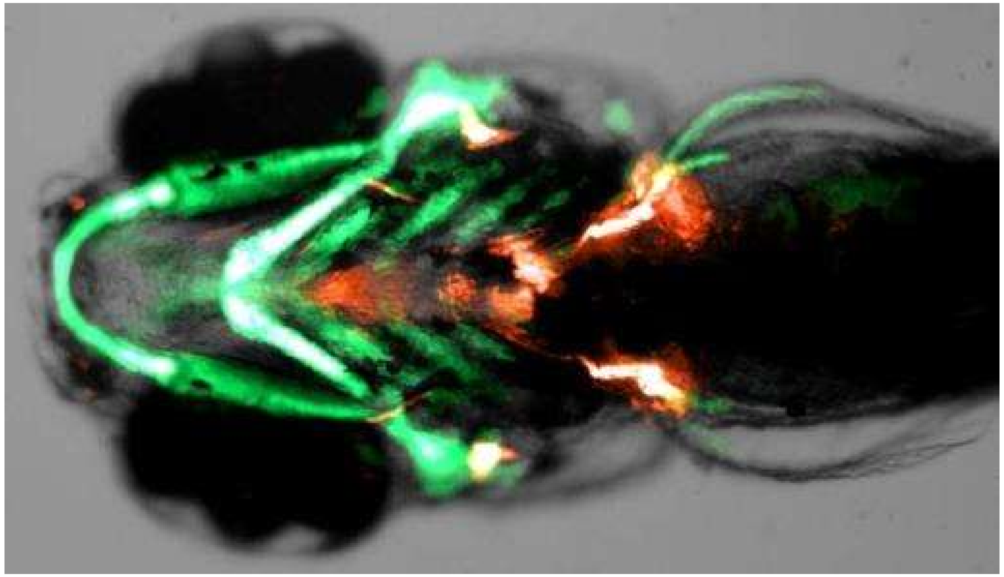
C. Central Nervous System

D. Olfactory Neurons

E. Motor Neurons



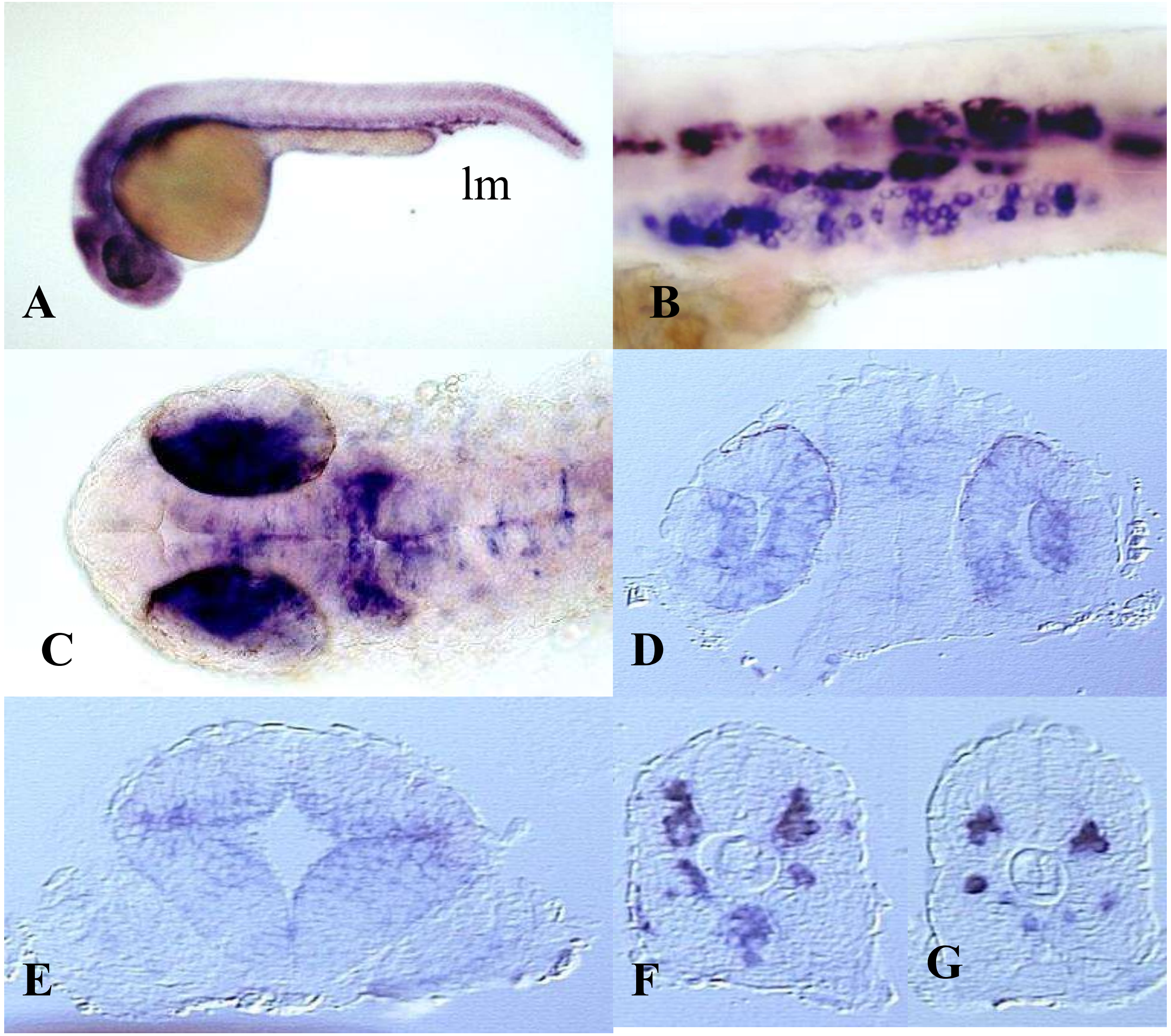
Double transgenic line 4.



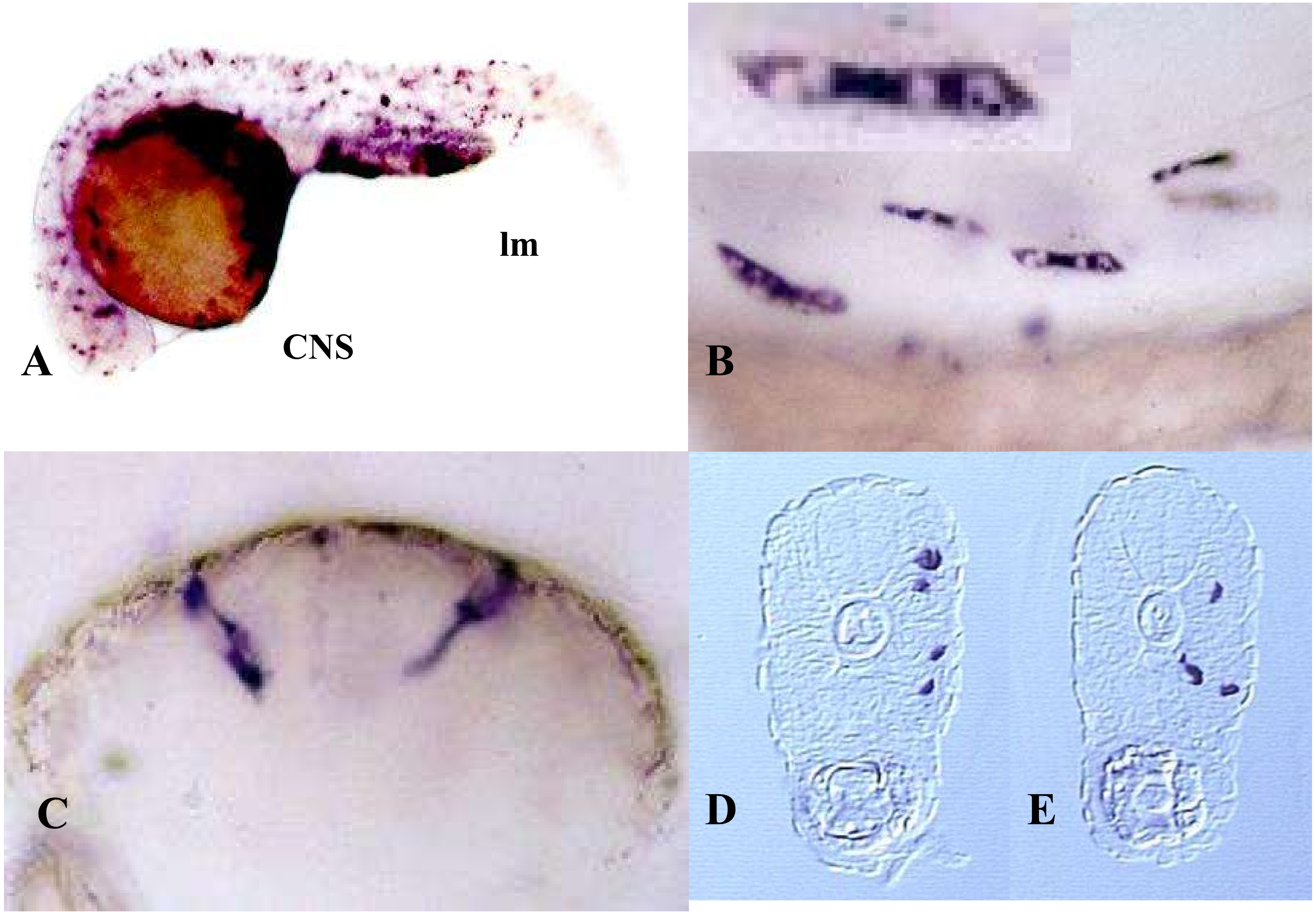
Expression in double transgenic embryo: eGFP in cartilage tissue and RFP in bone.

Heat shock inducible promoter expression

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM



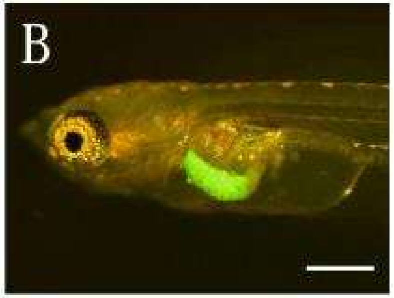
Heat shock inducted expression



Transgenic Bio-monitor Fish

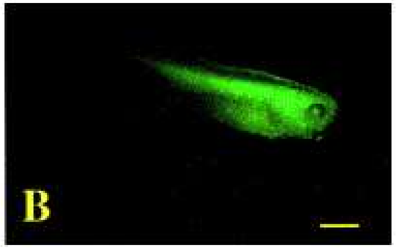
Environmental pollution detection:

Hormonal effects
Stress factors
Toxins
Heavy metals



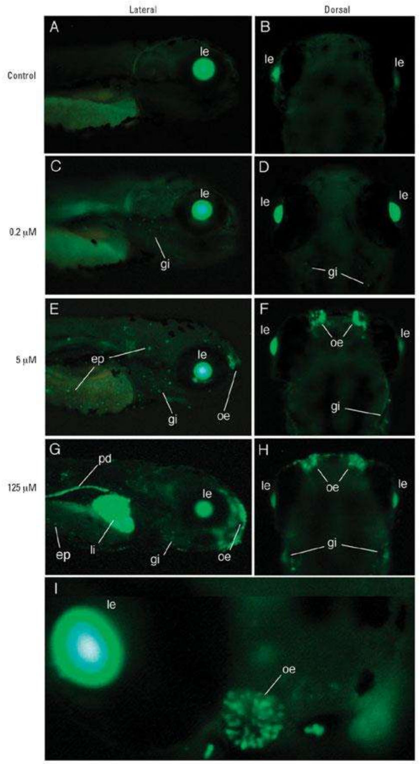
(Kurauchi et al. 2008)

Transgenic Medaka
in vivo estrogen effect
choriogenin H promoter + GFP



(Oofusa et al. 2003)

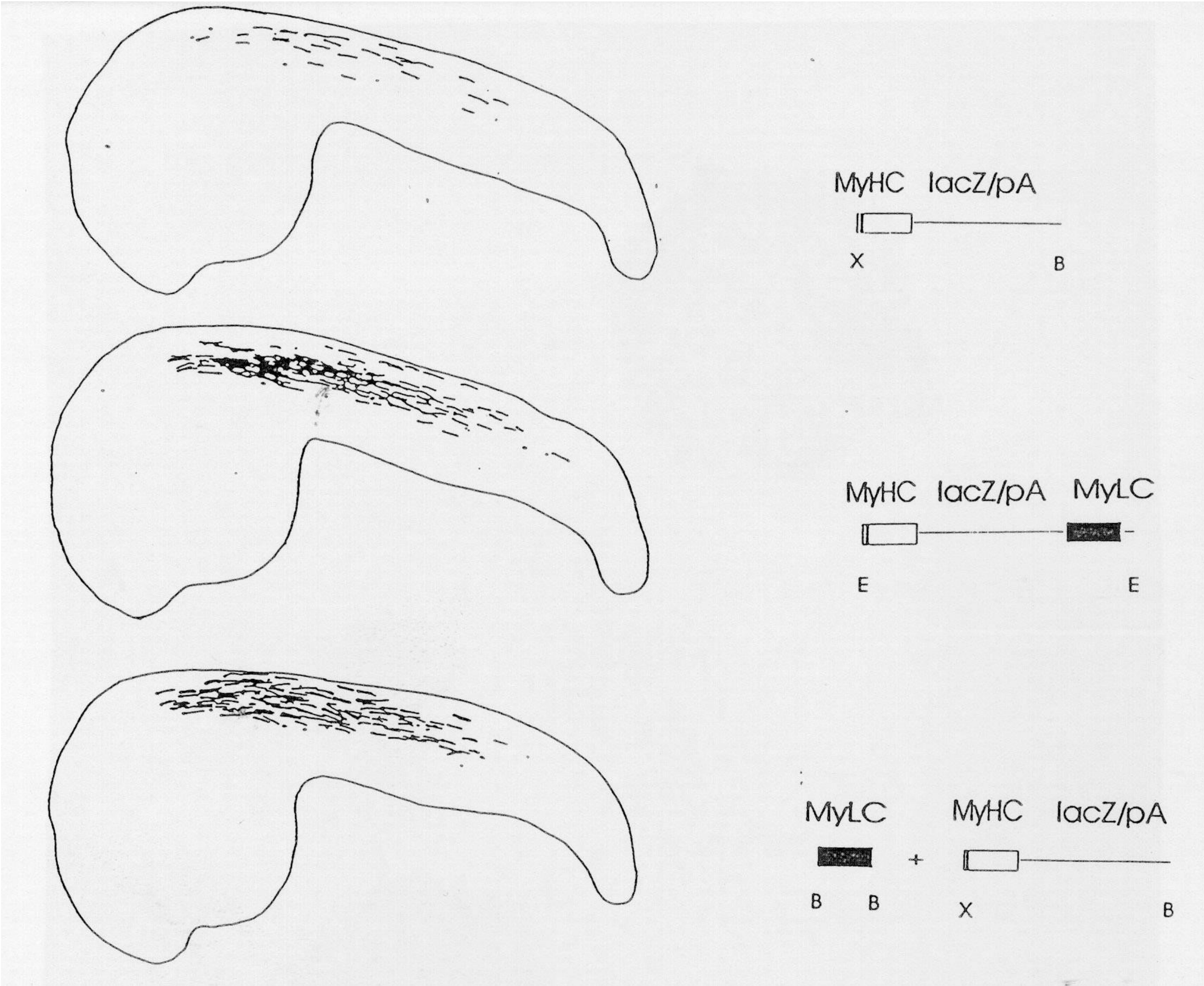
Transgenic Xenopus
Heavi metal detection
Mouse metallothionein-1
promoter+ EGFP



(Blechinger et al. 2002)

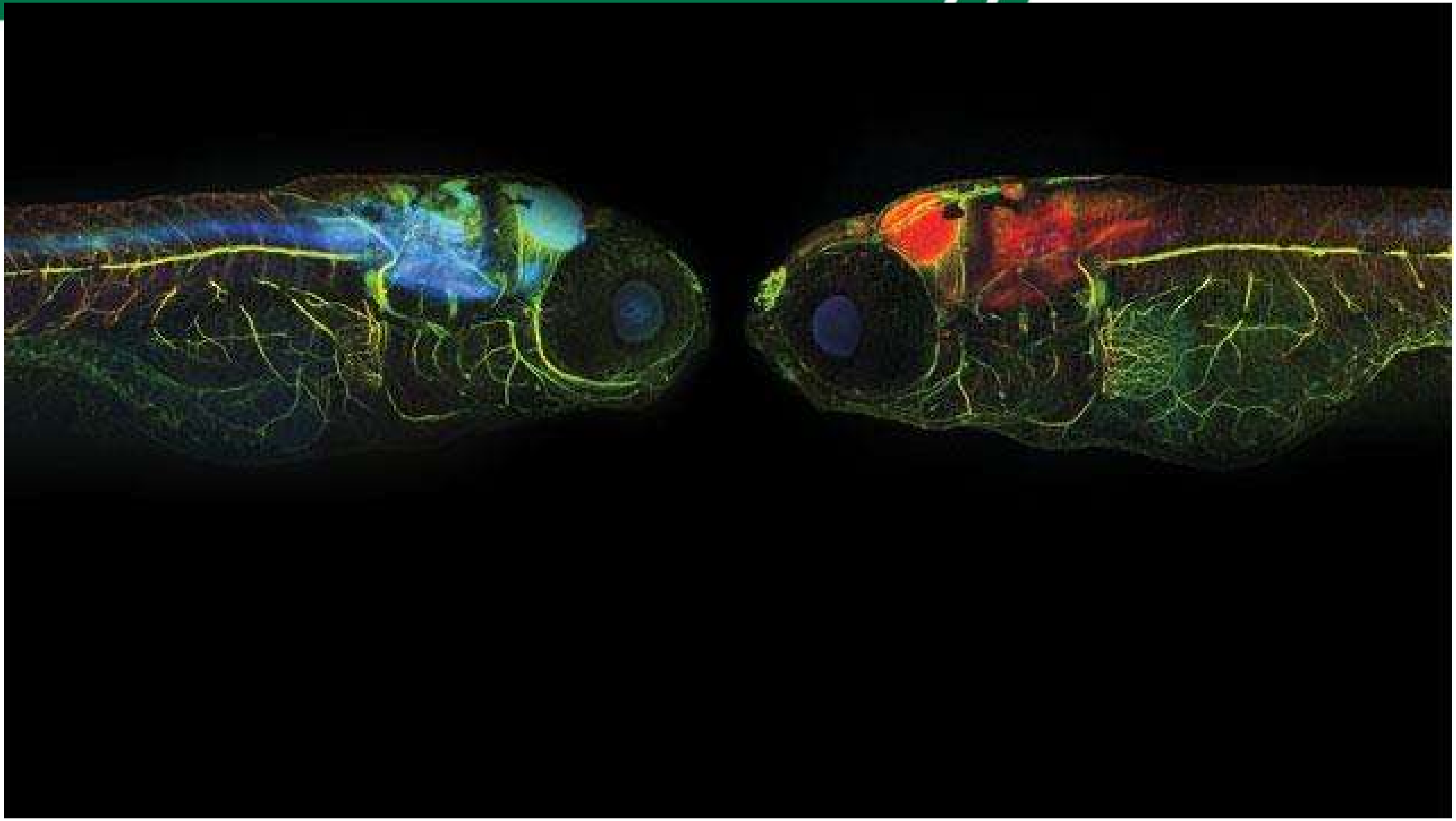
Transgenic zebrafish
Cadmium analysis
Hsp70 promoter + EGFP

Enhancer inducted change on expression



Special analyses

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

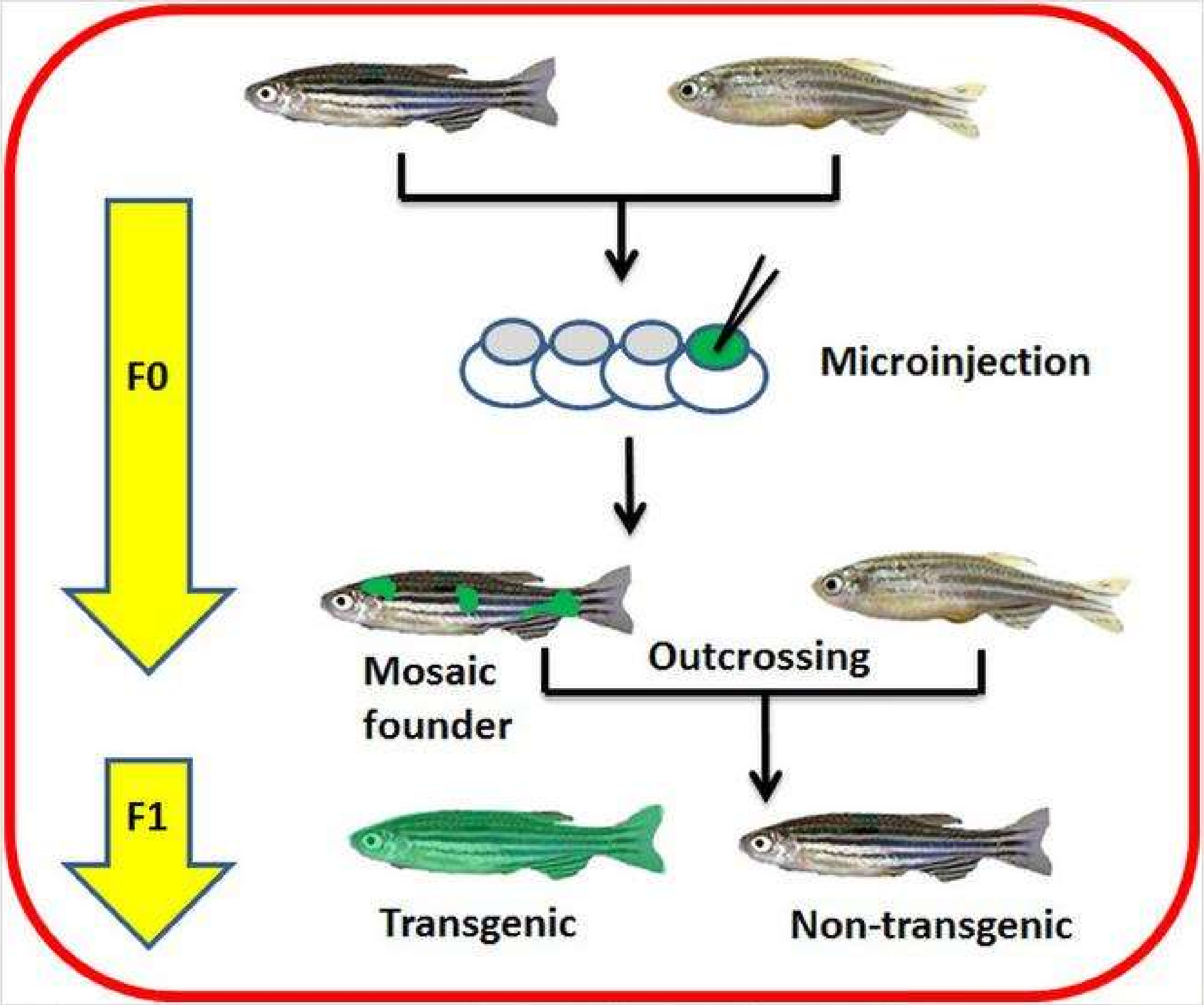


LOOKING INSIDE DISEASE: The wild-type zebrafish larva on the left is stained for the two neuronal proteins (green) and membrane-trafficking proteins expressed near synapses (blue). On the right, the neurons of a transgenic zebrafish larva produce the dementia-associated Tau protein (red), a disease-specific form of which is stained in blue. Tubulin is stained in green. COURTESY OF DOMINIK PAQUET, THE ROCKEFELLER UNIVERSITY, NEW YORK, USA

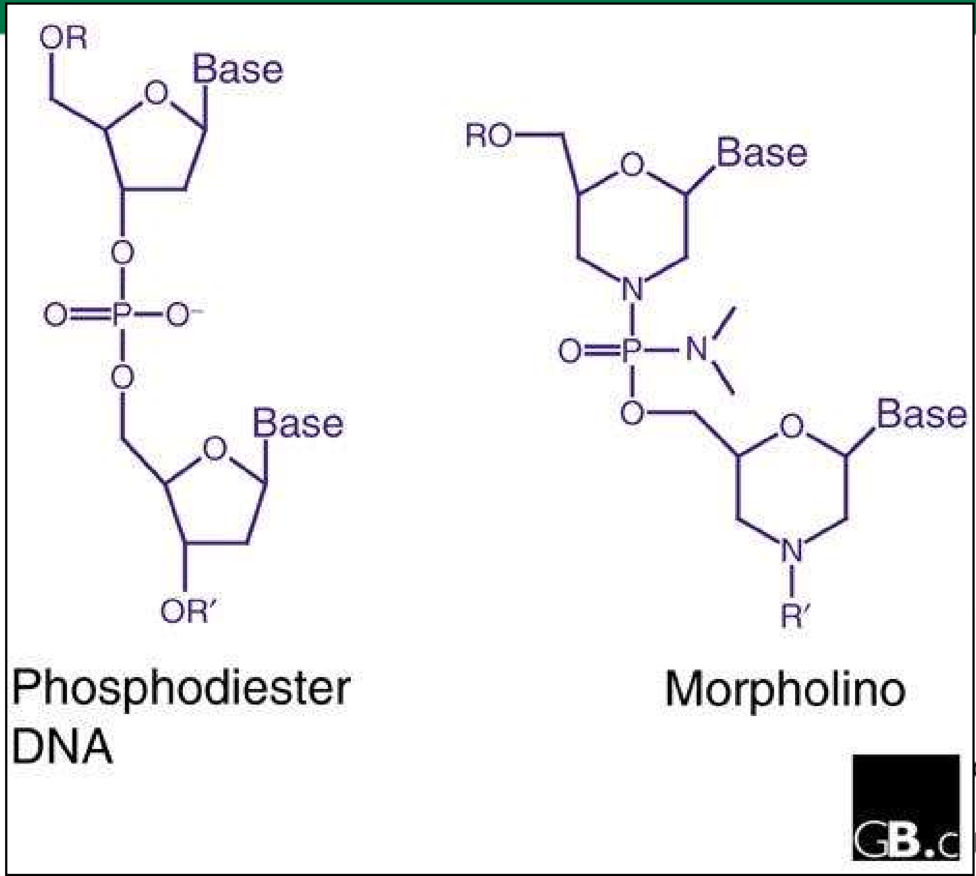
Transgenic zebrafish available as a service

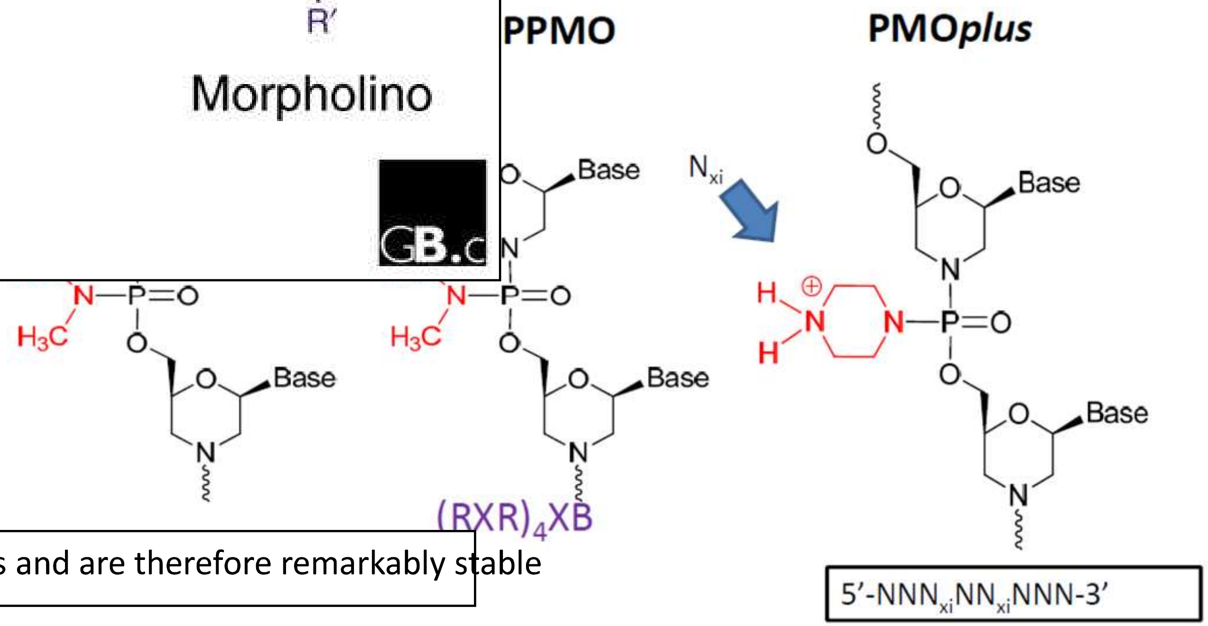
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

ZGENEBIO Transgenic Fish service SOP



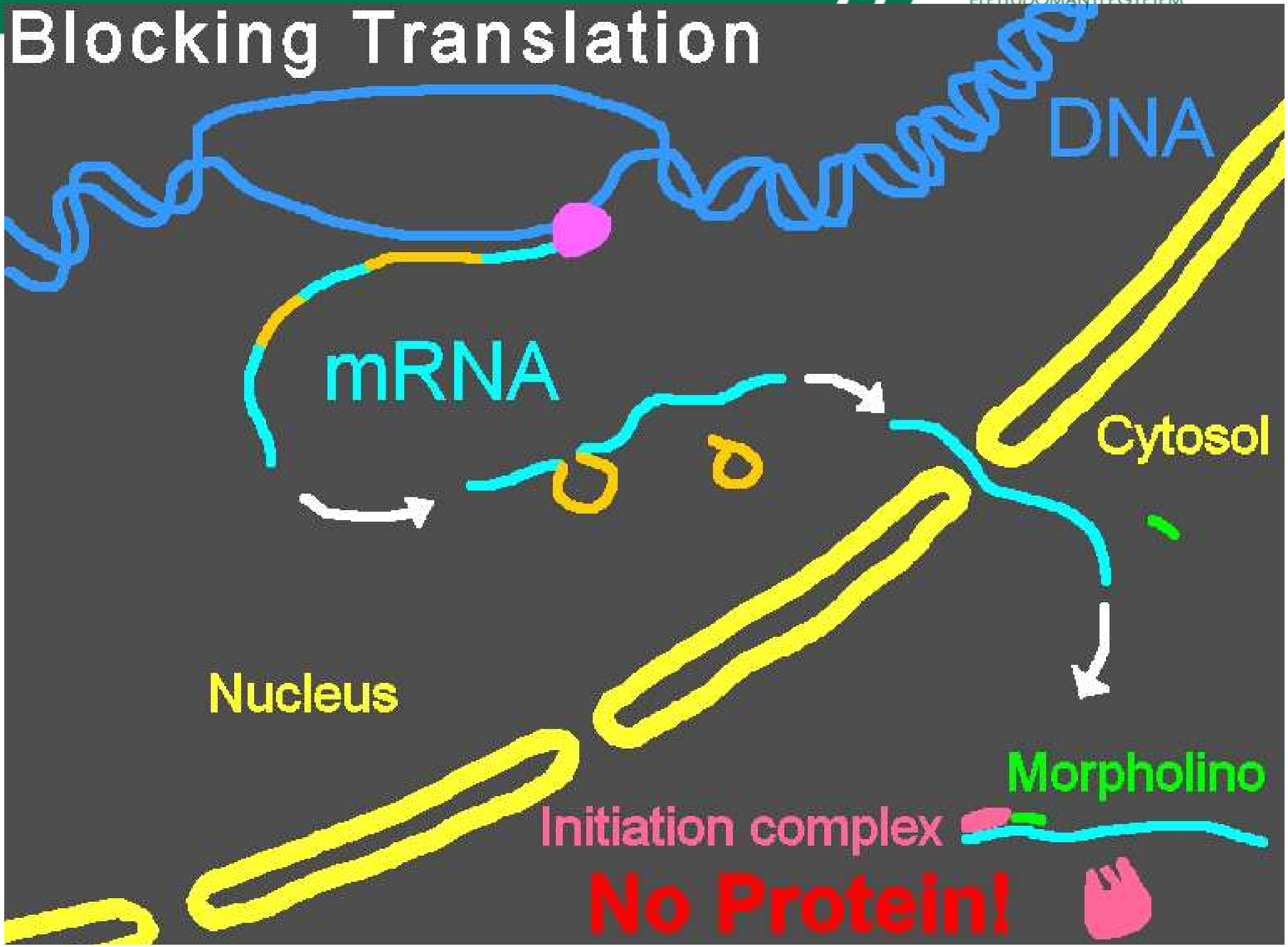
Morpholino



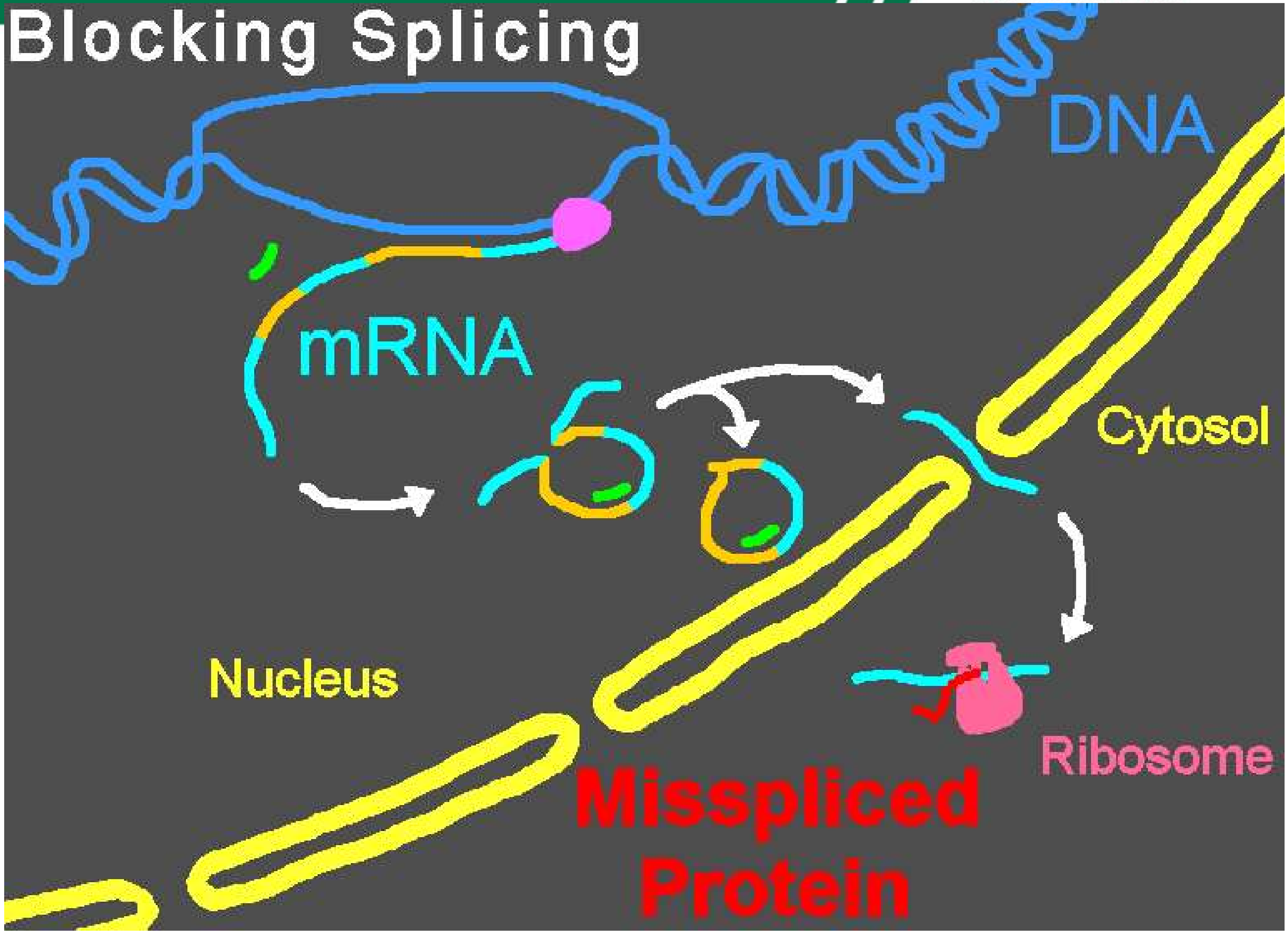


MOs are resistant to nucleases and are therefore remarkably stable

Morpholino 1



Morpholino 2



Antisens transgenic (knock down) fish

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

sGnRH-antisens transgenic rainbow trout

Aim: slow maturity or sterility

Results:

-reduced GnRH level

- no difference in maturity

(Uzbekova et al. 2000)

sGnRH-antiszensz transzgénikus carp

Results:

-reduced GnRH level

- Some sterile

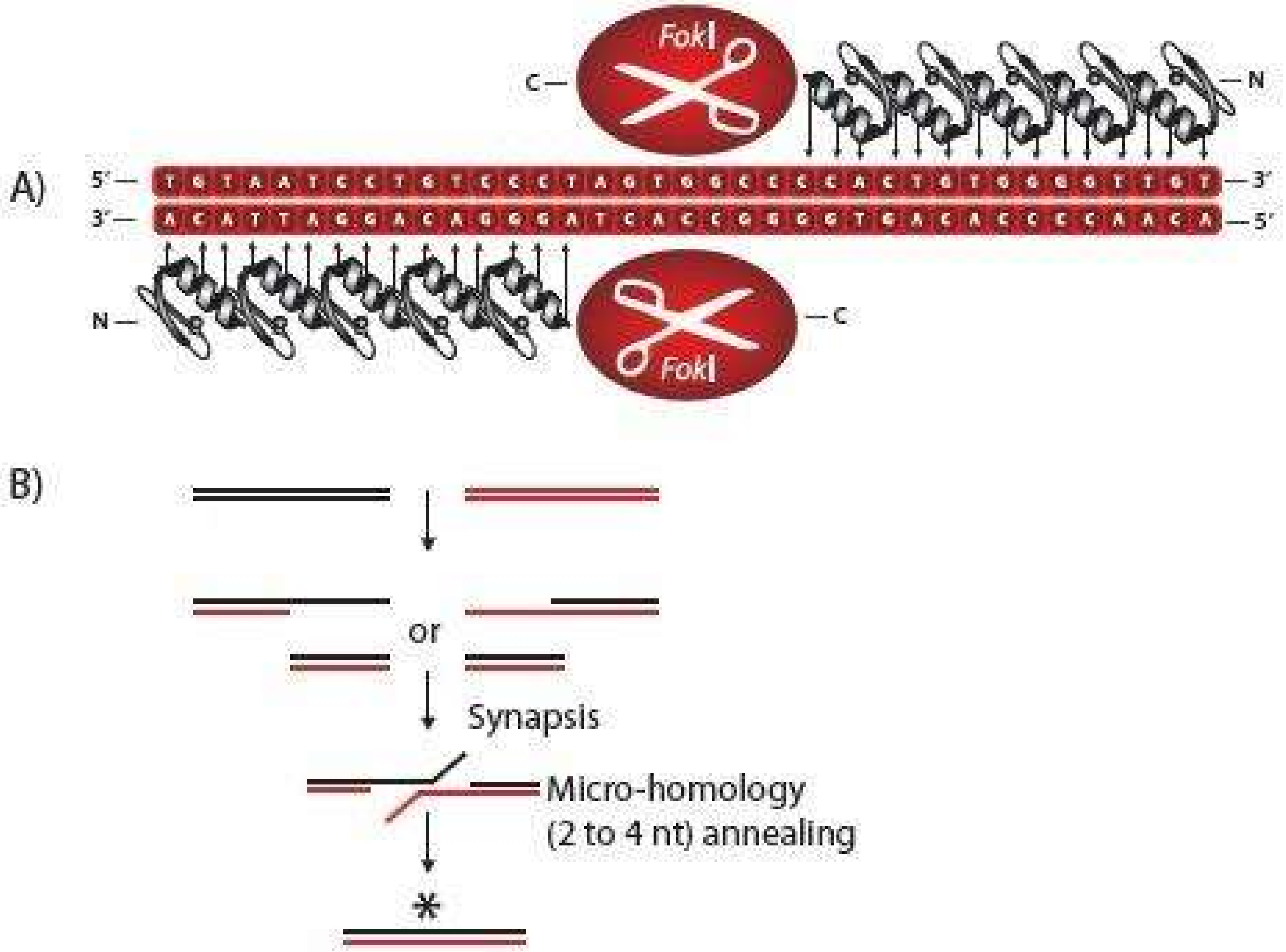
(Hu et al. 2007)

Myostatine-antisen transgenic rainbow trout

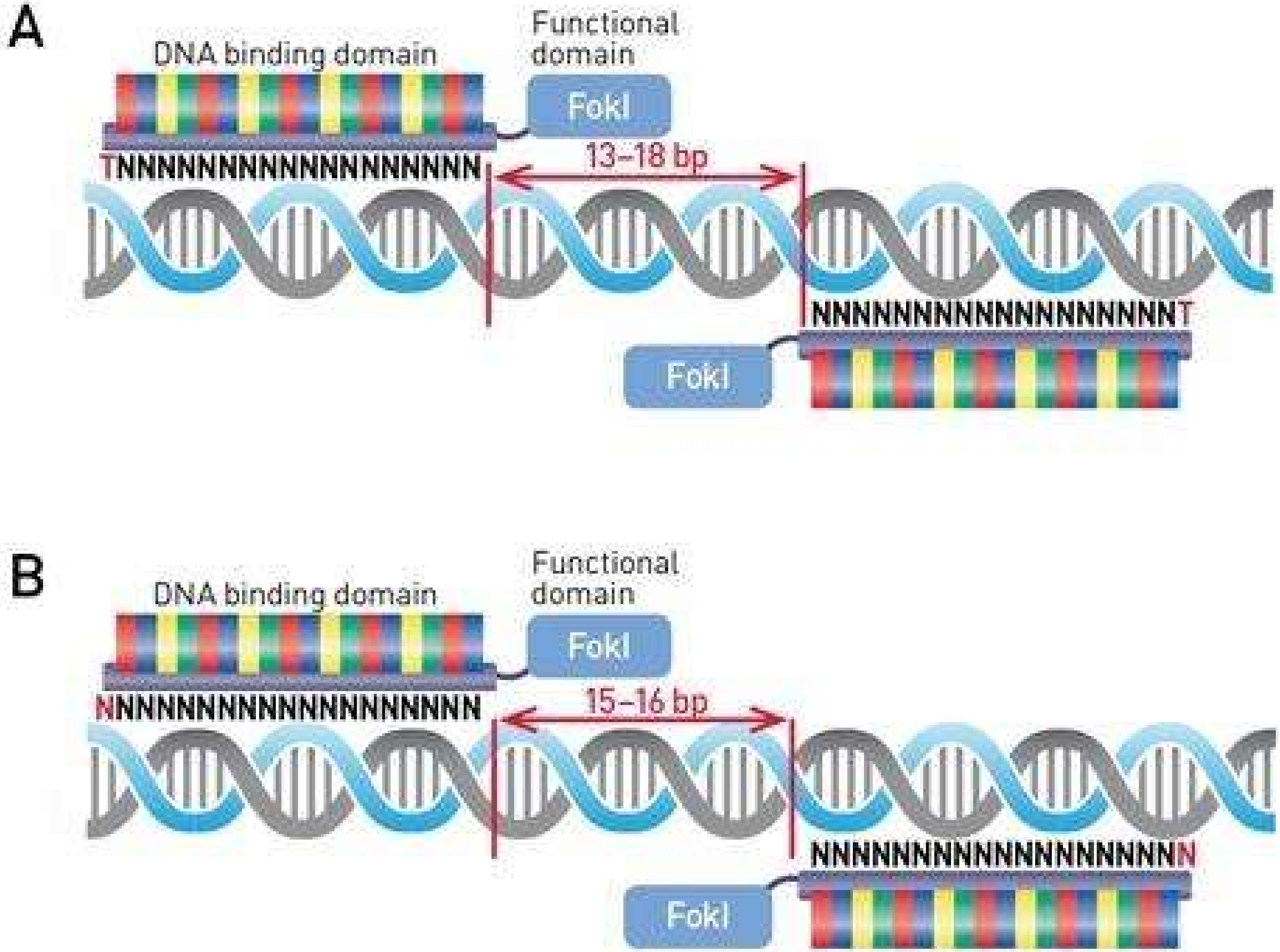
- 20-25% more muscle

(Terry Bradley. University of Rhode Island.2010)

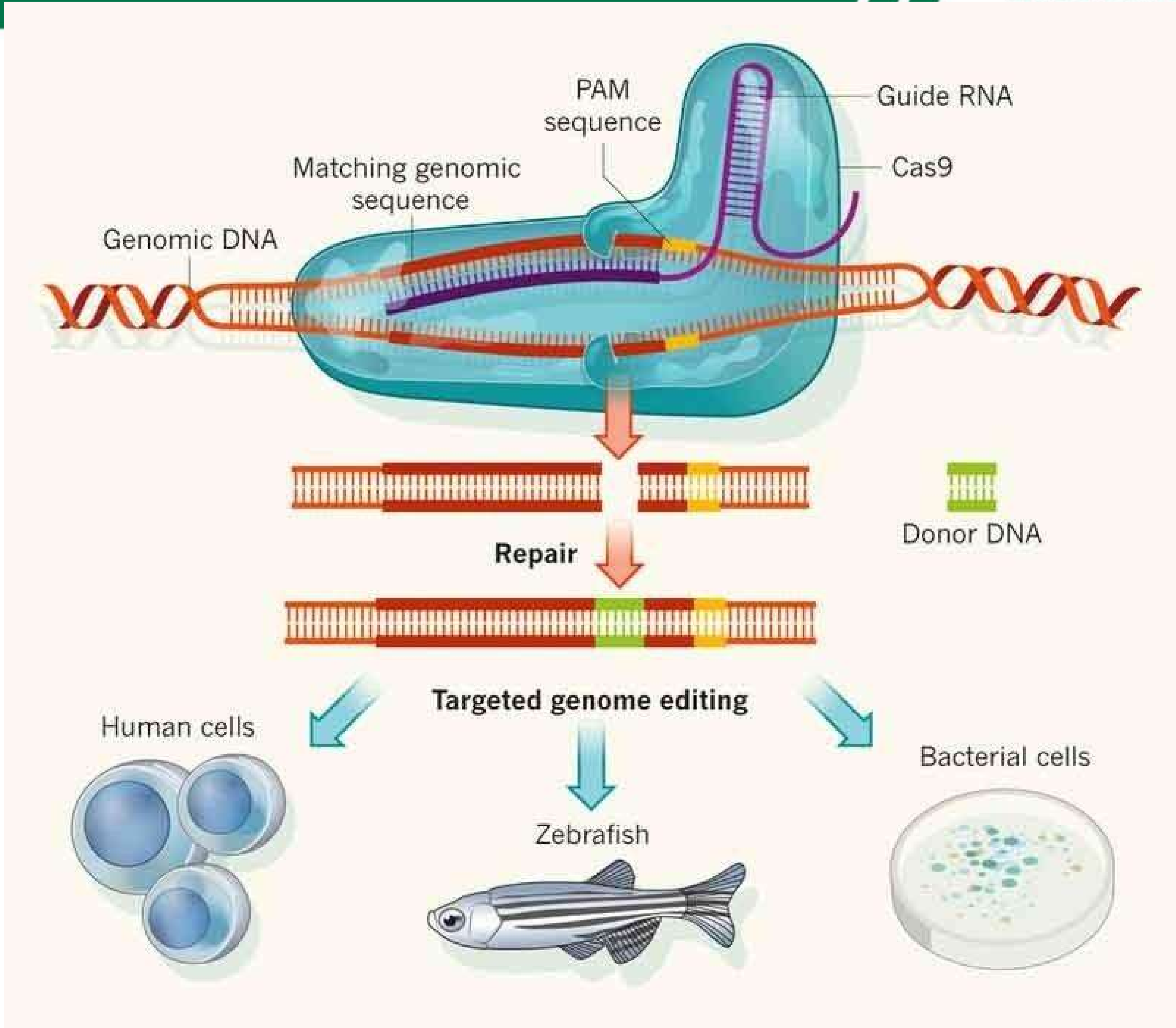
Zinc fingers



TALEN


A
DNA binding domain
Functional domain
FokI
TNNNNNNNNNNNNNNNNNNNN
13–18 bp
NNNNNNNNNNNNNNNNNNNNT
FokI
B
DNA binding domain
Functional domain
FokI
NNNNNNNNNNNNNNNNNNNNN
15–16 bp
NNNNNNNNNNNNNNNNNNNNN
FokI

CRISPR


PAM
sequence
Guide RNA
Cas9
Matching genomic
sequence
Genomic DNA
Donor DNA
Repair
Targeted genome editing
Human cells
Zebrafish
Bacterial cells

GM fishes

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

- Catfish
- Arctic Char
- Common Carp
- Indian Major Carps
- Flounder
- Goldfish
- Halibut
- Loach
- Atlantic Salmon
- Pacific Salmons such as Chinook and Coho
- Japanese Medaka
- Striped Bass
- Tilapia
- Turbot
- Cutthroat Trout
- Rainbow Trout
- Zebra fish

- Abalone
- Lobster
- Shrimp

AIMS of transgenesis

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

- Bigger body size
- Faster growing
- Better food conversion
- Lower fat content
- Tolerance of freezing
- Cold tolerance
- Faster sex development
- Slower sex development
- Resistance
- Bio-monitor fish
- Bio-reactor fish
- Metabolic changes
- Exam. of gene function

Growth hormone (Bigger body size, Faster growing, Better food conversion, Lower fat content)

Anti Freeze Protein (Tolerance of freezing, Cold tolerance)

GnRH, anti-GnRH (Faster sex development, Slower sex development)

Resistance genes

Stress promoters + reporter genes

Protein production for industry

Metabolic enzymes

Any gene

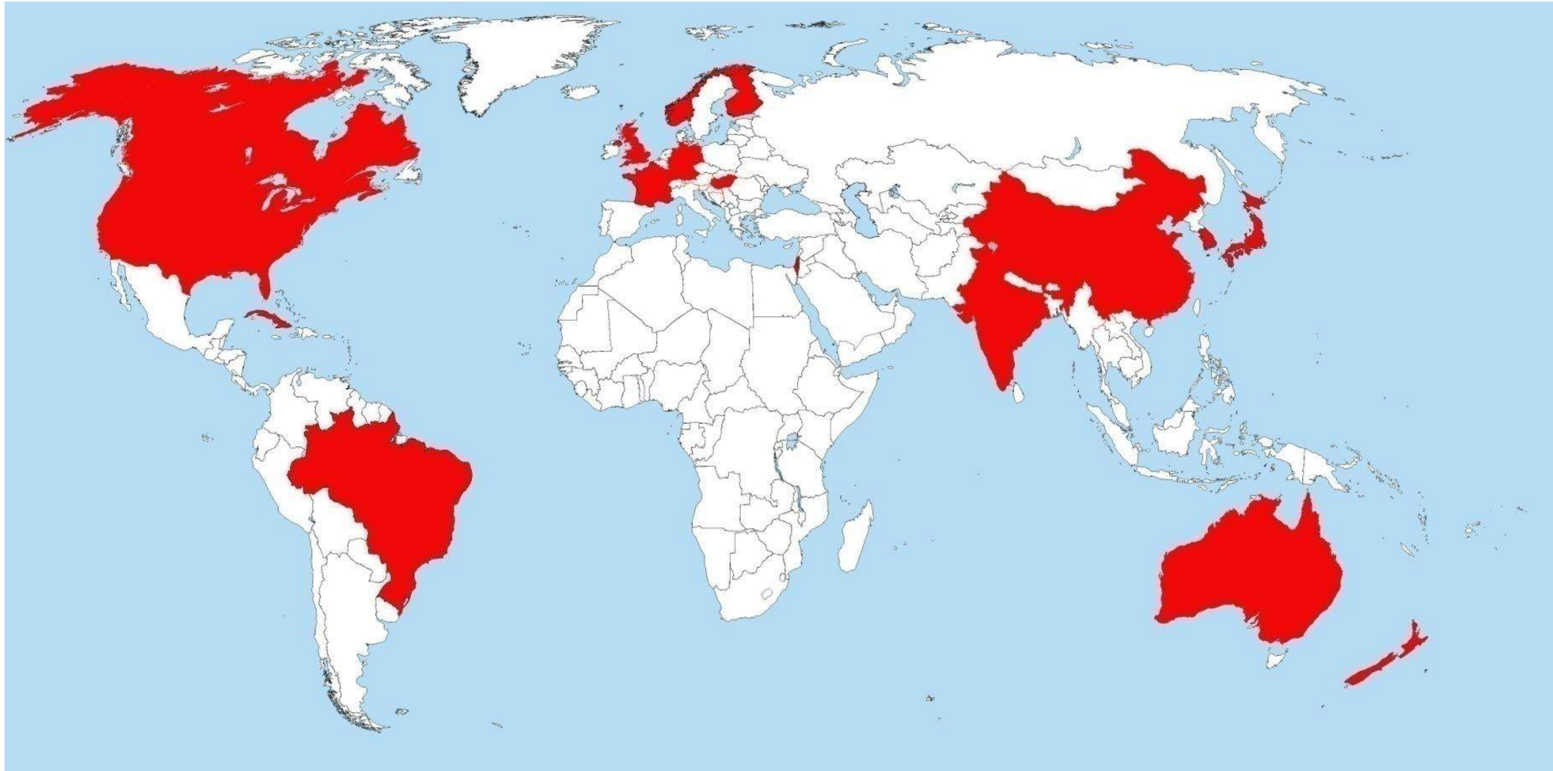
Species	Foreign gene	Desired effect and comments	Country
Atlantic salmon	AFP AFP salmon GH	Cold tolerance Increased growth and feed efficiency	United States, Canada
Coho salmon	Chinook salmon GH + AFP	After 1 year, 10- to 30-fold growth increase	Canada
Chinook salmon	AFP salmon GH	Increased growth and feed efficiency	New Zealand
Rainbow trout	AFP salmon GH	Increased growth and feed efficiency	USA, Canada
Cutthroat trout	Chinook salmon GH + AFP	Increased growth	Canada
Tilapia	AFP salmon GH	Increased growth and feed efficiency; stable inheritance	Canada, United Kingdom
Tilapia	Tilapia GH	Increased growth and stable inheritance	Cuba
Tilapia	Modified tilapia insulin-producing gene	Production of human insulin for diabetics	Canada
Salmon	Rainbow trout lysosome gene and flounder pleurocidin gene	Disease resistance, still in development	USA, Canada
Striped bass	Insect genes	Disease resistance, still in early stages of research	USA
Mud loach	Mud loach GH + mud loach and mouse promoter genes	Increased growth and feed efficiency; 2- to 30-fold increase in growth; inheritable transgene	China, Korea, Rep.
Channel catfish	GH	33% growth improvement in culture conditions	USA
Common carp	Salmon and human GH	150% growth improvement in culture conditions; improved disease resistance; tolerance of low oxygen level	China, USA
Indian Major carps	Human GH	Increased growth	India
Goldfish	GH AFP	Increased growth	China
Abalone	Coho salmon GH + various promoters	Increased growth	USA
Oysters	Coho salmon GH + various promoters	Increased growth	USA

AFP = anti-freeze protein gene (Arctic flatfish).
GH = growth hormone gene.

From the report - GENETICALLY MODIFIED ORGANISMS ANDFISHERIES,
by - Jacques Diouf, FAO Director-General 7 March 2000

Participant countries

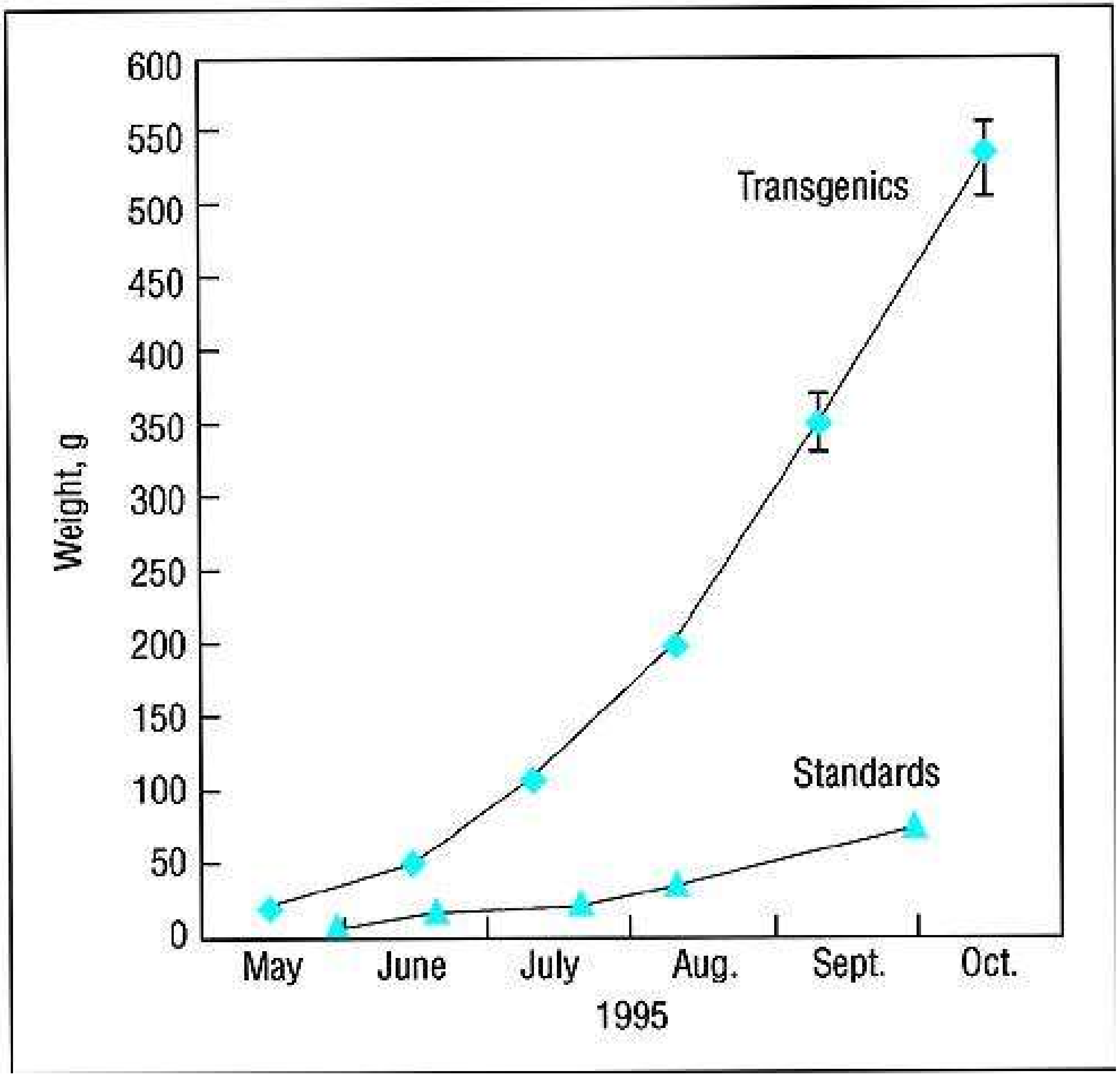
United States	China	Hungary
Canada	Korea Rep.	Norway
New-Zealand	India	Finland
United Kingdom	Germany	Brasil
Cuba	Israel	Japan

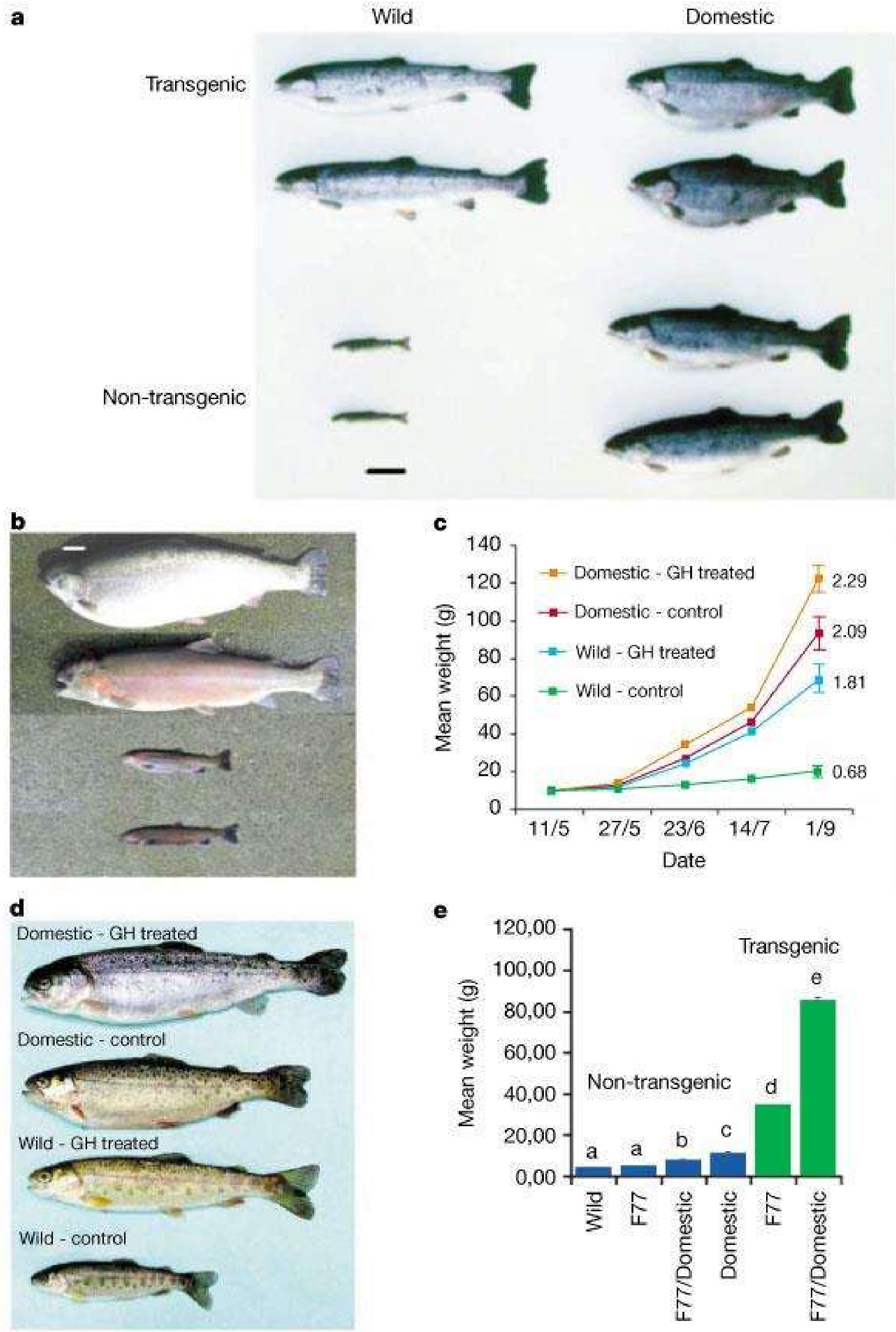


From the report - GENETICALLY MODIFIED ORGANISMS ANDFISHERIES,
by - Jacques Diouf, FAO Director-General 7 March 2000

Transgenesis 1.

Transgenesis 1.





a
Wild
Domestic
Transgenic
Non-transgenic
b
c
Mean weight (g)
Domestic - GH treated
Domestic - control
Wild - GH treated
Wild - control
2.29
2.09
1.81
0.68
11/5
27/5
23/6
14/7
1/9
Date
d
Domestic - GH treated
Domestic - control
Wild - GH treated
Wild - control
e
Mean weight (g)
Transgenic
Non-transgenic
a
a
b
c
d
e
Wild
F77
F77/Domestic
Domestic
F77
F77/Domestic

Chinook Salmon

Ocean Pout antifreeze gene promoter + chinook salmon growth hormone gene

1.2 kg v. 200 g 7.5 months old

From A/F Protein

Http://webhost.avin.net/afprotein/peidof.htm

Transgenesis 1.

MATE
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

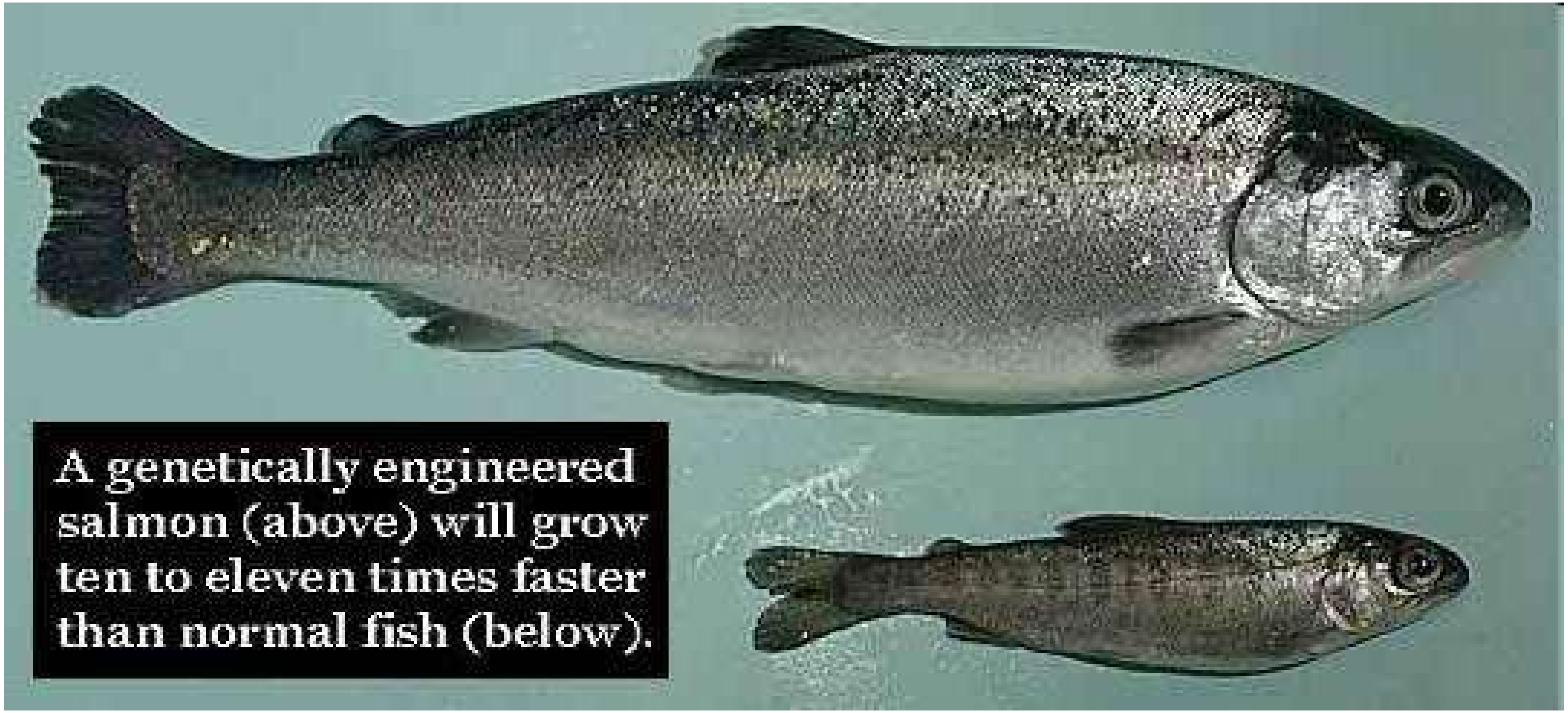
Transgenesis 1.

Transgenesis 1.

Transgenic Chinook salmon from the New Zealand King Salmon Company. The top 3 fish are transgenics: 11 months old with an average weight of 850g, while the bottom fish is a non-transgenic sibling of the same age, weighing 280g

Courtesy of Seumas Walker

Transgenesis 1.

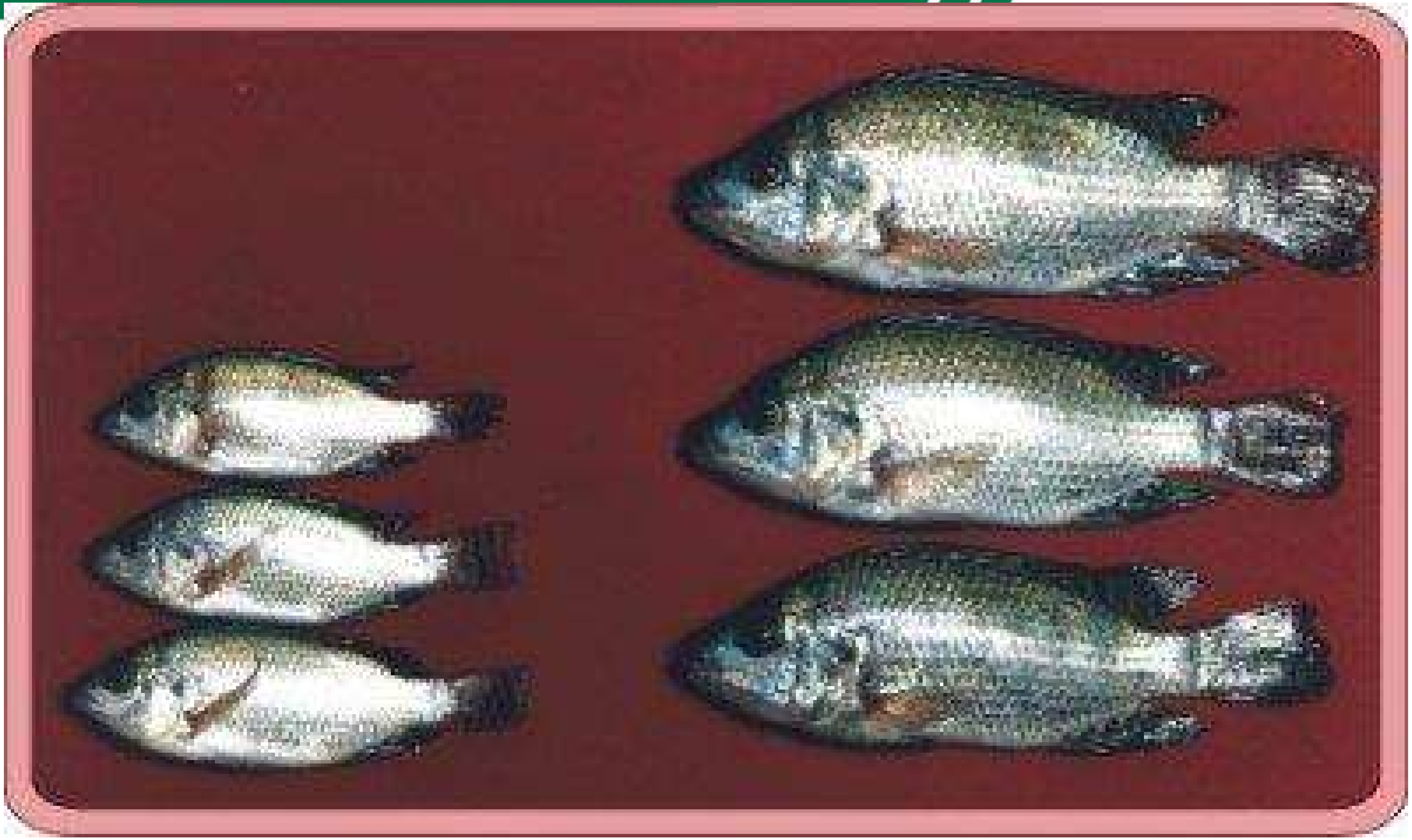


GH Transgenic Fish

(A) *Ictalurus punctatus*

(B) *Cyprinus carpio* (C) *Oreochromis niloticus*

Transgenesis 1.



Maclean, N., Rahman, M.A., Sohm, F., Hwang, G-L., Iyengar, A., Smith, A., Ayad, H. and Farahmand, H. (2002).
Transgenic tilapia and the tilapia genome. Gene. 295, 265-277

Mud loach

- -actin promoter linked to GH gene.
- Growth increase >30 fold.
- Gigantism.

Age = 6 months

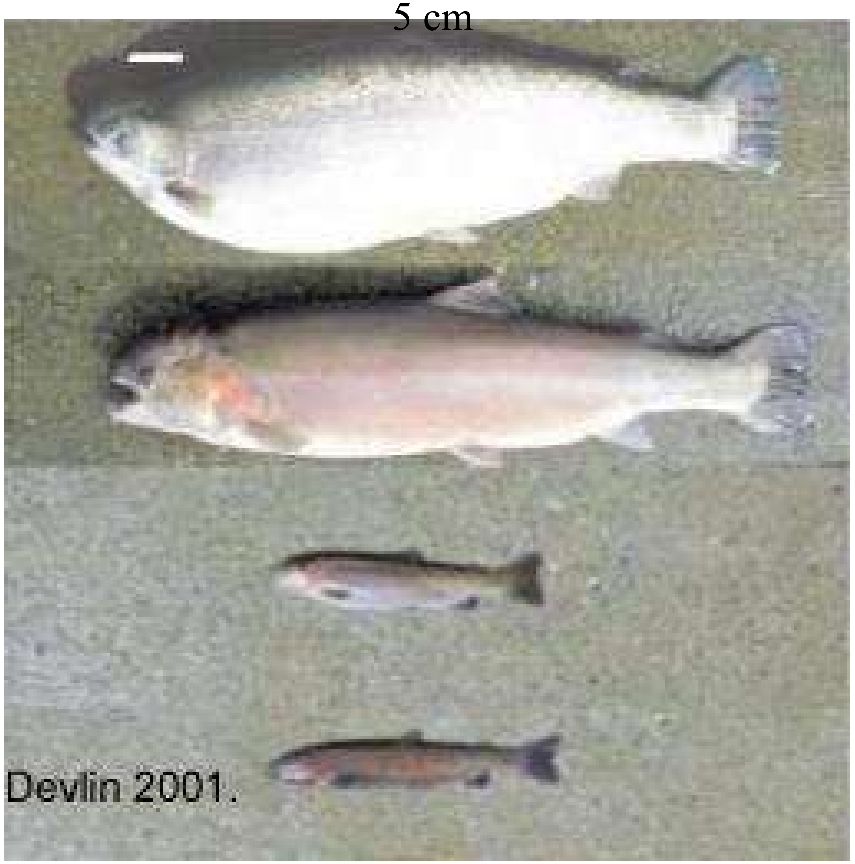
(Nam et al. 2001)

GH transzgenikus Rohu

Rohu (Labeo rohita)—a leginkább tenyésztett és a széleskörben fogyasztott halak között van indiában.
(Venugopal et al., 2004)

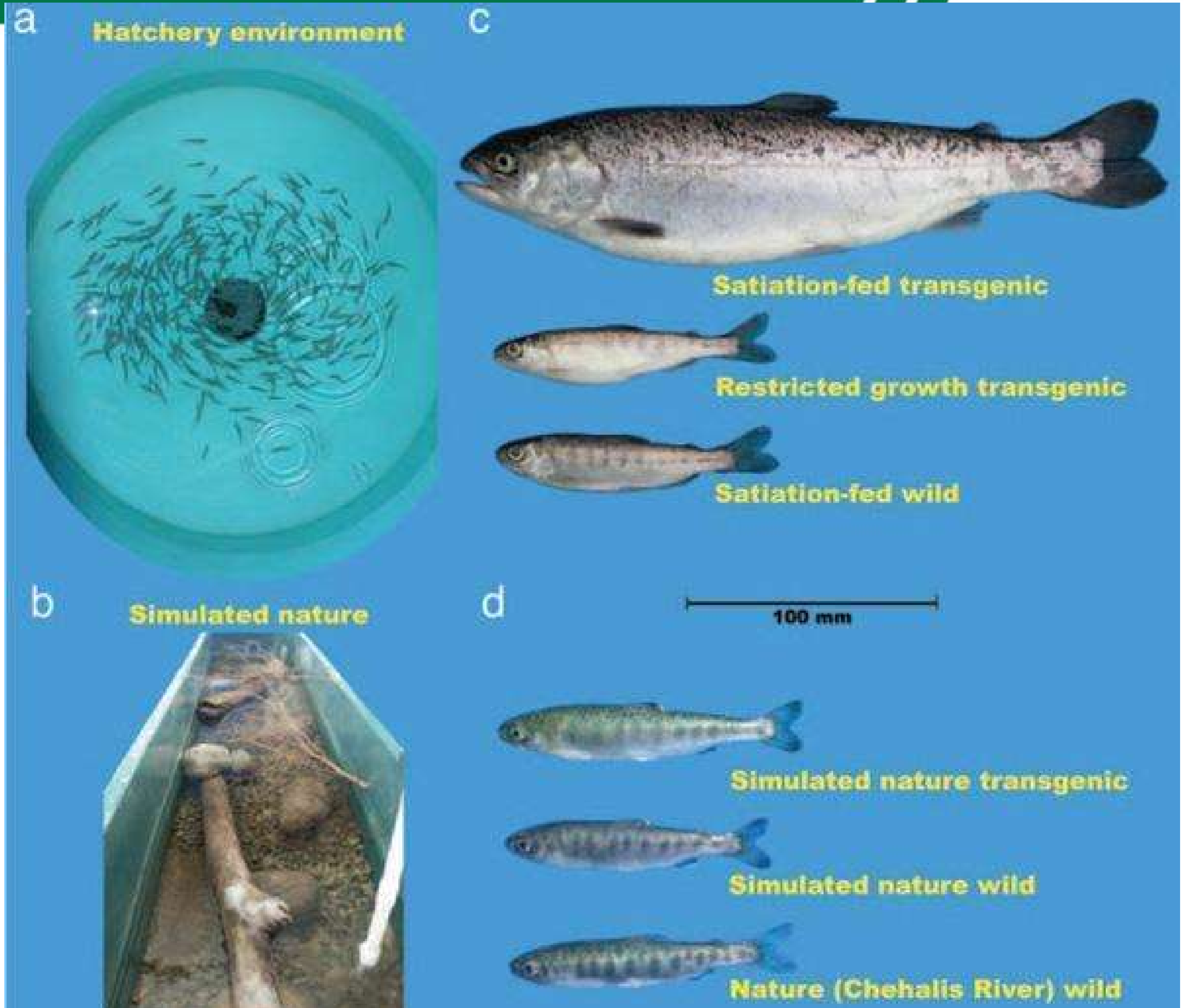
Transgenesis 1.

5 cm



Rainbow Trout transgenics are 37-83 X larger at sexual maturity and have lower juvenile viability

Environmental interaction



(Sundström et al. 2007),

Transgeniczgénikus „coho salmon” development disorders

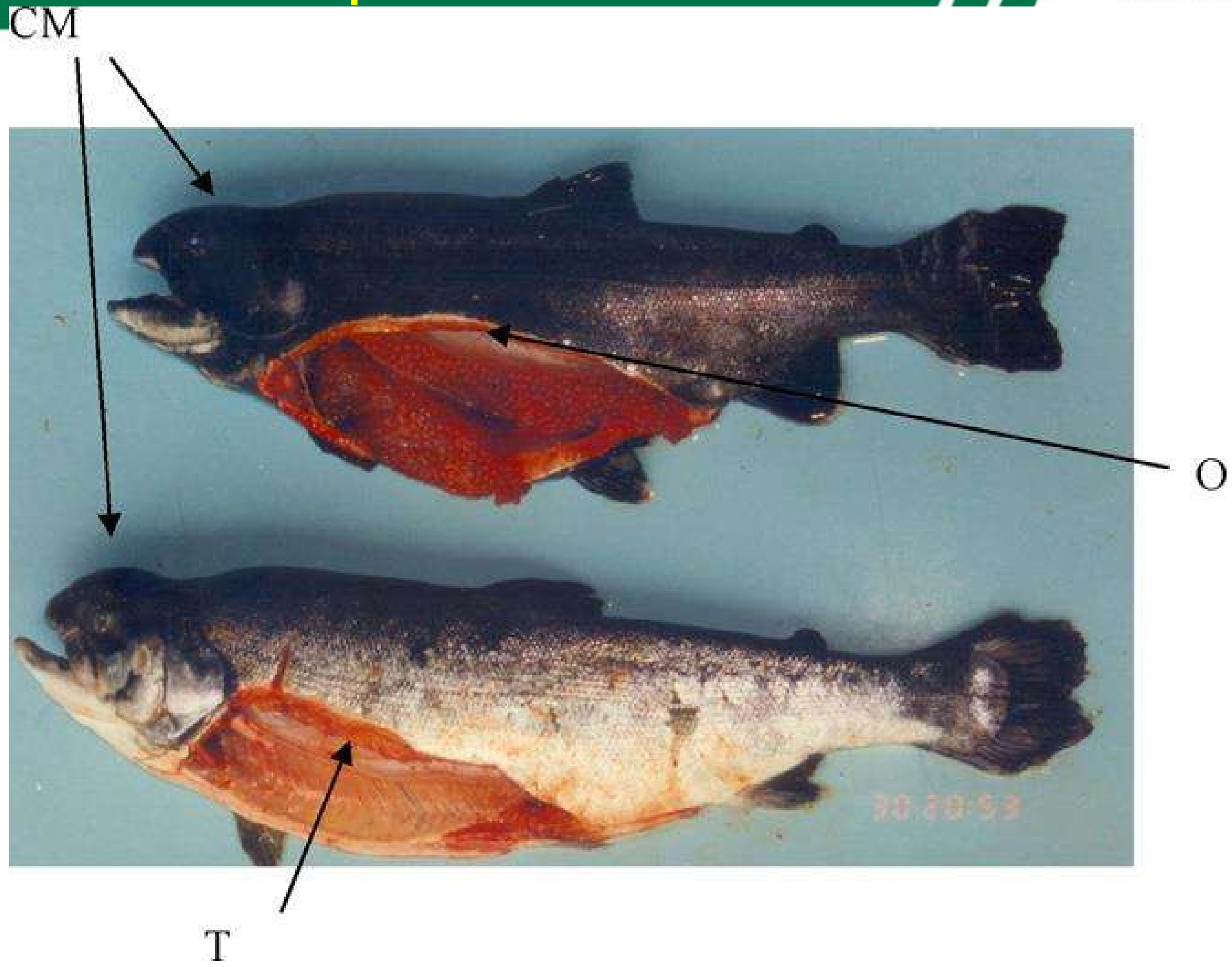
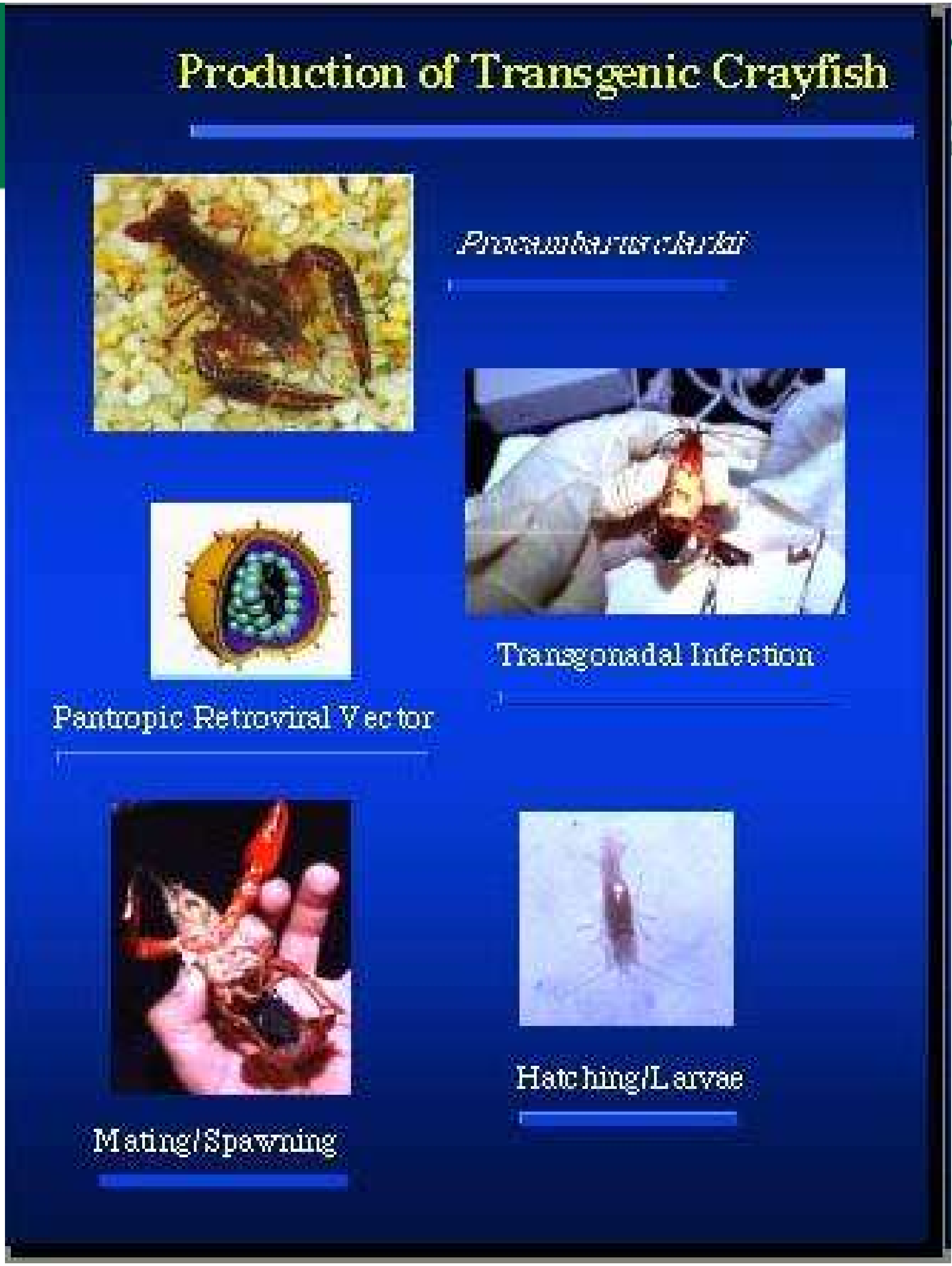
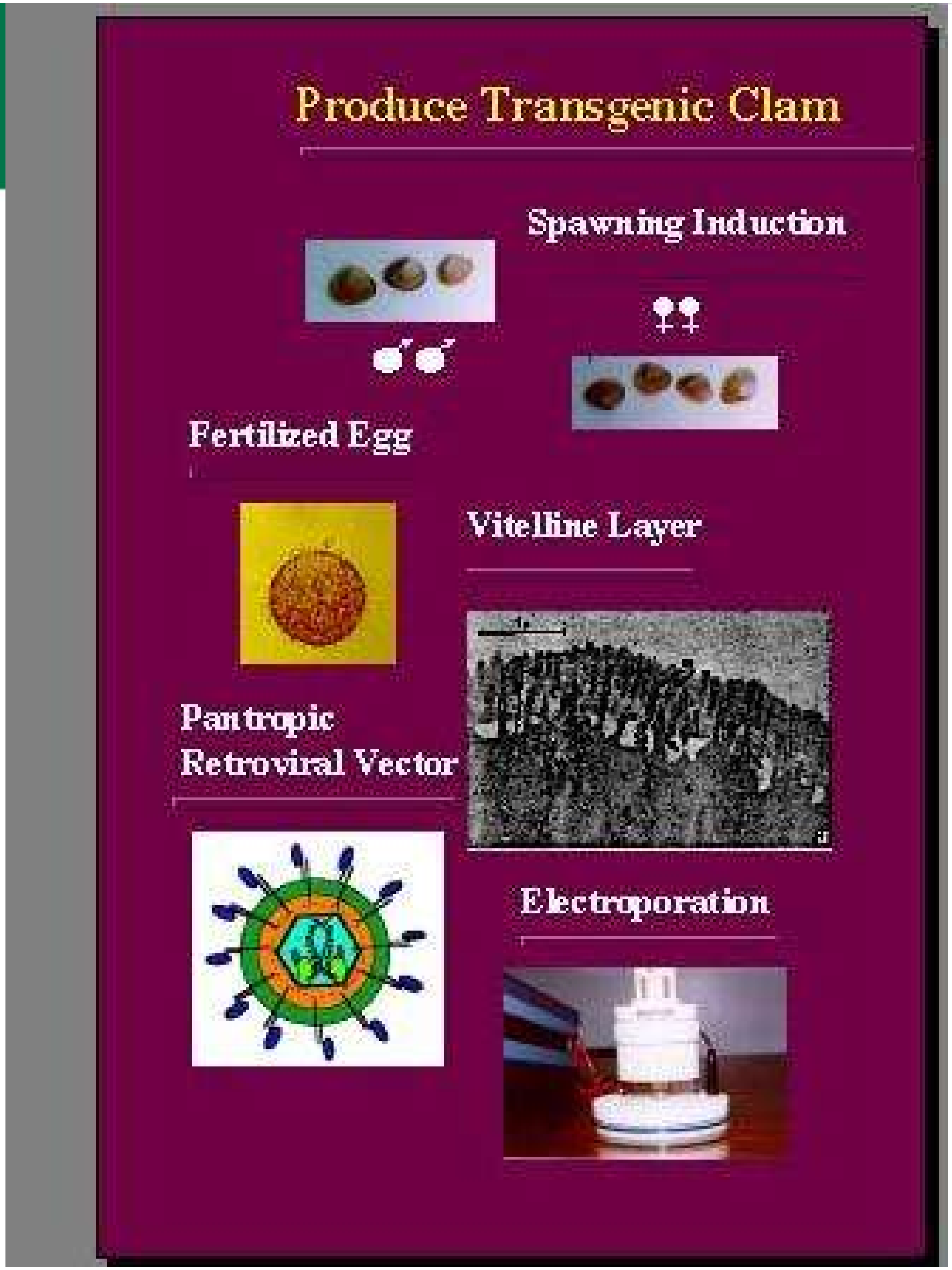


Fig. 1. Transzgenic OnMT-GH1 female (up) and milter ”coho salmon” individuals with scull deformation (CM) and maturity defects. The female have a well developed ovary (O), while the testis is underdeveloped in the males(T).


Production of Transgenic Crayfish
Procambarus clarkii
Transgonadal Infection
Pantropic Retroviral Vector
Hatching/Larvae
Mating/Spawning


Produce Transgenic Clam
Spawning Induction
Fertilized Egg
Vitelline Layer
Pantropic
Retroviral Vector
Electroporation

Antisense transgenic (knock down) Fish

sGnRH-antisense transgenic rainbow trout

aim: slow maturity or sterility

Results

-lower GnRH level.

- no effect on maturity

(Uzbekova et al. 2000)

sGnRH-antisense transgenic rainbow trout

aim: slow maturity or sterility

Results

-lower GnRH level.

- Some sterile fish

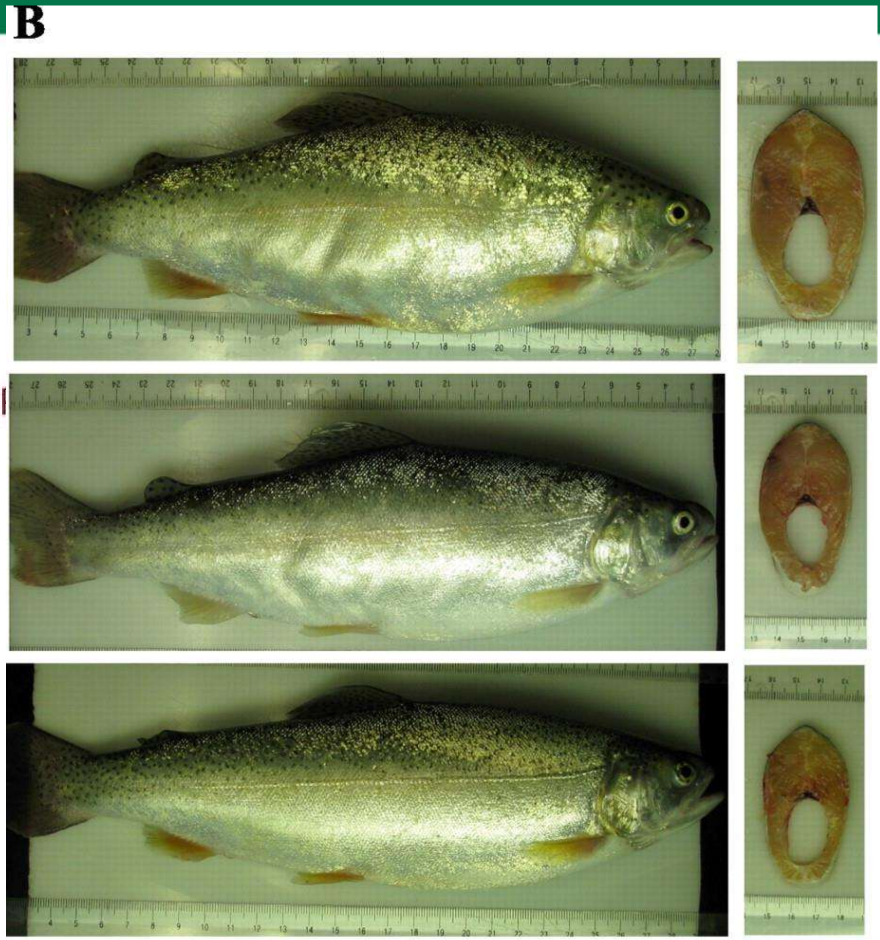
(Hu et al. 2007)

Myostatin-antisense transgenic rainbow trout

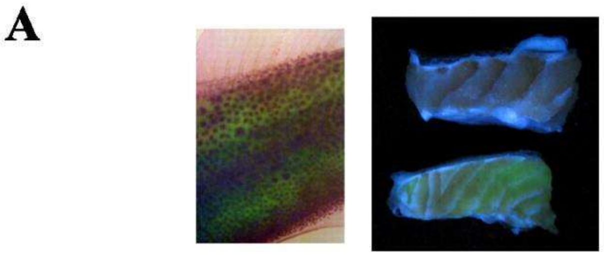
- 20-25% more muscle

(Terry Bradley. University of Rhode Island.2010)

Overexpression of follistatin in trout stimulates increased muscling



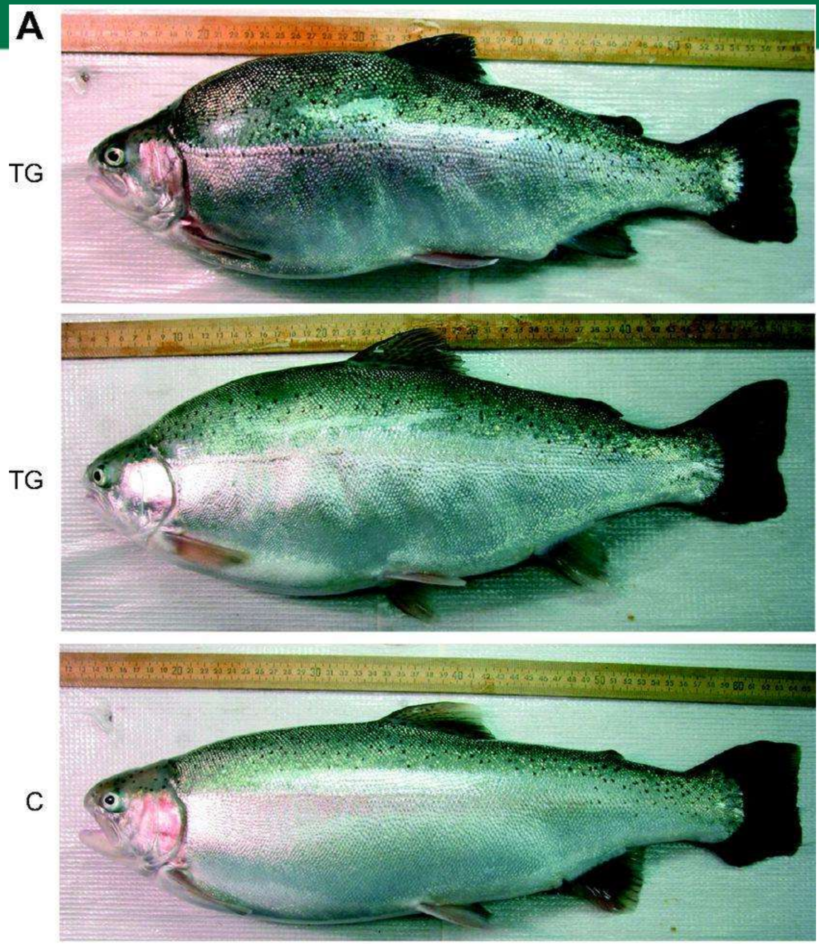
B: control (bottom) and transgenic trout overexpressing follistatin (top and middle). The whole body cross sections are from the respective individuals and were excised immediately anterior to the dorsal fin. All images were captured at an identical distance and camera settings.

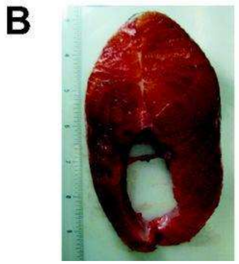


A: green fluorescent protein (GFP) larval muscle (left) and hypaxial muscle tissue from 2-yr-old control (top) and GFP transgenic trout photographed under 365 nm light and verifying expression of the vector.

Erika F. Medeiros et al. Am J Physiol Regul Integr Comp Physiol 2009;297:R235-R242

Activin II B receptor transgenic rainbow trout





Localized muscling exhibited by the P1 founder generation of rainbow trout. P1 transgenic individuals exhibit localized muscling in both the abdominal and epaxial regions of the musculature.

A:
TG - Morphology of 2-year-old *acvr2b*$^{\Delta}$ transgenic
C - Control

B: Cross-section of a P1 transgenic fish reveals asymmetrical skeletal muscle growth.

Michael P. Phelps et al. J Exp Biol 2013;216:3742-3750

GH-transgene effect on growth

Modest (10-50%)

Goldfish, carp, loach, channel catfish, Northern pike, rainbow trout

Moderate (100-200%)

Tilapia

Dramatic (to 1500%)

Salmo, Salvelinus, Oncorhynchus

NUS
National University of Singapore

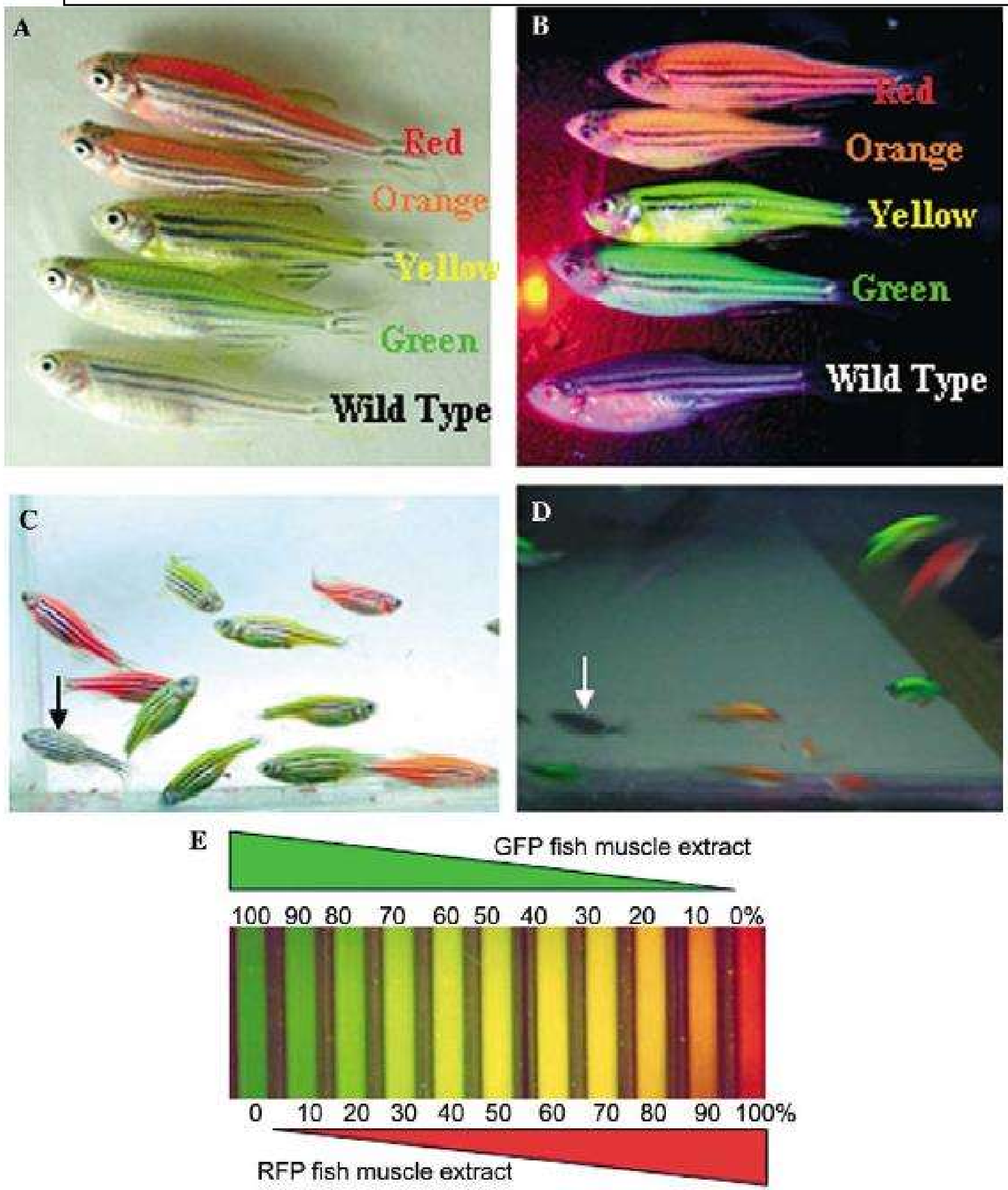
Zhiyuan Gong et.al. (2003)BBRC 308: 58-63

Environmental pollution sensitive fish:

For detection:

- estrogenic chemicals
- stress factors
- Metals
- Toxins
- Etc.

Temperature sensitive fish



Fluorescent transgenic zebrafish.

under daylight (A,C) and

385nm ultraviolet light (B,D).

Transgenic fish on the market 1.

GLOFISH®

http://www.glofish.com/

Fluorescent zebra fish for aquarium
Taiwan
USA

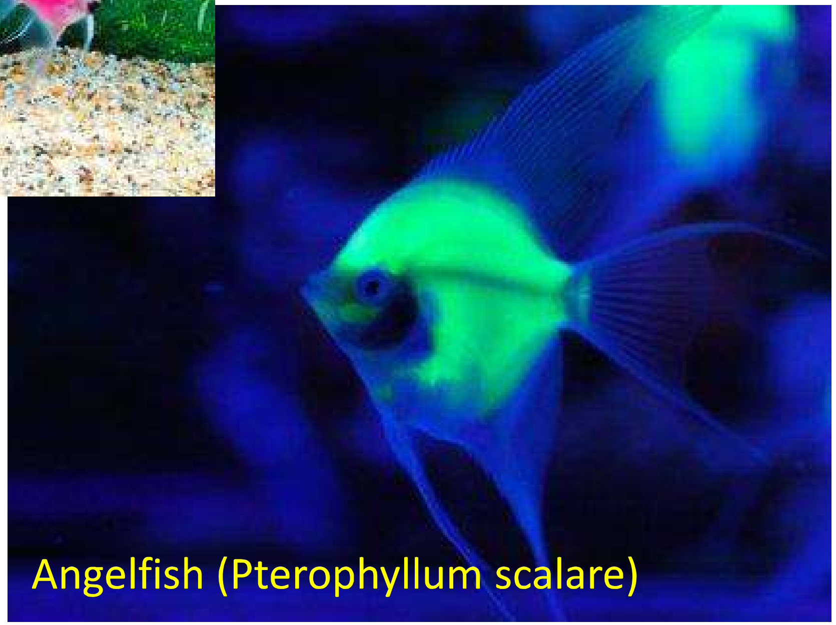
Can't buy *GloFish* in Australia,
Canada
or *Europe*

They prohibit the marketing of any genetically modified animals!

Convict cichlids (Amatitlania nigrofasciata)

Taiwan's Council of Agriculture
Announced:28 June 2010

Market introduction: 2012



Angelfish (Pterophyllum scalare)

GloFish

Glowing Sushi

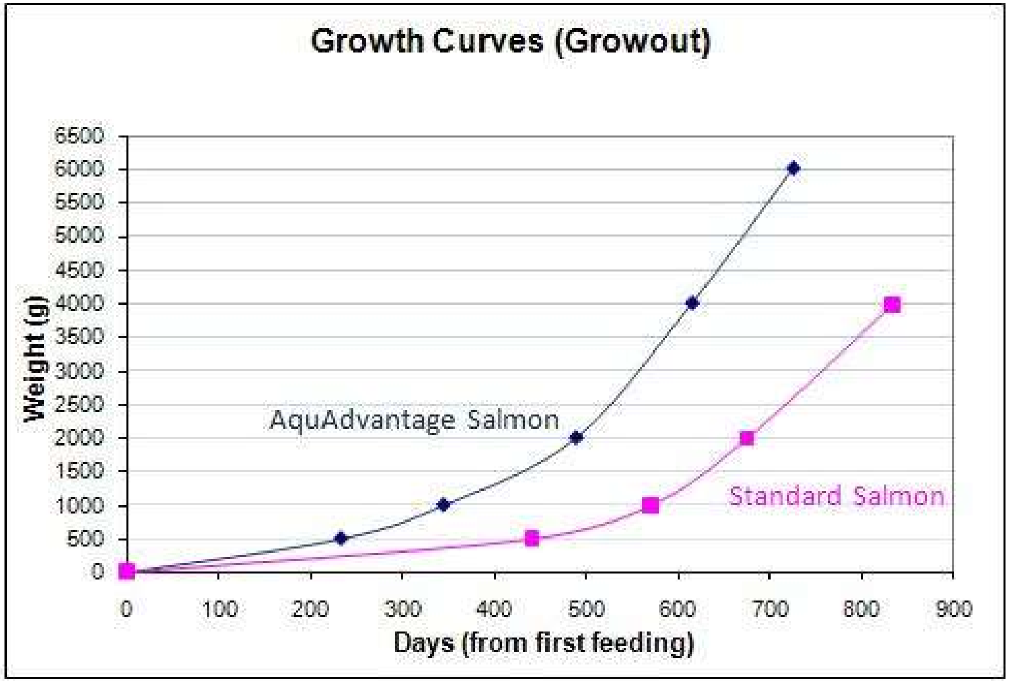
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Entis E. (1998) Taste testing at a top Canadian restaurant. Aqua Bounty Farms 1:1-4

AquAdvantage™ Atlanti salmon (Aqua Bounty Technologies Inc., Waltham, MA),
US Food and Drug Administration (FDA)
Applied for permission every year from 1999, (Logar and Pollock, 2005)

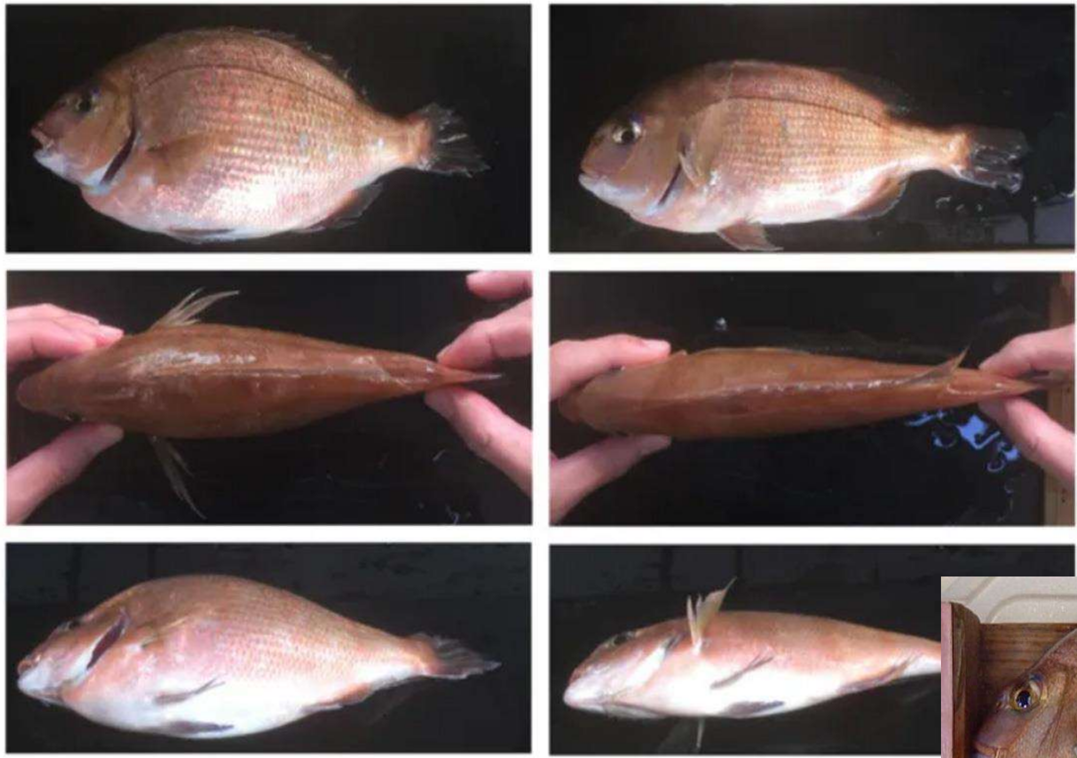
Ocean pout antifreeze gene promoter + chinook salmon growth hormone gene

Sterilized by triploidisation (second polar body retention+ cell sorter/flow cytometry)



Production of a breed of red sea bream *Pagrus major* with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9 (Aquaculture, Volume 495, 1 October 2018, Pages 415-42)

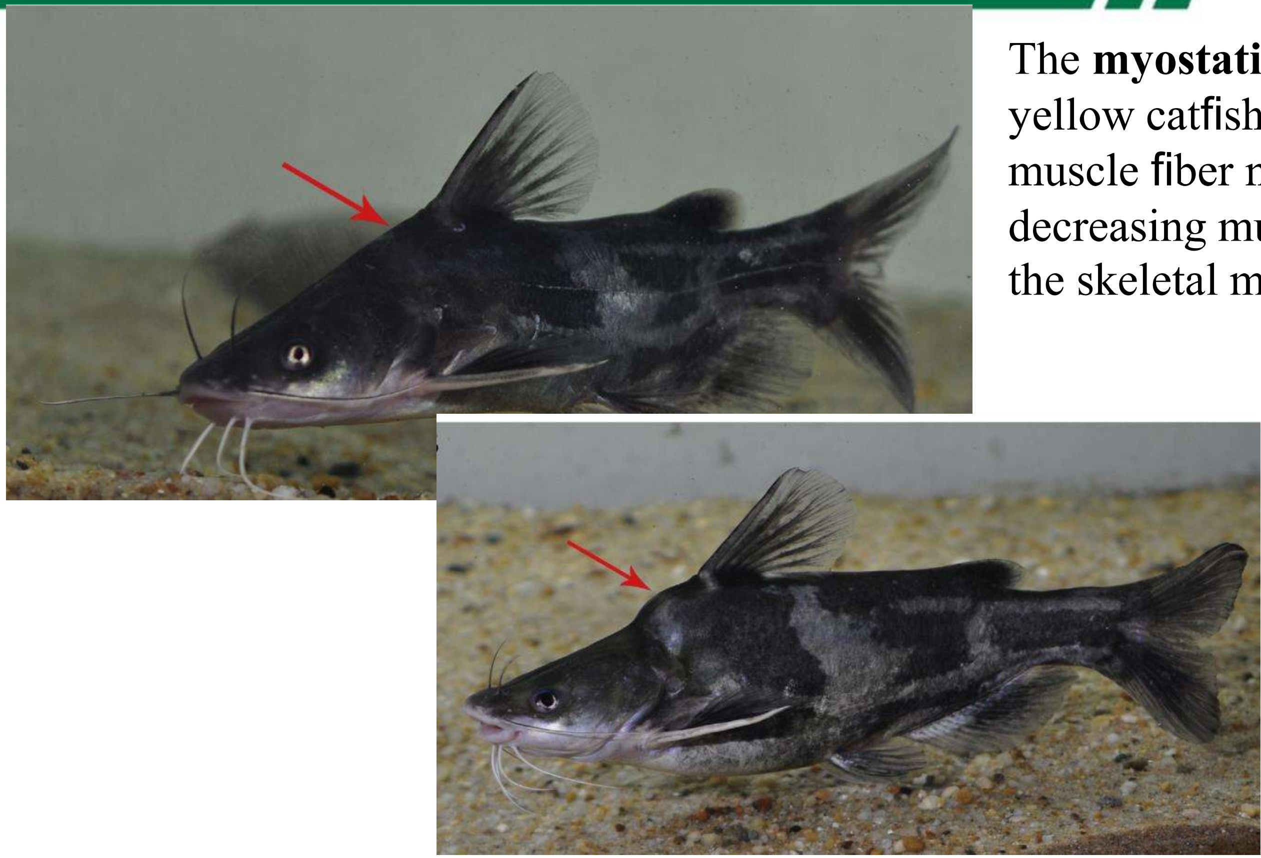
ÉLETTUDOMÁNYI EGYETEM



Myostatin (Pm-mstn) complete knockout

16% increas

The genome- edited-mstna yellow catfish (*Pelteobagrus fulvidraco*)



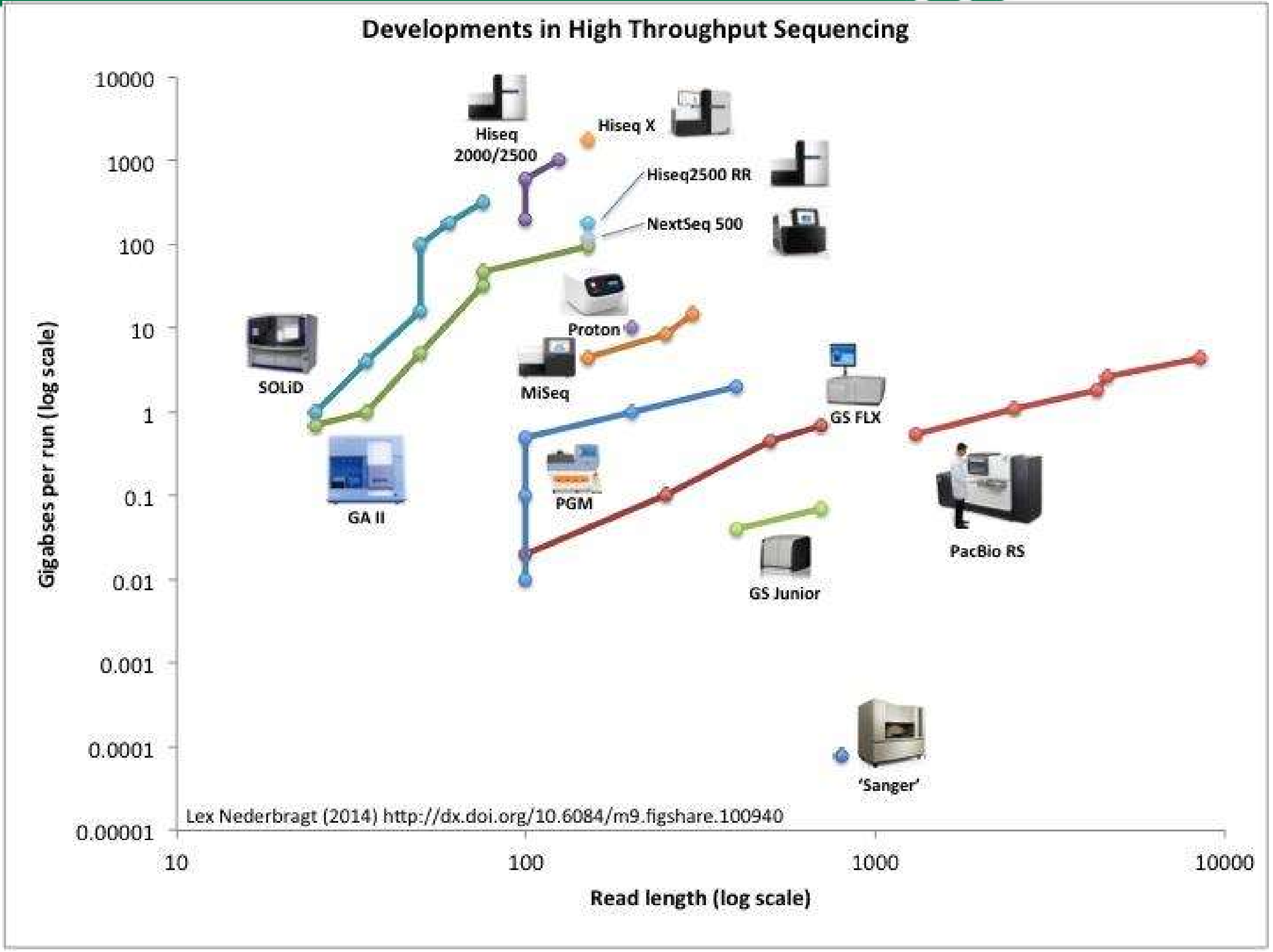
The **myostatin** knockout yellow catfish had increasing muscle fiber number but decreasing muscle fiber size in the skeletal muscle.

grew and bred normally,
37% gain

Aquaculture 523 (2020) 735187

Kyoto firm puts genome-edited tiger puffer on the table

In the genome-edited fish strain, a **leptin receptor** reducing appetite was removed, leading to growth at double speed and improved feed utilization efficiency. 1.9 times larger on average and 2.4 times larger than wild-type



Developments in High Throughput Sequencing
Gigabses per run (log scale)
Read length (log scale)
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
10
100
1000
10000
Hiseq 2000/2500
Hiseq X
Hiseq2500 RR
NextSeq 500
Proton
SOLiD
MiSeq
GS FLX
GA II
PGM
PacBio RS
GS Junior
'Sanger'
Lex Nederbragt (2014) http://dx.doi.org/10.6084/m9.figshare.100940

Genome Analysis

- *de novo* sequencing
- resequencing
- ChIP sequencing
- metagenome sequencing

Targeted Analysis

- exome sequencing
- custom targeted sequencing
- sequencing of amplicons and genomic fragments

Transcriptome Analysis

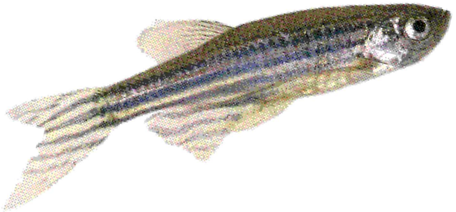
- mRNA sequencing: poly-A selection
- RNA sequencing: rRNA depletion
- strand specific RNA seq
- miRNA & small RNA sequencing
- deepCAGE / NanoCAGE sequencing

e!Ensembl Home

Aquatic Genome programs 1.

Fugu (*Takifugu rubripes*) 2002

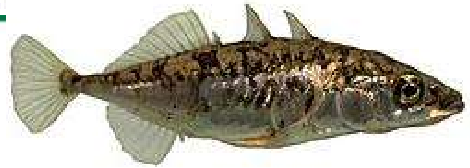
Green Spotted pufferfish (*Tetraodon nigroviridis*) 2004



Zebrafish (*Danio rerio*) 2005

Medaka (*Oryzias latipes*) 2007

Aquatic Genome programs 2.

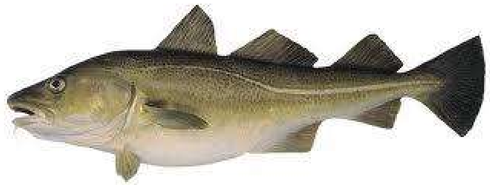


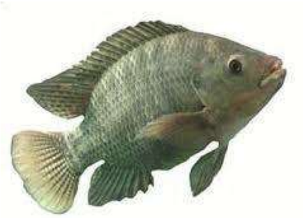
Strickleback (*Gasterosteus aculeatus*)

Lamprey (*Petromyzon marinus*)

Coelacanth (*Latimeria chalumnae*)

Atlantic cod (*Gadus morhua*),

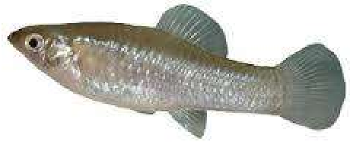




Tilapia (*Oreochromis niloticus*)

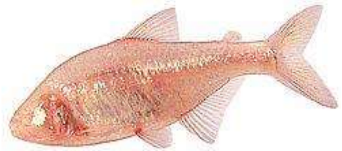
Spotted gar (*Lepisosteus oculatus*)

Amazon molly (*Poecilia formosa*)



Platyfish (*Xiphophorus maculatus*)

Cave fish (*Astyanax mexicanus*)



Ponty (*Cyprinus carpio*)

Szivárványos pisztráng
(*Oncorhynchus mykiss*)

Aquatic Genome programs 3.

2012: 11 +17 hal faj (public funded laboratories)

1	*Callorhinchus milii*	Elephant shark	Callorhinchidae	Chimaeriformes
2	*Leucoraja erinacea*	Little skate	Rajidae	Rajiformes
3	*Clupea harengus*	Atlantic herring	Clupeidae	Clupeiformes
4	*Ictalurus punctatus*	Channel catfish	Ictaluridae	Siluriformes
5	*Salmo salar*	Atlantic salmon	Salmonidae	Salmoniformes
6	*Oncorhynchus mykiss*	Rainbow trout	Salmonidae	Salmoniformes
7	*Nothobranchius furzeri*	Turquoise killifish	Notobranchiidae	Cyprinodontiformes
8	*Astatotilapia burtoni*	Burton's mouthbrooder	Cichlidae	Perciformes
9	*Maylandia zebra*	Zebra mbuna	Cichlidae	Perciformes
10	*Pundamila nyererei*	Nyererei cichlid	Cichlidae	Perciformes
11	*Neolamprologus brichardi*	Fairy cichlid	Cichlidae	Perciformes
12	*Rhamphochromis esox*	Tiger cichlid	Cichlidae	Perciformes
13	*Melanochromis auratus*	Golden mbuna	Cichlidae	Perciformes
14	*Mchenga conophoros*	Happy cichlid	Cichlidae	Perciformes
15	*Labeotropheus fuelleborni*	Blue mbuna	Cichlidae	Perciformes
16	*Dicentrarchus labrax*	European Seabass	Moronidae	Perciformes
17	*Thunnus orientalis*	Pacific bluefin tuna	Scombridae	Scombriformes

Bernardia et. al.:**The fishes of Genome 10K** Mar.Genomics, September 2012, Vol. 7 Pages 3–6

2017 FISH GENOMS

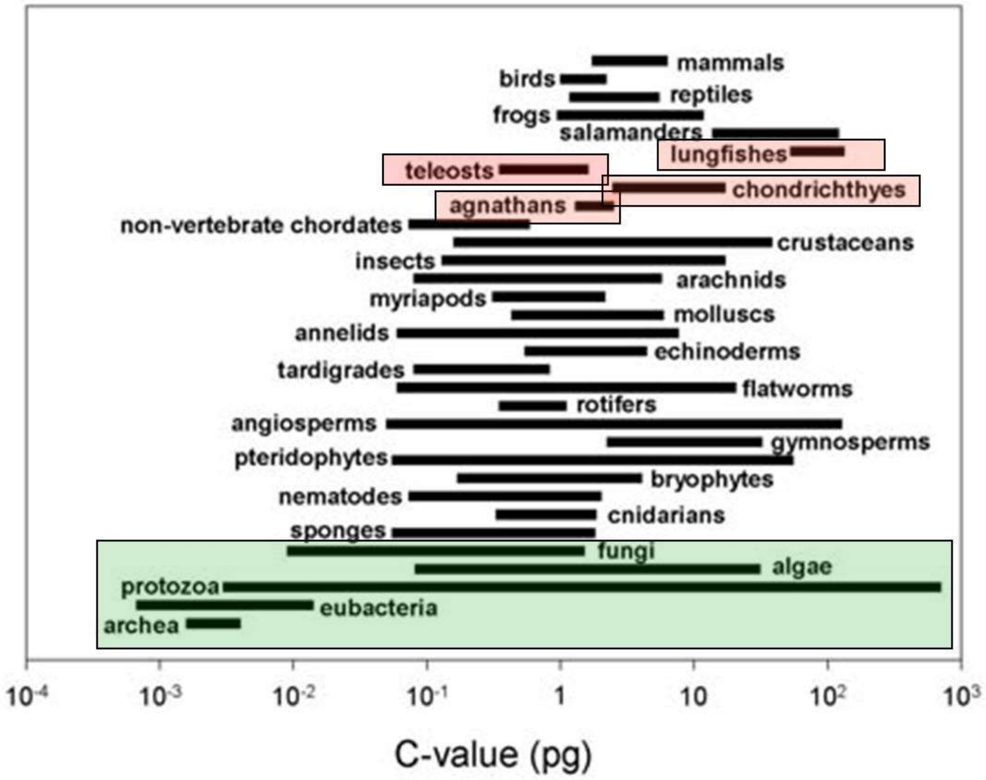
1 Cyprinus carpio
2 Cyprinodon nevadensis
4 Callorhinchus milii
5 Miichthys miiuy
6 Sebastes nigrocinctus
7 Sebastes rubrivinctus
8 Melanochromis auratus
9 Labeotropheus fuelleborni
10 Mchenga conophoros
11 Tetraodon nigroviridis
12 Cottus rhenanus
13 Squalius pyrenaicus
14 Sinocyclocheilus rhinocerous
15 Sinocyclocheilus anshuiensis
16 Periophthalmodon schlosseri
17 Periophthalmus magnuspinnatus
18 Pseudopleuronectes yokohamae
19 Amphilophus citrinellus
20 Rhincodon typus
21 Anguilla rostrata
22 Pampus argenteus
23 Poecilia reticulata
24 Kryptolebias marmoratus
25 Esox lucius

27 Lethenteron camtschaticum
28 Xiphophorus couchianus
29 Clupea harengus
30 Xiphophorus hellerii
31 Leuciscus waleckii
32 Labrus bergylta
33 Poecilia mexicana
34 Takifugu flavidus
35 Lates calcarifer
36 Scartelaos histophorus
37 Anguilla japonica
38 Thunnus orientalis
39 Pimephales promelas
40 Austrofundulus limnaeus
41 Cyprinodon variegatus
42 Stegastes partitus
43 Astyanax mexicanus
44 Poecilia formosa
45 Anoplopoma fimbria
46 Larimichthys crocea
47 Boleophthalmus pectinirostris
48 Cynoglossus semilaevis
49 Leucoraja erinacea
50 Anguilla anguilla
51 Xiphophorus maculatus

53 Morone saxatilis
54 Lepisosteus oculatus
55 Pygocentrus nattereri
56 Sinocyclocheilus grahami
57 Scleropages formosus
58 Mola mola
59 Pundamilia nyererei
60 Neolamprologus brichardi
61 Haplochromis burtoni
62 Latimeria chalumnae
63 Gadus morhua
64 Nothobranchius kuhntae
65 Nothobranchius furzeri
66 Rhamphochromis esox
67 Maylandia zebra
68 Fundulus heteroclitus
69 Oryzias latipes
70 Salmo salar
71 Petromyzon marinus
72 Ictalurus punctatus
73 Oreochromis niloticus
74 Gasterosteus aculeatus
75 Takifugu rubripes
76 Danio rerio

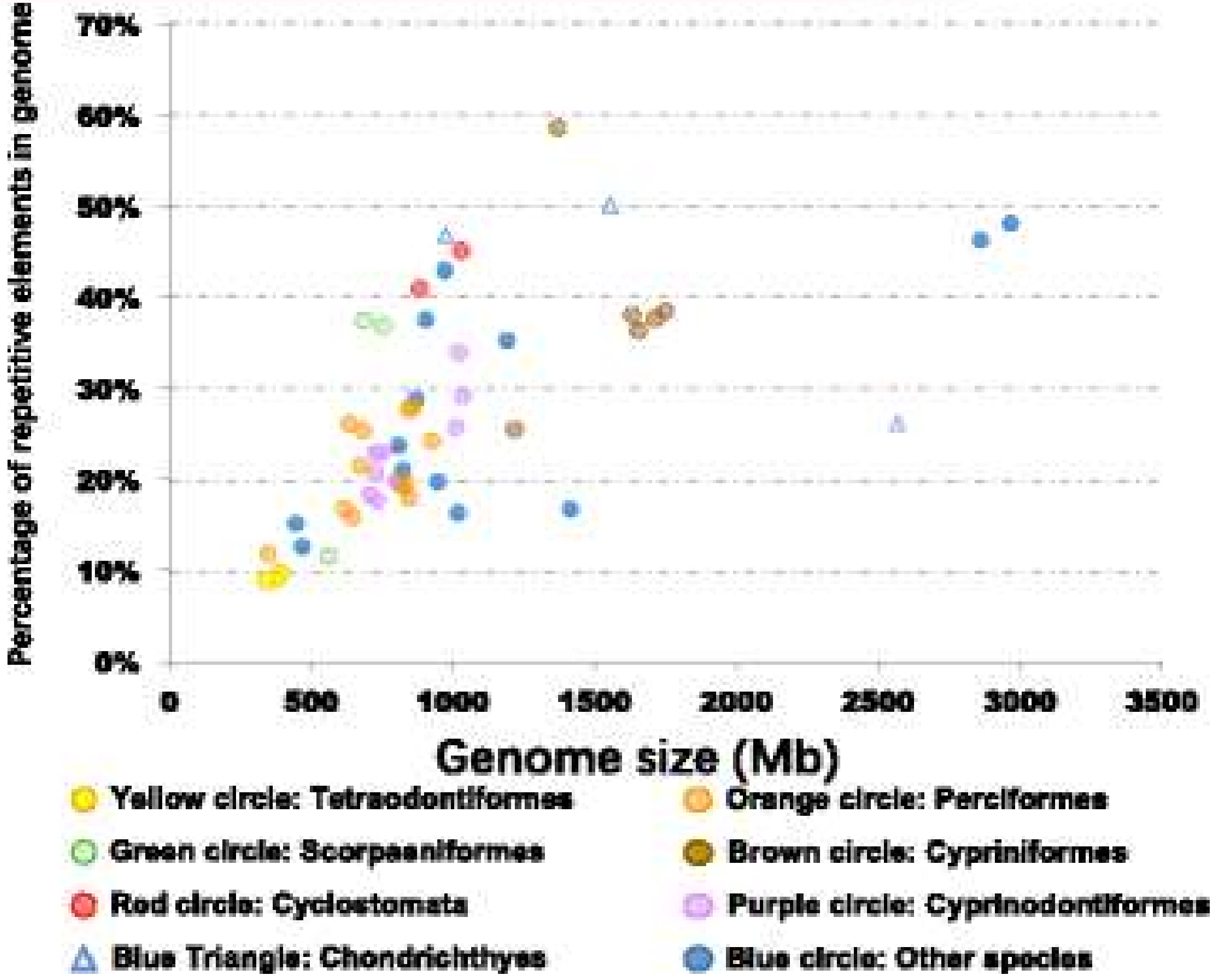
C value paradoxes

The C values are not correlate with the complexity level (number of the cells and cell types) of the species



Comparative genome analysis of 52 fish species

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

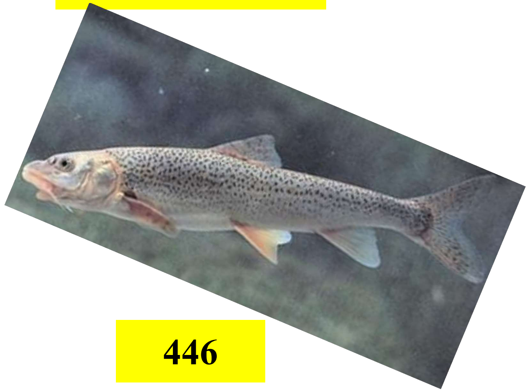


K value pardoxes

Acipenser brevirostrum

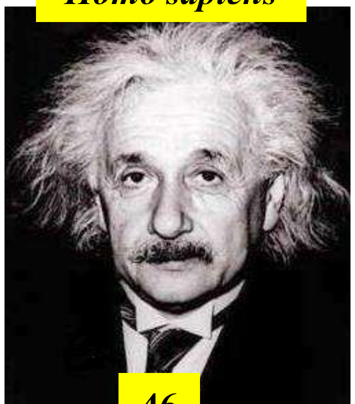
362 to 372

Ptychobarbus dipogon



446

Homo sapiens



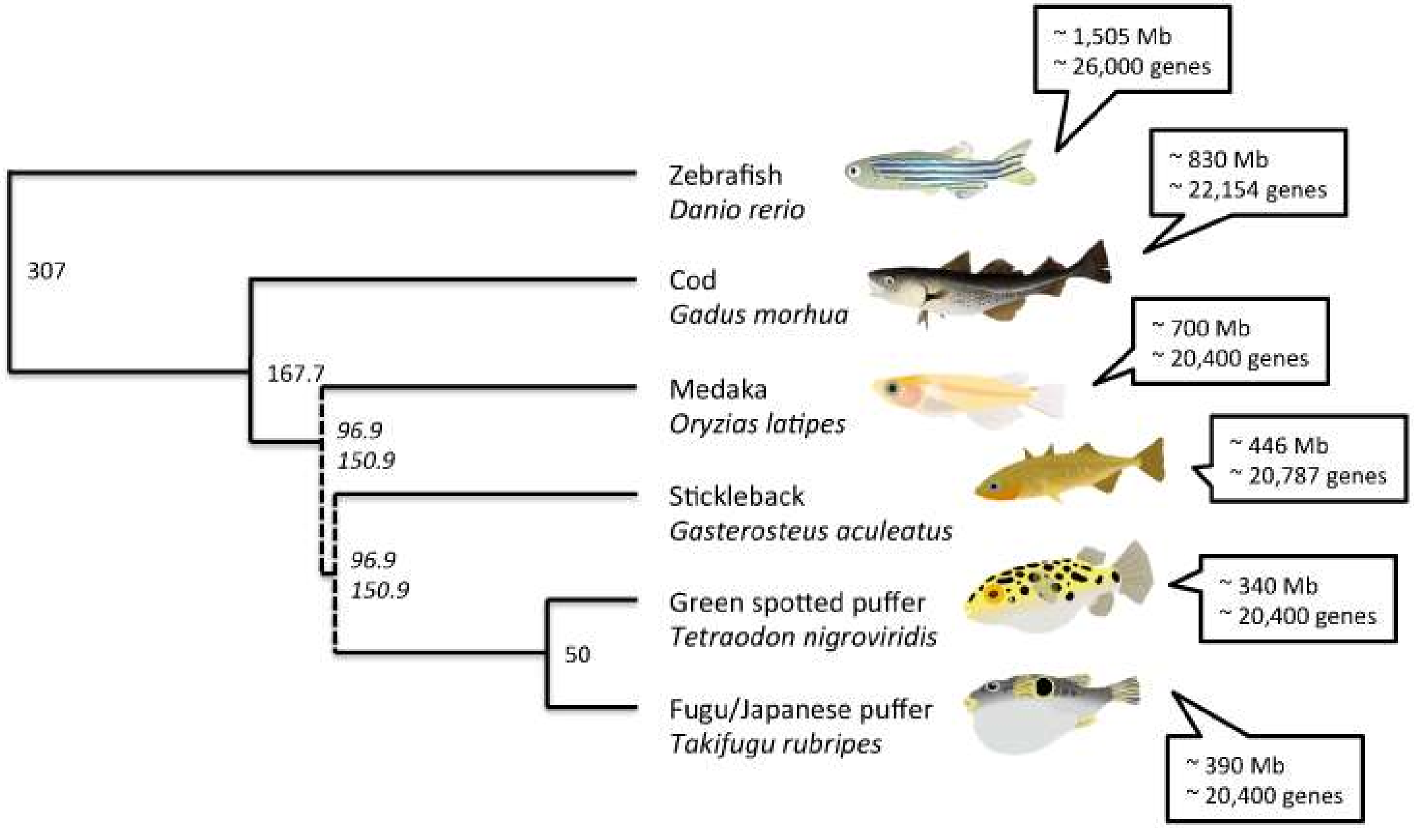
46

Notothenia neglecta

22

g value paradoxes

The number of the genes are not correlate with the complexity level (number of the cells and cell types) of the species



Fish Genom programs 2.

MATE
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

e! Ensembl *Genome* Browser. www.ensembl.org

	Zebrafish	FUGU	Medaka	Stickleback	Tetraodon
Gene build:	2007	2005	2005	2006	2007
protein-coding genes:	17 330	133	509	71	99
Projected protein-coding genes:	1 627	13762	13 893	15 143	13 772
Novel protein-coding genes:	2 365	3 728	5 284	5 573	5 731
Pseudo-genes:	98	162	1	52	147
RNA genes:	4 126	571	512	1 468	498
Genscan gene predictions:	45 287	29 699	123 380	44 884	23 832
Gene exons:	232 290	321 943	220 743	245 824	220 724
Gene transcripts:	31 841	48 027	24 662	27 629	23 289
Base Pairs:	1 527 000 581	393 312 790	700 369 883	446 627 861	342 419 788

1-3% coding region

	Species	Order	DNA/TcMar-Tc1	Microsatellites
Freshwater species	*Esox lucius*	Esociformes	35.9%	4.8%
	Fundulus heteroclitus	Cyprinodontiformes	22.6%	4.6%
	Xiphophorus hellerii	Cyprinodontiformes	22.7%	5.1%
	Xiphophorus couchianus	Cyprinodontiformes	22.3%	5.1%
	Amphilophus citrinellus	Perciformes	23.0%	6.2%
	Xiphophorus maculatus	Cyprinodontiformes	22.0%	6.2%
	Lepisosteus oculatus	Lepisosteiformes	10.5%	3.0%
	Pundamilia nyererei	Perciformes	18.7%	5.8%
	Haplochromis burtoni	Perciformes	19.6%	6.1%
	Maylandia zebra	Perciformes	16.8%	5.3%
	Neolamprologus brichardi	Perciformes	20.9%	6.7%
	Cyprinodon nevadensis	Cyprinodontiformes	7.6%	2.5%
	Poecilia formosa	Cyprinodontiformes	19.3%	6.5%
	Oreochromis niloticus	Perciformes	15.9%	5.4%
	Poecilia reticulata	Cyprinodontiformes	17.8%	6.1%
	Poecilia mexicana	Cyprinodontiformes	18.9%	6.7%
	Astyanax mexicanus	Characiformes	21.8%	8.0%
	Poecilia latipinna	Cyprinodontiformes	19.5%	7.4%
	Cyprinodon variegatus	Cyprinodontiformes	8.2%	3.4%
	Oryzias latipes	Beloniformes	5.0%	2.6%
	Ictalurus punctatus	Siluriformes	19.9%	14.1%
	Danio rerio	Cypriniformes	6.1%	5.9%
	Cyprinus carpio	Cypriniformes	6.4%	7.1%
	Sinocyclocheilus grahami	Cypriniformes	4.7%	5.7%
	Sinocyclocheilus rhinocerous	Cypriniformes	3.4%	6.0%
	Sinocyclocheilus anshuiensis	Cypriniformes	3.2%	6.2%
	Pimephales promelas	Cypriniformes	3.1%	6.7%
	Cottus rhenanus	Scorpaeniformes	0.9%	17.8%
	Tetraodon nigroviridis	Tetraodontiformes	1.3%	31.1%

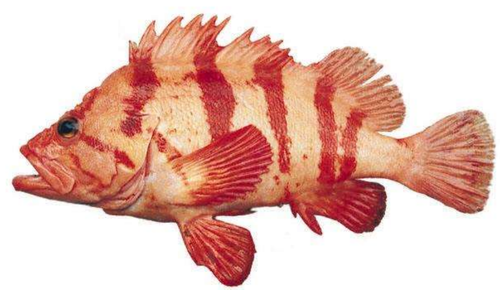
	Species	Order	DNA/TcMar-Tc1	Microsatellites
Diadromous species	*Salmo salar*	Salmoniformes	23.6%	7.5%
	Anguilla anguilla	Anguilliformes	11.9%	11.4%
	Anguilla rostrata	Anguilliformes	11.8%	13.9%
Marine species	*Thunnus orientalis*	Perciformes	3.6%	9.3%
	Pampus argenteus	Perciformes	5.6%	15.2%
	Gasterosteus aculeatus	Gasterosteiformes	4.1%	12.8%
	Miichthys miiuy	Perciformes	4.0%	14.7%
	Notothenia coriiceps	Perciformes	2.3%	9.5%
	Dicentrarchus labrax	Perciformes	2.4%	11.9%
	Larimichthys crocea	Perciformes	3.4%	17.7%
	Takifugu rubripes	Tetraodontiformes	3.3%	19.9%
	Sebastes nigrocinctus	Scorpaeniformes	1.1%	8.9%
	Cynoglossus semilaevis	Pleuronectiformes	2.7%	23.1%
	Takifugu flavidus	Tetraodontiformes	2.4%	21.8%
	Sebastes rubrivinctus	Scorpaeniformes	1.0%	9.1%
	Clupea harengus	Clupeiformes	3.0%	29.8%
	Latimeria chalumnae	Coelacanthiformes	0.0%	1.7%
	Gadus morhua	Gadiformes	0.4%	31.4%

Cyprinodon nevadensis

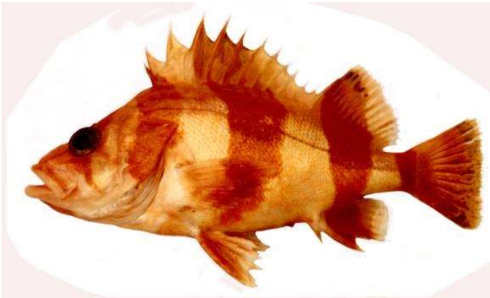
Miichthys miiuy

Melanochromis auratus

Sebastes nigrocinctus

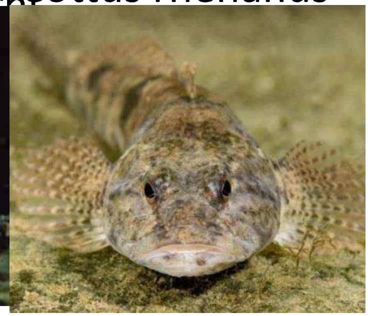


Sebastes rubrivinctus



Labeotropheus fuelleborni

Cottus rhenanus



Squalius pyrenaicus

Sinocyclocheilus rhinocerous

Sinocyclocheilus anshuiensis

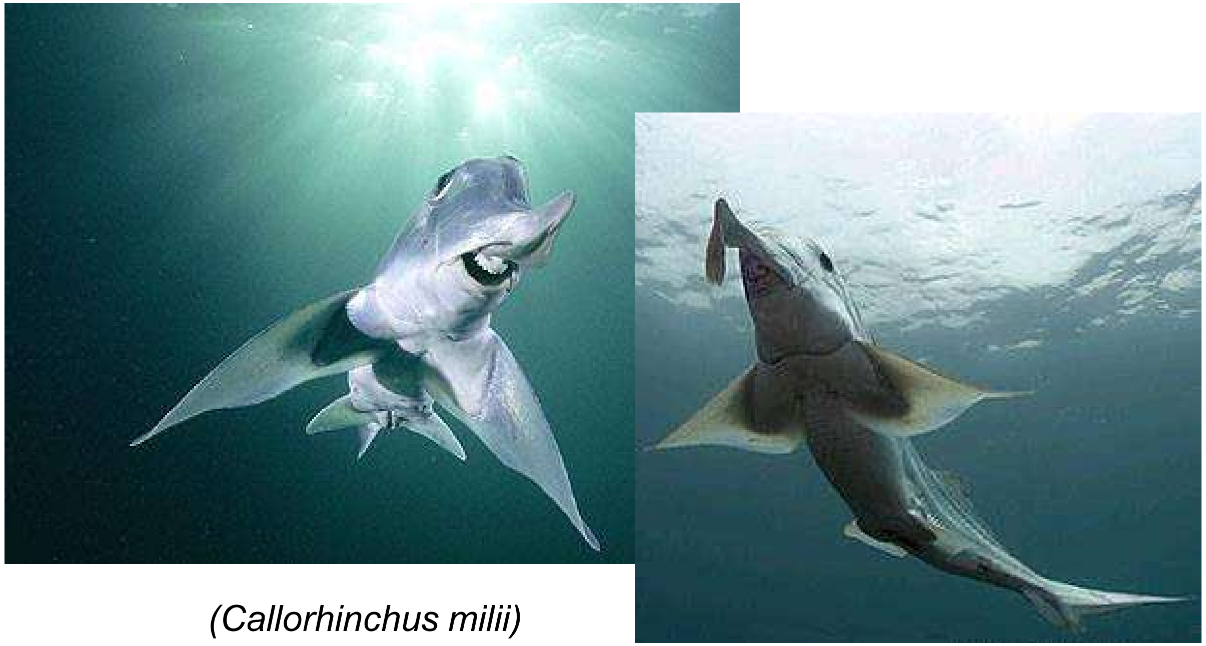
Astatotilapia burtoni

Zebra mbuna
(Maylandia zebra)

Tilapia Cichlid

Mchenga conophoros

Pundamila nyererei

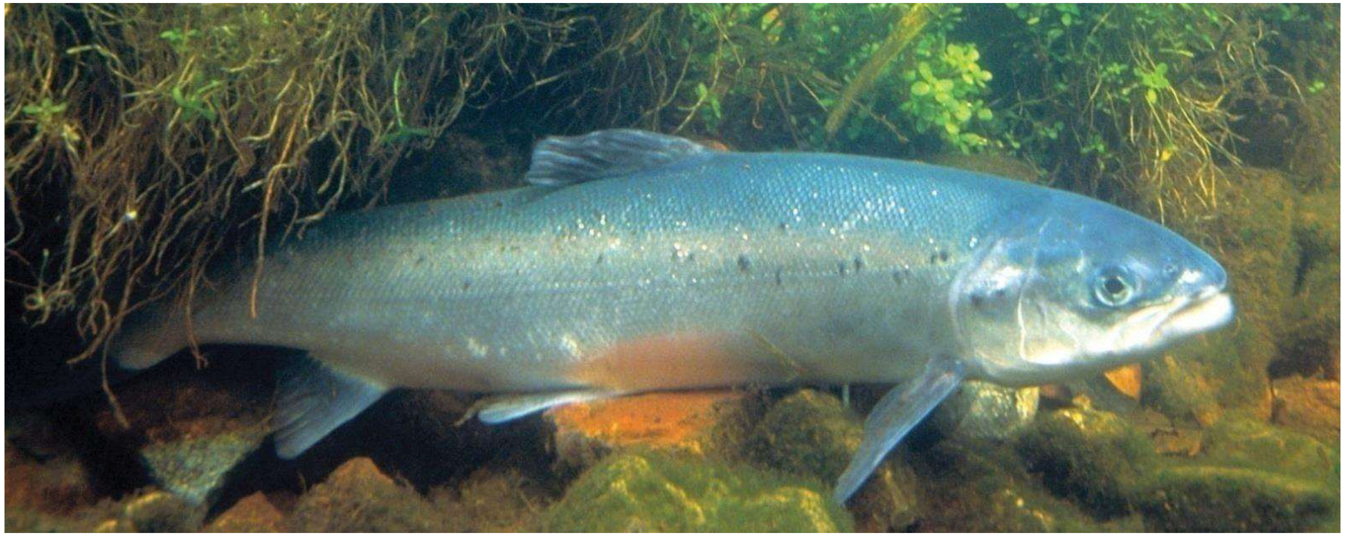


(Callorhinchus milii)

Venkatesh et al. 2005. *Curr. Biol.* 15: R82-R83

http://esharkgenome.imcb.a-star.edu.sg/

INTERNATIONAL SEQUENCING CONSORTIUM



Atlanti lazac

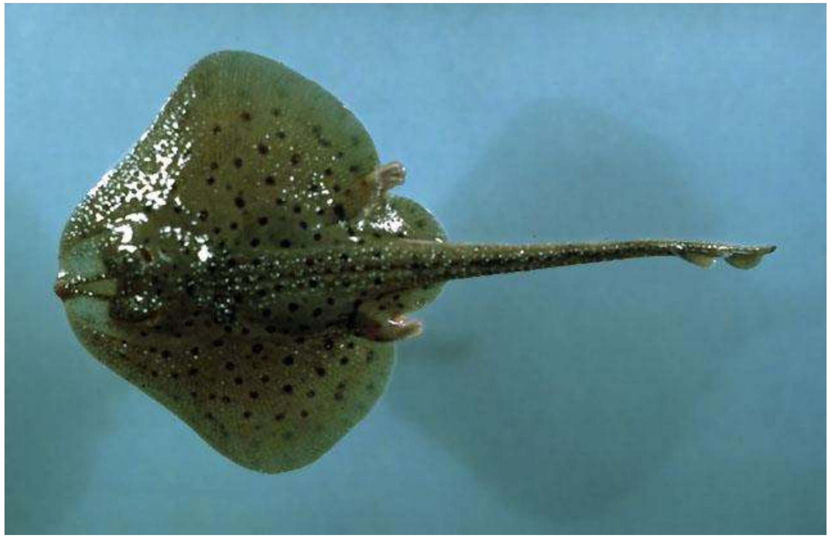
(Salmo salar)

Pettyes (csatorna) harcsa

(Ictalurus punctatus)

Little skate

(Leucoraja erinacea)



Aquatic Genome programs 5.

MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

GENOME 10K

2009

Cél: 10 000 gerinces genom

4000 hal genom

•Bernardia et. al.:**The fishes of Genome 10K** Mar.Genomics,

GENOME 10K

Aquatic Genome programs 5.

1	*Carcharodon carcharias*	Great white shark	Alopiidae	Lamniformes
2	*Polypterus senegalus*	Bichir	Polypteridae	Polypteriformes
3	*Acipenser sinensis*	Chinese sturgeon	Acipenseridae	Acipenseriformes
4	*Amia calva*	Bowfin	Amiidae	Amiiformes
5	*Scleropages formosus*	Golden arowana	Osteoglossidae	Osteoglossiformes
6	*Anguilla anguilla*	European freshwater eel	Anguillidae	Anguilliformes
7	*Aristichthys nobilis*	Bighead carp	Cyprinidae	Cypriniformes
8	*Megalobrama amblycephala*	Wuchang bream	Cyprinidae	Cypriniformes
9	*Hypophthalmichthys molitrix*	Silver carp	Cyprinidae	Cypriniformes
10	*Gobiocypris rarus*	Rare gudgeon	Cyprinidae	Cypriniformes
11	*Astyanax mexicanus*	Blind cave fish	Characidae	Characiformes
12	*Diaphus dumerilii*	Lanternfish	Myctophidae	Myctophiformes
13	*Hoplostethus atlanticus*	Orange roughy	Trachichthyidae	Beryciformes
14	*Hippocampus comes*	Tiger tail seahorse	Syngnathidae	Gasterosteiformes
15	*Monopterus albus*	Finless eel	Synbranchidae	Synbranchiformes
16	*Lateolabrax japonicus*	Japanese seabass	Moronidae	Perciformes
17	*Epinephelus coioides*	Grouper	Serranidae	Perciformes
18	*Sparus aurata*	Gilthead sea bream	Sparidae	Perciformes
19	*Pseudosciaena crocea*	Large yellow croaker	Sciaenidae	Perciformes
20	*Eleginops maclovinus*	Patagonian blenny	Eleginopidae	Perciformes
21	*Dissostichus mawsoni*	Antarctic toothfish	Nototheniidae	Perciformes
22	*Chaneocephalus aceratus*	Blackfin icefish	Channichthyidae	Perciformes
23	*Periophthalmodon schlosseri*	Giant mudskipper	Gobiidae	Perciformes
24	*Thunnus albacares*	Yellowfin tuna	Scombridae	Perciformes
25	*Pampus argenteus*	Pomfret	Stromateidae	Perciformes
26	*Paralichthys olivaceus*	Bastard halibut	Paralichthyidae	Pleuronectiformes
27	*Cynoglossus semilaevis*	Tongue sole	Cynoglossidae	Pleuronectiformes
28	*Mola mola*	Sunfish	Molidae	Tetraodontiformes

1. *Hydrolagus colliei*
2. *Sphyrna* sp.
3. *Pristis* sp.
4. *Torpedo californica*
5. *Erpetoichthys calabaricus*
6. *Polyodon spatula*
7. *Hiodon alosoides*
8. *Pantodon buchholzi*
9. *Notopterus notopterus*
10. *Megalops atlanticus*
11. *Albula vulpes*
12. *Gymnothorax mordax*
13. *Anchoa* sp.
14. *Sardinella* sp.
15. *Chanos chanos*
16. *Paedocypris progenetica*
17. *Cyprinus carpio*
18. *Catla catla*
19. *Botia kubotai*
20. *Serrasalmus*

maculatus

29. *Oncorhynchus tshawytscha*
30. *Coregonus clupeaformis*
31. *Esox lucius*
32. *Chauliodus* sp.
33. *Synodus*
34. *Benthosema pterotum*
35. *Lampris guttatus*
36. *Aphredoderus sayanus*
37. *Chilara taylori*
38. *Porichthys notatus*
39. *Lophius* sp.
40. *Thymichthys* sp.
41. *Ceratias* sp.
42. *Mugil cephalus*
43. *Odontesthes bonariensis*
44. *Exocoetus* sp.
45. *Hemiramphus* sp.
46. *Strongylura marina*
47. *Krytolebias*

armatus

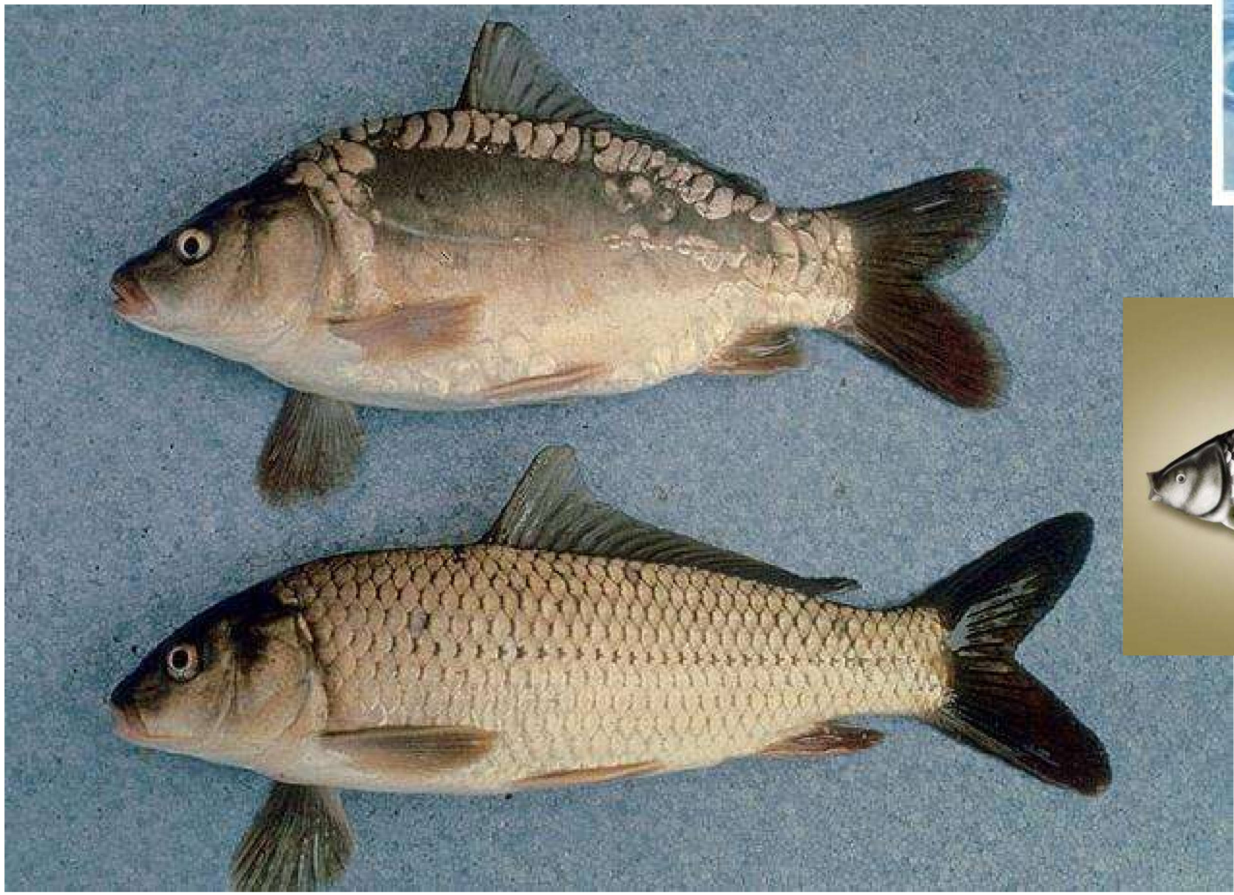
55. *Dactylopterus* sp.
56. *Sebastes chrysomelas*
57. *Pterois radiata*
58. *Platycephalus bassensis*
59. *Clinocottus analis*
60. *Aspidophoroides*
61. *Cyclopterus lumpus*
62. *Morone saxatilis*
63. *Pseudanthias squamipinnis*
64. *Micropterus salmoides*
65. *Sander vitreus*
66. *Sillago ciliata*
67. *Coryphaena hippurus*
68. *Caranx* sp.
69. *Lutjanus campechanus*
70. *Anisotremus virginicus*
71. *Polydactylus* sp.

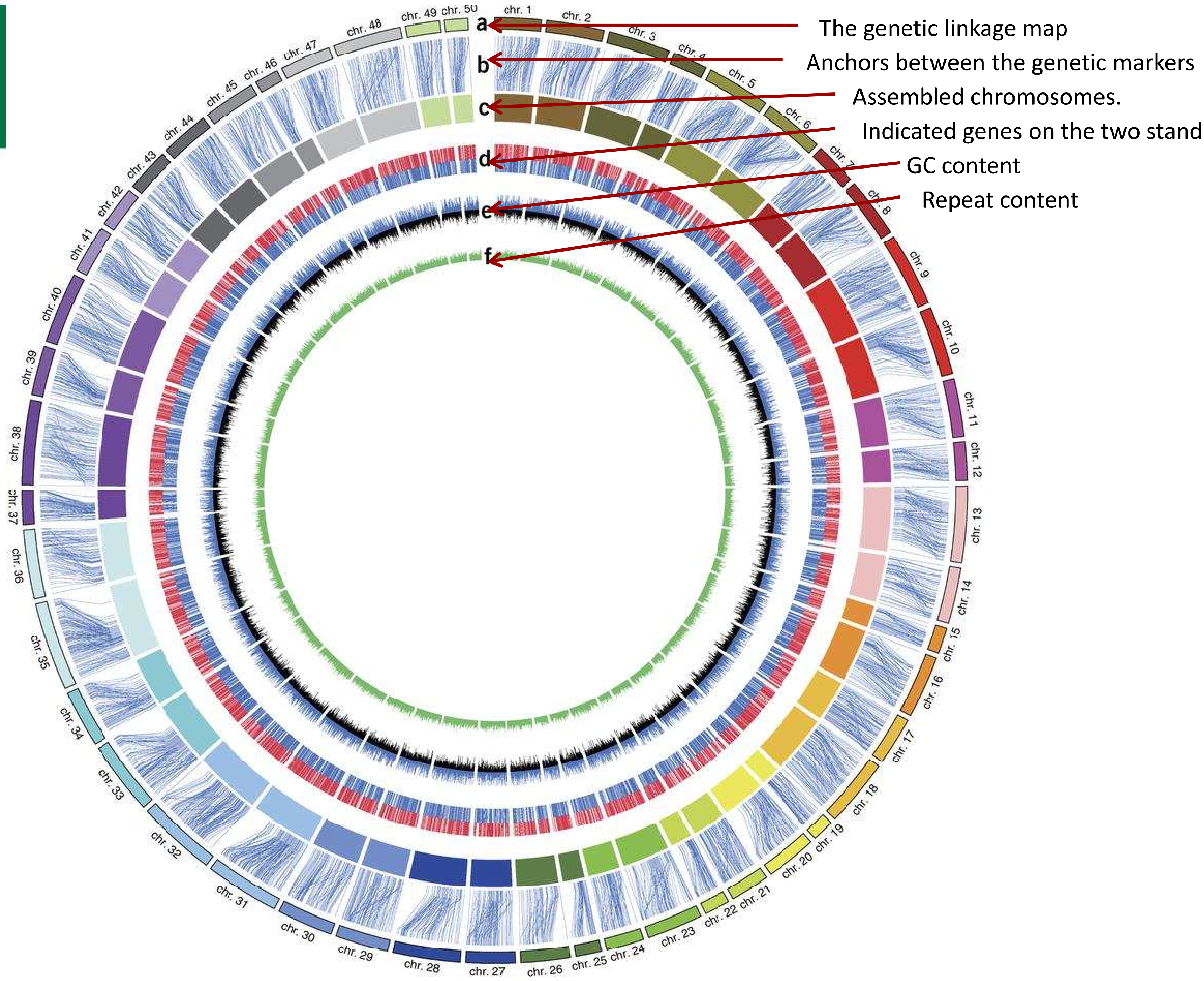
trimaculatus

79. *Hypsypops rubicundus*
80. *Semicossyphus pulcher*
81. *Halichoeres hortulanus*
82. *Scarus ghobban*
83. *Zoarces* sp.
84. *Thermarces cerberus*
85. *Dissostichus elegenoides*
86. *Ammodytes americanus*
87. *Gibbonsia montereyensis*
88. *Gobiesox maendricus*
89. *Bostrychus sinensis*
90. *Dormitator latifrons*
91. *Gillicthys mirabilis*
92. *Siganus luridus*
93. *Zanclus cornuta*
94. *Naso elegans*

Ponty (*Ciprinus carpio)*

Sequencing project 2009 - 2014

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chr. 49
chr. 50
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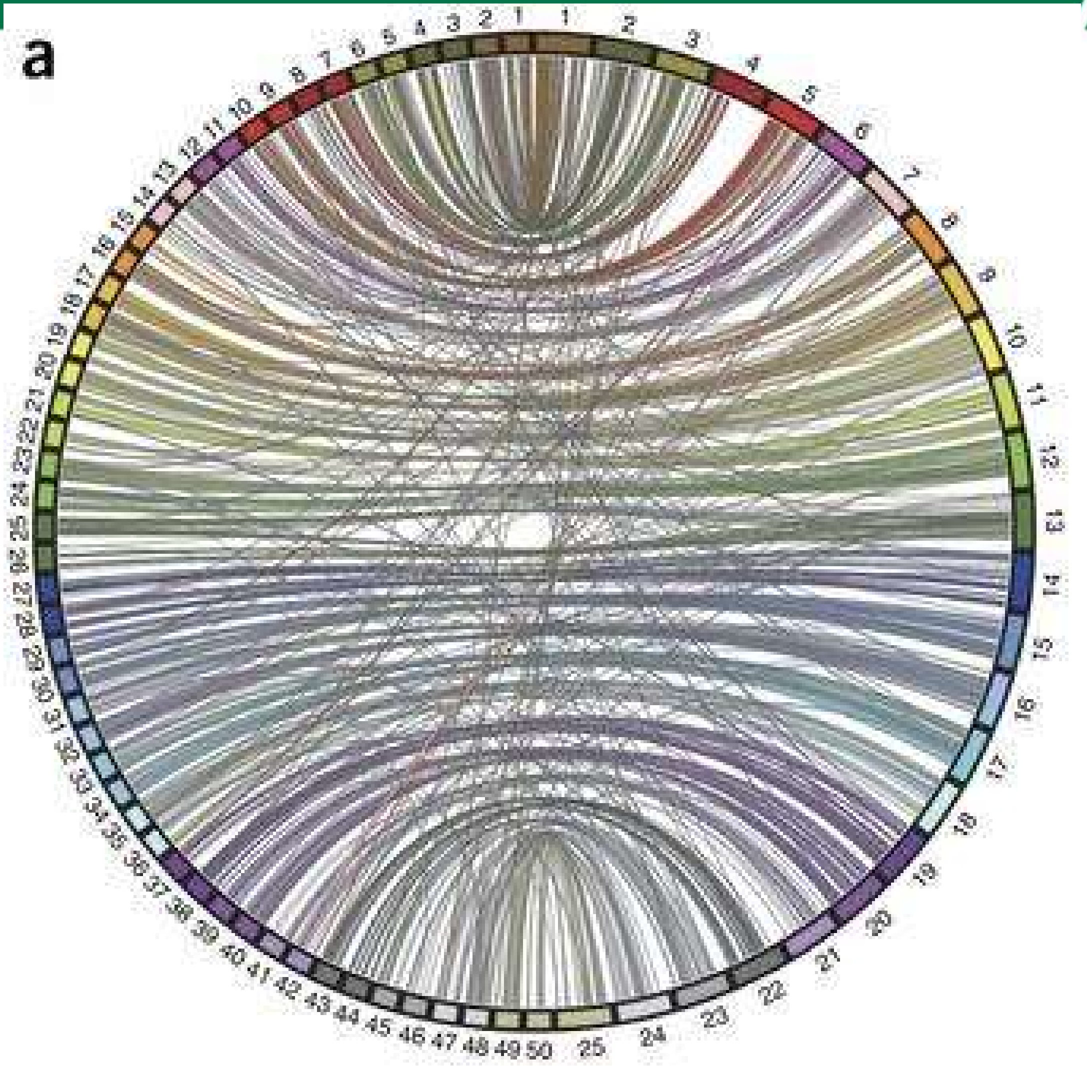
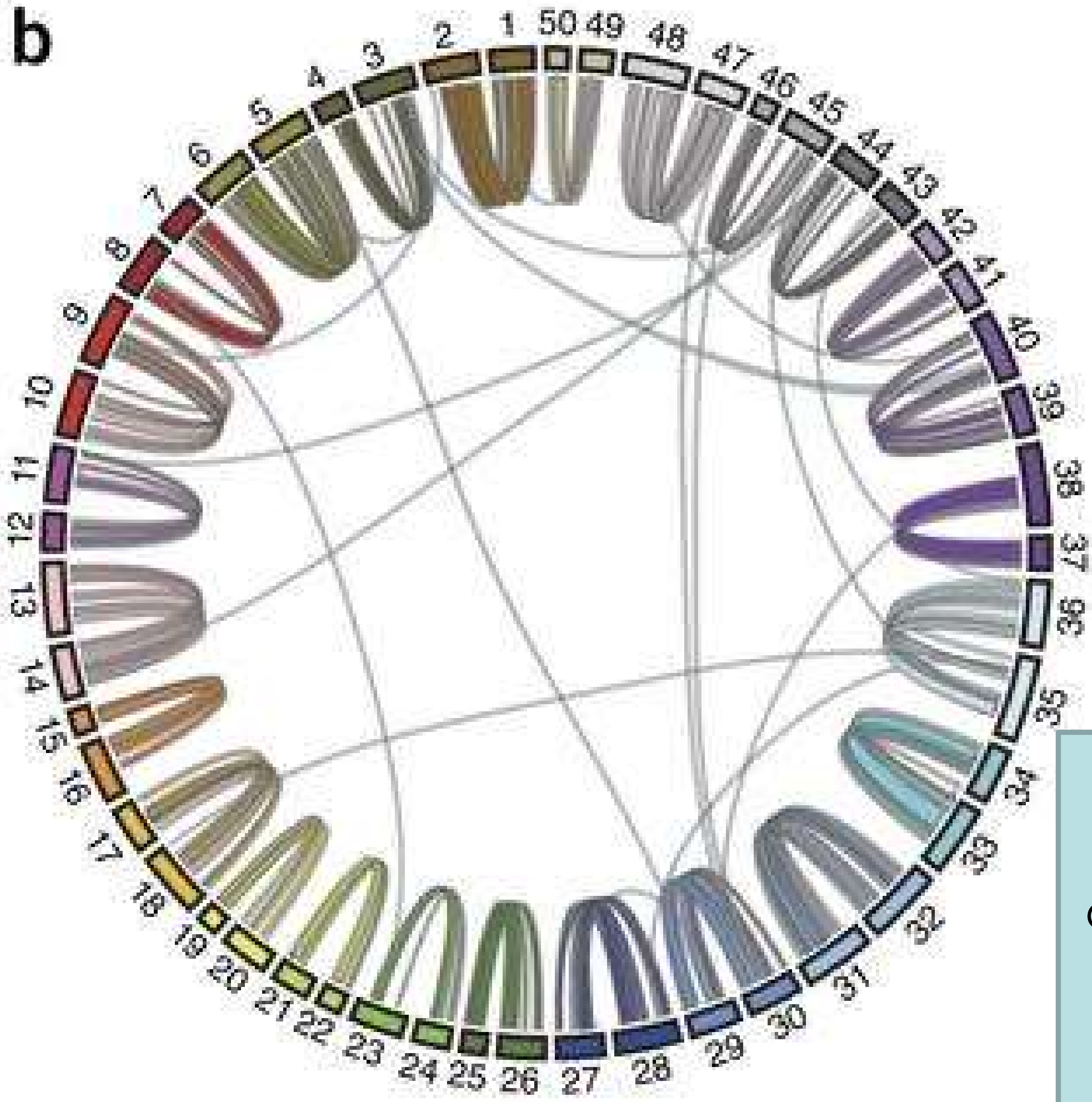
d
e
f
The genetic linkage map
Anchors between the genetic markers
Assembled chromosomes.
Indicated genes on the two stand
GC content
Repeat content



Nature Genetics 46, 1212–1219 (2014)

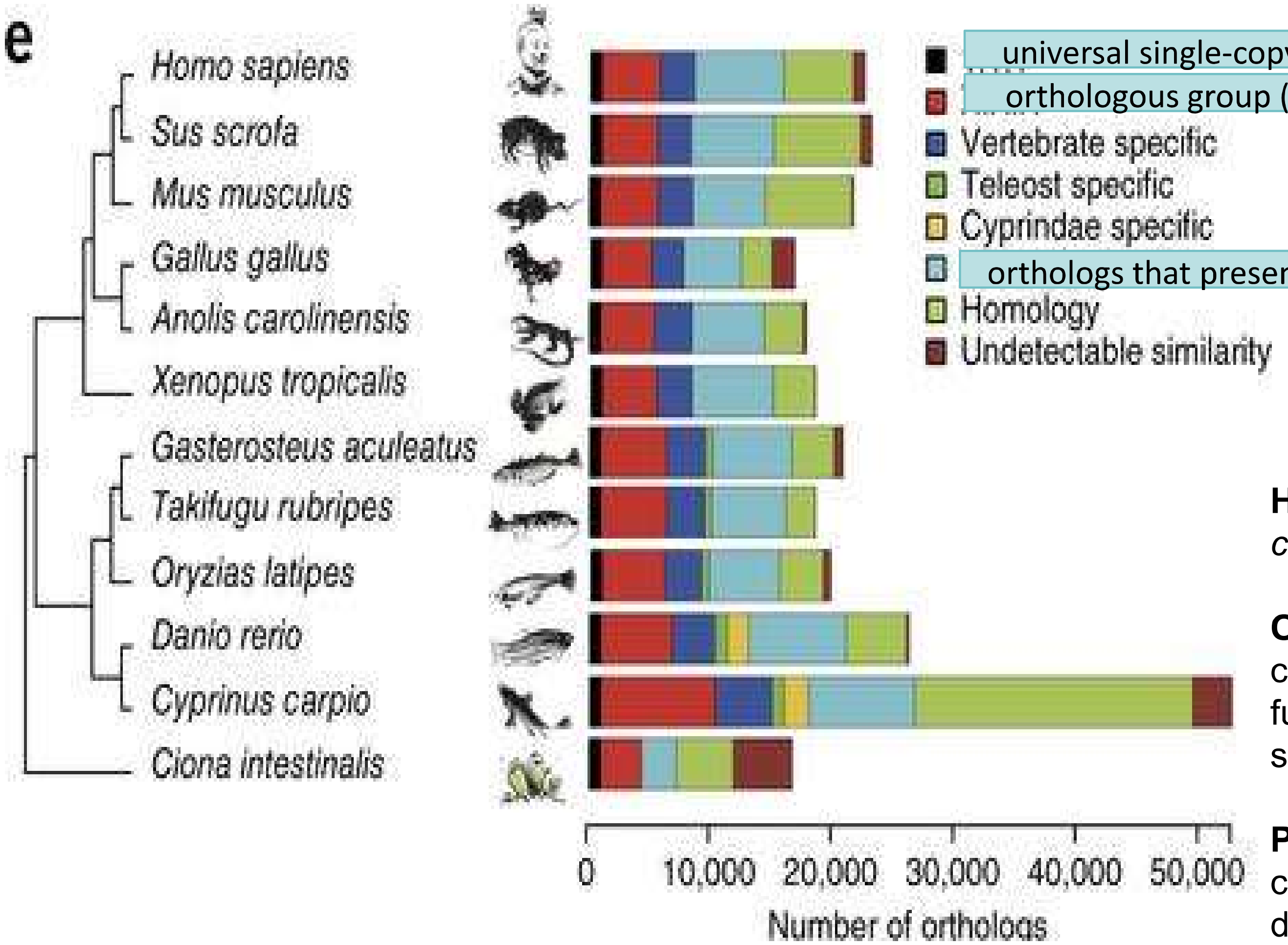
Comparison of the 50 chromosomes of carp with the 25 chromosomes of zebrafish (lines connecting the homolog genes)



Nature Genetics 46, 1212–1219 (2014)

Genome duplication on the Carp chromosomes (lines connecting the homolog genes)

Comparison of the gene repertoire



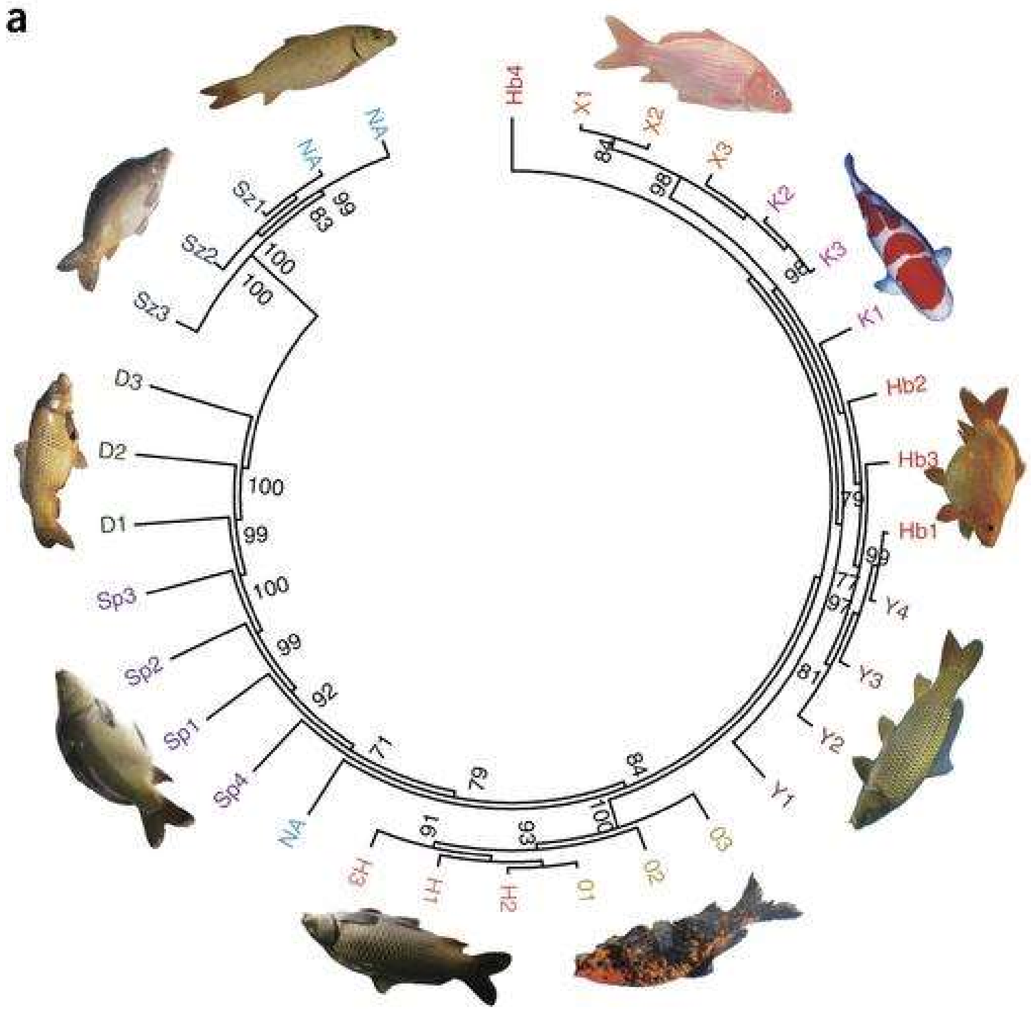
Homology = evolutionary *common origin*

Ortology: Genes from common ancestor with same functionality in different species

Paralogy: genes from common ancestor with different functions in a species

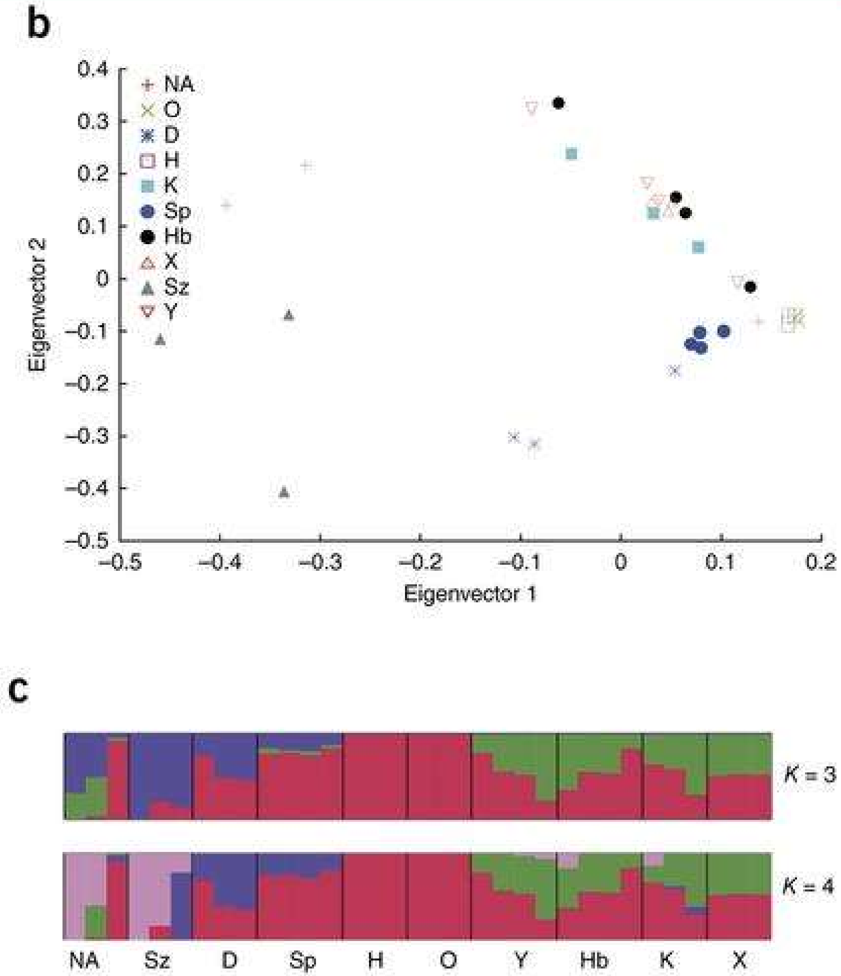
.

Nature Genetics 46, 1212–1219 (2014)



Sp, Songpu;
D, Danube;
Sz, Szarvas;
NA, North American;
Y, Yellow River;
H, Heilongjiang (Amur) ;
O, Oujiang color;
Hb, Hebao;
X, Xingguo;
K, koi.

Nature Genetics 46, 1212–1219 (2014)



Sp, Songpu;
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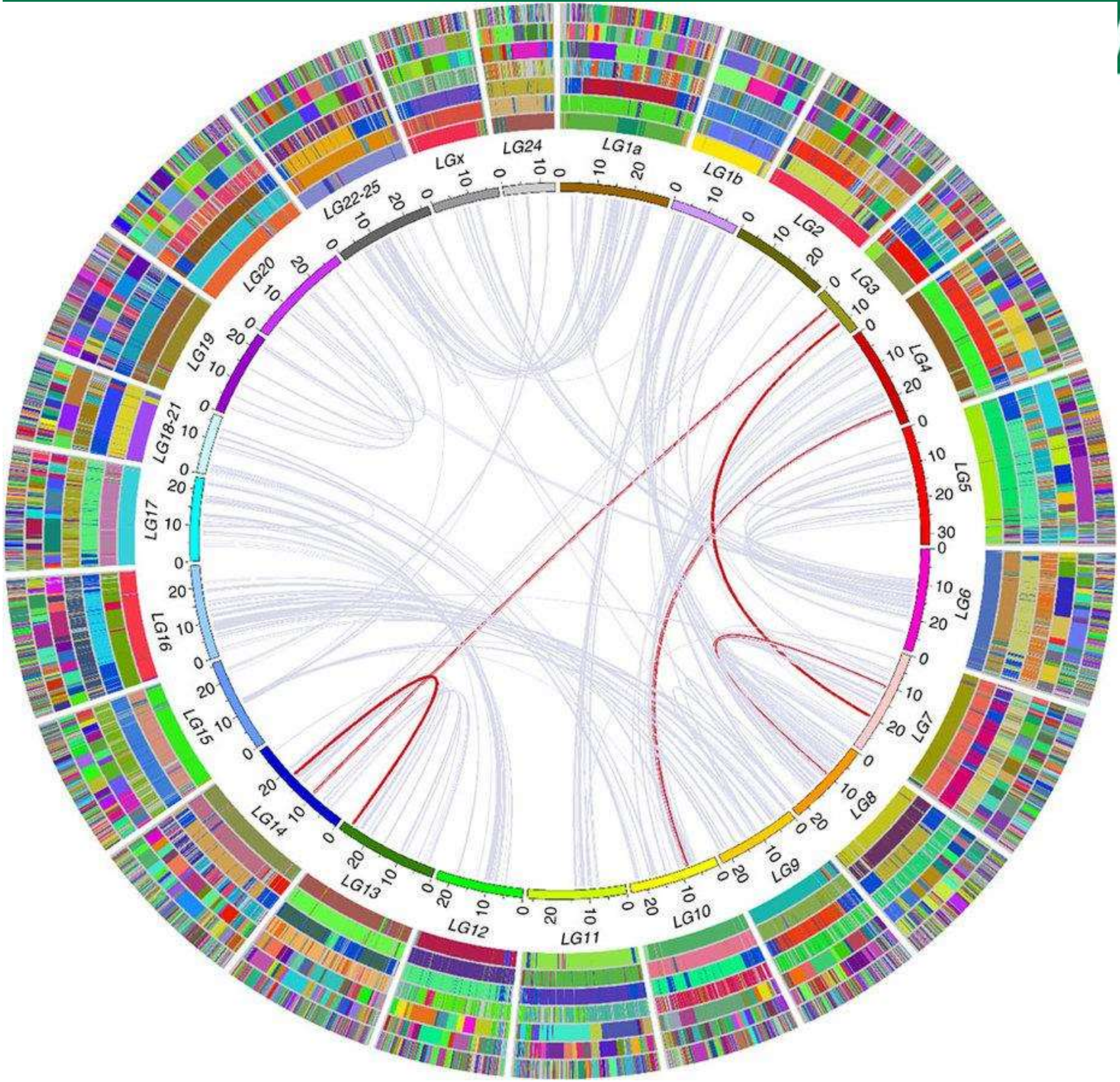
H, Heilongjiang;
O, Oujiang color;
Hb, Hebao;
X, Xingguo;
K, koi.

Nature Genetics 46, 1212–1219 (2014)

Gadus morhua genome

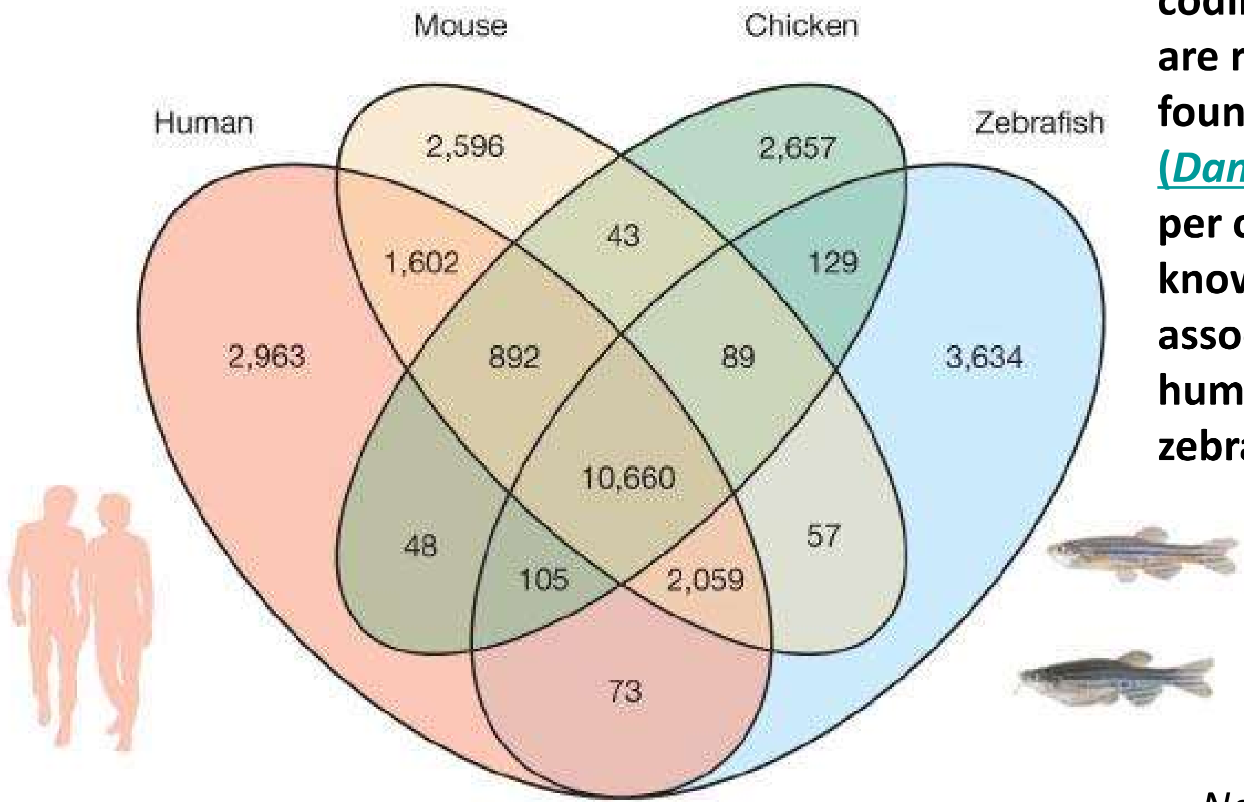
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM



From the inner to the outer layer: *G. aculeatus, O. latipes*, *T. nigroviridis, D. rerio, O. niloticus*, *T. rubripes* and *G. morhua*. Sea bass chromosomes (LGn) show conserved synteny with the assemblies of *G. aculeatus, O. latipes*, *T. nigroviridis* and *D. rerio*, while *O. niloticus*, *T. rubripes* and *G. morhua* are still scattered into many ungrouped scaffolds as reflected by tracks of different colours along the chromosomes. The colour code is species-specific. Blocks of collinearity between sea bass chromosomes are represented by grey inner links. Red inner links represent blocks of collinearity containing claudin genes

Nature Communications **5**,

Orthologue genes shared between the zebrafish, human, mouse and chicken genome (Kerstin Howe et al)



According to a [paper published in *Nature*](), 70 per cent of protein-coding human genes are related to genes found in the [zebrafish (*Danio rerio*)](), and 84 per cent of genes known to be associated with human disease have a zebrafish counterpart.

Nature 496, 498–503;

Aquatic Genome programs 4.