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AN INVENTORY OF TASTE IN CATERPILLARS: EACH SPECIES ITS OWN KEY*

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Food plant recognition in lepidopterous larvae is predominantly governed by the activity of eight taste neurones present in two sensilla styloconica located on each maxilla. This paper reviews the results of electrophysiological and behavioural studies made on various caterpillar species during the last 40 years. It appears that all species, even closely related ones, have different taste systems. Taste cells responding to general phagostimulants (e.g., carbohydrates) have been found in all species studied. In some species, highly specialized taste cells have been found that respond to plant taxon-specific secondary plant substances that act as 'token' stimuli for plant recognition. Taste cells responding to many different secondary plant substances occur in most species studied. Their activity deters feeding. Though the response profile of these taste cells is best described as generalized, they nevertheless show species-specific stimulus spectra. These generalist deterrent cells often play a crucial role in feeding behaviour, a conclusion which confirms JERMY's (1966) earlier inference on the preponderant role of inhibitory secondary plant substances in food-plant selection by herbivorous insects. The two most frequently studied neural coding mechanisms, 'labelled lines' and 'across-fibre patterning' have been inferred to operate in caterpillars. The first type is a likely coding mode in oligophagous species employing token stimulus receptors, whereas 'across-fibre patterning' most probably operates in all species confronted with choices between plant food of varying quality. The responses of each of the taste cell types to their specific stimuli may be modified by the presence of other plant constituents, indicating that a complex stimulus (plant sap) evokes a response that is unpredictable from knowledge of responses to single compounds. Variability in taste cell responsiveness is dependent on developmental stage, time of day, and feeding history. This indicates that caterpillar taste cells are not rigid systems, and even possess a 'peripheral memory'.

Key words: sensilla styloconica, chemoreception, Lepidoptera, electrophysiology

INTRODUCTION

A rich variety of green plants on this planet presents an abundant food source to herbivores. Myriads of insects have since their origin taken their share of it and, by doing so, probably contributed to the development of the unsurpassed chemical diversity hidden in the Plant Kingdom. Insects and plants, therefore, are more

* This paper has been prepared as a tribute to Dr. TIBOR JERMY, an esteemed scientist and dear friend, who, under many adverse circumstances, has maintained his scientific integrity and originality and has made a seminal and lasting contribution to the field of insect–plant biology.

firmly interwoven than has been thought for a long time. A conspicuous feature of herbivorous insects is a preponderance of food specialization in this group. Lepidoptera, comprising about 10 per cent of all animal species, form a striking illustration, since they are generally finicky eaters. The great majority of lepidopterous larvae are specialists with regard to the kind of food they accept: only one plant species, or a few species belonging either to the same genus (monophagy), or to the same family (oligophagy). Even generalist species are selective in their food choice and do show preferences for some plants over others.

Diet specialization is a fundamental aspect of an animal's biology and has at the same time far-reaching ecological implications. To properly value the ecological impact of food selection behaviour and its evolutionary significance, it is helpful, if not a dire necessity, to understand the underlying mechanism of this behaviour.

A plant's chemical composition is in many cases the most important source of information which herbivorous insects use to discriminate between host and non-host plants. Lepidopterous larvae have been known for a long time to use this kind of information (VERSCHAFFELT 1910). Caterpillars are in many respects also ideal insects to unravel some principles which govern food selection behaviour. Their size and amenability make them quite suitable for behavioural studies. Their sense of taste, an essential faculty in food recognition, is by a stroke of luck singularly simple and easily accessible to experimentation. Based on unique temporal patterns of firing it appeared possible to reliably discriminate between different taste cells in one and the same sensillum (PETERSON *et al.* 1993; GLENDINNING & HILLS 1997). Neurophysiological recordings from intact animals allow long-lasting experiments on individuals, which subsequently may be used for behavioural tests (GOTHILF & HANSON 1994). These characteristics have contributed to the fact that many efforts to elucidate the role of taste in herbivorous insects have employed caterpillars.

The aims of this paper are (1) to present an overview of our present knowledge of taste receptors in caterpillars, (2) to explain interspecific differences in their taste system in relation to food-plant preferences, (3) to discuss the concept of sensory codes which direct food selection behaviour, and (4) to indicate some avenues which may be pursued in order to obtain a full answer to the question why a caterpillar accepts certain plants while rejecting others.

After a brief discussion of the structure and function of all taste receptors in caterpillars this paper will focus on the responses of the two maxillary sensilla styloconica to a spectrum of chemicals known to occur in plants. The chemosensory properties of these sensilla will be discussed in relation to food-plant recognition.

THE SENSE OF TASTE: MORPHOLOGY AND FINE STRUCTURE

The sense of taste in lepidopterous larvae is located in sensilla on the maxillae and epipharynx (DETHIER 1937) and possibly in some receptors in the hypopharynx and deeper portions of the buccal cavity (KENT & HILDEBRAND 1987). Each maxilla has two lobes arising from the basal segment (palpiger). The medial lobe is the galea and the lateral three-segmented lobe is the maxillary palpus (Fig. 1).

Galea. Each galea bears two elongated blunt protuberances which each support a uniporous peg, commonly referred to as the medial and lateral sensilla styloconica (Fig. 2). Light microscopic and electronmicroscopic examination showed that each sensillum styloconicum is innervated by five bipolar neurones, one of which functions as a mechanoreceptor. The dendrites of the four remaining chemoreceptor cells extend into the lumen of the peg, and terminate at a short distance from a minute pore in the tip of the peg (SCHOONHOVEN & DETHIER 1966, DEVITT & SMITH 1982). A detailed description of the internal structure of the sensilla styloconica of *Mamestra configurata* is given by SHIELDS (1994). There is no evidence of close contacts or tight junctions between styloconic chemoreceptor cells, as has been observed in tarsal sensilla of adult cabbage root flies (ISIDORO *et al.* 1994).

In a beautifully illustrated report GRIMES and NEUNZIG (1986a) concluded from a comparative study on 41 species that on the outside both sensilla styloconica vary little among species and appear to be the most conservative structures among all (eight) sensilla on each galea.

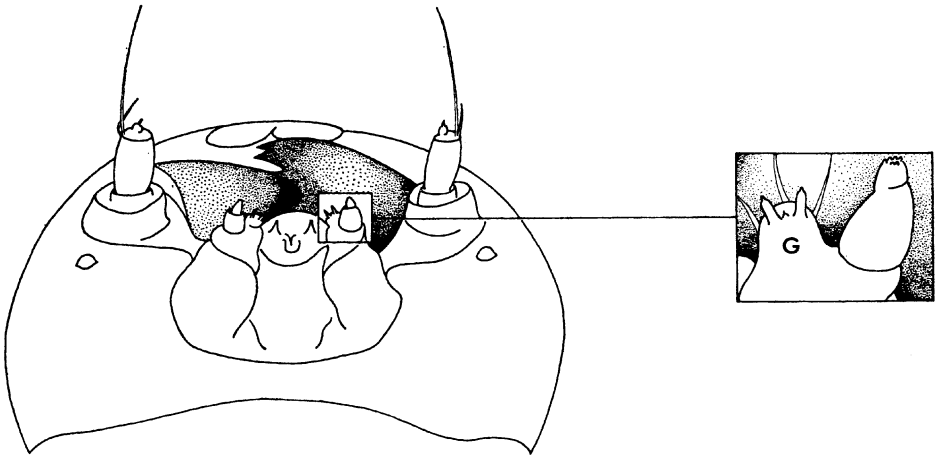

Fig. 1. Ventral view of a caterpillar head. G = galea, bearing two sensilla styloconica

Maxillary palpus. The third (distal) segment of the palpus bears a group of eight terminal sensilla basiconica, each innervated by 3–4 chemoreceptor neurones (SCHOONHOVEN & DETHIER 1966, DEVITT & SMITH 1982). Three of them have a grainy or pitted appearance, presumably due to the presence of pits which are entrances to underlying pores. It is speculated that these sensilla have an olfactory function, whereas the shape and smoothness of the remaining five uniporous sensilla indicate a role in contact chemoreception. In a more detailed study based on scanning electronmicroscopic images GRIMES and NEUNZIG (1986*b*) discern three different types of sensilla basiconica. They suggest that morphological differences observed between exophagous and endophagous species reflect functional differences with respect to taste and olfaction.

Epipharynx. One pair of dome-shaped epipharyngeal sensilla may be located on the epipharynx, the inner surface of the labrum. They have been described in larvae of *Bombyx mori* (GRANDI 1922, 1923), *Pieris brassicae* and *Manduca sexta* (MA 1972), *Malacosoma americana* (DETHIER 1975), and *Choristoneura fumiferana* (ALBERT 1980). Each sensillum is served by three neurones. These sensilla are not universally present in caterpillars, because they have been reported to be absent in *Mamestra brassicae* (BLOM 1978) and *Euxoa messoria* (DEVITT & SMITH 1982). The neurons innervating the epipharyngeal sensilla project on the frontal ganglion (MA 1972), tritocerebrum (MA 1976*a*, DE BOER *et al.* 1977) and suboesophageal ganglion (KENT & HILDEBRAND 1987).

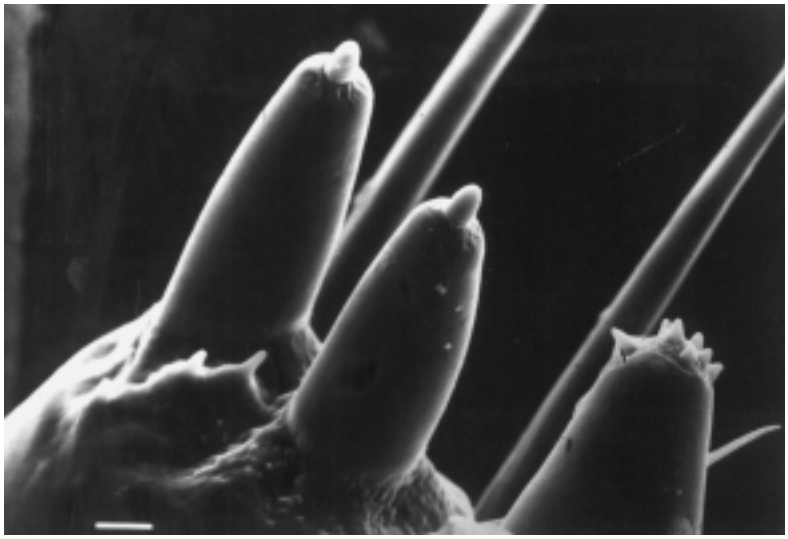

Fig. 2. Scanning electron micrograph of sensilla styloconica of *Spodoptera littoralis*. Scale = 10 μM (courtesy of W.M. BLANEY)

THE SENSE OF TASTE: BEHAVIOUR AND ELECTROPHYSIOLOGY

Physiological information on taste receptors located on the maxillary palps and epipharynx is pathetically limited.

Maxillary palpus. Behavioural experiments on *Lymantria dispar* (DETHIER 1937) and *B. mori* (ISHIKAWA *et al.* 1969) after removal of their maxillary palpi, provided evidence for the assumption that these appendages harbour olfactory receptors. This conclusion was confirmed unequivocally by recordings of increased neural activity in the palpi of *Manduca sexta* and *Hyalophora gloveri* in response to various plant odours (SCHOONHOVEN & DETHIER 1966, DETHIER & SCHOONHOVEN 1969). Similar results were obtained in *B. mori* (ISHIKAWA *et al.* 1969). Additionally, a gustatory role of some palpal sensilla could be inferred from the fact that topical application of some solutions caused changes in feeding behaviour (DETHIER 1937). Moreover, stimulation of the palpus with plant saps or dissolved chemicals elicited electrophysiological responses in *B. mori* (ISHIKAWA *et al.* 1969). More recently, GLENDINNING *et al.* (1998) published a detailed investigation of the responses from palpal chemoreceptors to compounds which deter feeding in *Manduca sexta* larvae.

Epipharynx. Early experiments in which the maxillae were surgically removed led to the unexpected observation that amputation of these appendages stimulated silkworms to eat plants that are normally rejected (TORII & MORII 1948). However, the fact that *Manduca sexta* caterpillars deprived of their maxillae still could discriminate between some host and non-host plants suggested the involvement of some hitherto unknown chemoreceptors in food selection behaviour (WALDBAUER & FRAENKEL 1961). When both maxillae as well as the antennae, bearing olfactory receptors, were extirpated, the ability to discriminate was still not completely lost (SCHOONHOVEN & DETHIER 1966). Therefore, it was hypothesized that the oral cavity also contains taste receptors. Only when, together with the maxillae and antennae, the labrum was removed, the loss of host discrimination appeared to be complete (DE BOER & HANSON 1987).

A taste function of the epipharyngeal sensilla has been proved by applying electrophysiological techniques in some taxonomically unrelated insect species. Thus, neural responses from these organs were obtained in *Pieris brassicae* (MA 1972), *Spodoptera exempta* (MA 1976a), *Manduca sexta* (DE BOER *et al.* 1977, GLENDINNING *et al.* 1999*b*, GLENDINNING *et al.* 2000), *Choristoneura fumiferana* (ALBERT 1980), *Bombyx mori*, and *Antheraea yamamai* (ASAOKA & AKAI 1991, ASAOKA & SHIBUYA 1995, ASAOKA 2000). All studies yielded evidence for the presence of three different taste cells, which were found to respond to deterrents and salts, and occasionally to other compounds as well.

SENSILLA STYLOCONICA: THE FUNCTIONS OF 8 TASTE CELLS

The two sensilla styloconica on each maxilla undoubtedly play a decisive role in hostplant selection behaviour. Therefore, though they contain only eight (paired) cells out of a total of approximately 59 (paired) chemoreceptory cells present in caterpillars (SCHOONHOVEN & DETHIER 1966), the function of these two sensilla has received more interest than all other chemoreceptors together. The main goal of most investigators has been to understand the sensory message which arises in these sensilla upon contact with a plant and which wholly or largely determines the insect's subsequent feeding response. To this end early students in this field recorded neural responses to saps from acceptable or unacceptable plants. To their disappointment they found these recordings, often showing impulses from all four cells, to be complex and too difficult to analyse to allow an in-depth analysis. Though different plant saps clearly evoked different impulse patterns, the character of the overall pattern of responses bore no orderly relationship to the acceptability or non-acceptability of the plants (ISHIKAWA 1966, SCHOONHOVEN & DETHIER 1966). Clearly, some detailed knowledge of the specificity spectra of all eight cells is needed before responses to complex stimuli, involving several receptors, may be understood. Thus, in the past decades most studies have concentrated on the identification of specific cells and their sensitivity to pure compounds or binary mixtures. Studies on responses to plant saps have been relatively rare (but see, e.g., SIMMONDS & BLANEY 1991).

Even the determination of the specificity ranges of individual cells has been found to be more difficult than anticipated. The analysis of a receptor's specificity range is impeded by the fact that many factors may affect a receptor's quantitative responsiveness. Factors that may exert profound effects on receptor sensitivity include larval stadium (e.g., differences between 4th and 6th instar: PANZUTO & ALBERT 1997, 1998), developmental stage (e.g., receptors of mid-instar larvae showing maximal responses: SIMMONDS *et al.* 1991), time of day (SCHOONHOVEN *et al.* 1991), nutritional status, and experience. Populations of different origin may also show qualitative and quantitative differences in responsiveness (e.g., WIECZOREK 1976). On top of that one usually encounters, also under fully standardized conditions, considerable interindividual variation, as well as marked intra-individual (i.e. measurements on the same individual) variation (e.g., SCHOONHOVEN 1976, SIMMONDS & BLANEY 1991, FRAZIER 1992, MENKEN & ROESSINGH 1998). Last but not least inhibitory or synergistic interactions commonly transmute receptor responsiveness when it is exposed to two (or more) compounds simultaneously. Despite this multitude of receptor modulating factors our knowledge of stimulus specificity and sensitivity of many taste cells has made ample

progress and provided fascinating new insights into their role of the process of food recognition, as will be shown in the following.

SPECIFICITY RANGES OF STYLOCONIC TASTE CELLS

In the course of years a typology of styloconic taste cells has been made for over 20 caterpillar species, inviting a comparison of the results (Table 1). It should be kept in mind, however, that different authors have often tested different sets of chemicals, thereby reducing the comparability of their results. There is also little uniformity in cell labelling. To establish whether or not two compounds stimulate one and the same cell, it is necessary to test them as a mixture. This has not been done for all possible combinations of stimulants on which Table 1 is based, and therefore some uncertainties remain.

Evidently the characterization of most taste cells is only partially complete. Even the taste spectra of some of the most thoroughly investigated species, e.g. *Pieris brassicae* and *Manduca sexta*, cannot be considered to be fully known. In spite of such gaps Table 1 shows some clear patterns among this not only taxonomically, but also behaviourally (specialists *versus* generalists) diverse group of insects. Responses to salt (NaCl and KCl) occur in all species tested. Some authors consider salt responses to originate in so-called deterrent (D) cells, another cell type of universal occurrence.

Receptor cells responding to one (sucrose, glucose or fructose; 'S', 'G' or 'F') or more ('sugars') carbohydrates likewise occur in all species tested. Many, but not all species have inositol cells. Specialized amino acid cells have been described only for some species, but not all listed species have been tested for their responsiveness to this group of chemicals. In some instances taste cells were found which specifically respond to host-plant specific secondary plant substances. For example, *Pieris brassicae* and *P. rapae* larvae possess two glucosinolate cells and most *Yponomeuta* species have dulcitol and/or sorbitol receptors, responding to the predominant carbohydrate in their host plants.

Sugars and, though to a lesser extent, inositol are general feeding stimulants. Clearly insects are equipped with taste cells which are tuned to detect feeding stimulants (sugars, inositol, amino acids, secondary plant substances occurring in their host plants) as well as cells (D cells and, to some extent, salt cells) signalling the presence and quantities of compounds which reduce or inhibit feeding.

A general pattern emerges when all cells are labelled according to their either stimulating or inhibiting effect on feeding, as suggested by BERNAYS and CHAPMAN (2001*a*). According to this classification each sensillum styloconicum in most cat-

Table 1. Typology of taste cells in the maxillary sensilla styloconica of selected lepidopterous larvae. AA = amino acids; CAT = catalpol; Chlor. A = chlorogenic acid; D = deterrents; gD = generalist deterrent cell; sD = specialized deterrent cell; glucosinol = glucosinolates; ar. glucosinol. = aromatic glucosinolates; PO = populin; SA = salicin; sugars = cell responds to >3 carbohydrates; S = sucrose; G = glucose; F = fructose

	Medial sensillum				Lateral sensillum			
	1	2	3	4	1	2	3	4
Adoxophyes orana (1)[a]			salt	salt	S	AA	inositol	phloridzin
Choristoneura fumiferana (2,3,4)	water	proline	salt	salt	sugars	AA	water	salt
Yponomeuta cagnagellus[b] (5)		dulcitol	D	salt	S	dulcitol	D	salt
Y. malinellus (5)			D	salt	S	sorbitol	D	salt
Chilo partellus (6)	water		Chlor.A	salt	S			salt
Eldana saccharina (6, 7)	water	S		salt	S		D	salt
Maruca testulalis (6)	water			salt	S			salt
Bombyx mori (8)	water	salt	D	salt	sugars	inositol	G	salt
Philosamia cynthia (9)	G	inositol	salt	salt	S	inositol	G	salt
Pieris brassicae (9,10,11,12,13)	sugars	ar. glucosinol	gD	salt	S	AA	glucosinol	sD
P. rapae (12,13,14,15)	sugars	ar. glucosinol	gD	salt	sugars	AA	glucosinol	sD
Operophtera brumata (9)	glycosides		salt	salt		inositol		
Manduca sexta (9,16, 17,18, 19)	G	inositol	D	salt	S/G	inositol	D	salt
Laothoe populi (1)	PO/SA		D		S	AA	PO/SA	
Grammia geneura (20,21,22)	sugars/AA/CAT		D	D	F	AA	D	D
Euproctis phaeorrhoea (9)	glycosides	inositol		salt	S	inositol		salt
Heliothis virescens (23,24)	S/alanine	inositol	D	D	S	alanine	D	D
H. armigera (23)	S	alanine	D	D	S	alanine	D	D
Mamestra brassicae (25,26)	sinigrin	inositol	D	salt	sugars	salt	D	salt

Table 1 (continued)

	Medial sensillum				Lateral sensillum			
	1	2	3	4	1	2	3	4
M. configurata (27)		inositol		salt	S	salt	sinigrin	salt
Spodoptera exempta (6,7,23, 28)	S	inositol	D		S	adenosine	D	
S. littoralis (23)	S	alanine	D	D	S	alanine	D	D
S. frugiperda (23)	S	alanine	D	D	S	alanine	D	D
S. litura (29)	F	inositol	salt	salt	sugars	water	D	salt
Trichoplusia ni (27)			sinigrin	salt	S		sinigrin	salt

[a]Data from: (1) SCHOONHOVEN 1973; (2) ALBERT 1980; (3) ALBERT & PARISELLA 1988*a*; (4) PANZUTO & ALBERT 1997, 1998; (5) VAN DRONGELEN 1979; (6) WALADDE *et al*. 1989; (7) DEN OTTER 1992; (8) ISHIKAWA 1963, 1967; (9) SCHOONHOVEN 1969*c*; (10) SCHOONHOVEN 1967, 1969*a*; (11) MA 1972; (12) VAN LOON 1990; (13) VAN LOON & SCHOONHOVEN 1999; (14) VAN LOON & VAN EEUWIJK 1989; (15) VAN LOON unpubl.; (16) SCHOONHOVEN & DETHIER 1966; (17) PETERSON *et al*. 1993; (18) GLENDINNING *et al.* 1999*b*; (19) FRAZIER 1986; (20) BERNAYS *et al.* 2000*a*; (21) BERNAYS & CHAPMAN 2001*b*; (22) BERNAYS & CHAPMAN 2001*a*; (23) SIMMONDS & BLANEY 1991; (24) BERNAYS & CHAPMAN 2000; (25) WIECZOREK 1976; (26) BLOM 1978; (27) SHIELDS & MITCHELL 1995; (28) MA 1976*a*, 1977*a*; (29) HIRAO *et al.* 1992

[b]Results on 7 other *Yponomeuta* species are presented in VAN DRONGELEN 1979

erpillar species contains two feeding stimulating and two deterrent cells (SIMMONDS & BLANEY 1991, BERNAYS & CHAPMAN 2001*a*). Such grouping, though satisfying our sense of unity in nature, hides the endless variation which exists among species. Detailed comparisons of their taste spectra show that each species is suited with a unique chemoreceptor system, as will be amplified in the next sections.

SUGAR CELLS

Plants generally contain sucrose and its constituent monosaccharides glucose and fructose as primary metabolites resulting from their photosynthetic activity. These compounds function as strong phagostimulants to most herbivorous insects, equipped with specialized receptors to detect sugars. Table 1 shows that all species tested have a sugar cell (cell #1 in the lateral taste hair) which responds to one or more kinds of sugar. In several instances a second sugar cell is found in the medial sensillum (cell #1). The stimulus ranges of the sugar cells have been investigated only in a limited number of species. Various mono-, di-, and trisaccharides may stimulate the sugar cell in some insects (Table 2), whereas in other species this receptor responds exclusively to sucrose (Table 3) or glucose ('G' in Table 1) or fructose ('F'). It should be noted, however, that a number of cells designated as sucrose ('S') cells in Table 1 have been identified by stimulation with sucrose only, and thus may possess a wider stimulus range. Though sucrose is a strong stimulant to most lepidopterous larvae, some species are insensitive to it. *Helicoverpa zea* does not electrophysiologically respond to sucrose, but it does to glucose (DETHIER & KUCH 1971). Other species are stimulated by sucrose only and appear insensitive to other sugars (DEN OTTER 1992).

Most caterpillar sugar cells have a threshold sensitivity of 0.1–1 mM and reach saturation at about 100 times higher concentrations. These cells thus are most sensitive over a range of about two orders of magnitude, a span that nicely covers the range of sugar levels generally present in green leaves, i.e., 10–50 mM/l (Table 4).

An important conclusion emerging from Tables 2 and 3 is, despite the limited information available, that the response properties of sugar cells vary widely between species. Also, when a species has two sugar-sensitive cells these cells appear to differ in their response characteristics with respect to quality and/or quantity of their stimuli (Tables 1–3).

A more detailed analysis of responsiveness to various sugars in relation to age showed that changes may occur with development. A striking example concerns the spruce budworm, *Choristoneura fumiferana*. The order of stimulating ef-

fectiveness of a number of sugars for fourth-instar larvae differs from that of sixth-instar larvae. This change is correlated with temporal changes in carbohydrate composition of their food plants (PANZUTO & ALBERT 1997).

Some non-sugar organic compounds which stimulate human sugar receptors appear to be ineffective when applied to caterpillar sugar cells. Saccharin in con-

Table 2. Stimulus spectra of sugar cells in the maxillary sensilla styloconica to various carbohydrates. M = medial and L = lateral sensillum

	P. brassicae (1)[a]		*P. rapae* (2)		*D. pini* (1)		*C. fumiferana* (3)		*B. mori* (4)	*M. brassicae* (5)
	M	L	M	L	M	L	M	L	L	L
Pentoses										
D-arabinose	o	o			+	o			++	+
L-arabinose	o	o	+	+	+	o	o	+	+	+
L-fucose	++	o	+++	+	+++	+++				+
D-ribose	o	o	+	o	o	+			±	
D-xylose	o	o	o	+	+++	o	o	+	±	
Hexoses										
D-fructose	+	o	++	o	+	o	o	+++	+	+
D-galactose	o	o	o	o	++	o	o	+	+	+
D-glucose	+	++	+	++	+++	+	o	++	++	+
D-mannose	o	o	o	o	+	o			±	o
L-sorbose	+	o	+	++	+	+			+/++	
Disaccharides										
Lactose	o	o	o	o	o	+			±	o
D-maltose	o	o	o	+++	o	o			+++	+
Sucrose	++	+++	++	+++	+	+++	o	+++	++++	+
D-trehalose	o	o	o	+			+		+	o
Melibiose							o	++	±	
Trisaccharides										
Melezitose	o	o			o	+			+	
Raffinose	o	o			o	o	o	++	+	
Polyhydric alcohols										
Inositol	o	o	o	o	+++	o	+++	+++	o	
D-sorbitol	o	o	o	o			+	+	±	

+++ = strong reaction, ++ = medium reaction, + mild reaction, ± little or no reaction, o = no reaction

[a](1) MA 1972; (2) VAN LOON unpubl.; (3) PANZUTO & ALBERT 1997; (4) ISHIKAWA 1967; (5) WIECZOREK 1976

centrations up to 10 mM does not stimulate taste cells in *Manduca sexta* or *Pieris brassicae*. Sodium cyclamate is also inactive in *Philosamia cynthia* and *P. brassicae* larvae, although in the latter another receptor cell (presumably the amino acid cell) is stimulated (SCHOONHOVEN 1974). This corresponds with the observation that saccharin does not stimulate feeding in behavioural tests (EGER 1937). The finding that in vertebrates non-sugar sweeteners act via a different transduction pathway from that used for sugars (BERNHARDT *et al.* 1996) suggests that this pathway is absent from insect sugar receptors. Likewise, thaumatin, a botanical protein very sweet to man, does not elicit responses from styloconic sugar cells in *P. brassicae* and gymnemic acid, a compound that suppresses sugar sensitivity in vertebrates, does not affect electrophysiological responses to sugar in *P. brassicae*

Table 3. Carbohydrates ranked in order of effectiveness for different sugar receptors. M = medial and L = lateral sensillum styloconicum

P. brassicae (1)	M:	fucose > sucrose > glucose > fructose (15 other sugars do not stimulate)
	L:	sucrose > glucose (17 other sugars do not stimulate)
P. rapae (2)	M:	fucose > sucrose = fructose > glucose = ribose = arabinose (8 other sugars do not stimulate)
	L:	sucrose = maltose > glucose = sorbose > arabinose = fucose = xylose (7 other sugars do not stimulate)
D. pini (1)	M:	xylose > glucose > fucose = inositol = galactose > sucrose > arabinose = fructose > mannose (8 other sugars do not stimulate)
	L:	sucrose > fucose > glucose > ribose = sorbose (12 other sugars do not stimulate)
C. fumiferana (3)	L (4th instar):	melibiose > sucrose > raffinose > fructose > inositol > glucose > L-arabinose = xylose = galactose > sorbitol
	L (6th instar):	sucrose > fructose > inositiol > raffinose > glucose = melibiose > sorbitol > L-arabinose > xylose = galactose
B. mori (4)	L:	sucrose > maltose > glucose = D-arabinose = rhamnose > sorbose > L-arabinose = fructose = galactose = trehalose = melezitose
E. saccharina (5)	M:	sucrose (12 other sugars do not stimulate)
	L:	sucrose (12 other sugars do not stimulate)
C. partellus (5)	L:	sucrose (12 other sugars do not stimulate)
M. testulalis (5)	L:	sucrose (12 other sugars do not stimulate)
S. exempta (5)	L:	sucrose (12 other sugars do not stimulate)

(1) MA 1972; (2) VAN LOON unpubl.; (3)PANZUTO & ALBERT 1997; (4) ISHIKAWA 1963; (5) DEN OTTER 1992

or *M. sexta*, again indicating basic differences in transduction processes (SCHOONHOVEN 1974, SCHIFFMAN 1997).

Few studies on herbivorous insects have addressed the causes of differences in stimulating capacity between different sugars for one receptor cell, or differences between receptor cells in their responsiveness to the same sugar. LAM and FRAZIER (1991) conclude from a structure-activity study that the difference in responsiveness to glucose between the glucose-sensitive cell located in the medial sensillum styloconicum of *M. sexta* and the cell in its lateral hair responding to su-

Table 4. Sensitivity thresholds and saturation levels of some sugar cells. L = lateral and M = medial sensillum

			Threshold	Plateau	Kb	
		Sugar	(mM)	(mM)	(mM)	Reference[a]
B. mori	L	sucrose	0.1	10		(1)
		glucose	2			
		fructose	5–10			
P. brassicae	L	sucrose	1	100	50	(2)
		glucose	10	300		
	M	sucrose	10			(2)
P. rapae	L	sucrose	0.1	20	0.5	(3)
		glucose	0.1	100	1.0	
	M	sucrose	0.5	32	1.0	(3)
D. pini	M	glucose	<0.5	50	30	(4)
M. sexta	M	glucose	0.5	100	8	(5)
C. fumiferana	L	sucrose	<0.5	50	1.5	(6)
S. exempta	L	sucrose	<0.1			(7)
M. testulalis	L	sucrose	<0.1	10		(7)
E. saccharina	L	sucrose	0.1	100		(7)
	M	sucrose	0.1	10		
C. partellus	L	sucrose	0.1	100		(7)
H. virescens	L	sucrose	0.5	10		(8)
H. subflexa	L	sucrose	0.5	10		(8)
G. geneura	M	sucrose	<0.1	50		(9)
		(serine)[b]	<0.1	10		
		(catalpol)[b]	0.01	5		

[a](1) ISHIKAWA 1963; ASAOKA 2000; (2) MA 1972; (3) VAN LOON unpubl.; (4) MENCO *et al*. 1974; (5) FRAZIER 1986; (6) ALBERT & PARISELLA 1988*a*; (7) DEN OTTER 1992; (8) BERNAYS & CHAPMAN 2000; (9) BERNAYS *et al.* 2000*a*

[b]Compounds stimulate sucrose-sensitive cell

crose and glucose can be attributed to differences in topographical binding-site characteristics.

Several studies have shown that feeding behaviour is quantitatively related to sensory input, as may be expected for a major and in most cases even dominant phagostimulant (e.g., BLOM 1978). The rank order of the major sugars for behavioural preferences in spruce budworm larvae correlates with that for firing frequency of the sugar-sensitive neuron in its lateral sensillum styloconicum (PANZUTO & ALBERT 1997), another indication of the importance of input from this cell to the gustation processing centre.

Table 5. Responses to inositol in medial (M) and lateral (L) sensilla styloconica

Species	M	L	Reference[a]
Cossus cossus (Cossidae)	+	–	(1)
Adoxophyes orana (Tortricidae)	–	+	(2)
Choristoneura fumiferana (Tortricidae)	+	+	(3)
Euchaetias egle (Oecophoridae)	–	–	(4)
Calpodes ethilus (Hesperiidae)	–	+	(5)
Dendrolimus pini (Lasiocampidae)	+	–	(6)
Malacosoma americana (Lasiocampidae)	+	+	(5)
Bombyx mori (Bombycidae)	–	+	(7)
Philosamia cynthia (Saturniidae)	+	+	(2)
Papilio troilus (Papilionidae)	+	–	(4)
P. glaucus (Papilionidae)		–	(4)
P. polyxenes (Papilionidae)	+	+	(4)
Pieris brassicae (Pieridae)	–	–	(6)
P. rapae (Pieridae)	–	–	(8)
Danaus plexippus (Danaidae)	–	+	(4)
Bupalus piniarius (Geometridae)	–	+	(6)
Operophtera brumata (Geometridae)	–	+	(2)
Celerio euphorbiae (Sphingidae)	+	+	(1)
Ceratomia catalpae (Sphingidae)	+	+	(4)
Manduca sexta (Sphingidae)	+	+	(2)
Sphinx ligustri (Sphingidae)	+	+	(1)
Estimene acrea (Arctiidae)	+	–	(4)
Grammia geneura (Arctiidae)	–	–	(9)
Isia isabella (Arctiidae)	+	+	(4)
Euproctis chrysorrhoea (Lymantriidae)	+	+	(1)
Lymantria dispar (Lymantriidae)	+	+	(4)

Table 5 (continued)

Species	M	L	Reference[a]
Leucoma salicis (Lymantriidae)	+	–	(1)
Episema caeruleocephala (Noctuidae)	+	–	(1)
Helicoverpa zea (Noctuidae)	+	–	(4)
H. virescens (Noctuidae)	+	–	(10)
H. subflexa (Noctuidae)	+	–	(10)
Mamestra brassicae (Noctuidae)	+	+	(6)
M. configurata (Noctuidae)	+	–	(11)
Spodoptera exempta (Noctuidae)	+	–	(12)
S. littoralis (Noctuidae)	+	+	(13)
Trichoplusia ni (Noctuidae)	–	–	(11)

[a](1) SCHOONHOVEN 1973; (2) SCHOONHOVEN 1969*c*; (3) PANZUTO & ALBERT 1997; (4) DETHIER 1973; (5) DETHIER & KUCH 1971; (6) MA 1972; (7) ISHIKAWA 1963; (8) VAN LOON unpubl.; (9) BERNAYS & CHAPMAN 2001*a*; (10) BERNAYS & CHAPMAN 2000; (11) SHIELDS & MITCHELL 1995; (12) MA 1977*a*; (13) SCHOONHOVEN *et al*. 1991

SUGAR ALCOHOL CELLS

Since ISHIKAWA's initiating paper (1963) on taste receptors in the silkworm, in which he reported the presence of, among others, a specific inositol-sensitive cell, many species of caterpillar have been found to possess a cell that vigorously responds to inositol (Table 5). In some cases, for instance in *Dendrolimus pini* (MA 1972, MENCO *et al.* 1974) and *Choristoneura fumiferana* (PANZUTO & ALBERT 1997), inositol stimulates the sugar cell. Usually, however, inositol stimulates one or even two specialized, so-called inositol cells. Their specificity spectra have been studied in a few species only, but it is generally assumed that they are highly specific. Thus, out of the nine stereo-isomeric configurations of inositol, only myo- and epi-inositol stimulated the inositol cell in *B. mori* (JAKINOVICH & AGRANOFF 1971, 1972). Both inositol cells in *M. sexta* are insensitive to the cyclitols mannitol, sorbitol, pinitol and quebrachitol (GLENDINNING *et al.* 2000).

The sensitivity of inositol cells is generally fairly high and comparable to threshold levels found in sugar cells, i.e., 0.1 mM (ISHIKAWA 1967, DEN OTTER 1992, BERNAYS *et al.* 1998). Their dynamic range (i.e., the steepest part of the concentration/response curve) overlaps with the known range of inositol concentrations in plant tissues, i.e., 0.5–10 mM (MORRÉ *et al.* 1990, NELSON & BERNAYS 1998). Thus, these cells are well equipped to quantitatively determine the presence of inositol in food plants.

The finding that many caterpillars devote out of their styloconic complement of only eight cells one or even two taste cells to inositol perception, suggests that inositol, an ubiquitous plant constituent (LOEWUS & MURTHY 2000), is an important signal in host-plant recognition and/or assessing plant nutritional value. It does indeed stimulate feeding behaviour when added to an artificial diet or smeared on an otherwise unacceptable plant leaf (e.g., SCHOONHOVEN 1969*c*, HAMAMURA 1970, GLENDINNING *et al.* 2000). However, the effects on feeding behaviour can only partially explain why caterpillars spend so much sensory input capacity on this simple compound. Inositol is known to occupy a multifunctional role in plant metabolism and to affect in manifold ways growth and development (LOEWUS & MURTHY 2000). Possibly inositol levels signal to the insect some important feature of a plant other than its nutritional value *per se* (NELSON & BERNAYS 1998, GLENDINNING *et al.* 2000).

Table 6. Presence of sorbitol-sensitive taste cells in medial (M) or lateral (L) sensillum styloconicum

Species	Feeding range[a]	Food plants	Sorbitol cell		Reference[b]
			M	L	
Malacosoma americana	O	mainly Rosaceae	o	+	(1)
Episema caeruleocephala	O	Rosaceae	o	+	(2)
Lymantria dispar	P	Rosaceous and other trees	+	o	(3)
Yponomeuta evonymellus	M	*Prunus padus* (Ros.)	o	+	(4)
Y. malinellus	M	*Malus* spp. (Ros.)	o	+	(4)
Y. padellus	O	Rosaceae	o	+	(4)
Y. mahalebellus	M	*Prunus mahaleb* (Ros.)	o	+	(4)
Y. cagnagellus	M	*Euonymus europaeus* (Celas.)	o	o	(4)
Y. irrorellus	M	*E. europaeus*	o	o	(4)
Y. plumbellus	M	*E. europaeus*	o	o	(4)
Y. rorellus	M	*Salix* spp. (Salic.)	o	o	(4)
Y. vigintipunctatus	M	*Sedum telephium* (Crass.)	o	o	(4)
Estigmene acrea	P	herbaceous plants	o	o	(3)
Choristoneura fumiferana	O	*Picea* spp. (Pinac.)	o	+	(5)
Dendrolimus pini	O	*Pinus* spp. (Pinac.)	o	o	(6)
Pieris brassicae	O	Cruciferae	o	o	(7)
Manduca sexta	O	Solanaceae	o	o	(8)
Papilio troilus	P	various trees	o	o	(1)

[a]O = oligophagous, P = polyphagous, and M = monophagous

[b](1) DETHIER 1973; (2) SCHOONHOVEN 1973; (3) DETHIER & KUCH 1971; (4) VAN DRONGELEN 1979; (5) PANZUTO & ALBERT 1997; (6) MENCO *et al.* 1974; (7) MA 1972; (8) GLENDINNING 2000

Some other sugar alcohols play a dominant role in certain caterpillar–host-plant relationships. Taste cells which typically respond to sorbitol or dulcitol, compounds which occur at high concentrations in Rosaceae and Celastraceae respectively, are present in species which feed exclusively or to a greater extent on plants belonging to these taxa, whereas species feeding on other plant taxa lack such receptors (Table 6). A well documented example of specialized sorbitol and dulcitol cells is provided in an electrophysiological inventory covering nine *Yponomeuta* species, all feeding specialists (VAN DRONGELEN 1979). Four species bound to rosaceous plant species possess sorbitol-specific taste cells, whereas the remaining five species lack such receptors. Three of the latter species feed monophagously on *Euonymus europaeus*, a shrub which is characterized by large amounts of dulcitol (a stereoisomer of sorbitol). These species are equipped with specific dulcitol-sensitive receptor cells in their lateral and, in two species, also in their medial sensilla styloconica (VAN DRONGELEN *l. c.*).

AMINO ACID CELLS

Since several amino acids stimulate feeding behaviour in various herbivorous insects (BERNAYS & SIMPSON 1982, ALBERT & PARISELLA 1988*b*, HIRAO & ARAI 1990) one may expect to find among an insect's chemoreceptor system neurons which respond either directly to amino acids, or whose responses to other compounds are modified by the presence of amino acids. Both modes of perception have been found to coexist in several caterpillar species.

Table 7 lists the results of studies in which the stimulus ranges of styloconic cells for a series of amino acids were determined in some detail. A glance at the table immediately reveals striking differences between species. Even two species considered to be closely related, *Pieris brassicae* and *P. rapae*, show several significant differences in their responses to the same set of amino acids. A second noteworthy feature is that all species respond to some nutritionally essential as well as non-essential amino acids. In both *Pieris* species the responses to the essential amino acids are stronger than to the non-essential ones, but this balance is reversed in, for instance, *Ecrisia acrea*. The signalling function of these compounds is presumably more important to the insect than exact knowledge of the presence of nutritionally relevant chemicals.

The picture arising from Table 7 is, however, very incomplete. It only presents data from one of the two sensilla styloconica, whereas it is known that in several cases the complementary sensillum also responds to one or more amino acids. Thus, in *P. brassicae* and *P. rapae* two of the dominant free amino acids in their

food plants, i.e., aspartic acid and glutamic acid, stimulate a cell in the medial sensillum rather than the amino acid cell in the lateral hair (VAN LOON & VAN EEUWIJK 1989). Another example is provided by larvae of *Choristoneura fumiferana*. All amino acids tested, except proline, stimulate a cell located in the other hair. Proline, on the other hand, evokes vigorous responses in the medial sensil-

Table 7. Amino acid receptors in maxillary chemosensilla of selected lepidopterous larvae[a]. L = lateral and M = medial sensillum styloconicum. Arterisks* indicate essential amino acids. (P.b.: *Pieris brassicae*; P.r.: *Pieris rapae*; H.z.: *Helicoverpa zea*; E.a.: *Ecrisia acrea*; M.a.: *Malacosoma americana*; D.p.: *Danaus plexippus*; P.p.: *Papilio polyxenes*; L.d.: *Lymantria dispar*; C.e.: *Calpodes ethlius*, A.o.: *Adoxophyes orana*; C.f.: *Choristoneura fumiferana*, G.g.: *Grammia geneura*)

	P.b. L (1,2)[c]	P.r. L (2,3)	H.z. L (3)	E.a. M (3)	M.a. M (3)	D.p. L (3)	P.p. L (3)	L.d. L (3)	C.e. L (3)	A.o. L (4)	C.f.[b] L (5)	G.g. L (6)
Arginine*	o	o	o	o	+	–	–	–	++		o	++
Histidine*	+++	+	o	o	o		o	o		+	+	+++
Isoleucine*	++	++	o	o	o	o	o	+		++		o
Leucine*	++	+++	++	+	o	o	o	o		+++	+	++
Lysine*	o	o										++
Methionine*	++	+++	++	+	o	++	o	–	++	+	+	++
Phenylalanine*	+++	+	o	++	o	o	o	o		o	+	o
Threonine*	+	o	o	+	o	++	++	+		o		+
Tryptophan*	++	+	o	+	o	o	+	o		+		o
Valine*	++	++	–	+	++	++	o	o		++	+	o
Alanine	++	++	o	+++	+++	++	o	o	–		+	+
Asparagine	++	++										o
Aspartic acid	o	o	o	o	+	o	o	o	++		+	+
Cysteine	+	o		++	o		–	o	++			
Cystine			++	o	+	+	o	o	+		+	o
Glutamic acid	o	o	o	++	++	o	–	o	o		+	++
Glycine	+	o	o	++	–	o	–	o			+	+
Proline	++	++	o	++	++	+++	o	o	++		o	o
Serine	++	++	o	+++	o	+++	++	o			+	o
Tyrosine	o	o	+	+	o	o	o	o			+	o

[a]+++ = strong reaction, ++ = medium reaction, + mild reaction, o = no reaction, – = inhibition as compared to control

[b]Different compounds were tested at different concentrations

[c]Data from (1) SCHOONHOVEN 1969*a*; (2) VAN LOON & VAN EEUWIJK 1989; (3) DETHIER & KUCH 1971; (4) SCHOONHOVEN 1973; (5) PANZUTO & ALBERT 1998; (6) BERNAYS & CHAPMAN 2001*a*

lum. In behavioural tests proline strongly stimulates feeding activity in this insect (PANZUTO & ALBERT 1998).

Some amino acids appear to stimulate 'sugar' cells. An interesting case of such a versatile receptor cell is present in *Grammia geneura* larvae. These insects have, in addition to an amino acid cell in their lateral sensillum styloconicum, in the other sensillum a neuron which responds to seven (out of 20) amino acids. The same cell can be stimulated by sucrose, glucose, and trehalose, and, remarkably, also by catalpol. The latter compound, an iridoid glycoside, occurs in a favoured food plant of this species. Because of its multiple specificity BERNAYS and CHAPMAN (2001*a*) named this cell a 'phagostimulatory cell', rather than a 'sugar' or 'amino acid' cell.

As has been described above for sugar cells the dose-response curves of some representative amino acids show a section of increasing responsiveness which spans a concentration range of about two orders of magnitude. In the case of *Pieris* the observed sensitivity ranges cover the concentrations of the compounds concerned as found in cabbage leaves (VAN LOON & VAN EEUWIJK 1989).

The relevance of amino acid receptors may be questioned in view of the fact that relatively small amounts of free amino acids occur in living plant tissues, and herbivores depend for their nitrogen requirements mainly on digestion of proteins. On the other hand, free amino acids are more readily available than proteins which need to be digested first, a process which involves energy. An indication of the fact that the amount of soluble nitrogen is important to an insect is deduced from better growth of *Pieris rapae* larvae on plants in which the tissues contained a greater proportion of the total nitrogen in soluble form than in control plants with similar levels of total nitrogen (SLANSKY & FEENY 1977). Furthermore, quantity and composition of the free amino acid pool may signal the nutritional status of a plant and as such form an important source of information to herbivores. In this context it is interesting to note that proline, though a non-essential amino acid for insects, is compared to other amino acids often a strong stimulus, which in *C. fumiferana* is even perceived via a separate channel. This very compound appears to play an important role in plants under water stress conditions and is known to accumulate in stressed plants (CYR *et al*. 1990). Conceivably, an insect obtains information on a plant's physiological status by measuring its proline level (PANZUTO & ALBERT 1998).

Several authors (e.g., DETHIER & KUCH 1971, HIRAO & ARAI 1990, BERNAYS & CHAPMAN 2001*a*) have reported that some amino acids may affect the impulse activity (positively or negatively) of various receptor types. Furthermore, cases are known in which a particular amino acid stimulates a deterrent cell (e.g., HIRAO & ARAI 1990). The observation that valine, though a strong stimulant of the amino

acid cell in *C. fumiferana*, appears to be a feeding deterrent in behavioural tests, may be attributed to its multiple effects on more than one cell type (PANZUTO & ALBERT 1998).

In conclusion, perception of amino acids is rarely if ever effected via a simple and highly specific chemosensory pathway. The finding that plant-like mixtures of amino acids stimulate two, and sometimes even three cells within the lateral sensillum of *Grammia geneura* (BERNAYS & CHAPMAN 2001*b*) fits into this inference. That multicomponent mixtures often evoke complex responses is hardly surprising in view of the fact that amino acids are structurally much more dissimilar than their common name suggests. Apart from that, their physiological roles in plants are multifaceted, and last but not least, their absolute and relative quantities vary greatly among plant species, as well as within plants, depending on developmental and physiological condition. Altogether, it is to be expected, also taking into account their different feeding habits, that amino acid perception among insect species shows little uniformity.

RECEPTORS FOR SIGN OR TOKEN STIMULI

In a pioneer study VERSCHAFFELT (1910) showed that certain specific secondary plant substances serve as cues used by some insects to recognise their food plants. It took half a century before the first chemosensory responses were recorded to one of the compounds which VERSCHAFFELT identified as a *sine qua non* for attack by insects specialized on these plants. He used sinigrin, a glucosinolate occurring in cruciferous plants, to entice *P. brassicae* larvae to feed on normally rejected plant species. Glucosinolates stimulate one neuron in each sensillum styloconicum of this insect, which thus function as phagostimulatory receptors for host-specific compounds (SCHOONHOVEN 1967). The cell located in the lateral sensillum responds to all tested glucosinolates with thresholds of ca. 0.1 mM. The cell in the medial sensillum reacts only to aromatic glucosinolates. This difference in specificity ranges allows the insect in principle to determine the ratio between total glucosinolates and aromatic glucosinolates. (Aromatic glucosinolates are induced in response to damage.)

After the elucidation of glucosinolates as pivotal in a crucifer-herbivore association the search for specific phagostimulants in other plant families was intensified. Although some striking cases have been reported, they are relatively rare in view of the interest in insect-plant relationships during the past decades (STÄDLER 1992, see also MÜLLER & RENWICK 2001). Within the Lepidoptera the most distinct case is found in the association of a number of insect species with Rosaceae.

The larvae of eight taxonomically diverse lepidopterans that feed only or at least mainly on rosaceous plants have receptors for sorbitol, the predominant soluble carbohydrate typical of this family, whereas such receptors do not occur in other insects (Table 6).

As discussed earlier the presence of sorbitol receptors in some *Yponomeuta* species specialized on rosaceous hosts is mirrored in dulcitol receptors in related *Yponomeuta* species, which feed only on *Euonymus europaeus*, a plant with dulcitol as its primary carbohydrate. These insects thus have, in addition to a sucrose receptor, sorbitol and/or dulcitol specific cells which signal the presence of a host-specific phagostimulant and which is at the same time an important nutrient (VAN DRONGELEN 1979). The dulcitol receptor cell responds to stimulus concentrations as present in its food plant with a maximum firing rate (Fig. 3). This indicates that it functions as a gauge which records the presence or absence of a sign stimulus, rather than measuring stimulus intensity.

An interesting taste cell type has been found in larvae of *Spodoptera exempta*. A cell which is specifically stimulated by adenosine and adenine provides a chemosensory basis for the fact that these compounds stimulate food uptake in this insect. This receptor is insensitive to purine or pyrimidine compounds or derivatives, including nucleotides and nucleosides (MA 1977*a*, MA & KUBO 1977). Whether or not we are dealing with an exceptional type of feeding stimulant

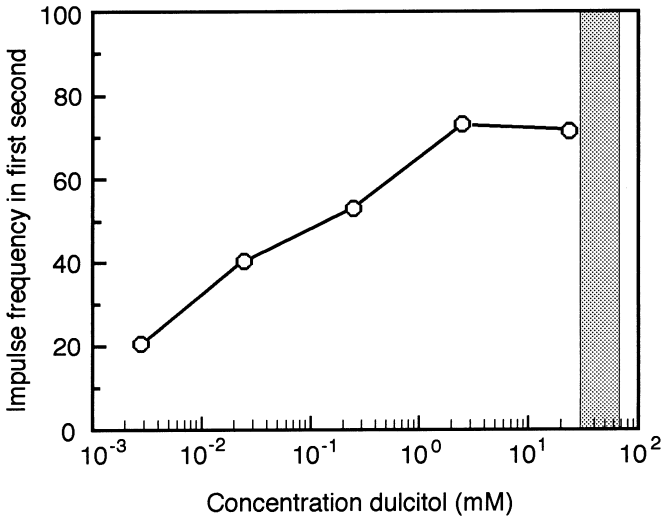

Fig. 3. Dose-response curve for dulcitol of the dulcitol-sensitive cell in the lateral sensillum styloconicum of *Yponomeuta cagnagellus*. The range of dulcitol concentrations in its host plant, *Euonymus europaeus*, lies within the shaded part of the figure (after MENKEN & ROESSINGH, 1998)

is unknown, because these compounds have rarely been included in feeding assays. Based on evidence from some related *Spodoptera* spp. MA (1977*a*) hypothesized that adenosine which occurs at a concentration of ca. 0.2 mMoles/1000g fresh maize leaves, is a more common phagostimulant for insects with grasses in their diet.

As referred to above, catalpol, a compound typically occurring in *Plantago* spp., stimulates at natural concentrations a phagostimulatory cell in *Grammia geneura*. This species, although polyphagous, shows a preference for *Plantago* over several other food plants. Catalpol, when added to a neutral substrate, stimulates feeding activity (BERNAYS *et al.* 2000*a*).

Table 8. Response spectra of deterrent neurones in four caterpillar species belonging to different food specialization categories, to four classes of secondary plant substances[a]

	B. mori (1)[b]	*P. brassicae* (2, 3, 4)	*M. sexta* (5)	*M. brassicae* (6, 7, 8)
	M[c]	O	O	P
Alkaloids				
Quinine	+	+	–	
Strychnine	+	+	–	+
Conessine	+	+	–	–
Caffein	+	-	+	
Nicotine	+	+	–	
Terpenoids/steroids				
Azadirachtin		+	–	–
ß-Ecdysone	+	+		+
Digitoxin		+		–
Phenolics, flavonoids				
Salicin	+	–	+	+
Rutin	+	–	–	
Quercitrin	+			–
Phloridzin	–	+	+	
Malvin		–	+	
Glucosinolates				
Glucocapparin		–	–	+
Glucotropaeolin		–	+	+

[a]Most chemicals were tested at concentrations of 1-10 mM or as saturated solutions

[b]Data taken from: (1) ISHIKAWA 1966; (2) MA 1969, 1972; (3) VAN LOON 1990; (4) VAN LOON & SCHOONHOVEN 1999; (5) SCHOONHOVEN 1973, 1981; (6) WIECZOREK 1976; (7) DESCOINS & MARION-POLL 1999; (8) VAN LOON unpubl.

[c]M = monophagous; O = oligophagous; P = polyphagous

The relative paucity of examples discovered up till now of host-plant specific compounds that serve as feeding stimulants may be due to the limited research capacity devoted to this subject, but it could also very well be that clear and simple relationships between insects and their host plants based on one or a few chemicals are less widespread than is often presumed. After all, many groups of minor plant compounds have fairly wide distributions, and relatively few have a sufficiently restricted distribution range to use them as a specific enough characteristic of a certain plant taxon (SWAIN 1972). This implies that when an insect cannot rely on a simple and unequivocal chemical flag, it would have to rely on a chemosensory system that obtains more subtle and at the same time more complex information of a plant's chemical composition to distinguish hosts from non-hosts.

DETERRENT CELLS

All herbivorous insects have deterrent (D) receptors which upon stimulation reduce or fully stop feeding activity. These cells fulfil a central role in host-plant recognition, or rather in identifying non-hosts, and have since their discovery (ISHIKAWA 1966) attracted much interest. The fairly extensive literature on the sensory coding of feeding deterrents in various insects is reviewed by FRAZIER (1986, 1992) and SCHOONHOVEN *et al.* (1992).

The apparent simplicity of host-nonhost discrimination by D cells hides a multifarious complexity. First, the response patterns of D cells vary greatly among species, even if they are closely related, both qualitatively (VAN DRONGELEN 1979) and quantitatively (Fig. 4) (DETHIER & KUCH 1971, VAN LOON 1990). Second, in those cases where a range of compounds has been tested, the D cell is sensitive to compounds belonging to more than one chemical class. At the same time, in none of these cases does this cell respond to all chemical classes tested or even to all the compounds within a class (Table 8). Thus, although the stimulus spectra of D cells are often remarkably broad with a seemingly capricious response pattern, they nonetheless display specificity.

Deterrent cells naturally vary in their specificity depending on species and stimulus type. When these cells indeed function as an identification device to perceive secondary plant compounds which may occur in non-hosts their specificity ranges are expected to overlap with the concentration ranges of these compounds as commonly encountered in plants. Table 9 shows threshold values and saturation levels for chemicals which are the strongest stimuli known for the insects mentioned. It may be concluded from this very limited set of data that threshold concentrations are commonly below 1 mM. In some cases this value is even about

1000 times lower and ranks among the lowest reported for insect taste cells (by comparison: human taste threshold for quinine is about 0.00075 mM).

There are many indications that food specialists feature a greater sensitivity to deterrents than polyphagous species (e.g., BERNAYS & CHAPMAN 1994, BERNAYS *et al.* 2000*b*) and, as JERMY more specifically stated, that "the sensitivity of chemoreceptors to deterrents is a general factor determining the host range of chewing phytophagous insects" (JERMY 1966, p. 9). However, to conclude that the data of Table 9 support this hypothesis would be premature, because the high sensitivities observed in the oligophagous species may also be due to the fact that the stimulus spectra of these species have been investigated much more thoroughly than those of the generalist species. It may very well be that much stronger deterrents for the latter species will be found when more compounds are tested.

The concentration range between threshold and maximum firing intensity spans two to three orders of magnitude, and is thus comparable to ranges as determined for sugar cells (Table 4).

An essential difference between D cells and phagostimulatory cells is found in the time characteristics of their responses. Deterrent cells generally show greater latency in their response than phagostimulatory cells (e.g., MA 1972, GLENDINNG & HILLS 1997, DESCOINS & MARION-POLL 1999). Two other features which de-

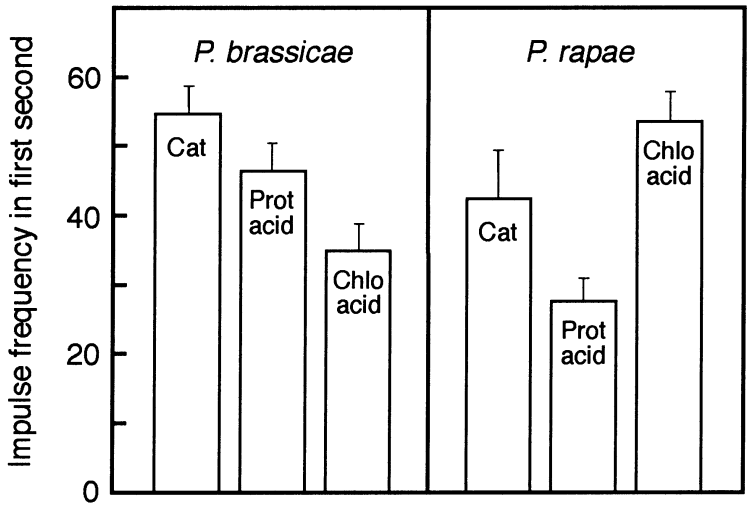

Fig. 4. Comparison of relative effectiveness of catechin (cat), protocatechuic acid (prot acid), and chlorogenic acid (chlo acid) on specialized deterrent cell in the lateral sensilla styloconica of two *Pieris* species. The three compounds were tested at 1 mM on *P. brassicae* and at 2.5 mM on *P. rapae* (data from VAN LOON 1990)

Table 9. Sensitivity thresholds and saturation levels of some deterrent cells. L = lateral and M = medial sensillum styloconicum; O = oligophagous and P = polyphagous

		Feeding range	Stimulus	Threshold (mM)	Plateau (mM)	References[a]
B. mori	M	O	strychnine	0.0001		(1)
P. brassicae	L	O	helveticoside	0.0002	0.03	(2)
	M	O	strychnine	0.001	0.02	(3)
M. sexta	L	O	aristolochic acid	0.0003		(4)
	M	O	caffeine	0.03	5	(4)
T. ni	L	P	sinigrin	0.02	5	(5)
	M	P	sinigrin	0.06	10	(5)
H. virescens	L	P	sinigrin	0.1	5	(6)
M. configurata	L	P	sinigrin	0.16	30	(5)
M. brassicae	L	P	sinigrin	0.5	500	(7)

[a]Data from (1) ISHIKAWA 1966; (2) VAN LOON & SCHOONHOVEN 1999; (3) MA 1972; (4) GLENDINNING *et al*. 1999*b*; (5) SHIELDS & MITCHELL 1995; (6) BERNAYS & CHAPMAN 2000; (7) WIECZOREK 1967

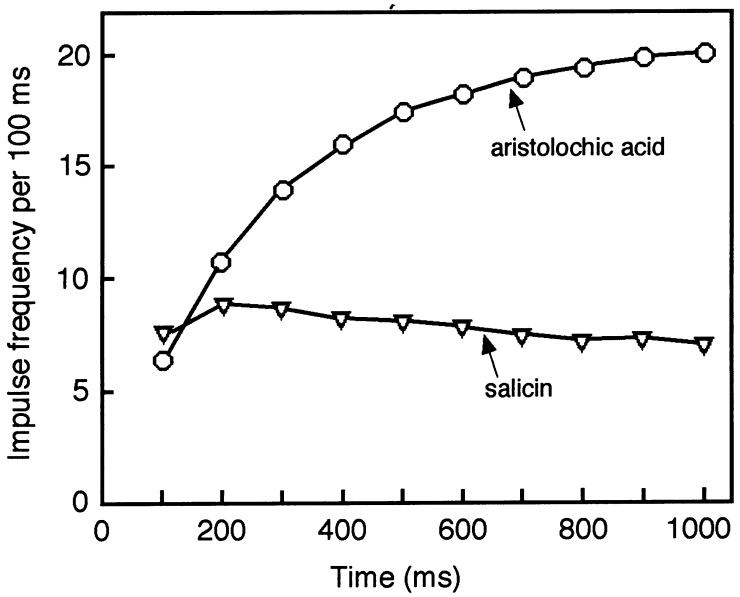

Fig. 5. Impulse frequencies elicited during the first second of stimulation by 10 µM aristolochic acid and 10 mM salicin in the D cell of the medial sensillum styloconicum of *Manduca sexta* (after GLENDINNING & HILLS, 1997)

terrent cells may show upon stimulation by certain compounds are a slow increase in impulse frequency (Fig. 5), and an increase in impulse amplitude with stimulus concentration (PETERSON *et al.* 1993, VAN LOON & SCHOONHOVEN 1999). Impulse amplitude changes are most likely irrelevant in the central integration process, but the slow start of impulse formation in D cells as compared to phagostimulatory cells is probably of importance. Another characteristic of D cells is their low adaptation rate (e.g., ISHIKAWA 1966, SCHOONHOVEN 1977, SHIELDS & MITCHELL 1995, DESCOINS & MARION-POLL 1999). The phasic-tonic relationship of a D cell, after it has reached its maximum activity in response to a given deterrent at a given concentration, differs from that of phagostimulatory cells (Fig. 6A), a characteristic which has a very marked effect on the ratio of impulse frequencies between the D cells and those responding to phagostimulants (Fig. 6B). As a consequence, the "taste" of a mixture of, for instance, sucrose and strychnine changes gradually, becoming more repulsive as time passes. Low levels of deter-

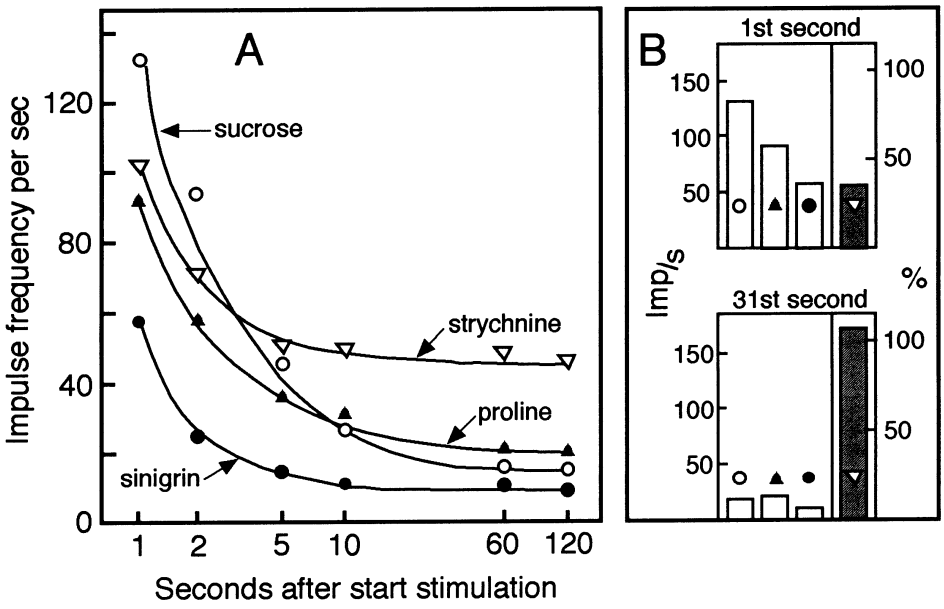

Fig. 6. (A) Adapatation curves of some chemoreceptory cells in the sensilla styloconica of *Pieris brassicae*. Stimuli: 0.003 mM strychnine, 100 mM proline, 10 mM sucrose, and 10 mM sinigrin. (B) Responses of four cell types during the 1st and 31st second of stimulation with a mixture of the same four chemicals, provided no interactions between the stimuli occur. The response intensity of the D cell (shaded) upon stimulation by strychnine is expressed as percentage of the summated impulse frequencies of the three phagostimulatory cells upon stimulation by sucrose, proline and siningrin, respectively (after SCHOONHOVEN 1977)

rents, though not preventive of feeding, may by way of this physiological mechanism reduce the lengths of feeding bouts. Shorter than normal feeding periods have often been recorded on deterrent foods that were eaten to some extent (BERNAYS *et al.* 2000*b*).

The decisive role of D cells in feeding behaviour is unambiguously supported by observations of a close correspondence between impulse frequencies recorded in D cells upon stimulation by a deterrent at various concentrations, and the feeding intensity on diets containing various amounts of the same compound (BLOM 1978, PETERSON *et al.* 1993, LUO *et al.* 1995, MESSCHENDORP *et al.* 1996, BERNAYS & CHAPMAN 2000).

SALT CELLS

Most if not all sensilla styloconica show neural activity when stimulated with salt solutions. KCl or NaCl are commonly used as an electrolyte enhancing electrical conduction of the recording electrode. These compounds, at the fairly high concentrations of 50 or 100 mM, stimulate one or two cells which have been labelled as 'salt' cells. Impulses from these cells are in most recordings characterized by relatively small amplitudes. ISHIKAWA (1963) suggested that they function as an anion and a cation cell. He found that the stimulating effect of the salts tested was dominated by the cations involved, and that monovalent cations were more effective stimuli than divalent cations.

Few studies report dose/response curves for one or more salts. A study on *Grammia geneura* shows increasing activity in two salt cells in response to KCl at concentrations ranging from 10 to 1000 mM. NaCl produced also responses in the two cells with slightly higher firing rates than KCl (BERNAYS & CHAPMAN 2001*a*). Interestingly, these authors conclude that salts and deterrents stimulate the same cells. PETERSON *et al.* (1993) also consider one of the two 'salt' cells in the medial sensillum of *Manduca sexta* to be in fact a D cell, and DETHIER (1973) reported that salicin, populin, and sinigrin tend to stimulate the 'primary salt cell' in the lateral sensillum of *Danaus plexippus*. Incongruent with these inferences is the fact that responses to salts display a temporal pattern quite different from that characterizing the majority of D cells (see previous section).

Obviously, sensory responses to salts have attracted only limited attention, which may be due to their supposedly minor role in host-plant selection. Moreover, salt responses appear often to be more or less suppressed when tested in mixtures with, for example, sucrose. It could be argued, therefore, that the role of salts

under natural conditions, that is as a component of leaf tissue sap, is of limited importance.

The view, recently expressed by BERNAYS and CHAPMAN (2001*a*), that salt cells have to be regarded as synonymous with D cells, opens a new perspective, which merits further investigation.

WATER CELLS

Water cells form perhaps the most mysterious cell type recognised so far among styloconic taste cells. Its existence has been reported in early papers on styloconic taste cells (ISHIKAWA & HIRAO 1963, SCHOONHOVEN & DETHIER 1966), although in some cases they have later been relabelled as salt cells. New instances of water cells have recently been described for several lepidopterous larvae (DEN OTTER 1992, PANZUTO & ALBERT 1998).

The presence of a water cell was concluded from stimulations with low salt concentrations or even distilled water (hence its sometimes used alternative name: 'low-salt cell'). Characteristically, water cells exposed to increasing salt concentrations are increasingly inhibited and may become fully suppressed, as was seen in *Bombyx mori* upon stimulation by 10 mM NaCl. Sugars and amino acids may inhibit this cell too (ISHIKAWA 1967, PANZUTO & ALBERT 1998).

It remains to be worked out whether 'water' cells under more natural conditions respond to compounds other than pure water, and then have to be renamed.

PLANT ACIDS

All plants contain organic acids, although the quantities and types of acid vary among species and with physiological state. Ascorbic acid is a common plant constituent, and, in contrast to other organic acids, an essential nutrient for most caterpillars. It stimulates feeding activity in, for instance, *Pieris brassicae* larvae (MA 1972). Therefore, any analysis of a caterpillar's gustatory sense should include responses to ascorbic acid and preferably some other common plant acids as well. However, few studies on this type of stimuli are available. DETHIER and KUCH (1971) and DETHIER (1973) have tested ascorbic acid, malic acid, oxalic acid, succinic acid and nicotinic acid on several caterpillar species. They noticed in several instances some increase of neural activity, and occasionally an inhibition of the salt cells as compared to control stimuli. No attempt was made to assign the observed action potentials to specific cell types. Caterpillars of *Choristoneura*

fumiferana respond to shikimic acid, a known feeding stimulant for this species. It is again unclear what cell type is involved (ALBERT 1980). Recently, BERNAYS *et al.* (1998) found that citric acid, oxalic acid and ascorbic acid may reduce salt responses and/or stimulate the D cells in *Manduca sexta* larvae. These responses, however, were largely ascribed to pH and not to specific effects of any of these compounds. Ascorbic acid at concentrations as it occurs in plant tissues consistently reduced responses to glucose and inositol.

The meagre information we have on sensory effects of plant acids does not provide evidence for the presence of a specific acid cell in caterpillars. At the present state of our knowledge it seems most likely that acids at natural concentrations primarily exert an effect on feeding behaviour by modulating the responses of various cell types. Besides, they may stimulate D cells at higher concentrations.

INHIBITORY AND SYNERGISTIC INTERACTIONS

When a styloconic sensillum is stimulated by a mixture of two compounds its neural response is often different from what would be expected on the basis of responses to the same compounds when tested singly. The presence of sucrose appeared to reduce the impulse frequency of the salt cell in *Bombyx mori*, and when the concentration of salt is increased, the intensity of responses of the sugar cell is reduced (ISHIKAWA 1963). Since ISHIKAWA's observation numerous examples of such negative interactions have been published. Thus, deterrents may inhibit sugar cells (Fig. 7) (e.g., FRAZIER 1986, VAN LOON 1990, HIRAO & ARAI 1991, MESSCHENDORP *et al.* 1996), and sugars and salts may inhibit D cells (e.g., SIMMONDS & BLANEY 1983, SHIELDS & MITCHELL 1995, GLENDINNING *et al.* 2000). Not only concentration of the inhibitory compound determines the degree of an inhibition, as shown in Fig. 7, but also exposure time. Three taste cells in *Pieris brassicae* responding to phagostimulants showed a gradual decrease of sensitivity when exposed to 1 mM polygodial, a drimane isolated from *Polygonum hydropiper*, for periods of up to 30 minutes. Its lateral glucosinolate receptor then showed a 20% lower sensitivity to sinigrin, while for the sugar cell and the amino acids cell reductions of about 50% were attained (SCHOONHOVEN & YAN 1989).

Another type of modification of normal chemosensory function caused by some feeding deterrents was first described by MA (1977*b*) after studying the effect of warburganal on phagostimulatory cells of *Spodoptera exempta*. This drimane compound appeared to distort the normal function of several cells, resulting in irregular patterns and eventually, depending on concentration and duration of exposure, to 'bursting' activity. The question, however, whether or not this type

of response, observed with relatively high concentrations and/or long stimulation periods, also occurs under natural conditions, remains to be solved (SCHOONHOVEN *et al.* 1992). The fact that tomatine, a constituent of one of the food plants of *Manduca sexta*, at low concentrations (i.e., 0.1 mM) causes within 30 seconds bursting activity in both sensilla, but is in behavioural experiments a (weak) phagostimulant (PETERSON *et al.* 1993) also seems inconsistent with the assumption that bursting patterns are normal physiological reactions.

Two compounds which both stimulate the same cell may when mixed also evoke a weaker response than expected from tests with the single compounds. A mixture of sucrose and glucose elicits a significantly lower impulse frequency than sucrose at the same concentration alone (ISHIKAWA 1967). Similar inhibitory interactions have been described for binary mixtures of compounds which stimulate, for instance, an amino acid cell [lysine and histidine (BERNAYS & CHAPMAN 2001*b*)] or deterrent cells [caffeine and salicin (SCHOONHOVEN 1978)].

Conversely, some combinations, for instance, inositol and glucose, or serine and alanine resulted in stronger than expected responses of respectively sugar and amino acids cells (MENCO *et al.* 1974, BERNAYS & CHAPMAN 2001*b*).

Deterrent compounds that on their own do not stimulate any neuron within a sensillum may also decrease the responsiveness of a cell responding to a nutrient, as exemplified by sinigrin inhibiting the inositol cell in *Heliothis virescens* (BERNAYS & CHAPMAN 2000).

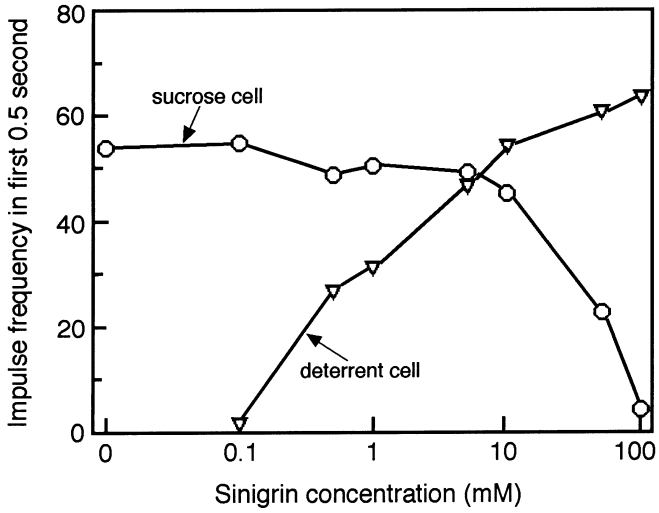

Fig. 7. Impulse frequencies of the sucrose-sensitive and deterrent cells in the lateral sensillum styloconicum of *Heliothis subflexa* upon stimulation with 5 mM sucrose mixed with different concentrations of sinigrin (after BERNAYS & CHAPMAN 2000)

Two compounds may also interact via a synergistic mechanism and induce a stronger neural response than each compound on its own would have done. Thus, the presence of sucrose greatly increased the response of the cell that in *Isia isabella* preferentially responds to sinigrin (DETHIER & KUCH 1971). Interestingly, a synergistic interaction between two chemicals at low concentrations may with increasing concentrations become reversed to an antagonistic interaction. This happens in the silkworm when strychnine at a fixed concentration is mixed with NaCl at varying concentrations. Increasing NaCl levels up till 40 mM NaCl induce an increase in firing frequency of the D cell in response to strychnine, whereas NaCl at a concentration of 100 mM and above inhibit the D cell (ISHIKAWA 1966).

The physiological mechanism underlying inhibitory (or excitatory) interactions when two cells are stimulated simultaneously is unknown. Mutual electrotonic influences may be involved, since in a dipterous insect direct contacts have been observed between chemosensory cell somata (ISIDORO *et al.* 1994). Direct physiological interactions between receptor cells occur in tibial chemosensilla of a grasshopper (WHITE *et al.* 1990).

The phenomenon of stimulus interactions resulting in inhibitions of one or more cells is reminiscent of lateral inhibition known from visual systems (HARTLINE *et al.* 1961). Likewise, inhibitory (and synergistic) relationships between chemicals may serve to sharpen the chemical image, the *Gestalt*, of a complex stimulus, thus providing the central nervous system (CNS) a partly pre-treated message.

The examples presented in this paragraph serve to stress the widespread occurrence of interactions between two or more chemicals, which probably take place at the receptor level. These mixture effects obviously hamper the analysis of responses to natural stimuli by the caterpillar's taste system because of the unpredictability of direction and degree of interplay between all contributing chemicals.

TASTE CELL CATEGORIES

Our approach of an analysis of taste cells in animals is coloured by the traditional concept of the four primary taste qualities: sweet, sour, salty, and bitter. The kinds of stimuli first chosen to investigate insect chemoreceptors and the denomination of cells responding to them reflect this background. Deterrent cells in caterpillars are still called by some researchers 'bitter' cells, since they often respond to compounds which taste bitter to humans.

In the foregoing paragraphs an attempt was made to categorize taste cells in lepidopterous larvae based on their responses to various ranges of chemicals (c.f. Table 1). Two distinct features of the caterpillar's gustatory apparatus have emerged. (1) Many more cell types can be distinguished than the four taste categories commonly recognised in man. Cells responding to a number (but not all) sugars have been found, but also cells narrowly tuned to glucose, or fructose, or sucrose, or inositol. Cells responding to many feeding deterrents occur side by side with cells responding to only some deterrents. Cation, anion, and water cells have been identified, as well as amino acid cells and glucosinolate cells. Though some of these types may on further analysis turn out to be equivalents, the perception of a multitude of taste cells remains. It thus appears impossible to classify caterpillar taste cells into a few discrete types, though the other extreme with each taste cell being unique in its properties defies our notion of phyletic relationships between caterpillars. (2) There is a pronounced variability of cell types across caterpillar species, the relevance of which may be appreciated especially in the context of sensory coding principles and evolutionary origins, both topics to be discussed later.

It should be noticed that the response specificity of even well-studied cells in most cases have not been tested exhaustively with a wide diversity of compounds. Examples of cells responding to apparently unusual stimuli underline the importance of testing ideally a broad range of chemicals on each cell. There is, as mentioned before, the cell responding to some sugars, several amino acids, and catalpol (BERNAYS & CHAPMAN 2001*a*). The observation of ribose stimulating the inositol cell in *Spodoptera exempta* (DEN OTTER 1992) contrasts with the generally found high specificity of inositol cells. Deterrent cells in particular may be stimulated by very differently structured compounds. Therefore, each gustatory receptor responds to a variety of compounds in a manner that is not constrained by chemical relationships. Nevertheless, the often used and convenient categorization is probably generally valid, although it clearly should not be regarded as implying an absolute and rigid classification.

Taste cells respond primarily to their specific stimuli, but are also affected by compounds which modulate receptor activity, as discussed in the previous section. These modulating compounds are to be regarded as 'latent' stimuli, which form a hidden fraction of the specificity range of a taste cell. Influences of these compounds come to the surface only during tasting complex stimuli as when contacting plant contents (SCHOONHOVEN 1987).

There is evidence that plant volatiles may also stimulate caterpillar taste cells (STÄDLER & HANSON 1975). Because the maxillae move rhythmically in coordination with mandible movements, the sensilla styloconica may be exposed several times per second to the surrounding air in alternation with contacts with plant ma-

terial. Plant volatiles conceivably modulate the sensory pattern elicited by the extruding leaf sap. This aspect needs further investigation to assess its significance for sensory coding.

Table 10. Effects of dietary history on sensitivity of maxillary taste cells. M = medial and L = lateral sensillum styloconicum

Species		Control food	Experimental food	Stimulus	Sensitivity change (%)[a]	Reference[b]
M. sexta	M	Tomato foliage	Art. diet	Tomato leaf sap	+35	(1)
	M	Art. diet	Art. diet + 10 mM inositol	50 mM inositol	–42	(1)
	L	Art. diet	Art. diet + 10 mM salicin	10 mM salicin	–34	(1)
	L	Art. diet	Art. diet + 5 mM caffeine (2 days)	5 mM caffeine	–70	(2)
	L	*Solanum* foliage	Tomato foliage	Solanum leaf sap	+72	(3)
S. littoralis	M	Art. diet	Art. diet + 20 mM nicotine	20 mM nicotine	–50	(4)
	L	Art. diet	Art. diet + 20 mM nicotine	20 mM nicotine	–42	(4)
	M	Art. diet	Art. diet + 0.01 mM azad. (2 days)	0.01 mM azadirachtin	–41	(5)
	L	Art. diet	Sugar-free art. diet (4-12 h)	100 mM sucrose	+170	(6)
	M	Cabbage	Art. diet	1 mM sinigrin	–56	(7)
S. exempta	M	Art. diet	Art. diet + 0.01 mM azad. (2 days)	0.01 mM azadirachtin	–52	(5)
P. brassicae	M	Cabbage	Art. diet	5 mM chlorogenic acid	–36	(8)
	L	Cabbage	Art. diet	5 mM chlorogenic acid	–32	(8)
	L	Cabbage	Art. diet	5 mM proline	–16	(8)

[a]Based on total impulse frequencies per sensillum

[b]Data from (1) SCHOONHOVEN 1969*b*; (2) GLENDINNING *et al.* 1999a; (3) STÄDLER & HANSON 1976; (4) BLANEY & SIMMONDS 1987a; (5) SIMMONDS & BLANEY 1983; (6) SIMMONDS *et al*. 1992; (7) SCHOONHOVEN *et al*. 1987; (8) VAN LOON 1990

CHANGES IN RECEPTOR SENSITIVITY

Chemoreceptors encode stimulus intensity in frequency of impulses which are propagated to the CNS. Curiously, in lepidopteran taste cells this frequency control process does not reflect a fixed and constant sensitivity, but appears to vary with the insect's feeding history. Table 10 summarizes some salient results of experiments in which the sensitivity of one identifiable cell or an assembly of cells has been compared between groups of caterpillars which were fed different food types. Insects reared on different host plants showed highly significant differences in their total impulse frequencies to plant saps. Also insects fed a standard artificial diet containing low levels of a feeding deterrent or phagostimulant (inositol) show desensitisation of the specific receptors for these compounds, whereas conditioning on a phagostimulant-deficient diet sensitised the, in this case, sugar receptor. Apparently this phenomenon is a general property of taste cells in caterpillars since it occurs across species and cell types. Sensitivity changes develop within periods of hours to days. A more detailed analysis of this process in the D cell of *Manduca sexta* revealed that exposure to caffeine reduced its sensitivity to caffeine as well as salicin, but not to aristolochic acid (SCHOONHOVEN 1969*b*, GLENDINNING *et al.* 1999*a*), indicating that different transduction pathways are involved.

In addition to an influence of feeding history many other variables may also alter taste cell sensitivity (BLANEY *et al.* 1986), including age (BLANEY & SIMMONDS 1987*a*), time of day (SIMMONDS *et al.* 1991), satiety level (SCHOONHOVEN *et al.* 1987), and nutritional requirements. Caterpillars which were fed protein-free food showed an increased sensitivity to stimulation with an amino acid mixture, while in insects fed carbohydrate-free food sensitivity to sucrose stimulation was increased (SIMMONDS *et al.* 1992). Obviously, nutrient-specific feedback mechanisms exist which render deprived insects more sensitive to specific food components, a form of 'specific hunger'. Probably nutrients in the receptor lymph, supposedly reflecting haemolymph composition, modulate taste cell responsiveness, as is the case for amino acids in locusts (SIMPSON & SIMPSON 1992) and sugars in blow flies (AMAKAWA 2001).

The phenomenon of sensitivity modulation in response to previous experience or physiological condition, though complicating the search for the sensory code, reveals a new perspective of sensory function. Rather than transmitting a constant and predictable message, the receptors modify the sensory input to the CNS in such a way that several factors, which need to be considered by the CNS when preparing its instruction for behavioural response, have already been taken into account. Evidently, decision processes are not restricted to the CNS, but in-

volve also other components of the neural system, i.e., receptors. This results in a more efficient use of the total neural assembly an insect possesses.

NEURAL INTEGRATION OF SENSORY INPUT

As may be concluded from the previous sections we have good information on sensory input to the CNS and a fair knowledge of behavioural output. This logically raises the question where and how chemosensory input is processed and integrated with other central neuronal activity, such as that associated with satiety level, prior to the initiation of motor output which drives feeding behaviour.

The axons of all maxillary taste receptors project directly without synapsing, into the suboesophageal ganglion (SOG) (KENT & HILDEBRAND 1987, MITCHELL *et al.* 1999). The SOG also provides motor output to those mouthparts immediately involved in the feeding process (BLANEY & SIMMONDS 1987*b*, GRISS *et al.* 1991, ROHRBACHER 1994). It is inferred that much of the central processing of various types of input (including that of the taste cells) takes place in the SOG. However, since inputs from other parts of the CNS, e.g., the frontal ganglion and olfactory lobes, also contribute to feeding behaviour including host-plant recognition, the assumption that a 'feeding centre' is situated in the SOG is still premature. Experimental evidence is badly needed.

The maxillary taste cells show, with the notable exception of the generalist D cells, rather narrow specificity ranges. Most cells generate a neural output that can be correlated in a dose-dependent manner with either acceptance or rejection of their adequate stimuli. Thus chemosensory input guiding feeding preferences consists of positive and negative signals from various chemical stimuli, and changes in the balance of these chemicals may change preference. (It is not *a priori* to be excluded that some cell types have a bimodal effect on food intake: low impulse frequencies lead to phagostimulation and high impulse frequencies cause feeding inhibition). This conflict between positive and negative input is in principle resolved in the CNS, though peripheral interactions have already contributed to the outcome. Thus 10 mM inositol totally counteracts the inhibitory effect of 10 mM caffeine in *Manduca sexta* through a central evaluation of gustatory input (in this case the compounds do not interact at the periphery) (GLENDINNING *et al.* 2000).

From experiments on different caterpillar species it may be concluded that information (as number of spikes per unit of time) from various cells reaching the CNS is here summated algebraically (SCHOONHOVEN & BLOM 1988, SIMMONDS *et al.* 1991). Presumably the cells signalling rejection are connected to a circuit with inhibitory synapses whereas acceptance signals enter circuits with excitatory

synapses. Such a simple model of sensory integration, based on electrophysiological and behavioural data obtained on *Pieris brassicae* larvae (BLOM 1978), appears to explain quite nicely the results of experiments with single chemicals (Fig. 8). When peripheral interactions occur, as commonly observed for mixtures of chemicals or plant saps, the input from different cells will be modified quantitatively, but this will not alter the principle of simple central summation, which still can lead to an appropriate response. In contrast, DE BOER (1993) concludes from behavioural studies employing selective ablations of various sensilla, that feeding on plant material involves a central processing mechanism based on differentially weighting of sensory inputs via different channels, instead of a simple additive procedure. The different opinions are probably due to differences in the methodol-

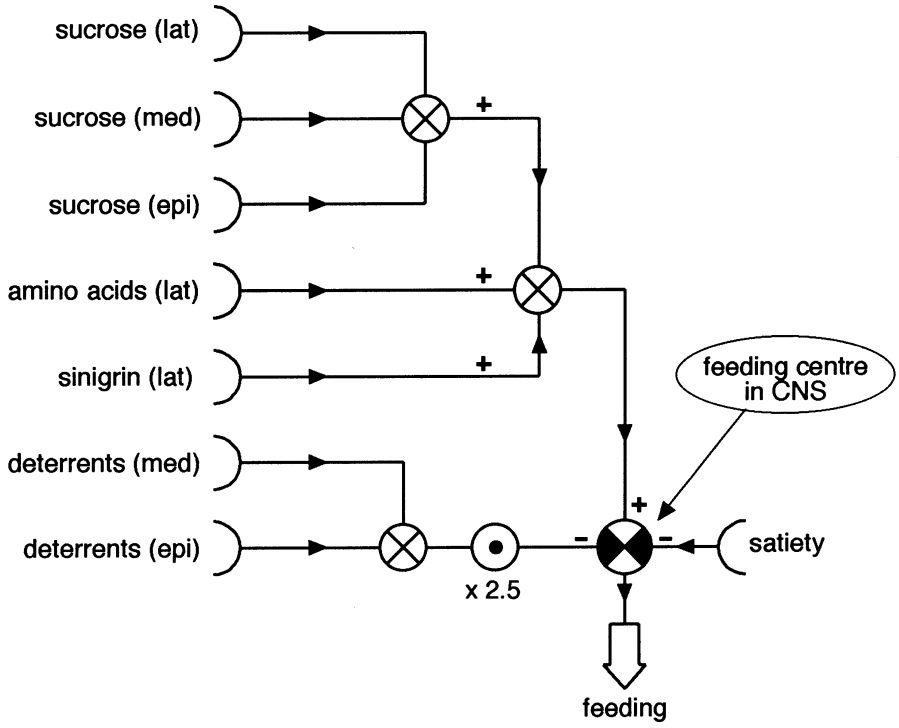

Fig. 8. Model of integration of sensory inputs from taste cells located in the lateral (lat) and medial (med) sensilla styloconica and the epipharyngeal organs (epi) as it might occur in the CNS of *Pieris brassicae* caterpillars. Inputs from the D cells would have negative effects and tend to inhibit feeding. One impulse reaching the CNS from the D cells neutralizes 2.5 impulses originating in one of the phagostimulatory receptors. Satiety has also an inhibitory effect. The balance between negative (inhibitory) and positive (stimulatory) inputs determines whether or not the insect will feed (after SCHOONHOVEN & BLOM 1988)

ogy used. The results from *Pieris brassicae* were based on experiments with single compounds, which are known to stimulate specific cells. The results from *Manduca sexta* were obtained with complex stimuli, i.e., plant material. Conceivably, neural integration depends on a simple arithmetic process, but when additional information via other lines is superimposed on the basic response, the origin of this information becomes important. This would explain the discrepant conclusions reached by BLOM (1978) and DE BOER (1993)

Another approach to unravelling the sensory code starts from impulse patterns evoked by plant saps. A simple comparison of total impulse frequencies in both sensilla styloconica showed that in *Manduca sexta* saps from acceptable plants stimulate the medial sensillum more strongly than the lateral sensillum, in contrast to unacceptable plants, which show a reversed ratio of neural activity (SCHOONHOVEN & DETHIER 1966). A more refined method employing computer techniques was chosen by DETHIER and CRNJAR (1982). These authors detected different temporal patterns in the responses of six receptors when stimulated by three different host-plant species. They suggest that these temporal response characteristics hold additional information for processing by the CNS, and eventual host discrimination.

Food-plant discrimination is submaximal or even absent for some plant combinations after unilateral removal of all chemoreceptors in *Manduca sexta*. DE BOER (1991) deduced from this observation that feeding decisions are based on both quantitative and qualitative aspects of chemosensory input. Unilateral maxillectomy in *Spodoptera exempta* larvae, likewise, produces an intermediate response in length of time before a non-food plant will be accepted, as compared to bilaterally or sham operated insects (MA 1976*b*). Apparently bilateral chemosensory input, rather than representing functional redundancy, provides useful information which is taken into account in the central decision process. The fact that some chemosensory pathways cross over to the contralateral side of the CNS (KENT & HILDEBRAND 1987) may result in the 'feeding centre' in unilaterally ablated insects receiving distorted or incomplete information.

Clearly our insight into the central integration process is still very primitive. It can only be expressed in rather general terms, for instance by saying that insects possess an innate profile of host-plant taste, or by using the metaphor of a 'key-lock' system, in which the key stands for a complex sensory pattern and the lock for an innate profile shaped to only accept neural patterns as elicited by host plants (SCHOONHOVEN 1987). This symbolization is incongruent with the model of simple algebraic summation of impulse frequencies, as this neglects their origins in specific cells. It seems unlikely that information on impulse origin will not be used in central decision processes. Further analysis of total response patterns to

simple mixtures and plant saps, as begun by DETHIER and CRNJAR (1982), in conjunction with behavioural studies, may prove to be a fruitful approach to understanding the mode of operation of the caterpillar's 'feeding centre'.

Discussions on coding in chemosensory systems often focus on three hypothetical codes for the representation of chemical messages sent to the CNS. They are (1) primary tastes exist, (2) taste and smells are represented in an analytical or labelled-line pattern, and (3) taste and smells are represented as a synthetic or across-fibre pattern of neural activity (FRAZIER 1992). It should be realized that these three models are not mutually exclusive and thus may operate within the same gustatory system. From the data presented on taste cells in caterpillars the labelled-line concept is applicable to several identified cell types, e.g., the D cells and sign stimulus cells. However, the coding mode based on across-fibre patterns seems also to operate to some extent in caterpillars and might help to better understand the roles of, for instance, the water and salt cells. Because olfactory cells in *Manduca sexta* larvae have very broad and overlapping response specificities (DETHIER & SCHOONHOVEN 1969, ITAGAKI & HILDEBRAND 1990), here across-fibre patterning must be the operative mechanism.

EVOLUTION

Of all organisms, the insects show the greatest diversity of diets. Among herbivorous species even generalists have marked preferences in diet (BRUES 1946, SCHOONHOVEN *et al.* 1998) and specialists are notorious for the most rigid restrictions. Their feeding habits have evolved amidst an unsurpassed diversity of green plants which harbour, unseen to the human eye, a still vaster diversity of chemicals. This could only happen when the gustatory sense is sufficiently versatile to adapt to the needs of each insect species. Conceivably, differences in food-plant selection between species are based on different central processing principles of invariable sensory inputs, or, alternatively, different feeding habits between species depend on differences in their gustatory systems, each adapted to a particular diet. Both models may also be combined to a third model, which appears to reflect the situation in caterpillars. The sense of taste (and smell), in close interplay with the central processing mechanism, is finely tuned to recognising a specific insect's host plant(s). The decisive role of the CNS is strikingly illustrated in a comparative study of two sister species, *Heliothis virescens*, a generalist feeding on plants from many families, and *H. subflexa*, which is restricted to one plant genus. The differences in diet breadth between the two species could in this case not be attributed to

different properties of their styloconic taste cells and thus must be due to differences in the central processing of sensory input (BERNAYS & CHAPMAN 2000).

The variation in gustatory profiles across species, as exemplified in Table 1, indicates great evolutionary flexibility of the sensory apparatus, which would make it relatively easy for a herbivore to switch to a new food source (provided this is not impeded by other constraints, e.g., nutritional inadequacy). The great inter-individual variation in both sensory responses and behavioural reactions observed under strict standardized experimental conditions, even if this variability is only partly genetic, provides ample opportunity for the evolution of new feeding preferences. The exploitation of new food sources could then result in the development of a new species. There is irrefutable evidence for speciation according to this scenario (MENKEN & ROESSINGH 1998, ROESSINGH *et al.* 1999).

The role of the D cells merits special attention, because host recognition is often primarily determined by the absence of deterrents, as the pioneering studies by JERMY (1958, 1966, 1984) have shown. In silkworms, a classic case of a strictly monophagous insect, mutant strains have been selected with a broader diet than parent lines. The D cell in these strains have lost their sensitivity to some, but not all deterrent compounds tested (ASAOKA 2000). The broad specificity ranges of D cells supposedly depend on the presence at the dendritic membrane of different receptor sites for the perception of different compounds (reviewed in FRAZIER 1992, SCHOONHOVEN *et al.* 1992).

Host-plant switching must be accompanied by a loss of sensitivity of the D cells to compounds which typically occur in the new host plant. This is nicely illustrated in *Yponomeuta rorellus* larvae, which are restricted feeders on *Salix* spp. Their D cells are significantly less sensitive to salicin than those of their sister species. Likewise, *Yponomeuta malinellus*, occurring on *Malus*, is insensitive to the *Malus*-specific compound phloridzin, that stimulates the D cell in eight related *Yponomeuta* species, which reject *Malus* (VAN DRONGELEN 1979). When *Y. cagnagellus* (host: *Euonymus*) was experimentally crossed with *Y. malinellus* (host: *Malus*) the D cells of their offspring showed an intermediate sensitivity to phloridzin (VAN DRONGELEN & VAN LOON 1980).

ASAOKA's (2000) observation of partial insensitivity of the D cell in the silkworm ties in with the demonstration of two excitatory transduction pathways in the D cells of *Manduca sexta* (GLENDINNING & HILLS 1997). In vertebrates, cells responding to bitter substances contain a large repertoire of different taste receptors, linked to gustducin, a G protein implicated in bitter signalling. Some gustducin-linked receptors have also been identified in insect cells (CHANDRASHEKAR *et al.* 2000) and may be operative in the systems just mentioned.

Loss of sensitivity to certain feeding deterrents is one aspect of a change in food-plant preference. Another step is developing a preference for a host-plant compound that usually acts as a deterrent (Fig. 9). Glucosinolates present a well-defined example. These compounds are general feeding deterrents to many insects which do not feed on cruciferous plants. Some polyphagous species which do feed on e.g., cabbage have deterrent cells which respond to sinigrin, but in the presence of sucrose and inositol these cells may be sufficiently inhibited to allow the insect to unreservedly feed on this plant (SHIELDS & MITCHELL 1995). In insects specialized on crucifers the D cell has become fully unresponsive to glucosinolates. Instead separate cells are now sensitive to these host-plant specific chemicals (SCHOONHOVEN 1967). Were the latter cells at one time D cells whose input in the CNS underwent a sign transformation at the synaptic level? Or did "loose receptor sites" (TALLAMY *et al.* 1999) on glucose-sensitive cells begin to accept glucosinolates as novel stimuli? With respect to the possibility of central nervous sign transformation, changes at the integrative level are known to occur. There is a silkworm strain that has normally functioning D cells, but nevertheless exhibits an expanded food-plant range (ISHIKAWA *et al.* 1963). There is no *a priori*

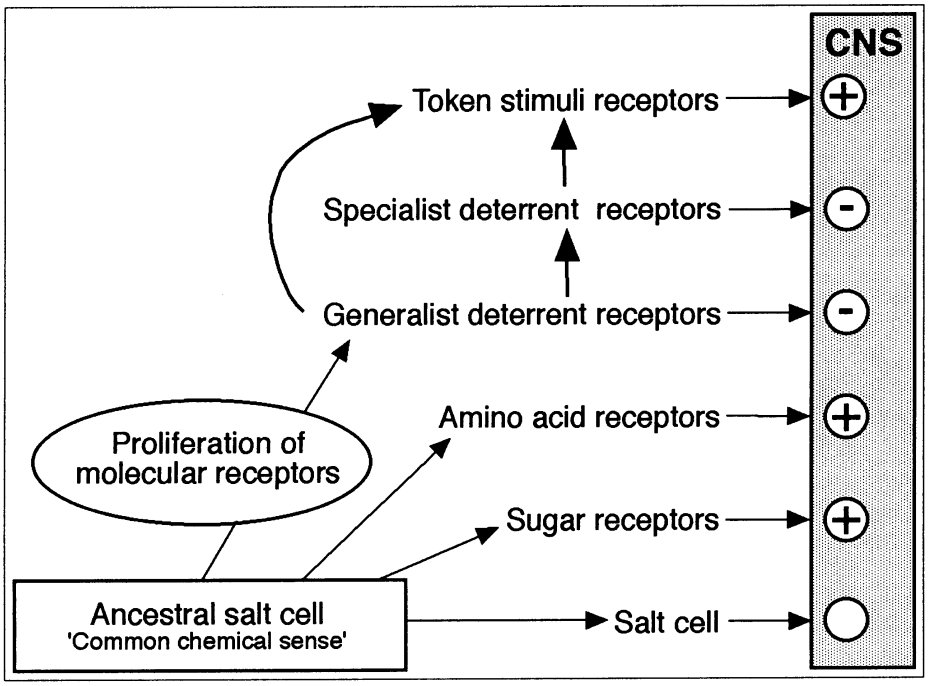

Fig. 9. Evolution of gustatory receptors in specialist herbivores

reason to expect that changes in the central processing mechanism are more difficult to realize than peripheral changes.

JERMY, in a thought-provoking review, stresses the prime role of heritable changes of chemoreceptors in insect evolution. He points out that evolution of host-plant specialization is not primarily due to ecological selective forces, but rather to a behavioural change governed by the insect's ability to recognise new potential food plants as a result of mutations (*sensu lato*) that change the function of an insect's sensory system. Only then selection starts and the new genotype may become successful if it can tolerate the many ecological and physiological constraints to which it will be subjected (JERMY 1993). The importance of this type of evolutionary mechanisms may once more be stressed by citing the concluding sentences of BERNAYS and CHAPMAN's book on host-plant selection by phytophagous insects (1994, p. 284): "The evolution of behavioral patterns is an evolution of properties of the nervous system. The precise details of how insects perceive the plant world, how they channel and integrate information, and finally how they behave in response to the information, will provide the details necessary to develop ideas further on how the behavior of host-plant choice in insects may have evolved."

CONCLUSIONS AND FUTURE DIRECTIONS

Lepidopterous larvae with their relatively simple gustatory sense offer an ideal system to analyse peripheral and central mechanisms governing feeding behaviour. Considering the bilateral eight-neuron taste system it is striking that subtle taste discrimination, evidently present in many caterpillar species, is allowed by so few cells. The lateral and medial sensilla have similar functionalities but yet show differentiation in their specificity ranges for both stimulant and deterrent receptors. In only few cases have the consequences for discrimination capacity been studied in detail (VAN LOON & SCHOONHOVEN 1999). Here we focussed on the maxillary sensilla styloconica, but it must be stressed that maxillary palp receptors as well as epipharyngeal sensilla undoubtedly contribute to even more subtle discrimination power (DE BOER & HANSON 1987, DE BOER 1993, GLENDINNING *et al.* 1998, VAN LOON unpubl.). The striking diversity of taste cell types, reflecting a great adaptability to the many plant substances nature offers, forms a crucial aspect of the role of herbivorous insects in terrestrial ecosystems.

The multitude of plant compounds acting as deterrents and recognised by JERMY (*in litt.*) as pivotal in many insect-plant interactions, stimulate some taste cells with broad, though well-defined specificity ranges. A molecular analysis of

their receptor sites is within sight, which aims at clarifying how these cells practise their impressive chemosensory repertoire, as is now being made in *Caenorhabditis elegans*, a nematode which with 20–30 chemosensory neurons can detect hundreds of chemicals (TROEMEL 1999).

Although the sense of taste in caterpillars has been studied during the past 40 years by just a few research groups, a clear picture has emerged, as the information presented in this paper shows. Of course intriguing and important questions remain to be solved. For instance, it is difficult to understand why inositol receptors receive the conspicuous position exhibited in most caterpillar species. These cells, from which recording is usually remarkably easy, merit further attention. Deterrent cells, in view of their clear-cut role in preference and rejection behaviour, are also most interesting elements of caterpillar taste systems. A comparative study on sensitivity thresholds in phylogenetically related monophagous and polyphagous species could answer the question whether diet breadth is related to receptor sensitivity (cf. Table 9) or depends on central decision-making processes in food-plant acceptance behaviour. Deciphering sensory codes from recordings of stimulations with plant saps is another line of research which will shed light on the mechanism of food selection behaviour; if the caterpillar brain can decode the sensory message, our computers should be able to do it as well.

The beguiling simplicity of chemoreception in caterpillars shrouds a captivating complexity of receptor diversity, neural interactions, temporal characteristics, and peripheral memory. The rich harvest of some decades of research opens exciting vistas on behavioural analysis in an ecological and evolutionary context, as well as on the molecular basis of the most universal sense in animals: chemoreception.

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