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Restricted diet breadth of the larvae of *Antheraea assamensis* and the role of the labrum-epipharynx and galeal sensilla

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Abstract

The silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae), grows primarily on *Persea bombycina* and *Litsea polyantha*. To understand if the restricted diet breadth is due to the specific role of gustatory sensilla of the larvae of *A. assamensis*, the same fifth instar larvae retaining only labrum-epipharynx or galeal sensilla were subjected to food choice tests. The foods used were leaves of two host-plant and two non-host-plant species. Mean per cent consumption and per cent of choosing larvae were used as parameters for drawing conclusions. The finding indicated involvement of the labrum-epipharynx for acceptance and galeal sensilla for rejection of a non-host-plant species. Scanning electron microscope studies revealed the presence of two sensilla on the galea, one lateral and one medial sensilla styloconicum and two gustatory sensilla in the epipharynx of *A. assamensis*. The study revealed the key role of galeal sensilla in the restrictive diet-breadth of *A. assamensis*.

Key words: food choice, galea, gustatory organs, labrum.

Introduction

Host-plant selection in phytophagous insects is dependent on the perception of phytochemicals by peripheral chemosensory organs located primarily on the mouthparts. Gustatory organs with their taste receptors play a key role in food discriminating behavior of insects (Hallem *et al*. 2006). The physiological basis of selective feeding has been examined mostly in *Manduca sexta* (Lepidoptera: Sphingidae) (Schoonhoven & Dethier 1966; Hanson & Dethier 1973; Stadler & Hanson 1975). Many specialist herbivores are reported to narrow their preferences as a result of experience (Jermy 1987). Although the underlying mechanisms are not known (Bernays & Weiss 1996), in some cases the reason may be attributed to increased or decreased sensitivity of their chemoreceptors to certain metabolites (Renwick & Lopez 1999). Styloconica on the galea and epipharyngeal sensilla on the labrum are reported as the primary gustatory organs in several lepidopteran insects (Schoonhoven & Dethier 1966; Stadler & Hanson 1975; De Boer *et al*. 1977; De Boer & Hanson 1987). On the molecular level the taste receptors were identified in both mammals (Hoon *et al*. 1999; Adler *et al*. 2000) and insects (Clyne *et al*. 2000) simultaneously only in the last decade of the 20th century. In *Drosophila*, 60 gustatory receptor-encoding genes have been identified (Clyne *et al*. 2000; Dunipace *et al*. 2001; Scott *et al*. 2001; Robertson *et al*. 2003), 76 such genes have been identified in *Anopheles gambiae* (Fox *et al*. 2001; Hill *et al*. 2002) and 65 genes in the gustatory receptor family have been identified in the silkworm *Bombyx mori* (Wanner & Robertson 2008). Extirpation of gustatory receptors

bearing chemosensory organs involved in feeding lead to acceptance or rejection of host or non-host-plant species (Schoonhoven & Dethier 1966; Hanson & Dethier 1973; Stadler & Hanson 1975). Therefore, the functional role of the chemosensory organs may be studied by ablation of the organs (Hanson & Dethier 1973; De Boer & Hanson 1987).

The muga silkworm, *Antheraea assamensis*, the producer of golden silk, is endemic and restricted to North Eastern India. The insect is polyphagous, but feeds primarily on two host-plants, *Persea bombycina* and *Litsea polyantha*, although they can thrive on nine other secondary host-plants belonging to different families. In contrast *Manduca sexta*, which has been used as model for studying the chemosensory basis of host-plant selection, is oligophagous and feeds mostly on plants belonging to Solanaceae family. No work has been carried out to probe into the chemosensory basis of such a narrow diet breadth in *Antheraea assamensis*, a behavior that is responsible for its restricted geographic affiliation. Although there is great demand for muga silk due to its durability, high tensile strength, UV light protecting capacity, etc., its production is hindered by the restricted feeding habit on only two primary host-plants and healthy growth only in semi-domesticated conditions. Because of their compulsory rearing in outdoor conditions, *A. assamensis* are susceptible to the adverse effects of nature. For increasing productivity, it is essential to understand all factors contributing to the feeding habits of this valuable bioresource.

The present investigation addresses questions regarding role of galeal sensilla styloconica and labrum-epipharyngeal sensilla in food selection by *Antheraea assamensis* larvae through food choice tests.

Materials and methods

Insects

***Antheraea assamensis* Helfer**

Larvae of *A. assamensis* were hatched from disease-free eggs obtained from the Government Sericulture Farms of Assam. Larvae were cultured on leaves of its primary host-plant, *Persea bombycina*, grown in the botanical garden within the campus of the Department of Life Sciences, Dibrugarh University, Assam, India.

Plants

For carrying out food choice tests, two host-plants of *A. assamensis* larvae, *Persea bombycina* Kostermans (Laurales: Lauraceae) and *Litsea polyantha* Juss, and two non-host plants, *Litsea grandifolia* Teschner (Laurales: Lauraceae) and *Ziziphus jujuba* Miller (Rosales: Rhamnaceae), were selected. All the plants were 3–4 years old and grown in the botanical garden of Department of Life Sciences, Dibrugarh University, Assam.

Plant extract preparation

As the host-plants and non-host-plants used dry out within hours of plucking, fresh thick water extracts were used to prepare food disks. Leaf extract was prepared by grinding 100 g of fresh leaves with 100 mL of distilled water in an electric grinder. Only extract prepared immediately before each bioassay was used. Leaf disks were prepared by soaking a Whatman fiber disk (GF/A, 14 mm diameter) in water extracts of the leaves of the plants considered or only with water.

Ablation of the sensory organ

Early 5th instar *A. assamensis* larvae were immobilized on ice for 15–30 minutes and the peripheral sense organs, antenna, maxillary palp, maxillary galea and labrum-epipharynx were removed selectively by microsurgery, keeping only the organ considered for study. Extirpations were performed on the two-day old 5th instar larvae under a dissecting binocular microscope (Olympus, http://www.olympus.co.uk) using fine dissecting scissors. After recovery, the larvae were allowed to feed normally on leaves of their primary host-plant. Larvae retaining only galeal sensilla styloconica were designated as GAL, larvae retaining only labrum-epipharynx as LAB, larvae retaining all chemosensory organs (both olfactory and gustatory) as ALL, larvae retaining none of the chemosensory organs (both olfactory and gustatory) as NONE and larvae retaining all organs (both olfactory and gustatory) unilaterally as UNI. As all the chemosensory organs are bilaterally represented, ablation of all chemosensory organs of one side should nullify the probable effect of surgery on the insect's normal activity. Hence all comparisons were made with the response of UNI. The study was done by ablating all other chemosensory organs leaving only either the galeal sensilla styloconica or labrum–epipharynx in order to be able to determine the functional significance of the organs in food preference or rejection.

SEM study of chemosensory organs of *A. assamensis*

Larvae of *A. assamensis* were immobilized by holding them at 4°C for 5–10 min and subsequently fixed in 2.5% glutaraldehyde solution in sodium cacodylate buffer (pH 7.2) overnight at 4°C. The samples were rinsed in buffer and subsequently fixed in 0.1% osmium tetroxide for 4 h at 4°C. Specimens were rinsed in buffer, dehydrated in an acetone series, air dried and mounted on aluminum stubs with carbon tape. The specimens were sputter-coated with platinum and observed under a FEI Quanta 200 3D scanning electron microscope (SEM). The SEM study was carried out

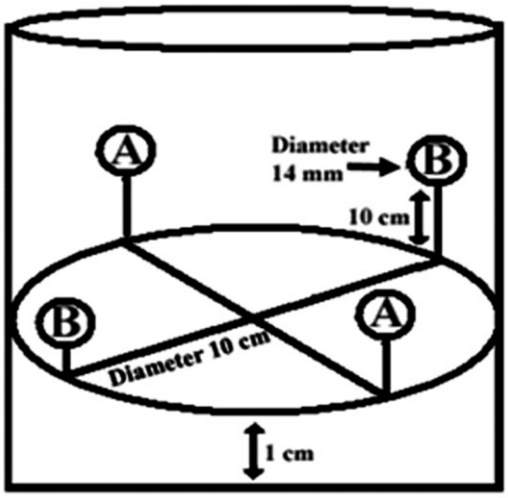

Figure 1 Scheme of leaf disk arrangement for food choice test.

at the National Chemical Laboratory (CSIR) Pune, Maharashtra, India.

Bioassay of food choice test

Bioassay was done through a food choice test carried out in two ways following the method of De Boer and Hanson (1984). The two-plant food choice test (Fig. 1) was carried out between a host-plant species, *P. bombycina* or *L. polyantha*, and a non-host-plant, *L. grandifolia* or *Z. jujuba*. The one-plant food choice test was carried out between a host-plant or non-host-plant and water in order to evaluate the degree of preference for different food plants by comparing the response to the plants with that of a neutral medium. Water was used as the neutral medium, as it was used in the preparation of the leaf extract.

In order to assay larval food preferences, four leaf disks (14 mm in diameter) of each plant species (A or B) were arranged alternately on the floor around the circumference of a transparent plastic container (10 cm diameter) (Fig. 1). The leaf disks were fitted to the distal end of bamboo sticks, whose proximal ends were fixed on hard cardboard kept at 1 cm above the bottom of the container. The bamboo sticks were used to hold the leaf disk like a stem of a plant and to provide crawling space for the larvae. All the larvae were not fed for 2–4 h before being subjected to the food choice test, and then were placed in the center of the floor of the container. When the larva had eaten about 50% of the area of one of the two plant species (A or B), the test was stopped. The time taken to consume 50% of the leaf disk (7 mm) was called T50, and it varied from 2 min to 1 h from the start of the test. Tests were repeated with a minimum of 10 larvae. The unit used to express consumption rate was percentage of area consumed per minute. The percentage of choosing larvae was based on the number of larvae in one group opting for a particular food. The two-plant choice test was carried out using the host-plant *vs* non-host-plants, as shown in Figure 1. If (A–A) was the host-plant then (B–B) was the non-host-plant and the single-plant choice test was carried out using the host/non-host *vs* water; if (A–A) was the host/non-host-plant then (B–B) was the water.

Statistical analysis

Differences in food choice between groups of larvae having different complements of remaining chemosensory organs were evaluated statistically by comparing the response of an individual group of larvae in one ablation group with those of UNI and control group. Preference based on mean per cent consumption per minute was analyzed by one ($P < 0.025$) and two ($P < 0.05$) tailed Mann–Whitney tests. A chi-squared test was performed between the per cent of choosing larvae to show orientation preference. All analysis was done by SPSS v17 (SPSS, Carey, NJ, USA).

Results

P. bombycina or *L. polyantha vs L. grandifolia*

Larvae were given a choice between host-plant *P. bombycina* and acceptable non-host-plant *L. grandifolia*. ALL, UNI, LAB and GAL larvae strongly preferred the host-plant ($P < 0.025$). The rate of consumption was significantly lower in GAL in comparison to UNI. (Fig. 2D). The per cent of larvae opting for *P. bombycina* was 100 in all larvae except NONE, which failed to differentiate between *P. bombycina* and *L. grandifolia*. Forty per cent of NONE opted for *P. bombycina*, while 60% opted for *L. grandifolia*. The consumption rate was higher for non-hosts ($P < 0.05$; Mann–Whitney ANOVA $U = 18.500$; $Z = -2.200$) (Fig. 2E) (Table 1).

In another case when larvae were given a choice between host *L. polyantha* and acceptable non-host *L. grandifolia*, ALL, UNI and GAL larvae opted for only *L. polyantha* ($P < 0.025$) Mean per cent consumption per minute was reduced in LAB and GAL in comparison to UNI ($P < 0.05$). The per cent of larvae opting for *P. bombycina* was 100 in all larvae except LAB and NONE, which failed to differentiate between *P. bombycina* and *L. grandifolia* (Fig. 2A,B,D). Eighty per cent of LAB opted for host-plant while 20% opted for non-host-plant (Fig. 2C). The mean per cent consumption per minute was higher in case of non-hosts ($P < 0.05$ Mann–Whitney ANOVA $U = 8.000$; $Z = -2.246$) (Table 1). Sixty per cent of NONE opted for *P. bombycina*, while 40% opted for *L. grandifolia*. The mean per cent consumption per minute was higher for non-host-plants ($P < 0.05$, Mann–Whitney ANOVA $U = 8.000$; $Z = -2.394$) (Fig. 2E) (Table 1).

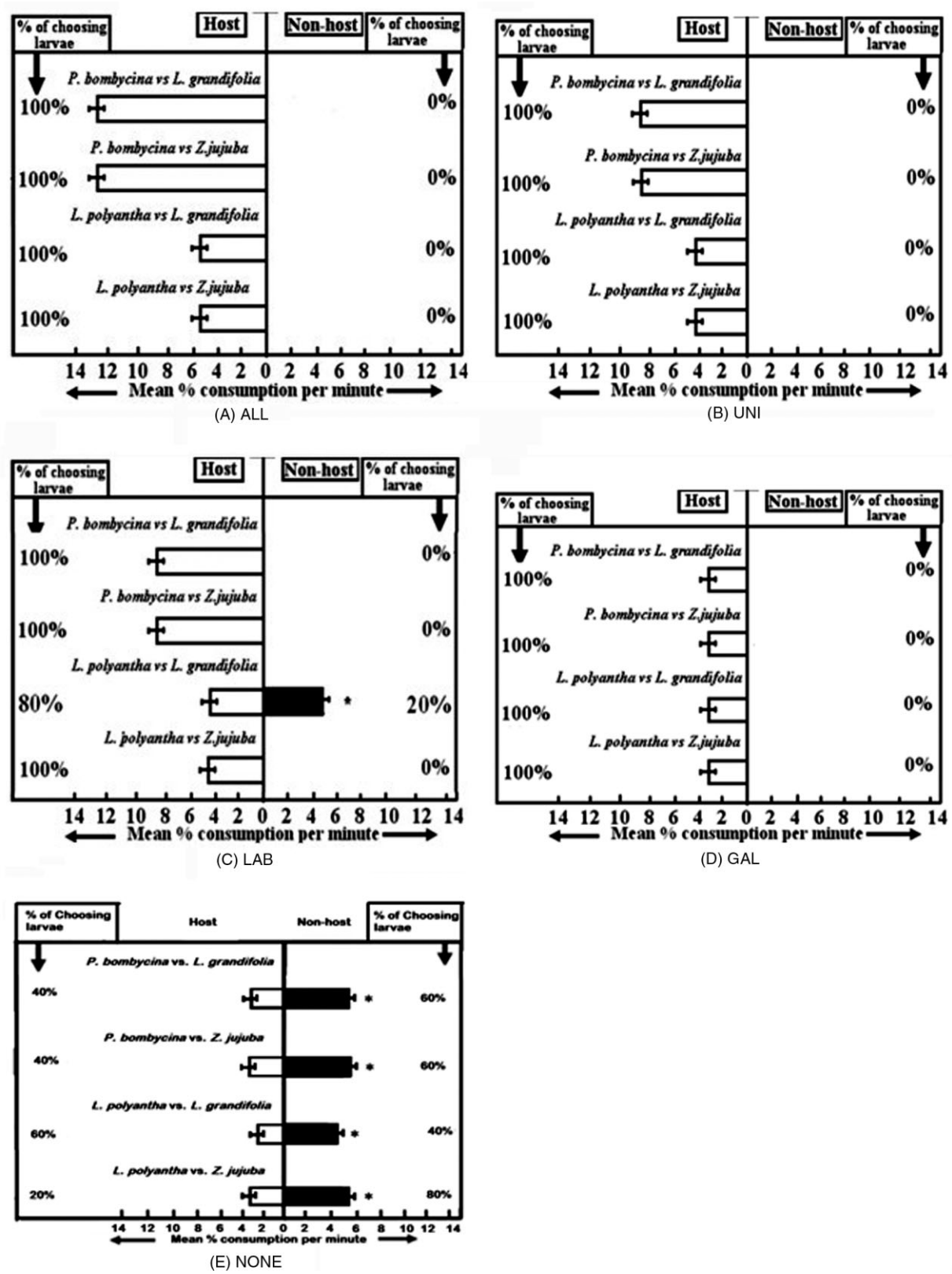

Figure 2 Mean per cent consumption per minute and per cent of choosing larvae in two-plant choice test (host-plant *vs* non-host-plant). For mean per cent consumption per minute, Mann–Whitney ANOVA test: $*P < 0.05$ (two tailed). ; $**P < 0.025$ (one tailed); ns: no significance.

Table 1 Mean per cent consumption per minute for gustatory organs in two-plant choice test (host-plants *vs* non-host-plants)

	P. bombycina (PB) *vs L. grandifolia* (LG)					*P. bombycina* (PB) *vs Z. jujuba* (ZJ)				
	PB	LG	*U*	*Z*	*P*	PB	ZJ	*U*	*Z*	*P*
ALL	13 ± 0.92	0		na		13 ± 0.82	0		na	
UNI	8.9 ± 0.74	0		na		8.9 ± 0.45	0		na	
GAL	3 ± 0.23	0		na		3 ± 0.21	0		na	
LAB	8.4 ± 0.78	0		na		8.4 ± 0.71	0		na	
NONE	3 ± 0.55	5 ± 0.48	18.500	–2.200	0.030	3 ± 0.54	5 ± 0.45	8.000	–2.246	0.043
	L. polyantha (LP) *vs L. grandifolia* (LG)					*L. polyantha* (LP) *vs Z. jujuba* (ZJ)				
	LP	LG	*U*	*Z*	*P*	LP	ZJ	*U*	*Z*	*P*
ALL	5.8 ± 0.67	0		na		5.8 ± 0.67	0		na	
UNI	4.2 ± 0.81	0		na		4.2 ± 0.45	0		na	
GAL	3 ± 0.22	0		na		3 ± 0.26	0		na	
LAB	4.2 ± 0.29	5.6 ± 0.50	8.000	–2.246	0.043	3.8 ± 0.26	0		na	
NONE	2.6 ± 0.42	4.2 ± 0.56	8.000	–2.394	0.026	3 ± 0.36	5 ± 0.59	18.500	–2.200	0.030

U, Mann–Whitney ANOVA; na, not applicable.

P. bombycina or *L. polyantha vs Z. jujuba*

Larvae were given the choice between the *P. bombycina* and *Z. jujuba*; ALL, UNI, LAB and GAL opted for *P. bombycina* and the per cent of larvae that opted for *P. bombycina* was 100 in each case ($P < 0.001$) (Fig. 2) Mean per cent consumption of *P. bombycina* by GAL was reduced in comparison to that of UNI (Fig. 2D). NONE could not differentiate between the host and non-host, 40% of the larvae opted for the host-plant while 60% opted for the non-host-plant. The mean per cent consumption per minute was higher for non-host-plant ($P < 0.05$, Mann–Whitney ANOVA $U = 8.000$; $Z = -2.246$) (Fig. 2E) (Table 1).

When larvae were given a choice between the *L. polyantha* and *Z. jujuba*, ALL, UNI, LAB and GAL opted for the *L. polyantha* and the per cent of larvae that opted for the *L. polyantha* was 100 in each case (Fig. 2). However, NONE failed to differentiate between the two choices ($P < 0.05$). Twenty per cent of NONE opted for the *L. polyantha* and 80% opted for *Z. jujuba*. Mean per cent consumption per minute was higher for *Z. jujuba* ($P < 0.05$, Mann–Whitney ANOVA $U = 18.500$; $Z = -2.200$) (Fig. 2E) (Table 1).

P. bombycina or *L. polyantha vs* water

Larvae were given a choice between *P. bombycina* and water; 90% of ALL, UNI and LAB, and 100% of GAL, preferred *P. bombycina* over water. Mean per cent consumption per minute was higher of *P. bombycina* than the water ($P < 0.05$) (Fig. 3A–D) (Table 2). Only 20% of NONE opted for *P. bombycina* and 80% opted for water. The variation in the mean per cent consumption per minute with respect to host-plant and water was not significant ($P > 0.05$, Mann–Whitney ANOVA $U = 44.000$; $Z = -1.934$) in the case of NONE (Fig. 3E) (Table 2).

When larvae were given a choice between the *L. polyantha* and water, 100% of ALL and UNI opted for *L. polyantha*. Ninety per cent of LAB and GAL preferred *L. polyantha* and the mean per cent consumption per minute of *L. polyantha* was higher than the water ($P < 0.05$) (Fig. 3C,D), while only 20% of NONE opted for *L. polyantha* and 80% opted for water. The variation in the mean per cent consumption per minute with respect to host-plant and water was not significant ($P > 0.05$, Mann–Whitney ANOVA $U = 73.500$; $Z = -1.947$) in the case of NONE (Fig. 3E) (Table 2).

L. grandifolia vs water

Larvae were given a choice between *L. grandifolia* and water. All of ALL, UNI and GAL preferred water, while 80% of LAB opted for water. However, the mean per cent consumption of water was higher than the non-host-plant by LAB ($P < 0.05$) (Fig. 4C). Eighty per cent of NONE opted for non-host-plant while 20% opted for water and the mean per cent consumption per minute for both the choices was significant ($P < 0.05$, Mann–Whitney ANOVA $U = 68.000$; $Z = -2.165$) (Fig. 4E) (Table 2).

Z. jujuba vs water

Larvae were given the choice between the *Z. jujuba* and water. ALL, UNI, LAB and GAL preferred only water, while

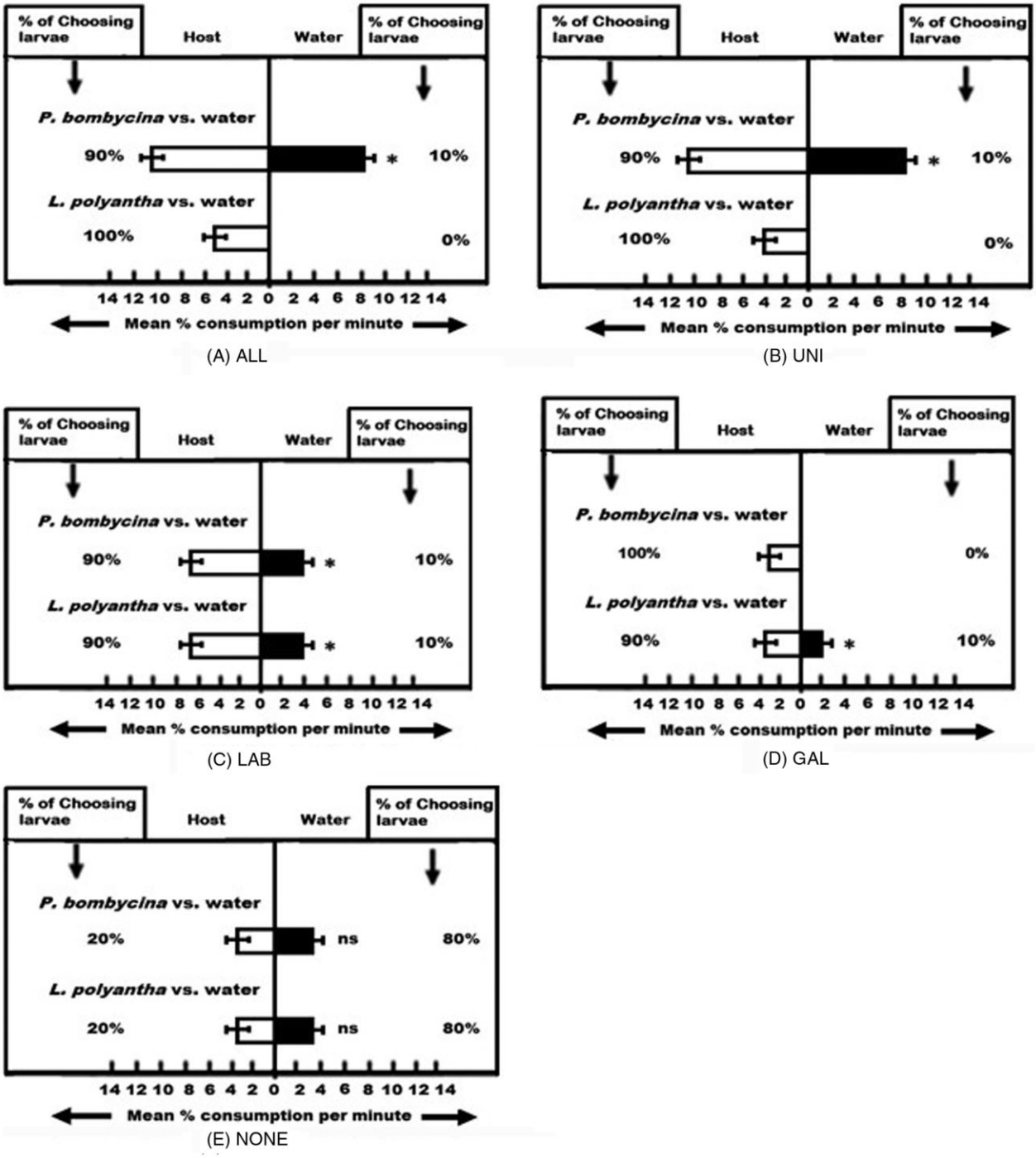

Figure 3 Mean per cent consumption per minute and per cent of choosing larvae in single-plant choice test (host-plant *vs* water). For mean per cent consumption per minute, Mann–Whitney ANOVA test: $*P < 0.05$ (two tailed). ; $**P < 0.025$ (one tailed); ns: no significance.

60% of NONE opted for non-host-plant and 40% opted for water. The mean per cent consumption per minute for both choices was significant ($P < 0.05$, Mann–Whitney ANOVA $U = 66.5.00$; $Z = -2.225$) (Fig. 4E) (Table 2).

Discussion

During foraging, herbivorous insects perceive different information available in the natural environment, and spe-

Table 2 Mean per cent consumption per minute for gustatory organs in single-plant choice test (host-plants/non-host-plants *vs* water)

	P. bombycina (PB) *vs* water (H_2O)					*L. polyantha* (LP) *vs* water (H_2O)				
	PB	H_2O	*U*	*Z*	*P*	LP	H_2O	*U*	*Z*	*P*
ALL	10.4 ± 0.86	8.4 ± 0.78	32.000	–2.166	0.035	5.6 ± 0.45	0		na	
UNI	10.2 ± 0.85	8.2 ± 0.45	49.000	–2.129	0.037	3.6 ± 0.24	0		na	
GAL	3 ± 0.21	0		na		3 ± 0.22	1.8 ± 0.11	56.000	–2.241	0.028
LAB	6.8 ± 0.61	4 ± 0.50	56.000	–2.241	0.028	6.8 ± 0.62	4 ± 0.40	32.000	–2.166	0.035
NONE	3.2 ± 0.11	3.2 ± 0.14	44.000	–1.934	0.060	2.4 ± 0.16	2.6 ± 0.19	73.500	–1.947	0.054
	L. grandifolia (LG) *vs* water (H_2O)					*Z. jujuba* (ZJ) *vs* water (H_2O)				
	LG	H_2O	*U*	*Z*	*P*	ZJ	H_2O	*U*	*Z*	*P*
ALL	0	12 ± 0.98		na		0	3.8 ± 0.12		na	
UNI	0	7 ± 0.51		na		0	3.6 ± 0.23		na	
GAL	0	7 ± 0.74		na		0	7 ± 0.71		na	
LAB	3 ± 0.31	5.6 ± 0.51	71.500	–2.030	0.045	0	5.6 ± 0.51		na	
NONE	3 ± 0.21	4 ± 0.42	68.000	–2.165	0.033	3 ± 0.19	4 ± 0.16	66.500	–2.225	0.027

U, Mann–Whitney ANOVA; na, not applicable.

cialist insects use specific biophysical and chemical cues provided by a host-plant. Insects use various sensory systems to locate their hosts (Chapman 2003; Bullas-Appleton *et al*. 2004; Hallem *et al*. 2006; Heisswolf *et al*. 2007; Jorgensen *et al*. 2007), and the degree of acceptability is likely based on qualitatively different perceptions owing to high chemosensory selectivity of output neurons (Dethier & Crnjar 1982; Reisenman *et al*. 2005). Bernays and Woods (2000) reported that the larvae of *M. sexta* in nature feed only for one-third of the total time they spend in their feeding exercise and the rest of the time is spent resting or grooming, and the patterns of feeding are not influenced directly by nutritional factors. The general feeding strategy of *A. assamensis* is similar to that of *M. sexta* except that they spend most of the non-feeding time moving about the feeding arena. They rest after several continuous bites and such periods of rest vary. The latter aspect of feeding behavior of *A. assamensis* can be attributed to the reasonably long time of 2 min to 1 h needed in the present study to determine T50 for the different groups of larvae. However, an insect's feeding rate may also vary due to adaptation for obtaining the maximum nutrients (Simpson 1995) and feeding arousal (Bernays & Singer 1998).

The contribution of olfactory receptors in food preference behavior of *A. assamensis* larvae was earlier discussed in order to probe the physiological basis of restricted diet breadth of the insect (Bora *et al*. 2013). SEM studies revealed the presence of gustatory sensilla in *A. assamensis* larvae that are similar to those of other lepidopteran insects. Although the gustatory organs are well known to be involved in food selection, it is not known if they are responsible for rejection of the non-host-plants in case of *A. assamensis*. Therefore, in the present study, food choice test was performed by ablating all other chemosensory organs, leaving only either the galeal styloconica or labrum–epipharynx in order to be able to determine the functional significance of the organs in host-plant preference or rejection by the larvae.

SEM studies showed that the galea of *A. assamensis* contains two sensilla, one lateral and one medial sensillum styloconicum (Fig. 5). The presence of certain hairy sensilla with a slender distally curved stem was reported by Hazarika and Bordoloi (1998). The styloconic sensilla located in the galea of maxilla were found to be better developed in terms of length of the whole sensilla and dimensions of the apical portion as compared to different strains of *Bombyx mori* (Asaoka 2000; Dey *et al*. 2011) and other lepidopteran larvae (Faucheux 1991). Each of the uniporous sensilla on the maxillary galea has four taste cells while the gustatory sensilla on the tip of the maxillary palpi have three to four cells each. In our earlier studies, electrophysiological characterization of the medial and lateral sensilla styloconica of *A. assamensis* larvae earlier revealed their sensitivity to a wide group of chemical compounds including sugars and sugar alcohols, salts and deterrents. The epipharyngeal sensilla responded to inositol, salts and deterrents such as salicin, nicotine and caffeine (Akulwad *et al*. 2011). In the present study, the epipharynx was also found to contain two gustatory sensilla. This is the first report of epipharyngeal sensilla in *A. assamensis*. In insects, gustatory chemoreceptors are reported to contain receptors for sugar, bitter compounds, hydrocarbons and even odorants like carbon dioxide (Wanner & Robertson 2008). In *Bombyx mori* 55

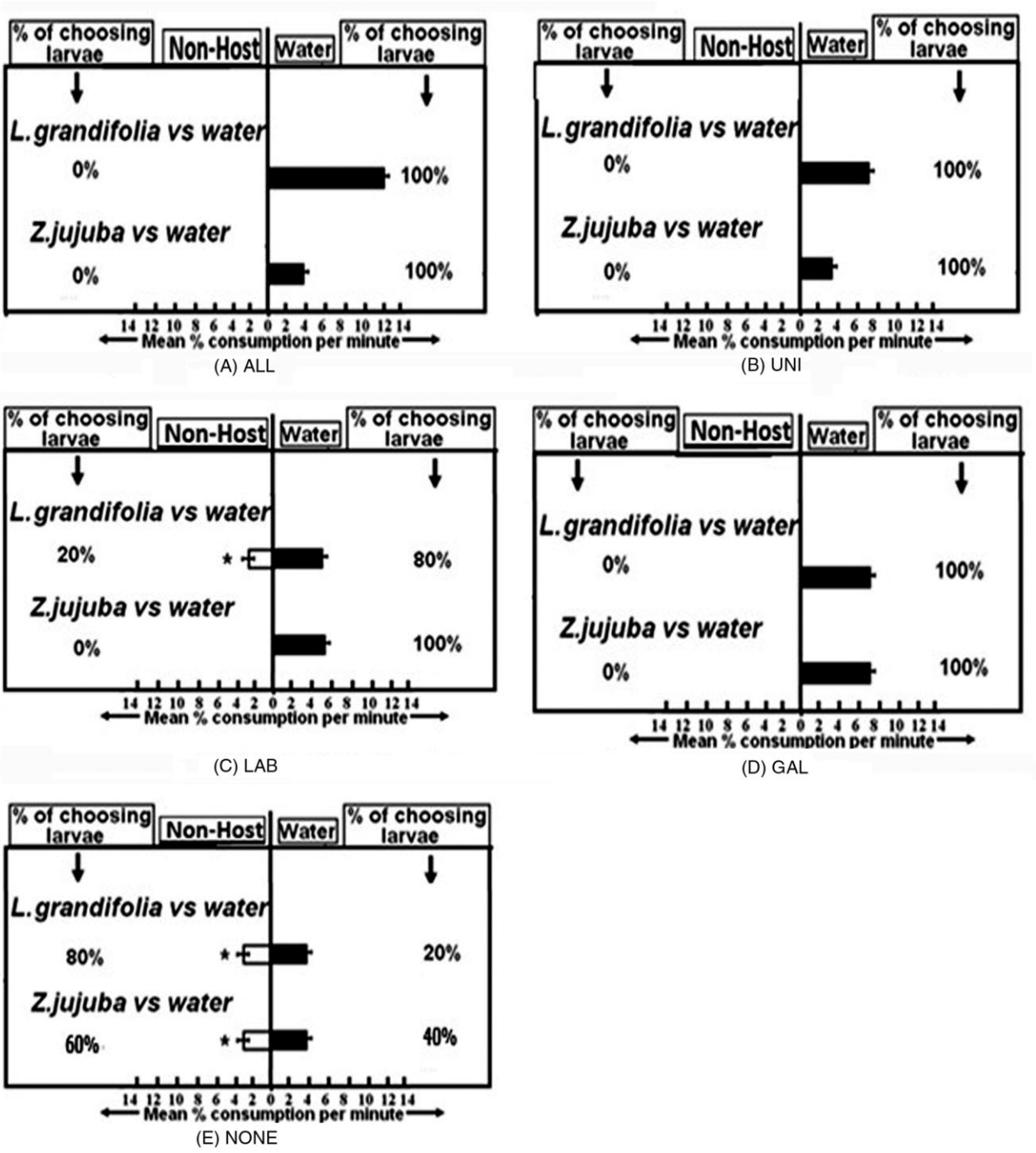

Figure 4 Mean per cent consumption per minute and per cent of choosing larvae in single-plant choice test (non-host-plant *vs* water). For mean per cent consumption per minute, Mann–Whitney ANOVA test: $*P < 0.05$ (two tailed).

gustatory receptors are proposed to be predominantly bitter receptors (Wanner & Robertson 2008). Such receptors may be responsible for perception of secondary plant metabolites.

In the study related to preference of host-plant over non-host-plant using both two-plant choice and single-plant choice tests, both GAL and LAB showed preferences for the host-plant, *P. bombycina*. Hence both galea and labrum-epipharynx were competent alone for mediating the host preference. However, for the other host-plant, *L. polyantha*, only GAL could differentiate between the host-plant

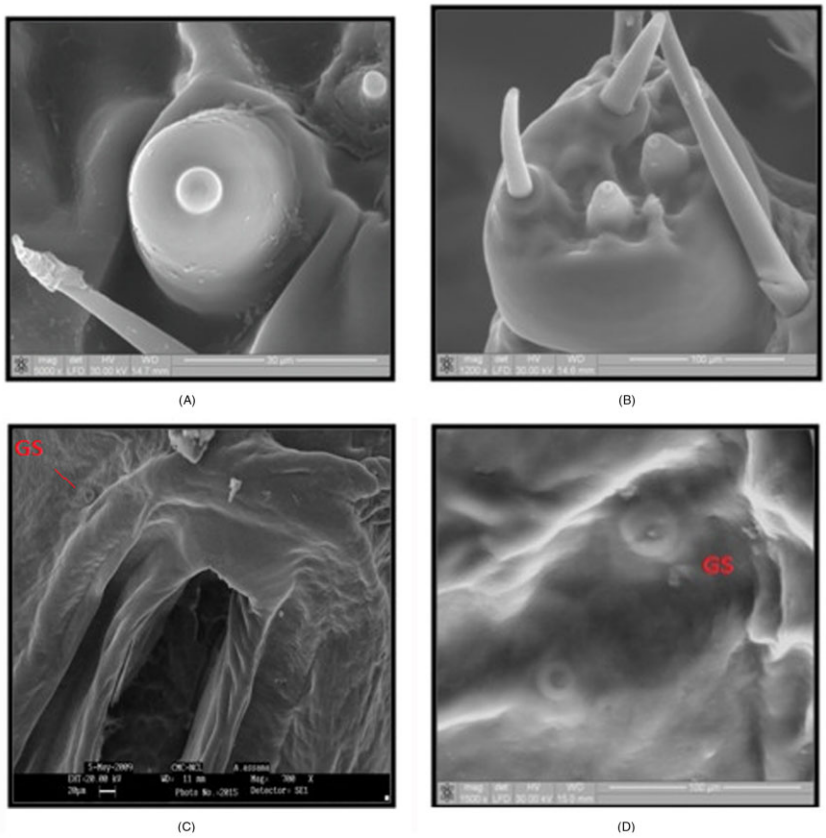

Figure 5 Scanning electron micrographs of chemosensory organs of *A. assamensis*. (A) Medial sensilla styloconica of galea; (B) galea-sensilla; (C) labrum-epipharynx; (D) epipharyngeal sensilla; GS, gustatory sensilla.

L. polyantha and non-host-plant, *L. grandifolia* while LAB chose both equally. The finding indicates that the labrum-epipharynx is involved in accepting a normally rejected non-host-plant but galeal sensilla are competent in rejecting the non-host-plants. Flowers and Yamamoto (1992) earlier reported the presence of deterrent receptors in galeal sensilla styloconica in *M. sexta*. Similarly Del Campo *et al*. (2001) showed that their host restricted feeding behavior was dependent on sensory input from both lateral and medial sensilla. Since only 20% of LAB opted for the non-host-plant, the larvae merely accepted the non-host-plant yet preferred the host. For a clear demonstration of preference for the host-plant, in the one-plant choice test, 90% of LAB opted for *L. polyantha* and only 20% opted for *L. grandifolia*. Similarly in the case of the single-plant choice test using host-plant, *P. bombycina* and water, 90% LAB opted for the host-plant. These observations reinforced the hypothesis that the labrum-epipharynx was not only competent in showing a preference for the host-plant, but also in acceptance of the normally rejected non-host-plant. Earlier in tobacco hornworm, *M. sexta*, either maxillary sensilla styloconica or epipharyngeal sensilla were reported to mediate aversion from food tested (De Boer & Hanson 1987; De Boer 1991).

NONE failed to differentiate between the host-plant and non-host-plant and the event was more prominent in the case of *L. polyantha* vs *L. grandifolia*, as 60% of NONE opted for *L. polyantha* and 40% opted for *L. grandifolia*. Further, the mean per cent consumption of the non-host-plant *L. grandifolia* was significantly higher than in the host-plant ($P < 0.05$). For a clearer demonstration, when the single-plant choice test was done, 20% of NONE opted for the host-plant and 80% for water but the mean per cent consumption was similar in both host-plants and water. In case of the non-host-plants, 80% NONE opted for *L. grandifolia* and 60% opted for *Z. jujuba* but the mean per cent consumption was significantly lower than that of water. The results clearly exhibited the principal involvement of galeal and labrum epipharyngeal sensilla in gustation by larvae of *A. assamensis* and the key role of galeal sensilla in its restrictive diet-breadth. In *Bombyx mori* the ligand carrier proteins encoded by odorant binding protein like gene (bmObpL1 and bmObpL3) were detected in the gustatory chemosensilla of the epipharynx and maxillary galea (Yoshizawa *et al*. 2011). In *Drosophila* also gustatory neurons are reported to express different gustatory receptors and detect different taste modalities, including sugar, bitter and water tastes (Liman *et al*. 2014). Further studies relating

to identification of receptor proteins, their distribution in galeal and epipharyngeal sensilla and the genes encoding them will help to understand the molecular mechanism of the chemosensory function of the gustatory sensilla in a restricted diet breadth of *A. assamensis*.

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References

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJP, Zuker CS (2000) A novel family of mammalian taste receptors. *Cell* **100**: 693–702.

Akulwad AK, Kasav A, Deka B, Bora DS, Sen A. Chemoreception in *Antheraea assama-Exorista sorbillans* complex: role of host plant chemicals. Presented at 59th Annual meeting of Entomological Society of America, Reno, Nevada, USA. 2011.

Asaoka K (2000) Deficiency of gustatory sensitivity to some deterrent compounds in "polyphagous" mutant strains of the silkworm, *Bombyx mori*. *Journal of Comparative Physiology* **186**: 1011–1018.

Bernays EA, Singer MS (1998) A rhythm underlying feeding behaviour in a highly polyphagous caterpillar. *Physiological Entomology* **23**: 295–302.

Bernays EA, Weiss M (1996) Induced food preferences in caterpillars: the need to identify mechanisms. *Entomologia Experimentalis et Applicata* **78**: 1–8.

Bernays EA, Woods HA (2000) Foraging in nature by larvae of *Manduca sexta* influenced by an endogenous oscillation. *Journal of Insect Physiology* **46**: 825–836.

Bora DS, Deka B, Sen A (2013) Host plant selection by larvae of the muga silk moth *Antheraea assamensis* and the role of antenna and maxillary palp. *Journal of Insect Science* **13**: 1–13.

Bullas-Appleton ES, Otis G, Gillard C, Schaafsma AW (2004) Potato leafhopper (Homoptera: Cicadellidae) varietal preferences in edible beans in relation to visual and olfactory cues. *Environmental Entomology* **33**: 1381–1388.

Chapman RF (2003) *Contact Chemoreception in Feeding by Phytophagous Insects*. Chapman and Hall, New York.

Clyne PJ, Warr CG, Carlson JR (2000) Candidate taste receptors in *Drosophila*. *Science* **287**: 1830–1834.

De Boer G (1991) Effect of diet experience on the ability of different larval chemosensory organs to mediate food discrimination by the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* **37**: 763–769.

De Boer G, Hanson FE (1984) Food plant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm *Manduca sexta*. *Entomologia Experimentalis et Applicata* **35**: 177–193.

De Boer G, Hanson FE (1987) Differentiation of roles of chemosensory organs in food discrimination among hosts and non-host plants by larvae of the tobacco hornworm, *Manduca sexta*. *Physiological Entomology* **12**: 387–398.

De Boer G, Dethier VG, Schoonhooven LM (1977) Chemoreceptors in preoral cavity of the tobacco hornworm, *M. sexta* and their possible function in feeding behaviour. *Entomologia Experimentalis et Applicata* **21**: 287–298.

Del Campo ML, Miles CI, Schroeder FC, Mueller C, Booker R, Renwick JA (2001) Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature* **411**: 186–189.

Dethier VG, Crnjar RM (1982) Candidate codes in the gustatory system of caterpillars. *Journal of General Physiology* **79**: 543–569.

Dey S, Singh S, Chakraborty R (2011) Surface ultrastructure of larval mouthpart sensilla of the muga silkmoth, *Antheraea assamensis*, an endemic species of north-east India. *Microscopy Research and Technique* **74**: 292–300.

Dunipace L, Meister S, McNealy C, Amrein H (2001) Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. *Current Biology* **11**: 822–835.

Faucheux MJ (1991) Morphology and distribution of sensilla on the cephalic appendages, tarsi and ovipositor of the European sunflower moth, *Homoeosoma nebulella* Den. & Schiff (Lepidoptera: Pyralidae). *International Journal of Insect Morphology and Embryology* **20**: 291–307.

Flowers RW, Yamamoto RT (1992) Feeding on non-host plants by partially maxillectomized tobacco hornworms (*Manduca sexta:* Lepidoptera: Sphingidae). *Florida Entomologist* **75**: 89–93.

Fox A, Pitts R, Robertson H, Carlson JR, Zwiebel L (2001) Candidate odor receptors from the malaria vector mosquito, *Anopheles gambiae*. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 14 693–14 697.

Hallem EA, Dahanukar A, Carlson JR (2006) Insect odor and taste receptors. *Annual Review of Entomology* **51**: 113–135.

Hanson FE, Dethier VG (1973) Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* **19**: 1019–1034.

Hazarika LK, Bordoloi S (1998) Antennal and mouth part sensilla of the muga silk worm *A. assama (*Lepidoptera: Saturniidae). *Sericologia* **38**: 55–63.

Heisswolf A, Gabler D, Obermaier E, Muller C (2007) Olfactory versus contact cues in host plant recognition of a monophagous *Chrysomelid* beetle. *Journal of Insect Behaviour* **20**: 247–266.

Hill CA, Fox AN, Pitts RJ *et al*. (2002) G protein-coupled receptors in *Anopheles gambiae*. *Science* **298**: 176–178.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJP, Zuker CS (1999) Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* **96**: 541–551.

Jermy T (1987) The role of experience in the host selection of phytophagous insects. In: Chapman RF, Bernays EA, Stoffolano JG (eds) *Perspectives in Chemoreception and Behavior*, pp 143–157. Springer-Verlag, New York.

Jorgensen K, Almaas TJ, Marion-Poll F, Mustaparta H (2007) Electrophysiological characterization of responses from

gustatory receptor neurons of sensilla chaetica in the moth *Heliothis virescens*. *Chemical Senses* **32**: 863–879.

Liman ER, Zhang YV, Montell C (2014) Peripheral coding of taste. *Neuron* **81**: 984–1000.

Reisenman CE, Christensen TA, Hilderbrand JG (2005) Chemosensory selectivity of output neurons innervating an identified, sexually isomorphic olfactory glomerulus. *Journal of Neuroscience* **25**: 8017–8026.

Renwick JA, Lopez K (1999) Experience-based food consumption by larvae of *Pieris rapae*: addiction to glucosinolates? *Entomologia Experimentalis et Applicata* **91**: 51–58.

Robertson HM, Warr CG, Carlson JR (2003) Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **100** (Suppl 2): 14 537–14 542.

Schoonhoven LM, Dethier VG (1966) Sensory aspects of host plant discrimination by lepidopterous larvae. *Archives Neerlandaises de Zoologie* **16**: 497–530.

Scott K, Brady R Jr, Cravchik A *et al*. (2001) A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104**: 661–673.

Simpson SJ (1995) Regulation of a meal: chewing insects. In: Chapman RF, De Boer G (eds) *Regulatory Mechanisms in Insect Feeding*, pp 137–156. Chapman and Hall, New York.

Stadler E, Hanson F (1975) Olfactory capabilities of the "gustatory" chemoreceptors of the tobacco hornworm larvae. *Journal of Comparative Physiology* **104**: 97–102.

Wanner KW, Robertson HM (2008) The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. *Insect Molecular Biology* **17**: 621–629.

Yoshizawa Y, Sato R, Tsuchihara K *et al*. (2011) Ligand carrier protein genes expressed in larval chemosensory organs of *Bombyx mori*. *Insect Biochemistry and Molecular Biology* **41**: 545–562.