Using modified Mendelian principles to determine the genotypic ratios of lethal mutations, epistasis and sex-linked mutations

BIO2133 Section B2

Demonstrators:

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Introduction

Part 1. Lethal mutations

Some mutations have the capability to interrupt processes that are essential for the organism to survive (Klug *et al,* 2012). They can significantly reduce longevity of that organism and may result in death in the homozygous state (Drouin *et al,* 2014).

Monohybrid cross L1

What is the pattern of inheritance in a monohybrid cross such that the mutation for star eyes (ST) is lethal when the fruit fly is homozygous? We hypothesize that when a female with star eyes is crossed with a homozygous wild type male, the resultant F_1 generation will yield an even split for the mutant and wild type (+) phenotypes – but the F2 generation will not have the same ratio as a monohybrid cross. The ratio we should see would be a 2 ST: 1 +, as the homozygous individuals for the star-eyed allele will not survive. We assume that the female is heterozygous for the lethal mutation, and that the gene is not sex-linked.

Predictions:

Parental cross: ♀ ST x ♂ + (♀ ST+ x ♂ ++)

♂ ♀	+
ST	ST+
+	++

Predicted phenotypic ratio of F_1: 1 ST: 1 + → (50%) wild (+) and (50%) mutant (ST)

F_1 cross: ♀ ST x ♂ ST (♀ ST+ x ♂ ST+)

♂ ♀	ST	+
ST	STST	ST+
+	ST+	++

Predicted phenotype of F_2: 2 ST: 1 + → (66.7%) mutant (ST) and (33.3%) wild (+), ¼ would not be observed; therefore the ratio was calculated out of 3.

Dihybrid cross L2

What is the pattern of inheritance of a dihybrid cross in which two different mutations are lethal in the homozygous condition? We hypothesize that when a female fruit fly with the lethal mutation of star eyes is crossed with a male that has a different lethal mutation of stubble bristles (SB), the F_1 generation will have a 1:1:1:1 ratio. We also hypothesize that the F_2 generation will show a 4:2:2:1 ratio as the possible gametes with the homozygous condition would have died. We assume that both parents are heterozygous for the mutant lethal alleles, and that these particular genes do not have an influence on one another.

Predictions

Parental cross: ♀ ST x ♂ SB (♀ ST+,++ x ♂ ++, SB+)

♂ ♀	+, +	+, SB
ST, +	ST+, ++	ST+, SB+
+, +	++, ++	++, SB+

Predicted phenotypic ratio of F_1: 1 ST: 1 SB: 1+: 1 ST,SB → (25%) mutant (ST), (25%) mutant (SB), (25%) double mutant (ST,SB) and (25%) wild (+).

F_1 cross: ♀ ST, SB x ♂ ST, SB (♀ ST+, SB+ x ♂ ST+, SB+)

♂ ♀	ST, +	ST, SB	+, +	+, SB
ST, +	STST, ++	STST, SB+	ST+, ++	ST+, SB+
+, SB	ST+, SB+	ST+, SBSB	++, SB+	++, SBSB
ST, SB	STST, SB+	STST, SBSB	ST+, SB+	ST+, SBSB
+, +	ST+, ++	ST+, SB+	++, ++	++, SB+

Predicted phenotypic of F_2: 4 ST, SB: 2 ST: 2 SB: 1 + → (44.4%) double mutant (ST,SB), (22.2%) mutant (ST), (22.2%) mutant (SB) and (11.1%) wild (+). 7/16 would not be observed; therefore this ratio was calculated out of 9.

Pearson Flylab was used to simulate these crosses; a female fly was selected to have star eyes (ST) and was bred with a male that was selected to have stubble bristles. We then chose 10,000 offspring to obtain the results for the F_1 and F_2 generations. A chi-squared analysis was conducted to compare our results to our observations.

Part 2. Epistasis

An interaction between genes such that their relationship is antagonistic – one gene can mask or interfere with the expression of another. Alleles that are being masked or silenced are known as hypostatic (OpenStax, 2013) while the genes that are doing the masking and are responsible for the phenotype are known as epistatic (Drouin *et al,* 2014).

What is the pattern of inheritance in a dihybrid cross in which the mutation of not having eyes (EY) on one allele, will hide the expression of the mutation for brown eyes (BW) on another allele in their homozygous condition (or vice versa)? We hypothesize that the F1 generation will yield all wild type offspring, but the F2 generation will have a 9:4:3 ratio depending on which mutation is epistatic and which is hypostatic. We must assume that the parents are heterozygous and that the wild type allele is dominant.

Predictions:

Parental cross: ♀ EY x ♂ BW (♀ EY+ x ♂ BW+)

♂ ♀	BW+
EY+	EY+, BW+

Predicted phenotypic ratio of F_1: 100% + (wild)

F_1 cross: ♀ + x ♂ + (♀ EY+,BW+ x ♂ EY+,BW+)

♂ ♀	EY, +	EY, BW	+, BW	+, +
EY, +	EYEY, ++	EYEY, BW+	EY+, BW+	EY+,++
EY, BW	EYEY, BW+	EYEY, BWBW	EY+, BWBW	EY+, BW+
+, BW	EY+, BW+	EYEY, BWBW	++, BWBW	++, BW+
+, +	EY+, ++	EY+, BW +	++, BW+	++, ++

Predicted phenotype of F_2: 9 +: 3 BW: 4 EY **or** 9 +: 3 EY: 4 BW
→ (60%) wild (+), (20%) mutant (BW), (30%) mutant (EY), this ratio suggests that the EY mutation is epistatic.
→ (60%) wild (+), (20%) mutant (EY), (30%) mutant (BW), this ratio suggests that the BW mutation is epistatic.

Part 3. Sex-linked mutations

A gene that is located on one of the sex chromosomes, X or Y is said to be a sex-linked gene. Sex-linked mutations occur on both of the sex chromosomes, this can affect the mode of inheritance for males and females differently, depending on which chromosome the mutation is found (Drouin *et al,* 2014). X linkage refers to genes that are located on the X chromosome (Klug *et al,* 2012) and will be the focus of this lab.

What is the pattern of inheritance in a monohybrid cross such that the mutation for having a sable body (S) is x-linked? We hypothesize that if we cross a female with a sable body with a wild type male, the F1 generation will yield a 1:1 ratio, but the female offspring will be wild type and the males will have a sable body. In this case, the F2 generation should also show a 1:1:1:1 ratio for each different combination of wild type and mutant (sable) offspring for each sex. We also hypothesize that if we conduct the reciprocal (in which the male has the sable body and is crossed with a wild type female), the ratio will be different. The F1 generation should show all wild type offspring for both sexes, and the F2 generation should have a 2:1:1 ratio, as the females should all be wild but the males could be either. We assume that the parental female is homozygous in both cases, and that the wild type allele is dominant.

Predictions:

Parental cross: ♀ S x ♂ + (♀X^S X^S x ♂ X^+Y)

♂ ♀	X^+	Y
X^S	X^S X^+	X^S Y

Predicted phenotypic ratio of F_1: (50%) ♀ + (wild), (50%) ♂ mutant (S).

F_1 cross: ♀ + x ♂ S (♀ X^S X^+ x ♂ X^S Y)

♂ ♀	X^S	Y
X^S	X^S X^S	X^S Y
X^+	X^+ X^S	X^+ Y

Predicted phenotype of F_2: 1 ♀ + : 1 ♀ S : 1 ♂ + : 1 ♂ S → (25%) ♀ wild (+), (25%) ♀ mutant (S), (25%) ♂ wild (+), (25%) ♂ mutant (S).

Reciprocal:

Parental cross: ♀ + x ♂ S (♀X^+ X^+ x ♂ X^SY)

♂ ♀	X^S	Y
X^+	X^+ X^S	X^+ Y

Predicted phenotypic ratio of F_1: (50%) ♀ + (wild), (50%) ♂ wild (+).

F_1 cross: ♀ + x ♂ + (♀ X^S X^+ x ♂ X^+ Y)

♂ ♀	X^+	Y
X^+	X^+ X^+	X^+ Y
X^S	X^S X^+	X^S Y

Predicted phenotype of F_2: 2 ♀ + : 1 ♂ + : 1 ♂ S → (50%) ♀ wild (+), (25%) ♂ wild (+), (25%) ♂ mutant (S).

Table 1. The results of the chi-squared analysis for a monohybrid cross with a lethal mutation, as well as a dihybrid cross with two lethal mutations. Team data is shown, with sample size (n) = 10,000.

Cross type	**Cross** (♀ x ♂)	**Predicted ratio**	**Observed ratio**	**Total X^2**	**DF**	**p**	***H_0***
Monohybrid L1	P (ST) x P (+)[1]	100%	100%	-	-	-	-
	F_1 (ST) x F_1 (ST)	2.00 (ST): 1.00 (+)	1.96 (ST): 1.00 (+)	0.84	1	0.36	Accepted
Dihybrid L2	P (ST) x P (SB)[1]	1.00 (+): 1.00 (SB): 1.00 (ST): 1.00 (SB,ST)	1.03 (+): 1.00 (SB): 1.02 (ST): 1.00 (SB,ST)	-	-	-	-
	F_1 (SB,ST) x F_1 (SB,ST)	1.00 (+): 2.00 (SB): 2.00 (ST): 4.00 (SB,ST)	1.00 (+): 2.01 (SB): 1.93 (ST): 4.02 (SB,ST)	2.49	3	0.48	Accepted

[1] (ST) refers to having the mutation of star eyes, (SB) refers to stubble bristles, and (+) refers to wild type traits.

Table 2. The results for the chi-squared analysis of two autosomal mutations in which the gene interaction is due to epistasis. Team data is shown, with sample size (n) = 10,000.

Cross type	**Cross** (♀ x ♂)	**Predicted ratio**	**Observed ratio**	**Total X^2**	**DF**	**p**	***H_0***
Dihybrid Epistasis	P (EY) x P (BW) [1]	100%	100%	-	-	-	-
	F_1 (+) x F_1 (+)	9.00 (+): 3.00 (BW): 4.00 (EY)	2.89 (+): 1.00 (BW): 1.28 (EY) [2]	0.11	2	0.95	Accepted

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[1] (EY) refers to the mutation of not having any eyes, (BW) refers to brown eyes and (+) refers to wild type eyes.

[2] The observed ratio differs from the predicted ratio in numbers, but the scale (although smaller) is the same.

Table 3. The results of the chi-squared analysis for the sex-linked mutation carrying the allele for a sable body, as well as its reciprocal. Team data is shown, with sample size (n) = 10,000.

Cross type	Cross (♀ x ♂)	Predicted ratio	Observed ratio	Total X^2	DF	p	*H_0*
Sex linked (S1)	P (S) x P (+)[1]	1.00 ♀ (+): 1.00 ♂ (S)	1.00 ♀ (+): 1.02 ♂ (S)	-	-	-	-
	F_1 (+) x F_1 (S)	1.00 ♀ (+): 1.00 ♀ (S): 1.00 ♂ (+): 1.00 ♂ (S)	1.02 ♀ (+): 1.05 ♀ (S): 1.02 ♂ (+): 1.00 ♂ (S)	2.51	3	0.47	Accepted
Sex linked reciprocal	P (+) x P (S)	1.00 ♀ (+): 1.00 ♂ (+)	1.00 ♀ (+): 1.02 ♂ (S)	-	-	-	-
	F_1 (+) x F_1 (+)	2.00 ♀ (+): 1.00 ♂ (S): 1.00 ♂ (+)	1.93 ♀ (+): 1.01 ♂ (S): 1.00 ♂ (+)	4.42	2	0.11	Accepted

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[1] (S) refers to having a sable body, while (+) refers to a wild type body

Discussion

Part 1. Lethal mutations

The results that we obtained from Flylab for the mono- and dihybrid crosses match what we originally predicted. The observed ratios in both cases, as well as for both generations, are very similar to our predicted ratios and the p values are relatively high (0.36 and 0.48 respectively). However, these ratios are different than what we would see if we were to conduct a normal mono- or dihybrid cross. Because we are dealing with lethal alleles, there are combinations that will result in offspring that will not be observed (homozygous individuals). In the normal mono- and dihybrid crosses that we experimented on in the previous lab, we used true-breeding parents. This could not be the case in this lab, as these individuals do not exist. This changes our F_1 ratio from 100% wild type to a 50/50% wild/mutant in a monohybrid cross, and a 1:1:1:1 ratio for a dihybrid cross (for each different combination of the two lethal alleles and the wild type). The F_2 generation will also be different. We would observe a 3:1 ratio for a normal monohybrid cross of wild vs mutant, but with a lethal allele present it would be a 2:1 ratio. The dihybrid cross changes from 9:3:3:1 to a 4:2:2:1. These changes represent the fact that with these lethal alleles present, homozygous individuals would not live. We also observe less wild type individuals, as it was not determined to be dominant.

Part 2. Epistasis

Our results obtained for epistasis closely follow what we hypothesized. Our observed ratios are very similar to the predicted ratios, and the p value was very high (0.95) making it very likely to occur in nature. FlyLab also allowed us to determine exactly which mutation was being masked. In this case, the mutation of not having eyes

(EY) was epistatic, and the mutation for brown eyes (BW) was hypostatic. The F_1 generation showed all wild type offspring, as both parents were heterozygous for the mutant traits. The ratio for the F_2 generation was 9 wild type, 3 brown eyes, and 4 eyeless. A classic dihybrid ratio as we have seen previously, is 9:3:3:1. This differs with epistasis because the alleles in question have an influence one another's expression (we assumed in the classic dihybrid cross, that the alleles were independent and did not effect the expression of the other). Since the wild type was dominant, the majority of offspring showed this phenotype; but when the trait for (EY) was present in its homozygous state it masked the expression of having either brown or wild type eyes. Thus alleles that are epistatic will change what we would observe in a normal dihybrid cross.

Part 3. Sex linked mutations

Our results for the sex linked mutations closely reflect what we have predicted. The S1 parental cross gave us a 1:1 ratio for our F_1 generation, the females were all wild type and the males all had sable bodies. The observed ratio for the F_1 cross was also very similar to our predictions, having a p value was 0.47. In the latter case, we crossed a female that was phenotypically wild type (but had the allele for a sable body) with a sable male. This particular cross allows for an even distribution of wild type and sable body expression for both females and males with a 1:1:1:1 ratio. When we conducted the reciprocal, the ratio changed. We predicted that when crossing a wild type female with a sable male, the F_1 generation would yield all wild type offspring. This was confirmed with FlyLab. The F_2 generation also showed no real difference between the ratios that we predicted and observed, having a p value of 0.11. Since the female had both alleles but the male only had the wild type allele, this gave us a ratio of having all wild type females but the males were both wild type and sable. These ratios differ from monohybrid crosses on autosomal chromosomes because the F_1 cross (even if the traits for the parents are switched for its reciprocal) will always yield a 3:1 ratio for wild type vs. mutant phenotypes. During sex linked mutations, the male and female contribute differently. The female has two X chromosomes and therefore has the possibility to carry two different alleles on each one (she can be a carrier). The male only carries one X chromosome, so it can contribute only one possible allele along with the Y chromosome.

References:

Drouin, G, Heinermann, P. and Perin. 2014. Bio2133 – Genetics Lab Manual. Department of Biology, University of Ottawa. P44-48

Klug, William S., Michael R. Cummings, Charlotte A. Spencer, and Michael A. Palladino. "4 Extensions of Mendelian Genetics." *Concepts of genetics, tenth edition*. San Francisco, Calif.: Benjamin Cummings ;, 2012. P77-80, 88-91. Print.

OpenStax College, *Biology.* OpenStax College. 30 May 2013. P344-345. http://cnx.org/content/col11448/latest/.

Appendix

Chi Square table for the F2 generation of the dihybrid (L2) cross

Phenotype	Star eyes, Wild type body	Star eyes, Stubble bristles	Wild type eyes, Wild type body	Wild type eyes, Stubble bristles	Total
Observed value	2123.00	4414.00	1099.00	2209.00	9845.00
Expected value	2187.77	4375.55	1093.88	2187.77	9845.00
Obs-Exp	-54.80	38.50	5.10	21.20	-
(Obs-Exp)2	2999.80	1478.40	26.20	450.70	-
(Obs-Exp)2/Exp	1.92	0.30	0.02	0.20	2.40

Chi Square table for Sex-linked cross (S1)

Phenotype	Female wild type	Male wild type	Female sable body	Male sable body	Total
Observed value	2487.00	2553.00	2488.00	2442.00	9970.00
Expected value	2492.50	2492.50	2492.50	2492.50	9970.00
Obs-Exp	-5.50	60.50	-4.50	-50.50	-
(Obs-Exp)2	30.30	3660.30	20.30	2550.30	-
(Obs-Exp)2/Exp	0.01	1.47	0.01	1.02	2.50

Raw Results from Flylab for Parts 1, 2 and 3

Lethal mutation (L1)

```
Results of Cross #1
Ignoring Sex
Parents
(Female: ST) x (Male: +)
Offspring
Phenotype           Number   Proportion   Ratio
+                     4986      0.5017        1.007
ST                    4953      0.4983        1.000
Total                 9939
----------------------------------------------------------------------
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```
Results of Cross #5
Ignoring Sex
Parents
(Female: ST) x (Male: ST)
Offspring
Phenotype               Number   Proportion    Ratio
+                          3382      0.3377          1.000
ST                         6634      0.6623          1.962
 Total                    10016
------------------------------------------------------------------------
Chi Square Hypothesis Using Cross #5
Ignoring Sex
Phenotype      Observed   Hypothesis   Expected        Chi-Square Term
+                  3382          1.0000          3338.6667       0.5624
ST                  6634          2.0000          6677.3333       0.2812
Total              10016        3.0000         10016.0000     0.8437

Chi-Squared Test Statistic = 0.8437
Degrees of Freedom = 1
Level of Significance = 0.3584
Recommendation: Do not reject your hypothesis
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Lethal mutation (L2)

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Results of Cross #2
Ignoring Sex
Parents
 (Female: SB;ST) x (Male: SB;ST)
Offspring
Phenotype               Number   Proportion    Ratio
+                          1099      0.1116         1.000
SB                          2209     0.2244          2.010
ST                          2123     0.2156          1.932
SB;ST                       4414     0.4483          4.016
 Total                     9845
------------------------------------------------------------------------
Results of Cross #3
Ignoring Sex
Parents
 (Female: ST) x (Male: SB)
Offspring
Phenotype               Number   Proportion    Ratio
+                           2523     0.2534         1.026
SB                          2459     0.2470          1.000
ST                          2509     0.2520          1.020
SB;ST                       2465     0.2476          1.002
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 Total                        9956
-------------------------------------------------------------------
Chi Square Hypothesis Using Cross #2
Ignoring Sex
Phenotype     Observed   Hypothesis   Expected      Chi-Square Term
+                 1099        1.0000      1093.8889      0.0239
SB                2209        2.0000      2187.7778      0.2059
ST                2123        2.0000      2187.7778      1.9180
SB;ST             4414        4.0000      4375.5556      0.3378
Total            9845        9.0000      9845.0000      2.4855

Chi-Squared Test Statistic = 2.4855
Degrees of Freedom = 3
Level of Significance = 0.4779
Recommendation: Do not reject your hypothesis
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Epistasis

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Results of Cross #4
Ignoring Sex
Parents
(Female: EY) x (Male: BW)
Offspring
Phenotype               Number   Proportion    Ratio
+                           9966     1.0000           1.000
 Total                    9966
----------------------------------------------------------------------
Results of Cross #5
Ignoring Sex
Parents
(Female: +) x (Male: +)
Offspring
Phenotype               Number   Proportion    Ratio
+                           5660     0.5588           2.889
BW                         1959     0.1934           1.000
EY                          2510     0.2478           1.281
Total                     10129
-------------------------------------------------------------------
 Chi Square Hypothesis Using Cross #6
Ignoring Sex
Phenotype     Observed   Hypothesis   Expected      Chi-Square Term
+                     5557       9.0000       5573.2500      0.0474
BW                   1863       3.0000      1857.7500      0.0148
EY                    2488       4.0000       2477.0000      0.0488
Total               9908     16.0000      9908.0000      0.1111

Chi-Squared Test Statistic = 0.1111
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Degrees of Freedom = 2
Level of Significance = 0.9460
Recommendation: Do not reject your hypothesis
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Sex-linked mutations (S1)

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Results of Cross #12
Parents
(Female: S) x (Male: +)
Offspring
Phenotype            Number   Proportion   Ratio
Female: +             4934      0.4956      1.000
Male: S               5021      0.5044      1.018
 Total                9955
-----------------------------------------------------------------------
Results of Cross #13
Parents
 (Female: +) x (Male: S)
Offspring
Phenotype            Number   Proportion   Ratio
Female: +             2487      0.2494      1.018
Male: +               2553      0.2561      1.045
Female: S             2488      0.2495      1.019
Male: S               2442      0.2449      1.000
 Total                9970
-----------------------------------------------------------------------
Chi Square Hypothesis Using Cross #13
Phenotype      Observed   Hypothesis   Expected       Chi-Square Term
Female: +        2487       1.0000     2492.5000      0.0121
Male: +          2553       1.0000     2492.5000      1.4685
Female: S        2488       1.0000     2492.5000      0.0081
Male: S          2442       1.0000     2492.5000      1.0232
Total            9970       4.0000     9970.0000      2.5119

Chi-Squared Test Statistic = 2.5119
Degrees of Freedom = 3
Level of Significance = 0.4731
Recommendation: Do not reject your hypothesis
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Sex linked mutations reciprocal (S1)

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Results of Cross #14
Parents
 (Female: +) x (Male: S)
Offspring
Phenotype            Number   Proportion   Ratio
Female: +             4949      0.4943      1.000
Male: +               5063      0.5057      1.023
```

```
 Total                          10012
-----------------------------------------------------------------------
Results of Cross #15
Parents
(Female: +) x (Male: +)
Offspring
Phenotype               Number   Proportion    Ratio
Female: +                 4924        0.4898         1.933
Male: +                     2582        0.2568         1.014
Male: S                      2547        0.2534         1.000
 Total                       10053
-----------------------------------------------------------------------
Chi Square Hypothesis Using Cross #15
Phenotype            Observed   Hypothesis     Expected          Chi-Square Term
Female: +               4924           2.0000            5026.5000       2.0902
Male: +                   2582           1.0000            2513.2500       1.8807
Male: S                    2547           1.0000            2513.2500       0.4532
Total                     10053          4.0000            10053.0000     4.4241

Chi-Squared Test Statistic = 4.4241
Degrees of Freedom = 2
Level of Significance = 0.1095
Recommendation: Do not reject your hypothesis
-----------------------------------------------------------------------
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