

Research Article

**INHERITANCE PATTERN OF SEX-LINKED TRAIT AND FEEDING
AND SEXUAL BEHAVIOUR OF *DROSOPHILA MELANOGASTER***

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ABSTRACT

This study was aimed to unravel the inheritance pattern of sex-linked trait (eye colour) and the feeding and sexual behaviour of *Drosophila melanogaster*. *D. melanogaster* was collected and identified the wild and mutant type flies; were kept in the culture vial and designed mating was done at a ratio of 1:5 male and female. In this experiment, the inheritance pattern of sex-linked trait (eye colour) was studied. The resulting offspring were counted and recorded, and chosen for further mating and study. Total number of wild type flies was higher than the mutant type flies. Moreover, the mutant type male flies were decreased in number in the subsequent generation due to selection against mutant type in each generation. It was observed that the desirable gene (wild type gene) frequencies were increased and undesirable gene (mutant type gene) frequencies were decreased significantly from 1st to 3rd generation. The feeding and sexual behaviour was observed by naked eye in different time interval. The larvae always attached with the culture and took the culture media as food up to pupa stage. However, the adults were stayed away from media; they were in media for half an hour after one to two hours interval. In case of mating behaviour, it was observed that the males tried to attract females by vibrating their wings as well as licking the female genitalia. After accepting the male by female drosophila, successful mating was observed for approximately 15 to 20 minutes.

Key words: *Drosophila*, selection, mating design, behaviour.

INTRODUCTION

The term sex-linked refers the gene carried by the X chromosome of an individual and its phenotypic expression related to the assortment of this chromosome (Elzinga *et al.*, 2007). Females have double copies of the X chromosome, and therefore two alleles of sex-linked genes are carried by them, one on each X chromosome while males have only one X chromosome which carries one allele of a sex-linked gene (Gardner *et al.*, 1991).

This implies that whatever allele is passed on the X chromosome of males will be expressed regardless of dominant or recessive nature. The expression of the alleles follows the dominant or recessive traits as females inherit two alleles for sex-linked genes (Elzinga *et al.*, 2007). Sex-linked traits are generally inherited with the autosomal chromosomes.

Sex linkage has been indicated for optic atrophy, juvenile glaucoma, myopia,

defective iris, color blindness, epidermal cysts, distichiasis, white occipital lock of hair, mitral stenosis, and some form of mental retardation in human (Gardner *et al.*, 1991). Sex-linked disease in Holstein cattle is a form of hairlessness with tooth problems, termed as “anhidrotic ectodermal dysplasia” (Drogemuller *et al.*, 2001). Sex-linked trait involves in the tortoise shell of cats and hemophilia in horse and barred colour in chicken. The explanation given for sex-linked inheritance in *Drosophila* applicable equally to traits in humans and livestock, poultry those are associated with genes on the X- chromosome (Gardner *et al.*, 1991).

Behaviour is the most complex phenotypes that are considered to be the action of an animal in response to its internal and external environment (Sokolwski, 2001). The feeding and sexual behaviour is very important for survive and reproduction of next generation, respectively. The feeding behaviour of drosophila was studied by Milisav (2009) and they reported that usually drosophila feed fermented products from the nature but when they are cultured in culture media they feed from it. The sexual behaviour was studied by William *et al.* (2000) and they observed that the female does not mate for about 10 to 12 hours after emerging from the pupa. Usually the male touches the female's body with his foreleg (tapping) for approaching for mating (Spieth, 1974).

Several studies were conducted regarding sex-linked inheritance (Elzinga *et al.*, 2007) and behavioural study (William *et al.*, 2000) of *Drosophila melanogaster*. However, very little information is available on sex-linked trait inheritance and behaviour of the fruit flies and other higher species (cattle, sheep, goat etc.) in Bangladesh. However, such study develops knowledge to delete and/or remedy of the effect of sex-linked recessive

gene and best individuals can be selected for the improvement of the economic traits. Sex-linked inheritance study is difficult to conduct in human and always in other animals (sheep, goat and cattle) due to their longer generation interval, gestation period, small litter size and also social and ethical manner. Furthermore, the feeding and sexual behaviour study for higher animal and human being is also sometimes impossible due to lack of proper facilities, time and also for the same biological, social and ethical constraints. Therefore, the study was aimed to identify the sex-linked trait and their inheritance pattern in *D. melanogaster* and to determine the feeding and sexual behaviour of *D. melanogaster*.

MATERIALS AND METHODS

A total of 100 drosophila were trapped in plastic bag where ripen fruits like banana and lemon were kept prior to the gathering of drosophila. Then the collected drosophila was cultured and let them to multiply by mating on standard culture media. For handling and observation of drosophila, the moving flies were anesthetized using lower temperature (4 °C) in a refrigerator leaving for 8-10 minutes. Then the sex of *D. melanogaster* was determined based on their body size, caudal end, anal plate, presence and absence of genital arch, sex comb and abdominal segments under 4x objectives of microscope (Olympus CH-2 Laboratory Microscope) in the Animal Genetics laboratory at Chittagong Veterinary and Animal Sciences University.

Mating Design

The mating unit comprised the male and female ratio at 1:5. This mating ratio was maintained for the sex-linked trait (eye colour) and the mating design is sketched below as:

P (Parents) X^+X^+ × X^wY
 Wild-type ♀ (Red eye) ↓ White-eyed ♂

F₁ ♂ Gametes
 X^w Y
 ♀ Gamete X^+

X^+X^w Wild-type ♀	X^+Y Wild-type ♂
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 X^+X^w × X^+Y
 Wild-type ♀ ↓ Wild-type ♂

F₂ ♂ Gametes
 X^+ Y
 ♀ Gametes X^+

X^+X^w Wild-type ♀	X^+Y Wild-type ♂
X^+X^w Wild-type ♀	X^wY White-type ♂

 X^w

Females : all wild-type /Red eye

Males : 1/2 wild-type /Red eye

1/2 white-eyed

Generation I : F₁ (Red eye ♀) × F₁ (White eye ♂)

Generation II : F₂ (Red eye ♀) × F₂ (White eye ♂)

Generation III : F₃ (Red eye ♀) × F₃ (White eye ♂)

Inheritance pattern of sex-linked trait

In this study sex-linked trait, eye color (red eye and white eye) was investigated. The identified adult male and female were placed into a culture vial at a ratio of 1:5, allowing them to mate to produce larvae. While the larval stage attained, the adults were transferred from the culture vial to another clean culture vial. The different eye colour individual was counted and inheritance trait was identified.

Feeding and sexual behavior of *Drosophila melanogaster*

The *Drosophila* (from egg to adult) was considered for the feeding and sexual behaviour study. During artificial culture, the feeding phenomenon of drosophila was observed to determine the time period and frequency of their feed intake from the culture media. Furthermore, during feeding whether they stood in a place and/or moved for feeding was also recorded.

Sexual behaviour was carefully observed and recorded either male or female approached their mate for mating, in what time they preferred for mating and how long they kept continuing their copulation. In addition, after completing their copulation how they behave each other was also observed and recorded.

Statistical analysis

The data for sex linked trait (eye colour) inheritance was collected. After collection the raw data was edited by using Microsoft Excel. χ^2 (Chi-square) test was performed to establish the agreement between observed and expected values.

The formula for Chi-square is:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where,

O = observed number of individuals

E = expected number of individuals

The significant differences tests were considered at 5% level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Sex-linked trait inheritance

The number of wild type (red type eye) and mutant type (white type eye) flies in different

generations from I to III of *D. melanogaster* are shown in Table 1. The successive generations up to third generation were produced by mating between the wild type and mutant type *D. melanogaster* which revealed that all four types of flies appeared up to 3rd generation (Table 1). In the case of eye colour the total number of wild type male and female offspring was 464 and 560, in F₁ generation. The frequency of both the alleles (wild and mutant) was equal and represented 50 percent in F₁ generation. But there were subsequent decline of mutant type from first to third generation (Table 2). This result indicates that all flies were wild type after mating between wild type and mutant type flies in F₁ generation. They were heterozygotes but their phenotypes were similar. Moreover, the number of female flies both wild and mutant type was higher than the male flies. The declining of white eye colour occurs due to effect of selection. In the current study selection of wild type trait (desirable gene) was done against the mutant type trait (undesirable gene) of *Drosophila*. Similar findings were reported by Merrell (1953) and Reed and Reed (1950) during their selective mating experiments against undesirable trait of *D. melanogaster*.

Table 1. Number of wild type and mutant type flies in different generation of *Drosophila melanogaster*.

Generation	Observed and expected Values	Sex-linked trait (Eye Colour)				Total	χ^2
		Red eye	White eye	Red eye	White eye		
		Female	Female	Male	Male		
I	Observed	330	278	322	230	1160	17.93*
	Expected	290	300	280	290		
II	Observed	310	270	370	120	1070	87.51*
	Expected	268	290	245	267		
III	Observed	316	258	314	92	980	116.11*
	Expected	245	250	240	245		

* Significant at 5% Level of Significance (P<0.05).

Table 2. Number of mutant males and the frequency of white gene in competition experiment of *Drosophila melanogaster*.

Generation	Eye colour	
	Male flies (n)	White eye colour allele frequency
I	552	41.67
II	490	24.49
III	406	22.66

The white eyed flies decreased in number in subsequent generation due to selective mating and a quick decline of mutant flies (white eye flies) were occurred in 3rd generation. Due to selection experiment the mutant type's males were less active in sexual performance than the wild types males. That's why rapid elimination of mutant gene occurred due to reduced sexual activity of mutant types males with less fecundity and viability. There were significant ($P < 0.05$) differences found between observed and expected values. Similar finding was reported for white eye gene in *D. melanogaster* by Singh (2012).

Feeding behaviour of *Drosophila melanogaster*

The life cycle of *D. melanogaster* comprised with the four stages including egg, larva, pupa and adult. The eggs are laid by *Drosophila* on the media, the larva after hatching from the egg, they were become intensely active and voracious. The feeding behaviour was observed in the adult stage up to producing egg and 1st instar larvae period. The larvae were situated on the media and they ate media all the time up to pupa stage. Then the pupa was crawl to dry place after 4 to 5 days later. The adult flies were observed for feeding studies for 24 hours. The flies were away from the media in maximum time. They came to touch in media for 20 to 30 minutes in 1 to 1.3 hours later. This pattern of feeding was repeated after that time interval. The flies took food from one

side or one place of the media. Sometimes they change their site for taking food otherwise they were stay at their feeding site in culture media. The flies consumed feed early in the morning and in the dawn than at the night. Moreover, the female flies feed more than male flies. Similar findings were reported by other studies (Ja *et al.*, 2007; Dubruille and Emery, 2008; Wong *et al.*, 2009).

Sexual behaviour of *Drosophila melanogaster*

The selected males and females were placed in culture vial for observing the mating pattern and time of copulation. At first the male came to close in female flies. The male was started to vibrate his wings for attracting the female. After 2 to 3 minutes the female allow the male. Then the male extends his proboscis and licks the female's genitalia. Then the male tried to grab the female body with his fore legs. After that male curled the tip of his abdomen which attempted to copulate. The female flies spread her wings allow the male to mount. The observed mating behaviour of male was similar with the other studies (Hall, 1994 and Greenspan, 1995). Usually the mounting is successful, both the male and female stay attach still for 15 to 20 minutes. These observations are closed with other findings (Yamamoto *et al.*, 1997; Spieth, 1974). The mating behaviour was observed frequently more during morning. The male flies were mated repeatedly to the female flies. The female

flies have also the acceptance or rejection power to choose mate partner. The mutant type males were less active in sexual activities than wild type male which are in agreement with the findings of other studies (Bastock, 1956; Elens, 1957).

CONCLUSION

This study revealed that the desirable gene (wild type gene) frequencies increased over undesirable gene (mutant type gene) from generation after generation. These findings were observed due to the selection of desirable trait over undesirable trait. The flies were more active in the early morning and in the dawn for feeding i.e. temperature has great effect on life cycle of *D. melanogaster* during the experimental period. This study will assist to identify and eliminate the undesirable genes from the population.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

REFERENCES

Bastock, M., 1956. A gene mutation that changes a behaviour pattern, *Evol.*, 10: 421-439.

- Drogemuller, C., Distl, O. and Leeb, T., 2001. Partial deletion of the bovine ED1 gene causes anhidrotic ectodermal dysplasia in cattle, *Genome-Research*, 11: 1699-1705.
- Dubruille, R. and Emery, P., 2008. A plastic clock: how circadian rhythms respond to environmental cues in *Drosophila*. *Mol. Neurobiol.*, 38: 129-145.
- Elens, A.A. 1957. Importance selective des differences d' activite antire males ebony etc. sauvage, dans les populations artificielles de *Drosophila melanogaster*, *Experientia*, 13: 293-294.
- Elzinga, C., Lawrence, S. M., Leege, L.M., Heidemann, M.K. and Straney, D.O. 2007. Biological Sciences 110: Laboratory Manual, 12th Edition, Plymouth, MI: Hayden-McNeil Publication, 2.
- Gardner, E.J., Simmons, M.J. and Snustad, D.P. 1991. Principles of Genetics. 8th Edition, John Wiley & Sons, INC, 84.
- Greenspan, R.J. 1995. Understanding the genetic construction of behavior. *Anim. Sci.*, 271: 72-78.
- Hall, J.C., 1994. The mating of a fly, *Sci.*, 264: 1702-1714.
- Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T. and Benzer, S. 2007. Prandiology of *Drosophila* and the CAFE assay. *Proc. Nat. Acad. Sci. USA*, 104: 8253-8256.
- Merrell, D.J. 1953. Selective mating as a cause of gene frequency changes in laboratory populations of *Drosophila melanogaster*. *Evol.*, 7: 287-296.
- Milislav, D., 2009. Biology of *Drosophila*. 10th edition, Cold Spring Harbor Laboratory; 1-2, (1950) Available at: <http://www.vguk.hr/novosti/akt/231-100.pdf> (Accessed March 03 2009), National Center for Biotechnology Information (NCBI). 2011. Genome Database: Retrieved 30/11/2011.

- Reed, S.C. and Reed, E.W., 1950. Natural selection in laboratory populations of *Drosophila* II, Competition between a white eye gene and its wild type allele, *Evol.*, 4: 34-42.
- Singh, A.K. 2012. Elimination of mutant types in selection experiment between wild type and mutant eye color in *Drosophila ananassae*. *J. Sci. Res.*, 56: 73-79.
- Sokolowski, M.B., 2001. *Drosophila*: Genetics meets behavior. *Nat. Rev. Genet.*, 2: 879-890.
- Spieth, H.T., 1974. Courtship behavior in *Drosophila*. *Annu. Rev. Entomol.*, 19: 385-405.
- Steel, R.G.D., Torrie, J.H. and Dickey, D.A., 1997. Principles and procedure of statistics - A biometrical approach. McGraw-Hill Co., Inc. NY and London, pp: 139-177.
- Williams, M., 2000. UAB center for community outreach development *Drosophila* Manual, University of Alabama at Birmingham Press: pp. 2-4.
- Wong, R., Piper, M.D., Wertheim, B. and Partridge, L., 2009. Quantification of food intake in *Drosophila*, *PLoS One*, 4: 60-63.
- Yamamoto, D., Jallon, J.M. and Komatsu, A., 1997. Genetic dissection of sexual behavior in *Drosophila melanogaster*. *Annu. Rev. Entomol.*, 42: 551-585.