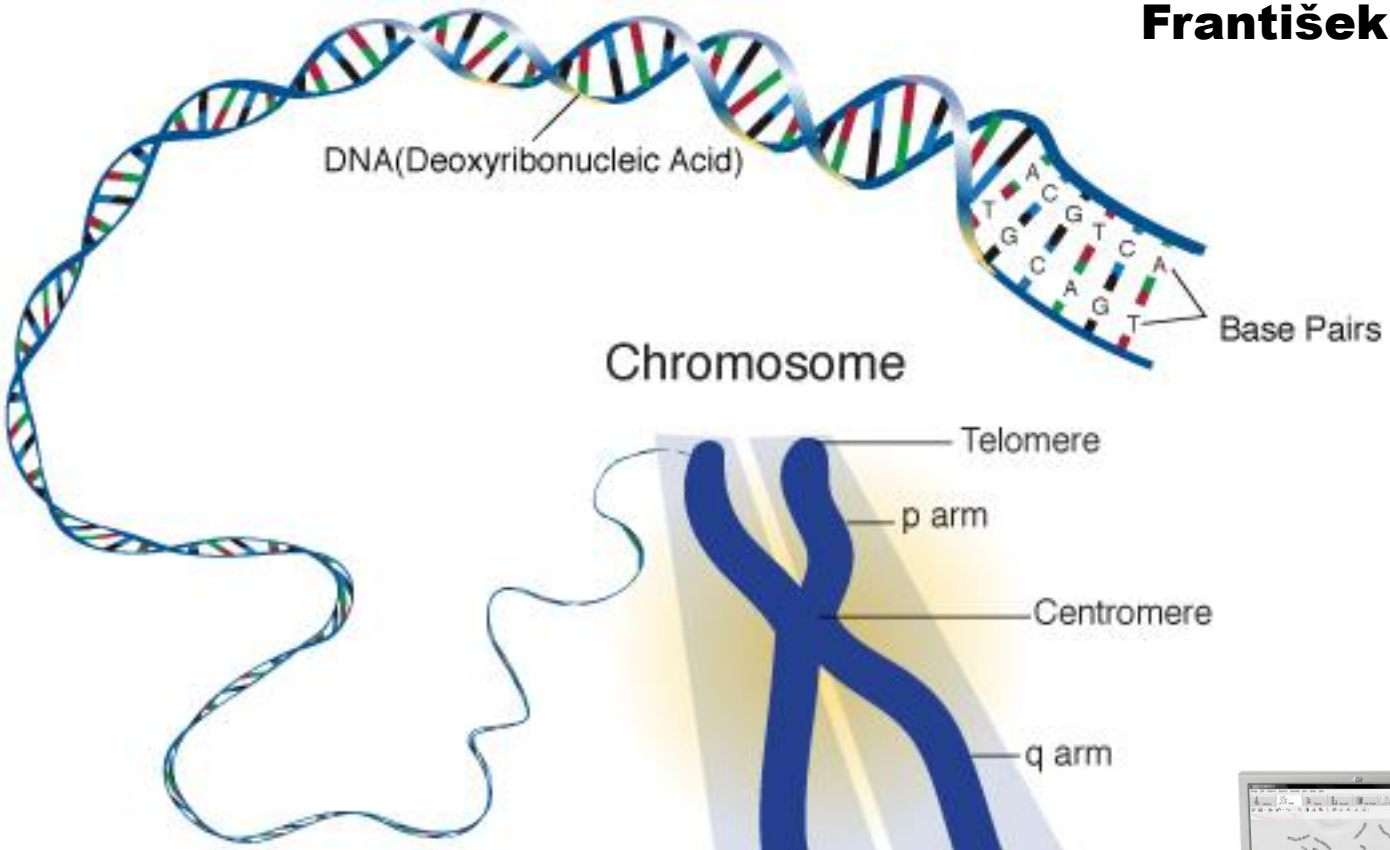
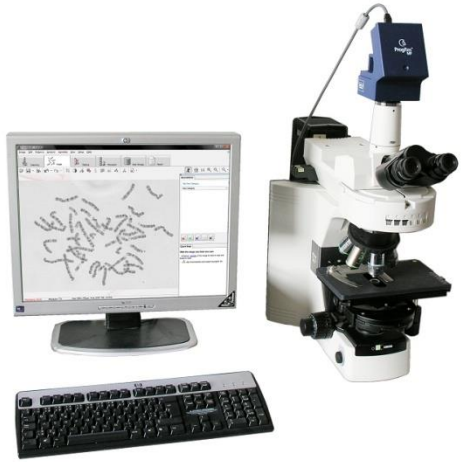
# Cytogenetic methods

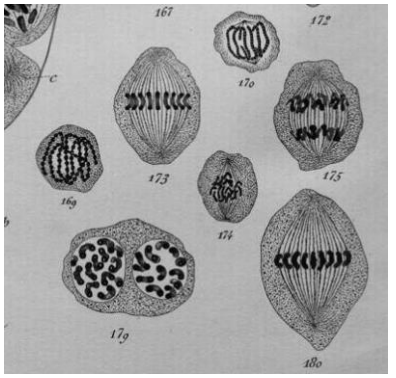
**František Šťáhlavský**



# Cytogenetic

is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes.

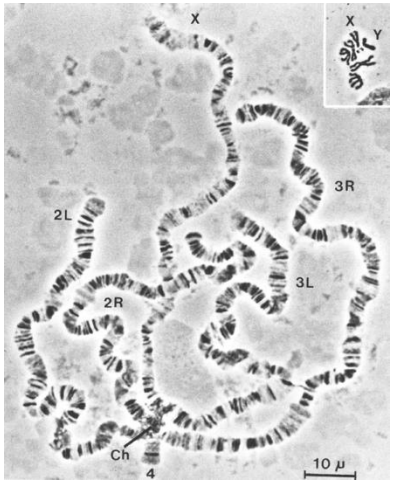


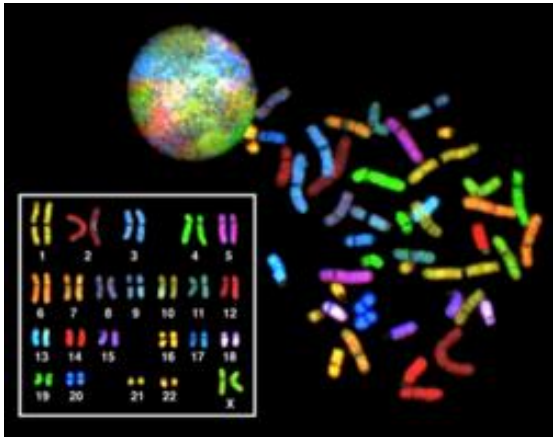
1842 – first observations of chromosomes
1888 – used term ***chromosome***

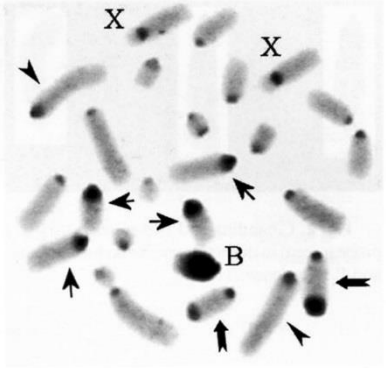
(*chroma*=colour , *soma*=body )

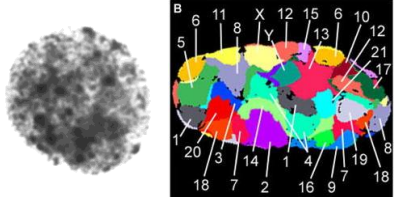
1902-04 – chromosomal inheritance theory

1950s – progress in methods (hypotonization,…
1956 – final determination of 2n in human
1968 – banding techniques
1990s – FISH techniques



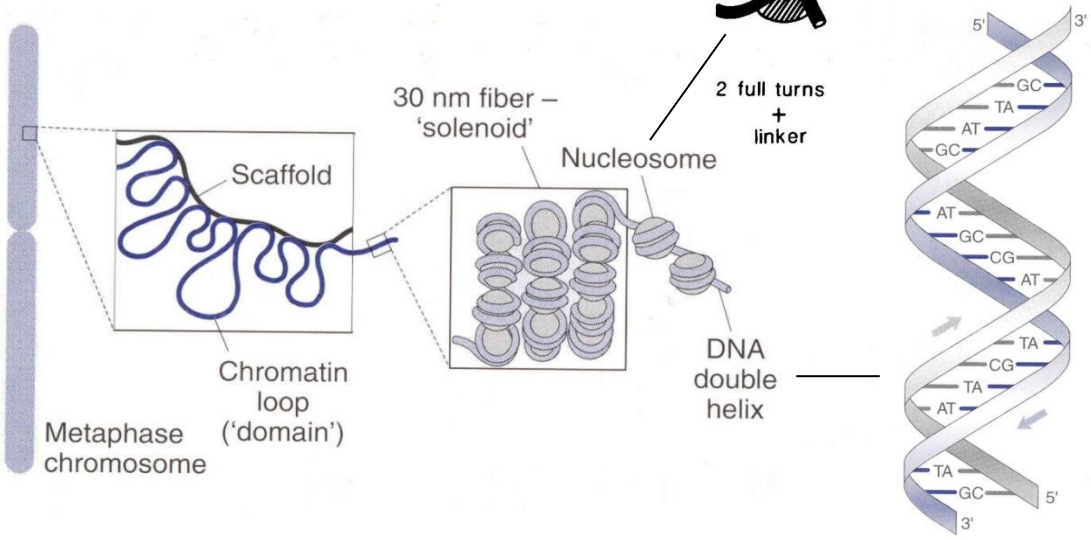







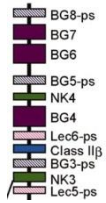


200b.p.
+
octamer (2x histons H2A, H2B, H3 a H4)
+
H1

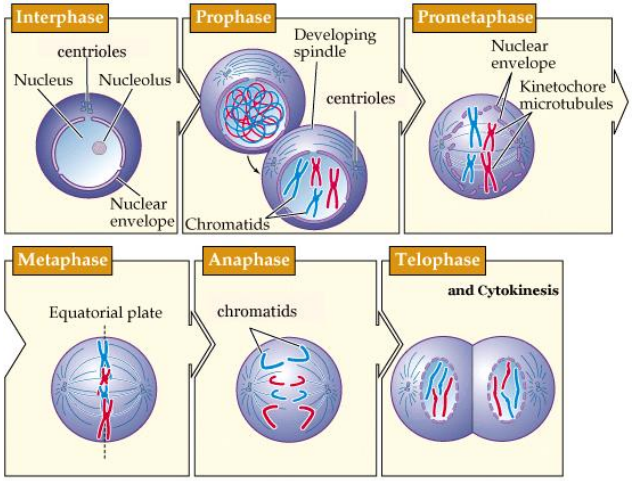



## Functions of chromosomes

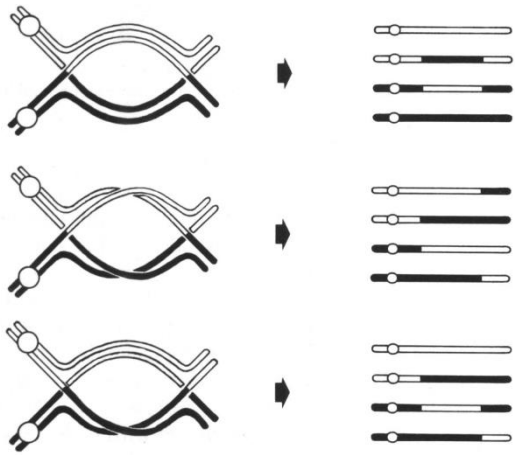
- spatial distribution of genes




- equal transport of genetic information during cell division

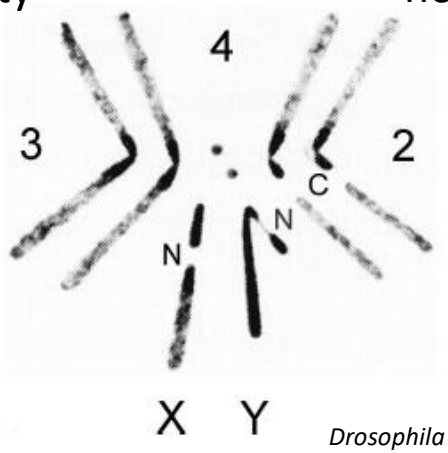



- crossing-over during meiosis, new genetic combinations



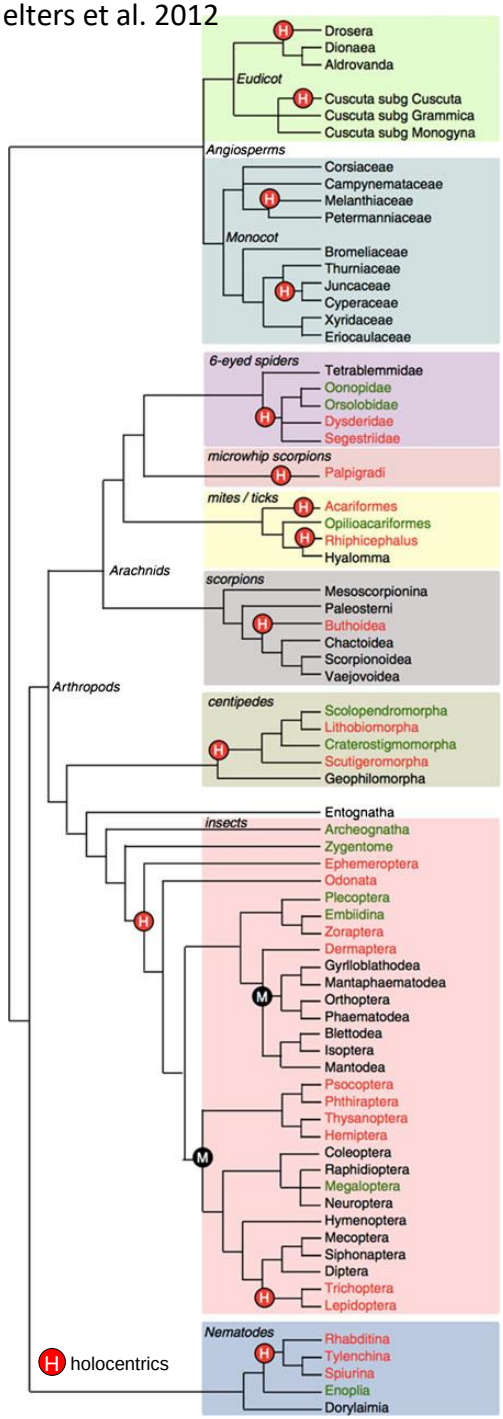
**EUCHROMATIN x HETEROCHROMATIN**

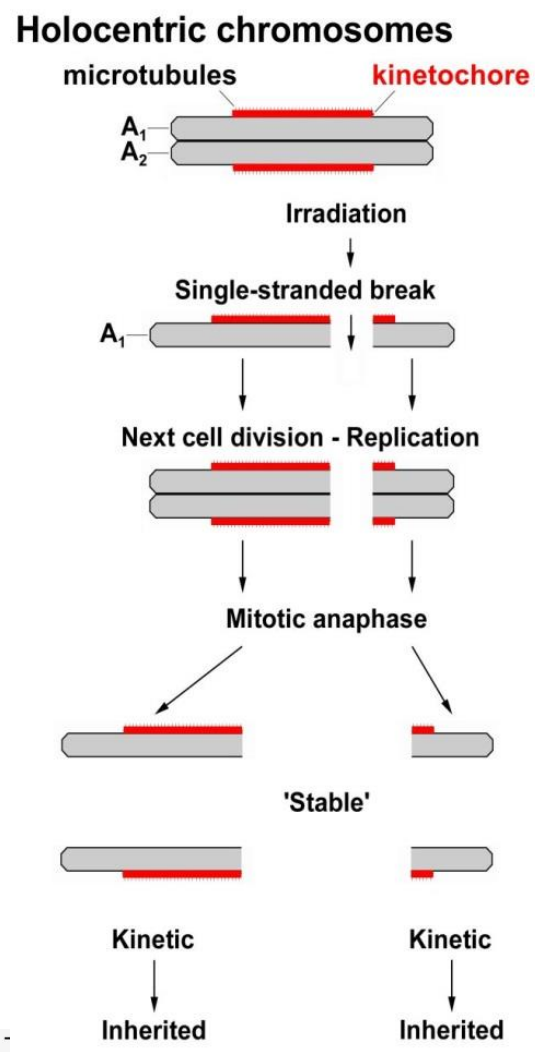
genetic activity — no genetic activity

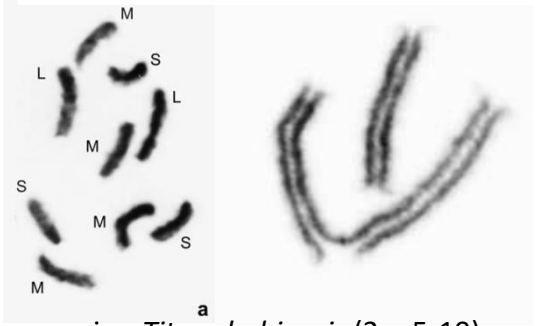



*Drosophila melanogaster* (2n = 8)

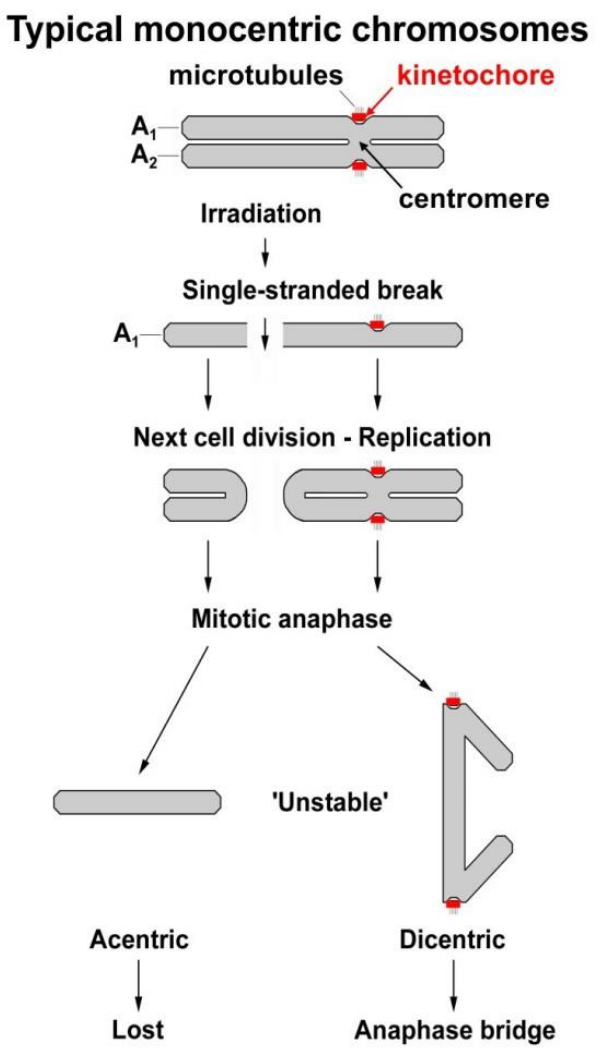
Melters et al. 2012

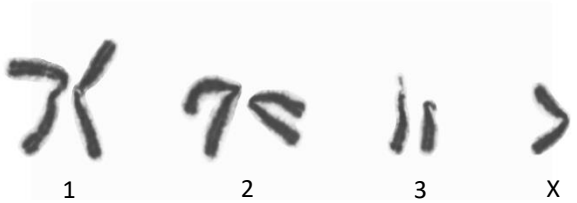


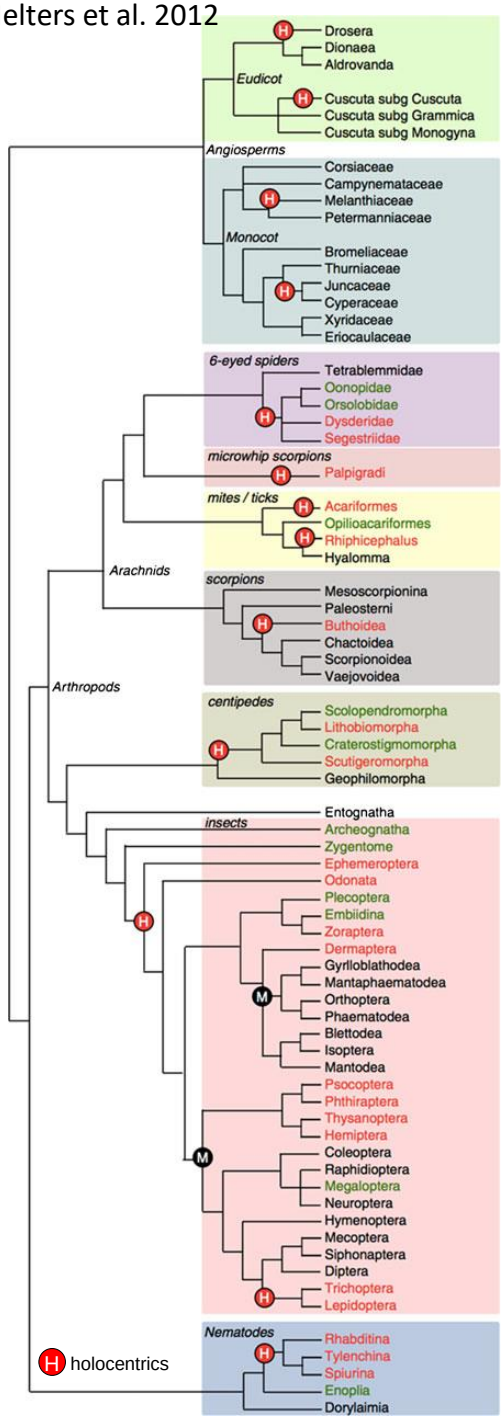

scorpion *Tityus bahiensis* (2n=5-19)

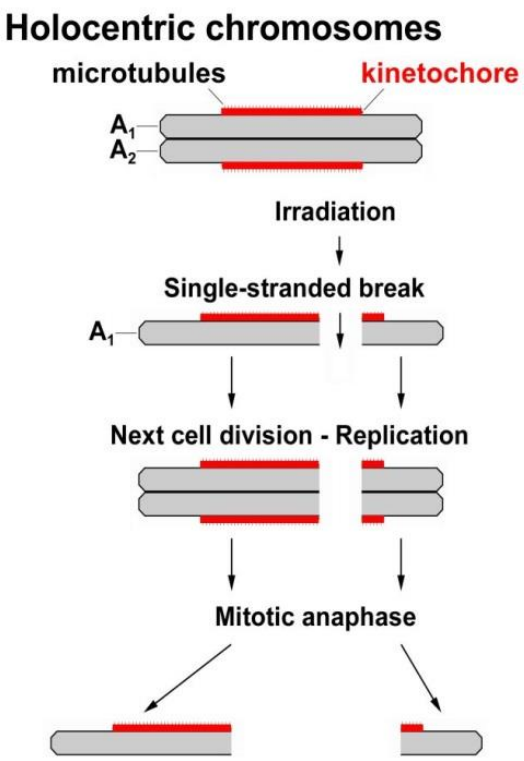


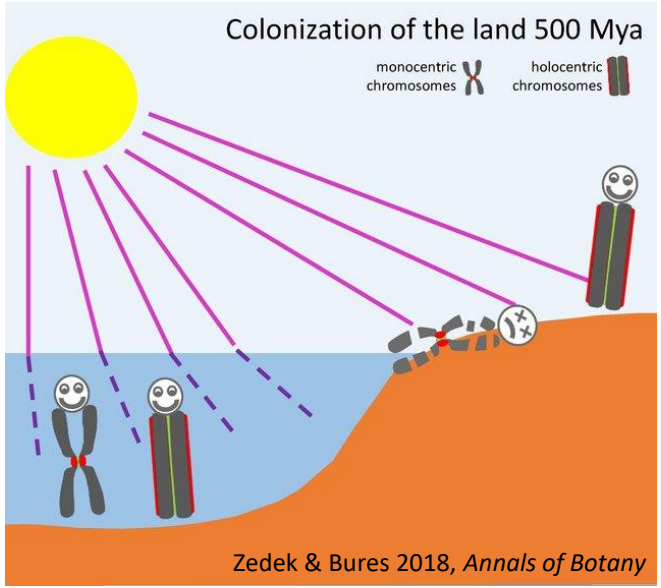



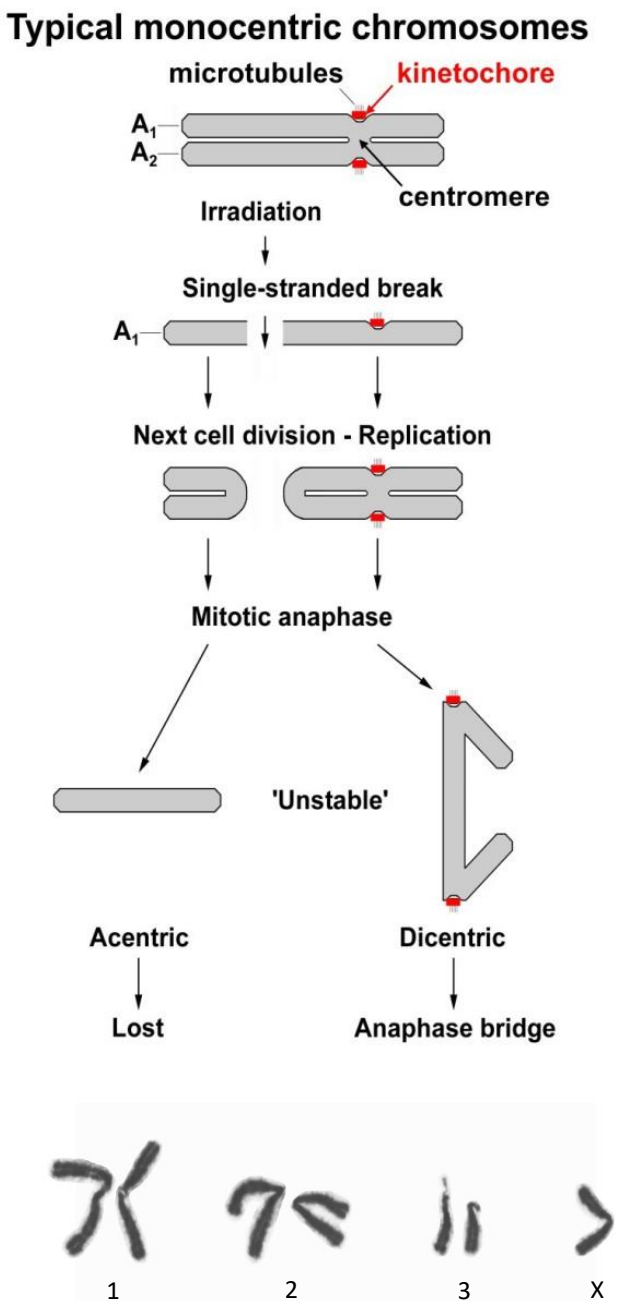

Pseudoscorpion: *Olpium turcicum*: 2n = 7, X0

Melters et al. 2012



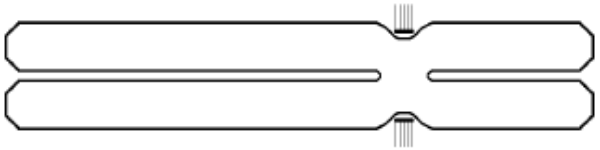





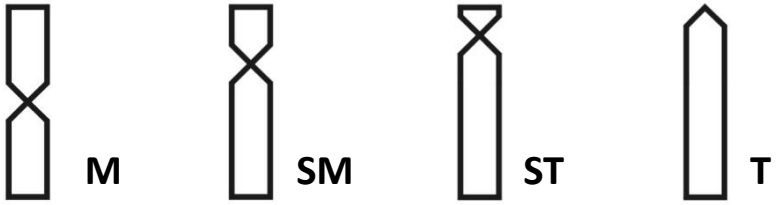


Pseudoscorpion: *Olpium turcicum*: 2n = 7, X0

Monocentric chromosomes

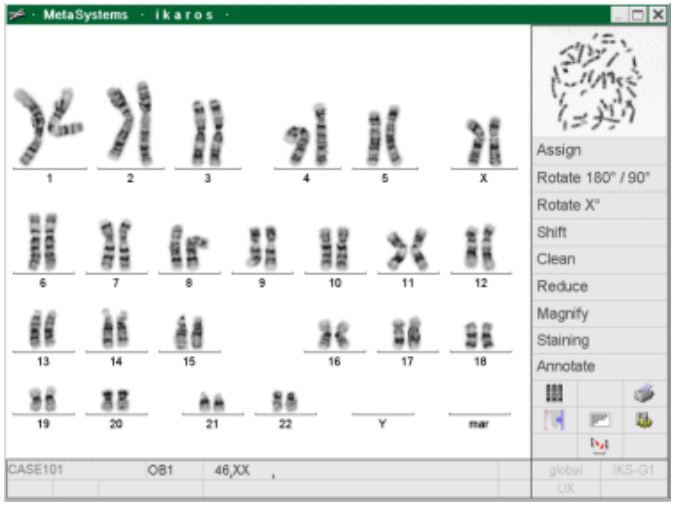


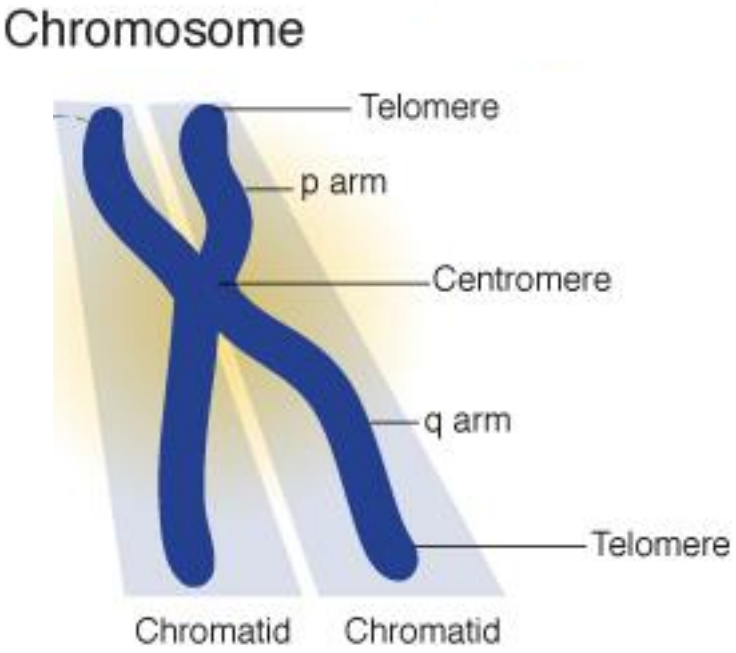
Holocentric (holokinetic) chromosomes



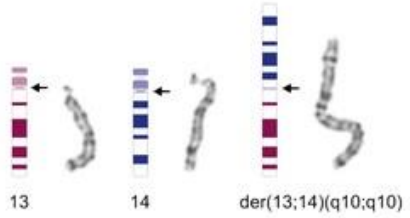

| Arm Ratio | Levan et al. (1964) | Green & Sessions (1991) |
|---|---|---|
| 1.0 | M (Metacentric) | m (Metacentric) |
| 1.0 ≤ x < 1.7 | m (Metacentric) | |
| 1.7 ≤ x < 3.0 | sm (Submetacentric) | sm (Submetacentric) |
| 3.0 ≤ x < 7.0 | st (Subtelocentric) | st (Subtelocentric) |
| 7.0 ≤ x < ∞ | t (Acrocentric) | t (Telocentric) |
| ∞ | T (Telocentric) | |

www.metasystems-international.com/ikaros




www.lucia.cz



https://karyotyper.com/

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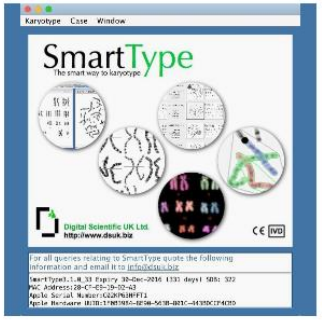


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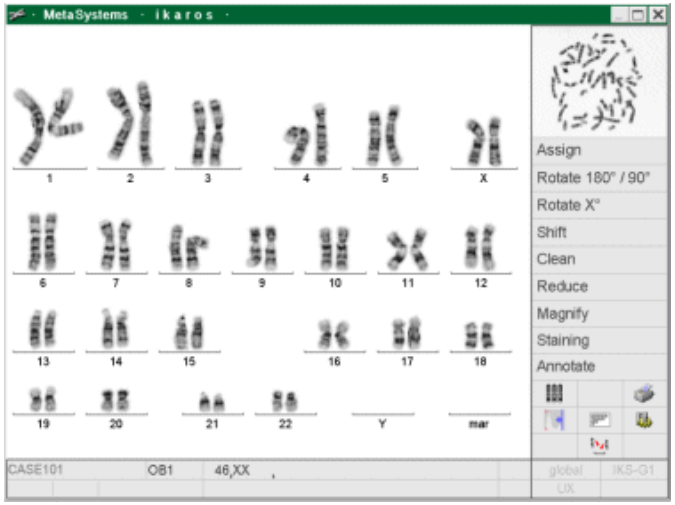
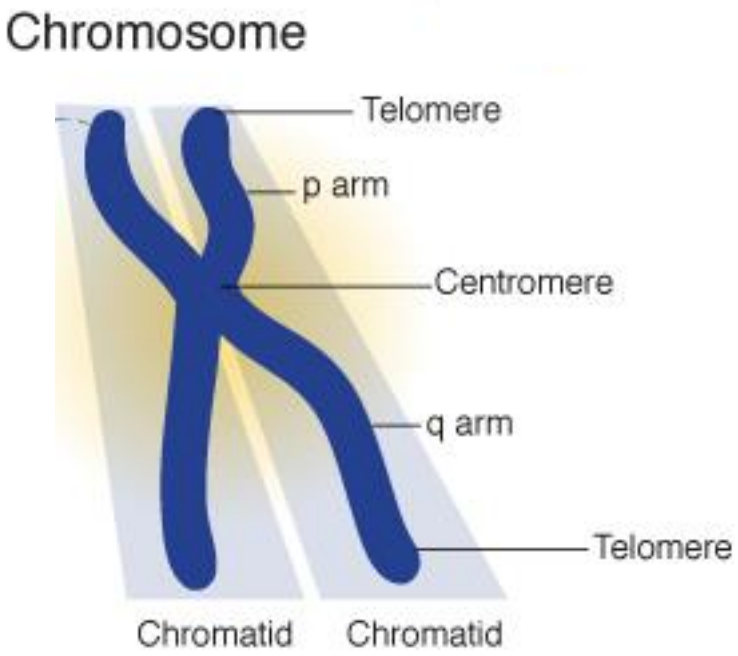
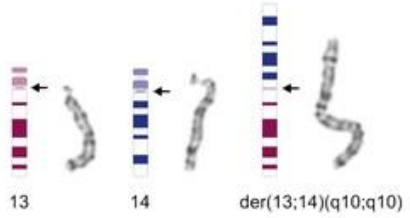


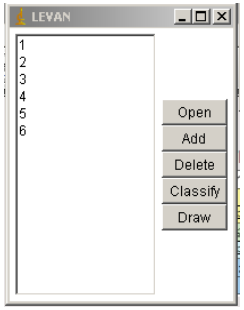



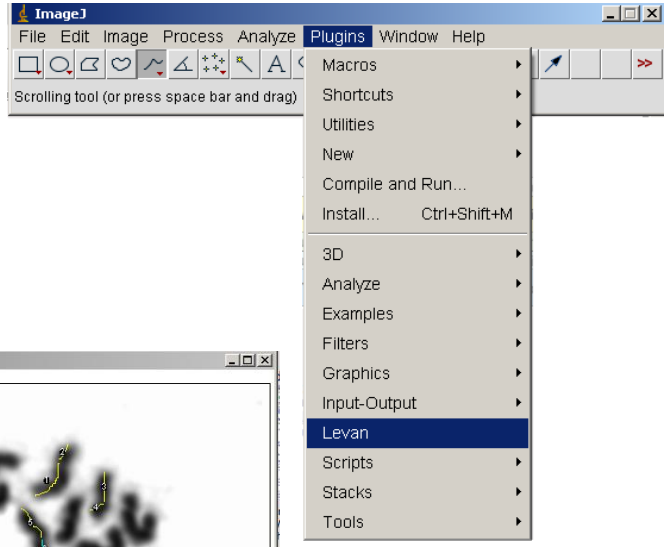

www.lucia.cz

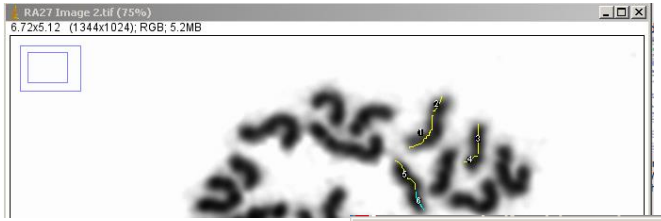


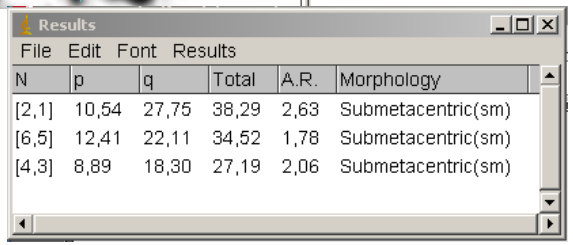
http://imagej.nih.gov/ij/
http://rsb.info.nih.gov/ij/plugins/levan/levan.html

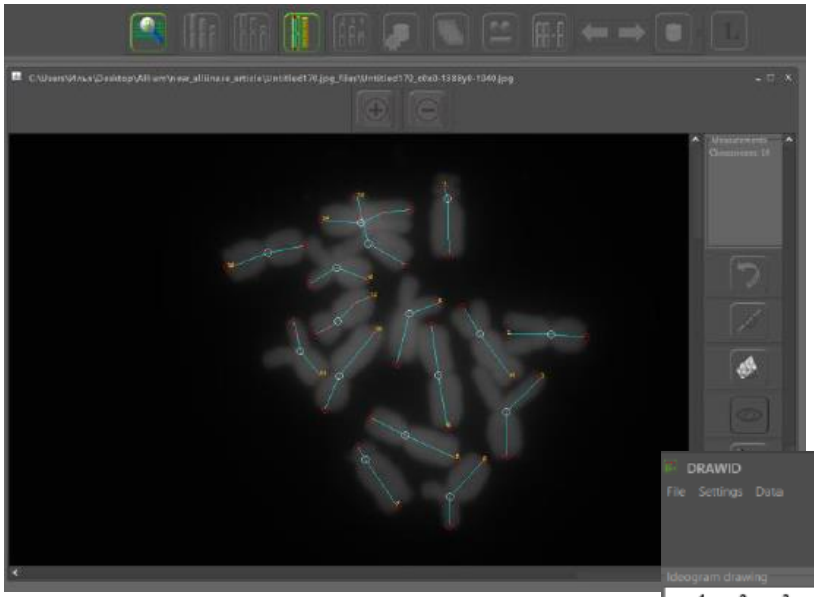
http://www.drawid.xyz



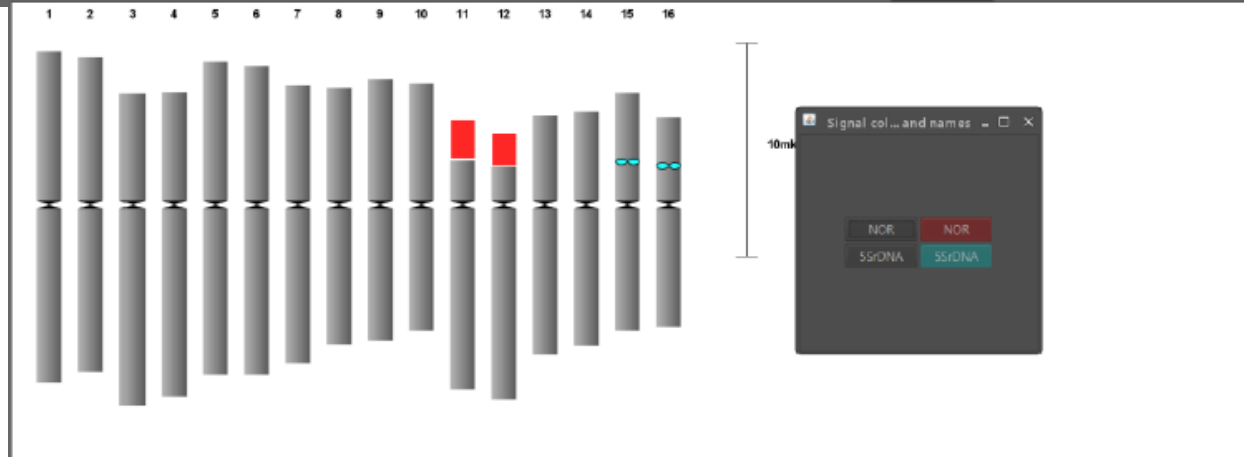



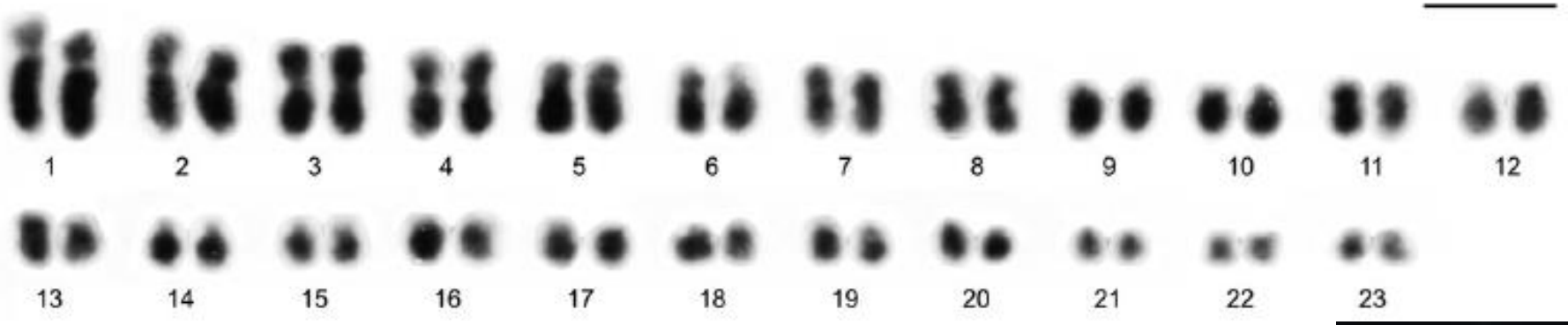



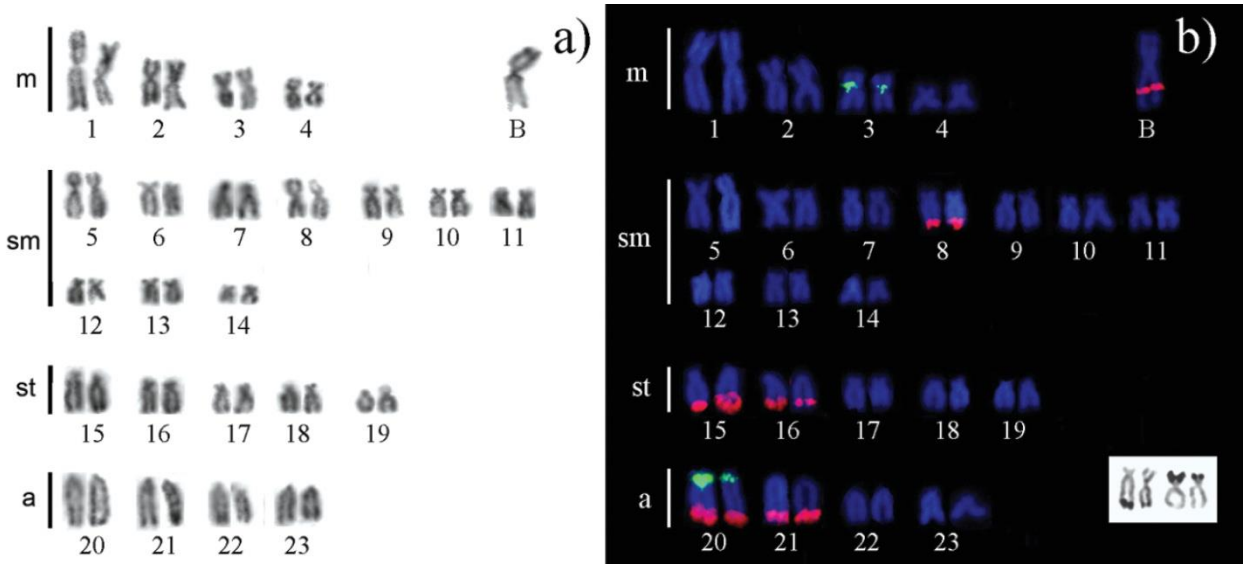





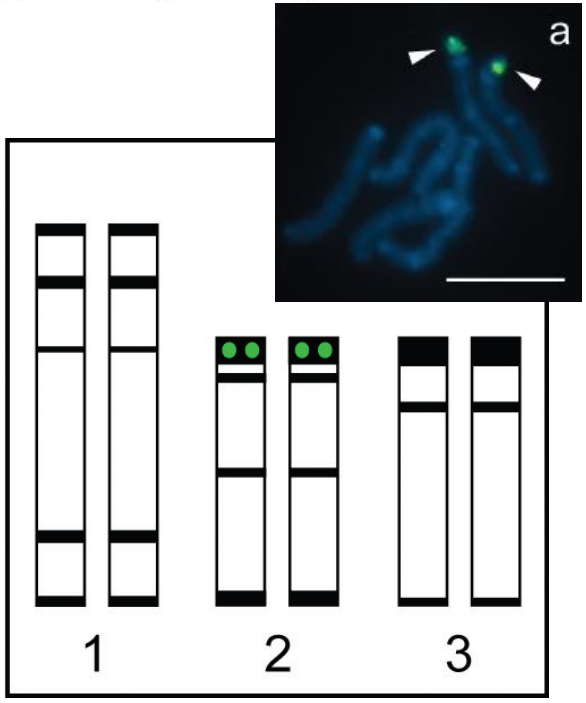





Karyogram of scirpion *Bothriurus rochensis*




Karyogram of *Astyanax fasciatus* deduced after conventional Giemsa staining and double FISH using 5S (green) and 18S rDNA (red) probes.

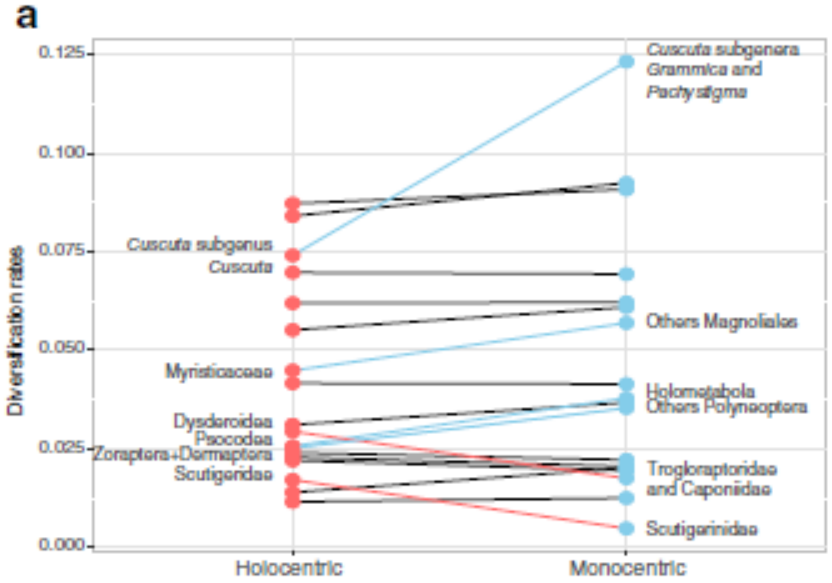



Idiogram (ideogram) of scorpion *Tityus trivittatus*

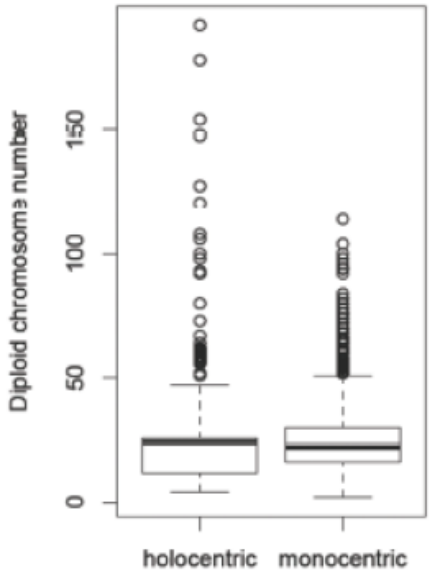
# number of chromosomes

from 2n=2
*Parascaris univalens,*
ants *Myrmecia pilosula, M. croslandi*,
(males n=1)

to 2n = 446 *Plebicula atlantica*




Márquez-Corro et al. 2018



# number of chromosomes

## Modal number of chromosomes

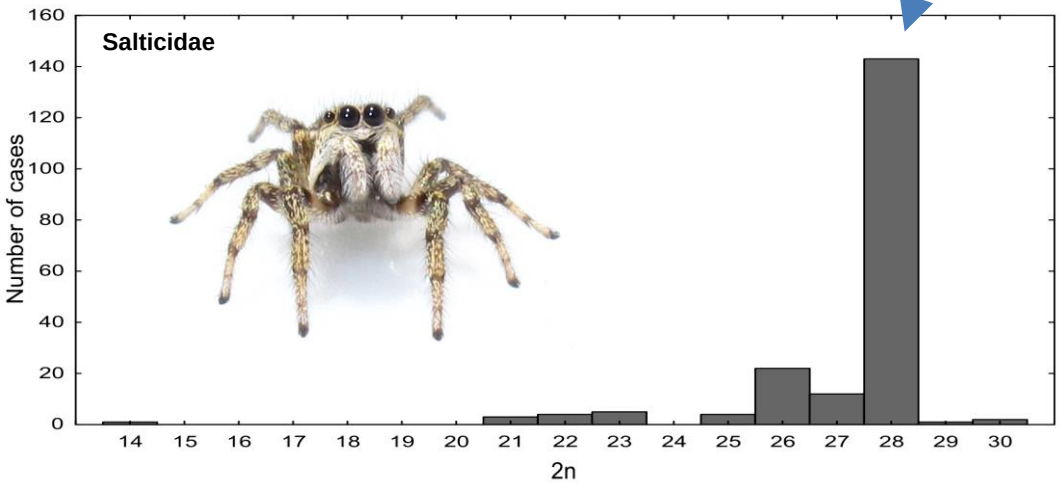
Odonata n=13

Lepidoptera n=28-32

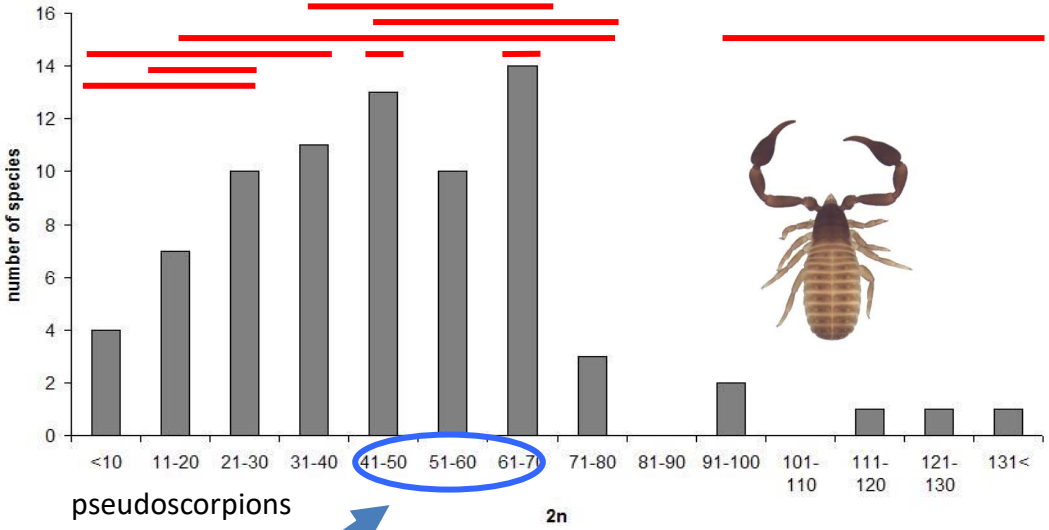
birds n=39-42

Diptera n=2-10

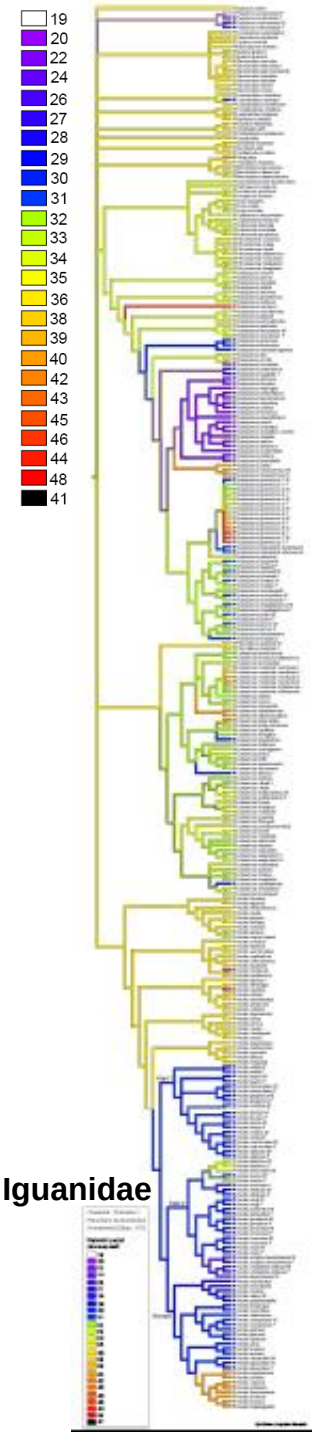
spiders




different families




pseudoscorpions

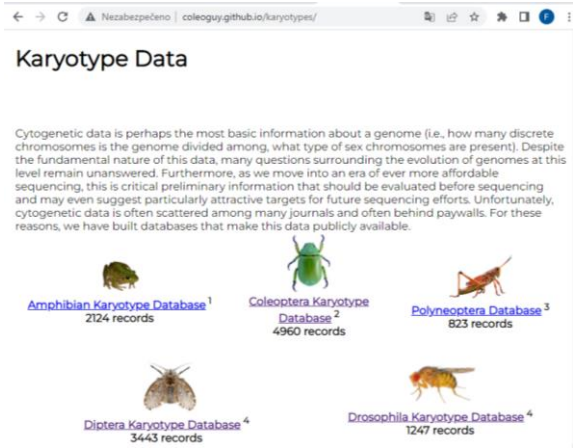


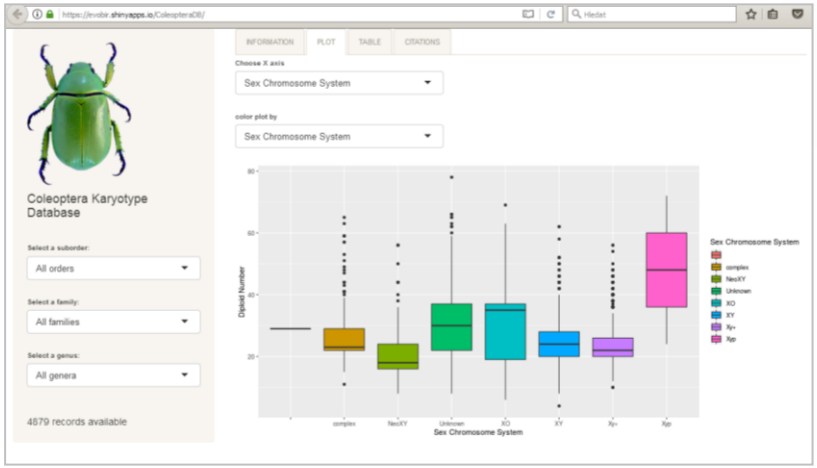
Zima 2000

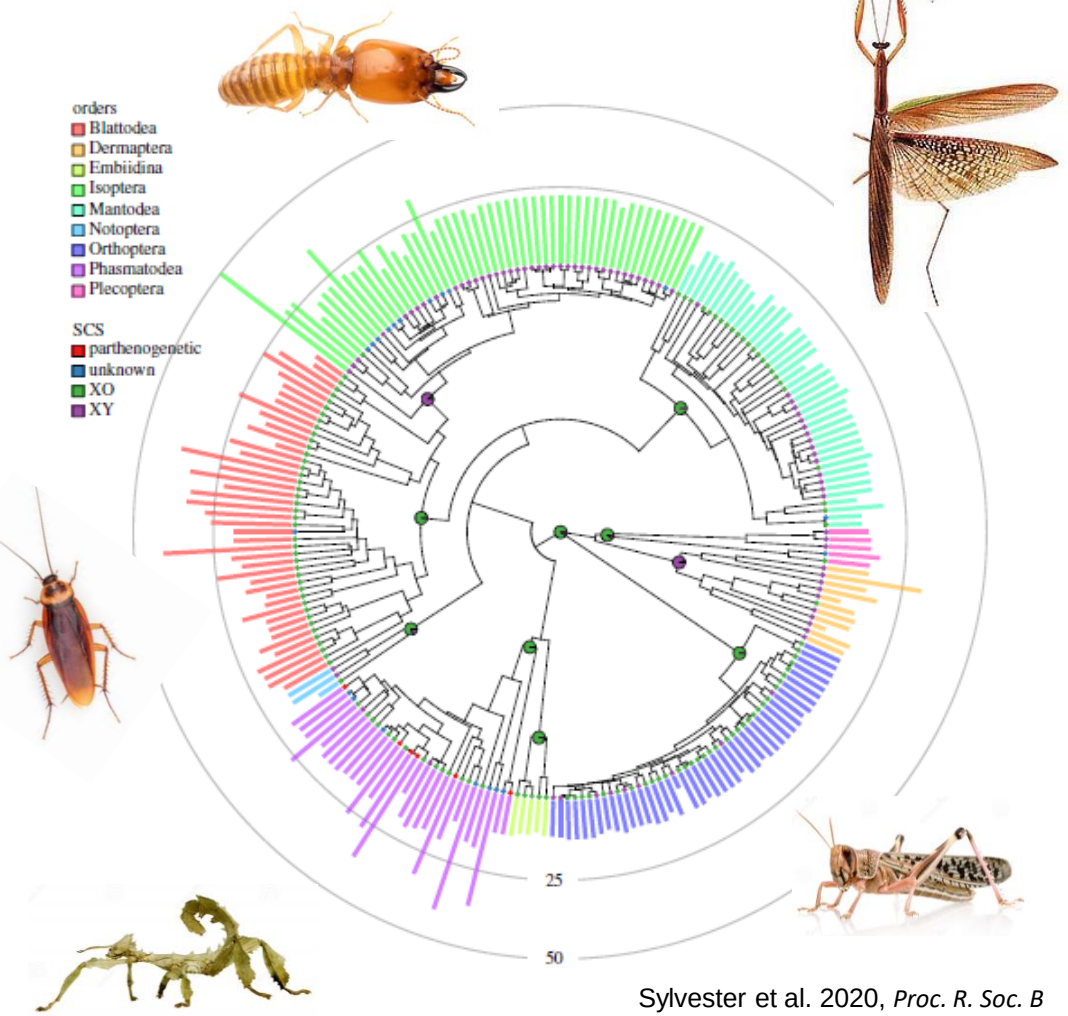
| | Palaearctic region | | Europe | |
|---|---|---|---|---|
| | total species | studied species | total species | studied species |
| Insectivora | 81 | 72 (88.9%) | 39 | 38 (97.4%) |
| Chiroptera | 73 | 57 (78.1%) | 37 | 36 (97.3%) |
| Rodentia | 299 | 240 (80.3%) | 106 | 105 (99.1%) |

Fish app. 30000 species
- 1700 karyotyped

http://coleoguy.github.io/karyotypes/




Sylvester et al. 2020, *Proc. R. Soc. B*

| Zima 2000 | Palaearctic region | | Europe | |
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Fish app. 30000 species
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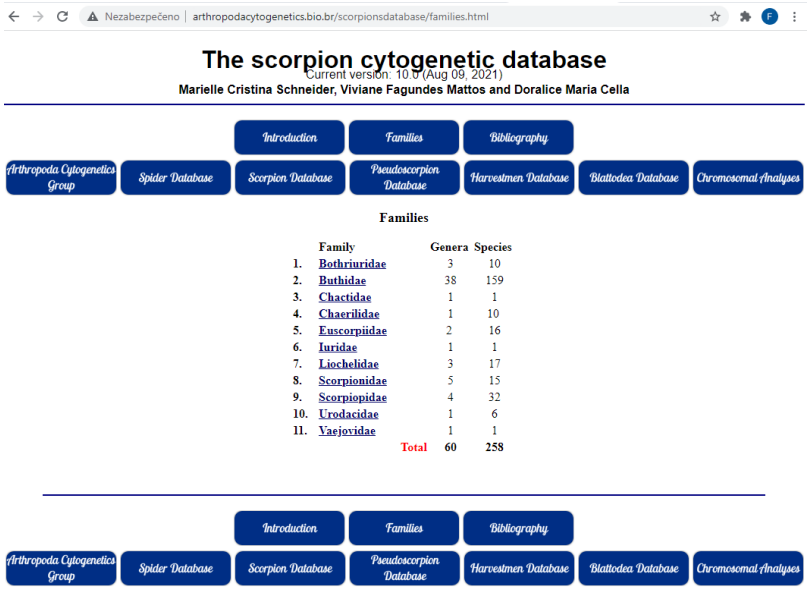
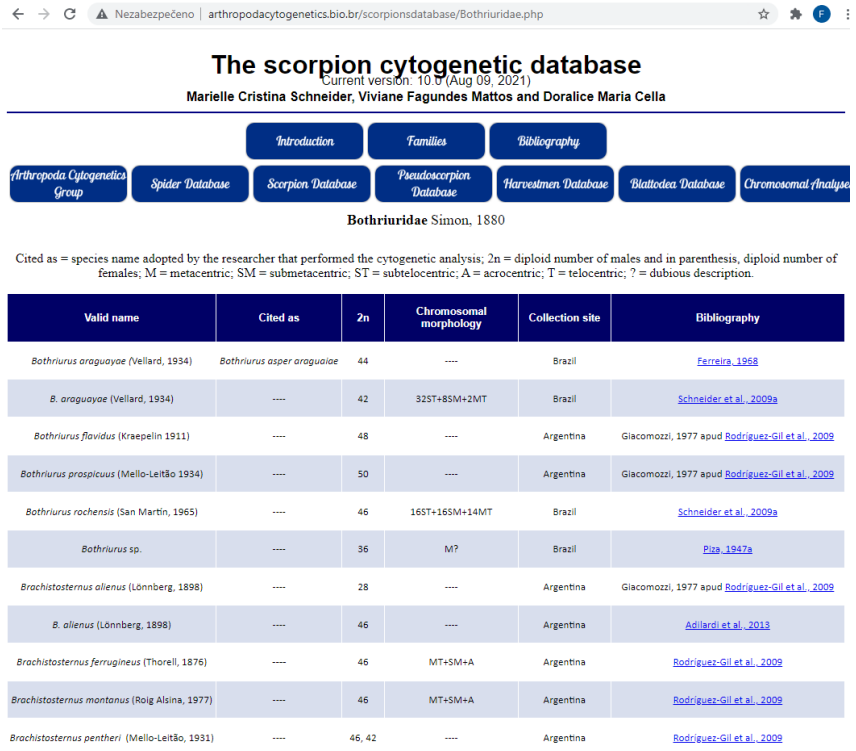
## Arachnids Karyotypes

https://arthropodacytogenetics.bio.br/

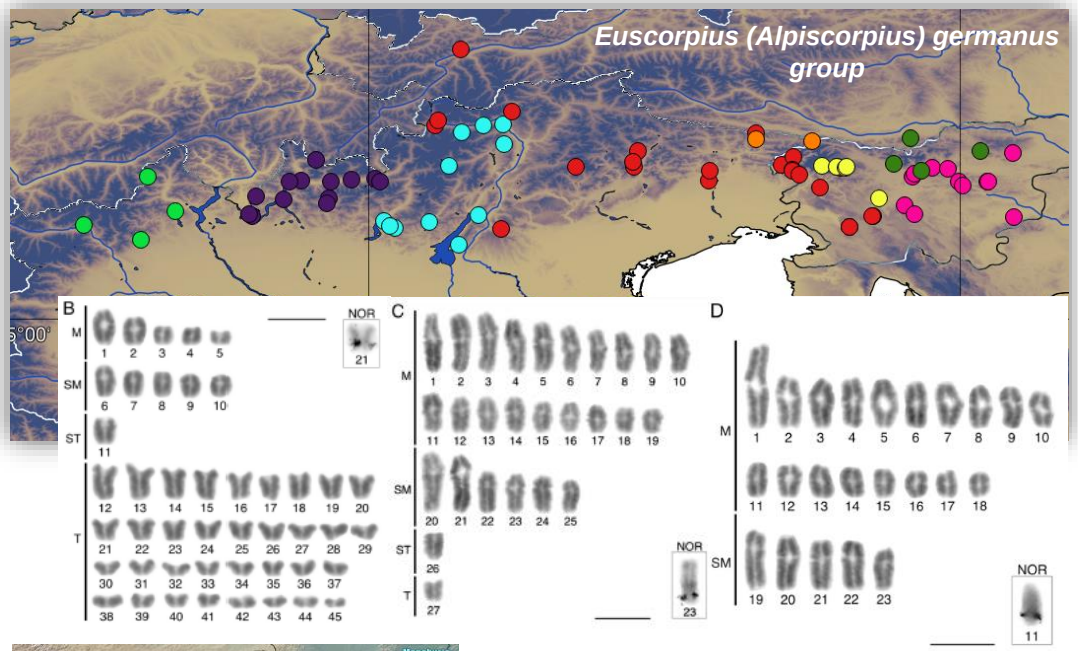


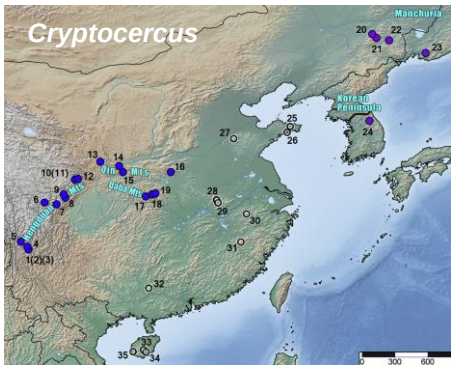



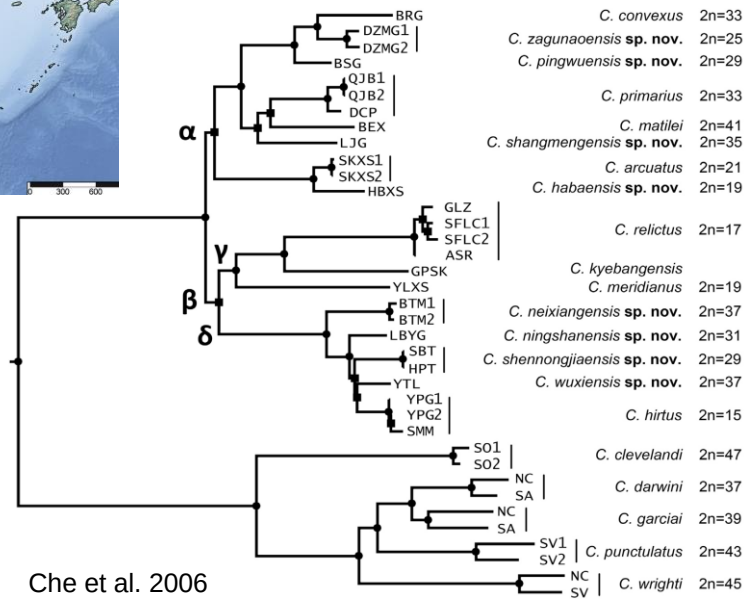
# Scorpiones




Štundlová et al. 2019



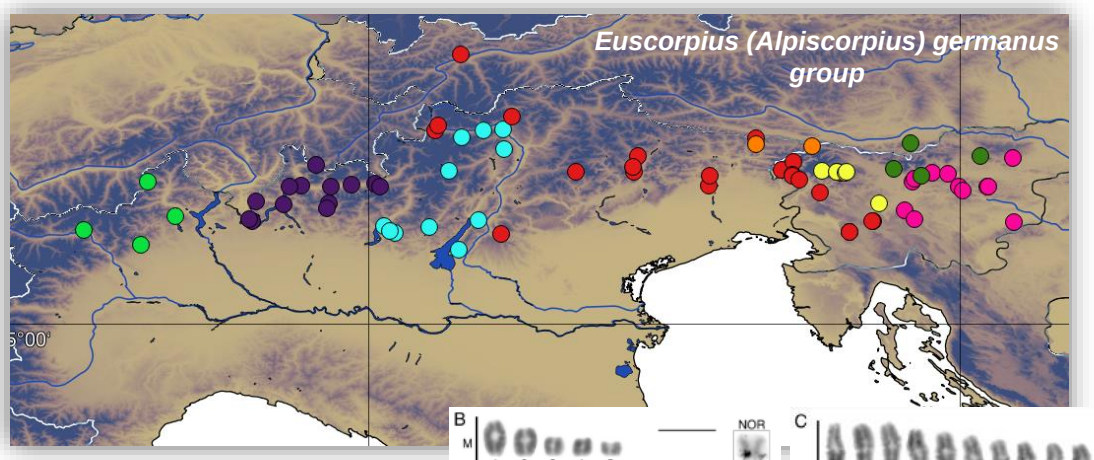



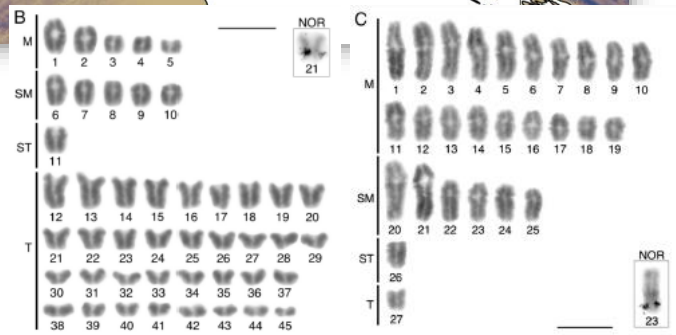

Che et al. 2006

# Scorpiones




Štundlová et al. 2019

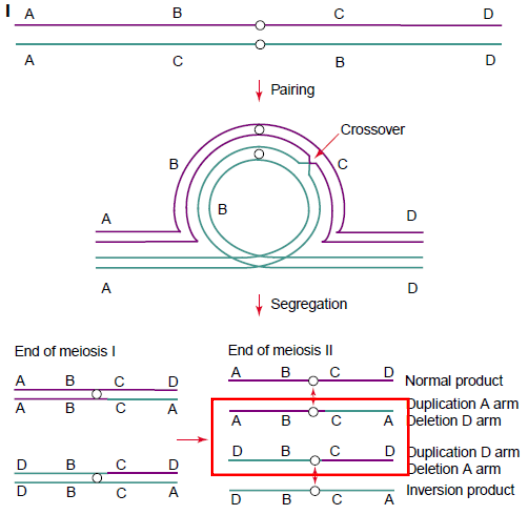


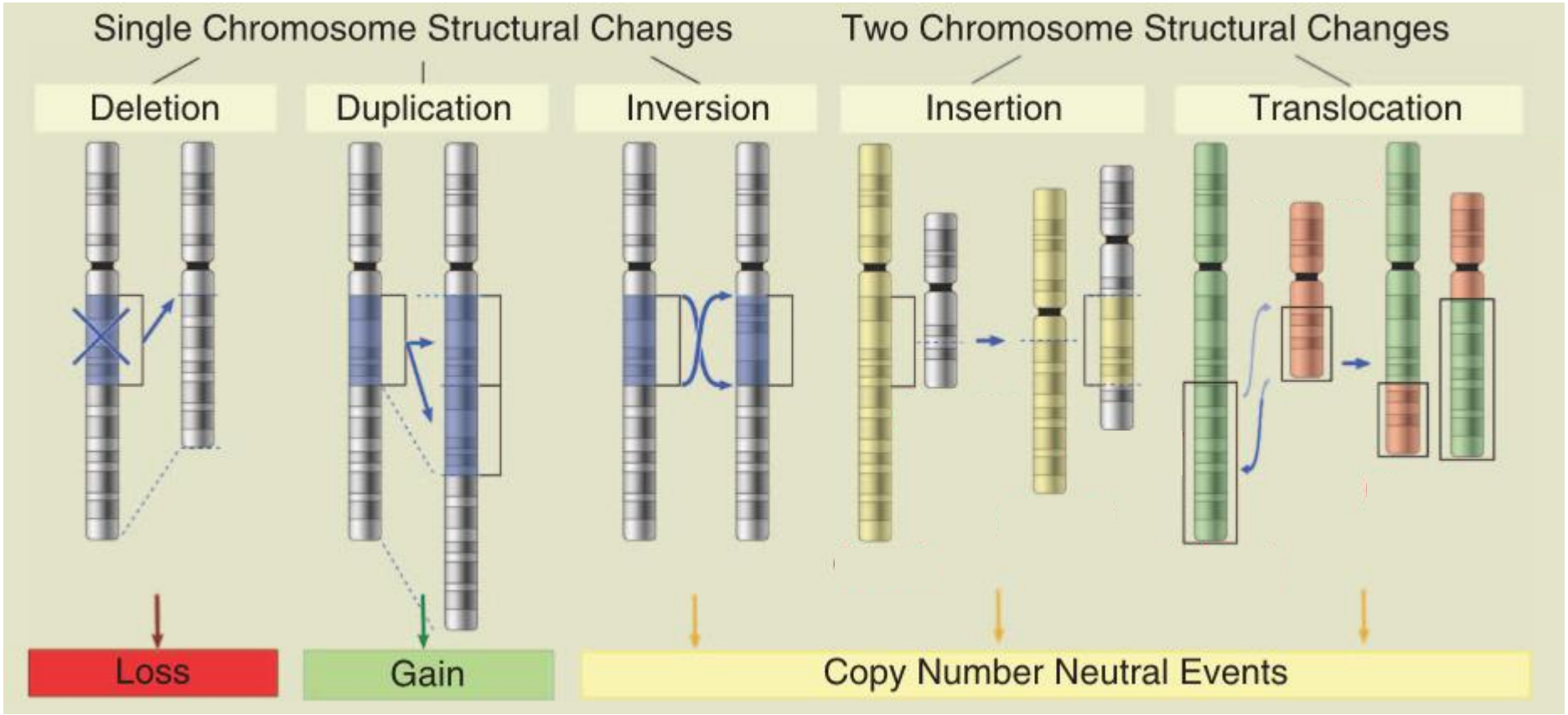

## chromosome speciation ?

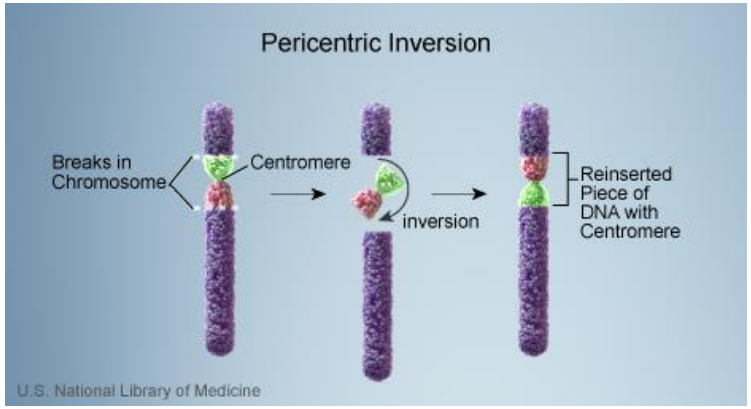
*“hybrid-sterility model”*
predicted that the karyotypic hybrids generate unbalanced gametes and thus reduce fertility.

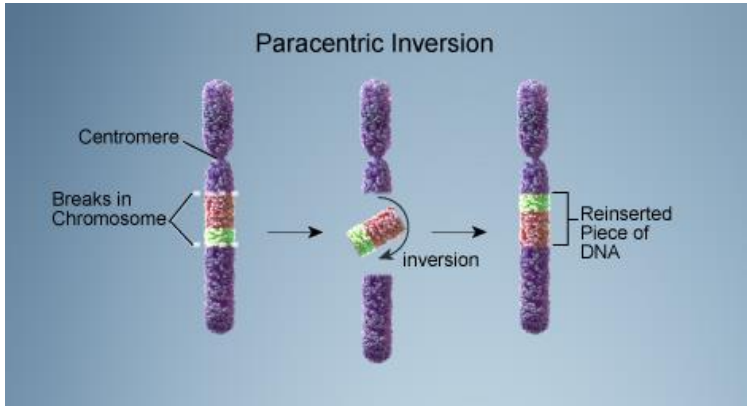
*“suppressed-recombination model”*
suggests that the rearrangements reduce recombination between chromosomes and lead to the divergence and speciation.



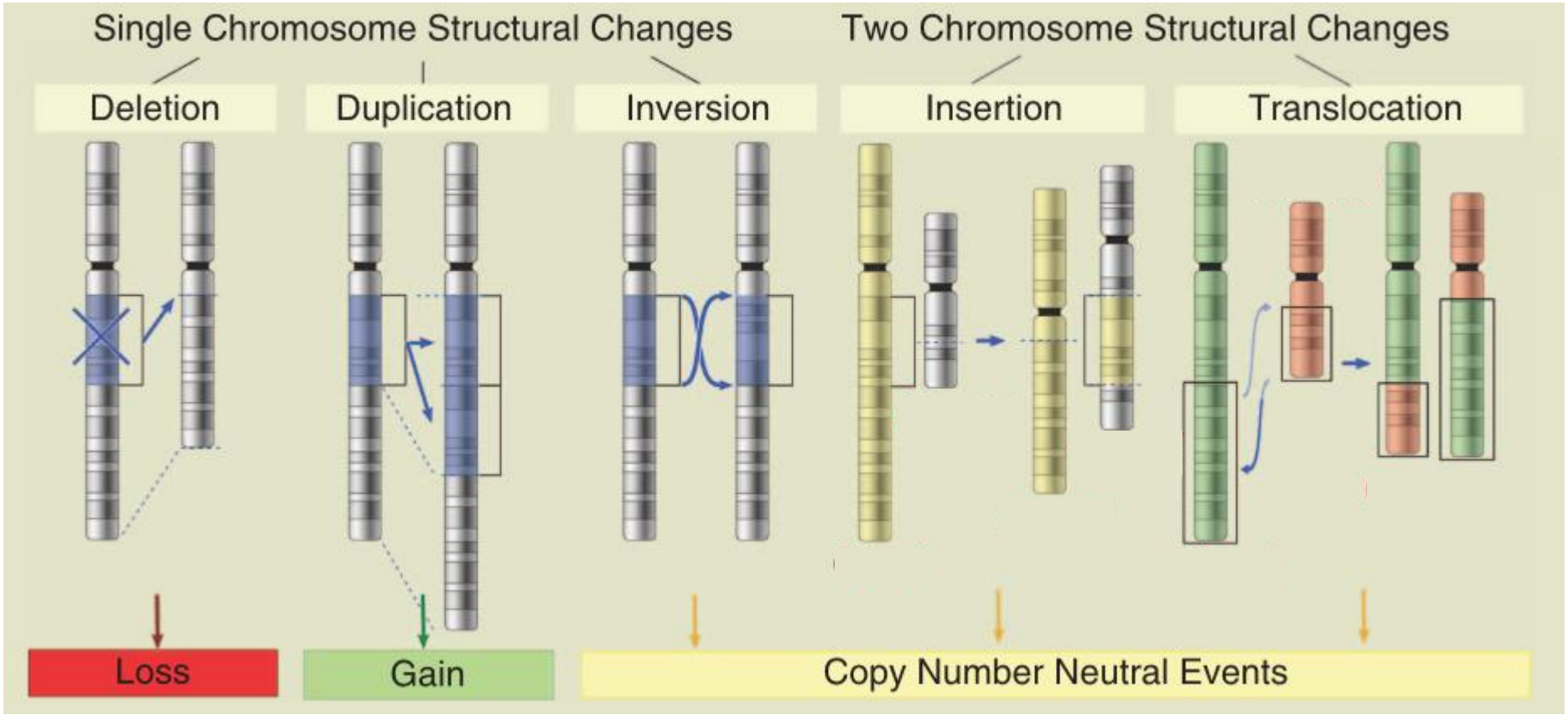
# types of chromosomal rearrangements



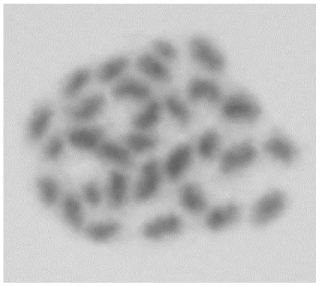



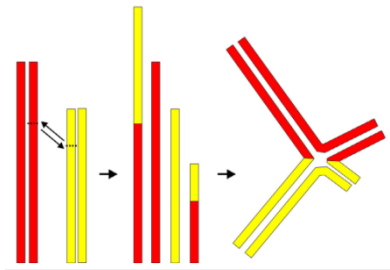



# types of chromosomal rearrangements

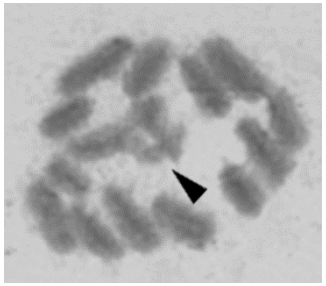


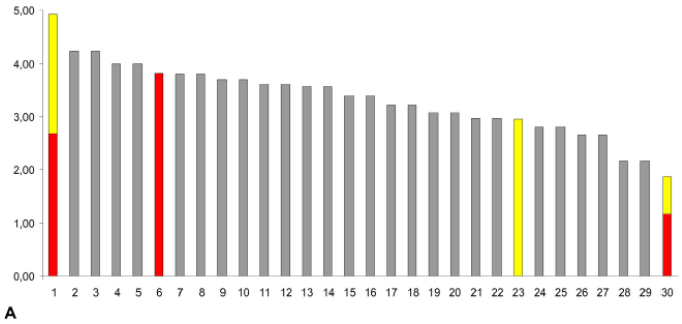

*Mitotic metaphase*





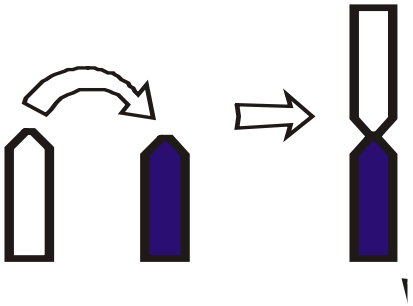
*Meiotic pachytene*

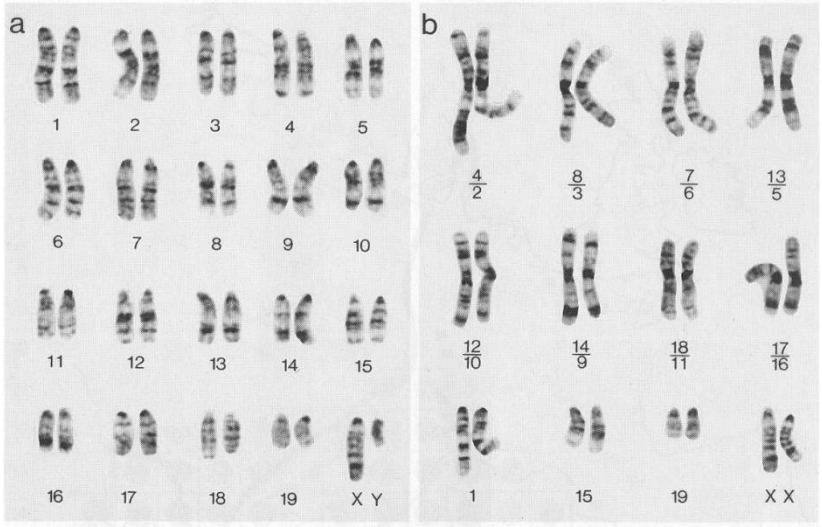





Scorpion *Gint gaitako*, 2n=30, Kovařík et al. 2019

# Centric fusions - Robertsonian translocations or fissions





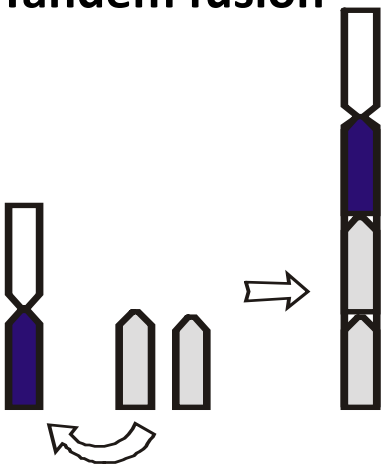

*Mus musculus domesticus*

Number fundamental

Phillips & Ráb 2001

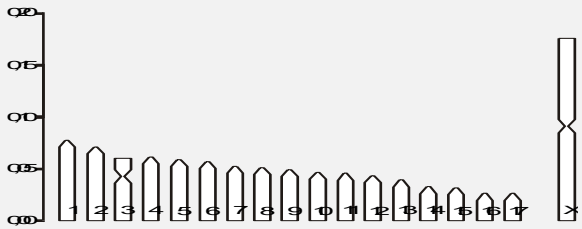
| Species | Common name | 2n | NF |
|---|---|---|---|
| Salmoninae (*cont.*) | | | |
| *Salvelinus* | | | |
| *confluentus* | Bull trout | 78 | 100 |
| *fontinalis* | Brook trout | 84 | 100 |
| *namaycush* | Lake trout | 84 | 100 |
| *leucomaenis* | Potted char | 84 | 100 |
| *pluvius* | Japanese char | 84–86 | 100 |
| *alpinus/malma* complex | Dolly Varden char | 82 | 98 |
| *albus* | White char | 78–80 | 98 |
| *alpinus* | Arctic char | 78 | 98 |
| *elgyticus* | Small mouthed char | 76–78 | 98 |
| *boganidae* | Boganid char | 76–78 | 98 |
| *kronicus* | Stone char | 78–82 | 100 |
| *taranetzi* | Eastern Arctic char | 76–78 | 98–100 |
| *levanidovi malma* | Levanidovi char | 78–80 | 98 |
| *lordi* | | | |
| *m. malma* | Dolly Varden char | 78 | 98 |
| *m. kraschennkovi* | Dolly Varden char | 82 | 98 |
| *Salvethymus* | | | |
| *svetovidovi* | Longfinned char | 56 | 98 |

# Tandem fusion

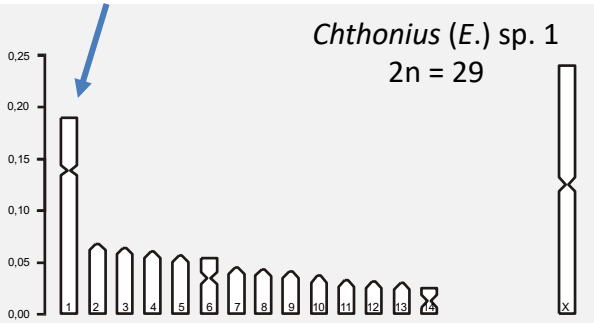


pseudoscorpions

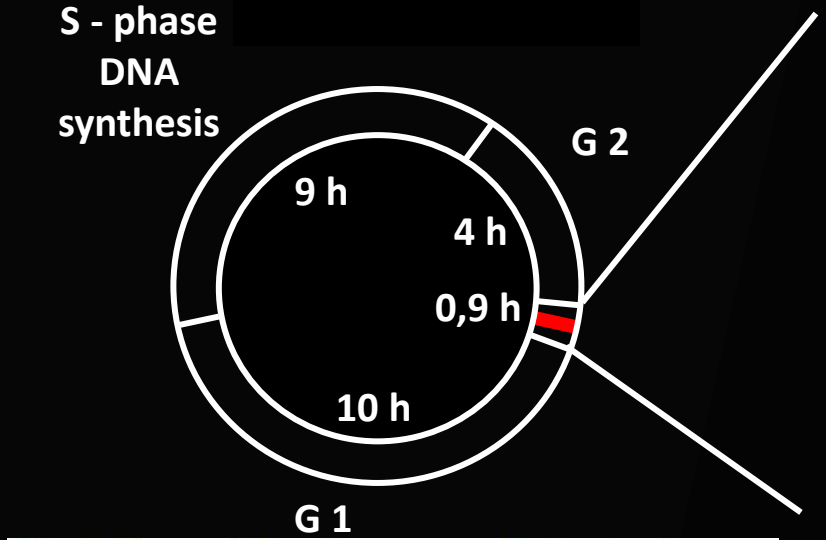
*Chthonius* (*E.*) *tetrachelatus*
2n = 35

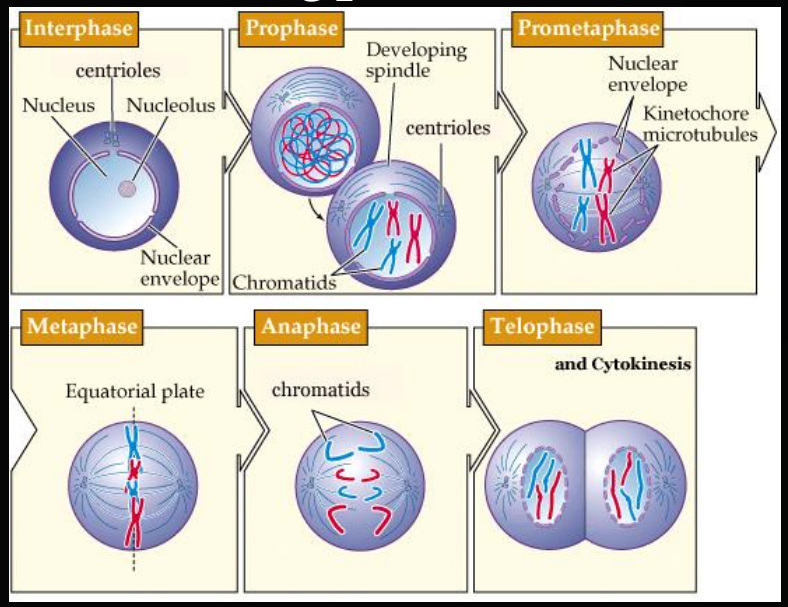


*Chthonius* (*E.*) sp. 1
2n = 29

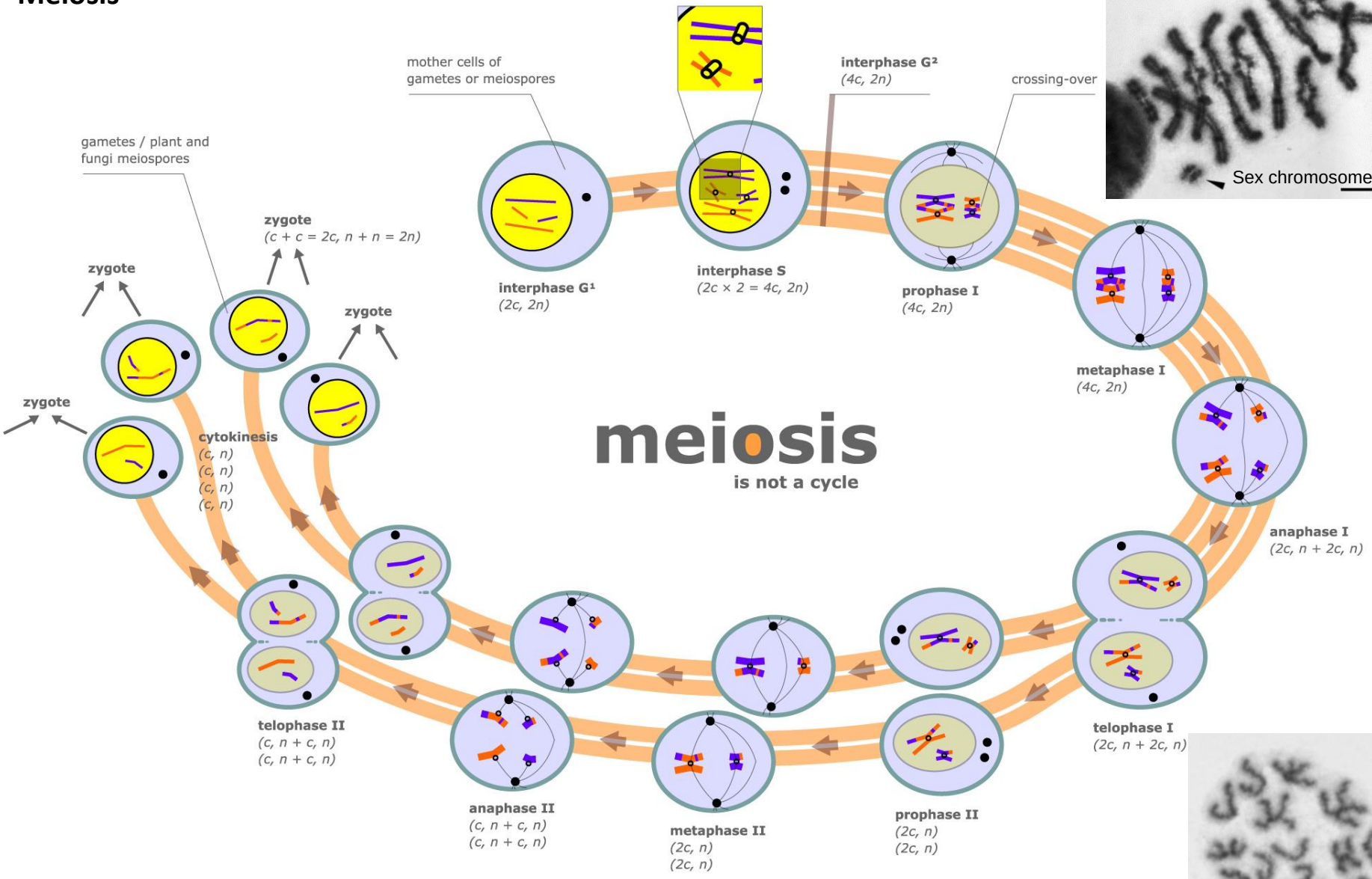


Mitosis


S - phase
DNA
synthesis
G 2
9 h
4 h
0,9 h
10 h
G 1


Interphase
centrioles
Nucleus
Nucleolus
Nuclear envelope
Prophase
Developing spindle
centrioles
Chromatids
Prometaphase
Nuclear envelope
Kinetochore microtubules
Metaphase
Equatorial plate
Anaphase
chromatids
Telophase
and Cytokinesis

# Meiosis


mother cells of
gametes or meiospores
interphase G²
(4c, 2n)
crossing-over
gametes / plant and
fungi meiospores
zygote
(c + c = 2c, n + n = 2n)
zygote
zygote
zygote
interphase G¹
(2c, 2n)
interphase S
(2c × 2 = 4c, 2n)
prophase I
(4c, 2n)
metaphase I
(4c, 2n)
meiosis
is not a cycle
anaphase I
(2c, n + 2c, n)
telophase I
(2c, n + 2c, n)
prophase II
(2c, n)
(2c, n)
metaphase II
(2c, n)
(2c, n)
anaphase II
(c, n + c, n)
(c, n + c, n)
telophase II
(c, n + c, n)
(c, n + c, n)
cytokinesis
(c, n)
(c, n)
(c, n)
(c, n)


Sex chromosome

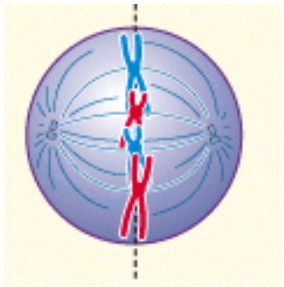
# Cytogenetic techniques

## The most important – good quality of chromosome preparation

### - Dividing cells

bone marrow, blood, amniotic fluid, cord blood, tumor, and tissues (including skin, umbilical cord, chorionic villi, liver, and many other organs)
In invertebrates very often salivary gland, embryo, testis

A mitotic inhibitor (**colchicine**, colcemid) is added to the culture. This stops cell division at mitosis which allows an increased yield of mitotic cells for analysis.



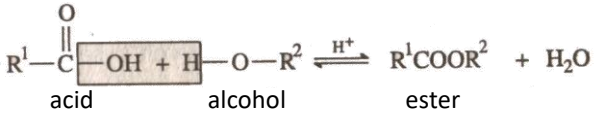
### - Hypotonic solution

Potassium chloride (KCl), Citric acid ($Na_3C_6H_5O_7$)

### - Fixation

methanol (or ethanol ) : glacial acetic acid (3:1)
Carnoy's fixative - ethanol : chloroform : glacial acetic acid (6:3:1)

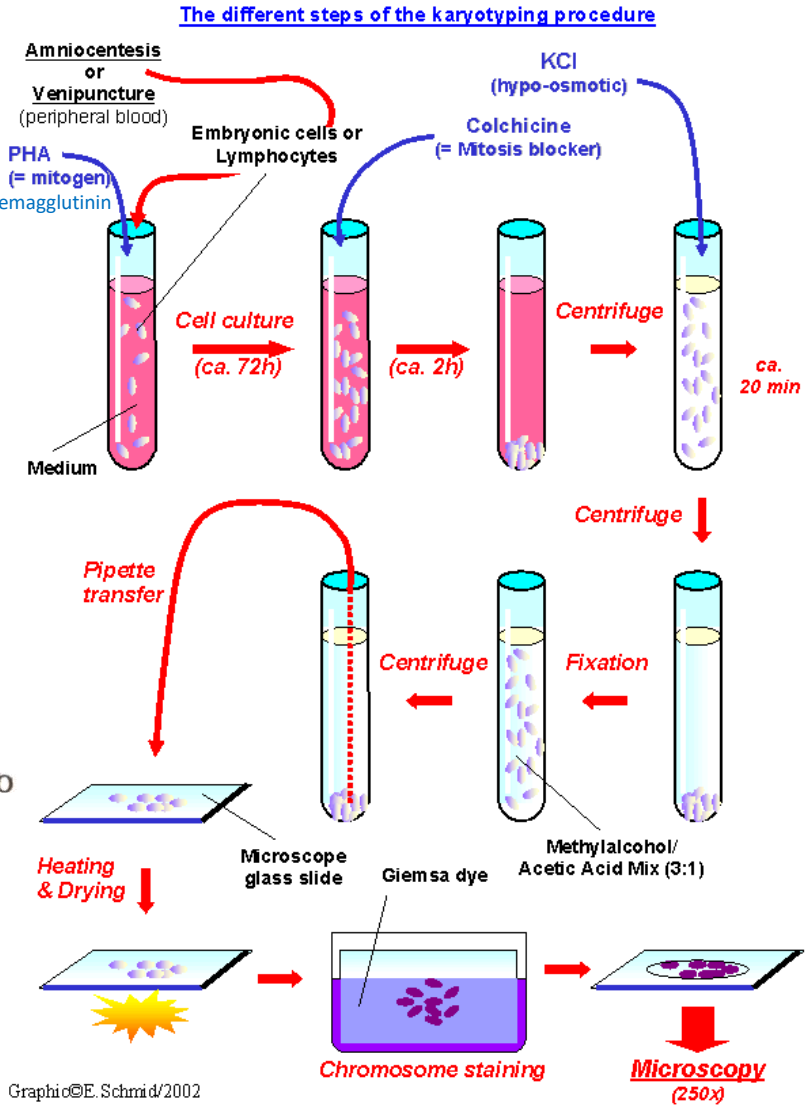
**allways fresh !! -**




### - Spreading

(good quality of microscope slides !!)

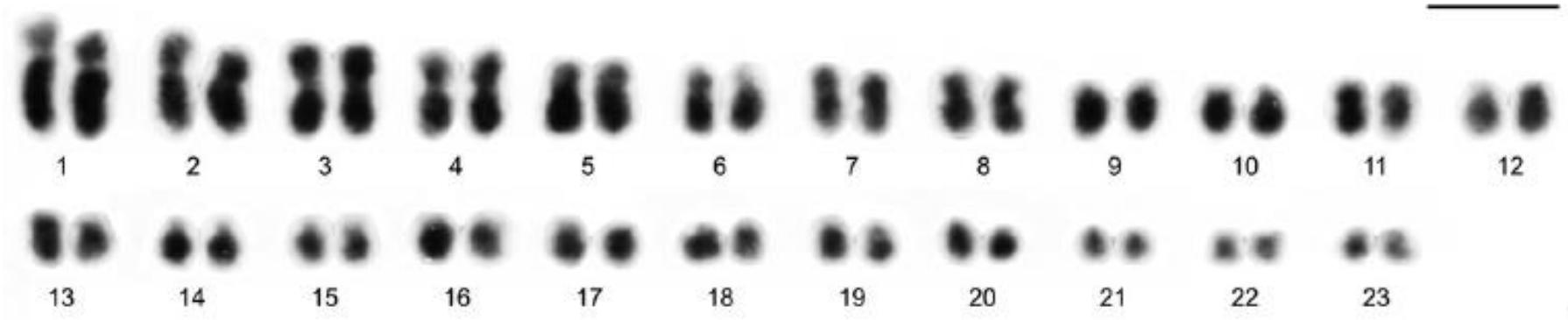
„dropping“
„squashing“
„plate spreading“

# Conventional staining – homogeneous staining

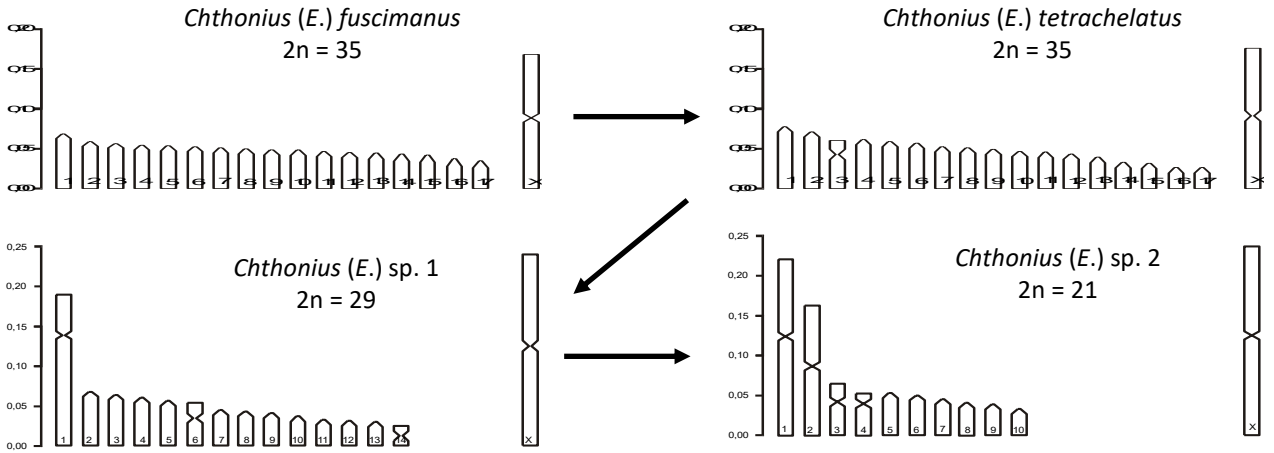
**Giemsa**
Haematoxylin
Acid-Schiff staining
Carbol fuchsin

Number, morfology and size of chromosomes




Scorpion: *Bothriurus rochensis*

pseudoscorpions:

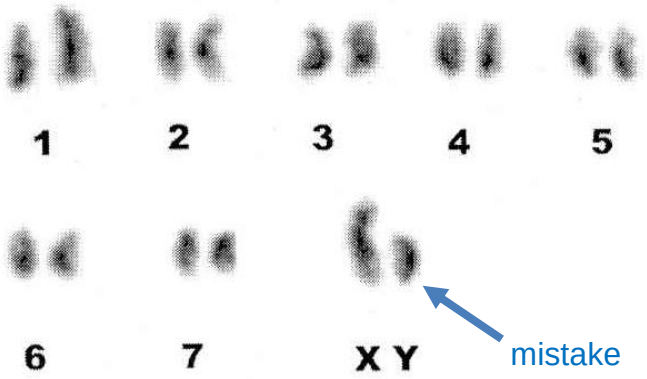



Šťáhlavský & Král 2004

# Conventional staining – homogeneous staining

## Giemsa

sex chromosomes, rearrangements

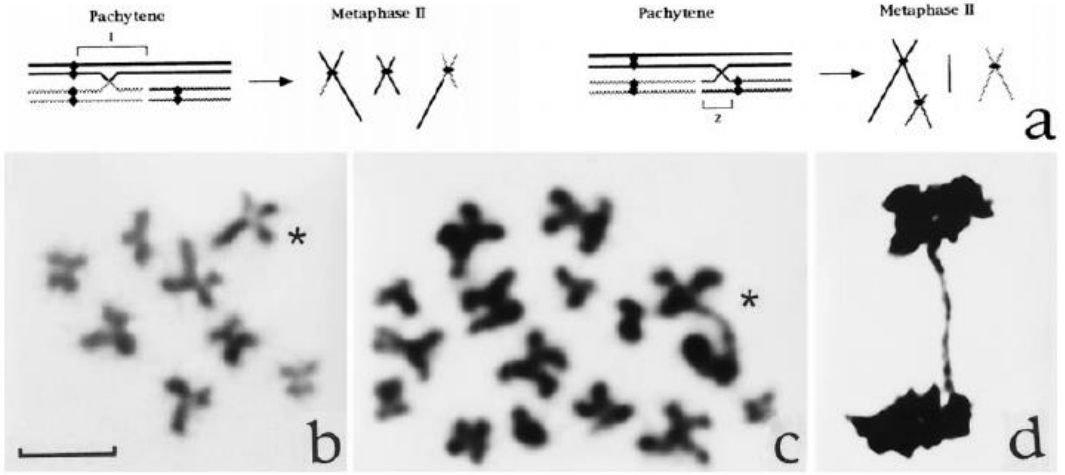



mistake

Scorpion: *Hottentotta judaicus*

no heteromorphic bivalent during pachytene

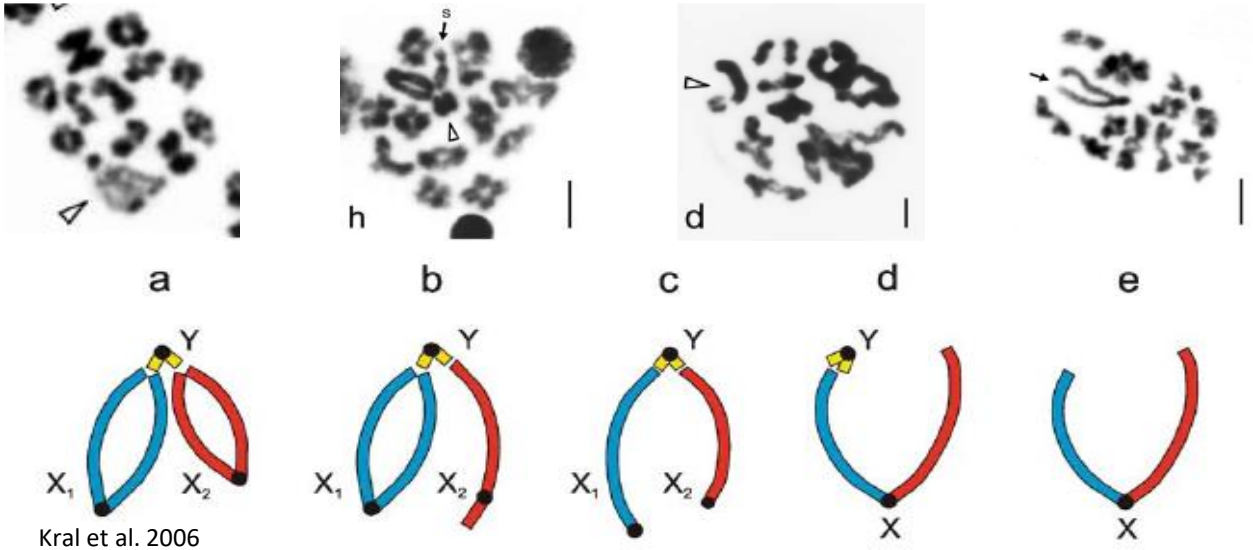
Qumsiyeh et al. 2013




Harvestmen: *Gagrellopsis nodulifera* (Gorlov & Tsurusaki 2000)

Spiders:

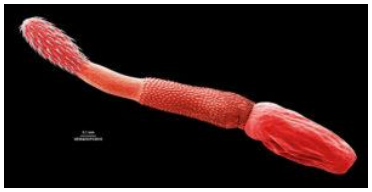
*Spermophora senoculata* *Pholcus phalangioides* *Diguetia albolineata* *Holocnemus caudatus*



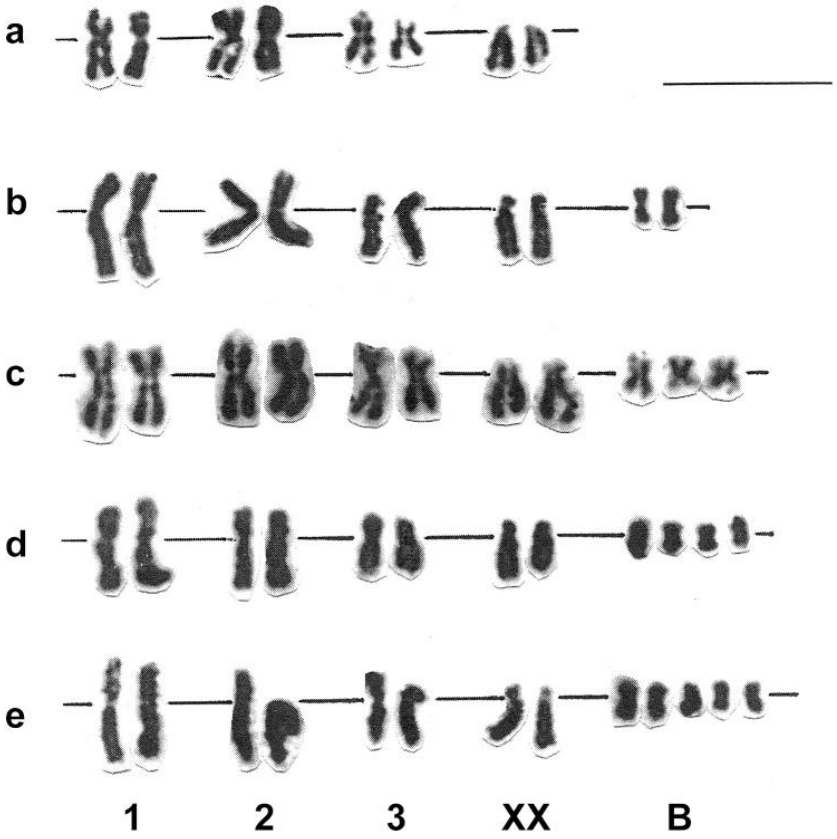

Kral et al. 2006

## Conventional staining – homogeneous staining

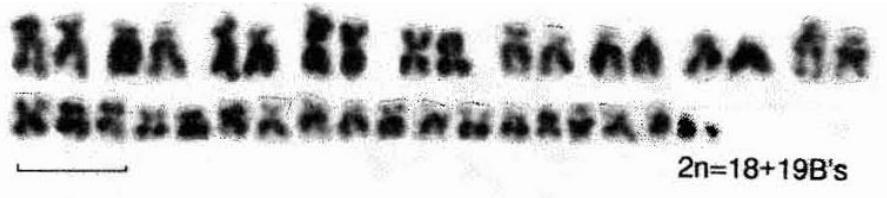
### Giemsa



B chromosomes




*Acanthocephalus lucii.* Chromosome sets of 5 female individuals. (a) 2n = 6 + XX; (b–e) 2n = 6 + XX + 2–5B (Špakulová et al. 2002)

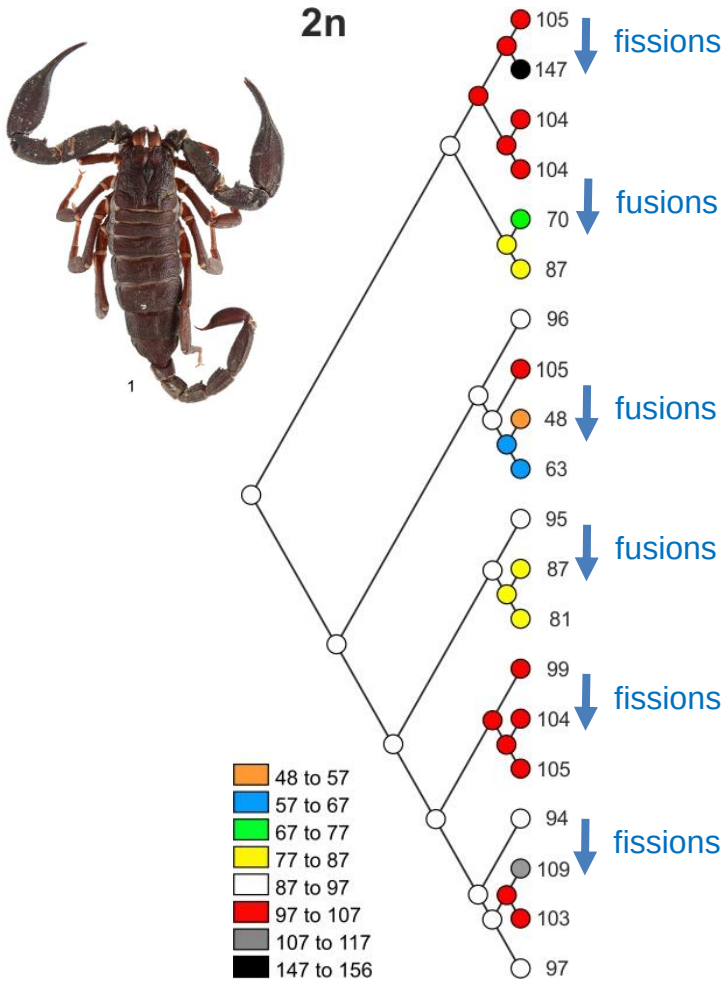



Hervestmen *Metagagrella tenuipes*
Tsurusaki 1993

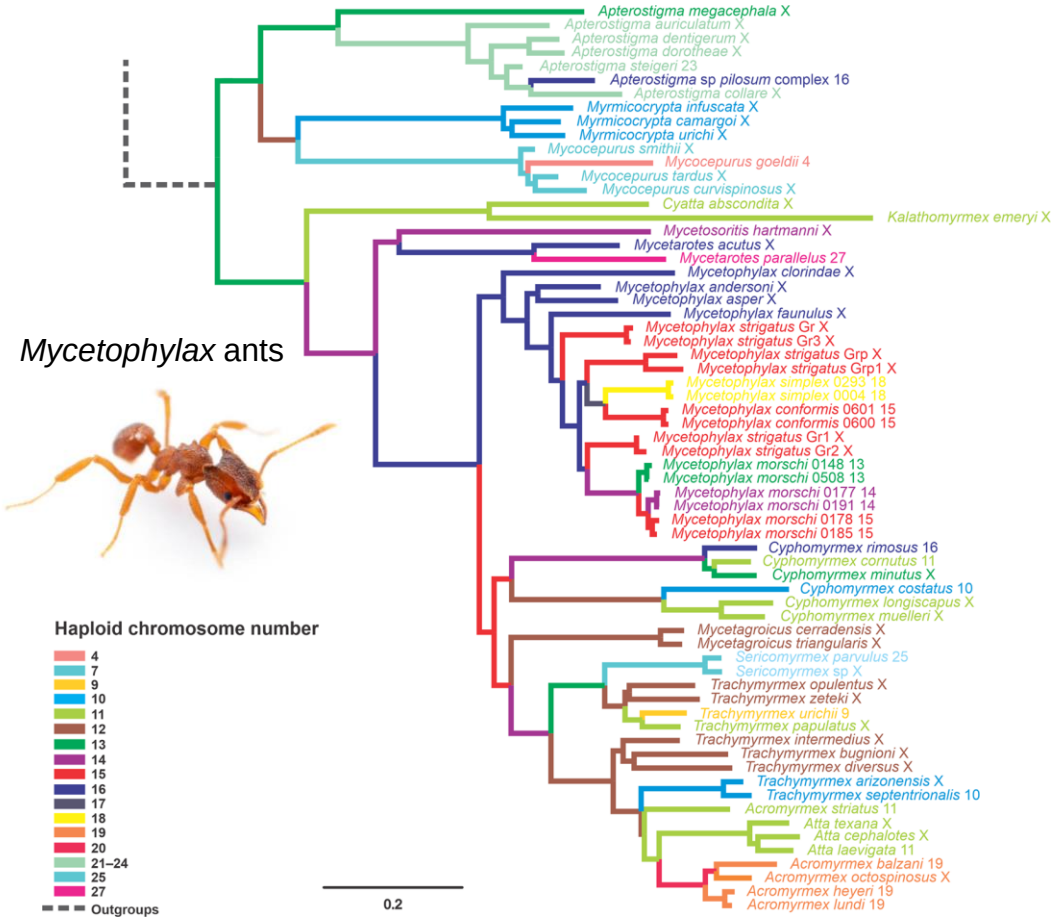
# Conventional staining – homogeneous staining

## Ancestral state

Mesquite
http://www.mesquiteproject.org/



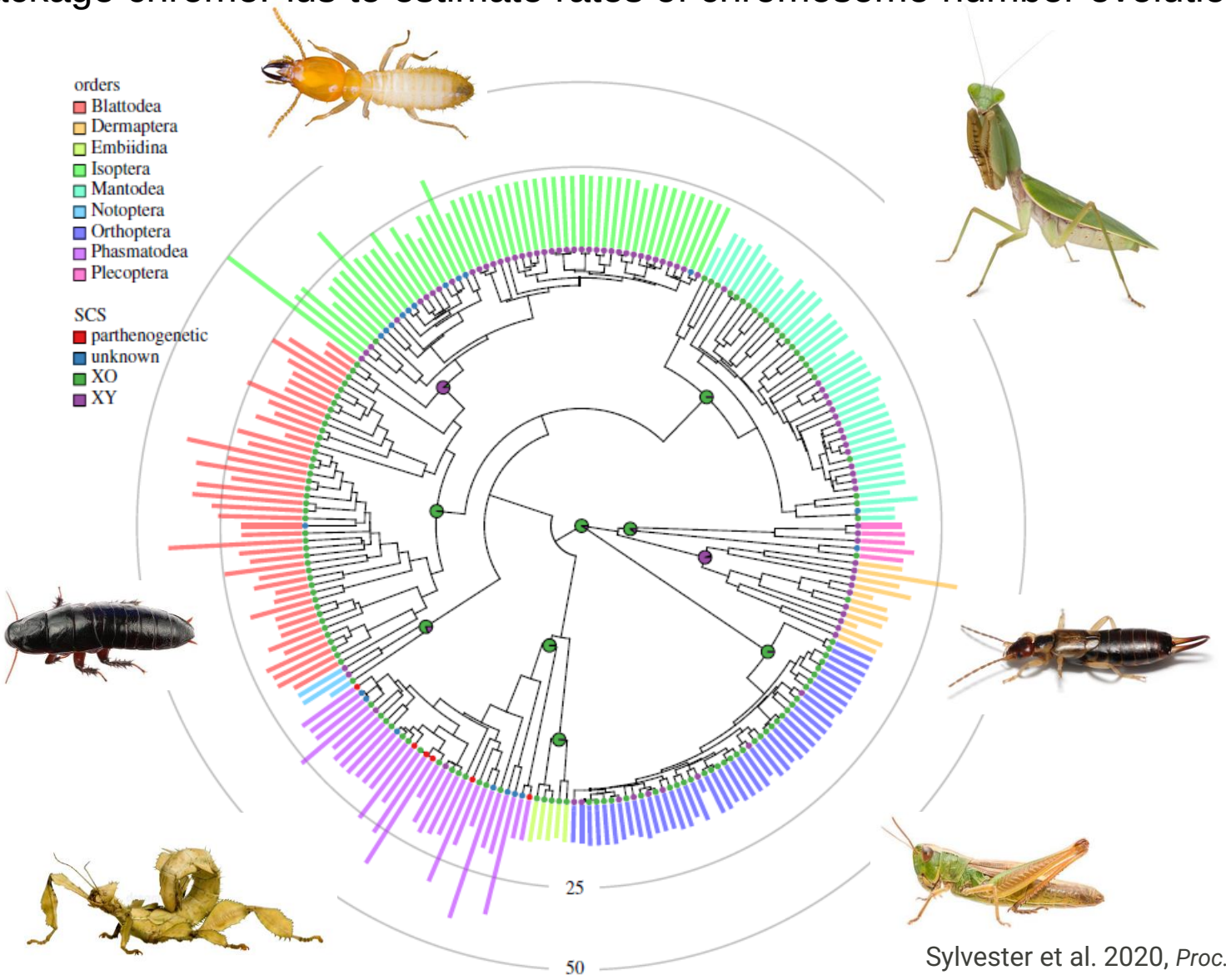

ChromEvol v. 2.0
http://www.tau.ac.il/~itaym ay/cp/chrom Evol/




Micolino et al. 2020 Sci. Rep.

## Conventional staining – homogeneous staining

R package chromePlus to estimate rates of chromosome number evolution

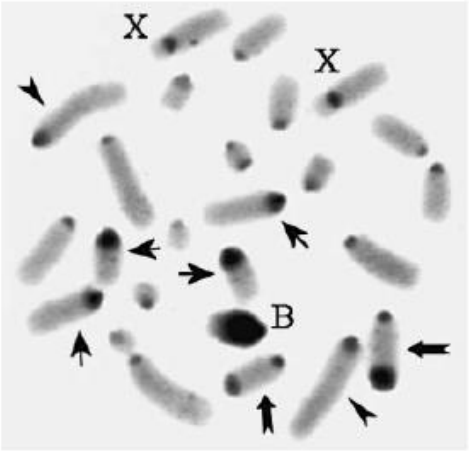



Sylvester et al. 2020, *Proc. R. Soc. B*

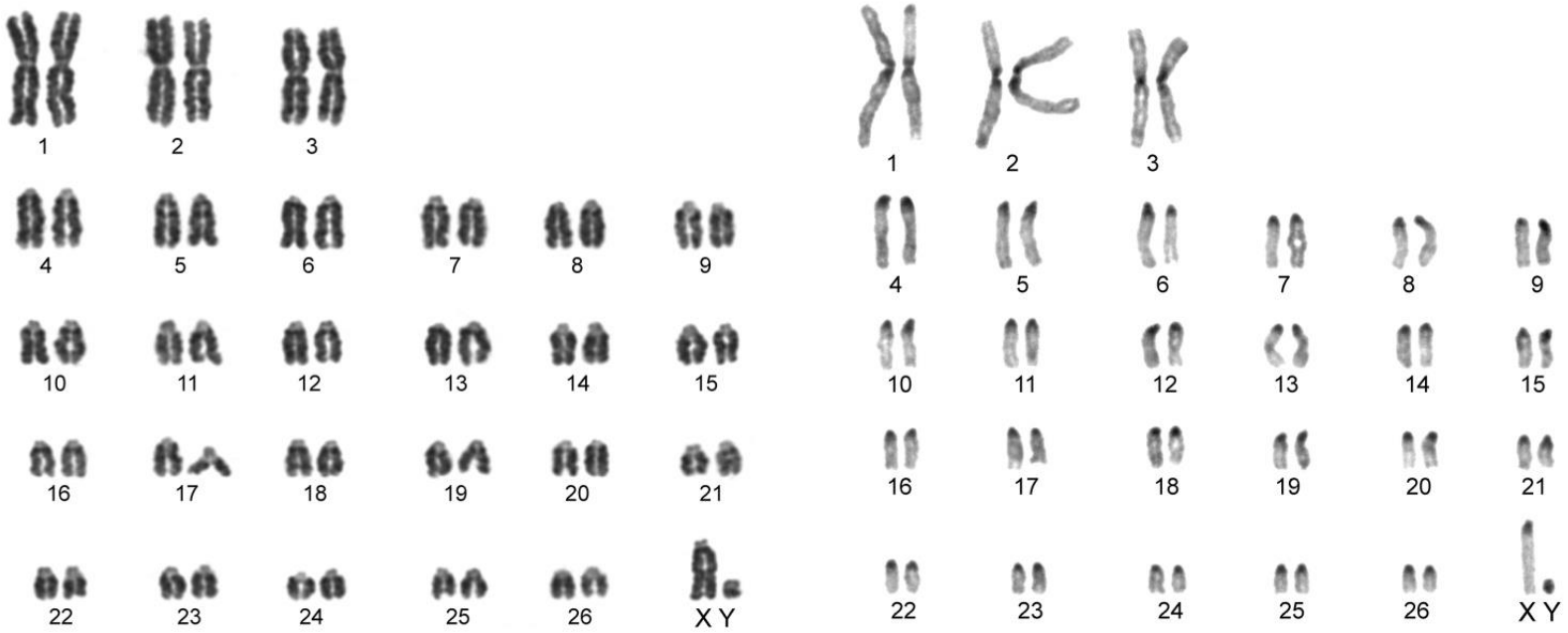
**Selective staining** – for specifique regions, large blocks

## **C- banding** - constitutive heterochromatin

0.2 M HCl for 20-45 min (depurination)
Rinse with DI water
4% $Ba(OH)_2$ (barium hydroxid) at 60 °C (denaturation)
Rinse with DI water
2x SSC at 60 °C for 20-75min (renaturation)
Rinse with DI water




*Podysma krylonensis*
Bugrov et al. 2004

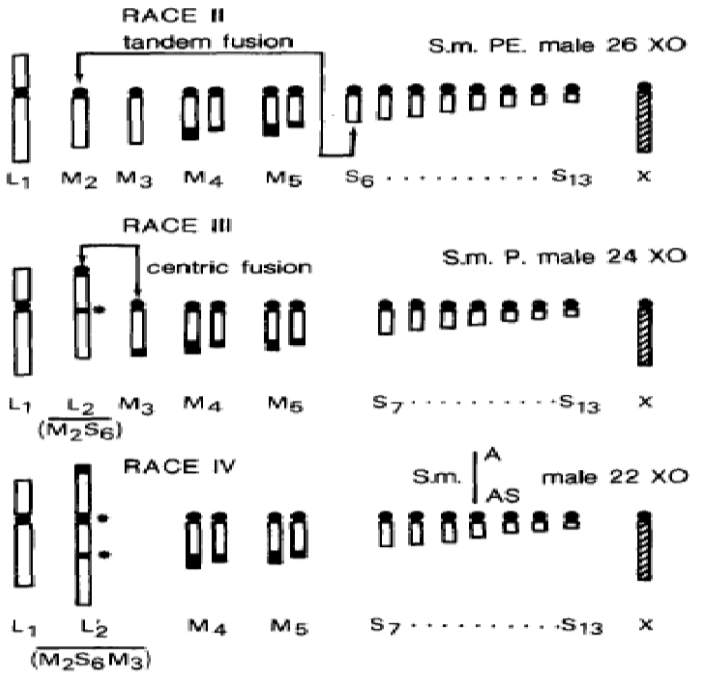


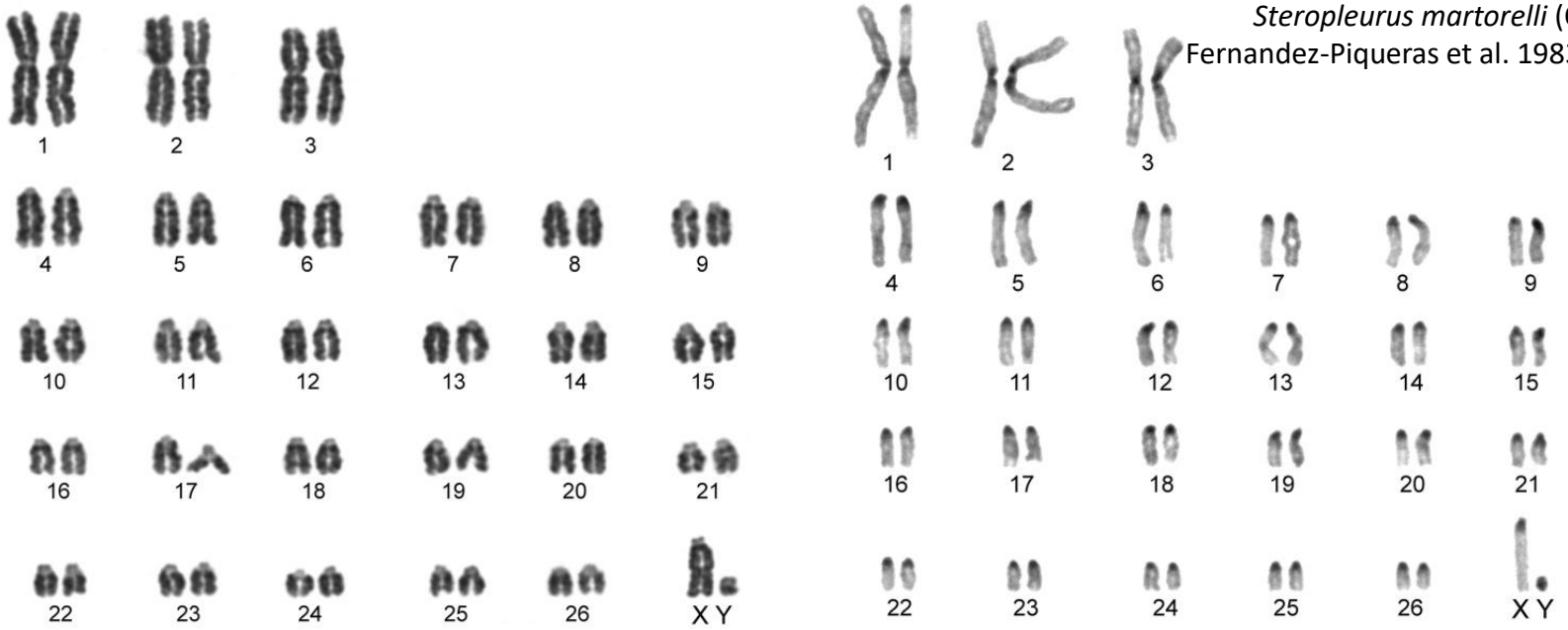

*Ovis orientalis anatolica* Arslan & Zima 2004

**Selective staining** – for specifique regions, large bloc

**C- banding** - constitutive heterochromatin

0.2 M HCl for 20-45 min (depurination)
Rinse with DI water
4% $Ba(OH)_2$ (barium hydroxid) at 60 °C (denaturation)
Rinse with DI water
2x SSC at 60 °C for 20-75min (renaturation)
Rinse with DI water



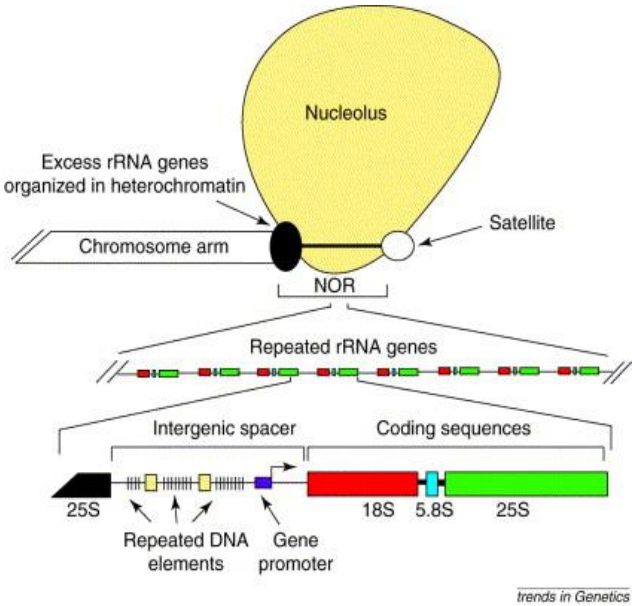

*Steropleurus martorelli* (Orthoptera)
Fernandez-Piqueras et al. 1983, Genetica

*Ovis orientalis anatolica* Arslan & Zima 2004

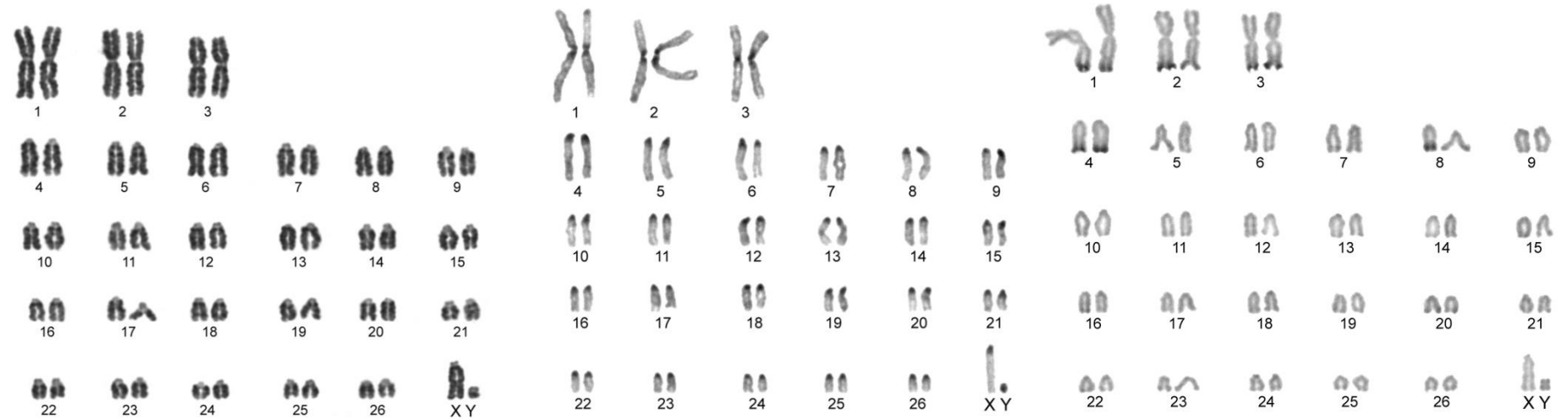
**Selective staining** – for specifique regions, large blocks

**Ag-NOR staining** - NOR = Nucleolar Organizing Region

The region contains several tandem copies of ribosomal DNA genes.




- 1 g of $AgNO_3$ in 1 mL of 0.02 g sodium citrate ($C_6H_5Na_3O_7 \cdot 2\ H_2O$) per 500 mL distilled water, adjusted to pH 3.0 with formic acid.
- Add 1-2 drops of the above solution onto the slides and place a cover slip over the preparation.
- Incubate slides in a moist chamber at 55–60°C for app. 30 min.



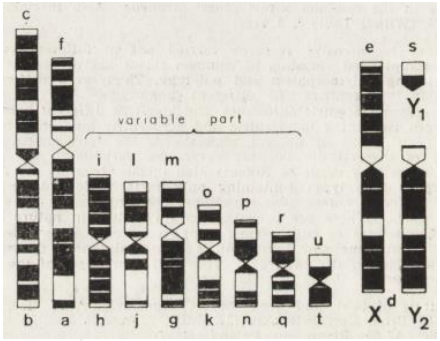

*Ovis orientalis anatolica* Arslan & Zima 2004

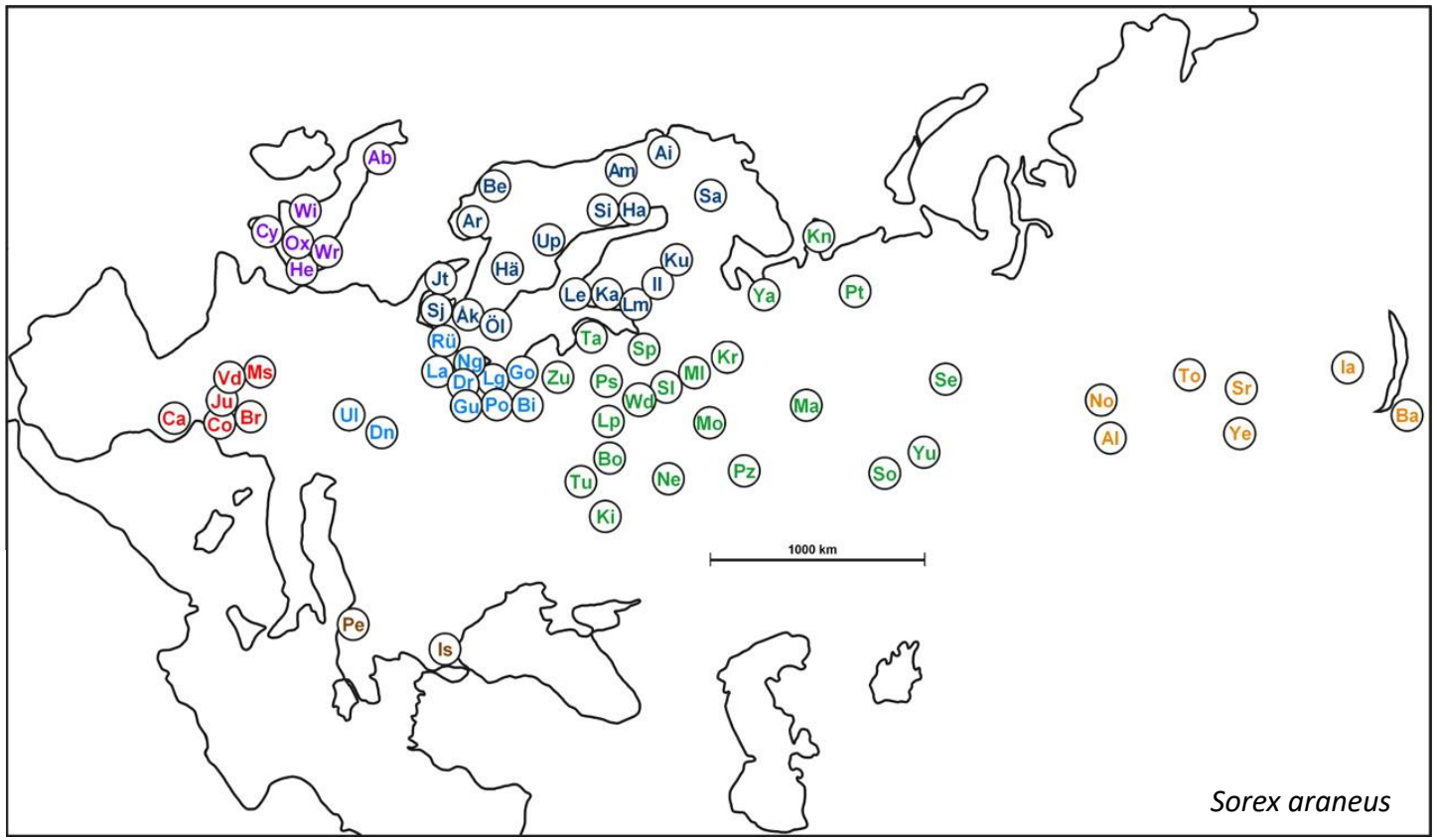
**Selective staining** – for specifique regions, large blocks

## G- banding

- obtained by the action of **trypsin** (10-20s  at room temperature in a fresh 0.25% trypsin and than washed in PBS to block the action of trypsin)
similar pattern also in **Q-banding** (satained by **quinacrin**)

*Sorex araneus*
2n=20-33
FN=40
Chrom. races=72



**Selective staining** – for specifique regions, large blocks

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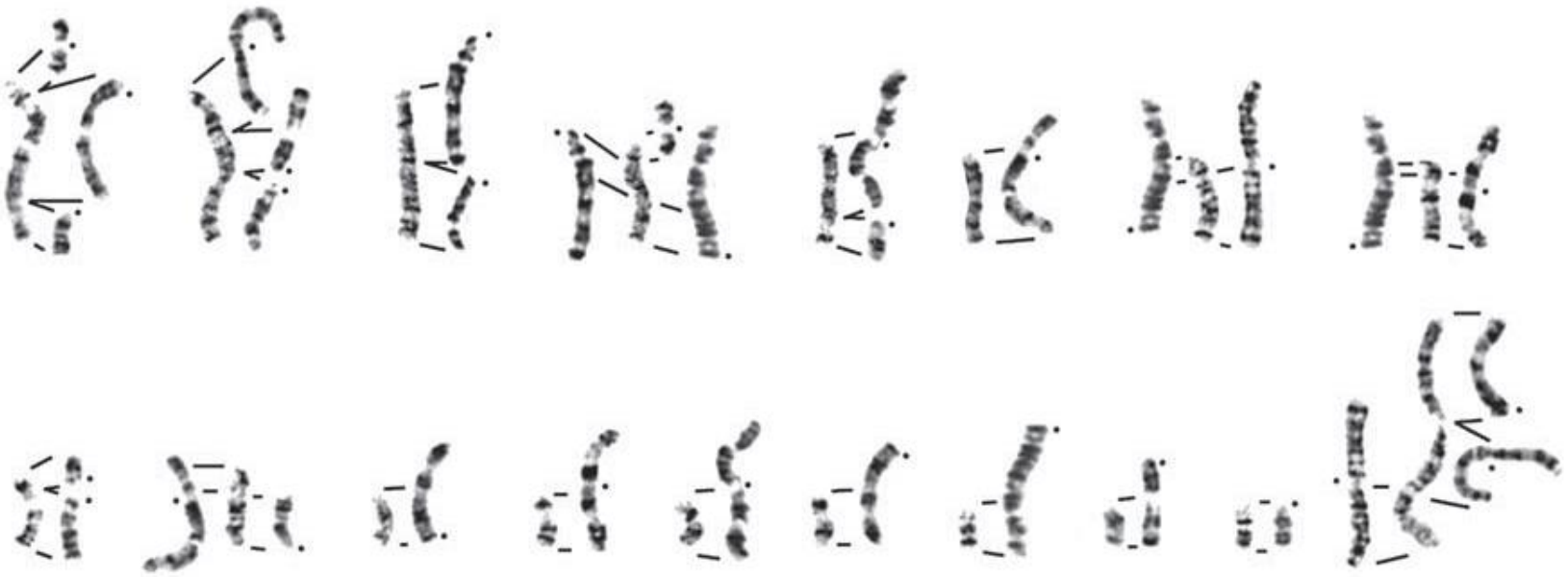
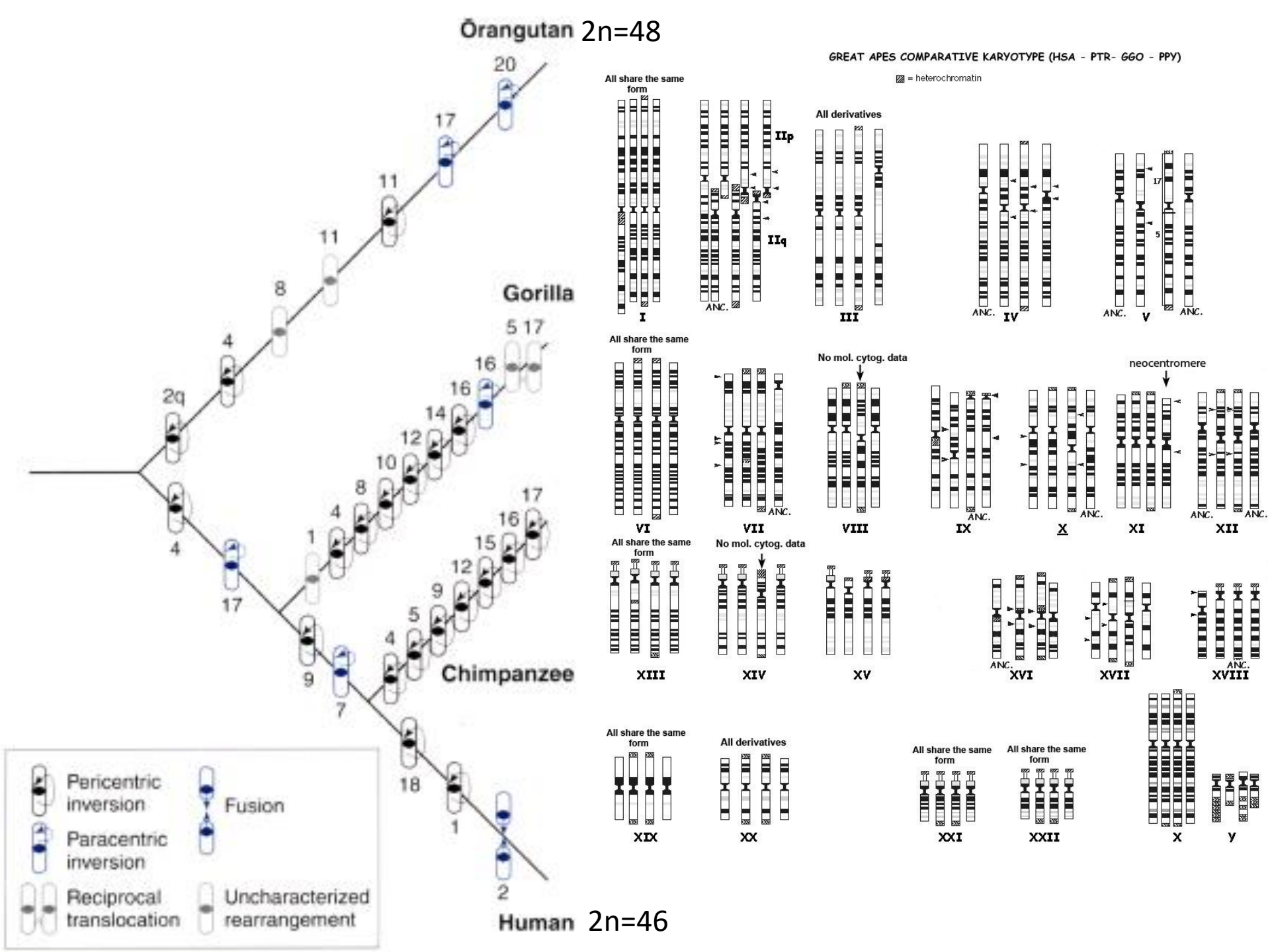


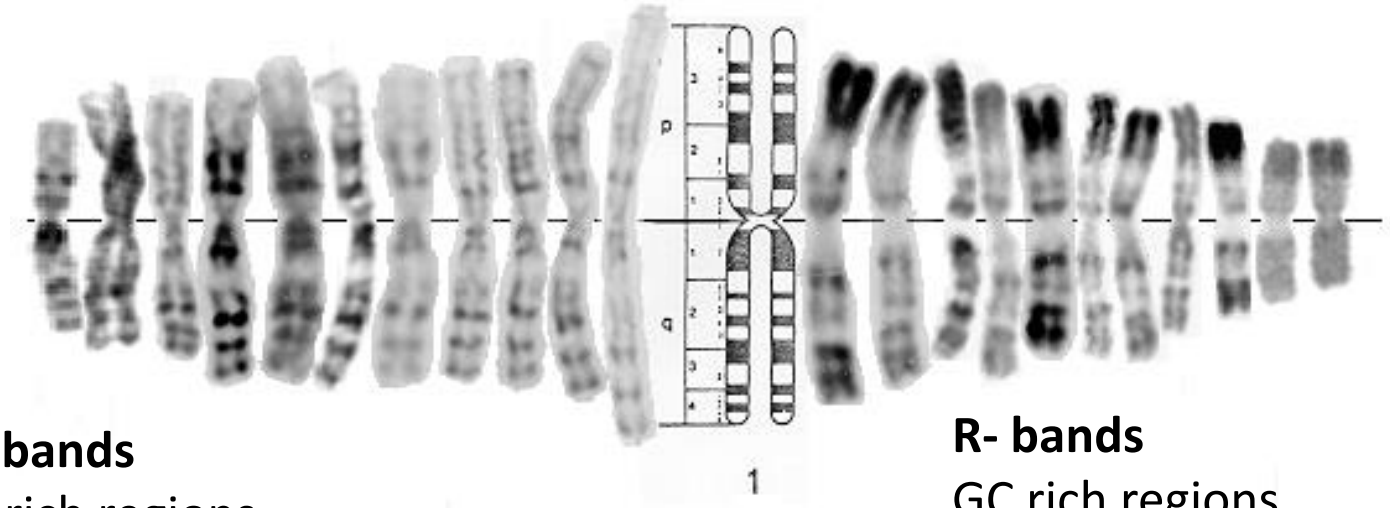
Comparison of G-banded chromosomes of *Sorex minutus* and *S. granarius*

Biltueva et al. 2011


Orangutan 2n=48
Gorilla
Chimpanzee
Human 2n=46
Pericentric inversion
Paracentric inversion
Reciprocal translocation
Fusion
Uncharacterized rearrangement
GREAT APES COMPARATIVE KARYOTYPE (HSA - PTR- GGO - PPY)
= heterochromatin
All share the same form
All derivatives
No mol. cytog. data
neocentromere
ANC.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
XX
XXI
XXII
X
y

**Selective staining** – for specifique regions, large blocks

**R- banding** – bands reverse to G-banding
- the thermal denaturation of chromosomes (30-90 minutes at 87°C)

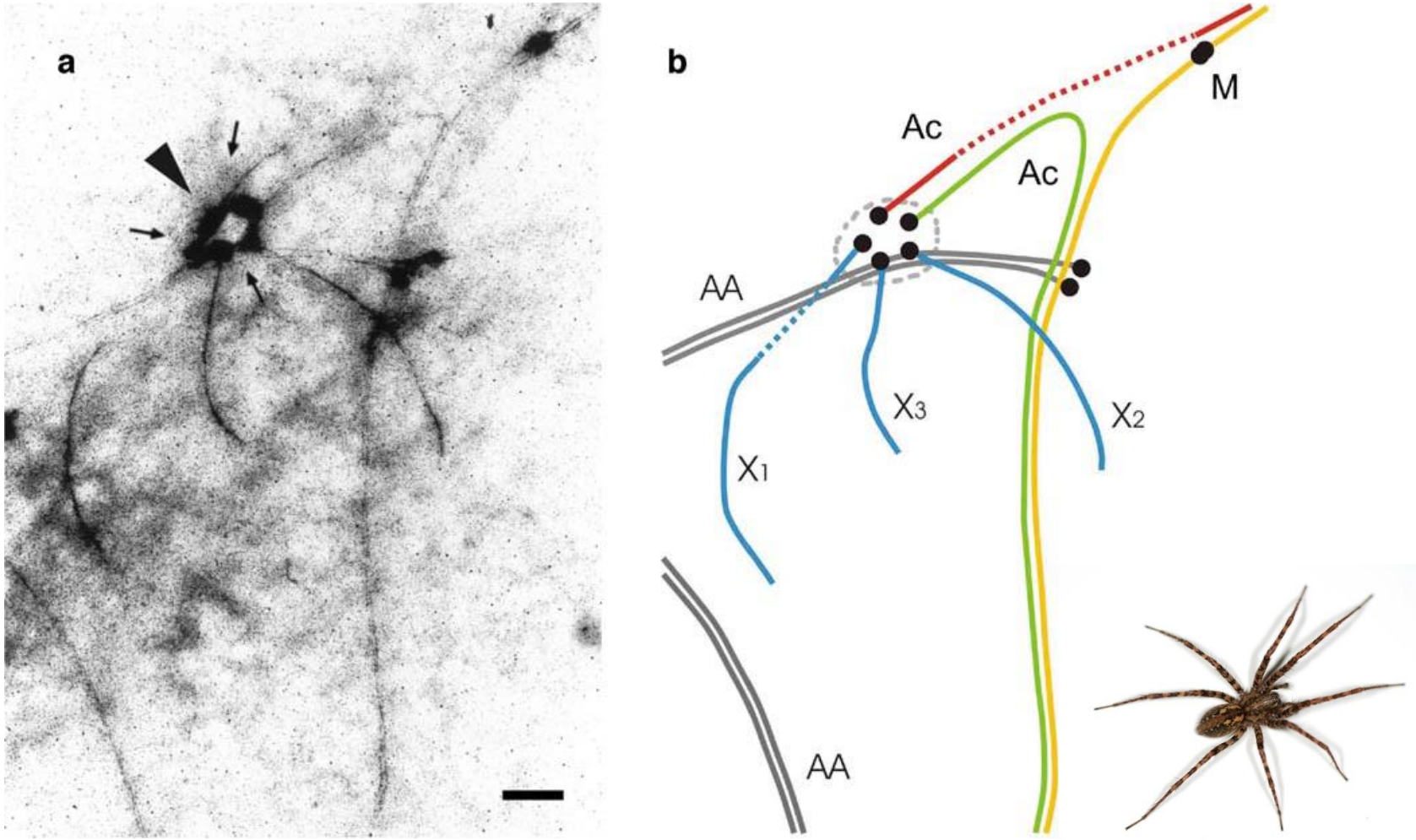



**G- bands**
AT rich regions

**R- bands**
GC rich regions

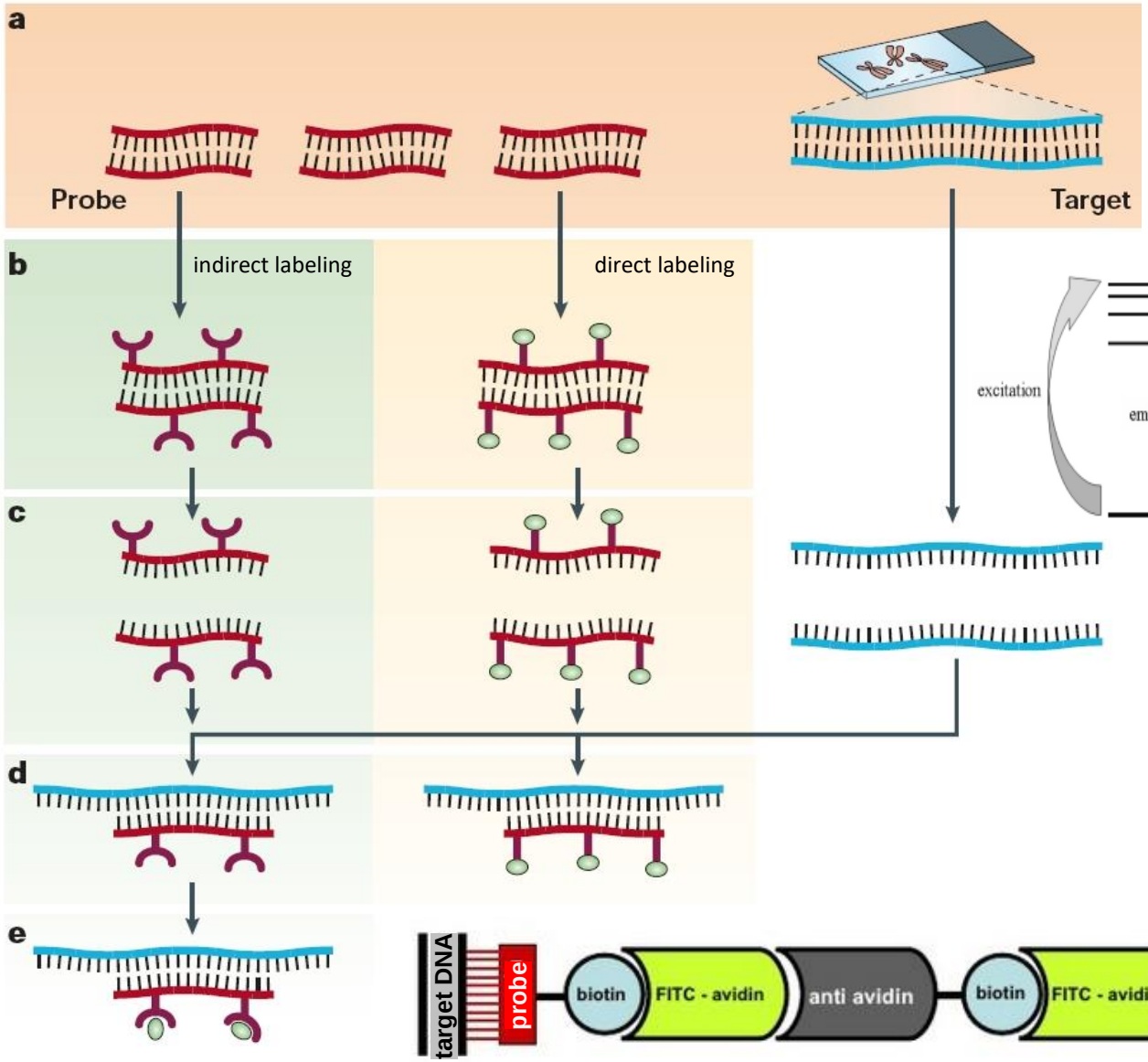
**Fluorochrome staining**
AT rich regions: DAPI (4',6-diamidin-2-fenylindol), chinakrin, Hoechst 33258
GC rich regions: chromomycin $A_3$, mithramycin, olivomycin

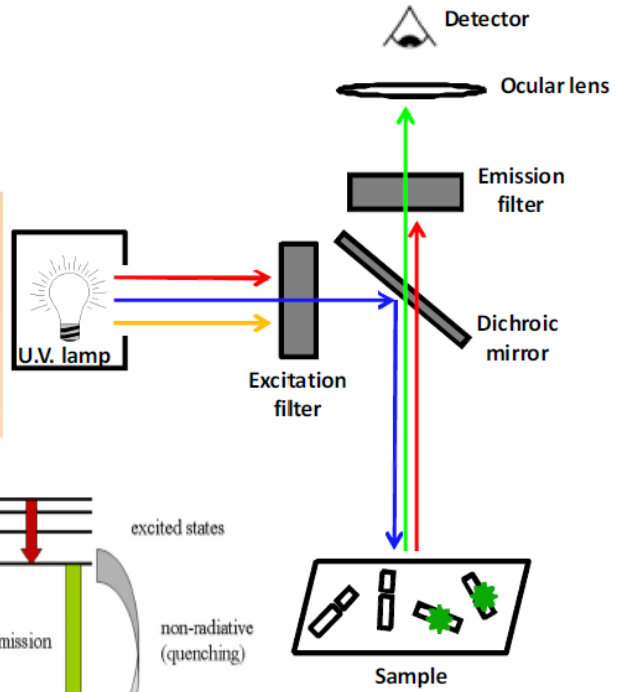
# Transmission electron microscopy (TEM)

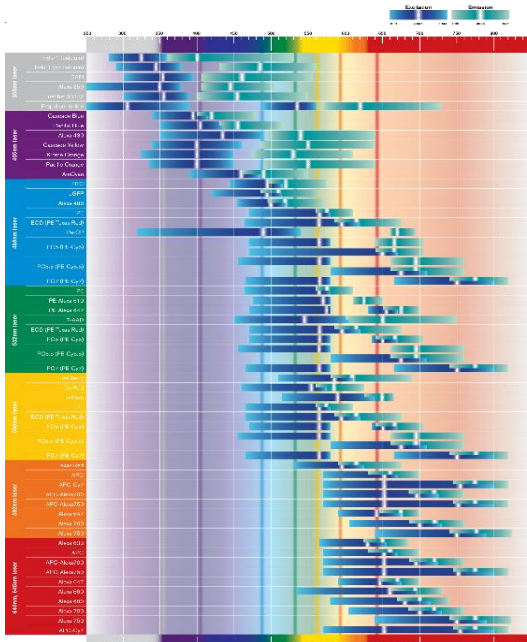


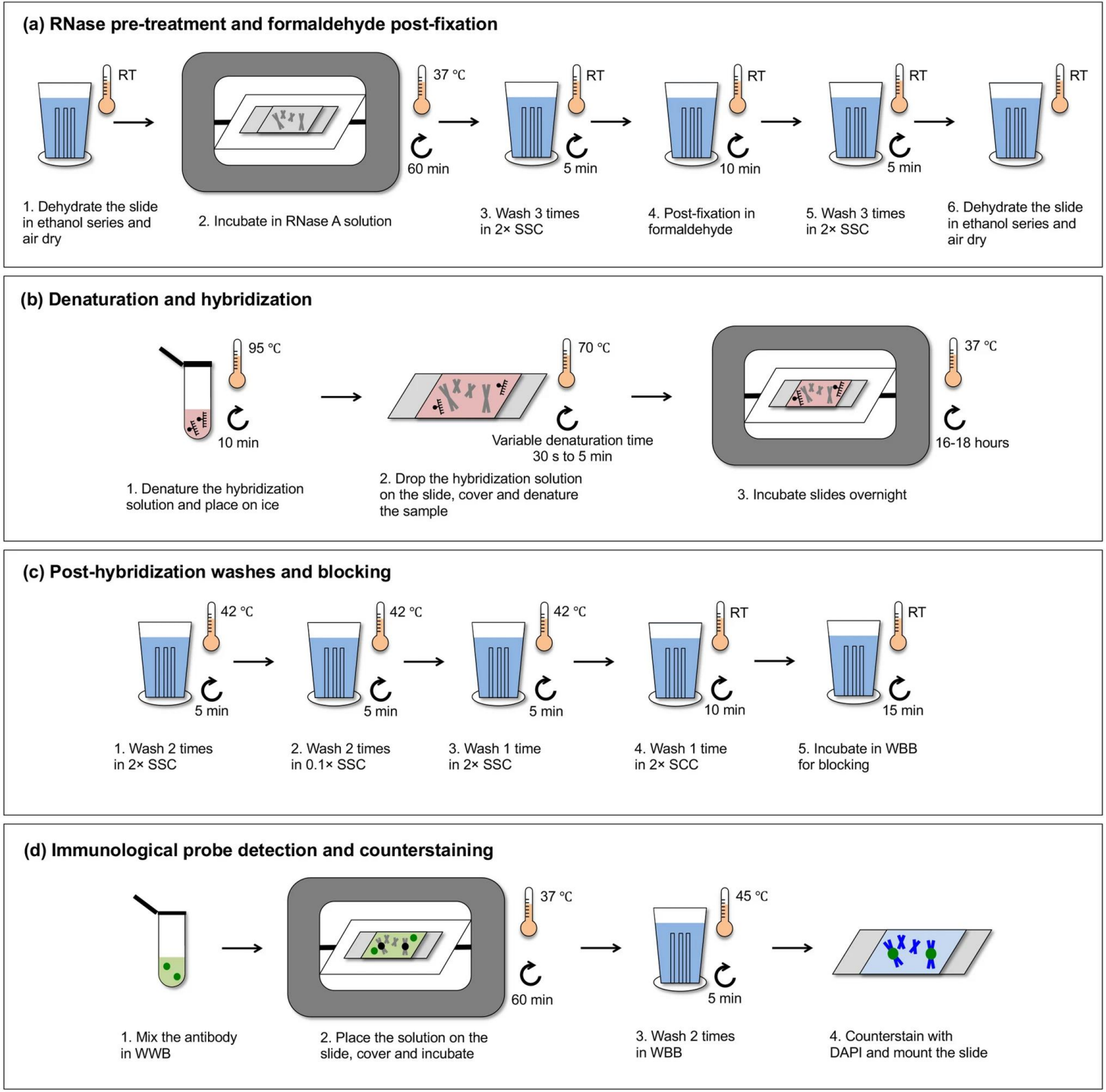

Ultrastructure of pairing of the X univalents with acrocentric chromosomes of the trivalent, *Malthonica ferruginea* male. (Král 2007)

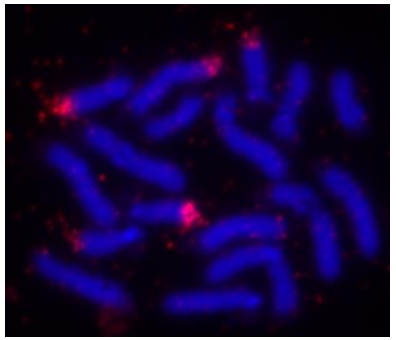
# **FISH** - **F**luorescence ***In Situ*** **H**ybridization

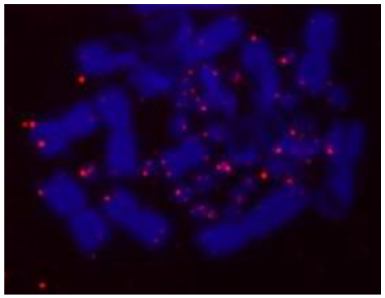




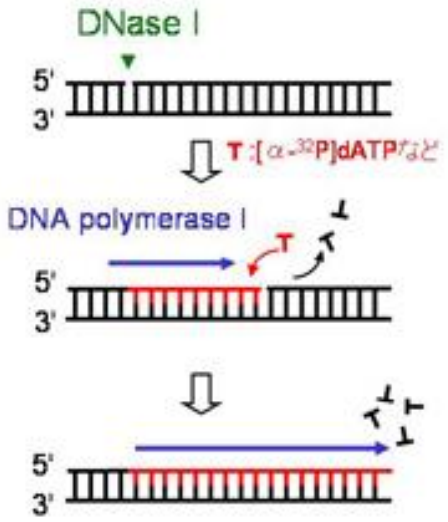





Cabral-de-Mělo & Mares 2021
*Mol. Genet. Genom.*
*https://doi.org/10.1007/s00438-021-01765-2*

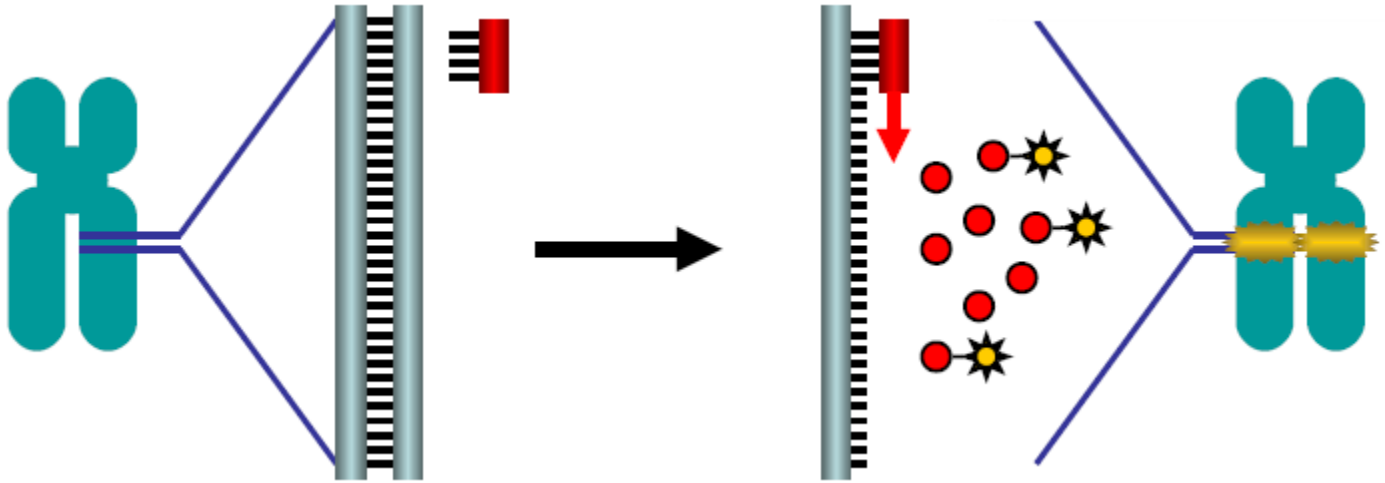
## NICK translation

- DNA Polymerase I is used to replace some of the nucleotides of a DNA sequence with their labeled analogues





## Primed *in situ* Labelling (PRINS)



**Centromere-FISH (ACM-FISH)**

**armFISH**

**Catalyzed Reporter Deposition-FISH (CARD-FISH)**

**Cellular Compartment Analysis of Temporal (Cat) Activity by Fish (catFISH)**

**Cytochalasin B (CB-FISH)**

**Chromosome Orientation (CO)-FISH**

**Combined Binary Ratio (COBRA)-FISH**

**Chromosome Orientation and Direction (COD)-FISH**

**Combinatorial Oligonucleotide (COMBO)-FISH**

**Comet-FISH**

**Cryo-FISH**

**Double Fusion FISH (D-FISH)**

**DNA Breakage Detection FISH (DBD-FISH)**

**e-FISH**

**Fiber-FISH**

**Flow-FISH**

**Fusion-Signal FISH**

**Halo-FISH**

**Harlequin-FISH**

**Immuno-FISH**

**Locked Nucleic Acids (LNAs)-FISH**

**Multiplex (M)-FISH**

**Multilocus or ML-FISH**

**Premature Chromosome Condensation (PCC)-FISH**

**Peptide Nucleic Acid (PNA)-FISH**

**Quantitative-FISH (Q-FISH)**

**Quantum Dot (QD)-FISH**

**Rainbow-FISH**

**Raman-FISH**

**Replicative Detargeting FISH (ReD-FISH)**

**Reverse-FISH**

**Recognition of Individual Genes (RING)-FISH**

**RNA-FISH**

**Cross Species Color Banding (Rx)-FISH**

**Split-Signal FISH**

**Stellaris RNA FISH (Single-Molecule RNA FISH)**

**T-FISH**

**3-D FISH**

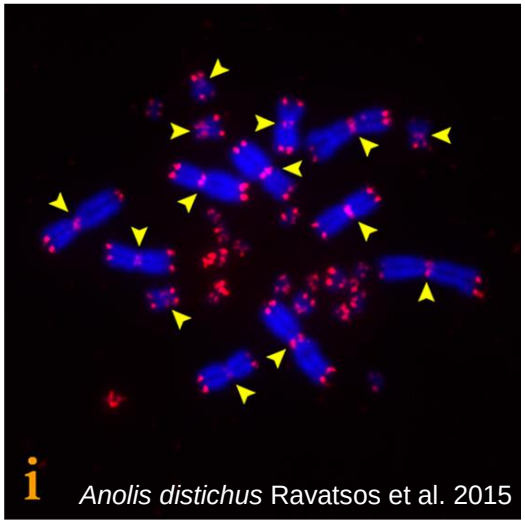
**Zoo-FISH**

## Types of probes

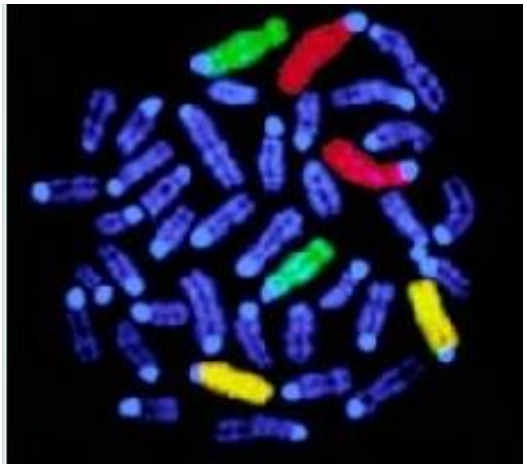
- Satelite DNA
  - centromeric
  - telomeric

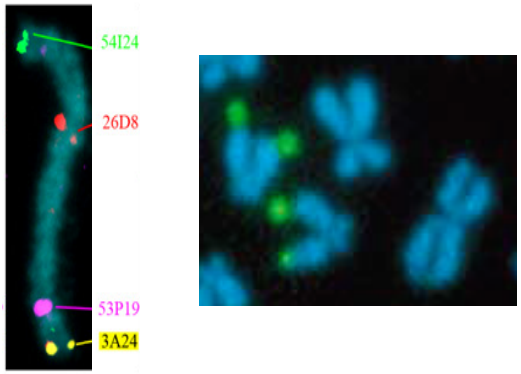
- painting probes

- locus specific




*Anolis distichus* Ravatsos et al. 2015

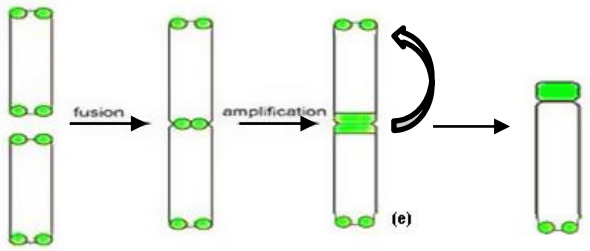




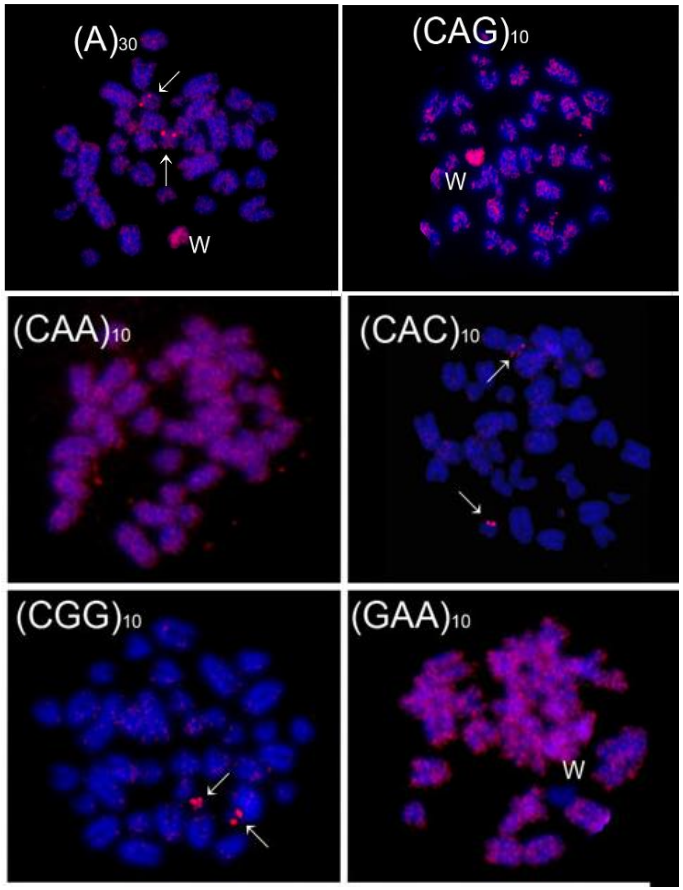



<u>telomere</u>

| | |
|---|---|
| Insects | (TTAGG)n |
| Vertebrates, Anellida, Mollusca | (TTAGGG)n |
| Nematoda | (TTAGGC)n |




het-ITS sutelo-ITS




*Eremias velox* Pokorná et al. 2011

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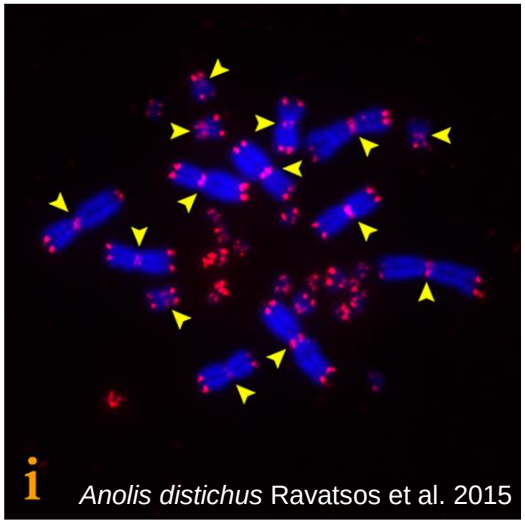


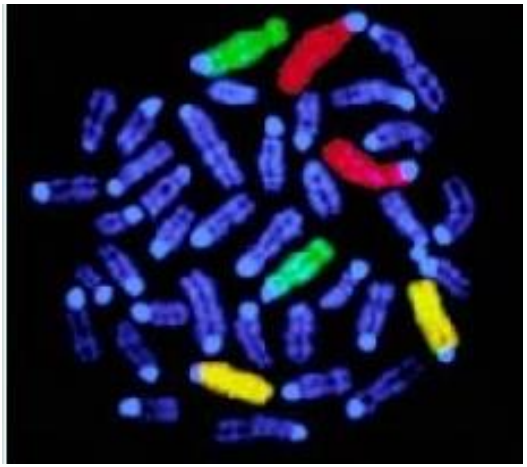

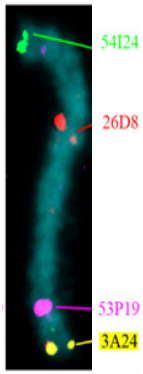


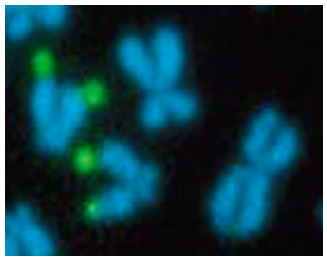

telomere

| | |
|---|---|
| Insects | (TTAGG)n |
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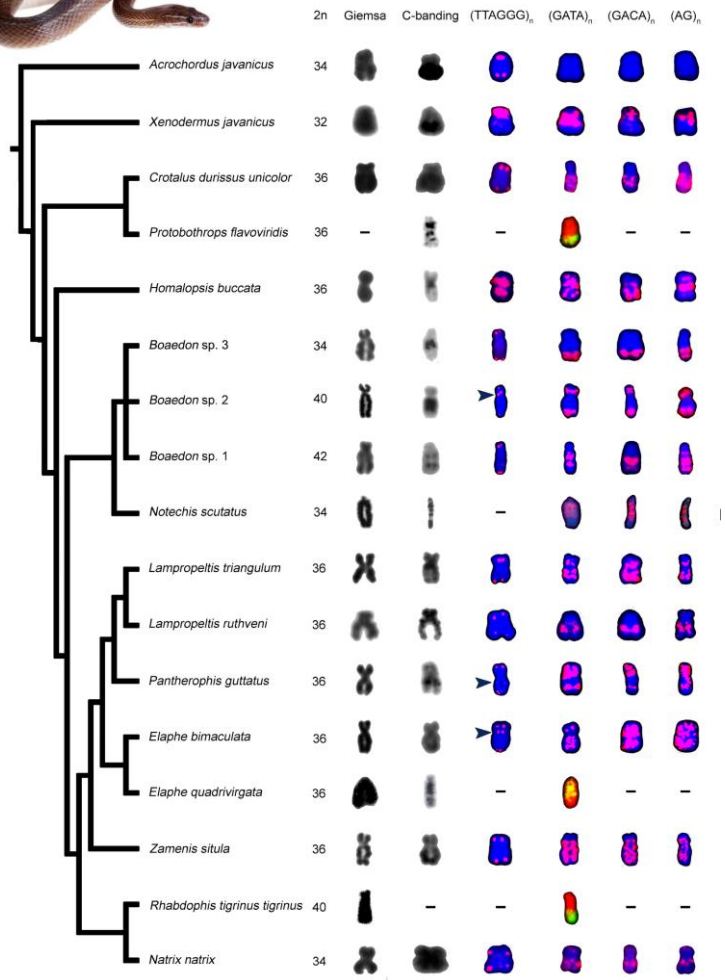
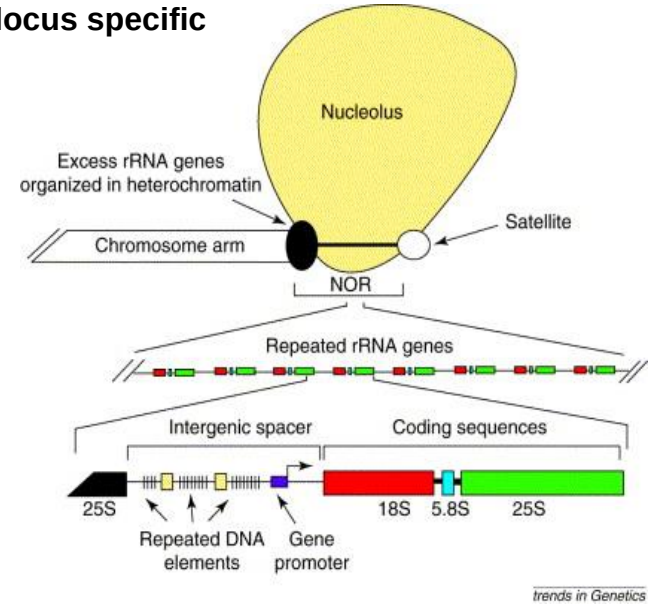


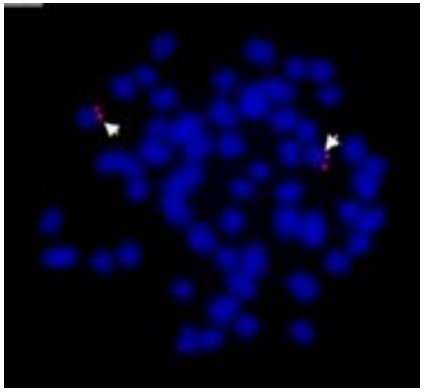

Augstenová et al. 2018, *Genes*

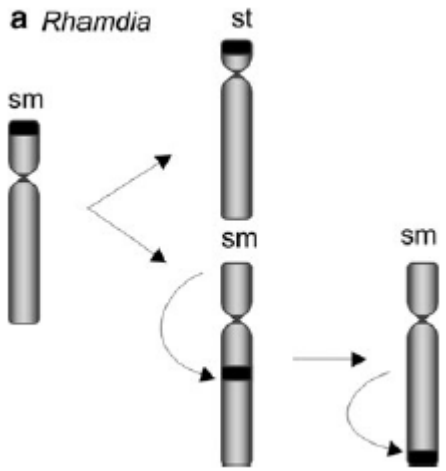
**locus specific**



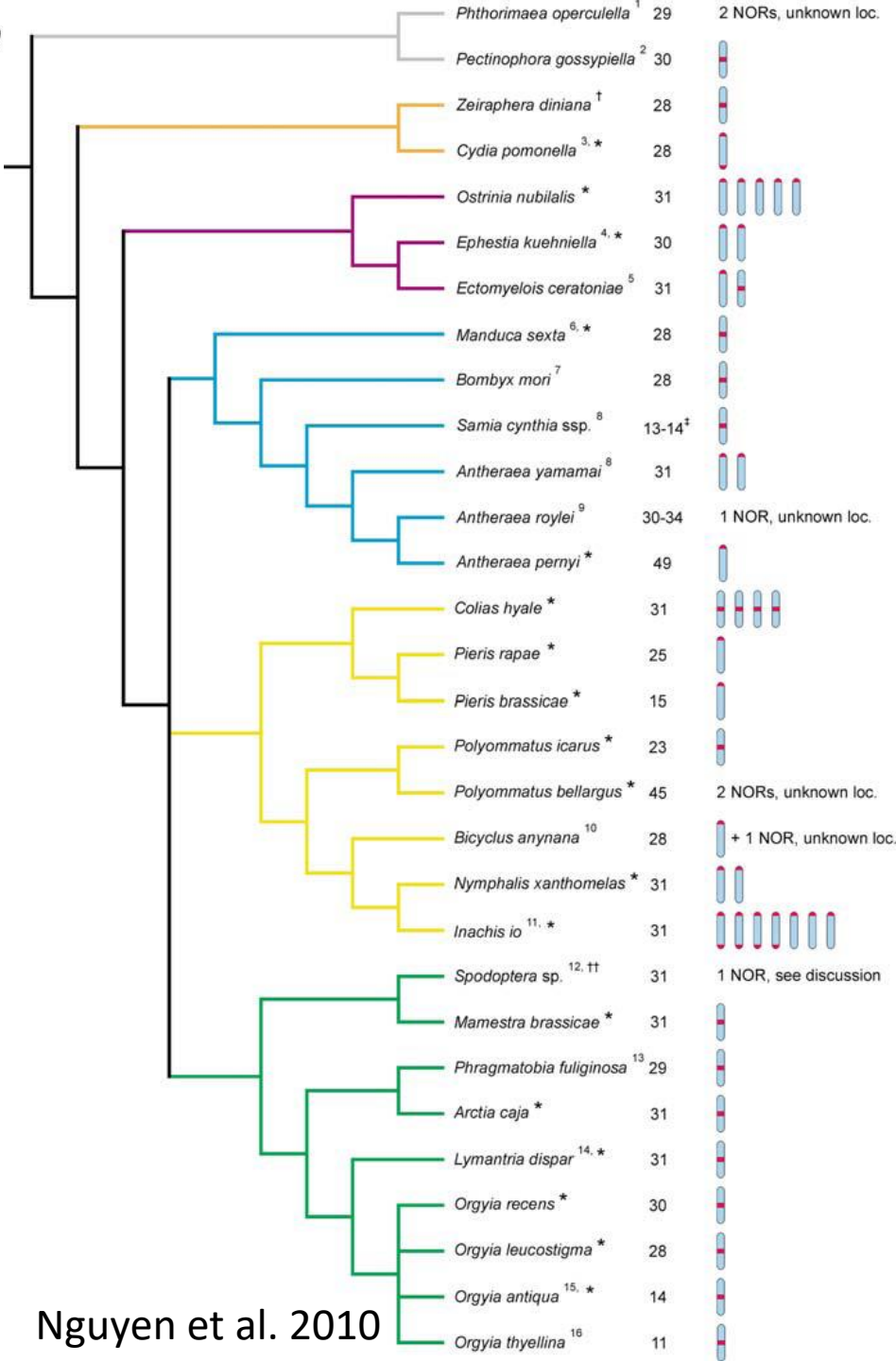

Ribosomal DNA (rDNA)
- loci encoding 5S and 45S (18S-5.8S-28S) rRNAs

18S rDNA probe



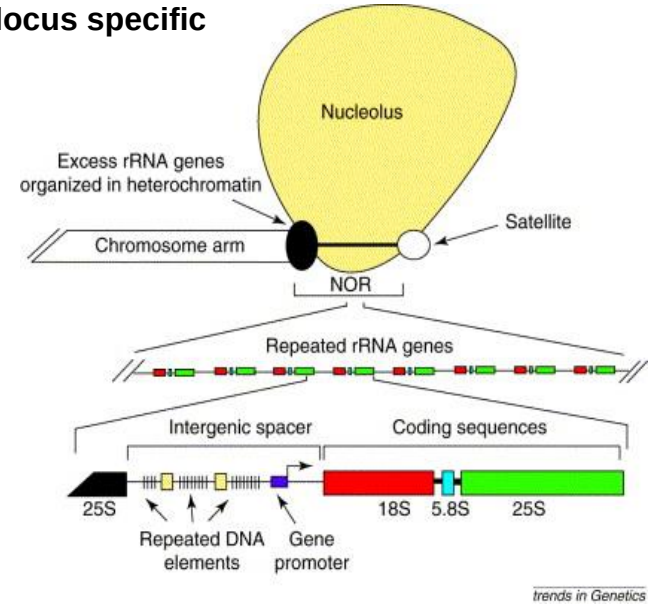



Borba et al. 2014




Nguyen et al. 2010

**locus specific**




Ribosomal DNA (rDNA)
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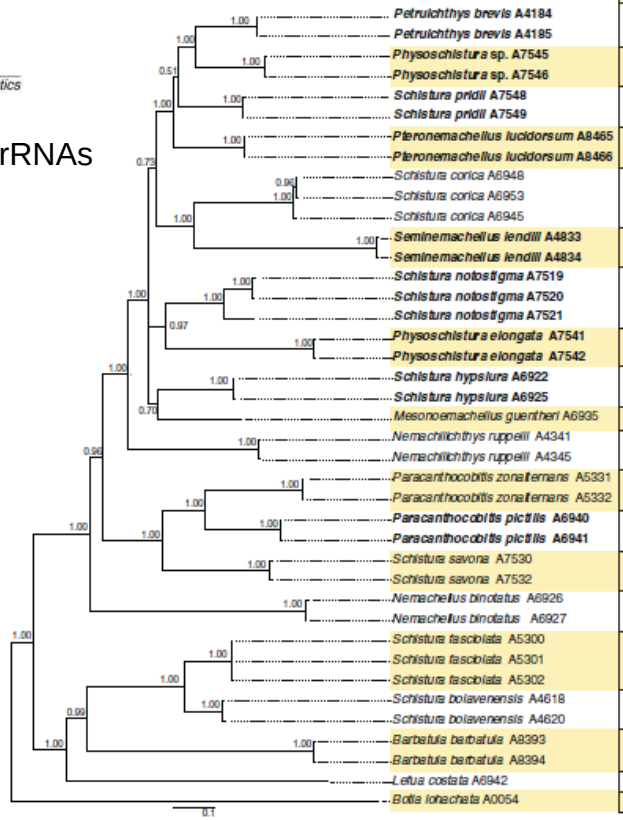
## Nemacheilidae

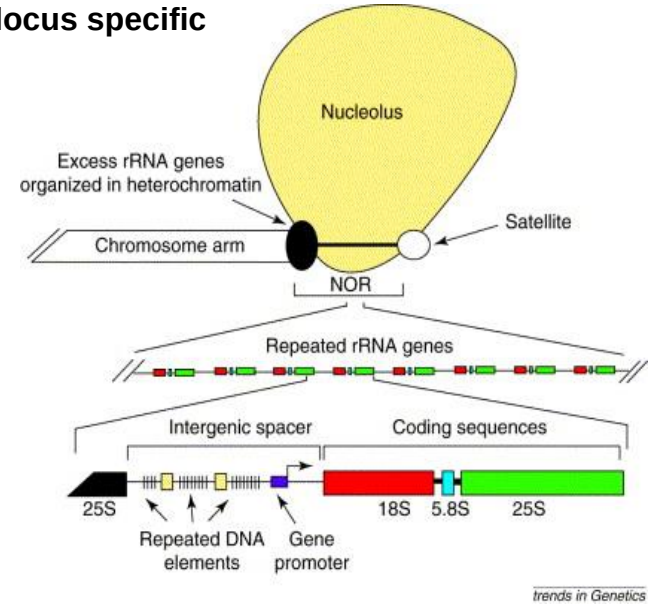
Sember et al. 2015




| 2n | Karyotype description | NF | Partial karyotypes with rDNA-bearing sites 45S 45/5S 5S |
|---|---|---|---|
| 50 | 8m - 16sm - 26st-a | 74 | |
| 50 | 10m - 12sm - 28st-a | 72 | |
| 50 | 8m - 18sm - 24st-a | 76 | |
| 50 | 8m - 12sm - 30st-a | 70 | |
| 50 | 12m - 18sm - 20st-a | 80 | |
| 50 | 16m - 24sm - 10st-a | 90 | |
| 44-48 | 20m - 18sm - 6st-a (NF=82) | | 18m - 14sm - 16st-a (NF=80) |
| 50 | 4m - 24sm - 22st-a | 78 | |
| 50 | 6m - 18sm - 26st-a | 74 | |
| 50 | 10m - 16sm - 24st-a | 76 | |
| 38 | 14m - 10sm - 14st-a | 62 | |
| 50 | 8m - 24sm - 18st-a | 82 | |
| 50 | 16m - 20sm - 14st-a | 86 | |
| 50 | 16m - 18sm - 16st-a | 84 | 45S: 2 - 4 signals, 5S: 2 - 8 signals (see the text) |
| 50 | 6m - 14sm - 30st-a | 70 | |
| 50-51 $XY_1Y_2$ | 8/9m - 22sm - 20st-a | 80-82 | |
| 50 | 8m - 24sm - 18st-a | 82 | |
| 50 | 6m - 12sm - 32st-a | 68 | |
| 50 | 4m - 20sm - 26st-a | 74 | |

45S rDNA sites  5S rDNA sites  [ ] variable polymorphic rDNA loci

**locus specific**

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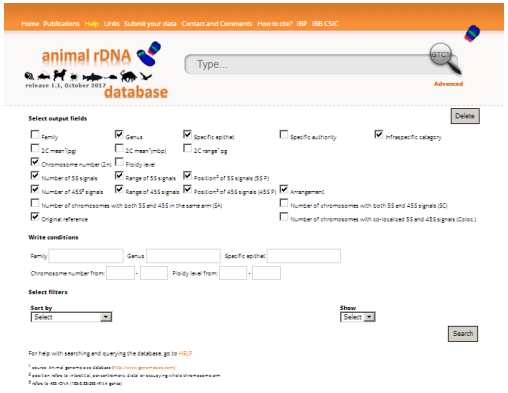
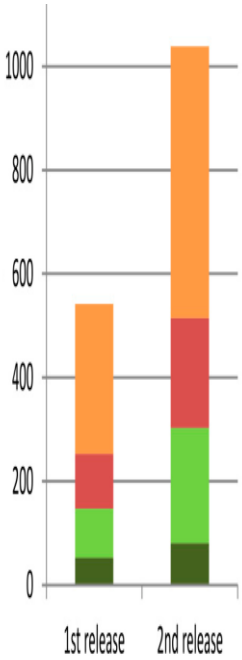



**http://www.animalrdnadatabase.com**

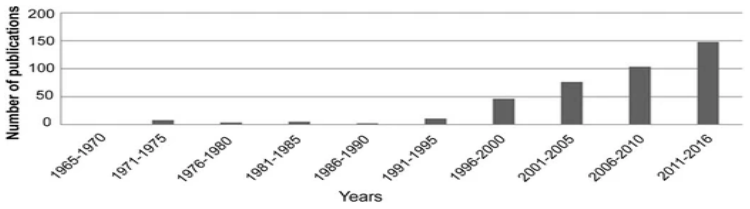
**2500 species**

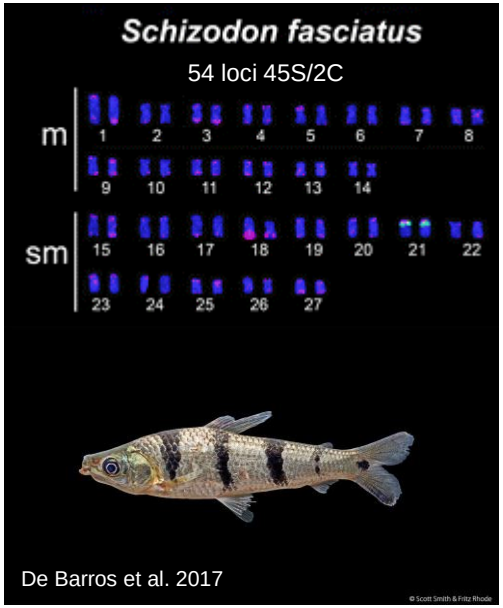
1068 Arthropods
653 fish

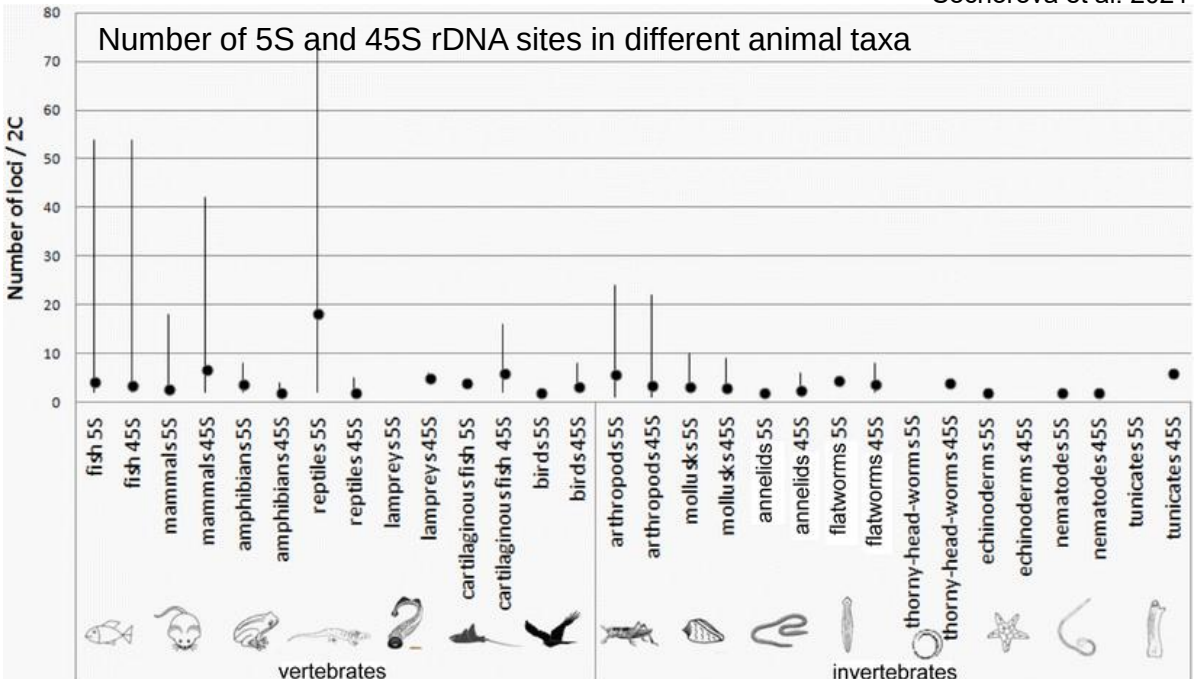



Sochorová et al. 2021

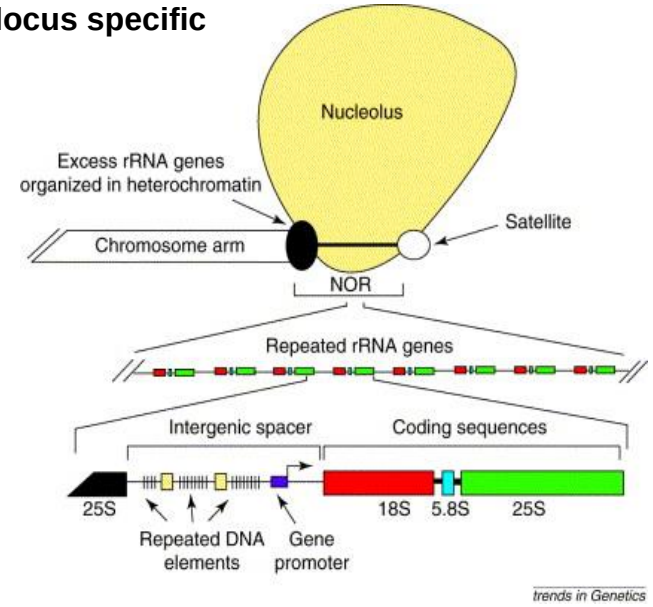
Sochorová et al. 2018



**locus specific**




Ribosomal DNA (rDNA)
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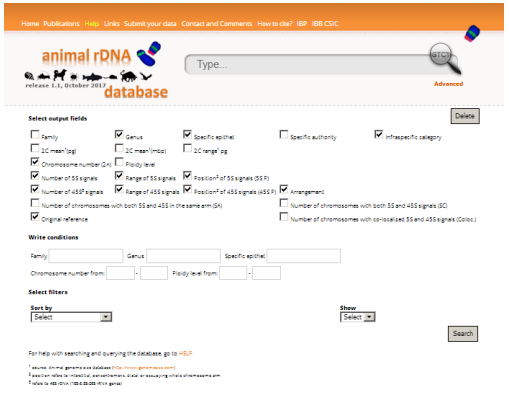
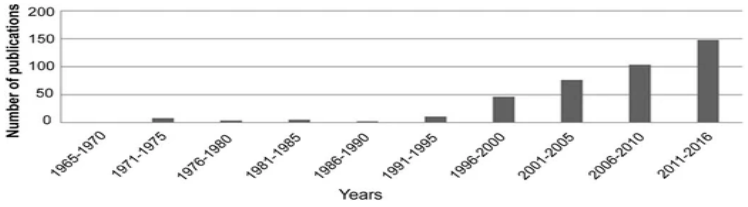



Sochorová et al. 2018

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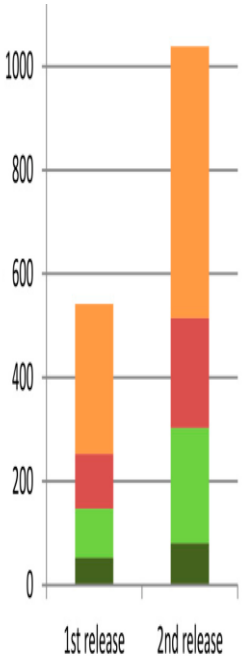
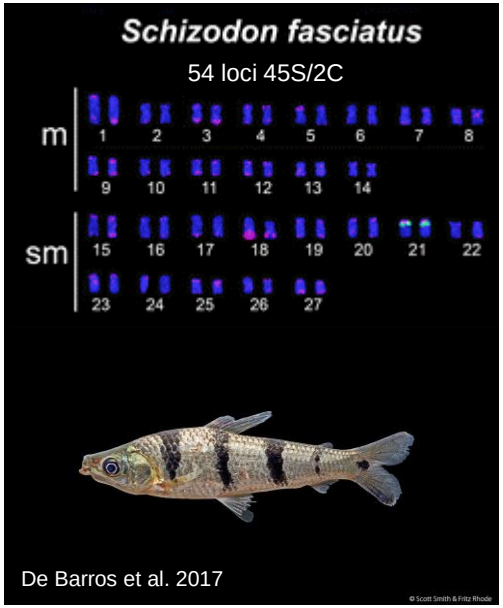
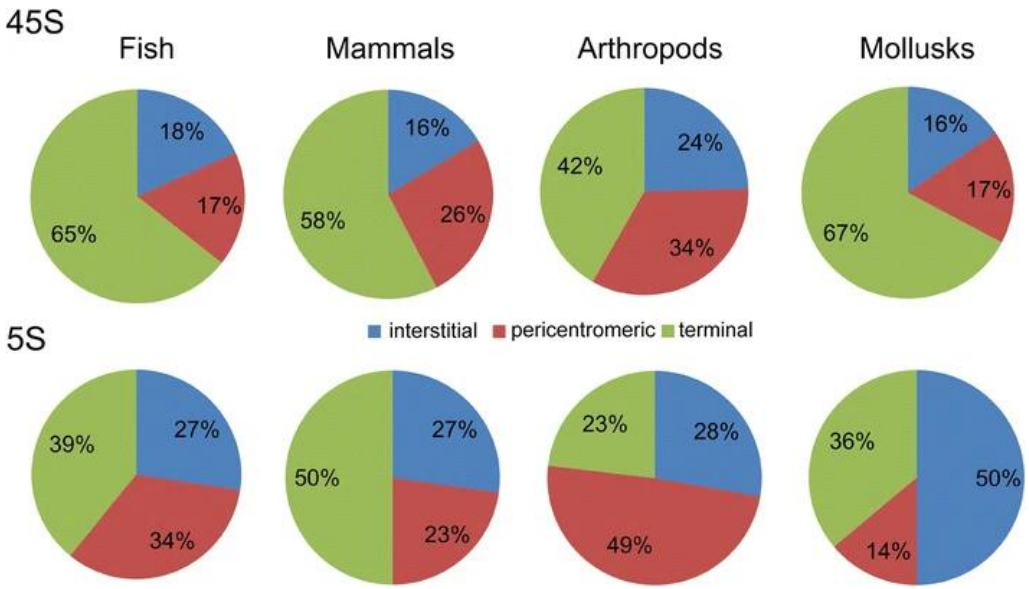



Sochorová et al. 2021



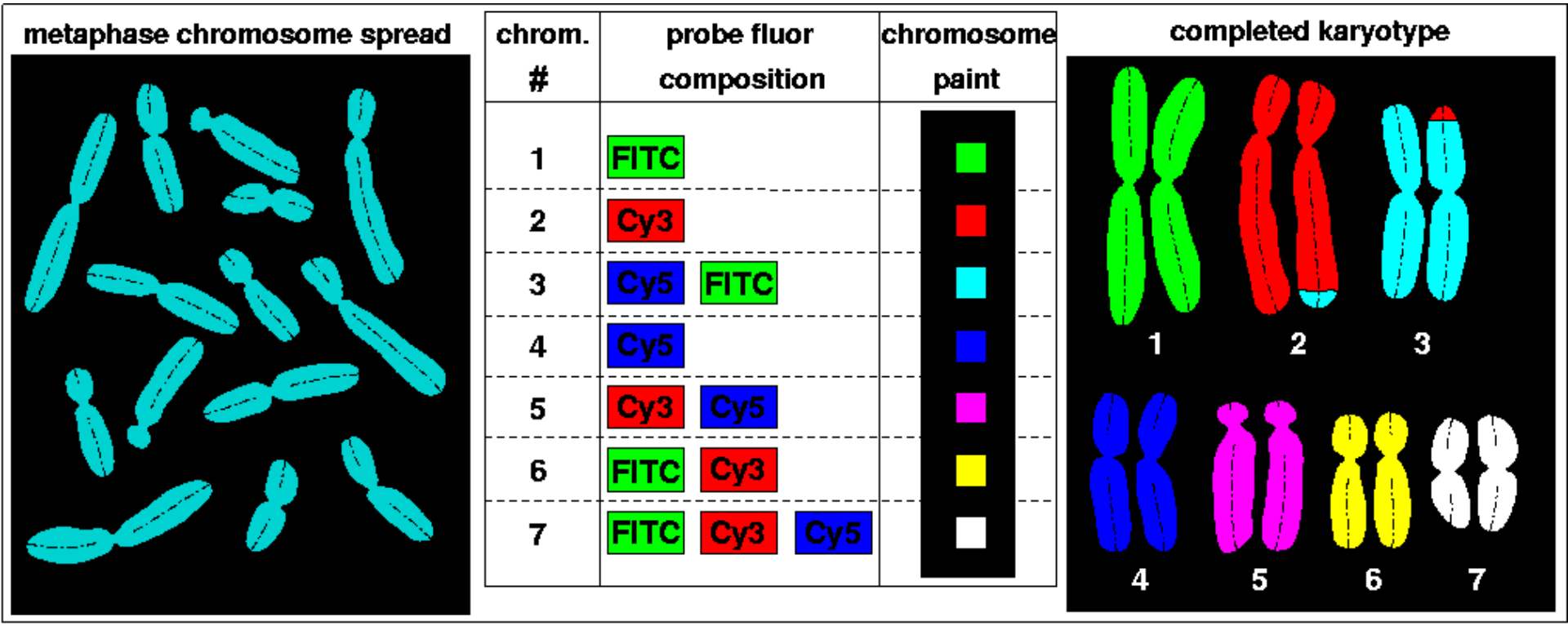

De Barros et al. 2017

Position of rDNA sites on chromosomes

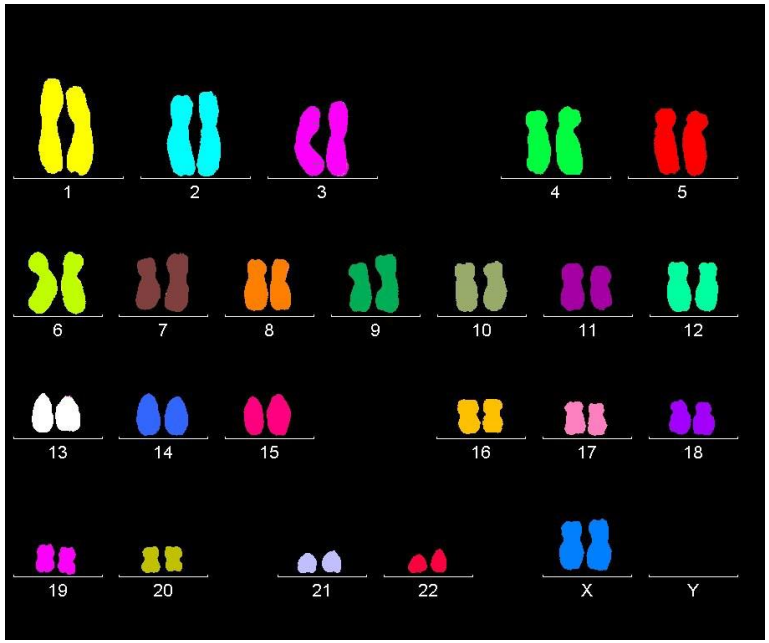


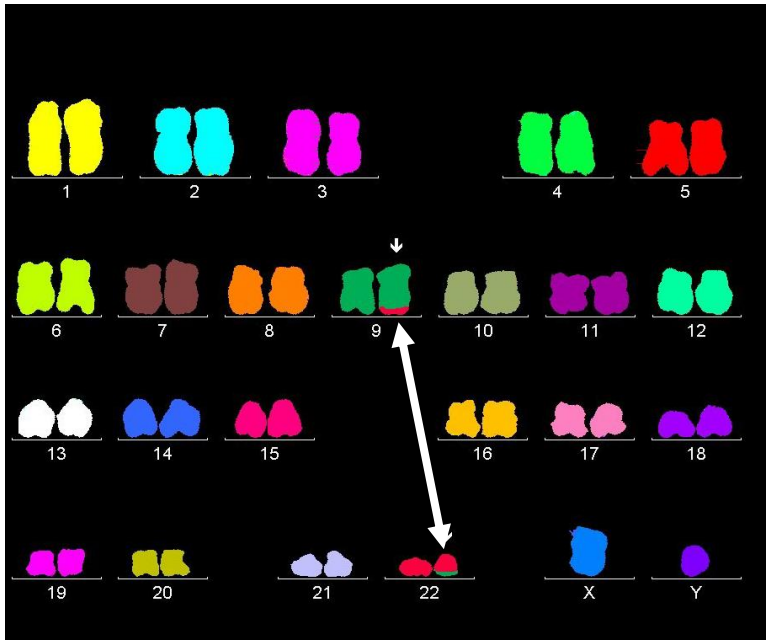
**M-FISH**

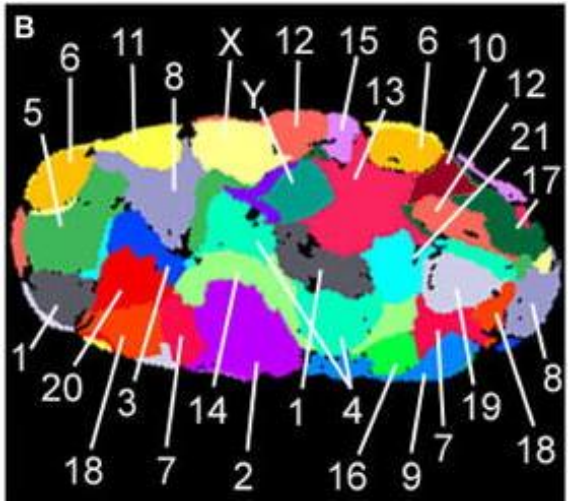
multiplex FISH or multicolour FISH

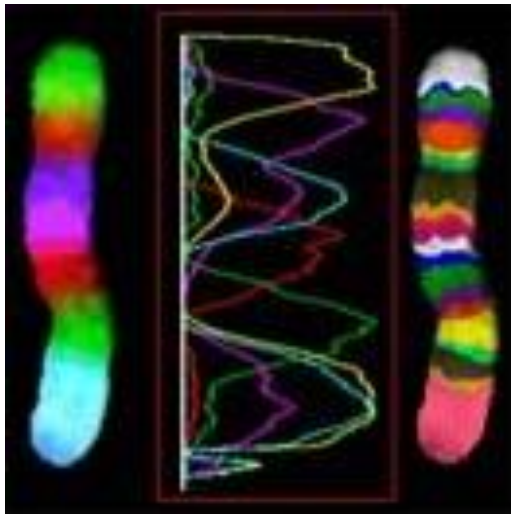


# M-FISH



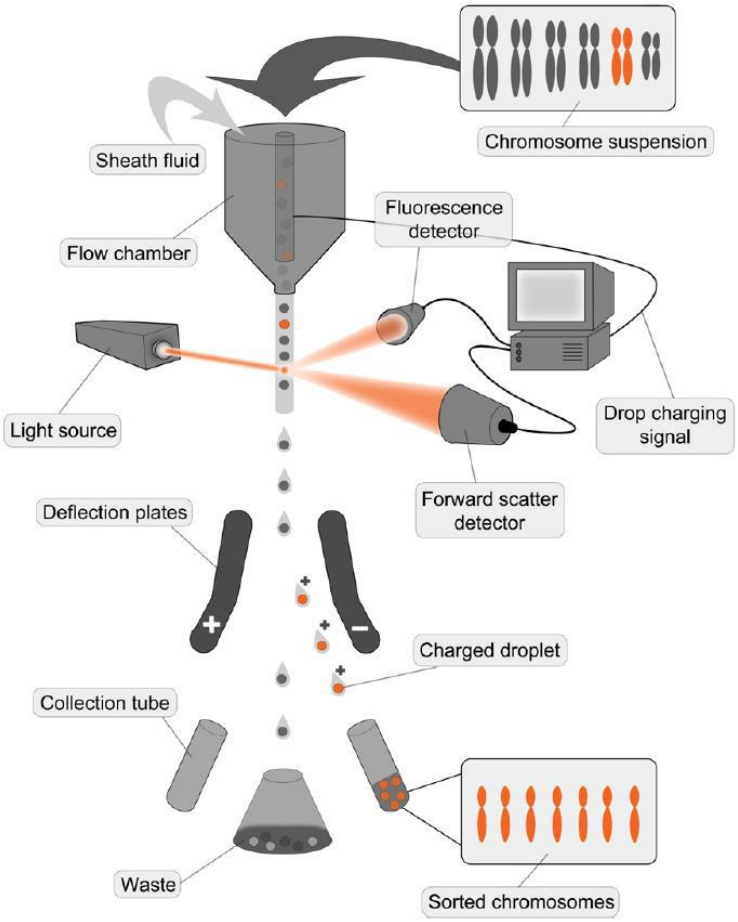



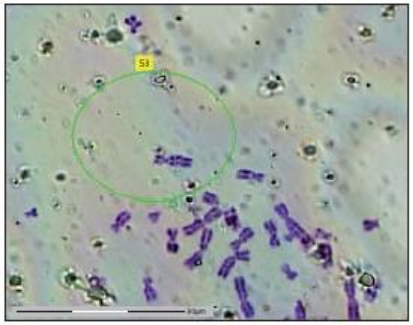



**Multicolour banding**
(mBAND)

# Chromosome sorting (flow cytometer)

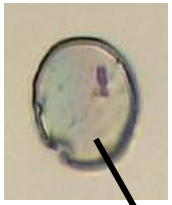



# Microdisection


I.


II.

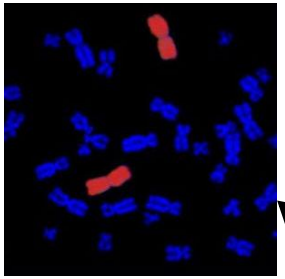

III.



*Taq* DNA polymerase

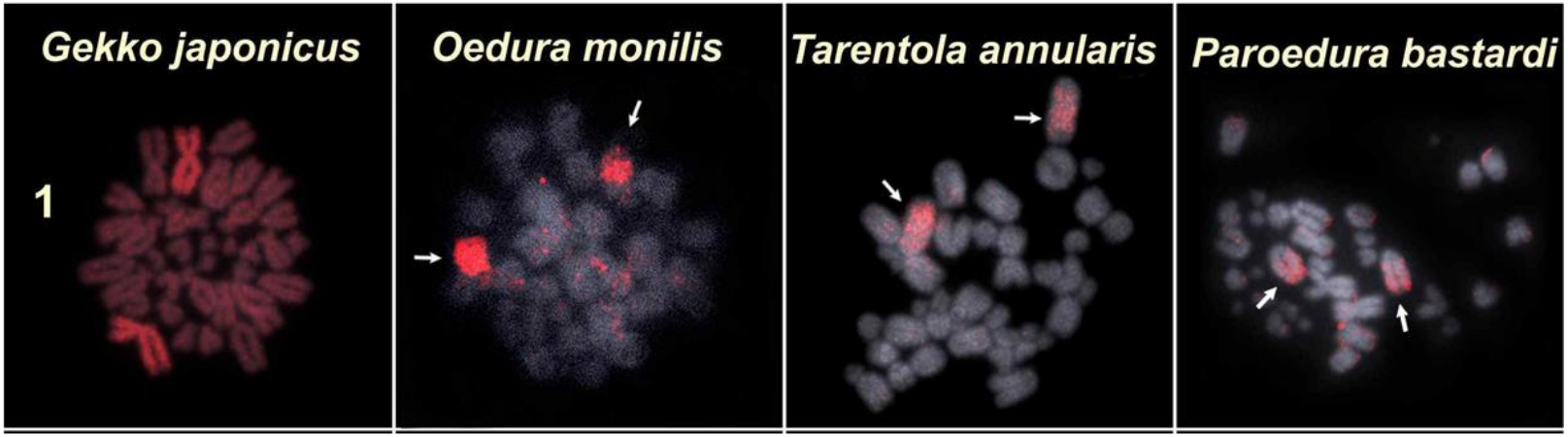
DOP primers

dNTPs

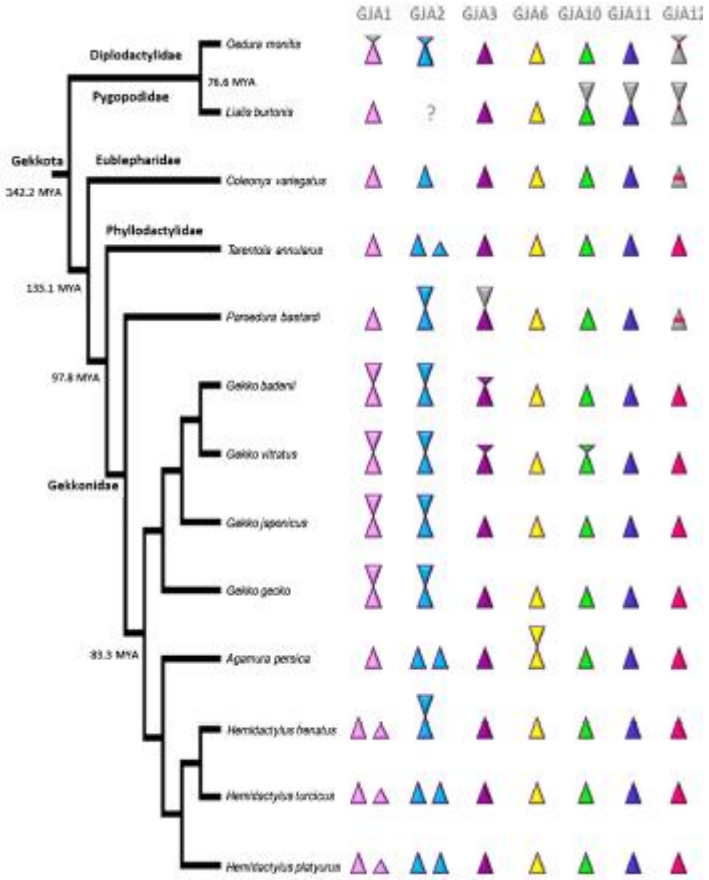


FISH labeling

DOP-PCR
(degenerated-oligonucleotide-primed PCR)

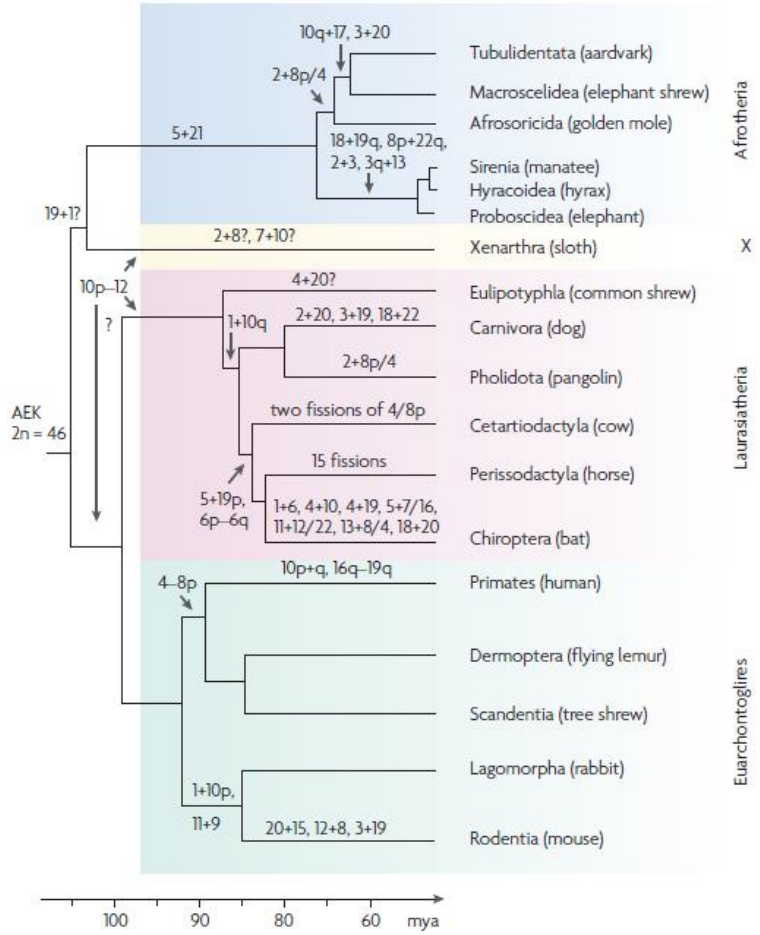



Pokorná et al. 2015, *Chrom. Res.*

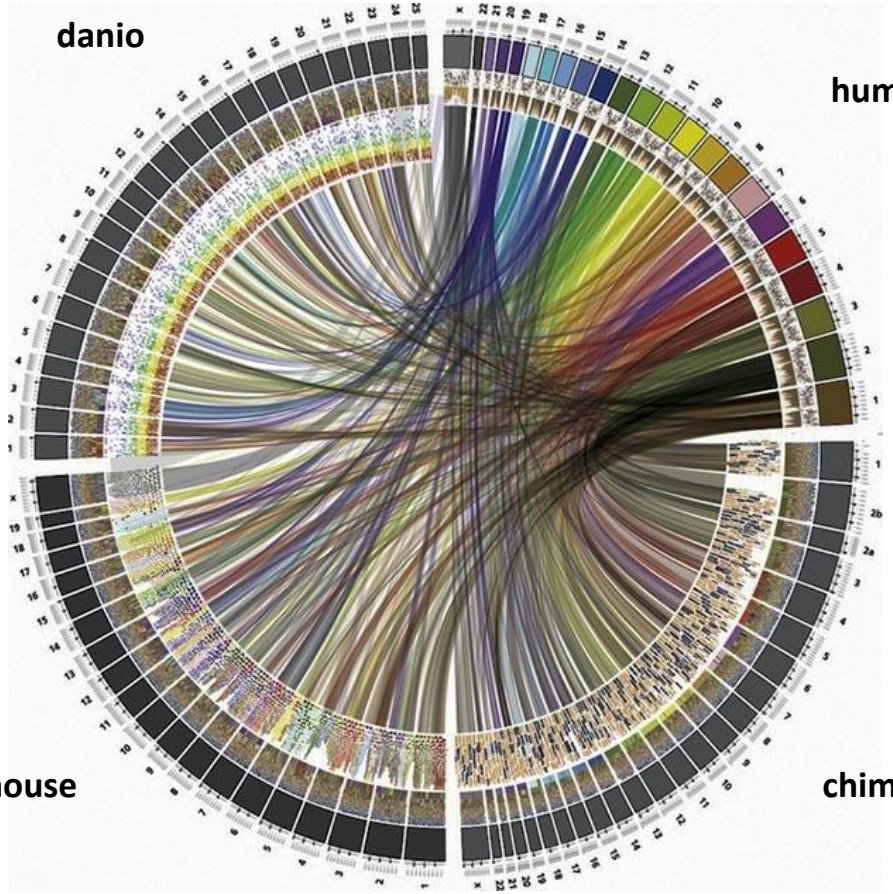


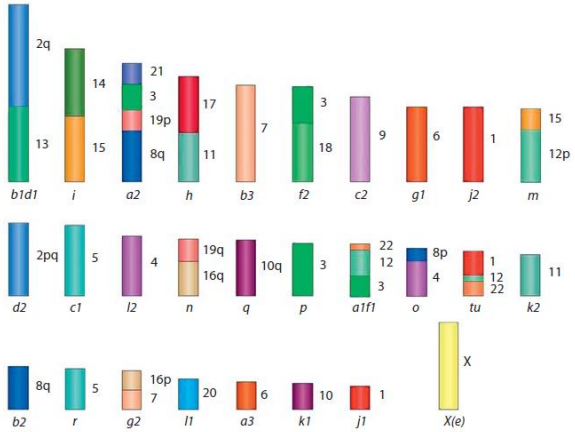
# ZOO-FISH

- cross-species chromosome painting, which uses painting probes specific for whole chromosomes, enables detecting homologous synteny blocks, the occurrence of which is evidence that species share a common ancestry and are related.




Ferguson-Smith & Trifonov 2007



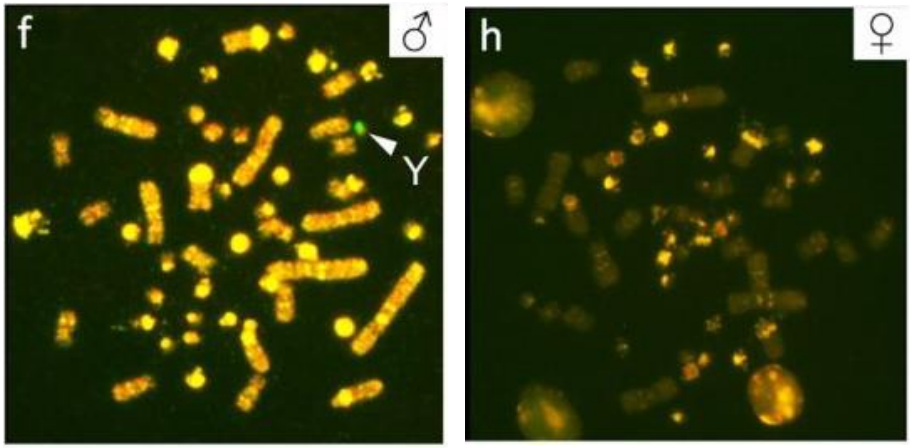



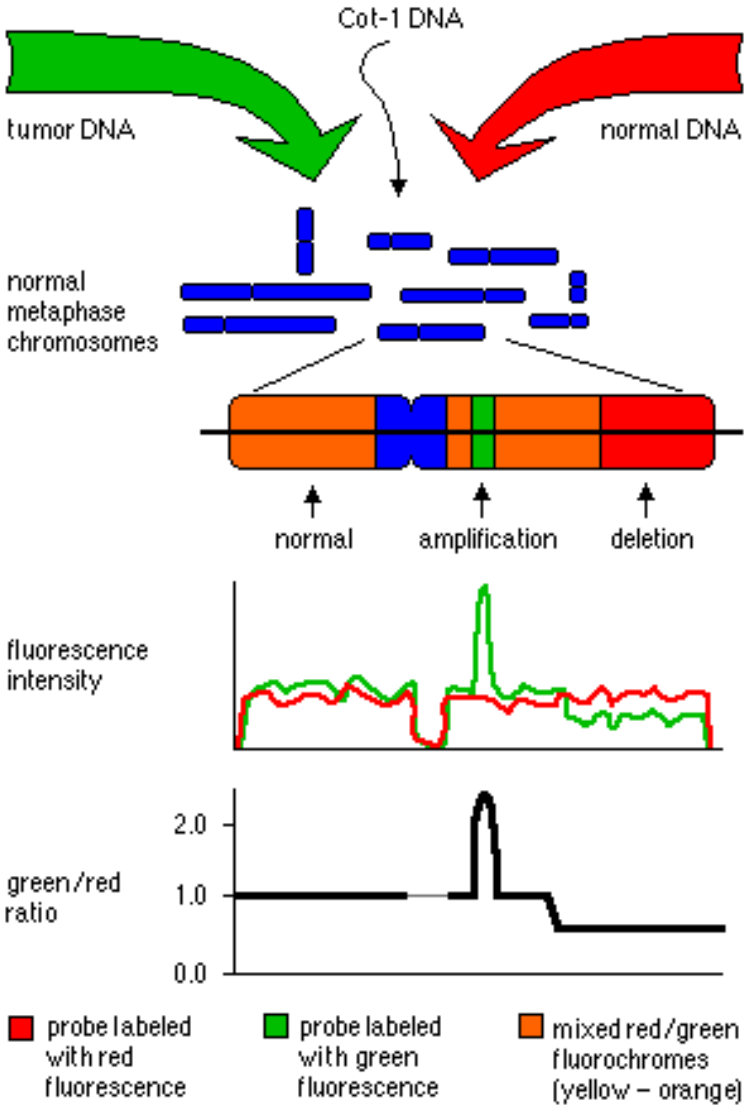

Biltueva et al. 2011

Ancestral karyotype of the genus *Sorex*
Used painting probes of human

# **CGH** – comparative genomic hybridization

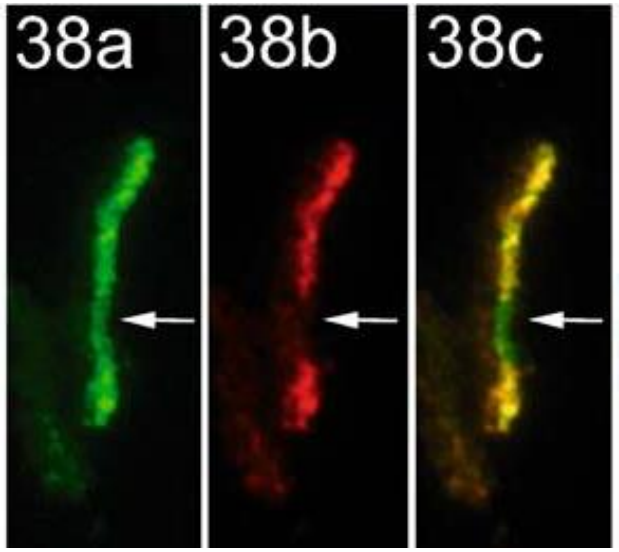
*Chelonida expansa*

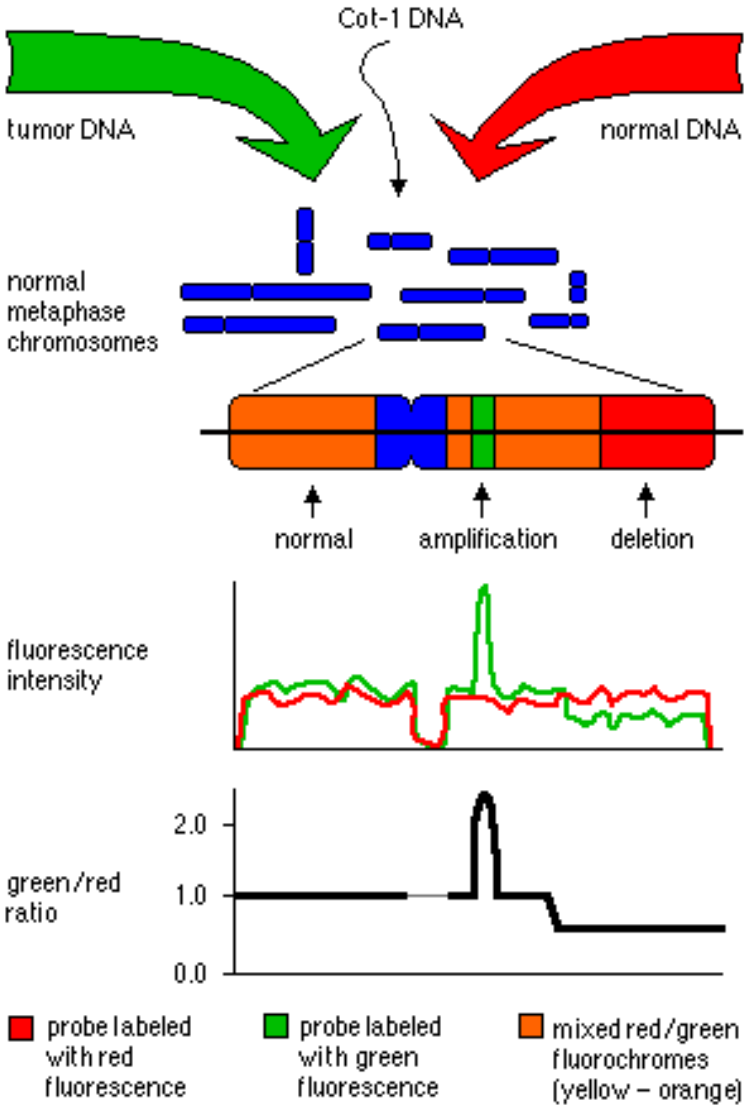




Mazzoleni et al. 2020, Sci. Rep.

# **CGH** – comparative genomic hybridization

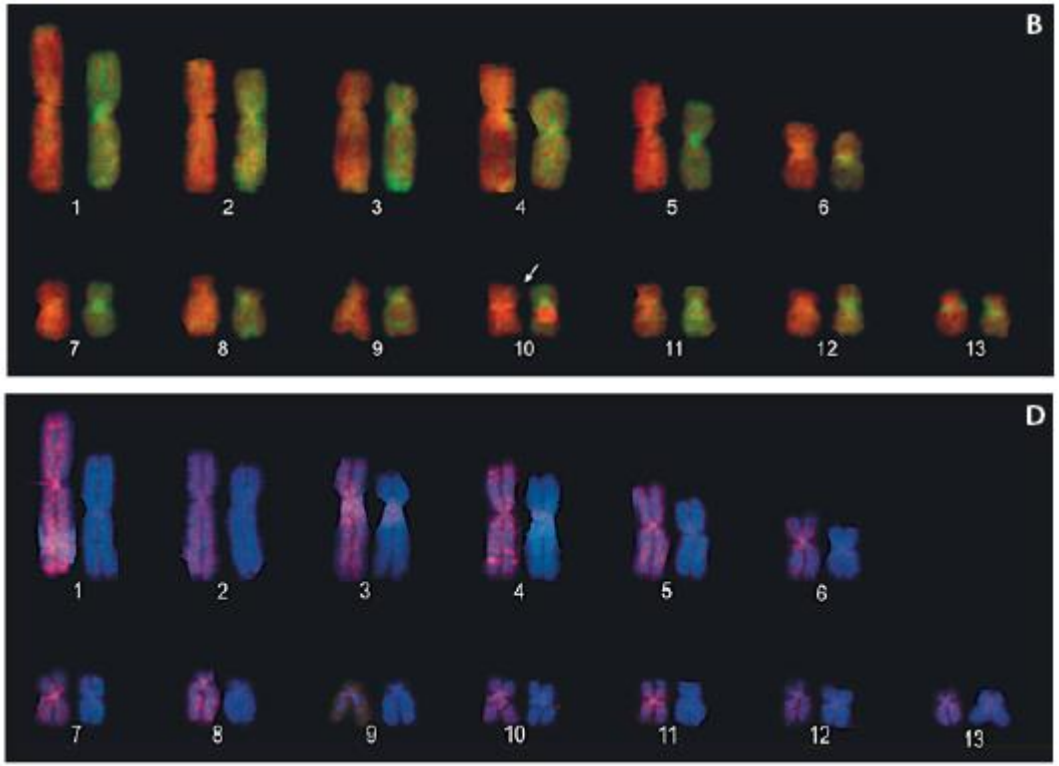


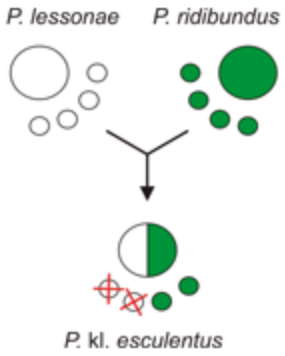

**Sex chromosomes**
CGH in pachytene of *Galleria mellonella*
female genomic probes were labelled with Alexa Fluor 488 (green)
male-derived genomic probes with Cy3 (red)
Arrow indicates a region of the W chromosome exclusively stained by female genomic probe.
Vítková et al. 2007



**GISH** – genomic *in situ* hybridization

- a type of FISH, uses total genomic DNA from one species as the labeled probe and unlabeled genomic DNA from another species at a much higher concentration as blocking DNA, substantially increasing the hybridization specifity




GISH on chromosomes of the water frog *Pelophylax esculentus* obtained from bone marrow of a single female.

**B.** Metaphase chromosomes hybridized with the Alexa Fluor 488-labeled genomic probe from *P. lessonae (green* signals); chromosomes were counterstained with PI (red)

**D.** Metaphase chromosomes hybridized with the Cy3-labeled genomic probe from *P. ridibundus (red* signals); chromosomes were counterstained with DAPI (blue). (Zalesna et al. 2011)

# Thank you for your attention