Paper ID: UGC 48846-804

THERMOTAXIS ASSAY ON DROSOPHILA MELANOGASTER USING HAIR DRYER GENERATED THERMAL DIVISION SETUP: SIMPLE INVESTIGATORY PROJECT FOR HIGH SCHOOL STUDENTS

G. Nagaraj
Zoology Section, Regional Institute of Education (NCERT), Manasagangothri, Mysuru-570006, Karnataka, India.

Abstract: Present study aims to make a comparative study on thermotaxis behavior among the three strains (OK, se and w) of adult Drosophila melanogaster using an improvised setup and to propose this simple investigation as a project for IX – XII class students. Using low-cost materials like OHP sheets, 90cm long tubes were prepared and thermal division / difference of 10 ºC (24.5 and 34.5 ºC) were created using hot air from hair dryer. With the standardised method thermal preference was assayed. Results show that all the three strains of flies were negatively themotaxic, avoiding higher and preferring lower temperature; and se flies were more sensitive than others. Further, presently improvised setup provides a new and simple method for investigating thermotaxis in Drosophila. Therefore, this experiment is proposed as a project for the students of IX to XII classes to develop inquisitive mind.

Key Words: Thermal gradient, improvisation, mutants, smart counting and bet-hedging strategy.

Introduction

Drosophila melanogaster is a poikilothermic organism that must sense and respond to both fine and coarse changes in environmental temperature for comfortable survival [1]. Even minor deviations in environmental temperature can have major impacts on development and lifespan [2] and ultimately the Darwinian fitness [3]. Drosophila uses multiple, redundant signaling pathways and neural circuits to execute thermotaxis behavior. Recent works has uncovered some of the key molecules mediating flies' thermo-sensation including the Transient Receptor Potential (TRP) channels [4], [5] and Gustatory receptor Gr28b [6]. Reference [7] found that for slow response TRPA1 is required and Gr28B is for rapid response. Studies have identified in the brain a set of warmth-activated anterior cell neurons [8], Dorsal Organ Cool Cells – a set of thermo-sensitive neuron for larval cool avoidance [9], [10]. Reference [11] stated that fine thermal discrimination of larva is depends on multiple rhodopsins and reference [12] found that projection neurons get excited by cooling, warmth or both in Drosophila. Similarly, reference [13] reported that, parallel mushroom body circuits- the β' and β-systems control temperature-preference in Drosophila during aging. On the other hand, reference [14] reported that histamine and its receptors modulate temperature-preference behavior and reference [15] demonstrated that neuropeptide diuretic hormone 31 (DH31) and Pigment Dispersing Factor Receptor (PDFR) contribute to regulate the preferred temperature decrease at night-onset. Variability in thermal preference may reflect an adaptive bet-hedging strategy [16]. Further, D melanogaster rely on behavioral strategies to stabilize their body temperature [17] and reference [18] opined that such thermoregulatory strategies may shape immune investment.

For in-depth understanding of thermotaxis behavior of Drosophila (larvae / adult) studies in the past used various methods and materials. Basically, in all those methods a gradient is typically created by heating and cooling opposite ends of a thermally conductive material [19], [20], [21], [22]. For instance, horizontal thermal gradient apparatus [23], [11], aluminium blocks containing temperature controlled circulating water [24], using lamp as

Paper ID: UGC 48846-804

a heat source thereby creating a photo-thermal gradient rather than just a thermal gradient [25], Peltier device, water tubes and air-cooling fans which are connected to a computer cooling system [26], tracking microscope with infrared laser spot light [27] are some of the complex and expensive instruments used in the past [28], [29]. In this circumstance, present study aims to make a comparative study on thermotaxis behavior among the three strains of adult Drosophila melanogaster using an improvised low-cost apparatus and to propose this simple investigation as a project for IX – XII class students.

Materials and Methods

Apparatus was designed using inexpensive, simple and easily available materials. Firstly, six numbers of transparent plastic tubes of 90 cm length and 2.5 cm diameter was prepared using OHP sheets as described in my earlier study [30]. They were graduated to 0-80 cm and marked in to four quarters of 20 cm length each. Using a hair dryer a difference of 10°C in temperature between left (0-40 cm) and right (41-80 cm) half of the tube was created by placing a thick card board (old file board) partition at the middle (at 40 cm) of the tube as shown in experimental setup (figure 1). A constant parallel light source was placed at about 100 cm height from the tube throughout the experiment. Left half of the tube had room temperature (24-25°C) and in the right part 10°C higher temperature (34-35°C) was maintained by standardised periodical ON and OFF of the hair dryer.

Figure 1. Experimental setup

Standardisation of the time required to maintain 35°C (±0.5°C)

To maintain temperature difference (in the tube) throughout the experiment, firstly the time required between ON and OFF of the hair dryer was standardised by the following two steps:

(a) Establishing thermal division (i.e. temperature difference)

A graduated transparent tube was placed horizontally on a thermo-coal sheet and six thermometers were kept at different areas viz. inside the left (B) and right (E) part of the tube, at left front (A) and back (C), right front (D) and back (F) of the tube as shown in figure 2. A cardboard sheet was placed in the centre to divide the tube in to two halves. Left and right ends of the tube were closed with an empty bottle and cotton respectively.

To test the applicability / efficiency of the partition board, a hair dryer was kept 20 cm away from right end of the tube and continuously ON for 1 hour. Initial (room) temperature in all the six thermometers (A to F) was 26°C and their temperature in every 10 minutes up to 1hour was noted and tabulated. Table 1 shows that in the 'A' thermometer area the temperature remained the same (26°C) but, in 'B' and 'C' thermometer area it reached to 29°C and 30°C respectively. On the otherhand, in the right area the three thermometers (D, E,

Paper ID: UGC 48846-804

and F) showed nearly about 10°C higher temperature i.e. 41, 39 and 42°C respectively after 1 hour. It is evident from the table that, the partition board helps to maintain temperature difference (thermal division) between left and right halves of the tube. It is also clear that, after switch ON of the hair dryer in the fifth minute 10°C temperature difference between left and right half of the tube was attained/ established. The same can be used as thermal gradient setup after removing partition board. Now, this difference has to be maintained throughout the experiment by periodical ON and OFF of the hair dryer.

(b) Determination of the time required between ON and OFF of the hair dryer

It may be observed from table 2 that, after attaining 34.5°C, the temperature reached to 35°C in 30 seconds and 35.5°C at 62 seconds. Then the hair dryer was put OFF and temperature reached to 35°C in 30 seconds, then 34.5°C in 60 seconds. Similarly, ten observations were made and found that 60 seconds was the time period required between ON and OFF of the hair dryer to maintain 35°C throughout the experiment.

Collection of Drosophila melanogaster

Two genetically defined homozygous mutants viz. sepia eye (se) and white eye (w) and a normal red eye (Oregon K (OK)) Drosophila melanogaster flies were brought from the University of Mysore. In 'rave-jaggery' medium [30] flies were cultured at room temperature (23-26 °C) for 12hrs dark and 12hrs light period. School students can collect wild fruit flies using banana fruits in bottles and perform the experiment.

Conducting thermotaxis assay

Unknown number of 2-7 days old OK flies was transferred to an empty bottle. Without anesthetisation of flies their number were counted with an improvised and smart technique using commonly available technology that is, using a smart phone. Photos were taken (in different angles) and counted the number of flies (smart counting) on the screen itself (figure 3). Bottle was kept under tube-light (40W) for 30 minutes for acclimatisation of flies.

Figure 3. Counting of flies in smart phone

Standardised apparatus was arranged and a bottle with counted (N=30) and acclimatised O.K flies were introduced at the left end of the tube. Hair dryer was put ON or OFF at standardised time interval (60 seconds) to maintain 35°C at the right half of the tube whereas, left half had room temperature i.e. 25°C. Simultaneously, similar to the experimental setup a control setup (figure 4) which is without partition board and hairdryer was maintained and assay was performed at room temperature (25°C).

Paper ID: UGC 48846-804

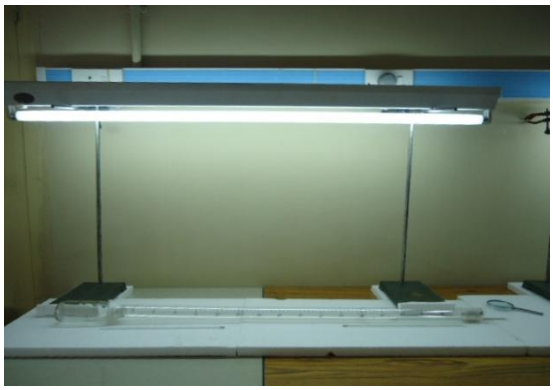


Figure 4. Control setup

Number of flies in each quarter (Q1-Q4) was noted for every 15 minutes for 5hrs in both control and experimental tubes. Percentages were calculated and results were tabulated and graphed. Likewise, the thermotaxis of other two mutants (se and w) was also studied in the next two days.

Result and Discussion

It is evident from the table 3 and figures 5 and 6 that, on average in control tube, 34.9% of OK flies were found in Q1 and 7.8, 3.4 and 11.3% in Q2, Q3 and Q4 respectively which may indicate distribution / movements of flies to the whole length of the tube. In case of experimental tube that was 54.6% in Q1 and 7.7% in Q2 but, almost nil in the warmer half i.e. Q2 and Q3. Further, almost first two hours the OK flies did not cross Q1 both in control and experimental tubes. Similarly, 57.4% of flies came out of control bottle in to the tube (remaining flies remained in the bottle) whilst 63.2% in experimental setup.

Likewise, average percentages of se flies in Q1, Q2, Q3 and Q4 of control tube were 36.2, 12.6, 3.6 and 12.3% respectively (table 4 and figures 7 and 8) whilst in experimental tube 46.5, 8.4, 0.3 and 0%. Totally 64.7% of se flies came out of the bottle in the control tube whereas that was 55.2% in experimental tube. Control flies after 30minutes reached to / found in whole length of the tube (Q1-Q4) but experimental flies after 180 minutes only moved to Q2 (if we ignore the only one fly which reached and remained in Q2 at 60 minutes).

Further, the average percentages of w flies were 48.5, 10.4, 8.7 and 8.2% in four quarters of the control tube respectively, whilst 41.2, 18.35, 1.5 and 0.3% in experimental tube (table 5 and figures 9 and 10). Totally 75.8% of w flies came out of the bottle in to the control tube whilst, 61.3% in experimental tube.

It is evident from the graph (figures 5-10) that, when time increases, all the three strains of flies in their control tube tend to be distributed equally and showed same pattern of distribution but, in case of experimental tube it was not. Further, it is also prominent that all the three flies are negatively sensitive to higher temperature (35^{o}C) and se flies are more sensitive than other two strains. In support, reference [14] found that the histaminergic mutants showed reduced tolerance for high temperature and enhanced tolerance for cold temperature. Furthermore, reference [3] reported that, peripheral nerves of Painless mutants show diminished response to high temperature (42^{o}C) stimulation, constant with a role for Painless in the detection of high temperatures. In addition, reference [16] observed that individual Drosophila melanogaster flies exhibit striking variation in light and temperature preference behaviors.

Regardless of genetic background, w^{1118}, yw and Canton S flies exhibits 1-1.5^{o}C increased temperature preference during day time [26]. Many experiments have found that, Drosophila aggregate at the edges of a gradient apparatus at uniform temperature [31], [32].

Paper ID: UGC 48846-804

Similarly, when exposed to a range of temperatures, the larva showed negative or positive thermotaxis in order to occupy an intervening preferred temperature range [33], [34], [35].

Reference [8] stated that, flies that selectively express dTrpA1 in the anterior cell neurons select normal temperatures whereas, flies in which dTrpA1 function is reduced or eliminated choose warmer temperature. Correspondingly, reference [5] and [2] opined that choose of ideal temperature (18^{o}C) over the other comfortable temperature (19 to 24^{o}C) depends on heterotrimetric guanine nucleotide-binding protein, phospolipase C and TRP channel. Similarly, reference [36] identified protein isoforms namely dTRPA1-C and dTRPA1-D and revealed a 37-aminoacid-long intracellular region that is critical for dTRPA1 temperature response.

Recently, reference [26] identified circadian rhythm of temperature preference in Drosophila in which preferred temperature of flies rises during day and falls during night; further, they also found that light affects fly's temperature preference independent of the circadian clock. Similarly, others observed the rhythmic fluctuation of body temperature of Drosophila over 24 hour period [37] and which decreases during night which is associated with sleep initiation [38].

Researches also explore the factors that affect the temperature preference of Drosophila. For example, reference [39] demonstrated that Drosophila prefers more or less 1^{o}C higher temperature when exposed to acute light rather than dark. Flies decreased their temperature preference (from 26.3^{o}C to 25.2^{o}C) when infected with Pseudomonas aeruginosa [40] and fungus [41]. Similarly, reference [28] reported that, females were more sensitive than males to higher temperatures and flies originating from high latitude and temperate region exhibited greater preference for cooler temperature. Other affecting factors are age [26], larval rearing temperature [23], high concentration of vitamin E [42] and defect in genes encoding histamine-gated chloride channel [14]. Further, larva achieves and maintains favorable temperature by regulating run length, size and direction of turns [27]. In general, insects will tend to spend more time at the cold end of the gradient simply because they move more slowly (or stop) in colder temperature [3] since their metabolic rates and rate of movements depends on environmental temperature [43].

In addition, this experiment was demonstrated to the Vth semester B. Sc. Ed teacher trainees of our institute (RIE, Mysuru) and they also practiced it. Further, all of them opined that this experiment can be performed by the high and higher secondary school students. They also expressed that they will give this experiment as a project to their students to go beyond text book.

Paper ID: UGC 48846-804

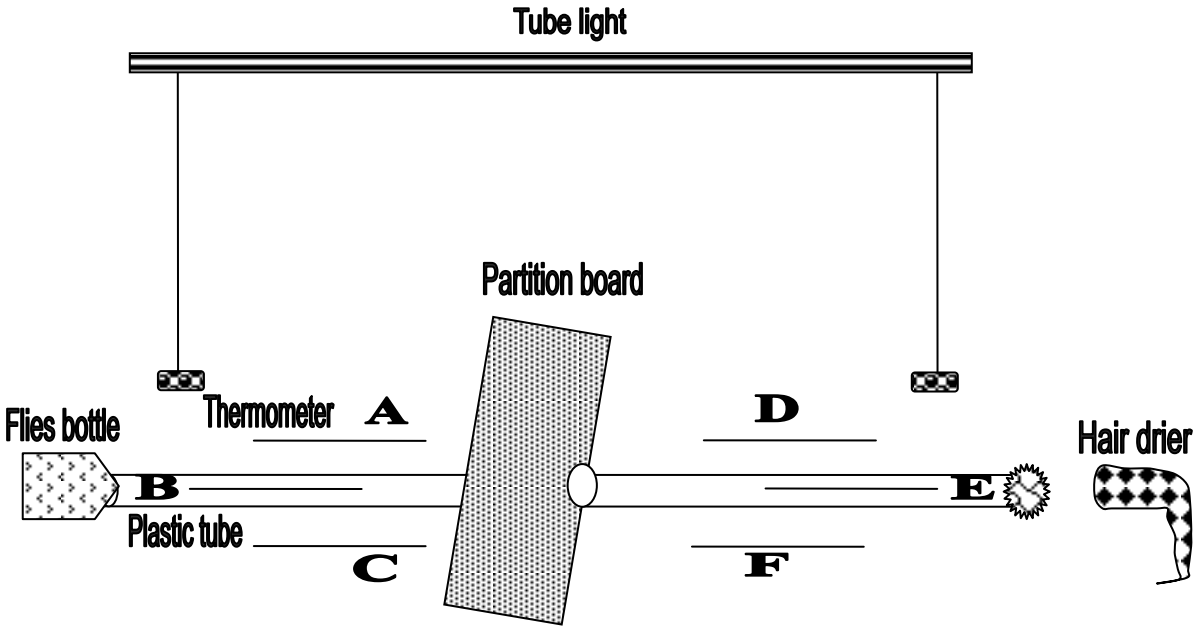


Figure 2. Place of six thermometers (A-F)

Table 1. Changes in temperature in thermometers (A to F) of different areas in relation to time

Thermometer (in different areas)	Time line (minutes)											
	0	1	2	3	4	5	10	20	30	40	50	60
	Temperature (°C)											
A	26	26	26	26	26	26	26	26	26	26	26	26
B	25.5	26	26	26.5	26.8	27	27.5	29	30	29.5	29	29
C	26	26	26.1	26.5	27	27.5	28	31	30.6	32	30	30
D	25.5	36.5	40	41	42	42	43	42.5	42	40	42	41
E	25	28.1	31.5	33	35	36	38	38.8	39	38	39	39
F	25.5	34	37.7	39	40.5	41	41	40	41	42	41	42

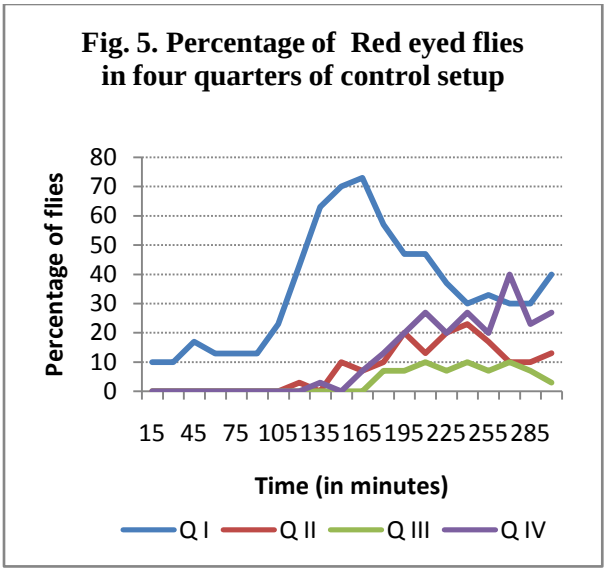
Table 2. Time required between ON and OFF of the hair dryer

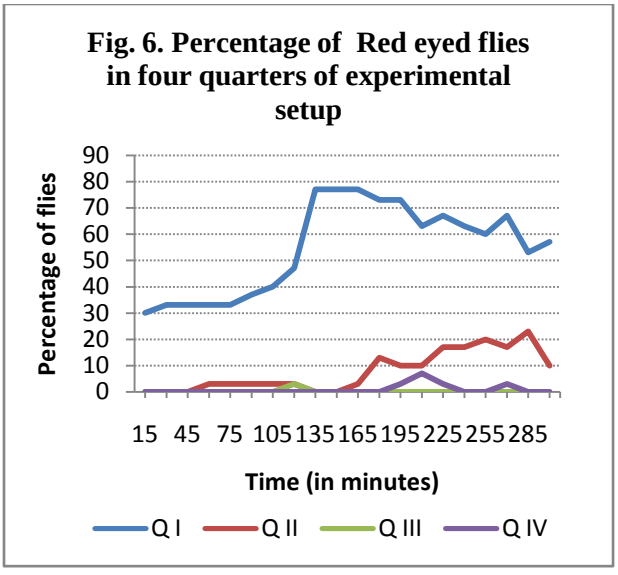
Time required for ON and OFF (in seconds)																					
ON	**34.5°C**	0	60	0	62	0	60	0	60	0	60	0	60	0	60	0	60	0	60	0	60
	35°C	30	30	32	31	30	30	31	30	31	30	30	31	30	30	31	30	30	30	30	31
OFF	**35.5°C**	62	0	62	0	60	0	60	0	62	0	60	0	60	0	60	0	60	0	60	0

Paper ID: UGC 48846-804

Table 3. Comparison of number and percentage of Red eyed (Oregon K) Drosophila melanogaster flies in four quarters (Q I-Q IV) of control and experimental setup

Time (minutes)	CONTROL (N=30)								EXPERIMENTAL (N=30)							
	Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)		Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15	3	10	0	0	0	0	0	0	9	30	0	0	0	0	0	0
30	3	10	0	0	0	0	0	0	10	33	0	0	0	0	0	0
45	5	17	0	0	0	0	0	0	10	33	0	0	0	0	0	0
60	4	13	0	0	0	0	0	0	10	33	1	3	0	0	0	0
75	4	13	0	0	0	0	0	0	10	33	1	3	0	0	0	0
90	4	13	0	0	0	0	0	0	11	37	1	3	0	0	0	0
105	7	23	0	0	0	0	0	0	12	40	1	3	0	0	0	0
120	13	43	1	3	0	0	0	0	14	47	1	3	1	3	0	0
135	19	63	0	0	0	0	1	3	23	77	0	0	0	0	0	0
150	21	70	3	10	0	0	0	0	23	77	0	0	0	0	0	0
165	22	73	2	7	0	0	2	7	23	77	1	3	0	0	0	0
180	17	57	3	10	2	7	4	13	22	73	4	13	0	0	0	0
195	14	47	6	20	2	7	6	20	23	73	3	10	0	0	1	3
210	14	47	4	13	3	10	8	27	19	63	3	10	0	0	2	7
225	11	37	6	20	2	7	6	20	20	67	5	17	0	0	1	3
240	9	30	7	23	3	10	8	27	19	63	5	17	0	0	0	0
255	10	33	5	17	2	7	6	20	18	60	6	20	0	0	0	0
270	9	30	3	10	3	10	12	40	20	67	5	17	0	0	1	3
285	9	30	3	10	2	7	11	23	16	53	7	23	0	0	0	0
300	12	40	4	13	1	3	8	27	17	57	3	10	0	0	0	0
Average%	**34.9**		**7.8**		**3.4**		**11.3**		**54.6**		**7.7**		**0.1**		**0.8**	

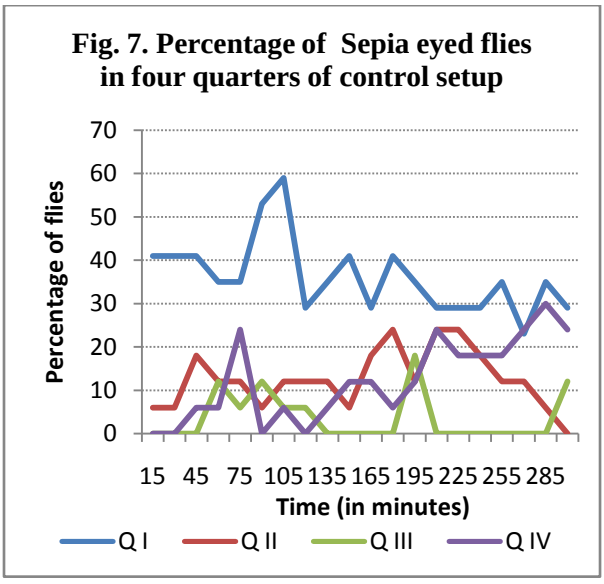


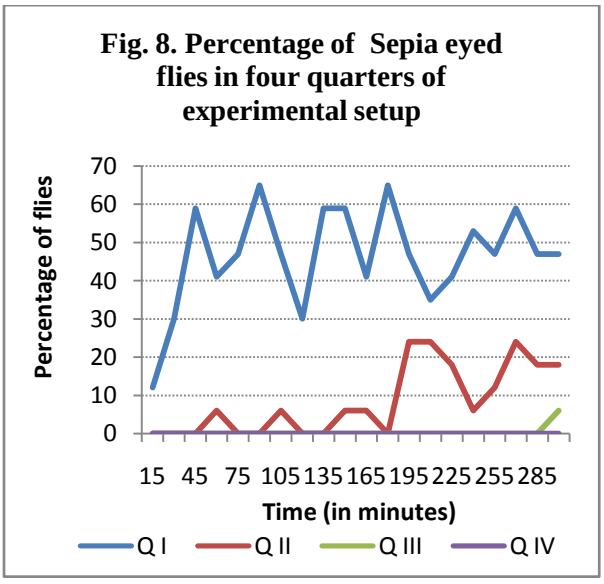


Paper ID: UGC 48846-804

Table 4. Comparison of number and percentage of Sepia eyed Drosophila melanogaster flies in four quarters (Q I-Q IV) of control and experimental setup

Time (minutes)	CONTROL (N=17)								EXPERIMENTAL (N=17)							
	Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)		Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15	7	41	1	6	0	0	0	0	2	12	0	0	0	0	0	0
30	7	41	1	6	0	0	0	0	5	30	0	0	0	0	0	0
45	7	41	3	18	0	0	1	6	10	59	0	0	0	0	0	0
60	6	35	2	12	2	12	1	6	7	41	1	6	0	0	0	0
75	6	35	2	12	1	6	4	24	8	47	0	0	0	0	0	0
90	9	53	1	6	2	12	0	0	11	65	0	0	0	0	0	0
105	10	59	2	12	1	6	1	6	8	47	1	6	0	0	0	0
120	5	29	2	12	1	6	0	0	5	30	0	0	0	0	0	0
135	6	35	2	12	0	0	1	6	10	59	0	0	0	0	0	0
150	7	41	1	6	0	0	2	12	10	59	1	6	0	0	0	0
165	5	29	3	18	0	0	2	12	7	41	1	6	0	0	0	0
180	7	41	4	24	0	0	1	6	11	65	0	0	0	0	0	0
195	6	35	2	12	3	18	2	12	8	47	4	24	0	0	0	0
210	5	29	4	24	0	0	4	24	6	35	4	24	0	0	0	0
225	5	29	4	24	0	0	3	18	7	41	3	18	0	0	0	0
240	5	29	2	18	0	0	3	18	9	53	1	6	0	0	0	0
255	6	35	3	12	0	0	3	18	8	47	2	12	0	0	0	0
270	4	23	2	12	0	0	4	24	10	59	4	24	0	0	0	0
285	6	35	1	6	0	0	5	30	8	47	3	18	0	0	0	0
300	5	29	0	0	2	12	4	24	8	47	3	18	1	6	0	0
Average%	**36.2**		**12.6**		**3.6**		**12.3**		**46.5**		**8.4**		**0.3**		**0**	

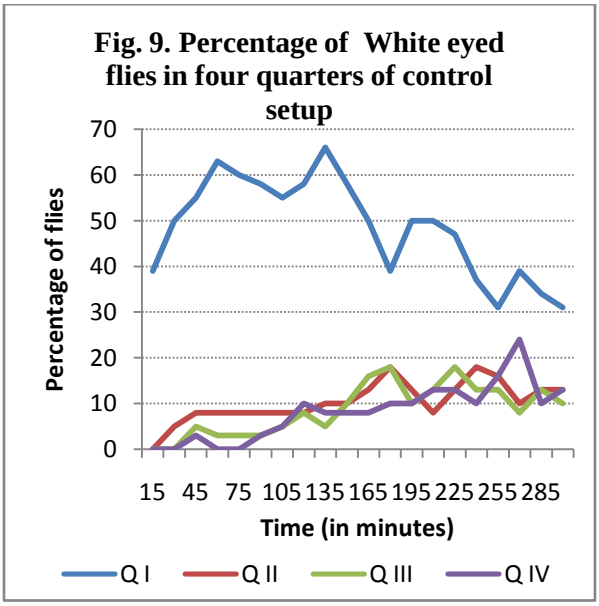


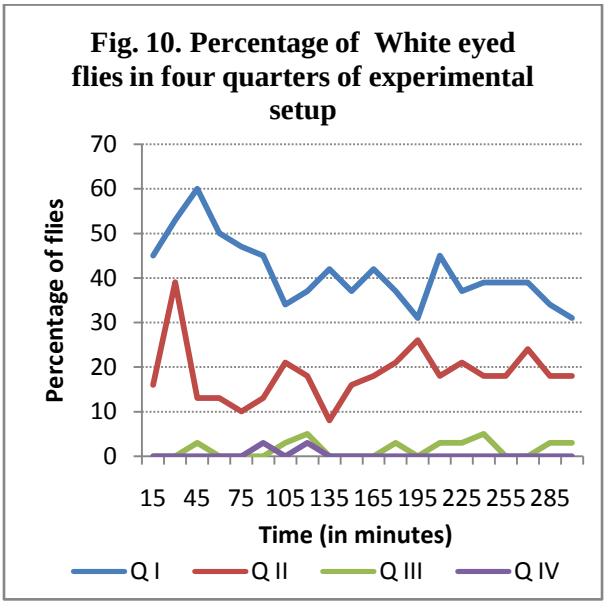


Paper ID: UGC 48846-804

Table 5. Comparison of number and percentage of White eyed Drosophila melanogaster flies in four quarters (Q I-Q IV) of control and experimental setup

Time (minutes)	CONTROL (N=38)								EXPERIMENTAL (N=38)							
	Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)		Q 1 (0-20 cm)		Q 2 (21-40 cm)		Q 3 (41-60 cm)		Q 4 (61-80 cm)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
15	15	39	0	0	0	0	0	0	17	45	6	16	0	0	0	0
30	19	50	2	5	0	0	0	0	20	53	15	39	0	0	0	0
45	21	55	3	8	2	5	1	3	23	60	5	13	1	3	0	0
60	24	63	3	8	1	3	0	0	19	50	5	13	0	0	0	0
75	23	60	3	8	1	3	0	0	18	47	4	10	0	0	0	0
90	22	58	3	8	1	3	1	3	17	45	5	13	0	0	1	3
105	21	55	3	8	2	5	2	5	13	34	8	21	1	3	0	0
120	22	58	3	8	3	8	4	10	14	37	7	18	2	5	1	3
135	25	66	4	10	2	5	3	8	16	42	3	8	0	0	0	0
150	22	58	4	10	4	10	3	8	14	37	6	16	0	0	0	0
165	19	50	5	13	6	16	3	8	16	42	7	18	0	0	0	0
180	15	39	7	18	7	18	4	10	14	37	8	21	1	3	0	0
195	19	50	5	13	4	10	4	10	12	31	10	26	0	0	0	0
210	19	50	3	8	5	13	5	13	17	45	7	18	1	3	0	0
225	18	47	5	13	7	18	5	13	14	37	8	21	1	3	0	0
240	14	37	7	18	5	13	4	10	15	39	7	18	2	5	0	0
255	12	31	6	16	5	13	6	16	15	39	7	18	0	0	0	0
270	15	39	4	10	3	8	9	24	15	39	9	24	0	0	0	0
285	13	34	5	13	5	13	4	10	13	34	7	18	1	3	0	0
300	12	31	5	13	4	10	5	13	12	31	7	18	1	3	0	0
Average%	48.5		10.4		8.7		8.2		41.2		18.35		1.55		0.3	





Paper ID: UGC 48846-804

Conclusion

All the three strains (OK, se and w) of flies were negatively themotaxic, avoiding higher temperature (34.5 °C) and preferring lower temperature (24.5 °C). Among them se flies showed relatively reduced tolerance for high temperature. Further, presently improvised setup provides a new and simple method for investigating thermotaxis in Drosophila. Therefore, this experiment is proposed as a project for the students of IX to XII classes to develop inquisitive mind.

Acknowledgements

Author sincerely thanks Dr. V. Tangpu, Zoology Section, the Head of DESM, the Principal, Regional Institute of Education, Mysore and the Director, National Council of Educational Research and Training, New Delhi for encouragement and support to carry out the study.

References

[1] Bellemer A. (2015). Thermotaxis, circadian rhythms, and TRP channels in Drosophila. Temperature (Austin), 11; 2(2):227-43.

[2] Kwon Y, Shim HS, Wang X, Montell C. (2008). Control of thermotactic behavior via coupling of a TRP channel to a phospholipase C signaling cascade. Nat Neurosci, 11(8):871-873.

[3] Dillonn ME, Wang G, Garrity PA, Huey RB. (2009). Thermal preference in Drosophila. J Thermal Biol, 34:109-119.

[4] Rosenzweig M, Brennan KM, Tayler TD, Phelps PO, Patapoutian A, Garrity PA. (2005).The Drosophila ortholog of vertebrate TRPA1 regulates thermotaxis. Genes Dev, 15; 19(4):419-24.

[5] Shen WL, Kwon Y, Adegbola AA, Luo J, Chess A, Montell C. (2011). Function of rhodopsin in temperature discrimination in Drosophila. Sci, 11; 331(6022):1333-1336.

[6] Barbagallo B, Garrity PA. (2015). Temperature sensation in Drosophila. Curr Opin Neurobiol, 34:8-13.

[7] Ni L, Bronk P, Chang EC, Lowell AM, Flam JO, Panzano VC, Theobald DL, Griffith LC, Garrity PA. (2013). A gustatory receptor paralogue controls rapid warmth avoidance in Drosophila. Nature, 29; 500(7464):580-584.

[8] Hamada FN, Rosenzweig M, Kang K, Pulver SR, Ghezzi A, Jegla TJ, Garrity PA. (2008). An internal thermal sensor controlling temperature preference in Drosophila. Nature, 10; 454(7201):217-20.

[9] Ni L, Klein M, Svec KV, Budelli G, Chang EC, Ferrer AJ, Benton R, Samuel AD, Garrity PA. (2016). The Ionotropic Receptors IR21a and IR25a mediate cool sensing in Drosophila. Elife. 29; 5.

[10] Klein M, Afonso B, Vonner AJ, Hernandez-Nunez L, Berck M, Tabone CJ, Kane EA, Pieribone VA, Nitabach MN, Cardona A, Zlatic M, Sprecher SG, Gershow M, Garrity PA, Samuel AD. (2015). Sensory determinants of behavioral dynamics in Drosophila thermotaxis. Proc Natl Acad Sci U S AJan 13; 1 12(2):E220-229.

[11] Okabe T, Chen HC, Luo J, Montell C. (2016). A Switch in Thermal Preference in Drosophila Larvae Depends on Multiple Rhodopsins. Cell Rep, 4;17(2):336-344.

[12] Liu WW, Mazor O, Wilson RI. (2015). Thermosensory processing in the Drosophila brain. Nature, 19; 519(7543):353-357.

[13] Krebs RA, Thompson KA. (2006). Direct and correlated effects of selection on flight after exposure to thermal stress in Drosophila melanogaster. Genetica, 128(1-3):217-225.

[14] Hong ST, Bang S, Paik D, Kang J, Hwang S, Jeon K, Chun B, Hyun S, Lee Y, Kim J. (2006). Histamine and its receptors modulate temperature-preference behaviors in Drosophila. J Neurosci, 5; 26(27):7245-7256.

[15] Goda T, Tang X, Umezaki Y, Chu ML, Hamada FN. (2016). Drosophila DH31 Neuropeptide and PDF Receptor Regulate Night-Onset Temperature Preference. J Neurosci, 16; 36(46):11739-11754.

Paper ID: UGC 48846-804

[16] Kain JS, Zhang S, Akhund-Zade J, Samuel AD, Klein M, de Bivort BL. (2015). Variability in thermal and phototactic preferences in Drosophila may reflect an adaptive bet-hedging strategy. Evolution, 69(12):3171-3185.

[17] Garrity PA, Goodman MB, Samuel AD, Sengupta P. (2010). Running hot and cold: behavioral strategies, neural circuits, and the molecular machinery for thermotaxis in C. elegans and Drosophila. Genes Dev, 1; 24(21):2365-82.

[18] Kutch IC, Sevgili H, Wittman T, Fedorka KM. (2014). Thermoregulatory strategy may shape immune investment in Drosophila melanogaster. J Exp Biol, 15; 217(Pt 20): 3664-9366.

[19] Barbour MG, Racine CH. (1967). Construction of performance of temperature gradient bar and chamber. Ecology, 48(5):861-863.

[20] Campbell RE. (1937). Temperature and moisture preferences of wireworms. Ecology, 18(4): 479-489.

[21] Chapman, RF. (1965). The behavior of nymphs of Schistocerca gregaria (Forskal) (Orthoptera,Acrididae) in a temperature gradient, with special reference to temperature preference. Behav , 24:283–321.

[22] Fogleman JC. (1978). A thermal gradient bar for the study of Drosophila. Drosophila Information Service, 53:212–213.

[23] Rajpurohit S. Schmidt PS. (2016). Measuring thermal behavior in smaller insects: A case study in Drosophila melanogaster demonstrates effects of sex, geographic origin, and rearing temperature on adult behavior. Fly (Austin), 10(4):1491-61.

[24] Young K, Hye-Seok S, Xiaoyue W, Craig M. (2017). Assaying thermotaxis behavior in Drosophila 3rd instar larvae using a two-way choice test: Protocol Exchange, 1-4.

[25] Prince GJ, Parsons PA. (1977). Adaptive behavior of Drosophila adults in relation to temperature and humidity. Australian J Zool, 25(2): 285–290.

[26] Goda T, Leslie JR, Hamada FN. (2014).Design and analysis of temperature pr eference behavior and its circadian rhythm in Drosophila. J Vis Exp, 13; (83): e51097.

[27] Luo L, Gershow M, Rosenzweig M, Kang K, Fang-Yen C, Garrity PA, Samuel AD. (2010). Navigational decision making in Drosophila thermotaxis. J Neurosci, 24; 30(12):4261-4272.

[28] Young K, Wei L. Shen, Hye-Seok S, Craig Montell. (2010). Fine thermotactic discrimination between the Optimal and Slightly Cooler Temperatures via a TRPV Channel in Chordotonal Neurons. J Neurosci. 4; 30(31): 10465–10471.

[29] Lixian Z, Andrew, Haidun Y, Ken H, Jessica R, Richard Y H, Geoffrey S P, Daniel TW, (2012). Thermosensory and non-thermosensory isoforms of Drosophila melanogaster TRPA1 reveal heat sensor domains of a thermoTRP channel. Cell Rep. 26; 1(1): 43–55.

[30] Nagaraj G. (2016). Comparison of phototaxis responses using improvised apparatus: a novel experiment for hands-on and minds-on learning. Global J Multi Dis Study, 5(9): 82-91.

[31] Waddington C, Woolf B, Perry M M. (1954). Environment selection by Drosophila mutants. Evolution, 8(2), 89–96.

[32] Fogleman JC. (1979). Oviposition site preference for substrate temperature in Drosophila melanogaster. Behav Genet, 9(5):407–412.

[33] Liu L, Yermolaieva O, Johnson WA, Abboud FM, Welsh MJ. (2003). Identification and function of thermosensory neurons in Drosophila larvae. Nat Neurosci, 6:267–273.

[34] Rosenzweig M, Brennan KM, Tayler TD, Phelps PO, Patapoutian A, Garrity PA. (2005). The Drosophila ortholog of vertebrate TRPA1 regulates thermotaxis. Genes Dev, 19: 419–424.

[35] Kwon Y, Shim HS, Wang X, Montell C. (2008). Control of thermotactic behavior via coupling of a TRP channel to a phospholipase C signaling cascade. Nat Neurosci, 11:871–873.

[36] Zhong L, Bellemer A, Yan H, Ken H, Jessica R, Hwang RY, Pitt GS, Tracey WD. (2012). Thermosensory and nonthermosensory isoforms of Drosophila melanogaster TRPA1 reveal heat-sensor domains of a thermoTRP Channel. Cell Rep, 26; 1(1):43-55.

[37] Refinetti R, Menaker M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. J Thermal Biol, 17: 129-133.

[38] Gilbert SS, Van den Heuvel CJ, Ferguson SA, Dawson D. (2004). Thermoregulation as a sleep signaling system. Sleep Med. Rev, 8: 81–93.

[39] Head LM, Tang X, Hayley SE, Goda T, Umezaki Y, Chang EC, Leslie JR, Fujiwara M, Garrity PA, Hamada FN. (2015). The influence of light on temperature preference in Drosophila. Curr Biol, 20; 25(8):1063-1068.

[40] Fedorka KM, Kutch IC, Collins L, Musto E. (2016). Cold temperature preference in bacterially infected Drosophila melanogaster improves survival but is remarkably suboptimal. J Insect Physiol, 93-94:36-41.

[41] Hunt VL, Zhong W, McClure CD, Mlynski DT, Duxbury EM, Keith Charnley A, Priest NK. (2016). Cold-seeking behaviour mitigates reproductive losses from fungal infection in Drosophila. J Anim Ecol, 85(1):178-186.

[42] Li Y, Li Y, Wu Q, Ye H, Sun L, Ye B, Wang D. (2013). High concentration of vitamin E decreases thermosensation and thermotaxis learning and the underlying mechanisms in the nematode Caenorhabditis elegans. PLoS One, 12: 8(8): e71180.

[43] Crill W, Huey R, Gilchrist G. (1996). Within and between generation effects of temperature on the morphology and physiology of Drosophila melanogaster. Evolution, 50(3): 1205–1218.