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***Research Article** (Open Access)*

FORAGING ASSOCIATED BEHAVIOURAL PATTERNS IN DROSOPHILA LARVAE

***K. N. Niladevi, Anmariya Joy and Elizabeth V. Mathew**
Department of Zoology, Union Christian College, Aluva, Kerala, India
**Author for Correspondence: niladevikn@uccollege.ac.in*

ABSTRACT
Drosophila larvae have emerged as a model organism for research on social interactions and curious behavioural patterns. The dynamics of collective foraging decisions including cluster formation, social digging and de-clustering have been well documented. The current study was undertaken to unveil the influence of food colour and odour in the selection of food by the *Drosophila* larvae and also to identify the major sensory route employed by the larvae during foraging. The larvae use both visual and olfactory cues to identify the food sources. With respect to the colour of the food, more preference was shown towards red coloured food source and least preference towards dark coloured food. Among the different fruity odours investigated, the odour of mango was observed to be the most preferred odour, followed by the odour of pineapple. Upon presentation of food sources with the preferred colour and odour, the larvae were found to select the food mostly based on odour which clearly indicated that the *Drosophila* larvae largely depend on their olfactory receptors to identify their food source. The temporary disruption of olfactory perception by exposing the larvae to chemical odorants resulted in a time lag in reaching the preferred food. Moreover, the disruption of olfactory perception resulted in peculiar behavioural patterns like head rising and head sweeping Among the various odorants tried, a perfect correlation between the exposure time and frequency of head sweeping behaviour was observed only with propionic acid.

***Keywords:** Drosophila Larvae, Foraging Behaviour, Colour Preference, Odour Preference, Chemical Odorants*

INTRODUCTION
Drosophila larvae have been a subject of intense behavioural studies for the past few decades. The ease of culturing the larvae and the recent developments in the field of neuroscience have resulted in the unveiling of many curious and complex behavioural patterns of this larva (Schneider-Mizell *et al.,* 2016, Larderet *et al.*, 2017, Clark *et al.*, 2018). Majority of the behavioural traits studied in Drosophila larvae are associated with foraging and feeding, as the larvae dedicate most of their lifetime for nutrient acquirement. The larvae are known to form aggregates and involve in social digging (Durisko *et al.*, 2014, Louis and Polavieja, 2017) and practice a process termed social digestion (Gregg *et al.*, 1990) in order to soften hard food. Moreover, social digging into the interior of food media provides them with the additional benefits of avoiding desiccation and predation (Carton and Sokolowski, 1992). The emergence of cooperative behavior within Drosophila larvae is observed as a more efficient way of digging (Dombrovski *et al.,* 2017) and the disruption of the clusters formed during cooperative behaviour has been attributed to the lack of access to air (Kim *et al.,* 2017). There has been a bounty of research works on the exploration of neuronal circuits in Drosophila larvae and the relationships between the neuronal networks and behavioural patterns (Guven-Ozkan and Davis, 2014, Ramdya *et al.*, 2015, Kohsaka *et al.*, 2017, Clark *et al.*, 2018). The study by Dombrovsky *et al.,* (2017) using mechanosensory mutant nompC and blind mutants NorpAP41 has shown that the vision and mechano-sensation play important roles in cluster formation. A more recent work led by the same authors has affirmed that the ability of an individual larva to participate in the cluster formation is influenced directly by the visual cues. The role of vision in the coordination of movements between pairs of larvae has also been revealed (Dombrovsky *et al.,* 2019).

The olfactory sensing apparatus of Drosophila larvae is the dorsal organ located at the tip of head and it is innervated by dendrites of 21 olfactory receptor neurons (Benton *et al.,* 2006, Vosshall & Stocker, 2007). Olfaction remains one of the important sensory inputs employed by the larvae to detect the environment, especially the food sources. Strong positive correlation has been reported between the olfactory neuronal activities and behavioural output in the larvae when they were exposed to different fruit odours (Dweck *et al.,* 2018). The larvae also show interesting head movements, runs and turns in response to chemical stimulants (Davies *et al.,* 2015). Apart from the pivotal role of olfactory sense in food preference and social behaviour, certain parts of the higher olfactory centers such as mushroom body has been shown to be involved in olfactory associative conditioning in larvae (Pauls *et al.,* 2010). Several workers have reported the conditioning of Drosophila larvae employing different positive and negative reinforcers (Dukas, 1999; Schipanski *et al.,* 2008; Honjo and Furukubo-Tokunaga, 2009, Khurana *et al.,* 2009) and the rate of memory decay was found to show considerable difference while employing attractive and aversive reinforcements (Honjo and Furukubo-Takunaga, 2009).
Despite the extensive research on the elucidation of visual and olfactory pathways of Drosophila larvae, it has been reported to detect only blue and green light using their photoreceptors while very little is known about the perception of other colours by Drosophila larvae (Farca Luna *et al.,* 2013). In the current study attempts were made to unveil the colour preference of larvae by exposing them to odour-less, differently coloured food sources. The relative roles of visual and olfactory receptors in identifying the preferred food by the larvae was also investigated on a temporal scale. Experiments on olfactive reception following temporary disruption of the receptors was also undertaken to arrive at significant and conclusive findings regarding foraging associated sensory perception and related behavioural patterns in Drosophila larvae.

MATERIALS AND METHODS

2.1. Influence of food colour on food preference by Drosophila larvae

The second instar larvae carefully collected from the culture medium was used for the study. The influence of colour of food on the foraging behaviour of *Drosophila* larvae was tested by providing them with foods of different colours. The colours used in the study were yellow, pink, red, green, dark green and coffee brown, where a colourless food source served as the control. (All the colours used for the study were of food grade quality, procured from local markets) The experiment was performed in a plate with a diameter of 20 cm. The plate was equally divided into 7 parts by using small cardboard pieces. The food source was prepared by using agar, starch, powdered yeast and sugar to get a suitable consistency. The prepared food was divided into seven equal portions and to each portion, one of the above-mentioned food - grade colours were added and mixed well to get six differently coloured media and a colourless control. Each medium was poured into one part of the plate and allowed for setting. The center portion of the plate was kept free. Once the media was set, 150 second instar larvae were introduced into the center portion of the plate and the various behaviours shown by the larvae were observed for 2 hours. Larval movements, attraction towards the colors and time taken to reach the food with different colours were noted.

*2.2. **Influence of odour on food preference by Drosophila larvae***

The influence of odour on the foraging behaviour of *Drosophila* larvae was tested by using different fruity odours. To obtain the same, the essence of fruits like Mango, guava, green apple, orange, pineapple and lemon were used (All procured from local markets). An odorless food source without any added fragrance served as the control. The experimental setup was same as the previous experiment except for that instead of coloured food sources, colourless food sources with different odours were used.

2.3. Interactive effect of colour and odour of food on Drosophila larvae

The experiment was performed in a petri plate. In the first run, the petri plate was divided into two equal parts by keeping the central portion of the plate free. One half of the plate was filled with red coloured food medium while the other half was filled with food medium containing mango essence. 50 numbers of freshly cultured second instar larvae were introduced into the center portion of the plate and observed for 1 hour.

In the second run, the petri plate was divided into three equal parts and the central portion remained free. One part of the plate was filled with red coloured media while the second part was filled with food containing mango essence. The third portion of the plate was filled with a medium that was red in colour and contained mango essence. 50 freshly cultured second instar larvae were introduced into the center portion of the plate and observed for 1 hour.

2.4. Conditioning of Drosophila larvae using chemical odorants

The influence of chemical odorants on the behavior of *Drosophila* larvae was studied by exposing the larvae to different chemicals such as n- Butyl alcohol, acetone, phenol, acetic acid and propionic acid. A small piece of cotton was dipped in 1:10 diluted n- Butyl alcohol. The cotton was placed in a Petri plate containing 20 second instar larvae and the plate was closed. Allowed the chemical to completely saturate the Petri plate for 2 minutes. Then the larvae were taken out and briefly dried on a filter paper and were placed at the center of another petri plate which contained red-coloured food medium, food media with mango odour and a control media with neither colour nor odour. Observations were made for 1 hour. The experiment was repeated by increasing the exposure time to chemical odorants from 2 minutes to 4, 6, 8 and 10 minutes. Same methodology was used for all the five chemical odorants under study.

RESULTS AND DISCUSSION

Drosophila larvae are currently used as model organisms for behavioural studies. Feeding associated behavioural patterns exhibited by these larvae are gaining more attention from the researchers, though, the picture is not yet complete. The present study mainly aimed at investigating the relative roles of food colour and odour in deciding the food preference of *Drosophila* larvae. Attempt was also made to observe the curious behavioural patterns exhibited by the larvae during foraging and feeding under different conditions.

3.1. Influence of colour of food on the foraging behaviour

The influence of colour of food on the foraging behaviour of larvae of *Drosophila* was tested by exposing the larvae to food media of different colours. The initial movement of the larvae from the center towards different colours took 4-11 minutes. However, a considerable number of larvae were observed to return to the center after visiting differently coloured food media. Majority of the larvae that entered coffee brown and dark green colour showed quick responses and returned back to the center at a faster rate. The rate of return was found to be low with the larvae that entered red and pink colours as majority of them stayed in the media. The larvae returned from other coloured foods took random movements until they finally settled down in their preferred colour and the entire process took 23 minutes.

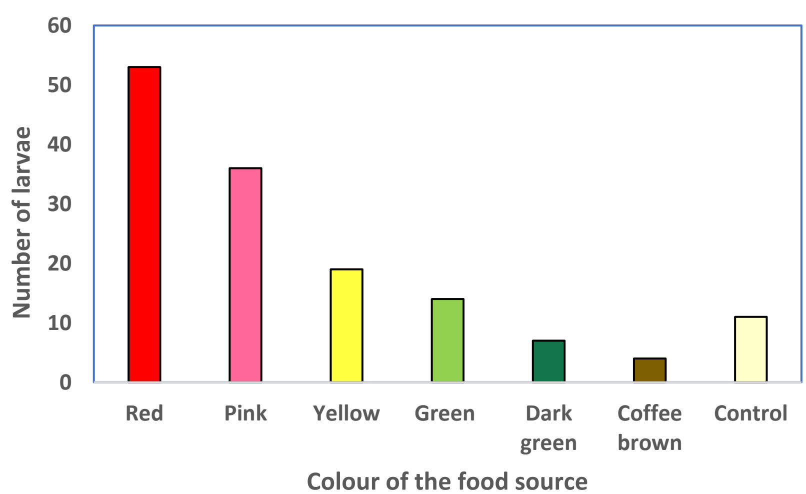

Figure 1. Influence of colour of food on the foraging behaviour of Drosophila larvae

Out of the 150 larvae introduced into the center of the plate, 53 larvae moved to the red colour and 36 larvae moved to the pink colour. 11 numbers of the larvae moved to the control while the movement of larvae to yellow, green, dark green and coffee brown were in the order of numbers 19, 14, 7 and 4 respectively (Figure 1). Six out of the 150 larvae failed to show any conspicuous movement and remained at the place of introduction. Those larvae moved to the red and pink colour were observed to show digging behaviour at a faster rate. The initial digging resulted in the disruption of the agar-based food media which seemed to facilitate faster digging by other larvae. It was observed that the larvae preferred such sites for digging rather than exploring new sites.

Results of the experiment conducted to study the influence of colour of food on the foraging behaviour has clearly indicated that 35 % of the larvae preferred red coloured food. This was followed by pink colour, in which 24 % of the larvae finally settled down. Most of the larvae that entered dark green and cofee brown coloured food, returned from those media to the center of the plate and then moved towards other colours. *Drosophila* larvae is generally considered as photonegative and has been reported to move away from the light source of varying wavelength ranging from UV to green (Humberg and Sprecher, 2017). The visual system of Drosophila larvae has been well elucidated and among the twelve photoreceptors (PRs) on each side, four PRs has been reported to express Rhodopsin-5 while eight express Rhodopsin-6 (Dombrovski *et al.*, 2019). In the present study, the larvae showed a preference towards red and pink-coloured foods while they moved away from green and dark coloured food sources. It has also been suggested that the Rh5 photoreceptor-type of *Drosophila* larvae is able to perceive UV up to green light (Humberg and Sprecher, 2017). Even though, there are reports on the photo receptive system of Drosophila larvae, there are no solid evidence regarding the extent to which they can distinguish different colours and hence, it will be impetuous at this stage to relate the colour preferences exhibited by the larvae in the present study to the well-studied photoreceptors of larvae as the precise role of these photoreceptors in colour discrimination has not yet been clearly established. It has been reported that the larvae prefer soft food than hard food as they can easily liquefy soft foods using their enzymes (Kudow *et al.*, 2019) and this could be the reason for the observed digging pattern exhibited by the larvae in which, the larvae preferred the sites on agar plates already explored by other larvae and the observations of the present study are in compliance with the reports of Kudow *et al* (2019).

3.2. Influence of odour of food on the foraging behaviour

The influence of different odours on the foraging behaviour of the larvae was tested by exposing them to food media having different fruity odours. The initial time taken for sensing the odour was 20-30 seconds within which the larvae showed movement towards different odours. The total time taken by the larvae to finally settle down in different media was found to be 14 minutes. Out of the 150 larvae introduced, 69 larvae (46 %) moved to mango and 38 (25.33 %) moved to pineapple. Almost equal preference was shown towards orange (10.66 %) and guava (9.33 %). Less preference was shown towards lemon (4.66 %), and green apple (4 %) (Figure 2). None of the larvae stayed in the control. Dweck *et al.*, (2018) has found that the most attractive fruit-headspace extracts for larvae are strawberry, passion fruit, mango and pineapple. Excluding strawberry and passion fruit, which were not used in the present study, the order of preference exhibited by larvae in the current study is very much in congruence with the early reports of Dweck *et al* (2018). The results also indicated that the *Drosophila* larvae can use their olfactory sense for the identification of food sources at a much faster rate (20-30 seconds) than they can do using the visual senses (4-11 minutes).

3.3. Interactive effect of colour and odour on Drosophila larvae

When freshly cultured larvae (50 numbers) were introduced into the petri plate, one half of which was filled with red coloured food medium and the other half with food medium containing mango odour, 88 % of the larvae were observed to move towards that portion of the petri plate containing food source with the odour of mango. The remaining larvae (12%) moved to red coloured food source (Figure 3). When an additional food source containing both the odour of mango and red colour was included in the study, the movement pattern remained almost the same with respect to red coloured food source (10 %) while majority of them

(54 %) preferred the food that contained both odour and colour. 36 % showed preference to food with mango odour alone. In both these experiments, the percentage of larvae that chose food based on colour fall in the narrow range of 10-12 % while the remaining larvae chose food with the odour of mango alone or in combination with red colour. Hence, it is logical to conclude that the larvae select their food mostly based on the odour of the food rather than colour. Schipanski *et al.*, (2008) has reported that the establishment and recall of olfactory memory in adult fruit flies are generally facilitated by light but similar studies on fruit fly larvae are scanty and the nature of interaction between visual and olfactory modalities of larvae needs to be studied in more detail. The results of the present study are valid proofs which points towards the fact that olfactory perception of *Drosophila* larvae is far ahead of the visual perception in the context of foraging.

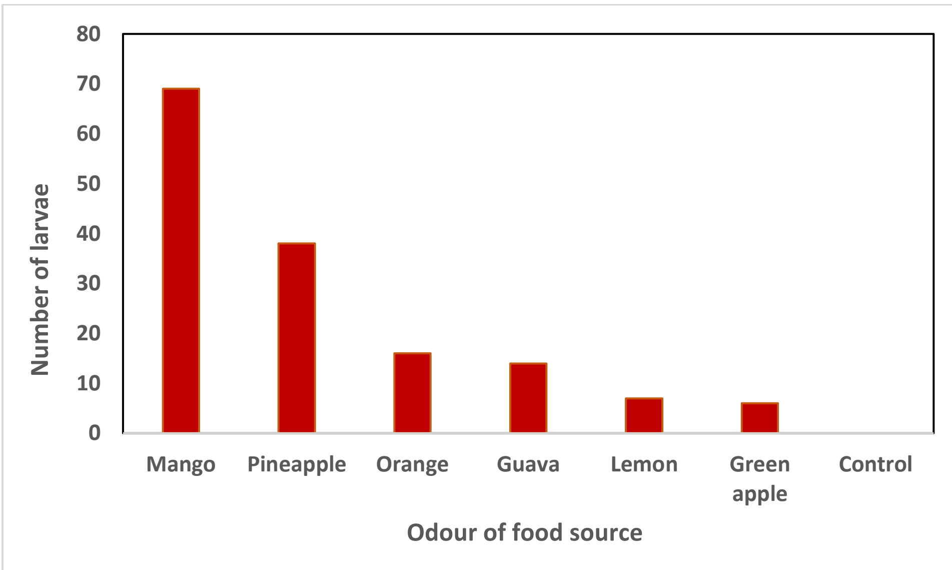

Figure 2. Influence of odour of food on the foraging behaviour of Drosophila larvae

Figure 3: Movement of Drosophila larvae towards the food source with red colour (A) and odour of mango (B) when both these food sources were presented together

3.4. Effect of chemical odorants on larval behaviour

When the larvae were exposed to different chemical odorants and subsequently introduced to food source, a positive linear relation was observed between the exposure time and the time taken by the larvae to identify the food source. In each case, when the exposure time was increased from 2 minutes to 4, 6, 8 and 10 minutes, the larvae took more time to identify the food source. The response of the larvae to different chemical odorants were in the order; phenol > acetone > acetic acid > n-butyl alcohol > propionic acid. With acetic acid, acetone and n butyl alcohol, the time taken for identification of food source had increased linearly with exposure time until 8 minutes of exposure. With an exposure time of 10 minutes, the larvae failed to show movement to the food source (Table 1) and the same pattern was observed in the case of three odorants. With phenol, the non-responsive behaviour was exhibited even at 4 minutes exposure time which was indicative of the fact that the response of larvae to odorants could vary considerably with the chemical nature of odorants. However, propionic acid was observed to have less effect on larvae and even at higher exposure time of 10 minutes, the larvae identified the food source within 26 minutes time. The chemotactic behaviour of Drosophila larvae towards various odorants has been well accounted and the larvae have been shown to exhibit two distinct modes of locomotion when exposed to chemical odorants (Khurana and Siddiqi, 2013).

Table 1: Time taken (in minutes) by the larvae to reach the food source over different periods of exposure (min) to selected chemical odorants

	Time of Exposure				
	2 min	**4 min**	**6 min**	**8 min**	**10 min**
Phenol	21	NE*	NE*	NE*	NE*
Acetone	9	16	29	41	NE*
Acetic acid	7	16	22	36	NE*
n- butyl alcohol	8	12	19	29	NE*
Propionic acid	5	8	18	21	26

**NE – Not estimated as the larvae failed to show any response*

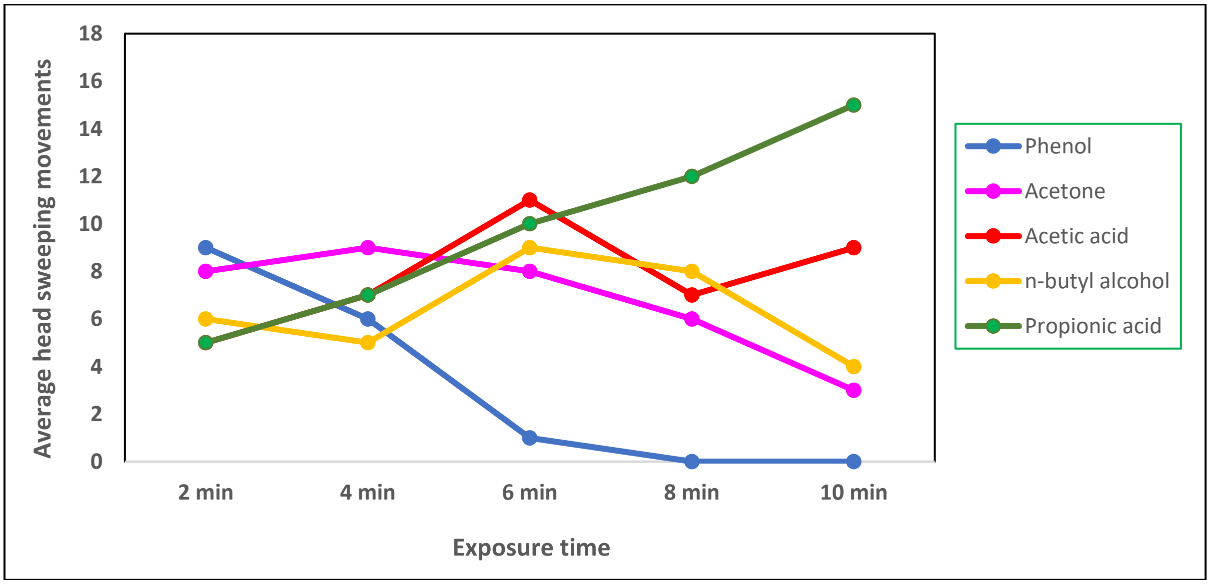

Figure 4: Average head sweeping behaviour exhibited by Drosophila larvae on exposure to chemical odorants over different periods of time

When the fresh larvae were introduced into the food medium for the first time, they started moving towards the preferred food within a time period of 2 minutes with minimal head casting movements while in the case of larvae those were exposed to chemical odorants and then reintroduced to food medium, they exhibited a type of characteristic 'head rising' movements followed by 'head sweeping' movements. However, no perfect correlation could be observed in the frequency of head sweeping behaviour to the exposure time to chemical odorants except in the case of propionic acid in which a linear relationship could be observed between the exposure time to odorants and the average number of head sweeping movements by the larvae (Figure 4).
It was obvious that exposure to strong chemical odorants have significant effect on the olfactory system of *Drosophila* larvae which deters them from quick identification of their food sources. The head rising and head sweeping behaviours were performed by the larvae as a means to overcome the effect of chemical odorants and at higher concentrations of odorants like phenol, the larvae failed to exhibit any sort of movement. It has been reported that the *Drosophila* larvae could compare the odour concentrations on either side, before performing odour taxis by using the two olfactory sense organs that are located one on each side (Davies *et al.*, 2015). Drosophila larvae have two olfactory receptors, one on each side and there are ambiguities over the efficiency of *Drosophila* larvae that have undergone unilateral surgical ablation of sensory organs to distinguish between the odour concentrations on left and right sides owing to the fact that the two receptors are placed so closely (Gomez-Marin *et al.*, 2010). It has been reported that staying near the odour source play a role in controlling the alterations between runs and turns in *Drosophila* larvae. The authors have also suggested that the larvae orient themselves towards higher odour concentrations (Gomez-Marin *et al.*, 2010). In the present study, a perfect correlation was observed between the exposure time to strong odours and time taken by the larvae to identify the food however, no such correlation could be observed between duration of exposure and head sweeping behaviour for majority of the odorants tested. It clearly shows that the *Drosophila* larvae have highly sensitive olfactory receptors that could be temporarily impaired on exposure to strong odorants which may hinder their ability to identify the food sources.

CONCLUSION

Drosophila is a model organism for research on social interaction and their larvae have been established as an excellent model for the study of learning and memory. The dynamics of collective foraging decisions by the larvae of *Drosophila* appears to be highly complex and the integration of multi-sensory information is very important for the identification of food. The larvae showed preference towards food having specific colour and odour and the olfactory perception proved to have major role in the identification of food as compared to the visual cues. When the olfactory receptors were temporarily disrupted by strong chemical odorants, there occurred a noticeable delay in the identification of food, accompanied by peculiar behavioural patterns like head rising and head sweeping. The chemical nature of the odorants also had a significant impact in the temporary disruption of olfactory senses of *Drosophila* larvae. The current study has provided valuable insights into the foraging behaviours of *Drosophila* larvae and paves the way for similar studies to explore the intricate behavioural patterns of other organisms as well.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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