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Olfactory Behaviour and Learning in Native versus Lab-bred *Drosophila melanogaster*

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Abstract : *The study was conducted to compare the olfactory behaviour of pure line Drosophila melanogaster (inbred upto 10 generation) and CsBz (inbred upto 1800 generation) with that of native Drosophila melanogaster. For this study, four experimental set-up were designed, named as control, experimental, avoidance and confirmatory set-up.Sixty larvae were used for each assay. Result showed that the larvae of native D. melanogaster were more attracted towards* 10^{-2} *concentration of Iso-Amyl Acetate than that of larvae of lab bred (inbred upto 10 generation) of Drosophila melanogaster and CsBz. Further, the learning and memory capacity of native Drosophila melanogaster was better developed than that of larvae of lab bred (inbred upto 10 generation) of Drosophila melanogaster and CsBz strain of D. melanogaster.*

Keywords: *Drosophila melanogaster, CsBz, Pureline, Iso-Amyl Acetate, Olfaction.*

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Introduction:

Drosophila melanogaster, also known as fruit fly or vinegar fly is a little insect about 3mm long, mostly found on spoiled, ripened or rotten fruit. Wild type fruit flies are yellow-brown with brick red eyes and transverse black rings across the abdomen. They exhibit sexual dimorphism, females are about 2.5 mm long, males are slightly shorter with darker backs.

Canton Special (CS) is one of the most-used wild-type strain in *Drosophila melanogaster* genetics studies. The CS stock was established by

C. B. Bridges and chosen because of its low mutation rate. Seymour Benzer introduced CS to what was to become neurogenetics in his landmark study in 1967, because of its strong fast-phototaxis response. The strain has been used as a control in neurogenetics studies ever since. This strain has been bred in the laboratory for over 1800 generations and has not faced the nature's selection pressure. Therefore, this strain of fly was used to compare its olfactory behaviour with that of native wild type flies.

Drosophila melanogaster is used for basic research work as it is easy to trap and breeds quickly (Vosshall and Stocker 2007, Gerber et al 2009). It has four pairs of chromosomes – the X/Y sex chromosome and autosomes 2, 3 and 4. It serves as a quick, efficient and powerful *in vivo* tool for the analysis of multiple human diseases. Because of their speed of development and sensitivity these models can serve as excellent *in vivo* models to test for drug efficiency and toxicity.

The flies develop attraction to odours to which they are exposed. The brain of adult *Drosophila melanogaster* however is complex, hence larvae were chosen for work because the larvae have adult like olfactory system and provide evidence for olfactory learning in individually assayed *Drosophila melanogaster* larvae (Scherer, Stocker and Gerber, 2003).

The present study aimed to test whether the olfactory system of native or lab-bred *Drosophila melanogaster* is most evolved. The olfactory system is more strongly and directly linked to the behaviour. These connections are sometimes innate but often also acquired and idiosyncratic. Odours possess the capacity to trigger strong memories immediately. Moreover, odorous stimuli provide a higher degree of memory retention than other sensory stimuli (Hughes 2004). Fruit flies are strongly attracted to fruity smell. Therefore, Iso-Amyl acetate (IAA) was used for the assay, because its smell resembles that of banana.

Materials and Methods:

Drosophila melanogaster were trapped from gardens. 250 ml of cornmeal medium was prepared and transferred into 6 bottles and then fruit flies were cultured. From cultured flies, a single gravid female was selected and transferred to 3-4 bottles each containing freshly prepared cornmeal medium.As soon as a layer of egg deposition was observed, the flies were transferred to another bottle immediately. The eggs were developed into adult flies. Now, from these adult flies again a gravid was selected and transferred to a new bottle containing media. The process was continued upto 10 generations to obtain lab bred pure line flies.

The 2^{nd} instar larvae of *Drosophila melanogaster* were harvested for performing olfactory assay. The larvae were attracted towards 10^{-2} concentration of Iso-Amyl Acetate (IAA), so this concentration of IAA was used as attractant for assay. Sixty same age group of 2^{nd} Instar larvae were taken for each set of the experiment and run on the agar Petri plate. It was covered with a black box and left for 2 minutes. The time was maintained with the help of a stop watch. After 2 minutes,the black box and the lid of the Petri plate was removed and the number of larvae on each side was counted. The detailed procedure was as follows :

1.CONTROL SET UP- For control set up, the agar plate was divided into two equal halves and one drop of distilled water was placed on each side with the help of a paintbrush. 60 larvae were kept in the middle of this set up, covered with a black box and after two minutes larvae on each side were counted.

2. EXPERIMENTAL SET UP-For experimental set up, similarly the agar Petri plate was divided into two halves and one drop of distilled water was kept on one half of the plate and IAA of the concentration 10^{-2} on the other side. 60 larvae were kept in the middle of this set up, covered with a black box and after two minutes larvae on each side were counted.

3. .AVOIDANCE REACTION SET UP- For this set up, the agar plate was cut in two halves and one half was removed. Now, 20 mM of NaCl was added to another, freshly prepared agar solution and heated. It was then poured into the half agar plate (whose one part was removed). One drop of distilled water was kept on one half of the plate and IAA of the concentration 10^{-2} on the other side which contained the NaCl.60 larvae were kept in the middle of this set up, covered with a black box and after two minutes larvae on each side were counted.

4. CONFIRMATION SET UP- For this set up, the agar Petri plate was divided into two halves and one drop of distilled water was kept on one half of the plate and IAA of the concentration 10^{-2} on the other side. 60 larvae were kept in the middle of this set up, covered with a black box and after two minutes larvae on each side were counted.

Results and Discussion:

Drosophila melanogaster is a temperature dependent organism. Table 1 shows monthly recordings of temperature and humidity of atmosphere and of Patna Women's College laboratory. Occurrence in relation to the temperature regime at three locations of Patna are shown in Tables 2 to 4. They were abundantly found during the time period of 2.00-4.00 pm at Danapur and Khazanchi Road and were found in least number during 10.00 am to 12.00 noon. However, in the Phulwarisharif area flies were abundant during all hours of the day.

The Olfactory Response Indices (ORI) of native and lab-bred (inbred upto 10 generations) *Drosophila melanogaster* towards different concentration of IAA are shown in Tables 5 and 6.The ORI of native *Drosophila melanogaster,* lab bred and CsBz towards IAA (concentration of 10^{-2}) are shown in tables 7 to 9. A comparative account of olfactory behaviour of the three groups of flies is shown in Table 10. Native and lab-bred (upto 10 generations) *Drosophila melanogaster* were most attracted towards IAA, while during the confirmatory test, only the native flies remembered the irritation caused by NaCl during the avoidance test.

Table 1. Occurrence of *Drosophila melanogaster* in Patna in relation to temperature and humidity.

MONTH	ATMOSPHERE		LABORATORY		Occurrence of *Drosophila*
	AVG. TEMP (°C)	HUMIDITY (%) (°C)	AVG. TEMP (°C)	HUMIDITY (%)	
JULY2015	34.00	80	32.50	90.2	-
AUG 2015	35.30	84.9	34.00	86	+
SEPT 2015	33.36	77	30.18	82.4	-
OCT 2015	33.07	73	31.09	75.88	-
NOV 2015	30.50	70	29.00	79.34	+
DEC 2015	25.00	72.8	22.82	80	+
JAN 2016	24.62	80.29	25.66	72	+
FEB 2016	28.79	76.44	27.28	70.50	+
MAR 2016	32.38	79.73	33.00	76	+
APR 2016	33.17	77.20	34.67	74.59	-
MAY 2016	40.93	75	39.08	70.18	-
JUN 2016	36.96	78.89	34.31	77	-

Table 2. Occurrence of *Drosophila melanogaster* at Danapur
Date- 15/01/2016 to 17/01/2016

	10:00 am			12:00 noon			02:00 pm			04:00 pm		
	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES
DAY1	16	69	02	18	65	02	23	47	04	24	42	06
DAY2	17	67	04	22	67	02	20	60	06	23	49	20
DAY3	16	63	10	18	63	16	22	48	25	23	42	30

Table 3. Occurrence of *Drosophila melanogaster* at Phulwarisharif
Date – 15/01/2016 to 17/01/2016

	10:00am			12:00 noon			02:00pm			04:00pm		
	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES
DAY1	13	88	22	20	68	35	24	47	67	25	47	43
DAY2	21	72	13	22	52	15	24	39	34	23	47	25
DAY3	13	82	20	19	64	26	22	45	32	22	50	43

Table 4.Occurrence of *Drosophila melanogaster* at Khazanchi Road
Date- 15/01/2016 to 17/01/2016

	10:00am			12:00 noon			02:00pm			04:00pm		
	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES	TEMP. ºC	HUMIDITY (%)	NO. OF FLIES
DAY1	18	77	04	18	69	02	25	47	02	24	48	03
DAY2	12	88	04	19	88	10	19	62	18	24	44	12
DAY3	13	82	08	19	64	14	21	47	16	23	47	23

Table 5. Olfactory response index for different concentration of IAA on 2nd instar larvae of native *Drosophila melanogaster*

DIFFERENT CONCENTRATION	ORI	MEAN	SD	SE (±)
DW	0,0.04,0.04	0.02	0.024	0.01
10^{-8} IAA	0.1,0.02,0.08	0.06	0.042	0.02
10^{-5} IAA	0.08,0.12,0.02	0.07	0.05	0.02
10^{-2} IAA	0.56,0.36,0.22	**0.38**	0.17	0.09

DW=DISTILLED WATER

Table 6.Olfactory response index for different concentration of IAA on 2nd instar larvae of lab-bred *Drosophila melanogaster*

DIFFERENT CONCENTRATION	ORI	MEAN	SD	SE (±)
DW	0.02,0	0.006	0.011	0.06
10^{-8} IAA	0.12,0.08,0.08	0.09	0.023	0.01
10^{-5} IAA	0.2,0.24,0.14	0.19	0.050	0.02
10^{-2} IAA	0.28,0.36,0.24	**0.29**	0.061	0.03
10^{-1} IAA	0.2,0.36,0.32	**0.29**	0.083	0.04

DW = Distilled Water ; ORI = Olfactory Response Index ; SD = Standard Deviation ; SE = Standard Error of mean

Table 7. Olfactory response index of 2nd Instar larvae of native *Drosophila melanogaster*

Larvae	DW vs DW	DW vs IAA	Avoidance Test	Confirmation Test
1ST Set	0.05	0.36	0.05	-0.01
2ND Set	0	0.15	-0.1	-0.06
3RD Set	0.03	0.2	0.11	-0.08

DW= Distilled water

IAA= Iso-Amyl Acetate (10^{-2} dilution)

Table 8. Olfactory response index of 2nd Instar larvae of lab-bred (upto 10 generations) *Drosophila melanogaster.*

Larvae	DW vs DW	DW vs IAA	Avoidance Test	Confirmation Test
1ST Set	0	0.36	0.21	0.13
2ND Set	0.01	0.18	0.2	0.15
3RD Set	0.01	0.2	0.15	0.23

DW= Distilled water

IAA= Iso-Amyl Acetate (10^{-2} dilution)

Table 9. Olfactory response index of 2nd Instar larvae of CsBz flies

Larvae	DW vs DW	DW vs IAA	Avoidance Test	Confirmation Test
1ST Set	0.01	0.13	0.06	0.15
2ND Set	0	0.08	0.06	0.13
3RD Set	0	0.1	0.05	0.15

DW= Distilled water

IAA= Iso-Amyl Acetate (10^{-2} dilution)

Table 10. Comparison of olfactory response index of 2nd Instar larvae of native *Drosophila melanogaster*, lab-bred *Drosophila melanogaster* and CsBz

	Native	Lab-bred (upto 10 generations)	CsBz (lab-bred for 48 years)
DW vs DW	0.02±0.01	0.006±0.003	0.003±0.003
DW vs IAA	0.23±0.06	0.24±0.05	0.10±0.01
Avoidance Test	0.02±0.06	0.18±0.01*	0.05±0.003
Confirmation Test	-0.05±0.03	0.17±0.05*	0.14±0.01*

Values are Mean ± S.E.

* $P<0.05$

Drosophila melanogaster activity seems to be temperature dependent. Under natural conditions, temperature rises gradually during the day. A thorough examination of such temperature cycles have shown that biological clocks are very sensitive to this stimulus and can entrain to a temperature cycle with an amplitude of 4º C (Currie et al., 2009). A rise in their number was found in the afternoon. Flies exhibit an afternoon peak when temperature is high, which might be actually an escape response (Vanin et al., 2012; De et al., 2013). *Drosophila* is poikilothermic and thus cannot internally regulate its body temperature. Instead, they move towards a preferred temperature (Hamada et al 2008).

Circadian (biological rhythms) are biological events that allow organisms to accurately predict rhythmic changes in their environment and thus increase their fitness (Tataroglu and Emery, 2014). In *Drosophila melanogaster*, it gates eclosion and courtship, determines the period of rest and activity, the timing of feeding and influence temperature preference (Zhang and Emery 2012). Besides controlling various behaviours, the *Drosophila* circadian clock also coordinate many rhythms in peripheral organs, such as olfactory and gustatory sensitivity rhythms (Krishnan et al 1999; Chatterjee et al 2010).

In the present experiment which was conducted at 10^{-2} concentration of Iso–Amyl Acetate, it was observed that larvae of native *Drosophila melanogaster* and of lab bred strain (inbred upto 10 generations in the laboratory) were most attracted towards the iso-amyl acetate concentration whose mean olfactory response indices were 0.23±0.06 and 0.24±0.05 respectively as compared to CsBz larvae having mean olfactory index 0.10±0.01.

Native flies were exposed to external environment and wide range of odour in comparison to the lab bred strain (inbred upto 10 generations in the laboratory) flies and CsBz flies which were confined to particular area and odour.

Larvae of native *Drosophila melanogaster* showed learning behaviour. When the 2nd instar larvae of native flies were treated with NaCl in the avoidance set up its mean olfactory response index was 0.02±0.06, it felt irritation so in confirmatory setup it move away from concentration side as it remembered the irritation felt in avoidance setup as its mean olfactory index was -0.05±0.02. But in CsBz larvae the condition was totally opposite. The mean olfactory response index in avoidance setup was 0.05±0.05 and in the confirmatory setup it was 0.14±0.006. Inspite of facing irritation in the avoidance setup, in confirmatory setup it went to the IAA side. In the case of lab bred strain (inbred upto 10 generations in the laboratory), the mean olfactory response index of avoidance setup was 0.18±0.01 and confirmatory setup was 0.17±0.03.

Hence, it can be said that learning and memory capacity of native *Drosophila melanogaster* larvae are more developed than the larvae of lab bred flies. But the learning and memory capacity of lab bred strain (inbred upto 10 generations in the laboratory) larvae was better than CsBz larvae as the former were lab-bred upto 10 generations but CsBz larvae have been lab-bred for about 1800 generations.

The primary cause for differences in the three sub strains of *Drosophila melanogaster* in this study might be either founder effect or epigenetic effect. This is because the larvae of *D. melanogaster* inbred upto 10 generations were the descendents of a single gravid female and larvae of CsBz are descendents of flies that have been lab-bred for about 1800 generations. Colomb and Brembs (2014) analysed the walking behaviour in five sub-strains of the standard laboratory wild-type *Drosophila melanogaster* Canton Special (CS) and concluded that founder effects but not laboratory selection likely caused the difference in their locomotor behaviour.

Conclusion:

From the results obtained, it can be concluded that the larvae of native *Drosophila melanogaster* were more attracted towards the 10^{-2} concentration of Iso-Amyl Acetate than the larvae of the lab bred strain (inbred upto 10 generations in the laboratory) and CsBz. Further, the learning and memory capacity of native flies was well developed than that of lab bred strain (bred upto 10 generations in the laboratory) and CsBz flies. The primary cause for differences in the three sub strains of *Drosophila melanogaster* in this study might be either founder effect or epigenetic effect.

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