Cytogenetics with special reference to domestic animals

Essential Genetics: a genomic perspective.
Seminar 4

Overview

- Studying chromosomes
- The normal karyotypes of domestic animals
- Chromosome abnormalities
- Chromosome abnormalities of domestic animals
Studying chromosomes

- Classical karyotyping
- Fluorescent in Situ Hybridization (FISH)
- Chromosome painting
- Molecular karyotyping
- Chromosome sorting

Classical karyotyping

- Obtaining and preparing cells for chromosome analysis
- Karyotyping and chromosome banding
Obtaining and preparing cells for chromosome analysis

- Cell source:
  - Blood cells
  - Skin fibroblasts
  - Amniotic cells / chorionic villi

- Increasing the mitotic index (proportion of cells in mitosis) using colcemid

- Synchronizing cells to analyze promoeptahase chromosomes

Karyotyping and chromosome banding

- Chromosome banding
- Molecular interpretation of chromosome bands
- Chromosome nomenclature
Chromosome banding

Box 2.2: Chromosome banding.

G-banding — the chromosomes are subjected to controlled digestion with trypsin before staining with Giemsa, a DNA-binding chemical dye. Paucely staining dark bands are known as G bands. Pale bands are G negative.

D-banding — the chromosomes are stained with a fluorescent dye which binds preferentially to AT-rich DNA, such as Quinacrine, DAPI (4′,6-diamidino-2-phenylindole) or Hoechst 33258, and viewed by UV fluorescence. Fluorescing bands are called D bands and mark the same chromosomal segments as G bands.

R-banding — is essentially the reverse of the G-banding pattern. The chromosomes are heat-denatured in saline before being stained with Giemsa. The heat treatment denatures AT-rich DNA, and R bands are G negative. The same pattern can be produced by binding G-staining dyes such as chromomycin A3, chromomycin A2, or acriflavine.

T-banding — identifies a subset of the R bands which are especially concentrated at the telomeres. The T bands are the most intensely staining of the R bands and are visualized by using either a particularly severe heat treatment of the chromosomes prior to staining with Giemsa, or a combination of dyes and fluorochromes.

C-banding — is thought to demonstrate constitutive heterochromatin, mainly at the centromeres. The chromosomes are typically exposed to denaturation with a saturated solution of barium hydroxide, prior to Giemsa staining.
Molecular interpretation

- G+ bands (= R- bands)
  - AT rich
  - Gene poor
  - LINE rich
  - Late replicating
  - SAR (Scaffold Attachment Regions) rich

From DNA duplex to metaphase chromosome

![Diagram showing DNA duplex and metaphase chromosome conversion](image)
Molecular interpretation

- G- bands (= R+ bands)
  - GC rich
  - Gene rich
  - SINE rich
  - Early replicating
  - SAR (Scaffold Attachment Regions) poor

Chromosome nomenclature

<table>
<thead>
<tr>
<th>Group</th>
<th>Chromosomes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1-3</td>
<td>Largest; 1 and 2 are submetacentric but 2 is subcentric.</td>
</tr>
<tr>
<td>B</td>
<td>4,5</td>
<td>Larger submetacentric with two arms very different in size.</td>
</tr>
<tr>
<td>C</td>
<td>6-12, X</td>
<td>Medium size, submetacentric.</td>
</tr>
<tr>
<td>D</td>
<td>13-15</td>
<td>Medium size, submetacentric with satellites.</td>
</tr>
<tr>
<td>E</td>
<td>16-18</td>
<td>Small, 16 is metacentric but 17 and 18 are submetacentric.</td>
</tr>
<tr>
<td>F</td>
<td>19-20</td>
<td>Small, metacentric</td>
</tr>
<tr>
<td>G</td>
<td>21-22, Y</td>
<td>Small, acrocentric, with satellites on 21 and 22 but not on the Y.</td>
</tr>
</tbody>
</table>

Autosomes are numbered from largest to smallest, except that chromosome 21 is smaller than chromosome 22.
Chromosome nomenclature

Box 2.3: Human chromosome nomenclature.

The International System for Human Cytogenetic Nomenclature (ISCN) is fixed by the Standing Committee on Human Cytogenetic Nomenclature (see Further reading). The basic terminology for human chromosomes was decided at a meeting in Paris in 1971, and is often referred to as the Paris nomenclature.

Short arm locations are labeled p (petite) and long arms q (queue). Each chromosome arm is divided into regions labeled p1, p2, p3 etc. and q1, q2, q3 etc., counting outwards from the centromere. Regions are demarcated by specific landmarks which are consistent and distinct morphological features, such as the ends of the chromosome arms, the centromere and certain bands. Regions are divided into bands labeled p11, p12 etc. and sub-bands p11.1, p11.2 etc. and sub-sub-bands for example p11.21, p11.22 in each case counting outwards from the centromere (Figures 2.22, 2.15).

Relative distance from the centromere is described by the words proximal and distal. Thus, proximal Xq means the segment of the long arm of the X that is closest to the centromere; while distal Xq means the portion of the short arm of chromosome 2 that is most distant from the centromere, and therefore closest to the telomere. Other common terms are as below.

When comparing human chromosomes with that of another species, the convention is to use the first letter of the genus name and the first two letters of the species name (e.g. HSA10 means human - Homo sapiens - chromosome 10).
Chromosome nomenclature

Classical karyotyping
Fluorescent in Situ Hybridization (FISH)
Chromosome painting
Molecular karyotyping
Chromosome sorting

Studying chromosomes

Figure 2-15 part 2 of 2. Human Molecular Genetics, 3e. 06 Baldwin Science 2004.
Fluorescence in situ hybridization (FISH)
Fluorescence in situ hybridization (FISH)

Studying chromosomes

- Classical karyotyping
- Fluorescent in Situ Hybridization (FISH)
- Chromosome painting
- Molecular karyotyping
- Chromosome sorting
Chromosome painting

Studying chromosomes
- Classical karyotyping
- Fluorescent in Situ Hybridization (FISH)
- Chromosome painting
- Molecular karyotyping
- Chromosome sorting
Molecular karyotyping

- Classical karyotyping
- Fluorescent in Situ Hybridization (FISH)
- Chromosome painting
- Molecular karyotyping
- Chromosome sorting

Studying chromosomes
Flow-sorting chromosome

Overview

- Studying chromosomes
- *The normal karyotypes of domestic animals*
- Chromosome abnormalities
- Chromosome abnormalities of domestic animals
The normal karyotype of domestic animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human, Homo sapiens</td>
<td>23</td>
</tr>
<tr>
<td>Cat, Felis catus</td>
<td>19</td>
</tr>
<tr>
<td>Horse, Equus caballus</td>
<td>32</td>
</tr>
<tr>
<td>Dog, Canis familiaris</td>
<td>39</td>
</tr>
<tr>
<td>Pig, Sus scrofa</td>
<td>19</td>
</tr>
<tr>
<td>Mouse, Mus musculus</td>
<td>20</td>
</tr>
<tr>
<td>Cattle, Bos taurus</td>
<td>30</td>
</tr>
<tr>
<td>Rat, Rattus norvegicus</td>
<td>21</td>
</tr>
<tr>
<td>Sheep, Ovis aries</td>
<td>27</td>
</tr>
<tr>
<td>Chicken, Gallus domestic</td>
<td>Ca. 39</td>
</tr>
</tbody>
</table>

(haploid chromosome number)

Pig
### Pig

<table>
<thead>
<tr>
<th>Abo</th>
<th>Bovine (Bos taurus)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Pig</td>
<td>Reference</td>
</tr>
</tbody>
</table>

#### Suidae

- **Pig**
  - Boar: Domestic pig
  - Wild boar: Sus scrofa
  - Asian wild pig: Sus scrofa
d
- **Wild boar**
  - Sus scrofa domesticus
  - Sus scrofa

#### Pig Breeds

- **Domestic pig**
  - Domestication: 10,000 BCE
  - Modern breeds: 1500 BCE

#### Pig Chromosomes

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Reference</td>
</tr>
</tbody>
</table>

### Cattle

![Cattle Image](image)

**Figure 1:** Chromosome map of the cattle genome, arranged according to the visuzel karyotype. (From: Smith, 1982, Genetics of the Domestic Animals.)

#### Cattle Breeds

- **Holstein-Friesian**
  - Origin: Netherlands
  - Characteristics: Milk production
- **Jersey**
  - Origin: New England
  - Characteristics: Dairy
- **Dairy Shorthorn**
  - Origin: Midwest
  - Characteristics: Dairy
- **Beef Shorthorn**
  - Origin: Midwest
  - Characteristics: Beef
- **Hereford**
  - Origin: Texas
  - Characteristics: Beef
- **Angus**
  - Origin: Scotland
  - Characteristics: Beef

#### Cattle Chromosomes

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-29</td>
<td>Reference</td>
</tr>
</tbody>
</table>

**Note:** The images and text in the document are not fully visible or legible due to the nature of the document format.
Cattle

Sheep: (Differences between species often involve Roberstonian fusions)
Conserved synteny between the human and mouse genomes. Regions from different mouse chromosomes (indicated by the colors of each mouse in B) show conserved synteny (gene order) with the indicated regions of the human genome (A). For example, the genes present in the upper portion of human chromosome 1 (orange) are present in the same order in a portion of mouse chromosome 4. Regions of human chromosomes that are composed primarily of short, repeated sequences are shown in black. Mouse centromeres (indicated in black in B) are located at the ends of chromosomes; no known genes lie beyond the centromere on any mouse chromosome. For the most part, human centromeres, indicated by constrictions, occupy more internal positions on chromosomes (see Figure 4–11). (Adapted from International Human Genome Sequencing Consortium, *Nature* 409:860–921, 2001.)

Overview

- Studying chromosomes
- The normal karyotypes of domestic animals
- *Chromosome abnormalities*
- Chromosome abnormalities of domestic animals
Chromosome abnormalities

- Types according to chromosome change:
  - Aberrant chromosome number
    - Aberrant euploidy (polyploidy)
    - Aneuploidy (trisomy, monosomy)
    - Mixoploidy
  - Aberrant chromosome structure (del, inv, dup, ins, R, mar, t, der)
  - Aberrant chromosome parental origin

- Types according to body extent:
  - Constitutional
  - Somatic (=>mosaic)

---

Aberrant euploidy (polyploidy)

![Diagram showing different types of euploidy](image-url)

Figure 2.19: Human Molecular Genetics, 3rd ed. © Garland Science (2004)
Aneuploidy

- Trisomy and monosomy
- Chromosomal non-disjunction or anaphase lag

Consequences of numerical chromosomal abnormalities

<table>
<thead>
<tr>
<th>Polyploidy</th>
<th>Aneuploidy (autosomes)</th>
<th>Aneuploidy (sex chromosomes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triplody (83, XXX, XXX or XYY)</td>
<td>Nullisomy (missing a pair of homologous chromosomes)</td>
<td>Additional sex chromosomes</td>
</tr>
<tr>
<td>Trisomy (one extra chromosome)</td>
<td>Monosomy (one chromosome missing)</td>
<td>缺失一个性染色体</td>
</tr>
</tbody>
</table>

1-3% of all conceptions, almost never live born, do not survive.

- Pre-implantation lethal
- Embryonic lethal

Usually lethal at embryonic or fetal stages, but trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) may survive to term and trisomy 21 (Down syndrome) may survive to age 40 or longer.

47, XXY (Klinefelter syndrome): presents relatively minor problems, with normal lifespan.

45X (Turner syndrome): About 95% of cases abort spontaneously; survivors have normal intelligence but interfere and show minor physical sign, 45X is not viable.
Mixoploidy:
Mosaicism versus chimerism

Chromosome abnormalities

- Types according to chromosome change:
  - Aberrant chromosome number
    - Aberrant euploidy (polyploidy)
    - Aneuploidy (trisomy, monosomy)
    - Mixoploidy
  - Aberrant chromosome structure (del, inv, dup, ins, R, mar, t, der)
  - Aberrant chromosome parental origin

- Types according to body extent:
  - Constitutional
  - Somatic (=> mosaic)
Nomenclature of chromosome abnormalities

Box 2.4: Nomenclature of chromosome abnormalities.

### Numerical abnormalities:

- **Trisomy**: 69.XX, 69.XXY, 69.XYY
- **Trisomy**: q.g. 47,XX,+18
- **Monosomy**: q.46
- **Monosomy**: q.47.XX/-, 46.XX

### Structural abnormalities:

- **Deletion**: q.46.XX(del)(p16.3)q13.3
- **Inversion**: q.46.XX(in)16pter-p11.2
- **Duplication**: q.46.XX.dup(16)(q12q22)
- **Inversion**: q.46.XX.inv(16)(p13q22)
- **Ring**: q.46.XX.ring(16)
- **Mero**: q.47,XX+mar

**Notes:**
- **Deletion** of a chromosome is indicated by a minus sign.
- **Terminal deletion** (breakpoint at q16.3) and **interstitial deletion** (p12-q28).
- **Deletion of a chromosome** with a specific breakpoint is shown.
- **Inversion** of a cell that contains a **marker chromosome** (an unidentifiable chromosome)
- **Balanced reciprocal translocation** with breakpoints in 2q35 and 8q13.1.
- **A balanced carrier of a t(14;21)** not only gives rise to a derivative chromosome, but indicates a **der(14)** present.
- **Translocation** T(14;21) can result in a **derivative** chromosome.

### Origin of structural abnormalities

Table 2.5: Structural abnormalities resulting from misrepair of chromosome breaks or recombination between homologous chromosomes.

<table>
<thead>
<tr>
<th>One chromosome involved</th>
<th>Two chromosomes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>One break</td>
<td></td>
</tr>
<tr>
<td><strong>Terminal deletion</strong> (termed by adding allele)</td>
<td><strong>Terminal deletion</strong></td>
</tr>
<tr>
<td><strong>Ring chromosome</strong> (Figure 2.24)</td>
<td><strong>Reciprocal translocation</strong> (Figure 2.21)</td>
</tr>
<tr>
<td><strong>Duplication</strong> or <strong>deletion</strong> by unequal sister chromatid exchange (Figure 11.17)</td>
<td><strong>Reciprocal translocation</strong> (Figure 2.21)</td>
</tr>
<tr>
<td><strong>Three breaks</strong></td>
<td><strong>Interchromosomal interchange</strong></td>
</tr>
<tr>
<td><strong>Various rearrangements</strong>, e.g. inversion, deletion, intrachromosomal insertion</td>
<td><strong>Interchromosomal interchange</strong> (direct or inverted)</td>
</tr>
</tbody>
</table>

This is a sheet nomenclature, a more complicated nomenclature is defined by the ISCN, that allows complete description of any chromosome abnormality—see Further reading.
Origin of structural abnormalities

Consequences of reciprocal translocations
Consequences of Robertsonian fusions

Chromosome abnormalities

- Types according to chromosome change:
  - Aberrant chromosome number
    - Aberrant euploidy (polyploidy)
    - Aneuploidy (trisomy, monosomy)
    - Mixoploidy
  - Aberrant chromosome structure (del, inv, dup, ins, R, mar, t, der)
  - Aberrant chromosome parental origin

- Types according to body extent:
  - Constitutional
  - Somatic (=>mosaic)
Aberrant parental origin

- Uniparental diploidy
  - Hydatiform moles
    - Paternal uniparental diploidy
    - Trophoblast hyperplasia
    - Risk of transformation in choriocarcinoma
    - Most moles are homozygous at all loci (chromosome doubling from single sperm)
  - Ovarian teratoma
    - Maternal uniparental diploidy
    - Disorganized embryonic tissue
    - Activation of uniovulated oocyte

---

Hydatiform mole
Aberrant parental origin

- Uniparental disomy:
  - Paternal or maternal
  - Isodisomy or heterodisomy
  - Matings between heterozygotes for reciprocal translocations
  - Trisomy or monosomy rescue
  - Anomalies if involved region contains imprinted genes.

Parental imprinting
Chromosome abnormalities

- Types according to chromosome change:
  - Aberrant chromosome number
    - Aberrant euploidy (polyploidy)
    - Aneuploidy (trisomy, monosomy)
    - Mixoploidy
  - Aberrant chromosome structure (del, inv, dup, ins, R, mar, t, der)
  - Aberrant chromosome parental origin

- Types according to body extent:
  - Constitutional
  - Somatic (=>mosaic)

Overview

- Studying chromosomes
- The normal karyotypes of domestic animals
- Chromosome abnormalities
  - Chromosome abnormalities of domestic animals
Pig

- Numerical aberrations
  - Cfr. Above
  - Chimerism
    - XX/XY chimerism
    - Does not necessarily result in intersexuality (vs bovine-free-martinism)
- Structural aberrations
  - Reciprocal translocations
    - « ... at least 50% of breeding boars removed due to lower than average litter size carried reciprocal translocation. »
  - Other: cfr. Above
- Sex reversal / (pseudo-)hermaphroditism

Reciprocal translocations of the pig
Pig

- Numerical aberrations
  - Cfr. Above
  - Chimerism
    - XX/XY chimerism
    - Does not necessarily result in intersexuality (vs bovine-Free-martinism)
- Structural aberrations
  - Reciprocal translocations
    - “... at least 50% of breeding boars removed due to lower than average litter size carried reciprocal translocation. »
  - Other: cfr. Above
- Sex reversal / (pseudo-)hermaphroditism

Bovine

- Numerical aberrations
  - Cfr. Above
  - Chimerism
    - Male-female twins
    - XX/XY chimerisms of blood and haematopoietic organs
    - Masculinization of females ⇔ AMH hormone of brother
- Structural aberrations
  - Reciprocal translocations
    - T(1;29)
Bovine recipr. translocations

Chromosomal abnormalities in domestic sheep
Association between Polled and intersexuality in goats

- In goats, Polled (absence of horns) is inherited as an autosomal dominant trait.
- It is always associated with sex reversal in females which is however transmitted as an autosomal recessive trait.
- The PIS locus has been mapped to 1q43 and the mutation has been identified by positional cloning as an 11.7 Kb deletion which is probably affecting the expression of the nearby FOXL2 gene.

http://dqa.jouy.inra.fr/lgbc/projets/PIS.html