SECOND LECTURE

More Mendelian Genetics

The dihybrid cross

When two genes are studied in the same cross the phenotype ratio *of the* F_2 generation is 9 : 3 : 3 :1. This represents the combination of two 3 : 1 ratios. The 9:3:3:1 ratio requires that the two genes are not involved in the same biochemical pathway nor situated close together on the same chromosome. When the Fl generation is crossed with the double recessive parent a ratio of 1:1:1:1 is obtained. The number and frequency of phenotypic classes from crosses involving any number of genes can be calculated by the expression $(3 :1)"$, where n is the number of genes involved.

Epistasis

The F_2 phenotypic ratios of the dihybrid cross are altered if the two genes concerned are involved in the same biochemical pathway. Several different alterations of the 9 :3:3:1 ratio are observed depending on how the genes interact. In all cases the number of phenotypic classes are reduced, and the new ratios are made up by summing certain of the classes from the original ratio.

Mend's laws

Two basic rules underpin transmission genetics in eukaryotes. These stem directly

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from Mendel's experiments. Alleles of the same gene separate to different gametes, and alleles of two different genes segregate (separate) independently of each other in gametogenesis.

Handling problems

When analyzing data obtained from crosses of this nature first look for the phenotypic ratios that are most likely to fit with the information you have. Then check the goodness-of-fit of each of these using the $\backslash^2$ statistic.

The dihybrid

Topic Cl showed how the 3 :1 monohybrid ratio could be used to explain the **cross** inheritance of a phenotype where only a single gene was involved. This is the basic Mendelian ratio and everything that follows depends upon it.

The obvious next step is to look at a situation where the inheritance of two different inherited characters are studied at the same time, **a dihybrid** cross. The simplest experimental system for this is to cross two pure-breeding strains of a species, one of which is homozygous for recessive alleles of two genes and the other homozygous for dominant alleles of the same two genes. Again Mendel was the first to carry out such experiments. In one of these he used pea plants that differed in two properties of the seed.

The choice of seed characteristics aided his work for two important reasons. It was easy to analyze large numbers of seeds accurately, thus improving the statistical basis of the work.

• By using seed characters, it was possible to reduce the length of time the experiments would take. The F_1 generation became the seeds produced by crossing the parental plants. When these seeds were planted out, grown on and allowed to self-fertilize, the F_2 generation was the seeds present in the pods. Thus he was

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saved the time (1 year) and effort of having to plant out these seeds and assess the characters in the plants that they produced.

Seed shape is determined by a single gene that has alleles for round, R (smooth), or wrinkled seeds, r. The round phenotype is completely dominant over wrinkled. Seed color can be yellow or green. Again this is determined by two alleles of a single gene. Yellow, *Y, is* completely dominant over green, y. The experiment is set out in *Fig.* 1. A cross between the two pure-breeding (homozygous) parental lines yielded an Fl generation which consisted only of round yellow seeds. As with the monohybrid cross the F_2 generation showed considerable diversity. Four different phenotypes could be identified. Of 556 seeds analyzed, he found 315 round yellow seeds, 108 round green seeds, 101 wrinkled yellow seeds and 32 round green seeds. This is close to a ratio of 9:3:3:1, which is referred to as the dihybrid ratio. Mendel obtained ratios close to this for several different combinations of pairs of genes, and since that time a great many other examples of this ratio have been demonstrated in crosses in plants, animals and fungi.

The predicted ratios of phenotypes and genotypes can be determined graphically using what is known as a Punnett square. Simple examples of this were given in Topic Cl to show the possible classes of progeny in the monohybrid cross. A Punnett square for the dihybrid cross is set out in Fig. 1. The 9:3:3:1 ratio is simply two 3:1 ratios combined, and shows that the alleles of the two genes behave (segregate) independently of each other. This is demonstrated more easily by looking at one gene at a time (Fig. 2). Take the seed shape gene first: in the F_2 generation a ratio of 3 round to 1 wrinkled would be expected. Now look at the seed color gene in those seeds that have the wrinkled phenotype. These should have a ratio of 3 yellow to 1 green. The same is true for wrinkled seeds, these should also have a ratio of 3 yellow to 1 green. If the ratios for the two phenotypes are multiplied across the 9:3:3:1 ratio is obtained.

As noted earlier the 3 :1 phenotypic monohybrid ratio can be distorted by factors such as incomplete dominance or lethal effects of certain alleles. These also affect the 9:3 :3 :1 ratio, but other factors can also modify this ratio. The 9:3:3:1 ratio depends on two conditions.

• The two different genes must not act on the same character. For instance if the proteins encoded by the two genes are involved in the same biochemical pathway then the ratios of phenotypes resulting from the genotypes in the F_2 generation will

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be altered. This is discussed in detail later in this section, under epistasis.

(a) Parent 1
produces
only round
yellow
seeds
(**RRYY**)

Parent 2
produces
only
wrinkled
green seeds
<***rryy)***

• If the two genes lie close together on the same chromosome the four classes of gamete are not produced at equal frequencies. This is the basis of gene mapping studies and is examined under **linkage** (see Topic C4).

As with the monohybrid cross it is also possible to conduct a testcross with the Fl generation of the dihybrid cross. If, in the round/wrinkled, yellow/green example of the 9:3:3:1 ratio, an Fl plant is crossed with the homozygous recessive parent for both genes the progeny fall into four phenotypic classes,

(b)   F$_1$ generation round yellow seeds
(**RrYy**)

Gametes	RY	Ry	rY	ry
RY	RRYY Round yellow	RRYy Round yellow	RrYY Round yellow	RrYy Round yellow
Ry	RRYy Round yellow	RRyy Round green	RrYy Round yellow	Rryy Round green
rY	RrYY Round yellow	RrYy Round yellow	rrYY Wrinkled yellow	rrYy Wrinkled yellow
ry	RrYy Round yellow	Rryy Round green	rrYy Wrinkled yellow	rryy Wrinkled green

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Phenotype class

Round, yellow seeds Round green seeds Wrinkled yellow seeds Wrinkled green seeds

Genotype cfass *RRYYW,* Rflyy (2), *RrYYia, RrYy* (4)

mnW.RwWi
n-W(1).n-yy(2) ">y(i)

Fig. 1. (a) The production of F1 plants. These can produce four different gametes RY, Ry, rY, ry. Tha matrix in (b) fPunnett squaw) snows a? the possible genotypes and pnenotypes that can arise when these plants are self-fertilized. The figures in brackets indicate the number of times each genotype appears in the Pumett square.

round yellow, round green, wrinkled yellow and wrinkled green. These classes occur with equal frequencies.

It would be a useful exercise for you to work this out for yourself. However this type of cross is most important in studies of gene mapping and linkage and is covered in detail in Topic C4.

At this point it is necessary to make a brief comment about systems where more than two genes are studied simultaneously. In the case of three genes, *** trihybrid cross,** in which complete dominance is observed for alleles of all three genes, the phenotypes observed fall into the ratio 27:9:9:9:3:3:3:1 (see Topic C12). The predicted ratios can be determined for crosses involving any number of genes by use of the expression (3:1)", where n is the number of genes.

Seed shape   **Seed color Final ratio**

                              Yellow   3 ——————————— 9

                Round   3

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Green   1 ——————— 3

Yellow   3 ——————— 3

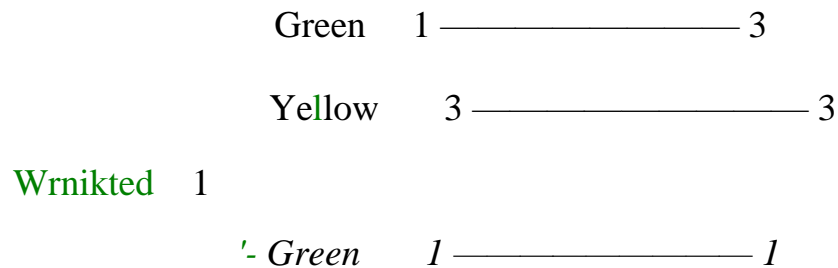Wrnikted   1

'- *Green*   *1* ——————— *1*

Fig. 2 Derivation of the 9 : 3: 3 :1 ratio. See text for explanation.

Epistasis:

Epistasis

Epistasis refers to situations where the expected ratio of phenotypes is not observed due to some form of physiological interaction between the genes involved. This is usually seen as a distortion of the 9:3:3 :1 ratio with a reduction in the number of different phenotypes observed. Many different ratios can be derived from the original 9:3:3:1 ratio. Three of these are shown in *Figs 3*, *4* and 5. Although each can be explained by specific examples from the literature it is simpler if the different ratios

are described here by reference to fictitious biochemical pathways in an unnamed plant species in which two enzymes, that are coded for by separate genes, are both involved in the production of petal pigments. The genes are denoted *A* and B with capital letters representing a functional dominant allele and lower case letters representing nonfunctional recessive alleles. All of the examples involve the F_2 generation produced from a doubly heterozygous *AaBb* F_1 generation. In working through these you may find it useful to construct a Punnett square for the genotypes, as shown in *Fig. 1,* and determine for yourself how the phenotypes are distributed.

In a strict sense 12: 3:1 is the only ratio which was originally referred to as epistasis, because the presence of the *A* allele can completely mask the genotype of the B gene, but the term is used now wherever genes interact to alter the expected

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ratios.

There are several other variations of the 9:3:3:1 ratio caused by interaction between the gene products. These include 9:6:1, 15:1 and 13:3. You should attempt to think of biochemical pathways that would yield these ratios. Remember that in every case the ratios are derived by summing together the four phenotype classes 9, 3, 3 or 1 of the basic ratio.

The examples described here are deliberately made simplistic but illustrate the basic principles. You should realize that the term phenotype is capable of different interpretations. A plant breeder may be happy to simply use flower color as we have done to describe phenotype. On the other hand a plant biochemist might wish to interpret the results differently and assay enzymes A and B *in vitro.* The phenotypic ratios determined in this way would be different from those given above. If this is not obvious to you, work them out for yourself. The genotypes would, of course, not change.

Mendel's law

The monohybrid and dihybrid ratios come directly from the work of Mendel.

Remember that this was carried out without any knowledge of chromosomes
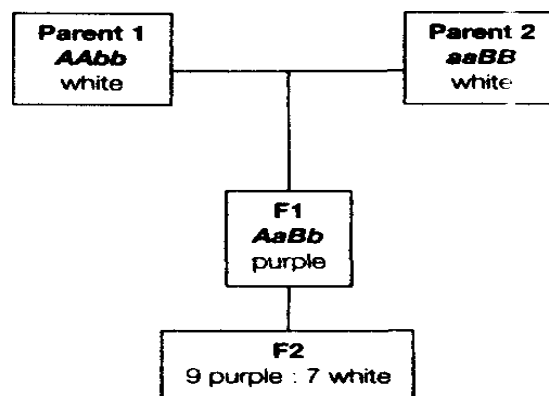
In this example the biochemical pathway woutd be a simple chain where enzyme A converts its substrate Into a white product which is. in turn. the substrate tor enzyme B which converts it to a purple product.

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	EnzyrneA	EnzymeB	
White substrate		White product	Purple product

F$_2$ phenotypes and genotypes
Purple 9
AABB (1). *ABAb* (2), *AaBB* (2), *AaBb(4)*
White 7
AAbb(1),Aabb(2}
aaBB(2),aaBb(1)

aabb (1)

have at least one functional copy of A and B and therefore can synthesize the purple pigment have only functional enzyme A and cannot complete synthesis of purple pigment **have only** tinctorial enzyme B and cannot complete **synthesis o< purple** pigment have no functional enzymes and cannot synthesize purple pigment

This is known as **complementation,** because the genes in the two white strains each complement the deficiency in the other strain, producing the purple product in the F$_1$.

Fig. 3. 9:7 ratio.

or DNA. Mendel's work is often expressed as the two laws or principles which he inferred to explain the inheritance of phenotypes.

The first of these states that in gamete formation the two alleles of the same gene segregate (separate) so that each gamete receives only one allele. This is clearly demonstrated in the monohybrid 3:1 ratio.

The second law states that alleles at any one gene segregate

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independently of alleles at any other gene. This derives from the dihybrid cross data where, in the example described in Fig. 1. the four alleles R, r, Y and y must act independently of each other so that the four different classes of gametes RY, Ry. rY and ry arise in equal numbers. These two principles form the basis of our knowledge of transmission genetics.

As either a student in an examination, or a geneticist carrying out research you may be faced with data obtained from Fl and F2 generations of crosses. You would need to be able to recognize ratios in order to decide how many genes are involved and whether or not epistasis is taking place. You may for instance be faced with the following example.

```
┌──────────────┐               ┌──────────────┐
│  Parent 1    │               │  Parent 2    │
│    AAbb      │───────┬───────│    aaBB      │
│    red       │       │       │   white      │
└──────────────┘       │       └──────────────┘
                       │
               ┌──────────────┐
               │     F1       │
               │    AaBb       │
               │   purple      │
               └──────────────┘
                       │
         ┌───────────────────────────┐
         │           F2              │
         │ 9 purple : 3 red : 4 white │
         └───────────────────────────┘
```
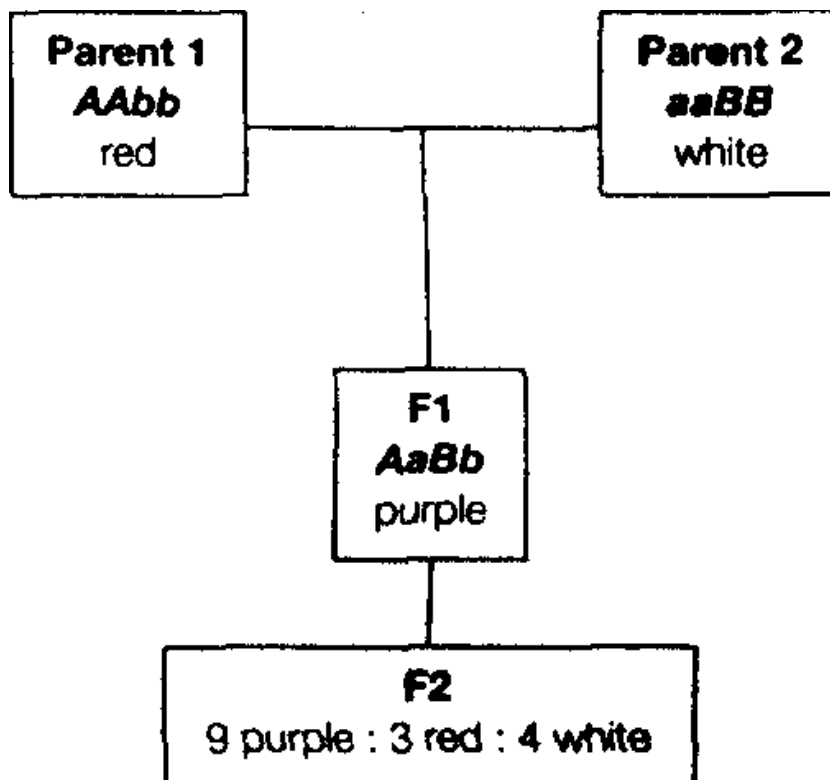
In this example the biochemical pathway would again be a simple chain, but in this case the product of enzyme A would be red in color

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Enzyme A   EnzymeB
   White substrate   Red product Purple product

F2 phenotypes and genotypes Purple*

AA8B (1). AABb (2). AaBB (2). AaBbW

Red 3
A4te(2)./tatob(1)

White 4
aaS8(2),aaSb(1)

ratio(i)

have at least one functional copy of both A and B and therefore can syntnesize the purple pigment have only functional enzyme A and produce red pigment but do not convert It to purple pigment

have no functional enzyme A and so cannot synthesize the red product that Is the substrate for enzyme B and will remain white

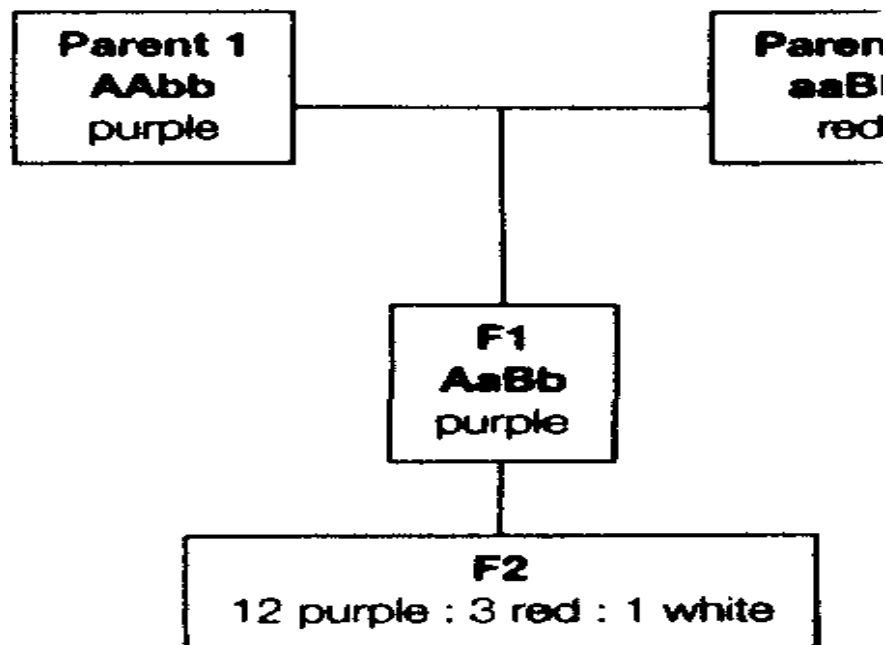have no functional enzymes and cannot synthesize any colored product

Fig. 4. 9:3:4 ratio.

A (toss between pure-breeding white-fruited and purple-fruited tomato plants produced an Fl generation in which all plants had purple fruit. In the subsequent F2 generation 160 plants were obtained, of these 99 had purple fruit, 25 had red fruit and 36 had white fruit. How would you approach such a problem?
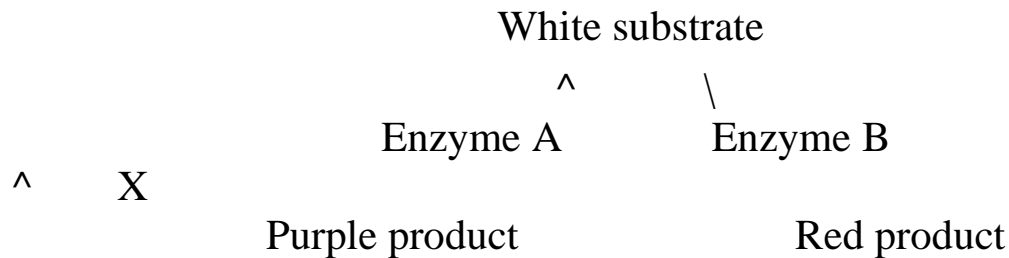
As you know nothing about the genes controlling fruit color in tomato you must first ask yourself the question, 'does the data fit any of the known Mendelian ratios?' The simplest way to proceed is to exclude those ratios which obviously do not apply. Clearly because there are

three different phenotypes in the F2 generation any ratio with only two classes such as 9: 7 or 3:1 are excluded. On examination of ratios with three phenotypic classes 9:3:4 looks a possible candidate, but a 1:2:1 ratio may also apply. How do you decide which ratio is the best fit to your data?

This is done using the x2 statistic, which is well suited to determining the goodness-of-fit to ratios. Detailed explanation, of this test is given (see Topic C13), but this example will be worked through here. The observed data is

```
┌─────────────────┐                          ┌──────────────
│    Parent 1     │                          │   Paren
│     AAbb        │──────────────┐           │   aaB
│    purple       │              │           │   red
└─────────────────┘              │           └──────────────
                                 │
                         ┌───────────────┐
                         │      F1       │
                         │     AaBb      │
                         │    purple     │
                         └───────────────┘
                                 │
                    ┌────────────────────────────┐
                    │            F2              │
                    │ 12 purple : 3 red : 1 white │
                    └────────────────────────────┘
```

In this case we need a more complex biochemical pathway. Here two enzymes compete (or the same substrate. Enzyme A which converts the substrate to a purple product has much higher affinity for the substrate than enzyme B which converts the substrate to a fed product. The difference in affinity for the substrate is so marked that enzyme B can only work effectively if no enzyme A is present.

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White substrate

^   \

Enzyme A   Enzyme B

^   X

Purple product   Red product

F2 phenotypes and genotypes Purple 12

AABB (1), ABA6 (2), AaBB (Z).

AaBb(A),AAbt>W,
/<abb(2)
Ftod3
aaBB(2),aafl6(1)
Whitel
aab6(1)
these have at least one functional a4ete A and convert all the substrate to purple product

lack any functional enzyme A. but have functional enzyme B which converts the substrate to a red product

have no functional enzymes and cannot synthesize any colored pigment

Fig. 5. 12 -.3:1 ratio.

compared with that which would have been predicted by the ratio. This is set out for both possible ratios.
Observed result: 99 purple   25 red   36 white   160 total Result

predicted by a 9:3:4 ratio

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%»x 160 30

'/. x   %.xl   , (99-90)2 (30-25)2

160   60   X = ——————— +

90   /v   rtrt

      90   30   40 = 2.13 with two degrees of

freedom This does not differ from the 9:3:4 ratio at the 5% level of

probability

Result predicted by a 1:2:1 ratio

      % x 160   % x 160 V<. x 160 40   80   40

, (40-25)2   (99-80)2   (40-39)2

Y- = ——————— + ——————— + ———————
•-40   80   40

= 10.53 with two degrees of freedom This is different from the 1 :2:1

ratio at the 5% level of probability.

On this basis the 1:2:1 ratio is rejected in favor of the 9:3:4 ratio.

Prof Dr Makhlouf M. M. Bekhit , Genetic and genetic engineering Department