Experimental Genetics I

Drosophila melanogaster

Protocol

11th of April 1997
through
6th of June 1997

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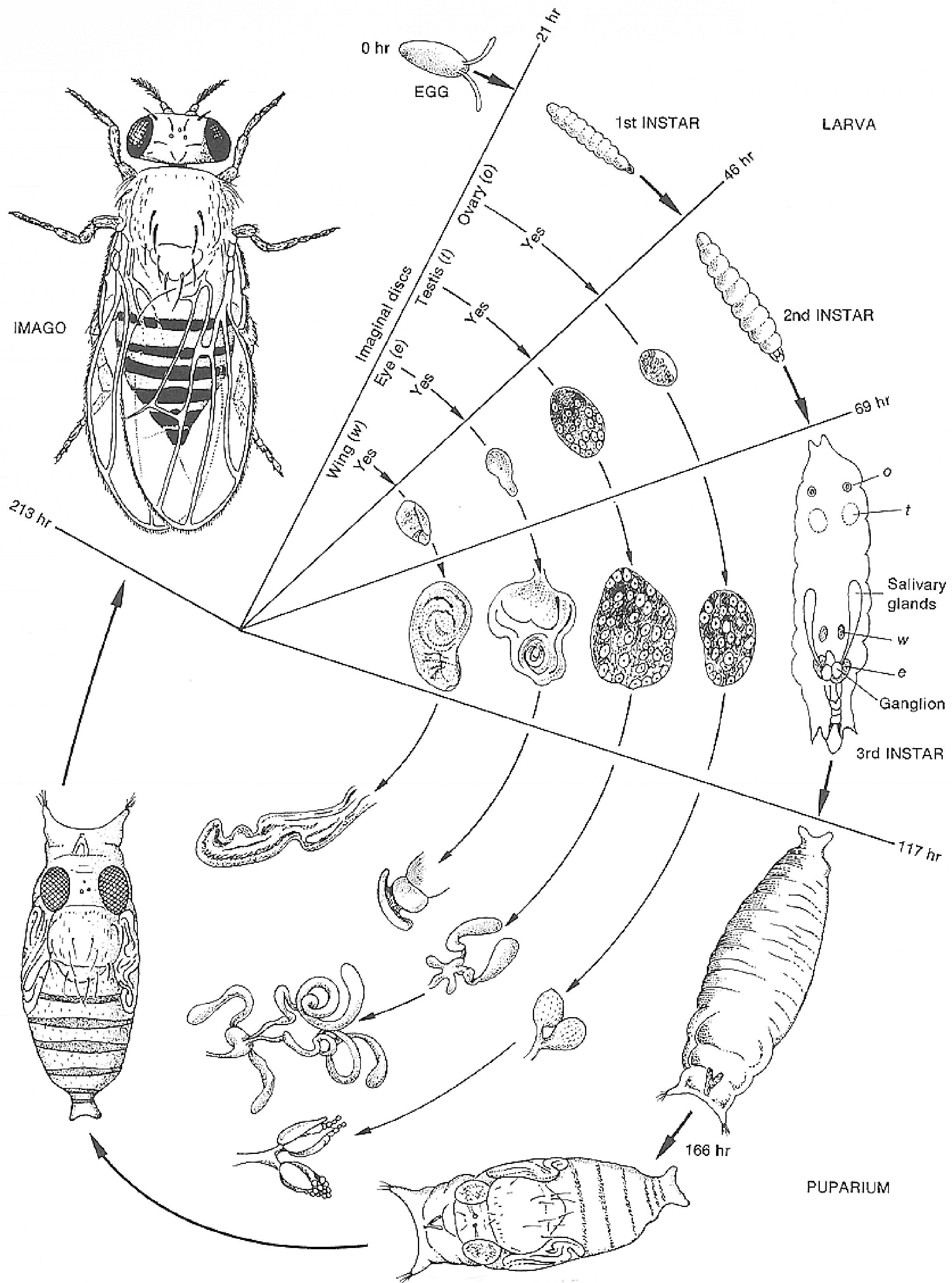
Salzburg, July 4th 1997

biophysics.sbg.ac.at/home.htm


0 hr
EGG
21 hr
1st INSTAR
LARVA
46 hr
Ovary (o)
Yes
Testis (t)
Yes
Imaginal discs
Eye (e)
Yes
Wing (w)
Yes
2nd INSTAR
IMAGO
69 hr
o
t
Salivary glands
w
e
Ganglion
3rd INSTAR
213 hr
117 hr
166 hr
PUPARIUM

Introduction

Drosophila melanogaster, commonly know as the fruit or vinegar fly, is well suited for laboratory research.

In addition, the large polytene chromosomes found in cell nuclei of the salivary glands of the third instar larval stage can be employed in cytological studies of chromosome aberrations.

Advantages of using ***Drosophila sp***. in experimental studies include the following:

1. Ease of culturing (small, inexpensively raised and handled, hence can be raised in simple culture media)
2. Short generation time (8 to 11 days at 25°C)
3. Prolific breeders (several hundred offspring from a single mating pair)
4. Small size (ease in handling and storage)

Life Cycle: The life cycle of *Drosophila* consists of four stages:

Egg: A fertilized adult fly starts to deposit eggs on the second day after emergence from the pupa. Each egg is about 0.5mm in length, ovoid in shape and white in color. Embryonic development of the egg takes about one day at 25°C, and hatching out of the egg case is the larva.

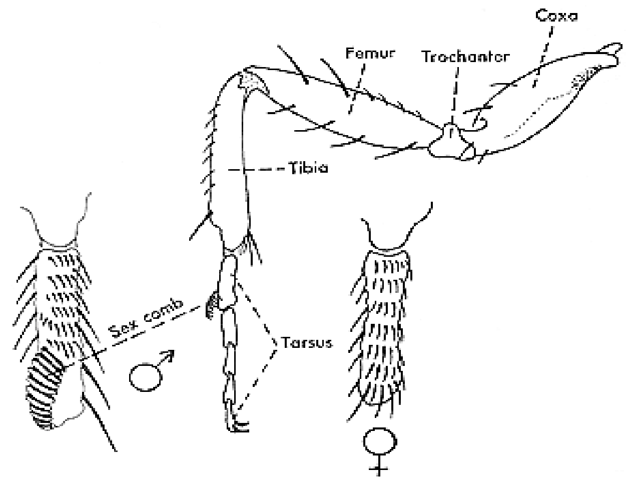
Larva: The larva is white segmented and wormlike. It has black mouth parts (jaw and hooks) in a narrowed head region. There are no eyes, lacks appendages, and breaths by trachea. This life cycle is of rapid eating and growing. There are three larval stages, called instars, separated from each other by molts. During the final (third) instar stage, the larva feeds until ready to pupate, then crawl out of the medium to a dry place, where it ceases to move. The larval stage takes about 4 days at 25°C for completion, at which time the third instar is about 4.5mm long.

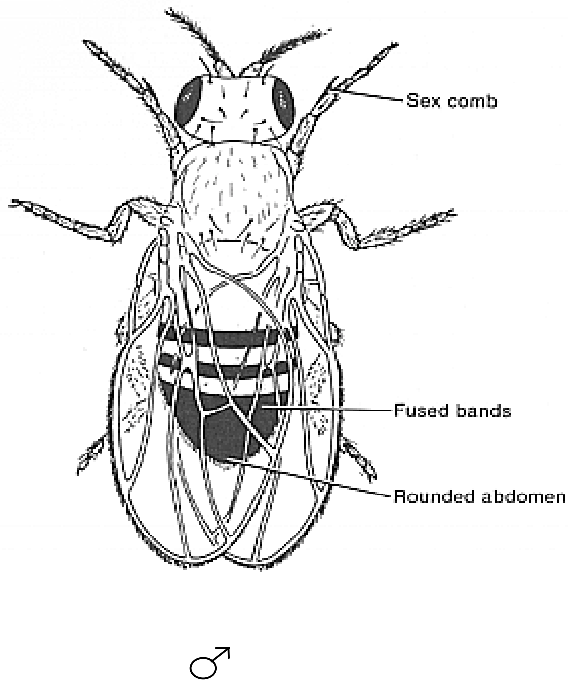
Pupa: A complex tissue reorganization (metamorphosis) occurs during pupation, and within four days at 25°C the adult emerges from the pupal case.

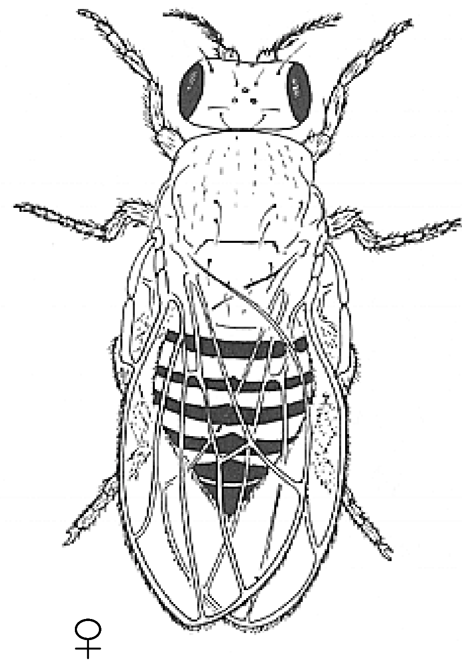
Adult: (Imago) Is considered the reproductive stage. At first the adult is greatly elongated and wings are unexpanded. Within an hour the wings expands and attains the more rotund form of the adult. The adults are light in color but darken within a few hours after hatching. Mating occurs after six hours of emergence from the pupal state. The sperm are stored in the spermatheceae and ventral receptacles of the female and are released gradually into the oviduct as eggs are produced and passed through the oviduct into the vagina. The female begins to deposit eggs about two days after it has emerged from the pupa. The average life span of an adult fly is 37 days at 25°C.

Sex Differences: Several criteria may be used to distinguish male and female flies:

	♂ **Male**	♀ **Female**
Adult size	smaller than female	larger than male
Size of Abdomen	male abdomen is rounded and much shorter	female abdomen curves to a point
Marking of Abdomen	alternating dark and light dorsal bands with the last few segments fused	alternating dark and light dorsal bands
Appearance of sex comb	tiny tuft of hairs on the basal tarsal segment of each leg	absent
External genitalia at the tip abdomen	claspers are darkly pigmented, arranged in circular form, and located just ventral to the tip.	ovipositor of the female is pointed
Sex organs during larval stage	large, white mass of testicular tissue	ovarian tissue constitutes a much smaller mass.



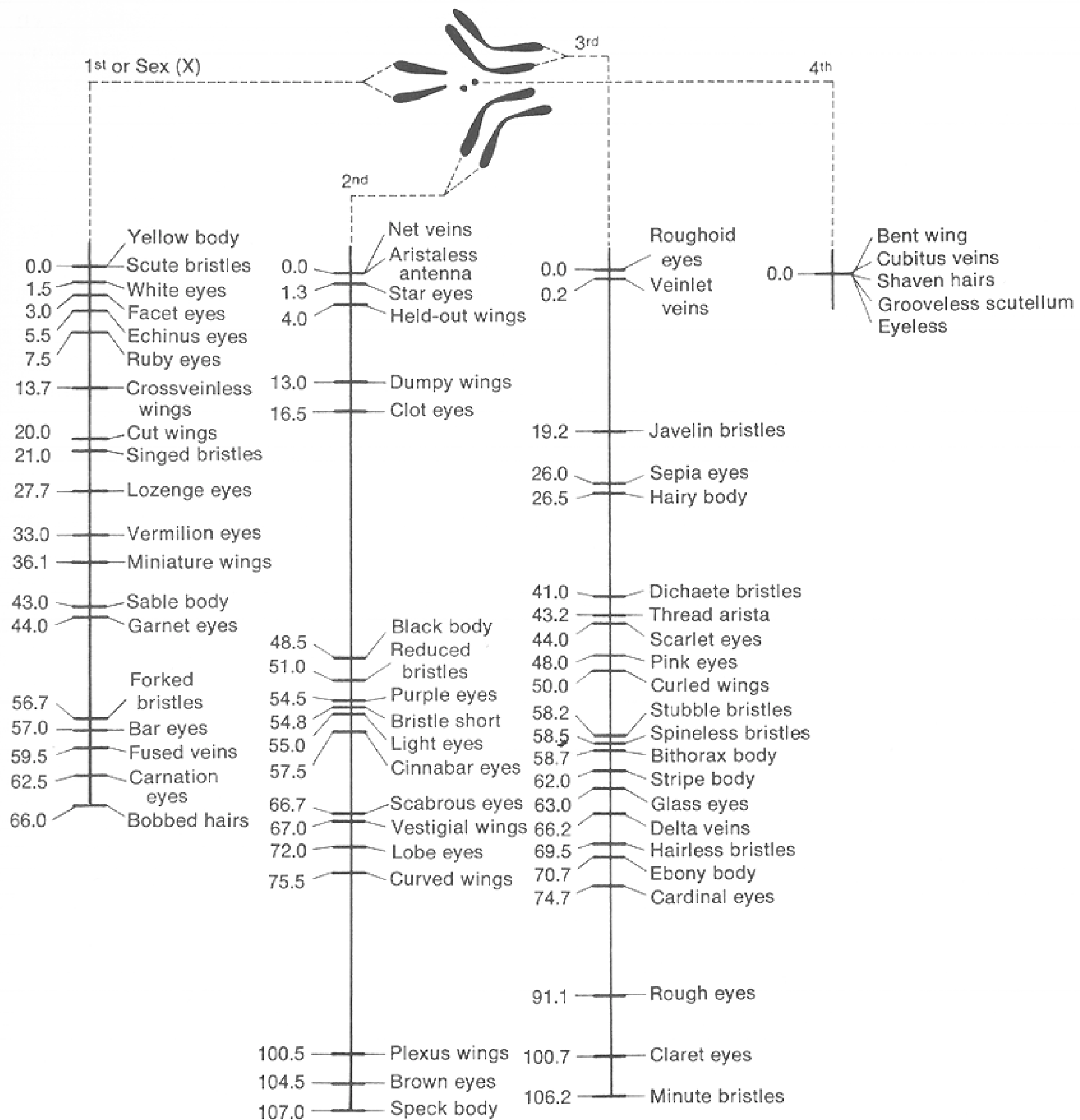




♂ ♀

Hereditary Traits: Before one observes their mutants, one needs to be familiar with the appearance of the wild-type *Drosophila*, the type found most often in natural populations of the organism. Although thousands of mutations in *Drosophila* are known, only those which are relevant to these exercises are listed.

	Wild-type (+)	**Mutant Type**
Eye	red, oval in shape, and many faceted	white, black, apricot, scarlet red, pink, or brown; changes in shape and number of facets
Wing	smooth edges, uniform venation, extend beyond the abdomen	changes in size and shape; absence of specific veins; changes in position in which wings are held when at rest
Bristle	fairly long and smooth (note distribution on head and thorax)	shortened, thickened, forked, or deformed (note changes in pattern of distribution)
Body Color	basically gray, with pattern of light and dark areas	black (in varying degrees), yellow (in doubtful cases color can often be determined clearly on wing veins and legs)



Symbols *Drosophila* Genetics: For convenience in listing, representative symbols are assigned to each mutant type. It is essentially an abbreviation in which it starts with an initial letter of the mutant name. The dominant wild-type allele is designated for b (black) b^+ , and the recessive wild-type allele of B (bar) is B^+.
„+“ always indicates the wild-type.

B	**Bar**: Eye restricted to a narrow vertical bar in males and in homozygous females. Heterozygous females has intermediate number of facets between homozygous females and wild-type; character is therefore considered **semidomonant**. Chromosome X - 57.0
bw	**brown**: Eye color in light brownish on emergence, darkening to garnet; testes and vasa colorless; Malpighian tubes somewhat paler than the wild-type Chromosome 2 - 104.5
f	**forked**: Bristles all shortened, gnarled, and bent, with ends split or bent sharply; effect on hairs similar, but detectable only with high-power magnification. Chromosome X - 56.7
se	**sepia**: Eye color on emergence transparent brownish red, darkening to sepia, and finally black. Ocelli remain wild-type in color. Chromosome 3 - 26.0
ore	**ore**: wild-type form of the organism investigated
v	**vermilion**: Eye color bright scarlet, not transparent; ocelli colorless. Chromosome X - 33.0
vg	**vestigial**: Wings and halteres are greatly reduced in size. Chromosome 2 - 67
y	**yellow**: Body color rich yellow; hairs and bristles brown with yellow tips; wing hairs and veins yellow; larval setae and mouth parts yellow to brown. Chromosome X - 0.0

1. Selected Experiments using *Drosophila*

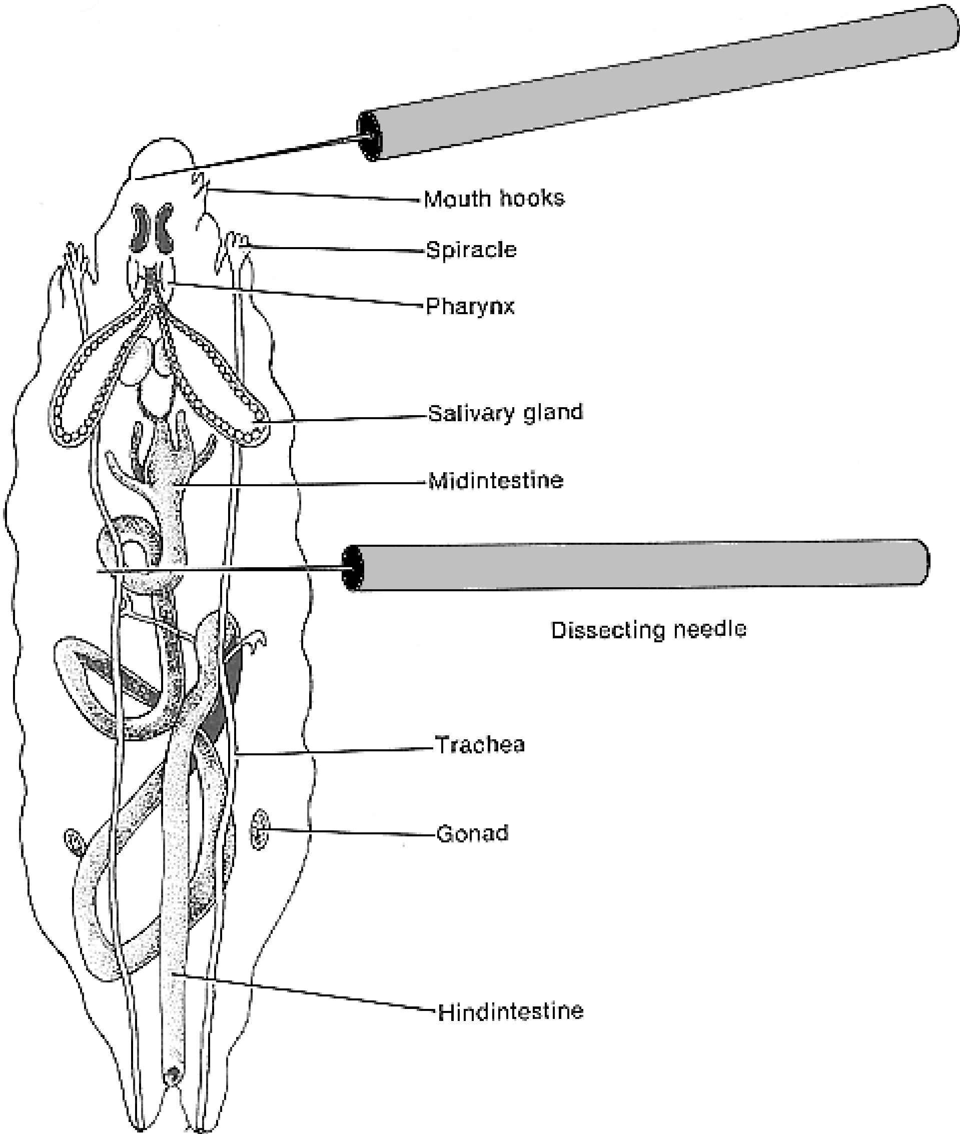
1.1 Salivary Gland and Chromosome Preparation - 2nd of Feb. 1997

material: 2 stainless steel dissecting needles
glass plate
Gurr's natural orcein
distilled water
microscope slides
cover slips
paper towel
compound microscope (x1000)
organism: *Drosophila* larvae (well fed)

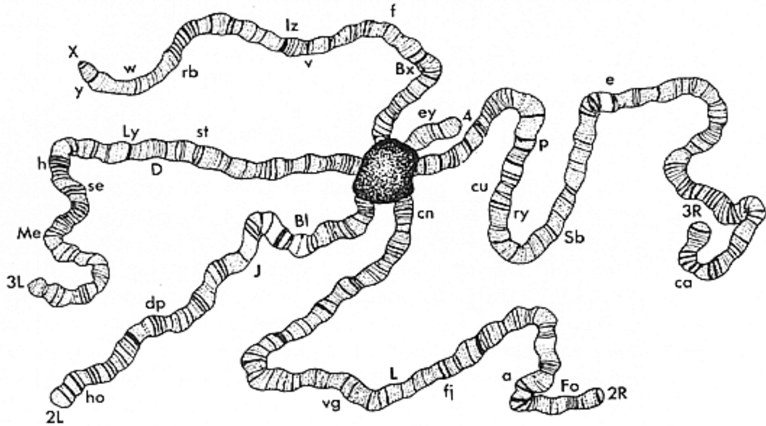
Purpose: A number of physical and mental abnormalities have been found to be the result of either the addition or subtraction of one of the chromosomes of the normal compliment. In the case of the fruit fly, the chromosomes of the larval salivary gland cells can be easily prepared and studied. In these cells, the homologous chromosomes are permanently synapsed. The cells of this tissues do not divide but only enlarge while the chromosomes are duplicated regularly. This process of chromosome duplication without cell division is called endomytosis, and the chromosomes are called polythene chromosomes (many stranded). This giant chromosomes are permanently arised by successive doubling of the original chromosomes, their are not constant in width. Especially striking is the occurrence of enlarged non-banded areas called puffs. Puffs are regions of chromosomes whose genes are involved in very active DNA transcription. The pattern of puffs of a particular chromosome varies with the type of tissue of the body.

Procedure: Transfer a fat, sluggish larvae from the side of the culture bottle to the glass plate.

- Place larvae in a drop of staining solution (optional NaCl-solution) on the plate; the dissection is done directly in the staining solution, using the 20x30 magnification of the binocular stereo dissecting microscope.
- Place one dissecting needle behind the black mouth hooks and the other near the posterior end of the larvae.
- Move the dissecting needle that is behind the mouth-hooks foreword very slowly.
- When the chitin begins to break, stop the foreword movement, hold the needle firmly in place, and move the other dissecting needle posteriorly; the internal structures of the larvae will be pulled out of the body.
- Identify the salivary glands. They appear as two long sausage-shaped bags with a characteristic fat body along one side. The glands should be bulbous and crystalline in appearance.
  Note: Keep the salivary glands always moist by adding extra stain or NaCl solution.
- Using your dissecting needle, separate the glands from the other tissues and transfer the glands to a drop of sustaining solution to the microscope slide.
- Place a cover slip over the glands, starting at one edge of the drop.
- Place the slide in a fold made of paper toweling. Cover the slide and press firmly with the ball of your thumb. The toweling will absorb the excess stain as it is forced from the space between the slide and cover slip.
- Check under your compound miscroscope for any band-shaped chromosomes using a magnification lower than 1000x.



Results and Evaluation:



1.2 Biochemical Separation of Eye Pigments - 16th of May 1997

material: chromatografic chamber
propanol
distilled water
ammonium hydroxide
chromatographic filter paper
glass rod with rounded ends
razor blade
dissecting needle
etherizer
UV-black light
organism: specimens of *Drosophila* eyes:
wild-type (ore)
brown
white
sepia
vermilion

Purpose: Chromatography is a method for the investigation of genetic pleiotrophy (the multiple effect on a single gene mutation) of *Drosophila* eye pigments and involves the use of paper-chromatography for the separation of various biochemical pigments located in wild-type and mutant eye tissue.
The wild-type eye pigments of *Drosophila* consist of two mayor components:

- Ommochromes (brown pigments), which are triptophan derivatives.
- Related pteridines, when present in wild-type eye alone with omochromes, result in red eyes.

The ommochromes and pepteridines are groups of naturally occurring compounds because they are gene-dependent for synthesis, and complement each other physiologically in the production of the eye color of *Drosophila*. Therefor, chromatography serves to determine:

1. Whether or not certain flies contain ommochromes.
2. Which of the seven known pteridines are present in given eye-color mutants.
3. Whether sex, age, structure of the fly-eye affect the presence of either the ommochromes or various pteridines.

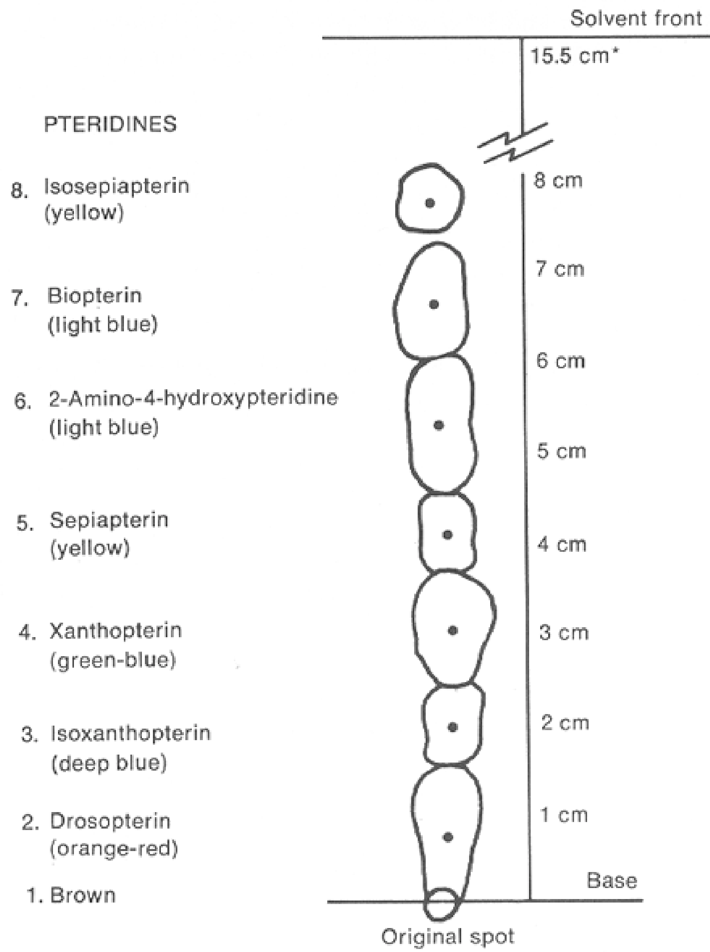
Examples of **Mutations**:

- Brown eye mutants lack the red pigments (pteridines);
- Classes of reddish eye mutants lack the brown pigments (homochromes);
- White eye mutants lack both pigments;
- Sepia contains abnormally large amounts of a particular pteridine, sepiapterine;

Procedure: Before preparing the samples mix propanol, water, and ammonium hydroxide in a 60:24:6 ratio and pour it into the chromatographic chamber until it reaches approx. 2.0 to 3.0cm in height. Place the chromatography paper on top of a clean sheet of notebook paper. Cut the paper with the razor blade into stripes 30 cm long and 4cm in width, draw a parallel line, 1cm from the lower edge with your pencil (indicates the start position). Etherize the flies (preferible from one sex only - either male or female), select three to four flies using a clean razor blade or dissecting needle, cut off the head of each of the samples. Place one head at a time and thoroughly crash one head with the glass rod. Allow the spot to dry before adding another head to the appropriate spot of the paper. Repeat the step with the other mutants (ore).
Touching only the edges of the paper, fold the paper on the opposite side of the printed samples in a way that the paper with the squeezed heads dips into the chromatographic solution. Seal the top of the chamber and place it at room temperature (away from direct heat or sunlight) at a safe location. After three to five hours, remove the chromatogram from the chamber and allow it to air-dry.

Note: Handle paper only by the edges, foreign matter, especially fingerprints, on the paper will affect the results of the experiment.

Results and Evaluation:



Spot	Color	Chemical	Mutant								ore	
			brown		sepia		white		verm.			
1	brown	ommochrome										
2	orange-red	drosopterin										
3	deep blue	isoxanthopterin										
4	green-blue	xanthopterin										
5	yellow	sepiapterin										
6	light blue	2-amino-4-hydroxypterine										
7	light blue	biopterin										
8	yellow	isosepiapterin										
			UV	DL	UV	DL	UV	DL	UV	DL	UV	DL

Legend: UV....seen under ultraviolet light
DL....seen under daylight conditions

2. Various crosses of mutant and wild-type *Drosophila*

Culture Media for *Drosophila*: The principle requirements of the medium are that it contains a sufficient amount of sugar to be used as food for larvae and for the growth of the yeast. The yeasts that are responsible for the fermentation constitute the whole diet of the fly. *Drosophila* can also be raised on soft fruit that is overripe and has begun to ferment. Therefore, *Drosohila* can be raised on any fermenting medium.

Typical Medium:		
	water	1l
	agar	8gr
	corn meal	60gr
	wheat germ	60gr
	sugar	70gr
	Methylparasept. (preservative)	4gr

Mix ingredients (except preservative) into the cool water and boil for at least 20 minutes while constantly stirring.
Dissolve preservative in ethanol and add it slowly to the steaming culture medium. While still hot pour the mixture into sterile 0.25l bottles until a 1cm thick layer is present.
Place a clean towel over the filled bottles and allow to cool; add a strip of paper toweling with one end into the food, to provide additional surface onto which the larvae may crawl to pupate. Seal the bottles with a spongy stopper.
Just before usage, the food is seeded with yeast, by adding a drop of thick suspension of fresh made yeast solution.

Care of cultures: Except when needed for counting or transferring, cultures should be kept in a constant temperature cabinet at 25°C: The need to sterilize the culture bottles before unplugging them for washing is evident when one considers that all culture bottles become reservoirs for contamination from molds, mites, and other *Drosophila* strains.

Etherization: To examine and count flies it is necessary to anaesthetize them with a light dose of ether. This is done by carefully and quickly transferring them from the culture bottle to a special etherizing bottle:
Caution: Ether is dangerously explosive, so there must be no flames or lighted cigarettes in the room.

- Dose the cotton pad that is stapled to the cork of the etherizing bottle with a few drops (do not over-saturate) of ether (do not place the stopper back in the bottle).
- Tap the culture bottle lightly on a pad of paper a few times until all the flies have been shaken down away from the mouth of the bottle.
- Quickly remove the cotton plug from the culture bottle, and in its place insert the mouth of the etherizing bottle. At this time the etherizing bottle should not contain ether fumes because ether, being heavier than air, will flow from the etherized bottle into the culture bottle, possibly killing larvae and pupal.
- Reverse the position of the bottles so that the etherizing bottle is now on the bottom, being careful to keep the mouths of the jars together (flies can escape). Note if the medium is not properly solidified, it may fall out of the culture bottle when inverted. In this case, do not invert the two bottles, but incline the etherizer up toward a light source. Flies are positively phototrophic and will move toward a light source.
- Holding the two jars together tightly with one hand, tap the side of the culture bottle with your other hand, or tap the bottom of the etherizing jar on a pad of paper. The flies will be dislodged and will fall into the etherizer.
- Quickly separate the two bottles and replace the cork on the etherizing bottle.
- After about one minute of etherization the flies will stop moving; wait about 20 seconds, then dump the flies out a 50 x 80 mm white index card. Flies that are dead from over-etherization extend their wings and legs at right angles to their bodies. Since some phenotypic traits change at death, dead flies should not be recorded.
- Flies usually remain etherized for 5 to 10 minutes. If it is necessary to reetherize, place the flies back into the etherizeer for a few seconds (reetherizing the flies for too many times will kill them).
- Flies that are to be discarded should be placed in a "morgue" (a bowl of mineral oil or water containing a household detergent).

Setting a Cross: In making experimental crosses it is often necessary to use <u>virgin female flies</u>. The easiest method of obtaining virgin females is based upon the fact that males rarely mate with females as early as 8 to 12 hours after emergence. Therefore, if all adult flies are emptied from the culture bottle and the bottle left for 10 hours or less, all females removed the second time should be virgin.

Males of any age may be used in a cross.

Note: Since etherized flies may become permanently stuck to the moist medium and die, it is important not to drop them onto the surface of the food. Instead, lay the culture bottle on its side and carefully slide the etherized flies from the index card to the glass surface. Do not place the bottle upright until the flies have revived.

The procedure of making a cross are:

1. Place three to five virgin females from the mutant strain with the corresponding number of wild-type males in a fresh culture bottle.
2. Mark the bottle with the nature of the cross and the date.
3. Record the crosses you have made on the Data Sheet provided.
4. Place the culture bottles in a 25°C constant temperature cabinet (away from the sunlight). If now larvae appears within five days, discard the culture and set up the cross once more in a fresh culture bottle.
5. If the culture is successful, remove the parents (usually after one week).

Note: All cultures should be labeled with your name, the cross (indicating phenotype of females and males), and the date of cross. About one week after making the cross, the adults should be removed so that they will not be confused with the progeny. Always be sure that plugs fit snugly into the mouth of the culture bottle; otherwise flies may escape.

Parental or **P** generation is a cross between stocks.
First filial generation or **F_1** are the offspring of the parental generation cross.
Second filial generation or **F_2** is a cross between two F_1 individuals in which will produce progeny.
Back-cross is a cross between an F_1 individual and a P individual.
Test-cross is any back-cross in which the recessive parental stock is involved. Some experiments require a test- cross because it is useful in confirming the conclusion drawn from the F_2 generation.

material needed for making crosses:

- 2 sterilized glass jars per cross filled with culture media
- bottle of ether
- stereo microscope (x40)
- glass plate
- dissecting needles
- 2 etherizers per cross
- dipper
- marker pen
- kleenex tissue
- solution of yeast
- morgue filled with ethanol

organism: *Drosophila melanogaster*

Chi square (χ^2) test - 23rd of May 1997

Purpose: This test is most used to compare experimentally obtained results with a given genetic model (hypothesis like 3:1, 9:3:3:1, 9:7, 1:1, and so on) and helps in the determination of linkage. The test generates a **p**-value (probability) that is the probability of obtaining by chance a specific deviation at least as great as the one observed, assuming that the hypothesis is correct.
The Chi square formula is:

χ, Greek letter for "chi"

$\chi^2 = \Sigma \{(X_{obs} - X_{exp})/X_{exp}\}$

X_{obs}, observed number of cases with a particular outcome
X_{exp}, expected number of cases with these particular outcome
Σ, Greek letter indicating the summation of

Before considering a number to use it is essential to consider another aspect of the χ^2-test, the degrees of freedom (**df**);
df takes into account the number of classes that are involved in a given cross. This is important, since the larger the number of classes, the more opportunities exist for chance deviations to occur. Such chance deviations will cause the χ^2 value to be very large when the number of classes is increased.

df = $X_{PT} - 1$ X_{PT}, number of phenotype

Both formulas are an integral part of each experiment. The performance of a statistical test is to decide whether the results obtained fit with the hypothesized genetic model.
It has generally been agreed that accepting a hypothesis as being compatible with the observed results if such deviations of the observed results from the ones expected would occur <u>5% or more</u> of the time, where the hypothesis true and the experiment repeated often. If the size of the deviation is so large, that they would be expected to occur <u>less than 5%</u> of the time, we then reject the hypothesis as being incompatible with the observed results.

Procedure:

1. State a simple hypothesis for the χ^2-test:
   - 3:1 ratio (monohybrid cross) resulting in heterozygous hybrid.
   - 9:3:3:1-ratio (dihybrid cross) resulting in heterozygous with respect of two pairs of alleles.
   - Lack of linkage which yields an expected ratio of 1:1:1:1 (null hypothesis)
2. Calculate the χ^2.
3. Estimate the probability **p** along with the df-value by using the χ^2-distribution table (below).
4. Reject (<5%) or accept (>5%) the hypothesis.

Probabilities of different χ^2 values for degrees of freedom 1 to 10

	(**p**) probabilities									
df	0.95	0.90	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.16	0.15	0.46	1.07	1.64	2.71	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	3.37	3.67	4.64	6.25	7.82	11.35	16.27
4	0.71	1.06	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.15	1.61	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.64	2.20	3.83	5.35	7.23	8.56	10.65	12.59	16.81	22.46
7	2.17	2.83	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.14
9	3.33	4.17	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.87	7.27	9.34	11.78	13.44	15.99	18.31	23.31	29.59

accept | reject

Results and Evaluation: See backside of data-sheets.

Linkage and Chromosome Linkage - 6th of June 1997

General: Each chromosome contains many genes. It was expected that the genes located in the same chromosome would be transferred from one generation to the next as a single group (said to be linked to one another - **chromosomes are linkage groups**). Thus *Drosophila* has four linkage groups. Linked genes are not always inherited as single unit. The frequency with which any two linked genes are inherited together varies with the particular pair of genes. T.H. Morgan postulated that the degree or strength of linkage depends on the distance between the linked genes in the chromosomes which lead to the construction of genetic or linkage maps of chromosomes. When the fly differ from the wild-type in two or more characteristics, the possibility always exists that two or more of the genes determining this characteristics are located in the same chromosomes. Genes that are located in the same chromosomes may:

1. always be inherited together and are said to be **completely linked** to one another revealing two to three phenotypes;
2. sometimes be inherited separately and are said to be **incompletely linked** to one another, thus showing four or more phenotypes;
3. if there is no linkage, one would expect to obtain 8 phenotypic classes with equal frequency, this would constitute a tri-hybrid test-cross (**independent assortment**).

If a fly, homozygous with three mutations is mated to a fly homozygous for the wild-type alleles of these genes (e.g.: vermilion, forked, yellow), all the offspring will have the normal phenotype but will be triple heterozygous. A test-cross of the F_1 females with triple heterozygotes to mutant males will yield different classes of offspring, depending on whether they are linked or not.

Linkage in the X-chromosome: The females exhibit recombinations for the genes located in the sex-chromosomes in the same manner as they do for the genes located in the autosomes.
In male *Drosophila* no crossing over at all occurs.

Interference and Coincidence: Is the quantitative estimate of the correspondents of double recombinants obtained to those expected:

$$\text{Coefficient of coincidence (CoC)} = \frac{\text{\% of double recombinant observed}}{\text{\% of double recombinants expected}}$$

It has been found that in
interference usually increases as the distance between loci becomes smaller, until a point is reached when no double crosses are found, CoC = 0. When the observed number of double recombinants is equal to the expected number, interference disappears and CoC is 1.

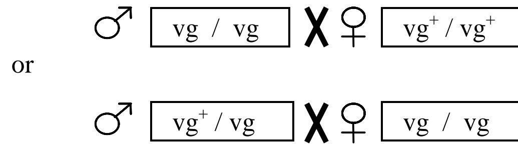
Procedure:

1. Set a cross with gene loci residing on one chromosome (e.g.: yellow, vermilion, forked).
2. Evaluate the F_2-generation once available by recording the various combinations of the y, v, f and wild type genes and record the following:
   - wild-type (y^+ ,v^+, f^+)
   - the mutants (y, v, f), the recombiants of cross-overs (6 more classes).
3. Determine the percentage of occurrence of each of the eight classes.
4. Calculate the percentage of crossing over or recombination between each pair of genes.
5. Draw a linkage map carrying the respective genes (mutants) based on the percentage of recombinants, with the distances between them in scale with the crossing-over values between them.
6. Compare the frequency of double crossover with that excepted. (CoC).
7. Record and explain the results.

Results and Evaluation: see back of the tri-hybrid cross (yellow-vermilion-forked) - data sheet

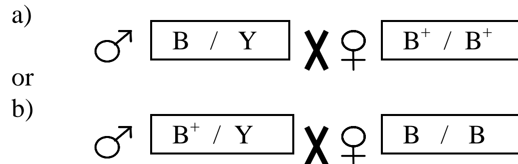
2.1 Executed Crosses - 11th of April through 26th of May 1997

1st Cross: Monohybrid single-autosomal recessive gene



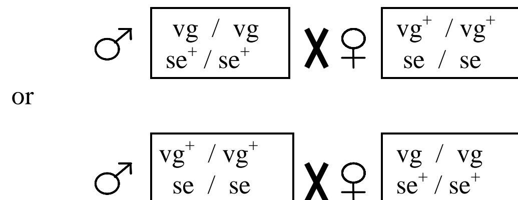
alleles like vestigial give the following hypothetical results:
P-cross ⇒ (F_1): all wild-type
F_1-cross ⇒ (F_2): 3 ore : 1 mutant
Testcross (F_1 x P): 1:1

2nd Cross: Monohybrid single-X-linked dominant gene



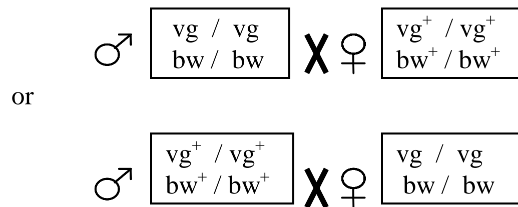
alleles like bar give the following hypothetical results:
P-cross ⇒ (F_1): a) ore♀ : mutant ♂
b) mutants only
F_1-cross ⇒ (F_2):a) 1 ore : 1 mutant
b) 3 mutants : 1 ore
Testcross. (F_1 x P): 1 ore : 1 mutant

3rd Cross: Dihybrid double-autosomal recessive gene (on separate chromosomes)



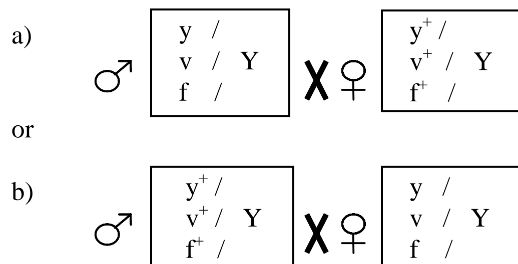
alleles like vestigial and sepia give the following hypothetical results:
P-cross ⇒ (F_1): wild-type
F_1-cross ⇒ (F_2): 9 wild-type
3 first mutant
3 second mutant
1 both mutant
Testcross. (F_1 x P): 1 : 1 : 1 : 1

4th Cross: Dihybrid double-autosomal recessive gene (on same chromosome)



alleles like vestigial and brown give the following hypothetical results:
P-cross ⇒ (F_1): wild-type
F_1-cross ⇒ (F_2): 9 wild-type
3 first mutant
3 second mutant
1 both mutant
Testcross. (F_1 x P): 1 : 1 : 1 : 1

5th Cross: Trihybrid double X-linked recessive gene



alleles like yellow, vermilion, and forked give the following hypothetical results:
P-cross ⇒ (F_1): a) all ore
b) ore ♀ : mutant ♂
F_1-cross ⇒ (F_2):8 phenotypes
Testcross. (F_1 x P): same as F_1-cross

Results and Evaluation: See respective data-sheets.

Monohybrid Cross ________ ♂ X ♀ ________ Chromosome # __________

Parental cross (P): ♂ ____/____ X ♀ ____/____ Date of cross______________

Date of removal of parents______________

Punnet Square of parental cross		
♀ \ ♂		

Count of parental progeny (F_1) Date

	Number	Phenotype ore	Phenotype mutants
♂			
♀			

Comments:

Siblings cross (F_1 x F_1): ♂ ____/____ X ♀ ____/____ Date of cross______________

Date of removal of parental F_1______________

Punnet Square of siblings cross		
♀ \ ♂		

Count of siblings progeny (F_2) Date

	Number	Phenotype ore	Phenotype mutants
♂ (expected)	( )	( )	( )
♀ (expected)	( )	( )	( )

Comments:

____/____ ____/____

Test cross (F_1 x P): Date of cross______________

Date of removal of parental F_1 x P_________________

Punnet Square of test cross				
♀ \ ♂				

Count of test cross progeny	Date	
	Number	Phenotype
		ore / mutants
♂		
♀		

Comments:

Calculation of χ^2 hypothesis:

phenotypes	observed	expected	(o - e)	$(o - e)^2$	$(o - e)^2 / e$
♂ ♀					
♂ ♀					
TOTALS ♂ ♀					

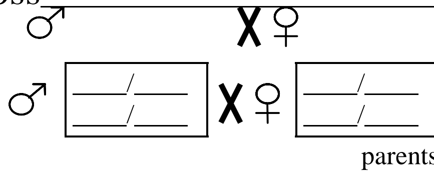
df = χ^2 =

p = / / ⇒ p = %

Conclusion:

Dihybrid Cross ________________ ♂ X ♀ Chromosome # ____________

Parental cross (P):



Date of cross____________

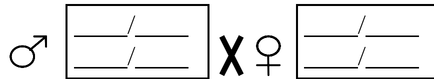
Date of removal of parents____________

Punnet Square of parental cross		
♀ \ ♂		

Count of parental progeny (F_1) Date			
	Number	Phenotype ore	Phenotype mutants
♂	______ ______	______ ______	______ ______
♀	______ ______	______ ______	______ ______

Comments:

Siblings cross (F_1 x F_1):



Date of cross____________

Date of removal of parental F_1____________

Punnet Square of test cross				
♀ \ ♂				
			____/____ ____/____	

Count of siblings progeny (F_2) Date			
	Number	Phenotype ore	Phenotype mutants
♂	______ ______ ______	______ ______ ______	______ ______ ______
(expected)	( )	( )	( )
♀ ____/____ ____/____	______ ______ ______	______ ______ ______	______ ______ ______
(expected)	( )	( )	( )

Comments:

Test cross (F_1 x P): Date of cross______________

Date of removal of parental F_1 x P__________________

Punnet Square of test cross				
♀ \ ♂				

Count of test cross progeny Date	Number	Phenotype	
		ore	mutants
	___ ___ ___	___ ___ ___	___ ___ ___
♂	___ ___ ___	___ ___ ___	___ ___ ___

♀

Comments:

Calculation of χ^2 hypothesis:

phenotypes	observed	expected	(o - e)	$(o - e)^2$	$(o - e)^2 / e$
♂ ♀					
♂ ♀					
♂ ♀					
♂ ♀					
TOTALS ♂ ♀					

df = χ^2 =

p = / / ⇒ p = %

Conclusion:

Trihybrid Cross________________________________ Chromosome # __________

Parental cross (P): ♂ ____/____ ____/____ ____/____ X ♀ ____/____ ____/____ ____/____

Date of cross______________

Date of removal of parents______________

Punnet Square of parental cross		
♀ \ ♂		

Count of parental progeny (F_1) Date			
	Number	Phenotype ore	mutants
♂	______ ______	______ ______	______ ______
♀	______ ______	______ ______	______ ______

Comments:

Siblings cross (F_1 x F_1): ♂ ____/____ ____/____ ____/____ X ♀ ____/____ ____/____ ____/____

Date of cross______________

Date of removal of parental F_1______________

Punnet Square of siblings cross		
♀ \ ♂		

Count of siblings progeny (F_2) Date			
	Number	Phenotype ore	mutants
♂	______ ______ ______ ______ ______ ______ ______ ______	______ ______ ______ ______ ______ ______ ______ ______	______ ______ ______ ______ ______ ______ ______ ______
(expected)	( )	( )	( )
♀	______ ______ ______ ______ ______ ______ ______ ______	______ ______ ______ ______ ______ ______ ______ ______	______ ______ ______ ______ ______ ______ ______ ______
(expected)	( )	( )	( )

Comments:

Results and Evaluation: from the tri-hybrid cross (yellow-vermilion-forked)

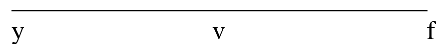
The data given below show eight phenotypic classes with different frequencies. It indicates that the cross dealt with three incompletely linked genes. Those testcross-offspring showing the original linkage pattern of the three genes are called partentals.
Those showing new linkage arrangements are called recombinants. The recombination of traits is due to a reciprocal breakage and exchange of segments of homologous chromosomes. The actual breakage and exchange of homologous chromosomes is called a chiasma.

	Phenotypes	Offspring	Recombinants		
no crossover	$\underline{y^+, v^+, f^+}$ y, v, f				
simple crossover	$\underline{y^+, v, f}$ y, v^+, f^+				
simple crossover	$\underline{y^+, v^+, f}$ y, v, f^+				
double crossover	$\underline{y^+, v, f^+}$ y, v^+, f				
	total number				
	F_R [%]				

When analyzing recombination data, one must consider only two genes at a time; expressed in the frequency of recombination (F_R)

F_R = 100·(number of mutants) / (total number) [%]

From these recombination data a genetic map can be constructed by using F_R as a map unit, which reflects the relative distance of the respective genes respectively.



y v f

Note: The distance between yellow and forked is shorter than calculated from the frequency of recombination between these genes. This discrepancy is due to the occurrence of double crossover, which makes widely separated genes appear closer together on the chromosome than they really are.

Glossary *Drosophila melanogaster*

Allele: The different, alternative forms of a gene that can exist at a single locus (see dominance).
Dominant: An allele that expresses its phenotypic effect even when heterozygous with a recessive allele; if *A* is dominant over *a*, then *AA* (homo-)and *Aa* (heterozygot) have the same pheno(wild)type.
Recessive: An allele whose phenotypic effect is not expressed, a mutant (e.g.: *aa*).
Autosome: A Look-alike chromosome other than a sex chromosome.
Backcross: A testcross between F2 generations of the recessive (*aa*-homo-) with the dominant (*Aa*-heterozygot) resulting in an equal display of recessive and dominant phenotypes.
Chiasma: (Gk. chiasma, cross) A cross-shaped structure commonly observed between nonsister chromatids during meiosis; the site of crossing over.
Chromosome: (G. chroma, color; soma, body) A linear end to end arrangement of genes and other DNA, sometimes with associated protein and RNA, found in Eukaryota.
Crossing Over: The exchange of corresponding chromosome parts between homologs (synapsis) by breakage and reunion (see also chiasma and meiosis).
Diploid: A cell that contains two copies of each type of chromosome (except sex chrom. compare haploid).
Dominance: An allele or corresponding phenotypic trait that is expressed in heterozygotes (see allele).
Codominance: The genetic situation in which both alleles in a heterozygote individual are fully equally expressed in the phenotype; no dominance of one allele over the other (bloodtype A x B = AB).
Incomplete D.: The genetic situation in which the phenotype of the heterozygote is intermediate between two homozygotes (red flowering plant x white f. p. = pink flowering plant).
Filial Generation: F_1, F_2, etc. In mendelian genetics the 1st, 2nd, etc. Generation in the line at descent.
Gene: The fundamental physical and functional unit of heredity, which carries information from one generation to the next; a segment of DNA, composed of a transcribed region and a regulatory sequence that makes possible transcription.
G. **Conversion**: A mitotic process of directed change in which one allele directs the conversion of a partner allele to its own form - altering the predicted outcome of Mendels 1st law from 2:2 to 3:1.
G. **Expression**: Synthesis of a polypeptide chain transcribed via the mRNA, tRNA, and rRNA using DNA as a template (see transcription).
G. **Dose**: The number of copies of a particular gene present in the genome (their number is directly proportional to the amount of proteins synthesized).
G. **Locus**: The specific place on a chromosome where a gene is located.
G. **Mapping**: Process of determining the location and distance between genes on a chromosome.
Genome: The entire complement of genetic material in a chromosome set (see gene dose, mutation).
Genotype: The specific allelic composition of a cell - either of the entire or, more commonly, for a certain gene or set of genes; genetic characteristics (makeup) that determine the structure and function of an organism (see also phenotype).
Haploid: Having only one copy of a chromosome (genome) set (compare diploid, polyploid).
Heterozygot: (Gk. heteros, different) Has two different alleles of a gene; one trait can be visible (dominant) while the other can be hidden (recessive), or visible both (codominant or incomplete dominant).
Homozygot: (Gk. homo, same) Has two identical alleles of a gene either *AA* (dominant) or *aa* (recessive).
Hybrid: An offspring resulting from mating between individuals of different genetic constitution.
Monohyb. cross: A cross between 2 individuals identically heterozygous at 2 loci (i.e.: *AaBb* x *AaBb*).
Dihyb. cross: A cross between 2 individuals identically heterozygous at one gene pair (i.e.: *Aa* x *Aa*).
Inbreeding: The breeding of closely related plants or animals; in plants, it is usually brought about by rtepeated self-pollination - as Mendel did.
Independent Assortment Structure: Mendels 2nd Law.
Life Cycle: All the stages by which an organism gives rise to others of its kind.
Linkage Group: Closely located genes on the same chromosome that tend to be transmitted as a single unit, hence, not following Mendel's 2nd law of independent assortment.

Meiosis: (Gk. replication) Two successive nuclear divisions (w/ corresponding cell division) that produce gametes (animals) or sexual spores (plants or fungi) having half (1n) the original genetic material.
Prior to **meiosis-I**, each chromosome is duplicated in the pre-meiotic S-phase to form a tetrad (synaptonemal complex) resulting in tetraploidity (4n); During prophase-I (synapsis) chiasma/ta are formed between non-sisterchromosomes resulting in crossing over; In metaphase-I the sister chromosomes are separated (2n - centromer still in tact); Ana-, and telophase-I similar to mitosis.
Meiosis-II follows (no interphase in-between) producing haploid cells (see mitosis (1n)).

Mendels laws: **1st**: Law of equal segregation; The two members of a genome-pair segregate from each other during meiosis; each gamete has an equal probability of obtaining either member of the gene pair (2:2). **2nd**: Law of independent assortment; unlinked or distantly linked segregating gene-pairs assort independently at meiosis (recombination).

Mitosis: (Gk. mitos, thread) A type of nuclear division (occurring at cell division) that produces two daughter nuclei identical to the parent nucleus; (di-, polyploid).
Prophase: (Gk. Pro, early; phasis, form) Early stage of nuclear division; nucleus disappears, mitotic spindle forms, chromosome condense and become visible.
Metaphase: (L. meta, half) Intermediate stage o.n.d.; chromosomes allign along the equatorial plane.
Anaphase: (Gk. ana, away) Spindle separates centromere, pulling chromatids apart to the opposed poles of the cell.
Telophhase: (Gk,. Telo, late) Late stage o.n.d.; spindle dissolves, nuclear envelope reappears daughter nuclei re-form (segregation and cytokinesis).

Mutant: An organism or cell carrying a mutation.

Mutation: (L. mutare, to change) A permanent change in chemical structure, organization, or amount of DNA; produces a gene or a chromosome set differing from the wild type, resulting in a faulty protein (loss or gain of function; gains and selection are the tools of evolution).
M. at DNA-level (DNA-sequence): Sequence of bases altered (not detectible w/ microscopic analysis).
M. at Protein level: particular aminoacids are altered resulting in different aminoacids or termination (non-detectible with microscopic analysis).
M. at Chromosome-level: Affect large / entire regions of chromosomes, hence location of genes (detectible with microscopic analysis).
M. at Genome level: Altering the chromosomal number (detectible with microscopic analysis).

Parental Generation: In mendelian genetics the individuals that give rise to the 1st filial generation F1.

Parental Type: In mendelian genetics, an offspring having the characteristics of one of the parents.

Phenotype: The physical appearance (makeup) of an organism controlled by its genes interacting with the environment; product of genotype (see dominant / recessive allele).

Pleiotrophy: The influence of a single gene on more than one trait (multiple expression).

Polygenetic Trait: Characteristics of a trait that varies in the quantity depending on the interaction of many genes; phenotypic traits controlled by more genetic loci (height: variations from short to tall).

Puff: A localized synthesis of RNA occurring at specific sites on giant chromosomes of Dipthera.

Punnet Square: A diagrammatic way of presenting the results of random fertilization from mating.

Puppate: The process of going from the larval stage to the adult stage in an insect.

Recombination: The formation of offspring by combination of genes that are present in either chromatid, resulting from the assortment of chromosomes and their genes during the production of gametes (meiosis) and their subsequent fertilization (ovum and testis) from different individuals. (the reshuffling of maternal and paternal chromosomes during meiosis, resulting in new genetic recombinations (compare complementation).

Recombinant Type: In mendelian genetics an offspring with characteristics different from that of the parents.

Recombination: The association in one individual of phenotypic traits from one of the parents.

Segregation: 1) Cytologically, the separation of homologous structures; 2) genetically, the production of two separate phenotypes, corresponding to two alleles of a gene, either in different individuals (meiotic segregation) or in different tissues (mitotic segregation).

Sex-Chromosome: Pairs of chromosomes when the member of the pairs are dissimilar and involved in sex determination, such as the X and Y chromosomes.
Sex-Linked: Characteristics of genes that are carried on these sex-chromosomes and therefore show different patterns of inheritance between male and female (colorblindness in humans is located on the X).

Synapsis: Close pairing of homologs (side by side) at meiosis (see also crossing over).

Synaptonemal Complex: A complex structure that unites homologs during the prophase of meiosis.

Test cross: see backcross.
Wild Type: The genotype or phenotype that is found in nature or in the standard laboratory stock for a given organism (see also mutant).
X-Chromosome: The sex chromosome found in two doses in female mammals and many other species.
Y-Chromosome: The sex chromosome found in a single dose in male mammals and many other species.

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