

Crossing true-breeding, virgin, *scarlet* female *Drosophila melanogaster* mutants with true-breeding, *apterous* male *Drosophila melanogaster* mutants for examining the Mendelian inheritance pattern.

Jessica T. Barber

The University of Wisconsin- La Crosse

BIO 306- 13L: Genetics Lab

Professor Werren

29 November 2021

Abstract

Female *scarlet* mutants were crossed with male *apterous* mutants in the Parental (P) generation for determining the phenotypic ratio of the Second filial (F2) generation of *Drosophila melanogaster*. The expected ratio for the cross of two autosomal recessive genetic mutations was 9 wild-type: 3 *scarlet*: 3 *apterous*: 1 *scarlet* and *apterous*. The self-crossed First filial (F1) generation resulted in an observed phenotypic ratio of 31.1 wild-type: 4.8 *scarlet*: 5.1 *apterous*: 1 *scarlet* and *apterous* in the F2 generation. The calculated Chi-square value of 66.300 (with three degrees of freedom, a p-value less than 0.05, and a Chi-square critical value of 7.815) indicated the results of the experiment as not statistically significant. In comparison to the critical value used in this Chi-square test, the higher Chi-square value prompted a rejected hypothesis for the experiment. The differences between the expected and observed phenotypic ratio for the F2 generation were not attributed to random chance alone.

Results

Drosophila melanogaster scarlet and *apterous* mutants have autosomal recessive genetic mutations on the *scarlet* gene (found on chromosome three) and *apterous* gene (found on chromosome two), respectively. If true-breeding, virgin, homozygous recessive, *scarlet* female mutants were crossed with true-breeding, homozygous recessive, *apterous* male mutants in the P cross, the expected phenotypic ratio would show a 9 wild-type: 3 *scarlet*: 3 *apterous*: 1 *scarlet* and *apterous* in the F2 generation.

After eight hours of hatching, *D. melanogaster* reach sexual maturity. *scarlet* females utilized in the P cross were separated and obtained from the initial vial within eight hours of hatching, to avoid fertilization by *scarlet* males from the same vial. For the P cross, five vials each contained two virgin, *scarlet* female mutants and four *apterous* male mutants. Once pupae

emerged, the P generation was eliminated from the vials. From the P cross, the expected phenotype of the F1 generation should have been wild-type, acquiring one recessive *scarlet* allele and one dominant *apterous* allele from the virgin females and one dominant *scarlet* allele and one recessive *apterous* allele from the males. During the F1 cross, considering the self-fertilization of mutant *D. melanogaster* with identical genotypes, the F1 cross did not require virgin females. Utilizing the offspring of the P cross, 12 vials with numerous males and female were created. By self-crossing the F1 generation, the heterozygotes for the *scarlet* and *apterous* genes should have, in theory, produced offspring that matched the expected phenotypic ratio. Once the F2 generation of pupae emerged within the vials, the F1 generation was disposed of. After the F2 generation hatched, *D. melanogaster* were observed and counted based on wild-type, scarlet, apterous, and scarlet and apterous phenotypes.

The observed F2 phenotype data consisted of 373 wild-type, 58 *scarlet*, 61 *apterous*, and 12 *scarlet* and *apterous* (Table 1). Furthermore, the observed phenotypic ratio of the F2 data amounted to 31.1 wild-type: 4.8 *scarlet*: 5.1 *apterous*: 1 *scarlet* and *apterous* (Table 1). In identifying whether the experiment yielded significant results, a Chi-square test was utilized. The Chi-square test of independence is one of the most valuable statistics for testing hypotheses by uncovering the significance of observed differences and identifying the categories that account for variances. In this particular experiment, the Chi-square equation determined the differences between the observed and expected ratios of the F2 *D. melanogaster*. In conducting the Chi-square significance test, the total number of counted F2 offspring (504) was multiplied by the expected phenotypic ratio of 9 wild-type: 3 *scarlet*: 3 *apterous*: 1 *scarlet* and *apterous* to determine the expected phenotypic data. The deduction of the observed phenotypic data from the expected phenotypic data was squared and the subsequent result was then divided by the

expected phenotype. The sum of all the Chi-squares calculated for each phenotypic class resulted in the total Chi-squared value of 66.300 (Table 1).

Table 1. Expected and observed phenotypic data and ratios for the F2 generation of *Drosophila melanogaster* with Chi-square calculation.

Phenotype	Expected (E)	Observed (O)	Expected phenotypic ratio	Observed phenotypic ratio	$[(O-E)^2 / E]$
Wild-type	283.5	373	9	31.1	28.255
<i>st</i>	94.5	58	3	4.8	14.098
<i>ap</i>	94.5	61	3	5.1	11.876
<i>st & ap</i>	31.5	12	1	1	12.071
Total	504	504	16	42	66.300

Discussion

Within this experiment, a dihybrid cross between *scarlet* and *apterous* *D. melanogaster* uncovered the phenotypic ratio of the F2 generation. Virgin, *scarlet* female mutants crossed with *apterous* male mutants, in the P generation, would have an expected 9 wild-type: 3 *scarlet*: 3 *apterous*: 1 *scarlet* and *apterous* phenotypic ratio in the F2 generation. Wild-type *D. melanogaster*, heterozygous for both the *scarlet* and *apterous* genes, should have emerged as the F1 progeny. After self-crossing the heterozygous wild-type F1 generation, 504 of the F2 offspring were identified and counted for determining the phenotypic ratio. Utilizing the Chi-square test, the results of the experiment were compared with the expected phenotypic ratio of the autosomal recessive traits. The Chi-square value, calculated using the F2 data found in

Table 1, was 66.300. This number indicates the significance between the expected and observed phenotypic ratio.

In computing the Chi-square value, three degrees of freedom and a critical Chi-square value of 7.815 were used. Considering that the calculated Chi-square of 66.300 was much higher than the critical Chi-square value of 7.815, the probability range was $0.01 > p$. The p-value and range refer to the likelihood that the results shown differ from the expected results due to chance. Since the probability of deviations due to chance are greater than 5%, the hypothesis for the experiment was rejected.

While the experiment data resulted in a rejected hypothesis, one possible methodical error that could have occurred could have been incorrect phenotypic counting of the F2 offspring. Wild-type *D. melanogaster* and *scarlet* mutants have similar phenotypic eye coloration. Distinguishing the difference between the two different eye colors was difficult at times. Another error that could have caused a rejected hypothesis involved failure to identify the phenotype (as wild-type) of every *D. melanogaster* self-fertilized during the F1 cross. In the future, in order to verify or contest the results of this investigation, another experiment could cross true-breeding *apterous* mutants with true-breeding *white* mutants. Utilizing *white D. melanogaster*, rather than *scarlet*, may assist researchers in less challenging visualization of the phenotypes of the F2 progeny. In addition, considering that the *vestigial* gene influences wing and haltere formation, similar to the *apterous* gene (1,2), an additional experiment could cross true-breeding, *vestigial* male *D. melanogaster* with true-breeding, virgin, *scarlet* female mutants to see if similar results are obtained. Studying the cross between *vestigial* and *scarlet* mutants would provide insight into whether *apterous* specifically, or all wing mutations have an influence

on the mating abilities of *D. melanogaster*. Comparing data from the two studies could provide further evidence to the significance of the current findings.

Considering the autosomal recessive nature of both mutated genes utilized during the dihybrid cross, a reciprocal cross would, in theory, provide similar results. Autosomal refers to the mutation genes on non-sex chromosomes. Furthermore, recessive refers to needing two mutated alleles for presenting the trait. Since sex had no influence on acquiring the traits studied in this experiment, true-breeding, virgin, *apterous* females crossed with true-breeding, *scarlet* males would produce equivalent findings. For the P cross, the vial that produced the most females within the eight-hour window indicated which mutants were chosen for the male and virgin female populations.

The *apterous* gene of *D. melanogaster* influences the wing, haltere and imaginal disc development (2,3). Imaginal disc, during larval stages, take form of an epithelial structure which develops into external structure of the head, thorax, limbs, and genitalia in pupae (3). Encoding a member of the LIM structural protein domain, *apterous* influences development of regulatory genes (2). Considering that *apterous* is required for transcriptional regulations of genes involved in wing and haltere development, the LIM protein domain functions as a transcription factor involved in transcribing DNA into RNA (2,4). In wild-type functioning *apterous* genes, the wings and haltere develop into visible and well-formed structures. *D. melanogaster* with *apterous* mutations and the resulting phenotype lack any wing structure (5). In addition, within *D. melanogaster*, wild-type eye coloring results from the work of two pathways: xanthommatin (brown) and drospterins (red) light screening pigments (6). The *scarlet* gene includes genetic information required for the generation of xanthommatin, a derivation of tryptophan. Of the four eye colored genes, *vermillion*, *cinnabar*, *scarlet*, and *white*, the functions of *scarlet* and *white*

lack significant evidence pertaining to the influence on the pathway (6). Wild-type functioning of the *scarlet* gene (as well as the other eye color genes) results in a red-brown, brick pigmentation within *D. melanogaster* eyes. If mutated, the *scarlet* gene would cause a bright red coloration within *D. melanogaster* eyes, since complications occur within the brown light screening pathway. Alteration of the *scarlet* gene causes an inability for tissues of the larval Malpighian tubules and the compound and simple eyes of adults to complete functions required for xanthommatin precursors, influencing the pathway (6).

Through this dihybrid cross of virgin, *scarlet* female *D. melanogaster* mutants with *apterous* male *D. melanogaster* mutants, the experimental data uncovered an observed phenotypic ratio of 31.1 wild-type: 4.8 *scarlet*: 5.1 *apterous*: 1 *scarlet* and *apterous*. A Chi-square significance test uncovered the non-statistical significance of the experiment based on the Chi-square value of 66.300 and a probability range of $0.01 > p$, using a critical Chi-square value of 7.815 and three degrees of freedom. Thus, the study data showed differing results than expected. Any differences in the data found in this experiment, compared to the expected findings, could not be attributed to chance alone. Future research should focus on crossing true-breeding *apterous* mutants with *D. melanogaster* with mutations affecting eye pigmentations, such as the *white* gene, that are more distinguishable from wild-type. Comparing the results of this experiment with a cross between males of other winged mutations and virgin, *scarlet* females could further validate the significance of this experiment and investigate the differences in mating abilities between *apterous* and other wing mutants.

Literature Cited

- (1) Williams, J.A., Bell, J.B. and Carroll, S.B. 1991. Control of *Drosophila* wing and haltere development by the nuclear *vestigial* gene product. *Genes & Development* 5(12B): 2481-2495.
- (2) Cohen, B., McGuffin, M.E., Pfeifle, C., Segal, D. and Cohen, S.M. 1992. *apterous*, a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. *Genes & Development* 6(5): 715-729.
- (3) Aldaz, S. and Escudero, L.M. 2010. Imaginal discs. *Current Biology* 20(10): PR429-R431.
- (4) Lambert, S.A., Jolma, A., Campitelli, L.F., Das, P.K., Yin, Y., Albu, M., Xiaoting, C., Taipale, J., Hughes, T.R., and Weirauch, M.T. 2018. The Human Transcription Factors. *Cell*. 175(2): 650-665.
- (5) Bieli, D., Kanca, O., Gohl, D., Denes, A., Schedl, P., Affolter, M. and Müller, M. 2015. The *Drosophila melanogaster* Mutants *ap^{blot}* and *ap^{Xasta}* Affect and Essential *apterous* Wing Enhancer. *Genetics* 5(6): 1129-1143.
- (6) Tearle, R.G., Belote, J.M., McKeown, M., Baker, B.S. and Howells, A.J. 1989. Cloning and Characterization of the *scarlet* Gene of *Drosophila melanogaster*. *Genetics* 122(3): 595-606.