ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 1(C), pp. 23023-23027, January, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

DO *DROSOPHILA* LARVAE PREFER LIGHT OR DARK?-AN IMPROVISED INVESTIGATORY PROJECT FOR HIGH SCHOOL STUDENTS TO GO BEYOND TEXT BOOK

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1383

ARTICLE INFO

Article History:

Received 15th October, 2017
Received in revised form 25th November, 2017
Accepted 23rd December, 2017
Published online 28th January, 2018

Key Words:

Phototaxis, practical classes, mutants, transition, rava-jaggery' medium.

ABSTRACT

Present study aims to explore the phototaxic behavior of larvae of three *Drosophila melanogaster* strains (namely Oregon K, sepia eye and white eye) with an improvised low-cost setup, which in turn may be a useful project for the school students of IX - XII classes. Simple light-dark preference test was carried out using a pair of glass Petri dishes with dark and light area, small cupboard and LED torch emitting 1000 lux light. Number of larvae in light and dark area was captured using a smart phone and their percentage was calculated. Results showed that all the three strains of larvae were negatively phototaxic and normal O.K larvae were more (84%) sensitive to light than the mutant sepia (70%) and white (71%) eyed larvae. There may exist a relation between phototaxis response and eye color. Further, as the pre and in-service teachers opined, this study can be an effective project for the higher secondary students for active learning by doing and to go beyond text book.

INTRODUCTION

Appropriate preference for light or dark can be crucial for an animal's survival (Gong *et al.*, 2010). Studies found two types of photo-receptor neurons (PRs) expressed by either Rhodopsin5 and Rhodopsin6 in the larval eye (Salcedo *et al.*, 1999, Sprecher *et al.*, 2007; Keene *et al.*, 2011; Hassan *et al.*, 2005) and Humber and Sprecher (2017) found that only the Rhodopsin5 subtype is essential for light avoidance. Gong *et al.* (2010) reported that two pairs of isomorphic neurons in the central brain switch the larval light preference. Further, Xiang *et al.* (2010) found that light-avoidance-mediating-photoreceptors tile the larval body wall. Carl Friendrich and Thomas (2017) mappd the larval eye's mind to understand the neural circuitary of larval vison. Further, Zhao *et al.* (2017) stated that turns with multiple and single head cast mediate *Drosophila* larval light avoidance. Studies also stated that, visual pathways which mediate *Drosophila* larval light avoidance and circadian clock entrainment are distinct (Keene *et al.*, 2011) and are developmentally related (Hassan *et al.*, 2005). Rodriguez and Campos (2009) reported that inactivation of DOPA decarboxylase neurons increases the response to light throughout larval development and Mapel *et al.* (2002) found the strong role of norpA-encoded phospholipase C signaling along with RH5 and Rh6 in both larval and adult extra-retinal circadian photoreception. Studies as well compared the larval and adult photoreception mechanisms and stated that larval and adult photoreceptors use different mechanisms to specify the same Rhodopsin fates (Sprecher *et al.*, 2007); and same transcription factors regulate diverse aspects of larval and adult photoreceptor development at different stages and in a context-dependent manner (Mishra *et al.*, 2013). Furthermore, Rodriguez and Campos (2009) stated that *Drosophila* larval photobehavior can be used to study the control of locomotion.

Practical classes bring the theory in action to verify Science concepts and provide empirical evidences. Not only that, they develop problem solving and reasoning ability, enhance mastering the subject, provide the scope for learning by doing, inculcates practical skills, trains in scientific methods and cultivates scientific temper (NCERT, 2006). Studies proved that, students learning with lots of experiments understand the concept well and get high marks in exams (Emerson and Taylor, 2004; Ball *et al.*, 2006). Correspondingly, Frank (1997) finds that, compared to students in a control class, students' homework scores increase when they participate in an experiment related to the homework topic.

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Teachers sometimes could not conduct effective practical classes because of non-availability of proper equipments or space especially in rural schools. In such situation low cost, improvised experiments which can be performed even at home can be very useful. In this context, present study aims to study the phototaxis behavior (light/ dark preference) of larvae of *Drosophila melanogaster* with an improvised low-cost setup, which in turn may be a useful experiment/ project for the school students of IX - XII class.

MATERIALS AND METHODS

Studies in the past used various highfy material and methods for deeper understanding of phototaxis. For example, high resolution computer based tracking analysis (Keene *et al.*, 2013; Gershow *et al.*, 2012; Hernandez-Nunez *et al.*, 2015) to quantify sensory motor structure of larvae. However, present study adapts/ improvises simple light-dark preference test as carried out by earlier studies (Gong, 2009; Gong and Gong, 2012; Farca Luna, 2013). A small cupboard (as dark box) having the dimension of 100x50x30 cm was taken and a white porcelain tile was kept at it's floor as shown in fig. 1.

Fig 1 Improvised experimental setup.

An LED torch was made hanging from the roof of the box and the torch emitted white light broad band spectra ranging from 450-600nm. The light falls on the larvae from 75cm height with 1000 lux intensity. Students can use even old carton boxes and small torch light. A pair of glass Petri dishes having the diameter of 15cm was taken and divided into four quarters. In each dish, opposite quarters and its' side wall was pasted with black paper as shown in fig. 2.

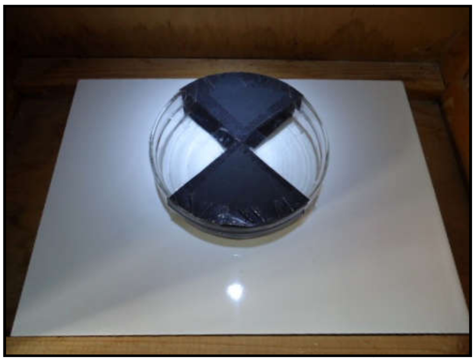


Fig 2 Two Petri dishes creates light and dark areas.

Three pure strains of *Drosophila melanogaster* viz. normal red eye (Oregon K) and mutants sepia eye and white eye flies were brought from the University of Mysore. These flies were cultured in glass bottle at room temperature with 12hr dark and 12hr light period in 'rava-jaggery' medium (Nagaraj, 2016). School students can collect the wild flies by keeping smashed banana in a bottle near the window. They can get larvae by rearing the flies in 'rava-jaggery' medium.

Mostly III instar larvae of O.K strain counting 10 numbers were collected from the side walls of the bottle using a brush. They were rinsed with Ringer's solution to remove the adhering food particles and kept in a plain Petri dish for 20 min for acclimatization. Meanwhile, one of the black paper pasted-Petri dishes was kept on the floor of the box. Then those larvae were transferred to the centre of this Petri dish and covered using another paper pasted Petri dish. The pasted black papers of two Petri dishes (upper and lower) were co-insides each other in order to create dark and light area (fig. 2) in the Petri dish. The torch light was ON and the door was closed in order to prevent the influence of external light, so that only the source light (torch light) effects the behavior of larvae. For every 5 minutes the door was opened very little and the position or number of larvae light and dark area was captured using a smart phone (without flash light) for about 2hrs. From the captured pictures the number and percentage of larvae in light and dark area was calculated and tabulated. Similar assay was performed for sepia and white eyed larvae in the next day.

RESULTS AND DISCUSSIONS

It is observed from the table 1 that, all the three strains of larvae showed negative phototaxis response. In support, to the present observation, other studies also found that the larvae are negatively photactic (Humberg and Sprecher, 2017; Sawin-McCormack *et al.*, 1995). Further, the average percentages of larvae in dark area shows that, among the three strains the normal O.K larvae are more (84%) sensitive (negatively) to light than the mutant sepia (70%) and white (71%) eyed larvae. Likewise, Sawin-McCormack *et al.*, (1995) observed that gl-mutant larva failed to respond to light during the foraging state and opined that this is likely due to lack of larval photoreceptors. Gong (2009) observed the preference of light to darkness by tim(01) larvae in the immediate light/ dark boundary passing test. Similarly, studies were also noted that, $norpA^{P41}$; cry^{b} double mutants were more affected than simple mutants in their entrainment to light-dark cycles (Emery *et al.*, 2000; Stanewsky *et al.*, 1998). In addition, average percentages also show that both mutants are equally sensitive (negatively) to light.

Moreover, the pattern of response which may be noted from the fig. 3-5 shows that, as the time progress red and sepia eyed larvae showed little inclination towards light (fig. 3 and 4), which may be due to acclimatisation / adjustment to light. On the contrary, white eyed flies initially moved towards light (not all) but, returned back to the dark as the time progress (fig. 5). It may be inferred from such response pattern that, there may exist a relation between phototaxis response and eye color (though proper eye was not developed). My previous study (Nagaraj, 2016) conducted with adult flies of same above three strain showed that, the flies are positively phototaxis and among them sepia eyed flies were fast in responding to light. Fascinatingly, studies also observed that, the larva in the earlier stage (I and II instar) was photo-negative (Mazzoni *et al.*, 2005; Scantlebury *et al.*, 2007; Yamanaka *et al.*, 2013), whereas in the later stage (III instar) it became photo-neutral

Table 1 Number and percentage of larvae in light and dark area

Strain	Red eye (N=10)				Sepia eye (N=10)				White eye (N=8)			
Time	Light		Dark		Light		Dark		Light		Dark	
(mins)	N	%	N	%	N	%	N	%	N	%	N	%
5	0	0	10	100	2	20	8	80	4	50	4	50
10	0	0	10	100	1	10	9	90	4	50	4	50
15	0	0	10	100	3	30	7	70	5	63	3	37
20	0	0	10	100	3	30	7	70	5	63	3	37
25	2	20	8	80	3	30	7	70	3	37	5	63
30	1	10	9	90	1	10	9	90	2	25	6	75
35	1	10	9	90	2	20	8	80	3	37	5	63
40	1	10	9	90	3	30	7	70	1	13	7	87
45	3	30	7	70	3	30	7	70	1	13	7	87
50	2	20	8	80	2	20	8	80	3	37	5	63
55	2	20	8	80	3	30	7	70	2	25	6	75
60	2	20	8	80	4	40	6	60	1	13	7	87
65	2	20	8	80	4	40	6	60	2	25	6	75
70	1	10	9	90	4	40	6	60	2	25	6	75
75	1	10	9	90	4	40	6	60	3	37	5	63
80	1	10	9	90	4	40	6	60	3	37	5	63
85	1	10	9	90	3	30	7	70	1	13	7	87
90	2	20	8	80	4	40	6	60	1	13	7	87
95	2	20	8	80	3	30	7	70	1	13	7	87
100	3	30	7	70	3	30	7	70	1	13	7	87
105	3	30	7	70	4	40	6	60	2	25	6	75
110	3	30	7	70	3	30	7	70	2	25	6	75
115	3	30	7	70	3	30	7	70	2	25	6	75
120	3	30	7	70	3	30	7	70	2	25	6	75
Average	2	16	8	84	3	30	7	70	2	29	6	71

Fig.3. Distribution of red eye larvae in light and dark areas over a preriod of time

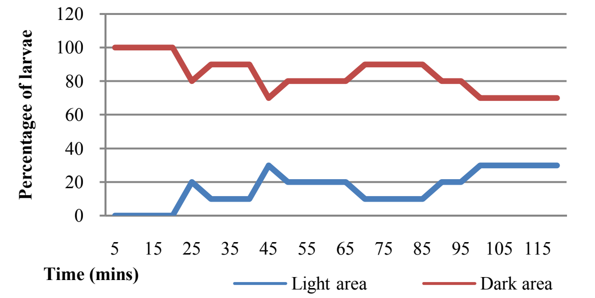


Fig.4. Distribution of sepia eye larvae in light and dark areas over a preriod of time

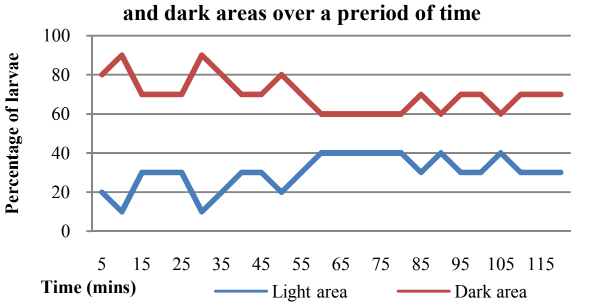
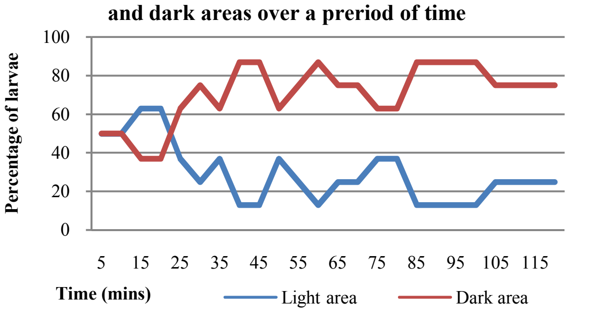


Fig.5. Distribution of white eye larvae in light and dark areas over a preriod of time



(Sawin-McCormack *et al.*, 1995; Rodriguez and Campos, 2009) and becomes photophilic with age (Godoy-Herrera *et al.*, 1984) or adult-hood (Gong *et al.*, 2010). Such transition may be due to the development from immersed foraging stage to active wandering stage in search of place for pupation (Humberg and Sprecher, 2017; Bainbridge and Bownes, 1981). In contrast, Yamanaka *et al.* (2013) reported that wandering larvae are photo-negative and prefer to pupate in darkness. Furthermore, von Essen *et al.* (2011) made an interesting observation that larva prefer food in darkness over food in light. Besides, Warrick *et al.* (1999) reported that photo-negativity of the larva can be elicited by light with wave length ranging from UV to green. Additionally, studies found that, changes in light intensity elicit turning behavior (Hassan *et al.*, 2000; Scantlebury *et al.*, 2007).

Further, to know the relevance of this experiment/ study to the school students, it was demonstrated to the pre-service teachers (of RIE, Mysuru) and in-service teachers (of JNVs). They opined that this can be an effective project for the higher secondary students to train them in scientific method and to go beyond text book.

CONCLUSION

It may be concluded from the above results that, all the three strains (O.K, se and wh) of larvae are negative phototaxic, and among them normal O.K larvae are more sensitive to light than the two mutants. There may exist a relation between phototaxis response and eye color. Further, as the pre and in-service teachers opined, this experiment can be an effective project for the higher secondary students for active learning by doing and to go beyond text book.

Acknowledgement

Authors sincerely thank Mr. Santhosh, Physics section, I/C Zoology section, Head DESM and the Principal of RIE, Mysuru and the Director, NCERT, New Delhi, for their moral support and encouragement.

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How to cite this article:

Nagaraj G.2018, Do Drosophila Larvae Prefer Light or Dark?-an Improvised Investigatory Project For High School Students To Go Beyond Text Book. *Int J Recent Sci Res.* 9(1), pp. 23023-23027.

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1383

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