

LARVAL LOCOMOTION BEHAVIOR IN THREE SPECIES OF *DROSOPHILA*

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Locomotion behavior of *Drosophila melanogaster*, *D. ananassae* and *D. bipectinata* was analyzed to know the pattern of locomotion for the respective species and to find interspecific relationship among them. Among the three species, *D. bipectinata* larvae passed the highest distance. In terms of turning per minute, *D. melanogaster* differed significantly from other species. Average bending number was also highest in this strain. The relationship of the three species has been discussed in terms of locomotion parameters and available phylogenetic trees relevant to these species.

Locomotion is an integral component of most animal behaviors. They rely on this behavior for searching food, mates and escaping from predators and respond to different stresses (Heckscher *et al.* 2012). Thus, understanding this behavior is helpful. As a fantastic model organism, *Drosophila* is being utilized in different behavioral studies (Reiter *et al.* 2001, Mcguire *et al.* 2005). Inter and intra-population digging behavior of larva have also been studied in *Drosophila* (Godoy-Herrera 1977). Larval foraging behavior, which includes locomotion, varies from species to species reflecting that this behavior can be differed from species to species within a same group (Godoy-Herrera and Connolly 2007). The richness in *Drosophila* species diversity also provides a great opportunity for extensive study (Singh 2015). Ten *Drosophila* species have so far been reported from Bangladesh (Sultana 1998). Among them, *D. melanogaster*, *D. bipectinata* and *D. ananassae* are commonly found. Previous work regarding locomotion behavior has been done on these species separately, not in the same experimental set-up (Green *et al.* 1983, Bauer and Sokolowski 1984). These three species have been collected, cultured and identified to explore larval locomotion behavior.

Procedure of the study: Adult flies collected from different places of Bangladesh, namely Dohar, Dhaka (23°37'06.5"N 90°07'10.1"E), Satchari, Sylhet (24°07'28.0"N 91°26'41.5"E) and Hiron Point, Sundarbans (21°48'58.0"N 89°27'46.6"E) were maintained on standard food. Three species of *Drosophila*

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were identified by following the established protocol (Markow and O'Grady 2005). Fly strains collected from Dohar, Hiron Point and Satchori were identified and labeled as *D. bipectinata* (DR), *D. melanogaster* (HP) and *D. ananassae* (SC), respectively.

Third instar larvae of all the three strains were placed in the middle of 2% agar plate over a background paper where each grid was of 0.5 cm. For each larva, video was captured for about 1 minute. For each species of *Drosophila*, the whole procedure was repeated for at least 20 times. When a larva changed its direction of movement by less than 90 degree and more than 90 degrees, it was considered as turning and bending, respectively. The number of grids it crossed revealed the distance travelled. Data were analyzed by using Microsoft excel.

Pattern of larval locomotion in the three strains of Drosophila species: Three major activities during larval locomotion, namely distance travelled, turning and bending were calculated and compared among these three strains of the *Drosophila* species shown in Table 1.

Table 1. Observation of three major activities during larval locomotion in three species of *Drosophila*

Sl. No.	Strain name	Identified species	Distance travelled (cm/min)	Turning number (number/min)	Bending number (number/min)
1	Dohar strain (DR)	<i>D. bipectinata</i>	4.225 ± 0.25	3 ± 0.42	0.7 ± 0.2
2	Hiron Point strain (HP)	<i>D. melanogaster</i>	2.125 ± 0.17	7.4 ± 0.68	1.65 ± 0.28
3	Shatchori strain (SC)	<i>D. ananassae</i>	2.75 ± 0.22	4 ± 0.73	1.05 ± 0.29

Distance travelled: *Drosophila bipectinata* larvae passed the highest distance of 4.225 ± 0.25 cm/min among the three species (Table 1). The distance travelled by the *D. bipectinata* was significantly different from all other species. On the other hand, *D. melanogaster* and *D. ananassae* larvae passed the distance of 2.125 ± 0.17 and 2.75 ± 0.22 cm/min, respectively. These two species did not show significant difference at 5% level.

Turning and bending: In *D. melanogaster*, turning number per minute was found 7.4 ± 0.68 which was the highest among the three species (Table 1). *D. bipectinata* and *D. ananassae* showed 3 ± 0.42 and 4 ± 0.73, respectively. These two species did not show significant difference. Mean bending number was highest in *D. melanogaster* and lowest in *D. bipectinata*. In *D. melanogaster*, the bending number was 1.65 ± 0.28 whereas 0.7 ± 0.2 was found in *D. bipectinata*. In *D. ananassae* the number was found 1.05 ± 0.29 showing no significant difference when compared to both *D. melanogaster* and *D. bipectinata*.

Analysis of turning and bending indicated that *D. melanogaster* had significant difference with other two species. However, in terms of travelled distance, it was different from *D. bipectinata* alone. Overall, *D. melanogaster* showed significant difference with *D. bipectinata* in all three parameters of locomotion behavior. On the other hand, *D. ananassae* shared common turning and bending pattern with *D. bipectinata* only and distance travelled with *D. melanogaster* only. Thus, *D. ananassae* can be placed in the middle of other two species as shown in the Fig. 1A. Therefore, it is revealed that interspecific differences existed among the three species of *Drosophila* in terms of the larval locomotion behavior parameters. Similar reports regarding *Drosophila* species existed, but the species of the present study were not covered in the same article. For instance, *D. melanogaster* and *D. pseudoobscura* were reported to have significant interspecific differences in the travelled distance ('path lengths')

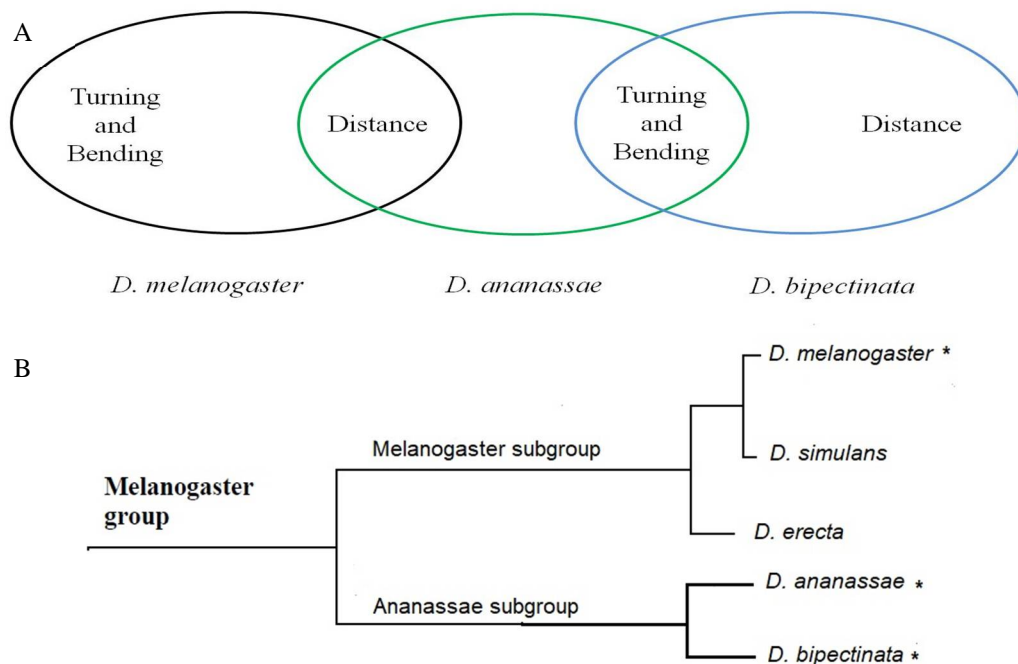


Fig. 1. A. Comparison of three *Drosophila* species in terms of three locomotion parameters. B. phylogenetic relationship among *Drosophila* species as adapted from Seetharam and Stuart (2013). Here, * indicates the species those were studied in the present study.

of foraging third instar larvae (Bauer and Sokolowski 1984). The larvae of *D. melanogaster* and *D. simulans* also showed interspecific differences in larval locomotion (Green *et al.* 1983). Additionally, evolutionary relationship showed that these three species belong to the melanogaster group (Fig.1B). Among them, *D. ananassae* and *D. bipectinata* were closer belonging to the ananassae

subgroup of melanogaster group whereas *D. melanogaster* was fallen into melanogaster subgroup of melanogaster group (Seetharam and Stuart 2013). This also reflects in the result of the present study as it was found that *D. ananassae* and *D. bipectinata* had no significant difference in terms of turning and bending numbers whereas *D. melanogaster* differed significantly from others (Fig.1A). However, absence of significant difference between *D. melanogaster* and *D. ananassae* may be attributed to their placing in the same melanogaster group as shown by Seetharam and Stuart 2013. Besides, *D. melanogaster* and *D. ananassae* were reported to have similar pupation site preferences (*i.e.* on the vial wall) whereas *D. bipectinata* preferred to pupate on the food surface in a laboratory condition (Alam *et al.* 2019). Thus, the larval locomotion data of the present study is in conformity with previous findings.

Overall, present observations confirm that larval locomotion behaviors differ from species to species in the *Drosophila* species. Besides, the behavioral differences can be related to their phylogenetic relationship as well. Thus, analyzing different locomotion behavior would be helpful for species identification, differentiation as well as studying evolution. Present work has provided baseline data that will be valuable in this regard. Further extensive study is needed for better understanding.

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