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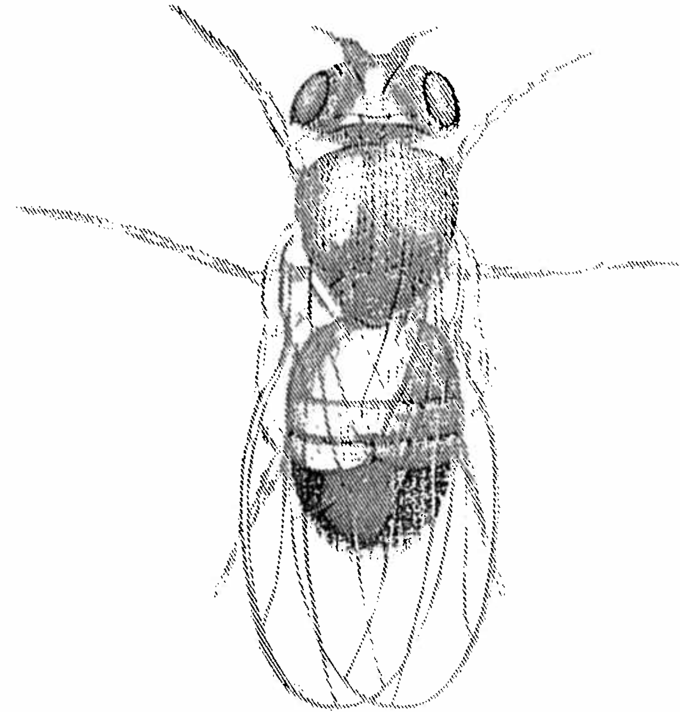
Practical Genetics with Drosophila Melanogaster



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PRACTICAL GENETICS
WITH
DROSOPHILA MELANOGASTER

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THE LIFE HISTORY OF DROSOPHILA MELANOGASTER

The Life Cycle is typical of the Diptera and its duration is dependent on temperature. The usually accepted optimum is 25°C, giving a cycle of 9-10 days. At 20°C the complete cycle takes 15 days, and normal development will of course continue at much lower temperatures (57 days at 10°C). Exposure to temperatures above 25° may produce adult phenocopies, these are non-inheritable variations in phenotype which while they are worth investigating as a separate project will confuse the issue in introductory experiments. Continued exposure to temperatures above 30°C will cause sterility or even death.

Approximate time schedule at 25°C

Day 0. egg laid
Day 1 larva emerges
Day 5 larva pupates
Day 9 adult emerges

N.B. Some less vigorous mutants, notably vestigial winged, are slower, taking as much as two days longer to emerge.

The eggs are oval, about 0.5mm in length and are laid on the surface of the food. Two curious paddle-shaped anterior projections prevent the egg from sinking into the medium. The larvae are voracious feeders, channelling their way through the food, often visible only by their black mouthparts. This 'working' of the larvae indicates a healthy culture. The larvae moult twice and the third instar achieves a length of 4.5mm.

When they are ready to pupate, the larvae emerge from the food and crawl to a relatively dry surface, such as the sides of the culture tube or bottle. The white larval skin hardens and darkens as development proceeds. Adults generally emerge in the early morning and are light in colour.

Larval faeces are retained for the first few hours and can be seen as a spot in the centre of the ventral side of the abdomen. This helps the identification of newly emerged flies, the body then gradually darkens. Newly emerged adults have elongated abdomens. The characteristic sex difference in abdominal shape are not evident for several hours after emergence. Thus young males do not show the relatively rounded, highly pigmented abdomen which so readily distinguishes older males. None the less the genitals are clearly visible under magnification. Care is essential when sexing flies within the first few hours after emergence. When in doubt call it "male"!

Mating normally occurs within 8-14 hours of emergence and egg laying begins on the second day and continues for at least another week so that up to 400-500 eggs may be laid, all fertilized by sperm from this initial mating. This is a crucially important consideration when selecting female flies for breeding experiments. If mating in fact occurs before segregation, subsequent mating with a male of different genotype will not produce heterozygous offspring within the normal experimental time. This is the largest cause of failure in experimental crosses. In a normal promiscuously breeding population, further mating and egg laying are likely, the average life span of the females being 3-4 weeks.

Contrary to popular belief, unmated females will lay eggs, though these are infertile.

APPARATUS AND BASIC TECHNIQUES

Apparatus List:

For each student or group of students

dissecting microscope (preferably stereo-binocular) or a good magnifier on a stand

white tile or card

30cm³ dropping bottle of ethoxyethane (ether)

etheriser

10cm. watchglass or glass petri dish with cotton wool for re-etherising (see diagram page 6)

fine sable or camelhair brush

glass-writing pencil

rubber or foam plastic pad

General Laboratory Apparatus and Equipment

Incubator — capable of maintaining 25°C. *Drosophila* can be reared in make shift cabinets over central heating radiators, or even at room temperature, but it must be strongly emphasised that in such uncontrolled conditions development is slow and time schedules so irregular and uncertain that it is virtually impossible to ensure correspondence with lesson times.

If food is to be prepared:

sterilising oven, autoclave or **pressure cooker** for sterilising bottles

aluminium saucepan with pouring lip (about 18cm diameter) refrigerator for storing food

Containers for flies

⅓ milk bottles (wide mouthed) for maintaining stocks

Tubes 100 x 25mm for crosses and temporary housing of segregated males and females etc.

Foam plastic bungs

Drosophila Medium (See FOOD.)

BASIC TECHNIQUES

CONTAINERS

The size of the flies makes it possible to keep stocks in third-pint wide-mouthed milk bottles. Tubes (100 x 25cm is a convenient size) serve as temporary containers for segregated males and females, and also for matings, their single generation and progeny. Bottles and tubes must be thoroughly washed in detergent, rinsed and dried. They are then plugged with plastic foam plugs and autoclaved at 15lbs/sq" for 10 mins. Alternatively sterilise the bottles only in an oven for 45 minutes at 160°C and steam the foam plugs, wrapped in foil. Note that throughout, though strict sterile technique cannot be maintained, the steps recommended here are important to minimise the danger of infection of cultures by fungi, bacteria and especially by **mites.** The latter are minute, pink creatures only just visible to the naked eye. Infection with mites can be avoided by keeping culture bottles standing on inverted half Petri dishes in a tray of oil.

FOOD

The larvae feed by burrowing through a soft medium, in nature this is usually rotting fruit, the adults probably feed mainly on the yeasts (and their fermentation products) growing on the fruit.

Many food media from crushed banana to wheatgerm, have been used, probably with equal success. The following recipe we find easy to prepare, and the ingredients readily obtainable.

180g fine oatmeal (coarser grades must be soaked first)

80g black treacle

15g powdered agar

15cm³ of 10% Nipagin (in 95% alcohol) see Appendix

1,400cm³ water

Mix the ingredients together and bring to the boil, stirring constantly. Boil for 12 minutes. Pour the medium carefully into the sterile containers to a depth of about 2cm, avoid spilling the medium down the sides of the tube or bottle as this will soil the plug and trap etherised flies. Replace the foam stopper. The ingredients given should be enough for 20 third-pint milk bottles or 100-110 tubes.

Alternatively, ready prepared medium, or 'Ready-mix' dried medium may be obtained (see Appendix). The latter is simply reconstituted with water.

Never use bottles or tubes with condensed water visible in them. Anaesthetised or newly emerged flies, or even active ones, particularly the less vigorous mutants like vestigial are easily trapped and drowned.

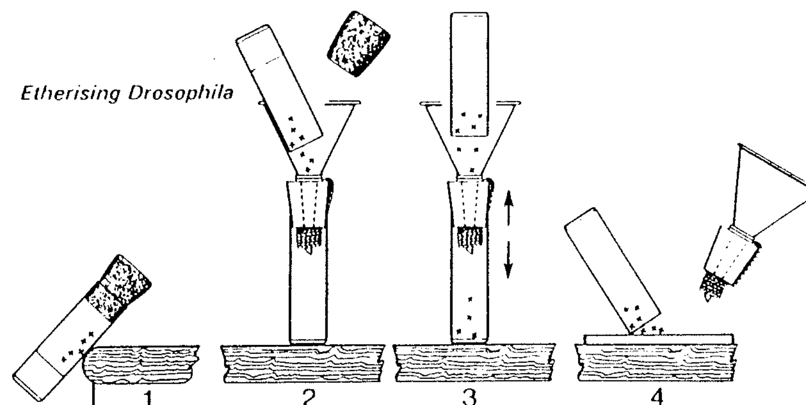
Before transferring flies to the tubes add one or two granules of dried yeast.

HANDLING

Never attempt to transfer flies without etherising. Escapees can survive and breed in laboratories and have a habit of turning up again surprisingly and in unwanted circumstances. We have even known them appear on embalmed dissection specimens stored in sealed plastic bags!

Etherisers (see diagram) — A wide variety of devices will serve. The string or wadding is moistened with 1-2cm³ of ether. (N.B. Ether vapour is harmless to human being in such small quantities, but it is still explosively inflammable!) Contact with **liquid** ether is immediately fatal to flies.

Transferring flies — Tap the side of the culture bottle or tube sharply but lightly on a rubber or foam pad as shown until all the flies adhering to the plug have been shaken off. Quickly remove the plug and insert the mouth of the tube into the funnel of the etheriser. Then tap the etheriser lighty on the pad as shown to knock the flies into the etheriser. **Do not etherise after all movement has ceased** (a few seconds only is needed). At the end of this time, quickly remove the culture tube, plug it, then remove the



funnel from the etheriser and immediately empty out the flies onto a white card or tile.

Check the bottle for remaining flies. If it is necessary to completely clear the culture, repeat the operation. Quick, deft handling is important to avoid

over-etherisation which may kill or temporarily sterilise the flies. Over-etherised flies can be recognised by their raised wings and curled abdomens, these flies should be discarded.

A simple emergency etheriser can be made by sellotaping cotton wool soaked in ether to the base of a petri dish and flies which start to wake up should be covered with this for a few seconds.

The flies are moved about with a fine brush. Any dead or unwanted specimens should be transferred to a morgue of 70% alcohol.

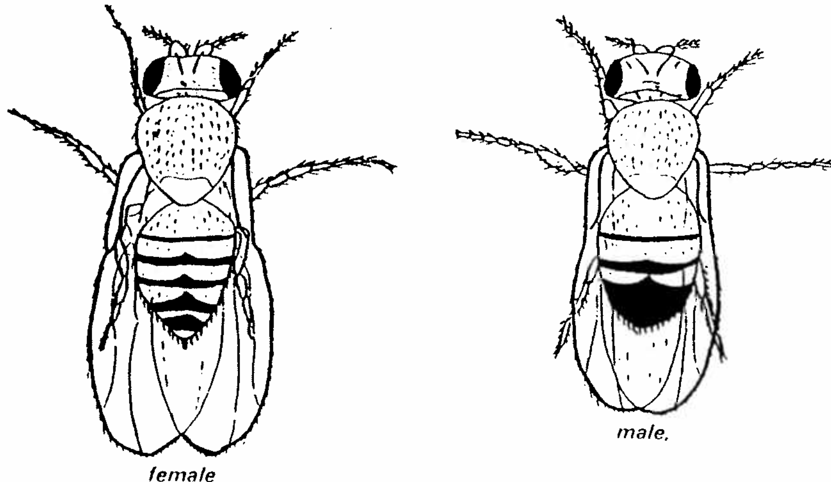
An emergency etheriser

After sorting, the flies should be brushed gently into culture tubes. The tube should be held on its side so that the flies do not land on the medium. Leave the tube on its side, until the flies recover, to prevent them becoming stuck in the medium.

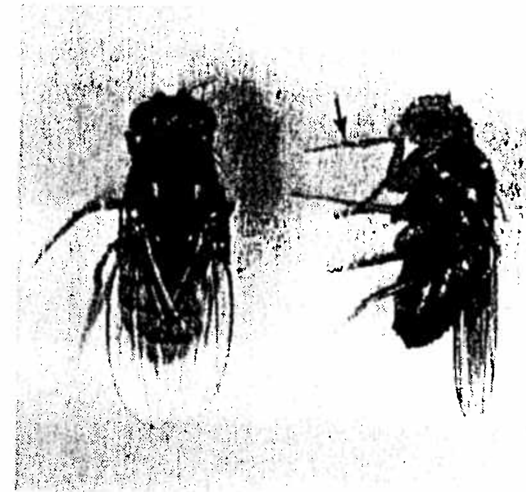
Important — each time an etheriser is used, always check that it is clear of flies both in the container and in the funnel. Failure to do this can lead to all sorts of problems if a stray fly is left behind.

SEXING

With mature flies at least, sex differences are obvious. The male has a distinctively shorter, blunter abdomen, and the black 'tail end', as seen in dorsal and lateral views, is clear even in the so-called 'black' or 'ebony' bodied mutant strains.



Really newly emerged flies may lack distinctive pigmentation and the difference in shape is much less marked. Here, checking of the genitalia under higher magnification will show the brown chitinous margins of penis and claspers in the male, and the distinctive 'parson's nose' of the anal plates of the female. Another distinguishing feature is the presence of the 'sex comb', a fringe of black hairs on the uppermost tarsal joint of the foreleg of the male, which is absent in the female.

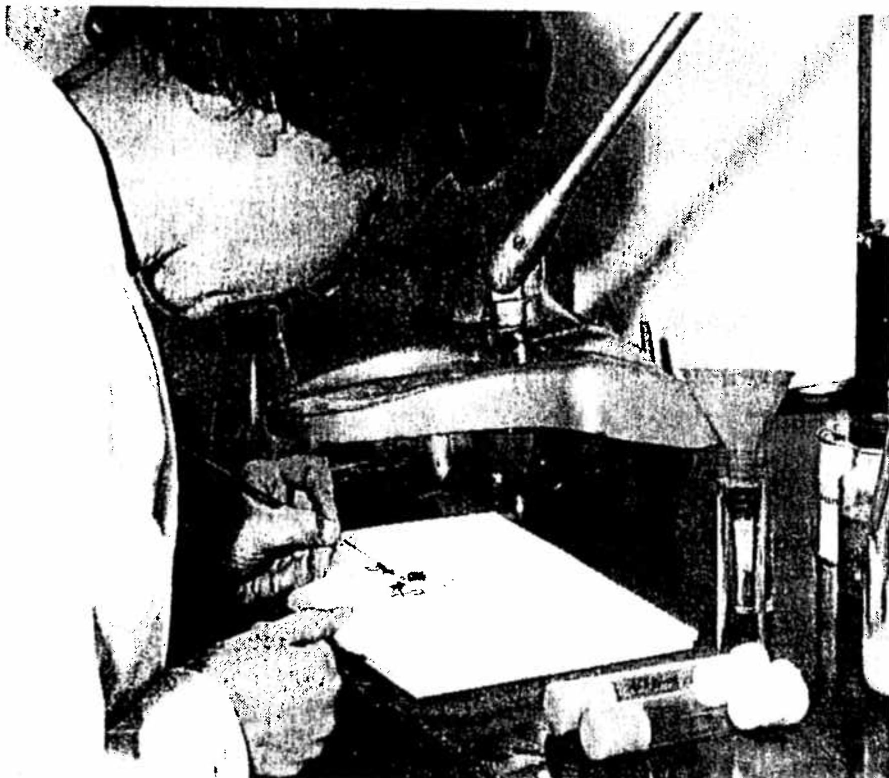


Wild type Drosophila, female dorsal view (left), male side view (right) showing sex comb (arrowed).

Virgin Selection

As outlined in Section 2, mating occurs during the first day after emergence and the sperm received fertilise all eggs laid for days afterwards, so that it is essential that the females used in experimental crosses between different strains shall be virgin. These are obtained from a culture with numerous papae near hatching.

Opinions differ on the length of time permissible between successive clearances to ensure virginity; some authorities stipulating not longer than 8 hours, others 12 or even 16. We suggest that the cultures should be cleared between 8.00am and 9.00am and again during the afternoon, each day. Virgin females should be obtained from the afternoon clearing. If more females are needed, the cultures could be cleared again in the evening. If this is done, the flies obtained the following morning will also be virgin.



Sexing Drosophila

Setting up Crosses

Symbols used: P —parental generation

F_1—first filial generation

F_2—second filial generation

a) for F_1: Accumulate sufficient males and virgin females (P) for your requirements. Etherise the accumulated males and virgin females and transfer 3 females and 3 to 6 males, or up to 5 pairs if numbers permit, to each culture tube (which should already contain food and yeast), Reciprocal crosses should be made, e.g. wild type males x vestigial females; vestigial males x wild females. Mark each tube with the details of the cross and the date.

b) for F_2: Set up in the same way except that the F_1 females used need not be virgin.

c) Backcrosses: Use virgin females from F_1 with P males and vice versa.

COLLECTING AND SCORING

See page 10 for general work schedule.

5-7 days after setting up the cross remove all flies to prevent mix ups. When F_1 adults begin to emerge remove, classify and record details of the progeny (remember to save some for further crosses or next generation). It is important to continue clearing and classifying flies for several days until all pupa cases are seen to be empty. Incomplete counts may give erroneous results e.g. an F_2 of nearer 4:1 from a wild x vestigial cross. Bear in mind that male flies and some mutant flies emerge more slowly than wild type females. The results of several experiments should be pooled and then analysed using the X^2 test to see if they fit the expected ratios. For information on statistical analysis please see Bibliography on page 19.

SUB-CULTURING

If stocks of Drosophila are to be maintained it is necessary to sub-culture them every 3 to 4 weeks. Have ready stoppered tubes or bottles containing fresh yeasted medium. Clear the flies by shaking out into an etheriser and then transfer 5 pairs per 100 x 25mm tube, or 15 pairs per third-pint bottle.

PRESERVING DROSOPHILA

It is a good idea to build up a stock of preserved Drosophila sorted into males and females and mutant types. Also, the results of various crosses could be retained. If flies are to be preserved, they should be etherised and transferred to a bottle of 2.5% formalin (bear in mind that eye pigments are soluable in alcohol.)

DISPOSAL

After use all bottles or tubes should be autoclaved to kill the flies and to sterilise and melt the medium. This may then be tipped into the sink and flushed away with plenty of water. The containers should then be washed thoroughly, rinsed and dried.

WORK SCHEDULES

General Timetable for all crosses at 25°C

	P stock cultures— • clear a.m. and p.m. to collect males and virgin females (page 7)
Day 0	set up crosses with reciprocals (page 8)
Day 5	remove parents
*Day 12-20	F_1 clear, classify and count (page 9)
Day 0	set up F_1 crosses in fresh tubes (virgins unnecessary) and if required backcrosses
Day 5	remove F_1 adults
*Day 12-20	F_2 clear, classify and count

*Allow a minimum of 3 days for counting, more if possible.

Drosophila mutants

The wild type *Drosophila* is designated +; the mutant types are referred to by the first one or two letters of their most recognisable characteristic. If the trait is dominant, upper case is used; for recessive, lower case, e.g. **vg** denotes vestigial wing (recessive); **B** denotes bar eyes (dominant). A full list of available mutants, and their symbols and chromosome locations are given in our current catalogue.

MONOHYBRID CROSS (no sex linkage)

This is an investigation of the inheritance of a single contrasting pair of characters.

Flies required: pure breeding wild type (+) and one of the following mutants:

vestigial wing (**vg**)

brown eye (**bw**)

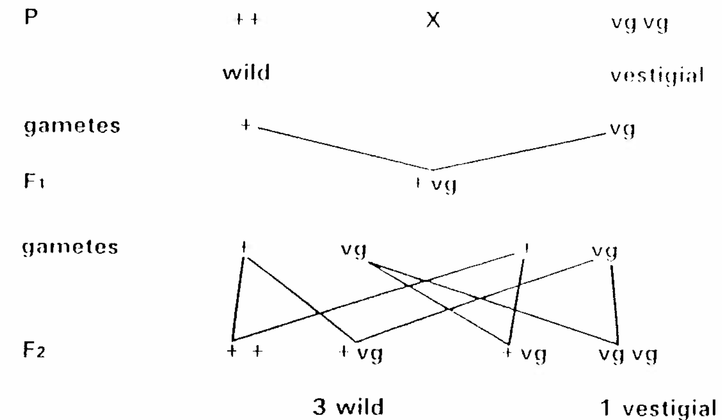
ebony body (**e**)

sepia eye (**se**)

1. Etherise some of the adults and be sure that you can distinguish males from females and the mutant type from the wild.

Note: Occasionally stock cultures obtained from us have a bad time in transit, particularly in low fluctuating temperatures, and may be dead on arrival. This is no cause for despair since all the cultures are checked for living larvae, so new adults will emerge in 7-10 days.

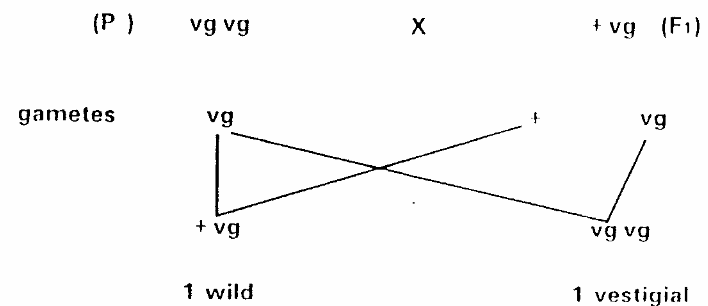
2. Follow the timetable for experimental crosses (on page 10) starting with the collection of males and virgin females.
3. According to **Mendel's First Law of segration**, the following diagram illustrates the events of this cross.

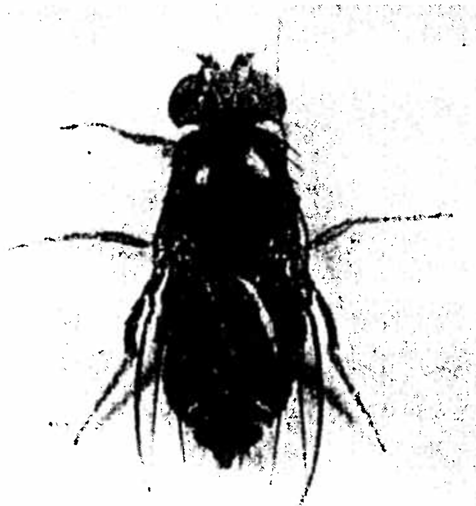


4. Use the X^2 test (see Bibliography) to analyse your results.

Backcross

This is done to confirm that the F_1 obtained from the monohybrid cross are in fact heterozygous. Cross the recessive parent with the F_1





Wild type Drosophila female (+)

Dumpy wing Drosophila female (dp)

Vestigial winged Drosophila female (vg)

DIHYBRID INHERITANCE (without linkage)

An investigation of the inheritance of two pairs of characters.

Flies required: wild type x dumpy wing/ebony body
or
dumpy wing (normal body) x ebony body (normal wing)

1. Examine adult flies.
1. Collect virgin females and set up reciprocal crosses. Follow the general schedule (page 10) to obtain F_2 flies.

P	$\frac{+\,+}{+\,+}$	X	$\frac{dp\ e}{dp\ e}$	OR	$\frac{+\ dp}{+\ dp}$	X	$\frac{e\ +}{e\ +}$
phenotype	wild		dumpy/ebony		dumpy		ebony
gametes	+ +		dp e				
F_1		$\frac{+\,+}{dp\ e}$					
phenotype		wild					

The genes for ebony and dumpy are found on different chromosomes and each member of one pair of alleles combines randomly with each member of the other pair giving four different combinations in the gametes. This is **Independent Assortment** (Mendel's Second Law). It is the result of the independent movement of homologous chromosomes to the two poles of the meiotic spindle during the first meiotic division.

Formation of gametes in F_1 flies giving gametes: ++, dp +, + e and dp e.

F2

gametes ♂ \ ♀	++	+dp	+e	dpe
++	++ / ++	++ / +dp	++ / +e	++ / dpe
	wild	wild	wild	wild
+dp	++ / +dp	+dp / +dp	++ / dpe	dp+ / dpe
	wild	dumpy	wild	dumpy
+e	++ / +e	++ / edp	+e / +e	+e / dpe
	wild	wild	ebony	ebony
dpe	++ / dpe	+dp / edp	+e / dpe	dpe / dpe
	wild	dumpy	ebony	dumpy/eb

= 9 wild : 3 dumpy : 3 ebony : 1 dumpy/ebony

AUTOSOMAL LINKAGE

Sometimes when a dihybrid cross is made, the results are not as shown above. This is due to the fact that two genes are present on the same chromosome and are usually inherited together. (Autosomes are those chromosomes not involved in sex determination).

However, crossing over takes place in a small proportion of gametes and when this occurs, the two alleles will be separated. The result of this is that most of the flies have the same combination of characteristics as the parents (parental combinations), but a few flies show new combinations (non-parental) of characters (recombinants). Thus, the ratios obtained vary according to the frequency of crossing over. This again depends on how far apart the genes are on the chromosome. If they are very close they will not be separated (i.e. recombinants will not occur) so often. Working out the percentage of recombinants gives us an idea of the distance between genes on a chromosome and this is the basis of genetic mapping.

The gametes produced by a dihybrid cross are most easily identified by a backcross*. Since this cross theoretically results in equal numbers of all 4 possible genotypes (if there is no linkage) the arithmetic is made much easier:

**This is a cross of F1 X double recessive parent.*

Flies required: A. wild type x black body/vestigial wing
and/or B. black body (normal wing) x vestigial wing (normal body)

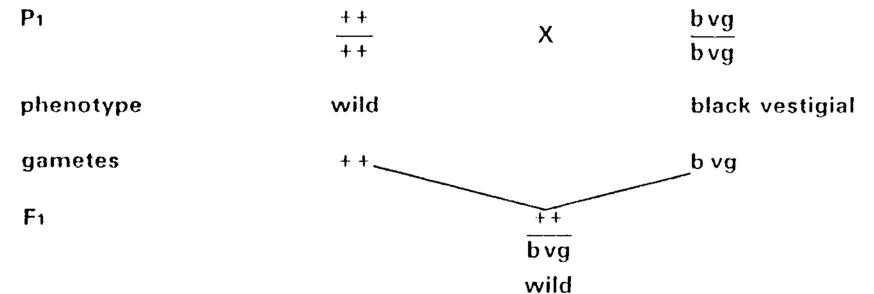
1. Examine adult flies
2. Follow the timetable for experimental crosses (page 10) starting with collection of males and virgin females and obtaining F_1.

Note

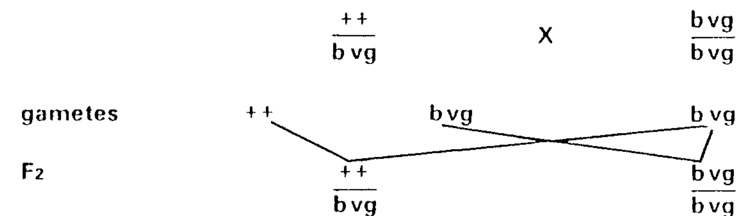
The alleles for black/wild body colour and normal/vestigial wing are on the *same* chromosome. The symbols for the parents (example A) are written $\frac{++}{++}$ and $\frac{b\,vg}{b\,vg}$ and the gametes contain $\underline{++}$ and $\underline{b\,vg}$ chromosomes.

Compare this with the example of dihybrid inheritance without linkage where the parents are symbolised as $\frac{++}{++}$ and $\frac{dp\,e}{dp\,e}$.

Example A



3. Backcross F_1 with black vestigial parent.



1 wild type : 1 black vestigial

plus a small number of recombinants:-
black body (normal wing) and vestigial wing (normal body)

Example B

P_1	$\frac{b+}{b+}$	x	$\frac{+vg}{+vg}$
Phenotype	black		vestigial
gametes	b+		+vg
F_1		$\frac{b+}{+vg}$ wild	

3. Cross F_1 with black bodies/vestigial winged flies (P_1 from example A).

	$\frac{b+}{+vg}$	x	$\frac{bvg}{bvg}$
gametes	b+ +vg		b vg
F_2	$\frac{b+}{bvg}$		$\frac{+vg}{bvg}$

1 black body/ normal wing : 1 normal body/ vestigial wing

plus a small number of recombinants wild type and black body/vestigial wings.

SEX LINKAGE

The female has two identical X chromosomes, while the male has one X and one Y chromosome. Genes which are carried on the X chromosome may not have alleles on the Y chromosome and therefore may be expressed in the phenotype even if they are recessive.

Flies required: A. wild type male x white eye female
B. wild type female x white eye male

1. Examine adult flies.
2. Follow the timetable for experimental crosses (only as far as F_1)

	A		B	
P_1	male wild	x female white eye	female wild	x male white eye
gametes	½ + ½ Y	w	+	½ w ½ Y
F_1	1 female wild	: 1 male white eye	1 female wild	: 1 male wild

Self fertilisation of the F_1 generation from each of these crosses results in a 1 : 1 : 1 : 1 ratio of wild type and white eyed males and females

APPENDIX A

Drosophila Service

We are able to offer a wide variety of mutants of Drosophila, sold as:

- Mutant Stocks
- Selected sets to illustrate simple Mendelian ratios and linkages
- Ready made crosses
- Segregated virgin females and males

for details please see our current catalogue.

Drosophila Beginners Kit

contents:
- 2 large stock cultures of different genotypes
- breeding tubes containing medium
- acrylic tiles
- sable brushes
- hand magnifiers
- wax pencils
- results pad
- booklet—"Practical Genetics with Drosophila melanogaster"

Sundry Supplies

M48700/0 Ready prepared Drosophila medium in tubes (10 tubes)
M48705/9 Ready prepared Drosophila medium in bottles (10 bottles)
M48710/3 Ready Mix dried food
C76500/7 Glass tubes 100mm x 25mm
B75120/7 Wide mouth bottles
B75560/2 Foam plastic plugs for tubes
B75640/0 Foam plastic plugs for bottles
B69340/2 Drosophila etheriser
M40921/9 Saccharomyces cerevisiae, dried active yeast
S60165/7 Nipagin 10% alcoholic solution
S60155/4 Nipagin 100g
M82135/0 results pad

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Experimental Biology Manual by G.D. Brown and J. Creedy 1970 published by Heinemann (~~available from us catalogue no. A92100/2~~).

Practical Genetics by P.M. Sheppard 1973 published by Blackwell Scientific (~~available from us catalogue no. A91050/8~~). This book contains more experiments with Drosophila and helps with statistical analysis and chromosome mapping. It also includes a wide range of genetics experiments with other animal and plant species — highly recommended.

Genetic Variations of Drosophila melanogaster by Linsey and Grell 1968 published by the Carnegie Institute of Washington (a detailed list of all the mutants of Drosophila).

Experiments in Genetics with Drosophila by M.W. Strickberger 1962 published by John Wiley & Sons. Contains more advanced practical work.

Invertebrate Types—Drosophila by B. Shorrocks published by Ginn & Co. An account of the biology of Drosophila.

Drosophila Guide by Demerec and Kaufmann 1967 published by Carnegie Institute of Washington.

Elementary Practical Statistics for Biologists, 1978 published by Philip Harris Biological (~~catalogue no. A90160/9~~).

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Work Schedules For Drosophila

VALUE PACKS A03451 TO A03542

1. Check cultures on receipt for active larvae (look for them 'working' in the food, often visible only by their black mouthparts) and perhaps pupae.

2. Clear the parents by shaking out into an etheriser. Make sure none remain. Examine the parents carefully and distinguish:
   a) The different phenotypes
   b) The sex difference

If now transferred to fresh tubes, these flies can be bred on for further work. It is essential to do this if back-crosses are to be made.
Occasionally, due to rough handling in transit, the flies become trapped by displaced food. There is no way of completely preventing this and we regret therefore that we can entertain no claim for free replacement in this event. Such cultures will, of course, still produce new adults.

3. Place the cleared cultures in the 25°C incubator or nearest equivalent, having noted the date of setting-up marked on them. Check progress daily. Adults may be expected to emerge from 9-10 days after setting-up, bearing in mind that cooling in transit retards development to some degree.

4. As soon as the first new adults appear, discard them, then clear the cultures night and morning. Segregate the sexes of each strain into separate food tubes, continuing for 3-4 days if necessary to accumulate sufficient parent flies for the crosses.

SEGREGATED MALES AND FEMALES A03591 TO A03761 START HERE

5. Etherise these accumulated virgin females and males and set up crosses in tubes with food and yeast: 3 females to 3/6 males, or up to 5 females if numbers permit. Ensure reciprocal crosses are included, e.g. wild type males x vestigial females, vestigial males x wild type females. Mark each tube with cross details and date.

READY MADE CROSSES A03773 TO A03827 START HERE

6. After 5 days, check for F_1 larvae and remove the parent flies. They may be discarded or transferred to new tubes for additional crosses.

7. On the tenth day (at 25°C) the F_1 should begin hatching. Every two days, clear the emergent flies, classify and count. Set up crosses for F_2, say 3 from each original, using similar numbers of males and females as in (5), BUT NOTE THAT THE LATTER NEED NOT BE VIRGIN. Discard into 'fly morgue' all F_1 flies not used in F_2 crosses.
   Alternatively, if back-crosses are required, clear F_1 night and morning to ensure virginity of females. Cross with recessive parent type bred on from original stock cultures (again, ensure reciprocals).

8. Whilst this work on the F_1 continues, obviously F_2 crosses should be checked daily. After 5 days, clear all adults and discard.

9. When the F_2 begins to emerge, clear, classify and count every other day for 8 days after the first emergence. This extended period is essential, not only for maximum numbers, but also to allow for slower emerging mutants such as vestigial.

10. Discard all F_2 flies.

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