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Behavioural correlates of phenotypic plasticity in mouthpart chemoreceptor numbers in locusts

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Abstract

Rearing locusts in an impoverished chemosensory environment leads to fewer chemoreceptors developing on the mouthparts and antennae as adults but the behavioural relevance of these changes remains unknown. To address this question, locusts were reared for the final two larval stadia on either a single, nutritionally near-optimal synthetic food ('plain' pretreatment), or a diet comprising two nutritionally complementary foods containing two added flavours ('mix' pretreatment). Insects reared on the 'mix' diet had a mean 20% more chemosensilla on the maxillary palps than those fed on the 'plain' diet. Following an equilibration period, when all newly moulted adults could feed on two nutritionally complementary foods, insects were food deprived for 2 or 6 h, and then given a test meal of a single balanced food at one of two dilutions whilst their behaviour was recorded. 'Mix'-pretreated locusts had a shorter latency to feed and were more likely to reject the test food upon first contact if deprived for only 2 h; but if they did take a meal it lasted longer and contained fewer pauses. Using sensilla number as a covariate removed the statistical significance of pretreatment regime, indicating that sensilla number, or some close correlate of it, can largely account for the variation in behaviour. This suggests that sensilla numbers are behaviourally relevant; particularly where locusts are not greatly food deprived and faced with marginally acceptable foods.

Keywords: *Locusta migratoria*; Sensilla; Taste; Gustation; Diet

1. Introduction

The significance of sensory experience to the development and functioning of sensory systems is now well known in both vertebrates (e.g. Rauschecker, 1995; Wang et al., 1995; Shatz, 1996; Berardi et al., 2000; Linkenhoker and Knudsen, 2002) and invertebrates (e.g. Murphey, 1986; Mimura, 1986, 1993; Volman and Camhi, 1988; Deruntz et al., 1994; Pflüger et al., 1994).

Several studies (Chapman and Lee, 1991; Rogers and Simpson, 1997; Bernays and Chapman, 1998) have reported changes in the number of chemosensory sensilla on the mouthparts and antennae of acridid grasshoppers with variation in the complexity of the chemosensory environment. Rogers and Simpson (1997) found that rearing *Locusta migratoria* L. for the last two nymphal stadia on nutritionally adequate but bland synthetic diets resulted in the development of fewer chemosensilla on the mouthparts and antennae relative to insects reared on plant foods. Enriching the chemosensory environment of insects reared on synthetic foods resulted in increased numbers of sensilla as adults. This enrichment was achieved either through the addition of allelochemical flavours or by forcing the insect to regulate its dietary intake by providing a choice of nutritionally unbalanced but complementary foods. Providing grass odour in the presence of a bland synthetic food resulted in a specific increase in the numbers of antennal olfactory sensilla but not gustatory sensilla, indicating a local effect of enrichment. Similar results were found by Bernays and Chapman (1998), who reared *Schistocerca americana* from hatching on plants or synthetic diets with and without added flavours and measured differences in antennal sensilla

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numbers. They found that the size of the effect on chemosensilla numbers varied with the type and location of sensilla on the antennae and the nature of the added compounds.

Although it is well established that chemosensory sensilla on the mouthparts and antennae of grasshoppers have an important role in the choice and regulation of food intake (Blaney and Chapman, 1970; Blaney and Duckett, 1975; Mordue (Luntz), 1979; Chapman, 1988, 1995) and that the numbers of chemosensilla that develop over larval life vary with chemosensory experience, it is not known whether differences in sensilla number have any behavioural consequences. Acridids possess high-density sensory fields consisting of up to several hundred chemosensory sensilla located on various mouthpart structures and the antennae (Thomas, 1966; Chapman, 1982). The dietary manipulations used by Rogers and Simpson (1997) and Bernays and Chapman (1998) resulted in local changes in sensilla numbers of 10–40%. Do such differences produce measurable effects on behaviour in a system that would appear to possess considerable redundancy (Chapman, 1988) compared to the chemosensory systems of other insects such as larval Lepidoptera, which achieve the same behavioural tasks as locusts using at most a few tens of chemosensilla (Schoonhoven and van Loon, 2002)?

Our aim in the present paper was to address this question. We generated differences in numbers of chemosensory sensilla on the maxillary palps of *L. migratoria* by rearing locusts for the final two nymphal stadia on either a plain or a chemically enriched diet. We show that there were marked differences in the feeding behaviour of locusts reared on these two diets. We then demonstrate that these differences in feeding behaviour can statistically be explained by the number of chemosensilla on the maxillary palps, both between and within dietary treatment groups. Our data therefore suggest that sensilla number and concomitant changes in chemosensory functioning have quantitative effects on feeding behaviour.

2. Materials and methods

2.1. Nymphal pretreatment regime

Nymphs of *L. migratoria* were obtained from the crowded stock culture maintained at the Department of Zoology, University of Oxford, reared on seedling wheat and wheat germ. Newly ecdysed fourth-instar nymphs (equal numbers of males and females) were selected over a staggered six-day period. Each insect was checked to ensure that all limbs, mouthparts and antennae were present and correctly formed. Locusts were placed in individual clear plastic boxes ($17 \times 12 \times 6$ cm) with an expanded aluminium perch, two 5.5-cm diameter Petri dishes containing artificial diet, and a plastic culture flask filled with de-ionised water. The experimental room was maintained at 29–31 °C under a 12:12 h light:dark regime. Food dishes were checked daily and replenished regularly. The insects were reared under these conditions throughout their fourth and fifth stadia until adult ecdysis (~16 days).

Locusts were assigned to one of two dietary regimes, 'mix' or 'plain', designed to generate differences in the number of chemosensilla on the maxillary palps. Locusts assigned to the 'mix' pretreatment were supplied with one food dish containing 35% protein (P) and 7% digestible carbohydrate (C) (35P:7C) flavoured with 1% amygdalin (Sigma Chemical Co.), and one dish containing 7P:35C flavoured with 1% tannic acid (Sigma Chemical Co.). Previous work has shown that locusts can detect both the chemical flavours at these concentrations but that they have no long-term effect on the amount of food eaten (Trumper and Simpson, 1994).

Locusts allocated to the 'plain' pretreatment were given two dishes of unflavoured diet containing 21P:21C. The latter is near optimal in nutrient composition (Chambers et al., 1995). These chemically defined synthetic foods were based on those of Simpson and Abisgold (1985) and further details may be found in Rogers and Simpson (1997).

2.2. Adult pretreatment regime

For the first two days after ecdysis, adults reared on both plain and mix pretreatments were allowed to standardise their nutritional state by self-regulating their intake of macronutrients, and also as an added precaution to ameliorate any direct affects of the allelochemicals in the diet of the 'mix'-pretreated insects (although there was no indication of any such effects). To achieve this, all adults were provided with two food dishes, one containing 35P:7C and the other 7P:35C neither of which contained allelochemicals.

2.3. Testing regime

On the third day after ecdysis, adult locusts were removed from their container at the end of a meal taken during ad libitum feeding and placed in individual $17 \times 12 \times 6$ cm clear plastic boxes inverted on the bench with their lids removed. These contained an expanded aluminium perch but no water bottle or food dish. Locusts were assigned to one of two food deprivation times (2 or 6 h). At the end of the allocated deprivation time, the box was gently lifted and a food dish containing either 7P:7C or 14P:14C was carefully slid underneath.

Table 1
Treatment combinations

Group	Pretreatment food	Deprivation (h)	Test food (P:C)	Replicates[a]
1	Mix	2	7:7	8 (3)
2	Mix	2	14:14	8 (6)
3	Mix	6	7:7	8 (7)
4	Mix	6	14:14	9 (9)
5	Plain	2	7:7	9 (8)
6	Plain	2	14:14	10 (10)
7	Plain	6	7:7	8 (8)
8	Plain	6	14:14	9 (9)

[a] Values in brackets indicate the number that took a full meal during the observation period; others ingested food but did not proceed to take a meal.

Once the test food was placed in the box, the behaviour of each locust was recorded onto a laptop computer every 15 s for 2 h, with observations of each insect in the group staggered by 1–2 s. Eighty-seven insects were observed (in groups of two to eight insects at a time), of which 69 ingested the test foods in the 2-h period and were thus included in analyses. A full breakdown of treatment combinations and numbers of replicates is provided in Table 1. After observations were complete, insects were labelled and stored in a freezer at −20 °C in readiness for histology.

2.4. Preparation of slides and sensilla counting

The maximum head width of each locust was measured using electronic callipers, prior to decapitation with a razor blade. The two maxillary palps were removed and placed in a dish containing 70% ethanol for 5 min, then soaked in 10% aqueous potassium hydroxide at 75 °C until transparent. They were next transferred to 100% ethanol for 5 min, washed in xylene for 5 min, rinsed once more in 100% ethanol for 5 min and cleared in eugenol. Finally, the palps were mounted on a microscope slide under a cover slip with Canada balsam.

The uniporous sensilla on the maxillary palp dome were examined and counted under a Zeiss microscope with *camera lucida* attachment at 400× magnification, as described in Rogers and Simpson (1997). The left and right palps of each locust were separately counted and the average was used in subsequent analyses.

2.5. Data analysis

The following behavioural variables were derived for each insect: (1) the latency to first period of committed ingestion, measured as the time from the start of the observation until the beginning of the first period of feeding lasting at least 15 s (i.e. two subsequent observation periods; see Simpson, 1995). The insect may well have contacted and rejected the food before finally commencing ingestion, but the sampling resolution was not sufficient to provide useful information on these brief events. (2) The percentage of insects that proceeded to take a meal after first ingesting the food. A meal was defined as a period of feeding exceeding 45 s, measured from the initiation of ingestion until the first continuous pause without ingestion exceeding 240 s (see Simpson, 1995). (3) The duration of the first meal. (4) The percentage time spent ingesting during the first meal, which typically consist of feeding bouts interspersed with short pauses.

Statistical analyses were conducted to assess the effects of pretreatment, deprivation time and treatment food on each of the behavioural variables, using general linear models in SPSS (release 11.0). If pretreatment was found to have a significant effect on feeding behaviour, either as a main effect or an interaction, the data were further analysed to assess more directly the contribution of sensilla number to these differences. Sequential mean square tests were used in all analyses such that interaction terms were considered after their main effects, to ensure that the models obeyed the principle of marginality. It was confirmed that all models met the parametric assumptions of homogeneity of variance, additivity, and normality of error.

3. Results

3.1. Effect of diet on sensilla number

The two pretreatment diets administered over the fourth and fifth nymphal stadia resulted in different numbers of chemosensilla on the mouthparts of adult locusts, with 'mix'-pretreated insects having on average 20% more sensilla than those pretreated on the 'plain' diet [Fig. 1; $F_{1,66} = 45.82$ ($P < 0.0005$)]. Sensilla number varied with insect size, as represented by head width [$F_{1,66} = 20.68$ ($P < 0.0005$)], but mean size did not differ between the two pretreatments [Fig. 1; $F_{1,67} = 0.009$ ($P = 0.923$)]. As reported in Rogers and Simpson (1997), there was no significant improvement in the model if the square of head width (proportional to area) was used instead of the linear dimension. Also, there was no significant effect of sex once size was accounted for, and thus sex was omitted as a factor from all subsequent analyses.

The significant effect of head width on sensilla number meant that the effect of size had to be compensated for in all subsequent analyses exploring the relationship between sensilla number and the behavioural variables. This was achieved by using the residuals from a regression of sensilla number against head width for all insects, henceforth termed 'residual sensilla number'. A residual sensilla number of greater than zero indicates more sensilla than average for that size of insect, while

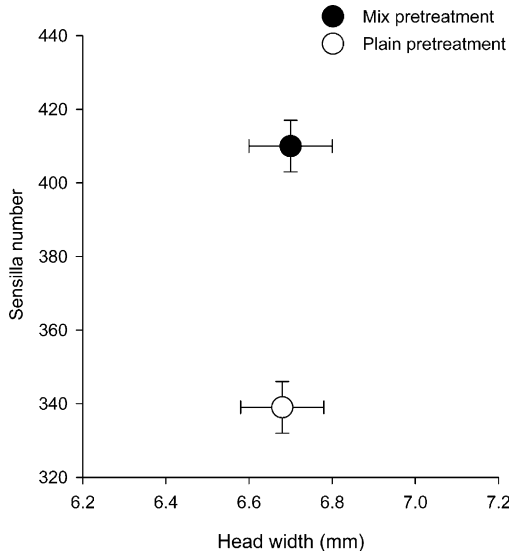

Fig. 1. Bicoordinate plots of means ± SEM head width versus numbers of maxillary palp dome chemosensilla on adult locusts that had been reared for the final two nymphal stadia on either a single, nutritious synthetic food ('plain' pretreated, open circle, $N = 36$), or one containing added flavours and nutritional variation ('mix' pretreated, filled circle, $N = 33$).

negative values mean that the locust possessed fewer sensilla than the average for its size.

3.2. Latency to the first period of sustained ingestion

Locusts that had been reared on the 'mix' pretreatment had a shorter latency to the start of feeding after being deprived for 2 h than did those reared on the 'plain' diet (Fig. 2). After 6 h of food deprivation, however, the latency to the start of feeding was shorter and similar in both pretreatment groups. Accordingly, there was a highly significant interaction between pretreatment and deprivation time [$F_{1,60} = 9.102$ ($P = 0.004$)]. There was no significant effect of treatment food (7P:7C vs. 14P:14C), either as a main effect [$F_{1,60} = 0.071$ ($P = 0.791$)] or as an interaction with deprivation [$F_{1,60} = 0.001$ ($P = 0.985$)] or pretreatment [$F_{1,60} = 1.155$ ($P = 0.287$)]. Nor was there a three-way interaction between pretreatment, deprivation and treatment food [$F_{1,60} = 0.721$ ($P = 0.399$)].

The next step was to establish to what extent the difference between 'mix' and 'plain' diet reared insects seen after 2 h food deprivation could be accounted for (or correlated with) differences in sensilla number, and whether pretreatment remained a significant factor when sensilla number was included in the analysis. When sensilla number was not included in an analysis of locusts experiencing 2 h deprivation, the effect of pretreatment diet was significant [$F_{1,31} = 7.12$ ($P = 0.012$)] as expected, accounting for 19% of the variance in latency to feed. When residual sensilla number was also included in the model, however, the effect of pretreatment rearing diet was no longer significant [$F_{1,30} = 1.782$ ($P = 0.192$)], but sensilla number was significant and accounted for 68% of the variance previously attributable to pretreatment [$F_{1,30} = 4.14$ ($P = 0.05$)]. The relationship between residual sensilla number and latency to feed is plotted in Fig. 3, which shows that the greater the number of sensilla, the shorter the latency to the start of feeding, both between and within pretreatment groups [Pearson's correlation coefficient = −0.451 ($P = 0.007$)].

3.3. Number of insects taking a full meal after the first period of sustained ingestion

The frequency of insects going on to take a full meal after beginning ingestion is plotted in Fig. 4. Locusts reared on the 'mix' pretreatment were more likely to reject the test food after initial sampling without embarking upon a full meal than were 'plain'-reared insects, especially after the shorter period of deprivation and when tested on 7P:7C.

3.4. Duration of the first meal

The duration of the first period of ingestion was significantly affected by pretreatment [$F_{1,50} = 6.818$ ($P = 0.012$)], but neither deprivation time nor test food was significant, either as a main effect or interaction term ($P > 0.162$ in all cases). Meals were consistently of longer duration in 'mix'-pretreated insects than in 'plain'-pretreated animals except when they were deprived for 6 h and fed 14P:14C (Fig. 5).

Accordingly, we next analysed the relationship between meal duration and residual sensilla number for 2-h deprived insects. When sensilla number was omitted from the analysis, the effect of pretreatment was significant [$F_{1,23} = 7.91$ ($P = 0.01$)], accounting for 26% of the variance in latency to feed. When residual sensilla number was included in the model, however, the effect of the pretreatment rearing diet was no longer significant [$F_{1,22} = 3.28$ ($P = 0.084$)]. Sensilla number, however, was significant [$F_{1,22} = 5.026$ ($P = 0.035$)], explaining 59% of the variance previously attributed to pretreatment. Thus, the difference in sensilla number that arises as a consequence of the pretreatment diets is a better predictor of the behavioural difference than the pretreatment diet per se. The relationship between residual sensilla number and the duration of the first meal is plotted in Fig. 6, which shows the positive correlation between residual sensilla number and meal duration both between and within pretreatments [Pearson's correlation coefficient = 0.565 ($P = 0.002$)].

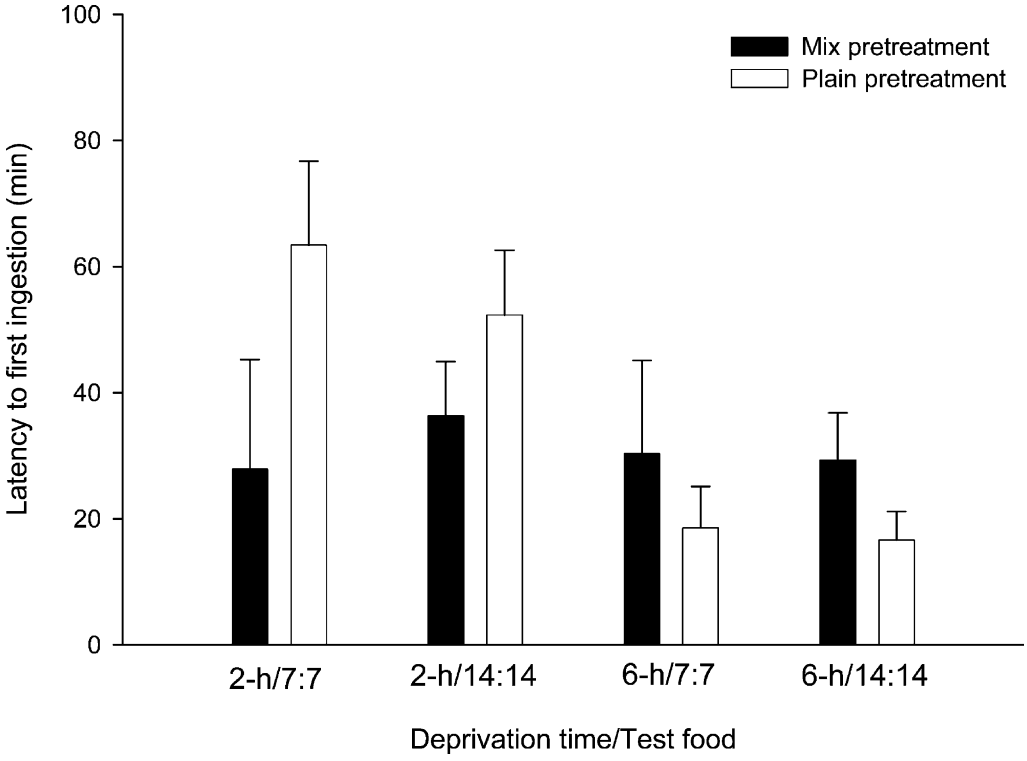

Fig. 2. Means ± SEM of the latency to the start of the first period of committed feeding in 'plain'- and 'mix'-pretreated locusts (white and black bars, respectively) that were deprived of food for either 2 or 6 h and tested on either a food containing 7% protein and 7% carbohydrate (P7:C7) or 14% protein and 14% carbohydrate (P14:C14). Numbers of replicates are listed in Table 1.

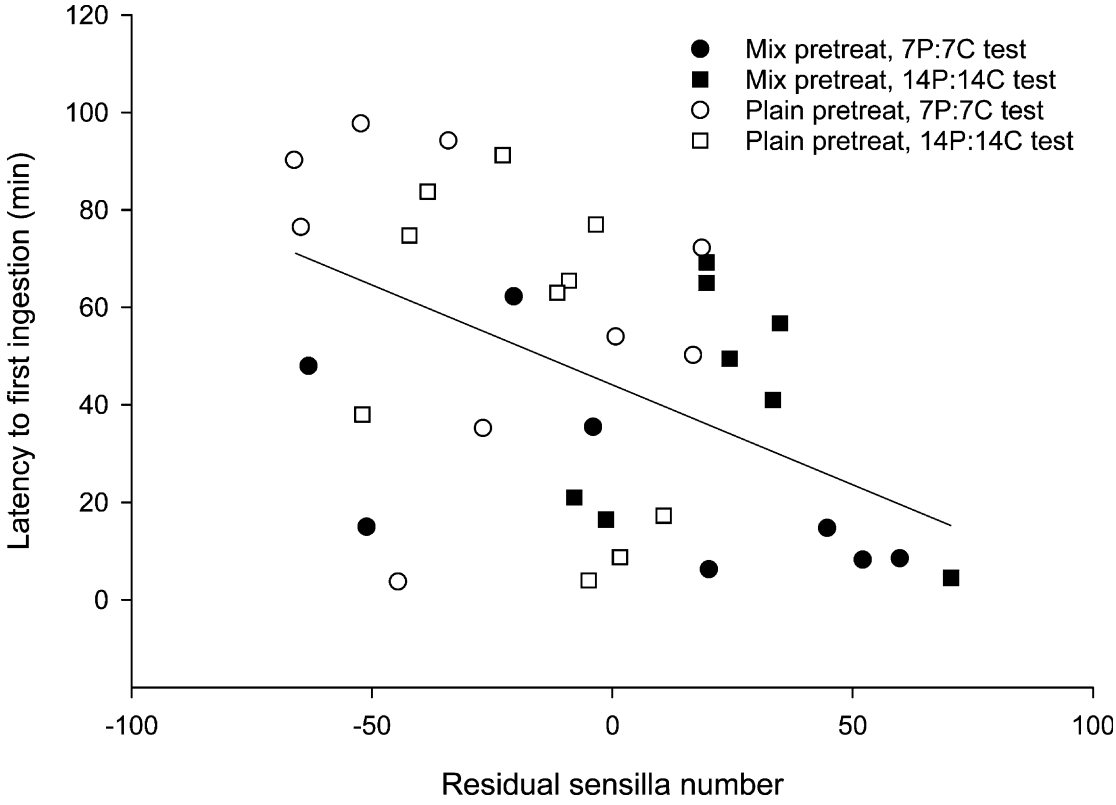

Fig. 3. Scatter plot of latency to the first period of ingestion versus residual (size-corrected) sensilla number for insects deprived of food for 2 h before testing. Filled symbols, 'mix'-pretreated locusts; open symbols, 'plain'-pretreated locusts; circles, tested on a food containing 7% protein and 7% carbohydrate; squares, tested on food containing 14% protein and 14% carbohydrate. The line is the fitted linear regression.

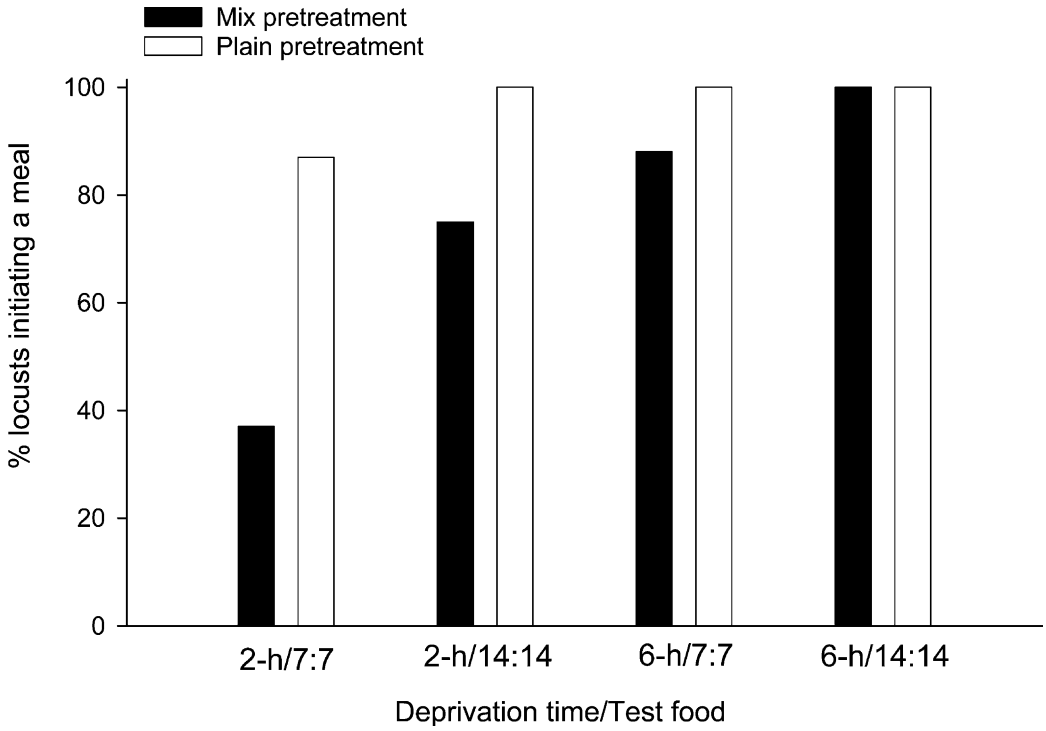

Fig. 4. The percentage of locusts that began feeding and went on to take a full meal. 'Plain'- and 'mix'-pretreated locusts (white and black bars, respectively) were deprived of food for either 2 or 6 h and tested on either a food containing 7% protein and 7% carbohydrate (P7:C7) or 14% protein and 14% carbohydrate (P14:C14). Numbers of replicates are listed in Table 1.

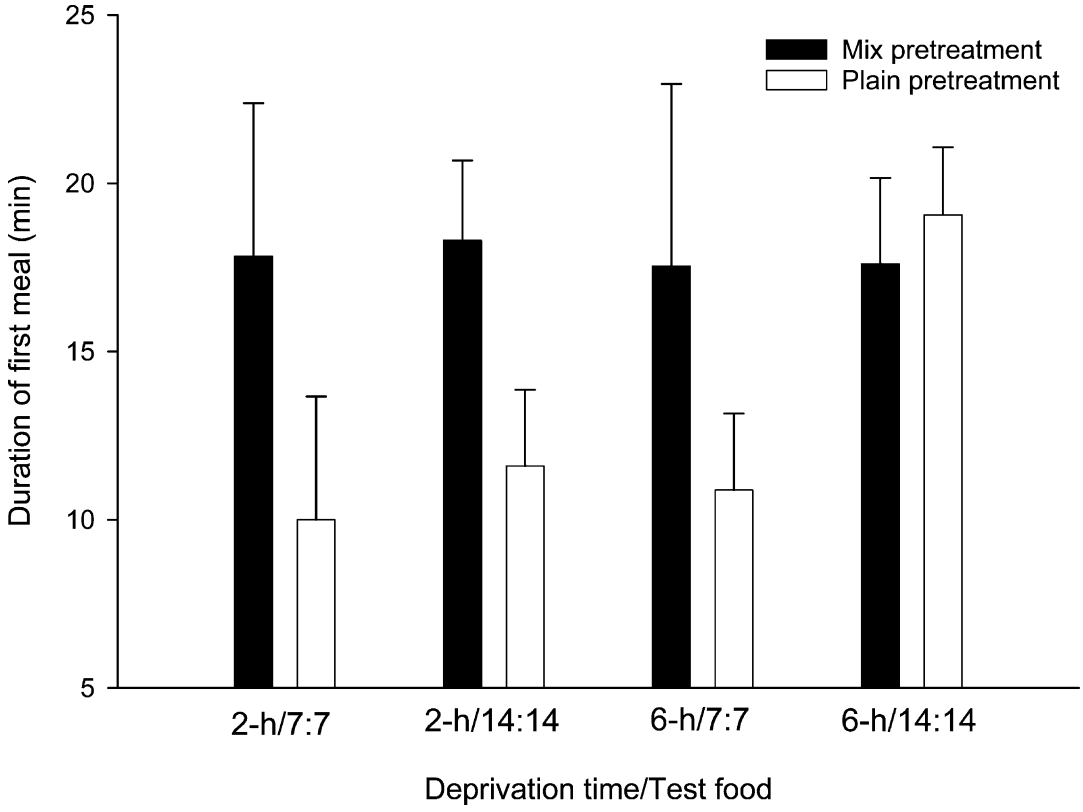

Fig. 5. Means ± SEM of the duration of the first meal in 'plain'- and 'mix'-pretreated locusts (white and black bars, respectively) that were deprived of food for either 2 or 6 h and tested on either a food containing 7% protein and 7% carbohydrate (P7:C7) or 14% protein and 14% carbohydrate (P14:C14). Numbers of replicates are listed in Table 1.

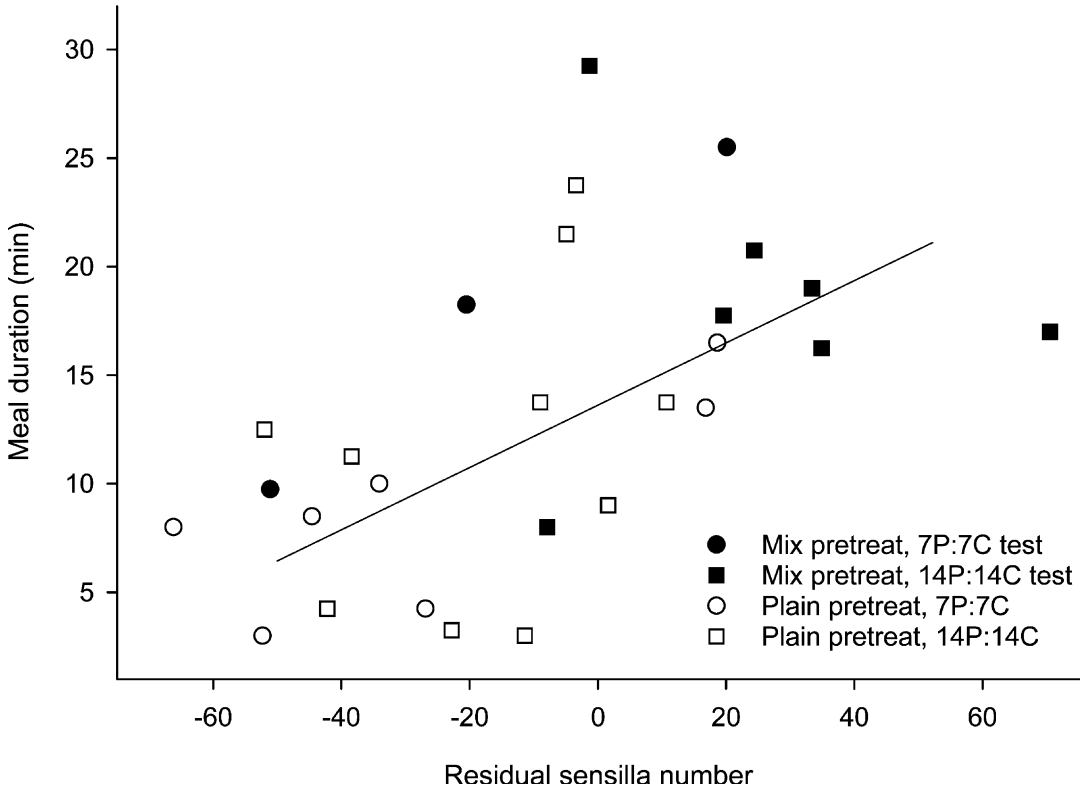

Fig. 6. Scatter plot of first meal duration versus residual (size-corrected) sensilla number for insects deprived for 2 h before testing. Filled symbols, 'mix'-pretreated locusts; open symbols, 'plain'-pretreated locusts; circles, tested on a food containing 7% protein and 7% carbohydrate; squares, tested on food containing 14% protein and 14% carbohydrate. The line is the fitted linear regression (see text).

3.5. *Percentage of time spent ingesting during the first meal*

When locusts feed, they typically take intra-meal pauses. The time spent pausing influences the rate at which food is ingested during the meal (Simpson, 1995). When the percentage of time spent ingesting during the first meal (arcsine transformed) was analysed, there were significant main effects of deprivation time [$F_{1,50} = 6.98$ ($P = 0.011$)] and test food [$F_{1,50} = 7.07$ ($P = 0.01$)]. There was also a significant interaction between pretreatment rearing diet and the test food [($F_{1,50} = 5.52$ ($P = 0.023$)]. This resulted from a difference between the 'mix' and 'plain'-pretreated insects in their response to diet 7P:7C, with the latter spending a lesser proportion of the meal feeding than the former (73% vs. 80%). The relationship between percentage time feeding and residual sensilla number is plotted in Fig. 7. Animals with residual sensilla numbers greater than −50 (Fig. 7) spent a high and constant proportion of the meal feeding (approximately 80%). Locusts with fewer sensilla than this threshold, however, showed a clear decline in percentage time spent ingesting during the meal with falling sensilla number [Pearson correlation, 0.561 ($P = 0.030$)]. Since the only insects with less than −50 residual sensilla were all 'plain' pretreated, these are the only insects to show a decline in percentage time spent ingesting with diminishing numbers of sensilla.

4. Discussion

There were pronounced behavioural differences, as well as differences in sensilla number, between adult locusts that were reared during their final two nymphal stadia on the 'plain' or the more chemically enriched 'mix' synthetic diets. 'Mix'-pretreated locusts had a shorter latency to first ingestion but were more likely to reject subsequently the test food without taking a full meal than 'plain'-pretreated locusts if they had been deprived for only 2 h. If 'mix'-pretreated locusts did take a meal, however, it lasted longer and contained fewer pauses. 'Mix'-pretreated locusts had 20% more sensilla on the maxillary palps than those insects reared on the 'plain' diet.

Our results suggest that locusts having larger numbers of maxillary palp chemosensilla showed greater responsiveness to nutrient taste stimuli both between the pretreatment groups and within them; the pretreatment would appear only to accentuate differences in sensilla number that naturally occur. Locusts with

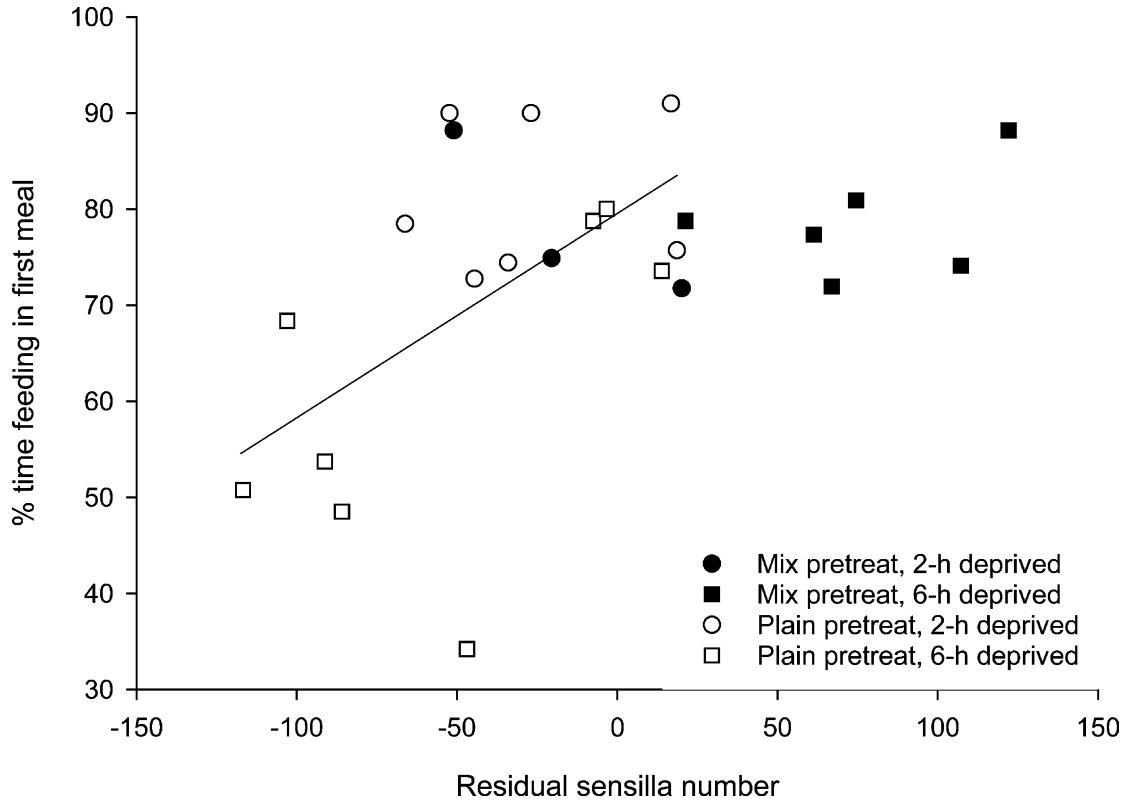

Fig. 7. Scatter plot of percentage time spent feeding rather than pausing during the first meal versus residual (size-corrected) sensilla number for insects tested on a food containing 7% protein and 7% carbohydrate. Filled symbols, 'mix'-pretreated locusts; open symbols, 'plain'-pretreated locusts; circles, deprived for 2 h before testing; squares, deprived for 6 h. The line is the fitted linear regression for 'plain'-pretreated insects (see text).

higher numbers of sensilla began feeding sooner, but were more ready to reject the marginal 7P:7C food, and having commenced a meal they paused less while feeding and ate for longer than insects with smaller numbers of sensilla. Thus, insects with more sensilla exhibit the properties of being more discerning in food choice and of receiving a greater excitatory chemosensory drive from the food, which promotes the continuation of feeding once it has started. These two roles of chemosensory sensilla, food selection and sensory drive, lead to the apparently counterintuitive result that mix-pretreated locusts are more likely to initiate feeding (increased excitatory input) but be subsequently more likely not to take a full meal on a marginal food (improved discrimination). These effects were noticeable when insects were deprived of food for 2 h, but not after 6 h of deprivation. Two hours is well within the range of free-feeding intermeal intervals demonstrated by most individuals (average about 60 min) (Simpson, 1995). It is therefore more representative of the timing at which feeding decisions are normally made than that at 6 h, by which time insects have reached their maximal response to deprivation. Meal size and ingestion rate on plant foods are known to increase with deprivation but have reached an asymptote after 6 h (Simpson et al., 1988), by which time differences due to sensilla number would most likely have been obscured. It would thus seem that differences in sensilla number are most behaviourally relevant under conditions where locusts are not greatly food deprived and are faced with marginally acceptable foods. It might be argued that the higher rejection rate in the 'mix'-pretreated insects might have been related to their shorter latency to commence ingestion. This cannot explain, however, why those locusts that did then proceed to take a meal fed with fewer pauses and for longer. Neither was there a significant relationship between latency to the start of feeding and the duration of the first feeding event [Pearson's correlation coefficient $= -0.155$ ($P = 0.373$) for 2 h deprived treatments].

The key issue is whether the behavioural differences reported here were the result of differences in numbers of chemosensilla, or some other tightly correlated effect of the pretreatment regimes. We have addressed this question by including sensilla numbers as a covariate in analyses of those behavioural variables that were significantly influenced by pretreatment. In each case, incorporation of residual sensilla number was itself statistically significant and removed the significance of pretreatment regime per se. In statistical terms, this indicates that residual (i.e. size-corrected) sensilla number, or something tightly correlated with it, can largely

account for the variation in behaviour attributable to pretreatment. This is analogous to the way that size explained the effect of sex on sensilla number. The sexes showed a highly significant difference in sensilla number, but when head width was included in the model as a covariate, the effect of sex disappeared.

Logically, then, either the behavioural effects of pretreatment regime were due to sensilla number, or to something (other than insect size) that was directly correlated with it. Although we only sampled the maxillary palp sensilla, matching changes in sensilla number are known to occur on the labial palps, labral sensilla fields and antennae, and these changes are therefore also expected to be tightly correlated to changes in maxillary sensilla (Rogers and Simpson, 1997). Our protocol was designed to minimise any possible collateral effects of nymphal pretreatment regime on adult behaviour. First, the nutritional compositions of the pretreatment diets sustained similar levels of growth, development and survivorship, as indicated by the lack of difference in adult size. Second, we chose the flavours and their concentrations in the mixed diet such that they had no measurable detrimental effect (Trumper and Simpson, 1994; Rogers and Simpson, 1997). Third, we standardised adult locusts before testing by placing them for two days with a choice of two nutritionally complementary foods lacking allelochemicals. Finally, we used two novel allelochemical-free test foods that differed in their nutritional composition to the pretreatment foods. Ultimately, a study of this kind can only indicate a correlative relationship between sensilla numbers and behaviour. It remains a small possibility, however, that changes in chemosensory or central physiology that run parallel to the differences in sensilla number could be the causal agents of the behavioural differences observed, although no such mechanism springs to mind.

If, as seems likely, the differences in behaviour measured in the experiment are directly attributable to sensilla numbers, the question arises as to how sensilla number affects behaviour. For example, is there an across the board reduction across all sensilla, or are specific sensilla types with similar chemosensory sensitivity profiles disproportionately reduced when locusts are reared in chemically impoverished environments? These questions require further investigation, and are related to the, as yet unknown, mechanism whereby chemosensory experience regulates sensilla number during post-embryonic development.

Our earlier experiments (Rogers and Simpson, 1997) suggested that the effect occurs locally within receptor fields, rather than involving a centrally co-ordinated mechanism. For example, if insects were reared in an environment perfused with grass odour but were unable to touch and taste the grass, antennal sensilla numbers were augmented, whereas palp contact chemosensilla stayed low in number. In the present study, even if the non-volatile flavours used in the 'mix' diet stimulate antennal olfactory receptors this will only serve to accentuate the total difference in overall chemosensilla numbers between the pretreatment groups.

Concomitant with the changes in sensilla number is a developmental change in the number of chemosensory afferents and possibly their connections onto central interneurones. Experimental alterations, such as ablation of sensilla (technically very difficult without damaging the soft palp dome) or covering part of the palp dome, would reduce the number of sensilla but would do nothing to the sensory neurones underlying them. The central nervous system would therefore be integrating a reduced amount of sensory information in a system that had developed with an increased sensory input. This situation strongly contrasts with locusts that have been reared under chemosensorily impoverished environments where sensory neurones fail to develop, leading to a reduced capacity for sensory processing rather than a sub-capacity reduction in total inputs such as would occur following ablation/covering.

What is known of the organisation of chemosensory afferent projections in the central nervous system would tend to support a prediction that more sensilla would lead to greater behavioural responsiveness. In the metathoracic ganglion of the locust sensory afferents from chemosensilla on the hind leg all project to the same tightly defined region of neuropil, defined by the somatotopic location of the sensillum on the leg rather than by modality or chemosensory specificity (Newland et al., 2000). Both mechanosensory and diverse chemosensory afferents make monosynaptic connections onto the same local interneurones (Newland, 1999; Rogers and Newland, 2002). There is also some evidence for considerable convergence of both gustatory and mechanosensory neurones from maxillary palp dome sensilla onto the same local interneurones within the sub-oesophageal ganglion (Rogers and Simpson, 1999; Rogers and Newland, 2003). It is very unlikely that the number of central neurones in the sub-oesophageal ganglion can increase post-embryonically (Shepherd and Bate, 1990). Therefore, if greater numbers of receptor neurones converge with the same individual output strength onto the same number of interneurones, it would be expected that behavioural responsiveness to gustatory (and mechanical) cues would be increased. Given the considerable convergence of chemosensory afferents onto a much smaller population of central neurones with very broad tuning, including mechanosensory sensitivity (Rogers and Newland, 2002), there are only limited opportunities for central remodelling. In the thoracic ganglia of locusts, contact chemosensory neurones effectively have

only one central somatotopically defined target. Plasticity can therefore be most readily accomplished through altering individual chemosensory neurone sensitivity, synaptic strengths or the total number of incoming neurones. We show that the latter occurs through changes in chemosensilla number and provide strong correlative evidence of its behavioural consequences, but this does not exclude the possibility of other types of sensory neurone plasticity.

Whilst experience-dependent differences in numbers of sensilla may influence relative behavioural sensitivity, this does not necessarily imply that the differences in sensilla number between stadia (Chapman and Thomas, 1978; Chapman, 1982), phenotypes (Greenwood and Chapman, 1984; Heifetz et al., 1994; Heifetz and Appelbaum, 1995), and species (Chapman and Fraser, 1989) necessarily follow the same trend of increasing chemosensitivity with increasing sensilla numbers. Clearly, other variables may compensate for differences in sensilla number. For example, the firing rate of gustatory receptors on the mouthparts of caterpillars, which possess fewer sensilla than grasshoppers (Chapman, 1982, 1995), seems to be higher than those reported in the literature for acridids (e.g. Schoonhoven and van Loon, 2002; Simpson and Simpson, 1992). In the thoracic ganglia of locusts, individual chemosensory action potentials elicit extremely small excitatory postsynaptic potentials in local interneurones (Newland, 1999); it is only the summated inputs from many different action potentials, both temporally and spatially (from different chemosensory neurones) that leads to substantial chemosensory mediated interneurone responses.

More is known in other modalities about how changing sensory input arising from differing numbers of sensory neurones reaching the central nervous system affect neuronal network properties. For example, the number of mechanosensory hairs on the cerci of orthopteroid insects increases dramatically over postembryonic development (>40-fold). Yet the output properties of postsynaptic interneurones and the escape response behaviours they elicit remain mostly unchanged throughout the lifetime of the insect (Chiba et al., 1992), although it has been suggested that there may be subtle changes in the signal-to-noise effectiveness of the system (Kämper and Murphey, 1994). Changes in several components of the network are required in order to scale the massive increase in the number of sensory neurones to a constant neuronal output. These are thought to include the mechanical properties of the sensilla hairs (Kämper, 1992); decreased sensitivity in individual sensory neurones; postsynaptic interneurone growth decreasing synaptic efficiency (Kämper and Murphey, 1994), and competition between afferents for limited space on their postsynaptic targets (Bacon and Blagburn, 1992). Clearly, similar, though as yet unknown, regulatory systems may be acting in the chemosensory system of the locust. The maxillary palps and other chemosensory fields differ functionally in an important respect from the cerci in that an escape response is essentially the same regardless of the size of the insect, but the food intake of an insect increases with size. If overall phagostimulatory input is important in regulating food intake, then the chemosensory system need not be so tightly regulated to the same strength of neuronal output, indeed to do so may serve to limit food intake in larger insects.

It would seem that the insects in the present study with higher numbers of maxillary chemosensilla fed with greater efficiency than those with fewer sensilla: they start feeding sooner, were more discriminating on the marginal food, and once committed to feeding, paused less and ate for longer. But increased sensitivity to stimuli need not always be beneficial. Bernays (1998) found that grasshoppers reared from hatching on a diet of six differently flavoured synthetic foods fed less efficiently than similar insects reared on nutritionally identical foods that contained only one of the flavours. The dietary regimes and allelochemical flavours used were the same as in Bernays and Chapman's (1998) study on antennal chemoreceptors. Some single allelochemicals (e.g. salicin) influenced sensilla numbers to a greater degree than others (e.g. citral and coumarin), but in general, grasshoppers reared on a single flavour had fewer antennal chemosensilla than those reared on the mix of six flavours.

Bernays has developed the 'neural hypothesis of diet breadth' based around the idea that increased complexity of sensory responses, although necessary to animals with a broad dietary range, serves to impede behavioural decision-making. Conversely, animals that can use simple or exaggerated sensory cues (e.g. dietary specialists) have a much simpler computational task to perform (Bernays and Wcislo, 1994; Bernays, 1996, 1998). The key word here is 'complexity' of sensory input, as distinct from the amount of any one type of input. When more sensilla provide more of the same input, then the expectation is that behavioural responses will be sensitised but not broadened. When more sensilla provide a wider range of types of input, then behavioural decision-making may indeed be impeded, provided that the different types of inputs are resolved within the CNS and not simply converged. The implication of Bernays' (1998) data is that grasshoppers reared on mixed flavours had developed qualitatively different responsiveness to chemical stimuli, as well as having quantitative differences (more sensilla). It is known in caterpillars that dietary experience influences the responsiveness of chemoreceptors to specific allelochemical cues (del Campo et al., 2001; Renwick, 2001; Schoonhoven and van Loon, 2002), and the same may

well also be true for acridids. Hence, were we to have tested our locusts on foods containing allelochemicals, rather than the unflavoured foods, we may have found a different outcome. Nevertheless, our data indicate that the complexity of the chemical environment influences the numbers of chemosensilla and that this in turn has detectable effects on behaviour. The next question to ask is whether the responses of individual sensilla of locusts reared under different chemosensory conditions have also been changed, either quantitatively or qualitatively.

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