Variable response of male *Helicoverpa armigera* moth to sex pheromone blends: A case of behavioural polymorphism?

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Sex pheromone traps used for monitoring *Helicoverpa armigera* (Hubner), a polyphagous pest of many crops cultivated across the length and breadth of India, are known to be highly inconsistent in trapping male moths. We report here, through replicated studies from five locations in Karnataka, India, two population parameters – density and polymorphism, as important factors that affect the trap catches of *H. armigera* males.

THE bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae), is a serious polyphagous pest of many cultivated crops in India. In order to help timely management of the pest, pheromone traps are widely recommended for its monitoring.

The sex pheromone of *H. armigera* is a multi-component system containing five chemicals, viz. (Z)-11-hexadecenal, (Z)-9-hexadecenal, (Z)-11-hexadecen-1-ol, hexadecenal and hexadecanol[1]. However, (Z)-11-hexadecenal is identified as the major sex-pheromone component[2] and (Z)-9-hexadecenal, the important minor component[3,4]. The species being an important pest of several crops in the Old World, blends of different combinations are being recommended in various countries[3–11]. However, in many countries including India, a binary mixture of (Z)-11-hexadecenal and (Z)-9-hexadecenal in a ratio of 97 : 3 respectively, is being recommended[6,7] as the most common blend for monitoring *H. armigera* populations. The same binary mixture is also being commercially marketed for use in India. But in the recent past, there were many unpublished reports of the poor performance of the commercial pheromone traps.

Trap catches are likely to vary depending on the parameters of the trap design, blend ratio of the components of the lure and environmental conditions. In addition, the population parameters such as density and genetic variation in the population might also contribute to the variable trap catches[12]. There could exist, for instance, genetic variation in the ability of individuals of a population to respond differentially to blend ratios of the pheromone. We explored the possibility of prevalence of such a behavioural polymorphism in *H. armigera* in responding to the lures by running traps provided with different blends of the five component chemicals of the pheromone.

Six different combinations selected on the basis of recommendations in different countries, of the five component chemicals, were used in four replications (Table 1). Blends prepared afresh from 95% pure chemicals imported from Natural Resources Institute, London were loaded at the rate of 2 mg per rubber septum, sealed and stored under refrigerated conditions. All the trials were started within one week from the date of preparation of the blends. Concurrent trials were run at Raichur, Dharwad, Ranebennur and Shimoga in cotton fields and at Bangalore (GKVK) in red-gram fields using freshly manufactured traps. All traps used at all locations were of the same design of baffle-funnel type manufactured by Bio-Pest Management. The traps were provided with dark red funnels of 5.5 cm height, mouth diameter 9.50 cm, bottom diameter 3.00 cm and were without a neck. The funnels were covered with a hood, provided with a slot for fixing the pheromone butt, of the same colour at a height of 3.50 cm above the funnel and supported by three sticks. The sticks also supported a baffle, each projecting into the funnel and directed towards the centre of the funnel. Each baffle was 4 cm wide and 3.5 cm high, sitting right above the funnel. When placed in the field the traps were provided with a clear plastic sleeve of 0.5 m length, attached to the outside of the funnel, such that moths entering the trap are led into the sleeve. The sleeve was closed at the bottom with the help of a rubber band in the field. The moths were collected from the sleeves by opening the same at the bottom. The funnels were also provided with a handle that helped fasten the traps to a stick.

Trials started during the third week of October 2000 and were terminated at all locations after 21 days. Traps were placed at 45 cm above crop canopy and the heights were adjusted weekly to accommodate for crop growth. At each location, each blend was replicated four times. Counts of male moths caught in each trap were recorded separately on daily basis and the data were pooled week-wise – WI, WII and WIII – representing the data of the three weeks, for statistical analysis. Data were analysed using a three-factor ANOVA after transforming the raw data into $\sqrt{x} + 0.5$.

It was observed that the trap catches varied greatly from location to location. Highest catches were at Raichur with a mean of 55 moths per week per treatment, and the lowest was at Bangalore with a mean of 5.6 moths per week per treatment. The ANOVA clearly indicated significant differences between the locations ($F_{4, 267} = 127.66$; $P < 0.01$), the blends ($F_{5, 267} = 239.53$; $P < 0.01$) and the weeks ($F_{2, 267} = 8.52$; $P < 0.05$) as also the various interaction effects, except the weeks × blends ($P > 0.05$). The results thus indicate locations representing mean trap catches from all traps irrespective of the blends, and blends irrespective of the locations, to vary greatly in their

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Table 1. Percentage of different components tested in six blends of sex pheromone of *Helicoverpa armigera*

Blend	(Z)-11-hexadecanal	(Z)-9-hexadecanal	Hexadecanal	Hexadecanol	(Z)-11-hexadecanol	Reference
P1	55.84	3.9	27.27	1.3	11.69	11
P2	87	3	4	6		7
P3	90.91	9.09				8–10
P4	92	8				4, 8
P5	97	3				3, 4, 6
P6	100					5

ability to trap male moths of *H. armigera*. Further, location × blend interaction ($F_{20,267} = 3.67$; $P < 0.05$) also suggested that the trap catches in different blends did not follow the same pattern in the five locations tested.

However, it is possible that the differences might be just the reflections of the differences in the absolute number of moths trapped in different localities, which showed a variation of up to 10-fold from locality to locality. Therefore, the data were normalized into relative measures. For this purpose, highest catches in any of the 24 traps (4 replications × 6 blends) on any day during the course of trapping in each locality was equated to 100, and all the other values were accordingly transformed. Therefore, when the means were taken for each week, the values were always less than 100, representing the percentile catches of the highest catch in a day in a trap for that location and are independent of the density differences across locations.

The differences observed, in trap catches, persisted even after normalization of the data. The differences were significant for the locations ($F_{4, 267} = 17.58$; $P < 0.01$), the blends ($F_{5, 267} = 204.03$; $P < 0.01$) and the weeks ($F_{2, 267} = 9.61$; $P < 0.05$). Further, the differences were also significant for the interaction between the location × blend ($F_{20, 267} = 3.08$; $P < 0.01$). This result clearly suggests differential response of moths to the six blends tested in different locations (Table 2). At the same time, the mean values of catches demonstrate the attraction of moths to all the blends in at least some of the locations tested, indicating the differential response of the moths in each population to different blends tested.

The extent of variation in the response of male moths to the various blends could possibly be a function of the size of the population. The extent of diversity among the male moths found in their response to the different blends was worked out using Shannon's diversity index (H'). Shannon's index was computed using the proportional catches in different blends at each locality following Magurran[13]. The diversity index was then correlated with the total number of moths trapped in each locality, as a measure of density. It was observed that the calculated diversities for different locations were not correlated to the density (mean moths per trap per day) of the moths ($r = 0.58$; $P < 0.05$). Thus the diversity of response pattern among male moths to different blends in different locations is independent of the local density of moths.

What is more important is the fact that location × blend interaction also showed significant differences, clearly indicating that the proportional response pattern of male *H. armigera* moths is not the same in all the populations tested. The proportional representation of moths trapped in the six combinations and five locations is indicated in Figure 1. The pie diagrams clearly indicate that even the most attractive combination, i.e. P_5 varied from as low as 61% in Raichur to as high as 89% in GKVK. Similar variations were also observed in other combinations such as P_1, P_2, P_3 and P_6. However, the striking differences were in the P_2 and P_6 combinations (see Table 2).

It is possible, however, that overwhelming catches observed in P_5 combination might underestimate the relative variation observed in the five localities to other blends. Therefore, to verify this aspect, an attempt was also made to reanalyse the data by removing values for P5. The results indicated continued significant location × blend interaction effect ($F_{16,222} = 1.99$; $P < 0.05$), suggesting that the differential response of the male moths is persistent even among the blends of lesser attraction and that the relative proportional attraction to these blends varied significantly from location to location.

The study revealed several important results. It was observed that the five locations varied significantly in the density of trappable moths. The density of moths in different locations might vary due to several factors. These include timing of the study, cropping pattern followed in the vicinity and climatic factors prevailing during the time of testing[14]. Contributions by these factors, however, were not investigated in the present study. However, the locational variation in trap catches to P_5, the recommended blend, suggests that the prevailing densities of moths in different locations might be the contributing factor for variable catches to the commercial traps.

Further, the study also revealed significant differences between location × blend combinations. This aspect is of particular importance because the differences persisted even after removing the effects of densities by normalizing the data for each location and also the most attractive blend. As a consequence, the results strongly indicate differential performance of the blends in different locations, indicating differences in the proportional representation of the moths that are attracted to different blends. Thus the results indicate behavioural polymorphism in *H. armigera* male moths to respond to

blends of their pheromonal components. The blends tested being very disparate combinations, the results are all the more interesting and suggest the possibility of a wide range of activity limits for *H. armigera* males to respond to their pheromonal blends. Similar polymorphism in their susceptibility to insecticides has been reported for populations of *H. armigera*[15] from South India.

Table 2. Percentage response of *Helicoverpa armigera* males to different combinations of pheromonal blends in different locations of Karnataka during *Kharif* 2000

	Raichur				Dharwad				Ranebennur			
Blend	WI	WII	WIII	Mean	WI	WII	WIII	Mean	WI	WII	WIII	Mean
P1	2.68 (8.14)	3.58 (9.29)	5.36 (11.56)	3.87 (9.66)	3.58 (5.98)	2.68 (6.88)	1.79 (5.73)	2.68 (6.20)	5.01 (9.50)	10.00 (16.00)	2.51 (5.04)	5.84 (10.18)
P2	21.88 (27.67)	16.97 (23.62)	20.98 (26.73)	19.94 (26.01)	3.57 (9.46)	4.47 (8.56)	4.47 (10.37)	4.17 (9.46)	5.01 (7.07)	0.01 (0.57)	0.01 (0.57)	1.68 (2.74)
P3	10.27 (16.35)	7.15 (12.99)	4.47 (10.29)	7.29 (13.21)	1.79 (4.30)	0.90 (3.15)	0.90 (3.15)	1.20 (3.54)	7.51 (11.54)	2.51 (5.04)	5.01 (7.07)	5.01 (7.88)
P4	4.91 (12.34)	5.30 (13.05)	7.14 (13.57)	5.79 (12.99)	4.46 (12.04)	2.68 (8.31)	1.79 (4.30)	2.98 (8.22)	0.01 (0.57)	12.50 (17.66)	0.01 (0.57)	4.17 (6.27)
P5	90.20 (74.02)	33.93 (35.58)	52.68 (46.37)	58.94 (51.99)	58.93 (50.67)	52.68 (47.21)	81.25 (68.35)	64.28 (55.41)	45.01 (42.27)	57.50 (49.55)	32.50 (34.28)	45.00 (42.03)
P6	6.25 (13.97)	0.90 (3.15)	0.01 (0.57)	2.39 (5.90)	0.90 (3.15)	2.68 (6.88)	1.79 (5.73)	1.79 (5.26)	5.01 (7.07)	12.50 (20.47)	5.01 (9.50)	7.50 (12.35)
Mean	22.70 (25.41)	11.30 (16.28)	15.11 (18.18)	16.37 (19.96)	12.21 (14.27)	11.02 (13.50)	15.33 (16.27)	12.85 (14.68)	11.26 (13.01)	15.84 (18.22)	7.51 (9.51)	11.53 (13.58)

	Shimoga				GKVK				Lure × Week			
Blend	WI	WII	WIII	Mean	WI	WII	WIII	Mean	WI	WII	W III	Mean ± SD
P1	1.40 (3.84)	3.17 (7.56)	0.01 (0.57)	1.53 (3.99)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	2.54 (5.61)	3.89 (8.06)	1.94 (4.70)	2.79 ± 2.57 (6.12)
P2	4.17 (8.56)	0.01 (0.57)	0.01 (0.57)	1.40 (3.24)	4.17 (8.67)	2.09 (4.62)	0.01 (0.57)	2.09 (4.62)	7.76 (12.28)	4.71 (7.59)	5.10 (7.76)	5.86 ± 7.35 (9.21)
P3	2.79 (7.11)	2.79 (7.11)	0.01 (0.57)	1.86 (4.93)	8.33 (14.55)	0.01 (0.57)	0.01 (0.57)	2.78 (5.23)	6.14 (10.77)	2.67 (5.77)	2.08 (4.33)	3.63 ± 3.23 (6.96)
P4	1.40 (3.84)	2.79 (7.11)	0.01 (0.57)	1.40 (3.84)	2.09 (4.62)	2.09 (4.62)	0.01 (0.57)	1.40 (3.27)	2.58 (6.68)	5.07 (10.15)	1.79 (3.91)	3.15 ± 3.26 (6.92)
P5	58.33 (53.67)	41.39 (39.84)	24.72 (28.56)	41.48 (40.69)	54.16 (51.12)	37.50 (37.50)	33.33 (34.31)	41.67 (40.98)	61.32 (54.35)	44.60 (41.93)	44.90 (42.37)	50.27 ± 16.45 (46.22)
P6	2.79 (7.11)	0.01 (0.57)	0.01 (0.57)	0.94 (2.75)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	2.99 (6.37)	3.22 (6.33)	1.37 (3.39)	2.53 ± 3.36 (5.37)
Mean	11.81 (14.02)	8.36 (10.46)	4.13 (5.24)	8.10 (9.91)	11.46 (13.35)	6.95 (8.08)	5.56 (6.20)	8.00 (9.21)	13.89 (16.01)	10.69 (13.31)	9.53 (11.08)	11.37 ± 4.54 (13.47)

Figures in parentheses are arc-sin transformed values; Details of treatments are given in Table 1. W, Week; P, Blend.

ANOVA				
Source of variation	DF	F	SEm	CD ($P = 0.05$)
Replication	3	8.52*	–	–
Location	4	17.58**	1.03	2.85
Blend	5	204.03**	1.13	3.12
Week	2	9.61*	0.80	2.21
Location × Blend	20	3.08**	2.52	6.98
Location × Week	8	3.72**	1.78	4.94
Blend × Week	10	2.48**	1.95	5.41
Location × Blend × Week	40	1.49*	–	–
Error	267	–	–	–

*$P < 0.05$; **$P < 0.01$.

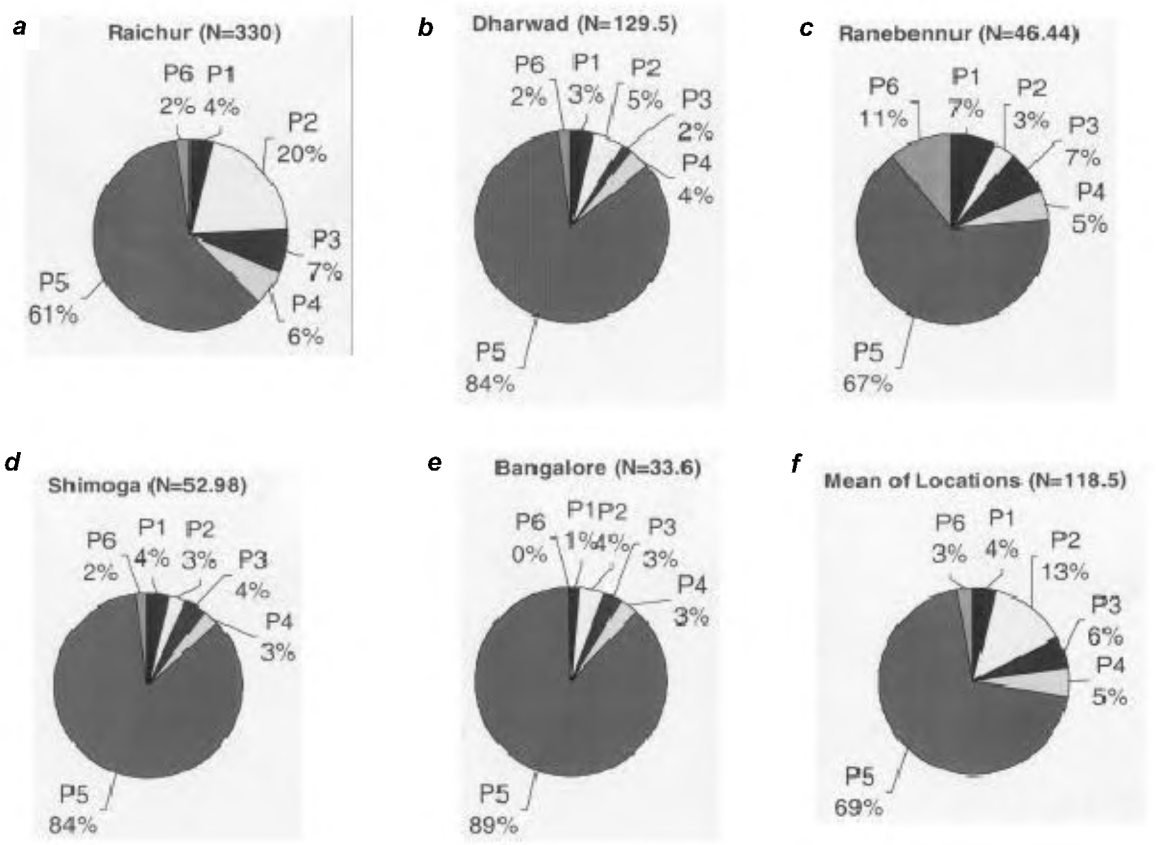

Figure 1 ***a–f.*** Per cent attraction of *Helicoverpa armigera* male moths to different blends of pheromonal components at five locations in Karnataka during *Kharif* 2000. P1 to P6 represent different blends as indicated in Table 1. *N* represents the mean total catches per week in each location.

In spite of the variable response of moths to pheromone blends, it was interesting to find that 97 : 3 mixture of (Z)-11-hexadecenal and (Z)-9-hexadecenal, as previously recommended, was the best combination of synthetic pheromone lure for trapping *H. armigera* male moths. This result was confirmed across all the locations tested. However, the proportional catches of *H. armigera* moths to this combination varied from as low as 61 to 89% in different locations. This is of particular importance because even when moth densities were highest as observed at Raichur, the above mixture managed to catch only 61% of the moths. As a consequence, it is difficult to use trap catches as indexes of moth densities and there is a need for calibrating the trap catches in each locality to reflect the densities of the moths. More so, because the per cent trap catches might vary even across small distances of a few tens of kilometres as observed between Ranebennur (67%) and Shimoga (84%).

Polymorphism is anticipated in *H. armigera* due to the fact that very disparate combinations of pheromonal components are suggested in different countries of the Old World. However, these were on a much larger geographical scale. The present study is a check on this aspect and on a much smaller geographical scale in India. The observed patterns in the response of *H. armigera* moths to pheromone lures provide us with an interesting model system to investigate the origin and maintenance of behavioural polymorphism. This is particularly appealing because, being a multicomponent pheromone, the lures can be blended in different ratios for manipulative field experiments. However, more controlled field and laboratory studies have to be carried out to obtain a complete picture of this polymorphism before addressing its evolutionary mechanisms in *H. armigera*.

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Methodological considerations in measurement of dominance in primates

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The strength of dominance hierarchy in a group of animals needs to be quantitatively measured since it influences many other aspects of social interactions. This article discusses three attempts made by previous researchers to measure the strength of hierarchy. We propose a method which attempts to rectify the lacunae in the previous attempts. Data are used from a group of Japanese macaques housed in a colony. A method to calculate strength of hierarchy has been illustrated and a procedure has been suggested to normalize the dominance scores in order to place the ranks of individuals on an interval scale.

AN animal of a species that lives a solitary life does often come into contact with another conspecific. Since animals in most species have rather limited home ranges, there is a good chance that the other conspecific which the animal encounters is the one that the animal would have met previously. On the other hand, animals that live in a social group know group members individually and their interactions are on a routine basis.

Bernstein and Gordon[1] describe interaction between any two animals as ignoring each other, attacking each other, or engaging in some common activity. The outcome of aggressive interactions could be understood by the encounter characteristics (such as age, sex: older individuals or males are more likely to win), location (an animal in its territory is more likely to win), or previous learning ('trained' winners or losers). However, 'when we note a regularity in the directionality of agonistic encounters, and such regularity cannot be explained by the course of the encounter itself, spatial determinants or broadly learned patterns such as trained winners or losers, then we describe the relationship governing agonistic encounters as a dominance relationship'[1].

Schjelderup-Ebbe[2] was the first biologist to identify and describe the presence of a hierarchical system in animal societies. Since then, hierarchies have been observed in all group-living species. A large number of research papers and reviews have been published, especially on primates, on the concept as well as on the mechanism, maintenance, reversal of dominance systems[3–6].

The members of a social group could be classified into a hierarchy in which the individual ranks are placed merely on an ordinal scale. At a more sophisticated level, the hierarchical difference could be quantified by placing individual ranks on an interval scale. The strength of dominance hierarchy refers to the strength of linearity in dominance relationships among members of a group. There are several types of hierarchies:

(a) *Despotism*: In which one individual may dominate all others with no difference of rank among rest of the group members.
(b) *Egalitarism*: In which each group member may be equally likely to win or lose in an encounter with any other member.
(c) *Complete linearity*: In which the dominance ranks are totally linear.

Most of the non-human primate societies range somewhere between complete egalitarism and complete linearity. A number of researchers have shown that the observed or expected outcome of dominant/subordinate interactions is related to many other aspects of social behaviour. It is therefore useful to determine the strength of a hierarchical system. It is for this reason that several attempts have been made to quantify the dominance relationship among members of a group in various species.

The quantification and analysis of dominance was first attempted by Murchison[7]. He measured social interactions in terms of categories of time and space, and subjected the data to the technique of co-variation. He demonstrated that the initial encounters could result in a polygonal form of dominance, but over a period of time,

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