Directional proboscis extension: A new paradigm for testing gustatory responses of *Drosophila melanogaster*

Sudipta Saraswati

Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India

When the taste organs on the legs of flies come in contact with acceptable stimuli, the fly extends its proboscis. This proboscis extension response has been routinely used to study the sense of taste in the fruitfly, *Drosophila melanogaster*. We observed that *Drosophila* exhibits a directionality in leg-mediated proboscis extension. The fly extends its proboscis in the direction of the stimulated leg when the stimulus is an attractant, and away from it when it is a repellent. Based on this observation, we have developed a new paradigm—directional proboscis extension test. This paradigm has been used for testing gustatory responses of *Drosophila* to different stimulants such as sucrose, NaCl, quinine and amiloride. The directional test is more sensitive than the standard proboscis extension test. The directional proboscis extension test may allow us to study features of gustatory behaviour that escape the standard tests.

THE fruitfly, *Drosophila melanogaster*, and other dipterans have taste organs on the mouthparts (i.e. proboscis), tarsal segments of legs and the anterior wing margins[1]. The response of flies to different tastants are measured by observing their behaviour and by recording the electrophysiological activity of the taste receptor cells[2–7]. *Drosophila* adults accept water, sugars and low concentrations of NaCl. Quinine, KCl and high concentrations of NaCl elicit avoidance response in feeding behaviour[4–6, 8–12].

The scientific investigation on the sense of taste in flies began in early twenties with a behavioural observation by Minnich. In 1921 he observed that when the tarsi of legs of nymphalid butterflies are placed in contact with a sugar solution, the proboscis is extended[13]. Minnich also reported that touching of a single long curved hair on the proboscis of the blowfly, *Phormia regina*, with sugar solution elicits extension of the proboscis[14]. Subsequently, Dethier and his colleagues used proboscis extension response to study different aspects of gustatory behaviour of the blowfly[15]. Similar studies have been carried out with *D. melanogaster*[4,8,9,16]. In all these studies the proboscis extension test has been carried out by applying a drop of solution containing the test chemical to tarsal segments of both the prothoracic legs. The response has been measured by scoring the presence or absence of proboscis extension. Yetman and Pollack reported directionality of the proboscis extension in *Phormia* by stimulating individual hairs on the proboscis[17].

We observed that *Drosophila* exhibits a directionality in leg-mediated proboscis extension. The fly extends its proboscis in the direction of the stimulated leg when the stimulus is an attractant, and away from it when stimulus is a repellent. Based on this observation, we have developed a variation of the proboscis extension test. In this test, both the prothoracic legs are simultaneously touched with equal-sized drops of water, one or both of which contain the stimulant. The test can be used either as a simple response test or a 'preference' test. This paper describes the use of the *directional proboscis extension test* and compares its sensitivity with other tests.

The Canton Special (CS) strain of *D. melanogaster* was used in the present study. Fly cultures were reared on standard cornmeal-yeast medium[18] at 24°C–25°C.

The proboscis extension response of *Drosophila* was measured using a variation of the protocol described by Deak[8]. Three to four day old flies were starved in the presence of moist tissue paper for 17–18 h and then anaesthetized by cooling on ice. The fly was mounted on a microscope slide, layered with an odour-free plasticine, ventral side up. It was immobilized with myristic acid. The preparation was kept in a moist chamber for 2–3 h to allow recovery. The prothoracic tarsal hairs of the fly were touched with deionized water. The interval between two consecutive stimulations was at least 3 min to avoid the possibility of habituation. The number of proboscis extensions in a single fly in response to five stimulations was scored. The flies that responded at least three times out of five stimulations were taken as responders. A set of nine flies was tested in each experiment.

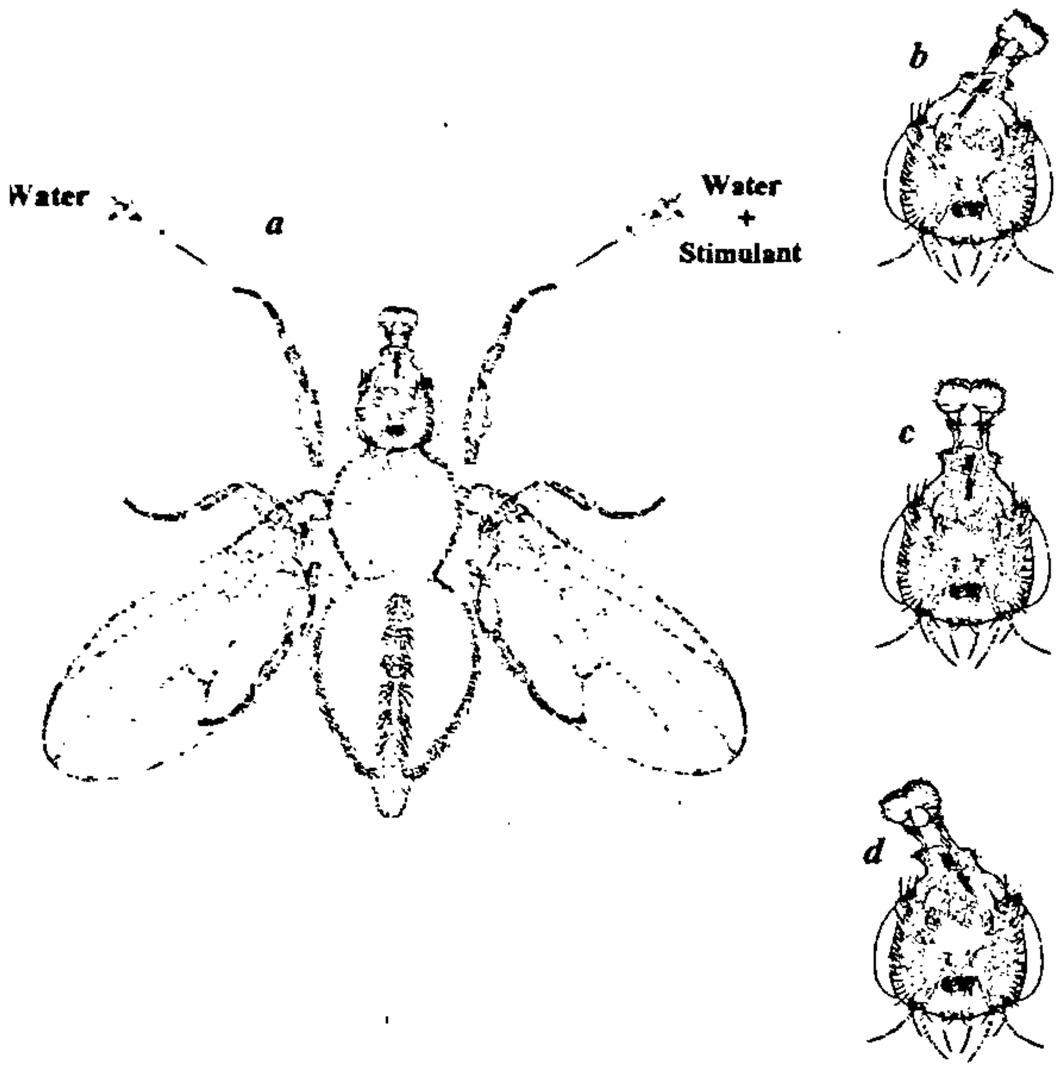
For the directional proboscis extension assay, the fly was prepared and mounted on a microscope slide as described above. Additionally, the wings, mesothoracic legs and metathoracic legs were also fixed onto the plasticine. In the usual form of the test, both prothoracic legs are simultaneously touched with water with at the tip of hypodermic needles, with or without the stimulating solute. The unilateral stimulus is randomized between the right and the left side. During the test, care was taken so that both legs touched the respective solutions at the same time and that neither of the two drops of the solution was ever accessible to the proboscis. The directions of proboscis extension in a single fly in response to five touches was scored. A schematic diagram of the test is outlined in Figure 1.

Table 1 shows the response of an unsatiated fly to deionized water. When one of the legs is touched with water (unilateral stimulus), 84.3 ± 5.09% of extensions are directed to the stimulated side and only 5.9 ± 3.29%

e-mail: sudipta@tifrvax.tifr.res.in

to the contralateral side. When both prothoracic legs are touched with water simultaneously, nearly half the extensions are medial, the remaining half are randomly directed.

We used the directional extension of the proboscis to examine responses to a number of stimulants, both attractant and repellent. In these experiments, the prothoracic legs were simultaneously touched with water on one side and a solution of the stimulant on the other side. The direction of proboscis extension was noted as medial (undirected), ipsilateral (towards the stimulated leg) and contralateral (away from the stimulated side). The results are summarized in Table 2.



'igure 1. Diagrammatic representation of directional proboscis extenion test. *a*, Bilateral stimulation of prothoracic legs. *b*, Ipsilateral (i.e. owards the stimulant). *c*, medial (i.e. undirected). *d*, Contralateral away from the stimulus) extension of the proboscis.

Table 1. Responses of unsatiated *Drosophila* to deionzsed water

	Per cent extensions			
timulus	Ipsilateral	Medial	Contralateral	N
nilateral	84.31 ± 5.09	9.80 ± 4.16	5.88 ± 3.29	51
ilateral	27.36 ± 4.32	48.11 ± 4.85	24.53 ± 4.17	106

alues are given as mean ± S.D.
, number of stimulations.

These results indicate that sucrose is a strong attractant. Standard proboscis extension and feeding preference tests also give similar results[12,19]. In the directional test, when the fly was subjected to simultaneous stimulation with 10^{-3} M sucrose on one side and 5×10^{-3} M sucrose on the other side, 78.33 ± 5.32% of extensions were directed towards the higher concentration. Thus the fly can discriminate between 10^{-3} M and 5×10^{-3} M sucrose sensed from two populations of nerves.

Response to NaCl shows that 7.5×10^{-2} M NaCl acts as an attractant to flies in the feeding preference test[12]. However, the fly avoids the same concentration of NaCl in directional proboscis extension test. When stimulated with water and aqueous solution of 10^{-4} M quinine simultaneously, *Drosophila* avoids quinine. In the standard proboscis extension test, an aqueous solution of 10^{-4} M quinine does not inhibit water-induced extension. Proboscis extension in response to deionized water is 91.87 ± 2.56. Extension towards an aqueous solution of 10^{-4} M quinine is 89.29 ± 2.95.

Among the three repellents tested, quinine is the strongest, amiloride is a weaker repellent than quinine but stronger than NaCl.

In a variety of species, amiloride, a pyrazine-based compound, has been shown to block amiloride-sensitive sodium channels that are involved in taste transduction[20]. The taste hair of *Drosophila* has four chemosensory cells two of which, L1 and L2, respond to salts. The third sensory neuron (S cell) responds to sugars while the fourth (W cell) is believed to detect water[3,4,21,22]. Electrophysiological recordings have shown that the L1 and S cells are not affected by amiloride while the W cell firing is inhibited[23]. We, therefore, examined the effect of amiloride on proboscis extension in response to water.

In the standard proboscis extension test, amiloride was found to have no inhibitory effect on the water-induced proboscis extension. In the directional proboscis extension test, however, when the prothoracic legs were

Table 2. Responses of unsatiated *Drosophila* to gustatory stimulants in bilateral stimulations

	Per cent extensions			
	Ipsilateral	Medial	Contralateral	N
Sucrose 10^{-3} M	92.00 ± 3.84	6.00 ± 3.35	2.00 ± 1.98	50
NaCl 7.5×10^{-2} M	13.59 ± 4.46	15.25 ± 4.67	71.18 ± 4.97	59
NaCl 1 M	1.43 ± 1.40	7.14 ± 3.08	91.43 ± 3.35	70
Quinine 10^{-4} M	2.66 ± 1.87	22.67 ± 4.83	74.67 ± 5.02	75
Amiloride 5×10^{-3} M	5.33 ± 1.82	10.67 ± 3.56	84.00 ± 4.23	75

Values are given as mean ± S.D.
N, number of stimulations.

simultaneously touched with water on one side and an aqueous solution of amiloride on the other side, a concentration-dependent avoidance to amiloride was observed (Figure 2).

The development of the directional proboscis extension assay – a single fly test for studying gustatory behaviour in *D. melanogaster* – has been described in this paper. In order to make use of the full potential of the neurogenetic approach in understanding behaviour, one should be in a position to measure behaviour in a variety of paradigms, since different behavioural paradigms measure different aspects of behaviour. Yetman and Pollack reported that in the blowfly directionality of proboscis extension depends on the identity of the stimulated hair on the proboscis[17]. However, the present study reports the directional sensitivity of chemosensory hairs on the tarsal segment of legs in *Drosophila*. This fact has been used to develop a paradigm that can be used as a simple response test or a 'preference' test. The sensitivity of this new assay is compared with that of the routinely used paradigms. The directional proboscis extension test described here may allow us to study features of gustatory behaviour which escape the standard tests.

The directional proboscis extension test is more sensitive than standard proboscis extension assay. In the standard assay, aqueous solution of 5×10^{-3} M amiloride failed to inhibit water-induced proboscis extension, but in the directional assay, the fly avoids amiloride at a much lower concentration. Similarly, 10^{-4} M quinine does not inhibit proboscis extension but affects the directional response. It is apparent from the above study that *Drosophila* exhibits a greater sensitivity to repellents that are presented as differential gustatory inputs to opposite prothoracic legs than to repellents that are presented as uniform input through both its legs.

In directional proboscis extension assay, 5×10^{-5} M amiloride (which inhibits frequency of W cell firing in tarsi[23]) does not show significant effect on directionality of proboscis extension. But the fly showed a strong avoidance to aqueous solution of 5×10^{-3} M amiloride. A possible explanation for this apparent discrepancy could be that the electrophysiological recordings were confined to a limited number of taste hairs on prothoracic legs. W cells with higher thresholds of amiloride inhibition might be present in other taste hairs.

Different behavioural paradigms need not elicit identical response for a given stimulant. It has been found that low concentrations of NaCl are attractive in the feeding preference test. Maximum attraction was observed at 7.5×10^{-2} M NaCl (refs 6, 12). However, no attraction is evident at any concentration of NaCl in proboscis extension test on water-satiated flies[11]. On the other hand, in the directional test 7.5×10^{-2} M NaCl evokes a strong avoidance. In feeding preference assay[5,6], sensory inputs may come from three different taste sensory structures: (i) taste hairs of proboscis, (ii) taste pegs of proboscis, and (iii) tarsal taste hairs of legs; whereas in the proboscis extension assay, the input is only from the tarsal hairs of legs. It is possible that the same concentration of NaCl may give rise to different sensory codes in different sensory structures. For example, the electrophysiological responses evoked by 7.5×10^{-2} M NaCl in chemosensory cells of the proboscis might carry the sensory code for attraction, whereas the same in the tarsal chemosensory cells might code for repulsion. Indeed, it appears that the genetic machinery regulating the NaCl-induced firing in proboscis and tarsi are different. In mutations like *gustE*, *gustJ* and *gustF*, NaCl-induced firing of the chemosensory neuron L1 of proboscis is specifically reduced[23,24,27]. In these mutants, tarsal counterpart of the salt neuron, T1 cell response does not show any alteration[23,27]. All three mutants show a reduction in NaCl-induced attraction response as measured in the feeding preference assay[23,24,27].

Behavioural experiments on the blowfly using attractants and repellents[25], imply that excitatory and inhibitory inputs are compared at some site in the central nervous system. A simple comparator model was postulated to explain gustatory behaviour of wild-type and mutant *D. melanogaster*[26]. Studies using directional proboscis

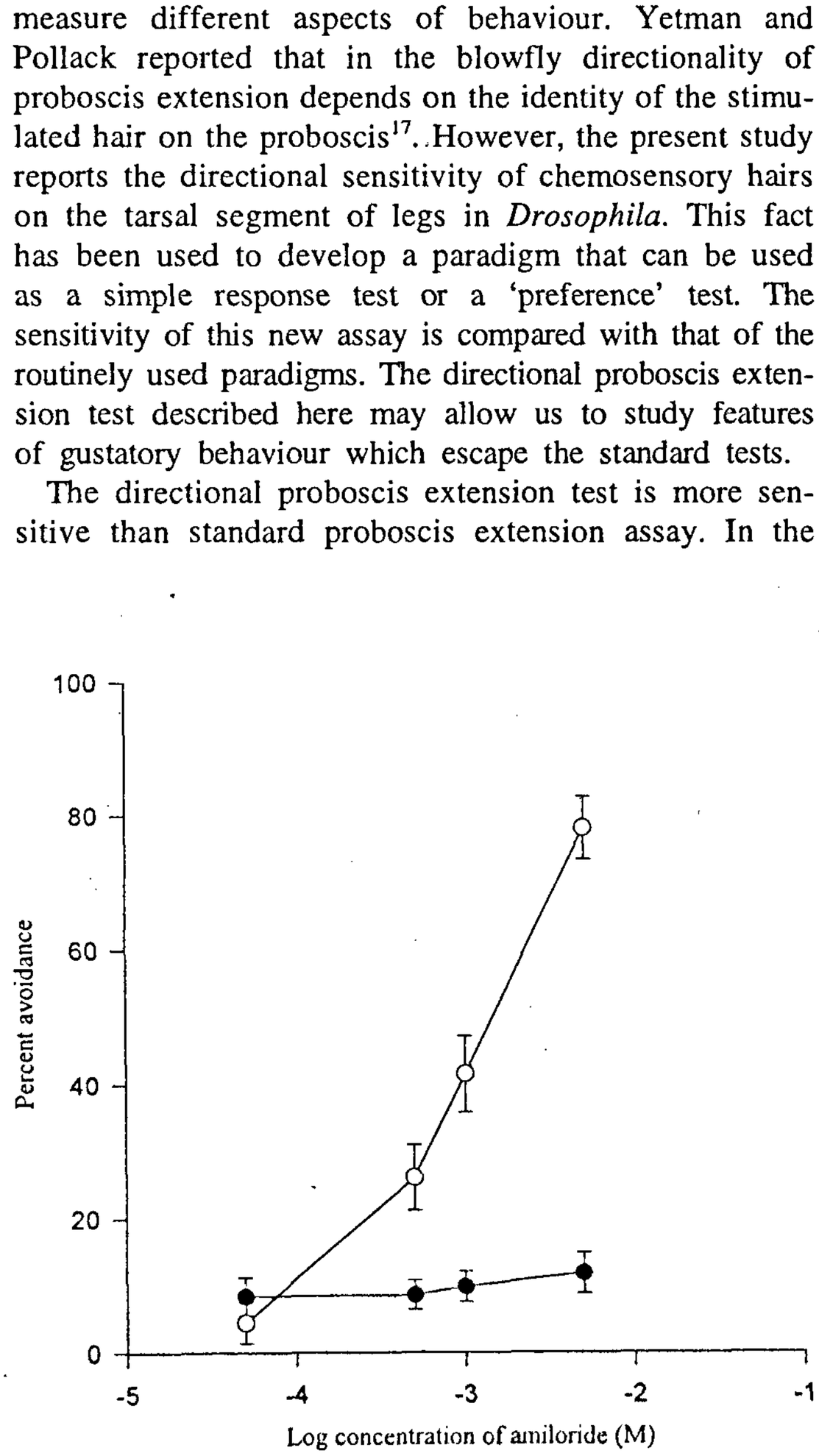


Figure 2. Effect of amiloride on water-induced proboscis extension as measured in the standard test (filled circle) and the directional test (open circle). Each point represents mean ± S.D. In case of directional proboscis extension test, each fly was stimulated five times and at least 45 such stimulations were used to calculate the mean and S.D. In case of standard proboscis extension test, each experiment comprised at least 9 flies and each fly was stimulated five times. Mean and S.D. were calculated from five such experiments.

extension response show that the fly discriminates between gustatory inputs from the left and right prothoracic legs. When the two legs are simultaneously stimulated by different concentrations of an attractant such as sucrose, the proboscis is extended towards the side of higher concentration. When water is paired with a repellent such as quinine, the fly preferentially extends its proboscis away from the repellent. These observations support the idea that chemosensory inputs are compared in the central nervous system of the fly. Moreover, these results imply that the comparison takes into account the chemical nature of the stimuli and the positional information of the stimuli.

Does the directional extension of proboscis play a significant functional role in the real life of the fly? An increase in the period of food deprivation is accompanied by an increase in the locomotor activity of the fly. Since tarsal hairs of legs are sensitive to taste stimuli, an increase in the locomotor activity allows the fly to explore a larger area in search for food[15]. In real life, while searching for food, the fly might have to orient itself towards the food source. Experiments on walking blowflies demonstrate that the fly has the ability to orient towards sucrose solution[15]. Our observations on *Drosophila* in the walking chamber also demonstrate that it is able to orient its proboscis as well as its whole body towards sucrose solution (unpublished results). This suggests that the directional extension of proboscis is part of foraging behaviour in *Drosophila*. The question is whether the directional extension of the proboscis plays any role in the orientation of the whole body towards the food source. One possibility is that the orientation of the body towards food depends on positive sensory input from the directionally-extended proboscis. In such case directional extension of proboscis should precede the orientation of the body. However, whether the orientation of proboscis precedes that of the fly, or both occur simultaneously, needs to be resolved.

1. Stocker, R. F., *Cell Tissue Res.*, 1994, **273**, 3–26.
2. Isono, K. and Kikuchi, T., *Jpn. J. Genet.*, 1974, **49**, 113–124.
3. Fujishiro, N., Kijima, H. and Morita, H., *J. Insect Physiol.*, 1984, **30**, 317–325.
4. Rodrigues, V. and Siddiqi, O., *Proc. Indian Acad. Sci.*, 1978, **87B**, 147–160.
5. Tanimura, T., Isono, K., Takamura, T. and Shimada, I., *J. Comp. Physiol. A.*, 1982, **147**, 433–437.
6. Arora, K., Ph D thesis, University of Bombay, Mumbai, India, 1985.
7. Wieczorek, H. and Wolff, G., *J. Comp. Physiol. A*, 1989, **164**, 825–834.
8. Deak, I. I., *Nature*, 1976, **260**, 252–254.
9. Tompkins, L., Cardosa, M. J., White, F. V. and Sanders, T. G., *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 884–887.
10. Falk, R., *J. Insect Physiol.*, 1979, **25**, 87–91.
11. Rodrigues, V., Ph D thesis, University of Bombay, Mumbai, India, 1981.
12. Siddiqi, O., Joshi, S., Arora, K. and Rodrigues, V., *Genome*, 1989, **31**, 646–651.
13. Minnich, D. E., *J. Exp. Zool.*, 1921, **33**, 173–203.
14. Minnich, D. E., *Anat. Rec.*, 1926, **34**, 126.
15. Dethier, V. G., *The Hungry Fly*, Harvard University Press, Cambridge, Massachusetts, 1976.
16. Stocker, R. F., *J. Comp. Physiol. A*, 1977, **115**, 351–361.
17. Yetman, S. and Pollack, G. S., *J. Comp. Physiol. A*, 1987, **160**, 367–374.
18. Lewis, E. B., *Drosophila Inf. Service*, 1960, **34**, 117–118.
19. Inamdar, M., VijayRaghavan, K. and Rodrigues, V., *J. Neurogenet.*, 1993, **9**, 123–139.
20. Lindemann, B., *Physiol. Rev.*, 1996, **76**, 719–766.
21. Falk, R., Bleiser-Avivi, N. and Atidia, J., *J. Morphol.*, 1976, **150**, 327–342.
22. Nayak, S. V. and Singh, R. N., *Int. J. Insect Morphol. Embryol.*, 1983, **12**, 273– 291.
23. Jayaram, V. C., Ph D thesis, University of Mumbai, Mumbai, India, 1997.
24. Sathe, S., Ph D thesis, University of Bombay, Mumbai, India, 1992.
25. Dethier, V. G., *Biol. Bull.*, 1953, **105**, 257–268.
26. Balakrishnan, R. and Rodrigues, V., *J. Exp. Biol.*, 1991, **157**, 161–181.
27. Saraswati, S., Ph D thesis, University of Mumbai, 1998.

ACKNOWLEDGEMENTS. I thank Professor O. Siddiqi for his encouragement and many valuable comments while this work was being done. I also thank Professor V. Rodrigues for helpful discussions. Thanks are due to C. Sasidhar for helping me with the diagram. The work described here has been supported by UNDP/World Bank/WHO Special Programme for Research Training in Tropical Diseases and by the Department of Biotechnology, Govt. of India Grant No. BT/R&D/09/29/93 to Prof. O. Siddiqi.

Received 3 February 1998; revised accepted 25 March 1998

Record of hepatopancreatic parvo-like virus in cultured penaeid shrimps of India

C. V. Mohan, K. M. Shankar, A. Hegde and P. M. Sudha

Fish Pathology Laboratory, Department of Aquaculture, College of Fisheries, Mangalore 575 002, India

This communication provides histopathological evidence for the occurrence of hepatopancreatic parvo-like virus (HPV) in the hypertrophied nuclei of midgut caecum and hepatopancreatic tubular epithelial cells of penaeid shrimps cultured in India.

In the last decade, commercial shrimp farming has taken firm root in several maritime states of India. Since July 1994, the shrimp industry has been bogged down with diseases in shrimps, especially of viral etiology[1]. So far, more than 12 viruses have been recorded in cultured shrimps of the world; a few of them being serious pathogens[2].

Scientific information on viral disease of cultured shrimps in India began to appear since 1995. However,