Cytogenetics

Early investigation of how genes contribute to phenotypes (whether it be in Mendelian or Non-Mendelian fashion) required no understanding of the material composition of genes themselves. Today we understand that genes are made of DNA, and in eukaryotes most genes are located on linear chromosomes. The compilation of chromosomes containing all of the genes for an organism makes up a **genome**. **Cytogenetics**, at its core, is the study of the large-scale structure of chromosomes within genomes and how variation in their structure and dosage can cause trait variation. This variation includes differences in chromosome size, shape, and number.

An important note on human disease

When talking about any application of genetics to human health, it is easy to de-humanize individuals with certain genetic conditions. Because of this, sections of this unit that review the impact of cytogenetic variation on human health will not focus on an exhaustive list of the phenotypic changes induced by genetic variation. Instead, the genetic variation will be introduced along with some of the phenotypic implications, and individuals with these conditions will be highlighted. The reason for this approach is to help readers recognize that individuals with lives very similar to their own possess these rare genetic conditions. In a later unit, the consequences of applying genetics to society without this humanizing perspective will be addressed within an ethical context.

Chromosome anatomy

We can orient ourselves regarding chromosomal structure by using a few anatomical terms. The ends of chromosomes are called **telomeres**. The "center" of a chromosome is called the centromere. "Center" is in quotes here because the center of a chromosome is not located in the exact middle- rather, the centromere is the constricted locus where the spindle fibers attach to the kinetocore during cell division. **Metacentric** chromosomes have centromeres located roughly in the middle of the chromosome, whereas **telocentric** chromosomes have centromeres located near the telomeres. Chromosome "arms" (the large region from the centromere to the telomere) are labeled as the **p** arm (short arm) and **q** arm (long arm). **Autosomes** are chromosomes that always have a homologous pair in a diploid organism. **Sex chromosomes** are chromosomes that do not have a homologous pair in the **heterogametic** sex (the sex with two different sex chromosomes: XY males or ZW females).

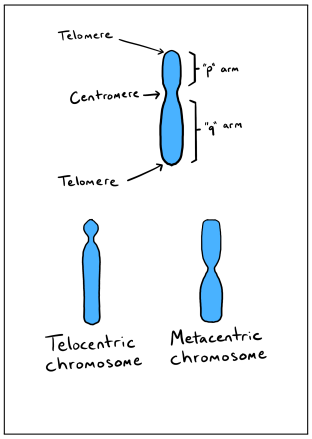

Figure 1: Depiction of chromosome arms and two chromosome types.

Chromatin

Chromosomes are made of **chromatin**, which is a combination of DNA and its associated histones (proteins that DNA wraps around). Although it is the DNA, not the histones, within chromatin that possesses the characteristic of genetic heritability, the histones influence **gene expression** (the transformation of DNA into a phenotype) by increasing/decreasing accessibility of the genes wrapped around them. In other words, while DNA is the molecule of genetic inheritance, histones are involved with **epigenetic** inheritance (a topic that will be covered further in the molecular genetics unit).

Chromosome packaging

There are two different forms of chromatin: **euchromatin** (not highly condensed, allows easy access for transcription) and **heterochromatin** (highly condensed, inhibits transcription). When the chromosomes prepare for cell division (be it mitosis or meiosis), the condensing of chromosomes results in all chromosomes being in a heterochromatin state. However, during the other phases of the cell cycle the chromosomes de-condense to allow for transcription of genes. However, regions of the chromosomes can retain their compact heterochromatin state, meaning that the transcription of the genes in these regions is inhibited.

The packaging of DNA around histones allows for the DNA to be of manageable size within the nucleus. If the DNA was completely unwound, it would be a massive mess within the 0.005 mm-wide nucleus (the DNA of a single human cell would stretch for nearly two meters if completely unwound). DNA doesn't wrap around a single histone. Rather histones join in clumps called **nucleosomes**. A single nucleosome contains eight core histone proteins (called an "octomer"), and the DNA wraps around this structure twice. A single "linker" histone locks the DNA wrap in place. Regularly-intersperced nucleosomes allow for the dense packing of DNA to form heterochromatin.

See this video for a visual representation of how DNA is packaged: `https://www.youtube.com/watch?v=gbSIBhFwQ4s`.

Chromosome Staining

Using recombination frequency isn't the only way to identify gene mapping information. Stains can be used to bind to areas with particular nucleotide composition. This is also how a **karyotype** (the complete set of chromosomes possessed by an organism) is discovered and how **karyograms** (the visual representation of a karyotype through microscopy) are created.

To stain chromosomes, cells collected in metaphase (when the chromosomes are condensed and aligned on the metaphase plate). Chromosomes are then stained with dies and photographed using a microscope. Using later computational analysis, chromosomes are then ordered based on banding pattern and size. Within a karyogram, chromosomes are ordered longest to smallest (i.e., the human chromosome "1" is the largest chromosome and chromosome "22" is the smallest).

One type of chromosome staining is known as **Giemsa staining**. Giemsa die binds to areas high in AT concentration (adenine and thymine), resulting in variable banding patterns along chromosomes based on differences in nucleotide composition. This variability can be seen under

a basic microscope, allowing for imaging and downstream karyotyping. Alternative methods include fluorescent staining, wherein fluorescent dies (e.g., "SKY" or "FISH") bind to areas based on nucleotide composition and images are taken using a fluorescent microscope. Analysis with computer software allows for visualization of diverse banding colors, which increases the accuracy of identification of homologous chromosomes compared to Giemsa staining.

Autosomes and Sex Chromosomes

Staining chromosomes facilitates the identification of homologous pairs, since the chromosomes with the same banding pattern are identified as homologous. This is simple for **autosomes** (chromosomes that have a homologous pair), but is not as straightforward for the **sex chromosomes** of the **heterogametic sex**. Sex chromosomes are those that contain genes involved in sex determination (X and Y in organisms where the male is the heterogametic sex, Z and W in organisms where the female is the heterogametic sex). The heterogametic sex is the sex with non-homologous sex chromosomes (XY males and ZW females). Although there are regions of sex chromosomes that contain the same genes and undergo recombination (known as pseudoautosomal regions), there are other regions that are unique. These unique areas will have their own unique banding pattern. Once all of the homologous chromosomes have been paired up in the heterogametic sex of diploid organisms, the remaining two chromosomes can be paired by process of elimination.

All mammals have XY sex determination (XY males and XX females), and all birds have ZW sex determination (ZW females and ZZ males). Squamate reptiles (snakes and lizards) have flipped back and forth between these sex-determination systems, and many species have XY determination with many others having ZW determination. Additionally, other squamate reptiles do not have "**heteromorphic**" sex chromosomes (meaning there is no difference in the chromosomes of males and females). Instead, sex in these organisms (along with turtles and crocodylians) is determined by their temperature during development in the egg. In organisms with heteromorphic sex chromosomes, biological sex is determined by much more than simply the presence/absence of a given sex chromosome. The presence of specific genes and their coordinated expression within a **gene cascade** (a series of sequential gene activations/suppressions among interacting genes and their products). Variation in the genes of these chromosomes and their expression can result in varying sex-related phenotypes. The association of genetic variation at the macro-scale (e.g., large-scale chromosomal alterations such as inversions, translocations, insertions, and deletions – each of which are introduced later in this unit) with phenotypic variation is attainable using techniques in cytogenetics. However, associating genetic variation of a smaller-scale (e.g., point mutations and smaller-scale insertions/deletions) is more challenging- we will discuss approaches to make these connections in a later unit on molecular genetics.

Bar Bodies

In therian mammal species, the homogametic sex (XX females and WW males) "turn off" one of their sex chromosomes (a process called **lyonization** after geneticist Mary F. Lyon who discovered this process). The "turned off" chromosomes express no genes; these chromosomes are known as **bar bodies**. The chromosome that gets turned into a bar body is randomly selected relatively early in embryo development, and all of the resulting daughter cells born through mitosis have the same chromosome as a bar body. Because which chromosome will be the bar body isn't decided in the zygote and is instead randomly chosen later during development, some

parts of the body will have one chromosome selected as the bar body and other parts of the body may have the other selected. Somatic heterogeneity, where some cells have different chromosomal configurations, is known as **mosaicism**. Because human somatic cells have different 'X' chromosomes selected as bar bodies (i.e., if you randomly selected two cells, you cannot with 100% certainty predict which of the 'X' chromosomes will be a bar body), XX females possess cellular mosaicism with respect to their 'X' chromosomes that are encoding gene products.

Practice Problem: Tortoiseshell Cats

An X-linked gene for cat coloration has two alleles: one that encodes black fur (X^B), and one that encodes orange fur (X^O). However, the color encoded by the given allele is only expressed in the immediate vicinity of the cell. Because of lyonization occuring in the late blastocyst stage, in adult cats sections of fur share the same allele expression (since they are all of the same mitotic origin). A tortoiseshell cat contains patches of orange and black. What is the genotype of these cats? Can they be of either sex?

Solution:

Genotype: X^OX^B; These cats can only be female (since only the females can be heterozygous, the males can only have genotype X^OY or X^BY)

Nondisjunction

Assymetry in Anaphase is **nondisjunction**; this is the outcome of homologous chromosomes failing to separate in Meiosis I or sister chromatids failing to separate in Mitosis or Meiosis II. Nondisjunction results in **aneuploidy**, which is when there is an incomplete set of chromosomes in a genome. When fertilization occurs between a euploid gamete and an aneuploid gamete, the resulting zygote is also aneuploid.

If nondisjunction in Meiosis of one parent resulted in one less chromosome in a gamete that undergoes fertilization with another gamete that has the standard number of chromosomes, then the resulting zygote is monosomic (2n-1). For example, Monosomy 13 is a genetic condition when a human cell has only one chromosome 13 instead of two. **Monosomy** is when there is one chromosome less than the typical number at a specific chromosome, and this condition is so lethal that only one scenario of monosomy allows an individual to survive past birth: this is Monosomy X (commonly called "**45, XO**" or "Turner's Syndrome"). Individuals with 45, XO are sterile due to incomplete ovary development and usually are shorter than average (along with multiple other symptoms), but with hormone therapy many individuals are able to lead regular lives. Approximately one in 2,500 female-assigned births are 45, XO.

If nondisjunction in Meiosis of one parent resulted in one extra chromosome in a gamete that undergoes fertilization with another gamete that has the standard number of chromosomes, then the resulting zygote is trisomic (2n+1). For example, Trisomy 18 is a genetic condition when a human cell has three has chromosomes 18 instead of two. **Trisomy** is when there is one chromosome more than the typical number at a specific chromosome, and this condition is so lethal that very few autosomal scenarios of trisomy allow an individual to survive past birth: these include Trisomy 21 (called "**47, +21**" or "Down Syndrome") and Trisomy 18 (called "**47, +18**" or "Edward's Syndrome"). Although 47, 18+ is highly lethal (roughly 95 percent of individuals with this condition don't survive to through year one), some individuals survive into childhood and in rare scenarios into adolescence/adulthood. This condition affects roughly one in 6,000

births. It is likely more common than that, as are other instances of nondisjunction, but most of these result in spontaneous abortion (which may occur in up to 26 percent of pregnancies, many of which go completely unnoticed).

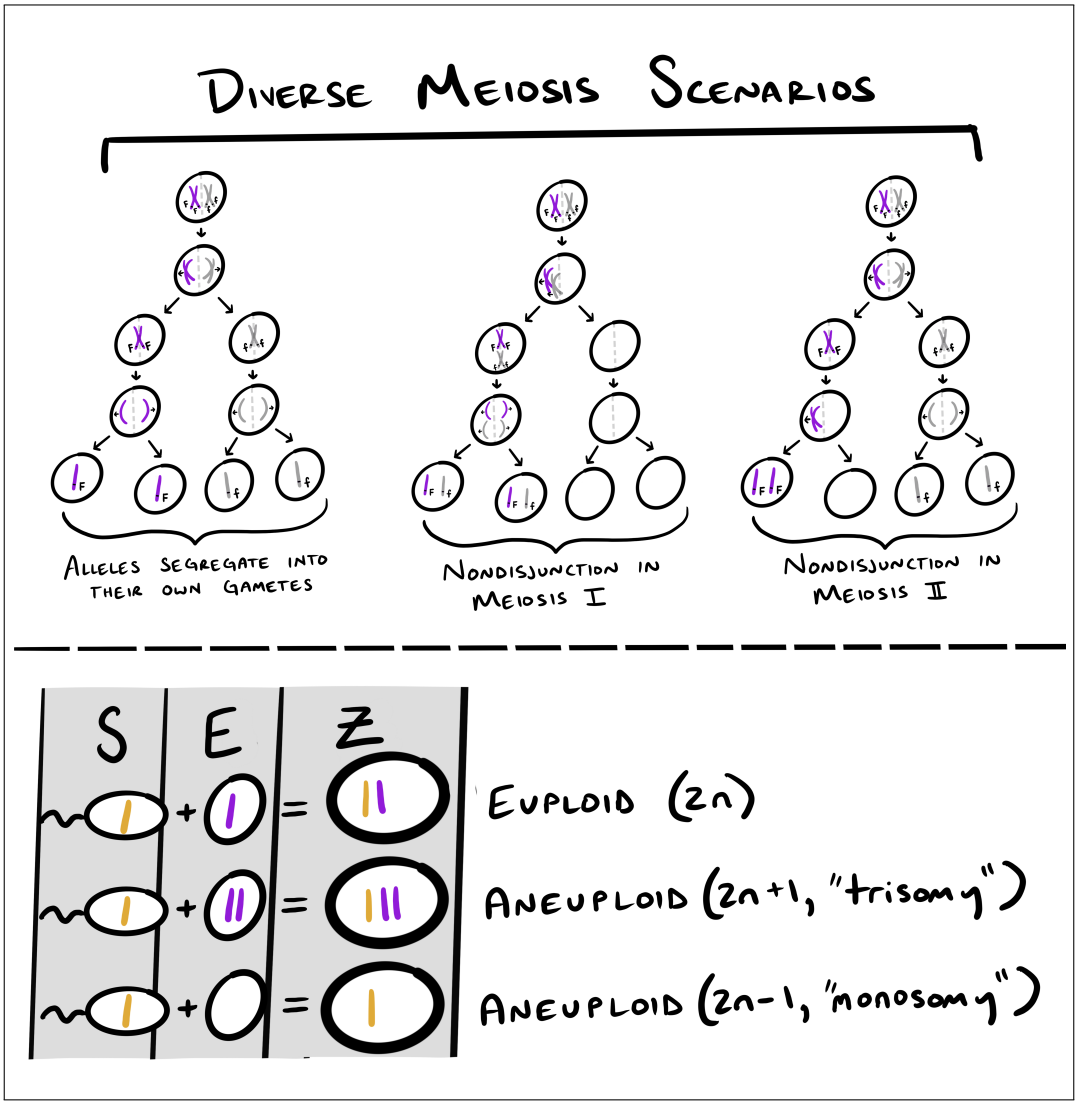

Figure 2: Visual explanation of how nondisjunction in either Anaphase I or Anaphase II (top) can lead to monosomy/trisomy upon fertilization of gametes that are affected by the nondisjunction (bottom). S = sperm, E = egg, Z = zygote.

Note: It is possible for some somatic cells of an individual to be aneuploid and others euploid. Because of the heterogeneity throughout the soma, this is known as "**mosaicism**"- and it is the result of nondisjunction in mitosis during development (rather than meiosis of the parental sex cells). In other words, non-mosaic aneuploidy results from nondisjunction in Meiosis of the pre-gametic sex cells of the parents, whereas mosaic aneuploidy results from nondisjunction happening in Mitosis during embryo growth. Roughly thirty percent of individuals with 45, XO have mosaic 45, XO (meaning that some of their cells have two complete genomes and other cells have one complete genome and another genome missing the 'X' chromosome).

Someone with 45, XO: Missy Marlowe
Olympian gymnast Melissa "Missy" Marlowe has 45, XO. Missy grew up in Salt Lake City, UT, and she competed as a gymnast for the University of Utah and in the 1988 Summer Olympics in Seoul, South Korea. In 1992 she was the NCAA all-around champion.

Of the diverse health conditions caused by chromosomal variation, 47, +21 is the most common (one in 600 births). 47, +21 causes reduced fertility (sterility in almost all XY males) and an increased likelihood of having children with 47, +21 in XX women with this condition. While it is a lifelong condition that varies in severity with effects on cognition and increases risk of some illnesses, many individuals can cope with the condition and excel in diverse areas of life with education and therapy.

Someone with 47, 21+: Pablo Pineda
Spanish award-winning actor and educator Pablo Pineda has 47, 21+. Pineda holds a Bachelor's Degree in educational psychology.

Trisomy of the sex chromosomes can also result in viable human health conditions. These include:

- **47, XXY** (Klinefelter's Syndrome)
- **47, XYY** (Jacob Syndrome)
- **47, XXX** (Triple X Syndrome)

It is estimated that 47, XXY affects one in 500 male-assigned births, yet 25 percent will never receive a diagnosis. This condition is often not diagnosed until adulthood, when a lower production of testosterone compared to XY males can result in the development of breast tissue. The commonality of late-stage diagnoses is because individuals with this condition live almost completely unaffected lives. While individuals with 47, XXY were all considered to be infertile, recent breakthroughs in testicular extraction can isolate viable sperm for subsequent succesful fertilization. Individuals with 47, XXY are more likely to be transgender than the general population, however bias of the reduced-frequency of diagnosis in the general population may skew this fact.

Someone with 47, XXY: Ryan Bregante
President and founder of "Living with XXY", an organization focused on helping individuals with 47 XXY. Before founding the organization, Ryan was a chef (graduate of the Culinary Institute of America) for ten years and an action sports photographer. Ryan enjoys snowboarding, camping, and hiking. See `https://livingwithxxy.org/` for more info.

It is estimated that 47, XYY affects one in 1000 male-assigned births, but this is probably an underestimate since many cases go undiagnosed. Individuals with 47, XYY are largely asymptomatic, but there are many myths about phenotypes caused by this condition (e.g., the myth of increased aggression).

Someone with 47, XYY: Edward R. Friedlander
Professor of Preclinical Sciences, pathologist, and award-winning health outreach educator. Ed enjoys flying planes, parachuting, and endurance swimming. See `http://www.pathguy.com/xyy.html` for more info.

It is estimated that 47, XXX affects one in 1000 female-assigned births, but this is probably an underestimate since many cases go undiagnosed. Individuals with 47, XXX are almost entirely asymptomatic.

Sexual Dimorphism

While this unit is not about the genetic underpinnings of sex differentiation, since most of the genetic conditions in the previous section on nondisjunction occured in the sex chromosomes and had implications for sex determinism and orientation it is appropriate to address the gene cascade that results in **sexual dimorphism** (the observable differences of males and females of the same species).

Although we refer to species with differential sex chromosomes as having "chromosomal sex determination", the chromosomes themselves aren't determining sex. Rather, *genes* on the chromosomes are responsible for the phenotypic differences that we call "sex" (e.g., genitalia). In fact, there are some species that don't have differential sex chromosomes, yet experiments show that their sexes are determined genetically (rather than environmentally such as the turtles and crocs described previously). Although there are no clear differences between the chromosomes that encode sex in these organisms, there are gene-level differences that encode the sexes.

The way genes control sex differentiation differs accross organisms, even when organisms may have the same sex-determination system. For example, both humans and fruit flies (*Drosophila*) have XY sex determination. Yet the gene that triggers sex differentiation in fruit flies (*DSX*) is on the 'X' chromosome, whereas the gene that triggers sex differentiation in humans (*SRY*) is on the 'Y' chromosome.

A superficial look at the human 'Y' chromosome may lead to an unimpressive impression. While the 'X' chromosome contains around 1,000-1,500 genes within its 155 million base pairs (mb), the 'Y' chromosome contains only 50-200 genes in its 57 mb. However, the genes contained therein are critical for sex development. Arguably the most critical is the gene "sex-determining region Y" (*SRY*). This gene activates *SOX9*, a transcription factor that activates *AMH*. *AMH* binds to the surface of the Müllerian ducts (the precursor to the female reproductive system which are present in both male and female embryos), and this binding induces programmed cell death for the cells of the Müllerian ducts. *SOX9* also increases expression of the gene product of *NR5A1*. *NR5A1* encodes the protein SF-1, whose increased expression leads to the development of testes. This is an extreme oversimplification and incomplete description of the gene pathway triggered by *SRY*, but it provides a glimpse into the function of the gene cascade in encoding sexual dimorphism.

A genetic variant in the *SRY* gene can inhibit development of the testes. Individuals with this variant develop ovaries, fallopian tubes, a uterus, and lack a penis due to the inhibited gene cascade that would cause male sex organ development. Although individuals with this variant have the typical 'X' and 'Y' chromosomes of a male, they are usually assigned as females at birth. This condition occurs in one in 80,000 female-assigned births, and is called **46, XY**. Although many individuals with 46, XY (along with many of the other chromosomal conditions discussed in this unit) are assigned one sex at birth, sometimes these assignment are complicated due to gonads/genitalia that aren't easily classified at birth as male or female- a condition known as **intersex**. Because of this, an individual's sex cannot be determined simply by looking at their karyotype. Although statistically this may be one way to guess the sex of an individual

(using patterns that are seen across large datasets; namely, that most men are 'XY' and most women are 'XX'), inferring an indvidual person's sex cannot be done by simply looking at which chromosomes they have.

Someone with 46, XY: Caster Semenya
South African middle distance runner who won two olympic gold medals in the 800 m. Caster was assigned female at birth and identifies as a woman.

Similar to 46, XY, **androgen insensitivity syndrome** (AIS) is the result of a variant in the *AR* gene, another gene involved in sex determination. Androgens are sex hormones that bind to androgen receptors (the gene product of the *AR* gene), and this binding is critical for the development of male external sex organs. Individuals with AIS commonly have the external characteristics of an 'XX' woman, but their internal sex organs are closer to that of an 'XY' male. For this reason, many individuals born with AIS are intersex and are assigned as females at birth. This condition affects one in 100,000 female-assigned births (this is highly likely to be an underestimate).

Someone with AIS: Sarah Gronert
German former professional tennis player.

While usually an XX individual develops the characteristics typical of an XX woman, a variant of the 'X' chromosome that includes a copy of the *SRY* gene (due to non-homologous recombination between the X and Y chromosomes) results in a condition called **46, XX** (or de la Chapelle Syndrome). Individuals with this condition are frequently born intersex and assigned male at birth, and it is not an uncommon condition in transgender women. It occurs in approximately one in 20,000 male-assigned births (but this is likely an underestimate). If you are wondering why all these conditions are "likely an underestimate", it is because most people don't know their own karyotype (do you?).

Chromosomal structural variation

Chromosomal structural variations encompass a wide range of alterations in the structure and arrangement of chromosomes in an organism's genome. These alterations include inversions, translocations, deletions, and insertions, and they can have significant impacts on evolution and disease.

Inversions involve the reversal of a locus, essentially flipping it within the chromosome. **Pericentric** inversions refers to inversions that include the centromere, whereas **Paracentric** inversions do not include the centromere. Both of these inversion types can disrupt the normal functioning of genes within the inverted region and impact the ability of recombination between homologous chromosomes, which can result in health issues. When an individual is homozygous for the inversion (i.e., both of the chromosome copies contain the inversion), recombination occurs as usual with no special adjustments. However, for heterozygous individuals an **inversion loop** forms. The need for this loop in order for recombination to occur results in decreased rates of recombination in these regions. Inversions are often associated with phenotypic variation within populations and between species, and sometimes constitute the most visible genetic differences between recently diverged species (thus involving them in hypotheses of the genetic drivers of speciation).

Inversions in human disease

"Inversion 16" or "inv(16)(p13.1q22)" is a chromosomal inversion on chromosome 16 associated with a subtype of acute myeloid leukemia (AML), a blood and bone marrow cancer. Individuals with AML carrying this inversion often have a relatively favorable prognosis and may respond well to certain treatments, though medical intervention is still necessary. This example highlights how chromosomal inversions can play a role in specific cancer types and associated human diseases.

Inversions in evolution

Theodosius Dobzhansky, a member of Thomas Hunt Morgan's fly lab, discovered that the gene variation between species of *Drosophila* flies was disproportionately contained within inversions that were responsible for the evolutionary divergence and phenotypic differences between the species. This genetic divergence resulted in reduced fitness and fertility when individuals with different karyotypes interbred, creating reproductive isolation through "hybrid breakdown." Additionally, these karyotypic forms were linked to different ecological niches, indicating adaptive advantages and niche specialization. Ultimately, Dobzhansky's work demonstrated how chromosomal inversions promote speciation by driving genetic differentiation, limiting interbreeding, and facilitating adaptation to diverse environments.

Translocations occur when a segment of one chromosome becomes attached to another non-homologous chromosome. Translocations can either be reciprocal (where genetic material swaps places between non-homologous chromosomes) or Robertsonian (where two chromosomes fuse). Translocations can disrupt gene sequences, gene expression, and **gene dosage** (the number of gene copies in a cell). Similar to inversions, translocations are sources of genetic diversity that have evolutionary significance.

Translocations in human disease

A Robertsonian translocation involving chromosomes 14 and 21 can lead to familial Down syndrome (47, 21+ that is inherited). In this scenario, a fusion between chromosomes 14 and 21 can lead to trisomy 21 due to a violation of the law of segregation that is not caused by nondisjunction. Individuals with a single 14+21 fused chromosome and only one other chromosome 14 and one other chromosome 21 are **carriers** of familial Down Syndrome (since they don't exhibit symptoms of the condition). Possible gametes from a carrier include three nonviable possibilities and three viable possibilities (one of which contains two copies of chromosome 21). If the viable gamete with two copies of chromosome 21 is involved in fertilization, it leads to a zygote with three copies of chromosome 21 (causing 47, 21+).

Translocations in evolution

The differences in karyotypes between humans and chimpanzees are the result of evolutionary processes, including a Robertsonian translocation event that occurred in the human lineage after our split from chimpanzees. This translocation fused two ancestral chromosomes into one, reducing our chromosome count from 24 pairs to 23 pairs, while chimpanzees retained the separate, unfused chromosomes. Notably, this fusion produced human chromosome 2, which is absent in chimpanzees and illustrates a significant chromosomal distinction between the two species. These chromosomal rearrangements exemplify how genetic changes over evolutionary time can lead to substantial differences in karyotypes among closely related species, contributing to their genetic diversity and uniqueness.

Deletions involve the loss of a segment of a chromosome. Depending on the size and location of the deletion, this can result in the loss of crucial genes, leading to genetic disorders. Deletions can also play a role in evolution by removing unnecessary or harmful genetic material, thereby streamlining the genome. **Insertions**, on the other hand, are the addition of genetic material to a chromosome. These can lead to gene duplications, which may provide the raw material for the evolution of new functions. However, insertions can also disrupt gene function and cause disease if they occur in critical regions.

Collectively, chromosomal structural variations can both contribute to human diseases and play a role in evolution. Some variations are detrimental and are associated with genetic disorders, while others can be advantageous and drive adaptation over generations. Understanding these variations and their impacts is crucial for both medical genetics and our understanding of the mechanisms behind evolutionary change.