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 – term chromosome (chromo-, some-)
1902-04 – chromosomal inheritance theory

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

1990s – FISH techniques
Functions of chromosomes
- spatial distribution of genes
- 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)
scorpion *Tityus bahiensis* (2n=5-19)

Olpium turcicum: 2n = 7, X0
Monocentric chromosomes

Holocentric (holokinetic) chromosomes

www.metasystems-international.com/ikaros

Monocentric
Submetacentric
Subtelocentric
Acrocentric
Telocentric

www.lucia.cz

Chromosome

www.lucia.cz
Idiogram (ideogram) of *Tityus trivittatus*

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

Karyogram of *Bothriurus rochensis*
number of chromosomes

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

2n = 446 Plebicula atlantica

Modal number of chromosomes
Odonata n=13
Lepidoptera n=28-32
birds n=39-42
Diptera n=2-10
Scorpiones

Euscorpius (Alpiscorpius) germanus group

Diagram showing various anatomical structures labeled as et, est, em, esb, eb, V12, V9, V6, V5.
Scorpiones

Gantenbein et al. 2000

Scherabon et al. 2000

Euscorpius (Alpiscorpius) germanus group
"hybrid-sterility models" predicted that the recombination among rearranged chromosomes in heterokaryotypic hybrids generate unbalanced gametes and thus reduce fertility.

"suppressed-recombination models" suggest that the rearrangements reduce recombination between chromosomes and lead to the divergence and speciation.
<table>
<thead>
<tr>
<th>Order</th>
<th>Palaeartic region</th>
<th>Europe</th>
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<tbody>
<tr>
<td></td>
<td>total species</td>
<td>studied species</td>
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<tr>
<td>Insectivora</td>
<td>81</td>
<td>72 (88.9%)</td>
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<tr>
<td>Chiroptera</td>
<td>73</td>
<td>57 (78.1%)</td>
</tr>
<tr>
<td>Rodentia</td>
<td>299</td>
<td>240 (80.3%)</td>
</tr>
</tbody>
</table>

Fish app. 30000 species
- 1700 karyotyped

Coleoptera Karyotypes
http://www.uta.edu/karyodb/

Arachnids Karyotypes
http://www.arthropodacytogenetics.bio.br/index.html
types of chromosomal rearrangements

Single Chromosome Structural Changes
- Deletion
- Duplication
- Inversion

Two Chromosome Structural Changes
- Insertion
- Translocation

Copy Number Neutral Events

Pericentric Inversion:
- Breaks in Chromosome
- Centromere
- Inversion
- Reinserted Piece of DNA with Centromere

Paracentric Inversion:
- Centromere
- Breaks in Chromosome
- Inversion
- Reinserted Piece of DNA
types of chromosomal rearrangements
Centric fusions - Robertsonian translocations or fissions

Mus musculus domesticus

Tandem fusion

Chthonius (E.) tetrachelatus  
2n = 35

Phillips & Ráb 2001

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>2n</th>
<th>NF</th>
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<tbody>
<tr>
<td>Salmo</td>
<td>Bull trout</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>fontinalis</td>
<td>Brook trout</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>taimen</td>
<td>Lake trout</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>leuciscus</td>
<td>Potted char</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>nissus</td>
<td>Japanese char</td>
<td>84–86</td>
<td>100</td>
</tr>
<tr>
<td>alpinus/malma complex</td>
<td>Dolly Varden char</td>
<td>82</td>
<td>98</td>
</tr>
<tr>
<td>alpinus</td>
<td>White char</td>
<td>78–80</td>
<td>98</td>
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<td>arcticus</td>
<td>Arctic char</td>
<td>78</td>
<td>98</td>
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<td>elgicus</td>
<td>Small mouthed char</td>
<td>76–78</td>
<td>98</td>
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<tr>
<td>boygianus</td>
<td>Boganiid char</td>
<td>78–82</td>
<td>100</td>
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<tr>
<td>toranski</td>
<td>Stone char</td>
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<td>98–100</td>
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<tr>
<td>levandovski</td>
<td>Eastern Arctic char</td>
<td>78–80</td>
<td>98</td>
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<td>malma</td>
<td>Levandovski char</td>
<td>78–80</td>
<td>98</td>
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<td>m. m.</td>
<td>Dolly Varden char</td>
<td>78</td>
<td>98</td>
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<tr>
<td>malma</td>
<td>Dolly Varden char</td>
<td>82</td>
<td>98</td>
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<tr>
<td>longimundus</td>
<td>Longfinsed char</td>
<td>56</td>
<td>98</td>
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<tr>
<td>Chthonius (E.) sp. 1</td>
<td>2n = 29</td>
<td></td>
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</tr>
</tbody>
</table>
Mitosis

- S-phase: DNA synthesis
- G1, G2, 4h, 9h, 10h

Interphase (centrioles, nucleus, nuclear envelope, chromatids)
- Prophase (developing spindle, centrioles)
- Prometaphase (nuclear envelope, kinetochore microtubules)
- Metaphase (equatorial plate, chromatids)
- Anaphase (chromatids)
- Telophase and Cytokinesis
Meiosis
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$_2$C$_6$H$_5$O$_7$)

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

- Spreading
  “dropping”
  “squashing”
  “plate spreading”
Conventional staining – homogeneous staining

**Giemsa**

**Haematoxylin**

**Acid-Schiff staining**

**Carbol fuchsin**

Bothriurus rochensis

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**Chthonius (E.) fuscimanus**

2n = 35

**Chthonius (E.) tetrachelatus**

2n = 35

**Chthonius (E.) sp. 1**

2n = 29

**Chthonius (E.) sp. 2**

2n = 21

Šťáhlavský & Král 2004

Number, morphology and size of chromosomes
Conventional staining – homogeneous staining

**Giensma**

- **Hottentotta judaicus**
  - Qumsiyeh et al. 2013

**sex chromosomes, rearrangements**

- *Gagrellopsis nodulifera* (Gorlov & Tsurusaki 2000)
  - Kral et al. 2006

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

Number, morphology and size of chromosomes

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>Ref.</th>
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<td>(FM)</td>
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<tr>
<td>Eublepharis macularius</td>
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<td>(FM)</td>
</tr>
<tr>
<td>Holodactylus africanus</td>
<td>38</td>
<td>(FM)</td>
</tr>
<tr>
<td>Hemitheconyx caudicinctus</td>
<td>38</td>
<td>(FM)</td>
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<tr>
<td>Goniurosaurus lichtenfelderi</td>
<td>38</td>
<td>(FM)</td>
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<td>Goniurosaurus araneus</td>
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<td>(FM)</td>
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<td>Goniurosaurus splendens</td>
<td>24</td>
<td>(FM)</td>
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<tr>
<td>Goniurosaurus kuroiwaee</td>
<td>24</td>
<td>(FM)</td>
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<td>Coleonyx brevis</td>
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<td>Coleonyx variegatus</td>
<td>32</td>
<td>(FM)</td>
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<td>Coleonyx switaki</td>
<td>24</td>
<td>(M)</td>
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<tr>
<td>Coleonyx elegans</td>
<td>32</td>
<td>(F) / 31</td>
</tr>
</tbody>
</table>

Pokorná et al. 2010
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)$_2$ 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
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 AgNO₃ in 1 mL of 0.02 g sodium citrate (C₆H₅Na₃O₇·2 H₂O) 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.
Selective staining – for specific 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-band**ing (stained by **quinacrin**)

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

Biltueva et al. 2011
Selective staining – for specific 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 A₃, 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 - Fluorescence *In Situ* Hybridization

**Figure a:** Diagram showing the process of FISH. Probes bind to the target, and labeling can be indirect or direct.

**Figure b:** Indirect labeling process.

**Figure c:** Direct labeling process.

**Figure d:** Hybridization process involving biotin and avidin.

**Figure e:** Diagram showing the flow of fluorescence excitation, emission, and non-radiative quenching.
FISH - Fluorescence *In Situ* Hybridization

**a**

Probe → indirect labeling → direct labeling → Target

**b**

Probe → indirect labeling → direct labeling → Amplification

**c**

Probe → direct labeling → Amplification

**d**

Probe → Amplification

**e**

cloned DNA probe

**FISH-TSA**

- Biotin
- Streptavidin
- Horseradish Peroxidase
- Tyramide
- Fluorophore
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)
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td>Centromere-FISH (ACM-FISH)</td>
<td>Immuno-FISH</td>
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<tr>
<td>armFISH</td>
<td>Locked Nucleic Acids (LNAs)-FISH</td>
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<tr>
<td>Catalyzed Reporter Deposition-FISH (CARD-FISH)</td>
<td>Multilocus or ML-FISH</td>
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<tr>
<td>Cellular Compartment Analysis of Temporal (Cat) Activity by Fish (catFISH)</td>
<td>Premature Chromosome Condensation (PCC)-FISH</td>
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<tr>
<td>Cytochalasin B (CB-FISH)</td>
<td>Peptide Nucleic Acid (PNA)-FISH</td>
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<td>Chromosome Orientation (CO)-FISH</td>
<td>Quantitative-FISH (Q-FISH)</td>
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<td>Combined Binary Ratio (COBRA)-FISH</td>
<td>Quantum Dot (QD)-FISH</td>
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<td>Chromosome Orientation and Direction (COD)-FISH</td>
<td>Rainbow-FISH</td>
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<td>Combinatorial Oligonucleotide (COMBO)-FISH</td>
<td>Raman-FISH</td>
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<td>Comet-FISH</td>
<td>Replicative Detargeting FISH (ReD-FISH)</td>
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<td>Cryo-FISH</td>
<td>Reverse-FISH</td>
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<td>Double Fusion FISH (D-FISH)</td>
<td>Recognition of Individual Genes (RING)-FISH</td>
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<td>DNA Breakage Detection FISH (DBD-FISH)</td>
<td>RNA-FISH</td>
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<td>e-FISH</td>
<td>Cross Species Color Banding (Rx)-FISH</td>
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<td>Fiber-FISH</td>
<td>Split-Signal FISH</td>
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<td>Flow-FISH</td>
<td>Stellaris RNA FISH (Single-Molecule RNA FISH)</td>
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<td>Fusion-Signal FISH</td>
<td>T-FISH</td>
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<td>Halo-FISH</td>
<td>3-D FISH</td>
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<td>Harlequin-FISH</td>
<td>Zoo-FISH</td>
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Types of probes

- Satellite DNA
  - centromeric
  - telomeric

- painting probes

- locus specific

telomere
Insects
Vertebrates, Anellida, Mollusca
Nematoda

(RTAGG)n
(RTAGGG)n
(RTAGGC)n

Pokorná et al. 2011
locus specific

18S rDNA probe

Nguyen et al. 2010

Borba et al. 2014
locus specific

http://www.animalrdnadatabase.com

Sember et al. 2015

Nemacheilidae

<table>
<thead>
<tr>
<th>2n</th>
<th>Karyotype description</th>
<th>NF</th>
<th>Partial karyotypes with rDNA-bearing sites</th>
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<tbody>
<tr>
<td>48</td>
<td>18m - 24m - 16st-a</td>
<td>62</td>
<td>18m - 24m - 16st-a (NF-62)</td>
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<tr>
<td>50</td>
<td>58 - 16m - 26st-a</td>
<td>74</td>
<td>45S: 2.4 signals, 55S: 2.8 signals (see text)</td>
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<tr>
<td>50</td>
<td>68 - 16m - 26st-a</td>
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<td>78 - 16m - 26st-a</td>
<td>74</td>
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<td>88 - 16m - 26st-a</td>
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<tr>
<td>50</td>
<td>108 - 16m - 26st-a</td>
<td>74</td>
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Legend:
- 45S DNA sites
- 55S DNA sites
- Variable polymorphic rDNA sites

http://www.animalrdnadatabase.com
M-FISH
multiplex FISH or multicolour FISH

<table>
<thead>
<tr>
<th>chrom. #</th>
<th>probe fluor composition</th>
<th>chromosome paint</th>
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<tr>
<td>1</td>
<td>FITC</td>
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<td>2</td>
<td>Cy3</td>
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<td>3</td>
<td>Cy5, FITC</td>
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<td>Cy3, Cy5</td>
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<tr>
<td>7</td>
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</table>

completed karyotype

metaphase chromosome spread
M-FISH

Multicolour banding (mBAND)
Chromosome sorting

Fig. 1. Schematic of a flow cytometer capable of sorting two different populations of chromosomes.

Microdissection

DOP-PCR (degenerated-oligonucleotide-primed PCR)

FISH labeling

Taq DNA polymerase
DOP primers
dNTPs

![Karyotype images](image)

Pokorná et al. 2012, *Chromosoma*

![Phylogenetic tree](image)
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.

Ancestral karyotype of the genus *Sorex* Biltueva et al. 2011
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 specificity

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