Biology 2250 Laboratory 3
2011

The Virtual Fly
(Mendelian Genetics using a simulated *Drosophila melanogaster* online program)

Name: __________________ _____________________
MUN # ______________________________________
Lab Slot______________________________________

The Virtual Fly Laboratory uses a computer program that simulates the results of *Drosophila melanogaster* crosses for 29 common morphological variants (bristles, eye colour, body colour, wing size, etc.) based on the known patterns of dominant or recessive inheritance and the linkage relationships of the alleles and gene loci for these traits. **For information on *Drosophila melanogaster* that you will need for this lab, print off the appropriate sections of the <u>Biology 2250 Course notes and lab manual</u> on the Biology 2250 course web site and bring them to the lab! Also read pages 339-343 of your text book.**

Also, parts of this lab MUST be completed before coming to lab and will be checked by the lab staff. Those parts are noted throughout the lab exercises.

The principle advantages of a computer simulation for our purposes is that crosses and the resulting progeny can be produced in only a few seconds (instead of several days), and that large number of progeny can be produced in each cross, which increases the statistical reliability of experimental results. Further advantages are that computer simulations are not subject to escaped flies; attempts to mate flies of identical sex, misidentification of variants, and do not require preparations of malodorous media or any other joys that afflict experiments with living organisms. The Virtual Fly Website can be found at:

http://www.biologylabsonline.com

***You need to purchase the Fly Lab manual from the bookstore to obtain a **login name** and **password**.

For each cross, follow the instructions on the computer to select the phenotypes of the parents. Record the phenotypes and results in the tables provided.

1. Click on the box ***Design*** for each fly.

Select the appropriate variant(s) for your cross using the characteristics listed on the left hand side. **All P_1 parents are HOMOZYGOUS for whatever variant you are dealing with (the Default flies are automatically set as homozygous wild-type for both parents).** Also note that the program does not use the standard notation for *Drosophila* variants; you are expected to figure out whether the variants in the crosses are due to dominant or recessive alleles.
Use the correct notation (lower and upper case, and "+") to write the genotypes. In each experiment, carefully distinguish *phenotype, genotype, genes* and *alleles.*

2. After you have designed both the female and male fly, click on the box that says ***Mate.***

You will be given the F_1 results of the cross by clicking on the box that says ***Analyze Results***: record the phenotypes and/ or numbers of the F_1 males and females. You have the option here to ***Use Sex*** or to ***Ignore Sex*** depending on what you require for recording. To go back to the cross, click on ***Return to Lab***.

3. To produce the second generation, click on the ***Select*** boxes below the diagrams of the F_1 offspring, this brings these F_1 offspring up to the top so you can then click on ***Mate*** to produce F_2 offspring. You will then be given the results of the F_2 generation: record them in the table provided. If any calculations are required, they should be done before performing another cross. If you repeat a cross, the computer will give you a new set of data.

Use the ***New Mate Box*** icon to return to the beginning of the program to perform another cross.

1 - MONOHYBRID CROSS - AUTOSOMAL
LOBE EYES x WILD-TYPE EYES

Before coming to lab, complete the cross diagrams below to determine the **Expected Results.** Refer to the **Introduction to Drosophila Genetics and Appendix C** in the Biology 2250 Course Notes and Lab Manual for the correct way to write genotypes and use the **correct symbols** (use **Appendix B)** for the variants involved.

	CROSS A		**CROSS B**	
PARENT PHENOTYPE	FEMALE	MALE	FEMALE	MALE
	LOBE EYES	WILD TYPE EYES	WILD TYPE EYES	LOBE EYES
P_1 GENOTYPE				
P_1 GAMETES				
F_1 GENOTYPE				
F_1 PHENOTYPE				
F_1 GAMETES				

1 mark total for this table

PUNNETT SQUARES to determine **expected F_2 results** using the **F_1 gametes**:

♀ \ ♂	**CROSS A**			♀ \ ♂	**CROSS B**	

0.5 mark total for this table

EXPECTED F_2 PHENOTYPE RATIOS:

0.25 Cross A:

0.25 Cross B:

In lab, complete the Cross below using the FlyLab online Computer program and record the **actual results** below:

1 - MONOHYBRID CROSS - AUTOSOMAL
LOBE EYES x WILD-TYPE EYES

	CROSS A		CROSS B	
PARENTS PHENOTYPE	FEMALE	MALE	FEMALE	MALE
	LOBE EYES	WILD-TYPE EYES	WILD-TYPE EYES	LOBE EYES
F_1 PHENOTYPE				
F_2 PHENOTYPE	# FEMALES	# MALES	# FEMALES	# MALES
WILD-TYPE EYE				
LOBE EYE				

0.5 mark total for this table

Observed F_2 Phenotype Ratio **(round to one decimal place)**:

0.5 Cross A ____________ Lobe eyes: _________ wild eyes
Cross B ____________ Lobe eyes: _________ wild eyes

QUESTIONS

0.5 1.Which of the alleles of the **Lobe** gene for eye shape is dominant? _____________
Based on the results, how do you know?
____
____

0.25 2.Why doesn't it make any difference to the F_2 generation if the variant (i.e. Lobe) allele is carried by the male or female parent?
____

2 - MONOHYBRID CROSS - SEX-LINKED
WILD-TYPE EYE COLOUR x WHITE EYES

Before coming to lab, complete the cross diagrams below to determine the **Expected Results.** Refer to the **Introduction to Drosophila Genetics and Appendix C** in the Biology 2250 Course Notes and Lab Manual for the correct way to write genotypes and use the **correct symbols** (use **Appendix B)** for the variants involved.

	CROSS A		**CROSS B**	
PARENT PHENOTYPE	FEMALE	MALE	FEMALE	MALE
	WILD TYPE EYES	WHITE EYES	WHITE EYES	WILD TYPE EYES
P_1 GENOTYPE				
P_1 GAMETES				
F_1 GENOTYPE				
F_1 PHENOTYPE				
F_1 GAMETES				

1 mark total for this table

PUNNETT SQUARES using the F_1 gametes

♂ / ♀	**CROSS A**			♂ / ♀	**CROSS B**	

0.5 mark total for this table

EXPECTED F_2 PHENOTYPE RATIOS **(include sex)**

0.25 Cross A:

0.25 Cross B:

In lab, complete the Cross using the FlyLab online Computer program and record the **actual results** below:

2 - MONOHYBRID CROSS - SEX-LINKED
WILD-TYPE EYE COLOUR x WHITE EYES

PARENTS PHENOTYPE	**CROSS A**		**CROSS B**	
	FEMALE	MALE	FEMALE	MALE
	WILD-TYPE EYES	WHITE EYES	WHITE EYES	WILD-TYPE EYES
F_1 PHENOTYPE				
F_2 PHENOTYPE	# FEMALES	# MALES	# FEMALES	# MALES
WILD-TYPE EYES				
WHITE EYES				

0.5 mark total for this table

Observed F_2 Phenotype Ratio **(round to one decimal place):**

0.5 Cross A: _____wild-type female: ____wild-type male: _______ white eyed female: _____ white eyed male

Cross B: _____wild-type female: ____wild-type male: _______ white eyed female: _____ white eyed male

QUESTIONS:

0.25 1. Which of the alleles of the **White** gene for eye colour is dominant? ___________________

0.25 2. Are the results of a cross involving a sex-linked trait the same for reciprocal crosses? _____________

3. Look for the variants lobe eye and white eyes on the *Drosophila* gene map (Appendix B). On which chromosome is the **lobe eye** locus located? _________________
0.25 On which chromosome is the **white eye** locus located? ________________

3 - DIHYBRID CROSS - AUTOSOMAL INDEPENDENT
EBONY BODY x DUMPY WING

Before coming to lab, complete the cross diagrams below to determine the **Expected Results.** Refer to the **Introduction to Drosophila Genetics and Appendix C** in the Biology 2250 Course Notes and Lab Manual for the correct way to write genotypes and use the **correct symbols** (use **Appendix B)** for the variants involved.

PARENT PHENOTYPE	EBONY BODY COLOUR & WILD-TYPE WING	WILD-TYPE BODY COLOUR& DUMPY WING
P_1 GENOTYPE		
P_1 GAMETES		
F_1 GENOTYPE		
F_1 PHENOTYPE		
F_1 GAMETES		

1.0 mark total for this table

PUNNETT SQUARE:

♀ \ ♂				

0.5 mark total for this table

0.25 Expected F_2 Phenotype Ratio:

In lab, complete the Cross using the FlyLab online Computer program and record the **actual results** below:

3 - DIHYBRID CROSS - AUTOSOMAL INDEPENDENT
EBONY BODY x DUMPY WING

PARENTS PHENOTYPE	**CROSS A**		**CROSS B**	
	FEMALE	MALE	FEMALE	MALE
	WILD-TYPE BODY COLOUR & DUMPY WING	EBONY BODY & WILD-TYPE WING	EBONY BODY & WILD-TYPE WING	WILD-TYPE BODY COLOUR & DUMPY WING
F_1 Phenotypes				
F_2 Phenotypes	# FEMALES	# MALES	# FEMALES	# MALES
WILD TYPE				
DUMPY WING				
EBONY BODY				
DUMPY WING & EBONY BODY				

0.5 mark total for this table

Observed F_2 Phenotype Ratio (exclude sex):

Cross A: _____ wild-type:_____ dumpy wing: _______ ebony:_____ dumpy wing & ebony

0.5

Cross B: _____ wild-type:_____ dumpy wing: ______ebony:______ dumpy wing & ebony

1. Looking at the results of cross 3, determine if the variant alleles are **dominant** or **recessive** to the wild type allele. Therefore:

0.5 The variant allele of the **dumpy wing** gene is________________to the wild-type allele.
The variant allele of the **ebony body** gene is _________________to the wild-type allele.

<u>Chi Square (Goodness of Fit) Analysis of Results:</u>

(**Refer to Appendix A on the Chi Square Test before completing the Chi Square analysis**).

0.5 1. Use the results from **Cross 3 Cross A** to perform a Chi Square test to determine if these results fit the expected results for a dihybrid, autosomal independent cross.

Null Hypothesis (i.e. what is the ratio expected?): ________________________________
____

1.5 Table 1. Chi Square Test for Autosomal Independence of the Alleles for **Dumpy Wing** and **Ebony Body** in *Drosophila melanogaster*.

Phenotype	Observed #	Expected #	(O – E)	$(O – E)^2$	$(O – E)^2/E$
wild					
dumpy wing					
ebony					
dumpy/ebony					
Total #					$*\chi^2$ =

# of degrees of freedom (d.f.) = ________

$* \ \chi^2 = \sum [(O - E)^2 / E]$

1.5 Interpretation of Chi Square test:

TEST CROSS

A test cross is a cross used to evaluate a particular hypothesis. Such a test cross can be used, for example, to determine whether an individual who shows a dominant trait is a homozygous or a heterozygous. This is done by crossing the individual to a **tester** individual that is known to be homozygous recessive for that trait. If all the offspring show the dominant phenotype, the individual in question is homozygous dominant. However, if the offspring show a 1:1 ratio of the dominant and recessive phenotypes, the individual in question is heterozygous.

4 - DIHYBRID CROSS -test cross

SHAVEN BRISTLES & CURVED WING X **WILD TYPE**

PARENTS PHENOTYPE	**CROSS A**		***CROSS B**	
	FEMALE	MALE	FEMALE	MALE
	SHAVEN BRISTLES & CURVED WING	WILD-TYPE	SHAVEN BRISTLES & CURVED WING	Redo CROSS A – use F_1 males
F_1 Phenotypes			X	X
F_2 Phenotypes	# FEMALES	# MALES	**F_1:** # FEMALES	**F_1**: # MALES
WILD-TYPE				
SHAVEN BRISTLES				
CURVED WING				
SHAVEN BRISTLES & CURVED WING				

1 mark total for this table

****NOTE:** To do Cross B, you need to start over and redo Cross A **BUT** instead of mating the F_1 male with a F_1 female as in Cross A, you redesign the F_1 female to have a shaven bristles and curved wings (2 recessive traits).

In the Table for Cross B, when recording data for Cross B, you don't go to the F_2. The # of males and # of females you record is for the **F_1 of THAT** cross.

F_1 Phenotype Ratio for the TEST CROSS (Cross B) (**excluding sex)**:

0.25 ____ wild-type: ____ shaven bristles: ____ curved wings:____ shaven bristles and curved wings

Based on the results of the test cross on the previous page, are the F_1 wild type males heterozygous or homozygous dominant? **Explain** how you know.

0.5 ____

____

____

____

____

____

LINKAGE AND CROSSING-OVER-DIHYBRID CROSSES

Cross 5- DIHYBRID CROSS - AUTOSOMAL LINKED (trans)

PURPLE EYE x BLACK BODY

PARENTS PHENOTYPE	**CROSS A**		**CROSS B**	
	FEMALE	**MALE**	**FEMALE**	**MALE**
	WILD EYE &BLACK BODY	PURPLE EYE & WILD BODY	PURPLE EYE & WILD BODY	WILD EYE & BLACK BODY
F_1 Phenotypes				
TEST CROSS	PURPLE EYE & BLACK BODY	X **F_1**	**F_1** X	PURPLE EYE & BLACK BODY
	#s of:		#s of:	
WILD-TYPE				
PURPLE EYE				
BLACK BODY				
PURPLE EYE & BLACK BODY				

1.0 mark total for this table

<u>Determination of % Recombination for Cross 5</u> (Ignoring Sex):

	Cross A			Cross B	
PHENOTYPE	total #	%		total #	%
WILD-TYPE					
PURPLE EYE					
BLACK BODY					
PURPLE EYE & BLACK BODY					
Overall TOTAL					

0.5 total mark for this table

0.5 Recombination %: Cross A_________________ Cross B_________________

*In order to determine if the F_1 gamete types are Parentals or Recombinants in the table below, compare the F_1 gamete phenotype to the parents' phenotype in the Cross. Any F_1 gamete phenotypes not like the parental phenotypes are recombinants while those exactly like the parents are parental type gametes:

F_1 gamete types	State if Parental, Recombinant or N/A:	State if Parental, Recombinant or N/A:
	Cross A:	**Cross B:**
WILD-TYPE		
PURPLE EYE		
BLACK BODY		
PURPLE EYE & BLACK BODY		

0.5 mark total for this table

CROSS 6 – DIHYBRID CROSS – AUTOSOMAL LINKED (cis)
PURPLE EYE & BLACK BODY X WILD TYPE

PARENTS PHENOTYPE	**CROSS A**		**CROSS B**	
	FEMALE	**MALE**	**FEMALE**	**MALE**
	WILD TYPE	PURPLE EYE & BLACK BODY	PURPLE EYE & BLACK BODY	WILD TYPE
F_1 Phenotypes				
TEST CROSS	PURPLE EYE & BLACK BODY	X F_1	F_1 X	PURPLE EYE & BLACK BODY
	#s of:		#s of:	
WILD-TYPE				
PURPLE EYE				
BLACK BODY				
PURPLE EYE & BLACK BODY				

1.0 mark total for this table

<u>Determination of % Recombination for Cross 6</u> (Ignoring Sex):

PHENOTYPE	**Cross A** #	%		**Cross B** #	%
WILD-TYPE					
PURPLE EYE					
BLACK BODY					
PURPLE EYE & BLACK BODY					
TOTAL					

0.5 mark total for this table

0.5 Recombination %: Cross A_______________ Cross B_________________

F_1 gamete types	State if Parental, Recombinant or N/A:	State if Parental, Recombinant or N/A:
	Cross A:	**Cross B:**
WILD-TYPE		
PURPLE EYE		
BLACK BODY		
PURPLE EYE & BLACK BODY		

0.5 mark total for this table

<u>MAP DISTANCE</u>

The map distance =percent of crossing over = the percent recombination (1% recombination = 1 map unit or 1 centimorgan (cM)).

So, for example, if the map distance is 10 map units then we expect 10% of the F_1 gametes will be recombinant and hence there will be two classes of recombinant gametes of 5% each and the remaining 90% will be divided by the parental classes of gametes.

The frequency of crossing over depends on the distance between loci. The number of flies showing cross-over phenotypes can be used to determine how far apart the loci are.

1. Find the total number of flies showing the recombinant phenotypes**.** Then, calculate the map distance between purple eye and black body, **using Cross B's results** from **Cross 6** (the cross with the two variant phenotypes in **cis.)**

$$\frac{\text{Total recombinants}}{\text{Total}} \times 100 = \%\text{ cross over}$$

1% cross over = 1centimorgan (cM),

0.25 so the Map distance between purple eye and black body (i.e. from **pr to bl)** = ________ cM

0.25 **2**. From the gene map, what is the actual distance between the **pr** and the **bl** loci?_____cM

3. Use the computer program (Flylab) to do a **test cross** to calculate % recombinants with loci located different distances apart, in the **cis** configuration, for the 3 gene pairs below. For each gene pair, start by crossing a wild-type male with a female showing the 2 recessive traits listed and then select the F_1 female to use in the test cross with a redesigned male also showing the 2 recessive traits.

0.5

Gene pair	% crossover phenotypes	Map distance from **actual** gene map (Appendix B)
black body-purple eye (use results from previous page)		
black body- brown eyes		

0.5 How does the amount of crossing over change as the distance between the loci changes?

____

CROSS 7– TRIHYBRID CROSS – AUTOSOMAL LINKED (cis)
MINIATURE WING, YELLOW BODY& CROSSVEINLESS WINGS x WILD TYPE

Complete the following crosses in this table using the computer program:

	FEMALE	MALE
Parent Phenotype	WILD X	miniature, yellow, crossveinless
Test Cross	F_1 X	miniature, yellow, crossveinless

*****Before you fill in the table on the next page**, you will determine the correct order of the 3 loci using the results from the test cross above:

<u>HOW TO DETERMINE THE CORRECT ORDER OF LOCI :</u>

Example:

A cross is made between homozygous wild-type female *Drosophila* (**$a^+a^+b^+b^+ c^+c^+$**) and triple-mutant males (**aa bb cc**) (the order here is arbitrary). The F_1 (**$a^+a\ b^+b\ c^+c$**) females are test crossed back to the triple-mutant males and the F_2 phenotypic ratios are as follows:

"a^+ b c"	18
"a b^+ c"	112
"a b c"	308
"a^+ b^+ c"	66
"a b c^+"	59
"a^+ b^+ c^+"	321
"a^+ b c^+"	102
"a b^+ c^+"	15
	1000

The gene order can be determined by examination of the relative frequencies of the F_2 phenotypes.

a. Because linked loci tend to stay together, the non-crossover (NCO) or parental phenotypes should be most frequent (and equal in number). In this case

a$^+$b$^+$c$^+$ (321) and **a b c** (308)

b. Because simultaneous crossovers between the outside and middle loci are unlikely, the double-crossover (DCO) genotypes should be the least frequent. We observe

a$^+$ b c (18) and **a b$^+$c$^+$** (15)

c. Then, to determine the physical order of loci, compare the parental and double-crossover phenotypes. ***The marker that appears to "switch places" is in the middle*** [technically, this marker is said to be "out of phase"]. Here, the **a$^+$b$^+$c$^+$** NCO and **a b$^+$c$^+$** DCO phenotypes indicate that the **a** locus falls between the **b** and **c** loci. The correct order of the loci is **b a c**. [Note that this order is equivalent to **c a b**, and that the order of the outside markers is arbitrary].

1. Based on the results for Cross 7, what is the correct order for the 3 loci in that Cross (miniature wing, yellow body and crossveinless wings)?

0.5____

____

____

<u>Now that you know the correct order of the 3 loci, fill in the table below</u>:

CROSS 7 – TRIHYBRID CROSS – AUTOSOMAL LINKED (cis)
MINIATURE WINGS, YELLOW BODY & CROSSVEINLESS WINGS x WILD TYPE

	FEMALE	**MALE**	
Parent Phenotype	WILD X	miniature, yellow, crossveinless	
Parent Genotype			
Test Cross	F_1 X	miniature, yellow, crossveinless	
F_1 gamete types (from the testcross) (Ignore sex)	F_1 gamete Genotypes (from the testcross)	Number	State if Parental, single or double recombinant
wild			
miniature wings			
crossveinless wings			
miniature & crossveinless			
yellow body			
miniature & yellow body			
crossveinless & yellow body			
miniature, yellow, crossveinless			

2.5 marks total for this table

<u>MAKING A GENE MAP</u>

It is possible to develop a gene map, showing the order of the loci and the distance between them by observing the number of offspring showing recombinant phenotypes. The first step is to determine the correct order of the loci, which has already been completed.

The next step in the development of a gene map is to calculate the map distance between the gene pairs. For a trihybrid cross, the recombination fraction (and therefore the map distance) for any of the **gene pairs** is calculated as:

RF = <u># in the SCO phenotypes + # in the DCO phenotypes</u> X 100
Total # of offspring

Note: SCO = Single Crossovers and DCO =Double Crossovers

*For the **total length** you can use the formula above but add **all** the SCO's + **2** (DCO) together over the total # X 100.

2. Calculate the recombination fraction (%RF) for the 3 gene pairs from the trihybrid cross (Cross 7). Put the gene pairs and their recombination fraction in the table below. **Show your calculations in the space provided.**

Gene Pair	% RF

1.5

3. Based on the recombination fractions **you calculated above**, draw a gene map of the 3 genes and **also** draw the **actual** gene map for the 3 genes using the *Drosophila* gene map in Appendix B. (i.e. draw 2 maps).

0.5 **Gene map based on your calculations:**

0.5 **Actual gene map:**