Phenotypic plasticity in numbers of antennal chemoreceptors in a grasshopper: effects of food

Abstract Grasshoppers, Schistocerca americana, reared from hatching on artificial diet had fewer sensilla on the antennae in the final larval stage than insects reared on lettuce. This was true of basiconic and coeloconic sensilla (presumed olfactory) and trichoid sensilla (presumed gustatory). The degree of difference varied along the antenna and with sensillum type. Adding salicin to the diet restored the numbers of all types of sensillum to levels equal to, or approaching, those in lettuce-fed insects. The addition of some volatile compounds – carvone (monoterpene), chalcone (flavonoid), citral (monoterpeno) and guaiacol (phenolic) – resulted in slight increases in number, but coumarin (phenylpropanoid) had no effect. None of the compounds, either singly or in combination, produced more sensilla than were present in plant-fed insects.

Key words Phenotypic plasticity · Chemoreceptors · Antenna · Grasshopper · Salicin

Introduction

Previous studies have established that phenotypic variation in numbers of antennal sensilla occurs in a number of acridid species primarily in relation to the degree of crowding of the insects (Greenwood and Chapman 1984; Heifetz et al. 1994; Heifetz and Appelbaum 1995). In an earlier study it was shown that some variation occurred in Schistocerca americana in relation to the complexity of the odor or food to which the insects were subjected, but the effects were slight (Chapman and Lee 1991). More recently, Rogers and Simpson (1997) observed that feeding Locusta migratoria on artificial diet produced a more marked reduction in antennal sensillum numbers compared with plant-fed insects. Here, we report a similar effect in S. americana and examine the effects of adding various chemicals to the diet with a view to obtaining some insight into the mechanisms involved in regulating sensillum numbers.

The antennae of acridids bear five types of sensillum: large and small basiconic sensilla, two types of coeloconic sensilla, and trichoid sensilla. Most coeloconic sensilla have an olfactory function, but a few, about 4% in L. migratoria (Altner et al. 1981), are hygroreceptors. Both types of basiconic sensilla are multiporous (Ameismeier 1987) with a presumed olfactory function. The trichoid sensilla are uniporous and are presumed to be contact chemoreceptors.

Our specific objectives were to determine (1) how the numbers of different types of sensilla are affected by feeding the insects on artificial diet, as opposed to green plant, (2) how the numbers are affected by the addition of different plant compounds, some of which are volatile, others not, to the artificial diet, and (3) if changes in abundance of the different types of sensilla are correlated, suggesting the possibility of a common regulatory mechanism.

Materials and methods

The insects used in the experiments were obtained from a stock culture of S. americana normally reared in crowds on Romaine lettuce and wheat bran. For the experiments, grasshoppers were reared from hatching until the 3rd or 4th day of the sixth larval stadium (approximately 1 month) on lettuce or complete artificial diets that contained all essential nutrients in suitable proportions, including 14% carbohydrate, and 14% protein on a dry weight basis (Abisgold and Simpson, 1987). In addition, rutin, a flavonoid glycoside that stimulates feeding on these diets was included at 0.5% dry weight (Bernays and Raubenheimer 1991). The dry diets were made into moist cakes with 1% agar and kept in the refrigerator for up to a week. Small plastic dishes were filled with the moist diet and presented to the insects fresh daily. Insects fed lettuce were given fresh leaf pieces daily.
The early larval stages were maintained in cylindrical Perspex cages (10 cm diameter and 25 cm high) with screen tops. Cages contained a strip of wire mesh to ensure suitable roosts for resting and molting. Twenty neonates of unknown sex were placed in each cage. There were seven treatments besides those given plain artificial diet or lettuce. In six of these seven treatments, a single plant chemical was added to the artificial diet. In the seventh treatment, the insects were presented with six dishes of artificial diet each laced with a different chemical. This is called the mixed treatment.

The chemicals added to the diets were:
1. Guaiacol, a phenolic compound which is probably widely distributed in plants (Gibbs 1974) and which is often a major component of the volatile compounds present in the feces of both *L. migratoria* and *Schistocerca gregaria*. It is a component of the aggregation pheromone in these species (Fuzeau-Braesch et al. 1988; Toro et al. 1994).
2. Salicin, a phenolic glycoside probably restricted to the Salicaceae and a few other plant taxa.
3. Coumarin, a phenylpropanoid widely distributed in plants.
4. Chalcone, is representative of the flavonoid group which is quite widely distributed in plants although chalcone itself probably does not occur naturally (Gibbs 1974).
5. Citral is a monoterpen compound found in a number of plant families.
6. Carvone is another monoterpen found in Lamiaceae and representatives of a few other families.

Pilot studies were carried out to determine the appropriate concentrations of the compounds. Insects were provided with a choice of two food dishes: one of plain diet and one containing a particular concentration of the compound being tested. Each compound was tested at a range of concentrations (five to eight) deemed relevant from their effects on feeding in a related species of grasshopper (Chapman and Bernays 1977). Usually the higher concentrations deterred feeding while the lower concentrations stimulated feeding, so the concentrations selected were those intermediate ones that resulted in no measurable effect on food intake — that is the test and plain diets were eaten in similar amounts (data not shown). The concentrations used (as percentage dry weight of diet) were: carvone 0.05%, chalcone 0.2%, citral 0.05%, coumarin 0.1%, guaiacol 0.1%, and salicin 0.25%. The chemicals were added to the diet at the time of mixing the dry diet with agar and the mixture stirred until gelling began.

As individuals molted to the fourth stadium they were moved to larger cages; plastic 101-tubs with screen sections in the lids. The cylinders and tubs were kept in a rearing room at an air temperature of 23–25 °C, with fresh air turnover of 20 room changes per hour. They were arranged around 100-W tungsten lamps so that during photophase (LD 12 h:12 h) individual insects could regulate their body temperatures behaviorally.

When insects reached the sixth stadium they were weighed and sexed. The left antennae were removed and placed in 5% KOH at room temperature until the internal tissues had been cleared but the cuticular structures still showed different degrees of transparency so that the main sensillum types were readily distinguished. The antennae were then dehydrated in ethanol and mounted in polyvinyl alcohol mounting medium (BioQuip).

Counts and counts were made with a light microscope using an eyepiece scale for antennal and annulus lengths and an eyepiece grid for counts of sensilla. All counts were done at x40 magnification. It was not always possible to differentiate large and small basiconic sensilla and their numbers are pooled, but their relative proportions were assessed on some preparations. The two types of coeloconic sensilla cannot be distinguished under the light microscope.

Antennal annuli were numbered from distal to proximal. There is some variation in the number of annuli in any one developmental stage and, because new annuli are added basally, numbering from the tip ensures homology between the more distal annuli of different individuals (Chapman and Greenwood 1986). Most sensilla occur on the 15 most distal annuli; more proximally the numbers of olfactory sensilla are very low. For this reason, counting was limited to the 16 distal annuli. Counts were made only on female insects with intact antennae, resulting in six to eight insects from each treatment.

One-way ANOVA was used to look for significant effects of treatment (different diets) on the numbers of basiconic, coeloconic and trichoid sensilla in different regions of the antennae. To simplify the presentation we have considered the total numbers of each type of sensillum in different regions of the antennal flagellum rather than the numbers on individual annuli. The numbers of basiconic and coeloconic sensilla on annuli 1 and 2 do not change during development (R.F. Chapman, unpublished observations) and we consider them together as a unit. The next three annuli (3–5) do not divide after hatching although the numbers of sensilla do increase; these we also consider as a unit. The more proximal annuli divide and show periodic increases in sensillum number (R.F. Chapman, unpublished observations). Annuli 6–10, which have large numbers of both types of olfactory sensilla, are treated as a third unit, and annuli 11–16, through which the numbers of olfactory sensilla decline sharply, as a fourth. Because trichoid sensilla increase on all annuli after hatching, the numbers on annuli 1–5 are treated together for purposes of analysis.

Where relevant, post hoc Bonferroni tests were used to identify significant differences among treatments. Regression analysis was used to detect relationships among numbers of different sensillum types. Since size can influence sensillum numbers, insect weights, antennal lengths, numbers of annuli, and lengths of the individual annuli were compared across treatments by one-way ANOVA.

**Results**

**Effects of diet**

The antennae of insects reared on plain artificial diet (no added chemicals) had fewer sensilla of all types compared with those of insects reared on lettuce, but the effects varied with sensillum type along the length of the antenna (Fig. 1). The numbers of basiconic and coeloconic sensilla on the first two annuli were not affected by treatment and were similar to those on first stage larvae (R.F. Chapman, unpublished observations). There were fewer basiconic sensilla along the rest of the antenna in insects reared on plain diet, but the greatest reduction was on annuli 6–10 where the diet-reared insects had less than 60% of the sensilla of plant-reared insects. More distally and more proximally, the figures were close to 70% and 65%, respectively. In contrast, there were no differences in numbers of coeloconic sensilla in the distal and proximal regions, but insects reared on plain diet had only 75% of those on plant-reared insects on annuli 6–10. Finally, the numbers of trichoid sensilla only differed on the proximal annuli (11–16) where diet-reared insects had only 75% of the sensilla of plant-reared insects.

The addition of single chemicals to the artificial diet had different effects on the three sensillum types. The number of basiconic sensilla on annuli 3–5 was restored to the level of plant-reared insects by all the chemicals except coumarin (Fig. 2), but only carvone and salicin had a partial restorative effect (the numbers were significantly different from those on both plant-reared and plain-diet-reared insects) over annuli 6–10, while salicin and guaiacol had at least partial restorative effects on the number on the proximal annuli (no significant difference from plant-reared or plain-diet-reared insects).