**Determination of an Unknown Genotype Using Chi-Square**

Ngan Lam, Daniel Chanelo, Emily Quick, Mohamad Chwiki

February 15, 2018

PBC 3063L

Section: 10

**Materials and Method**

*Culture vial preparation:*

The Materials needed for this preparation contained a clean vial, dry fly food, a glass bowl, a foam plug, a spatula, yeast, culture netting and water (Garey et al., 2018). First of all, the culture vial was created by were combined in a coordinated proportion utilizing a spatula. The amount of water was added to the dry food until the point that the sustenance presented a mashed potato consistency (Garey et al., 2018). If the food prepared with a lot amount of water would cause the flies drowning, and preparing sustenance unreasonably dry would cause lack of hydration. As soon as the right proportion of sustenance to water was accomplished, around one inch of nourishment was set at the base of the vial (Garey et al., 2018). Following this, five yeast granules were included to the vial best of the nourishment (Garey et al., 2018). At that point, fly netting was placed inside the vial and in the wake of being collapsed vertically and the vial was placed horizontally, it was help to avoid the fly get stuck in the food (Garey et al., 2018). This procedure was rehashed three or more circumstances totaling four fly culture vials.

*Anesthetization the Flies:*

We used FlyNap to anesthetize our flies. This briefly puts them to sleep to allow us to sex them and separate them under a microscope. Anesthetizing D. melanogaster: A vial containing male D. melanogaster with an obscure genotype was marginally tapped on a lab seat so the flies accumulated at the highest point of the froth plug would move down (Garey et al., 2018). At this point, a wand dunked in FlyNap was immediately put in the vial for 45 second and removed, and the vial was then laid on its side with the goal that oblivious flies would not fall in the sustenance (Garey et al., 2018). When every one of the flies quit moving, the FlyNap wand was expelled and the flies were then expelled from the vial (Garey et al., 2018). Also, vials containing flies with genotype sepia/sepia and vestigial/vestigial separately were acquired and every vial was anesthetized like above and the females of every vial were scored under a microscope and gathered.

*Setting up a genetic cross:*

15 males D. melanogaster with an unknown genotype were gathered subsequent to being anesthetized (Garey et al., 2018). At that point, the vial containing female D. melanogaster with the genotype sepia/sepia was acquired, and subsequent to being anesthetized 12 flies were gathered (Garey et al., 2018). Three obscure male flies were then put in a fly culture vial with four sepia/sepia female flies; and one more vial contained the same amount with three obscure male and four sepia/sepia female flies. A moment the two fly culture vial additionally containing three obscure guys and four sepia/sepia females was then arranged. Moreover, a vial containing anesthetized female D. melanogaster with the genotype vestigial/vestigial was acquired; and two more fly culture vials were then gotten and three obscure guys and two vestigial/vestigial females were put in every vial. After setting up every vial, the vials were set on its side until the point that the flies ended up cognizant to keep the flies from falling and getting to be stuck in the sustenance (Garey et al., 2018). When every one of the flies were cognizant, the vials were set upright in a hatchery warmed to 25°C (Garey et al., 2018).

*Evacuating grown-up D. melanogaster before posterity show up:*

The next week, the hatchlings in every vial was watched. The grown-up flies exhibit in the vials were then anesthetized with FlyNap, expelled from the vials and after that set in a fly mortuary (Garey et al., 2018). The vial containing the hatchlings was then stopped with a froth top and put in a 25°C incubator (Garey et al., 2018).

*Choosing phenotype of offspring:*

The vials containing the offsprings were obtained and every vial was anesthetized with FlyNap (Garey et al., 2018). Each fly was then scored under an intensifying point of convergence for the phenotype concurring the check made on its vial (Garey et al., 2018). The vials which contained the group of the male with the obscure genotype and the sepia/sepia females were scored in context of sepia eyes versus wildtype eyes. In like way, the vials that contained the successors of the male with the obscure genotype and the vestigial/vestigial females were scored in context of wildtype wings and immaterial wings. Resulting to scoring every one of the flies, the information was recorded and the flies were set in fly entombment benefit homes (Garey et al., 2018).

**Result**

The target of this investigation was to decide the obscure genotype of male D. melanogaster by intersection them with known genotypes of female D. melanogaster. Following two weeks of getting ready hereditary crosses and isolating the grown-ups from posterity; on week three the phenotypes of the posterity was scored and after that a conclusion on the genotype of the obscure male was come to.

|  |  |  |
| --- | --- | --- |
|  | + | + |
| Se | +/Se | +/Se |
| Se | +/Se | +/Se |

Table 1: the above cross demonstrates a conceivable genotype of the male fly. As needs, be, because of this cross, all the posterity will delineate a wildtype phenotype, which is red eyes.

|  |  |  |
| --- | --- | --- |
|  | + | Se |
| Se | +/Se | Se/Se |
| Se | +/Se | Se/Se |

Table 2: A conceivable genotype of the male fly; which brings about the offspring communicating a 50:50 phenotypic proportion of wildtype eyes to sepia eyes

|  |  |  |
| --- | --- | --- |
|  | + | + |
| Vg | +/Vg | +/Vg |
| Vg | +/Vg | +/Vg |

Table 3: The cross the offspring imparting wildtype wings as their phenotype if the male fly crossed with the homozygous vestigial female is homozygous wildtype.

|  |  |  |
| --- | --- | --- |
|  | + | Vg |
| Vg | +/Vg | Vg/Vg |
| Vg | +/Vg | Vg/Vg |

Table 4: the cross shows a conceivable genotype of the male fly; which brings about the offspring communicating a 50:50 expected phenotypic proportion of wildtype wings to vestigial wings

The Chi-square (x2) result of the experiment:

The chi –square below analyzing for each cross when testing the known phenotype and unknown. The purpose of this test was to determine whether any deviations from what expected.

|  |  |  |  |
| --- | --- | --- | --- |
| Phenotypes | Observed | Expected | (O/E)2 |
| Wildtype | 203 | 203 | 0 |
| Sepia | 0 | 0 | 0 |
| **Total:** | 203 | 203 | 0 |

 Table 5: The above table showed he chi-square calculation of cross between sepia female with unknown phenotype. The ratio of the test was not support the hypothesis that expected as 4:0. The probability values associated with X2 value are 0.975<P<0.995.

|  |  |  |  |
| --- | --- | --- | --- |
| Phenotypes | Observed | Expected  | (O/E)2 |
| Wildtype | 28 | 25 | 0.36 |
| Vestigial | 22 | 25 | 0.36 |
| **Total:** | 50 | 50 | 0.72 |

Table 6: The table above showed the chi-square calculation of the cross between Vestigial female and unknown phenotype. The ratio of the test was support the hypothesis that expected 2:2. The probability values associated with X2 values are 0.250<P<0.500.

Besides, tables five and six above delineate the chi-square esteems figured from doing the two crosses. After ascertaining the chi-square esteems, the p esteems for the two arrangements of information were resolved; and comes about proposed that the two crosses took after Mendelian hereditary qualities.

**Discussion**

The two principle objectives of this trial were to decide the obscure genotype of male D. melanogaster and to decide whether the alleles of its posterity took after Mendelian legacy designs (Garey et al., 2018). As appeared in tables one through four, there were four anticipated genotypes for the male D. melanogaster. In the wake of gathering and breaking down the information appeared in tables five and six, the anticipated genotypes of the male flies were x+/y, heterozygous wildtype (+/se) for eye shading and homozygous wildtype (+/+) for wings. The information gathered from intersection the male with a minimal female (vg/vg) brought about all the posterity having wildtype wings and no minimal wings, as appeared in table six. This information unequivocally coordinates the anticipated male genotype in table two, since like the watched information, when a homozygous wildtype (+/+) male was crossed with a homozygous minimal (vg/vg) female, the subsequent posterity just had wildtype wings. Thusly, comes about proposed that the male fly was homozygous wildtype (+/+) for wings. Besides, as appeared in table five, in the wake of intersection the obscure male with a homozygous sepia (se/se) female, the subsequent posterity gave an around 50:50 proportion of wildtype eyes to sepia eyes. This arrangement of information best fits the anticipated male genotype in table three since when a heterozygous wildtype (+/se) male is crossed with a homozygous sepia (se/se) female, the subsequent posterity introduces a 50:50 proportion of wildtype eyes to sepia eyes. When every one of the information was gathered and contrasted with the crosses foreseeing the genotype of the male flies, comes about recommended that the male D. melanogaster's genotype was homozygous wildtype for wings (+/+) and heterozygous wildtype for eyes (+/se). Besides, the sex of the posterity was not considered in light of the fact that we were just taking a gander at the posterity's wings and eye shading which are non-sex connected attributes. However, the x+/y genotype of the male fly was resolved in light of the fact that while watching the fly, it had red eyes.

Moreover, in the wake of deciding the genotype of the male D. melanogaster and breaking down the phenotypic proportions of the posterity from the two crosses, a chi-square esteem was computed for each cross. A chi-square test decides whether the information gathered from a hereditary cross is reliable with Mendelian legacy designs (Brooker, 2012). A chi-square test additionally uncovers if the exploratory information is of solid match to the hypothetical or expected esteems (Garey et al., 2018). As appeared above in table nine, the chi-square an incentive for the cross between the homozygous sepia female and the anticipated homozygous wildtype male was figured to be zero. Along these lines, the cross between the homozygous vestigial female and the anticipated heterozygous wildtype male had a chi-square estimation of 0.72, as appeared in table ten. In the wake of computing the chi-square esteem the p esteem can be resolved; the p esteem is the likelihood that the variety given by the chi-square esteem is because of arbitrary shot (Brooker, 2012). The hereditary cross including wildtype and sepia eyes had one level of opportunity, and a chi-square estimation of zero. Since the watched information definitely coordinated the normal information, the chi-square estimation of zero shows that no deviation happened between the watched and expected esteems. Similarly, the hereditary hybrid of wildtype and vestigial wings additionally had one level of flexibility, and with a chi-square of 0; it is proposed that there is a 20-5% chance that the deviations watched were because of arbitrary shot.

Albeit the two crosses display chi-square esteems that recommend that Mendelian legacy is being taken after, there could at present be wellsprings of mistake that happened all through the test. For example, if the flies were not anesthetized effectively the tally of the flies could be off in light of the fact that it is a probability that the flies either fell in the sustenance and kicked the bucket, or were not completely oblivious and took off. Another wellspring of mistake could have been if the flies were not tallied and phenotypically scored effectively. Generally the analysis achieved its objectives; in any case, results may have strayed because of conceivable blunders.

**References**

Brooker, Richard. 2012. Genetics: Analysis and Principles 4th Ed. McGraw-Hill Higher Education. New York, NY. 33 pp.

CMMB Department. 2018. *Mendelian and Molecular Genetics: Investigation of Wing Development and Pigment Biosynthesis Pathways in Drosophila melanogaster.* Genetics Laboratory Manual PCB 3063L, Spring 2018. University of South Florida. Pro-Copy, Tampa, FL. Print.