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HOST ACCEPTANCE BEHAVIOUR OF AN APHID PARASITOID *LIPOLEXIS OREGMAE* [GAHAN] [HYMENOPTERA: BRACONIDAE]

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ABSTRACT: The present study examines the combined effect of external and internal cues that regulate the host-acceptance behaviour of *Lipolexis oregmae* (Gahan) (Hymenoptera: Braconidae) which is the third phase [following host habitat location and host finding] in the process of host selection by the parasitoids. During this phase, the female parasitoids may use physical and/or chemical cues in host recognition. These cues include host size, shape, surface texture, and movement, and the presence of volatile or nonvolatile chemicals. The role of following factors in host acceptance by *L. oregmae* were studied in detail: role of aqueous extract of host, role of cornicle secretion of host, role of host age [instars], role of female size and age, influence of host mobility, the effect of colour, and site of attack. The data furnish considerable insight into the host-selection process of *L. oregmae*. They show that *L. oregmae* first finds the host-habitat and then locates the host proper and finally accepts it for oviposition. Olfaction plays significant role in habitat location while the host finding and acceptance is brought about mostly by olfaction and taction stimuli coordinated by gustation and vision. Parasitoid's antennae play a major role in this process.

Keywords: *Lipolexis oregmae*, *Aphis gossypii*, host acceptance, parasitoid, biological control.

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1. INTRODUCTION

The host-selection process and final acceptance by aphid parasitoids begin with the analysis of external cues and culminate with probing and subsequent oviposition; this complex behavioural sequence to assess host suitability is regulated by a variety of chemical, physical and nutritional factors [1-3]. The role of these factors on host acceptance behaviour of the several aphid parasitoids

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have been studied in much detail [4-13] particularly the external cues. Briefly, physical factors play an important role in host acceptance, as the visual detection of the host, an attack response by the parasitoid even without physical contact, and colour is of key importance [11, 13-15] and semiochemicals [9, 16-18]. However, the host acceptance behaviour of *Lipolexis oregmae* (Gahan) (Hymenoptera: Braconidae: Aphidiinae) is not known, though the parasitoid was extensively used to control the population of brown citrus aphid [19]. Its host habitat location and host finding behaviour was studied earlier by me [20-21]. In biological control, host instar selection by a parasitoid is among the most important factors that affect its potential to reduce an aphid population, since it affects both aphid and parasitoid population increase [8]. The host suitability has been considered to be a linear function of host size, but, although it is true in hosts that do not feed, it is not always the case in aphid parasitoids [22]. The optimal foraging theory predicts that host selection and acceptance aims at maximising the profits of the next parasitoid generation [23]. That is, the female takes care to ensure the most favourable environment for improving its progeny fitness, so that to increase its reproductive success rate. Host individuals may be suitable in terms to satisfy a minimum threshold for growth and development for the parasitoid larva but are presumably of different quality because different hosts can cover the nutritional needs of the developing parasitoid larva at a different success rate [24]. The quality of the host could affect the size, sex ratio, longevity and fecundity of the adult parasitoid [8, 22, 25]. Our knowledge on the factors that influence *L. oregmae* host selection is not known and it mainly concerns preference for different aphid species [11, 26, 27]. However, a foraging parasitoid when searching in an aphid colony encounters different types of host and therefore, studies that examine parasitisation rate and preference on different host types that are simultaneously present, are relevant to the natural field situation. Foraging theory predicts that optimal host selection aims to maximise the profits for the next parasitoid generation [23]. Taking into account that the host represents a finite food resource, acceptable host individuals should satisfy the minimum resource threshold for the parasitoid larva's growth [24]. In fact, the finite resources available during parasitoid development result in a close association between host size and offspring size [28]. Therefore, fitness of the offspring is largely dependent on the mother's ability to accurately assess the suitability of the host [29]. Accepted host individuals apparently satisfy a minimum quality threshold for the parasitoid larva's growth and development but presumably should cover its nutritional needs at a different success rate [24]. However, apart from host traits, host selection is also based on behavioural and physiological properties of the foraging female, such as her physiological state, age [24, 30, 31], previous experience [32] and search time allocation [33]. Host choice decisions among hosts of dissimilar size may be affected by the size of the foraging female. Parasitoid female size is related to reproductive potential as well as to foraging efficiency [34-37]. In particular, large females of *Aphidius colemani* Viereck [Hymenoptera: Braconidae: Aphidiinae] encounter hosts faster than small females [38]. Host selection is also

affected by host defensive capability, which might increase the parasitoid's risk during host manipulation and reduce the time available for searching. Thus, it may be advantageous for females to be large as they are more successful at parasitizing large hosts. Evidence for this is derived from studies on mortality risk [24] and handling time [5, 39] during host manipulation and search time allocation [33], which reveal positive correlations with host size. Despite this association between maternal size and host selection this topic has received a little attention. The existence of positive association between female parasitoid size and host size has earlier been demonstrated [40]. In both these studies, small and large females were produced by rearing the parasitoid in a small or a large host. Therefore, it remains unclear whether an effect of female size on host selection occurs in sympatric females. In fact, such an approach would examine to what extent intra-population female size variation could alter the frequencies of different hosts attacked. Searching for hosts occurs on plants and thus plant traits might affect host searching and handling. For example, characteristics of plants such as trichomes can interfere with the foraging effectiveness of parasitoids [41]. In the present study, the hypothesis was tested that large parasitoid females could be more efficient than small ones in overcoming the difficulties posed by the structure of plant substrates. The present study aims to fill this gap, by studying both the combined effect of external and internal cues that regulate the host-acceptance behaviour of *L. oregmae*.

2. MATERIALS AND METHODS

2.1. Insect rearing

A culture of *L. oregmae* was initiated from *Aphis gossypii* Glover (Hemiptera: Aphididae) mummies collected on *Solanum melongena* L. (Solanaceae) in Gorakhpur a month before the start of the experiments. During this period, the parasitoid culture was kept under its rearing conditions in the laboratory. The parasitoid culture was developed on the potted eggplants infested with *Aphis gossypii*. The aphid was reared on eggplants and was initially collected on eggplants in the campus of the university. They were kept in wood framed cages 120×60×100 cm, in the field laboratory with average day temperature $22.0 \pm 2.5^\circ\text{C}$ under natural light. Potted egg plants bearing high infestations of *A. gossypii* were covered separately with a plastic cage and were placed in a growth chamber with $25 \pm 0.5^\circ\text{C}$, $70 \pm 5\%$ R.H. and a 14L : 10D photoperiod. Each cage was 15 cm in diameter and 30 cm in height. It was made of a PVC leaf [0.4 mm thick] with two lateral openings each of 10×10 cm, which together with the top opening of the cage, were covered with fine muslin cloth. Then, 5–10 newly emerged parasitoids were released in each cage. At the emergence of the first parasitoids of the new generation drops of dilute mixture of honeydew and honey were placed on the muslin at the top of the cage, since it is a highly suitable food for survival of *L. oregmae* adult [42]. After two days the parasitoids emerged in the cage were collected by insect aspirator, and released to another plastic cage containing an egg plant with aphids, for maintenance of the parasitoid rearing. In the

experiments naïve (which had not a previous contact with aphids) parasitoids of less than 12 h old were used. Egg plant leaves bearing only mummies of *A. gossypii* were introduced in a plastic cage of 20 cm in diameter and 30 cm high, otherwise similar to that described above. Also feeding and conditions inside the cabinet were identical. After emergence, the parasitoids were allowed to copulate in similar cage and kept there for another 24 h, to mate. Females were then carefully collected.

2.2. Behavioural bioassays

L. oregmae females used in all experiments emerged from mummies isolated in glass vials [8×60 mm]. The mummies were checked twice a day to isolate newly-emerged adults, which were immediately fed with honey and tested the next day.

2.2.1. Role of olfaction

2.2.1.1. Role of aqueous extract of host on the response of the parasitoid

Ten aphids were crushed on a blotting paper [Whatman No.1] and the cuticles were removed, thereafter the filter paper [having the soaked extract] was exposed to a single female parasitoid in a Petri dish [5.5 cm in diameter x 1 cm high]. In another set of experiment the cuticle was thoroughly washed with water to remove any adhering fluid and then was exposed to the parasitoid by keeping it on the filter paper. The behaviour of the parasitoid was observed and recorded in two conditions under stereoscopic binocular microscope.

2.2.1.2. Role of cornicle secretion of host on the response of the parasitoid

The cornicle secretion of *A. gossypii* was obtained by gently stimulating the aphids with forceps and then rapidly soaking the filter paper [Whatman No. 1] with the exudate. The piece of cornicle secretion soaked filter paper was put in the centre of the Petri dish [5.5 cm in diameter x 1 cm high] on another filter paper and a female parasitoid was released to observe its response under stereoscopic binocular microscope. The behaviour of the parasitoid was recorded.

2.2.2. Role of host age [instars]

The oviposition preference of *L. oregmae* females was investigated when provided simultaneously with equal numbers of all instars of *A. gossypii* to test the hypothesis that all instars of the aphids are equally accepted as host for the parasitoid. The experiments were conducted in plastic Petri dishes 10 cm in diameter and 1 cm in height. A layer of moistened filter paper on the base of the dish was placed on which a leaf of eggplant was put upside down. Ten individuals of each instar [first to fourth] of *A. gossypii* were carefully transferred with a fine camel hair brush and left for about 1 h to settle down on the leaf. Then, a female parasitoid subjected to the procedure described above, was introduced in the dish. After the first aphid was attacked, the dish was placed in a growth cabinet with conditions as above. The parasitoid was left in the dish for 1 h.

After 48 h of their parasitisation, the aphids were treated with a mixture of chloral hydrate-phenol [saturated solution] mixture for 24-36 h to detect the presence of eggs under the light microscope. The parasitoid eggs in each aphid were counted. Because of the large number of trials involved, different cohorts of aphids and parasitoids were used for the six choice combinations; however, the sequence of trials involving different instar-combinations was arbitrary, with trials interspersed in time. Using dichotomous choice tests, the hypothesis was tested that females of *L. oregmae* do not distinguish between the four instars of the aphid, *A. gossypii*. The four instars were compared two at a time with 20 individuals of each instar, for a total of six sets of replicated [10 times] choice tests: first instar vs second instar, first instar vs third instar, first instar vs fourth instar, second instar vs third instar, second instar vs fourth instar, and third instar vs fourth instar. A convenient index of preference is the ratio of the proportions of available and accepted host types, or the ratio of eggs laid in different host types [43, 44]. Both indices yield similar results if the number of eggs laid is correlated with the number of hosts parasitised [44]. Females of *L. oregmae* never deposit multiple egg clutches during a single ovipositor insertion and superparasitised usually when the female parasitoid was exposed for a longer period to a small host patch. However, to avoid any biased estimate of preference on the basis of number of eggs laid, in this experiment, therefore, the numbers of aphids parasitised was estimated as a measure of preference. If parasitised hosts are not replaced in choice tests, the probability increases that the less preferred host type is accepted as the preferred hosts become parasitised and are rejected by the female to avoid superparasitism; relative preference eventually can no longer be detected [44]. In preliminary tests, we determined that discrimination between host types would be maximised if each parasitoid female was provided with 30 aphids, 15 of each instar, and allowed to forage for approximately 30 min. Older instars in each test had the distal end of one antenna amputated as a biomarker [45]. Females were placed individually in a glass Petri dish (5.5 cm in diameter x 1 cm high), which was lined with a Whatman No. 1 filter paper. At the end of a trial, the aphids were transferred to a screened cage containing a fresh brinjal shoot. After 48 h of their parasitisation, the aphids were treated with a mixture of chloral hydrate-phenol (saturated solution) mixture as mentioned above to estimate the number of aphids parasitised.

2.2.3. Role of female size and age

The above experiment showed that females of *L. oregmae* accepted all four aphid instars as hosts but preferred the relatively smaller aphids [second and third instars] among those available. Because all experimental females were standardised for size and age, other hypotheses were tested that host choice is determined by female size and female age. If a preference for small hosts simply reflects some physical constraint of the female, such as ovipositor length [46, 47]. Large females, as opposed to small females, should be more successful attacking large hosts, whereas small females should be more (or equally) successful as large females attacking small hosts. This proposition was tested by

rearing two cohorts of *L. oregmae*; one cohort was reared on first instars and the other on fourth instars of *A. gossypii*. Because parasitoid size is a positive function of host size, parasitoids developing in small aphids are significantly smaller on average than counterparts developing in large aphids [48]. Using the same design as mentioned above, both small [n = 10] and large females [n = 10] were provided with a choice between second and fourth instars [20 individuals of each] in a Petri dish; females were allowed to forage for 30 min. After 48 h of their parasitisation, the aphids were treated with a mixture of chloral hydrate-phenol [saturated solution] mixture for 24-36 h to detect the presence of eggs under the light microscope. The parasitoid eggs in each aphid were counted. Old as opposed to young, females can maximise their life-time reproductive success by searching host patches more thoroughly and accepting a broader range of host types [31, 49-52]. We tested the hypothesis that host choice in *L. oregmae* varies with female age, predicting that older females lacking reproductive success and approaching death would be less selective than younger females. Two cohorts of naïve females differing in age were given a choice between 20 second- and 20 fourth instar *A. gossypii* in a Petri dish; one cohort of females was 1 days old [n = 10] and the other cohort was 6 days old [n = 10]; females rarely live longer than 8-10 days in the laboratory. All other procedures were the same as in experiment mentioned for host age preference.

2.2.4. Influence of host mobility

The hypothesis that host choice in *A. gossypii* is influenced by aphid mobility and defensive behaviour was tested. Especially first instars are generally less mobile and have fewer effective anti-parasitoid defenses than older instars, which are larger and more active [53]. If large *A. gossypii* are difficult to capture and subdue by females of *L. oregmae*, anaesthetised aphids that are unable to move and escape should be accepted equally, independent of their size (small vs large). Aphids were anaesthetised with CO₂ for 5 min (second instar) and 10 min (fourth instar), respectively. A cohort of females was divided into two groups; females in the treatment group (n = 10) were given a choice between anaesthetised aphids, while those in the control group (n = 10) were given a choice between unanaesthetised aphids of the same size and age. Each trial included 20 aphids, 10 second and 10 fourth instars, and lasted 30 min. After 48 h of their parasitisation, the aphids were treated with a mixture of chloral hydrate-phenol (saturated solution) mixture for 24-36 h to detect the presence of eggs under the light microscope. The parasitoid eggs in each aphid were counted.

2.2.5. The effect of colour

The effect of colour was tested by following experiments: 25 third instar nymphs of aphid were placed in a Petri dish and a single female parasitoid was introduced into it for 15 min. The Petri dish was then put in the sun light. Different such sets were also kept in sun light separately covered with green, blue, yellow, red coloured plastic papers and with black paper. The number of hosts parasitised were determined by the method described above.

2.2.6. Site of attack

The abdomen was designated as lateral, middle and posterior portions [Fig. 1]. About 100 third instar nymphs of the aphid were kept in a Petri dish and a single female parasitoid was introduced. The site of prick on host was noted with the help of the figure. Total 250 such pricks were observed.

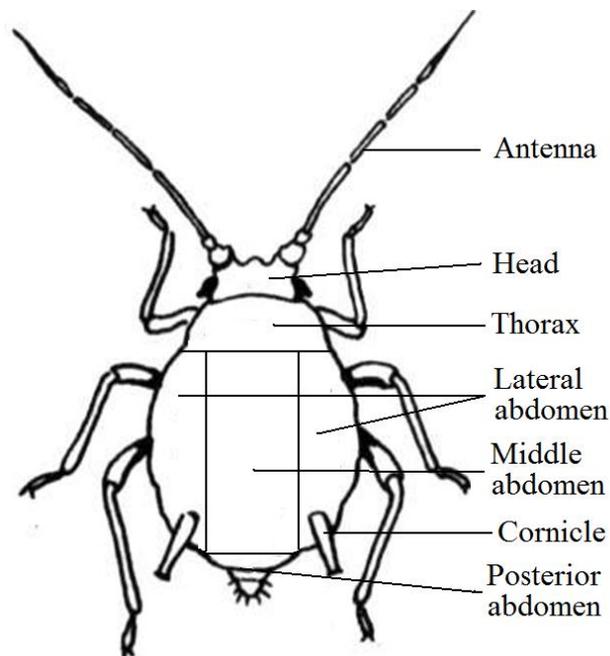


Fig. 1. Division of *Aphis gossypii* for locating the attack site of the parasitoid *Lipolexis oregmae* for oviposition.

2.3. Statistical analysis

Parasitisation data were analyzed using a 1 or 2-way ANOVA. Data on oviposition rate were root-square transformed whereas data on the percentage of aphids parasitised were arcsin transformed before analysis. Means were separated using the Tukey-Kramer HSD test [$P = 0.05$].

The preference of each parasitoid among instars of each aphid species, was also evaluated using the Manly's index [54-56]. Manly's preference index was estimated as:

$$\beta_j = \frac{\ln\left(\frac{r_j}{A_j}\right)}{\sum_{j=1}^4 \ln\left(\frac{r_j}{A_j}\right)}, j=1, 2, 3, 4$$

where β_j = Manly's Beta for hosts in aphid instar j , r_j = the number of hosts in aphid instar j not selected by the parasitoid, A_j the number of hosts in aphid instar j available to the parasitoid. This index is based on the assumptions that all host types are encountered at random and the preference is independent of density. However, in cases that host exploitation is not negligible this is appropriate

index [43, 56]. The Manly's index values for each aphid instar were examined for significant difference that expectation based on the numbers of each aphid instar available using a t-test.

Preference in dichotomous choice tests was estimated as the ratio of the relative proportion of younger hosts accepted as compared with the proportion of older hosts accepted among those available:

$$J_{\text{young/old}} = \frac{\frac{a}{(a+b)}}{\frac{c}{(c+d)}}$$

where a and b are the total numbers of accepted and rejected hosts among the younger instar, and c and d are the total numbers of accepted and rejected hosts among the older instar, pooled between trials. The error variance of J was calculated following Bliss [57]:

$$\text{var}(J) = \frac{1}{a} - \frac{1}{a+b} + \frac{1}{c} - \frac{1}{c+d}$$

The 95% confidence interval of J is also calculated. An absence of preference is indicated if the 95% confidence interval includes the value of 1.

3. RESULTS AND DISCUSSION

3.1. Role of olfaction

3.1.1. Role of aqueous extract of host on the response of the parasitoid

The parasitoids when introduced in the Petri dish having aqueous extract of *A. gossypii* moves for a while and orientate herself towards the host patch. She frequently touches the patch with her antennae and frequently tries to prick it like a host aphid. However, such pricks never culminate in oviposition. However, unlike the above observation, the parasitoid when introduced in the Petri dish having water washed integument of *A. gossypii*, occasionally touches the integument with her antennae but never try to prick the same as she does with the host patch. Pricking posture of the female parasitoid is considered as a positive response for host acceptance. The above results demonstrated that the female *L. oregmae* uses olfactory stimulus present inside the host [the host seeking stimulant] for its acceptance. The cuticle of the host (the physical characteristics of the host) did not induce the female parasitoid a host acceptance response.

3.1.2. Role of cornicle secretion of host on the response of the parasitoid

The behaviour of the female *L. oregmae* was the same as observed in case of host extract experiment. The result demonstrated that the source of host seeking stimulant is present in the secretion of cornicle.

3.2. Role of host age [instars]

L. oregmae accepted different instars of the host *A. gossypii* differently as host for egg deposition. The effect of instar was significant in the parasitoid oviposition on *A. gossypii* ($F_{3,36} = 55.59$, $P < 0.001$). The number of third instar nymphs parasitised was the highest differing significantly to that of other instars followed by second, fourth and first instar, respectively (Table 1). Significantly more aphids were parasitised in the third than second instar and finally, the number of second instar parasitised was significantly higher than in the fourth and first instar. The values of Manly's preference index for each instar of *A. gossypii* are presented in Table 1. This index was significantly higher than the expected in the second and third instars whereas the preference for first and fourth instar aphids was significantly lower than the expected ones.

Table 1. Preference of different developmental stages of *Aphis gossypii* as host by *Lipolexis oregmae*.

| Host stage | Total number of aphids presented | Number of aphids parasitised | Percentage of aphids parasitised | Manly's preference index |
|--------------------------------------------------------------------------|----------------------------------|------------------------------|----------------------------------|--------------------------|
| I instar | 400 | 9.0±2.2a | 22.0±5.1a | 0.056* |
| II instar | 400 | 28.0±5.7c | 69.3±14.1c | 0.268** |
| III instar | 400 | 37.2±4.8d | 93.0±10.7d | 0.605*** |
| IV instar | 400 | 10.6±2.9b | 26.8±5.8b | 0.071* |
| F _{3,36} =4.878, P < 0.01; * P < 0.05, **P < 0.01, ***P < 0.001 | | | | |

The results of dichotomous choice tests are displayed in Table 2. The numbers of aphids parasitised ($F_{11,108} = 34.91$, $P < 0.001$) by females of *L. oregmae* varied between the six instar combinations (Table 2). Pooling within instars across combinations, the mean number of aphids parasitised by each female were declined with host instar: First instar (Mean ± S.E. = 10.70±0.48 aphids), second instar (15.30±0.24 aphids), third instar (17.13±0.28 aphids), and fourth instar (10.23±0.56 aphids), in that order. Parasitism was not instar-specific, however. Females laid the most eggs in trials including second and third instar hosts and the fewest eggs in trials including first and fourth instar hosts (Table 2). When given a choice between two equally available host types, wasps accepted more second or third instars, regardless of the combination (paired-z test, first vs second instar : $t = 9.05$, $P < 0.001$; first vs third instar: $t = 19.18$, $P < 0.001$; first vs fourth instar : $t = 2.30$, $P > 0.05$; second vs third instar : $t = 6.16$, $P < 0.001$; second vs fourth instar: $t = 15.85$, $P < 0.001$; third vs fourth instar : $t = 35.35$, $P < 0.004$). For all six combinations, the index of preference J was significant except between first and fourth instars, indicating the following ranked order of preference: third > second > fourth > first instar (Table 2).

Table 2. Preference pattern of *Lipolexis oregmae* for different instars of the aphid *Aphis gossypii* in dichotomous choice tests.

| Choice | Instar | Number of aphids parasitised | t-values | J [95% confidence interval] |
|--------|------------|------------------------------|----------|-----------------------------|
| 1 | I instar | 10.7±0.6 | 9.05* | 0.745 [0.873-0.618] |
| | II instar | 14.6±0.6 | | |
| 2 | I instar | 10.7±0.6 | 19.18* | 0.635 [0.716-0.554] |
| | III instar | 16.9±0.3 | | |
| 3 | I instar | 10.7±0.4 | 2.30+ | 0.963 [1.115-0.811] |
| | IV instar | 11.6±0.9 | | |
| 4 | II instar | 15.0±0.6 | 6.16* | 0.885 [0.967-0.804] |
| | III instar | 17.0±0.4 | | |
| 5 | II instar | 16.3±0.5 | 15.85* | 1.738 [2.021-1.456] |
| | IV instar | 9.7±0.5 | | |
| 6 | III instar | 17.5±0.3 | 35.35* | 1.900 [2.105-1.695] |
| | IV instar | 9.4±0.5 | | |

Abbreviations: J - index of preference (95% confidence interval) for the younger over the older host instar. Values for which variation is shown are means ± SE. Differences between means within choice combinations are statistically significant by paired t-test (*P < 0.05). +Not significant.

3.3. Role of female size and age

3.3.1. Female size

Females developing in fourth instar aphids were approximately 1.5 times as large as their counterparts developing in first instar aphids. Large and small females did not differ in the total number of aphids parasitised (large females, 14.70 ± 0.70 aphids; small females, 13.80 ± 0.42 aphids; t = 1.85, P > 0.05). Also, host preference did not vary with host body size in that both large and small females parasitised significantly more second instar than fourth instar aphids (Small females, t = 18.38, P < 0.001; large females, t = 9.35, P < 0.001) (Fig. 2A), with the two preference indices overlapping in their 95% confidence intervals (large females, $J_{\text{second/fourth instar}} = 1.77$; 95% CI, 1.498-2.046; small females, $J_{\text{second/fourth instar}} = 1.75$; 95% CI, 1.542-1.957).

3.3.2. Female age

One day old females parasitised more aphids (16.20± 0.44 aphids) than 6 day old females (9.5 ± 0.62 aphids) that had no prior host experience (t = 3.35, P < 0.001); Although both groups of females

parasitised significantly more second instar than fourth instar aphids that were equally available (paired t-test, young females, $t = 14.43$, $P < 0.001$; old females, $t = 9.45$, $P < 0.001$) (Fig. 2B).

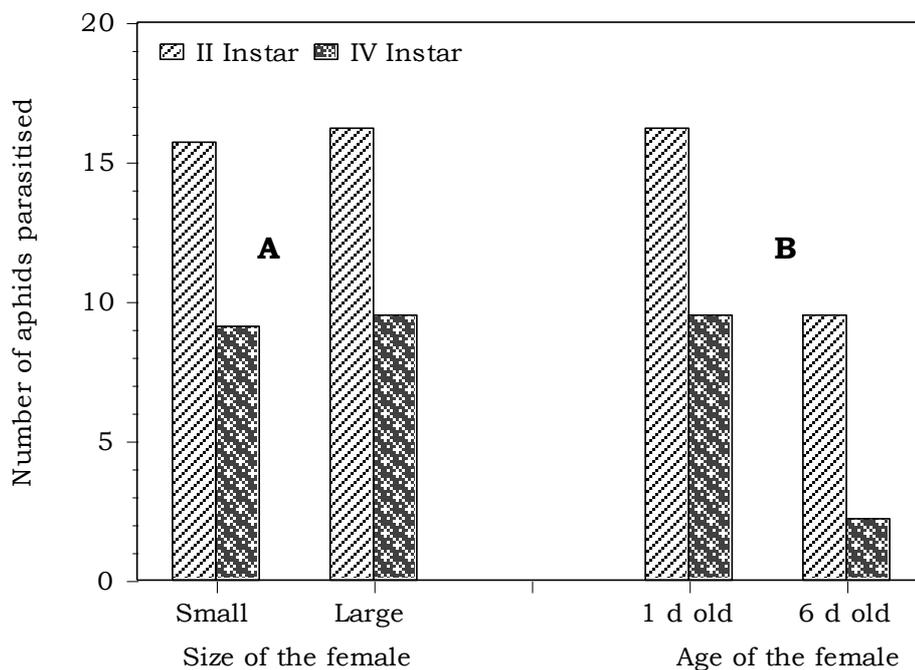


Fig. 2. A. Influence of body size of *Lipolexis oregmae* as measured by length on host choice by naïve females. Females were 1 d old. Large [2.13 mm] and small females [1.52 mm] were given a choice between 20 second and 20 fourth instar *Aphis gossypii* for 30 min. Columns represent mean numbers of aphids parasitised by each female based on 10 individuals/instar and trial. B. Influence of age on host choice in naïve females of *Lipolexis oregmae*. Young [1 d old] and females [6 d old] were given a choice between 20 second and 20 fourth instar *Aphis gossypii* for 30 min. Columns represent mean numbers of aphids parasitised by each female based on 10 individuals/instar and trial.

3.4. Host mobility

Naïve females of *L. oregmae* accepted both anaesthetised aphids and normal aphids which are able to move and defend themselves. Slightly more anaesthetised aphids than normal aphids were parasitised in total (anaesthetised aphids, $= 11.60 \pm 0.31$ aphids; normal aphids, 11.1 ± 0.49 aphids; $t = 1.76$, $P > 0.05$); Also, aphid behaviour did not affect the direction of preference in that females parasitised more second instar than fourth instar hosts (Fig. 3); but almost equal proportion of second instar were parasitised among anaesthetised hosts (anaesthetised aphids, $J_{\text{second/fourth instar}} = 1.85$, 95% CI = 1.533-2.172; normal aphids, $J_{\text{second/fourth instar}} = 1.84$, 95% CI = 1.781-1.907).

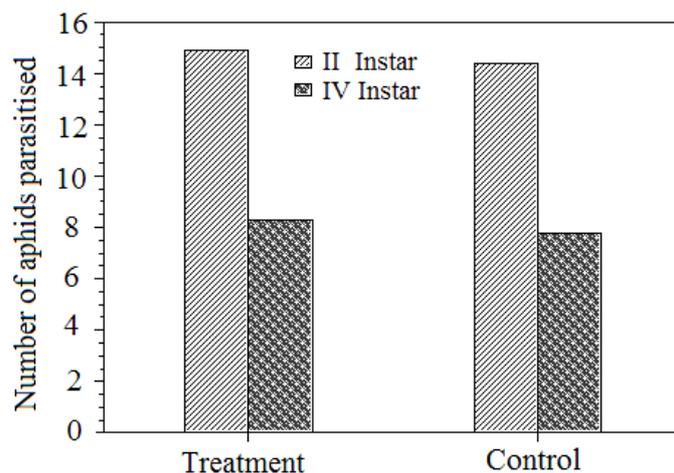


Fig. 3. Influence of host mobility on host choice in *Lipolexis oregmae*. One day old, naïve females were given a choice between 20 second and 20 fourth instar *Aphis gossypii* for 30 min. Females in the treatment group received anaesthetised aphids, whereas those in the control group received normal aphids capable of movement and defence. Columns represent mean numbers of aphids parasitised by each female based on 10 individuals per instar and trial.

3.5. The effect of colour

Total darkness greatly reduces the host acceptance (30% parasitisation, Table 3), whereas maximum parasitisation occurs in sun light. Different coloured light do not significantly influence the rate of parasitisation.

Table 3. Effect of different light colour on the acceptance of *Aphis gossypii* as host by *Lipolexis oregmae*.

| Light quality | Number of aphids parasitised | Percentage of aphids parasitised |
|----------------|------------------------------|----------------------------------|
| Sunlight | 36.8±4.9b | 73.6±9.7 |
| Total darkness | 17.4±4.3a | 34.8±8.7 |
| Green | 32.8±3.6b | 65.6±7.1 |
| Blue | 31.6±3.6b | 63.2±7.3 |
| Yellow | 32.2±4.6b | 64.4±9.2 |
| Red | 31.2±5.4b | 62.4±10.8 |

3.6. Site of attack

Though laterals of the host body are the most preferred site for pricking, other parts of the body of the host are also attacked by the ovipositor. It has been observed that the abdomen of the host is maximally pricked (85.6%) of which lateral parts are the most vulnerable (57.2%) followed by the middle of the abdomen (21.2%). The head and thorax are sparingly attacked (2.4% and 12.0%, respectively). Antennae and legs of the host are also occasionally attacked by *L. oregmae* (0.4 and

2.7%, respectively) while the cornicles are not attacked at all. Only 42.8% of the stings culminate in oviposition. Eggs have never been found in antennae, head and legs of the host (Table 4).

Table 4. Site of attack on *Aphis gossypii* by the ovipositor of *Lipolexis oregmae* and number of eggs of the parasitoid in different parts of the host.

| Body parts | | No. of pricks | No. of eggs | Egg/prick ratio | % of prick |
|------------------|-----------|---------------|-------------|-----------------|------------|
| Cephalic region | Head | 4 | 0 | 0 | 1.6 |
| | Antennae | 2 | 0 | 0 | 0.42 |
| Thoracic region | Thorax | 21 | 7 | 0.33 | 8.40 |
| | Legs | 9 | 0 | 0 | 2.71 |
| Abdominal region | Laterals | 143 | 74 | 0.52 | 57.20 |
| | Middle | 53 | 21 | 0.40 | 21.20 |
| | Posterior | 18 | 5 | 0.28 | 7.20 |
| | Cornicles | 0 | 0 | 0 | 0 |
| Total | | 250 | 107 | 42.80 | - |

4. DISCUSSION

4.1. Role of olfaction

The analysis of the behavioural responses by *L. oregmae* females to the aphid aqueous extract, water-washed integument, cornicle secretion and developmental stages of the host indicates that a proper integration of external and internal cues is a key issue in the modulation of the final response. The experimental data obtained demonstrate that the parasitoid reacts to internal cues present in the host haemolymph and the cornicle secretion and that the intensity of the response is co-modulated by specific external cues. Two types of chemosensilla have been observed to be associated with the ovipositor valves in aphid parasitoids [9]. Upon contact with the patch containing host extract, they detect host acceptance signals present therein. Chemosensilla associated with the ovipositor of parasitic wasps have been often described as small cuticular pores, pits or small pegs set in cupules [58-60]. More specifically, the occurrence of pores on the tip of the ovipositor has been already reported in Aphidiinae, with *Aphidius uzbekistanicus* Luzhetski showing four pores of small diameter (0.1–0.2 µm) associated with each of the paired lower valves. Furthermore, other putative sensory structures have been observed on Aphidiines ovipositor, such as basiconica sensilla in *Ephedrus plagiator* (Nees) [61]. These sensilla detects the internal chemical cues, whereas the external kairomones are likely detected with antennae (gustatory and/or olfactory sensilla). The egg laying is then controlled by the integration of the sensorial inputs detected by these two organs. The reaction to haemolymphatic kairomones is not a novel issue and it was demonstrated for several species of parasitic Hymenoptera. It not only elicited oviposition response but also enhanced the

reproductive potential of the aphid parasitoid [62-64].

4.2. Role of host age [instars]

Females of *L. oregmae* attacked and laid eggs in all four nymphal instars of the brinjal aphid, *A. gossypii*; however, when given a choice, they always accepted third instars followed by second, fourth and first instars (Table 1). However, when given a choice between younger and older stages, *L. oregmae* The magnitude of preference for second and third instar aphids was influenced, apparently, by the relative difference in size between the available host types (Tables 1, 2). *L. oregmae* had a higher preference on the second and third instar nymphs than on others. Earlier, *L. oregmae* was observed to oviposit and develop in all four instars of *Toxoptera citricida* Kirkaldy [65] but parasitised more second instar hosts than others, however, the progeny were larger when develop into fourth instar hosts than smaller/younger hosts. It also suggested that defensive behaviour in these instars had no significant effect on parasitism. Similarly, the parasitoid wasps, *Binodoxys indicus* (Subba Rao and Sharma) [11], *Lysiphlebia mirzai* Shujauddin [25, 66] and *Trioxys pallidus* (Haliday) [67] preferred the third instar nymphs of its host aphids for oviposition. In contrast, *Binodoxys angelicae* (Haliday) [68] and *Aphidius colemani* (Viereck) had a preference on the first instar nymphs of *Aphis pomi* de Geer and *Acyrtosiphon pisum* Harris [5], respectively. *Trioxys cirsi* (Curtis) and *Monoctonus pseudoplatani* (Marshall) parasitoids of platanus aphid, *Drepanosiphum platanoides* (Shrank) oviposit in younger nymphs and rarely parasitise the older nymphs [69]. The first instar nymphs of green peach aphid, *Myzus persicae* (Sulzer) were most easily parasitised by *Ephedrus cerasicola* Stary, in addition to the handling time and aphid defensive behaviour [70], but *Trioxys pallidus* (Haliday) had some problems in parasitising the first instar nymphs of *Chromaphis juglandicola* Kalt. [67]. Although fourth instar hosts contain more nutritional resources for offspring development and growth, they may be more difficult to capture than second and third instar hosts [65]. Especially in parasitoids that physically attack and subdue their hosts, host choice is expected to reflect a trade-off between the female's risk of injury or loss of opportunity time (if a large host escapes) and her gain in fitness from offspring growing to a larger size and/or developing faster. Second and third instars are generally more abundant than fourth instars and adult aphids in natural populations [71]. The relatively high mortality observed among first instars [72] could indicate that small aphids are susceptible to wasp venom; however, *A. gossypii* stung by *L. oregmae* recover relatively quickly from the paralysis, including first instars [5]. If hosts are equally suitable but vary in size, females should favour medium-sized hosts and avoid both very small hosts (which may be suboptimal for the immature parasitoid) and very large hosts (which may be risky to attack for the female). However, *Monoctonus paulensis* (Ashmead), preferred the smallest available hosts, which is unexpected and counter to this prediction [5]. The hypothesis that host choice varies with parasitoid size was also tested. Because females of *L.*

oregmae deposit their eggs in a specific location, the physical size of a female, especially the length of her ovipositor, may constrain the size of the aphids that she can parasitise [46]. Although large females had nearly 1.5 the body mass of small females, and presumably had a correspondingly longer ovipositor, both kinds of female showed the same degree of preference for the smaller over the larger instars [Fig. 2A]. The results indicate that second instar aphids were more likely to be parasitised, regardless of the size of the parasitoid. However, large females appeared more effective than the small females in parasitising the larger hosts, [Fig. 2A]. Therefore, results support earlier findings [5, 40] that report a positive relationship between female size and that of the hosts mummified. In this study, aphid size and age was used synonymously. As size increases with age, the results could equally well support the hypothesis that *L. oregmae* prefers relatively younger rather than smaller aphids. In *Megoura viciae* Buckton, the allocation pattern of nutritional resources to somatic and gonadal tissues changes as aphids approach reproductive age [73]. Such physiological changes in the host, in turn, may alter host quality and ultimately influence host choice. However, females of *M. paulensis* were indiscriminate and oviposit even in aphids that are unsuitable for offspring development [5, 74], a behaviour also observed in *Monoctonus crepidis* (Haliday) [75]. Moreover, in a direct test of the relative importance of aphid age versus size, the ranking order of different host types was dependent on their size and not age [33]. Comparing starved pea aphids and aphids feeding normally on broad beans, females of *Ephedrus californicus* Baker attacked and parasitised more starved (i.e., small) than non-starved (i.e., large) aphids of the same age; however, differences in host size had no influence on parasitoid attack rates when aphids of the same age were anaesthetised so that they could not escape or defend themselves [33].

4.3. Role of female size and age

Second, another hypothesis was tested, if host choice is affected by female age. Older females are expected to be less choosy than younger counterparts [50]. Contrary to this prediction, 7 day old, as opposed to 2 day old, females attacked fewer aphids per unit of time and parasitised proportionally more first instar than third instars [5]. Two explanations that are not mutually exclusive could account for these results. One explanation is that egg maturation was affected by host deprivation. If this is true, older females that were deprived of hosts had probably fewer eggs available for oviposition than younger counterparts. For example, in *Aphidius smithi* Sharma and Subba Rao, a parasitoid of the pea aphid, total fecundity declined when females were limited to less than 20 aphids per day throughout life [44]. However, Aphidiinae are not known to resorb eggs and cannot, therefore, repartition energy resources to prolong longevity in the absence of suitable hosts. An alternative explanation is that females of *M. paulensis* with age became less effective in subduing and handling pea aphids, in particular any larger instars. It had been shown that older females of *Lysiphlebus cardui* Marshall, a parasitoid of *Aphis fabae cirsiacanthoides* Scopoli needed more time than younger females for host handling and oviposition [31]. Third, the hypothesis that

parasitoid preference reflects differences in handling costs and oviposition success between host types was tested [47, 53, 76]. Anti-parasitoid behaviour including dropping from the feeding site and kicking with the hind legs are generally better developed in older instars and adult aphids than in younger instars.

4.4. Host mobility

Using anaesthetised aphids, the influence of host mobility and defensive behaviour on parasitoid choice was evaluated. Contrary to expectations, it was found that parasitoids accepted anaesthetised aphids and their normal counterparts equally and, furthermore, preferred second over fourth instars under both conditions [Fig. 3] similar to *M. paulensis* [5]. *A. gossypii* while feeding remain almost quiet which facilitates oviposition by *L. oregmae*. However, in case of moving aphids, the parasitoids follow them, attempt to sting frequently (mostly the ovipositor fails to make contact with host) but the moment the host stops (may be for feeding or due to some injury) she quickly oviposits. It is due to pricking mutilation [71, 77] that the host gets injured and stops but not due to the paralysing effect of the sting of *M. paulensis* [4] like some aphid parasitoids [75, 78-82]. Therefore, the movement of *A. gossypii* has nothing to do in host acceptance by *L. oregmae*. However, in some other parasitoids, host movement has been suggested as an important stimulus in the induction of oviposition [83].

4.5. The effect of colour

In addition, to chemical cues, visual cues also play a role in host acceptance and recognition, with colour being an important short-range cue. Colour alone stimulated attack responses; *Aphidius ervi* Haliday females attacked green aphids in preference to brown aphids and showed attack responses toward yellow paint pigments enclosed in glass capillary tips [84].

4.6. Site of attack

Though laterals of the host body are the most preferred site for pricking by *L. oregmae*, other parts of the host are also attacked. The abdomen of the host is maximally pricked of which lateral parts are the most vulnerable (Table 4) like *B. indicus* [11] but unlike *A. matricariae* [82, 85]. The greater number of eggs in the abdomen is possibly in the interest of the parasitoid as it provides sufficient food as well as space to accommodate the developing larvae.

5. CONCLUSION

The data presented furnish considerable insight into the host-selection process of *L. oregmae*. It shows that *L. oregmae* first finds the host-habitat and then locates the host proper and finally accepts it for oviposition. Olfaction plays significant role in habitat location while the host finding and acceptance is brought about mostly by olfaction and taction stimuli co-ordinated by gustation and vision. Parasitoid's antennae play a major role in this process.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

I declare that no financial interest or any conflict of interest exists.

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