Autosomal/X-Linked Inheritance (W1)

Terms

- **Trait**: Characteristic of an organism, eg. Seed colour
- **Phenotype**: Physical **appearance** of an organism eg. Yellow seed coat
- **Genotype**: **Genetic composition** of the individual eg. YY, Yy or yy
- **Gene**: Unit of heredity (**region of DNA**) influencing a trait, eg. Gene for seed colour
- **Allele**: **Alternate version** of the same gene; Y or y
- **Locus**: Specific place on a chromosome occupied by a gene
- **Homozygous**: An organism possessing two of the **same alleles** at a locus
- **Heterozygous**: An organism possessing two **different alleles** at a locus
- Mutations (variant) <1% and Polymorphisms >1% prevalence

3 Mendelian Principles of Laws of Inheritance

1. Principle of **segregation** (pairs of gene variants separate into reproductive cells)
2. Principle of **uniformity** (dominance, recessive)
3. Principle of **independent assortment** (each pair of alleles segregate independently of other alleles during meiosis)

Testcross

- Cross between dominant phenotype (DD, Dd) and **homozygous recessive** (dd)
  - DD x dd gives 4Dd
  - Dd x dd gives 2 Dd 2 dd

Autosomal Recessive Inheritance (dd)

- Usually **unaffected parents**. Asymptomatic carriers (Dd, heterozygous)
- Increased incidence of parental **consanguinity**
- Roughly **equal** in males and females. Tends to **skip** generations

Autosomal Dominant Inheritance (Dd or DD)

- At least **one affected parent**
- Roughly **equal** in males and females. Usually appears **every** generation
- Males pass to both females and males

X-Linked Recessive Inheritance (X^a,Y or X^a,X^a)

- Affects mainly **males**
- Usually **unaffected parents**. Usually affected male relatives
- There is **no male-to-male transmission**. Males are Hemizygous

X-Linked Dominant Inheritance ((X^A,Y or X^A,X^a or X^A,X^A)

- Affects both sex, but often **excess** of **females**
- Usually **one parent affected**
- **Females** have more **mild symptoms** due to **X-inactivation**
- Affected female has 50% chance of having affected child
- **No male-to-male transmission**
- Usually result from new mutations or germ-line mutations

X chromosome inactivation

- One X chromosome is randomly inactivated early in embryonic development
- Ensures female produce X-linked gene products in **quantities** roughly **similar** to those in **males**: **dosage compensation**
- Other X chromosome becomes a Barr body

SRY (sex determining region)

- Y-linked inheritance

Rules of Probability

- **Product Rule**: Pr of independent events occurring together is:
  - Pr(A and B) = Pr(A) * Pr(B)
- **Sum rule**: Pr of either of two mutually exclusive events occurring is:
  - Pr(A or B) = Pr(A) + Pr(B)

Extensions to Mendelian Inheritance (W2)

1.1 Different types of dominance

1. Complete dominance
2. Incomplete dominance (mix)
3. Codominant (show both colours, e.g. stripes, blood group)

1.2 Multiple Alleles

- ABO blood group
  - A and B alleles are **codominant**, O allele is **recessive**
  - O is universal donor. Glycosyl-transferase enzyme adds sugars
  - I^A and I^B alleles are codominant because both transferases are active
- **Heterozygote advantage**: Heterozygote for sickle cell anaemia allele have better resistance to malaria than homozygote. Sickled cells rupture when infected by malaria parasite

1.3 New Mutation

- Cause appearance of genetic disease w/ no previous family history
- May cause autosomal or X-linked **dominant** genetic diseases
- **Mosaicism**: one cell in embryo that contains mutation
  - **Somatic** mosaicism: present in some tissues, not gametes
  - **Germ-line** mosaicism: restricted to gamete lineage

1.4 Lethal Alleles

- Allele that has potential to cause death of an organism
- Variants in essential genes
- Expected genotype 3:1 ratio can appear as 2:1 instead (death of 1 group)
  - A^Y is dominant for coat colour but recessive for lethality
  - A^Y A^Y kills while A^Y A doesn't kill
- e.g. Allele could be lethal in homozygous. Homozygous dies, heterozygous lives

1.5 Penetrance

- Same genotype, different phenotype
- Frequency of expression of a phenotype <100%, gene shows reduced or incomplete penetrance
- Retinoblastoma: 90% penetrance, 90% chance you will develop disease
- Age-dependent

1.6 Variable expressivity

- Severity of expression of the phenotype with same genotype
- Can occur with different types of alleles at same disease locus
- Modified by X-inactivation

1.7 Pleiotropy

- One variant has multiple phenotypic effects
- One gene affects multiple traits. Most variants are pleiotropic

1.8 Genetic Heterogeneity

- Single disease phenotype caused by variants at different alleles (same gene) or different loci in different families
- **Allelic heterogeneity**: many different variants within one gene result in disease
- **Locus heterogeneity**: same clinical phenotype can result from more than one gene. May be due to epistasis
- AAbb (different genes) x aaBB -> progeny wild type, complementation
- a1a1 (same gene) x a2a2 -> progeny mutant phenotype, fail to complement

1.9 Anticipation

- Strongly expressed at earlier stage
- Common in disease caused by dynamic mutations (trinucleotide repeat that expands during gametogenesis and interferes with gene expression)
- Huntington disease: expansion of CAG triplet in gene

Dihybrid cross: between YYRR x yyrr. Dihybrid cross gives **F2 9:3:3:1**

2.1. Complementary Gene Action

- **F2 9:7 dihybrid ratio**
- Peas: Normally purple, white varieties. CCpp x ccPP
- F1: (CcPp) all purple
- F2: C_;P_ : C_;pp or cc;P_ or cc;pp in 9:7 ratio
- Need a **dominant** allele for **both gene** to produce trait (purple)

2.2 Epistasis

- Phenotype produced by a variant of one gene (epistatic allele) blocks/masks the phenotype produced by alleles of another gene
- Recessive epistasis: **F2 9:3:4**. homozygous ee is epistastic to B gene
  - Yellow -(E gene)—> brown —(B gene)-> black. ee stops at yellow
  - Black: 9. Brown: 3. Yellow: 4
- Dominant epistasis: **F2 12:3:1**
  - Genotypes A_;bb = yellow fruit (3)
  - Genotype aa;bb = green (1)
  - A_;B_ or aa;B_ = white fruit (B is epistatic to A) (12)

2.3 Duplicate genes

- **F2 15:1 dihybrid ratio**
- 15: T_;_ _ or _ _; V_
- 1: tt;vv

2.4 Effect of environment on phenotype

- Temperature: Tyrosinase (melanin production) in cats work best at room temperature. Extremities are darker in hair colour
- Chemical: Phenylketonuria (PKU) Autosomal recessive. Less tyrosine is made with PKU as enzyme phenylalanine hydroxylase is not functional
- Chemical: congenital alactasia, A/R, can't break down lactose

2.5 Genomic (parental imprinting)

- One allele is transcriptionally inactive (imprinted), heavily methylated

2.6 Sex-influenced and sex-limited traits

- Baldness is autosomal dominant condition in males, recessive in females
- Ovary development and milk yield in females; sperm in males
- Allele B behaves dominant in males and recessive in females

2.7 Cytoplasmic inheritance

- mitochondrial DNA is cytoplasmically inherited, not segregated at mitosis
- Matrilineal: through the mothers line
- Mitochondrial DNA is maternally inherited, high mutation rate
- Homoplasmy: every mitochondrial genome carries the causative mutation
- Heteroplasmy: mixed population of normal and mutant genomes
- Autosomal pattern, present in all generations
- Affects males and females equally
- Affected females: 100% of children will be affected
- Affected males: 0% of children will be affected

Monohybrid: differ in one trait. Fully recessive line: **test cross**. AABB x aabb: **dihybrid cross. True breeding**: produces same characteristic on self pollination. F2 progeny is cross backed to either parent, progeny produced is called **backcross progeny**

Genetic Counselling, Linkage, Recombination and Mapping (W3)
Genetic Counselling
Ethical Implications
- Research merit and integrity
  - Potential benefit, well designed, follows principles of research conduct
- Justice: Fair recruitment, access of benefits
- Beneficence: Benefit must outweigh harm. Non-maleficence: avoid harm
- Respect: Autonomy of participants, respect privacy, confidentiality and culture beliefs.

Genetic counselling
1. Interpretation of medical and family histories to assess chance of developing disease
2. Education about inheritance, testing, management, prevention

Linkage
- If two genes are linked on same chromosome, will not assort independently
- Genes on same chromosome are linked
- Linkage is rarely ever complete: crossover between non-sister chromatids occur
- Linked genes can be separated by recombination during meiosis
  - Independent assortment: 1AB:1Ab:1aB:1ab
  - Complete linkage: 1AB:1ab
  - Recombination: ?
- Coupling phase: AB/ab. Repulsion phase: Ab/aB

Recombination Frequency
- Proportion of recombinant gametes will vary depending on crossover rates
- If crossovers never occur, RF = 0%
- If one crossover always occurred then RF = 50% (independent assortment)
- RF = (Number of recombinants) / Total progeny x 100
- 50% max because nearly always two non-crossover gametes
- Test cross used to measure RF
  - Phenotypes of zygotes - genotypes of heterozygous parents
  - pr+vg+/pr vg x pr vg/pr vg (homozygous recessive)
- Parental always highest amounts. Recombination lowest amounts. (542, 537, 76, 75)

Map distance
- Very close together = small chance of recombination (small RF)
- Far apart = high chance of recombination (large RF)
- 1 map unit (mu) = 1cM = 1% recombination between them

Double crossovers
- Cancel out recombinants for distant markers
- Remain is same position
- RF > 25%, need to do statical test
- Hypothesis: assume genes are unlinked to see if accept (unlinked) or reject (linked)
  - If p>0.05 then support hypothesis (unlinked)

Three point trihybrid test crosses - mapping human genes
- Calculating outer loci including double crossover:
- Double crossover progeny are added twice because they each represent 2 crossovers
- RF% = [((number of double crossovers x 2) + recombinant already counted)/ (total progeny)]*100

Interference and Coincidence
- **Interference**: one crossover affects the chance of another occurring nearby
- Formula to calculate **Coefficient too Coincidence (CoC)**
  - Chance of crossover = **RF** or map units **/ 100**
  - Calculate **expected** number of **double crossovers**:
    - (Chance of crossover between A and B) x (chance of crossover between B and C) x total progeny
  - Calculate observed number of double crossovers:
    - Count the number of double crossovers between A and C
  - Calculate coefficient of coincidence
    - **(Observed number of double crossovers) / (expected number of double crossovers)**
- Interference = 1 - (CoC)
- CoC = (number of all DCOs)/([(RF/100 of distance1)(RF/100 of distance2)(total prog.)
- **Positive Interference**: Obs. < Exp. C<1 I>0
- **Negative Interference**: Obs. > Exp. C>1 I<0
- **No interference**: Obs = Exp. C=1 I=0
- **Complete Interference**: No double cross overs. C=0. I=1

DNA Variation, Detection Methods, Marking and Profiling (W4)
DNA Variation and Detection Methods
DNA variants
- Require allelic differences in specific DNA sequences
- Include: Single Nucleotide Polymorphisms (**SNP**), Deletion-Insertion (**InDel**) and Short Tandem Repeats (**STR**)

Sequencing vs Genotyping
Sequencing:
- Up to all 3 billion bp in genome. Find new variants and rare diseases
- Targeted (info on individual genes)
- Exome, whole genome
- 24hrs - 2/3 weeks. Few thousand AUD

Genotyping:
- Up to 5 million bp (usually 500-600k). Find known/common variants
- Targeted (individual genes to a few SNPs)
- Small scale (dozen-hundreds SNPs), medium scale (thousands), large
- Few days, 50 AUD

Single Nucleotide Polymorphisms (SNPs)
- Simple variant of a DNA sequence of a one base pair difference
- Affects ability of restriction enzyme to cut the surrounding sequence
  - Restriction Fragment Length Polymorphism (**RFLP**) Less bands
- RFLP can be detected using PCR from genomic DNA, followed by restriction enzyme digestion and compare bands on gel.

Allele Specific Oligonucleotide (ASO)
- High-throughput genotyping
- Can detect small InDels

Why SNPs are good for mapping
- Very common in the genome - once every 300 bp. Around 10 million SNPs
- Limitation: SNPs usually have only 2 alleles. Micro/mini satellite loci have many

Next Generation Sequencing (NGS)
1. DNA fragmentation
2. Library preparation (primers, adapters)
3. Template amplification (PCR)
4. Sequencing
5. Assembly

DNA Markers, Molecular Mapping and DNA Profling
Micro- and mini-satellite loci
- Short DNA sequences that occur in a variable number of **tandem repeats**
- 2-10bp repeat: **micro-satellite** (Short Tandem Repeat, **STR**)
- 10-100bp repeat: **mini-satellite** (Variable Number of Tandem Repeats, **VNTR**)
- VNTR detection: using **southern hybridisation** using repeat sequence as probe
- STR detection: PCR, using sequence on each side of repeat as primers

Mapping using satellite markers
- 300,000 satellite loci in genome
- Many alleles, many repeats and high level of polymorphism
- Used in DNA profiling, forensics, high resolution genetic mapping

Mapping with DNA Markers
- Many alleles, high level of polymorphism, ease of scoring, Many detected
- High resolution map has >1 DNA marker loci per map unit

Benefits of Genetic Mapping
- Tell if disorder is caused by one gene or different genes
- Identifying disease loci and whole exome/genome sequencing
- Genetic counselling

DNA Profilling
- Establish identity or relationships
- Possible because: genomic DNA is stable, all cells in body have some DNA, DNA is unique (high level of variation)
- Genetic diversity in humans: 99.9% identical at DNA sequence level
  - 0.1% difference = 3 million base pairs. Difference in coding & non-coding reg.

Mini satellite loci DNA fingerprinting
- Digest genomic DNA with restriction enzyme, perform Southern blot. Use probe complementary to the repeat to detect all loci. Unique fingerprint on blot
- Higher number of loci = increased probability pattern will be unique
- However, requires several micrograms of DNA, and DNA must be intact

DNA profiling
- 10-15 unlinked micro-satellite loci, 4bp repeats. Highly polymorphic
- Primers bound outside of repeat, DNA at one locus is amplified
- Advantages: PCR is extremely sensitive, little starting material required, can genotype partially degraded DNA samples
- Disadvantages: contaminating DNA easily amplified
- Pr that suspect is innocent and has exact same DNA profile is 0.0000009%

Source of error
- False inclusion: relatives, some alleles more frequent in certain areas
- False exclusions: technical problems (low amounts DNA), contamination, human error

DNA **fingerprinting**
- **Mini-satellites**, restriction digest followed by southern blotting
- Single probe detects alleles from **several loci**
- Compare 'fingerprints' of southern blot

DNA **profling**
- **Micro-satellites,** multiplex PCR detects alleles from a **single locus**
- Combined independent (unlinked) micro-satellite markers
- Calculate probability

Applications
- Clinical, Forensics, Legal, Conservation Biology