Using Flylab to study the patterns of inheritance in monohybrid, dihybrid and trihybrid crosses and determine the genotype of a F1 generation with a dominant phenotype using a testcross.

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INTRODUCTION

An autosomal mutation is one where the sex of the organism does not matter; it affects males and females equally. The chromosome that is affected is not a sex chromosome (Kelsell, 1987).

Monohybrid Predictions:

The question being studied is what is the pattern of inheritance in a monohybrid cross? The hypothesis for this is that there will be more offspring carrying the dominant phenotype. Three assumptions for this lab are that the parents are purebred and true breeding flies. The wild allele is dominant over the mutant allele for all 3 genes. Finally that the genes in question we are looking at are not sex-linked.

F1:

Phenotype of parents: ♀ AP x ♂ + Genotype of parents: ♀ APAP x ♂ ++

	♂ ++	♂ ++
♀ AP	AP+	AP+
♀ AP	AP+	AP+

Predicted Phenotypic Ratio of F1: 1+ (100% wild type, (100% wild type, assuming that the wild allele is dominant over the mutant allele)

F2:

	♂AP+	♂AP+
♀AP+	AP+	AP+
♀AP+	AP+	AP

Predicted phenotypic Ratio of F2: 3 + : 1 (75% wild type, 25% mutant)

Dihybrid Predictions:

The question being studied is what is the pattern of inheritance in a dihybrid cross? Three assumptions we make are that the two genes are not linked, the parents are pure-bred (homozygous for both traits). The wild allele is dominant over the mutant allele in both genes. The two genes are not sex-linked. The hypothesis is that there will be more offspring carrying the dominant phenotype.

The overview of this experiment was done on the online program called Flylab. We are selecting various mutations given to us on our male and female flies respectively. Crossing them to produce generation one (F1) and crossing those children once more to create generation two (F2). Once these two crosses were done we calculated the ratios of the phenotypic outcomes and the chi-squared value is recorded, along with degrees of freedom and the statistical significance value.

F1:

Phenotype of parents: ♀ AP+ x ♂ +, SV Genotype of parents: ♀ APAP, ++ x ♂ ++, SVSV

	♂ +,SV	♂ +,SV
♀ AP,+	AP+,SV+	AP+,SV+
♀ AP,+	AP+,SV+	AP+,SV+

Predicted Phenotypic Ratio of F1: 1+ (100% wild type, assuming that the wild allele is dominant over the mutant allele in both genes)

F2:

	♂AP,SV	♂AP,+	♂+,SV	♂+,+
♀AP,SV	APAP,SVSV	APAP,SV+	AP+,SVSV	AP+,SV+
♀AP,+	APAP,SV+	APAP,++	AP+,SV+	AP+,++
♀+SV	AP+,SVSV	AP+,SV+	++,SVSV	++,SV+
♀+,+	AP+,SV+	AP+,++	++,SV+	++,++

Predicted phenotypic Ratio of F2: 9 +, + : 3 AP, + : 3 +, SV : 1 C, BL. 9/16 (56%) wild (+), 3/16 (19%) mutant AP, 3/16 (19%) mutant SV and 1/16 (6%) double mutant (AP,SV)

Trihybrid Predictions:

The question being studied is what is the pattern of inheritance in a trihybrid cross? Three assumptions we make are that the three genes are not linked, the parents are pure-bred (homozygous for both traits). The wild allele is dominant over the mutant allele in both genes. The two genes are not sex-linked. The hypothesis is that that there will be more offspring carrying the dominant phenotype.

F1:

Phenotype of parents: ♀ +,+,+ x ♂ AP,SV,SE

Genotype of parents: ♀ ++,++,++ x ♂ APAP,SVSV,SESE

	♂ AP,SV,SE
♀ +,+,+	AP+,SV+,SE+

Predicted Phenotypic Ratio of F1: 1+ (100% wild type, assuming that the wild allele is dominant over the mutant allele in all three genes)

F2:

	♂AP,+,+	♂AP,+,SE	♂AP,SV,+	♂AP,SV,SE,	♂+,+,+	♂+,+,SE	♂+,SV,+	+,SV,SE
♀AP,+,+	APAP,++,++	APAP,++,SE+	APAP,SV+,++	APAP,SV+,SE+	AP+,++,++	AP+,++,SE+	AP+,SV+,++	AP+,SV+.SE+
♀AP,+,SE	APAP,++,SE+	APAP,++,SESE	APAP,SV+,SE+	APAP,SV+,SESE	AP+,++,SE+	AP+,++,SESE	AP+,SV+,SE+	AP+,SV+,SESE
♀AP,SV,+	APAP,SV+,++	APAP,SV+,SE+	APAP,SVSV,++	APAP,SVSV,SE+	AP+,SV+,++	AP+,SV+,SE+	AP+,SVSV,++	AP+,SVSV,SE+
♀AP,SV,SE	APAP,SV+,SE+	APAP,SV+,SESE	APAP,SVSV,SE+	APAP,SVSV,SESE	AP+,SV+,SE+	AP+,SV+,SESE	AP+,SVSV,SE+	AP+,SVSV,SESE
♀+,+,+	AP+,++,++	AP+,++,SE+,	AP+,SV+,++,	AP+,SV+,SE+	++,++,++	++,++,SE+	++,SV+,++	++,SV+,SE+
♀+,+,SE	AP+,SV+,SE+	AP+,++,SESE	AP+,SV+,SE+	AP+,SV+,SESE	++,++,SE+	++,++,SESE	++,SV+,SE+	++,SV+,SESE
♀+,SV,+	AP+,SV+,++	AP+,SV+,SE+	AP+,SVSV,SE+	AP+,SVSV,SE+	++,SV+,++	++,SV+,SE+	++,SV+,++	++,SVSV,SE+
♀+,SV,SE	AP+,SV+,SE+	AP+,SV+,SESE	AP+,SVSV,SE+	AP+,SVSV,SESE	++,SV+,SE+	++,SV+,SESE	++,SVSV,SE+	++SVSV,SESE

Predicted phenotypic ratio: 27 +, +, + : 9 AP, +, + : 9 +, SV, + : 9 +, +, SE : 3 AP, SV, + : 3 AP, +, SE: 3 +, SV, SE: 1 AP, SV, SE→ 27/64 (42%) wild phenotype (+), 9/64 (14%) mutant AP, 9/64 (14%) mutant SV, 9/64 (14%) mutant SE, 3/64 (4.7%) double mutant (AP, SV), 3/64 (4.7%) double mutant (AP, SE), 3/64 (4.7%) double mutant (SV, SE) and 1/64 (1.6%) triple mutant (AP, SV, SE).

Where in all examples given above AP is apterous wings, SV is shaved bristles and SE are sepia eyes.

Test Cross Predictions:

The test cross is used to identify the genotypes responsible for the phenotypes of the progenies after we conduct a cross. This means that a test cross is made between an organism exhibiting the dominant trait and carrying the unknown dominant genotype and another organism displaying the recessive trait. If the recessive trait is displayed in any of their offspring it means that the unknown genotype is heterozygous dominant. However, if the dominant trait is detected in all offspring the unknown genotype is homozygous dominant. The accuracy the test cross results are dependent on a large statistical sample (RÖSSLER, 1982).

The question being studied is what is the genotype of a F1 generation that display the dominant phenotype? Three assumptions we make are that the three genes are not linked, the parents are pure-bred (homozygous for both traits) or can be heterozygous. The wild allele is dominant over the mutant allele in both genes. The two genes are not sex-linked. The hypothesis is that the genotype we are testing for will reveal to be ether homozygous or heterozygous. C is curved wings and E is ebony body. The experimental design for this part of the lab was done by having one parent with one mutation at one gene and another parent with one mutation at another gene assigned on Flylab. Then we conduct the cross between the two parents in order to obtain the F1 generation. All of this is done on the Flylab program online in the lab.

F1 Predictions if parents are homozygous:

Predicted phenotype of parents: ♀+ x ♂CE

Predicted genotype of parents: ♀++++ x ♂CECE

	♂CECE
♀++	C+,E+

Predicted phenotypic ratio for F1 Testcross: 1+ (100% wild type)

F2 Predictions if parents are heterozygous:

Predicted phenotype of parents: ♀+ x ♂CE

Predicted genotype of parents: ♀CC+,EE+ x ♂CECE

	♂ CE
♀CE	CE,CE
♀C+	CC,E+
♀E+	C+,EE
♀++	C+,E+

Predicted phenotypic ratio for F1 Testcross: 1+, E:1, C:1, C,E:1

Summary of experimental design: Using the Flylab online program and chi-squared goodness of fit test we designed two flies and crossed them together. One of their offspring were selected and crossed with a designed homozygous recessive fly. This test confirmed that the flies were heterozygous for C and E alleles.

RESULTS

Table 1: Phenotype of parents and offspring, observed ratio and predicted ratio after crossing, chi-squared value, degrees of freedom, p value and the validity of the hypothesis for the monohybrid, dihybrid and trihybrid crosses

Cross Type	Cross (♀ x ♂)	n	Predicted ratio	Observed ratio	Total X^2	DF	p	H_0
Monohybrid	P (AP) x P (+) F1 (+) x F1 (AP)	9987	3.00:1	3:1	0.00082	1	0.97	Accepted
Dihybrid	P(AP+)x (+, SV) F1(AP+)xF1(+, SV)	9976	8.50:2.80:3:1	9:3:3:1	6.86	3	0.076	Accepted
Trihybrid	P(+,+,+) x P (AP,SV,SE) F1(AP+,SV+, SE+) x F1 (AP+,SV+,SE+)	10092	26:8.4:8.6:8.60:3.20:2.90:2.90:1	27:9:9:9:3:3:3:1	7.40	7	0.34	Accepted

Table 2: Phenotype of parents and offspring, observed ratio and predicted ratio after crossing, chi-squared value, degrees of freedom, p value and the validity of the hypothesis for test cross

Testcross (♀ x ♂)	n	Predicted ratio	Observed ratio	Total X^2	DF	p	H_0
♀(+) x ♂(CE)	10247	1:1:1:1	1:1:1:1	1.5	3	0.68	Accepted

DISCUSSION

In the monohybrid cross we can see the ratio is 1 in the F1 offspring, thus proving that there is a 100% chance the offspring will get the wild type gene; which is dominant in this case. The possible genotype for the F1 offspring is AP+, in F2 it is AP+ or AP, the ratio for this cross was a 3:1, with three AP+ and 1 AP. The degrees of freedom was 1 and our chi-squared value was 0.00082 with a statistical significance of 0.97. In conclusion our predicted ratio was identical to our observed ratio in a total population of 9987, and our hypothesis was accepted. In the dihybrid cross we had the predicted phenotypic ratio of F1: 1+ (100% wild type, assuming that the wild allele is dominant over the mutant allele in both genes). In F2 the predicted phenotypic ratio was 9 +, + : 3 AP, + : 3 +, SV : 1 C, BL. 9/16 (56%) wild (+), 3/16 (19%) mutant AP, 3/16 (19%) mutant SV and 1/16 (6%) double mutant (AP,SV). The degrees of freedom was 3 and our chi-squared value was 6.86 with a statistical significance of 0.076. In conclusion our observed ratio was 9:3:3:1 which was close to our predicted ratio of 8.50:2.80:3:1. We also had accepted the hypothesis. Trihybrid crosses are done by mating 2 individuals with different gene pairs that determine three differnet specific traits and we follow and track those traits in a punnet square (Yao,2014). In the trihybrid cross the predicted phenotypic ratio of F1 was: 1+ (100% wild type, assuming that the wild allele is dominant over the mutant allele in all three genes). In F2 the predicted phenotypic ratio: 27 +, +, + : 9 AP, +, + : 9 +, SV, + : 9 +, +, SE : 3 AP, SV, + : 3 AP, +, SE: 3 +, SV, SE: 1 AP, SV, SE→ 27/64 (42%) wild phenotype (+), 9/64 (14%) mutant AP, 9/64 (14%) mutant SV, 9/64 (14%) mutant SE predicted , 3/64 (4.7%) double mutant (AP, SV), 3/64 (4.7%) double mutant (AP, SE), 3/64 (4.7%) double mutant (SV, SE) and 1/64 (1.6%) triple mutant (AP, SV, SE). The degrees of freedom was 7 and our chi-squared value was 7.40with a statistical significance of 0.34. In conclusion, we accepted our hypothesis as our observed ratio of 27:9:9:9:3:3:3:1 was close to our predicted ratio 26:8.4:8.6:8.60:3.20:2.90:2.90:1.

In the test cross the parents were heterozygous the predicted genotype of the parents was: ♀CC+,EE+ x ♂CECE. In the test cross our predicted phenotype of parents was ♀+ x ♂CE. With the predicted phenotypic ratio for F1 Testcross: 1+ (100% wild type). This was assuming that the parents were homozygous. The degrees of freedom was 3, the statistical significance was 0.68 and the hypothesis was accepted. The chi-squared value was 1.5 and the predicted ratio was identical to the observed ratio, 1:1:1:1.

Overall conclusion:

The genotypic and phenotypic ratios of these flies can be predicted using an online simulation of fruit fly breeding where we observe the effects of a dominant wild type gene and the recessive mutant gene on 2 generations of offspring (Zhu and Zhang, 2007). We observed these mutations in a monohybrid, dihybrid and trihybrid cross. And we calculated the possible genotypic ratios of the parents using a test cross.

APPENDIX

Sample chi-squared calculation done on a dihybrid cross:

$$X^2 = \sum \frac{(Obs - Exp)^2}{Exp}$$

Phenotype	+,+	AP,+	SV+	AP,SV	Total
Observed Value	5534	1955	1835	652	9976
Expected Value	5611.5	1870.5	1870.5	623.5	9976
Obs-Exp	-77.5	84.5	-35.5	28.5	...
(Obs-Exp)^2	6006.25	7140.25	1260.25	812.25	...
(Obs-Exp)^2 /Exp	1.07	3.82	0.67	1.30	6.86=X^2[a]

[a] Values based on the degrees of freedom: 4-1=3, the p value: 0.076 and that the hypothesis was accepted.

Refferences

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