**The neurobiology of gustation in insect disease vectors:
progress and potential**

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Summary

For insect vectors of human diseases, mealtimes are a key moment of infection. Understanding how and when such species decide on what to feed is both an interesting problem in sensory neurobiology and a source of information for intervention of these behaviors to control spread of infectious agents. Here I review the current knowledge of the molecular and cellular mechanisms of gustation in insect disease vectors, covering blood-feeders as well as scavengers that spread pathogens indirectly. I also consider how these behaviors are modulated over short and long timescales, and describe efforts to artificially modulate them. Though a relatively nascent field, gustatory neurobiology in insect vectors has much promise for future fundamental discoveries and practical applications.

Introduction

Transmission of pathogens from insect vectors to humans (or other animals) – and vice versa – occurs most directly through bites by blood-feeding species such as mosquitoes [1], sand flies [2], fleas [3] and triatomine (or “kissing”) bugs [4] (Table 1). Disease spread by insects can also be more indirect: blowflies and cockroaches, for example, may inadvertently inoculate our food with harmful microorganisms carried on or in their bodies [3] (Table 1). Understanding on what, when and how insect vectors feed is therefore not only of interest to chemical ecologists and sensory neurobiologists, but also has enormous potential for the development of intervention strategies to reduce disease transmission.

Investigations of food-seeking behaviors by insect vectors over the last century have revealed enormous diversity in feeding preferences [5-7]. Some hematophagous species are highly restricted in their source of blood meals: the body louse *Pediculus humanus humanus* is an obligate parasite that feeds only on humans [8]. A few mosquito subspecies are also highly specialized for human blood (e.g., *Anopheles gambiae* sensu stricto and *Aedes aegypti aegypti*), while others display a broader host range, such as *Culex* species, which are anthropophilic in the tropics but feed on birds and various mammals in other

regions [1,9]. Regardless of the source, blood meals are essential for females of these species to produce eggs, likely due to their high protein content [5]. In non-hematophagous species, the propensity to spread pathogens is, for some, like blowflies, due to their feeding on microbe-rich carrion and dung; for others, such as cockroaches, it may be linked to their omnivorous diet and indiscriminate foraging. Feeding behaviors, particularly in hematophagous insects, are also highly plastic, such as the profound suppression of feeding exhibited by female mosquitoes for several days after a blood meal [1].

Despite the central role of gustatory behaviors for survival and reproduction of insect disease vectors, knowledge of the underlying neurobiology is still fragmentary, and lags some way behind that of odor-guided behaviors [10,11]. However, recent work, employing genomic, molecular, physiological, pharmacological and neurogenetic approaches has begun to provide insights into the molecular and neuronal basis of tasting and feeding in these species. In this review, I consider some of these advances, and discuss open questions and future priorities.

Gustatory sensory organs and neurons: variations on common themes

The sensory structures that insects use to assess non-volatile chemicals from food/host sources have been studied anatomically and functionally for decades in many different species, most famously reviewed in Dethier's monograph on the blowfly *Phormia regina* [6]. In the last 15 years, systematic investigations in the (non-vector) vinegar fly *Drosophila melanogaster* have extended the insights first obtained in other insects to provide a broad (though still incomplete) understanding of chemical detection and discrimination by the gustatory system [12]. Despite the enormous diversity of salient chemicals for different species, and overt morphological distinctions in insects' mouthparts [5], there are many commonalities in the properties of taste sensors: chemicals are detected by gustatory sensory neurons housed within cuticular hairs (sensilla), which decorate various appendages including the labellum of the proboscis (Figure 1a-b), legs, wing margins, and ovipositor; other sensilla line the pharyngeal lumen through which food passes prior to digestion [13,14]. In some species, such as the triatomine bug *Rhodnius prolixus*, sensilla on the antenna (an olfactory organ in

most insects) also appear to contribute to non-volatile chemical detection [13]. Gustatory sensilla have a pore at their tip to allow movement of chemicals from the environment into the sensillar lymph fluid that bathes the ciliated endings of sensory dendrites. Individual sensilla house stereotyped clusters of neurons (typically 2-5), which exhibit physiological sensitivity to a specific class of appetitive (e.g., sugars, low salt concentrations, water) or aversive (e.g., bitter compound/high salt concentrations) stimuli [6,12]. These properties led to an initially simple model of taste coding where a limited number of broadly-tuned sensory channels (e.g., “sweet” and “bitter” neurons) are tightly coupled to acceptance or rejection behaviors.

Recent efforts in mosquitoes have started to examine this model in more detail through comparisons of responses to a panel of ecologically-relevant sugars. Within the labellar sensilla analyzed, “sweet” neurons were found to be most sensitive physiologically to sucrose and melezitose, as well as mixtures of fructose and glucose (but not these monosaccharides alone), with concomitant behavioral preference for – and survival of animals on – these sugars [15,16]. Such observations suggest the existence of refined mechanisms for perception and discrimination of these compounds, as is the case in *D. melanogaster* [17] and blowflies [6]. Bitter compound detection also appears to be complex in mosquitoes, involving activation of the “bitter neuron” and inhibition of the physiological sensitivity of both the “sweet” and water neurons [15,18,19], as well as contributions from internal gustatory organs [20]. In triatomine bugs, behavioral studies reveal their capacity to discriminate different bitter tastants [21]. Moreover, these compounds can inhibit feeding behavior both prior to ingestion, through activation of antennal bitter neurons, and after their first mouthful, potentially through pharyngeal sensilla [13]. Thus, contrary to simple models, these observations imply that individual tastant classes act through multiple neural pathways.

It remains unclear whether acceptance and rejection of sweet and bitter tastants in blood-feeding insects is related to host choice rather than, for example, assessment of carbohydrate-rich plant nectar. There are anecdotal claims that humans with higher skin secretions of sugars are more attractive to mosquitoes, but such a link (if true) could be due to indirect influences, for example, the skin microbiota, which are a source of many odiferous molecules

[22]. Intravital imaging of feeding in *An. gambiae* has revealed prolonged probing of skin by the proboscis both prior to and after penetration [23], suggesting that both superficial and intradermal cues are assessed. However, the relevant signals and sensory pathways are unclear, and receptors for known blood-derived feeding stimulants, such as ATP for *Ae. aegypti* [24], have not been identified. This might be because blood normally comes into contact only with internal gustatory sensory organs (as it is ingested), which are much harder to analyze by electrophysiological methods [20]. It is also possible that olfactory attractants, such as L-lactic acid [25], are sensed by the gustatory system. Finally, the contribution of chemosensory sensilla on other appendages, such as legs, wing margins [26,27] and the ovipositor [28] to feeding or other behavioral decisions, is poorly understood.

Divergent (and predominantly orphan) taste receptors

Molecular receptors for taste stimuli are also starting to be characterized, building upon their initial identification in *D. melanogaster* [12]. These receptors comprise two large repertoires of presumed ligand-gated ion channels, the Gustatory Receptors (GRs) and Ionotropic Receptors (IRs), as well as certain members of the Pickpocket and Transient Receptor Potential families [12]. Perireceptor gustatory-expressed “Odorant” Binding Proteins (OBPs) may also function in initial chemical recognition [29], but their role in sensory transduction remains unclear.

Within the increasing deluge of genomes and transcriptomes of insect disease vectors, homologous chemosensory receptor and OBP genes have been eagerly sought [30-37]. Comparative genomic and phylogenetic analyses with *D. melanogaster* receptors of known function have allowed predictions about roles of particular receptors, for example, of a clade of GRs that detect sugars [38]. Notably, these receptors appear to be lacking in tsetse flies [36], which may be related to the obligate hematophagous nature of both females and males in these species (contrasting with mosquitoes, where males feed on nectar). For many taste receptors, clear orthologous relationships have been difficult to define [30,39], which probably reflects the evolution of distinct taste preferences of different species.

Tissue-specific RNA-sequencing analyses in mosquitoes have also started to reveal in which chemosensory organs different receptor genes are expressed [40-43], although cellular level (co)expression properties have not yet been described. Furthermore, despite phylogenetically-guided predictions, soberingly few taste ligand/receptor relationships have been defined for GRs and IRs. This is in large part due to the inability to express these receptors in heterologous systems where they can be functionally interrogated. The lack of progress stands in contrast to the relative ease of deorphanization of Odorant Receptors (ORs) [44,45], and implies that taste receptors function only in very specific (but still-undefined) combinations and/or require additional co-factors or a particular cellular environment. Recently, an antennal neuron population in *D. melanogaster* was proposed as a potential “GR decoder” system, in which heterologous expression of *An. gambiae* GR25a (an ortholog of the *D. melanogaster* fructose receptor GR43a [46]) was observed to confer sugar sensitivity [47]. However, it is unclear whether the ligand specificity of receptors heterologously expressed in this decoder always faithfully reflects their endogenous properties [48].

It is important to recognize that the anthropomorphic definition that distinguishes gustatory and olfactory pathways in mammals [49] is only partly relevant to insects. Although olfaction is mediated by neurons in the antennae and maxillary palps that project to the antennal lobe, the olfactory receptor repertoires (ORs and a subset of IRs) are molecularly related to the two main taste receptor families [50,51]. The strict segregation of taste and olfaction is further blurred in mosquitoes (and probably many other insects [52]): in *An. gambiae*, neurons housed in the proboscis can respond to volatile chemicals (i.e., without direct contact with the stimulus source) [53] (although see [16]), and a subset of ORs is expressed in this organ in several mosquito species [42,53-56]. Expression of some *Ae. aegypti* ORs is also detected in the legs [42]. Intriguingly, ORs that can detect certain blood volatiles (amongst other non-blood chemicals) are found in the piercing/sucking stylet of *Ae. aegypti* [55]. However, it is unclear whether these receptors sense blood before or only after the stylet has penetrated the skin, and equivalent OR-expressing stylet neurons have not been observed in *An. gambiae* [57]. Finally, new transgenic neural tracing tools in *An. gambiae* have revealed that OR-expressing neurons in the proboscis project to the primary taste center, the subesophageal zone (SEZ) [57], and not – as

previously suggested [53] – the antennal lobe (Figure 1c). However, because of the paucity of knowledge of the roles of taste-organ expressed ORs *in vivo*, it remains unclear whether such anatomical projections underlie sensory integration of olfactory and gustatory stimuli in the SEZ or, perhaps more simply, that these ORs function essentially as taste receptors.

Plasticity in feeding behaviors over multiple timescales

As in all animals, the feeding behaviors of insect disease vectors are flexible, adapting on both short and long-timescales according to the internal state of the individual and the selection pressures in the environment. Beyond the basic scientific interest, understanding these processes is of substantial epidemiological significance, because of their influence on the temporal and spatial dynamics of pathogen transmission.

Mosquitoes provide a striking example of cyclical feeding behavior: in female *Ae. aegypti*, for example, following a blood meal, host-seeking and blood-feeding are suppressed for three days, permitting egg production, fertilization and oviposition, after which a new round of host-seeking and blood-feeding begins [1]. The orchestration of this dramatic switch in feeding behavior was found, nearly 50 years ago, to depend upon a humoral substance [58]. Subsequently, Head Peptide-I (HP-I), a short neuropeptide F-related molecule, apparently present in the nervous system, was proposed as a key molecular component of this substance [59]. However, recent expression analyses did not detect HP-I in the brain (but rather in the male accessory gland, from where it might be transferred to females during copulation) [60]. Moreover, an *in vitro* pharmacologically defined neuropeptide Y-like receptor for HP-I, NPYLR1, is dispensable for any feeding-related behaviors [61]. Thus, the contribution and site of action of HP-I are unresolved.

While the precise determinant(s) of the dramatic behavioral switch are unknown, neurotranscriptomic analysis of female *Ae. aegypti* before and after a blood meal has revealed co-incident up- or down-regulation of many hundreds of genes [42]. (Gene expression changes pre- and post-blood feeding have also been described in *An. gambiae*, in more limited analyses of olfactory organs [62,63]). These modulated genes include those encoding chemosensory

receptors, neuropeptides and neurotransmitters, consistent with a substantial reweighting of gustatory and olfactory detection and processing capacities of these animals. However, the relationship of these transcriptional changes with behavioral phenotypes remains to be determined.

Shorter-term modulation of gustatory-dependent behaviors in both sexes is likely to be prevalent (e.g., circadian control [64]). In addition, observations of non-associative and associative learning of taste stimuli in mosquitoes [65,66] and triatomine bugs [13,21] indicate that these animals also rely on prior experience to guide host choice. The underlying molecular mechanisms are largely obscure, but probably involve conserved neurotransmitter and neuropeptide signals, such as serotonin [67] and allostetins [68], acting in central circuits. Recent studies also hint at gustatory sensory neurons being a direct target for neuromodulation: in *Ae. aegypti*, a receptor for kinin-family neuropeptides (which have multifunctional roles in the alimentary canal) was found to be expressed in labellar and leg taste neurons, and pharmacological treatment (through topical application or feeding) of non-degradable kinin analogs suppressed sugar detection and feeding [69]. The natural context for the action of this peripheral neuropeptide signal is unclear, but these observations are consistent with evidence from *D. melanogaster* that sensory neurons are an important target for pathways modulating taste perception and behavior [12].

Beyond endogenous control mechanisms, one intriguing way in which disease vector feeding (amongst other behaviors) might be modulated is by the infective agent itself. For example, in sandflies (*Lutzomyia longipalpis*), the protozoan parasite *Leishmania* causes the insect to persist with blood feeding and to bite multiple mammalian hosts [70]. In mosquitoes, diverse influences of the parasite have been reported [71]. In some cases, infection is simply detrimental to the insect host (and parasite): *Culex pipiens*' starvation resistance is compromised by the presence of *Plasmodium* [72]. In *Anopheles* mosquitoes, the presence of the infectious sporozoites (but not the non-infectious oocysts) can increase female probing and biting persistence, while decreasing blood meal size (which may lead to multiple feeds on different host individuals) [71]. While it is appealing to credit the parasite with devious manipulative abilities, it is unclear to what extent these effects are truly adaptive (representing "extended phenotypes"); complementary field studies are required to determine the

significance of the observations made in controlled laboratory assays to the efficiency of parasite transmission in nature. Moreover, some changes in mosquito feeding can also be induced with heat-killed *E. coli*, potentially linked to alterations in gut insulin signaling [73-75]. These observations raise the possibility that many of the consequences of parasite infection – though potentially beneficial for the parasite – may simply be the by-product of a general immune response to microbial infection.

Insects can also exhibit plasticity over evolutionary time scales – facilitated by their relatively large population size and short generation time – which allows them to adapt their behavior to novel selective pressures in the environment. Peripheral chemosensory systems display particularly strong genetic signatures of selection over short timescales in insects [76]. In the olfactory system, the evolution of preference of *Ae. aegypti* strains to selectively target humans is linked to increases in the expression and sensitivity of an OR for sulcatone, a particularly abundant component of human odor [77] (although mosquito behavioral responses to this volatile appear complex [78]). In the gustatory system, the species-specificity of GRs and IRs strongly implies adaptation of taste preferences to particular ecological niches [30,39], but the dearth of knowledge about specific ligand/receptor relationships (as well as limited knowledge of the chemical ecology of species-specific taste stimuli) precludes further insight.

One fascinating example of sensory evolution at the neuronal level in the gustatory system was discovered in the cockroach *Blattella germanica*. Within natural populations, “glucose-averse” strains have been identified, which exhibit physiological sensitivity to glucose (but not other sugars) in the canonical bitter-sensing taste neuron [79,80]. The molecular basis for this physiological trait is unknown, but is thought to reflect a broadening of tuning of bitter-neuron receptors, rather than “ectopic” expression of sugar-sensing GRs in these cells [79]. Although this trait comes at fitness cost – due to impaired nutritional intake [81] – this unusual sensory coding mechanism enables cockroaches to avoid human-laid toxic baits in which glucose is used as a primary phagostimulant. Recent anthropogenically-imposed selection pressures may therefore have driven the global spread of glucose-averse cockroach populations [82].

Manipulation of feeding: desired and undesired effects

One motivation to study the chemosensory biology of insect disease vectors is to allow the development of rational strategies to manipulate host feeding and disease spread. As introduced above, attractive sugar toxic baits (ASTBs) represent a common approach [83], which relies on most vectors' innate feeding on sweet substances to induce their consumption of toxins. Beyond poisoning insects, the most widely used chemical manipulator of their behavior is *N,N*-Diethyl-*meta*-toluamide (DEET), which was developed in a systematic effort more than 50 years ago; many others have been discovered serendipitously [84,85]. For any of these compounds, their mechanism of action – and even whether they all act as “repellents” – remains incompletely understood, but it appears that the most effective, such as DEET, operate on multiple sensory pathways [84,85]. DEET has important effects in the vapor phase, but precisely how it influences the olfactory system is still-debated [84-88]. Soon after its initial development, however, DEET was also shown to be a direct blood-feeding deterrent for *Ae. aegypti* [89]; this finding was later generalized to other commonly-used “repellents” and different mosquito species [90], as well as to sugar-feeding behavior in *D. melanogaster* [91]. Electrophysiological analyses revealed DEET (and certain other repellents) could activate bitter-sensing labellar neurons in diverse mosquitoes and in *D. melanogaster* [18,19,91,92]. Moreover, genetic studies in *D. melanogaster* demonstrated that the physiological and behavioral responses to DEET in the gustatory system depend, in part, upon specific GRs [91]; how these receptors are targeted by this chemical is unknown. These observations support a model in which DEET (and perhaps other repellents) act by stimulating aversive gustatory pathways that counteract the activity of appetitive gustatory channels. However, it is unclear how easily information on the impact of DEET on sugar-feeding behavior in *D. melanogaster* can be extrapolated to mosquitoes, where skin landing and blood consumption are likely to involve a more complex set of positive gustatory cues and chemosensory appendages.

While a better understanding of taste-evoked behaviors in disease vectors might enable development of more effective, selective, and safer repellents, artificial application of chemicals can of course have unanticipated consequences

in nature. In some cases, as in cockroaches' response to toxic baits described above, species may adapt and subvert our efforts [79]. In other cases, our interventions cause serious ecological damage, as is the case for the neonicotinoid family of insecticides, whose widespread use has been linked to devastating impact on important pollinators such as honey bees [93]. Unexpectedly, sublethal doses of neonicotinoids (in sugar solutions) promote feeding preferences by bees [94], even though their consumption ultimately disrupts learning, navigation and motor function. The preference does not appear to be due to sensory detection of these compounds [94], suggesting a central site of action of neonicotinoids. Importantly, this implies that bees (unlike cockroaches' avoidance of poisoned baits) may be unable to rely on peripheral gustatory mechanisms to avoid ingesting these toxic chemicals.

Conclusions

Despite the broad appreciation of gustatory-related behaviors in diverse insect vectors, our current knowledge of the underlying neurobiology remains quite superficial, and – as illustrated here – often dispersed across many different species. Key future priorities should include comprehensive analyses of the physiological responses of gustatory neurons and the *in situ* expression patterns of candidate taste receptors, as well as the development of heterologous systems for receptor deorphanization. Comparative genomic and functional data across closely-related species with distinct feeding preferences should help researchers home in on important sensory pathways. Moreover, identification of receptors for ecologically important phagostimulants will provide us with targets for potential pharmacological manipulation in nature.

Encouragingly, the establishment of genetic tools to visualize and manipulate neural circuits in several disease vectors – notably mosquitoes – now makes it conceivable to derive causal relationship between gustatory cues, neural representations and behaviors. Genetic approaches will also be essential to cleanly dissect the relative contributions of gustatory, olfactory and other sensory inputs to feeding behaviors, as well as for interrogating the poorly understood neuromodulatory mechanisms by which internal state and experience influence them.

Finally, it will be interesting to expand these investigations to non-volatile chemical signals that control social interactions (e.g., cuticular hydrocarbons [95], which function as sex pheromones in drosophilids [96]) or oviposition site selection [97,98]. Such behaviors are equally important for the success – and so devastating impact – of insect disease vectors.

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Legends

Table 1. Insect disease vectors

A non-comprehensive overview of the insect vectors discussed in this article. “Biological vectors” are those in which the infectious agent must spend part of its life cycle within the insect host; “mechanical vectors” are passive carriers of the infectious agent [3]. Host ranges may vary within species (e.g., *An. gambiae* and *Ae. aegypti*; see text). Genus names are given for bacteria where multiple species may be pathogenic.

Figure 1. Chemosensory organs and neurons in *Anopheles gambiae*

(a) Light sheet fluorescence microscopy image of the head of a female *An. gambiae* showing the main chemosensory organs. Some chemosensory neurons (in both olfactory and gustatory appendages) are transgenically labeled with GFP (green) using the Q-system (genotype: *Orco-QF2, QUAS-mCD8:GFP*) [57]. Image kindly provided by Olena Riabinina and Christopher Potter (Johns Hopkins University School of Medicine).

(b) Scanning electron micrograph showing a dorsal view of the extremity of the proboscis of a female *An. gambiae*. Scale bar, 50 μ m. Reproduced from [15] under a Creative Commons license.

(c) Top: Confocal image of a whole mount brain of a female *An. gambiae* (*Orco-QF2, QUAS-mCD8:GFP*) – stained with anti-GFP (green) and the neuropil marker nc82 (magenta) – showing the projection of these neurons to the primary olfactory center (antennal lobe; neurons from the antennae and maxillary palps) and the gustatory center (subesophageal zone; neurons from the proboscis). Higher magnification images of the chemosensory centers are shown below. Reproduced from [57] under a Creative Commons license.

Table 1. Insect disease vectors

Species	Common name	Vector type	Blood meal source	Parasite (disease)
<i>Anopheles gambiae</i>	malaria mosquito	Biological	mammals, birds	<i>Plasmodium</i> (malaria)
<i>Aedes aegypti</i>	yellow fever mosquito	Biological	mammals, birds	yellow fever virus (yellow fever), dengue virus (dengue fever), Zika virus (Zika fever, microcephaly)
<i>Culex pipiens</i>	common house mosquito	Biological	birds, mammals	West Nile virus (West Nile fever); Japanese encephalitis virus (Japanese encephalitis), Zika virus (Zika fever, microcephaly)
<i>Lutzomyia longipalpis</i>	sandfly	Biological	mammals	<i>Leishmania</i> (Leishmaniasis)
<i>Pediculus humanus humanus</i>	body louse	Biological	humans	<i>Rickettsia prowazekii</i> (epidemic typhus), <i>Borrelia recurrentis</i> (relapsing fever)
<i>Rhodnius prolixus</i>	triatomine/kissing bug	Biological	mammals, birds	<i>Trypanosoma cruzi</i> (Chagas disease/American trypanosomiasis)
<i>Blattella germanica</i>	cockroach	Mechanical	N/A	<i>Salmonella</i> (salmonellosis), <i>Streptococcus</i> (pharyngitis etc.)
<i>Phormia regina</i>	blowfly	Mechanical	N/A	<i>Salmonella</i> (salmonellosis)