

Using Mendel's laws of inheritance to determine the phenotypes of fruit fly offspring in mono-, di- and tri-hybrid crosses, as well as using a test cross to discover an unknown genotype.

BIO2133 Section B2

Demonstrators:

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Introduction

Part 1. Mono-, di and tri-hybrid crosses

An autosomal mutation is a mutation that occurs in the autosomal chromosomes, thus it would not be a sex-linked mutation (Klug *et al*, 2012). They can be either inherited dominant autosomal mutations - in which the first generation (F_1) will express the phenotype, or they can be autosomal recessive mutations in which it could remain unseen for many generations – resulting in a phenotype only when there is a homologous condition present (Klug *et al*, 2012).

Monohybrid cross

What is the pattern of inheritance in a monohybrid cross between a female fruit fly with wild type (+) eyes and a male with the mutation of having brown eyes (BW)? We hypothesize that a cross between these two parents with different alleles, will result in offspring that follow Mendel's first law (law of segregation): the gamete will randomly receive its own pair of alleles, but the dominant allele will determine the expression of that trait (Klug *et al*, 2012). In this circumstance the F_1 generation will have wild type eyes and be heterozygous, but the second generation (F_2) should have a 3:1 ratio of wild type and brown eyes. In order to model this experiment we have made some assumptions; the wild type allele (+) is dominant over the mutant allele (BW), and both parents are homozygous for their given trait (Drouin *et al*, 2014).

Predictions:

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

| | | |
|---|---|-----|
| | ♂ | BW |
| ♀ | | |
| + | | +BW |

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

F_1 cross: ♀ + x ♂ + (♀ BW+ x ♂ BW+)

| | | | |
|----|---|------|-----|
| | ♂ | BW | + |
| ♀ | | | |
| BW | | BWBW | BW+ |
| + | | BW+ | ++ |

Predicted phenotype of F_2 : 3 +: 1 BW → (75%) wild (+) and (25%) mutants (BW)

Dihybrid Cross

What is the pattern of inheritance of a dihybrid cross between a female fruit fly that has brown eyes and a wild type body, with a male that has wild type eyes and a ebony body (E)? We hypothesize that the offspring will follow Mendel's first law (see above), as well as the second law (law of independent assortment): separate genes for different traits will be passed on in an independent fashion (Klug *et al*, 2012). Thus, we should see heterozygote offspring in the F_1 generation, but the F_2 generation should follow the 9:3:3:1 ratio of wild type to mutant traits. It is assumed that these two genes are not linked, and that they do not have an influence on the others expression (Drouin *et al*, 2014). This cross was simulated by using the Pearson Flylab computer program. The female and male fruit flies were selected to display different phenotypes (brown eyes and ebony body), while wild type phenotypes were left as default. They were then bred to produce the F_1 generation. Mating two offspring from the F_1 generation produced the F_2 generation. In both the parental and F_1 crosses, the number of offspring to produce was 10,000. A chi-squared analysis was used to test the predicted phenotypic ratios by obtaining the observed ratios, as well as there X^2 and p-value.

Predictions:

Parental cross: ♀ BW x ♂ E (♀ BWBW, ++ x ♂ ++, EE)

| | | |
|---|---|------|
| | ♂ | +, E |
| ♀ | | |

| | |
|-------|---------|
| BW, + | BW+, E+ |
|-------|---------|

Predicted phenotypic ratio of F₁: 100% + (wild)

F₁ cross: ♀ + x ♂ + (♀BW+, E+ x ♂ BW+, E+)

| | | | | |
|-------|----------|----------|---------|---------|
| ♀ \ ♂ | BW, E | BW, + | +, E | +, + |
| BW, E | BWBW, EE | BWBW, E+ | BW+, EE | BW+, E+ |
| BW, + | BWBW, E+ | BWBW, ++ | BW+, E+ | BW+, ++ |
| +, E | BW+, EE | BW+, E+ | ++, EE | ++, E+ |
| +, + | BW+, E+ | BW+, ++ | ++, E+ | ++, ++ |

Predicted phenotypic of F₂: 9 + : 3 BW : 3 E : 1 BW,E → (56%) wild (+), (19%) mutant BW, (19%) mutant (E), and (6%) double mutant (BW,E).

Trihybrid cross

What is the pattern of inheritance in a trihybrid cross between a female fruit fly that has wild type eyes, bristles and body – with a mutant male that has brown eyes, shaven bristles (SV) and an ebony body? We hypothesize that each trait (gene) will adhere to both of Mendel's laws of inheritance, the F₁ generation should be heterozygous for all traits and the F₂ generation should follow the trihybrid ratio of 27:9:9:9:3:3:3:1 (Klug *et al*, 2012). To determine an appropriate model, we must assume that the genes are all on separate chromosomes, and that any one gene will not exert an influence on the expression of any of the others (Drouin *et al*, 2014).

Predictions:

Parental cross: ♀ + x ♂ SV, BW, E (♀ ++, ++, ++ x ♂ SVSV, BWBW, EE)

| | |
|---------|--------------|
| ♀ \ ♂ | SV, BW, E |
| +, +, + | SV+, BW+, E+ |

Predicted phenotypic ratio of F₁: 100% + (wild)

F₁ cross: ♀ + x ♂ + (♀ SV+, BW+, E+ x ♂ SV+, BW+, E+)

| ♀ \ ♂ | SV, +, + | SV, +, E | SV, BW, + | SV, BW, E | +, +, + | +, +, E | +, BW, + | +, BW, E |
|-----------|---------------|---------------|----------------|----------------|--------------|---------------|---------------|---------------|
| SV, +, + | SVSV, ++, ++ | SVSV, ++, E+ | SVSV, BW+, ++ | SVSV, BW+, E+ | SV+, ++, ++ | SV+, ++, E+ | SV+, BW+, ++ | SV+, BW+, E+ |
| SV, +, E | SVSV, ++, E+ | SVSV, ++, EE | SVSV, BW+, E+ | SVSV, BW+, EE | SV+, ++, E+ | SV+, ++, EE | SV+, BW+, E+ | SV+, BW+, EE |
| SV, BW, + | SVSV, BW+, ++ | SVSV, BW+, E+ | SVSV, BWBW, ++ | SVSV, BWBW, E+ | SV+, BW+, ++ | SVSV, BW+, E+ | SV+, BWBW, ++ | SV+, BWBW, E+ |
| SV, BW, E | SVSV, BW+, E+ | SVSV, BW+, EE | SVSV, BWBW, E+ | SVSV, BWBW, EE | SV+, BW+, E+ | SV+, BW+, EE | SV+, BWVW, E+ | SV+, BWBW, EE |
| +, +, + | SV+, ++, ++ | SV+, ++, E+ | SV+, BW+, ++ | SV+, BW+, E+ | ++, ++, ++ | ++, ++, E+ | ++, BW+, ++ | ++, BW+, E+ |
| +, +, E | SV+, ++, E+ | SV+, ++, EE | SV+, BW+, E+ | SV+, BW+, EE | ++, ++, E+ | ++, ++, EE | ++, BW+, E+ | ++, BW+, EE |
| +, BW, + | SV+, BW+, ++ | SV+, BW+, E+ | SV+, BWBW, ++ | SV+, BWBW, E+ | ++, BW+, ++ | ++, BW+, E+ | ++, BWBW, ++ | ++, BWBW, E+ |
| +, BW, E | SV+, BW+, E+ | SV+, BW+, EE | SV+, BWBW, E+ | SV+, BWBW, EE | ++, BW+, E+ | ++, BW+, EE | ++, BWBW, E+ | ++, BWBW, EE |

Predicted phenotypic ratio of F₂: 27+: 9 SV: 9 BW: 9 E: 3 SV, BW: 3 SV, E: 3 BW, E: 1 SV, BW, E → (42%) wild +, (14%) mutant SV, (14%) mutant BW, (14%) mutant E, (4.7%) double mutant (SV, BW), (4.7%) double mutant (SV, E), (4.7%) double mutant (BW, E), (1.6%) triple mutant (SV, BW, E).

Part 2. Testcross

A test cross is a tool that can be used to determine an unknown genotype in an organism displaying a dominant phenotype, it is carried out by crossing this organism with its opposite sex that has a known homozygous, recessive genotype (Miko, 2008). Thus the ratio of the offspring will show whether it is homozygous or heterozygous dominant.

What is the genotype of the F₁ generation if they display the dominant phenotype?

We hypothesize that if we cross a female that has an unknown but dominant phenotype, with a male that is homozygous recessive for both traits – then the ratio of the F₂ generation will determine the genotype of F₁. The female displays apterous wings (AP) and wild type eyes while the male has wild type wings and sepia eyes (SE). We assume that each gene is on a separate chromosome, and that the expression of one gene will not exert an effect on the other (Drouin *et al*, 2014).

Predictions:

Parental cross: ♀ AP x ♂ SE (♀ unknown genotype x ♂ unknown genotype)

| | |
|-------|------|
| ♀ \ ♂ | SE |
| AP | +, + |

Predicted genotypic ratio: unknown genotype

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

The F_2 generation can have two possible outcomes:

F_1 cross: ♀ + x ♂ AP, SE (♀ AP+, SE+ x ♂ APAP, SESE)

| | |
|--------|------------|
| ♀ \ ♂ | AP, SE |
| AP, SE | APAP, SESE |
| AP, + | APAP, SE+ |
| +, SE | AP+, SESE |
| +, + | AP+, AP+ |

Predicted genotypic ratio: *See table above*

Predicted phenotypic ratio of F_2 : 25% each 1:1:1:1

F_1 cross: ♀ + x ♂ + (♀ ++, ++ x ♂ APAP, SESE)

| | |
|-------|----------|
| ♀ \ ♂ | AP, SE |
| +, + | AP+, SE+ |

Predicted genotypic ratio: 100% heterozygous

Predicted phenotypic ratio of F_2 : 100% + (wild)

This experiment was conducted using Flylab, we selected a parental cross between a female fruit fly with apterous wings and a male with sepia eyes. The ratio was determined and recorded. An F_1 female was to mate with a homozygous recessive male (AP,SE) to generate the ratio for the F_2 generation. The number of offspring was selected to be 10,000. A chi-square test of fitness was conducted using this software to determine

the observed ratio in order to determine the genotype of the F₁ generation.

Results

Table 1 The chi-squared analysis of a mono-, di and tri-hybrid cross.

| Cross type | Cross | Predicted Ratio ¹ | Observed ratio | Total X ² | DF ² | p | H ₀ |
|------------|--|--|---|----------------------|-----------------|------|----------------|
| Monohybrid | P(+) x P(BW) | 100% | 100% | - | - | - | - |
| | F ₁ (+) x F ₁ (+) | 3.00(+): 1.00(BW) | 3.04(+): 1.00(BW) | 0.45 | 1 | 0.50 | Accepted |
| Dihybrid | P(BW,+) x P(+, E) | 100% | 100% | - | - | - | - |
| | F ₁ (+, +) x F ₁ (+, +) | 9.00(+): 3.00(BW): 3.00(E): 1.00(BW,E) | 8.90(+): 2.93(BW): 3.06(E): 1.00(BW,E) | 1.82 | 3 | 0.61 | Accepted |
| Trihybrid | P(+,+,+) x P(SV,BW,E) | 100% | 100% | - | - | - | - |
| | F ₁ (+,+,+) x F ₁ (+,+,+) | 27.00(+): 9.00(SV): 9.00(BW): 9.00(E): 3.00(SV,BW): 3.00(SV,E): 3.00(BW,E): 1.00(SV,BW,E) | 28.91(+): 9.60(SV): 9.48(BW): 9.22(E) 3.07(SV,BW): 3.08(SV,E): 3.12(BW,E): 1.00(SV,BW,E) | 3.54 | 7 | 0.83 | Accepted |

¹ The parental cross (P) was left as a percentage rather than the ratio

² Degrees of freedom (DF) was measured as the number of phenotypes - 1

Table 2 The chi-squared analysis of the testcross in order to determine an unknown genotype.

| Testcross | Predicted ratio | Observed ratio ¹ | Total X^2 | DF | p | H_0 |
|---------------------------------|-----------------|-----------------------------|-------------|----|------|----------|
| F ₁ (+,+) x P(AP,SE) | 1.00(AP,SE): | 9.34(+,+): | 2.18 | 3 | 0.54 | Accepted |
| | 1.00(AP,+): | 3.07(+,SE): | | | | |
| | 1.00(+,SE): | 3.18(AP,+): | | | | |
| | 1.00(+,+) | 1.00(AP,SE) | | | | |

¹The observed value relates to a cross between two heterozygous offspring, thus being very different from the predicted ratio.

Discussion

Part 1: Mono-, di and tri-hybrid crosses

Using the Chi-square test of hypothesis, we observed offspring ratios that closely reflect the ratios that we predicted. We predicted that the parental cross would result in heterozygous (F_1) offspring in all three cases, and the observed value confirmed this – being 100%. The observed ratio for the F_2 generation of the monohybrid cross was really close to our predicted ratio of 3:1, and had a reasonable p-value of 0.50 which shows us that it is highly likely to have occurred. The dihybrid cross followed our predictions as well. The observed ratio was 8.90:2.93:3.06:1, with an even higher p-value. Lastly, when we compare the trihybrid cross with our predictions, the observed values are more similar than the other two crosses. It has the highest p-value of all three (0.83).

Part 2: Testcross

When we defined a test cross, we concluded that it was a method in which an unknown genotype could be determined. In order to carry this experiment out correctly, we had to cross a female fruit fly of the F_1 generation showing the wild type phenotype, with a male that was homozygous recessive for both traits. If the resulting offspring showed the wild type phenotype, then the female should have been homozygous dominant. If the female was heterozygous for the wild type and recessive traits, the ratio of the offspring should have shown an even split (~25%) between the four different phenotypic combinations. We failed to properly determine the F_1 generation, as we selected two F_1 offspring to breed instead of breeding an $F_1 (+,+)$ with a homozygous male (AP,SE). Our results for the chi-squared analysis also reflect this, in which we used the hypothesis for a monohybrid cross (9:3:3:1). The important thing to take away from

this is that it is very important to check over the data before proceeding. We could have easily changed our mistake by selecting the correct male to mate with our F_1 female, and then by using the appropriate hypothesis in Flylab in order to obtain the predicted results for this test cross.

Appendix

Chi Square table for the dihybrid cross

| Phenotype | Wild type eyes, Wild type body | Wild type eyes, Ebony body | Brown eyes, Wild type body | Brown eyes, Ebony body | Total |
|-----------------------------|-----------------------------------|-------------------------------|-------------------------------|---------------------------|-------|
| Observed value | 5598 | 1924 | 1848 | 629 | 9999 |
| Expected value | 5625.4375 | 1874.8125 | 1874.8125 | 624.9375 | 9999 |
| Obs-Exp | -27.4375 | 49.1875 | -26.8125 | 4.0625 | - |
| (Obs-Exp) ² | 752.82 | 2419.41 | 718.91 | 16.50 | - |
| (Obs-Exp) ² /Exp | 0.1338 ¹ | 1.2904 | 0.3834 | 0.0264 | 1.834 |

¹ Value was calculated using a CASIO fx-300MS, this calculation is slightly different than the Chi-square analysis using FlyLab.

Raw Results from Flylab for Parts 1 and 2

Results for the monohybrid cross

Results of Cross #1

Ignoring Sex

Parents

(Female: +) x (Male: BW)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 10120 | 1.0000 | 1.000 |
| Total | 10120 | | |

Results of Cross #2

Ignoring Sex

Parents

(Female: +) x (Male: +)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 7590 | 0.7529 | 3.047 |
| BW | 2491 | 0.2471 | 1.000 |
| Total | 10081 | | |

Chi Square Hypothesis Using Cross #2

Ignoring Sex

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|-----------|-----------------|
| + | 7590 | 3.0000 | 7560.7500 | 0.1132 |
| BW | 2491 | 1.0000 | 2520.2500 | 0.3395 |

Total 10081 4.0000 10081.0000 0.4526

Chi-Squared Test Statistic = 0.4526

Degrees of Freedom = 1

Level of Significance = 0.5011

Recommendation: Do not reject your hypothesis

Results for the Dyhybrid cross

Ignoring Sex

Parents

(Female: BW) x (Male: E)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 9980 | 1.0000 | 1.000 |
| Total | 9980 | | |

Results of Cross #4

Ignoring Sex

Parents

(Female: +) x (Male: +)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 5598 | 0.5599 | 8.900 |
| BW | 1848 | 0.1848 | 2.938 |
| E | 1924 | 0.1924 | 3.059 |
| BW;E | 629 | 0.0629 | 1.000 |
| Total | 9999 | | |

Chi Square Hypothesis Using Cross #4

Ignoring Sex

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|-----------|-----------------|
| + | 5598 | 9.0000 | 5624.4375 | 0.1243 |
| BW | 1848 | 3.0000 | 1874.8125 | 0.3835 |
| E | 1924 | 3.0000 | 1874.8125 | 1.2905 |
| BW;E | 629 | 1.0000 | 624.9375 | 0.0264 |
| Total | 9999 | 16.0000 | 9999.0000 | 1.8246 |

Chi-Squared Test Statistic = 1.8246

Degrees of Freedom = 3

Level of Significance = 0.6096

Recommendation: Do not reject your hypothesis

Results for the trihybrid cross

Results of Cross #5

Ignoring Sex

Parents

(Female: +) x (Male: SV;BW;E)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 10193 | 1.0000 | 1.000 |
| Total | 10193 | | |

Results of Cross #6

Ignoring Sex

Parents

(Female: +) x (Male: +)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|--------|
| + | 4250 | 0.4284 | 28.912 |
| SV | 1411 | 0.1422 | 9.599 |
| BW | 1393 | 0.1404 | 9.476 |
| SV;BW | 451 | 0.0455 | 3.068 |
| E | 1356 | 0.1367 | 9.224 |
| SV;E | 452 | 0.0456 | 3.075 |
| BW;E | 460 | 0.0464 | 3.129 |
| SV;BW;E | 147 | 0.0148 | 1.000 |
| Total | 9920 | | |

Chi Square Hypothesis Using Cross #6

Ignoring Sex

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|-----------|-----------------|
| + | 4250 | 27.0000 | 4185.0000 | 1.0096 |
| SV | 1411 | 9.0000 | 1395.0000 | 0.1835 |
| BW | 1393 | 9.0000 | 1395.0000 | 0.0029 |
| SV;BW | 451 | 3.0000 | 465.0000 | 0.4215 |
| E | 1356 | 9.0000 | 1395.0000 | 1.0903 |
| SV;E | 452 | 3.0000 | 465.0000 | 0.3634 |
| BW;E | 460 | 3.0000 | 465.0000 | 0.0538 |
| SV;BW;E | 147 | 1.0000 | 155.0000 | 0.4129 |
| Total | 9920 | 64.0000 | 9920.0000 | 3.5379 |

Chi-Squared Test Statistic = 3.5379

Degrees of Freedom = 7

Level of Significance = 0.8312

Recommendation: Do not reject your hypothesis

Results for the testcross

Results of Cross #7

Ignoring Sex

Parents

(Female: AP) x (Male: SE)

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 10036 | 1.0000 | 1.000 |
| Total | 10036 | | |

Results of Cross #8

Ignoring Sex

Parents

(Female: +) x (Male: +)¹

Offspring

| Phenotype | Number | Proportion | Ratio |
|-----------|--------|------------|-------|
| + | 5587 | 0.5625 | 9.343 |
| SE | 1840 | 0.1853 | 3.077 |
| AP | 1907 | 0.1920 | 3.189 |
| SE;AP | 598 | 0.0602 | 1.000 |
| Total | 932 | | |

Chi Square Hypothesis Using Cross #8

Ignoring Sex

| Phenotype | Observed | Hypothesis | Expected | Chi-Square Term |
|-----------|----------|------------|-----------|-----------------|
| + | 5587 | 9.0000 | 5586.7500 | 0.0000 |
| SE | 1840 | 3.0000 | 1862.2500 | 0.2658 |
| AP | 1907 | 3.0000 | 1862.2500 | 1.0753 |
| SE;AP | 598 | 1.0000 | 620.7500 | 0.8338 |
| Total | 9932 | 16.0000 | 9932.0000 | 2.1750 |

Chi-Squared Test Statistic = 2.1750

Degrees of Freedom = 3

Level of Significance = 0.5369

Recommendation: Do not reject your hypothesis

References

¹ The testcross was completed incorrectly, we selected two F₁ offspring instead of the wild type female with the homozygous recessive male.

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