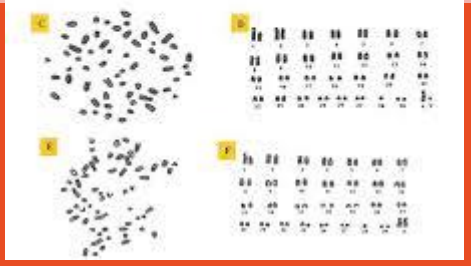
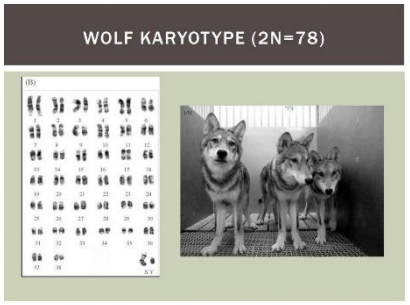
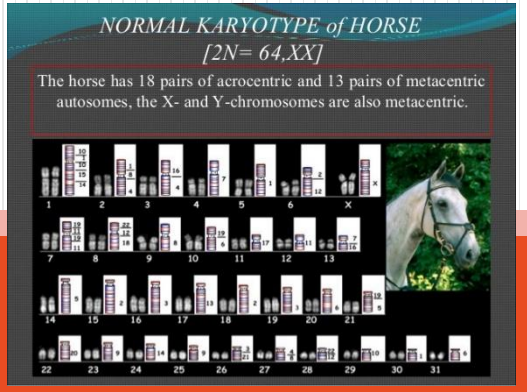






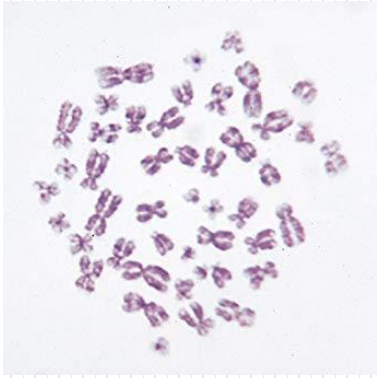
Chromosomes
Karyotypes

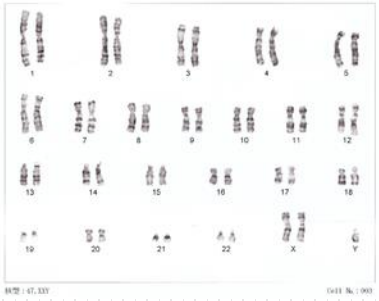
Lecture 7

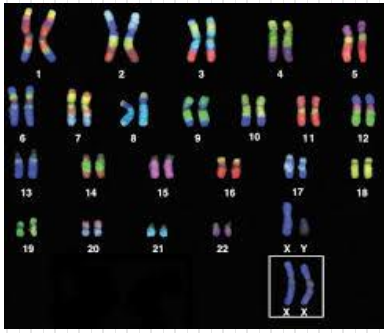
SLE254 Genetics

Concepts of Genetics (12th ed)

Pages 40f, 54, 54f & 618f







Karyology – the study of whole sets of chromosomes

- **Karyotype**
  - A complete set of chromosomes from a cell that has been photographed during cell division and arranged in a standard sequence
- Important for cell biology and genetics, and the results may be used in evolutionary biology and medicine
- Why use them?
  - Study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events

Devil Facial Tumour Disease (DFTD)

"Infectious" – direct contact req

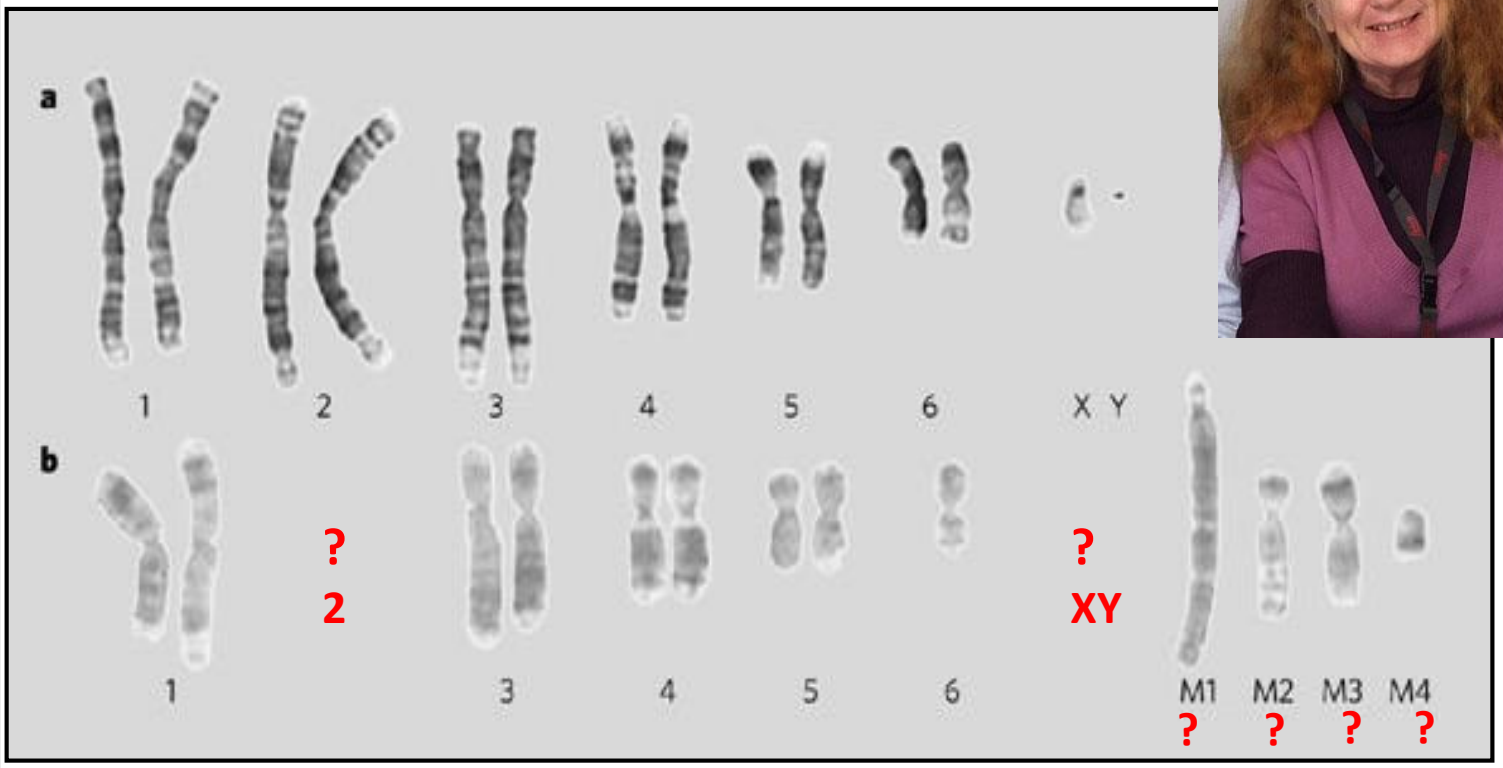
How does it spread?

Via viruses????????

DFTD is a CONTAGIOUS cancer- transfer of LIVE cancer cells

Tumours from ALL individuals share the same complex karyotype rearrangements

a. Normal Devil chromosomes



Anne-Maree Pearse

b. Tumour chromosomes

Pearse & Swift (2006) Nature, 439:549

How many chromosomes does a normal diploid human cell contain?

- Karyology has helped solve this issue
  - However, it took a while…
- In 1912, Hans von Winiwarter reported 47 chromosomes in spermatogonia and 48 in oogonia
- Theophilus Painter in 1922 favoured 48 chromosomes
- Albert Levan in 1956 – the karyotype of humans includes only 46 chromosomes

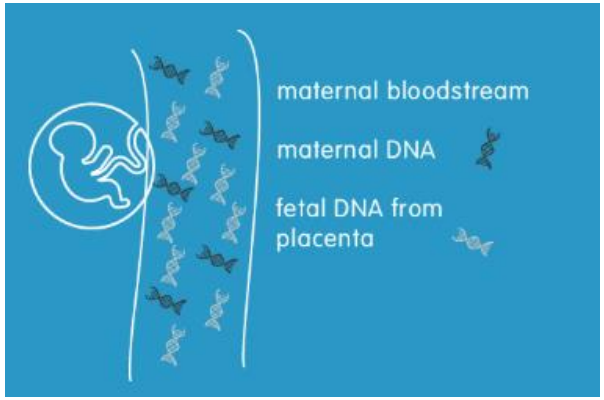
Obtaining cells for chromosome studies

- Any nucleus can be used to make karyotype
  - Lymphocytes, skin cells, cells from biopsies, tumour cells

- Sampling cells before birth

Methods to test for Down Syndrome in high risk mothers

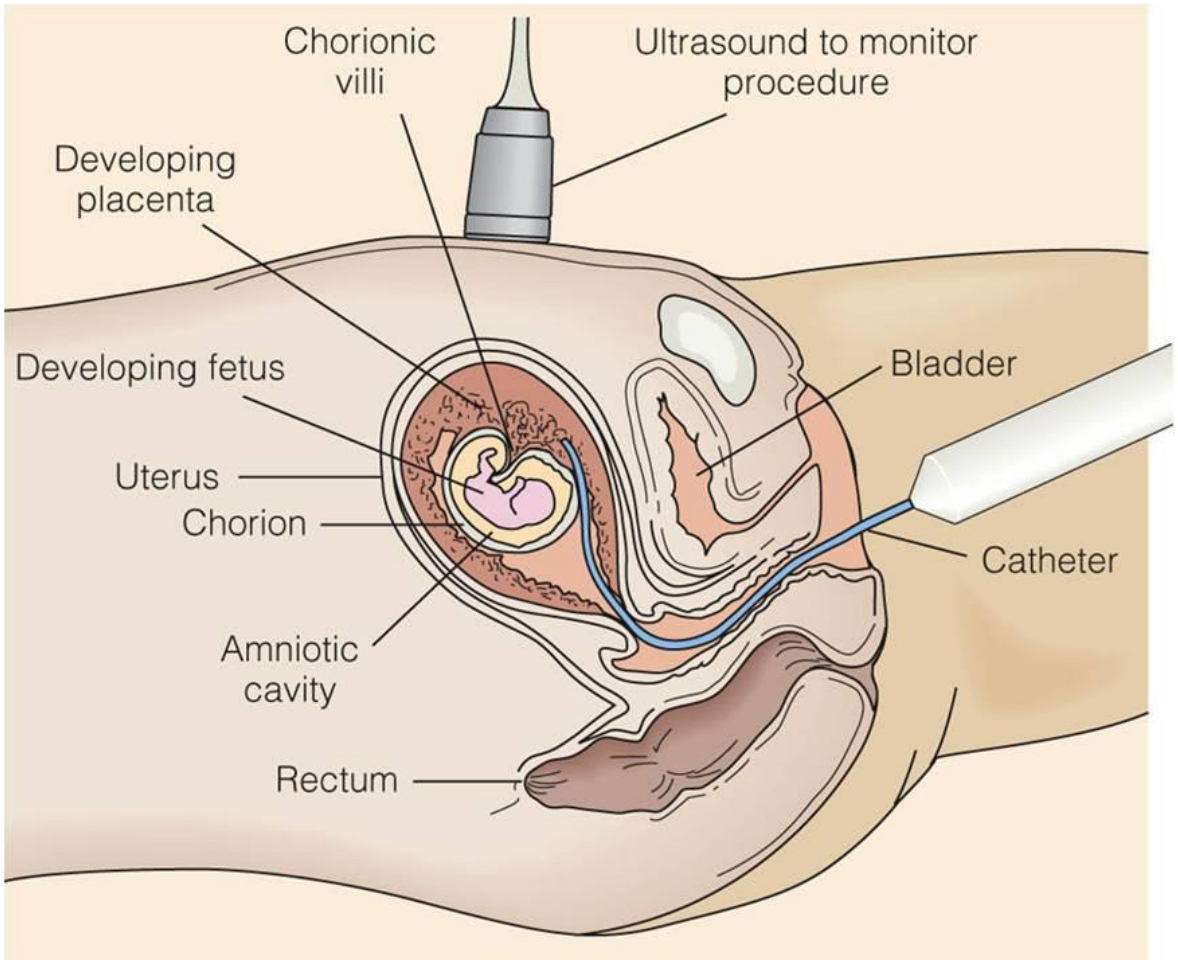
  - Amniocentesis
  - Chorionic villus sampling (CVS)
  - 'percept' cfDNA (cell free fetal DNA) from 10 weeks, ideal between 11-16 weeks



Chorionic Villus Sampling (CVS)

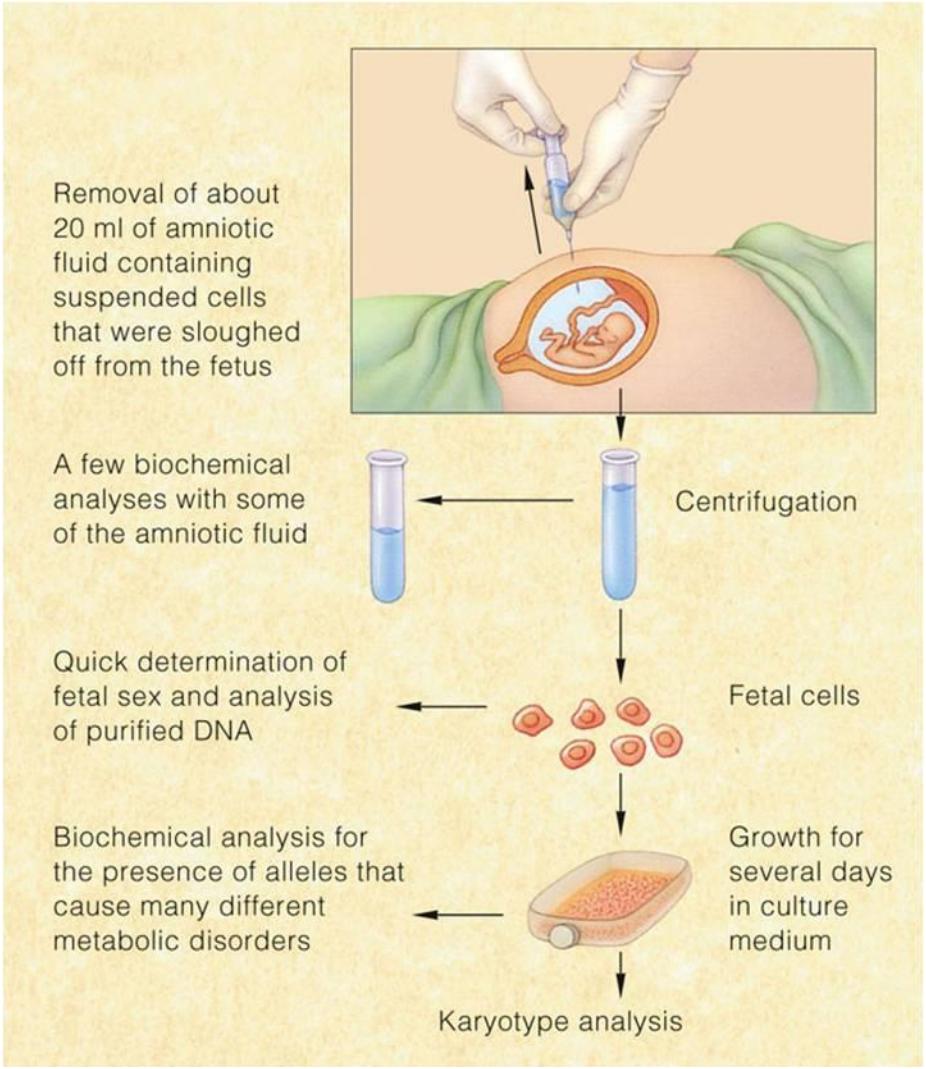
- A method of sampling foetal chorionic cells by inserting a catheter through the vagina or abdominal wall into the uterus
  - Used in diagnosing biochemical and cytogenetic defects in the embryo
  - Usually performed in the eighth or ninth week of pregnancy (earlier than amniocentesis).

Chorionic Villus Sampling (CVS)



The **chorion** is the outermost fetal membrane and the **villi** are **projections from this membrane** that can be sampled without harming the fetus.

Amniocentesis



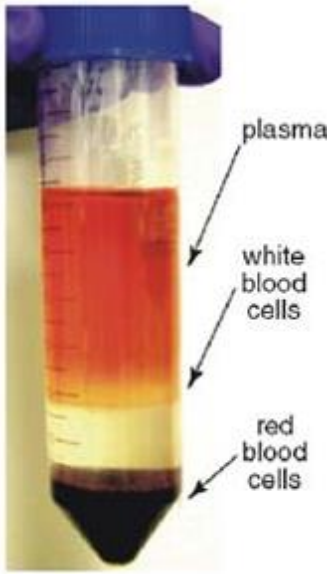
Fetal skin cells come off and float in the amniotic fluid.

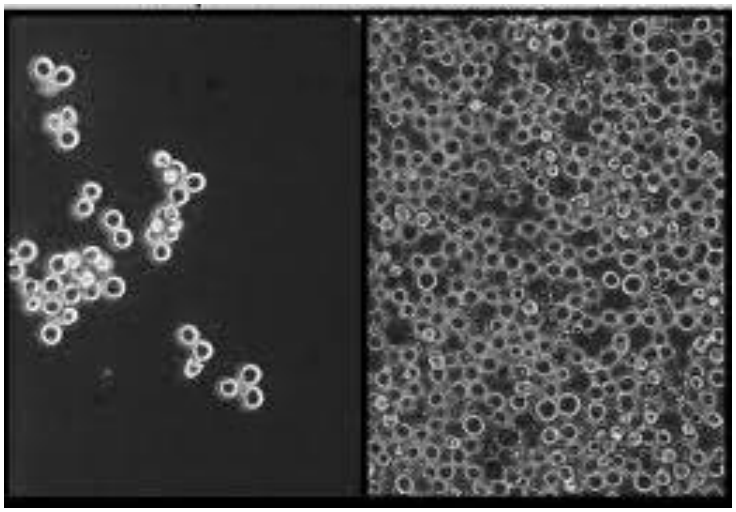
These can be sampled, grown and analysed for chromosomes, enzymes or DNA.

Usually performed in the 14th to 18th week of pregnancy

Performing a karyotype from blood

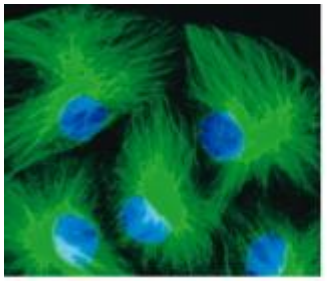
- Blood is drawn from body and treated to stop coagulation
- Mononuclear cells (lymphocytes and monocytes*) are purified from the blood by centrifugation
- The purified cells are cultured for 3-4 days in the presence of a **mitogen**, which stimulates the lymphocytes to proliferate



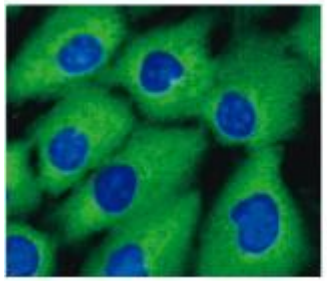


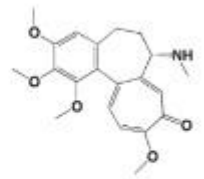
Performing a karyotype from blood

- The cells are treated with a drug such as colcemid, which disrupts mitotic spindles and prevents completion of mitosis
  - Enriches the population of metaphase cells
- Cells are harvested and treated briefly with a hypotonic solution.
  - This makes the nuclei swell osmotically

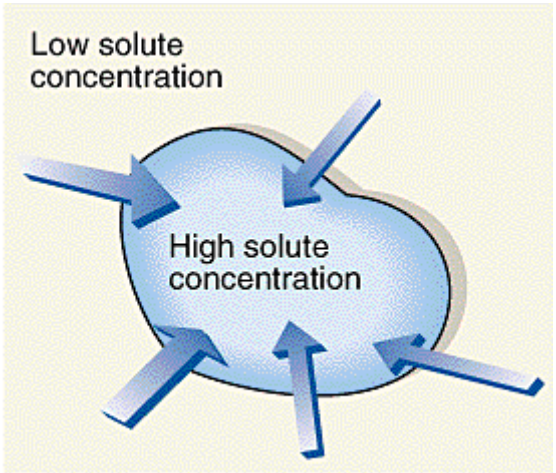


Control



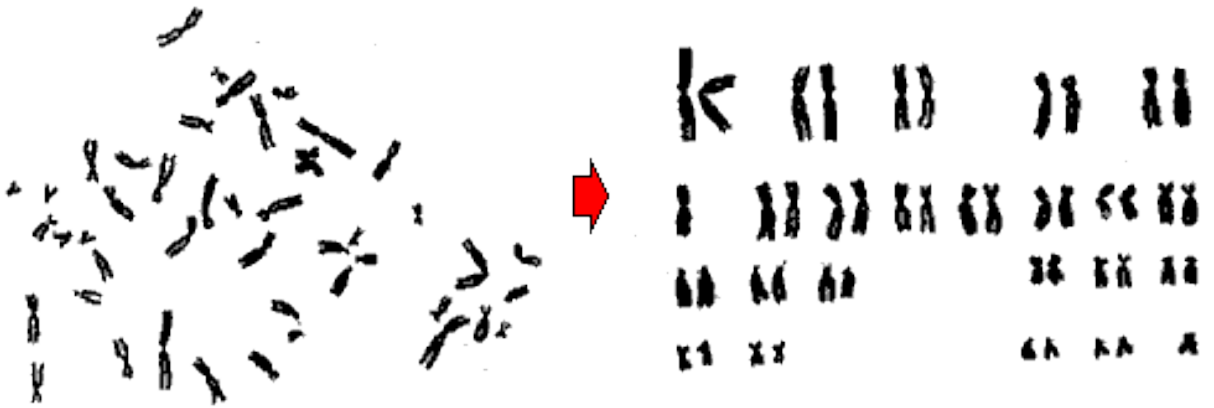


Colcemid
(desacetyl-N-methyl cholchicine)

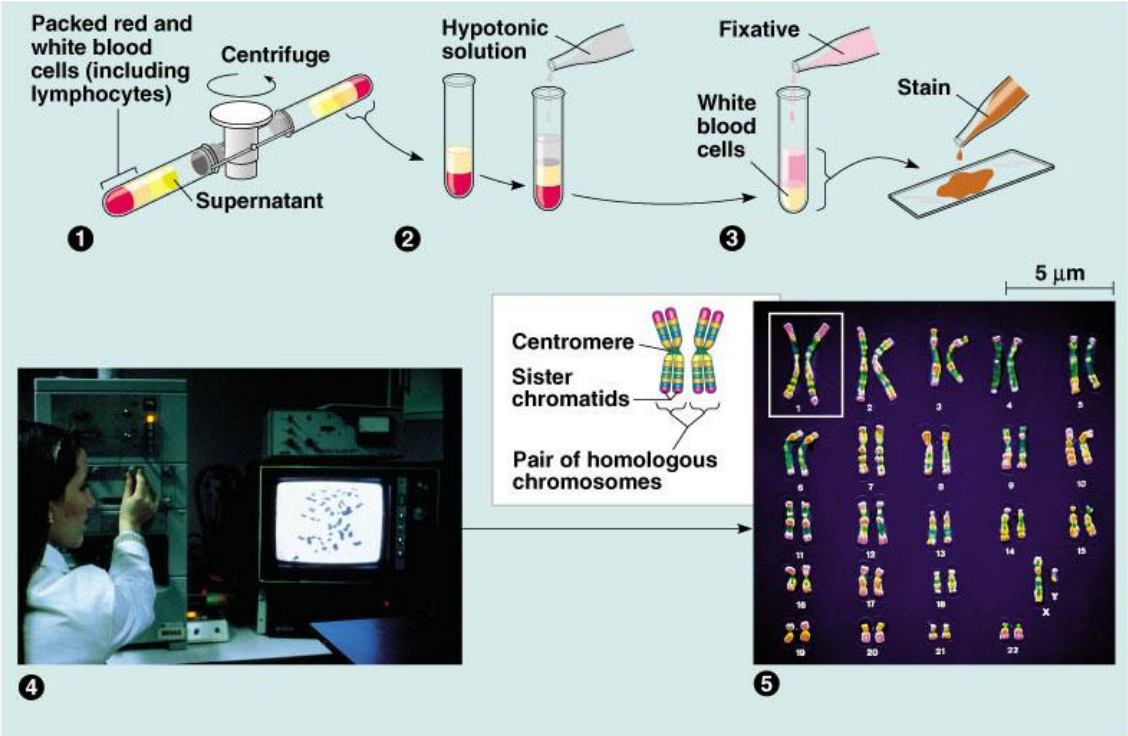


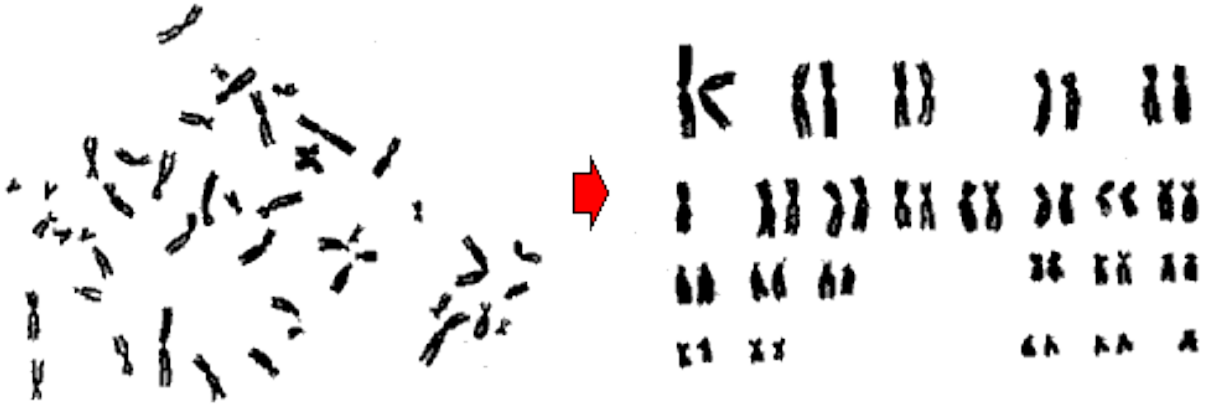
Performing a karyotype from blood

- The swollen cells are fixed, dropped onto a microscope slide and dried
- Slides are stained to induce a banding pattern
- The slides are scanned to identify a clear chromosome spread, and then photographed.
- Previously the image was cut up and rearranged in standard format – now days software is used



Performing a karyotype from blood





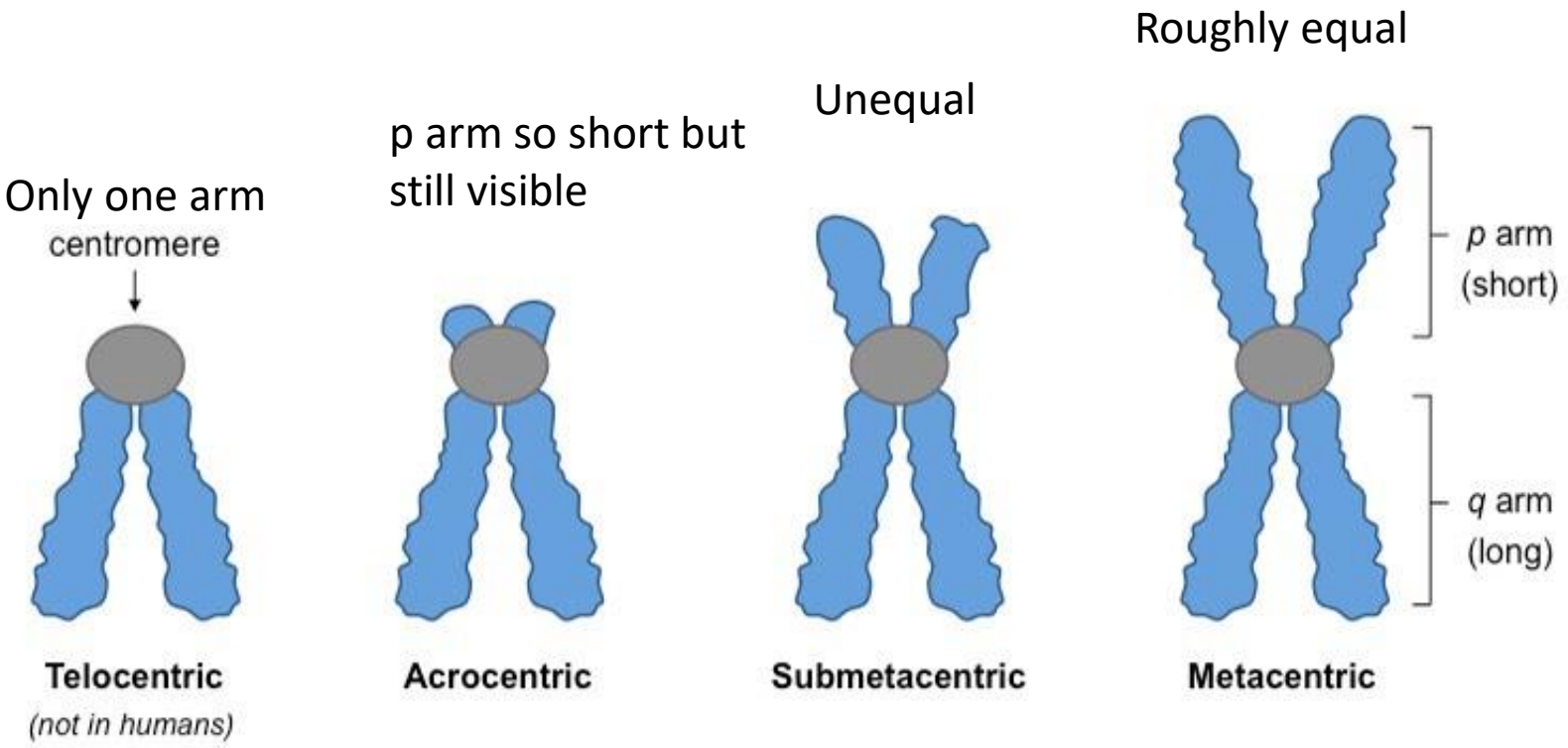
Identifying chromosomes

- Three key features are used to identify chromosomal similarities and differences
  - Size. This is the easiest way to tell two different chromosomes apart
  - Centromere position. Centromeres are regions in chromosomes that appear as a constriction
  - Banding pattern. The size and location of stained bands on chromosomes make each chromosome pair unique

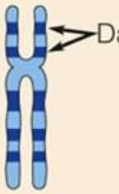
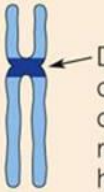
Centromere location

- Metacentric
  - A chromosome that has a centrally placed centromere
- Submetacentric
  - A chromosome whose centromere is placed closer to one end than the other
- Acrocentric
  - A chromosome whose centromere is placed very close to, but not at, one end
- Telocentric
  - A chromosome whose centromere is placed at one end of the chromatid and hence only one arm
  - Not seen in humans

Centromere location- recall one chromosome that has replicated during mitosis: 2 sister chromatids

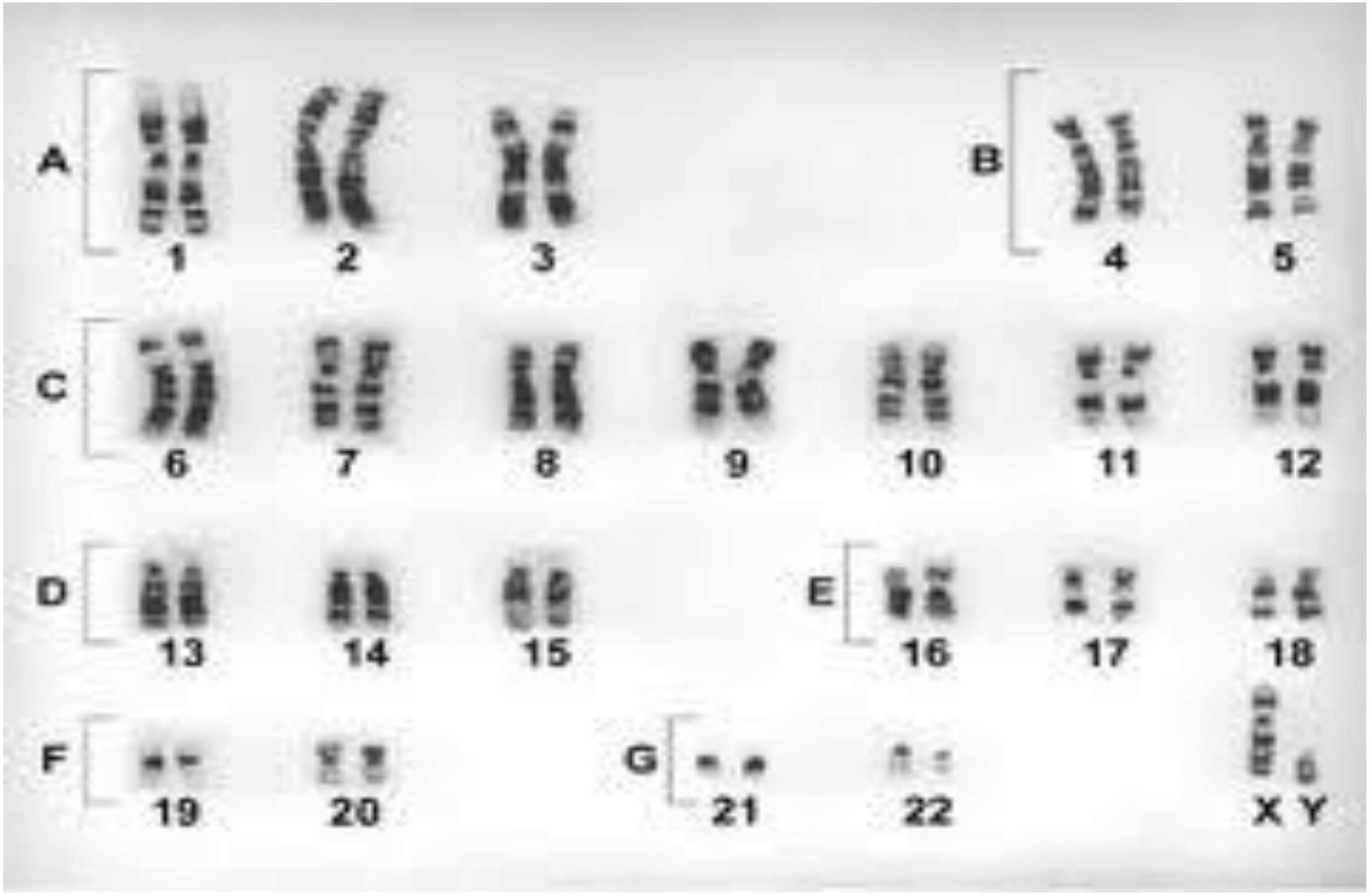


Chromosome staining procedures

Banding technique	Appearance of chromosomes
G-banding — Treat metaphase spreads with enzyme that digests part of chromosomal protein. Stain with Giemsa stain. Observe banding pattern with light microscope.	 Darkly stained G bands.
R-banding — Heat metaphase spreads at high temperatures to achieve partial denaturation of DNA. Stain with Giemsa stain. Observe with light microscope.	 Darkly stained R bands correspond to light bands in G-banded chromosomes. Pattern is the reverse of G-banding.
C-banding — Chemically treat metaphase spreads to extract DNA from the arms but not the centromeric regions of chromosomes. Stain with Giemsa stain and observe with light microscope.	 Darkly stained C band centromeric region of the chromosome corresponds to region of constitutive heterochromatin.
Q-banding — Treat metaphase spreads with the chemical quinacrine mustard. Observe fluorescent banding pattern with a special ultraviolet light microscope.	 Bright fluorescent bands upon exposure to ultraviolet light; same as darkly stained G bands.

Darkest bands where most DNA is- stain intercalates

Human karyotype



Chromosome Groups

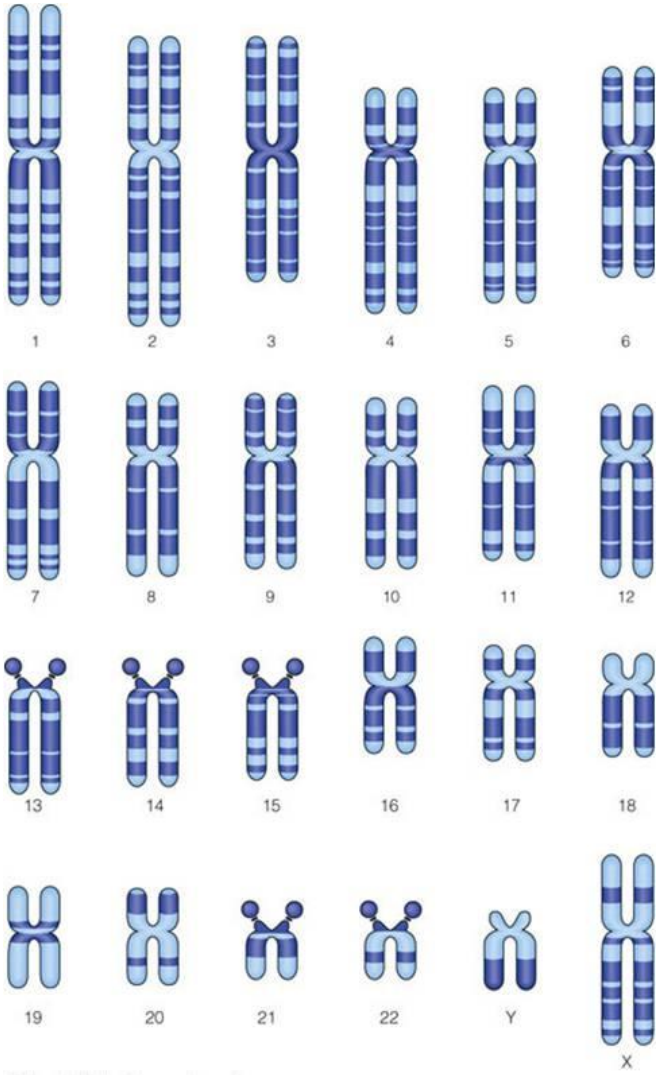
Group	Chromosomes	Description
A	1–3	Largest; 1 and 3 are metacentric but 2 is submetacentric
B	4,5	Large; submetacentric with two arms very different in size
C	6–12,X	Medium size; submetacentric
D	13–15	Medium size; acrocentric with satellites
E	16–18	Small; 16 is metacentric but 17 and 18 are submetacentric
F	19,20	Small; metacentric
G	21,22,Y	Small; acrocentric, with satellites on 21 and 22 but not on the Y
Autosomes are numbered from largest to smallest, except that chromosome 21 is smaller than chromosome 22.		

Mazen Zahama Molecular Biology 1/2009

Source: http://www.slideserve.com/broderick/human-chromosomes-identification-by-g-banding-1

Karyogram: chromosome banding patterns

A **karyogram** (or ideogram) is a diagram of the chromosomes, showing the banding pattern

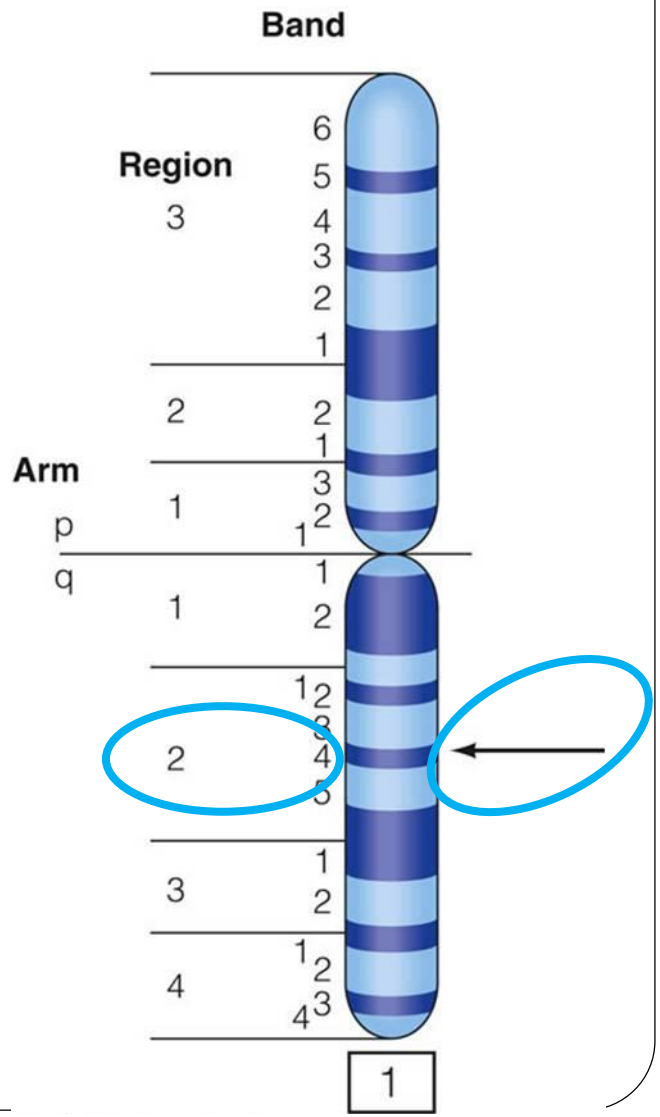


Human metaphase chromosomes contain about 550 bands

More bands can be detected in later prophase chromosomes

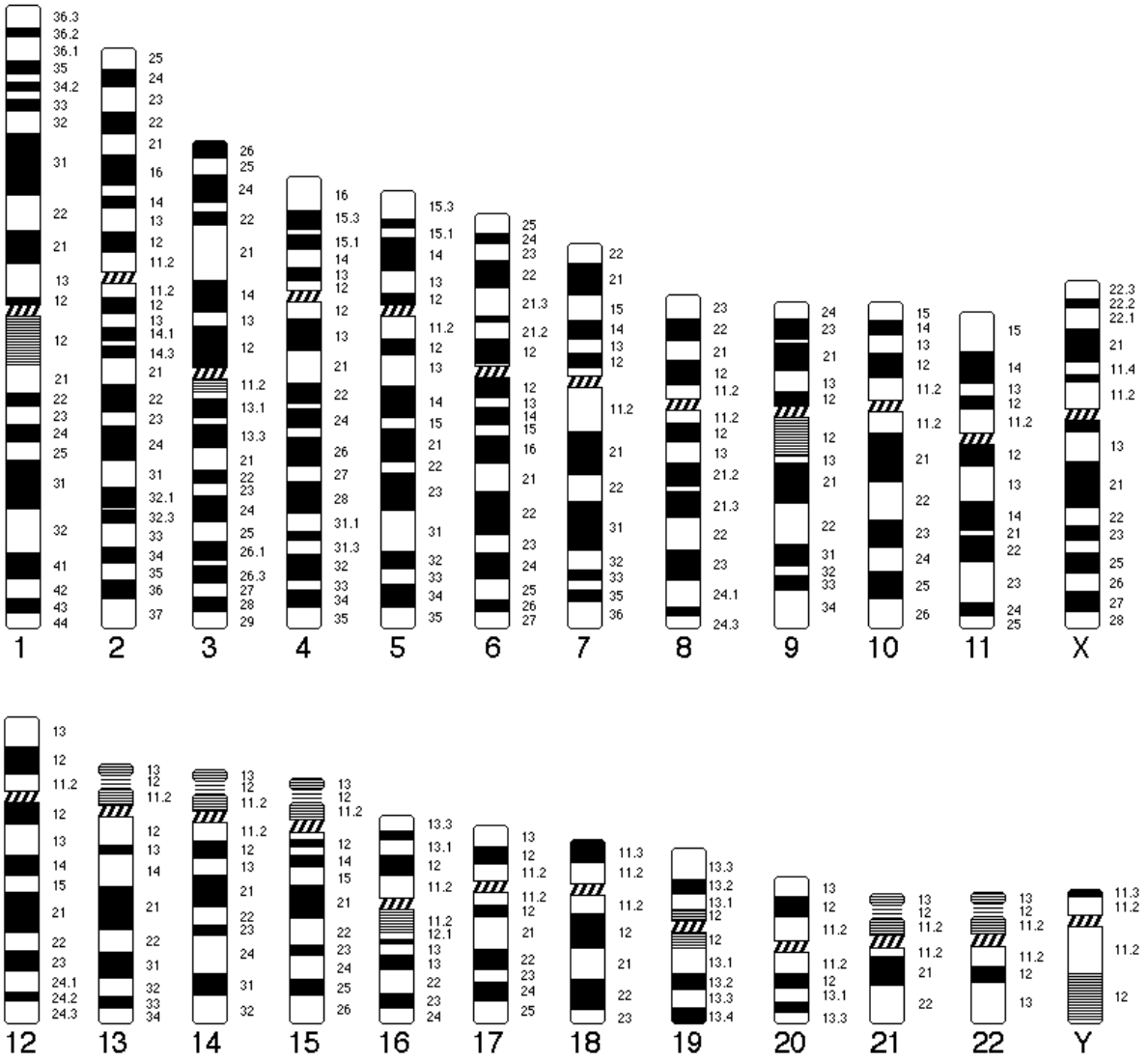
System of naming chromosome bands

- Allows any region to be identified by a **descriptive address** (chromosome number, arm, region, and band)



The short arm is p and the long arm is q
Each arm is divided into regions, with bands within the region numbered.
Arrow: 1q2.4 (chromosome 1, long arm, region 2 band 4)

Human karyogram



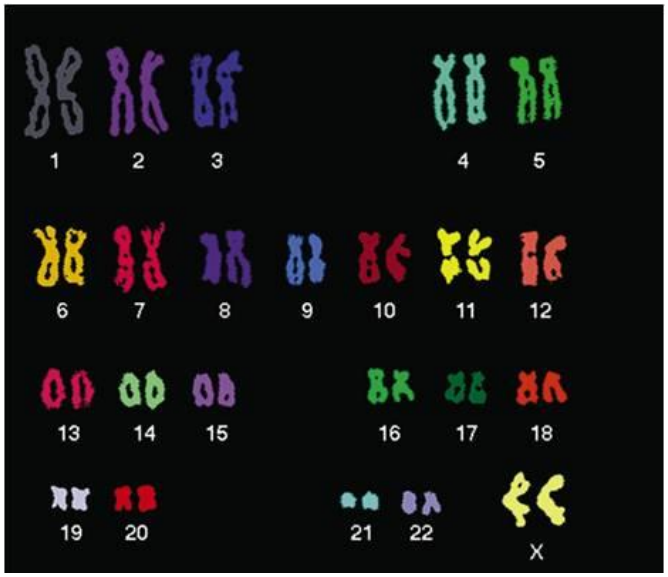
Karyotype symbols

- An alpha-numerical karyotype is the designation of the chromosome constitution
  - **46, XX.**
- del =deletion,
- dup = duplication
- t = translocation
- inv = inversion
- p = short arm of chromosome (p= petit)
- q = long arm of chromosome (q=queue)
- + = addition of entire chromosome
- - = loss of entire chromosome

Karyotype symbols

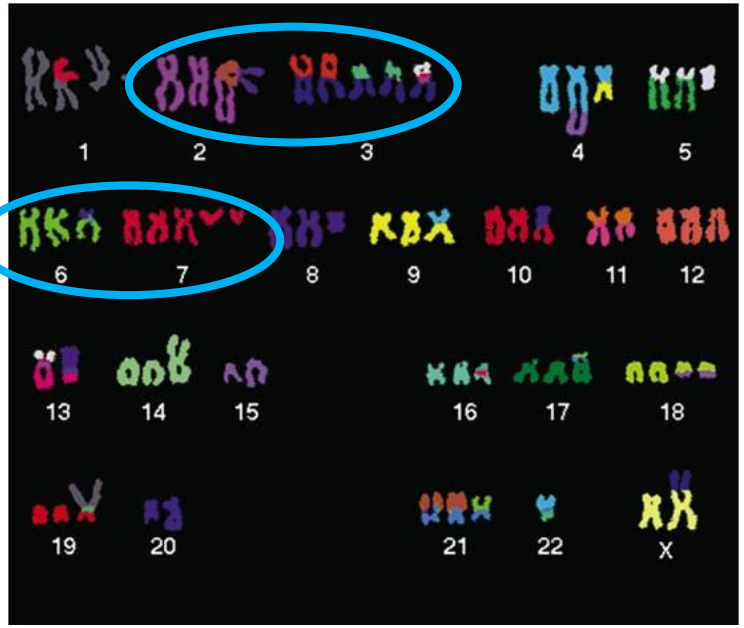
Chromosomal Abnormality		Syndrome Phenotype
46,del(4p)	Wolf-Hirschhorn syndrome	Mental retardation; midline facial defects consisting of broad nose, wide-set eyes, small lower jaw, and cleft palate; heart, lung, and skeletal abnormalities common; severely reduced survival
46,del(11)(p13)	WAGR syndrome	Tumors of the kidney (Wilms tumor) and of the gonad (gonadoblastoma); aniridia (absence of the iris); ambiguous genitalia; mental retardation
46,t(9;22)(q34q11)	CML (chronic myelogenous leukemia)	Enlargement of liver and spleen; anemia; excessive, unrestrained growth of white cells (granulocytes) in the bone marrow
46,t(8;14)	Burkitt's lymphoma	Malignancy of B lymphocytes that mature into the antibody-producing plasma cells; solid tumors, typically in the bones of the jaw and organs of the abdomen

Chromosome painting



Chromosome-specific DNA probes are labelled with a fluorescent dye. Using combinations of probes and dyes can produce unique colours for each chromosome.

Chromosome painting is a very powerful method for detecting chromosomal abnormalities in a cancer cell. Note the translocations and triploidy and tetraploidy.



Devil Facial Tumour Disease (DFTD)

"Infectious" – direct contact req

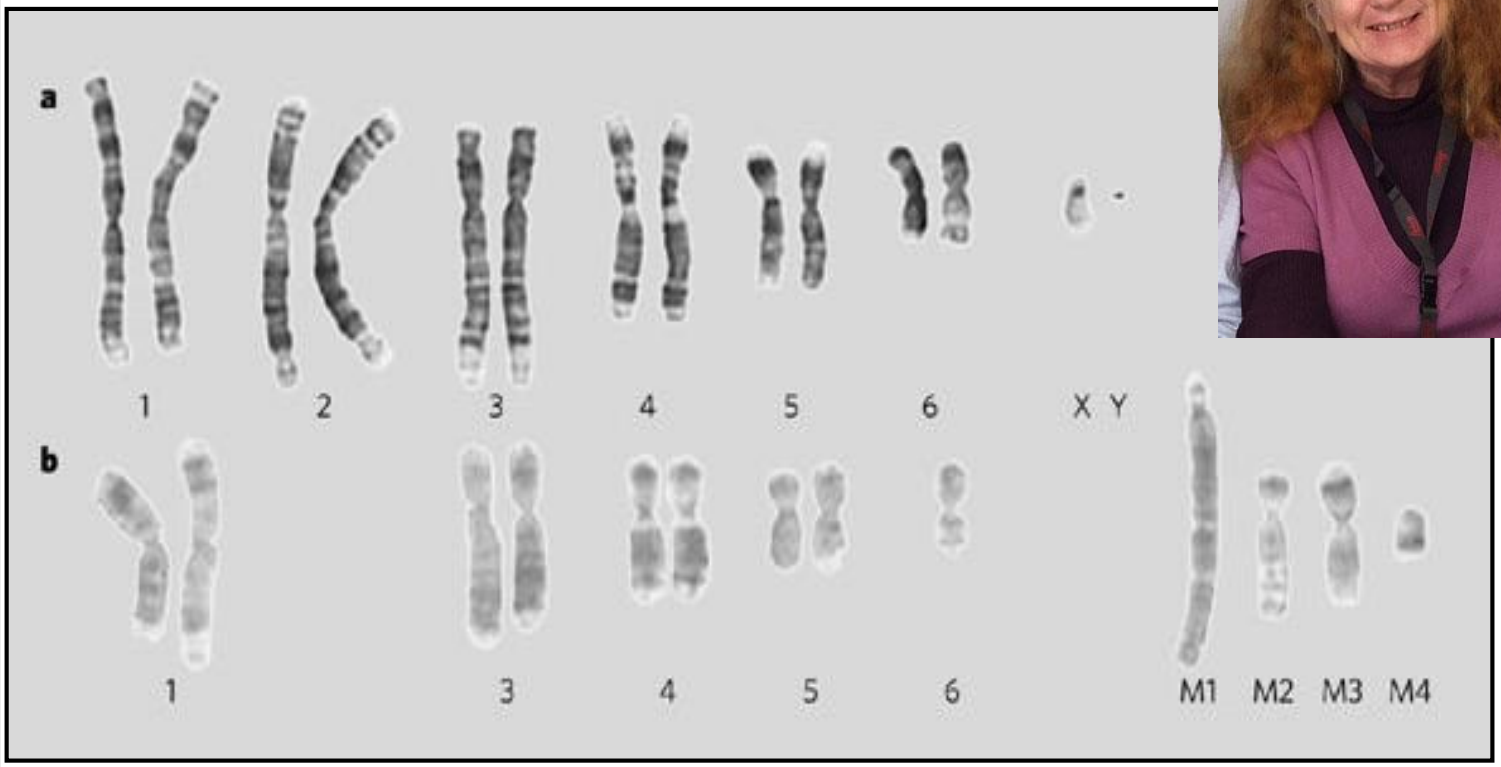
How does it spread?

Via viruses?

DFTD is a CONTAGIOUS cancer- transfer of LIVE cancer cells

Tumours from **ALL** individuals share the same complex karyotype rearrangements

a. Normal Devil chromosomes

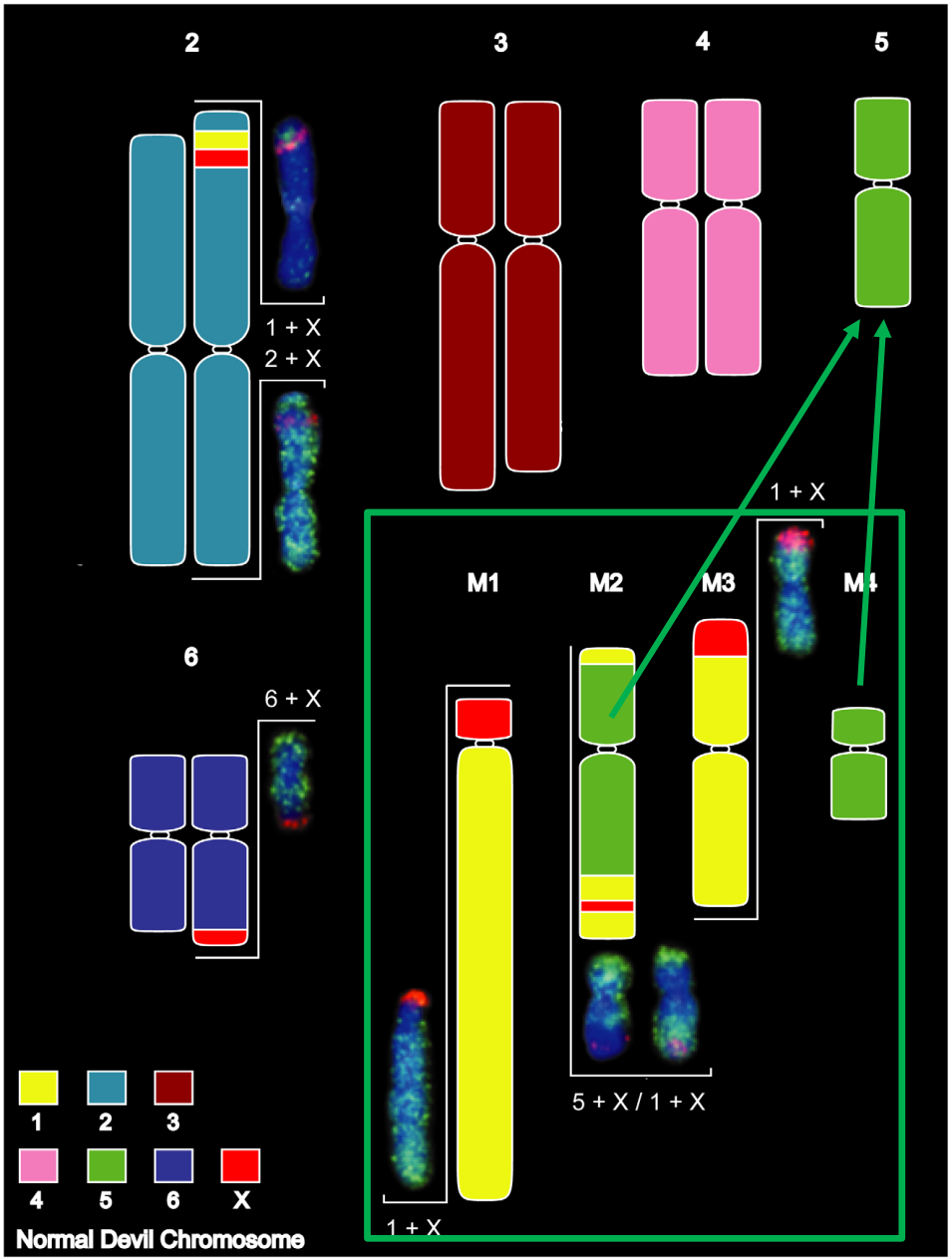


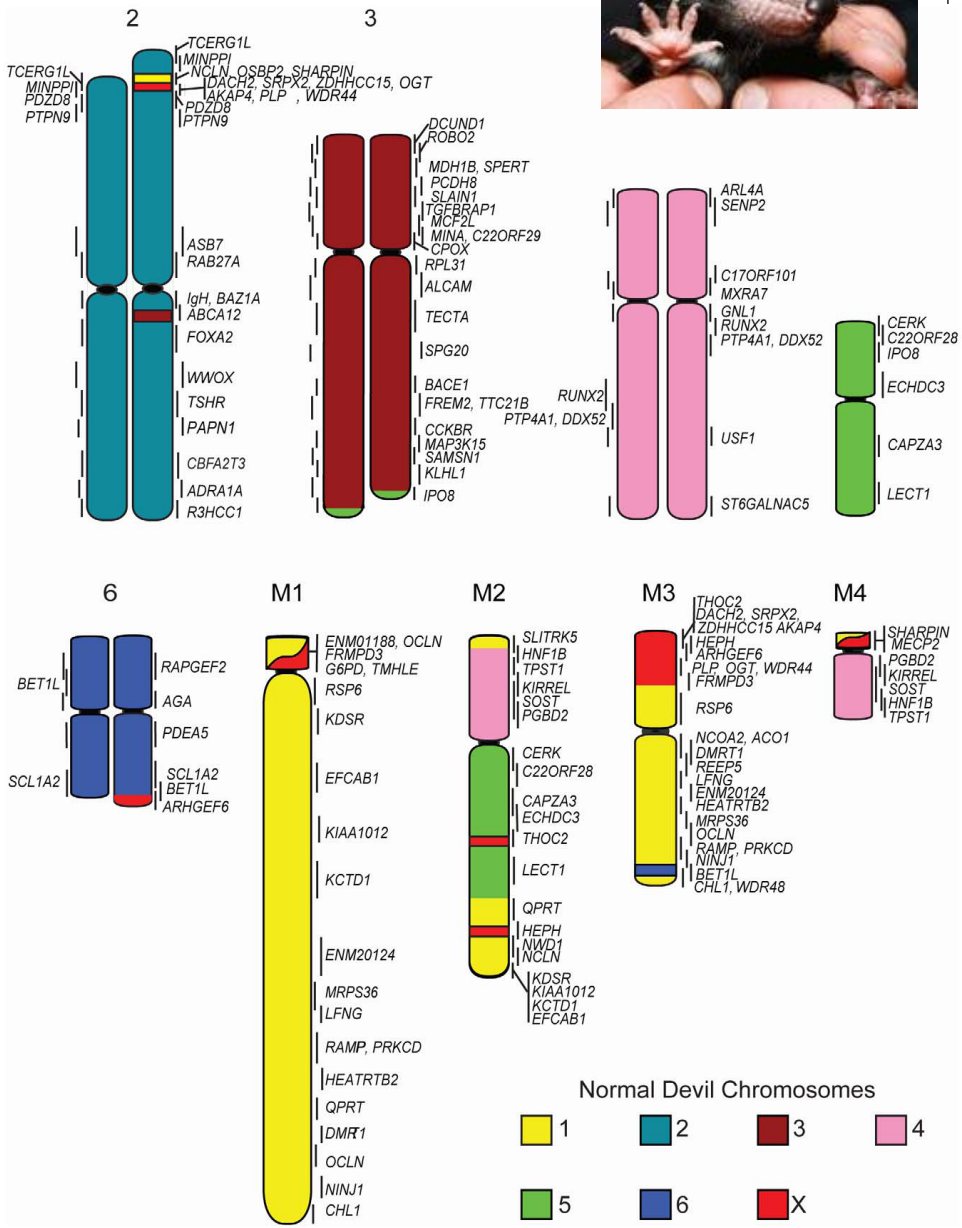
Anne-Maree Pearse

b. Tumour chromosomes

Pearse & Swift (2006) Nature, 439:549

Chromosome paintings of DFTD chromosomes

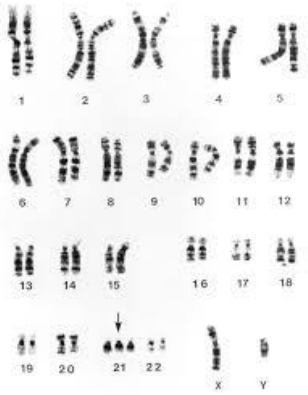




Analysing karyotypes

- Karyotypes reveal variations in chromosomal structure and number
  - 1959: Discovery that Down syndrome is caused by an extra copy of chromosome 21

- Chromosome banding and other techniques can identify small changes in chromosomal structure



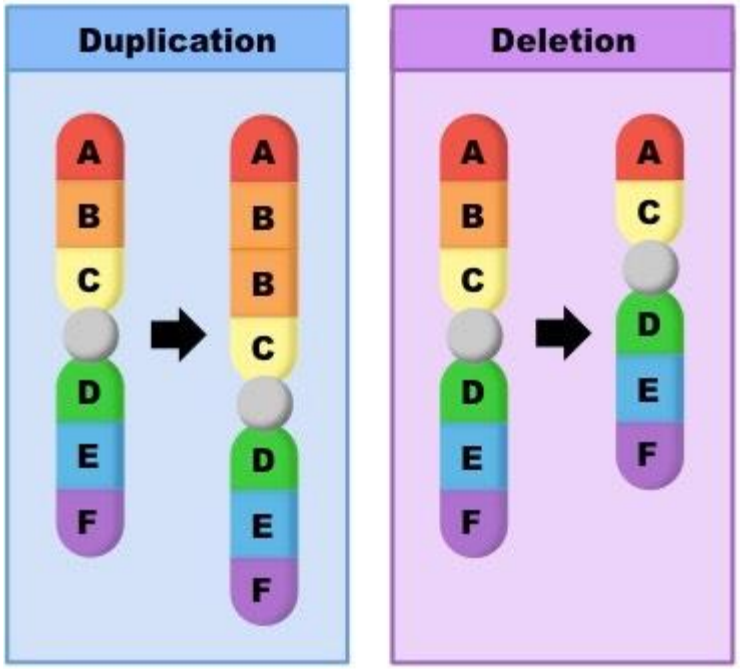
Information from a karyotype

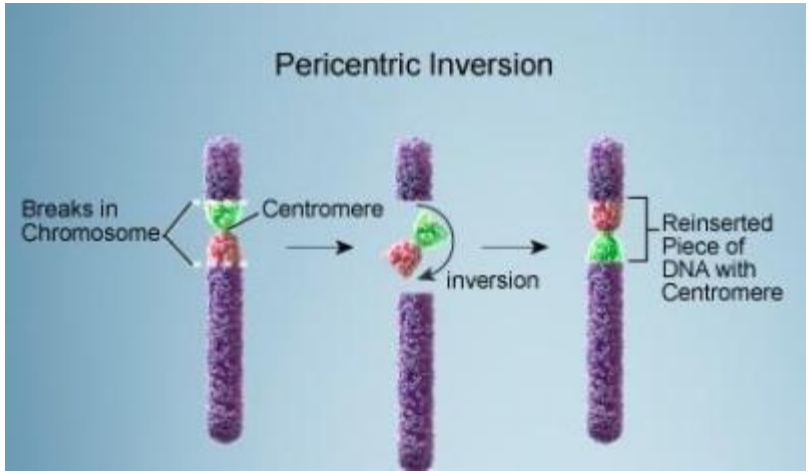
- Six different characteristics of karyotypes are usually observed and compared

- 1. Differences in absolute sizes of chromosomes
  Chromosomes can vary in absolute size by as much as twenty-fold between genera of the same family
  - *Lotus tenuis* and *Vicia faba* (legumes), both have six pairs of chromosomes yet *V. faba* chromosomes are many times larger

Information from a karyotype

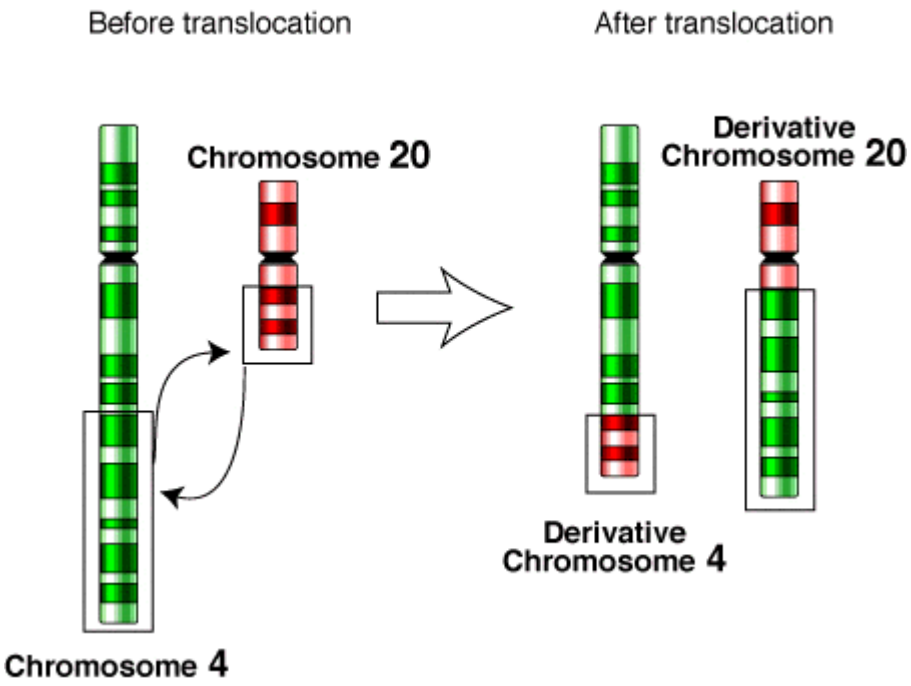
- 2. Differences in the position of centromeres
  - This can be brought about by unequal translocations, pericentric inversion, and centric fusion and fission





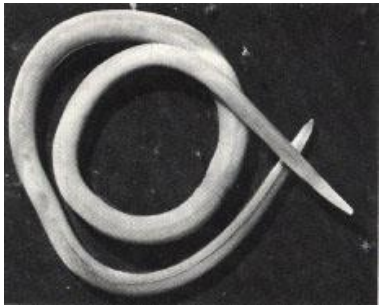
Information from a karyotype

- 3. Differences in relative size of chromosomes
  - Can only be caused by translocations of unequal lengths



Information from a karyotype

- 4. Differences in basic number of chromosomes
- Chromosome number can change due to a number of reasons
  - Chromosome elimination during development
    - Seen in the sciarid flies
  - Chromatin diminution found in some roundworms, portions of the chromosomes are cast away in particular cells
  - Aneuploidy where the chromosome number in the cells is not the typical number for the species
  - Polyploidy – more than two sets of chromosomes
    - Can be normal for some species



Differences in basic number of chromosomes

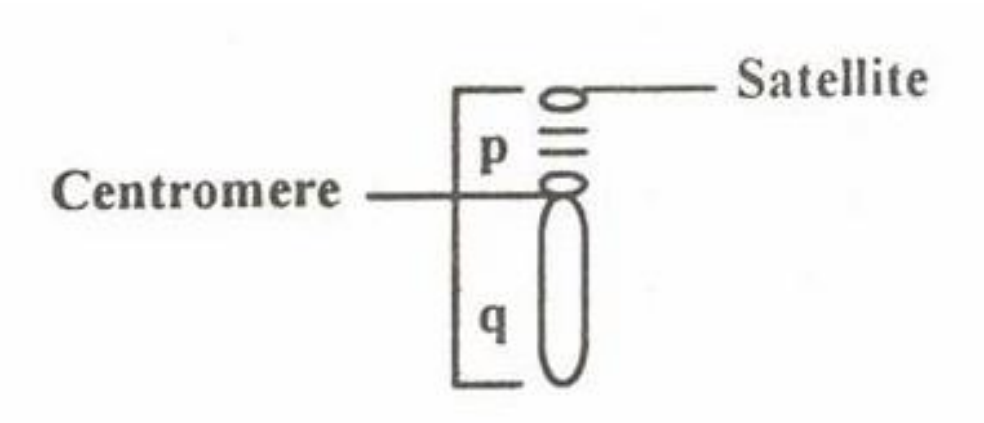
- The number of chromosomes in the karyotype between (relatively) unrelated species is hugely variable
  - The low record is held by an ant: The “Jumper ant” *Myrmecia pilosula,* a primitive group of ants found only in Australia. Males are **haploid n = 1.**
  - The high record would be the Adder's Tongue Fern *Ophioglossum* with an average of **1262 chromosomes**

Differences in basic number of chromosomes in same genus

- The genus *Muntiacus* has 12 recognised species
  - The Indian Muntjac (*Muntiacus muntjak*) is the mammal with the lowest recorded chromosome number: The male has a diploid number of 7, the female only 6 chromosomes.
  - The Chinese Muntjac (*Muntiacus reevesi*), in comparison, has a diploid number of 46 chromosomes

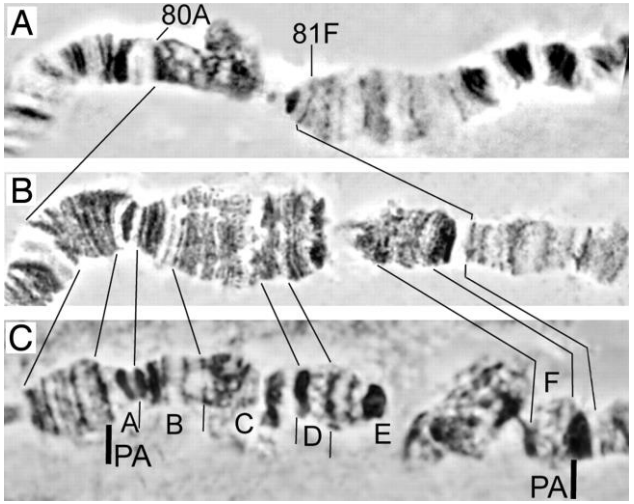
Information from a karyotype

- 5. Differences in number and position of satellites
- Small bodies attached to a chromosome by a thin thread of chromatin
- The secondary constrictions are always constant in their positions and can be used as markers



Information from a karyotype

- 6. Differences in degree and distribution of heterochromatic regions
  - Heterochromatin stains darker than euchromatin in G banding (opposite in R banding), indicating tighter packing, and mainly consists of genetically inactive repetitive DNA sequences.



https://www.pnas.org/content/104/31/12819

Let's make a karyotype

https://www.youtube.com/watch?v=rtyDt_mc6Kw 7 min

- Let's make a karyotype

http://learn.genetics.utah.edu/content/basics/karyotype/

Did you manage to make a karyotype? If not, check out the solution on:

https://www.youtube.com/watch?v=suYzexiv_s4

- Check this out, if you are a nerd (like me) and want to meditate while watching human karyotypes:

https://www.youtube.com/watch?v=E0WkZr819UU