MBLG2072 – Semester 2

L1: Classical and Molecular Genetics I

Mendel's experiments

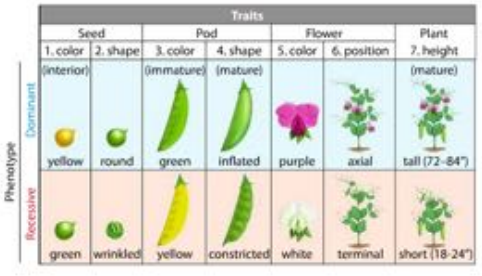
(1) controlled crosses

(2) use of pure breeding strains (*progeny are either homozygous dominant or recessive*)

(3) use of dichotomous traits (*non-continuous*)

(4) counting results

(5) replicate, reciprocal and test crosses



**All progeny have phenotypic ratio of 3:1*

Definitions	Meaning
Phenotype	The observable characteristics of an individual
Genotype	Genetic constitution of an individual organism
Allele	≥2 alternative forms of a gene that arise by mutation and are found at the same place on a chromosome
Homozygous (XX) or (xx)	Particular gene that has identical alleles on both homologous chromosomes
Heterozygous (Xx)	Pair of genes where one is dominant and one is recessive
Recessive	Character only observed in the homozygous state
Dominant	Character expressed when heterozygous state is indistinguishable from the homozygous state
F1 progeny	1st filial generation of offspring of distinctly different parental types
F2 progeny	offspring from allowing the F1 individuals to interbreed

*Geneticists apply phenotype (i.e. dominant or recessive) to alleles of genes

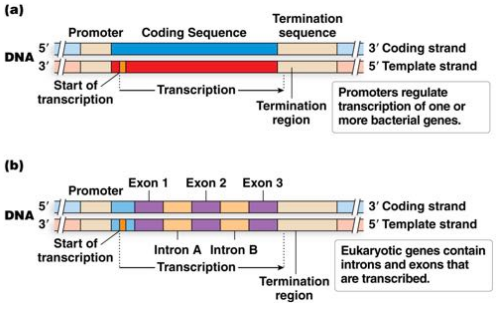
Mutant phenotypes are <u>due</u> to

a) Mutations in genes

b) The phenotype is the result of gene expression

c) The type of dominance depends on how the mutation affects gene expression

**NB. Within a diploid cell there are only ever 2 alleles.*



Know the rules for gene nomenclature

Genes often named after the **mutant phenotype** first described. Some genes and phenotypes:

Drosophila :	
Curly (Cy)	Wing shape
grim	Cell death
hedgehog (hh)	Bristle number
Adh	Enzyme activity

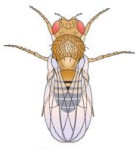
Mouse :	
Sonic hedgehog (SHH)	Embryo development
White spotting (W)	Coat colour pattern

Arabidopsis :	
APETALA2 (AP2)	Reduced petals
SUPERMAN (SUP)	Increased male reproductive organs

***USE italics for gene symbols**

Wild-type is the genotype or phenotype found in nature or standard laboratory stocks, e.g. in Drosophila the vestigial wings locus has the symbol vg^+

Mendelian notation:	• Dominant alleles: *A* • Recessive alleles: *a*
Genetic notation	• *A*, a^+ or + may be the annotation for the normal or wild-type allele → • Wingless (wg) is the gene → wg^1 *and* wg^{SP-1}: recessive mutant alleles • *Cy*: **dominant mutant allele** • Cy^+: **wild-type allele**



Wild type

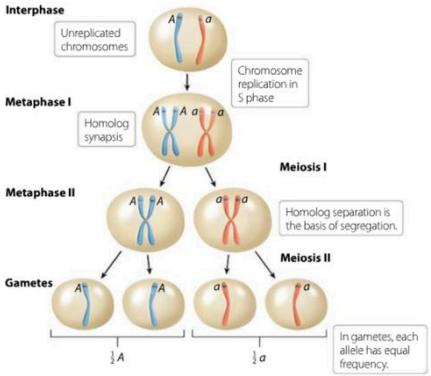
vg^+/vg^+ or $+/+$

Vestigial wings

vg/vg

Understand and apply Mendel's 1st law of equal segregation

1. The 2 alleles for a trait separates during gamete formation where each allele has **equal probability** to be included in the gamete
2. The random union of gametes, **one from each parent at fertilization**, will produce progeny in ratios determined by chance



Use a Punnett square to determine the outcome of a cross (phenotype and genotype)

Know what a monohybrid cross and a test cross is

	Monohybrid Cross:	**Test Cross**
Definition	Cross between individuals differing in **a single trait** e.g. flower colour in peas (purple vs. white)	Cross **unknown genotype** with **homozygous recessive (*pp*).**
Punnett Square	Female gametes: $\frac{1}{2}P$, $\frac{1}{2}p$ Male gametes: $\frac{1}{2}P$, $\frac{1}{2}p$	Tester: $\frac{1}{2}p$, $\frac{1}{2}p$ Unknown: $\frac{1}{2}P$, $\frac{1}{2}$?
Phenotypic Ratio	**3:1**	If **unknown was**: • heterozygous (*Pp*) = 1:1 phenotype ratio • homozygous (*PP*) = ONLY 1 phenotype (*Pp*)
Genotypic ratio	**1:2:1**	

**These events are 2 mutually exclusive (independent) events are occurring together*

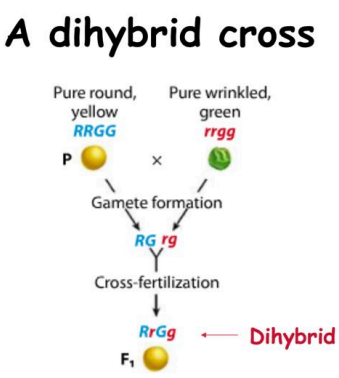
******Sum rule or addition rule*** *- the probability of the occurrence of* ***either*** *of 2 or more mutually exclusive events is the* ***sum*** *of their individual probabilities.*

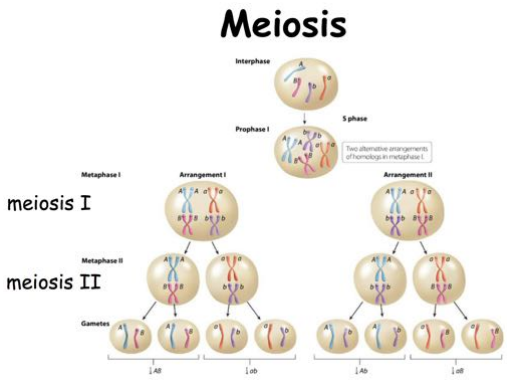
L2: Classical and Molecular Genetics II

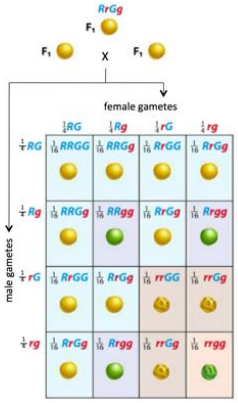
Determine segregation ratios in a dihybrid cross

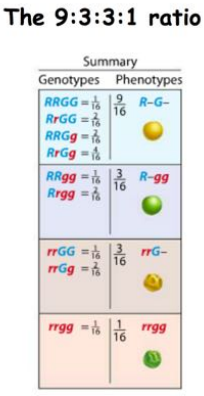
Mendel's 2ND Law or The Law of Independent Assortment

During gamete formation segregation of the alleles of one gene is independent of the segregation of the alleles of another gene









Mendel's dihybrid-cross experiment produced a 3:1 ratio for each trait and a 9:3:3:1 ratio for the combined phenotypes.

Use the χ^2 test to establish goodness of fit with hypothesised ratios

Goodness of fit

How do you tell whether the observed numbers agree with the hypothesised ratio?

- When each observation falls into one of 2 or more mutually exclusive categories, can use χ^2 test.
- First need **null hypothesis (H_0)** e.g. 9:3:3:1

$$\chi^2 = \sum_{\text{all classes}} \frac{(O - E)^2}{E}$$

O and E are Observed and Expected NUMBERS.
(not fractions or percentages)

CASE EXAMPLE:

Cross: RrGg (round, yellow) x RrGg (round, yellow)

Null hypothesis: H_0 =9:3:3:1

Class	Observed	Expected	O – E	$\frac{(O-E)^2}{E}$
Round, yellow	315	312.8	2.2	0.015
Round, green	108	104.2	3.8	0.139
Wrinkled, yellow	101	104.2	-3.2	0.098
Wrinkled, green	32	34.8	-2.8	0.225
Total	556	556		$X^2 = 0.477$

How do you determine the significance of the result?

- Tables of χ^2 show probability (P) values
- **Degrees of freedom = number of categories – 1**
  [in this case 4, hence d.f. = 3]

$\chi^2_{[3]} = 0.477$, P > 0.05; not significantly different from expected; therefore fail to reject the null hypothesis.

Probability (*P*) value

df	0.95	0.90	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.016	0.15	0.46	1.07	1.64	2.17	3.84	6.64	10.83
2	0.10	0.21	0.71	1.39	2.41	3.22	4.61	5.99	9.21	13.82
3	0.35	0.58	1.42	2.37	3.67	4.64	6.25	7.82	11.35	16.27
⋮										
13	5.89	7.04	9.93	12.34	15.12	16.99	19.81	22.36	27.69	34.53
14	6.57	7.79	10.82	13.34	16.22	18.15	21.06	23.69	29.14	36.12
15	7.26	8.55	11.72	14.34	17.32	19.31	22.31	25.00	30.58	37.70
	Fail to reject chance hypothesis							Reject chance hypothesis		

Understand the difference between dominant and recessive

Definitions	Meaning
Recessive	Character only observed in the homozygous state
Dominant	Character expressed when heterozygous state is indistinguishable from the homozygous state

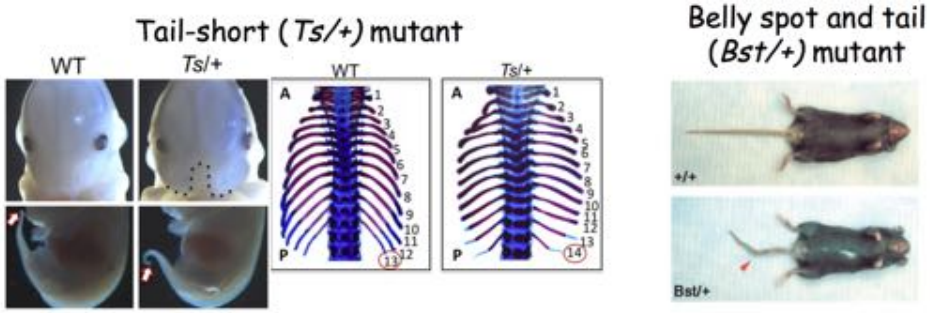
Be able to describe when a wild-type gene is haplo-sufficient and when a wild type gene is haplo-insufficient

Example 1: Wild type allele is dominant	**Example 2: Wild type allele is recessive**
40 units of enzyme R Substrate → → → Product	18 units of enzyme T Substrate → → → Product
• Wild type gene R^+ produces 50 units of R • Mutant gene r produces no units of R	• Wild type gene T^1 produces 10 units of T • Mutant gene T^2 produces 5 units of T
This means that: • R^+/R^+ = 100 units *[produces product]* • R^+/r = 50 units *[produces product]* • r/ r = 0 units	This means that: • T^1/T^1 = 20 units *[produces product]* • T^1/T^2 = 15 units • T^2/T^2 = 10 units
In this case: • R^+ is dominant and r is recessive The **R^+ wild type allele** is **haplo-sufficient**	In this case (example of **albinism**) • T^2 is dominant and T^1 is recessive The **T^1 wild type allele** is **haplo-insufficient** **NOTE: Ribosomal protein mutants are haplo-insufficient**

Case Examples:

1) Dominant mutations in ribosomal protein genes in mice

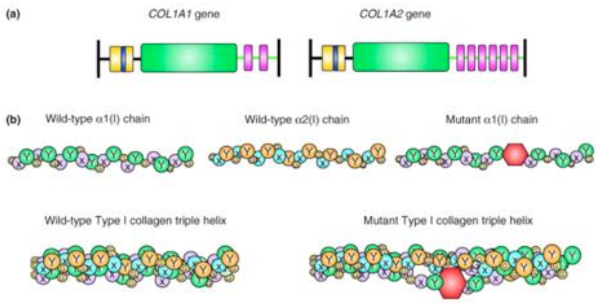
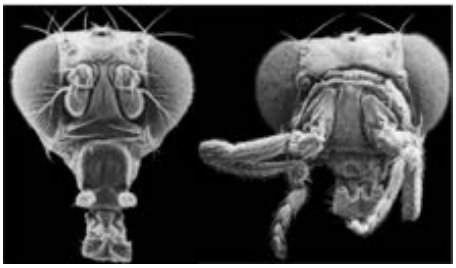
- These mutant genes are dominant but homozygous lethal, hence homozygous expression would be lethal



2) Dominant mutations in ribosomal protein genes in Humans**: Diamond-Blackfan Anemia**

- DBA prevents bone marrow from producing sufficient RBCs → causing fatigue, weakness and pale appearance
- Sufferers tend to have small head size and a low frontal hairline with 1/3 having slow growth → short stature.
- Approximately 50-65% of individuals with DBA have identified mutations in ribosomal protein genes (RPS19, RPL5, RPL11, RPL35A, RPS7, RPS10, RPS17, RPS24, or RPS26) → hence non-functional ribosomes

Understand the difference between loss-of- function and gain-of-function mutations

	Loss-of-function mutations	Gain-of-function mutations
Types	• **Amorphic (null)** mutation (complete loss) • **Hypomorphic (leaky)** mutation (partial loss) • **Dominant negative** mutations (altered gene product → due to premature stop codon)	• **Hypermorphic** mutation (Increased gene activity) *For these mutations the homozygous is more severe than the heterozygous* • **Neomorphic** mutation (Novel function) → gene expressed in areas not usually expressed
Effect	**Decrease or complete loss of** the functional activity of a gene product	New function or have increased levels of expression
Mutation	usually **recessive**	often **dominant**
		
Example	• **Osteogenesis imperfecta (brittle bone disease)** is a dominant negative mutation on the COL1A1 and COL1A2 genes • Genes encode type I collagen → essential for bone flexibility and strength • Sufferers easily prone to fracture due to fragile bones Others include = cystic fibrosis, sickle-cell aneamia	• Drosophila Antennapaedia (Antp) mutant has **legs in the place of antennae** • Dominant mutations with expression of a gene in the wrong place or at the wrong time and cannot be compensated by wild-type allele

L3: Classical and Molecular Genetics III

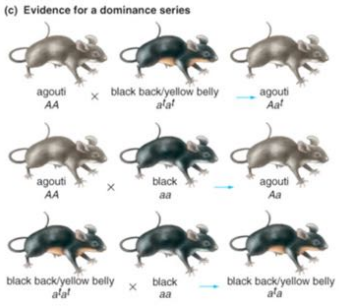
Understand pedigrees and how to use these to identify recessive and dominant traits

Allelic series

- Diploid organisms have 2 alleles at a locus
- Populations or groups of organisms can have > 2 alleles
- An order of dominance between alleles can form an allelic series
- Mouse agouti gene allelic series A > a^t >a

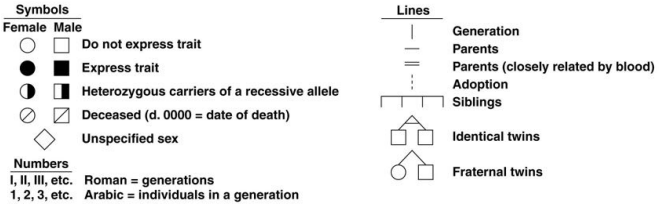
(b) Alleles of the *agouti* gene

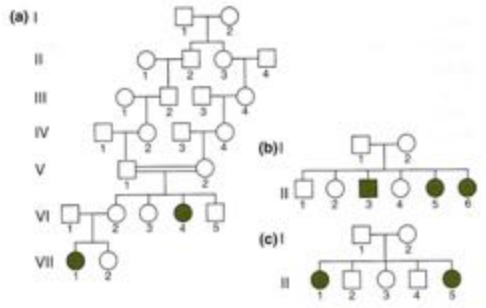
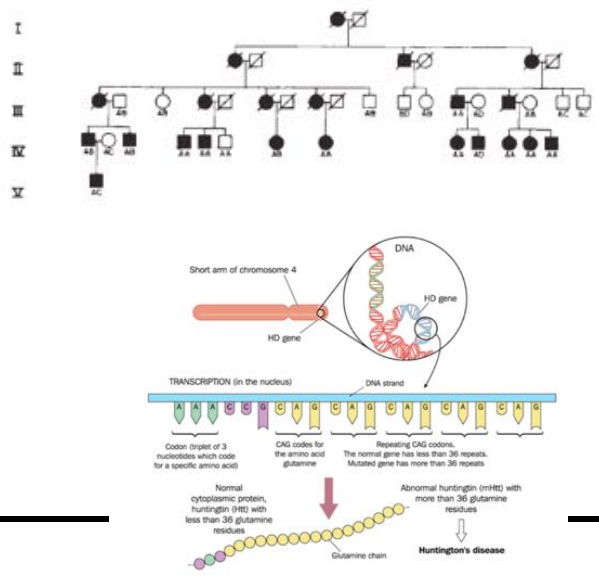
Genotype	Phenotype
A–	agouti
$a^t a^t$	black/yellow
aa	black



Pedigrees (family trees)

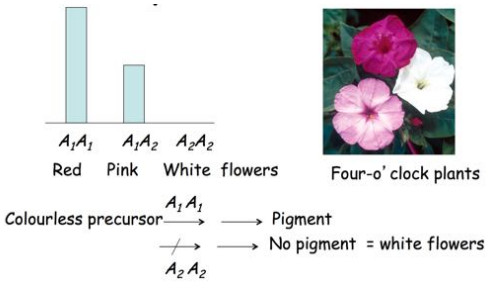
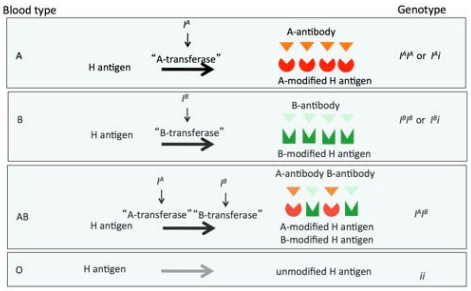
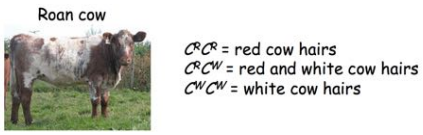
- trace inheritance of traits in humans and some animals



Autosomal Recessive Inheritance	Autosomal Dominant Inheritance
1) Individuals who have the disease are often born to parents who **do not** 2) If **only 1 parent** has the disorder the risk that a child will have it depends on the genotype of the other parent 3) If **both parents** have the disorder, all children will have it 4) The sex ratio of affected offspring is expected to be equal 5) The disease is not usually seen in each generation but if an affected child is produced by unaffected parents, the risk to subsequent children is 1⁄4 6) If disease is rare in the population, unaffected parents of affected children are likely to be related to one another	1. Each individual who has the disease has at least one affected parent 2. Males and females are affected in equal numbers 3. Either sex can transmit the disease allele 4. In crosses where one parent is affected and the other is not, ≈ half the offspring have the disease 5. Two unaffected parents **will not** have any children with the disease 6. Two affected parents may produce unaffected children
Cystic Fibrosis → Genetic Mapping →Gene for CF: CFTR Found  Genetic mapping is possible due to recombination • Polymorphisms show the genetic variation of individuals within a population. • Polymorphisms that are close together on a chromosome tend to be inherited together.	**Huntington's Disease** (usually adult onset → neurodegeneration) 

Know the different types of dominance and how to distinguish between incomplete dominance and codominance

There are several genetic outcomes for dominant mutations:

	Incomplete dominance or partial dominance	**Co dominance**
Effect	Phenotype is intermediate of parent's phenotype	Characteristics of alleles from <u>both parents</u>
Example		**ABO blood type** 
		

Know some of the factors that affect Mendelian ratios. What are they?

<u>Factors affecting observed phenotype and genotype</u>

1. **Lethal alleles**
2. **Pleiotropy** → single gene affects 2 or more characters
3. **Penetrance and expressivity**
4. **Interactions among genes** (epistasis)
5. **Sex-linked inheritance**

Describe how to determine if a gene is embryo lethal

The A (agouti) gene → Many alleles, determining the extent of black and yellow pigment in the hair shaft

- AA and Aa = wild type, black hair with yellow band agouti
- aa = non-agouti, solid black hair
- A^YA = yellow, solid yellow hair

yellow X agouti → 1 yellow : 1 agouti

A^YA AA → A^YA AA

yellow X yellow → 1 agouti : 2 yellow : 1 lethal

A^YA A^YA → AA A^YA A^YA^Y

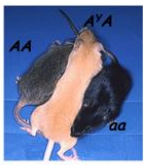
<u>Key Points</u>:

- <u>Impossible</u> to obtain a pure breeding yellow line from yellow offspring (as embryological lethal)
- Time of death may vary i.e. before or after birth
- Also <u>semilethal alleles</u> cause reduced viability + lead to altered genetic ratios e.g. Aa X Aa 90% A- : 10% aa

Appreciate the relationship between gene and phenotype

- one gene / many phenotypes
- one phenotype / many genes

Pleiotropy: A single gene affects two or more characters



A^Y in mice affects:

coat colour ⟶ A^YA Yellow

weight ⟶ A^YA Obese

***W* white spotting gene** in mice affects:

migration of melanocytes ⟶ white spotting

germ cells ⟶ sterility

blood precursor cells ⟶ anemia

W/+ mouse

Many genes can influence a single phenotype

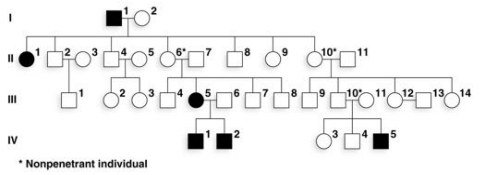
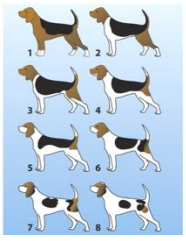
Coat colour

Gene 1: *agouti*: distribution of colour on each hair multiple alleles
A- banded, agouti
a - solid black
A^Y - solid yellow
a^t - black back, yellow belly

Gene 2: dark colour of hair black or brown
B - black
b - brown

Gene 3: albino or pigmented
C - coloured depending on alleles at *A* and *B*
c - recessive, homozygotes are pure white

Understand the difference between penetrance and expressivity. Why are these factors important?

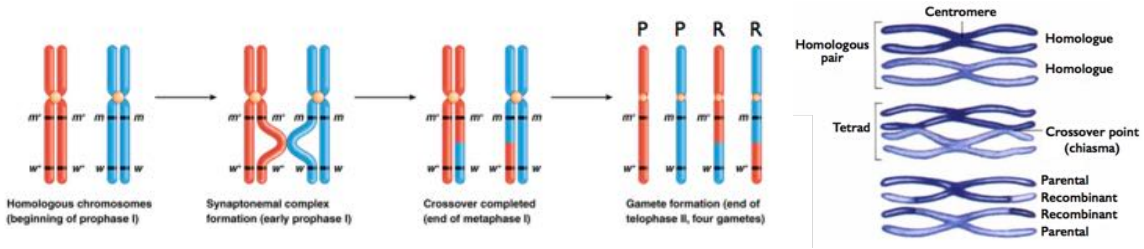
TERM	Definition	EXAMPLE	Effect
Penetrance	Proportion of individuals of a specific genotype that exhibit the **corresponding phenotype**	For recessive mutation *a* *aa* ⟶ 100% phenotype penetrance = 1.0 For recessive mutation *d* *dd* ⟶ 80% phenotype penetrance = 0.8 ⟶ 20% normal	1. **MODIFICATION** of segregation ratios or apparent inheritance of an allele 2. Changing the phenotype between individuals
	Example: Polydactyl • Single dominant gene with **incomplete penetrance** • At least 1 in 4 people with the mutation have 5 digits	 * Nonpenetrant individual	
Variable expressivity	Extent to which a phenotype is expressed	 Piebald spotting in beagles. Spotting is due to a dominant gene S^p with variable expressivity.	

Note: Variable phenotypes can be caused by a number of factors:

- modifier genes
- environmental factors
- allelic variation
- complex genetic and environmental interactions

Use recombination frequencies to calculate map distances and interference

Recombination (crossing over)



- **Recombination,** or **crossing over** separates alleles of linked genes → to produce new allelic combinations
- **Chiasmata =** site of crossing over
- Occurs during **prophase I** of **meiosis**
- Crossing over occurs between **chromatids** of homologous chromosomes
- Crossing over creates **parental (P)** and **recombinant (R)** gametes after segregation

Quantifying linkage

- Recombination frequency determines the arrangement of elements along a chromosome
- **Recombination rate** is a measure of **genetic distance**

$$\textbf{Recombination frequency } (\mathbf{r}) = \frac{\#\ of\ recombinant\ offspring}{total\ \#\ of\ offspring}$$

$$\textbf{Genetic distance} = \mathbf{r} \times 100 = \text{ map unit (m.u.) or centiMorgans (cM)}$$

Appreciate the use of molecular markers for linkage mapping and disease diagnosis

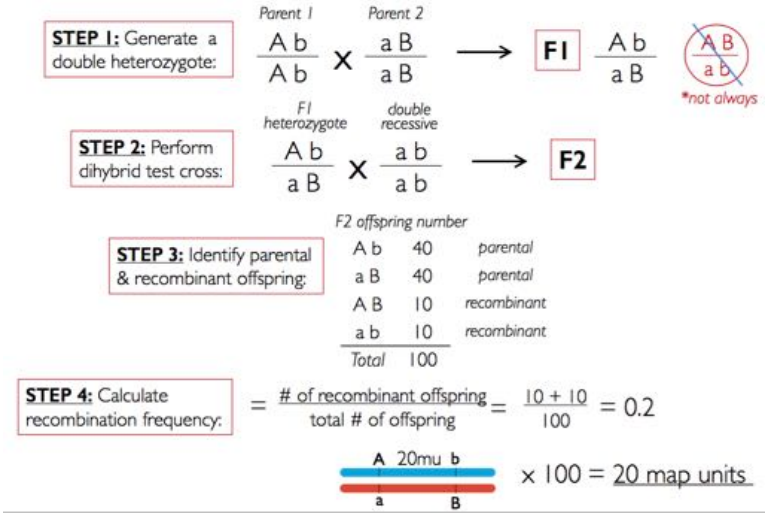
Linkage maps

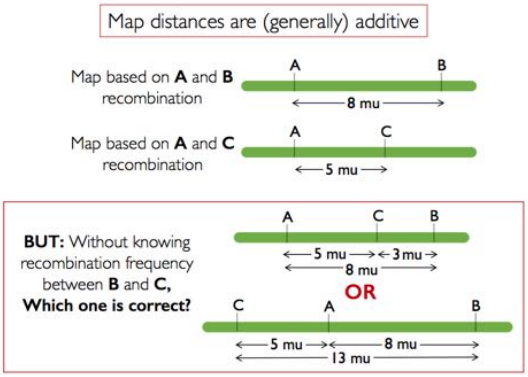
- Mapping genetic markers by recombination frequency
- The further 2 genes are apart = ↑ chance of recombination = ↑recombinant progeny
- Closer 2 genes are = ↓ chance of recombination = ↓ recombinant progeny
- Genes **> 50 m.u. apart** undertake so much recombination between them, they are deemed to be **unlinked and assort independently**

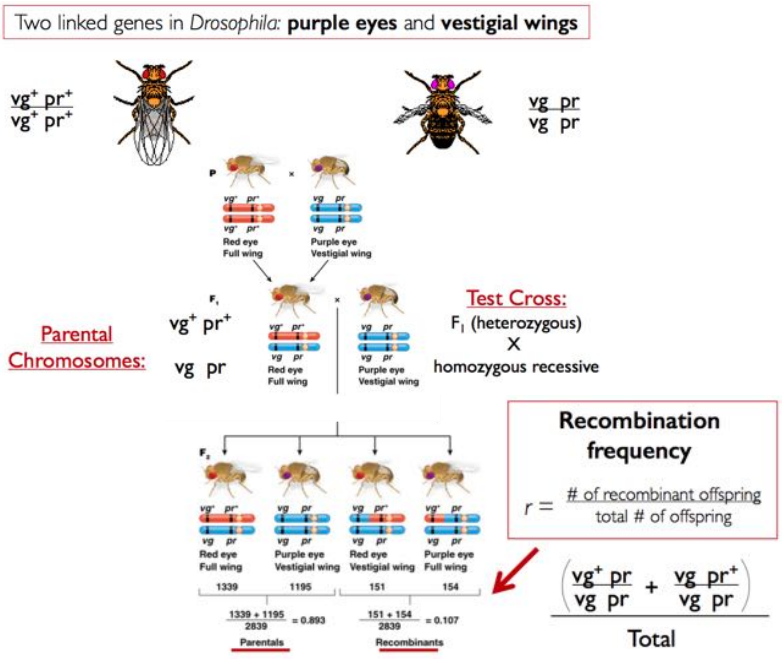
Dihybrid test cross: (heterozygote x double-recessive individual)

AaBb X aabb

- **ONLY** 1 type of gamete is produced for the double recessive individual (**ab**) → gametes won't contribute to offspring phenotype
- 4 types of gametes are produced from the heterozygous parent (**AB, ab, Ab, aB**)
- Phenotype of dihybrid cross progeny are derived from the gametes of the heterozygous parent
- 2 gametes are **parental** and 2 are **recombinant**







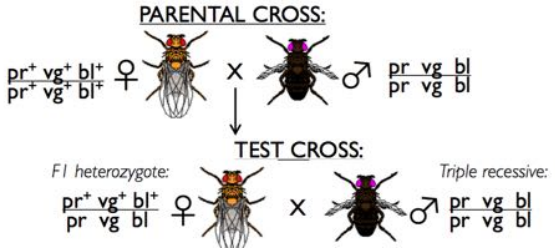
3-point test cross

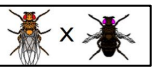
Important Note:

- 3-point test cross determines if 3 genes are **linked**, the **gene order**, and **map distance**
- **DO NOT** assume that all genes are linked
- **DO NOT** assume that the order of writing is the order of the genes on the chromosome

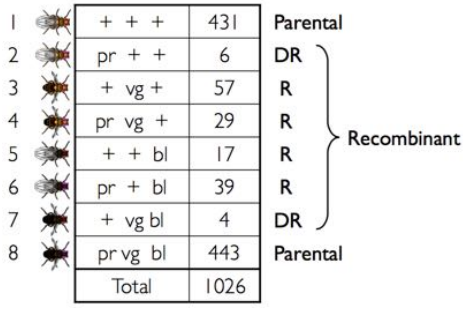
EXAMPLE:

Three genes in *Drosophila*: **purple eyes, vestigial wings** and **black body**



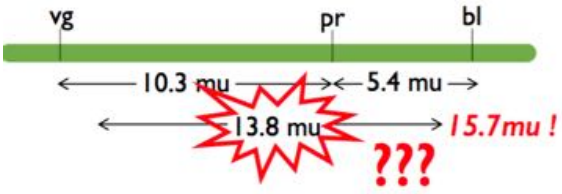
3-point test cross: $\frac{+\ +\ +}{pr\ vg\ bl} \times \frac{pr\ vg\ bl}{pr\ vg\ bl}$ 

Progeny phenotypes: 8 classes of progeny: **Parental (P), Single Recombinant (R)** and **Double Recombinant (DR)**

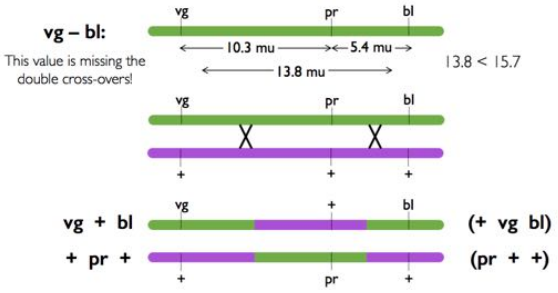


1	+ + +	431	Parental
2	pr + +	6	DR
3	+ vg +	57	R
4	pr vg +	29	R
5	+ + bl	17	R
6	pr + bl	39	R
7	+ vg bl	4	DR
8	pr vg bl	443	Parental
	Total	1026	

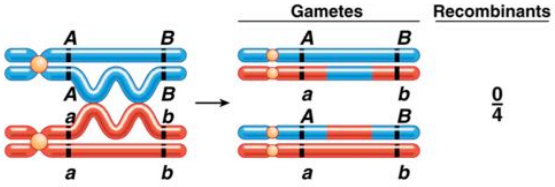
Recombinant

Step 1: Determine the gene order:	• Find **double crossover** classes (i.e. *the classes with the fewest recombinants)* • *This is row 2 and row 7* pr; +; + *and* +; vg; bl • The marker that is **different** from the **parental configuration** is the **middle marker** vg – pr – bl *or* bl – pr – vg
STEP 2: Consider markers in all possible pairwise combinations:	**pr** and **vg** recombination frequency: (2, 3, 6, 7) $\frac{6+57+39+4}{1026} = \frac{106}{1026}$ $r = 0.103$ $\times 100 = 10.3$ mu **pr** and **bl** recombination frequency: (2, 4, 5, 7) $\frac{6+29+17+4}{1026} = \frac{56}{1026}$ $r = 0.054$ $\times 100 = 5.4$ mu **vg** and **bl** recombination frequency: (3, 4, 5, 6) $\frac{57+29+17+39}{1026} = \frac{142}{1026}$ $r = 0.138$ $\times 100 = 13.8$ mu
Step 3: Put the map together!	 **Why not the same distance?** Due to effects of double recombination appearing as non-recombinants

Step 4: Factor in double recombination



DR classes *appear* non-recombinant for vg and bl but actually have 2 cross-over events → which are not visible in progeny since recombination events occur between flanking markers

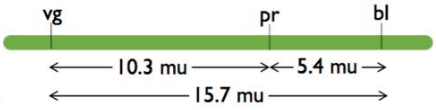


NEED TO CONSIDER: Actual **vg** and **bl** recombination frequency (with DRs) by adding **TWICE**:

$$\frac{6+6+57+29+17+39+4+4}{1026} = \frac{162}{1026}$$

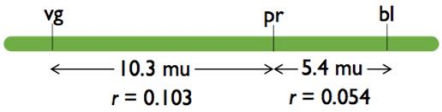
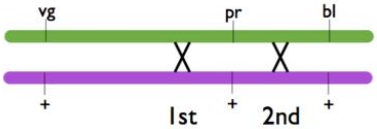
$r = 0.157$

$\times 100 = 15.7$ mu



Interference

- Crossover events in adjacent regions of the chromosome may not be independent
- **Interference *(I)*** occurs when DR events happen more or less frequently than expected
- The **coefficient of coincidence *(c)*** is the **observed** number of double recombinants divided by the **expected**
- Use the **recombination frequency *(r)*** of single recombinants to calculate expected double recombinants
- **Interference *(I)*** = **1 - *c***

STEP 1: Calculate expected double recombinants 	(*r* of vg–pr) x (*r* of pr–bl) x total progeny 0.103 x 0.054 x 1026 = 5.7 is expected double recombinants
STEP 2: Calculate Coefficient of Coincidence:	$c = \frac{\text{Observed DRs}}{\text{Expected DRs}} = \frac{10}{5.7} = 1.7$
STEP 3: Calculate Interference 	$I = 1 - c$ $= 1 - 1.7$ $= -0.7$ **Interference is negative (*I* < 0):** A cross-over in one region **promotes** crossing over (recombination) in adjacent region **Interference is positive (*I* > 0):** A cross-over in one region **interferes** with crossing over in adjacent region

L14: Mobile Genetic Elements

Transposable genetic elements: Transposase

- **Transposable genetic elements are** DNA sequences that move via **transposition**
    - Many forms, lengths, copy numbers
- Can cause a mutation via **insertional inactivation**

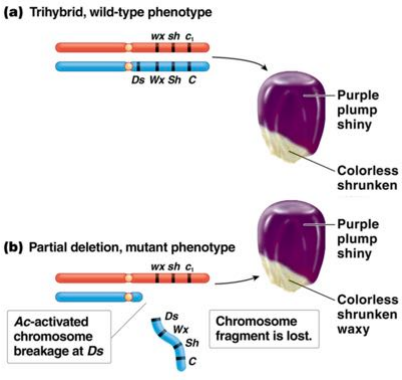
TRANSPOSABLE ELEMENTS:

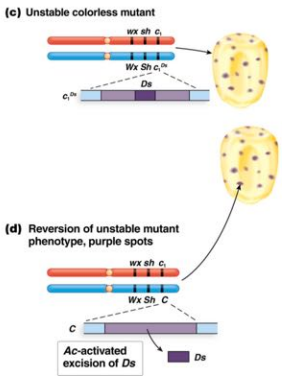
- Transposition requires the **enzyme transposase** encoded by genes carrying some transposable elements
- Some transposable elements carry other genes WHILE some contain only repetitive sequences

Dissociation (*Ds*) element	Site of chromosome breakage
Activator (*Ac*) element	2nd element required for chromosome breakage

Transposable element type	Contains Transposase gene?	Function
Autonomous	Yes	All DNA sequences that conduct transposition (e.g. *Ac*)
Non-autonomous	No	• May lack the sequences needed for transposition (e.g. *Ds*) • CANNOT move → unless transposase is provided by an autonomous element elsewhere in the genome

McClintock's transposable genetic element hypothesis was that the unstable mutant phenotype resulted when a **transposable element (DS)** created a mutation by its insertion into the **C** allele and led to reversion when the expression of **Ac allele** led to its removal.





- Barbara McClintock discovered transposition in maize
- Noticed that some sectors lacked colour, were shrunken and waxy

WHY?

- Colourless sectors had terminal deletion of one chromosome 9 homologue
- Purple sectors, intact chromosome 9

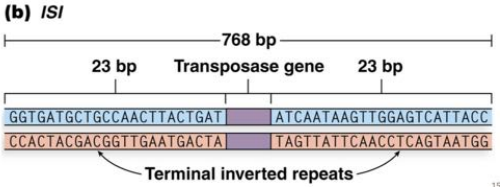
- Colourless kernels have varied purple spotting
- **Unstable mutant alleles** = Transposable element *Ds* insertion into the *C* locus produces a kernel lacking pigmentation (the mutation is called c_1^{DS})
- Rare transposition of *Ds* out of the gene
- Reversion to wild type
- Array of purple spots on the kernels

Bacterial genomes • Insertion sequences • Transposons • Transposition

2 categories of transposable elements (insertion sequences and transposons)

IS (insertion sequence) elements (≈ 1000bp)

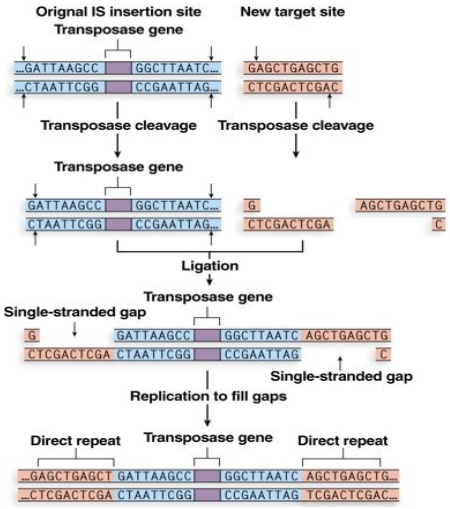
- Only contains genes required for autonomous transposition
    - Transposase gene flanked by a **short, inverted repeat (IR) sequence** → (needed for transposition)
- **Different IS elements** have **different IR sequences**



IS (insertion sequence) transposition

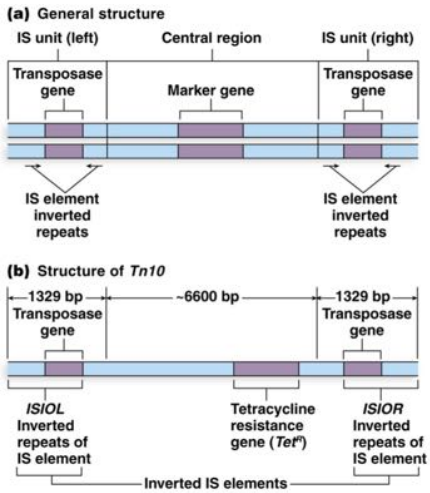
How do insertion sequences work?

1. Cleavage of IS elements ends
2. Transposase recognizes and cleaves a sequence at the new integration site
3. IS element is ligated into the new site
4. Gaps filled by DNA replication
5. Pair of **direct repeats** is produced upon integration



Transposons → most genes carried by transposons give **antibiotic resistance**

Transposons (Tn) type	Length	Structure	Transposase gene?	Other features
Composite	Long (kB)	Complete IS elements with inverted repeats at each end	Yes (at least one)	• One or more functional genes • *E.g. transposon Tn10*
Simple	Short (<50bp)	Enzyme encoded by the simple transposon itself	No transposase in IRs	Additional genes in the central element



Transposition Mechanisms

- Requires duplication of the target site (i.e. **direct repeats to either side of the inserted element**)
- 2 mechanisms of transposition that lead to target site duplication

Transposition Mechanisms	**(1) Conservative Transposition**	**(2) Replicative Transposition**
Method	• Excises a transposable element from one position and inserts it into a new location • Cut-and-paste	• Copying of the transposable element • Initiation of replication of the element in a plasmid
Movement	• Moves transposable elements around the genome	• Transposase facilitates formation of a **cointegrate** – a temporary fusion of the plasmids • Recombination can resolve cointegrate • Both plasmids have a copy of the element
Increase in # of elements per genome	No	Yes

Question: What is the main enzyme responsible for excising and copying transposable genetic elements from chromosomes and inserting them into new locations?

Transposase

Eukaryotic genomes: DNA transposons, Retrotransposons

2 groups of eukaryotic transposable elements

DNA transposons Transposed through conservative or replicative transposition

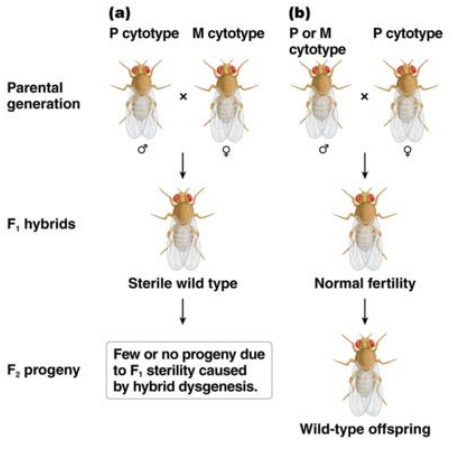
Retro-transposons
- Transcribed
- RT produces a dsDNA copy → inserted into the genome

P-Element Structure (≈ 2900 bp) in Drosophila

- **Transposable element with gene encoding transposase** (≈ 31 bp inverted repeats)
- **Evidence of RAPID EVOLUTION** → *all Drosophila after 1960s have P elements but prior do not*
- Nonfunctional elements with no transposase

Hybrid Dysgenesis

- *P* elements produce **hybrid dysgenesis**
- *Hence, P* elements = biological mutagens
  - M cytotype females have no *P* elements (lab strain)
  - P strain males contain ≈ 30 *P* elements (WT strain)



Cross between M cytotype females and P stain males

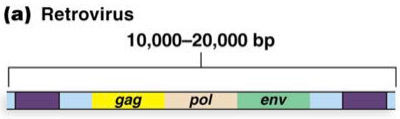
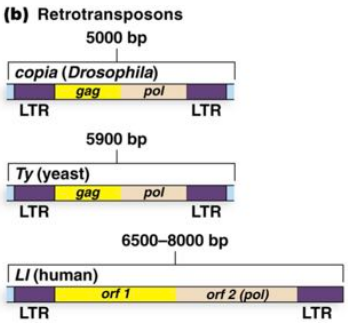
RESULT: Causes **sterility** in the F_1 progeny due to widespread transposition of multiple *P* elements resulting in **hybrid dysgenesis** in the offspring

WHY? Females without P elements in their genome canot recognize the P elements in the male thus cannot stop hyperactive P element insertion into genome

What does the model of hybrid dysgenesis predict for the F1 and F2 generations when an M-cytotype male is crossed to a P-cytotype female?

F1 and F2 generations will all be wild type and display normal fertility. This is because females with the P-elements recognize the presence of other P elements such that there is a suppression of the hyperactivity, transposing and mutagenesis of the inserting P elements.

Transposition Modifies Eukaryotic Genomes

	Retroviruses	Retrotransposons
Mechanism	• Infect eukaryotic cells with ssRNA genome → transcribed into dsDNA by **reverse transcriptase**	
Viral particles synthesised	YES (***gag* and *env*** *for capsid formation while* ***pol*** encodes reverse transcriptase)	NO No capsid formation
Host Dependent	Yes	Yes (more parasitic → manipulate genome but does not disseminate)
STRUCTURE	 Flanked by **long terminal repeats** (**LTRs**)	 NO *env* gene

LINE and SINE Elements of Humans

- **45% of the human genome** derived from former transposable elements ⟶ are permenantly fixed
- However, some transposable elements are still functional

	# per genome	Size	Function
LINES	600,000	6.5–8.0kb (LONG)	Cause mutation
SINES	1.2 million	100–300bp (SHORT)	

MBLG2072 – Semester 2

L15: Gene transfer in Bacteria I

Methods of gene transfer

Method of gene transfer	***Description***
Conjugation	Transfer of replicated DNA from a donor to a recipient
Transformation	Uptake of DNA from the environment
Transduction	Transfer of DNA from one bacterium to another by a viral vector

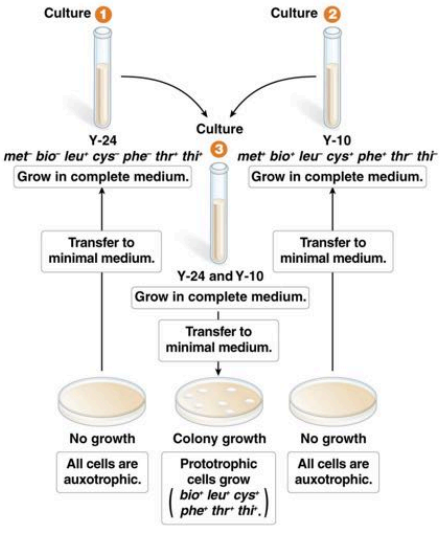
Bacterial Genome and Plasmid Differences

Bacterial Genome	**Plasmid**
Covalently closed circle	Covalently closed circle
dsDNA	dsDNA
One copy of each gene (Haploid)	**Multiple copies**
Essential genes	**Non-essential genes**

F plasmids and mating

Plasmid Type	**Usage**
F (fertility) plasmid	Contains transfer genes
R (resistance) plasmid	• Carries antibiotic resistance genes • Easily modifiable → recombinant DNA use
High copy number plasmids (≈ 50 copies/cell)	Replicate independently from the chromosome
Low copy number plasmids (≈ 1-2 copies/cell)	Replicate with the chromosomes

Cross	**Is exconjugant converted to donor state?**	**Has donor bacterial genes been transferred to the exconjugant?**
F^+ X F^-	**Yes (F^- → F^+)**	**No**
Hfr X F^-	**No**	**Yes**
F' X F^-	**Yes (F^- → F')**	**Yes**



Conjugation of F^+ and F^- cells (one-way transfer of F factor) → (F^+ x F^-)

- Donor cells (F^+ with plasmid) → recipients (F^- without plasmid)
    - **Exconjugant** cell (recipient cell with modified genetic info after receiving DNA from the donor cell)
- Donor cells transfer bacterial genes to recipient cell via F (fertility) factor
    - F factor integrates into the bacterial chromosome to form an **episome**
- F factor = 100 kb in length 40 genes that control conjugation

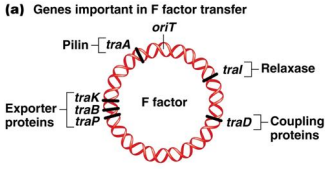
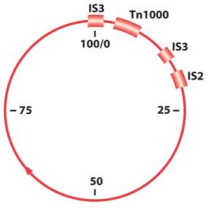
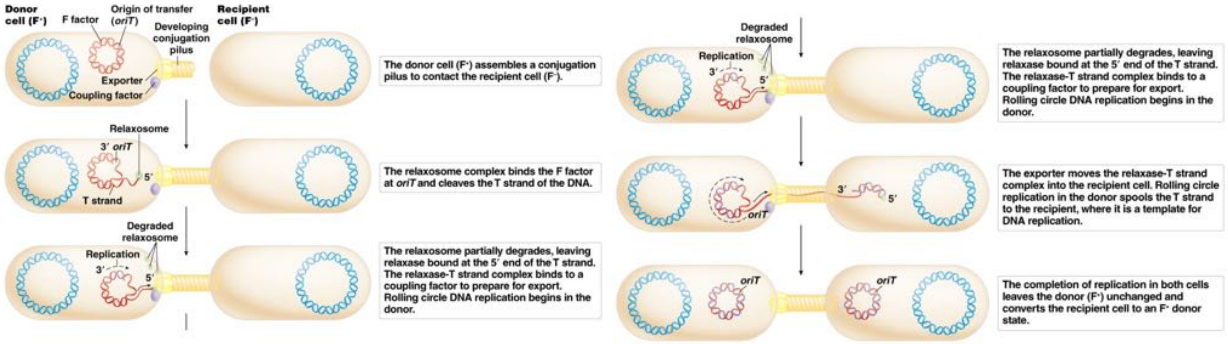


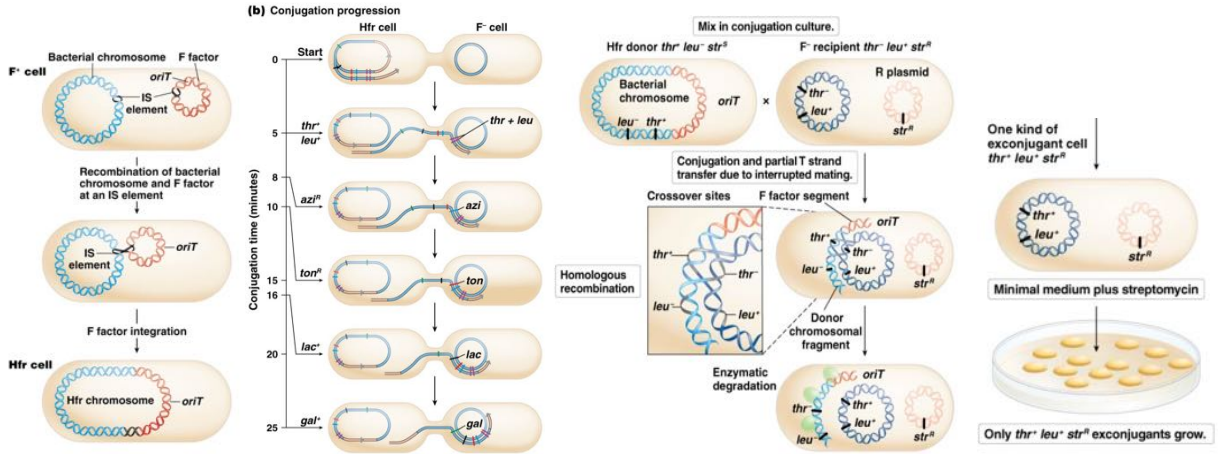
Fig 13.8 IS elements on the F plasmid





High frequency of transfer (Hfr) mating → (Hfr x F^-)

- Gene transfer by **rolling circle replication**
- The segment of T strand DNA enters the recipient and is used to generate a double-stranded linear fragment



Key Outcomes of Hfr × F^- Mating

1) Transfer of 1 or more donor alleles into the recipient chromosome via **homologous recombination** to form **exconjugant chromosome**
2) Incomplete transfer of chromosome → F factor is not fully transferred during the mating = recipient cell **NOT CONVERTED** into a donor cell
3) Therefore, the recipient cell **is NOT CONVERTED** into a donor cell

Chromosome (time of entry) **mapping using conjugation**

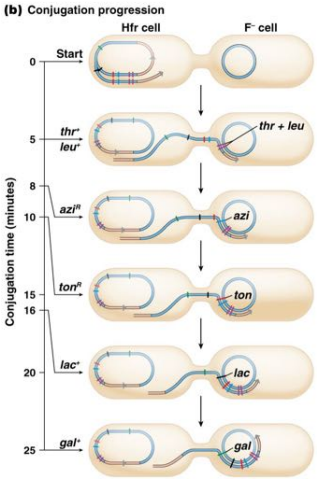
Interrupted Mating Analysis Produces Time-of-Entry Maps

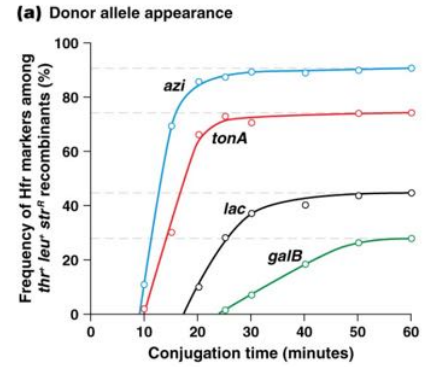
METHODOLOGY:

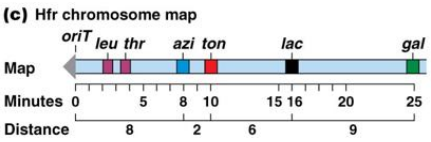
- Breaking conjugation tube to stop mating before Hfr chromosome is completely transferred from donor to recipient

Time-of-entry mapping

- Transfer genes in a **specific order**
- Distance of the gene from the **origin of transfer (*oriT*)** is related to *time* of transfer
- Genes closest to *oriT* will transfer earlier

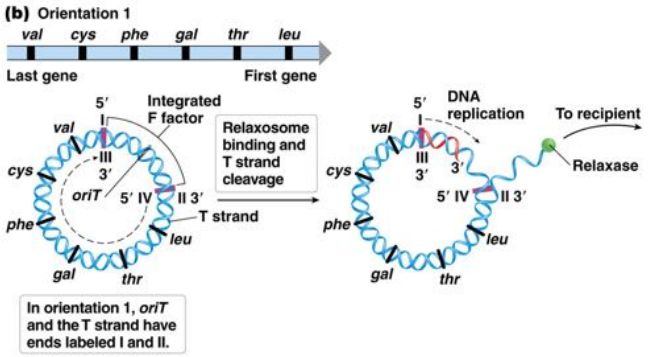


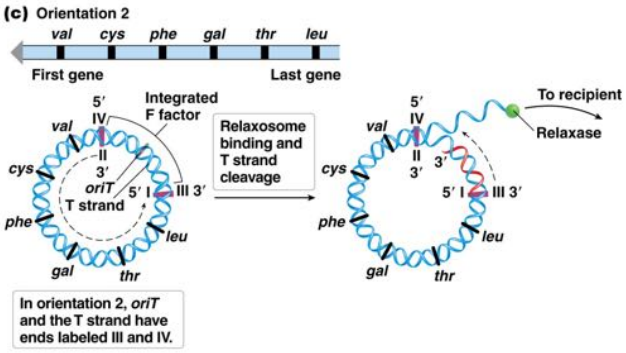




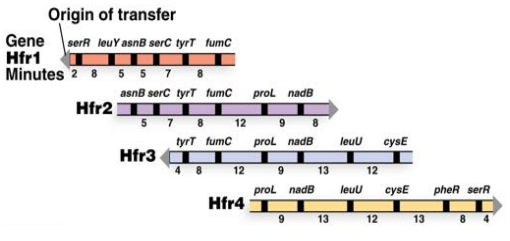
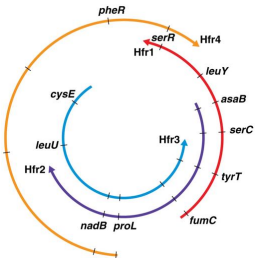
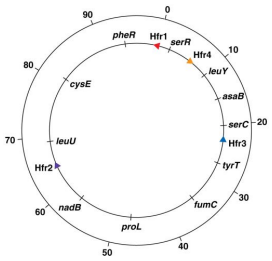
F Factor Integration

- F factor integration occurs at any IS element
- **2 possible directions** for each integration → Remains constant for that Hfr strain





Constructing chromosome (time-of-entry) map:

Step 1: Construct Overlapping Linear Maps using multiple HFR strains	Step 2: Construct Circular Map of donor chromosome	Step 3: Construct Complete donor chromosome
Data from each Hfr strain can create partial overlapping maps	Linear maps are used to create an overall **circular map**	
		

F’ mating/conjugation

- **Imperfect excision** of the F factor from an Hfr chromosome produces **F’ factor**
- **F′ factor** = all its own DNA + a segment of the bacterial chromosome (still functional)

RESULT OF F’ x F⁻

- Exconjugants with **F′ factor** have **partial diploids (***i.e. contain 2 copies of the bacterial chromosome genes found on the F’ factor)*
- Partial diploidy is stable through replication and cell division
- Can be used to study bacterial genes