

EXPERIENCE-DEPENDENT CHANGES IN THE NUMBER OF CHEMOSENSORY SENSILLA ON THE MOUTHPARTS AND ANTENNAE OF *LOCUSTA MIGRATORIA*

S. M. ROGERS* AND S. J. SIMPSON

Department of Zoology and University Museum, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

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Summary

The effect of diet on the number of gustatory and olfactory sensilla was investigated in locusts. Insects fed for the final two stadia on nutritionally adequate synthetic foods have fewer sensilla on the maxillary palps and antennae than insects fed on the usual rearing diet of seedling wheat. This effect was seen irrespective of the nutritional balance of the foods and was independent of the concentration of nutrients present or the size of the insect. Supplying wheat odour to locusts fed synthetic foods had no effect on the number of sensilla present on the palps, but completely reversed the decrease in the number of olfactory sensilla of the antennae and partially reversed the decrease in the number of antennal uniporous trichoid sensilla. Locusts that were allowed to select between two nutritionally unbalanced but complementary synthetic foods had a higher number of sensilla on the maxillary

palps than those fed individual synthetic foods, providing the two foods differed substantially in their protein:carbohydrate ratios. Insects also developed more sensilla if they were fed on two foods of identical nutritional composition but with different added flavours (1% tannic acid or amygdalin). Exposing locusts to synthetic foods for a single stadium did not cause any significant decrease in sensilla number. The results suggest that the number of sensilla that develop in a given sensory field is influenced by the variety of chemical stimuli experienced and the chemical complexity of the environment as provided by the presence of distinct individual sources of stimulation.

Key words: locust, *Locusta migratoria*, sensilla, diet, variability, plasticity, development.

Introduction

The proper development and functional integrity of sensory systems is known in many cases to be partly dependent on the sensory input that is received by those systems. This principle is well established in vertebrates for several modalities, particularly in the visual and somatosensory systems. The modifications seen, which may include hypertrophy of regions dealing with specific aspects of the sensory environment as well as degeneration in the absence of appropriate sensory stimulation, all affect the central nervous system (CNS) (Blakemore and Cooper, 1970; Purves and Lichtman, 1985; Diamond *et al.* 1994; Rauschecker, 1995; Wang *et al.* 1995; Shatz, 1996). There is an increasing number of examples of similar events occurring in insects and other invertebrates (Murphey, 1986), again for a range of modalities, that in some cases may not only affect the CNS but can also influence the sensitivity, form and number of the sense organs themselves (Matsumoto and Murphey, 1977; Bloom and Atwood, 1981; Hertel, 1983; Mimura 1986, 1993; Volman and Camhi, 1988; Deruntz *et al.* 1994; Pflüger *et al.* 1994).

The Acridoidea as a group are characterised by the possession of many contact chemosensory sensilla. These occur both as a sparse scattering over the entire surface of the

insect, but also as well-defined sensory fields often containing hundreds of sensilla. The principal fields are located on the tarsi, the terminals of the maxillary and labial palps, the inner surface of the labrum and the antennae (Thomas, 1966). In contrast to groups such as larval Lepidoptera, which maintain a small and constant number of sensilla (Baker *et al.* 1986), the number of sensilla in the Orthopteroidea is highly variable. The number is related to several factors including the size of the species, the developmental stage and even the size of an individual insect within a stadium (Chapman, 1982), with small species generally having fewer sensilla than larger species, and with new sensilla being added to existing fields at each moult. Differences in the number of sensilla on the antennae have also been shown between solitary and gregarious forms of three species of grasshopper (Heifetz and Applebaum, 1995; Chapman and Lee, 1991; Greenwood and Chapman, 1984). However, the sensilla population does show some degree of correlation with the dietary habit of different species, with scavengers having the most and the monophagous *Boettia argentatus* the fewest (Chapman and Fraser, 1989), and with polyphagous and oligophagous species ranged in between (reviewed in Chapman, 1982, 1995).

*e-mail: stephen.rogers@zoology.ox.ac.uk.

Short-term changes in the responsiveness of gustatory sensilla are well-documented and form an essential part of the mechanisms by which locusts and other insects regulate the intake and composition of their diet (Bernays *et al.* 1972; Abisgold and Simpson, 1988; Simpson *et al.* 1991; Simpson and Simpson, 1992; Simmonds *et al.* 1992; Simpson and Raubenheimer, 1993*a*, 1996). In addition to such short-term changes, there arises the possibility that longer-term effects of the sensory environment may manifest themselves in variation in sensilla number. As demonstrated by Chapman and Lee (1991), the range of available food plants or even an enriched olfactory environment had various effects on the numbers of both olfactory and trichoid uniporous (contact) chemoreceptors on the antennae of *Schistocerca americana*.

The present study examined the effect of dietary experience on the number of sensilla on the mouthparts and antennae of *Locusta migratoria* over the course of one or two stadia. Specifically, it looked at the differences between locusts fed the standard rearing diet of seedling wheat and wheat germ, and those fed chemically defined synthetic diets. These diets contain all the nutrients necessary to sustain growth but are devoid of the plethora of non-nutrient chemicals produced by the primary and secondary metabolism of plants. The ease with which the nutrient content of the diets can be varied, and the known behavioural consequences of doing so, which in part are mediated by the chemoreceptors (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993*b*; Simpson *et al.* 1995), allowed the testing of several hypotheses regarding the nature of the observed differences.

Materials and methods

The study was undertaken as a series of experiments, each designed to examine a different aspect of sensilla number plasticity, and summaries of the specific diets and treatments used in each experiment are given with the results. Most of the features of rearing, experimental and analytical design were common to all and are detailed here.

Insects and rearing

Locusta migratoria (Reiche and Fairmaire) used in the experiments were taken from the crowded culture maintained at the Department of Zoology, University of Oxford, where the insects are fed on seedling wheat and wheat germ. Unless stated otherwise, insects were collected on the day of moulting to the fourth stadium and checked to ensure that all the limbs, mouthparts and antennae were present and correctly formed. Equal numbers of male and female insects were used in all experiments. The locusts were subsequently reared in individual clear plastic boxes 175 mm×105 mm×50 mm in dimension, in a constant-temperature room at 29–30 °C under a 12h:12h light:dark photo regime, which are conditions similar to those experienced by the stock culture. Each box contained an expanded aluminium perch and food and water containers as appropriate. The boxes were laid out in a rectangular grid in near proximity to each other amongst which

individuals from each diet or treatment group were evenly dispersed.

Diets

Most of the experiments in this study involved comparisons between insects fed on whole-plant diets and a range of chemically defined synthetic foods. Plant-fed insects were given 8- to 9-day-old fresh wheat seedlings, cut just above the roots, and dry wheat germ contained in the two separate halves of a small rectangular plastic box (75 mm×45 mm×20 mm). Cut wheat was replaced every 2 days.

The synthetic foods used were dry and granular. Consequently, a separate source of drinking water was required. For insects fed on a synthetic diet, a circular feeding dish (as in Raubenheimer and Simpson, 1993) and a rectangular drinking bottle, made from a plastic culture flask bored with two 15 mm holes, were used. The manufacture of the synthetic diets was as in Simpson and Abisgold (1985). Each food contained a constant proportion (4%) of lipids, minerals and vitamins, but variable proportions of protein (casein, peptone and egg albumen in a 3:1:1 ratio) and digestible carbohydrate (sucrose and dextrin in a 1:1 ratio). The remainder was made up with indigestible cellulose. Actual protein:carbohydrate percentages used are detailed in the methods of the individual experiments. Synthetic diets are designated by two numbers, the first is the percentage content of protein (P), the second the percentage content of digestible carbohydrate (C). Synthetic foods were changed every 3 days.

Subsequent processing

Insects that failed to moult properly, becoming either deformed or losing a limb in the process, were discarded from the experiments. Moulting failure or mortality removed up to 25% of the insects set up in each experiment. Following their terminal moult, the insects were weighed to the nearest 1 mg, then killed by placing in a freezer at –40 °C and were subsequently stored in tubes of 70% ethanol. Locusts were decapitated and the maximum head width and length measured. The maxillae, labium and labrum and front of the head were removed with a razor blade. The detached mouthparts were then brought to absolute ethanol *via* 70% ethanol, macerated in hot (60–70 °C) 10% (w/v) aqueous KOH for 10 min or until transparent, degreased in xylene and cleared in eugenol, rinsing between stages in 100% ethanol. Finally, the mouthparts were mounted on microscope slides with Canada Balsam.

Sensilla and counting

The sensilla were counted by drawing a map of each of the sensory fields being sampled using a Zeiss microscope with *camera lucida* attachment. The labral fields, consisting of uniporous basiconic sensilla (Chapman, 1982), and antennal fields were drawn at 100× magnification. The antennae contain a variety of sensilla: coeloconic sensilla, small and large basiconic sensilla, which are all multiporous and have an olfactory function, and a smaller number of uniporous trichoid

sensilla, similar in size and structure to the terminal palp sensilla and usually judged as having a contact-chemoreceptive function (Greenwood and Chapman, 1984). Because of the difficulty of distinguishing unambiguously between the olfactory sensilla in unstained preparations at low magnifications, all the coeloconic and basiconic sensilla were counted together, although this will have masked any differences in the relative proportions of these sensilla types that might have occurred (also, in practice, the small basiconic sensilla and many of the large basiconic sensilla were not resolvable at 100 \times and consequently were not included). The distinctive trichoid antennal sensilla were counted separately. The uniporous trichoid sensilla of the terminal maxillary and labial fields were drawn at 400 \times magnification by slowly advancing the level of focus through the thickness of the palp and drawing all the focused sensilla at each level. The total number of sensilla drawn in each field was then counted using a click-counter.

Statistical analysis

The suitability of the data for parametric analysis was determined by examining frequency distributions of the raw data and plots of residuals against fitted values. It was infrequently found that certain sensilla fields were obviously malformed, either in the distribution of sensilla over the surface or in overall shape, and occasionally fields were damaged during the maceration process. Such outliers, if more than 2.5 standard deviations from the mean number of sensilla for the treatment, were discarded from the analyses.

As size is known to be an important determinant of the number of sensilla for many sensory fields (Chapman, 1982), maximum head width, as a measure of size, is taken as a covariate in analyses of covariance (ANCOVAs) between diet (as the main effect) and the number of sensilla present (dependent variable) from the various fields in all the analyses that follow. The results of the analyses were similar whether the size variable used was adult mass or a linear size measurement. As *F*-ratios for linear measurements of size were consistently larger than for mass, the former were kept as the preferred covariate, being a better predictor of the number of sensilla. Maximum head width was taken throughout as a representative linear measurement. The results were also robust in the face of transformations (e.g. squaring head width) designed to equilibrate linear and volume measurements to a putatively area-dependent variable such as the number of sensilla.

The covariate \times treatment interaction terms of the models (head width \times diet) were found to be non-significant in all cases and have been omitted from the analyses. The sex of the animal had no effect on the number of sensilla that could not be accounted for through differences in size in any of the sensilla groups, with a single exception (see below). The lack of significant interaction terms in the models and the near-parallel slopes of the regressions when plotted argued against a strongly non-linear relationship. Non-linear regressions when fitted to the data all tended to straight lines.

Table 1. Results of an analysis of covariance examining the effect of prolonged feeding on wheat or synthetic foods on the number of sensilla present in adult locusts with maximum head width taken as covariate

Field	d.f. residual	Head width	Treatment
		d.f.=1 <i>F</i> -ratio	d.f.=3 <i>F</i> -ratio
Maxillary palp dome	32	9.275**	4.111*
Eleventh antennal annulus trichoid	28	4.529*	15.678***
Eleventh antennal annulus olfactory	28	0.182	4.438*

In each treatment, locusts were reared for two stadia on one of four different treatments: fresh wheat, dried wheat, P14:C14 synthetic food alone or P14:C14 synthetic food in the presence of wheat odour.

Significant results are marked as follows: **P*<0.05; ***P*<0.01; ****P*<0.001.

Results

The effect of synthetic food, wheat and wheat odour on sensilla number

To examine how different feeding regimes affected the number of sensilla present on adult insects, 48 fourth-instar locusts were reared for two stadia in a constant-temperature room (29–30 °C) with an air-regulating unit that provided a constantly replenished and largely odour-free air supply. Insects were divided equally between four different feeding regimes. (1) Fresh wheat and wheat germ (the usual rearing diet). (2) Dried wheat (placed for 72 h in a desiccating oven at 40 °C and subsequently stored at –40 °C until needed) and wheat germ. Drinking water was also provided. (3) P14:C14 synthetic food and water, a food having a near optimal balance in the proportions of protein and carbohydrate needed for locusts (Chambers *et al.* 1995). (4) P14:C14 synthetic food and water, but in an atmosphere replete with wheat odour. This was provided by filling a small (50 ml) pot with a perforated zinc panel in its lid with finely chopped fresh wheat and placing it in the rearing box. The wheat was replaced on alternate days. Ten locusts either died or failed to moult properly over the course of the experiment and one further individual was later discarded because it possessed a deformed palp, leaving a total of 37 individuals for the analysis. These losses were distributed evenly over all the treatment groups. The number of sensilla on the right maxillary palp and the trichoid and olfactory sensilla of the right eleventh antennal annulus (counting from the base) of the adults were counted.

An ANCOVA analysis of the results is presented in Table 1. All three sensory fields showed a significant effect of treatment on the number of sensilla. The numbers of sensilla on the maxillary palp and the trichoid antennal sensilla also exhibited a significant correlation with head width. The results of a *post-hoc* analysis (Student–Newman–Keuls test) of the effects of treatment on the different sensory fields is given below, and the treatment effects can also be seen in Fig. 1.

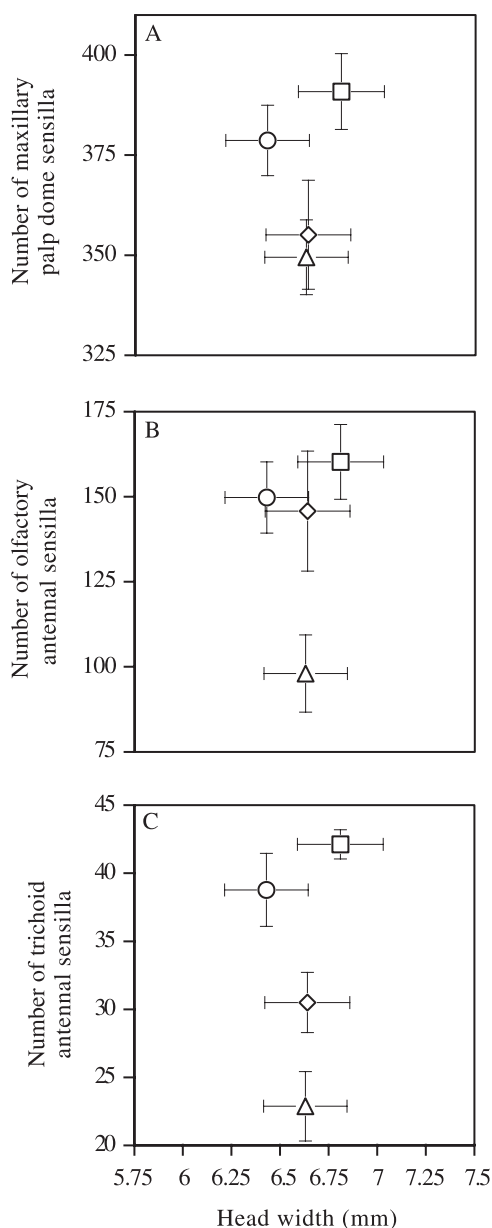


Fig. 1. The means and standard errors of the number of sensilla on the maxillary palps and antennae plotted against the means and standard errors of maximum head widths of locusts fed: fresh wheat (\square ; $N=8$), dried wheat (\circ ; $N=9$), P14:C14 synthetic food alone (\triangle ; $N=10$) or in conjunction with fresh wheat odour (\diamond ; $N=10$) for the last two nymphal stadia on (A) the maxillary palp dome, (B) the olfactory antennal sensilla of the eleventh flagellar annulus or (C) the trichoid antennal sensilla of the eleventh flagellar annulus.

Both groups of locusts fed on synthetic food had approximately 11 % fewer sensilla on the maxillary palps (Fig. 1A) than those fed the usual rearing diet of wheat. The mean number of sensilla on the palps of locusts fed dried wheat was slightly lower (but not significantly so) than those fed fresh wheat, but this difference could be explained by the smaller size of the insects. The number of olfactory sensilla on the antennae (Fig. 1B) was similarly depressed in locusts fed on

the synthetic food compared with locusts fed either of the wheat treatments. However, this effect was reversed in the presence of wheat odour, and the number of sensilla on insects fed synthetic foods in the presence of wheat odour was indistinguishable from that of insects fed wheat. The trichoid sensilla of the antennae exhibited an intermediate effect (Fig. 1C): both wheat treatments produced similarly high sensilla numbers, and the locusts fed synthetic food alone exhibited a 40 % decrease in the number of sensilla, but whilst wheat odour presented in conjunction with the synthetic food raised the number of sensilla compared with that found in insects fed synthetic food in isolation (ANCOVA comparing synthetic diet fed with synthetic diet+wheat-odour-fed locusts; $F_{1,13}=2.747$, $P=0.1214$ for head width as covariate, $F_{1,13}=6.690$, $P=0.0226$ for treatment as main effect), the number was still significantly lower than that for insects fed on wheat (ANCOVA with the synthetic diet+wheat-odour-fed and wheat-fed insects as treatment groups; $F_{1,22}=8.556$, $P=0.0078$ for head width as covariate, $F_{1,22}=15.326$, $P=0.0007$ for treatment as main effect).

The effect of nutrient concentration and nutrient balance on the number of chemosensory sensilla on the major sensory fields of the head

The influence of both the concentration and balance of macronutrients on the number of sensilla was investigated by feeding a total of 105 fourth-instar locusts a range of synthetic foods varying in protein and digestible carbohydrate content. The number of sensory fields sampled was increased to include all the major chemosensory fields of the head. Groups of 15 insects were used in seven different treatments, falling into three categories. (1) The overall concentration of protein and carbohydrate present was varied in four different treatments; P7:C7, P14:C14, P21:C21 and P35:C35; all having the same near-optimal protein:carbohydrate ratio (Chambers *et al.* 1995). (2) Two further treatment groups were fed P7:C21 or P21:C7, which were nutritionally highly unbalanced with respect to their protein and carbohydrate content in relation to the optimal dietary composition for *L. migratoria* nymphs. (3) A final group was fed fresh wheat and wheat germ and served as a control and comparison with the previous experiment.

In the adult insects, both the left and right fields of the major sensory areas of the head were counted, namely the terminal sensilla of the maxillary and labial palps, the A1, A2 and A3 labral fields of the cibarium, and the olfactory and trichoid sensilla of the eleventh antennal annulus.

There were no significant differences in the numbers of sensilla on the left and right maxillary palps, labial palps or antennae, and the mean number of sensilla across both sides for each field was used in the analysis. All three labral fields, however, were found to have consistent asymmetries between sides, and each side was therefore analysed separately (paired *t*-test, $P<0.001$ for each). The high degree of asymmetry found in the cibarial fields may reflect the asymmetry of the mandibles underlying the labrum, creating an irregular space in which the sensilla must be positioned. The results of an

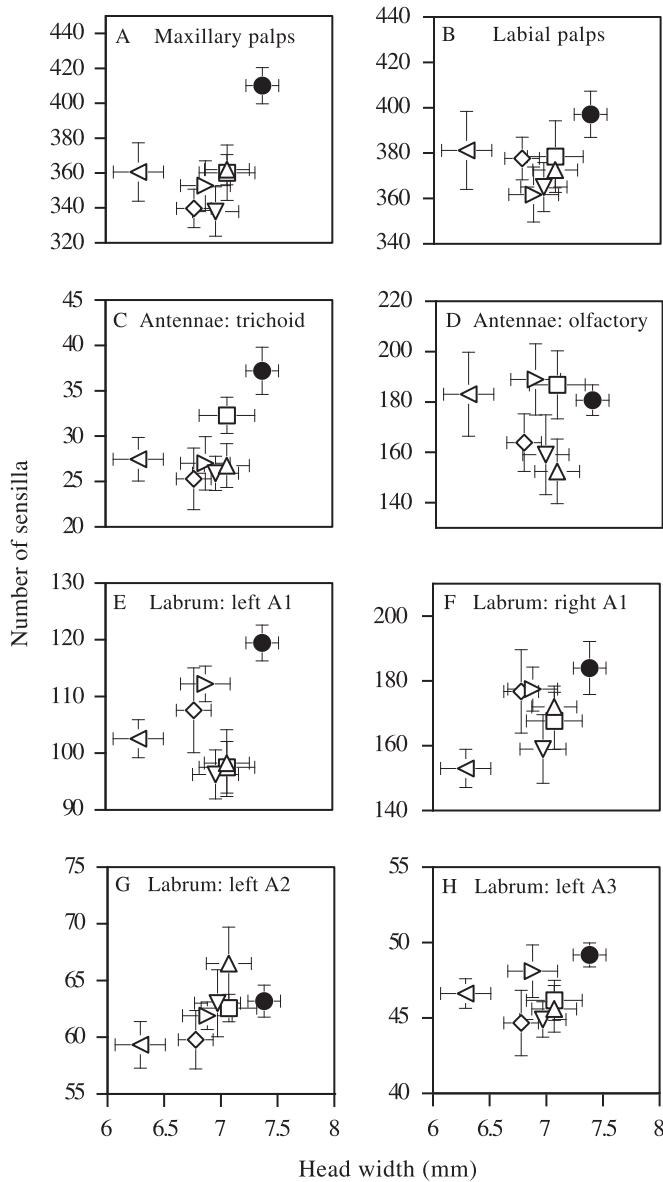


Fig. 2. The means and standard errors of the numbers of sensilla present on the main sensory fields of the locust head plotted against the means and standard errors of maximum head width after being fed various foods from the fourth stadium to adulthood. Four of the foods, P7:C7 (\triangle ; $N=10$), P14:C14 (∇ ; $N=10$), P21:C21 (\triangleright ; $N=10$) and P35:C35 (\triangleleft ; $N=9$), had a near-optimal protein:carbohydrate ratio, but varied in overall concentration. Two of the foods, P7:C21 (\diamond ; $N=9$) and P21:C7 (\square ; $N=7$), were highly unbalanced in their protein:carbohydrate ratio. The final group was fed fresh wheat and wheat germ (\bullet ; $N=11$).

ANCOVA analysis are presented in Table 2 (and see also Fig. 2). The maxillary palps, labial palps, trichoid antennal sensilla and the left A1 labral field showed a significant effect of diet ($P=0.0001$, 0.0202 , 0.0064 and 0.0094 respectively, see Table 2 for details; also Fig. 2A–C,E). None of the other fields showed any diet-related effect, although head width was significant as a covariate (Table 2) for all the sensilla fields

Table 2. Results of an analysis of covariance for an experiment in which the effects of nutrient concentration and balance on the number of sensilla present in adult locusts were examined with maximum head width taken as covariate

Sensory field	d.f. residual	Head width d.f.=1 <i>F</i> -ratio	Diet d.f.=7 <i>F</i> -ratio
Maxillary palps	58	47.249***	4.721***
Labial palps	57	49.251***	2.756*
Left A1 labral	50	6.321**	3.222**
Right A1 labral	47	20.373***	1.084
Left A2 labral	55	19.223***	0.965
Right A2 labral	52	9.255**	0.564
Left A3 labral	56	0.357	1.276
Right A3 labral	55	0.065	0.576
Eleventh antennal annulus trichoid	54	3.264*	3.402**
Eleventh antennal annulus olfactory	54	0.176	1.819

Locusts were reared for two stadia on one of seven different diets, P7:C7, P14:C14, P21:C21, P35:C35, P7:C21, P21:C7 or fresh wheat.

The numbers of sensilla on each of the major sensory fields of the head were compared.

Significant results are marked as follows: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

with the exception of the olfactory antennal sensilla (Fig. 2D) and the A3 labral fields (Fig. 2H).

A *post-hoc* analysis (Student–Newman–Keuls) applied to the fields showing a treatment effect indicated that wheat-fed locusts had consistently more maxillary palp (Fig. 2A) and trichoid antennal (Fig. 2C) sensilla than locusts fed any of the single synthetic foods. Moreover, none of the insects fed single synthetic foods differed from each other, regardless of the food composition.

Although a significant effect of treatment was indicated for the labial palps, the *post-hoc* test failed to isolate any differences between the different diets, suggesting that the effect of treatment on sensilla number was far weaker on the labial palps (Fig. 2B) than on the maxillary palps. For the left A1 labral field, only three of the foods, P7:C7, P14:C14 and P21:C7, led to significant differences in sensilla number compared with wheat-fed locusts in the *post-hoc* test ($P<0.05$ in the Student–Newman–Keuls test, values calculated as $P=0.0022$, 0.0009 and 0.0016 , respectively, in a Bonferroni–Dunn test on the same data; see also Fig. 2E). The decreased number of sensilla on the left A1 labral field of locusts fed a synthetic diet is difficult to explain, given the lack of effect of diet on the right A1 field (Fig. 2F) or on any of the other labral fields. This field was also the only field to show a significant effect of sex on sensilla number (ANCOVA with sex added as an effect $F_{1,58}=8.103$, $P<0.01$). The left A2 (Fig. 2G) and A3 (Fig. 2H) are depicted as representative of the labral fields that showed no effect of diet on sensilla number.

In contrast to the previous experiment, there appeared to be no significant effect of treatment on the olfactory sensilla of the antennae (Table 2). Closer examination of the data indicated that, as before, P14:C14-fed locusts had fewer sensilla than wheat-fed individuals, as did the locusts fed the other low-protein foods, P7:C7 and P7:C21. However, locusts fed the high-protein foods P21:C21, P35:C35 and P21:C7 all had elevated numbers of sensilla and were indistinguishable from those fed wheat (see Fig. 2D). Re-analysing the data with dietary protein concentration as a factor rather than diet gave a significant effect of treatment (ANCOVA; head width as covariate, $F_{1,54}=0.137$, $P>0.05$; dietary protein as main effect $F_{4,54}=2.7$, $P<0.05$). High-protein diets are considerably more odiferous than those with lower protein concentrations, and this may have promoted sensilla development. The odour effect of protein may also have been amplified because the experiment was carried out in a constant-temperature room which lacked the high-efficiency air-filtering unit used previously and, consequently, the atmosphere was considerably more odour-rich.

The effect of dietary choice

Different aspects of dietary choice were investigated in 75 fourth-instar locusts divided between five treatments in which the insects were fed the following combinations of synthetic foods with a combined protein and carbohydrate content of 28%. (1) Two dishes of highly nutritionally unbalanced but complementary foods, P23.2:C4.8 with P5.3:C22.7, chosen because the optimal diet composition lies exactly midway between them (Chambers *et al.* 1995). (2) Another pair of symmetrically unbalanced complementary foods, but much closer in composition, P14.8:C13.2 with P9.8:C18.2. (3) A food with the ideal protein:carbohydrate ratio for locusts, P12.3:C15.7. (4) Two dishes of the ideal food as above, but with a different flavour added to each. 1% dry mass of either tannic acid or amygdalin (Sigma) was sieved onto the finished foods and stirred thoroughly prior to use. Previous work had shown that locusts can detect these flavours at the concentrations used but they do not have a long-term effect on the amount of food eaten (Trumper and Simpson, 1994). (5) A single dish of the ideal food, unflavoured. The sensilla on the right maxillary palp of the adults were counted.

An ANCOVA analysis (head width as covariate, $F_{1,46}=37.05$, $P<0.001$; treatment as main effect $F_{4,46}=3.83$, $P<0.01$) indicated a significant effect of treatment, and a *post-hoc* (Student–Newman–Keuls) test of the results (see also Fig. 3) indicated that locusts allowed a choice of two nutritionally unbalanced complementary foods, providing those foods were sufficiently heterogeneous, had more sensilla than those fed a single food. The locusts given a choice of foods more similar in composition had sensilla numbers no different from those fed a single food. The addition of flavours to the synthetic food also served to elevate the number of sensilla (a significant difference, $P<0.05$, in number of sensilla compared with locusts fed either the ideal food or the two foods of close nutrient content was found in the Student–Newman–Keuls

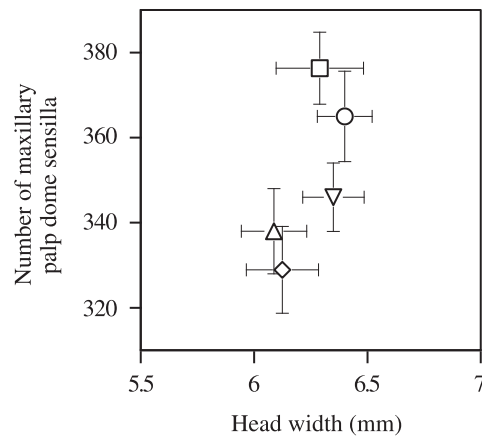


Fig. 3. The effect of dietary choice on the mean numbers of maxillary palp dome sensilla, in relation to maximum head width, for locusts fed widely divergent complementary foods varying in protein and digestible carbohydrate content: P23.8:C4.8/P5.3:C22.7 (□; $N=9$); narrowly divergent complementary foods, P14.8:C13.2/P9.8:C18.2 (◇; $N=12$); an ideal food (P12.3:C15.7) from either one (△; $N=9$) or two (▽; $N=12$) food dishes; or two ideal foods with added flavours, 1% tannic acid in one and 1% amygdalin in the other (○; $N=11$). Both the flavoured and widely divergent treatments have significantly more sensilla than the other treatments ($P<0.05$ in a Student–Newman–Keuls test). Values are means \pm the standard error of the mean.

test) on the palp to values similar to those for individuals given nutritionally very heterogeneous foods. The number of feeding dishes used to supply food to the insect had no effect on the number of sensilla.

The time course of expression

The time necessary for differences in sensilla number to become apparent was examined in two groups of 20 locusts that were reared for a single stadium, either from the fourth to the fifth stadium or from the fifth stadium to adulthood. Half the locusts in each group were fed P21:C21 synthetic food, the other half fresh wheat and wheat germ. A control group of 20 insects was reared from the fourth stadium to adulthood. The number of terminal sensilla on the right maxillary palp was counted for all groups.

Analysis of the results showed that neither the insects reared from the fourth to the fifth instar nor from the fifth instar to adulthood showed any significant effects of diet on sensilla number (ANCOVA: for fourth to fifth instar, with head width as covariate, $F_{1,13}=4.597$, $P<0.05$; for sensilla number as main effect, $F_{1,13}=3.301$, $P>0.05$; for fifth instar to adult, with head width as covariate, $F_{1,17}=6.772$, $P<0.05$; for sensilla number as main effect, $F_{1,17}=0.085$, $P>0.05$). The control group showed similar decreases in sensilla number when fed synthetic foods as found in previous experiments.

Discussion

The results of these experiments show that the

chemosensory environment locusts experience during the last two stadia has a role in determining the number of sensilla present in certain sensory fields in the adult. Locusts fed nutritionally adequate, but chemically simple, synthetic foods had fewer sensilla than insects fed wheat, whilst enriching the sensory environment of insects fed synthetic foods served to promote sensilla development. Two distinct aspects of the sensory environment, which may be termed chemical diversity and chemical heterogeneity, both of which could have a role in promoting sensilla development, were manipulated in these experiments.

The chemical diversity is the number of different detectable chemicals available to the locust. Wheat, although lacking the extensive secondary metabolism found in many plants, contains a host of nutritive and non-nutritive chemicals, and locusts fed wheat experienced a greatly enhanced chemical diversity compared with those fed synthetic foods. The addition of flavours to synthetic foods also caused a significant increase in the number of sensilla. Chemical concentration, however, appeared to be relatively unimportant in determining sensilla number since there were no differences in the number of palp sensilla between locusts fed foods showing an overall fivefold variation in the concentration of the two major macronutrients. This conclusion is further supported by the similarity in the number of sensilla found in locusts fed fresh and dried wheat, between which the relative proportions of volatile and non-volatile chemicals will have varied considerably. The only exception was found in the olfactory sensilla of the antennae, since locusts fed odiferous high-protein foods had more of these sensilla than those fed low-protein foods.

Although in these experiments chemical diversity was presented predominantly in the form of different foods, it was also shown that manipulating the olfactory environment alone could affect the numbers of antennal sensilla. Enriching the atmosphere with non-food-plant-associated chemicals was shown by Chapman and Lee (1991) similarly to increase the number of antennal sensilla, and it is possible that contact-chemosensory stimuli provided in the absence of foods could increase the number of palp sensilla. The labral sensory fields were not greatly affected by diet. These fields represent the last opportunity to sample potential foods before ingestion, and as such their numbers may be more strongly defended. As the cibarial sensilla only have access to chemicals once the insect has bitten a food, they are also presumably subjected to a more restricted range of stimuli than the external sense organs.

The second environmental factor that influenced the number of sensilla, chemical heterogeneity, can be defined as the availability of different sources of distinct stimulation. Our results show that locusts presented with a choice of two highly unbalanced but complementary foods had more sensilla than those fed either a single highly unbalanced food alone or a single food mixed to the protein:carbohydrate ratio that locusts will eat and defend if given a choice (Simpson and Raubenheimer, 1993b; Simpson *et al.* 1995). In each case, the total number of different chemicals available to the sensory

system was the same but the choice treatments were qualitatively different in that they provided a source of sensory heterogeneity.

Heterogeneity is pervasive in the natural environment. Plants, as well as being a source of many different kinds of chemical, are also highly complex systems, with the chemicals being concentrated in various tissues. Their chemical constituents can also vary considerably over time because of factors such as water stress, previous insect attacks or, as in the case of cut wheat, decomposition (Bernays and Lewis, 1986; Roessingh *et al.* 1985). Appropriate selection from a range of potential foods to form a balanced diet is an important role for the gustatory system (Bernays and Simpson, 1982; Simpson *et al.* 1995), but potential foods are just some of the sources of stimulation that locusts will encounter, some or all of which may have a role in affecting the final sensilla numbers.

For several reasons, it is unlikely that the plasticity in sensilla number observed in the experiments reported here was the result of a nutritional deficit. First, olfactory sensilla numbers could be manipulated independently of diet. Second, diet affected some but not all sensory fields; malnutrition either from a generally inadequate diet or through the lack of a specific micro-nutrient would be expected to affect all sensilla fields similarly; and finally, the number of sensilla on most of the affected fields did not differ between insects fed nutritionally balanced and strongly unbalanced or diluted synthetic foods. The hypotheses that the total time spent palpating, or the total area available for sampling, led to increased sensilla production can also be rejected because increasing the number of dishes in which a food was given had no effect on sensilla number. The synthetic foods also differed considerably from wheat in their fibre content and texture. However, the elevated sensilla number in the choice treatments and the lack of differences in number of sensilla between locusts fed on any of the synthetic foods, which ranged from 26 to 82 % cellulose and differed widely in consistency from hard granules to looser flocculent mixtures, argues against texture being a major factor determining sensilla number.

The influence of the number of sensilla on the sensory physiology and behaviour of locusts is unclear. Insects that experienced a deprived sensory environment still had a considerable number of sensilla on the maxillary palps as adults (approximately 350), many more than are present on wheat-fed fifth-instar nymphs (approximately 250 sensilla), yet there are no known differences in the sensory or discriminatory abilities of normal adults and nymphs (Chapman, 1988, 1995). The percentage differences in maxillary palp sensilla numbers seen in this study are half the magnitude of the smallest differences reported between labral sensilla populations of similarly sized polyphagous, oligophagous and monophagous species of grasshopper (Chapman and Fraser, 1989). It is possible that longer periods of exposure to synthetic foods would have increased the magnitude of the difference between synthetic- and wheat-fed locusts, and might have affected more of the sensory fields of the head. Nevertheless, the induced

differences in sensilla number reported here, whilst not as great as those between species, raise the possibility that differences between species with different dietary preferences could be the result of a complex interaction between genes, experience and behaviour. In such a case, the number of sensilla would be in part both a determinant and a consequence of dietary choice.

Cuticular structures can only be changed at ecdysis, but it is unknown how sensory experience can influence the number of sensilla in subsequent instars. It was beyond the scope of the present study to determine whether the observed decreases were due to the disappearance of already existing sensilla, the failure to produce new sensilla or a combination of both. Under normal circumstances, new sensilla are added to the population of a sensory field at each moult, with existing sensilla being maintained from one instar to the next (Blaney *et al.* 1971). Whilst there is a sizeable literature on the development of new sensilla, this seems exclusively dedicated to sensillogenesis in embryos, on new cercal annuli, or that occurring during pupation when there is a complete remodelling of the entire cuticle (Gnatzy, 1978; Ameismeier, 1985; Seidl, 1991; Lienhard and Stocker, 1991; Ray *et al.* 1993) but very little is known of the genesis of new sensilla in an already existing field.

The different numbers of sensilla present on the antennae and palps when both the olfactory and gustatory environments were altered independently is consistent with there being no systematic determination of the number of sensilla, but rather that the sensory experience of individual sensilla is critical to the future development of the sensilla within the different fields. Thus, for olfactory sensilla, volatile stimulation alone is enough to maintain normal numbers; the terminal palp sensilla, used predominantly in close proximity to potential foods, need actual contact with suitable surfaces to elevate sensilla number; and the trichoid antennal sensilla, whilst structurally similar to those on the palp dome, have a mode of use that probably involves some sampling of volatile substances, and the number of these sensilla is affected, although not strongly, by the presence of wheat volatiles in the air. Under normal conditions, the detection of the wide range of nutritive and non-nutritive chemicals present in different plants is an integral part of food selection and feeding, and it appears that exposure to these chemicals and to a suitably complex environment may be an essential part of the normal development, and perhaps functioning, of the chemosensory systems.

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