Chemosensory receptors in the tobacco hawkmoth *Manduca sexta*

Dissertation

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Introduction

Chemosensation, the sense of smell and taste, allows animals to assess the chemical properties of their environment. They use it to identify food sources, avoid harmful substances, find mating partners, escape from predators and to find places suitable for their offspring. Understanding the chemosensory system means, to understand how animals assess their environment, such as: Which chemicals are attractive, and why? How do animals find their mating partners? What do they like to eat, what to avoid. Insects are a suitable model to study chemosensation because of the following: Their chemosensory circuits in the nervous system are easy accessible and well-studied. Insects are adapted to nearly every ecosystem on our planet. Finally, they have an important impact on human life in many ways; from agricultural pests to parasites and disease vectors, from pollinators to food sources. We employed the tobacco hawkmoth *Manduca sexta* (Lepidoptera: Sphingidae) to study the molecular basis of chemosensation in the context of the insect's environment. *M. sexta* served as a model organism in several biological disciplines like behavioral research, immunology, electrophysiology, biochemistry and chemical ecology. There is plenty of information available about this insect in regards to the ecological context as well as morphology and electrophysiology of the chemosensory system and olfactory guided behavior. In this dissertation, we established the molecular basis of chemoreception and formulated hypotheses. More specifically, we investigated the influence of the host plants on the larvae and oviposition in the females.

Our model organism, *M. sexta*, and its environment

The natural habitats of *M. sexta* are arid regions from South to North America. Adult moths feed on nectar and thereby pollinate their host plants. The larvae of *M. sexta* are specialized to feed on plants of the nightshade family like *Nicotiana* and *Datura*. Especially the interaction between *M. sexta* and *Nicotiana attenuata* has been studied in detail (Baldwin, 2001). If the plant is attacked by herbivores such as *M. sexta* then the plant produces high amounts of a toxic alkaloid, nicotine, as well as protease inhibitors to deter herbivores from feeding on the plant (Pohlon and Baldwin, 2001; Steppuhn and Baldwin, 2007). Additionally, the plant emits volatile compounds that can attract predators of *M. sexta* (Kessler and Baldwin, 2001). Of course, *M. sexta* larvae are not defenseless: They can tolerate high amounts of alkaloids like nicotine in their host plants (Wink and Theile, 2002), and female moths avoid ovipositioning on attacked plants (Baldwin, 2001; Kessler and Baldwin, 2001).

The chemosensory tissue

An advantage of our model system *M. sexta* is the rich information about the morphology of the chemosensory system. The main olfactory organ of an adult moth is the antenna. It consists of three parts, namely scape, pedicle and flagellum. The flagellum is subdivided in about 80 'little rings', which are collectively called the annuli. Each annulus is covered with hair like structures, the sensilla. The *M. sexta* antennae are dimorphic: in cross section female antennae have are oval, male antennae are key hole shaped. Male antennae bear long trichoid sensilla which can be seen with the naked eye (Keil, 1989). The male trichoid sensilla are arranged in a u-shaped pattern (Keil, 1989) while the female trichoid sensilla are much shorter and do not have this u-shape distribution (Shields and Hildebrand, 1999a). Next to the trichoid sensilla there are basiconic and coeloconic sensilla which are olfactory as well (Shields and Hildebrand, 1999a, 1999b). Every sensillum houses one to five olfactory sensory neurons (OSNs) (Shields and Hildebrand, 1999a, 1999b) whose dendrites extend into the sensillum shaft that is filled with the sensillum lymph (Keil and Steinbrecht, 1984). Olfactory sensilla have pores where the odor molecules can enter (Keil and Steinbrecht, 1984). In the aqueous sensillum lymph there are odorant binding proteins (OBPs), which are assumed to carry the often lipophilic molecule to the receptors on the dendritic membrane of the OSN (Vogt and Riddiford, 1981). The axon of the OSN synapses in a specific part of the brain, the antennal lobe which has a glomerular structure (Strausfeld and Hildebrand, 1999). All axons of OSNs expressing a certain chemoreceptor converge into the same glomerulus and synapse there onto local interneurons and projection neurons which send their axons into higher brain centers (Couto et al., 2005; Vosshall et al., 2000). Therefore the number of glomeruli correlates with the number of different kinds of OSNs (Vosshall et al., 2000).

The major gustatory organs on the head of adult *M. sexta* are the antennae and the proboscis. On the *M. sexta* antenna are chaeticonic sensilla (Lee and Strausfeld, 1990), which are gustatory on *Heliothis virescens* (Lepidoptera: Noctuidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae) antenna (Jiang et al., 2015; Jørgensen et al., 2006). There are styloconic sensilla on the *M. sexta* proboscis (Reiter et al., 2015) which are gustatory in *H. virescens* (Jørgensen et al., 2006). Gustatory neurons of the proboscis and the antenna extend their axons into a brain region called the ipsilateral subesophageal zone (Jørgensen et al., 2006; Reiter et al., 2015).

Additionally, it is known from some adult Lepidoptera that they employ tissues like the tarsi and the female ovipositor for chemosensation, especially linked to the oviposition behavior

(Renwick and Chew, 1994). *M. sexta* has putative chemosensory sensilla on the tarsi and the ovipositor (Eaton, 1986; Kent and Griffin, 1990). However, their function in chemosensation is not confirmed in *M. sexta* so far.

The main olfactory organ of the larvae is the antenna. It has three segments with olfactory sensilla on the second and third segment (Dethier and Schoonhoven, 1966). Additional olfactory sensilla are found on the maxillary palp of caterpillars (Dethier and Schoonhoven, 1966). The OSNs project to a brain region called the larval antennal center where they synapse on local interneurons and projection neurons (Itagaki and Hildebrand, 1990). Gustatory sensilla are located on the maxillary palp and the galea of larvae (Dethier and Schoonhoven, 1966). Gustatory neurons project into the ipsilateral subesophageal ganglion of the larva (Kent and Hildebrand, 1987).

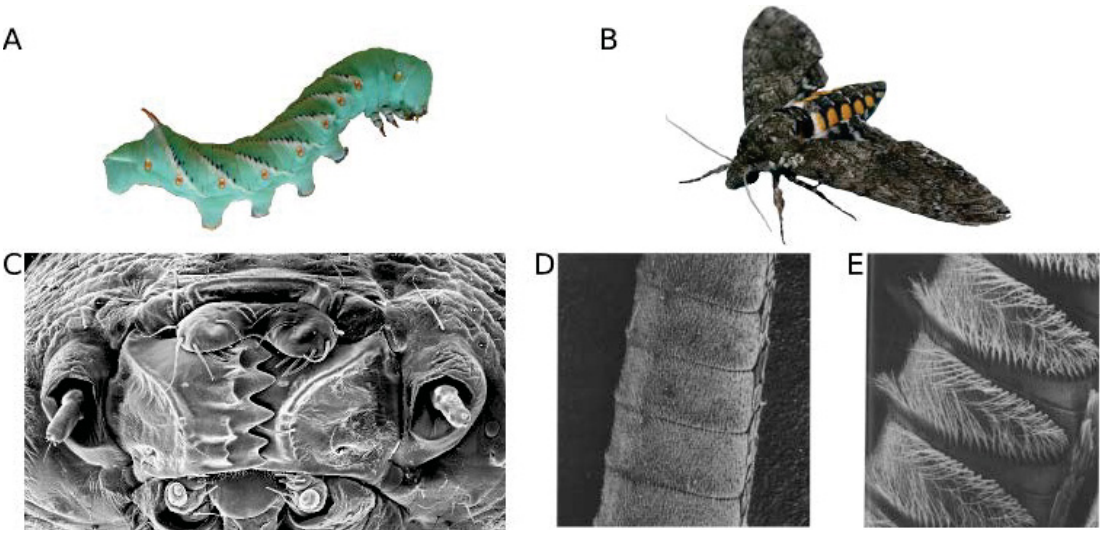

Fig.1: Structure of chemosensory organs of *M. sexta*. (A) Picture of a caterpillar. (B) Picture of an adult hawkmoth. (C) Scanning electron microscopy image of a larval head (picture source: jeremyswan.com). (D) Scanning electron microscopy image of a female antenna (picture source: Keil, 1989). (E) Scanning electron microscopy image of a male antenna (picture source: Keil, 1989).

Insect chemosensory receptors

The major classes of chemosensory receptors in insects are the gustatory receptors (GRs), olfactory receptors (ORs) and ionotropic receptors (IRs). Insect ORs have been first identified in *Drosophila melanogaster* (Diptera: Drosophilidae) by their antennal expression and their predicted structure: they have seven trans-membrane domains like mammalian ORs

(Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). They are not related to mammalian ORs (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999) but they came up during the evolution of insects (Missbach et al., 2014). In contrast to mammalian ORs, insect ORs are inserted atypically into the membrane with an internal N-terminus and an external C-terminus (Benton et al., 2006). Additionally, ORs are coexpressed with the coreceptor ORCo (Vosshall et al., 2000). ORCo and the OR form heteromeric complexes in the membrane (Neuhaus et al., 2005). The OR transfers the ligand specificity (Dobritsa et al., 2003; Störtkuhl and Kettler, 2001) whereas ORCo functions as chaperone which guides the OR to the membrane (Larsson et al., 2004). The ORCo-OR complex acts as ligand gated cation channel (Sato et al., 2008; Wicher et al., 2008).

GRs were discovered in *D. melanogaster* in a similar way like ORs: they are seven trans-membrane proteins and are expressed in gustatory organs (Clyne et al., 2000). GRs are inserted into the membrane like ORs, with an internal N-terminus and an external C-terminus (Zhang et al., 2011). GRs are mainly involved in gustation but there are GRs involved in olfaction and thermosensing (Ni et al., 2013). In *D. melanogaster* neurons responding to CO_2 coexpress Gr21a and Gr63a (Jones et al., 2007; Kwon et al., 2007; Suh et al., 2004). The CO_2 receptors form a subgroup conserved in neopteran insects including lepidopteran species like *Bombyx mori* (Lepidoptera: Bombycidae) (Wanner and Robertson, 2008). Further conserved functional subgroups of GRs are sugar receptors (Kent and Robertson, 2009), fructose receptors (Miyamoto et al., 2012), bitter receptors and, in *D. melanogaster*, pheromone receptors (Montell, 2009). In contrast to ORs the ligands of several *D. melanogaster* GRs are still unknown.

IRs are derived from ionotropic glutamate receptors and some members of the family are antennally expressed in *D. melanogaster* as well (Benton et al., 2009). Two IRs, IR8a and IR25a, are expressed together with other IRs and therefore are assumed as coreceptors (Benton et al., 2009). IRs are supposed to detect mostly water soluble compounds like acids and amines (Abuin et al., 2011; Benton et al., 2009), but can play a role in taste as well: in *D. melanogaster* the IR20a clade is expressed in taste neurons (Koh et al., 2014; Stewart et al., 2015) and IR76b is involved in salt tasting (Zhang et al., 2013).

Chemosensory receptor gene evolution occurs via a birth-and-death model (Nei et al., 2008). In a relatively short time receptors are duplicated and eliminated. There are often lineage specific expansions. And with every speciation step, the new species duplicate different receptors. This can explain why there are no direct orthologs of ORs between insect families like Diptera and Lepidoptera but ORCo (Hansson and Stensmyr, 2011; Krieger et al., 2003).

ORCo is presumably the origin of all ORs and could have evolved with the ability to fly (Missbach et al., 2014). Among GRs there are subgroups with representatives in several insect families like the sugar receptors. They are found in basal insects and therefore date back at least until the separation from the basal insects (Missbach et al., 2014). IRs are found in olfactory organs of other lines of arthropods and nematodes, too and are therefore assumed to date back to the origin of Protostomia (Croset et al., 2010).

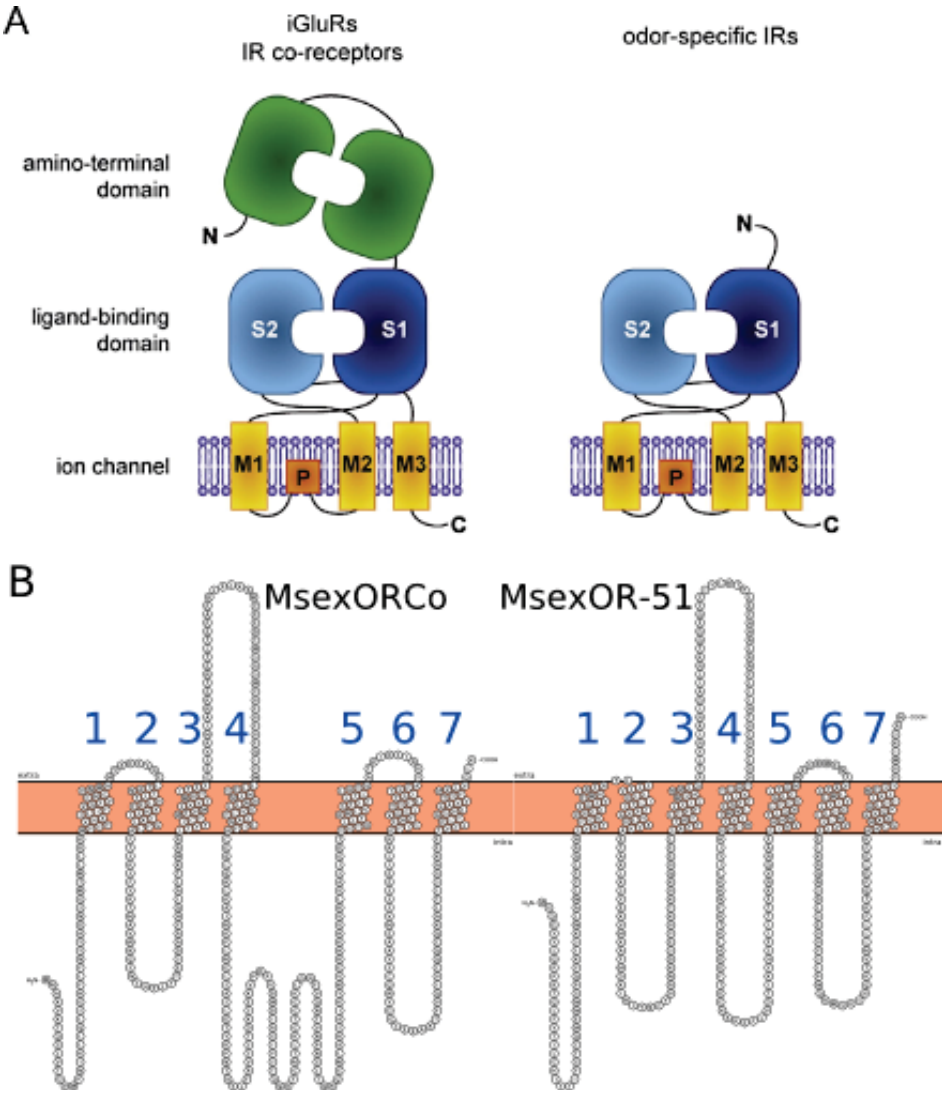

Fig.2: Structures of chemosensory receptors: (A) Schematic structure of IRs: They form tetramers consisting of IR coreceptors and odor-specific IRs (picture source: Rytz et al., 2013). (B) Predicted transmembrane structure of the amino acid sequence of olfactory coreceptor MsexORCo and MsexOR-51, a putative sex pheromone receptor, which is expressed in male and larval *M. sexta*. ORs have seven transmembrane domains and form functional complexes with the coreceptor ORCo in which the OR transfers the ligand specificity. The transmembrane domains are predicted by TOPCONS (http://topcons.cbr.su.se) and displayed with PROTTER (http://wlab.ethz.ch/protter).

The molecular basis of olfaction in *M. sexta* was established by identifying ORs in cDNA libraries (Grosse-Wilde et al., 2010; Patch et al., 2009). Subsequently, transcriptome sequencing of antennae led to the identification of 64 ORs, six IRs and two GRs (Grosse-Wilde et al., 2011; Howlett et al., 2012). We conclude that at least six ORs are unknown, because *M. sexta* adults have about 70 glomeruli in the antennal lobe (Grosse-Wilde et al., 2011). The majority of IRs and GRs is presumably unknown, because *B. mori* has 18 IRs and 65 GRs (Croset et al., 2010; Wanner and Robertson, 2008).

Chemosensory receptors for finding mating partners: The Lepidoptera pheromone system

The Lepidoptera pheromone system is a classic example of chemical ecology since the discovery of the first pheromone, bombykol, from *B. mori* (Butenandt et al., 1959). Female lepidopterans emit a pheromone blend to attract males over large distances. Female *M. sexta* produce a pheromone blend which consists of several compounds (Tumlinson et al., 1989). Two of these, bombykal (E,Z-10,14:16-Ald) and E,E,Z-10,12,14:16-Ald are sufficient to attract males in the wind tunnel (Tumlinson et al., 1989). Male specific trichoid sensilla on the antenna house at least three types of OSNs for detecting three compounds of the pheromone bland, namely bombykal, E,E,Z-10,14,14:16-Ald and E,E,E-10,14,14:16-Ald (Kaissling et al., 1989). But only two male specific ORs have been identified (Grosse-Wilde et al., 2010; Patch et al., 2009) thus, a third candidate is still elusive. Both of them belong into a subgroup of ORs which is specific for Lepidoptera, the pheromone receptor clade. Pheromone receptor candidates have a higher similarity among each other than to other ORs (Krieger et al., 2004). All Lepidoptera pheromone receptors for which the ligands have been identified belong into this clade (Xu et al., 2015; Zhang and Löfstedt, 2015). However, there are ORs in this subgroup which do not detect pheromones (Bengtsson et al., 2014; Jordan et al., 2009).

Chemosensory receptors for finding a good place for the offspring

If females place their eggs on suitable host plants the larvae can develop faster. Therefore, finding a good place for oviposition should result in higher offspring survival and become an important trait during evolution (Thompson and Pellmyr, 1991). Choosing a place for oviposition involves vision, chemosensation and mechanosensation (Sparks and Cheatham, 1970; Yamamoto and Fraenkel, 1960). In several Lepidoptera species direct contact of tarsi and ovipositor to the plant is important (Renwick and Chew, 1994). During this procedure mechanosensory and gustatory properties can be assessed by the female. Since *M. sexta* is not landing on the plant for oviposition, this behavior is especially important. Volatile cues

transfer information about the identity and the quality of the host plant (Späthe et al., 2013). *M. sexta* females can detect 119 compounds from *Nicotiana attenuata* and *Datura wrightii* (Späthe et al., 2013). Based on the odor profile of the plant females prefer to oviposit on *Datura wrightii* over *Nicotiana attenuata* (Späthe et al., 2013). Females prefer plants that are not damaged by herbivores over damaged ones by volatiles emitted after the plant was attacked by herbivores (Allmann et al., 2013; Baldwin et al., 2001; Kessler and Baldwin, 2001; Späthe et al., 2013).

Induced preference in *M. sexta* larvae

After feeding on solanaceous plants *M. sexta* larvae prefer to feed on the same plant species in choice experiments (Boer and Hanson, 1984). After ablation of all chemosensory organs, larvae do not discriminate between the plant species they were reared on and other host plant species (de Boer, 1991). When larvae were reared on tomato and then placed on a non-solanaceous species, some of the larvae prefer to starve to death instead of eating the new host plant (del Campo et al., 2001). Electrophysiological recordings of gustatory neurons demonstrated, that the response to substances like potassium chloride, glucose and indioside D is different between larvae reared on artificial diet and larvae reared on solanaceous plants (del Campo et al., 2001). The mechanism underlying this effect is unknown.

Methodology

We aimed for identification of the complete *M. sexta* chemosensory receptor set to find candidates with important function for mating, ovipositioning and larval host plant choice. Furthermore we sought to understand how *M. sexta* larvae can adapt to a variety of host plants and how the induction of preference works. We performed a large scale expression analysis of chemosensory genes using RNAseq to obtain transcriptomes of several chemosensory as well as detoxification tissues. Thus, we made use of two developments: The *M. sexta* genome project gave us the opportunity to search for chemosensory receptor genes in the genome and additionally this project allowed us to test RNAseq as a new method for gene expression profiling.

RNAseq utilizes a consequence of next generation sequencing (Cloonan et al., 2008; Mortazavi et al., 2008). After total RNA isolation the mRNA is purified and fragmented. The fragments are bound on a plate. The fragmented mRNA is transcribed into DNA using a reverse transcriptase followed by second strand synthesis. Adapters are ligated to the cDNA fragments and the cDNA fragments are amplified via PCR resulting in the sequencing library. This library is sequenced by synthesis using fluorescence labeled dNTPs (Illumina sequencing) and the so called sequencing reads are obtained. The reads are mapped to a

reference gene set; in our case the official gene set of the *M. sexta* genome project. The library construction is quantitative. Higher expressed genes will cause more reads in the sequencing results. Additionally, longer mRNA transcripts result in more reads, too. Thus, the number of reads per transcript correlates with the number of transcripts and depends on the length of the transcripts. In the RNAseq analysis the number of obtained reads is corrected by the number of mapped reads and the gene length by calculating the RPKM value (Mortazavi et al., 2008). This procedure has proven to be as accurate as standard gene expression profiling methods like microarrays and qPCR (Marioni et al., 2008). We created data sets of female and male antennae, female ovipositors, larval antennae and maxillae, larval gut, larval labial glands and whole larvae.

In manuscript (1) we report how we used this data to correct the gene models generated by an automated annotation pipeline as part of the *M. sexta* genome project to build a reference set of chemosensory receptor genes. We used the corrected and several new gene models to find sex specifically expressed genes and to characterize the chemosensory repertoire of larvae. In manuscript (2) we report expression of chemosensory receptors in the ovipositor. We identified putative olfactory sensilla on the ovipositor and using electrophysiological recording we identified possible ligands which could be important for oviposition site selection. In manuscript (3) we challenged *M. sexta* larvae by rearing them on host and non-host plants. We checked the expression of detoxification, immune system related and chemosensory genes to assess the ability of *M. sexta* larvae to adapt to their environment.

Overview of Manuscripts

Manuscript 1

A reference gene set for chemosensory receptor genes of *Manduca sexta*

Christopher Koenig, Ariana Hirsh, Sascha Bucks, Christian Klinner, Heiko Vogel, Aditi Shukla, Jennifer H. Mansfield, Brian Morton, Bill S. Hansson, Ewald Grosse-Wilde

Insect Biochemistry and Molecular Biology, *accepted for publication September 8, 2015*

In the first manuscript we used RNAseq and the *M. sexta* genome to build a reference gene set for chemosensory receptor genes, namely ORs, IRs and GRs. We identified additional male and female specific ORs and the larval OR repertoire. This data will be the foundation for all further studies on chemosensory genes in *M. sexta*.

Built on an idea conceived by all authors.
Designed experiments: C. Koenig (50%), A. Hirsh, S. Bucks, A. Shukla, J. H. Mansfield, B. Morton, E. Grosse-Wilde
Collected samples for RNAseq: C. Koenig (100%)
Performed bioinformatics analysis: C. Koenig (50%), A. Hirsh, S. Bucks, A. Shukla, J. H. Mansfield, B. Morton, E. Grosse-Wilde
Performed and analyzed molecular experiments: C.Koenig (70%), S. Bucks, C. Klinner, E. Grosse-Wilde
Wrote the manuscript: C. Koenig (50%), H. Vogel, J. H. Mansfield, B. Morton, B. S. Hansson, E. Grosse-Wilde

Manuscript 2

Olfaction in the ovipositor of *Manduca sexta*

Christian Klinner, Christopher Koenig, Kevin C. Daly, Bill S. Hansson, Ewald Grosse-Wilde

Chemical senses, *in preparation*

In the second manuscript we investigate the role of the female ovipositor in olfaction. Transcriptomic data indicated the presence of chemosensory receptors. Using electron microscopy we found olfactory sensilla and recorded from them. The detected compound could play an important role in oviposition site selection.

Built on an idea conceived by all authors.
Designed experiments: C. Klinner, C. Koenig (10%), E. Grosse-Wilde
Performed and analyzed SSR experiments: C. Klinner, K. C. Daly, E. Grosse-Wilde
Performed bioinformatics analysis: C. Koenig (90%), E. Grosse-Wilde
Performed and analyzed molecular experiments: C. Koenig (90%), E. Grosse-Wilde
Wrote the manuscript: C. Klinner, C. Koenig (10%), K. C. Daly, B. S. Hansson, E. Grosse-Wilde

Manuscript 3

The plastic response of *Manduca sexta* to host and non-host plants

Christopher Koenig*, Anne Bretschneider*, David G. Heckel, Ewald Grosse-Wilde, Bill S. Hansson, Heiko Vogel

* These authors contributed equally

Insect Biochemistry and Molecular Biology, Volume 63, August 2015, Pages 72–85
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In the third manuscript we reared larvae on different host and a non-host plant and performed RNAseq on several tissues. We found plant depended differential gene expression of one OR and several OBPs. This could play an important role for inducing preference for certain host plants in the larvae.

Built on an idea conceived by all authors.
Designed experiments: C. Koenig (40%), A. Bretschneider, H. Vogel
Performed and analyzed feeding assay: C. Koenig (50%), A. Bretschneider, H. Vogel
Collected samples for RNAseq: C. Koenig (100%)
Performed bioinformatics analysis: C. Koenig (40%), A. Bretschneider, H. Vogel
Wrote the manuscript: C. Koenig (40%), A. Bretschneider, D. G. Heckel, E. Grosse-Wilde, B. S. Hansson, H. Vogel

Manuscript 1

A reference gene set for chemosensory receptor genes of *Manduca sexta*

Christopher Koenig, Ariana Hirsh, Sascha Bucks, Christian Klinner, Heiko Vogel, Aditi Shukla, Jennifer H. Mansfield, Brian Morton, Bill S. Hansson, Ewald Grosse-Wilde

Insect Biochemistry and Molecular Biology, *accepted for publication September 8, 2015*

Abstract

The order of Lepidoptera has historically been crucial for chemosensory research, with many important advances coming from the analysis of species like *Bombyx mori* or the tobacco hornworm, *Manduca sexta*. Specifically *M. sexta* has long been a major model species in the field, especially regarding the importance of olfaction in an ecological context, mainly the interaction with its host plants. In recent years transcriptomic data has led to the discovery of members of all major chemosensory receptor families in the species, but the data was fragmentary and incomplete. Here we present the analysis of the newly available high-quality genome data for the species, supplemented by additional transcriptome data to generate a high quality reference gene set for the three major chemosensory receptor gene families, the gustatory (GR), olfactory (OR) and antennal ionotropic receptors (IR). Coupled with gene expression analysis our approach allows association of specific receptor types and behaviors, like pheromone and host detection. The dataset will provide valuable support for future analysis of these essential chemosensory modalities in this species and in Lepidoptera in general.

Keywords: olfaction, taste, Lepidoptera, ionotropic receptor, pheromone receptor, gustatory receptor

1. Introduction

Chemosensation allows assessment of the chemical composition of the environment, making it an extremely important sensory modality for most species. In insects chemosensation is usually involved in a large number of essential behaviours, such as foraging, mating and oviposition. Insects employ three groups of chemosensory receptor genes: olfactory receptors (ORs), variant ionotropic receptors (IRs) and gustatory receptors (GRs) (Cande et al., 2013; Hansson and Stensmyr, 2011; Nei et al., 2008).

Insect ORs and mammalian ORs are not directly related and evolved independently (Benton et al., 2006). The likely origin of insect ORs are GRs with which they form a superfamily (Dunipace et al., 2001; Robertson et al., 2003). Both are seven transmembrane domain receptors (Clyne et al., 2000; Vosshall et al., 1999), that in contrast to other such proteins exhibit an inverted topology, i.e. with an intracellular N-terminus (Benton et al., 2006; Zhang et al., 2011). In *Drosophila melanogaster* ORs are housed in the dendritic membrane of olfactory sensory neurons (OSNs) in the antenna and the maxillary palp (Vosshall et al., 2000). ORs form a heteromultimeric complex with a coreceptor (ORCo) (Vosshall and Hansson, 2011) in which the OR conveys the ligand specificity (Benton et al., 2006; Hallem et al., 2004; Neuhaus et al., 2005; Sato et al., 2008; Wicher et al., 2008). Most OSNs express only a single OR gene. The axons of OSNs project to substructures in the antennal lobe called glomeruli, with each glumerulus innervated by neurons expressing the same OR (Vosshall et al., 2000). Evolutionarily, ORCo predates ORs, which appeared in winged insects (Missbach et al., 2014).

A special case of OR-based olfaction is the Lepidoptera pheromone system, a classical model system of olfaction and chemical ecology (Hansson and Stensmyr, 2011; Hansson, 1995). In many moth species including *M. sexta*, females emit a single compound or a blend of odors as an efficient long distance mating cue for males. The male moths have highly specific ORs to detect the conspecific female signal. In comparison to other ORs these pheromone receptors are more conserved, forming a subgroup within the OR family (Engsontia et al., 2014; Krieger et al., 2004; Mitsuno et al., 2008). The ligands of pheromone receptors have been identified in several species, for example *Bombyx mori* (Nakagawa et al., 2005; Sakurai et al., 2004), *Heliothis virescens* (Grosse-Wilde et al., 2007; Kurtovic et al., 2007; Wang et al., 2011), and *Spodoptera littoralis* (Montagné et al., 2012).

The other members of the superfamily, the GRs, are generally expressed in gustatory receptor neurons. In contrast to ORs, several GRs are usually expressed in a single neuron. While GRs likely also form heteromeric complexes, they do not have a common coreceptor which would be comparable to ORCo (Hallem et al., 2006). Most of the GRs are receptors

for non-volatile substances, i.e. tastants (Montell, 2009), with the notable exception of GR21a and GR63a in *D. melanogaster*, which detect CO_2 (Jones et al., 2007). Additionally, some GRs convey temperature sensitivity (Ni et al., 2013). GRs are ancient, predating even the split of Deuterostomia and Protostomia. However, it is unclear when the family was recruited into the sensory system and there is no evidence for a chemosensory role outside of arthropods. In the deuterostomian linage an involvement of GRs in development has been demonstrated, indicating that this might be their ancient function (Saina et al., 2015).
IRs are likely derived from ionotropic glutamate receptors; a subset of the family is also involved in the detection of odorants (Benton et al., 2009). Antennal IRs form complexes consisting of one of two IR coreceptors, IR8a and IR25a, together with ligand binding IRs (Rytz et al., 2013) and they are conserved across the Protostome linage and hence more ancient than ORs (Croset et al., 2010). One member of this gene family, IR76b, is involved in salt tasting in *D. melanogaster* (Y. V Zhang et al., 2013).
M. sexta is a model organism for insect chemosensation, and has been well described from an anatomical, electrophysiological, behavioral and ecological perspective (for example Allmann et al., 2013; Ghaninia et al., 2014; Riffell et al., 2009; Shields and Hildebrand, 2001; Stengl, 2010). In recent years some members of the different receptor families involved in chemosensation have been identified (Grosse-Wilde et al., 2011, 2010; Howlett et al., 2012; Patch et al., 2009). The newly available genome data of this model species allowed us to extend this analysis, identifying complete sets of the involved receptor families and analyzing their expression in different chemosensory tissues with the goal to predict function.
We found new putative ORs and were able to correct and reject some of the formerly reported sequences originating from transcriptomic data, as they were either duplicates or different types of receptors, not belonging to the OR family. Using RT-PCR and *in situ* hybridization we found sex specific expression of two ORs, which were not investigated before. One of the two candidates, MsexOR-51, is a candidate for the elusive third pheromone receptor, while MsexOR-15 is the first female specific OR belonging to the pheromone receptor clade. Using RNAseq we analyzed the expression of all ORs in male, female and larval antennae. Additionally, *M. sexta* has a repertoire of 21 IRs, the largest antennal IR repertoire reported for a lepidopteran species so far. The GR gene repertoire exhibits an expansion of bitter receptors, which is typical for Lepidoptera. By combining genome and transcriptome data our analysis establishes a high quality reference set of *M. sexta* chemosonsory genes, providing a crucial basis for further studies linking ecology, behavior and genomics.

2. Material and Methods

2.1. Animal rearing

M. sexta animals were taken from a colony reared at the Max Planck Institute for Chemical Ecology. *M. sexta* larvae were kept on artificial diet (46 g of agar, 144 g of wheat germ, 140 g of corn meal, 76 g of soy flour, 75 g of casein, 24 g of Wesson's salt mixture, 36 g of sugar, 5 g of cholesterol, 12 g of ascorbic acid, 6 g of sorbic acid, 3 g of methyl paraben, 9 ml of linseed oil, 60 ml of 3.7% formalin, 30 mg of nicotinic acid, 15 mg of riboflavin, 7 mg of thiamine, 7 mg of pyridoxine, 7 mg of folic acid, and 0.6 mg of biotin per 1.8 L of water) in climate chambers (26° C, 75 % humidity, and 16 h light: 8 h dark). Larvae were placed on fresh food three times per week. Wandering last instar larvae were placed in wood blocks for pupation. The pupae were sorted into paper bags for hatching, and male pupae were transferred to a separate climate chamber.

2.2. Total RNA isolation

Antennae and maxillae of ten *M. sexta* larvae were dissected and each tissue sample was directly transferred to liquid nitrogen. Samples were then homogenized with a micro pistil. After evaporation of liquid nitrogen, RL buffer (innuPREP RNA Mini Kit, Analytik Jena, Jena, Germany) was added. Samples were stored at -20° C until they were processed further. For adults, antennae of single female or male *M. sexta* moth were removed and homogenized with two 3 mm steal beads (Qiagen, Hilden, Germany) in RL buffer (innuPREP RNA Mini Kit, Analytik Jena, Germany) using a Tissuelyser (Qiagen, Hilden, Germany) for 5 min at 50 Hz. Samples were stored at -20° C. Finally, RNA isolation was performed with the innuPREP RNA Mini Kit (Analytik Jena, Jena, Germany) according to the manufacturer's instructions.

2.3. cDNA synthesis

RNA samples were treated with TurboDNAse (Ambion, TX) according to the manufacturer's instructions. DNAse was removed using Trireagent (Sigma-Aldrich, MO) following the instructions of the producer. RNA was dissolved in 25 µl RNA storage solution (Ambion, TX). For cDNA synthesis 1 µg total RNA per sample was used as template for the Super Script III kit (Invitrogen, CA). For cloning purposes, cDNA was synthesized using a template of equal parts RNA from male, female and larval samples.

2.4. RT-PCR

RT-PCR reactions consisted of 18.5 µl water, 2.5 µl colored reaction buffer (Qiagen, Hilden, Germany), 1 µl dNTPs (Thermo Fisher Scientific, Lithuania), 1 µl Primer A, 1 µl Primer B, 0.5 µl taq DNA Polymerase (Qiagen, Hilden, Germany) and 0.5 µl cDNA with a final volume of 25 µl. Primer A and Primer B are placeholders for gene specific primer pairs. Reaction was done in a thermocycler (GeneAmp PCR System 9700, PE Applied Biosystems) with 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 45 s. The final step was an incubation at 72°C for 10 min. To check the results samples were loaded on an 1 % agarose gel.

2.5. Cloning

PCR products were extracted from the agarose gel using the EZNA Gel Extraction Kit (Omega, GA) following the instructions. For TA-cloning the TA Dual Promotor Cloning Kit (Invitrogen, CA) was used according to the instructions of the manufacturer. Transformation into competent cells was performed as described by Hanahan (Hanahan, 1983). Afterwards cells were plated on LB agarose plates containing 100 mg/l ampicillin (Roth, Karlsruhe, Germany) and 80 mg/l X-gal (Roth, Karlsruhe, Germany) and allowed to grow over night in an incubator at 37°C.

2.6. Plasmid Isolation and Sequencing

Plasmid Mini Preparation was performed with the EZNA Plasmid DNA Miniprep Kit (Omega, GA), following the manufacturer's instructions. Samples were sequenced on a 3730XL DNA Analyser (Applied Biosystems) at the Max Planck Institute for Chemical Ecology.

2.7. Fluorescence *in situ* hybridization

Enzymatic cutting of plasmids containing MsexOR-15 and MsexOR-51 followed standard protocols with restriction enzymes. After this step samples were purified using agarose gel electrophoresis and gel extraction was performed with the EZNA Gel Extraction Kit (Omega, GA) as described previously.

To synthesize the probe, 150ng purified DNA was added to 2 µl transcription buffer, 2 µl DIG RNA Labeling Mix (11 277 073 910, Roche, Switzerland)and 2 µl Polymerase Sp6 or T7 (Roche, Switzerland), and the total volume adjusted to 20 µl using aqua dest. The sample was incubated for 3 h at 37ºC and precipitated with 2.5 µl Pink Coprecipitant (Bioline, London, UK) and 75 µl 96% Ethanol over night at -20ºC.

After centrifugation for 30 min at 4ºC at 16.1 rcf and discarding the supernatant, the pellet was washed with 50 µl 70% Ethanol and centrifuged again for 30 min at 4ºC at 16.1 rcf. The supernatant was removed through pipetting, the pellet air dried and then dissolved in 50 µl water.

Trimming of the probe to a length of 600 bases was performed by adding 25 µl probe to 25 µl carbonate buffer (80 mM $NaHCO_3$, 120 mM Na_2CO_3, pH 10.2) at 60ºC for a defined time calculated as described (Angerer and Angerer, 1992). Afterwards the reaction was stopped with 5 µl acetic acid and 250 µl hybridization buffer (50% formamide, 2X SSC (0.15 M NaCl, 0.015 M Na-citrate, pH 7.0), 10% dextran sulfate, 20 mg/ml yeast t-RNA, 0.2 mg/ml herring sperm DNA).

Antennae used for *in situ* hybridization were embedded in O.C.T. compound (VWR, Belgium) and frozen at -60°C in a cryostat. Cross sections were received by cutting at -25°C with a thickness of 18 µm. Cryosections were thaw-mounted onto SuperFrost slides (Menzel, Braunschweig, Germany) and air dried for 30 min.

After Fixation with 4% paraformaldehyde in 0.1 M $NaCO_3$ (pH 9.5) for 30 min at 4°C the slides were washed in PBS (0.85% NaCl, 1.4 mM KH_2PO_4, pH 7.1) for 1 min. Denaturation of proteins was done for 10 min in 0.2 M HCl followed by a washing step for 2 min in PBS with 1% Triton X-100 and two 30 s washes in PBS. At last the slides were incubated for 10 min at 4°C in a solution of 50% formamide with 5X SSC. Probes were diluted in hybridization buffer 1:50. This mixture was pipetted onto the slides, which were then incubated over night at 55°C in a box humidified using 50% formamide in water.

After hybridization the slides were incubated in 0.1X SSC at 60°C for 30 min and then washed 1 min with TBS (100 mM Tris, pH 7.5, 150 mM NaCl). Unspecific binding sites were saturated with blocking solution (TBS with 0.03 % Triton X-100 and 1% blocking reagent) by incubation for 30 min at room temperature in a box humidified with water. The antibody anti-Dig (Roche, Switzerland) was diluted 1:500 in blocking solution. The blocking solution was removed from the slides and replaced with the diluted antibody. After 60 min incubation at 37°C in a humidified box the slides were washed three times for 5 min in TBS with 0.05% Tween 20. The slides were rinsed with detection buffer (100 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, pH 8.0). Then 100 µl of the HNPP/FastRedTR detection set (Roche, Switzerland) per slide was applied for 30 min for detection of anti-Dig conjugates. After 3 further wash steps with TBS with 0.05% Tween 20 for 5 min the slides were rinsed with aqua dest. Slides were sealed with PBS/Glycerol 3:1 and signals visualized with a LSM 510 Meta confocal microscope (Zeiss, Oberkochen, Germany).

2.8. RNAseq

Library construction and sequencing was performed by the Max Planck Genome Center Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). 1 µg of total RNA from male antennae and female antennae was used for one TruSeq library each. TruSeq libraries were generated from poly-A enriched mRNA. The library was sequenced with an Illumina HiSeq 2500 sequencer and 10 million of 100 bp single reads per sample were obtained. For larval antennae and maxillae we used the data from a previous study (Koenig et al., 2015).
For mapping we used the official gene set (OGS) 2 of *M. sexta* and replaced the OR sequences with our own corrected sequences. The reads were mapped to this reference and the expression values were calculated as RPKM (Reads per kilo base per million mapped reads) using CLC Genomics Workbench v8.0 (http://www.clcbio.com). The *M. sexta* OGS2 data can be accessed from https://i5k.nal.usda.gov/Manduca_sexta or ftp://ftp.bioinformatics.ksu.edu/pub/Manduca/OGS2/.

2.9. Receptor Sequences

As basis for *M. sexta* OR sequences we used the automated annotation of the genome, corrected manually in a first pass. Additionally we performed BLAST searches using this sequences in the scaffolds of the genome. As basis for nomenclature we referred to the published data of *M. sexta* chemosensory genes (Grosse-Wilde et al., 2011, 2010; Howlett et al., 2012; Patch et al., 2009). We assigned all known OR transcripts to genes in the genome. Where sequences were available that had been verified by sequencing of RT-PCR products we preferred those over genomic sequencing data. In some cases manual correction of gene models was assisted by sequencing of RT-PCR products obtained from antennal cDNA.
We collected OR sequences from several species of Lepidoptera with published genome data: *B. mori* (Krieger et al., 2005; Nakagawa et al., 2005; Tanaka et al., 2009; Wanner et al., 2007), *D. plexippus*, *H. melpomene*, and *P. xylostella*. For BmorOR-9, BmorOR-21, BmorOR-25, BmorOR-26, BmorOR-30, BmorOR-33, BmorOR-34, BmorOR-35, BmorOR-37, BmorOR-38, BmorOR-45, BmorOR-47, BmorOR-57 the NCBI annotation release 101 from June 2 2015 was used. Additionally we added OR sequences from transcriptomic data of *S. littoralis* (Jacquin-Joly et al., 2012; Poivet et al., 2013) and *S. litura* (J. Zhang et al., 2013).
In total we used 71 ORs of *B. mori*, 66 ORs of *D. plexippus*, 70 ORs of *H. melpomene*, 74 ORs of *M. sexta*, 95 ORs of *P. xylostella*, 46 ORs of *S. littoralis*, and 3 ORs of *S. litura* for alignment. However, we removed several ORs from the analysis since they could not be aligned with the majority of ORs, indicating potentially problematic gene models or

annotations. This affected 5 ORs of *B. mori*, 6 ORs of *D. plexippus*, 4 ORs of *H. melpomene*, 3 previously published ORs of *M. sexta*, and 1 OR of *P. xylostella*. The amino acid sequences of the remaining 405 ORs were aligned using MAFFT version 7.017 (Katoh et al., 2002) with default parameters, option "--auto". The alignment was edited by hand for five partial receptors which were not aligned properly.

We collected ionotropic glutamate receptor sequences from *D. melanogaster, S. littoralis* and species of Lepidoptera with published genome data: *B. mori*, *D. plexippus*, *H. melpomene*, and *P. xylostella*. Additionally to the already published ionotropic glutamate receptor sequences (Croset et al., 2010; Olivier et al., 2011; Poivet et al., 2013; Zhan et al., 2011) we performed BLAST searches for ionotropic glutamate receptors in all four Lepidoptera species as well as in the *M. sexta* OGS2. Sequences shorter than 100 amino acid residues were excluded from further analysis. We removed several ionotropic glutamate receptor candidates from the analysis because they could not be aligned together with the majority of ionotropic glutamate receptors. After cloning MsexIR87a its predicted amino acid sequences was added since it is not contained in the OGS2 of *M. sexta*. The amino acid sequences of 249 ionotropic glutamate receptors were aligned using MAFFT version 7.017 (Katoh et al., 2002) with default parameters.

GR sequences for *B. mori* were obtained from (Wanner and Robertson, 2008); for *D. plexippus* and *H. melpomene* from (Briscoe et al., 2013) with additional *D. plexippus* sequences downloaded from the Monarch Genome Project (http://monarch.umassmed.edu/biology/chemoreception.html). We used these sequences for BLAST searches in the scaffolds of the *M.sexta* genome. Where possible we curated the annotation of GR candidates. Amino acid sequences were aligned using MAFFT version 7.017 (Katoh et al., 2002) with default parameters.

All DNA sequences of *M. sexta* were submitted to the European Nucleotide Archive under the accession number LN885098-LN885134 for cloned sequences and LN885135-LN885236 for predicted sequences.

2.10. Phylogenetic analysis

For phylogenetic analysis we applied the approximate maximum-likelihood method with the Jones, Taylor, Thornton substitution model in FastTree version 2.1.7 (Price et al., 2010). FastTree calculates local support values for each branch. The OR and GR Trees were displayed rerooted on the conserved co-receptor ORCo using Figtree version 1.4.1.

3. Results and Discussion

3.1. *Manduca sexta* OR gene family

Initially we focused on the identification of all members of the OR coding gene family in the genome data. Previous publications reported 63 *M. sexta* ORs and the coreceptor ORCo. However, most previously reported OR coding sequences were incomplete. Using the genome data of *M. sexta* we were able to identify 73 ORs of which 70 are included in the OGS2 (supplement 1, 2, 8). The three additional ORs were identified after official lock down of the OGS2. In the course of the analysis we were able to identify ten cases where previously reported OR gene fragments thought to be independent actually belonged to the same gene, and rejected the respective names.

Both to assist in the annotation and to facilitate analysis of expression in the different chemosensory tissues we generated additional RNAseq data of female and male antennae, and larval antennae plus maxillae. Initially we used this data to verify and correct the chemosensory gene annotations of the *M. sexta* genome. In 20 cases this transcriptomic data allowed us to identify errors in gene models. In ten cases the concerned OR gene sequences were not covered completely by the genomic data; for six of these we could complete the sequences either using transcriptomic data or by PCR amplification from antennal cDNA, followed by cloning and subsequent sequencing. However, for a few receptor types sequences were too similar to reliably assign or reconstruct individual gene models using either approach. Accordingly, based on comparison with known OR genes of other species we estimate that 17 of the 73 OR sequences are still partial. Three of these (MsexOR-82, MsexOR-83, MsexOR-85) we identified as pseudogenes based on incomplete gene structure, i.e. missing exons.

We performed an approximate-maximum-likelihood phylogenetic analysis (Price et al., 2010) of a multiple sequence alignment of predicted Lepidopteran OR protein sequences derived from genomic data and transcriptomic data in case of *S. littoralis* and *S. litura* (Fig. 1). For only a small number of ORs orthologs existed in every investigated species (MsexOR-18, 22, 27, 29, 41, 43, 62, 64). Each species had at least one species specific OR clade expansion.

ORs with similar amino acid sequences potentially detect very similar ligands (Bohbot et al., 2011; McBride and Arguello, 2007). Accordingly, the two orthologs of the linalool-detecting BmorOR-19 (Anderson et al., 2009), MsexOR-5 and MsexOR-6, have been speculated to differentiate between the (+) and the (-) form of linalool (Grosse-Wilde et al., 2010), a sensory function described previously for the species (Reisenman et al., 2009). However, there are other ORs in *B. mori* responding to linalool (Tanaka et al., 2009). BmorOR-29, an

ortholog of MsexOR-18, responds to linalool, citral and linalyl acetate (Tanaka et al., 2009). BmorOR-42, an ortholog of MsexOR-24, responds to linalool and linalyl acetate (Tanaka et al., 2009).

M. sexta also has two orthologs to SlituOR-12, which detects cis-3-hexenyl acetate (J. Zhang et al., 2013), namely MsexOR-42 and MsexOR-66. The change from cis-3-hexenyl-acetate to trans-2-hexenyl acetate emission is a signal emitted by the plant after a herbivore attack (Allmann et al., 2013). *M. sexta* is able to differentiate the two isomers thereby avoiding oviposition on attacked plants emitting trans-2-hexenyl acetate (Allmann et al., 2013). Thus, we hypothesize that MsexOR-42 and MsexOR-66 detect the two isomers of hexenyl acetate. BmorOR-24, which is broadly tuned and detects for example hexyl acetate, 2-hexenyl acetate and 2-hexenal (Tanaka et al., 2009), has six orthologues in *M. sexta*, which could detect similar ligands. Two closely related ORs of a *B. mori* specific expansion, BmorOR-45 and BmorOR-47, have highly similar ligand profiles, too (Anderson et al., 2009).

As previously reported all known Lepidoptera pheromone receptors sorted into one clade (Krieger et al., 2005, 2004; Nakagawa et al., 2005; Sakurai et al., 2004). Similar to results from *P. xylostella* (Engsontia et al., 2014) the support value of the pheromone receptor clade was low (aLRT = 73). However, we note that for *M. sexta* only two receptors are present within this clade, although physiological data suggests the presence of at least three (Kaissling et al., 1989). We therefore suggest an expansion of the clade to the next node, which is well supported in our phylogeny (Shimodaira-Hasegawa test, P = 0.999). The pheromone receptor clade would then contain two additional receptor candidates of *M. sexta*, MsexOR-15 and MsexOR-51. The pheromone receptor clade comprises non pheromone receptors as well, which could explain the high number of *P. xylostella* ORs in this clade (Bengtsson et al., 2014; Engsontia et al., 2014). To be considered as pheromone receptor candidates, at least one of them should exhibit male-specific expression associated with trichoid sensilla.

3.2. Additional sex specific ORs in *Manduca sexta*

In total four receptors of *M. sexta* belong to this extended pheromone receptor clade. Of these, only MsexOR-1 and MsexOR-4 belong to the 'classical' pheromone receptor clade. Both have previously been reported as putative pheromone receptors based on their phylogenetic position as well as male-biased expression, and association of cells expressing these receptors with long trichoid sensilla, known to be the structural element involved in pheromone detection (Grosse-Wilde et al., 2010; Kaissling et al., 1989; Patch et al., 2009). Both are orthologs of functionally characterized pheromone receptors of *B. mori* (Grosse-

Wilde et al., 2006; Nakagawa et al., 2005; Patch et al., 2009, Fig. 1).
To test for a potential identity as pheromone receptor candidates we analyzed sex-specific expression using RT-PCR of the two other receptor types present in the expanded clade, MsexOR-15 and MsexOR-51. MsexOR-15 transcripts were only detected in cDNA preparations derived from adult female antennal tissue (Fig. 2A). In RNA *in situ* hybridization experiments, signals indicating MsexOR-15 expression were localized underneath trichoid sensilla of female antennae (Fig. 2B,C). While suggesting a role in a female-specific behavior, these results indicate that MsexOR-15 is not involved in detection of female released pheromones in the male antenna. In contrast, RT-PCR indicated expression of MsexOR-51 in the antennae of male but not female adults (Fig. 2D). For this receptor type fluorescent RNA *in situ* hybridization elicited signals that were localized close to the base of trichoid sensilla in male antennae (Fig. 2E,F). In comparison to earlier data from similar experiments with probes directed against MsexOR-1 and MsexOR-4 (Grosse-Wilde et al., 2010), the total number of MsexOR-51 expressing cells per section was lower. In total, these results suggest that MsexOR-51 is the elusive third pheromone receptor candidate, with the reduced number of MsexOR-51 expressing cells roughly matching the lower number of OSNs in trichoid sensilla detecting the minor component E10,E12,E14-16:Al (Kaissling et al., 1989). Based on these data the suggested expansion of the pheromone receptor clade seems warranted, with the strong support this clade exhibited in our analysis indicating one common ancestor for the whole clade in contrast to Engsontia et al. 2014 (Engsontia et al., 2014).
MsexOR-15 has no clear orthologue in *B. mori*, whereas MsexOR-51 is an orthologue to BmorOR-6, which exhibits similar male biased or male specific expression (Tanaka et al., 2009; Wanner et al., 2007). So far ligands for this receptor are unknown.

3.3. Distribution of OR genes in the genome

Most of the OR genes were found as singletons in the genome. In seven loci two ORs were found directly next to each other, and one gene cluster on scaffold 267 contained three ORs in a row (Fig. 3). We also found one unique case of gene duplication on scaffold 24, which contains a cluster of seven OR genes, six of which are closely related by sequence (Fig. 3). We investigated the synteny of the two larger OR gene clusters of *M. sexta* with the loci containing the closest homologs in the *B. mori* genome (Fig. 3) by comparing flanking genes on both sides of each OR gene cluster in the two genomes. Based on this analysis the *M. sexta* OR cluster on scaffold 24 is organized similarly to a region of the *B. mori* chromosome 28 which contains BmorOR-11, BmorOR-23 and BmorOR-24. Whereas for BmorOR-11

there is no orthologue gene in *M. sexta*, six of the genes present in *M. sexta* are all most similar in sequence to BmorOR-24, a broadly tuned receptor (Tanaka et al., 2009). We conclude that the gene has undergone recent lineage-specific duplications. BmorOR-23, the last OR gene present in the *B. mori* cluster is the orthologue of MsexOR-84.

The *M. sexta* OR cluster on scaffold 267 is orthologous to a region on *B. mori* chromosome 9, which contains the genes encoding BmorOR-20 and the linalool-detecting BmorOR-19 (Anderson et al., 2009). The three *M. sexta* OR-coding genes in the corresponding cluster, MsexOR-9, MsexOR-26 and MsexOR-65 are closest in sequence to BmorOR-20, with the previously reported two orthologs of BmorOR-19, MsexOR-5 and MsexOR-6 situated on scaffold 200, indicating a likely translocation.

The synteny of the *M. sexta* scaffolds 24 and 267 to the *B. mori* chromosomes 28 and 9 is in agreement with results from chromosome comparisons: Using BAC-FISH analysis with conserved genes Yasukochi and coworkers did not find genome rearrangements between the two species on the respective chromosomes (Yasukochi et al., 2009).

3.4. Expression of ORs in larval, female and male antennae

Beyond initial assessment of the pheromone receptor candidates, analysis of the expression of all ORs in the antennae of adult male and female as well as the larva is important for any initial assessment of putative functions. To this end we employed RNAseq, generating at least ten million Illumina reads for each tissue. As reference for the mapping analysis we used *M. sexta* OGS2, again replacing the putative OR genes with our corrected gene models and adding OR genes that were newly identified. After mapping of the sequencing data against the reference, expression values were calculated as RPKM and are shown for all OR genes (Fig. 4). In all three tissues ORCo is expressed at levels that are similar or exceeding most highly expressed OR genes, which can be expected of a coreceptor for all ORs. However, we did not find a 1:1 relation of the expression values of ORCo and the sum of all ORs as previously reported for *D. melanogaster* by Menuz and coworkers (Menuz et al., 2014).

Using this approach we were able to identify several sex-specific or sex-biased (fold difference > 10) receptors for males (6 ORs) as well as for females (7 ORs). Three male specific ORs (MsexOR-1, MsexOR-4, MsexOR-51) belong to the expanded pheromone receptor clade (Fig.1, Fig.2). Of these, MsexOR-51 is also expressed in larval antennae, with the expressing cells associated to the pheromone sensitive sensilla trichodea in the male adult. Expression of this putative pheromone receptor in larval chemosensory tissues might indicate a behavior in *M. sexta* that is similar to *S. littoralis.* Larvae of *S. littoralis* are

attracted by extracts of female pheromone glands, and possess sensilla on their antennae that house pheromone-sensitive OSNs (Poivet et al., 2012). There are three additional male specific receptors (MsexOR-16, MsexOR-83, and MsexOR-77), which do not belong to the pheromone receptor group. Our RNAseq data reflects previous results on the female specific expression of MsexOR-5, MsexOR-6 (Grosse-Wilde et al., 2010) as well as MsexOR-15 (Fig. 2). MsexOR-5 and MsexOR-6 are orthologs of BmorOR-19, a female specific OR detecting linalool (Anderson et al., 2009). Of the additional female-specifically expressed ORs, MsexOR-87 is an ortholog of BmorOR-19, for which expression was not detected in antennal tissue (Tanaka et al., 2009). For the remaining three, MsexOR-15, MsexOR-85, and MsexOR-86, there are no clear orthologs in *B. mori.*

For four receptor coding genes, MsexOR-20, MsexOR-73, MsexOR-80, and MsexOR-82 we were not able to identify expression in our RNAseq analysis, although we were able to clone MsexOR-20 and MsexOR-80 from antennal cDNA. MsexOR-73 and MsexOR-82 are predicted transcripts of OGS2 based on BLAST searches and RNAseq data from several tissues. It is possible that the other three genes are either pseudogenes, too, or are expressed in other than the sampled tissues, perhaps the palps or even non-chemosensory tissues. Expression value differences of ORs were in agreement with observations in *D. melanogaster* where a 170-fold range between RPKM expression values of ORs was detected (Menuz et al., 2014). Interestingly, the neuron with the highest spike amplitude in a single sensillum has the highest expression value of its OR gene in most cases in *D. melanogaster* (Menuz et al., 2014). With few exceptions we lack information about the number of expressing OSNs for most ORs in *M. sexta,* as well as their distribution over the antenna. In all pheromone-sensitive sensilla there is one bombykal responding neuron, which has the highest spike amplitude (Kaissling et al., 1989). Based on the fact that MsexOR-4 is the OR with the highest expression level, we conclude that it is likely the bombykal receptor. The *B. mori* bomkykal receptor, BmorOR-3 (Grosse-Wilde et al., 2006; Nakagawa et al., 2005), is orthologous to MsexOR-1 (Fig. 1), and the putative bombykal receptor, MsexOR-4, is orthologous to the *B. mori* bombykol receptor, BmorOR-1 (Grosse-Wilde et al., 2006; Nakagawa et al., 2005). Thus, it would seem likely for the orthologous genes MsexOR-4 and BmorOR-1 to detect the major compound of the pheromone blend. This is bombykal for *M. sexta* (Tumlinson et al., 1989) and bombykol for *B. mori* (Butenandt et al., 1959). Thus, we hypothesize pheromone receptor sequences, in contrast to regular OR sequences, form subgroups based on their function as detector for the major or minor compounds rather than the chemical structure of the detected ligand. This would also fit the grouping of pheromone receptors based on detecting major or minor compounds as proposed by Xu and colleagues

(Xu et al., 2015).

3.5. Ionotropic glutamate receptors for chemosensation

We performed BLAST searches against the official gene sets of *M. sexta*, *B. mori*, *H. melpomene*, and *P. xylostella* using known ionotropic glutamate receptor and IR sequences of *D. melanogaster*, *B. mori*, and *D. plexippus* (Croset et al., 2010; Zhan et al., 2011). Hits with a length of less than 300 bases were ignored. For BmorIR87a we were not able to find an orthologue in *M. sexta* OGS2. Therefore we expanded the search and included the genome scaffold sequence information, where we were able to identify a putative MsexIR87a gene. To verify expression of this gene, we amplified the candidate transcript from an antennal cDNA of *Manduca* using PCR, sequencing the product. The respective gene sequence was added to the list of ionotropic glutamate receptors and IRs (supplement 3, 4, 8). All sequences were aligned and we performed an approximate-maximum-likelihood (Price et al., 2010) phylogenetic analysis (Fig.5).

In the *M. sexta* genome we found orthologs for all but three (DmelIR92a, DmelIR76a, DmelIR84a) antennal *D. melanogaster* IRs. This is in agreement with data from the *D. plexippus* genome (Zhan et al., 2011). Additionally, we identified orthologs for IR87a and the Lepidoptera-specific IR143. IR87a orthologs are antennally expressed only in Lepidoptera but not in *D. melanogaster.* In our analysis the genes encoding both IR87a and IR143 are intron-less both in the genome of *M. sexta* and *B. mori*. There is no functional data available for either of the receptors. We found two orthologs for IR75p in *M. sexta.* This is likely a lineage-specific duplication since it is not reported from any other species of Lepidoptera so far.

We identified two new putative IRs and called them MsexIR3 and MsexIR4. For MsexIR3 we did not find orthologs in the other investigated species. The separating node of this receptor is well supported (Shimodaira-Hasegawa test, P = 0.942). MsexIR4 has orthologs in *H. melpomene* and *P. xylostella*, but the separating node of this group is less supported (Shimodaira-Hasegawa test, P = 0.889).

Surprisingly we found an orthologue to the Lepidoptera-specific IR1 in *B. mori* and *H. melpomene* indicating that this IR is not restricted to noctuids as previously reported (Olivier et al., 2011). In *S. littoralis,* IR1 is expressed not only in the antennae but also in other tissues, for example the brain (Olivier et al., 2011).

The antennal lobe of adult *M. sexta* comprises 70 +/-1 glomeruli in male individuals and 68 glomeruli in female individuals (Grosse-Wilde et al., 2011). Each glomerulus is innervated from OSNs expressing one OR (Couto et al., 2005) or IR gene (Rytz et al., 2013). Therefore

the number of glomeruli correlates with the number of ORs and IRs. We found and 73 ORs, which 7 are female-specific, 6 are male-specific and 3 could be pseudogenes. We found 21 IRs including the two coreceptors. In total we identified about the same number of OR and IR genes as there are glomeruli, keeping in mind that a) nor all ORs are expressed in adult antennae and b) IR expressing neuronal populations are not associated 1:1 with expressed IR genes (Rytz et al., 2013).

3.6. Identification of Gustatory Receptors

We identified a total of 45 GR genes in the *M. sexta* genome based on sequence similarity to *B. mori* GRs (supplement 5, 6, 8). Of these, 17 contain full length coding sequences. 28 sequences are partial and lack either a stop or a start codon. Two GRs (MsexGR1 and MsexGR2, now named MsexGR41 and MsexGR6) had previously been identified by transcriptome sequencing, and those sequences were extended here (Grosse-Wilde et al., 2011; Howlett et al., 2012). Alternative splicing was predicted for 3 receptors (MsexGR11, MsexGR15 and MsexGR18) based on predicted exon structure of the genomic sequence, but could not be confirmed by transcriptome sequencing.

Next we generated a phylogenetic tree of the complete GR family as identified from four species of Lepidoptera with sequenced genomes and published GR sequences: *M. sexta*, *B. mori* and the butterflies *D. plexippus* and *H. melpomene* (Fig. 6). Similar to other insects, the *M. sexta* GR family is rapidly evolving. A majority of *M. sexta* GRs (27, or 61%) were most closely related to another *M. sexta* GR, indicating that they arose from duplications after the *M. sexta* lineage diverged from the other Lepidoptera. Additionally, 33 of the 45 (75%) *M. sexta* GRs apparently arose from duplications that occurred after moths and butterflies diverged.

3.7. Putative functional classes of *M. sexta* GRs

Four classes of insect GRs have been described: CO_2, fructose, non-fructose sugar receptors, and bitter/other receptors (Sánchez-Gracia et al., 2009). Based on clustering with genes previously classified into these groups we can putatively assign a functional class to most *M. sexta* GRs. The CO_2 receptors are among the most conserved. *M. sexta* has 3 CO_2 receptors, with apparent ortholog relationships among the lepidopteran genes. In fact, these three CO_2 genes are also conserved in Diptera and Coleoptera (Robertson and Kent, 2009).

The fructose receptors were defined in *B. mori* by their similarity to *D. melanogaster* GR43a (Wanner and Robertson, 2008). DmelGR43a is required for fructose response *in vivo*, and also functions in the brain to detect blood fructose levels, which in turn regulates feeding

behavior (Miyamoto et al., 2012). It was recently shown that DmelGR43a can in fact respond to a range of sugars (Freeman et al., 2014). This GR family is represented by 2 genes in *B. mori* and one in each butterfly genome. There are 4 fructose receptor family genes in *M. sexta*, with the phylogeny suggesting two ancestral moth genes that each underwent duplication. One pair of duplicates, MsexGR9.1 and MsexGR9.2, were among those represented in transcriptome libraries: both are expressed in the larval fat body, ovaries and testes, and MsexGR9.2 is additionally expressed in brain, all of which could be consistent with a role in detecting metabolic cues (see supplement 7).

The sugar receptors (SRs) are represented by the *D. melanogaster* GR5/63/61 family of 8 genes, and respond to a variety of non-fructose sugars (Freeman et al., 2014; Kent and Robertson, 2009). There is substantial overlap in SR response profiles and *in vivo* function, and these receptors have been proposed to interact as heterodimers. Insect genomes encode variable SR numbers, with the four Lepidopteran species ranging from 5 (*M. sexta*) to 11 (*D. plexippus*). In *M. sexta* we identified MsexGR4, MsexGR5, MsexGR6, MsexGR6.2 and MsexGR7. Analysis of SRs in many insect orders suggested that there were 2 ancestral SR genes in Lepidopterans that gave rise to 2 clades; one clade is distinguished by the presence of a novel, and variable exon that inserts several amino acids into the extracellular region between the 5th and 6th transmembrane domains (Kent and Robertson, 2009). We also observed two major SR clades in our expanded Lepidopteran phylogeny (hereafter, SR1 and SR2); the node is well supported (Shimodaira-Hasegawa test, P = 0.901, Fig. 6). However, 6 of the 28 SR genes cluster outside these clades in our phylogeny (BmorGR7, MsexGR7, HmelGR7, DpleGr1, DpleGR40, BmorGR8).The novel exon is present in SR1 and absent from SR2 genes (Fig. 6, Fig. 7), consistent with the proposal of two ancestral lepidopteran SRs. Its presence in both butterflies and moths further supports the hypothesis that the novel exon is basal to Lepidopterans. However, both BmorGR7 and BmorGR8 contain a novel exon, which either indicates multiple exon gains or losses, or that the two *B. mori* receptors are in fact monophyletic with SR1. Exon boundaries were confirmed by transcriptome sequencing for *B. mori* and *H. melpomene* genes and are predicted for *M. sexta* and *D. plexippus* (Briscoe et al., 2013; Kent and Robertson, 2009).

The remaining 35 *M. sexta* GRs cluster with predicted bitter/other receptors. Lineage specific expansions are common in bitter receptors, and this is evident in all four species shown here (Fig. 6 and see Briscoe et al., 2013; Wanner and Robertson, 2008). Four large clusters, which included 18/45 *M. sexta* GR genes contained only *M. sexta* and *B. mori* genes, suggesting an origin in the moth lineage. There was a single group of bitter receptor genes that were apparently conserved and unduplicated in all four species (the cluster

containing MsexGR41). The function of these receptors is unknown in any species, but MsexGR1 expression was detected in gut, fat body, gonads and brain (see supplement 7). We detected expression of 2 CO2 receptors (MsexGr2, MsexGR3), one SR (MsexGR6), three Fructose receptors (MsexGR9.1, MsexGR9.2, MsexGR10.2) and two other GRs (MsexGR41, MsexGR42) in our transcriptome of adult antenna. Expression of a SR in the adult antenna is reported in *D. melanogaster* as well (Fujii et al., 2015). In the larval transcriptome of antenna and maxilla we detected expression of all three CO2 receptors, two SR (MsexGR5, MsexGR6), one Fructose receptor (MsexGR10.1) and two other GRs (MsexGR34, MsexGR41). In additional transcriptome libraries of the *M. sexta* genome project we detected expression of MsexGR41 as well as MsexGR6 and demonstrated the expression of another 4 GR genes (see supplement 7). We found expression of a SR in the head of adults and the brain of larvae. In the brain of larvae a Fructose receptor was expressed, too. Expression of a SR and a fructose receptor in the brain is reported from *D. melanogaster* adults (Fujii et al., 2015).

3.8. Chromosomal clustering of *M. sexta* GRs

In *M. sexta* the 5 SRs are chromosomally clustered (Fig. 6, yellow dots). GR4 is separated by an unrelated coding sequence from the other four SRs, which are adjacent..
Most of the bitter receptor expansions that occurred within the *M. sexta* lineage were also chromosomally clustered. One chromosomal region with 7 GRs (Fig. 6, green dots) contained members clustering into two groups in the phylogeny. The largest *M. sexta*-specific GR expansion (Fig. 6, blue and purple dots) contains 12 genes located in 3 chromsosomal clusters, each containing 3-5 genes. The phylogeny did not reveal any clear paralog relationships that would indicate the duplication history of these clusters.

4. Conclusions

We established a reference set of chemosensory genes, namely ORs, IRs and GRs, for the model species *M. sexta*. Based on our findings regarding the expression of MsexOR-51 (male-specific and close to sensilla trochoidea) we propose expanding the pheromone receptor clade in Lepidoptera to include this receptor. The expression of MsexOR-51 in the larvae of *M. sexta* could indicate a general mechanism in Lepidoptera, allowing the larvae to recognize plants visited by adult females by using a pheromone component as a marker. These selected plants could be beneficial for larvae. Both behavioral experiments to test if the larvae are attracted to the pheromone blend, and heterologous expression to verify detection of the pheromone component are still needed.

Furthermore we report the so far largest repertoire of antennal IRs for a species of Lepidoptera. This raises the question for which behavioral functions this extended repertoire is required. A connection to the use of solanaceous hostplants is likely and needs to be investigated. In this context, the expansion of the IR75p subgroup is especially interesting. In all available Lepidoptera genomes we find lineage-specific expansions in the OR/GR superfamily. So far, however, we lack functional data on all of these expansions, and until now no one has tested if the expansions are specific for the genus or the species. This could be done by sequencing the genome of several species of one genus. These expansions might play an important role in insect speciation.

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Figures

Fig.1: Maximum likelihood phylogenetic tree of Lepidoptera ORs, rooted to the coreceptor ORCo. ORCo subgroup is highlighted in yellow. The pheromone receptor group (PRs) according to Engsontia (Engsontia et al., 2014) is highlighted in dark blue. A subgroup containing additional sex specific ORs is highlighted in light blue. (Picture sources: *Danaus plexippus* from http://www.morguefile.com/archive/display/226596, *Heliconius melpomene* was obtained from the Smithsonian Institution, *Plutella xylostella* from Donald Hobern, *Spodoptera littoralis* from https://www.flickr.com/photos/gails_pictures/5256990553/, *Spodoptera litura* from Amy Carmichael)

Fig.2: Sex specific expression of two ORs belonging to the additional pheromone receptor group: (A) RT-PCR using male and female cDNA with Primers for RL31 (positive control) and MsexOR-15. MsexOR-15 detection is detected in female antennae only. (B) fluorescence *in situ* hybridization of female antennae using a RNA probe against MsexOR-15 transcripts. (C) only the fluorescence channel of (B). (D) RT-PCR using male and female

cDNA with Primers for RL31 (positive control) and MsexOR-51. MsexOR-51 expression is detected in male antennae only. (E) fluorescence *in situ* hybridization of male antennae using a probe against MsexOR-51. Cells expressing MsexOR-51 are located underneath long trichoid sensilla. (F) only the fluorescence channel of (E). Scale bar is 50 µm.

Fig.3: Synteny analysis of two scaffolds of *M. sexta* and the respective chromosomes of *B. mori*. *M. sexta* has six orthologs to BmorOR-24. The neighboring genes are also orthologs to the respective *B. mori* genes, indicating more extensive microsynteny. *M. sexta* has three orthologs to BmorOR-20. The neighboring genes are orthologs in both species. But the orthologues of BmorOR-19 are located on a different scaffold in *M. sexta*, indicating a genomic rearrangement in the evolution of *M. sexta*.

Fig.4: Expression profile of ORs in male, female and larval tissue. Expression values are in RPKM; the y axis is logarithmic. Among the ORs male specific, female specific and larval specific receptors can be identified. MsexOR-51, a male specific OR belonging to the pheromone receptor group, is expressed in larval antennae too.

Fig.5: Maximum likelihood phylogenetic tree of Lepidoptera and *D. melanogaster* IRs rooted to the NMDA receptors and IR25a as well as IR8a. We found orthologues for every reported antennal IRs in *M. sexta*. For IR75p *M. sexta* has a lineage specific expansion. iGluRs are marked yellow and are presented without names. (Picture sources: *Danaus plexippus* from http://www.morguefile.com/archive/display/226596, *Heliconius melpomene* was obtained from the Smithsonian Institution, *Plutella xylostella* from Donald Hobern, *Spodoptera littoralis* from https://www.flickr.com/photos/gails_pictures/5256990553/, *Spodoptera litura* from Amy Carmichael)

Fig.6: Maximum likelihood phylogenetic tree of Lepidoptera GRs. SR1 and SR2 stands for sugar receptor group 1 or 2 (Picture sources: *Danaus plexippus* from http://www.morguefile.com/archive/display/226596, *Heliconius melpomene* was obtained from the Smithsonian Institution)

Fig.7: Alignment of the predicted amino acid sequence of putative Lepidoptera sugar receptors (SR). SR1: These SRs are characterized by an additional exon (start and end marked by arrows). SR2: These SRs miss that exon.

References

Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T., Hansson, B.S., 2013. Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. Elife 2013. doi:10.7554/eLife.00421

Anderson, A.R., Wanner, K.W., Trowell, S.C., Warr, C.G., Jacquin-Joly, E., Zagatti, P., Robertson, H., Newcomb, R.D., 2009. Molecular basis of female-specific odorant responses in *Bombyx mori*. Insect Biochem. Mol. Biol. 39, 189–97. doi:10.1016/j.ibmb.2008.11.002

Angerer, L.M., Angerer, R.C., 1992. *In situ* hybridization to cellular RNA with radiolabeled RNA probes., in: DG, W. (Ed.), In Situ Hybridization. IRL Press, Oxford, p. 17.

Bengtsson, J.M., Gonzalez, F., Cattaneo, A.M., Montagné, N., Walker, W.B., Bengtsson, M., Anfora, G., Ignell, R., Jacquin-Joly, E., Witzgall, P., 2014. A predicted sex pheromone receptor of codling moth *Cydia pomonella* detects the plant volatile pear ester. Front. Ecol. Evol. 2, 1–11. doi:10.3389/fevo.2014.00033

Benton, R., Sachse, S., Michnick, S.W., Vosshall, L.B., 2006. Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. PLoS Biol. 4, e20. doi:10.1371/journal.pbio.0040020

Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136, 149–62. doi:10.1016/j.cell.2008.12.001

Bohbot, J.D., Jones, P.L., Wang, G., Pitts, R.J., Pask, G.M., Zwiebel, L.J., 2011. Conservation of indole responsive odorant receptors in mosquitoes reveals an ancient olfactory trait. Chem. Senses 36, 149–160. doi:10.1093/chemse/bjq105

Briscoe, A.D., Macias-Muñoz, A., Kozak, K.M., Walters, J.R., Yuan, F., Jamie, G. a., Martin, S.H., Dasmahapatra, K.K., Ferguson, L.C., Mallet, J., Jacquin-Joly, E., Jiggins, C.D., 2013. Female Behaviour Drives Expression and Evolution of Gustatory Receptors in Butterflies. PLoS Genet. 9. doi:10.1371/journal.pgen.1003620

Butenandt, A., Beckmann, R., Stamm, D., Hecker, E., 1959. Über den Sexuallockstoff des Seidenspinners *Bombyx mori* - Reindarstellung und Konstitution. Zeitschrift für Naturforsch. B 14, 283–284.

Cande, J., Prud'homme, B., Gompel, N., 2013. Smells like evolution: The role of chemoreceptor evolution in behavioral change. Curr. Opin. Neurobiol. doi:10.1016/j.conb.2012.07.008

Clyne, P.J., Warr, C.G., Carlson, J.R., 2000. Candidate taste receptors in *Drosophila*. Science 287, 1830–1834. doi:10.1126/science.287.5459.1830

Couto, A., Alenius, M., Dickson, B.J., 2005. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr. Biol. 15, 1535–1547. doi:10.1016/j.cub.2005.07.034

Croset, V., Rytz, R., Cummins, S.F., Budd, A., Brawand, D., Kaessmann, H., Gibson, T.J., Benton, R., 2010. Ancient Protostome Origin of Chemosensory Ionotropic Glutamate Receptors and the Evolution of Insect Taste and Olfaction. Plos Genet. 6. doi:10.1371/journal.pgen.1001064

Dunipace, L., Meister, S., McNealy, C., Amrein, H., 2001. Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. Curr. Biol. 11, 822–835. doi:10.1016/S0960-9822(01)00258-5

Engsontia, P., Sangket, U., Chotigeat, W., Satasook, C., 2014. Molecular Evolution of the Odorant and Gustatory Receptor Genes in Lepidopteran Insects: Implications for Their Adaptation and Speciation. J. Mol. Evol. 79, 21–39. doi:10.1007/s00239-014-9633-0

Freeman, E.G., Wisotsky, Z., Dahanukar, A., 2014. Detection of sweet tastants by a conserved group of insect gustatory receptors. Proc. Natl. Acad. Sci. U. S. A. 111, 1598–603. doi:10.1073/pnas.1311724111

Fujii, S., Yavuz, A., Slone, J., Jagge, C., Song, X., Amrein, H., 2015. *Drosophila* Sugar Receptors in Sweet Taste Perception, Olfaction, and Internal Nutrient Sensing. Curr. Biol. 25, 621–627. doi:10.1016/j.cub.2014.12.058

Ghaninia, M., Olsson, S.B., Hansson, B.S., 2014. Physiological Organization and Topographic Mapping of the Antennal Olfactory Sensory Neurons in Female Hawkmoths, *Manduca sexta*. Chem. Senses. doi:10.1093/chemse/bju037

Grosse-Wilde, E., Gohl, T., Bouche, E., Breer, H., Krieger, J., 2007. Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. Eur. J. Neurosci. 25, 2364–2373. doi:10.1111/j.1460-9568.2007.05512.x

Grosse-Wilde, E., Kuebler, L.S., Bucks, S., Vogel, H., Wicher, D., Hansson, B.S., 2011. Antennal transcriptome of *Manduca sexta*. Proc. Natl. Acad. Sci. U. S. A. 108, 7449–7454. doi:10.1073/pnas.1017963108

Grosse-Wilde, E., Stieber, R., Forstner, M., Krieger, J., Wicher, D., Hansson, B.S., 2010. Sex-specific odorant receptors of the tobacco hornworm *Manduca sexta*. Front. Cell. Neurosci. 4. doi:22 10.3389/fncel.2010.00022

Grosse-Wilde, E., Svatos, A., Krieger, J., 2006. A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem. Senses 31, 547–555. doi:10.1093/chemse/bjj059

Hallem, E. a, Dahanukar, A., Carlson, J.R., 2006. Insect odor and taste receptors. Annu. Rev. Entomol. 51, 113–135. doi:10.1146/annurev.ento.51.051705.113646

Hallem, E.A., Ho, M.G., Carlson, J.R., 2004. The molecular basis of odor coding in the *Drosophila* antenna. Cell 117, 965–979.

Hanahan, D., 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166, 557–580. doi:10.1016/S0022-2836(83)80284-8

Hansson, B.S., 1995. Olfaction in Lepidoptera. Experientia. doi:10.1007/BF01946910

Hansson, B.S., Stensmyr, M.C., 2011. Evolution of Insect Olfaction. Neuron 72, 698–711. doi:10.1016/j.neuron.2011.11.003

Howlett, N., Dauber, K.L., Shukla, A., Morton, B., Glendinning, J.I., Brent, E., Gleason, C., Islam, F., Izquierdo, D., Sanghavi, S., Afroz, A., Aslam, A., Barbaro, M., Blutstein, R., Borovka, M., Desire, B., Elikhis, A., Fan, Q., Hoffman, K., Huang, A., Keefe, D., Lopatin, S., Miller, S., Patel, P., Rizzini, D., Robinson, A., Rokins, K., Turlik, A., Mansfield, J.H., 2012. Identification of chemosensory receptor genes in *Manduca sexta* and knockdown by RNA interference. BMC Genomics 13, 211. doi:10.1186/1471-2164-13-211

Jacquin-Joly, E., Legeai, F., Montagné, N., Monsempes, C., François, M.C., Poulain, J., Gavory, F., Walker, W.B., Hansson, B.S., Larsson, M.C., 2012. Candidate chemosensory genes in female antennae of the noctuid moth *Spodoptera littoralis*. Int. J. Biol. Sci. 8, 1036–1050. doi:10.7150/ijbs.4469

Jones, W.D., Cayirlioglu, P., Kadow, I.G., Vosshall, L.B., 2007. Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. Nature 445, 86–90. doi:10.1038/nature05466

Kaissling, K.E., Hildebrand, J.G., Tumlinson, J.H., 1989. Pheromone Receptor Cells in the Male Moth *Manduca sexta*. Arch. Insect Biochem. Physiol. 10, 273–279.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066. doi:10.1093/nar/gkf436

Kent, L.B., Robertson, H.M., 2009. Evolution of the sugar receptors in insects. BMC Evol. Biol. 9, 41. doi:10.1186/1471-2148-9-41

Koenig, C., Bretschneider, A., Heckel, D.G., Grosse-Wilde, E., Hansson, B.S., Vogel, H., 2015. The plastic response of *Manduca sexta* to host and non-host plants. Insect Biochem. Mol. Biol. 63, 72–85. doi:10.1016/j.ibmb.2015.06.001

Krieger, J., Grosse-Wilde, E., Gohl, T., Breer, H., 2005. Candidate pheromone receptors

of the silkmoth *Bombyx mori*. Eur. J. Neurosci. 21, 2167–2176. doi:10.1111/j.1460-9568.2005.04058.x

Krieger, J., Grosse-Wilde, E., Gohl, T., Dewer, Y.M.E., Raming, K., Breer, H., 2004. Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). Proc. Natl. Acad. Sci. U. S. A. 101, 11845–11850. doi:10.1073/pnas.0403052101

Kurtovic, A., Widmer, A., Dickson, B.J., 2007. A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature 446, 542–546. doi:10.1038/nature05672

McBride, C.S., Arguello, J.R., 2007. Five *Drosophila* genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. Genetics 177, 1395–1416. doi:10.1534/genetics.107.078683

Menuz, K., Larter, N.K., Park, J., Carlson, J.R., 2014. An RNA-Seq Screen of the *Drosophila* Antenna Identifies a Transporter Necessary for Ammonia Detection. PLoS Genet. 10, e1004810. doi:10.1371/journal.pgen.1004810

Missbach, C., Dweck, H.K.M., Vogel, H., Vilcinskas, A., Stensmyr, M.C., Hansson, B.S., Grosse-Wilde, E., 2014. Evolution of insect olfactory receptors. Elife 2014, 1–22. doi:10.7554/eLife.02115

Mitsuno, H., Sakurai, T., Murai, M., Yasuda, T., Kugimiya, S., Ozawa, R., Toyohara, H., Takabayashi, J., Miyoshi, H., Nishioka, T., 2008. Identification of receptors of main sex-pheromone components of three Lepidopteran species. Eur. J. Neurosci. 28, 893–902. doi:10.1111/j.1460-9568.2008.06429.x

Miyamoto, T., Slone, J., Song, X., Amrein, H., 2012. A fructose receptor functions as a nutrient sensor in the *Drosophila* brain. Cell 151, 1113–1125. doi:10.1016/j.cell.2012.10.024

Montagné, N., Chertemps, T., Brigaud, I., François, A., François, M.-C., de Fouchier, A., Lucas, P., Larsson, M.C., Jacquin-Joly, E., 2012. Functional characterization of a sex pheromone receptor in the pest moth *Spodoptera littoralis* by heterologous expression in Drosophila. Eur. J. Neurosci. 36, 2588–96. doi:10.1111/j.1460-9568.2012.08183.x

Montell, C., 2009. A taste of the *Drosophila* gustatory receptors. Curr. Opin. Neurobiol. 19, 345–353. doi:10.1016/j.conb.2009.07.001

Nakagawa, T., Sakurai, T., Nishioka, T., Touhara, K., 2005. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307, 1638–1642. doi:10.1126/science.1106267

Nei, M., Niimura, Y., Nozawa, M., 2008. The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. Nat. Rev. Genet. 9, 951–963.

doi:10.1038/nrg2480

Neuhaus, E.M., Gisselmann, G., Zhang, W.Y., Dooley, R., Stortkuhl, K., Hatt, H., 2005. Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. Nat. Neurosci. 8, 15–17. doi:10.1038/nn1371

Ni, L., Bronk, P., Chang, E.C., Lowell, A.M., Flam, J.O., Panzano, V.C., Theobald, D.L., Griffith, L.C., Garrity, P. a, 2013. A gustatory receptor paralogue controls rapid warmth avoidance in *Drosophila*. Nature 500, 580–4. doi:10.1038/nature12390

Olivier, V., Monsempes, C., François, M.-C., Poivet, E., Jacquin-Joly, E., 2011. Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol. Biol. 20, 189–99. doi:10.1111/j.1365-2583.2010.01057.x

Patch, H.M., Velarde, R.A., Walden, K.K.O., Robertson, H.M., 2009. A Candidate Pheromone Receptor and Two Odorant Receptors of the Hawkmoth *Manduca sexta*. Chem. Senses 34, 305–316. doi:10.1093/chemse/bjp002

Poivet, E., Gallot, A., Montagné, N., Glaser, N., Legeai, F., Jacquin-Joly, E., 2013. A comparison of the olfactory gene repertoires of adults and larvae in the noctuid moth *Spodoptera littoralis*. PLoS One 8, e60263. doi:10.1371/journal.pone.0060263

Poivet, E., Rharrabe, K., Monsempes, C., Glaser, N., Rochat, D., Renou, M., Marion-Poll, F., Jacquin-Joly, E., 2012. The use of the sex pheromone as an evolutionary solution to food source selection in caterpillars. Nat. Commun. 3, 1047. doi:10.1038/ncomms2050

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One 5. doi:10.1371/journal.pone.0009490

Reisenman, C.E., Riffell, J.A., Hildebrand, J.G., 2009. Neuroethology of oviposition behavior in the moth *Manduca sexta*. Ann. N. Y. Acad. Sci. 1170, 462–7.

Riffell, J. a, Lei, H., Hildebrand, J.G., 2009. Neural correlates of behavior in the moth *Manduca sexta* in response to complex odors. Proc. Natl. Acad. Sci. U. S. A. 106, 19219–26. doi:10.1073/pnas.0910592106

Robertson, H.M., Kent, L.B., 2009. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. J. Insect Sci. 9, 19. doi:10.1673/031.009.1901

Robertson, H.M., Warr, C.G., Carlson, J.R., 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. A. 100 Suppl , 14537–42. doi:10.1073/pnas.2335847100

Rytz, R., Croset, V., Benton, R., 2013. Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. Insect Biochem. Mol. Biol. 43, 888–97. doi:10.1016/j.ibmb.2013.02.007

Saina, M., Busengdal, H., Sinigaglia, C., Petrone, L., Oliveri, P., Rentzsch, F., Benton, R., 2015. A cnidarian homologue of an insect gustatory receptor functions in developmental body patterning. Nat. Commun. 6, 6243. doi:10.1038/ncomms7243

Sakurai, T., Nakagawa, T., Mitsuno, H., Mori, H., Endo, Y., Tanoue, S., Yasukochi, Y., Touhara, K., Nishioka, T., 2004. Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc. Natl. Acad. Sci. U. S. A. 101, 16653–16658. doi:10.1073/pnas.0407596101

Sánchez-Gracia, A., Vieira, F.G., Rozas, J., 2009. Molecular evolution of the major chemosensory gene families in insects. Heredity (Edinb). 103, 208–216. doi:10.1038/hdy.2009.55

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., Touhara, K., 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452, 1002–U9. doi:10.1038/nature06850

Shields, V.D.C., Hildebrand, J.G., 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth *Manduca sexta*. Microsc. Res. Tech. 55, 307–329. doi:10.1002/jemt.1180

Stengl, M., 2010. Pheromone transduction in moths. Front. Cell. Neurosci. 4. doi:10.3389/fncel.2010.00133

Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R., Touhara, K., 2009. Highly Selective Tuning of a Silkworm Olfactory Receptor to a Key Mulberry Leaf Volatile. Curr. Biol. 19, 881–890. doi:10.1016/j.cub.2009.04.035

Tumlinson, J.H., Brennan, M.M., Doolittle, R.E., Mitchell, E.R., Brabham, A., Mazomenos, B.E., Baumhover, A.H., Jackson, D.M., 1989. Identification of a Pheromone Blend Attractive to *Manduca sexta* (L.) Males in a Wind Tunnel. Arch. Insect Biochem. Physiol. 10, 255–271.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., Axel, R., 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96, 725–736.

Vosshall, L.B., Hansson, B.S., 2011. A Unified Nomenclature System for the Insect Olfactory Coreceptor. Chem. Senses 36, 497–498. doi:10.1093/chemse/bjr022

Vosshall, L.B., Wong, A.M., Axel, R., 2000. An Olfactory Sensory Map in the Fly Brain. Cell 102, 147–159. doi:10.1016/S0092-8674(00)00021-0

Wang, G., Vásquez, G.M., Schal, C., Zwiebel, L.J., Gould, F., 2011. Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. Insect Mol. Biol. 20, 125–33. doi:10.1111/j.1365-2583.2010.01045.x

Wanner, K.W., Anderson, a R., Trowell, S.C., Theilmann, D. a, Robertson, H.M., Newcomb, R.D., 2007. Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. Insect Mol. Biol. 16, 107–19. doi:10.1111/j.1365-2583.2007.00708.x

Wanner, K.W., Robertson, H.M., 2008. The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. Insect Mol. Biol. 17, 621–629. doi:10.1111/j.1365-2583.2008.00836.x

Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., Hansson, B.S., 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452, 1007–U10. doi:10.1038/nature06861

Xu, W., Papanicolaou, A., Liu, N.-Y., Dong, S.-L., Anderson, A., 2015. Chemosensory receptor genes in the Oriental tobacco budworm *Helicoverpa assulta*. Insect Mol. Biol. 24, 253–263. doi:10.1111/imb.12153

Yasukochi, Y., Tanaka-Okuyama, M., Shibata, F., Yoshido, A., Marec, F., Wu, C., Zhang, H., Goldsmith, M.R., Sahara, K., 2009. Extensive conserved synteny of genes between the karyotypes of *Manduca sexta* and *Bombyx mori* revealed by BAC-FISH mapping. PLoS One 4. doi:10.1371/journal.pone.0007465

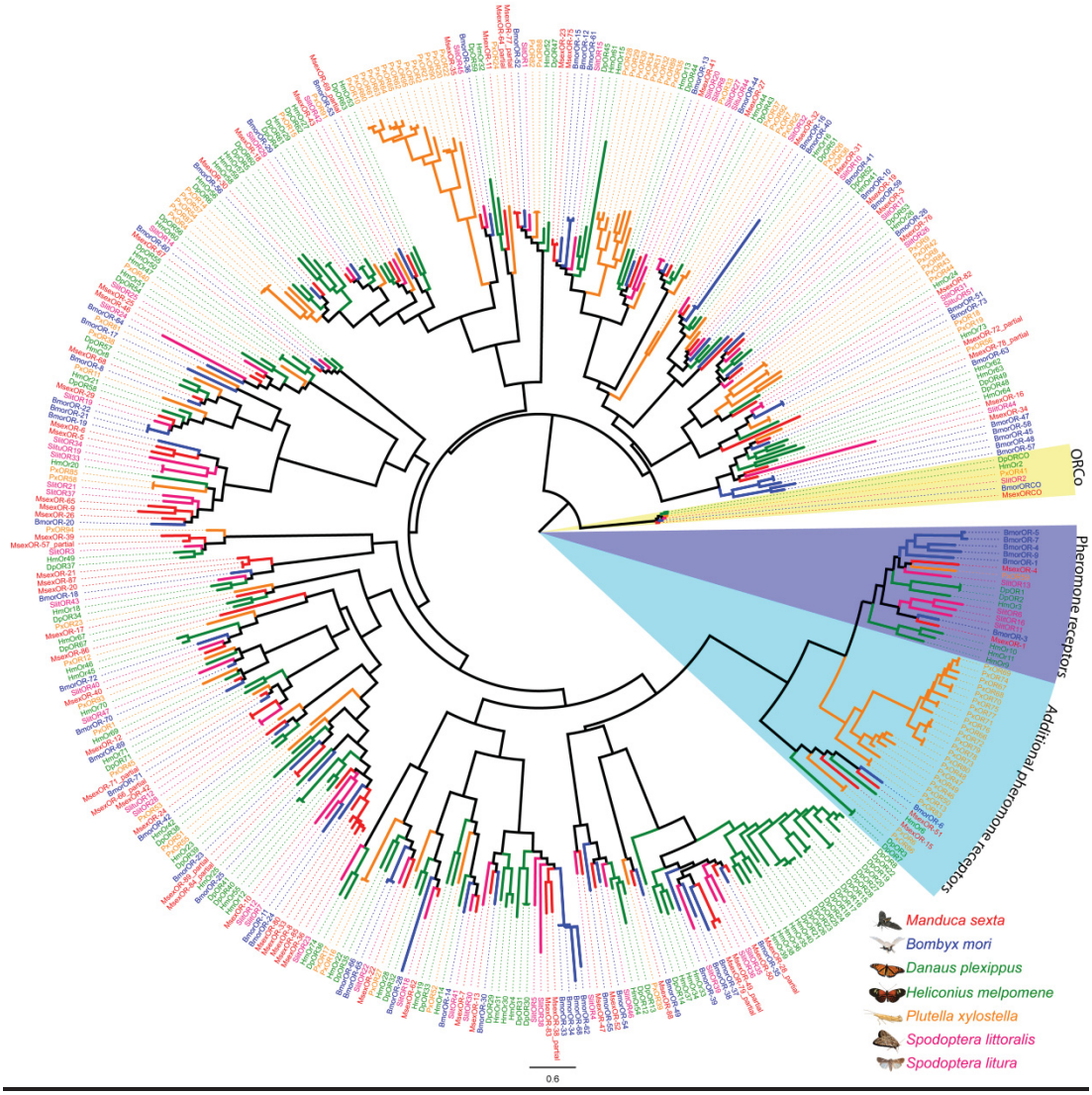
Zhan, S., Merlin, C., Boore, J.L., Reppert, S.M., 2011. The monarch butterfly genome yields insights into long-distance migration. Cell 147, 1171–85. doi:10.1016/j.cell.2011.09.052

Zhang, H.J., Anderson, A.R., Trowell, S.C., Luo, a. R., Xiang, Z.H., Xia, Q.Y., 2011. Topological and functional characterization of an insect gustatory receptor. PLoS One 6, 1–10. doi:10.1371/journal.pone.0024111

Zhang, J., Liu, C.C., Yan, S.W., Liu, Y., Guo, M.B., Dong, S.L., Wang, G.R., 2013. An odorant receptor from the common cutworm (*Spodoptera litura*) exclusively tuned to the important plant volatile cis-3-Hexenyl acetate. Insect Mol. Biol. 22, 424–432. doi:10.1111/imb.12033

Zhang, Y. V, Ni, J., Montell, C., 2013. The molecular basis for attractive salt-taste coding in *Drosophila*. Science 340, 1334–8. doi:10.1126/science.1234133

Fig.1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Pheromone receptors
Additional pheromone receptors
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Spodoptera litura
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Fig.2

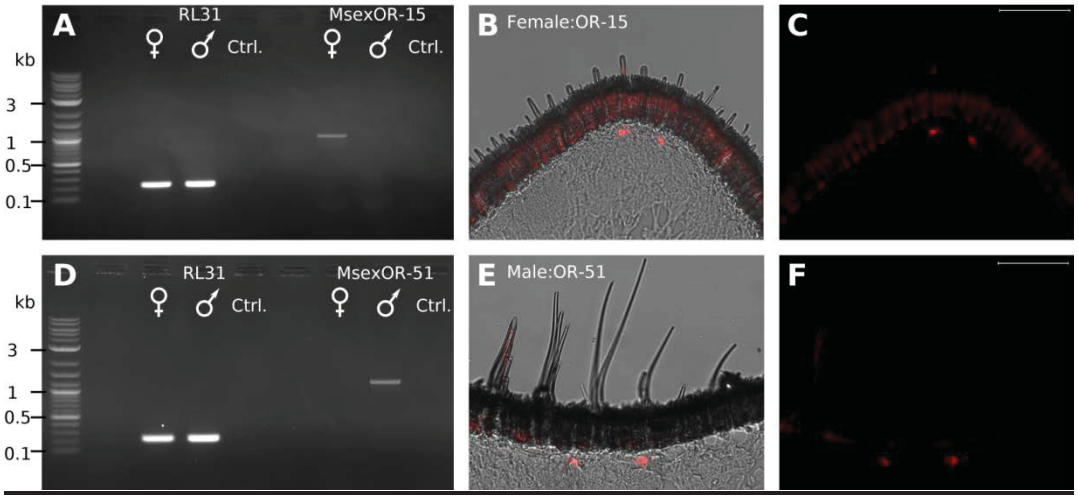
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Fig.3

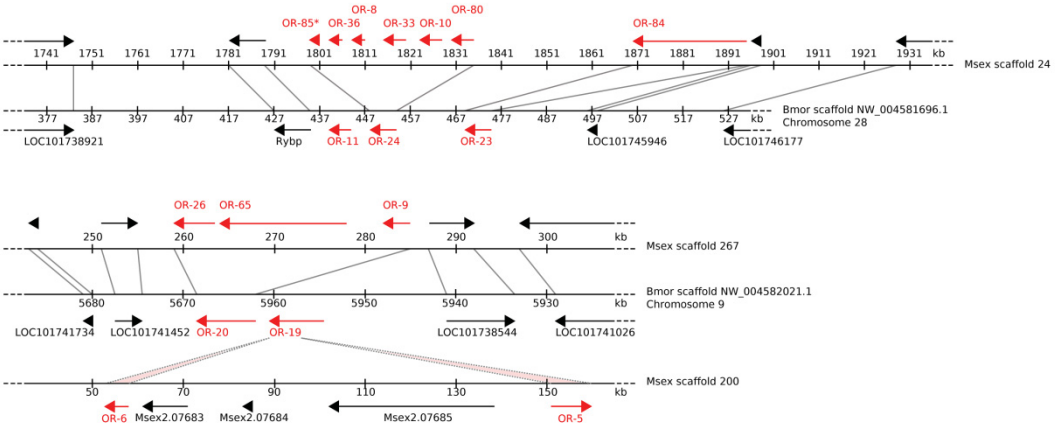
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Fig.4

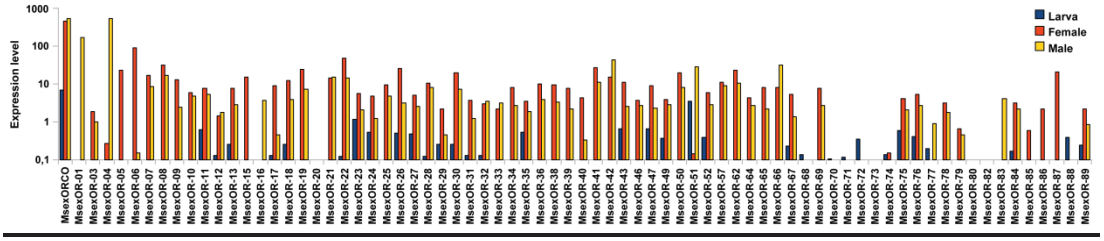
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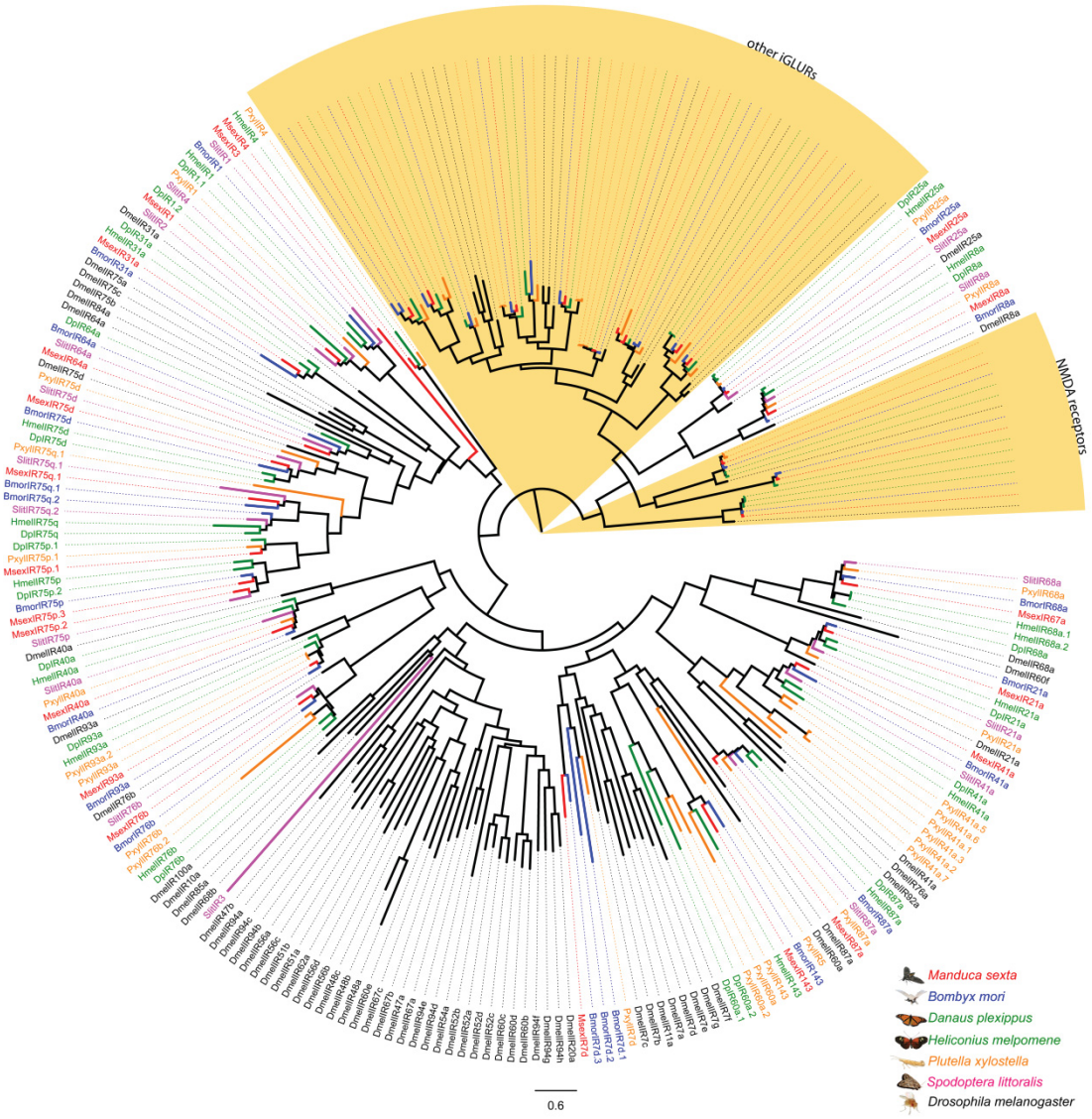
Fig.5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NMDA receptors
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Danaus plexippus
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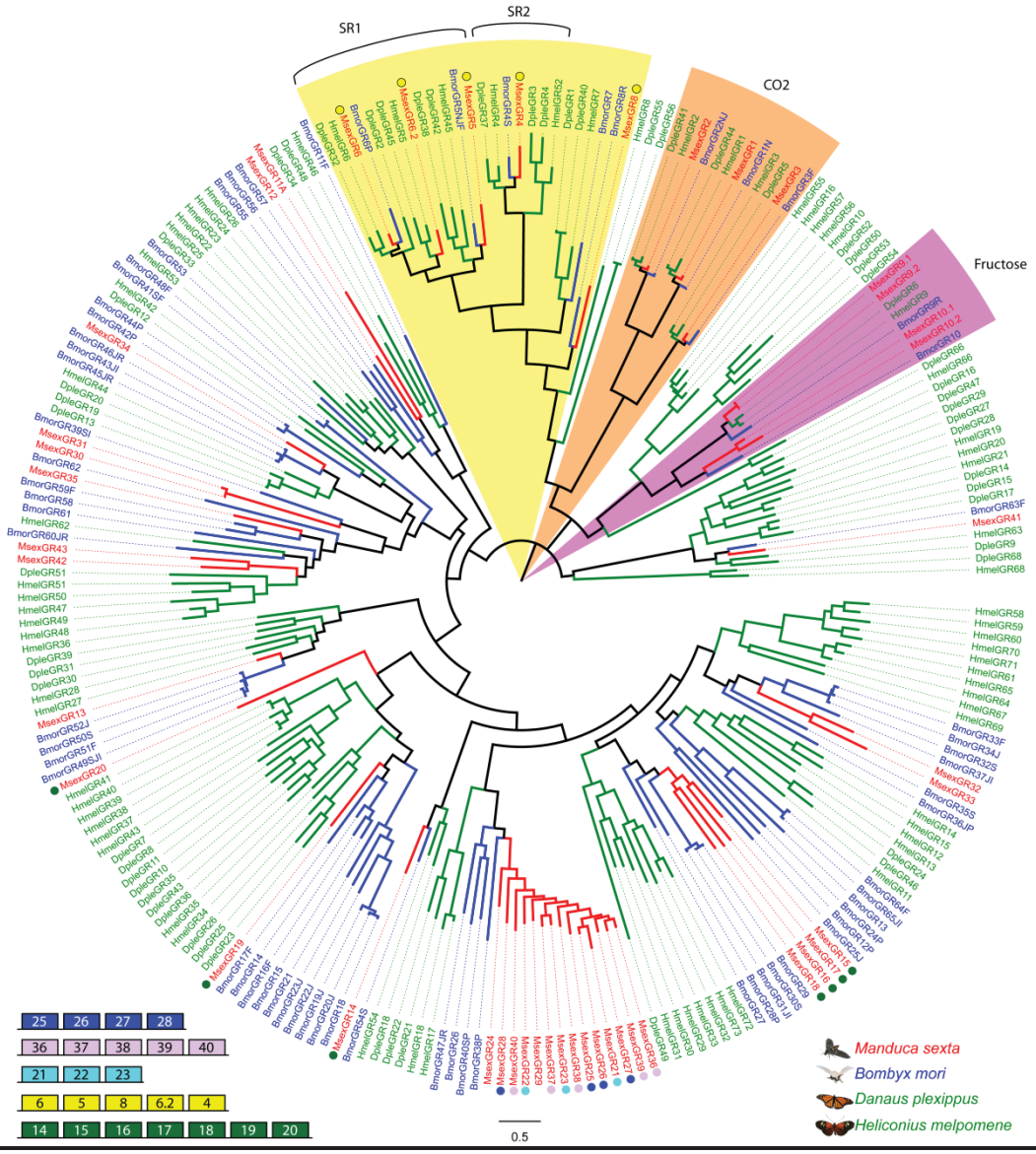
Drosophila melanogaster

Fig.6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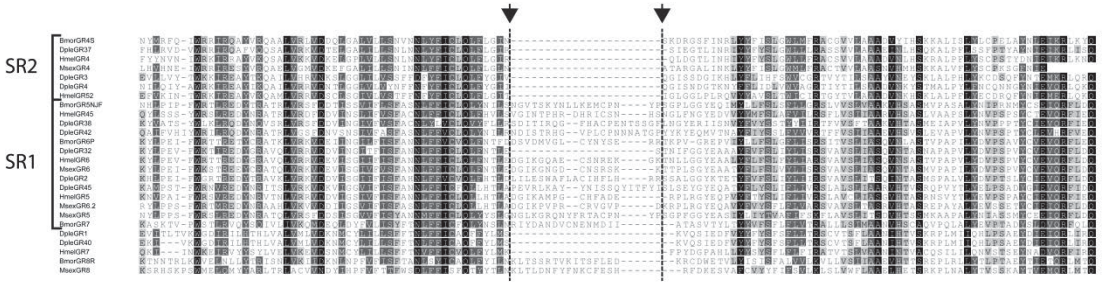
Bombyx mori
Danaus plexippus
Heliconius melpomene

Fig.7



Supplement

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4	Msex2.13403-RA	41a	Msex2.03121	4	Msex2.01718
5	Msex2.07686-RB	21a	Msex2.03713	5	Msex2.01731
6	Msex2.07682-RA	64a	Msex2.05776	6	Msex2.01729
7	Msex2.07660-RA	75p.3	Msex2.07446	7	Msex2.04097
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9	Msex2.09282-RA	75p.1	Msex2.09079	9	Msex2.05028
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12	Msex2.03330-RA	8a	Msex2.10447	12	Msex2.06155
13	Msex2.05711-RB	40a	Msex2.11104	13	Msex2.05778
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25	Msex2.08017-RA			24	Msex2.00832
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27	Msex2.02512-RA			26	Msex2.01738
28	Msex2.12520-RA			27	Msex2.01740
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38	Msex2.00624-RA			36	Msex2.07485
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Msex OR	OGS2 name	MsexIR	OGS2 name	Msex GR	OGS2 name
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47	Msex2.04331			43	Msex2.11166
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Gene name	GR subfamily	Tissue sample
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MsGR2	SR	abdomen_larva4_late abdomen_larva5_early abdomen_larve5_prewander abdomen_larve5_wander brain_larva2_early brain_larva3_early brain_larva4_early, fat_pupa head_2d_5 head_adult_1d, midgut_larva2, , testes_adult_1d testes_pupal_15d testes_pupal_3d
MsGR30	SR	fat_body_adult_1d, malpighian_tubules_adult_1d, testes_adult_1d testes_pupal_15d
MsGR34	bitter	fat_body_pupal_15d, ovaries_pupal_15d testes_adult_1d testes_pupal_15d testes_pupal_3d
MsGR35	fructose	abdomen_larve5_prewander, fat_body_larva5_prewander, ovaries_pupal_15d testes_adult_1d testes_pupal_15d
MsGR38	fructose	brain_larva2_early brain_larva3_early, fat_body_adult_1d fat_body_adult_7d, malpighian_tubules_adult_1d malpighian_tubules_adult_3d, midgut_adult_3d ovaries_adult_1d ovaries_pupal_15d testes_adult_1d testes_pupal_15d

Expression of GRs identified in transcriptome libraries of the *Manduca* Genome Project.

Manuscript 2

Olfaction in the ovipositor of *Manduca sexta*

Christian Klinner, Christopher Koenig, Kevin C. Daly, Bill S. Hansson, Ewald Grosse-Wilde

Chemical senses, *in preparation*

Abstract

The tobacco hornworm *Manduca sexta* is a classical model organism in the fields of olfaction and chemical ecology. A major research focused has been the interaction with its host plants for oviposition. However, there is very little information on olfactory functions involved in this process in organs outside the antennae.
Here we present an analysis of olfaction in the ovipositor of *M.sexta*. We have characterized the expression of several chemosensory receptors in the tissue. Scanning electron microscopy allowed us to identify and visualize 7 putative olfactory sensilla, that are similar in appearance to sensilla basiconica. Finally, functional characterization of receptor neurons in these sensilla, using a broad panel of 142 odorants, clearly revealed responses elicited by stimulation with odorants. Four functional distinct OSN types could be identified based their response profiles. Overall, the data clearly demonstrates for the first time an olfactory function of the *M.sexta* ovipositor. Furthermore our results strongly suggest a function in the identification of oviposition sites as well as mating.

1. Introduction

The sensory perception and assessment of volatile cues from the environment is critical for the lifestyle of many insects. The information provided by odours is oftentimes guiding many crucial behaviors, e.g. orientation to food sources, intraspecific communication, and location of suitable oviposition sites (Baker, 1989; Renwick, 1989). Especially *M.sexta* as a classical olfactory model organism has been studied intensively. One well analysed behaviour here is the choice of an optimal host plant for ovipositing; it is of utmost importance to ensure offspring survival. Ovipositing females are exposed to different stimuli for orientation towards potential host plants including visual-, olfactory- and contact cues (Yamamoto et al., 1969).

M.sexta preferentially selects solanacaeous plants (Mechaber and Hildebrand, 2000) e.g. tobacco (*Nicotiana spec.*), tomato *(Solanum lycopersicum))* (Mechaber et al., 2002) and *Datura wrightii* (Raguso et al., 2003a; Riffell et al., 2008) for oviposition and larval feeding. Host plants emit a variety of olfactory cues (Fraser et al., 2003; Mechaber et al., 2002; Raguso et al., 2003b; Späthe et al., 2013). Of importance are green leaf volatiles (GLVs); they comprise defensive volatiles and herbivore-induced plant volatiles (HIPV) that are released after herbivore damage and are attractant to predators of *M. sexta* (Hare, 2010; Köllner et al., 2008; Rasmann et al., 2005). Female moths avoid oviposition on herbivore damaged plants (Reisenman et al., 2009).

In general, volatiles from specific microbial communities can affect moth oviposition (Davis et al., 2013). For example, fungal infection reduced attraction and oviposition by the grapevine moth *Lobesia botrana* (Tasin et al., 2012). This indicates that the animal is able to identify changes in the chemical profile of the plants headspace caused by an infection. Volatiles are used as indicators of plant quality by moths when searching for host plants and suitable oviposition sites (Tasin et al., 2011). All this demonstrates that the ovipositing females perception of a highly dynamic olfactory environment is crucial for the survival of the offspring.

In contrast to the antennae, the primary olfactory organ in insects (Hansson and Stensmyr, 2011), other organs with a proposed olfactory function are not well characterized.

In *M.sexta* outside the antennae putative chemosensory sensilla have been described in legs (Kent and Griffin, 1990), labial palps (Kent et al., 1986) and ovipositor (Eaton, 1986). Oviposition is a complex behavior that relies input from multiple sensory modalities (Ramaswamy, 1988; Yamamoto et al., 1969).

The ovipositor of *M.sexta* consists of the extended terminal segment, the paired papillae anales, which is covered by many trichoid sensilla (Eaton, 1986), which are supposedly involved in mechano- and contact chemosensation. Putative olfactory sensilla on the ovipositor have not yet been described. In two other species of Lepidoptera, *Monopis crocicapitella* and *Homoeosoma nebulella,* Faucheux (Faucheux, 1988, 1991) proposed an olfactory role of a multiporous sensillum basiconicum on each of the anal papillae. The importance of mechano- and contact chemosensory hairs on abdominal segments in host plant selection for oviposition was described in several lepidopteran insects (Baker and Ramaswamy, 1990a; Chadha and Roome, 1980; Valencia and Rice, 1982; Waladde, 1983).
Multiporous sensilla are essential in sensing olfactory molecules. The pores allow odor molecules to reach dendrites of olfactory sensory neuron (OSNs) in the interior (Keil and Steinbrecht, 1984). Receptors detecting these compounds are embedded in the dendritic membrane (Wicher, 2015). Three types of chemosensory receptors are available in the detection of volatiles: olfactory receptors (ORs) (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999), ionotropic receptors (IRs) (Benton et al., 2009) and CO_2 receptors, which belong to the gustatory receptor gene family (GRs) (Jones et al., 2007; Kwon et al., 2007).
ORs form a heteromultimeric complex composed of the OR-X protein, which determines ligand-specificity, and the co-receptor ORCo (Hallem et al., 2004; Neuhaus et al., 2005; Vosshall and Hansson, 2011) that acts as an ion channel (Sato et al., 2008; Wicher et al., 2008). ORCo also functions as a chaperon for the transfer of ORs to the OSN dendrites (Benton et al., 2006). IRs are variant ionotropic receptors related to ionotropic glutamate receptors (iGluRs). The subfamily of antennal IRs were recently identified as odorant detecting (Benton et al., 2009); they form complexes with one of two IR coreceptors, IR8a and IR25a (Abuin et al., 2011; Rytz et al., 2013).
In Lepidoptera, expression of IRs, ORs and ORCo in the ovipositor has been reported for *Sesamia nonagrioide* (Glaser et al., 2013), indicating a potential presence of olfactory detection in this organ. Similarily, 2 ORs, but no ORCo were found in *Chilo suppressalis* (Xia et al., 2015).
Here we present an analysis of the expression of chemosensory receptor genes in the ovipositor of *M.sexta* females. Furthermore we performed scanning electron microscopy to visualize and identify putative olfactory sensilla. Finally, we performed single sensillum recordings, demonstrating for the first time the presence of olfactory sensory neurons in

the ovipositor of a lepidopteran species, identifying potentially ecologically relevant ligands from a broad panel of odors.

2. Materials and methods

2.1. Animals

Larvae of the hawkmoth *M.sexta* (Lepidoptera, Sphingidae) were reared on artificial diet (46 g of agar, 144 g of wheat germ, 140 g of corn meal, 76 g of soy flour, 75 g of casein, 24 g of Wesson's salt mixture, 36 g of sugar, 5 g of cholesterol, 12 g of ascorbic acid, 6 g of sorbic acid, 3 g of methyl paraben, 9 mL of linseed oil, 60 mL of 3.7% formalin, 30 mg of nicotinic acid, 15 mg of riboflavin, 7mg of thiamine, 7mg of pyridoxine, 7mg of folic acid, and 0.6 mg of biotin per 1.8 L of water), under a photoperiod of 16 h light/8 h dark with relative humidity of 75% at 26 °C.

Two day-old virgin female adults were allowed to mate for one day and used for electrophysiological measurements afterwards. Virgin female adults were used after 2-3 days after eclosion.

2.2. RNAseq

Abdominal segments 8-11 of three mated and three virgin *M. sexta* females were dissected and directly transferred to Tri Reagent (Sigma-Aldrich, USA). Samples were homogenized for 5 min at 50 Hz with two 3 mm steal beads (Qiagen, Germany) using a Tissuelyser (Qiagen, Germany). Samples were stored at -20° C. RNA isolation was performed according to the manufacturer's instructions.

Library construction and sequencing was performed by the Max Planck Genome Center Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). 1 µg of total RNA from each sample was used for TruSeq library preparation. TruSeq libraries were generated from poly-A enriched mRNA. The library was sequenced with an Illumina HiSeq 2500 sequencer and 10 million 100 bp single reads per sample were obtained.

For mapping we used the official gene set 2 of *M. sexta* and replaced the OR, IR and GR sequences as reported (Koenig et al, 2015). The *M. sexta* official gene set 2 data can be accessed from https://i5k.nal.usda.gov/Manduca_sexta or ftp://ftp.bioinformatics.ksu.edu/pub/Manduca/OGS2/. The reads were mapped to the reference and the expression values were calculated as RPKM (Reads per kilo base per million mapped reads) using CLC Genomics Workbench v8.5 (http://www.clcbio.com).

2.3. RT-PCR

Total RNA was isolated from single virgin or mated female ovipositors as described for RNAseq. RNA samples were treated with TurboDNAse (Ambion, TX). Three biological replicates were obtained for each. The RNA samples served as template for cDNA synthesis using the SuperScript III Reverse Transcriptase (Invitrogen, CA) and oligo-dT Primers according to the manufacturer's instructions. PCRs were performed using Advantage 2 Polymerase Mix (Clontech, CA) and gene specific primers (MsexOR-26: ATG GCA AGC TAC GAA GGA AAC AAA AC and TCA ATA TAA AAG CGA AAG CAC TGA ATA CG) under the following conditions: 1 min 95°C, 35 cycles (30 s 95°C, 30 s 60°C, 2.5 min 68°C), 3 min 68°C.

Samples were loaded on 1% agarose gel. Resulting bands were sequenced on 3730XL DNA Analyser (Applied Biosystems) at the Max Planck Institute for Chemical Ecology.

2.4. Scanning electron microscopy

The extended female ovipositors were cut between the seventh and eighth abdominal segment. The ovipositors were fixed in 4% glutaraldehyde (Sigma-Aldrich, USA) at 4°C overnight, dehydrated in ascending ethanol series (70%, 80%, 90%, 96% and 3x100% ethanol) and finally critical point dried (BAL-TEC CPD 030, Bal-Tec Union Ltd., Liechtenstein). The samples were mounted on a metal holder covered with adhesive tape and sputter coated with gold prior to examination with a scanning electron microscope (LEO 1530, Zeiss, Wetzlar, Germany), which was set at 8 kV and 8-11 mm working distance.

2.5. Physiology

2.5.1. Preparation

M.sexta females (2-3 days after eclosion) were gently pushed into a 5ml pipette tip (Biozym, Hess. Oldendorf) with the tip cut open after cutting wings, legs and antennae. The female ovipositor was extended by gently pushing the abdomen, fixed with dental wax to glass slides and wrapped tightly with Parafilm® (Pechiney Plastic Packaging, Chicago, USA).

2.5.2. Single-sensillum recordings

Sensilla were achieved with electrolytically sharpened tungsten electrodes (Harvard Apparatus Ltd, Edenbridge, UnitedKingdom) using piezoelectric micromanipulators PM-10 (Märzhäuser Wetzlar GmbH, Wetzlar, Germany). Under a stereomicroscope, the recording electrode was inserted into either the shaft or the base of individual sensilla. A

silver wire, which was placed into the eye of the moth, was used as reference electrode. The signal was amplified 10 times by Syntech Universal AC/DC Probe, sampled (10,667.0. samples/s), filtered (100Hz–3kHz with 50/60 Hz suppression) and digitized through IDAC-4 -USB-interface (Syntech, Hilversum, Netherlands) to the computer. Recordings were visualized and analyzed using Autospike v. 3.7 (Syntech, Hilversum, Netherlands). Responses of individual neurons were quantified by subtracting the counted number of spikes 3 s after and before stimulus delivery, which last 0.5 s. Heatmap and bar-diagrams were prepared with PAST (Palaeontological Statistics, (http://folk.uio.no/ohammer/past/). Figures have been edited in Adobe Illustrator CS6 (Adobe Systems, San Jose, USA).

2.5.3. Odorant stimulation

The chemicals used for single sensillum recordings (SSR) are listed in Supplementary Table 1. All compounds were diluted to a final concentration of 10^{-2} according to their vapor pressure in hexane (<1.5mm Hg, 25°C) or mineral oil (>1.5 mm Hg, 25°C). 10 µl of the diluted odors were pipetted onto a small piece of filter paper and placed inside a glass Pasteur pipette. A constant charcoal-filtered/humidified air flow (0.5 m⁄s) was delivered through a metal tube. Its outlet was positioned in a distance of 5-10mm to the preparation and a stimulus controller delivered the compounds with a blowing air puff through a hole into the air stream.

3. Results:

3.1. Morphology

We performed scanning electron micrographs (SEM) to visualize putative olfactory sensilla on the ovipositor of *M.sexta*. The ovipositor of *M. sexta* consists of the extended terminal segment, the paired papillae anales (Fig.1A). It is covered with short- pointed microtrichia and tubercles, bearing one larger sensillum each (Eaton, 1986) (Fig.1B). The ovipositor surface carries many sensilla trichoidea. Between these we identified ~7 sensilla on each of the bilaterally paired papillae (white arrows, Fig.1A) which are blunt-tipped and exhibit a porous surface (Fig.1D), indicating a likely function in chemosensation. For comparison, the other sensilla end in bent, fine pointed tips and have no pores. The sockets of the longer, non-porous sensilla enclose the base tighter in comparison to the putative chemosensory sensilla (Fig.1C).

The longer sensilla all appear to be tactile receptors; they vary in length, whereas the putative chemosensory sensilla are consistently approximately 110µm long. The longer

sensilla can be named sensilla trichoidea according to (Eaton, 1986; Faucheux, 1988, 1991; Valencia and Rice, 1982); the morphology of the putative chemosensory sensilla is reminiscent of sensilla basiconica on the antennae, where they house olfactory sensory neurons.

3.2. Expression Analysis

We performed RNAseq to assess gene expression in ovipositors from virgin and mated *M. sexta* females, focusing on chemosensory receptors genes. Using our previously reported reference gene set (Koenig et al., 2015) we were able to detect expression of MsexGR3, MsexIR25a, MsexIR75p.2 and MsexIR76b as expressed more or less equally in virgin and mated females (Fig. 2 and Supplementary Table 2). Several chemosensory receptors exhibit higher expression in virgin than in mated females, namely MsexGR2, MsexIR1, MsexIR7d, MsexIR8a, MsexIR68a and MsexIR75d (Fig. 2 and Supplementary Table 2). MsexOR-26 expression was detected only in virgin females. The expression of the olfactory coreceptor MsexORCO was low in virgin females (virgin: RPKM = 0.2; mated: RPKM = 0.0), but we confirmed its expression by RT-PCR (data not shown).

3.3. Physiology

Based on their morphology and the expression of IR and OR genes in the ovipositor we speculated that at least some of the identified sensilla basiconica will house olfactory sensory neurons. To test this, we performed electrophysiological measurements of these sensilla on the ovipositor of *M.sexta*, stimulating with a broad panel of 142 odorants. Odorants where chosen both to match the ecology of the animal, and to cover chemical space. Accordingly the panel consisted of acids, alcohols, aldehydes, esters, amines, aromatics, ketones, lactones, quinones, pheromones and terpenes (Supplementary Table 1). Single unit recordings clearly showed functionally distinct neuronal units within the sensilla, with spiking behvaiour matching that of antennal OSNs (Fig. 3). Furthermore, they revealed at least two distinct sensilla types, which house one or two OSNs, respectively (Fig.3).

Seventeen of 49 odor-responsive sensilla showed two distinct spike amplitudes, indicating the presence of two OSNs (A and B) in each sensillum (Fig.3a, b). Distinct responses of two OSNs could be recognized in 9 of these 17 sensilla (Fig.3a; Fig.4 first nine columns). In contrast, an odor-responsive OSN was paired with a non-responding neuron in 8 of these 17 sensilla (Fig. 3b). In 32 cases only a single OSN was present in the recorded

sensillum. (Fig.3c). All responses from the 58 responsive OSNs, measured in 49 sensilla, are summarized in Figure 4. Virgin and mated females were analyzed together because they did not exhibit marked differences in electrophysiological measurements.

OSNs could be classified based on their response profiles (Fig.5), identifying four distinct functional OSN types. Six odour- responsive OSNs that exhibited slight variations in their response spectra were not included in this classification (S15b, 17a, 21a, S29a, S31a, 31b). The first functional type (Fig. 5a, type A), comprised of OSNs from four measurements, responds to compounds belonging to several chemical classes but mainly to alcohols. The highest response was consistently elicited by 4-methyl-1-pentanol; strong to medium responses were obtained with cis-2-pentenol, benzyl alcohol, trans-2-hexen-1-ol, prenol, methionol, 2-phenyl ethanol and cis-3-hexenol. Examples of responses to alcohols (4-methyl-1-pentanol and cis-2-pentenol) are shown in Fig 3a. No other identified OSN type detected these compounds. Furthermore, colocalized OSNs of this class always exhibited strong responses to lactones (γ-hexalactone and γ-valerolactone) and acids (hexanoic acid); low to medium responses to butyric acid, 2,5-hexanedione, 4-ethylguaiacol and (1S)-(-)-verbenone.

The functional type B (Fig. 5b) is the most common, 50% of the recorded OSNs responded best to γ-hexalactone and (S)-verbenol and elicited strong to medium responses to γ-heptalactone, γ-valerolactone and p-toluquinone.

Thirteen OSNs revealed narrow response spectra and showed small to medium responses to quinones (p-toluquinone and p-benzoquinone) as well as pyrrolidine (Fig.5c, type C).

Out of 58 odour- responsive OSNs, 6 OSNs exhibit strong excitation to γ-hexalactone exclusively, and were grouped into one functional type (Fig.5d, type D). The strongest response of all types of OSNs was observed when 4-methy-1-pentanol was presented to the first OSN type (Fig.5a, ~70 spikes/3s) and γ-hexalactone to of type B (Fig.5b, ~64 spikes/3s). The OSNs belonging to the narrowly tuned types C and D, displayed lower spiking activity maximum (γ-hexalactone, ~29 spikes/3s, (C) and ~24 spikes/3s p-toluquinone, (D), respectively).

In general, the OSNs displayed none to low spontaneous activity of 0-3 spikes/s; the non-responding neurons (Fig.3b) with more than 15 spikes/s spontaneous activity were the only exception.

The majority of the OSNs showed tonic responses when presented individual odorants (Figs. 3a-c). In comparison, the responses after quinone (p-toluquinone and p-

benzoquinone) stimulation terminated relatively abruptly, indicating phasic response (Fig.3a).

4. Discussion

Here we present for the first time evidence for an olfactory function of the ovipositor of *M.sexta*.

Initially, we employed scanning electron microscopy to visualize presence of 7 putative olfactory sensilla, that are similar in appearance to sensilla basiconica. Using RNAseq we demonstrated expression of several IRs, one OR and two GRs in ovipositor tissue of *M.sexta*, further suggesting detection of volatile and non-volatile chemical cues.

Among the expressed receptors are 8 putative IRs, including the two IR-coreceptors, IR8a and IR25a, which in antennae are generally present in IR expressing OSNs (Abuin et al., 2011; Rytz et al., 2013). The presence of both IR coreceptors, IR8a and IR25a was reported in the ovipositor of *Sesamia nonagrioides* (Glaser et al., 2013) and IR25a alone in *Spodoptera littoralis* (Olivier et al., 2011). The presence of IRs together with the co-receptors IR8a and IR25a implies olfactory function in the ovipositor of *M. sexta*.

Finally, electrophysiological measurements on the identified sensilla clearly showed responses elicited by stimulation with odorants. Out of a broad panel of 142 odorants covering both the ecology of the animal, and chemical space, active compounds were predominantly alcohols, lactones and quinones. Four functional distinct OSN types could be identified based on their response profiles.

One OSN type (Fig.4A) was responsive to several chemical classes, mostly to alcohols, and can detect specific GLVs e.g. cis-3-hexenol, trans-2-hexenol and benzyl alcohol. After feeding damage *Nicotiana attenuata* constantly emits a set of GLVs including cis-3-hexenol to attract predators. This emission also leads to reduced oviposition in *M. quinquemaculata* and *M.sexta* (Kessler and Baldwin, 2001; Späthe et al., 2013).

Furthermore, ovipositing *M.sexta* females can differentiate between (Z)-3- and (E)-2- GLVs and in field experiments laid more eggs on *Datura wrightii* plants perfumed with the Z-isomer, than on plants perfumed with the E- isomer (Allmann and Baldwin, 2010; Allmann et al., 2013). The detection of GLVs and (E)-2- and/or ratios of both isomers provides crucial information for host plant selection about plant damage, active plant defenses, predation and competition risks for the survival off the offspring. Obviously, detection of these compounds is crucial for oviposition behaviour in *M. sexta*. All the GLVs, e.g. cis-3-hexenol, trans-2-hexenol and alcohols (cis-2-pentanol and 4-methyl-pentanol) that

elicited responses in olfactory ovipositor sensilla are also detected by *M.sexta* antenna (Ghaninia et al., 2014; Shields and Hildebrand, 2001). It thus can be speculated that the detection of the same molecule on different organs provides additional information for the moth. We speculate that detection on the antennae provides more general and broad information about long-range host location and choice. In contrast, detection on the ovipositor will provide "last chance" information on the quality of the exact spot for egg laying, potentially helping to avoid mistakes that could be fatal for the offspring.
A similar mechanism was postulated for the amounts of GLV-isomers emitted by the plant; (Z)-3 might be relevant for long-range host location and (E)-2 valuable for short distances (Allmann and Baldwin, 2010; Allmann et al., 2013).

The specific response to quinones by one of the 4 functional types of OSNs further supports this speculated mechanism (Fig.5C). Naturally occurred quinones are secondary metabolites and widespread in flowering plants, bacteria, fungi and insects (Thomson, 2012, 1991). 1,4 benzoquinone and toluquinone act as defensive compounds in other insect species, for example *Tribolium castaneum* (Unruh et al., 1998). An ecological relevant function of quinones in the habitat of *M.sexta* has not been reported so far, but information about host plant – microbe relationships might be provided, i.e. oviposition site health: infestation with microbes.

Beyond oviposition another behaviour potentially involving olfactory detection by the ovipositor could be mating. Here, detection over short distances of volatiles emitted by males could guide copulation attempts. One indication for this is that several chemosensory genes exhibit higher expression in the ovipositor of virgin females than in mated females (MsexGR2, Msex7d, MsexIR1, MsexIR8a, MsexIR68a, Msex75d and MsexOR26). Downregulation of the sole OR expressed in the tissues, MsexOR-26, suggests a function of this receptor in mating. However, virgin and mated females did not exhibit marked difference in electrophysiological measurements, suggesting that the receptor responds to odorants not included in our already broad panel. This of course further supports the notion of its involvement in reproduction, which would likely necessitate a narrow response profile similar to pheromone receptors.

We could detect expression of IR75d in the *M.sexta* ovipositor, its orthologue in *Drosophila melanogaster* is expressed in antennae and responds to pyrrolidine (Rytz et al.,

2013). SSR recordings of olfactory sensilla on the *M.sexta* ovipositor revealed that some OSNs responded to pyrrolidine as well (Fig.4), likely due to expression of MsexIR75d.
We also detected expression of IR76b, which is discussed as a salt tasting receptor in *D. melanogaster* (Zhang et al., 2013). In this context, it was shown that chemosensitive sensilla on the ovipositor of *Chilo partellus* and *Eldana saccharina* responded to salts (Waladde, 1983). Stimulation with salt elicited responses in the gustatory sensilla on the ovipositor of the butterfly *Pieris brassicae* as well (Klijnstra and Roessingh, 1986).
From the OR coding gene family, in the ovipositor of virgin females expression was only detected of MsexOR-26 and the coreceptor ORCo. The presence of both OR and ORCo suggests that at least one OR-employing OSN exists in the tissue. Presence of several ORs and ORCo was also reported in female ovipositors of *Sesamia nonagrioides* (Glaser et al., 2013). *In Chilo suppressalis* two ORs, but no ORCo were found expressed in the ovipositor (Xia et al., 2015). In *Heliothis virescens* the pheromon receptors HR6 and HR13 were detected in the ovipositor (Grosse-Wilde et al., 2007; Wang et al., 2011; Widmayer et al., 2009).
The high responses to γ-hexalactone, elicited by three of the 4 functional types of OSNs might be interesting in this context. Several lactones were tested to examine effects of ring size and side chain length; five- (γ) membered rings elicited response exclusively (γ-valero, γ-hexa- and γ-heptalactone). A lactone sensitive OR of *Anopheles gambiae*, AgOR48 has been recently described eliciting responses also, to a lesser extent to γ-hexalactone and γ-heptalactone (Pask et al., 2013). Several ORs of *Drosophila melanogaster* also detect γ-hexalactone (Hallem and Carlson, 2006). However, responses to lactones are existent in ovipositor OSNs also in mated *M. sexta*, excluding involvement of ORs in this function. This clearly suggests that an IR is responsible for this detection. Since no described IR in non-lepidopteran species provides this function it seems likely to be coupled to the lepidoptera-specific IR1.
Expression of the two GRs, MsexGR2 and MsexGR3, orthologs of the highly conserved CO_2 receptors, were also detected in the ovipositor. Their expression in adult antenna and, together with the third CO_2 receptor in larval antenna and maxilla, was recently reported (Koenig et al., 2015). *M.sexta* posses large labial palp pit organs that sensitively detect CO_2 (Kent et al., 1986). In this context, it was shown that CO_2 fluctuations affect the oviposition behavior in *M. sexta* (Abrell et al., 2005). This indicates that CO_2, emitted by host plants at night might be detected by *M. sexta* also via the ovipositor.

Using scanning electron micrographs (SEM) we could also visualize putative chemosensory sensilla, most likely sensilla basiconica on the ovipositor of *M. sexta*.
The presence of a small group of sensilla, most likely sensilla basiconica, was described on the ovipositor of *M.sexta* (Eaton, 1986). The reported location was ventrolateral to the anal papillae suggesting a contact chemosensory function, due to appropriate positioning to contact the oviposition substrate (Eaton, 1986). In contrast, we identified ~7 putative chemosensory sensilla with a porous surface located on each of the bilaterally paired papillae surrounded by about 100 trichoid sensilla (Eaton, 1986).
Our findings provide evidence of the proposed olfactory role of a multiporous sensillum basiconicum, originally described on each of the anal papillae of *Monopis crocicapitella* and *Homoeosoma nebulella* (Faucheux, 1988, 1991). On the ovipositor of *H. hippophaecolus,* three types of sensilla were detected including sensilla basiconica with abundant pores and a terminal pore (Wang et al., 2015). So far, morphological studies revealed tactile mechano- and contact chemosensory function on abdominal segments in several lepidopteran insects: *Phtorimaea operculella* (Valencia and Rice, 1982), *Chilo partellus*, *Spodoptera littoralis* (Chadha and Roome, 1980), *Eldana saccharina* (Waladde, 1983), *Heliothis virescens* and *Heliothis subflexa* (Baker and Ramaswamy, 1990b). Tactile and bimodal taste/tactile sensilla were also found in *Ostrinia Nubilalis* (Marion-Poll et al., 1992), *Plutella xylostella* (Qiu et al., 1998), *Choristoneura fumiferana* (Banga et al., 2003) and *Ephestia kuehniella* (Anderson and Hallberg, 1989). The detection of fructose and sucrose by contact chemoreceptors was shown in *Lobesia botrana* (Maher and Thiery, 2004; Maher et al., 2006). Until now, only mechanosensory- and contact chemosensory modalities were functionally characterized in lepidopteran species (Banga et al., 2003; Klijnstra and Roessingh, 1986; Maher and Thiery, 2004; Maher et al., 2006; Qiu et al., 1998; Waladde, 1983; Yamaoka et al., 1971). Single unit recordings revealed that we could distinguish two morphologically distinct types of putative olfactory sensilla on the ovipositor. Based on their spike amplitudes, they are separated by housing either one or two OSN in each sensillum (Fig3A).
Nearly all OSNs exhibited excitatory response but on the contrary, did not show inhibitory responses. This might be explained by very low spontaneous activity of the OSNs, in most cases 0-3 spikes/s. In few cases non-responding B-neurons (Fig.3B) showed higher spontaneous activity of more than 15 spikes/s, but they were clear exceptions. Since these neurons did not respond to any odorants presented, it is possible that they contribute to a different sensory modality. Possible are detection of CO_2, or mechano-, gustatory- or

hydroreceptive function. Alternatively, suitable olfactory ligands might have been absent from our odour panel. This is possibily also true for the OSNs of the narrowly tuned types C and D (Fig.5), which exhibited relatively low spiking activity even when presented with their best ligand from our odorant set.

Based on these results we could show for the first time that the olfactory system of *M.sexta* is present and functioning on the ovipositor as well. This will be the base for further experiments to examine in more detail the process of choosing an optimal oviposition site, which is a complex and challenge task, and many factors need to be assessed.

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Figures

Figure 1.

Scanning electron micrographs (SEM) of the ovipositor of *M. sexta* and its associated sensilla. **A)** Overview of the extended terminal segment of the *M. sexta* ovipositor. Most abundant on the ovipositor are non-porous sensilla, which vary in length. White arrows indicate putative chemosensory sensilla on each of the bilaterally paired papillae. Seven were counted on average on each papilla. **B)** Medial view on one side of the papillae. The ovipositor is covered with short-pointed microtrichia and tubercles. White arrows indicate three putative blunt-tipped chemosensory sensilla. **C)** The sockets of the putative chemosensory sensilla are less tight than for non-porous sensilla (white arrows). Asterisks indicate putative chemosensory sensilla. **D)** Detailed view of the putative olfactory sensilla. The shaft of the putative olfactory sensillum is porous (Arrows).

Scale bars: **A**: 100 µm; **B**: 20 µm; **C**: 20 µm; **D**: 1 µm

Figure 2.

Expression values of chemosensory receptor genes in *M. sexta* ovipositor tissue. Data from virgin females highlighted in blue; mated in violet.

Depicted are only chemosensory receptor genes with an RPKM value > 0.5 in at least one of the two samples.

Figure 3.

Representative single sensillum recording (SSR) traces of *M. sexta* ovipositor sensilla containing two neurons per sensillum (**A** and **B**) or a single neuron (**C)**. The response pattern of two neurons housing in basiconic sensilla to several compounds are shown in (**A**). The response pattern of an odor-responsive A-neuron paired with a non-responding B-neuron is shown in (**B**).

Colocalized OSN pairs. In the first, the A-neuron (large amplitude) exhibits weak and the B-neuron (small amplitude) medium spiking activity in response to stimulation with γ-octalactone. γ-hexalactone elicits strong responses in the A-neuron, but also stimulates the B-neuron to a lesser degree. Toluquinone elicits medium- in the A-neuron and small spiking activity in the B-neuron. 4-methyl-1-pentenol and cis-2-pentenol eliciting high excitatory response in B-neurons whereas A-neurons show no spiking. The last trace depicts the non-response to stimulation with hexane alone. The presented measurements are from the same sensillum.

Excitatory response of an A-neuron (large spike) to γ-hexalactone. The B-neuron did not respond to any of the odours tested, but exhibited generally high spontaneous activity. Neither neuron responds to hexane.

Representative traces from a sensillum potentially housing a single OSN. γ-hexalactone elicits spiking activity; the hexane control (below) does not.

All compounds were tested as dilutions of 10^{-2} in hexane or mineral oil. Stimulus bar = 0.5s. Spike amplitude classes, namely A, B represent the number of OSNs innervating individual sensilla as identified from random spiking activity. A, B, and C depict traces from one distinct individual each.

Figure 4.

Color coded odorant response profiles of 58 individual OSNs in response to 142 individually presented odorants from 49 sensilla which surround the ovipositor of female *M.sexta* moths. All compounds were presented at a dilution of 10^{-2} (in hexane or mineral oil). Responses are color coded to represent the percentage of peak spike rate for the neuron: red indicates the maximum response given by the OSN across all stimulants;

yellow, medium excitation (50%); light blue, no response and dark blue indicates that spiking activity was reduced compared to baseline. Based on their spike amplitude, individual olfactory sensilla contain single OSN (N=32; single circles) or two OSNs (N=17; paired circles). Nine pairs of OSNs designated A (light grey) and B (grey) showed response pattern for both neurons. In 8 sensilla (38, 7, 10a, 10b, 16a, 18, 21c and 22) an odour-responsive OSN was paired with a non-responsive OSN. From sensilla housing two responsive OSNs measurments have been clustered to indicate colocalization. Circles below the names symbolize OSNs housing in a sensillum, with circle color code referring to the bar chart of figure 4 to facilitate association with functional neuron classes. Designations: Numbers represent number of the recordings; S= small amplitude; M=mated; a, b, c represent first-, second- or third recorded sensilla of the same animal.

Figure 5.

Odor response spectra from olfactory sensilla on the ovipositor of *M.sexta*, classified by response spectrum. Data was clustered manually.

Classification of response profiles suggests 4 functional types of OSNs (**A-D**). Depicted are 20 representative odours eliciting response in at least one functional type.

Error bars represents standard deviation.

References

Abrell, L., Guerenstein, P.G., Mechaber, W.L., Stange, G., Christensen, T. a., Nakanishi, K., and Hildebrand, J.G. (2005). Effect of elevated atmospheric CO2 on oviposition behavior in Manduca sexta moths. Glob. Chang. Biol. *11*, 1272–1282.

Abuin, L., Bargeton, B., Ulbrich, M.H., Isacoff, E.Y., Kellenberger, S., and Benton, R. (2011). Functional architecture of olfactory ionotropic glutamate receptors. Neuron *69*, 44–60.

Allmann, S., and Baldwin, I.T. (2010). Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. Science *329*, 1075–1078.

Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T., and Hansson, B.S. (2013). Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. Elife *2013*, 1–23.

Anderson, P., and Hallberg, E. (1989). Structure and distribution of tactile and bimodal taste/tactile sensilla on the ovipositor, tarsi and antennae of the flour moth, Ephestia kuehniella (Zeller) (Lepidoptera : Pyralidae). Int. J. Insect Morphol. Embryol. *99*, 13–23.

Baker, T.C. (1989). Sex pheromone communication in the Lepidoptera: New research progress. Experientia *45*, 248–262.

Baker, G.T., and Ramaswamy, S.B. (1990a). Tarsal and ovipositor sensilla of Heliothis virescens and H. subflexa (Lepidoptera : Noctuidae). Proc. Entomol. Soc. Washingt. *92*, 521–529.

Baker, G.T., and Ramaswamy, S.B. (1990b). Tarsal and ovipositor sensilla of Heliothis virescens and H. subflexa (Lepidoptera : Noctuidae). Proc. Entomol. Soc. Washingt. *92*, 521–529.

Banga, N., Albert, P.J., Kapoor, N.N., and McNeil, J.N. (2003). Structure, distribution, and innervation of sensilla on the ovipositor of the spruce budworm, Choristoneura fumiferana, and evidence of a gustatory function for type II sensilla. Can. J. Zool. Can. Zool. *81*, 2032–2037.

Benton, R., Sachse, S., Michnick, S.W., and Vosshall, L.B. (2006). Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo. PLoS Biol. *4*, e20.

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell *136*, 149–162.

Chadha, G.K., and Roome, R.E. (1980). Oviposition behaviour and the senilla of the ovipositor of Chilo partellus and Spodoptera littoralis (Lepidoptera: Noctuidae). J. ZooL Lond. *192*, 169–178.

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J., and Carlson, J.R. (1999). A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron *22*, 327–338.

Davis, T.S., Crippen, T.L., Hofstetter, R.W., and Tomberlin, J.K. (2013). Microbial Volatile Emissions as Insect Semiochemicals. J. Chem. Ecol. *39*, 840–859.

Eaton, J.L. (1986). Morphology of Abdominal Segments Eight and Nine of the Female Tobacco Hornworm, Manduca sexta (Lepidoptera: Sphingidae). Ann. Entomol. Soc. Am. *79*, 629–635.

Faucheux, M.J. (1988). Multiporous sensilla on the ovipositor of Monopis Crocicapitella clem. (Lepidoptera:tineidae). Int. J. Insect Morphol. Embryol. *17*, 473–475.

Faucheux, M.J. (1991). Morphology and distribution of sensilla on the cephalic appendages, tarsi and ovipositor of the European Sunflower moth, Homoeosoma nebulella Den.&Schiff. (Lepidoptera: Pyralidae). Int. J. Insect Morphol. Embryol. *20*, 291–307.

Fraser, A.M., Mechaber, W.L., and Hildebrand, J.G. (2003). Electroantennographic and behavioral responses of the sphinx moth Manduca sexta to host plant headspace volatiles. J. Chem. Ecol. *29*, 1813–1833.

Gao, Q., and Chess, a (1999). Identification of candidate Drosophila olfactory receptors from genomic DNA sequence. Genomics *60*, 31–39.

Ghaninia, M., Olsson, S.B., and Hansson, B.S. (2014). Physiological Organization and Topographic Mapping of the Antennal Olfactory Sensory Neurons in Female Hawkmoths, Manduca sexta. Chem. Senses.

Glaser, N., Gallot, A., Legeai, F., Montagné, N., Poivet, E., Harry, M., Calatayud, P.A., and Jacquin-Joly, E. (2013). Candidate chemosensory genes in the stemborer sesamia nonagrioides. Int. J. Biol. Sci. *9*, 481–495.

Grosse-Wilde, E., Gohl, T., Bouché, E., Breer, H., and Krieger, J. (2007). Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. Eur. J. Neurosci. *25*, 2364–2373.

Hallem, E. a, and Carlson, J.R. (2006). Coding of odors by a receptor repertoire. Cell *125*, 143–160.

Hallem, E. a, Ho, M.G., and Carlson, J.R. (2004). The molecular basis of odor coding in the Drosophila antenna. Cell *117*, 965–979.

Hansson, B.S., and Stensmyr, M.C. (2011). Evolution of Insect Olfaction. Neuron *72*, 698–711.

Hare, J.D. (2010). Ontogeny and season constrain the production of herbivore-inducible plant volatiles in the field. J. Chem. Ecol. *36*, 1363–1374.

Jones, W.D., Cayirlioglu, P., Grunwald Kadow, I., and Vosshall, L.B. (2007). Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature *445*, 86–90.

Keil, T. a, and Steinbrecht, R.A. (1984). Mechanosensitive and Olfactory Sensilla of Insects. Insect Ultrastruct. Vol. 2 477–516.

Kent, K.S., and Griffin, L.M. (1990). Sensory organs of the thoracic legs of the moth Manduca sexta. Cell Tissue Res. *259*, 209–223.

Kent, K.S., Harrow, I.D., Quartararo, P., and Hildebrand, J.G. (1986). An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in Manduca sexta and certain other sphinx moths and silk moths. Cell Tissue Res. *245*, 237–245.

Kessler, a, and Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. Science *291*, 2141–2144.

Klijnstra, J.W., and Roessingh, P. (1986). Perception of the oviposition deterring pheromone by tarsal and abdominal contact chemoreceptors in Pieris brassicae J. Entomol. Exp. Appl. *40*, 71–79.

Koenig, C., Hirsh, A., Bucks, S., Klinner, C., Vogel, H., Shukla, A., Mansfield, J.H., Morton, B., Hansson, B.S., and Grosse-Wilde, E. (2015). A reference gene set for chemosensory receptor genes of Manduca sexta. Insect Biochem. Mol. Biol.

Köllner, T.G., Held, M., Lenk, C., Hiltpold, I., Turlings, T.C.J., Gershenzon, J., and Degenhardt, J. (2008). A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell *20*, 482–494.

Kwon, J.Y., Dahanukar, A., Weiss, L. a, and Carlson, J.R. (2007). The molecular basis of CO2 reception in Drosophila. Proc. Natl. Acad. Sci. U. S. A. *104*, 3574–3578.

Maher, N., and Thiery, D. (2004). Distribution of chemo- and mechanoreceptors on the tarsi and ovipositor of female European grapevine moth , Lobesia botrana. Entomol. Exp. Appl. *110*, 135–143.

Maher, N., Thiery, D., and Städler, E. (2006). Oviposition by Lobesia botrana is stimulated by sugars detected by contact chemoreceptors. Physiol. Entomol. *31*, 14–22.

Marion-Poll, F., Guillaumin, D., and Claudine, M. (1992). Sexual dimorphism of tarsal receptors and sensory equipment of the ovipositor in the European corn borer , Ostrinia nubilalis. Cell Tissue Res. *267*, 507–518.

Mechaber, W.L., and Hildebrand, J.G. (2000). Novel, non-solanaceous hostplant record for Manduca sexta (Lepidoptera: Sphingidae) in the Southwestern United States. Ann. Entomol. Soc. Am. *93*, 447–451.

Mechaber, W., Capaldo, C., and Hildebrand, J. (2002). Behavioral responses of adult female tobacco hornworms, Manduca sexta, to hostplant volatiles change with age and mating status. J. Insect Sci. *2*, 1–8.

Neuhaus, E.M., Gisselmann, G., Zhang, W., Dooley, R., Störtkuhl, K., and Hatt, H. (2005). Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. Nat. Neurosci. *8*, 15–17.

Olivier, V., Monsempes, C., François, M.-C., Poivet, E., and Jacquin-Joly, E. (2011). Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol. Biol. *20*, 189–199.

Pask, G.M., Romaine, I.M., and Zwiebel, L.J. (2013). The Molecular Receptive Range of a Lactone Receptor in Anopheles gambiae. Chem. Senses *38*, 19–25.

Qiu, Y., Loon, J.J.A. Van, and Roessingh, P. (1998). Chemoreception of oviposition inhibiting terpenoids in the diamondback moth Plutella xylostella. Entomol. Exp. Appl. *87*, 143–155.

Raguso, R., Henzel, C., Buchmann, S., and Nabhan, G. (2003a). Trumpet Flowers of the Sonoran Desert : Floral Biology of Peniocereus Cacti and Sacred Datura. Int. J. Plant Sci. *164*, 877–892.

Raguso, R., Levin, R., Foose, S., Holmberg, M., and McDade, L. (2003b). Fragrance chemistry, nocturnal rhythms and pollination “syndromes” in Nicotiana. Phytochemistry *63*, 265–284.

Ramaswamy, S.B. (1988). Host finding by Moths: Sensory modalities and Behaviours. J. Insect Physiol. *34*, 235–249.

Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., and Turlings, T.C.J. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature *434*, 732–737.

Reisenman, C.E., Riffell, J. a, and Hildebrand, J.G. (2009). Neuroethology of oviposition behavior in the moth Manduca sexta. Ann. N. Y. Acad. Sci. *1170*, 462–467.

Renwick, J. a. a. (1989). Chemical ecology of oviposition in phytophagous insects. Experientia *45*, 223–228.

Riffell, J. a, Alarcón, R., Abrell, L., Davidowitz, G., Bronstein, J.L., and Hildebrand, J.G. (2008). Behavioral consequences of innate preferences and olfactory learning in hawkmoth-flower interactions. Proc. Natl. Acad. Sci. U. S. A. *105*, 3404–3409.

Rytz, R., Croset, V., and Benton, R. (2013). Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in Drosophila and beyond. Insect Biochem. Mol. Biol. *43*, 888–897.

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., and Touhara, K. (2008). Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature *452*, 1002–1006.

Shields, V.D.C., and Hildebrand, J.G. (2001). Responses of a population of antennal olfactory receptor cells in the female moth Manduca sext a to plant-associated volatile organic compounds. J. Comp. Physiol. A Sensory, Neural, Behav. Physiol. *186*, 1135–1151.

Späthe, A., Reinecke, A., Olsson, S.B., Kesavan, S., Knaden, M., and Hansson, B.S. (2013). Plant species- and status-specific odorant blends guide oviposition choice in the moth Manduca sexta. Chem. Senses *38*, 147–159.

Tasin, M., Betta, E., Carlin, S., Gasperi, F., Mattivi, F., and Pertot, I. (2011). Volatiles that encode host-plant quality in the grapevine moth. Phytochemistry *72*, 1999–2005.

Tasin, M., Knudsen, G.K., and Pertot, I. (2012). Smelling a diseased host: grapevine moth responses to healthy and fungus-infected grapes. Anim. Behav. *83*, 555–562.

Thomson, R. (2012). Naturally occurring quinones (Elsevier).

Thomson, R.H. (1991). Distribution of naturally occurring quinones. Pharm. Weekbl. Sci. Ed. *13*, 70–73.

Unruh, L.M., Xu, R., and Kramer, K.J. (1998). Benzoquinone levels as a function of age and gender of the red flour beetle, Tribolium castaneum. Insect Biochem. Mol. Biol. *28*, 969–977.

Valencia, L., and Rice, M. (1982). Contact chemoreceptors on the ovi- positor of the potato moth, Phthorimaea operculella (Zell.) (Lepidoptera: Gelechiidae). Int. J. Insect Morphol. Embryol. *I*, 121–128.

Vosshall, L.B., and Hansson, B.S. (2011). A unified nomenclature system for the insect olfactory coreceptor. Chem. Senses *36*, 497–498.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., and Axel, R. (1999). A spatial map of olfactory receptor expression in the Drosophila antenna. Cell *96*, 725–736.

Waladde, S.M. (1983). Chemoreception of adult stem-borers: tarsal and ovipositor sensilla on Chilo partellus and Eldana saccharina. Int. J. Trop. Insect Sci. *4*, 159–165.

Wang, G., Vásquez, G.M., Schal, C., Zwiebel, L.J., and Gould, F. (2011). Functional characterization of pheromone receptors in the tobacco budworm Heliothis virescens. Insect Mol. Biol. *20*, 125–133.

Wang, R., Zhang, L., Xu, L., Zong, S., and Luo, Y. (2015). Sensilla on the Antennae and Ovipositor of the Sea Buckthorn Carpenter Moth , Holcocerus hippophaecolus Hua et al (Lepidoptera : Cossidae). Syst. Morphol. Physiol. 68–76.

Wicher, D. (2015). Olfactory Signaling in Insects. In Progress in Molecular Biology and Translational Science, R. Glatz, ed. (Burlington: Academic Press), pp. 37–54.

Wicher, D., Schäfer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature *452*, 1007–1011.

Widmayer, P., Heifetz, Y., and Breer, H. (2009). Expression of a pheromone receptor in ovipositor sensilla of the female moth (Heliothis virescens). Insect Mol. Biol. *18*, 541–547.

Xia, Y.-H., Zhang, Y.-N., Hou, X.-Q., Li, F., and Dong, S.-L. (2015). Large number of putative chemoreception and pheromone biosynthesis genes revealed by analyzing transcriptome from ovipositor-pheromone glands of Chilo suppressalis. Sci. Rep. *5*, 7888.

Yamamoto, R.T., Jenkins, R.Y., and McClusky, R.K. (1969). Factors determining the selection of plants for oviposition by the tobacco hornworm Manduca sexta. Entomol. Exp. Appl. *12*, 504–508.

Yamaoka, K., Hoshino, M., and Hirao, T. (1971). Role of sensory hairs on the anal papillae in oviposition behaviour of Bombyx mori. J. Insect Physiol. *17*, 897–911.

Zhang, Y. V, Ni, J., and Montell, C. (2013). The Molecular Basis for Attractive Salt-Taste Coding in Drosophila. Science (80-.). *340*, 1334–1338.

Supplementary Table 1

Chemical Class	Chemical	CAS number	Company	Purity
Acid	hexanoic acid	000142-62-1	Aldrich	=99.5%
Acid	decanoic acid	000334-48-5	FLUKA	purum, =98.0% (GC)
Acid	phenyl acetic acid	103-82-2	Aldrich	=99%, FCC
Acid	2-oxopentanoic acid	1821-02-9	Aldrich	=98.0% (T
Acid	butyric acid	107-92-6	FLUKA	puriss., =99.5% (GC)
Acid	propionic acid	79-09-4	FLUKA	puriss. p.a., standard for GC, =99.8% (GC)
Acid	acetic acid	64-19-7	Aldrich	=99.99%
Acid	myristic acid	544-63-8	Sigma	Sigma Grade, 99-100%
Acid	palmitic acid	57-10-3	Sigma	SigmaUltra, ~99%
Acid	palmitoleic acid	373-49-9	Sigma	analytical standard =99%
Acid	oleic acid	112-80-1	Sigma	reagent grade, ~99% (GC)
Acid	linoleic acid	60-33-3	Aldrich	=99%
Acid	p-coumaric acid	501-98-4	Sigma	=98.0% (HPLC)
Acid	3-(4-hydroxyphenyl)propionic acid	501-97-3	Aldrich	98%
Acid	3,4-dihydroxyhydrocinnamic acid	1078-61-1	Aldrich	98%
Acid	ferulic acid	537-98-4	Aldrich	99%
Alcohol	cis-2-pentenol	001576-95-0	Aldrich	>=96%
Alcohol	4-methyl-1-pentanol	626-89-1	Aldrich	97%
Alcohol	prenol	000556-82-1	Aldrich	99%
Alcohol	cis-3-hexenol	000928-96-1	Aldrich	purum, =98.0% (GC)
Alcohol	trans-2-hexen-1-ol	000928-95-0	FLUKA	purum, =95.0% (GC)
Alcohol	1-hexanol	111-27-3	FLUKA	puriss. p.a., standard for GC, =99.9% (GC)
Alcohol	linalool	000078-70-6	Aldrich	97%
Alcohol	benzyl alcohol	000100-51-6	Aldrich	anhydrous, 99.8%

Alcohol	2-phenyl ethanol	000060-12-8	FLUKA	purum, =99.0% (GC)
Alcohol	3-octanol	000589-98-0	Aldrich	99%
Alcohol	methionol	505-10-2	Aldrich	=98%, Kosher, FG
Alcohol	2,3-butanediol	513-85-9	Aldrich	
Alcohol	furfuryl alcohol	98-00-0	Aldrich	=98%
Aldehyde	hexanal	000066-25-1	Aldrich	98%
Aldehyde	benzaldehyde	000100-52-7	Sigma	=99%
Aldehyde	cis-3-hexenal	006789-80-6	Aldrich	50weight% in triacetin, Kosher;0.10% alpha-tocopherol, synthetic as antioxidant
Aldehyde	trans-2-hexenal	6728-26-3	Aldrich	98%
Aldehyde	decanal	000112-31-2	FLUKA	
Aldehyde	nonanal	000124-19-6	FLUKA	purum, =95.0% (GC)
Aldehyde	citral	005392-40-5	Aldrich	95%
Aldehyde	Phenylacetaldehyde	000122-78-1	Aldrich	Aldrich
Aldehyde	cinnamaldehyde	104-55-2	Aldrich	=98%, FCC, Kosher
Amine	pyrrolidine	000123-75-1	Aldrich	99%
Amine	1,4-diamino butane	110-60-1	Aldrich	99%
Amine	cadaverine	462-94-2	FLUKA	purum, =97.0% (GC)
Amine	ammonia		Sigma	28% in H2O, =99.99%
Amine	L(-)-nicotine	54-11-5	Riedel-de Haën	PESTANAL®, analytical standard
Aromatic	1,2,4-trimethyl benzene	000095-63-6	FLUKA	puriss., =98.5% (GC)
Aromatic	pyrrole	000109-97-7	ACROS	
Aromatic	3-propyl toluene	001074-43-7	ABCR	99%
aromatic	methyl salicylate	000119-36-8	Sigma	ReagentPlus®, =99% (GC)
aromatic	4-ethyl guaiacol	002785-89-9	Aldrich	=98%, FCC, Kosher, FG
aromatic	Eugenol	97-53-0	FLUKA	purum, =99.0% (GC)
aromatic	4-ethyltoluene	622-96-8	FLUKA	purum, =95.0% (GC)
aromatic	o-cymene	527-84-4	Aldrich	99%
Ester	ethyl acetate	000141-78-6	Aldrich	anhydrous, 99.8%
Ester	cis-2-penten-1-ol acetate	042125-10-0	Provided by Ales Svatos	99,3%

			MPI-CE	
Ester	trans-2-hexenyl acetate	002497-18-9	Aldrich	=98%, FCC, Kosher
Ester	ethyl butyrate	000105-54-4	Sigma	99%
Ester	cis-3-hexenyl acetate	003681-71-8	Aldrich	=98%, FCC, Kosher
Ester	butyl acetate	000123-86-4	FLUKA	puriss. p.a., =99.0% (GC)
Ester	methyl hexanoate	000106-70-7	FLUKA	=99.8% (GC)
Ester	ethyl hexanoate	000123-66-0	Aldrich	=99%
Ester	methyl benzoate	000093-58-3	Aldrich	99%
Ester	benzyl acetate	000140-11-4	Aldrich	=99.0%
Ester	cis-3-hexenyl valerate	035852-46-1	Grau-aromatics	98,2%
Ester	benzyl butyrate	000103-37-7	Alfa Aesar	98%
Ester	cis-3-hexenyl propionate	033467-74-2	Aldrich	>97%, Kosher
Ester	cis-3-hexenyl butyrate	016491-36-4	Aldrich	=98%, Kosher
Ester	geranyl acetate	000105-87-3	Sigma	98%
Ester	ethyl benzoate	000093-89-0	Aldrich	=99%
Ester	propyl benzoate	002315-68-6	Aldrich	99%
Ester	benzyl propionate	000122-63-4	Alfa Aesar	99%
Ester	benzyl salicylate	000118-58-1	FLUKA	purum, =99.0% (GC)
Ester	cis-3-hexenyl benzoate	025152-85-6	Aldrich	=97%
Ester	triacetin	000102-76-1	Aldrich	=98.5%, FCC, FG
Ester	amyl acetate	628-63-7	Aldrich	=99%
Ester	nonyl acetate	143-13-5	Aldrich	FCC
Ester	ethyl 2-methylbutyrate	7452-79-1	Aldrich	99%
Ester	3-acetoxy-2-butanone	4906-24-5	alfa aesar	98%
Ester	(-)-ethyl L-lactate	687-47-8	Aldrich	98%
Ester	cis-2-penten-1-yl acetate		Provided by Ales Svatos MPI-CE	99,3%
Ester	ethyl lactate	97-64-3	Aldrich	natural, =98%, FCC, FG
Ester	ethyl heptanoate	106-30-9	Aldrich	99%
Ketone	2-heptanone	000110-43-0	Sigma	99%

Ketone	(-)-menthone	014073-97-3	FLUKA	puriss. p.a., terpene standard for GC, =99.0% (sum of enantiomers, GC
Ketone	acetophenone	000098-86-2	FLUKA	puriss. p.a., =99.0% (GC)
Ketone	6-methyl-5-hepten-2-one	000110-93-0	Aldrich	99%
Ketone	6,10,14-trimethyl-2-pentadecanone	000502-69-2	chemos	98%
Ketone	geranyl acetone	000689-67-8	Aldrich	=97%, FG
Ketone	cis-jasmone	000488-10-8	FLUKA	purum, analytical standard, =99.0% (sum of isomers, GC
Ketone	benzyl acetone	2550-26-7	Aldrich	98%
Ketone	2,5-hexanedione	110-13-4	Aldrich	=98%
Ketone	ß-Ionone	79-77-6	alfa aesar	96%
Lactone	γ-octalactone	104-50-7	Aldrich	=97%, FCC
Lactone	γ-hexalactone	695-06-7	Aldrich	=98%, FCC, Kosher, FG
Lactone	γ-nonalactone	104-61-0	Aldrich	=98%, FCC, Kosher
Lactone	γ-undecalactone	104-67-6	Aldrich	99%
Lactone	δ-decalactone	705-86-2	Aldrich	=98%, Kosher, FCC
Lactone	γ-decanolactone	706-14-9	Aldrich	99%
Lactone	γ-valerolactone	108-29-2	Aldrich	99%
Lactone	δ-dodecalactone	713-95-1	SAFC	=98%, FCC, FG
Lactone	γ-heptalactone	105-21-5	SAFC	=98%, FCC, FG
Lactone	γ-dodecalactone	2305-05-7	Aldrich	=97%, FCC, FG
Lactone	(±)-mevalono-lactone	674-26-0	Sigma	~97% (titration)
Others	Hexane		VWR-Chemicals	99%
Others	mineral oil		Aldrich	
others	Henkel 100		Henkel	Mixture containing 93 chemicals.
others	Iodoform	75-47-8	Aldrich	99%
Pheromone	(E,Z)-10,12-hexadecadienal		Provided by Ales Svatos MPI-CE	
Pheromone	(Z)-11-hexadecenal		Provided by	

			Ales Svatos MPI-CE	
Pheromone	(E,E)-10,12-hexadecadienal		Provided by Ales Svatos MPI-CE	
Pheromone	(E,Z)-11,13-pentadecadienal		Provided by Ales Svatos MPI-CE	
Quinone	p-benzoquinone	106-51-4	Riedel-de Haën	=99% (GC)
Quinone	p-toluquinone	553-97-9	FLUKA	purum, =98.0% (HPLC)
Terpene	(S)-(-)-limonene	005989-54-8	Aldrich	96%
Terpene	ocimene	029714-87-2	Aldrich	90+ % (mixture of isomers)
Terpene	ß-myrcene	000123-35-3	FLUKA	purum, =95.0% (GC)
Terpene	(R)-(+)-limonene	005989-27-5	FLUKA	analytical standard, for terpene analysis
Terpene	β-humulene	116-04-1	Aldrich	technical, =90% (GC
Terpene	farnesol	004602-84-0	Aldrich	95%
Terpene	nerol	000106-25-2	Aldrich	FCC, =97%, Kosher
Terpene	geraniol	000106-24-1	FLUKA	purum, =96.0% (GC)
Terpene	trans-nerolidol	40716-66-3	FLUKA	BioChemika, =85% (GC)
Terpene	ß-caryophyllene	000087-44-5	FLUKA	puriss., =98.5% (sum of enantiomers, GC
Terpene	valencene	4630-07-3	Aldrich	natural, =65%
Terpene	carvacrol	499-75-2	Aldrich	natural, 99%, FG
Terpene	α-humulene	6753-98-6	Aldrich	=96.0% (GC)
Terpene	3-carene	13466-78-9	Aldrich	90%
Terpene	ß-pinene	18172-67-3	Aldrich	99%
Terpene	farnesene	502-61-4	Aldrich	0.10% alpha-tocopherol, synthetic as antioxidant
Terpene	(+;-)-phytol	7541-49-3	Aldrich	97%, mixture of isomers
Terpene	nootkatone	4674-50-4	Aldrich	=99.0% (GC)
Terpene	α-terpineol	10482-56-1	Aldrich	natural,Kosher
Terpene	(-)-camphene	5794-04-7	FLUKA	technical, =90% (sum of camphene and

				fenchene, GC
Terpene	(+)-camphene	5794-03-6	Aldrich	=80%, FCC, Kosher
Terpene	(±)-citronellal	106-23-0	FLUKA	analytical standard =98.0% (GC);
Terpene	(±)-sabinene	3387-41-5	ROTH	rotichrom GC
Terpene	1,8-cineole	470-82-6	FLUKA	=99.7% (GC)
Terpene	(S)-verbenol	19890-02-9	Aldrich	95%
Terpene	(-)-caryophyllene-oxide	1139-30-6	Sigma	=99.0% (sum of enantiomers, GC)
Terpene	(+)-carvone	2244-16-8	Aldrich	96%
Terpene	1-r-fenchone	7787-20-4	Aldrich	=98%
Terpene	ß-cyclocitral	432-25-7	Aldrich	=90%, FG
Terpene	γ-terpinene	99-85-4	Aldrich	=95%
Terpene	(-)-α-cedrene	469-61-4	FLUKA	puriss., =99.0% (sum of enantiomers, GC)
Terpene	(+)-ß-cedrene	546-28-1	FLUKA	purum, =95.0% (sum of enantiomers, GC)
Terpene	(1S)-(-)-verbenone	1196-01-6	Aldrich	94%

Supplementary Table 2

Chemosensory receptor	**RPKM (Virgin)**	**RPKM (Mated)**
MsexGR02	5.3	0.1
MsexGR03	0.9	0.9
MsexIR01	3.4	0.2
MsexIR04	0.6	0.2
MsexIR25a	0.9	1.0
MsexIR68a	1.6	0.2
MsexIR75d	1.8	0.5
MsexIR75p.2	0.6	0.6
MsexIR76b	5.0	4.5
MsexIR7d	13.2	1.4
MsexIR8a	0.7	0.1
MsexOR-26	1.3	0.0

Supplementary Table 2: Expression values of chemosensory receptor genes in *M.sexta* ovipositor tissue. Depicted are only chemosensory receptor genes with an RPKM value > 0.5 in at least one of the two samples

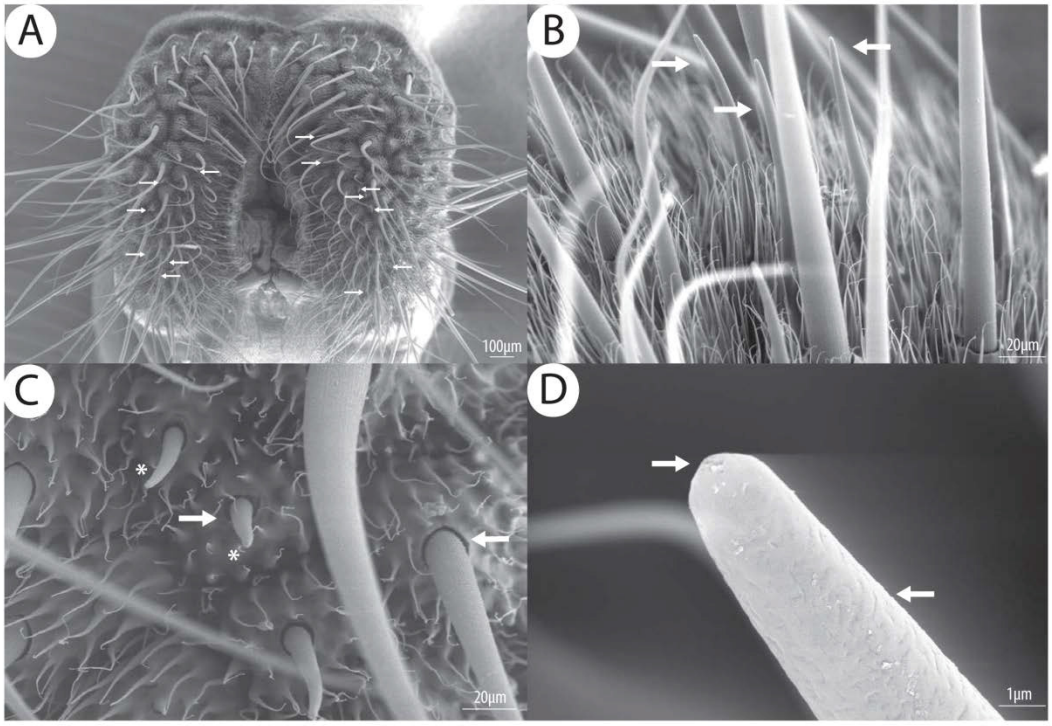

Figure 1.

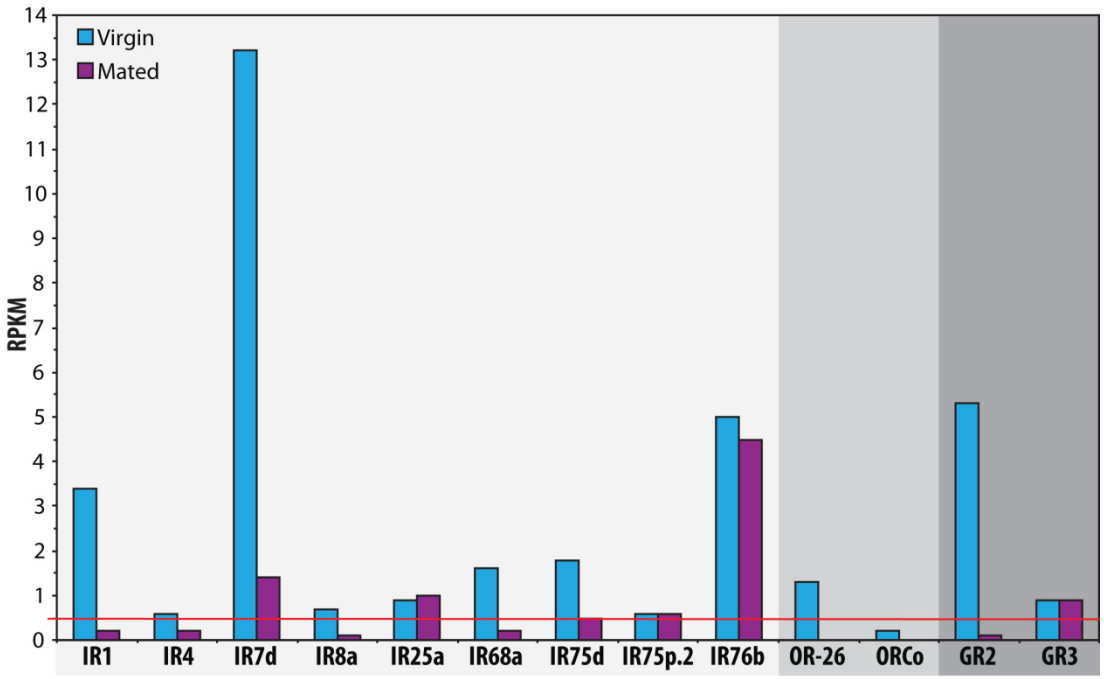
Virgin
Mated
RPKM
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
IR1
IR4
IR7d
IR8a
IR25a
IR68a
IR75d
IR75p.2
IR76b
OR-26
ORCo
GR2
GR3

Figure 2

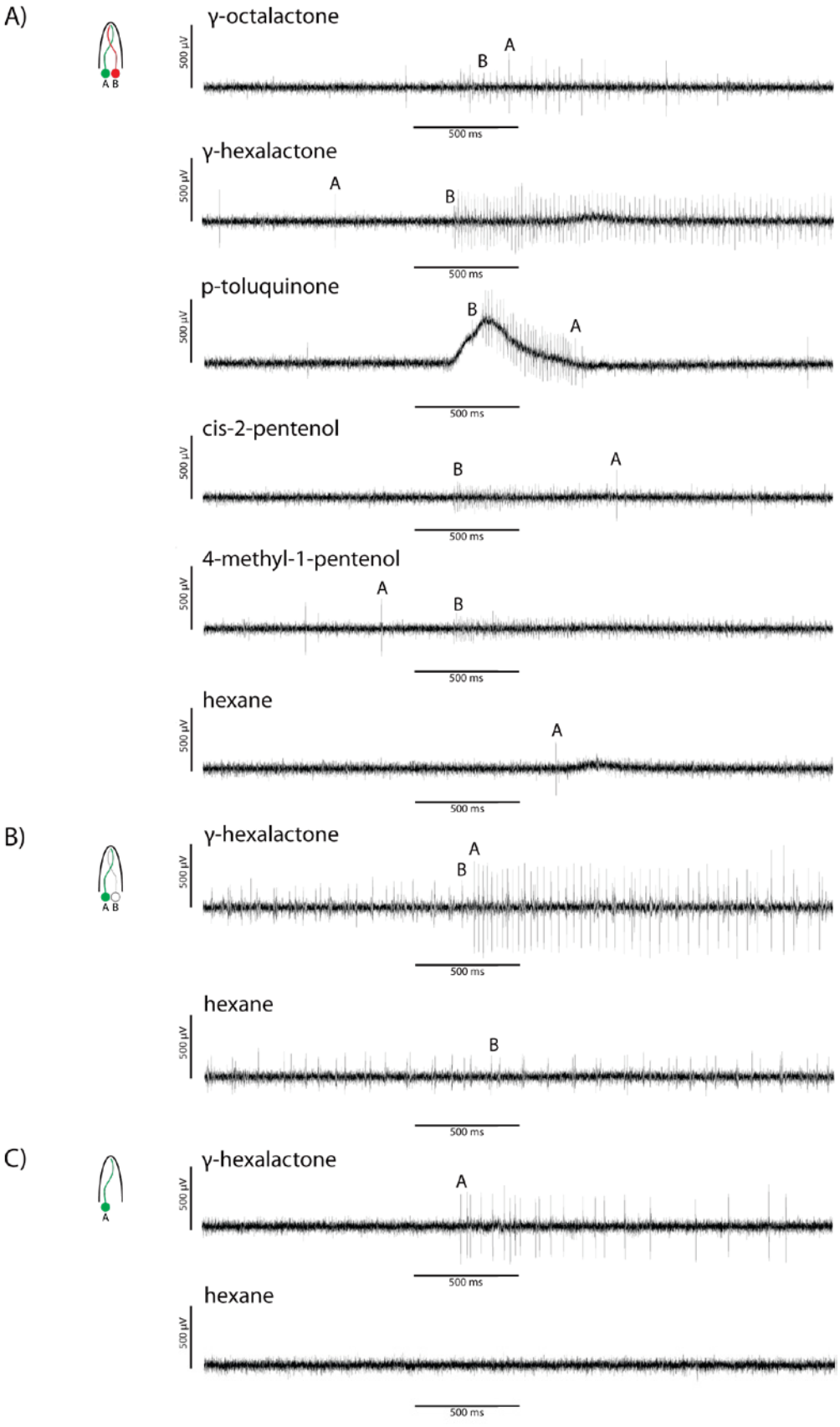
A)
γ-octalactone
γ-hexalactone
p-toluquinone
cis-2-pentenol
4-methyl-1-pentenol
hexane
B)
γ-hexalactone
hexane
C)
γ-hexalactone
hexane
A
B
500 µV
500 ms

Figure 3.

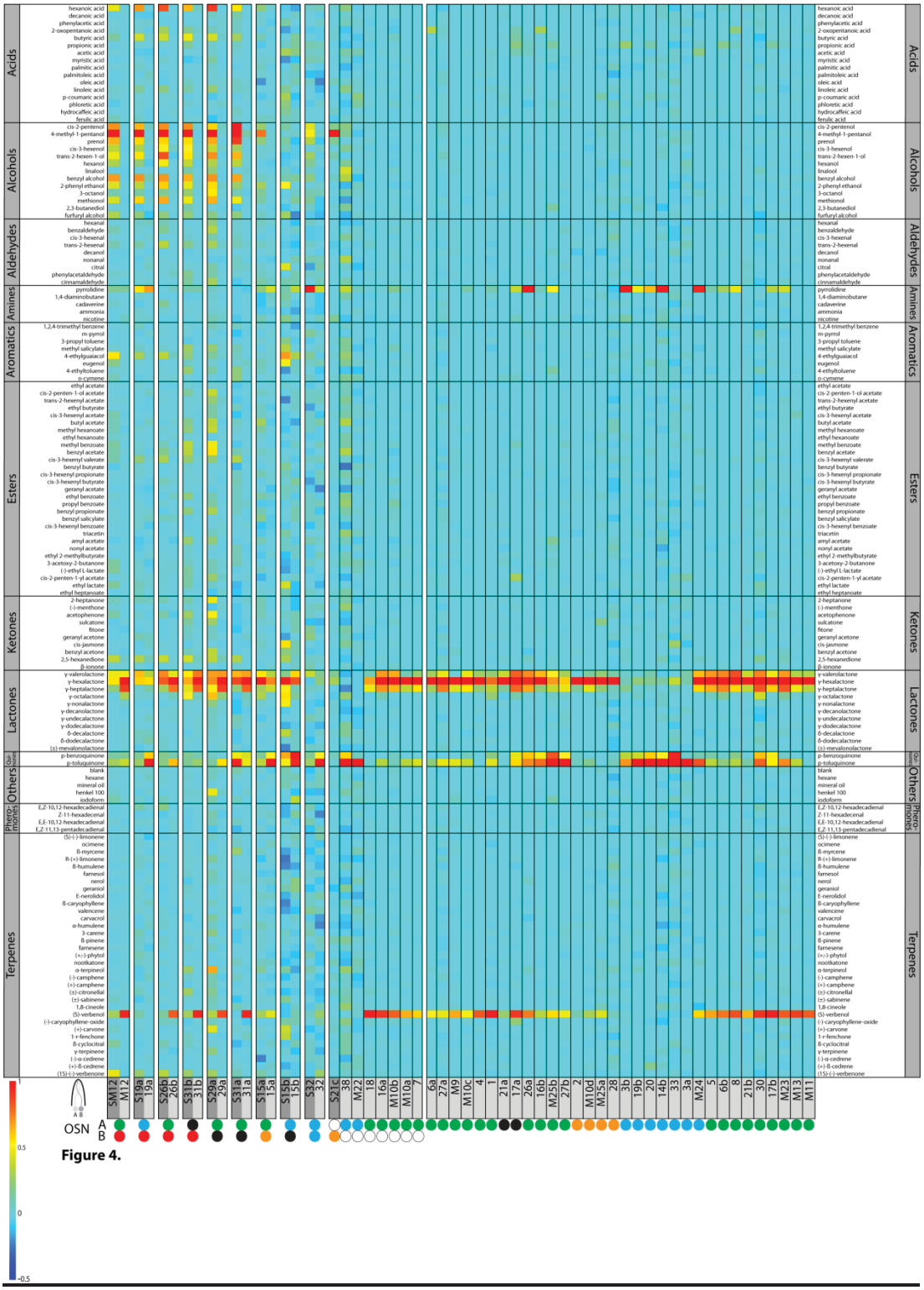
Acids
Alcohols
Aldehydes
Amines
Aromatics
Esters
Ketones
Lactones
Others
Pheromones
Terpenes
OSN
A
B
1
0.5
0
-0.5

Figure 4.

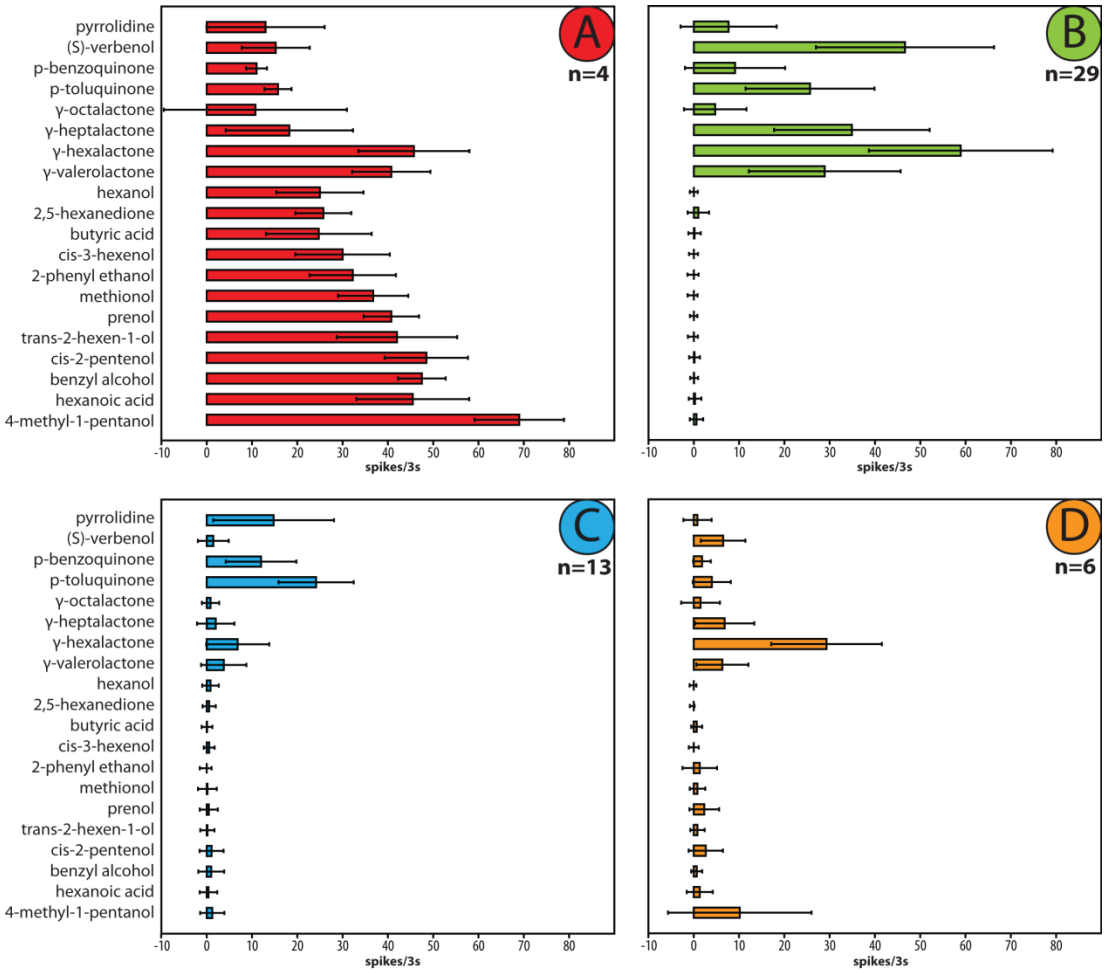
pyrrolidine
(S)-verbenol
p-benzoquinone
p-toluquinone
γ-octalactone
γ-heptalactone
γ-hexalactone
γ-valerolactone
hexanol
2,5-hexanedione
butyric acid
cis-3-hexenol
2-phenyl ethanol
methionol
prenol
trans-2-hexen-1-ol
cis-2-pentenol
benzyl alcohol
hexanoic acid
4-methyl-1-pentanol
A
n=4
B
n=29
C
n=13
D
n=6
-10 0 10 20 30 40 50 60 70 80
spikes/3s

Figure 5.

Manuscript 3

The plastic response of *Manduca sexta* to host and non-host plants

Christopher Koenig*, Anne Bretschneider*, David G. Heckel, Ewald Grosse-Wilde, Bill S. Hansson, Heiko Vogel

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The plastic response of *Manduca sexta* to host and non-host plants

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ABSTRACT

Specialist insect herbivores have evolved efficient ways to adapt to the major defenses of their host plants. Although *Manduca sexta*, specialized on Solanaceous plants, has become a model organism for insect molecular biology, little is known about its adaptive responses to the chemical defenses of its hosts. To study larval performance and transcriptomic responses to host and non-host plants, we conducted developmental assays and replicated RNAseq experiments with *Manduca* larvae fed on different Solanaceous plants as well as on a Brassicaceous non-host plant, *Brassica napus*. *Manduca* larvae developed fastest on *Nicotiana attenuata*, but no significant differences in performance were found on larvae fed on other Solanaceae or the non-host *B. napus*. The RNAseq experiments revealed that *Manduca* larvae display plastic responses at the gene expression level, and transcriptional signatures specific to the challenges of each host- and non-host plant. Our observations are not consistent with expectations that specialist herbivores would perform poorly on non-host plants. Instead, our findings demonstrate the ability of this specialized insect herbivore to efficiently use a larger repertoire of host plants than it utilizes in the field.

1. Introduction

The tobacco hornworm (*Manduca sexta,* henceforth called *Manduca)* has become an important model system in insect science (Baldwin, 2001; Riddiford et al., 2003; Shields and Hildebrand, 2001; Späthe et al., 2013). There are many studies on its biochemistry, behavior and physiology, and many studies on its host plant *Nicotiana*, yet few studies have investigated *Manduca* host-plant interactions at the molecular level. Although *Manduca* larvae specialize on nightshade (Yamamoto and Fraenkel, 1960), they can be reared on artificial diet as well as on non-solanaceous plants, such as *Brassica* spp. (Brassicaceae) under laboratory conditions (Boer and Hanson, 1984). Although such behavior would classify *Manduca* as an oligophagous species rather than a specialist species, the broad host plant range accepted in the lab is not well documented in the field.

In this study, we focused on three typical host plants of *Manduca*: *Nicotiana attenuata* (coyote tobacco), *Solanum lycopersicum* (tomato) and *Datura wrightii* (sacred datura), all belonging to the Solanaceae (nightshade) family, as well as the non-host plant *Brassica napus* (rapeseed). While the solanaceous host plants differ in their secondary metabolites and proteinaceous effectors, *B. napus* also uses a different class of metabolites, glucosinolates, as its major chemical defense (Fahey et al., 2001).

Plants of the nightshade family employ alkaloids, phenylpropanoids, flavonoids, and protease inhibitors to deter herbivores: *Nicotiana,* for example, produces the alkaloid nicotine, as well as trypsin inhibitors. Both of these have been shown to be effective defenses especially against generalist herbivores but can also impact specialist performance (Steppuhn and Baldwin, 2007). *Nicotiana* plants as well as artificial diet containing high nicotine concentrations inhibit the growth of both *Manduca* and the

Abbreviations: AM, antennae and maxillae; G, gut with Malpighian tubules; SG, silk gland (labial gland); W, whole insect; P450, cytochrome P450 monooxygenase; GST, glutathione S-transferase; UGT, UDP-glycosyl transferase; ABC, ATP-binding cassette; IMD, immune deficiency; JAK, janus kinase; STAT, signal transducers and activators of transcription; JNK, c-Jun N-terminal protein kinases; SOCS, suppressor of cytokine signaling; AMP, antimicrobial peptide; WAP, whey acid proteins; AFP, antifungal proteins; MAPK, MAP kinase; OR, olfactory receptor; OBP, odorant binding protein; OSN, olfactory sensory neuron.

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0965-1748/

polyphagous *Helicoverpa zea* (Harvey et al., 2007; Voelckel et al., 2001), and trypsin inhibitors from sweet potato have been shown to affect the growth of and therefore confer resistance to *Spodoptera litura* (Yeh et al., 1997). *S. lycopersicum* uses tomatine, chlorogenic acid, polyphenol oxidase, and proteinase inhibitors to deter herbivorous insects (Kennedy, 2003). Tomatine, a glycoalkaloid, has strong negative effects on the growth rate of *Spodoptera exigua* as well as of *H. zea*, both of which are generalist species that naturally occur on tomato (Bloem et al., 1989). *Datura wrightii* synthesizes the alkaloids scopolamine and hyoscyamine (Hare and Walling, 2006; Parr et al., 1990). Scopolamine prolongs the development and enhances the mortality of *Spodoptera frugiperda*, another generalist from the Noctuidae family (Alves et al., 2007).

Unlike the alkaloid-based chemical defenses of Solanaceae, brassicaceous plants use a system that is activated by two components, glucosinolates and myrosinase enzymes, as their major chemical defense (Bruce, 2014). Highly adapted insect herbivores can feed with impunity on their glucosinolate-containing host plants, but most polyphagous herbivores are negatively affected by high levels of glucosinolates (Arany et al., 2008; Kliebenstein et al., 2005; Winde and Wittstock, 2011).

Although transcriptional responses of generalist herbivores to different host plants or isolated toxins and of generalist and specialist herbivores to individual host plants have been analyzed, studies on large-scale transcriptional responses of herbivorous insects to a range of host plants are scarce or focused on aspects other than herbivore-host plant interactions (Zhan et al., 2011). Not only does host plant chemistry have an impact on detoxification-related gene expression, but secondary metabolites can be crucial for continued larval feeding. The chemical perception of the environment provides information on, for example, food sources and mating partners (Hanson and Dethier, 1973). Olfaction is important for larval plant discrimination and differences in host plant chemistry could potentially be reflected at the level of larval gustatory and olfactory gene expression. Likewise, plant secondary metabolites can influence an insect's immune system, resulting in the differential expression of immune-related genes. Immune defense strategies might also vary with the breadth of an organism's diet (Barthel et al., 2014; Lee et al., 2008; Ponton et al., 2013). Similarly, differences in both types and densities of host plant-associated bacteria can have an impact on innate immunity in herbivorous insects (Freitak et al., 2007).

The goal of this study was to compare the performance of larvae feeding on host and non-host plants as well as global changes in the gene expression of *Manduca* larval tissues elicited by feeding on these plants. To investigate these transcriptional responses, we used a replicated RNAseq approach combined with the official *Manduca* Gene Set (OGS2). In our analyses of the transcriptional responses of *Manduca* larvae, we mainly focused on putative detoxification-related, immune-related and olfactory genes. Here we show that *Manduca* larvae grow fastest on *Nicotiana*, one of the main host plants of this specialized insect. However, *Manduca* larvae performed equally well when fed on the non-host plant *Brassica* as when they fed on other host plants. We report specific changes in the expression of genes related to detoxification, immunity and olfaction as a consequence of feeding on different plants, providing insights into the plastic response of an herbivorous insect with a restricted repertoire of host plants.

2. Material and methods

2.1. Biological material and Manduca sexta rearing

Wild types of the following plant species were used for the experiments: *N. attenuata*, *Datura wrightii*, *S. lycopersicum* (cv. Balcony Magic) and *B. napus* (cv. Dwarf Essex). All plants were grown in a greenhouse maintained at 26 °C, 75% humidity, and a 16 h light and 8 h dark cycle.

Manduca larvae were fed on artificial diet (46 g of agar, 144 g of wheat germ, 140 g of corn meal, 76 g of soy flour, 75 g of casein, 24 g of Wesson's salt mixture, 36 g of sugar, 5 g of cholesterol, 12 g of ascorbic acid, 6 g of sorbic acid, 3 g of methyl paraben, 9 mL of linseed oil, 60 mL of 3.7% formalin, 30 mg of nicotinic acid, 15 mg of riboflavin, 7 mg of thiamine, 7 mg of pyridoxine, 7 mg of folic acid, and 0.6 mg of biotin per 1.8 L of water). Insects were kept at 26 °C, 75% humidity, and a 16 h light and 8 h dark cycle.

2.2. Feeding assay

For the feeding assay, larvae were reared on artificial diet up until shortly before reaching the third instar (L3), when they were transferred to one of the four host (*Nicotiana*, *Datura, Solanum*) or non-host (*Brassica*) plants, respectively, or fed the artificial diet. Plants were 5–10 weeks old (flowers had been removed) and larvae were allowed to feed on whole plants or the artificial diet for eight consecutive days (Fig. 1A). Larval weight was recorded every second day. Differences in the development were statistically analyzed using ANOVA (in R). From the average weight per treatment, we calculated the relative growth rate, since it represents the proportional increase in mass per unit time and adjusts for initial size and the nonlinear patterns of growth over time.

Silk (labial) glands (SG), as well as guts together with Malpighian tubules (G), were dissected from L4 non-molting larvae at Zeitgeber time 8–12. Antennae together with maxillae (AM) were collected from L5 non-molting larvae at Zeitgeber time 8–12. Insects were dissected in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5). In addition, whole insects (L4 non-molting larvae) (W) were ground in liquid nitrogen at Zeitgeber time 8–12 for RNA isolation. Dissected tissue was kept at −20 °C in lysis buffer (innuPREP RNA Mini Kit, Analytik Jena, Germany) until being used for RNA isolation.

2.3. RNA isolation and illumina sequencing

RNAseq experiments were carried out with RNA isolated from larvae reared on artificial diet or different host plants. For SG, G, and W samples, three larvae were pooled for each RNA sample (biological replicate). For AM samples, ten larvae were pooled to receive a sufficient amount of RNA. Three replicates were created from each tissue. Total RNA was extracted according to the manufacturer's instructions (innuPREP RNA Mini Kit, Analytik Jena, Germany).

Library construction and sequencing was performed by the Max Planck Genome Center Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). 1 μg of total RNA was used for a TruSeq RNA library and mRNA enrichment was performed. The library was sequenced with an Illumina HiSeq2500 sequencer. Approximately 10 million 100 bp single-end reads per biological replicate, per treatment, and for each of the tissue samples were obtained. Quality control measures, including filtering high-quality reads based on the score given in fastq files, removing reads containing primer/adaptor sequences and trimming read length, were carried out using CLC Genomics Workbench v6.5 (http://www.clcbio.com).

2.4. Gene annotation

The *Manduca* OGS2-predicted gene set was annotated using BLAST, Gene Ontology and InterProScan searches using BLAST2GO PRO v2.6.1 (www.blast2go.de) (Conesa and Götz, 2008). For BLASTX searches against the non-redundant NCBI protein database (NR database) up to 20 best NR hits per transcript were retained, with

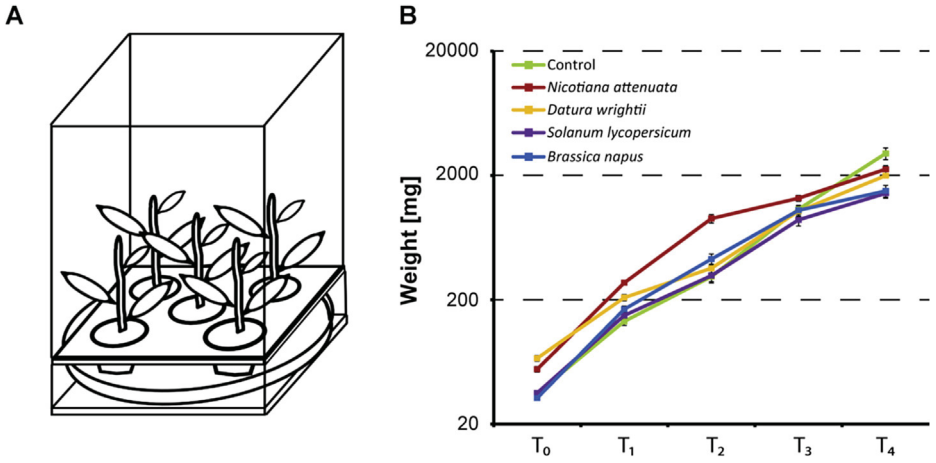

Fig. 1. A) Experimental setup of feeding assay. Larvae were reared on five non-flowering plants (both host and non-host plants) for eight consecutive days. B) *M. sexta* larval development on host and non-host plants. P-value: * < 0.05, ** < 0.01, *** < 0.001 (ANOVA); N = 20/plant or diet. T: timepoint.

an E-value cut-off $\leq 10^{-1}$ and a minimum match length of 15 amino acids to obtain the best homologue also for predicted short polypeptides. Annex (Myhre et al., 2006) was used to optimize the GO term identification further by crossing the three GO categories (biological process, molecular function and cellular component) to search for name similarities, GO term relationships and enzyme relationships within metabolic pathways (Kyoto Encyclopedia of Genes and Genomes). The *Manduca* OGS2 data can be accessed from https://i5k.nal.usda.gov/Manduca_sexta or ftp://ftp.bioinformatics.ksu.edu/pub/Manduca/OGS2/.

2.5. Digital gene expression analysis

Digital gene expression analysis was carried out by using QSeq Software (DNAStar Inc.) to remap the Illumina reads from all samples (each of the replicates of all samples was mapped individually) onto the reference backbone (*Manduca* Official GeneSet2) and then by counting the sequences to estimate expression levels, using previously described parameters for read mapping and normalization (Vogel et al., 2014a). Biases in the sequence datasets and different transcript sizes were corrected using the RPKM algorithm (reads per kilobase of transcript per million mapped reads) to obtain correct estimates for relative expression levels. To control for the effect of global normalization using the RPKM method, we also analyzed a number of highly-conserved housekeeping genes that are used as control genes for quantitative PCR. These included several genes encoding ribosomal proteins (rpl3, rpl4, rpl13, rpl15, rps2, rps8, rps12, rps15a, rps18 and rps24), elongation factor 1alpha and eukaryotic translation initiation factors 4 and 5. The corresponding genes were inspected for overall expression levels across samples and treatments, and were found to display expression level differences (based on RPKM values) lower than 1.3-fold between samples, indicating they were not differentially expressed and validating their housekeeping function. In Supplementary data Table 1 (Table S1) all of the gene names mentioned in the tables and figures of the main manuscript as well as Supplementary Data are linked to the respective Msex2 official gene identifiers.

Table 1
Growth rate of *Manduca sexta* larvae fed on host- and non-host plants, as well as on artificial control diet. T: timepoint.

Treatment	T_1	T_2	T_3	T_4
Control	0.28	0.18	0.27	0.22
Nicotiana attenuata	0.35	0.26	0.08	0.12
Datura wrightii	0.24	0.13	0.23	0.13
Solanum lycopersicum	0.30	0.16	0.22	0.10
Brassica napus	0.36	0.20	0.20	0.08

3. Results

3.1. Growth rate on different plants

All larvae (L3) were weighed at day 0 and every second day following. Control animals were fed artificial diet and exhibited consistent growth and development, showing that this is a suitable control.

The larvae developed differently on the respective plants, both at the beginning and during the course of the experiment. The start weight of animals fed on *Datura* and *Nicotiana* was significantly different from the start weight of animals fed on the other plants (time point 0; p-value < 0.001; Fig. 1B; S1). Until the fourth day, larvae fed on *Nicotiana* were significantly heavier than those fed on all other plants (time point 2; p-value < 0.001). However, two days later (time point 3) there was no significant difference between the larvae fed on the plants and those fed on the control diet. At the last time point sampled, larvae performed equally well when fed on the control diet and on *Nicotiana* (p-value > 0.05) and better than those fed on all the other plants (p-value < 0.05). Even though larvae fed on *Datura* were significantly heavier at the beginning of our measurements, they were unable to retain their body mass to the degree that larvae fed on the other plants were. After four days, larvae fed on *Datura* were as heavy as those fed on tomato or rapeseed. Both the tomato host plant and the non-host rapeseed, however, led to a similar development (measured as growth rate) over time of *Manduca* larvae. Interestingly, larvae fed on *Brassica* show the highest growth rate at time point 1, which subsequently significantly decreased (Table 1). The control individuals fed on artificial diet developed consistently throughout the measured time points.

In summary, *Manduca* larvae have a faster initial growth rate when fed on *Nicotiana* and attain a higher final weight when fed on artificial diet. However, *Manduca* performs nearly as well when fed on *Brassica*, an acceptable non-host plant compared to the host plants *Datura* and *Solanum*.

3.2. Genome expression profile and GO enrichment analysis

We mapped the sequencing reads obtained from the individual samples (tissues and whole larvae fed on different host plants) to the official gene set of the *Manduca* genome project containing

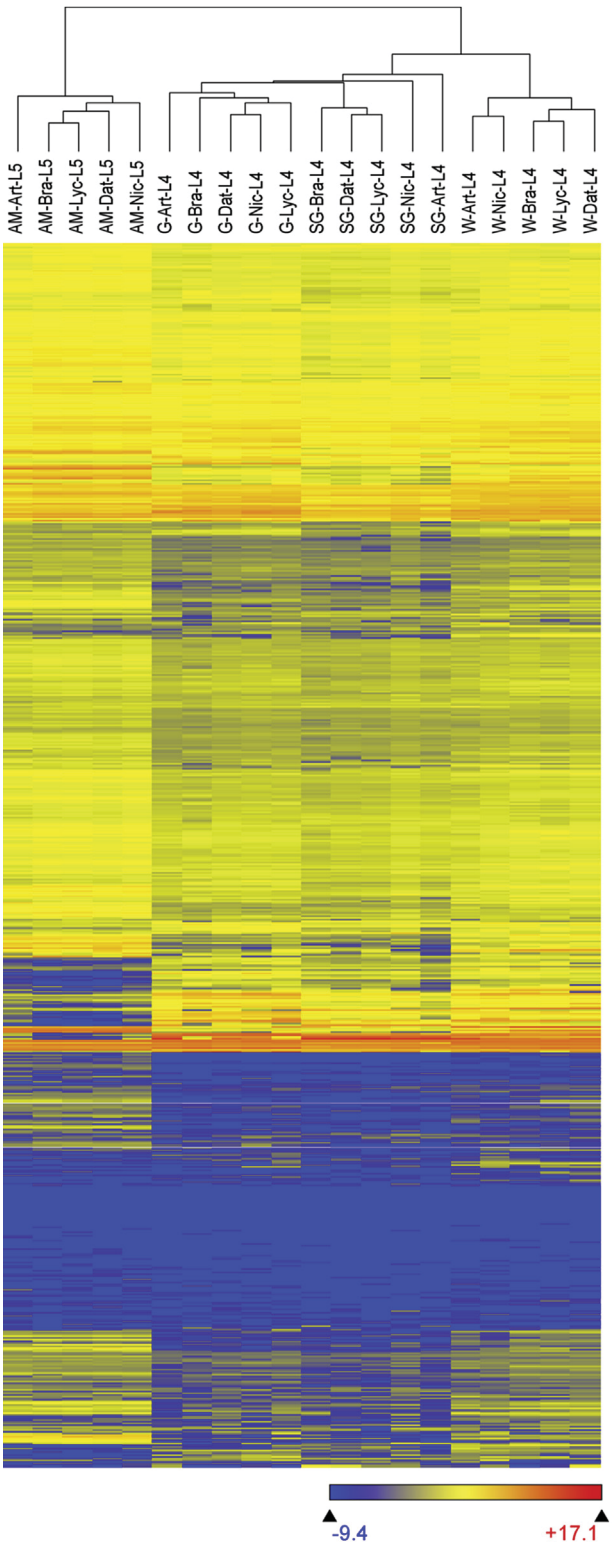

Fig. 2. Heatmap depicting all identified and mapped *M. sexta* OGS2 genes across tissues and treatments. The different tissues cluster each together in individual clades. log2-transformed RPKM values are shown (blue resembles low expressed genes, while red represents high expressed genes). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

15,540 predicted genes (excluding splice variants). The sequence for cytochrome P450 monooxygenase CYP6B46 was added manually, as it was not present in the official gene set but is known to be regulated when *Manduca* feeds on *Nicotiana* (Kumar et al., 2014). Fig. 2 shows a heat map of the log2-transformed expression values (RPKM) of all genes for all investigated tissues. We performed hierarchical clustering using Euclidean distance metric and the centroid linkage method. As expected, each tissue forms an individual cluster based on its gene expression profile. Samples from larvae fed on artificial diet form a distant branch separate from larvae fed on plants with regard to the antennae/maxillae tissue (AM), silk (labial) gland tissue (SG), and gut/Malpighian tubules tissue (G). As a notable exception to the above patterns, in the samples of whole insects (W), larvae reared on *Nicotiana* and artificial diet cluster together, suggesting that feeding on these two diets seems to influence global gene expression patterns in the same way.

Using as a conservative cut-off a minimum 8-fold change between samples, we compared the frequencies of gene ontology (GO) terms of larvae fed on plants to the frequencies of those reared on artificial diet. Larvae grown on each of the plants exhibited an increase in GO terms linked to vesicle-mediated transport, structural composition of cuticle, and transcription factors. In addition, in larvae reared on *Brassica*, GO terms such as fatty acid synthase complex, tetrapyrrole binding, cell differentiation, enzyme regulator activity, and actin filament based process were overrepresented (Fig. S2A). In larvae fed on *Datura*, GO terms such as tetrapyrrole binding, neurotransmitter binding, monooxygenases, oxidoreductases, cell differentiation, glutathione metabolic process, and enzyme regulator activity were overrepresented (Fig. S2B). In larvae fed on *Solanum,* GO terms such as tetrapyrrole binding, actin-filament-based process, and locomotion are overrepresented (Fig. S3A), and in larvae fed on *Nicotiana*, GO terms such as transferase activity and locomotion were overrepresented in addition to the ones mentioned earlier (Fig. S3B).

The total number of differentially expressed genes across all tissues is the greatest in the G samples, especially genes that are related to detoxification (Table 2). However, except for one tissue and category (olfaction in SG), genes of the three functional categories we focused on are regulated in response to plant feeding in a tissue and treatment-specific manner.

Next, we compared gene expression in the W samples from individuals fed on the four plants to gene expression in individuals fed on artificial diet. We found 322 genes at least 4-fold upregulated (p-value < 0.05) in the samples of larvae fed on plants (Table 3).

3.3. Detoxification-related genes

The detoxification and metabolism of most xenobiotics likely occurs via a common set of detoxification-related enzymes, all of which belong to multigene families. Phase I enzymes, including cytochrome P450 proteins (P450s), participate in the functionalization step of xenobiotic detoxification, whereas Phase II enzymes, such as glutathione S-transferases (GST) and UDP-glycosyltransferases (UGTs), convert lipophilic xenobiotics into more hydrophilic compounds to facilitate excretion, for example by ATP binding cassette (ABC) transporters, or sequestration during phase III.

3.3.1. Glutathione S-Transferases

Glutathione S-transferases (GST), which are part of Phase II detoxification, catalyze the conjugation of glutathione to xenobiotics (Eaton and Bammler, 1999). The total number of predicted GSTs in the *Manduca sexta* genome was 42 and included several

Table 2
Total number of differentially expressed genes in all tested tissues (P value < 0.05). AM: antennae and maxillae; G: gut with Malpighian tubules; SG: silk gland (labial gland); W: whole insect.

Tissue	Total# of DEGs	Related function		
		Detoxification	Immunity	Olfaction
AM	269	4	2	6
Gut	1711	47	16	1
SG	325	2	3	0
Whole larvae	758	44	5	6

subfamilies, such as the delta, epsilon, omega, sigma, theta, zeta and microsomal (MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) domain containing) GSTs. We were able to identify 40 transcripts in our data set.

Overall, samples show that GST gene expression patterns cluster in a tissue-specific manner, except for in one case (W-Dat-L4, Fig. S4). Thus, GST expression patterns are quite homogeneous across the samples, with notable exceptions. For example, GST delta4 is up-regulated in the G tissue of *Manduca* larvae fed on *Datura* and in the SG samples of *Manduca* larvae fed on *Nicotiana* (Fig. S4). This gene could be important for successful larval development on *Datura* or *Nicotiana*, and it could either be involved in xenobiotic detoxification or be a more general cellular stress response.

In general, members of the epsilon subfamily are related to the detoxification of xenobiotics (Corona and Robinson, 2006). *Manduca* GSTs Epsilon2 and 3 are similarly up-regulated across all G samples derived from plant-fed larvae, unlike in the G samples from larvae fed on artificial diet. In larvae fed with artificial diet, none of the GSTs were up-regulated compared to samples from plant-fed larvae. This expression pattern implies that genes of this family and especially the two above-mentioned epsilon subfamily members do not have a clear plant-specific function.

The microsomal GST MAPEG1 is most expressed in AM tissue, whereas MAPEG2 is equally expressed in all samples. Since those two genes do not show a treatment-specific up-regulation, they might not be involved in host-plant detoxification; instead, they might play a role in more general cellular processes.

The GST Sigma1 is thought to be involved in oxidative stress response in *Drosophila melanogaster* (Singh et al., 2001). Our data show that, although the *Manduca* orthologue is generally up-regulated in the AM and G tissue of larvae fed on plants, this up-regulation is more pronounced in the *Brassica*-fed larvae. Feeding on *Brassica*, the only non-host plant used in this assay, might lead to higher oxidative stress levels in *Manduca* larvae. The expression profile of GST Sigma 5 is similar to that of GST Sigma 1. It remains to be elucidated whether those two genes are specifically involved in the ability to develop on *Brassica* or whether they are involved in a more general stress response and are induced as a result of the breakdown of glucosinolates into their toxic end products.

3.3.2. ABC transporters

ATP-binding cassette (ABC) transporters constitute one of the most abundant protein families in all organisms (Sturm et al., 2009). Those transmembrane proteins can be divided into different subfamilies (A-H) with different functions. They are involved in cellular transport mechanisms and Phase III detoxification, as well as in general cellular processes (Sturm et al., 2009).

We identified 54 distinct contigs in the *Manduca* transcriptome that are part of ABC transporters (53 of which were annotated in OGS2). The annotated gene model for ABC-D1 and ABC-D2 was split in OGS2; therefore the N- and C-terminal halves were mapped individually. Many members of the subfamilies identified showed a treatment- or tissue-specific expression profile (Fig. 3). In contrast to the distinct tissue-specific clustering observed for the GST genes, clustering of the ABC transporter genes is less clear. For example, SG samples from larvae fed on plants do not form a separate cluster, but rather display treatment-specific gene expression patterns. In contrast, AM samples form a distinct cluster separately from all other samples. While most ABC genes display a rather homogeneous expression pattern within a specific tissue and across treatments, there are some notable exceptions.

Members of subfamilies B and C are involved in detoxification processes and multidrug resistance (Liu et al., 2011). Transcripts homologous to ABC-B1 and ABC-B3 are significantly more highly expressed (p-value $_{B1} = 0.016$; p-value $_{B3} = 0.028$) in the G of insects fed on plants than in the G of larvae fed on artificial diet. ABC-B1, also known as PGP or MDR1, is known for conferring resistance to many different xenobiotics (Holland et al., 2003). Since it is up-regulated most in insects fed on *Datura* and *Solanum*, this plant-specific expression pattern might be an adaptation of *Manduca* larvae to those plants. ABC-B3 is important for antigen processing in mammals (Holland et al., 2003). However, although its function in insects is still unknown, it is highly expressed in the G when larvae are fed on plant material (Fig. 3) and might thus have an important role when insects are exposed to plant secondary metabolites or to general and abundant plant compounds. The transcripts of ABC-C3 and ABC-C11 are most highly expressed in the AM tissue but do not display treatment-specific expression patterns (p-value = 0.665). ABC-C3 is known for its ability to remove toxic organic ions (Zelcer et al., 2001), and its high expression in AM samples compared to other tissues suggests that either the detoxification of xenobiotics or more general transport processes starts within the larval mouth and that this response might be general and dietary-independent. ABC-C2 and ABC-C6 were more expressed in specific tissues and in a treatment-specific way, suggesting these genes could be involved in the development of larvae fed on *Brassica Datura* or on *Solanum* respectively (Fig. 1B). In addition to the above ABC-C transporters, ABC-C4 (Fig. 3), a transporter which has been shown to confer resistance to several xenobiotics (Russel et al., 2008), displays a peak in gene expression (7652 up-regulated) in the W and G samples from *Manduca* larvae fed on *Solanum*, and may be an important developmental adaption to this specific host plant.

Proteins belonging to the ABC-D subfamily transport long-chained-fatty acids (Theodoulou et al., 2006). The respective *Manduca* ABC-D genes are mainly expressed in the mouth parts as well as in the G tissue (Fig. 3) and display overlapping expression profiles with the ABC-A genes, which are involved in cholesterol transport. Those two subfamilies interact in the fatty-acid digestion by transporting these fatty acids to the gut cells; there they are reassembled into triglycerides and coated with cholesterol before being transported into the blood stream (Dean and Annilo, 2005).

Table 3
Differential gene expression from whole (W) larvae reared on plants or on artificial control diet.

Comparison	W-Bra vs W-Art	W-Dat vs W-Art	W-Lyc vs W-Art	W-Nic vs W-Art	total
DEG 4 fold, p < 0.05	174	256	170	49	322
Treatment specific	9	83	29	11	132

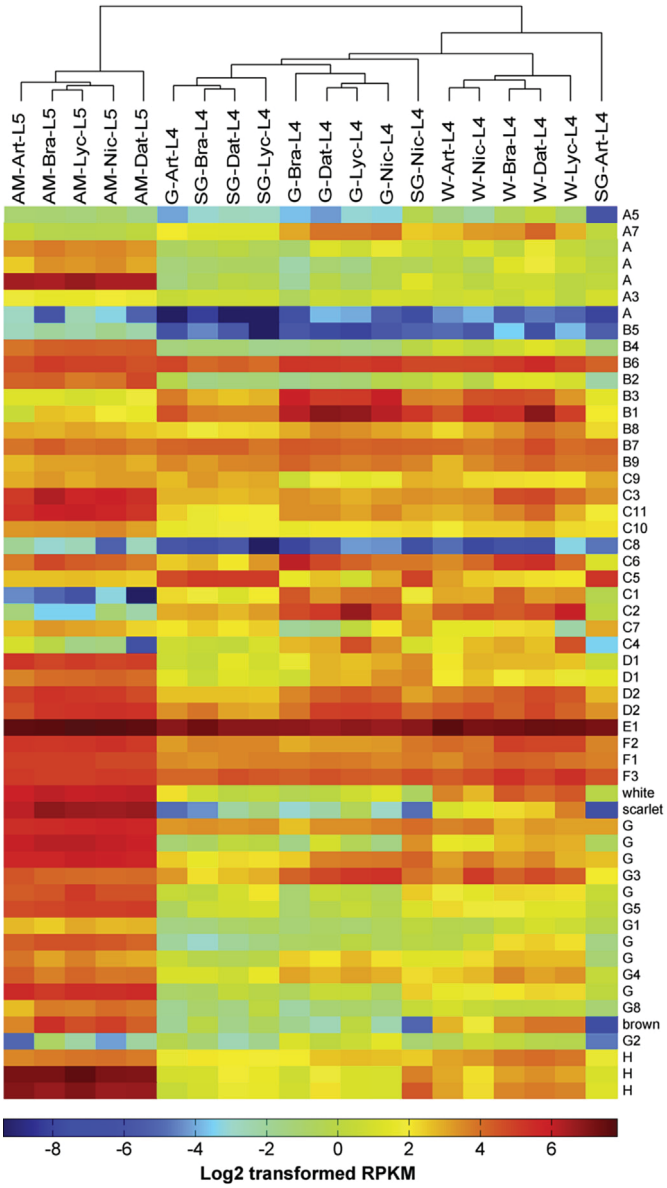

Fig. 3. Gene expression of ABC transporters when *Manduca sexta* larvae were shifted from control diet to plants. Values are based on log2-transformed RPKM values relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: gut with Malpighian tubules; SG: silk gland (labial gland); W: whole insect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Members of subfamily G, which has many functions besides detoxification (Holland et al., 2003), were found to be mostly expressed in the mouth parts. The major eye pigment precursor transporters *white*, *brown* and *scarlet* (Chen et al., 1996), as well as ABC-G5 and ABCG8, both of which are involved in sterol transport (Lee et al., 2001), are mainly expressed in the AM samples. Although the function of the transporter ABC-G3 is not yet understood, it is mainly expressed in the G tissue of insects fed on plants and therefore might exhibit a general function for developing on plants. The function of ABC transporters belonging to subfamily H is not yet known. In *Manduca*, ABC-H transporters are mainly expressed in the AM tissue, which suggests a general function rather than specific involvement, for example in detoxification (Fig. 3). Overall, ABC transporters might be an important part of larval *Manduca* plastic response and adaptation to the different host plants.

3.3.3. UDP-glycosyltransferases

UDP-glycosyltransferases (UGTs) transfer sugars to endogenous and exogenous compounds. They catalyze the biotransformation of xenobiotics, which supports insects in detoxifying dietary metabolites (Ahmad and Hopkins, 1993). Additionally, the transfer of sugars play a role in processes such as pigmentation, cuticle formation and olfaction (Wang et al., 1999).

The OGS2 of *Manduca* comprises 42 annotated UGTs. We identified three additional candidate genes in the transcriptome but none of these were expressed in the investigated tissues. UGTs are expressed in all investigated tissues (Fig. S5). The expression pattern of UGTs is similar in G and SG samples. The expression of three UGTs (39B3, 40H2, 40L2) was focused on the AM samples. The complete UGT gene expression data showed that the tissues generally cluster together, independent of the treatment. However, the G and SG samples from larvae fed on artificial diet are distant from samples of larvae fed on plants. Two UGTs (40C2, 340A3) are up-regulated in the G tissue depending on the treatment (p-value < 0.05) and might thus play a role in the detoxification of plant-specific xenobiotics. In addition to seven other UGTs (p-value < 0.05; 33C, 40C1, 40E2, 40J1p, 42A3, 42A4, 50A3), UGTs 40C2 and 340A3 also exhibit a differential expression pattern in the W samples. Although generally expressed at low levels, one UGT (33H1) is differentially expressed in the AM tissue (p-value < 0.05). We did not detect statistically significant differences in the expression of UGTs in the SG tissue between larvae fed on plants and larvae fed on artificial diet.

3.3.4. Cytochrome P450 monooxygenases

Cytochromes P450, which are membrane-bound enzymes involved in the metabolism of a variety of molecules such as vitamins and hormones, are probably best known for their ability to metabolize xenobiotics and have frequently been shown to be inducible when insects are exposed to certain plant metabolites (De la Paz Celorio-Mancera et al., 2011; Feyereisen, 1999; Hung, 1997; Yamamoto et al., 2010). The official gene set of the *Manduca* genome contains 99 annotated P450 sequences. We added the sequence of CYP6B46 because it has recently been reported as being induced when *Manduca* larvae are fed on *Nicotiana* wild-type plants which contain the ability to produce nicotine (Kumar et al., 2014). While performing a BLAST search with the transcriptome, we identified 17 additional candidates; Figure S6 thus shows the expression profiles of 117 putative P450 genes.

Although several P450s were not expressed in any of the tissues and samples analyzed, a number of P450s were specifically expressed in the AM, SG, or G tissues (not, however, in a clan-specific manner). Several P450s displayed high expression levels only in the W samples, suggesting that they are expressed in tissues other than those investigated (Fig. S6). All AM as well as W samples showed similar expression profiles and cluster together. The G as well as the SG tissue from larvae fed on *Brassica*, *Datura*, and *Solanum* cluster together, but the samples from larvae fed on *Nicotiana* and artificial diet are distinct. These P450 gene expression patterns match the patterns observed for the larval development on the respective plants (Fig. 1B).

19 and 22 P450s were differentially expressed in the G and in W samples, respectively, depending on which plants larvae fed on (p-value < 0.05). Although four of these P450s were not characterized further, three belong to the CYP4 clan; this clan plays an important role in biosynthesizing hormones and pheromones as well as in metabolizing plant toxins such as isoquinoline alkaloids (Danielson et al., 1998). Seven P450s belong to the CYP3 clan, members of

which have been shown to metabolize a broad spectrum of plant compounds such as sesquiterpenes, flavonoids, and insecticides (Feyereisen, 2006; Hung, 1997). One of these CYP3 P450s is also differentially expressed in the SG samples (p-value = 0.0475). Four P450s belong to the clan of mitochondrial P450s, which are typically up-regulated when larvae are exposed to xenobiotics (Yamamoto et al., 2010); a differentially expressed P450 belongs to the CYP2 clan involved in ecdysteroid metabolism in insects.

In larvae fed on *Brassica*, cytochrome P450 CYP332A4 was more than 4-fold up-regulated. Although this gene has already been identified in the *Manduca* midgut transcriptome (Pauchet et al., 2010), its function in relation to the detoxification of host plant metabolites is unknown.

CYP6B46 was previously reported to be induced in the guts of *Manduca* larvae feeding on *Nicotiana* (Kumar et al., 2014). Although we could confirm the up-regulation of CYP6B46 in larvae fed on nicotine (p-value = 0.0477), this P450 gene is expressed even more highly in larvae fed on all other tested plants compared to larvae fed on both *Nicotiana* and artificial diet. Unlike most of the other genes identified in the tissues of larvae fed on all types of plants, expression of P450s seem not to be affected in the AM tissue of the larvae that fed on any of the plants.

3.4. Immunity

The microbiota and potential pathogens which are encountered by *Manduca* larvae when feeding on different plant species can be quite variable and have to be dealt with by their immune system. The multilayered innate immune system of insects is made up of pattern recognition (pattern recognition receptors (PRRs)), signal transduction pathways, and antimicrobial peptides (AMPs) (Casanova-Torres and Goodrich-Blair, 2013) and other defense-related proteins (Hoffmann, 1995; Ratcliffe et al., 1985: Kanost et al., 2004) whose release can be effective against pathogenic challenges (Boutros et al., 2002; Kanost et al., 2004; Ma and Kanost, 2000). In insects, these processes are regulated by the Toll, JAK/STAT, IMD and JNK pathways (Boutros et al., 2002; Dostert et al., 2005; Lemaitre et al., 1996, 1995; Zhong et al., 2012). These pathways help insects defend themselves against invading microorganisms; the Toll pathway is stimulated by gram-positive bacteria as well as by fungi, whereas the IMD pathway is mainly stimulated by gram-negative bacteria (Lemaitre et al., 1997). The hierarchical clustering of the immune-related gene data resulted in 3 main blocks containing AMs, G and SGs, as well as W samples (Fig. 4).

In addition to genes from the Toll and IMD pathway, we could identify larger parts of the JAK/STAT pathway, such as domeless, hopscotch, STAT and SOCS. Genes from those pathways were found to be expressed evenly and at moderate levels in all larval tissues and regardless of what plants larvae fed on (Fig. 4). However, for a number of those signaling pathway genes, we observed high expression levels both in W as well as in the AM samples.

Unlike the even and moderate expression levels of the pattern recognition and signaling pathway genes in larvae, most of the antimicrobial peptides (AMPs) are expressed at low levels in most of the tissues, display a more dynamic regulation and are differentially expressed in a diet-dependent way. Our quantitative analysis of transcripts showed that the majority of the AMPs were detectable in the W samples, which includes the integument and the fat body, and is the major organ for AMP synthesis during systemic immune responses (Ferrandon et al., 2007). In general, all the tissues of larvae fed artificial diet treatments displayed the overall lowest expression of AMPs.

Several AFPs - antifungal proteins - were found to be most highly expressed in the larval mouthparts when larvae fed on plants (Fig. 4), but these increases were not specific to any one plant. However, in other tissues, such as the SG as well as in W samples, a number of AFPs, attacins, a cecropin and gallerimycin displayed statistically significant differences between treatments; in other words, host plant-specific expression patterns were observed. Compared to other AMPs, the whey acid proteins (WAPs) are highly expressed in the G and SG, and display expression differences related to dietary differences of the larvae in a complex tissue and host-plant specific way.

Members of the MAP kinase (MAPK) family are involved in stress response and the regulation of immune responses (Botella et al., 2001). A total of 16 MAPK pathway genes are present in the *Manduca* OGS2, eight of which are expressed in our transcriptome data (Fig. 4). Two of those MAPK genes were differentially expressed in the G samples (p-value < 0.05).

An important aspect of the immune system is melanization and wound healing. Phenoloxidases (POs) are involved in the biosynthesis of melanin and are therefore considered to be essential components of the insect immune system (Sugumaran, 2002). The *Manduca sexta* genome includes two phenoloxidases, both of which are mainly expressed in the W and AM samples and are not significantly differentially expressed between treatments (p-value$_1$ = 0.827; p-value$_2$ = 0.952).

3.5. Olfaction

Two major gene families involved in insect olfaction code for olfactory receptors (ORs) and odorant-binding proteins (OBPs) (Hansson and Stensmyr, 2011). ORs are expressed by olfactory sensory neurons (OSNs) in the antennae, where the OR proteins are situated in the dendritic membrane housed by the olfactory sensilla (Vosshall et al., 2000). ORs detect volatile ligands, mediating a neuronal response. In all cases to date, ORs are co-expressed with the olfactory receptor coreceptor ORCo (Vosshall and Hansson, 2011); ORs and ORCo form a functional, heteromultimeric complex (Sato et al., 2008; Wicher et al., 2008). OBPs are globular, water-soluble proteins that are secreted into the sensillum lymph surrounding the OSN dendrite. They supposedly bind to lipophilic odorant molecules, enabling them to transition into the sensillum lymph and therefore to the OR. OBPs have also been reported to be involved in non-olfactory functions in other body parts (Pelosi et al., 2006).

3.5.1. Odorant receptors

We annotated 70 OR coding genes as well as the ORCo gene within the genome data of *Manduca* (Koenig et al., forthcoming). The sequences of MsexOR-10 and MsexOR-15 were added to the *Manduca* gene set, because although they are annotated in the genome, they were missing in the final OGS2. Using the RNAseq dataset presented here, we analyzed the expression of the respective gene families in larval tissues, as well as differences in gene expression between larvae fed on different diets. In total, 20 OR genes and ORCo are expressed in the AM tissue of larvae (Fig. 5; RPKM ≥0.25: MsexOR-11, 18, 23, 24, 26, 27, 29, 30, 32, 35, 47, 51, 52, 57, 67, 71, 72, 74, 75, 76). Especially noteworthy among the expressed ORs was MsexOR-51, which in adult antennae is male-specific and belongs to the Lepidoptera pheromone clade (Koenig et al., forthcoming). Although no response of *Manduca* larvae to female emitted pheromones has yet been reported, such a response has been described for *Spodoptera littoralis* (Poivet et al., 2012). The other two reported pheromone receptor candidates, MsexOR-1 and MsexOR-4 (Grosse-Wilde et al., 2010; Patch et al., 2009), are not expressed in the larvae. Regarding other expressed receptors, we can only speculate on function. MsexOR-30, for example, is an orthologue of BmOR-56, the OR in *Bombyx mori* that responds to cis-jasmone and mediates the attraction of the larva to mulberry

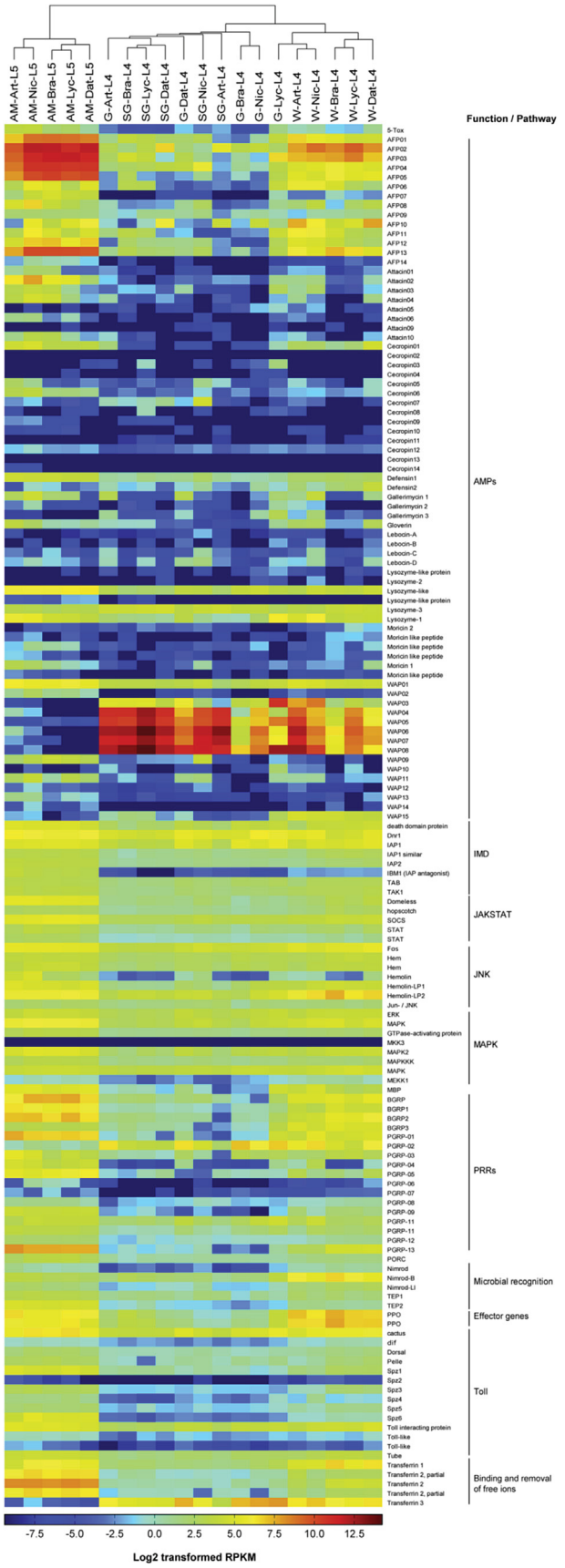

Fig. 4. Expression of immunity-related genes. Gene expression levels in the different tissues are based on log2-transformed RPKM values, and responses are expressed relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: guts with Malpighian tubules; SG: silk gland (labial gland); W: whole insect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

leaves (Tanaka et al., 2009). Other *Manduca* ORs are also orthologues of larval *B. mori* ORs (see Table 4 for receptors and detected compounds). For four ORs (MsexOR-42, 47, 57, 66), expression could also be detected (RPKM $\geq$ 0.25) in non-olfactory larval tissue. RPKM values for all OR genes are low, indicating low transcript abundance within the tissue when compared with all other gene families analyzed. Notably, the antennae of *Manduca* larvae contain presumably seven olfactory sensilla (six basiconic and one styloconic), and the maxilla contains thirteen presumably olfactory sensilla (nine basiconic on the palp, two basiconic and two stylocinic on the galea) (De Boer et al., 1977; Dethier and Schoonhoven, 1966; Kent and Hildebrand, 1987). In total, 30–35 OSNs are present in the tissues, which fits the number of ORs expressed according to our data. The number is comparable to the number of ORs expressed in larval chemosensory tissues of *S. littoralis* (20 ORs) (Poivet et al., 2013) and *B. mori* (23 ORs) (Tanaka et al., 2009). Although the RPKM values fluctuated between the samples from larvae on different host plants, the differences are only statistically significant for MsexOR-27 (p-value = 0.032).

3.5.2. Odorant-binding proteins

There were 49 annotated OBP coding genes in the genome. Expression of eleven OBPs was restricted to the AM tissue in larvae (ABP2, 4, 6, OBP5, 11, 12, 13, 14, 15, 31, 37). Twelve OBPs were expressed in all investigated tissues (ABP1, 7, 8, x, OBP21, 22, 25, 26, 30, 35, 38, 40). For ten OBPs, we did not detect expression in any of the analyzed tissues (ABP3, OBP1, 2, 3, 4, 16, 23, 24, 27, 29). Unlike most ORs, several OBPs were present in G, as well as SG tissue (Fig. 6).

Five OBPs (ABP6, OBP17, OBP28, OBP32, and OBP38) were differentially expressed in the AM depending on larval diet (p-value < 0.05). OBP33 was up-regulated in G tissue when larvae fed on *Solanum* (p-value < 0.05). Six OBPs (ABP7, OBP14, 17, 30, 32, 38) exhibited differential expression in W samples (p-value < 0.05). We did not detect significant expression changes for any OBPs present in the SG samples.

4. Discussion

The aim of this study was to compare *Manduca* larval developmental as well as transcriptional responses to host- and non-host plants. To address this, a developmental assay was combined with a replicated RNAseq approach, using *Manduca* larvae fed on different diets. Our results confirm that *Manduca* is capable of developing on different host and non-host plants. Furthermore, we demonstrate that overall transcriptional signatures of larvae fed on different host plants are quite specific for each host plant and larval tissue combination, suggesting larvae are capable of a plastic and adaptive response to each plant diet.

To feed successfully on host plants, larvae actually require several adaptations; these include host-plant perception and continued feeding (non-deterrency) through olfactory and/or gustatory cues, digestion and plant nutrient uptake processes, detoxification of plant secondary metabolites and countermeasures against other plant defenses, and the management of plant-associated microbiota. In addition to the tissues of whole larvae, we focused our transcriptomic analyses on a selected set of larval tissues, namely antennae and maxillae, which are the first tissues interfacing with plant material, followed by salivary glands and guts.

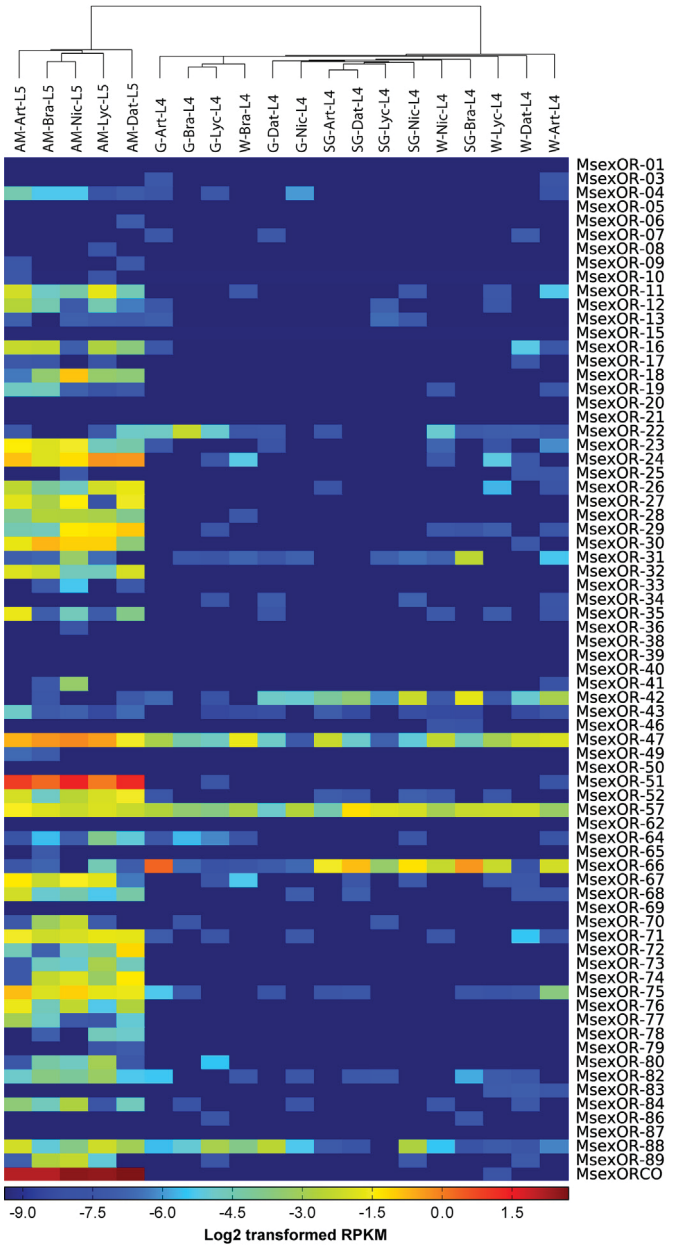

Fig. 5. Expression patterns of olfactory receptors (ORs). Values are based on log2-transformed RPKM values relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: guts with Malpighian tubules; SG: silk gland (labial gland); W: whole insect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Under laboratory conditions, *Manduca* can be reared on artificial diet and on plants belonging to several different plant families (Boer and Hanson, 1984). Our larval growth and performance assay suggests that *N. attenuata* is the best host plant for *Manduca*, better than *Datura wrightii*, *S. lycopersicum*, and the non-host plant *B. napus*. In our set of tested plants, the larvae grew fastest on *Nicotiana*. Artificial diet is rich in carbohydrates and low in toxic substances compared to a diet of plants and should therefore be considered the optimal "diet"; yet initial growth rates of larvae fed on *Nicotiana* were even higher than the values of those fed on artificial diet. A possible explanation could be that *Nicotiana* foliage contains optimally balanced nutrients required for larval development compared to the other tested plants or the artificial diet. Alternatively, growth differences could be due to the effect of hormesis, whereby a low amount of defensive compounds can have positive effects on the growth rate of insects (Harvey et al., 2007; Kaiser, 2003; Voelckel et al., 2001). Such a hormetic effect has been observed at the developmental as well as transcriptional level in *Helicoverpa armigera*, when larvae are fed low gossypol concentrations (De la Paz Celorio-Mancera et al., 2011). Plants of the *Nicotiana* genus are known to produce large amounts of the secondary metabolite nicotine as well as trypsin inhibitors, both of which inhibit the growth of many insect species (Baldwin, 2001; Steppuhn and Baldwin, 2007). However, the specialized insect *Manduca* seems to have overcome this barrier; not only do P450s probably form the basis for the metabolism of nicotine (Kumar et al., 2014), but ABC transporter-like mechanisms excrete nicotine into the hemolymph (Gaertner et al., 1998; Wang et al., 2005). In addition to data for *Manduca*, *high expression of a UGT was significantly linked to nicotine resistance in D. melanogaster* (Marriage et al., 2014). However, despite a likely role of secondary metabolites in *Manduca* larval performance, we cannot exclude the possibility that *Nicotiana* contains elevated levels of substances such as nitrogen-containing compounds, which have been shown to be essential for insect development.

Our data also confirm that *Manduca* can feed and develop normally on *Brassica*, a non-host plant, under laboratory conditions as previously reported (Boer and Hanson, 1984). Thus this species, which is specialized on Solanaceous plants, may have the potential to switch to a new plant or extend its host-plant range. However, under field conditions, host-plant extensions have not been reported for *Manduca* larvae. One possible explanation for this discrepancy between larval performance in the lab and field might be that *Brassica* is simply not chosen for oviposition by female moths. In laboratory experiments, *Manduca* shows no oviposition preference between *Brassica oleracea* and *Nicotiana* (Späthe et al., 2013). However, since oviposition in *Manduca* is guided by olfactory stimuli, and contact chemostimulation elicits deposition of eggs (Yamamoto et al., 1969), it may be that *B. napus* is not accepted for oviposition in the field. As a result there is only a modest chance of its being accepted as a host plant, since larvae show a clear preference for the plants they have previously eaten when given a choice (Jermy et al., 1968).

Based on our RNAseq data, the GO enrichment analysis identified plant-specific overrepresented GO terms. The overrepresentation of such GO-terms related to e. g. chitin binding in larvae fed on all plants but not on artificial diet suggests that a specialized insect such as *Manduca* activates general pathways which are necessary for larval development on plants, irrespective of the plant species the larvae feed on. Chitin is an important component of the peritrophic matrix lining the midgut and is responsible for the strength, elasticity and permeability of this structure, which is an important physical and biochemical barrier (Hegedus et al., 2009; Terra, 2001). It has been hypothesized that the peritrophic matrix is involved in protecting larvae from ingested toxins (Abedi and Brown, 1961; Devenport et al., 2006). An overrepresentation of chitin-binding related genes thus might imply that these genes are another line of defense in *Manduca*

Table 4
ORs expressed in *M. sexta* larvae which have orthologues in *B. mori*, where their best ligands were identified using the expression of heterologues in frog oocytes (Tanaka et al., 2009). All listed *B. mori* ORs are also expressed in the larvae (Tanaka et al., 2009).

M. sexta OR	*B. mori* OR orthologue	Ligands for *B. mori* OR
MsexOR-30	BmOR-56	cis-jasmone
MsexOR-52	BmOR-54	cis-jasmone, Henkel 100
MsexOR-24	BmOR-42	linalool
MsexOR-18	BmOR-29	linalool, citral, linalyl aceteate

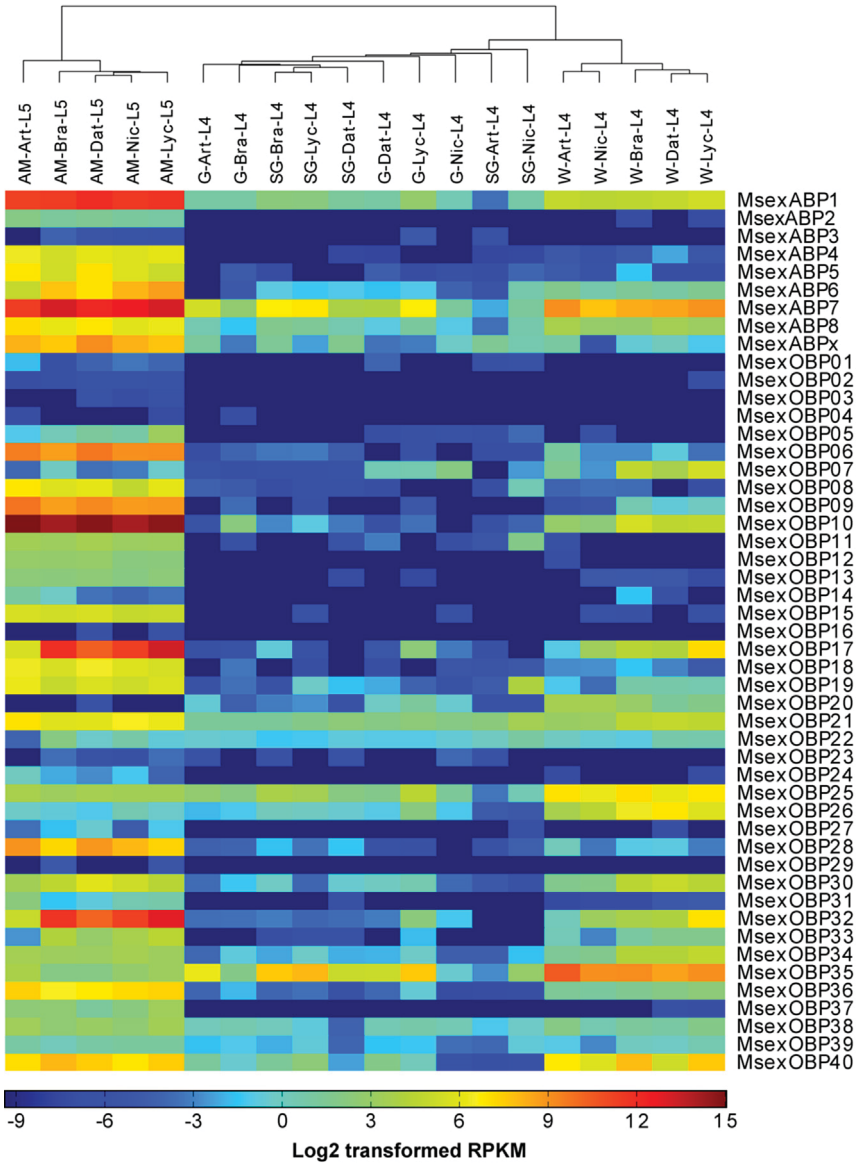

Fig. 6. Expression of odorant-binding proteins (OBP). OBP expression is highest in the antennae and maxillae samples, but there is some expression in the gut/Malpighian tubules and labial gland samples. Values are based on log2-transformed RPKM values relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: guts with Malpighian tubules; SG: silk gland (labial gland); W: whole insect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

larvae, in addition to genes that assist in active detoxification as well as those that provide immunity.

Although we tested several plants to identify differentially expressed genes specific to a certain host plant the larvae fed on, it may be that some of the identified genes perform multiple roles (Vogel et al., 2014b). For example, CYP6B46, initially identified as strongly up-regulated in larvae feeding on diet containing nicotine, was proposed to be linked to nicotine-uptake or -transport processes in the larval guts of *Manduca* (Kumar et al., 2014). However, we could demonstrate that CYP6B46 is up-regulated in larvae fed on all of our tested plants (e.g. note the 19-fold change in G samples of *Brassica*-fed insects). This level of up-regulation implies a more general function for CYP6B46, instead of a clear but limited correlation with nicotine detoxification or transport as has been suggested (Kumar et al., 2014).

Most of the research in insect communication and behavioral changes has investigated adult stages, although several studies have shown that larvae can also respond to volatile cues (Del Campo, 2003; Del Campo et al., 2001; Glendinning et al., 2009). This study provides the first insight into the molecular olfactory repertoire of *Manduca* larvae. We detect the expression of 20 ORs in the AM tissue, including the putative pheromone receptor MsexOR-51 (Koenig et al., forthcoming), and MsexOR-30, an orthologue of a *B. mori* OR that is necessary for host targeting (Tanaka et al., 2009). Additionally, we report the expression of a large number of OBPs in all investigated tissues; these OBPs likely function as transporters of lipophilic substances.

We report changes in the expression of OR and OBP genes that could contribute to the response of larvae grown on different plants rather than artificial diet (Glendinning et al., 2009). If specific OBPs are missing, the response of a receptor to its ligands is decreased (Pophof, 2002). On the other hand, the majority of ORs seems to function independently of the presence of OBPs (Hallem and Carlson, 2006; Hallem et al., 2004; Swarup et al., 2011). Up- or

down-regulation of OBPs could be one mechanism larvae use to adapt the periphery of the olfactory system to specific odors. Since OBPs also interacted with gustatory receptors, the observed regulation of OBP could also influence gustation (Galindo and Smith, 2001; Jeong et al., 2013; Swarup et al., 2014). It is possible that there is a change in gene expression in other chemosensory tissues such as the labrum. We did not dissect this tissue, but its ablation is known to alter the host plant selection of *Manduca* larvae (De Boer, 1991; Del Campo et al., 2001). However, other reports emphasize the importance of the antennae and maxillae for host plant selection (De Boer, 2006, 1993; Hanson and Dethier, 1973; Waldbauer and Fraenkel, 1961). Furthermore, other chemosensory receptor types, such as gustatory receptors and ionotropic receptors, may be involved. Additionally, it is possible that the expression of other genes in the signaling cascade, rather than the expression of the receptors, is responsible for the induction events in the chemosensory tissue; or it may be that induction simply does not involve the regulation of gene expression. Therefore the effect of induction on the chemosensory tissue (Glendinning et al., 2009) may be different from the effect of changes in gene expression.

Although the direct negative effects of plant allelochemicals on larval growth and detoxification-related gene expression seem obvious, the secondary effects of plant allelochemicals on other aspects of insect physiology, such as innate immunity, have been less thoroughly investigated. In interactions among herbivorous insects, microorganisms, parasitoids and host plants, secondary compounds can have profoundly different effects on the outcome of these interactions and ultimately on insect performance and survival (Berenbaum, 1988; Mumm and Dicke, 2010; Diamond and Kingsolver, 2011; Del Campo et al., 2013). Secondary compounds can either reduce the toxicity of pathogens by reducing consumption rates of insects, or increase the toxicity of pathogens by adding extra stress to an insect's metabolism (Berenbaum, 1988; Castro et al., 2009, 2008; Navon et al., 1993). The different host plants to which *Manduca* larvae are exposed could also harbor different, possibly pathogenic microorganisms. The composition of the microbial community on both the surface and the interior of the plant leaf, locations which are known to contain diverse and dense bacterial communities, vary among conspecific plants as well as among different leaves and parts of the same plant (Meyling and Eilenberg, 2006; Monier and Lindow, 2004; Vodovar et al., 2005). Because larvae are naturally exposed to microbes via consumption, this diversity adds to the list of novel niche conditions to which herbivorous insects' immune system must adapt (Freitak et al., 2009a, 2009b, 2007). In order to maintain organismal homeostasis, insect herbivores have to respond to both the challenges posed by host plant secondary metabolites as well as to the respective microbiota they harbor. For a number of immune signaling pathway genes we observed high expression levels in the AM samples. This could indicate that the early detection of pathogens or of high microbial loads in the diet is important and starts in the mouthparts.

Consistent with the induction of immune-related genes by plant-associated microbes and/or plant secondary metabolites, most of the AMPs, although generally expressed at rather low levels, display a dynamic regulation and are differentially expressed in a diet-dependent way. Low expression levels of the AMPs compared to e.g. signaling pathway genes is plausible, since AMPs, as part of the humoral response, are regulated by and downstream of the IMD, JNK and the Toll pathway (Boutros et al., 2002; Casanova-Torres and Goodrich-Blair, 2013; Gunaratna and Jiang, 2013; Lemaitre et al., 1996, 1995). Because mounting an immune response is costly, most AMPs are expressed at measurable levels only when microbial patterns are detected and the respective signaling pathways activated. While AMP expression was overall lowest in all the tissues of larvae fed artificial diet, most AMPs also display expression differences in a complex tissue and host-plant specific way. Thus, although we could not identify a single plant with a clear differential expression pattern of immune-related genes, both host plant-associated microbiota as well as host plant chemistry does affect immune gene expression in different larval tissues in a context-dependent manner, with each host plant inducing a distinct set of immune-related genes.

Taken together, our findings demonstrate the ability of larvae of a specialized insect herbivore to efficiently use a larger repertoire of host plants than it commonly utilizes in the field. *Manduca* larvae display plastic responses at the gene expression level and offer transcriptional signatures specific to the challenges of each host- and non-host plant. In order to understand why, despite the overall positive effect of all plants tested on larval performance, *Manduca* specializes only on the nightshade family, we have to take into account the complexity of an ecosystem. *Manduca*'s preference for the nightshade family is not necessarily based on the insect's limited ability to successfully feed and develop on other plants; instead, larval preference could simply be connected to adult preference. Alternatively, larvae that are restricted from feeding extensively on these plants may receive developmental advantages in a more complex ecological context, such as host plant-dependent resistance to pathogens or parasites.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmb.2015.06.001.

References

Abedi, Z.H., Brown, A.W.A., 1961. Peritrophic membrane as vehicle for DDT and DDE excretion in Aedes aegypti larvae. Ann. Entomol. Soc. Am. 54, 539–542. http://dx.doi.org/10.1093/aesa/54.4.539.

Ahmad, S.A., Hopkins, T.L., 1993. β-Glucosylation of plant phenolics by phenol β-glucosyltransferase in larval tissues of the tobacco hornworm, Manduca sexta(L.). Insect Biochem. Mol. Biol. 23, 581–589. http://dx.doi.org/10.1016/0965-1748(93)90031-M.

Alves, M.N., Sartoratto, A., Trigo, J.R., 2007. Scopolamine in Brugmansia suaveolens (Solanaceae): defense, allocation, costs, and induced response. J. Chem. Ecol. 33, 297–309. http://dx.doi.org/10.1007/s10886-006-9214-9.

Arany, a. M., De Jong, T.J., Kim, H.K., Van Dam, N.M., Choi, Y.H., Verpoorte, R., Van Der Meijden, E., 2008. Glucosinolates and other metabolites in the leaves of Arabidopsis thaliana from natural populations and their effects on a generalist and a specialist herbivore. Chemoecology 18, 65–71. http://dx.doi.org/10.1007/s00049-007-0394-8.

Baldwin, I.T., 2001. An ecologically motivated analysis of plant-herbivore interactions in native tobacco. Plant Physiol. 127, 1449–1458. http://dx.doi.org/10.1104/pp.010762.

Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D.G., Groot, A.T., 2014. Immune defence strategies of generalist and specialist insect herbivores. Proc. Biol. Sci. 281, 20140897. http://dx.doi.org/10.1098/rspb.2014.0897.

Berenbaum, M.R., 1988. Allelochemicals in insect-microbe-plant interactions: agents provocateurs in the coevolutionary arms race. In: Barbosa, P., Letourneau, D.K. (Eds.), Novel Aspects of Insect-plant Interactions. John Wiley & Sons, New York, pp. 97–124.

Bloem, K.A., Kelley, K.C., Duffey, S.S., 1989. Differential effect of tomatine and its alleviation by cholesterol on larval growth and efficiency of food utilization

inHeliothis zea andSpodoptera exigua. J. Chem. Ecol. 15, 387–398. http://dx.doi.org/10.1007/BF02027799.
Boer, G., Hanson, F.E., 1984. Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm Manduca sexta. Entomol. Exp. Appl. 35, 177–193. http://dx.doi.org/10.1111/j.1570-7458.1984.tb03378.x.
Botella, J.A., Baines, I.A., Williams, D.D., Goberdhan, D.C.I., Proud, C.G., Wilson, C., 2001. The Drosophila cell shape regulator c-Jun N-terminal kinase also functions as a stress-activated protein kinase. Insect Biochem. Mol. Biol. 31, 839–847. http://dx.doi.org/10.1016/S0965-1748(01)00029-7.
Boutros, M., Agaisse, H., Perrimon, N., 2002. Sequential activation of signaling pathways during innate immune responses in Drosophila. Dev. Cell 3, 711–722. http://dx.doi.org/10.1016/S1534-5807(02)00325-8.
Bruce, T.J.A., 2014. Glucosinolates in oilseed rape: secondary metabolites that influence interactions with herbivores and their natural enemies. Ann. Appl. Biol. 164, 348–353. http://dx.doi.org/10.1111/aab.12128.
Casanova-Torres, Á.M., Goodrich-Blair, H., 2013. Immune signaling and antimicrobial peptide expression in Lepidoptera. Insects 4, 320–338.
Castro, D.P., Figueiredo, M.B., Genta, F. a., Ribeiro, I.M., Tomassini, T.C.B., Azambuja, P., Garcia, E.S., 2009. Physalin B inhibits Rhodnius prolixus hemocyte phagocytosis and microaggregation by the activation of endogenous PAF-acetyl hydrolase activities. J. Insect Physiol. 55, 532–537. http://dx.doi.org/10.1016/j.jinsphys.2009.01.013.
Castro, D.P., Figueiredo, M.B., Ribeiro, I.M., Tomassini, T.C.B., Azambuja, P., Garcia, E.S., 2008. Immune depression in Rhodnius prolixus by seco-steroids, physalins. J. Insect Physiol. 54, 555–562. http://dx.doi.org/10.1016/j.jinsphys.2007.12.004.
Chen, H., Rossier, C., Lalioti, M.D., Lynn, A., Chakravarti, A., Perrin, G., Antonarakis, S.E., 1996. Cloning of the cDNA for a human homologue of the Drosophila white gene and mapping to chromosome 21q22.3. Am. J. Hum. Genet. 59, 66–75.
Conesa, A., Götz, S., 2008. Blast2GO: a comprehensive suite for functional analysis in plant genomics. Int. J. Plant Genomics 2008. http://dx.doi.org/10.1155/2008/619832.
Corona, M., Robinson, G.E., 2006. Genes of the antioxidant system of the honey bee: annotation and phylogeny. Insect Mol. Biol. 15, 687–701. http://dx.doi.org/10.1111/j.1365-2583.2006.00695.x.
Danielson, P.B., Foster, J.L.M., McMahill, M.M., Smith, M.K., Fogleman, J.C., 1998. Induction by alkaloids and phenobarbital of Family 4 Cytochrome P450s in Drosophila: evidence for involvement in host plant utilization. Mol. Gen. Genet. MGG 259, 54–59. http://dx.doi.org/10.1007/s004380050788.
De Boer, G., 1991. Effect of diet experience on the ability of different larval chemosensory organs to mediate food discrimination by the tobacco hornworm, Manduca sexta. J. Insect Physiol. 37, 763–769. http://dx.doi.org/10.1016/0022-1910(91)90111-C.
De Boer, G., 1993. Plasticity in food preference and diet-induced differential weighting of chemosensory information in larval Manduca sexta. J. Insect Physiol. 39, 17–24. http://dx.doi.org/10.1016/0022-1910(93)90013-H.
De Boer, G., 2006. The role of the antennae and maxillary palps in mediating food preference by larvae of the tobacco hornworm, Manduca sexta. Entomol. Exp. Appl. 119, 29–38. http://dx.doi.org/10.1111/j.1570-7458.2006.00397.x.
De Boer, G., Dethier, V.G., Schoonhoven, L.M., 1977. Chemoreceptors in the preoral cavity of the tobacco hornworm, Manduca sexta, and their possible function in feeding behaviour. Entomol. Exp. Appl. 21, 287–298. http://dx.doi.org/10.1007/BF00291791.
De la Paz Celorio-Mancera, M., Ahn, S.-J., Vogel, H., Heckel, D.G., 2011. Transcriptional responses underlying the hormetic and detrimental effects of the plant secondary metabolite gossypol on the generalist herbivore Helicoverpa armigera. BMC Genomics. http://dx.doi.org/10.1186/1471-2164-12-575.
Dean, M., Annilo, T., 2005. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Genet., Annu. Rev. Genomics Hum. Genet. 6, 123–142. http://dx.doi.org/10.1146/annurev.genom.6.080604.162122.
Del Campo, M.L., 2003. Chemosensory tuning to a host recognition cue in the facultative specialist larvae of the moth Manduca sexta. J. Exp. Biol. 206, 3979–3990. http://dx.doi.org/10.1242/jeb.00626.
Del Campo, M.L., Miles, C.I., Schroeder, F.C., Mueller, C., Booker, R., Renwick, J., 2001. Host recognition by the tobacco hornworm is mediated by a host plant compound. Nature 411, 186–189. http://dx.doi.org/10.1038/35075559.
Del Campo, M.L., Halitschke, R., Short, S.M., Lazzaro, B.P., Kessler, A., 2013. Dietary plant phenolic improves survival of bacterial infection in *Manduca sexta* caterpillars. Entomol. Exp. Appl. 146, 321–331.
Dethier, V., Schoonhoven, L.M., 1966. Sensory aspects of host-plant discrimination by Lepidopterous larvae. Arch. Néerl. Zool. 16, 497–530. http://dx.doi.org/10.1163/036551666X00057.
Devenport, M., Alvarenga, P.H., Shao, L., Fujioka, H., Bianconi, M.L., Oliveira, P.L., Jacobs-Lorena, M., 2006. Identification of the aedes aegypti peritrophic matrix protein AeIMUCI as a heme-binding protein. Biochemistry 45, 9540–9549. http://dx.doi.org/10.1021/bi0605991.
Diamond, S.E., Kingsolver, J.G., 2011. Host plant quality, selection history and trade-offs shape the immune responses of *Manduca sexta*. Proc. Biol. Sci. 278, 289–297.
Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J.A., Imler, J.L., 2005. The JAK-STAT signaling pathway is required but not sufficient for the antiviral response of Drosophila. Nat. Immunol. 6, 946–953.
Eaton, D.L., Bammler, T.K., 1999. Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol. Sci. 49, 156–164. http://dx.doi.org/10.1093/toxsci/49.2.156.
Fahey, J.W., Zalcmann, a T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. http://dx.doi.org/10.1016/S0031-9422(00)00316-2.
Ferrandon, D., Imler, J.-L., Hetru, C., Hoffmann, J.A., 2007. The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. Nat. Rev. Immunol. 7, 862–874. http://dx.doi.org/10.1038/nri2194.
Feyereisen, R., 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–533. http://dx.doi.org/10.1146/annurev.ento.44.1.507.
Feyereisen, R., 2006. Evolution of insect P450. Biochem. Soc. Trans. 34, 1252–1255. http://dx.doi.org/10.1042/BST0341252.
Freitak, D., Heckel, D.G., Vogel, H., 2009a. Dietary-dependent trans-generational immune priming in an insect herbivore. Proc. Biol. Sci. 276, 2617–2624. http://dx.doi.org/10.1098/rspb.2009.0323.
Freitak, D., Heckel, D.G., Vogel, H., 2009b. Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (Trichoplusia ni). Front. Zool. 6, 7. http://dx.doi.org/10.1186/1742-9994-6-7.
Freitak, D., Wheat, C.W., Heckel, D.G., Vogel, H., 2007. Immune system responses and fitness costs associated with consumption of bacteria in larvae of Trichoplusia ni. BMC Biol. 5, 56. http://dx.doi.org/10.1186/1741-7007-5-56.
Gaertner, L.S., Murray, C.L., Morris, C.E., 1998. Transepithelial transport of nicotine and vinblastine in isolated malpighian tubules of the tobacco hornworm (Manduca sexta) suggests a P-glycoprotein-like mechanism. J. Exp. Biol. 201, 2637–2645.
Galindo, K., Smith, D.P., 2001. A large family of divergent Drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla. Genetics 159, 1059–1072.
Glendinning, J.I., Foley, C., Loncar, I., Rai, M., 2009. Induced preference for host plant chemicals in the tobacco hornworm: contribution of olfaction and taste. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 195, 591–601. http://dx.doi.org/10.1007/s00359-009-0434-7.
Grosse-Wilde, E., Stieber, R., Forstner, M., Krieger, J., Wicher, D., Hansson, B.S., 2010. Sex-specific odorant receptors of the tobacco hornworm Manduca sexta. Front. Cell. Neurosci. 4 http://dx.doi.org/10.3389/fncel.2010.00022.
Gunaratna, R.T., Jiang, H., 2013. A comprehensive analysis of the *Manduca sexta* immunotranscriptome. Dev. Comp. Immunol. 39, 388–398.
Hallem, E. a, Carlson, J.R., 2006. Coding of odors by a receptor repertoire. Cell 125, 143–160. http://dx.doi.org/10.1016/j.cell.2006.01.050.
Hallem, E.A., Ho, M.G., Carlson, J.R., 2004. The molecular basis of odor coding in the Drosophila antenna. Cell 117, 965–979.
Hanson, F.E., Dethier, V.G., 1973. Rôle of gustation and olfaction in food plant discrimination in the tobacco hornworm, Manduca sexta. J. Insect Physiol. 19, 1019–1031. http://dx.doi.org/10.1016/0022-1910(73)90028-0.
Hansson, B.S., Stensmyr, M.C., 2011. Evolution of insect olfaction. Neuron 72, 698–711. http://dx.doi.org/10.1016/j.neuron.2011.11.003.
Hare, J.D., Walling, L.L., 2006. Constitutive and jasmonate-inducible traits of Datura wrightii. J. Chem. Ecol. 32, 29–47. http://dx.doi.org/10.1007/s10886-006-9349-8.
Harvey, J.A., Van Dam, N.M., Witjes, L.M.A., Soler, R., Gols, R., 2007. Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. Ecol. Entomol. 32, 15–23. http://dx.doi.org/10.1111/j.1365-2311.2006.00838.x.
Hegedus, D., Erlandson, M., Gillott, C., Toprak, U., 2009. New insights into peritrophic matrix synthesis, architecture, and function. Annu. Rev. Entomol. 54, 285–302. http://dx.doi.org/10.1146/annurev.ento.54.110807.090559.
Hoffmann, J.A., 1995. Innate immunity of insects. Curr. Opin. Immunol. 7, 4–10. http://dx.doi.org/10.1016/0952-7915(95)80022-0.
Holland, I.B., Cole, S.P.C., Kuchler, K., Higgins, C.F. (Eds.), 2003. ABC Proteins: from Bacteria to Man. Academic Press, London, UK.
Hung, C., 1997. Isolation and characterization of CYP6B4, a furanocoumarin-inducible cytochrome P450 from a polyphagous caterpillar (Lepidoptera: Papilionidae). Insect Biochem. Mol. Biol. 27, 377–385. http://dx.doi.org/10.1016/S0965-1748(97)00009-X.
Jeong, Y.T., Shim, J., Oh, S.R., Yoon, H.I., Kim, C.H., Moon, S.J., Montell, C., 2013. An odorant-binding protein required for suppression of sweet taste by bitter chemicals. Neuron 79, 725–737. http://dx.doi.org/10.1016/j.neuron.2013.06.025.
Jermy, T., Hanson, F.E., Dethier, V.G., 1968. Induction of specific food preference in Lepidopterous larvae. Entomol. Exp. Appl. 11, 211–230. http://dx.doi.org/10.1111/j.1570-7458.1968.tb02046.x.
Kaiser, J., 2003. Hormesis. Sipping from a poisoned chalice. Science. http://dx.doi.org/10.1126/science.302.5644.376.
Kanost, M.R., Jiang, H., Yu, X.-Q., 2004. Innate immune responses of a lepidopteran insect, *Manduca sexta*. Immunol. Rev. 198, 97–105.
Kennedy, G.G., 2003. Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus Lycopersicon. Annu. Rev. Entomol. 48, 51–72. http://dx.doi.org/10.1146/annurev.ento.48.091801.112733.
Kent, K.S., Hildebrand, J.G., 1987. Cephalic sensory pathways in the central nervous system of larval Manduca sexta (Lepidoptera: Sphingidae). Philos. Trans. R. Soc. Lond. B. Biol. Sci. 315, 1–36.
Kliebenstein, D.J., Kroymann, J., Mitchell-Olds, T., 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. Curr. Opin. Plant Biol. http://dx.doi.org/10.1016/j.pbi.2005.03.002.

Kumar, P., Pandit, S.S., Steppuhn, A., Baldwin, I.T., 2014. Natural history-driven, plant-mediated RNAi-based study reveals CYP6B46's role in a nicotine-mediated antipredator herbivore defense. Proc. Natl. Acad. Sci. U. S. A. 111, 1245–1252. http://dx.doi.org/10.1073/pnas.1314848111.

Lee, K.P., Simpson, S.J., Wilson, K., 2008. Dietary protein-quality influences melanization and immune function in an insect. Funct. Ecol. 22, 1052–1061. http://dx.doi.org/10.1111/j.1365-2435.2008.01459.x.

Lee, M.H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A.K., Salen, G., Dean, M., Patel, S.B., 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat. Genet. 27, 79–83. http://dx.doi.org/10.1038/83799.

Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J.M., Hoffmann, J. a, 1995. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the Drosophila host defense. Proc. Natl. Acad. Sci. 92, 9465–9469. http://dx.doi.org/10.1073/pnas.92.21.9465.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J., Hoffmann, J., 1996. The Dorsoventral Regulatory gene cassette controls the Potent antifungal response in Drosophila adults. Cell 86, 973–983. http://dx.doi.org/10.1016/S0092-8674(00)80172-5.

Lemaitre, B., Reichhart, J.-M., Hoffmann, J.A., 1997. Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc. Natl. Acad. Sci. 94, 14614–14619. http://dx.doi.org/10.1073/pnas.94.26.14614.

Liu, S., Zhou, S., Tian, L., Guo, E., Luan, Y., Zhang, J., Li, S., 2011. Genome-wide identification and characterization of ATP-binding cassette transporters in the silkworm, Bombyx mori. BMC Genomics 12. http://dx.doi.org/10.1186/1471-2164-12-491.

Ma, C., Kanost, M.R., 2000. A beta1,3-glucan recognition protein from an insect, *Manduca sexta*, agglutinates microorganisms and activates the phenoloxidase cascade. J. Biol. Chem. 275, 7505–7514.

Marriage, T.N., King, E.G., Long, A.D., Macdonald, S.J., 2014. Fine-mapping nicotine resistance loci in Drosophila using a multiparent advanced generation intercross population. Genetics 198, 45–57. http://dx.doi.org/10.1534/genetics.114.162107.

Meyling, N.V., Eilenberg, J., 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113, 336–341. http://dx.doi.org/10.1016/j.agee.2005.10.011.

Monier, J.M., Lindow, S.E., 2004. Frequency, size, and Localization of bacterial Aggregates on Bean leaf surfaces. Appl. Environ. Microbiol. 70, 346–355. http://dx.doi.org/10.1128/AEM.70.1.346-355.2004.

Mumm, R., Dicke, M., 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defenseThe present review is one in the special series of reviews on animal–plant interactions. Can. J. Zool. http://dx.doi.org/10.1139/Z10-032.

Myhre, S., Tveit, H., Mollestad, T., Lægreid, A., 2006. Additional Gene Ontology structure for improved biological reasoning. Bioinformatics 22, 2020–2027. http://dx.doi.org/10.1093/bioinformatics/btl334.

Navon, A., Hare, J.D., Federici, B.A., 1993. Interactions amongHeliothis virescens larvae, cotton condensed tannin and the CryIA(c)?-endotoxin ofBacillus thuringiensis. J. Chem. Ecol. http://dx.doi.org/10.1007/BF00980685.

Parr, A.J., Payne, J., Eagles, J., Chapman, B.T., Robins, R.J., Rhodes, M.J.C., 1990. Variation in tropane alkaloid accumulation within the solanaceae and strategies for its exploitation. Phytochemistry 29, 2545–2550. http://dx.doi.org/10.1016/0031-9422(90)85185-I.

Patch, H.M., Velarde, R.A., Walden, K.K.O., Robertson, H.M., 2009. A candidate pheromone receptor and two odorant receptors of the Hawkmoth manduca sexta. Chem. Senses 34, 305–316. http://dx.doi.org/10.1093/chemse/bjp002.

Pauchet, Y., Wilkinson, P., Vogel, H., Nelson, D.R., Reynolds, S.E., Heckel, D.G., Ffrench-Constant, R.H., 2010. Pyrosequencing the Manduca sexta larval midgut transcriptome: messages for digestion, detoxification and defence. Insect Mol. Biol. 19, 61–75. http://dx.doi.org/10.1111/j.1365-2583.2009.00936.x.

Pelosi, P., Zhou, J.J., Ban, L.P., Calvello, M., 2006. Soluble proteins in insect chemical communication. Cell. Mol. Life Sci. http://dx.doi.org/10.1007/s00018-005-5607-0.

Poivet, E., Gallot, A., Montagné, N., Glaser, N., Legeai, F., Jacquin-Joly, E., 2013. A comparison of the olfactory gene repertoires of adults and larvae in the noctuid moth Spodoptera littoralis. PLoS One 8, e60263. http://dx.doi.org/10.1371/journal.pone.0060263.

Poivet, E., Rharrabe, K., Monsempes, C., Glaser, N., Rochat, D., Renou, M., Marion-Poll, F., Jacquin-Joly, E., 2012. The use of the sex pheromone as an evolutionary solution to food source selection in caterpillars. Nat. Commun. 3, 1047. http://dx.doi.org/10.1038/ncomms2050.

Ponton, F., Wilson, K., Holmes, A.J., Cotter, S.C., Raubenheimer, D., Simpson, S.J., 2013. Integrating nutrition and immunology: a new frontier. J. Insect Physiol. http://dx.doi.org/10.1016/j.jinsphys.2012.10.011.

Pophof, B., 2002. Moth pheromone binding proteins contribute to the excitation of olfactory receptor cells. Naturwissenschaften 89, 515–518. http://dx.doi.org/10.1007/s00114-002-0364-5.

Ratcliffe, N.A., Rowley, A.F., Fitzgerald, S.W., Rhodes, C.P., 1985. Invertebrate immunity: basic Concepts and Recent Advances. Int. Rev. Cytol. 97, 183–350. http://dx.doi.org/10.1016/S0074-7696(08)62351-7.

Riddiford, L.M., Hiruma, K., Zhou, X., Nelson, C. a, 2003. Insights into the molecular basis of the hormonal control of molting and metamorphosis from Manduca sexta and Drosophila melanogaster. Insect Biochem. Mol. Biol. 33, 1327–1338. http://dx.doi.org/10.1016/j.ibmb.2003.06.001.

Russel, F.G.M., Koenderink, J.B., Masereeuw, R., 2008. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol. Sci. 29, 200–207. http://dx.doi.org/10.1016/j.tips.2008.01.006.

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., Touhara, K., 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452, 1002–1006. http://dx.doi.org/10.1038/nature06850.

Shields, V.D.C., Hildebrand, J.G., 2001. Responses of a population of antennal olfactory receptor cells in the female moth Manduca sexta to plant-associated volatile organic compounds. J. Comp. Physiol. A 186, 1135–1151. http://dx.doi.org/10.1007/s003590000165.

Singh, S.P., Coronella, J.A., Benes, H., Cochrane, B.J., Zimniak, P., 2001. Catalytic function of Drosophila melanogaster glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. Eur. J. Biochem. 268, 2912–2923. http://dx.doi.org/10.1046/j.1432-1327.2001.02179.x.

Späthe, A., Reinecke, A., Olsson, S.B., Kesavan, S., Knaden, M., Hansson, B.S., 2013. Plant species- and status-specific odorant blends guide oviposition choice in the moth Manduca sexta. Chem. Senses 38, 147–159. http://dx.doi.org/10.1093/chemse/bjs089.

Steppuhn, A., Baldwin, I.T., 2007. Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. Ecol. Lett. 10, 499–511. http://dx.doi.org/10.1111/j.1461-0248.2007.01045.x.

Sturm, A., Cunningham, P., Dean, M., 2009. The ABC transporter gene family of Daphnia pulex. BMC Genomics 10, 170. http://dx.doi.org/10.1186/1471-2164-10-170.

Sugumaran, M., 2002. Comparative biochemistry of Eumelanogenesis and the Protective roles of phenoloxidase and melanin in insects. Pigment. Cell. Res. 15, 2–9. http://dx.doi.org/10.1034/j.1600-0749.2002.00056.x.

Swarup, S., Morozova, T.V., Sridhar, S., Nokes, M., Anholt, R.R.H., 2014. Modulation of feeding behavior by odorant-binding proteins in Drosophila melanogaster. Chem. Senses 39, 125–132. http://dx.doi.org/10.1093/chemse/bjt061.

Swarup, S., Williams, T.I., Anholt, R.R.H., 2011. Functional dissection of Odorant binding protein genes in Drosophila melanogaster. Genes. Brain. Behav. 10, 648–657. http://dx.doi.org/10.1111/j.1601-183X.2011.00704.x.

Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R., Touhara, K., 2009. Highly selective tuning of a silkworm olfactory receptor to a Key Mulberry leaf volatile. Curr. Biol. 19, 881–890. http://dx.doi.org/10.1016/j.cub.2009.04.035.

Terra, W.R., 2001. The origin and functions of the insect peritrophic membrane and peritrophic gel. Arch. Insect Biochem. Physiol. 47, 47–61. http://dx.doi.org/10.1002/arch.1036.

Theodoulou, F.L., Holdsworth, M., Baker, A., 2006. Peroxisomal ABC transporters. FEBS Lett. 580, 1139–1155. http://dx.doi.org/10.1016/j.febslet.2005.12.095.

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., Boccard, F., Lemaitre, B., 2005. Drosophila host defense after oral infection by an entomopathogenic Pseudomonas species. Proc. Natl. Acad. Sci. U. S. A. 102, 11414–11419. http://dx.doi.org/10.1073/pnas.0502240102.

Voelckel, C., Krügel, T., Gase, K., Heidrich, N., van Dam, N.M., Winz, R., Baldwin, I.T., 2001. Anti-sense expression of putrescine N-methyltransferase confirms defensive role of nicotine in Nicotiana sylvestris against Manduca sexta. Chemoecology 11, 121–126. http://dx.doi.org/10.1007/PL00001841.

Vogel, H., Badapanda, C., Knorr, E., Vilcinskas, A., 2014a. RNA-sequencing analysis reveals abundant developmental stage-specific and immunity-related genes in the pollen beetle Meligethes aeneus. Insect Mol. Biol. 23, 98–112. http://dx.doi.org/10.1111/imb.12067.

Vogel, H., Musser, R.O., Celorio-Mancera, Paz, de la, M., 2014b. Transcriptome responses in herbivorous insects towards host plant and toxin feeding. In: Annual Plant Reviews. John Wiley & Sons, Ltd, pp. 197–233. http://dx.doi.org/10.1002/9781118829783.ch6.

Vosshall, L.B., Hansson, B.S., 2011. A Unified Nomenclature system for the insect olfactory coreceptor. Chem. Senses 36, 497–498. http://dx.doi.org/10.1093/chemse/bjr022.

Vosshall, L.B., Wong, A.M., Axel, R., 2000. An olfactory sensory map in the Fly Brain. Cell 102, 147–159. http://dx.doi.org/10.1016/S0092-8674(00)00021-0.

Waldbauer, G.P., Fraenkel, G., 1961. Feeding on normally Rejected plants by Maxillectomized larvae of the tobacco hornworm, Protoparce sexta (Lepidoptera, Sphingidae). Ann. Entomol. Soc. Am. 54, 477–485.

Wang, J.-S., Markowitz, J.S., Donovan, J.L., Devane, C.L., 2005. P-glycoprotein does not actively transport nicotine and cotinine. Addict. Biol. 10, 127–129. http://dx.doi.org/10.1080/13556210500122995.

Wang, Q., Hasan, G., Pikielny, C.W., 1999. Preferential expression of biotransformation enzymes in the olfactory organs of Drosophila melanogaster, the antennae. J. Biol. Chem. 274, 10309–10315. http://dx.doi.org/10.1074/jbc.274.15.10309.

Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., Hansson, B.S., 2008. Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452, 1007–1011. http://dx.doi.org/10.1038/nature06861.

Winde, I., Wittstock, U., 2011. Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. Phytochemistry. http://dx.doi.org/10.1016/j.phytochem.2011.01.016.

Yamamoto, R.T., Fraenkel, G.S., 1960. The specificity of the tobacco hornworm, *Protoparce sexta*, to solanaceous plants. Ann. Entomol. Soc. Am. 53, 503–517.

Yamamoto, K., Ichinose, H., Aso, Y., Fujii, H., 2010. Expression analysis of cytochrome P450s in the silkmoth, Bombyx mori. Pestic. Biochem. Physiol. 97, 1–6. http://dx.doi.org/10.1016/j.pestbp.2009.11.006.

Yamamoto, R.T., Jenkins, R.Y., McClusky, R.K., 1969. Factors determining the selection of plants for oviposition by the Tobacco hornworm Manduca sexta. Entomol. Exp. Appl. 12, 504–508. http://dx.doi.org/10.1111/j.1570-7458.1969.tb02548.x.

Yeh, K.-W., Lin, M.-I., Tuan, S.-J., Chen, Y.-M., Lin, C.-J., Kao, S.-S., 1997. Sweet potato (Ipomoea batatas) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against Spodoptera litura. Plant Cell. Rep. 16, 696–699. http://dx.doi.org/10.1007/s002990050304.

Zelcer, N., Saeki, T., Reid, G., Beijnen, J.H., Borst, P., 2001. Characterization of drug transport by the human multidrug resistance protein 3 (ABCC3). J. Biol. Chem. 276, 46400–46407. http://dx.doi.org/10.1074/jbc.M107041200.

Zhan, S., Merlin, C., Boore, J.L., Reppert, S.M., 2011. The monarch butterfly genome yields insights into long-distance migration. Cell 147, 1171–1185. http://dx.doi.org/10.1016/j.cell.2011.09.052.

Zhong, X., Xu, X.X., Yi, H.Y., Lin, C., Yu, X.Q., 2012. A Toll-Spätzle pathway in the tobacco hornworm, *Manduca sexta*. Insect Biochem. Mol. Biol. 42, 514–524.

Supplementary data:

The plastic response of *Manduca sexta* to host and non-host plants

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Supplementary Table Legend:

Table S1: Gene names mentioned in text and figures and respective *Manduca sexta* OGS2 gene IDs.

Supplementary Figure Legends:

Figure S1: Larval development on each host or non-host plant for five consecutive time points (T1-T5). P-value: n.s. > 0.05, * < 0.05, ** < 0.01, *** < 0.001 (ANOVA); N = 20 / plant or diet, respectively.

Figure S2: Differential GO term representation between larvae fed on control diet or plant material. Differences are shown as the percentage of sequences associated with a specific GO category in the reference set (*Manduca sexta* OGS2) against the test set (number of differentially expressed genes with a fold change of at least 8 between control and treatment) using Fisher's exact test (Blast2GO) **A)** *Brassica napus* **B)** *Datura wrightii.*

Figure S3: Differential GO term representation between larvae fed on control diet or plant material. Differences are shown as the percentage of sequences associated with a specific GO category in the reference set (*Manduca sexta* OGS2) against the test set (number of differentially expressed genes with a fold change of at least 8 between control and treatment) using Fisher's exact test (Blast2GO) **A)** *Solanum lycopersicum* **B)** *Nicotiana attenuata.*

Figure S4: Expression of genes belonging to the glutathione S-transferase (GST) family. Expression levels are dependent on the food source and the tissue type. Gene expression levels in the different tissues are based on log2-transformed RPKM values and responses are expressed relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: gut with Malpighian tubules; SG: silk gland (labial gland); W: whole insect

Figure S5: Expression patterns of UDP-glycosyltransferase (UGT) genes. Gene expression levels in the different tissues are based on log2-transformed RPKM values and responses are expressed relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: guts with Malpighian tubules; SG: silk gland (labial gland); W: whole insect

Figure S6: Differential expression of Cytochromes P450 in *Manduca* larvae feeding on different plants. Values are based on log2-transformed RPKM values relative to the median intensity of all contigs (blue = down-regulation; red = up-regulation). AM: antennae and maxillae; G: guts with Malpighian tubules; SG: silk gland (labial gland); W: whole insect

Gene class	Msex OGS2 number	Gene name
GST	Msex2.00305	MAPEGZ1
	Msex2.00306	MAPEGZ2
	Msex2.00537	Delta-OLF1
	Msex2.00538	Delta-OLF2
	Msex2.00809	Delta5
	Msex2.01219	Zeta3
	Msex2.02313	Theta1
	Msex2.02374	Omega3
	Msex2.02375	Omega2
	Msex2.02376	Omega1
	Msex2.02751	Epsilon5a
	Msex2.02752	Epsilon5b
	Msex2.02753	Epsilon6b
	Msex2.03348	Sigma1
	Msex2.03349	Sigma2
	Msex2.03350	Sigma3
	Msex2.03351	Sigma4b
	Msex2.03352	Sigma5b
	Msex2.03353	Sigma6
	Msex2.03355	Sigma8b
	Msex2.04941	Epsilon3
	Msex2.05056	Epsilon2
	Msex2.08395	Epsilon8
	Msex2.08396	Epsilon4
	Msex2.08503	Delta4
	Msex2.08504	Delta3
	Msex2.09785	MAPEG1
	Msex2.09786	MAPEG2
	Msex2.09787	MAPEG3
	Msex2.11063	Omega4
	Msex2.11554	Zeta2
	Msex2.11763	Zeta1
GST	Msex2.11862	Delta1
	Msex2.11863	Delta2
	Msex2.15508	Epsilon6a
	Msex2.15509	Epsilon7
	Msex2.15513	Sigma4a
	Msex2.15514	Sigma5a
	Msex2.15515	Sigma8a
	Msex2.15517	Epsilon1
	Msex2.11862	Delta1
	Msex2.11863	Delta2
	Msex2.15508	Epsilon6a
	Msex2.15509	Epsilon7
	Msex2.15513	Sigma4a
	Msex2.15514	Sigma5a
	Msex2.15515	Sigma8a
	Msex2.15517	Epsilon1
ABC Transporter	Msex2.01019	white
	Msex2.01020	scarlet
	Msex2.01581	A5
	Msex2.02789	G
	Msex2.02920	G
	Msex2.02921	G
	Msex2.02959	G3
	Msex2.03566	A7
ABC Transporter	Msex2.03567	A
	Msex2.03568	A
	Msex2.04170	A
	Msex2.04213	D1
	Msex2.04981	C9
	Msex2.05212	D1
	Msex2.05814	H
	Msex2.05815	H
	Msex2.06707	C3
	Msex2.06970	F2
	Msex2.07270	B5
	Msex2.07664	C11
	Msex2.07869	C10
	Msex2.08166	B4
	Msex2.08194	C8
	Msex2.08825	B6
	Msex2.09106	B2
	Msex2.09107	B3
	Msex2.09200	H
	Msex2.09295	E1
	Msex2.09773	B1
	Msex2.09797	C6
	Msex2.09851	G
	Msex2.10064	B8
	Msex2.10395	F1
	Msex2.10847	G5
	Msex2.11130	C5
	Msex2.11196	G1
	Msex2.11687	D2
	Msex2.11695	G
	Msex2.11947	B7
	Msex2.12147	A3

Gene class	Msex OGS2 number	Gene name
ABC Transporter	Msex2.12351	A
	Msex2.12541	G
	Msex2.12542	G4
	Msex2.12678	C1
	Msex2.12679	C2
	Msex2.12701	G
	Msex2.12844	B9
	Msex2.13212	G8
	Msex2.13266	brown
	Msex2.13803	G2
	Msex2.13929	F3
	Msex2.15061	C7
	Msex2.15073	D2
	Msex2.15134	C4
UGT	Msex2.00532	UGT42A3
	Msex2.00533	UGT42B3
	Msex2.00534	UGT42A4
	Msex2.01742	UGT33G1
	Msex2.01842	UGT39B3
	Msex2.02747	
	Msex2.02748	UGT40A2
	Msex2.04831	UGT46A5
	Msex2.05185	UGT44A3
	Msex2.05263	UGT34A4
	Msex2.06759	UGT45A1
	Msex2.08124	UGT46C1
	Msex2.09140	UGT48B1
	Msex2.09875	UGT40E1
	Msex2.09876	UGT40J1p
	Msex2.09877	UGT40H2
	Msex2.09878	UGT40C1
	Msex2.09879	UGT40C3
UGT	Msex2.09880	UGT40L2
	Msex2.10200	UGT33L1
	Msex2.10777	UGT41C1
	Msex2.11004	UGT47A3
	Msex2.11077	UGT33H2
	Msex2.11078	UGT33H1
	Msex2.11079	UGT33E1
	Msex2.11080	UGT340B1
	Msex2.11081	UGT340A3
	Msex2.11082	UGT340A4
	Msex2.12063	UGT50A3
	Msex2.12523	UGT33E3
	Msex2.12524	UGT33C
	Msex2.12525	UGT33P1
	Msex2.12598	UGT40A2
	Msex2.13845	UGT340A1
	Msex2.13846	
	Msex2.13847	UGT340A2
	Msex2.13848	UGT340A5p
	Msex2.14488	UGT50A3
	Msex2.15377	UGT50A3
	Msex2.15495	UGT33G2
	Msex2.15496	UGT33G3
	Msex2.15497	UGT40E2
	Msex2.15498	UGT33E2
	Msex2.15529	UGT40C2
	Msex2.15545	
P450	missing	CYP6B46
	Msex2.00093	CYP428A
	Msex2.01198	CYP339A
	Msex2.01282	CYP6A
	Msex2.01466	CYP337B
P450	Msex2.01467	CYP337A
	Msex2.01638	CYP4AU
	Msex2.01639	CYP4AU
	Msex2.01872	CYP333A3
	Msex2.01884	CYP354A5
	Msex2.02491	CYP6B45
	Msex2.02492	CYP6B
	Msex2.02933	CYP4CG
	Msex2.02935	CYP4CG
	Msex2.02936	CYP4CG1
	Msex2.03303	CYP332A
	Msex2.03304	CYP332A
	Msex2.03305	CYP332A
	Msex2.03681	CYP303A
	Msex2.04085	CYP321A
	Msex2.04412	CYP302A
	Msex2.04540	CYP4G4
	Msex2.04949	CYP4C
	Msex2.04950	CYP340A
	Msex2.04951	CYP340A
	Msex2.04952	CYP4C
	Msex2.04955	CYP4C
	Msex2.05089	CYP367B
	Msex2.05090	CYP4C
	Msex2.05506	CYP18A
	Msex2.05508	CYP18A
	Msex2.05509	CYP306A
	Msex2.05640	CYP4G
	Msex2.05641	CYP4G49
	Msex2.05642	CYP4G
	Msex2.05754	CYP338A
	Msex2.05854	CYP49A

Gene class	Msex OGS2 number	Gene name	Gene class	Msex OGS2 number	Gene name	Gene class	Msex OGS2 number	Gene name
P450	Msex2.06102	CYP341A	P450	Msex2.10890	CYP333B	P450	Msex2.13589	CYP4C
	Msex2.06392	CYP6AE		Msex2.10891	CYP333B		Msex2.13751	CYP6BD
	Msex2.06452	CYP4S		Msex2.10893	CYP333B		Msex2.13820	CYP341B
	Msex2.06453	CYP4S		Msex2.10894	CYP333B11		Msex2.13906	CYP6AE
	Msex2.06640	CYP315A		Msex2.10895	CYP333B		Msex2.14281	CYP341B
	Msex2.06944	CYP6AU		Msex2.10896	CYP333B		Msex2.14397	CYP6BD
	Msex2.06945	CYP6B		Msex2.11047	CYP6AE		Msex2.14567	CYP6BD
	Msex2.07258	CYP49A		Msex2.11048	CYP6AE		Msex2.14593	CYP340C
	Msex2.07259	CYP301A		Msex2.11105	CYP9G		Msex2.14600	CYP340C
	Msex2.07399	CYP305B		Msex2.11106	CYP9G		Msex2.14709	CYP6BD
	Msex2.07561	CYP324A		Msex2.11107	CYP9G		Msex2.14778	CYP6AE
	Msex2.07562	CYP324A		Msex2.11402	CYP307A		Msex2.15113	CYP307A
	Msex2.07563	CYP324A		Msex2.11700	CYP6AB13		Msex2.15162	CYP333B
	Msex2.08007	CYP304F		Msex2.11774	CYP6AN		Msex2.15204	CYP366D
	Msex2.08108	CYP6CT		Msex2.11778	CYP9A		Msex2.15240	CYP6BD
	Msex2.08297	CYP314A		Msex2.11779	CYP9A		Msex2.15476	CYP6BD
	Msex2.08561	CYP6AB		Msex2.11790	CYP9A	OBP	Msex2.00460	MsexOBP09
	Msex2.08562	CYP6AB		Msex2.11791	CYP9A		Msex2.00461	MsexOBP10
	Msex2.09027	CYP6AX		Msex2.11907	CYP341B		Msex2.00462	MsexABP1
	Msex2.09686	CYP9AJ		Msex2.11908	CYP341B		Msex2.00463	MsexOBP14
	Msex2.09894	CYP337A		Msex2.11909	CYP341B		Msex2.00464	MsexOBP11
	Msex2.09933	CYP341B		Msex2.11957	CYP6AN		Msex2.00465	MSexABP2
	Msex2.09934	CYP4C		Msex2.12312	CYP366D		Msex2.00466	MsexOBP12
	Msex2.09935	CYP341B		Msex2.13217	CYP6AE32		Msex2.00467	MsexABP4
	Msex2.10033	CYP4M1		Msex2.13219	CYP6AE		Msex2.00468	MsexOBP13
	Msex2.10034	CYP4M		Msex2.13220	CYP6AE		Msex2.01454	MsexOBP15
	Msex2.10035	CYP4M2		Msex2.13221	CYP6AE		Msex2.01707	MsexOBP16
	Msex2.10036	CYP4M		Msex2.13294	CYP6AN5		Msex2.01708	MsexABP8
	Msex2.10215	CYP6AE31		Msex2.13295	CYP6AN		Msex2.02108	MsexABPx
	Msex2.10327	CYP15A		Msex2.13515	CYP4C		Msex2.02300	MsexOBP18
	Msex2.10760	CYP9A		Msex2.13552	CYP4L		Msex2.02656	MsexOBP20
	Msex2.10889	CYP333B		Msex2.13553	CYP333B		Msex2.02657	MsexOBP19

Gene class	Msex OGS2 number	Gene name	Gene class	Msex OGS2 number	Gene name	Gene class	Msex OGS2 number	Gene name
OBP	Msex2.02658	MsexOBP38	OBP	Msex2.15032	MsexOBP37	OR	Msex2.07161	MsexOR-86
	Msex2.02698	MsexOBP21	OR	missing	MsexOR-10		Msex2.07660	MsexOR-07
	Msex2.03248	MsexOBP22		missing	MsexOR-15		Msex2.07682	MsexOR-06
	Msex2.03518	MsexOBP23		Msex2.00565	MsexOR-82		Msex2.07686	MsexOR-05
	Msex2.03519	MsexOBP24		Msex2.00624	MsexOR-38		Msex2.07920	MsexOR-75
	Msex2.03888	MsexOBP07		Msex2.00707	MsexOR-24		Msex2.07921	MsexOR-23
	Msex2.03889	MsexABP6		Msex2.01521	MsexOR-36		Msex2.07922	MsexOR-41
	Msex2.03890	MsexOBP17		Msex2.01522	MsexOR-08		Msex2.08006	MsexOR-22
	Msex2.03891	MsexOBP33		Msex2.01523	MsexOR-33		Msex2.08017	MsexOR-25
	Msex2.03892	MsexABP7		Msex2.01524	MsexOR-80		Msex2.08018	MsexOR-46
	Msex2.03893	MsexOBP32		Msex2.01525	MsexOR-84		Msex2.08303	MsexOR-64
	Msex2.04082	MsexOBP26		Msex2.01571	MsexOR-42		Msex2.08305	MsexOR-77
	Msex2.05759	MsexOBP06		Msex2.01618	MsexOR-66		Msex2.08399	MsexOR-19
	Msex2.05760	MsexOBP03		Msex2.02252	MsexOR-67		Msex2.08680	MsexOR-34
	Msex2.05761	MsexOBP01		Msex2.02512	MsexOR-27		Msex2.08681	MsexOR-16
	Msex2.05762	MsexOBP04		Msex2.02514	MsexOR-29		Msex2.08682	MsexOR-78
	Msex2.05763	MsexOBP02		Msex2.02515	MsexOR-68		Msex2.09025	MsexOR-74
	Msex2.07317	MsexOBP25		Msex2.02754	MsexOR-69		Msex2.09038	MsexOR-87
	Msex2.07430	MsexOBP05		Msex2.02755	MsexOR-43		Msex2.09279	MsexOR-26
	Msex2.07487	MsexABP5		Msex2.03330	MsexOR-12		Msex2.09281	MsexOR-65
	Msex2.08382	MsexOBP27		Msex2.04326	MsexOR-62		Msex2.09282	MsexOR-09
	Msex2.09425	MsexABP3		Msex2.04330	MsexOR-52		Msex2.09918	MsexOR-32
	Msex2.10958	MsexOBP28		Msex2.04331	MsexOR-47		Msex2.09919	MsexOR-31
	Msex2.11541	MsexOBP29		Msex2.04542	MsexOR-70		Msex2.09996	MsexOR-57
	Msex2.12548	MsexOBP39		Msex2.04689	MsexOR-71		Msex2.09997	MsexOR-39
	Msex2.13730	MsexOBP30		Msex2.04798	MsexOR-01		Msex2.10957	MsexOR-51
	Msex2.13843	MsexOBP08		Msex2.04835	MsexOR-83		Msex2.11073	MsexOR-30
	Msex2.14143	MsexOBP31		Msex2.05573	MsexOR-17		Msex2.11103	MsexOR-35
	Msex2.14192	MsexOBP34		Msex2.05711	MsexOR-13		Msex2.11504	MsexOR-11
	Msex2.14323	MsexOBP35		Msex2.05788	MsexOR-40		Msex2.11915	MsexOR-21
	Msex2.14420	MsexOBP40		Msex2.06100	MsexOR-72		Msex2.11916	MsexOR-20
	Msex2.14586	MsexOBP36		Msex2.06834	MsexOR-73		Msex2.12027	MsexOR-76

Gene class	Msex OGS2 number	Gene name
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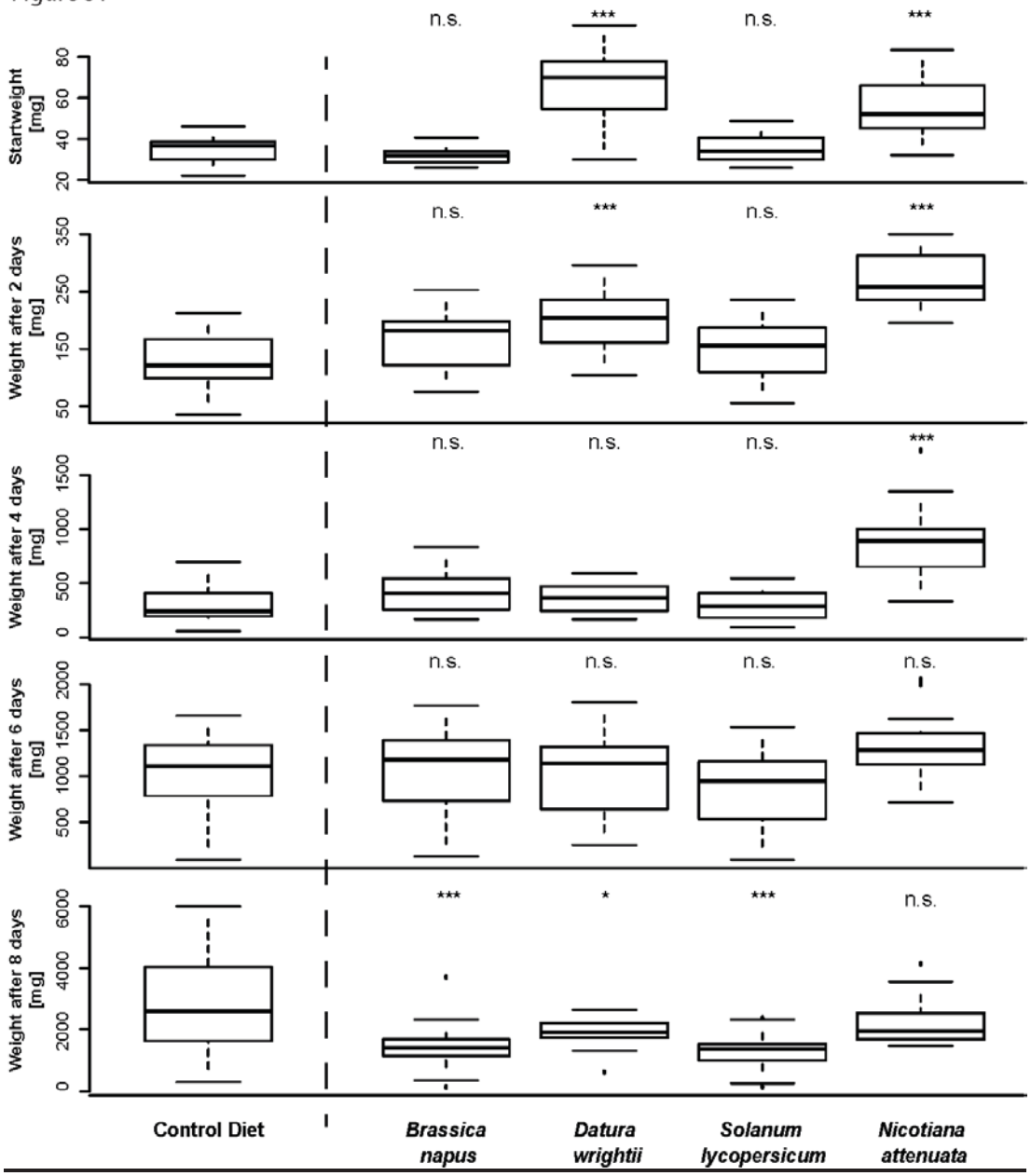
	Msex2.12521	MsexOR-50
	Msex2.12779	MsexORCO
	Msex2.12902	MsexOR-88
	Msex2.13403	MsexOR-04
	Msex2.14357	MsexOR-03
	Msex2.14612	MsexOR-89
	Msex2.14943	MsexOR-18
	Msex2.14948	MsexOR-79
	Msex2.15272	MsexOR-49
n. spec.	Msex2.12103	5-TOX
AMP	Msex2.01301	Lebocin-A
	Msex2.01302	Lebocin-B
	Msex2.01303	Lebocin-D
	Msex2.01305	Lebocin-C
	Msex2.02244	Defensin2
	Msex2.04820	Lysozyme-2
	Msex2.04821	Lysozyme-like protein
	Msex2.04822	Lysozyme-1
	Msex2.04906	WAP01
	Msex2.04907	WAP02
	Msex2.05149	WAP03
	Msex2.05150	WAP04
	Msex2.05151	WAP08
	Msex2.05152	WAP05
	Msex2.05153	WAP06
	Msex2.05154	WAP07
	Msex2.05155	WAP09
	Msex2.05156	WAP10
	Msex2.05157	WAP11
	Msex2.05158	WAP12
	Msex2.05159	WAP13
AMP	Msex2.05160	WAP14
	Msex2.05161	WAP15
	Msex2.05594	Moricin like peptide
	Msex2.05595	Moricin 1
	Msex2.05596	Moricin 2
	Msex2.05597	Moricin like peptide
	Msex2.05598	Moricin like peptide
	Msex2.05599	Moricin like peptide
	Msex2.06539	Defensin1
	Msex2.08540	Attacin06
	Msex2.08541	Attacin02
	Msex2.08542	Attacin03
	Msex2.08543	Attacin10
	Msex2.08544	Attacin01
	Msex2.08545	Attacin04
	Msex2.08785	Cecropin07
	Msex2.08787	Cecropin09
	Msex2.08788	Cecropin10
	Msex2.09992	Lysozyme-like protein
	Msex2.09994	Lysozyme-3
	Msex2.09998	Lysozyme-like
	Msex2.11074	Gloverin
	Msex2.12105	Gallerimycin 2
	Msex2.12106	Gallerimycin 3
	Msex2.12108	Gallerimycin 1
	Msex2.13057	AFP13
	Msex2.13789	Cecropin01
	Msex2.13830	AFP03
	Msex2.13831	AFP02
	Msex2.13832	AFP04
	Msex2.13833	AFP05
	Msex2.13834	AFP06

Gene class	Msex OGS2 number	Gene name
AMP	Msex2.13835	AFP07
	Msex2.13836	AFP08
	Msex2.13837	AFP09
	Msex2.13839	AFP11
	Msex2.13907	Cecropin02
	Msex2.13910	Cecropin05
	Msex2.13912	Cecropin06
	Msex2.13920	Attacin05
	Msex2.13921	Attacin09
	Msex2.14460	Cecropin13
	Msex2.14461	Cecropin14
	Msex2.14466	Cecropin12
	Msex2.14467	Cecropin11
	Msex2.14877	AFP14
	Msex2.15011	AFP01
	Msex2.15057	AFP12
	Msex2.15307	AFP10
	Msex2.15523	Cecropin08
	Msex2.15547	Cecropin03
	Msex2.15548	Cecropin04
Effector genes	Msex2.09885	PPO
	Msex2.11367	PPO
IMD	Msex2.01596	IAP1 similar
	Msex2.01715	IBM1 (IAP antagonist)
	Msex2.02602	TAK1
	Msex2.05476	death domain protein
	Msex2.05545	TAB
	Msex2.05604	IAP2
	Msex2.05607	IAP1
	Msex2.06959	Dnr1
JAKSTAT	Msex2.07815	Domeless
	Msex2.08880	hopscotch
JAKSTAT	Msex2.10552	STAT
	Msex2.11519	SOCS
	Msex2.12153	STAT
JNK	Msex2.01399	Hemolin-LP2
	Msex2.02705	Hem
	Msex2.05111	Jun- / JNK
	Msex2.09018	Hemolin-LP1
	Msex2.09332	Hem
	Msex2.09807	Hemolin
	Msex2.09858	Fos
MAPK	Msex2.04292	ERK
	Msex2.04304	MAPK
	Msex2.07041	GTPase-activating protein
	Msex2.07813	MKK3
	Msex2.08319	MAPK2
	Msex2.08712	MAPKKK
	Msex2.09629	MAPK
	Msex2.10253	MEKK1
MBP	Msex2.06179	MBP
Microbial recognition	Msex2.07575	Nimrod
	Msex2.07576	Nimrod-B
	Msex2.07580	Nimrod-LI
	Msex2.12427	TEP1
	Msex2.13097	TEP2
PORC	Msex2.12453	PORC
PRR	Msex2.03605	PGRP-03
	Msex2.03606	PGRP-04
	Msex2.03607	PGRP-02
	Msex2.09015	PGRP-08
	Msex2.09398	PGRP-11
	Msex2.10091	PGRP-09
	Msex2.10092	PGRP-01

Gene class	Msex OGS2 number	Gene name
PRR	Msex2.10093	PGRP-13
	Msex2.10096	PGRP-06
	Msex2.10097	PGRP-07
	Msex2.11527	PGRP-12
	Msex2.11767	PGRP-11
	Msex2.13760	BGRP1
	Msex2.13829	BGRP3
	Msex2.14130	BGRP2
	Msex2.14131	BGRP
	Msex2.15530	PGRP-05
Toll	Msex2.02793	cactus
	Msex2.02909	Spz1
	Msex2.03391	Spz4
	Msex2.04007	Toll-like
	Msex2.04433	Spz3
	Msex2.07379	Spz6
	Msex2.08663	Tube
	Msex2.09986	Spz2
	Msex2.11498	dif
	Msex2.11500	Dorsal
	Msex2.11537	Toll interacting protein
	Msex2.12529	Spz5
	Msex2.13883	Toll-like
	Msex2.14106	Pelle
Binding and removal of free ions	Msex2.02637	Transferrin 1
	Msex2.10790	Transferrin2, partial
	Msex2.10792	Transferrin 2
	Msex2.10793	Transferrin2, partial
	Msex2.12754	Transferrin 3

Figure S1


Startweight [mg]
Weight after 2 days [mg]
Weight after 4 days [mg]
Weight after 6 days [mg]
Weight after 8 days [mg]
n.s.
***
*
Control Diet
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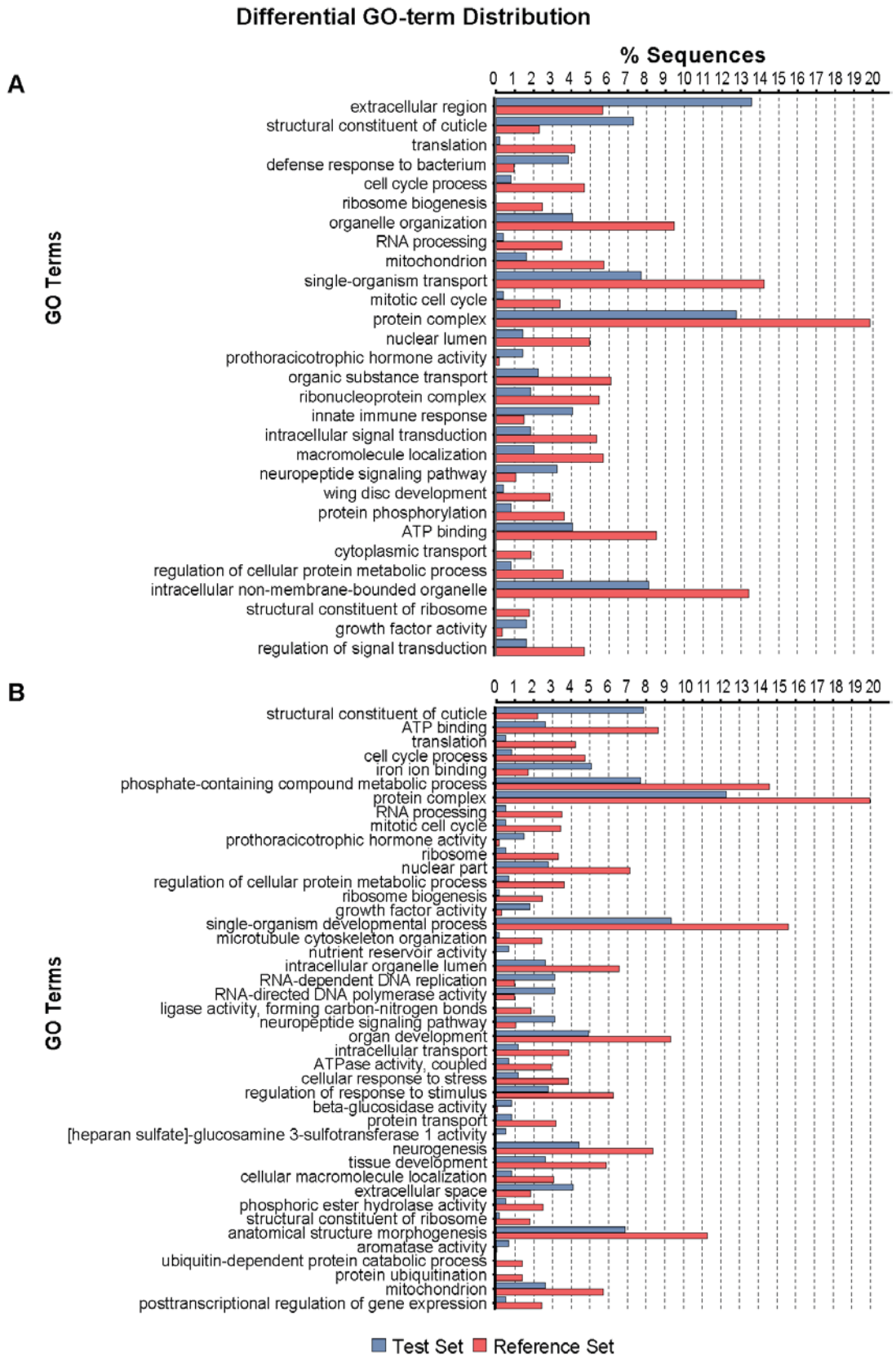
Datura wrightii
Solanum lycopersicum
Nicotiana attenuata

Figure S2


Differential GO-term Distribution
A
% Sequences
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GO Terms
extracellular region
structural constituent of cuticle
translation
defense response to bacterium
cell cycle process
ribosome biogenesis
organelle organization
RNA processing
mitochondrion
single-organism transport
mitotic cell cycle
protein complex
nuclear lumen
prothoracicotrophic hormone activity
organic substance transport
ribonucleoprotein complex
innate immune response
intracellular signal transduction
macromolecule localization
neuropeptide signaling pathway
wing disc development
protein phosphorylation
ATP binding
cytoplasmic transport
regulation of cellular protein metabolic process
intracellular non-membrane-bounded organelle
structural constituent of ribosome
growth factor activity
regulation of signal transduction
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
GO Terms
structural constituent of cuticle
ATP binding
translation
cell cycle process
iron ion binding
phosphate-containing compound metabolic process
protein complex
RNA processing
mitotic cell cycle
prothoracicotrophic hormone activity
ribosome
nuclear part
regulation of cellular protein metabolic process
ribosome biogenesis
growth factor activity
single-organism developmental process
microtubule cytoskeleton organization
nutrient reservoir activity
intracellular organelle lumen
RNA-dependent DNA replication
RNA-directed DNA polymerase activity
ligase activity, forming carbon-nitrogen bonds
neuropeptide signaling pathway
organ development
intracellular transport
ATPase activity, coupled
cellular response to stress
regulation of response to stimulus
beta-glucosidase activity
protein transport
[heparan sulfate]-glucosamine 3-sulfotransferase 1 activity
neurogenesis
tissue development
cellular macromolecule localization
extracellular space
phosphoric ester hydrolase activity
structural constituent of ribosome
anatomical structure morphogenesis
aromatase activity
ubiquitin-dependent protein catabolic process
protein ubiquitination
mitochondrion
posttranscriptional regulation of gene expression
Test Set
Reference Set

Figure S3

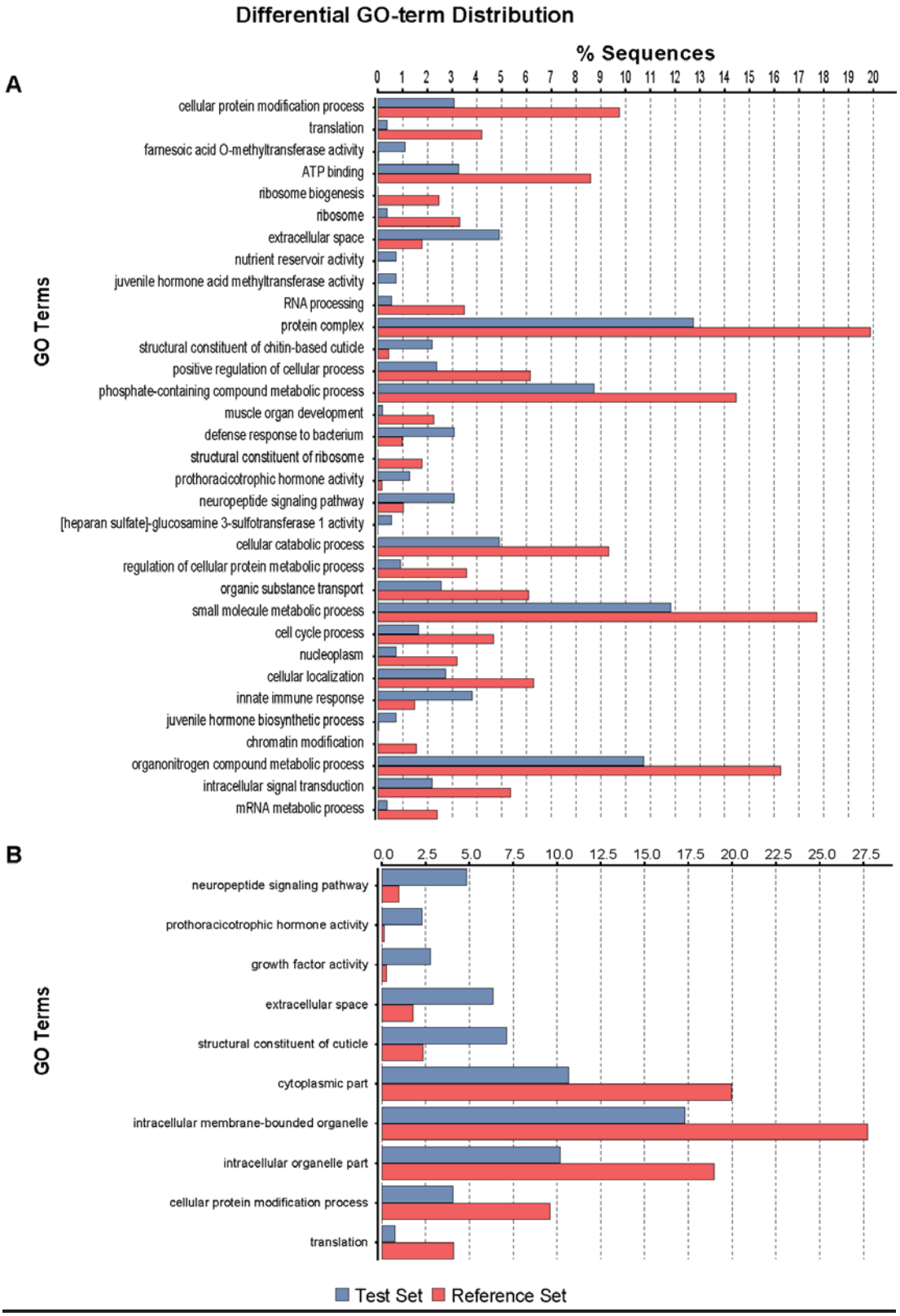
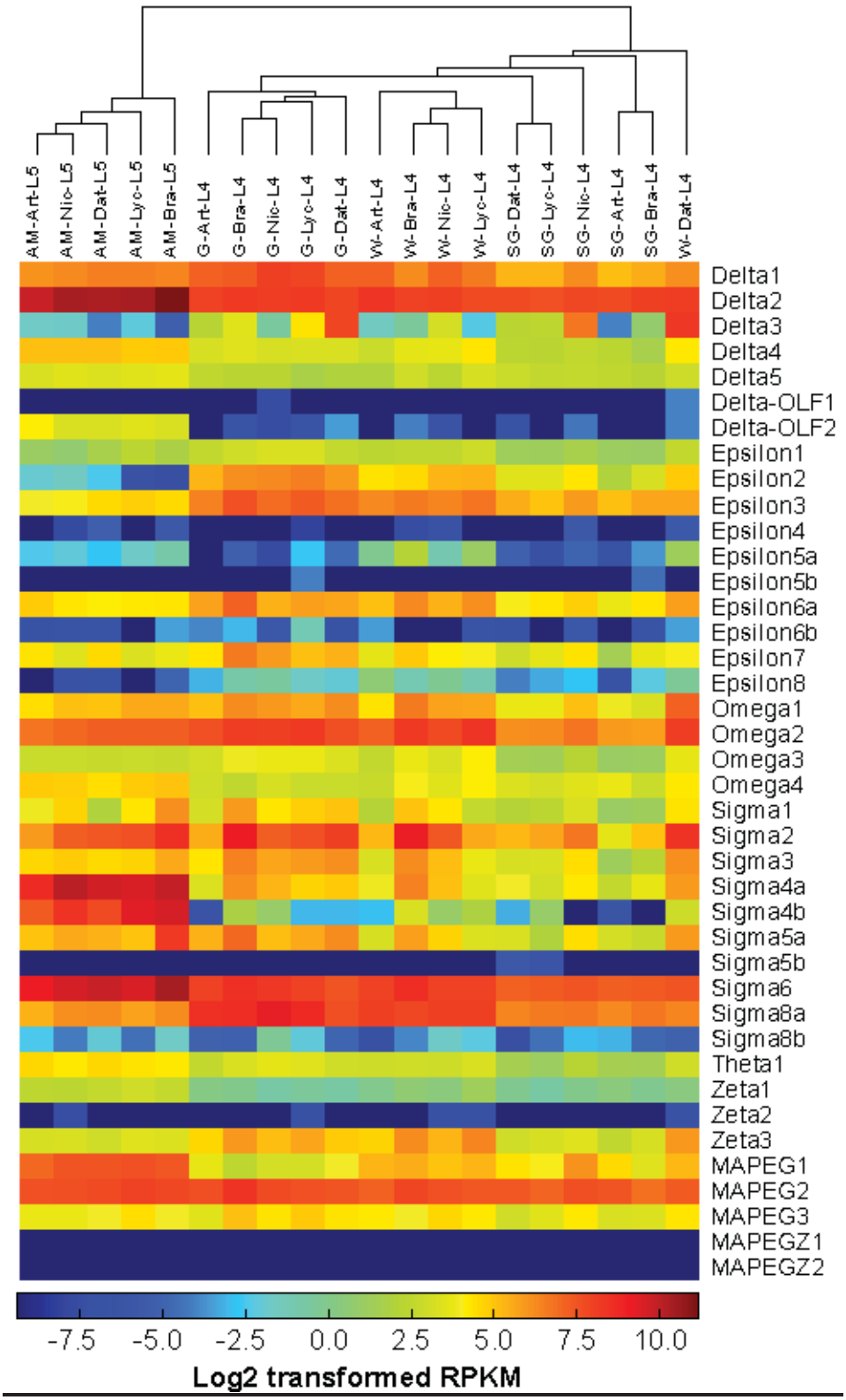

Differential GO-term Distribution
A
% Sequences
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
GO Terms
cellular protein modification process
translation
farnesoic acid O-methyltransferase activity
ATP binding
ribosome biogenesis
ribosome
extracellular space
nutrient reservoir activity
juvenile hormone acid methyltransferase activity
RNA processing
protein complex
structural constituent of chitin-based cuticle
positive regulation of cellular process
phosphate-containing compound metabolic process
muscle organ development
defense response to bacterium
structural constituent of ribosome
prothoracicotrophic hormone activity
neuropeptide signaling pathway
[heparan sulfate]-glucosamine 3-sulfotransferase 1 activity
cellular catabolic process
regulation of cellular protein metabolic process
organic substance transport
small molecule metabolic process
cell cycle process
nucleoplasm
cellular localization
innate immune response
juvenile hormone biosynthetic process
chromatin modification
organonitrogen compound metabolic process
intracellular signal transduction
mRNA metabolic process
B
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5
GO Terms
neuropeptide signaling pathway
prothoracicotrophic hormone activity
growth factor activity
extracellular space
structural constituent of cuticle
cytoplasmic part
intracellular membrane-bounded organelle
intracellular organelle part
cellular protein modification process
translation
Test Set
Reference Set

Figure S4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AM-Nic-L5
AM-Dat-L5
AM-Lyc-L5
AM-Bra-L5
G-Art-L4
G-Bra-L4
G-Nic-L4
G-Lyc-L4
G-Dat-L4
W-Art-L4
W-Bra-L4
W-Nic-L4
W-Lyc-L4
SG-Dat-L4
SG-Lyc-L4
SG-Nic-L4
SG-Art-L4
SG-Bra-L4
W-Dat-L4
Delta1
Delta2
Delta3
Delta4
Delta5
Delta-OLF1
Delta-OLF2
Epsilon1
Epsilon2
Epsilon3
Epsilon4
Epsilon5a
Epsilon5b
Epsilon6a
Epsilon6b
Epsilon7
Epsilon8
Omega1
Omega2
Omega3
Omega4
Sigma1
Sigma2
Sigma3
Sigma4a
Sigma4b
Sigma5a
Sigma5b
Sigma6
Sigma8a
Sigma8b
Theta1
Zeta1
Zeta2
Zeta3
MAPEG1
MAPEG2
MAPEG3
MAPEGZ1
MAPEGZ2
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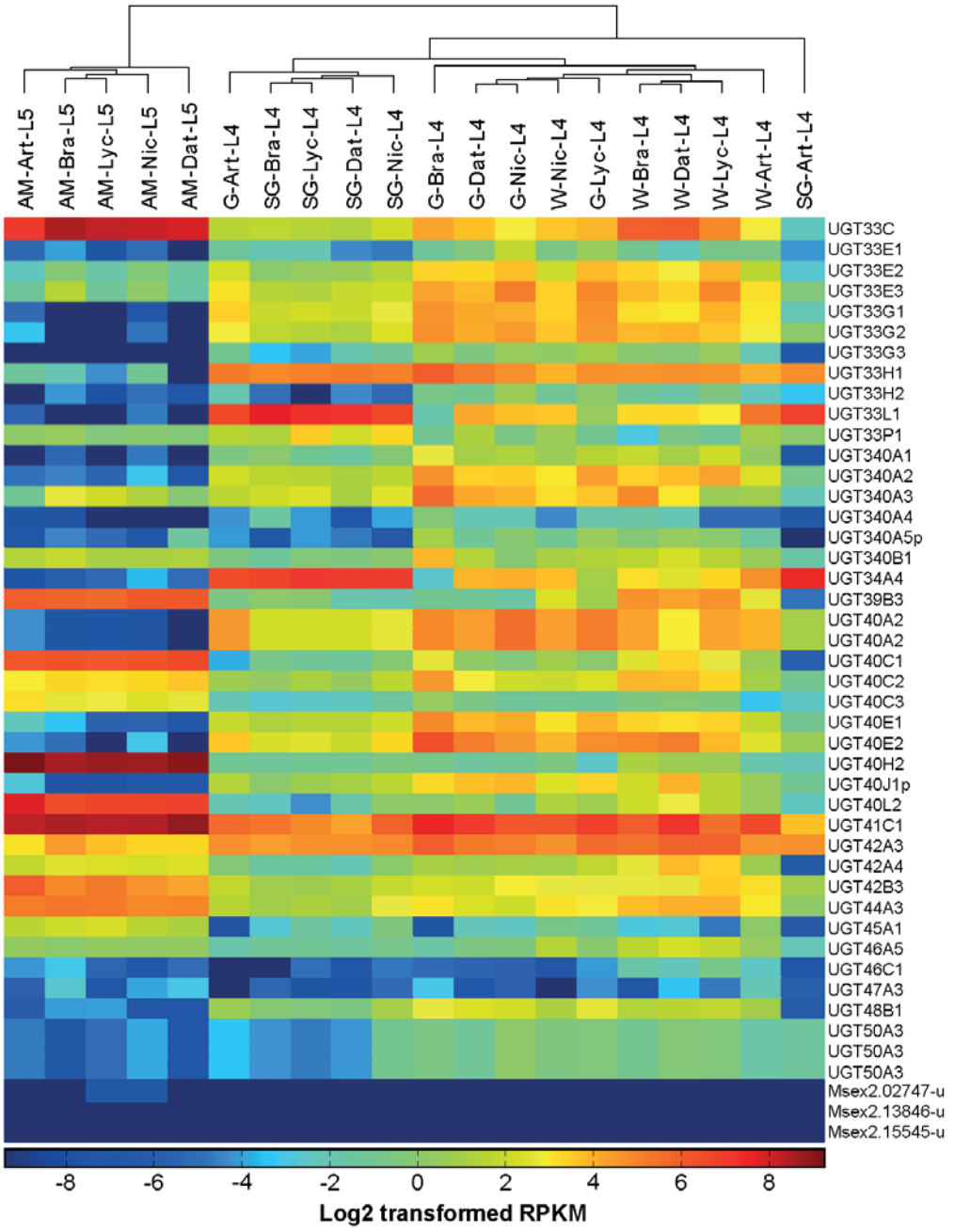
-5.0
-2.5
0.0
2.5
5.0
7.5
10.0
Log2 transformed RPKM

Figure S5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AM-Bra-L5
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AM-Nic-L5
AM-Dat-L5
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SG-Dat-L4
SG-Nic-L4
G-Bra-L4
G-Dat-L4
G-Nic-L4
W-Nic-L4
G-Lyc-L4
W-Bra-L4
W-Dat-L4
W-Lyc-L4
W-Art-L4
SG-Art-L4
UGT33C
UGT33E1
UGT33E2
UGT33E3
UGT33G1
UGT33G2
UGT33G3
UGT33H1
UGT33H2
UGT33L1
UGT33P1
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UGT340A3
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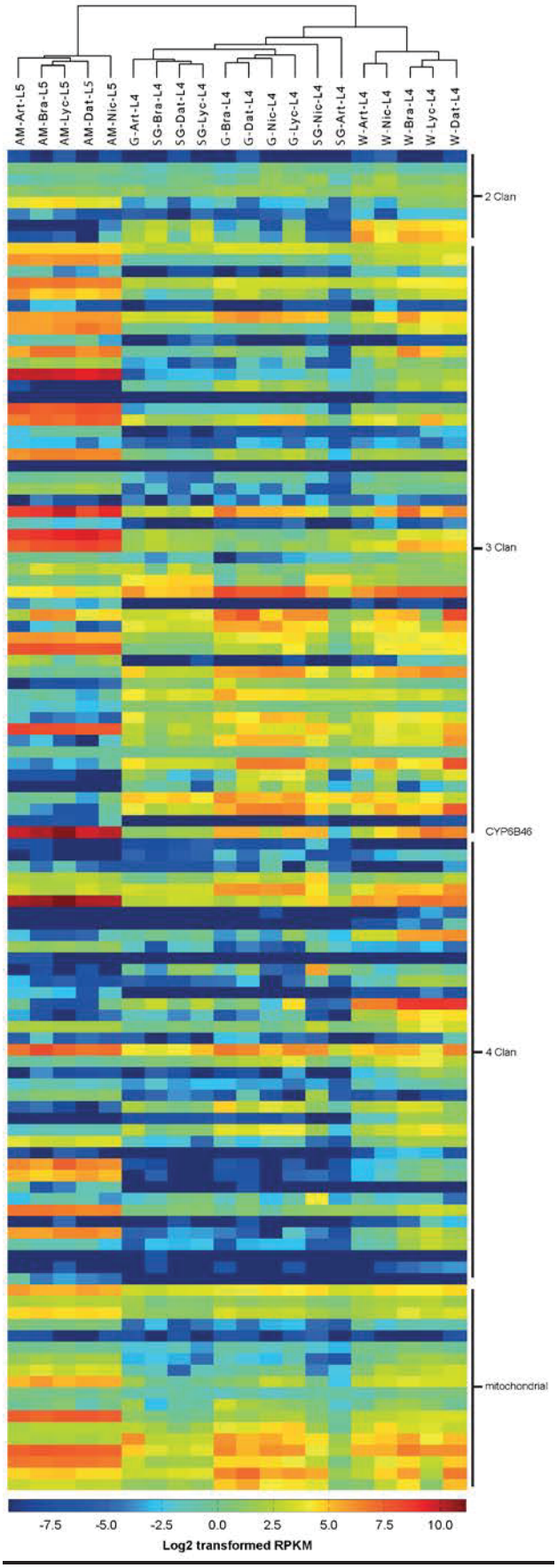
UGT40H2
UGT40J1p
UGT40L2
UGT41C1
UGT42A3
UGT42A4
UGT42B3
UGT44A3
UGT45A1
UGT46A5
UGT46C1
UGT47A3
UGT48B1
UGT50A3
UGT50A3
UGT50A3
Msex2.02747-u
Msex2.13846-u
Msex2.15545-u
-8
-6
-4
-2
0
2
4
6
8
Log2 transformed RPKM

Figure S6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AM-Bra-L5
AM-Lyc-L5
AM-Dat-L5
AM-Nic-L5
G-Art-L4
SG-Bra-L4
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SG-Lyc-L4
G-Bra-L4
G-Dat-L4
G-Nic-L4
G-Lyc-L4
SG-Nic-L4
SG-Art-L4
W-Art-L4
W-Nic-L4
W-Bra-L4
W-Lyc-L4
W-Dat-L4
2 Clan
3 Clan
CYP6B46
4 Clan
mitochondrial
-7.5
-5.0
-2.5
0.0
2.5
5.0
7.5
10.0
Log2 transformed RPKM

Discussion

We employed *M. sexta* as a model system to study the chemosensory receptor repertoire in the context of chemical ecology. Initially, we created a reference gene set of chemosensory genes using the recently available genome combined with our RNAseq data of chemosensory tissues. By expression profiling of males, females and larvae we identified sex specific and stage specific receptors. They emphasize the role of chemosensation for finding a mating partner, choosing suitable sites for ovipositioning and finding suitable host plants. We challenged the gene expression of *M. sexta* larvae by placing them on different host and non-host plants to study their response at the transcriptomic level.

The reference set for chemosensory receptor genes in *M. sexta*

We first aimed to make use of the *M. sexta* genome to create a reference set for chemosensory receptor genes. The automated annotations of the *M. sexta* genome were inaccurate for most of these genes, and several were not identified at all. Chemosensory receptor genes evolve fast and their sequences are highly divergent limiting the success of automated homology based annotation algorithms. We used our RNAseq data sets of chemosensory tissues to manually curate the automated annotation of ORs, IRs and GRs. In addition, we cloned 40 ORs in full length to verify our corrected gene models. Compared to other Lepidoptera genome projects the gene models of *M. sexta* are fairly good. For most of the IRs and GRs however, we did not detect an expression level high enough to predict new gene models. We did not find any expression for two genes (MsexOR-20 and MsexOR-80) in the RNAseq data, although we were able to clone them from antennal cDNA. We conclude that there is a limitation in detecting rare transcripts which could be overcome by deeper sequencing obtaining more than 10 million reads (Mortazavi et al., 2008).

We expended a lot of effort into the reference gene set, because expression analyses depend on reliable references. Inaccurate gene predictions lead to biased expression values in RNAseq studies. If a gene model contains an intron, the RPKM value of this gene will be lower, because the number of mapped reads will be related to the length of the gene. Whereas, if a gene model contains a repetitive sequence which is not present in the real transcript, more reads are mapped and the RPKM value increases. This could be the reason for the differences in the larval gene expression in manuscript 1 compared to manuscript 3: in manuscript 1 the improved gene set was used, in manuscript 3 the gene set with automated annotations was used. Our manually curated annotations will be of use for any follow up genome sequencing of Lepidoptera. Annotation programs like Augustus can use data from

related species to predict genes in genomes (Stanke et al., 2006), therefore, our annotations of chemosensory genes will assist in annotating homologous genes in other species of Lepidoptera.

Male specific ORs: key players in finding a mating partner

A male specific task in Lepidoptera is finding the females by detecting their pheromone blend. Pheromone receptors of Lepidoptera are mostly male specific and have a higher sequence similarity to each other than regular ORs (Krieger et al., 2004; Mitsuno et al., 2008; Xu et al., 2015). All functionally characterized Lepidoptera pheromone receptors belong into one subgroup of ORs (Xu et al., 2015; Zhang and Löfstedt, 2015). Males of *M. sexta* have three types of OSNs in male specific trichoid sensilla to detect three compounds of the female pheromone blend (Kaissling et al., 1989). But only two male specific OR genes have been described so far (Grosse-Wilde et al., 2010; Patch et al., 2009). We identified a novel gene, MsexOR-51, which is expressed in males and larvae, as a putative third receptor for one of the *M. sexta* pheromone compounds. Using fluorescence *in situ* hybridization we showed that the expressing cells are located near to the pheromone sensitive sensilla. Additionally, this candidate belongs into the subgroup of Lepidoptera pheromone receptors. However, there are ORs in the clade which do not respond to pheromone compounds: *Cydia pomonella* (Lepidoptera: Tortricidae) OR3 responds to pear ester (Bengtsson et al., 2014) and *Epiphyas postvittana* (Lepidoptera: Tortricidae) OR1 responds among other compounds to methyl salicylate (Jordan et al., 2009). However, both of the ORs are expressed in males and females (Bengtsson et al., 2014; Jordan et al., 2009) in contrast to our candidate MsexOR-51, which is not expressed in females. Therefore we propose that MsexOR-51 is the missing third pheromone receptor. Our results support the subgroup of pheromone receptors in Lepidoptera and we assume a monophyletic origin of these ORs in the Lepidoptera lineage.

Female specific ORs and their possible function

While both males and females have to find plants for nectar feeding, only females have to identify plants as host for their offspring. Plants attacked by herbivores increase their level of defense substances and emit attractants for parasitoids of the herbivores (Kessler and Baldwin, 2001). Therefore, females avoid ovipositing on these plants (Baldwin, 2001; Kessler and Baldwin, 2001; Späthe et al., 2013). Female specific receptors could be linked to this task. Two ORs are known to be female specific, MsexOR-5 and MsexOR-6 (Grosse-Wilde et al., 2010). They are duplicates of an orthologous *B. mori* receptor responding to

linalool and could be detecting the enantiomers of linalool (Grosse-Wilde et al., 2010; Tanaka et al., 2009). *M. sexta* can differentiate between the enantiomers and probably use this cue to assess the quality of a host plant (Reisenman et al., 2009). We identified an additional female specific OR, MsexOR-15. Female biased ORs in *B. mori* respond to compounds like linalool, benzoic acid, 2-phenylethanol, benzaldehyde and methyl benzoate (Anderson et al., 2009).

There is an alternative explanation for female specific ORs. MsexOR-15 belongs into the pheromone clade. Therefore, it is possible that one of the female specific receptors is responsible for detecting male emitted compounds. Males of *M. sexta* have scent brushes on the second abdominal segment (Birch et al., 1990). Females respond to volatiles emitted by this gland (Grant, 1971). The emitted compounds are still unknown as well as their behavioral consequences. Females of *H. virescens* detect odorants from male hairpencils with their antennae (Hillier and Vickers, 2004) and the detection of the male emitted pheromone by the female is necessary for normal mating success (Hillier and Vickers, 2004). This mechanism could apply for *M. sexta* as well.

Chemosensory receptor genes in the ovipositor

Using scanning electron microscopy we found seven putative chemosensory sensilla on each of the ovipositor papillae (manuscript 2). According to the morphology we detected expression of several IRs, two GRs (both from the CO2 group), one OR (MsexOR-26) as well as ORCo in ovipositors of virgin female *M. sexta*. We demonstrated that the ovipositor of *M. sexta* responds to compounds playing a role in host plant selection. Benzyl alcohol is a green leave volatile which is important in host plant choice by *M. sexta* (Späthe et al., 2013). Cis-3-hexene-1-ol is another herbivore induced plant volatile that allows *M. sexta* to discriminate between damaged and undamaged plants (Späthe et al., 2013). This compound attracts generalist predators which feed on *M. sexta* eggs (Kessler and Baldwin, 2001). The decision of oviposition determines whether the offspring will have a good host plant for development or a bad one where they will struggle to survive.

By comparing RNAseq data from virgin and mated female *M. sexta* we found a down regulation of MsexOR-26 after mating. Since we did not find changes in the electrophysiological response to host plant odors between virgin and mated animals we conclude that MsexOR-26 is detecting other compounds. This could mean that this OR is only necessary in virgin females and therefore could be linked to the mating procedure: either as detector for the female emitted pheromone or for the male emitted compounds. In

females of *H. virescens* expression of a pheromone receptor, HR13, is detected in cells located underneath sensilla on their ovipositor (Widmayer et al., 2009). The function could be to provide a feedback for a more controlled release of the pheromone (Widmayer et al., 2009). Conspecific females could stop calling for the males if they sense other females nearby (Harari et al., 2015). We did not detect expression of a putative pheromone receptor in the ovipositor in *M. sexta*. But since we do not know the ligands of MsexOR-26, we cannot exclude that it detects a compound of the pheromone blend. Alternatively, MsexOR-26 could respond to male emitted compounds from the scent brushes on the abdomen (Birch et al., 1990).

The larval chemosensory gene set

Larvae of *M. sexta* do not depend completely on their mother's choice, since they can migrate to other plants. Additionally, plants like *Nicotiana attenuata* do not offer enough foliage for complete development of a *M. sexta* larvae from the egg to the pupal stage. Therefore, the larvae need a chemosensory system to identify host plants. We report the expression of 20 ORs in the antennae and maxillae of larval *M. sexta* (manuscript 3). After curating the gene models of several ORs we found expression of 23 ORs (RPKM >=0.15; manuscript 1). Our result is in the scope of the results from other Lepidoptera larvae. The larval repertoire of *B. mori* for example comprises 23 ORs (Tanaka et al., 2009) and the one of *Spodoptera littoralis* (Lepidoptera: Noctuidae) 22 ORs (Poivet et al., 2013). We found two ORs exclusively expressed in larva. In *B. mori* six ORs are larvae specific (Tanaka et al., 2009), whereas in *S. littoralis* no larval specific ORs are reported. The data in *S. littoralis* is based on transcriptome data so it is likely that some receptors remain unidentified (Poivet et al., 2013). The low number of larval specific receptors in *M. sexta* compared to *B. mori* could be explained by the different life styles of these two species: *M. sexta* adults feed on the nectar of nightshade plants and the larvae grow up on nightshade plants. This means, the host plants are similar in both stages. In *B. mori* the adults do not feed at all and only the larvae and the females need to identify host plants.

From behavioral experiments we know, that *M. sexta* larvae can find host plants only by the use of olfaction (Glendinning et al., 2009). If they were reared on potato foliage they approached disks perfumed with the odor of potato foliage with higher probability (Glendinning et al., 2009). After removing the larval antennae this effect was gone (Glendinning et al., 2009). However, the key molecules which are detected by the larvae are elusive. One larval receptor, MsexOR-30, is the ortholog of BmorOR56 (manuscript 1 and 3). BmorOR56 responds to cis-jasmone, a compound from mulberry (Tanaka et al., 2009).

Cis-jasmone is also emitted by *Nicotiana attenuata* leaves after they have been attacked by *M. sexta* (Von Dahl and Baldwin, 2004). Based on their sequence similarity we hypothesize that MsexOR-30 is detecting cis-jasmone, and therefore could have a behavioral relevance for *M. sexta* as feeding stimulant.

Another larval receptor is the pheromone receptor candidate MsexOR-51 (manuscript 1 and 3). The question arises whether the ORs detects one of the pheromone compounds of *M. sexta* or has a different ligand. Larvae of *S. littoralis* are attracted by the female pheromone (Poivet et al., 2012). One of the *S. littoralis* pheromone binding proteins is expressed in the larval antenna (Poivet et al., 2012). In *M. sexta* larvae we did not detect expression of one of the pheromone binding proteins OBP1, OBP2, OBP3 or OBP4 (manuscript 3, Vogt et al., 2015). But the binding characteristics of OBPs are partially overlapping. A larval OBP of *Spodoptera exigua* is binding the major sex pheromone compound of this species (Jin et al., 2015). We assume that the ability to detect the pheromone is present in *M. sexta* larvae, too. Electrophysiological recording from larval antenna showed a response to bombykal and the complete pheromone blend (Itagaki and Hildebrand, 1990). Behavioral experiments are necessary to investigate whether *M. sexta* larvae are attracted by any of the compounds of the female pheromone blend.

Induced preference in *M. sexta* larvae

M. sexta larvae do not have an innate preference for solanaceous plants. They can be reared on artificial diet and plants from other families like *Brassica napus* (Crucifereae) and *Vigna sinensis* (Leguminosae) until the final larval instar (Boer and Hanson, 1984). Our feeding assay demonstrates that the growth rate of larvae on *Brassica napus* was similar to the growth rate of larvae on *Datura wrightii* and *Solanum lycopersicum* (manuscript 3). However, *M. sexta* larvae develop a preference for solanaceous host plants after feeding on them (Boer and Hanson, 1984; del Campo et al., 2001; Glendinning et al., 2009). They even prefer to starve to death instead of feeding on non-solanaceous plants (del Campo et al., 2001). This effect is termed ‘induced preference’ and the underlying mechanism is still not understood (Bernays and Weiss, 1996). If indioside D which is present in *Solanum lycopersicum* plants is added to the foliage of *Vigna sinensis M. sexta* larvae reared on solanaceous plants accept the non-solanaceous plant (del Campo et al., 2001). Indioside D is detected by gustatory neurons of the larvae and the response to this compound is stronger in induced larvae (del Campo et al., 2001). Also the electrophysiological response of gustatory neurons to potassium chloride and glucose changes because of the consumption of solanaceous plants (del Campo et al., 2001; Glendinning et al., 2009). We were interested in

the molecular basis of this effect. Thus, we reared larvae on solanaceous plants (*Nicotiana attenuata*, *Datura wrightii*, *Solanum lycopersicum*), a non-solanaceous plant (*Brassica napus*) and artificial diet. We dissected the antennae and maxillae for RNAseq analysis. In this RNAseq data set one OR, MsexOR-27, and five OBPs are differentially expressed in antennae and maxillae of larvae feeding on different host plants and artificial diet (manuscript 3). MsexOR-27 expression was down regulated in larvae feeding on *Solanum lycopersicum*. Four of the OBPs were upregulated in larvae feeding on any of the plants compared to larvae feeding on artificial diet. One OBP was down regulated in *Solanum lycopersicum* and *Brassica napus* compared to the other plants and artificial diet. One OBP was down regulated in *Nicotiana attenuata* and *Brassica napus* compared to the other plants and artificial diet. The down regulation of the OR could lead to a weaker spike response of the OSN to odorants like it is reported from GRs to certain tastants in *M. sexta* (Glendinning et al., 2009). In *Estigmene acrea* larvae (Lepidoptera; Arctiidae) the exposure to a pyrrolizidine alkaloid leads to a loss of gustatory response to this compound (Bernays et al., 2003). In *D. melanogaster* exposure to high odor concentrations for a long time leads to down regulation of an OR (von der Weid et al., 2015). We propose that MsexOR-27 detects a compound which is present in *Solanum lycopersicum* headspace but not in headspace of *Nicotiana attenuata*, *Datura wrightii* and *Brassica napus*. MsexOR-27 belongs to a clade of ORs which is present in all our investigated Lepidoptera species (manuscript 1). Therefore, it could be an important OR for all Lepidoptera species. Up and down regulation of OBPs could increase or decrease the response of sensory neurons to odorants. For example, down regulation of OBPs decreases the response to odors in *Anopheles gambiae* (Rund et al., 2013). OBPs are also present in gustatory sensilla (Galindo and Smith, 2001). Therefore, we conclude that the expression changes of OBPs could be responsible for the increased or decreased response of solanaceous fed larvae to certain tastants and that gene expression regulation at the periphery could contribute to the effect of induced preference.

Importance of host plant choice

One advantage of induced preference could be that the larvae have adapted their metabolism to this specific plant and changing the plant would require sever changes again. By comparing the RNAseq data of larvae reared on different plants or artificial diet we found numerous changes in the gene expression in gut and labial gland tissue (manuscript 3). The used artificial diet did not contain any toxic substances and was rich in carbohydrates. The plants employ toxins as defense mechanism and therefore, the larvae have to deal with the toxins. The gene expression is up or down regulated depending on the host plant. We

conclude that *M. sexta*, albeit an oligophagous species, employs a very specific way of detoxification and reacts in a flexible way to the uptake of toxins. Therefore it is possible, that induced preference prevents larvae from changing the host plant and thereby changes the gene expression pattern again. However, larvae reared on *Brassica napus* do not prefer this plant over solanaceous plants or other non-host plants (Boer and Hanson, 1984), therefore additional experiments may be required to assess this effect. Obviously, *M. sexta* larvae can survive on several very different host plants. But in nature they occur mostly on plants of the nightshade family. Although they can survive on other plants, the decision of the females prevailed during evolution. We still do not know how *M. sexta* is able to tolerate high nicotine doses. A P450 cytochrome suspected to play a role in the detoxification of nicotine specifically (Govind et al., 2010; Kumar et al., 2014). It is up regulated in larvae feeding on *Datura wrightii*, *Solanum lycopersicum* and *Brassica napus* as well (manuscript 3). If this cytochrome is down regulated by RNAi, the larvae have a lower amount of nicotine in their hemolymph, exhale smaller amounts via the spiracles and are more susceptive to predation (Kumar et al., 2014). We argue that the same enzyme is used for detoxification or sequestration of alkaloids of *Datura wrightii* and *Solanum lycopersicum*, too and consequentially protects *M. sexta* larvae against predation.

We found differences in growth rate of *M. sexta* depending on the host plant (manuscript 3). Larvae feeding on *Nicotiana attenuata* exhibited the fastest growth. The growth rate on *Datura wrightii*, *Solanum lycopersicum* and *Brassica napus* were similar but slower than on *Nicotiana attenuata*. A fast growth rate should be favorable, because it indicates a good host plant. Interestingly, female *M. sexta* prefer *Datura wrightii* over *Nicotiana attenuata* in oviposition assays (Späthe et al., 2013). Fast growth reduces the change of parasitism, but on the other hand, *Datura wrightii* plants offer more foliage and maybe this is the reason, why this preference in the females was favorable in evolution in the absence of predation or risk of parasitism.

It is possible, that females oviposit on several other plants. But larvae are consumed or parasitized in early stages. Hence, there are less reports of *M. sexta* on non-nightshade plants. However, more likely is that females prefer plants where the survival rate of the offspring is higher. Due to larger number of offspring these preference will dominate in the population.

Concluding remarks

In the present thesis I have established a reference gene for chemosensory receptor genes in *M. sexta*, which will facilitate future Lepidoptera genome projects. By expression profiling of the genes I could formulate hypotheses about their role in the life of female and male moths as well as larvae. I have successfully used this gene set to investigate how these genes are regulated depending on the life style of the *M. sexta* larvae. My results demonstrate the importance of the oviposition choice and how the ovipositor chemosensory receptor repertoire can assess host plants. My data can be used to investigate many more aspects of *M. sexta* olfaction as the role of additional chemosensory tissues, including tarsi and proboscis.

Summary

Chemosensation, the sense of smell and taste, enables insects to assess the quality of their environment. They use it to find mating partners, to select oviposition sites, for foraging and to avoid dangers. Insects possess three major classes of chemosensory receptors: olfactory receptors (ORs), ionotropic receptors (IRs) and gustatory receptors (GRs). All three gene families are highly divergent because they evolve quickly and are essential for adaptation of the insect to its environment. We utilized the tobacco hawkmoth *Manduca sexta* as a model organism to study the molecular basis of chemosensation in the context of mating, ovipositing and larval adaptation to different host plants. Our objectives were the following: How does the chemosensory receptor repertoire adapt to the different needs of males, females and larvae? How can the larvae adapt to their different host plants?

Using RNAseq data of chemosensory tissues from male and female antenna as well as larval antennae and maxillae we curated the automated gene models of the *M. sexta* genome project and we cloned the majority of ORs to confirm our gene models (manuscript 1). We created a reference gene set for chemosensory receptor genes comprising 73 ORs, 21 IRs and 45 GRs. This gene set will facilitate the identification and annotation of chemosensory receptor genes in following Lepidoptera genome project and allowed use to formulate hypothesis for future projects.

By comparing RNAseq data from male, female and larval antennae we identified an additional OR not expressed in female antenna, MsexOR-51, as a third putative pheromone receptor (manuscript 1). The candidate belongs into a conserved group of ORs in Lepidoptera, the pheromone receptor group. We assume that they originated early in the evolution of Lepidoptera since all known Lepidoptera pheromone receptors belong into this group. MsexOR-51 is also expressed in larvae and could enable the larvae to detect the female emitted pheromone. This has been demonstrated in *Spodoptera littoralis* and could be a common principle in Lepidoptera.

Additionally we identified a third female specific receptor which belongs into the pheromone receptor group (manuscript 1). This receptor could detect the volatiles of the male scent brush whose identity remains unknown.

We assume that females use their ovipositor for close range testing whether a plant is suitable for ovipositing or not. Therefore we investigated the chemosensory role of the ovipositor (manuscript 2). Using scanning electron microscopy we identified putative chemosensory sensilla on the ovipositor. Electrophysiological recordings identified ligands

that can be related to herbivore induced volatiles of plants and volatiles emitted by other insects. We created RNAseq data sets of female ovipositors and detected expression of MsexOR-26, several IRs and two GRs in the ovipositor of virgin females. Additionally, we found down regulation of the expression of MsexOR-26 in the ovipositor after mating. This could imply a function of this OR in the mating process. MsexOR-26 could detect a male emitted volatile or a compound of the female emitted pheromone for a feedback regulation.

M. sexta female oviposit mostly on solanaceous plants. Albeit *M. sexta* larvae do not have an innate preference for these plants, a preference is induced after feeding on them. This process ties the larvae to its first host plant. We were interested in the molecular basis of this process (manuscript 3). In a vast RNAseq study we reared *M. sexta* larvae on typical host plants (*Nicotiana attenuata*, *Datura wrightii*, *Solanum lycopersicum*), a non-host plant, *Brassica napus*, and the standard artificial diet. We dissected the antennae and maxillae, the gut, the labial glands as well as whole larvae for use in sequencing. We report changes in OR and odorant binding protein expression depending on the larval diet. Additionally the detoxification system adapts specifically to every host plant. Both effects may contribute to the observed induction of preference. In a feeding assay larvae grew fastest on *Nicotiana attenuata* which we therefore assume as the best host plant. The growth on the non-host plant *Brassica napus* was not worse than on the host plants *Datura wrightii* and *Solanum lycopersicum* demonstrating that *M. sexta* could feed on more plants than reported from nature. We conclude that host plant choice is not limited be the abilities of the larvae because they are able to feed on several more plants. The restriction to solanaceous species may originate in the oviposition of the females further manifested by the induced preference in larvae.

This thesis provides insights into the molecular basis of chemosensation in different ecological contexts, following the life cycle of a lepidopteran species from mating and oviposition throughout the larval stage. The principles studied here in *M. sexta* can be applied and generalized to other insects and will facilitate further research in chemical ecology and molecular biology.

Zusammenfassung

Chemosensorik, also der Geruchs- und Geschmacksinn, befähigt Insekten, die Qualität ihrer Umwelt einzuschätzen. Sie benutzen sie, um Sexualpartner und Eiablegeplätze zu finden, sowie zur Nahrungssuche und um Gefahren aus dem Weg zu gehen. Insekten haben drei Hauptklassen von Chemorezeptoren: Olfaktorische Rezeptoren (ORs), ionotrope Rezeptoren (IR) und gustatorische Rezeptoren (GRs). Alle drei Genfamilien sind hoch divergent, da sie sich schnell entwickeln und essentiell zur Anpassung an die Umwelt sind. Wir haben den Tabakschwärmer *Manduca sexta* als Modellorganismus verwendet, um die molekularen Grundlagen der Chemosensorik im Kontext von Paarung, Eiablage und der Anpassung der Raupe an ihre Wirtspflanze zu erforschen. Unsere Ziele lassen sich so formulieren: Wie ist das Repertoire der Chemorezeptoren an die unterschiedlichen Bedürfnisse von Männchen, Weibchen und Raupen angepasst? Wie kann sich die Raupe an ihre Wirtspflanze anpassen?

Mit Hilfe von RNAseq-Daten aus chemosensorischem Gewebe von Männchen, Weibchen und Raupen korrigierten wir automatisiert erstellte Genmodelle des *M.-sexta*-Genom-Projektes und bestätigten die korrigierten Modelle durch Klonierung eines Großteils der ORs (Manuskript 1). Wir erstellten einen Referenzsatz von Chemorezeptorgenen, der 73 ORs, 21 IRs sowie 45GRs umfasst. Dieser Referenzsatz wird die Identifikation und Annotation von Chemorezeptoregenen in späteren Lepidoptera-Genomprojekten erleichtern. Er erlaubte uns, Hypothesen für zukünftige Projekte auszustellen.

Durch den Vergleich der RNAseq-Daten von Männchen-, Weibchen- und Raupenantennen identifizierten wir einen weiteren OR, der nicht in Weibchen exprimiert wird, MsexOR-51, als einen dritten putativen Pheromonrezeptor (Manuskript 1). Der Kandidat gehört in die konservierte Gruppe von Lepidoptera-Pheromonrezeptoren. Wir gehen davon aus, dass diese früh in der Evolution von Lepidopteren entstanden sind, weil alle bekannten Lepidoptera-Pheromonrezeptoren in diese Gruppe gehören. MsexOR-51 ist auch in Raupen exprimiert, und ermöglicht dadurch vielleicht die Detektion des weiblichen Pheromons. Dies wurde für *Spodoptera littoralis* gezeigt und könnte ein generelles Prinzip in Lepidoptera sein.

Zusätzlichen identifizierten wir einen dritten OR, der nur in Weibchen exprimiert wird, aber auch in die Gruppe der männlichen Pheromonrezeptoren gehört (Manuskript 1). Möglicherwiese ermöglicht dieser Rezeptor dem Weibchen den Duft zu detektieren, den das Männchen abgibt. Um welche chemische Verbindung es sich dabei handelt, ist noch nicht bekannt.

Wir nehmen an, dass Weibchen ihren Ovipositor benutzen, um im Nahbereich die Eignung einer Pflanze zur Eiablage einschätzen zu können. Daher untersuchten wir, ob der Ovipositor an der Chemosensorik beteiligt ist. Mit Hilfe von Elektronenmikroskopie fanden wir Sensillen auf dem Ovipositor, die olfaktorisch sein könnten. Durch elektrophysiologische Ableitungen detektierten wir Antworten auf Düfte, die von Pflanzen nach Herbivorenbefall abgegeben werden und Düfte, die von anderen Insekten abgegeben werden. Wir haben RNA von Ovipositoren sequenziert und konnten die Expression von MsexOR-26, einigen IRs und zwei GRs in ungepaarten Tieren nachweisen. In der Probe von gepaarten Tieren wurde MSexOR-26 nicht exprimiert. Daher könnte dieser OR eine Rolle bei der Paarung spielen. Er könnte zum Beispiel auch Moleküle vom Männchen detektieren oder aber, als Feedback, die weibliche Pheromonausschüttung regulieren.

M. sexta Weibchen legen Eier meist an Solanaceae ab. Obwohl die Raupen keine angeborene Präferenz für diese Pflanzen haben, wird diese Präferenz induziert, wenn sie an ihnen fressen. Dadurch wird die Raupe an ihre erste Wirtspflanze gebunden. Wir interessierten uns für die molekularen Ursachen für diesen Prozess (Manuskript 3). In einer breit angelegten RNAseq-Studie hielten wir *M.sexta* Raupen auf typischen Wirtspflanzen (*Nicotiana attenuata, Datura wrightii, Solanum lycopsericum*), auf einer Wirtpflanze, auf der sie in der Natur nicht vorkommen (*Brassica napus*) sowie auf dem üblichen Kunstfutter. Wir sezierten Antennen und Maxillen, den Darm, die Labialdrüsen und ganze Tiere und sequenzierten die RNA. Je nach Nahrungsquelle der Raupen änderten sich die Expression eines ORs sowie einiger Odorant-Binde-Proteine. Außerdem wurde die Expression von Detoxifikationsgenen ebenso je nach Nahrungsquelle angepasst. Beide Beobachtungen könnten zum beschriebenen Effekt der Induktion von Präferenz beitragen. In einem anderen Experiment ermittelten wir die Wachstumsrate der Raupen auf den unterschiedlichen Nahrungsquellen. Das schnellste Wachstum ermöglichte *Nicotiana attenuata*, weshalb wir diese Pflanze als die beste Nahrungsquelle ansehen. Das Wachstum auf *Brassica napus*, was keine natürliche Wirtspflanze ist, war nicht langsamer als auf den natürlichen Wirtspflanzen *Datura wrightii* und *Solanum lycopersicum*. Wir schlussfolgern, dass *M. sexta* Raupen mehr Nahrungsquellen erschließen könnten, als sie es in der Natur tatsächlich tun. Die Beschränkung auf Nachtschattengewächse wird vermutlich durch die Eiablage bedingt sowie durch die induzierte Präferenz manifestiert.

Diese Doktorarbeit gibt Einsicht in die molekularen Grundlagen der Chemosensorik unter verschiedenen ökologischen Gesichtpunkten. Dabei bin ich den Lebenszyklus des Tabakschwärmers von der Paarung zur Eiablage und durch das larvale Stadium gefolgt. Die

hier an *M. sexta* untersuchten Prinzipien können verallgemeinert und auf andere Insekten angewandt werden. Sie werden weitere Forschung im Bereich chemische Ökologie und Molekularbiologie unterstützen.

References

Abedi, Z.. H., Brown, A.W.A., 1961. Peritrophic Membrane as Vehicle for DDT and DDE Excretion in *Aedes aegypti* Larvae. Ann. Entomol. Soc. Am. 54, 539–542. doi:10.1093/aesa/54.4.539

Abrell, L., Guerenstein, P.G., Mechaber, W.L., Stange, G., Christensen, T. a., Nakanishi, K., Hildebrand, J.G., 2005. Effect of elevated atmospheric CO2 on oviposition behavior in *Manduca sexta* moths. Glob. Chang. Biol. 11, 1272–1282. doi:10.1111/j.1365-2486.2005.00989.x

Abuin, L., Bargeton, B., Ulbrich, M.H., Isacoff, E.Y., Kellenberger, S., Benton, R., 2011. Functional architecture of olfactory ionotropic glutamate receptors. Neuron 69, 44–60. doi:10.1016/j.neuron.2010.11.042

Ahmad, S.A., Hopkins, T.L., 1993. β-Glucosylation of plant phenolics by phenol β-glucosyltransferase in larval tissues of the tobacco hornworm, *Manduca sexta*(L.). Insect Biochem. Mol. Biol. 23, 581–589. doi:10.1016/0965-1748(93)90031-M

Allmann, S., Baldwin, I.T., 2010. Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. Science 329, 1075–1078. doi:10.1126/science.1191634

Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T., Hansson, B.S., 2013. Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. Elife 2013. doi:10.7554/eLife.00421

Alves, M.N., Sartoratto, A., Trigo, J.R., 2007. Scopolamine in *Brugmansia suaveolens* (Solanaceae): defense, allocation, costs, and induced response. J. Chem. Ecol. 33, 297–309. doi:10.1007/s10886-006-9214-9

Anderson, A.R., Wanner, K.W., Trowell, S.C., Warr, C.G., Jacquin-Joly, E., Zagatti, P., Robertson, H., Newcomb, R.D., 2009. Molecular basis of female-specific odorant responses in *Bombyx mori*. Insect Biochem. Mol. Biol. 39, 189–97. doi:10.1016/j.ibmb.2008.11.002

Anderson, P., Hallberg, E., 1989. Structure and distribution of tactile and bimodal taste/tactile sensilla on the ovipositor, tarsi and antennae of the flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera : Pyralidae). Int. J. Insect Morphol. Embryol. 99, 13–23.

Angerer, L.M., Angerer, R.C., 1992. *In situ* hybridization to cellular RNA with radiolabeled RNA probes., in: DG, W. (Ed.), In Situ Hybridization. IRL Press, Oxford, p. 17.

Arany, a. M., De Jong, T.J., Kim, H.K., Van Dam, N.M., Choi, Y.H., Verpoorte, R., Van Der Meijden, E., 2008. Glucosinolates and other metabolites in the leaves of *Arabidopsis thaliana* from natural populations and their effects on a generalist and a specialist herbivore. Chemoecology 18, 65–71. doi:10.1007/s00049-007-0394-8

Baker, G.T., Ramaswamy, S.B., 1990. Tarsal and ovipositor sensilla of *Heliothis virescens*

and *H. subflexa* (Lepidoptera: Noctuidae). Proc. Entomol. Soc. Washingt. 92, 521–529.

Baker, T.C., 1989. Sex pheromone communication in the Lepidoptera: New research progress. Experientia 45, 248–262.

Baldwin, I.T., 2001. An Ecologically Motivated Analysis of Plant-Herbivore Interactions in Native Tobacco. Plant Physiol. 127, 1449–1458. doi:10.1104/pp.010762

Baldwin, I.T., Halitschke, R., Kessler, A., Schittko, U., 2001. Merging molecular and ecological approaches in plant-insect interactions. Curr. Opin. Plant Biol. 4, 351–8. doi:10.1016/S1369-5266(00)00184-9

Banga, N., Albert, P.J., Kapoor, N.N., McNeil, J.N., 2003. Structure, distribution, and innervation of sensilla on the ovipositor of the spruce budworm, *Choristoneura fumiferana*, and evidence of a gustatory function for type II sensilla. Can. J. Zool. Can. Zool. 81, 2032–2037. doi:10.1139/Z03-175

Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D.G., Groot, A.T., 2014. Immune defence strategies of generalist and specialist insect herbivores. Proc. Biol. Sci. 281, 20140897. doi:10.1098/rspb.2014.0897

Bengtsson, J.M., Gonzalez, F., Cattaneo, A.M., Montagné, N., Walker, W.B., Bengtsson, M., Anfora, G., Ignell, R., Jacquin-Joly, E., Witzgall, P., 2014. A predicted sex pheromone receptor of codling moth *Cydia pomonella* detects the plant volatile pear ester. Front. Ecol. Evol. 2, 1–11. doi:10.3389/fevo.2014.00033

Benton, R., Sachse, S., Michnick, S.W., Vosshall, L.B., 2006. Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors *in vivo*. PLoS Biol. 4, e20. doi:10.1371/journal.pbio.0040020

Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136, 149–62. doi:10.1016/j.cell.2008.12.001

Berenbaum, M.R., 1988. Allelochemicals in insect-microbe-plant interactions: Agents provocateurs in the coevolutionary arms race, in: Barbosa, P., Letourneau, D.K. (Eds.), Novel Aspects of Insect-Plant Interactions. John Wiley & Sons, New York, pp. 97–124.

Bernays, E.A., Rodrigues, D., Chapman, R.F., Singer, M.S., Hartmann, T., 2003. Loss of gustatory responses to pyrrolizidine alkaloids after their extensive ingestion in the polyphagous caterpillar *Estigmene acrea*. J. Exp. Biol. 206, 4487–4496. doi:10.1242/jeb.00705

Bernays, E.A., Weiss, M.R., 1996. Induced food preferences in caterpillars: the need to identify mechanisms. Entomol. Exp. Appl. 78, 1–8. doi:10.1111/j.1570-7458.1996.tb00759.x

Birch, M.C., Poppy, G.M., Baker, T.C., 1990. Scents and Eversible Scent Structures of Male Moths. Annu. Rev. Entomol. 35, 25–54. doi:10.1146/annurev.en.35.010190.000325

Bloem, K.A., Kelley, K.C., Duffey, S.S., 1989. Differential effect of tomatine and its alleviation by cholesterol on larval growth and efficiency of food utilization in *Heliothis zea* and *Spodoptera exigua*. J. Chem. Ecol. 15, 387–98. doi:10.1007/BF02027799

Boer, G., Hanson, F.E., 1984. Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm *Manduca sexta*. Entomol. Exp. Appl. 35, 177–193. doi:10.1111/j.1570-7458.1984.tb03378.x

Bohbot, J.D., Jones, P.L., Wang, G., Pitts, R.J., Pask, G.M., Zwiebel, L.J., 2011. Conservation of indole responsive odorant receptors in mosquitoes reveals an ancient olfactory trait. Chem. Senses 36, 149–160. doi:10.1093/chemse/bjq105

Botella, J.A., Baines, I.A., Williams, D.D., Goberdhan, D.C.I., Proud, C.G., Wilson, C., 2001. The *Drosophila* cell shape regulator c-Jun N-terminal kinase also functions as a stress-activated protein kinase. Insect Biochem. Mol. Biol. 31, 839–847. doi:10.1016/S0965-1748(01)00029-7

Boutros, M., Agaisse, H., Perrimon, N., 2002. Sequential Activation of Signaling Pathways during Innate Immune Responses in *Drosophila*. Dev. Cell 3, 711–722. doi:10.1016/S1534-5807(02)00325-8

Briscoe, A.D., Macias-Muñoz, A., Kozak, K.M., Walters, J.R., Yuan, F., Jamie, G. a., Martin, S.H., Dasmahapatra, K.K., Ferguson, L.C., Mallet, J., Jacquin-Joly, E., Jiggins, C.D., 2013. Female Behaviour Drives Expression and Evolution of Gustatory Receptors in Butterflies. PLoS Genet. 9. doi:10.1371/journal.pgen.1003620

Bruce, T.J.A., 2014. Glucosinolates in oilseed rape: secondary metabolites that influence interactions with herbivores and their natural enemies. Ann. Appl. Biol. 164, 348–353. doi:10.1111/aab.12128

Butenandt, A., Beckmann, R., Stamm, D., Hecker, E., 1959. Über den Sexuallockstoff des Seidenspinners *Bombyx mori* - Reindarstellung und Konstitution. Zeitschrift für Naturforsch. B 14, 283–284.

Cande, J., Prud'homme, B., Gompel, N., 2013. Smells like evolution: The role of chemoreceptor evolution in behavioral change. Curr. Opin. Neurobiol. doi:10.1016/j.conb.2012.07.008

Casanova-Torres, Á.M., Goodrich-Blair, H., 2013. Immune signaling and antimicrobial peptide expression in Lepidoptera. Insects 4, 320–338. doi:10.3390/insects4030320

Castro, D.P., Figueiredo, M.B., Genta, F. a., Ribeiro, I.M., Tomassini, T.C.B., Azambuja, P., Garcia, E.S., 2009. Physalin B inhibits Rhodnius prolixus hemocyte phagocytosis and microaggregation by the activation of endogenous PAF-acetyl hydrolase activities. J. Insect Physiol. 55, 532–537. doi:10.1016/j.jinsphys.2009.01.013

Castro, D.P., Figueiredo, M.B., Ribeiro, I.M., Tomassini, T.C.B., Azambuja, P., Garcia, E.S., 2008. Immune depression in *Rhodnius prolixus* by seco-steroids, physalins. J. Insect Physiol. 54, 555–562. doi:10.1016/j.jinsphys.2007.12.004

Chadha, G.K., Roome, R.E., 1980. Oviposition behaviour and the senilla of the ovipositor of *Chilo partellus* and *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. ZooL Lond. 192, 169–178.

Chen, H., Rossier, C., Lalioti, M.D., Lynn, A., Chakravarti, A., Perrin, G., Antonarakis, S.E., 1996. Cloning of the cDNA for a human homologue of the *Drosophila* white gene and mapping to chromosome 21q22.3. Am. J. Hum. Genet. 59, 66–75.

Cloonan, N., Forrest, A.A.R.R., Kolle, G., Gardiner, B.B.A., Faulkner, G.J., Brown, M.K., Taylor, D.F., Steptoe, A.L., Wani, S., Bethel, G., Robertson, A.J., Perkins, A.C., Bruce, S.J., Lee, C.C., Ranade, S.S., Peckham, H.E., Manning, J.M., McKernan, K.J., Grimmond, S.M., 2008. Stem cell transcriptome profiling via massive-scale mRNA sequencing. Nat Meth 5, 613–619. doi:10.1038/NMETH.1223

Clyne, P.J., Warr, C.G., Carlson, J.R., 2000. Candidate taste receptors in *Drosophila*. Science 287, 1830–1834. doi:10.1126/science.287.5459.1830

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J.H., Carlson, J.R., 1999. A novel family of divergent seven-transmembrane proteins: Candidate odorant receptors in *Drosophila*. Neuron 22, 327–338.

Conesa, A., Götz, S., 2008. Blast2GO: A comprehensive suite for functional analysis in plant genomics. Int. J. Plant Genomics 2008. doi:10.1155/2008/619832

Corona, M., Robinson, G.E., 2006. Genes of the antioxidant system of the honey bee: annotation and phylogeny. Insect Mol. Biol. 15, 687–701. doi:10.1111/j.1365-2583.2006.00695.x

Couto, A., Alenius, M., Dickson, B.J., 2005. Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr. Biol. 15, 1535–1547. doi:10.1016/j.cub.2005.07.034

Croset, V., Rytz, R., Cummins, S.F., Budd, A., Brawand, D., Kaessmann, H., Gibson, T.J., Benton, R., 2010. Ancient Protostome Origin of Chemosensory Ionotropic Glutamate Receptors and the Evolution of Insect Taste and Olfaction. Plos Genet. 6. doi:10.1371/journal.pgen.1001064

Danielson, P.B., Foster, J.L.M., McMahill, M.M., Smith, M.K., Fogleman, J.C., 1998. Induction by alkaloids and phenobarbital of Family 4 Cytochrome P450s in *Drosophila* : evidence for involvement in host plant utilization. Mol. Gen. Genet. MGG 259, 54–59. doi:10.1007/s004380050788

Davis, T.S., Crippen, T.L., Hofstetter, R.W., Tomberlin, J.K., 2013. Microbial Volatile Emissions as Insect Semiochemicals. J. Chem. Ecol. 39, 840–859. doi:10.1007/s10886-013-0306-z

de Boer, G., 2006. The role of the antennae and maxillary palps in mediating food preference by larvae of the tobacco hornworm, *Manduca sexta*. Entomol. Exp. Appl. 119, 29–38. doi:10.1111/j.1570-7458.2006.00397.x

de Boer, G., 1993. Plasticity in food preference and diet-induced differential weighting of chemosensory information in larval *Manduca sexta*. J. Insect Physiol. 39, 17–24. doi:10.1016/0022-1910(93)90013-H

de Boer, G., 1991. Effect of diet experience on the ability of different larval chemosensory organs to mediate food discrimination by the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 37, 763–769. doi:10.1016/0022-1910(91)90111-C

de Boer, G., Dethier, V.G., Schoonhoven, L.M., 1977. Chemoreceptors in the preoral cavity of the tobacco hornworm, *Manduca sexta*, and their possible function in feeding behaviour. Entomol. Exp. Appl. 21, 287–298. doi:10.1007/BF00291791

de la Paz Celorio-Mancera, M., Ahn, S.-J., Vogel, H., Heckel, D.G., 2011. Transcriptional responses underlying the hormetic and detrimental effects of the plant secondary metabolite gossypol on the generalist herbivore *Helicoverpa armigera*. BMC Genomics. doi:10.1186/1471-2164-12-575

Dean, M., Annilo, T., 2005. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu. Rev. Genomics Hum. Genet., Annual Review of Genomics and Human Genetics 6, 123–42. doi:10.1146/annurev.genom.6.080604.162122

del Campo, M.L., 2003. Chemosensory tuning to a host recognition cue in the facultative specialist larvae of the moth *Manduca sexta*. J. Exp. Biol. 206, 3979–3990. doi:10.1242/jeb.00626

del Campo, M.L., Halitschke, R., Short, S.M., Lazzaro, B.P., Kessler, A., 2013. Dietary plant phenolic improves survival of bacterial infection in *Manduca sexta* caterpillars. Entomol. Exp. Appl. 146, 321–331. doi:10.1111/eea.12032

del Campo, M.L., Miles, C.I., Schroeder, F.C., Mueller, C., Booker, R., Renwick, J. a, 2001. Host recognition by the tobacco hornworm is mediated by a host plant compound. Nature 411, 186–9. doi:10.1038/35075559

Dethier, V., Schoonhoven, L.M., 1966. Sensory Aspects of Host-Plant Discrimination By Lepidopterous Larvae. Arch. Néerlandaises Zool. 16, 497–530. doi:10.1163/036551666X00057

Devenport, M., Alvarenga, P.H., Shao, L., Fujioka, H., Bianconi, M.L., Oliveira, P.L., Jacobs-Lorena, M., 2006. Identification of the *Aedes aegypti* peritrophic matrix protein AeIMUCI as a heme-binding protein. Biochemistry 45, 9540–9549. doi:10.1021/bi0605991

Diamond, S.E., Kingsolver, J.G., 2011. Host plant quality, selection history and trade-offs shape the immune responses of *Manduca sexta*. Proc. Biol. Sci. 278, 289–297. doi:10.1098/rspb.2010.1137

Dobritsa, A. a., Van Der Goes Van Naters, W., Warr, C.G., Steinbrecht, R.A., Carlson, J.R., 2003. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 37, 827–841. doi:10.1016/S0896-6273(03)00094-1

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J.A., Imler, J.-L., 2005. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *Drosophila*. Nat. Immunol. 6, 946–953. doi:10.1038/ni1237

Dunipace, L., Meister, S., McNealy, C., Amrein, H., 2001. Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. Curr. Biol. 11, 822–835. doi:10.1016/S0960-9822(01)00258-5

Eaton, D.L., Bammler, T.K., 1999. Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol. Sci. 49, 156–164. doi:10.1093/toxsci/49.2.156

Eaton, J.L., 1986. Morphology of Abdominal Segments Eight and Nine of the Female Tobacco Hornworm, *Manduca sexta* (Lepidoptera: Sphingidae). Ann. Entomol. Soc. Am. 79, 629–635.

Engsontia, P., Sangket, U., Chotigeat, W., Satasook, C., 2014. Molecular Evolution of the Odorant and Gustatory Receptor Genes in Lepidopteran Insects: Implications for Their Adaptation and Speciation. J. Mol. Evol. 79, 21–39. doi:10.1007/s00239-014-9633-0

Fahey, J.W., Zalcmann, a T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. doi:10.1016/S0031-9422(00)00316-2

Faucheux, M.J., 1991. Morphology and distribution of sensilla on the cephalic appendages, tarsi and ovipositor of the European Sunflower moth, *Homoeosoma nebulella* Den.&Schiff. (Lepidoptera: Pyralidae). Int. J. Insect Morphol. Embryol. 20, 291–307.

Faucheux, M.J., 1988. Multiporous sensilla on the ovipositor of *Monopis Crocicapitella* clem. (Lepidoptera: Tineidae). Int. J. Insect Morphol. Embryol. 17, 473–475.

Ferrandon, D., Imler, J.-L., Hetru, C., Hoffmann, J.A., 2007. The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. Nat. Rev. Immunol. 7, 862–874. doi:10.1038/nri2194

Feyereisen, R., 2006. Evolution of insect P450. Biochem. Soc. Trans. 34, 1252–1255. doi:10.1042/BST0341252

Feyereisen, R., 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–33. doi:10.1146/annurev.ento.44.1.507

Fraser, A.M., Mechaber, W.L., Hildebrand, J.G., 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. J. Chem. Ecol. 29, 1813–1833.

Freeman, E.G., Wisotsky, Z., Dahanukar, A., 2014. Detection of sweet tastants by a conserved group of insect gustatory receptors. Proc. Natl. Acad. Sci. U. S. A. 111, 1598–603. doi:10.1073/pnas.1311724111

Freitak, D., Heckel, D.G., Vogel, H., 2009a. Dietary-dependent trans-generational immune priming in an insect herbivore. Proc. Biol. Sci. 276, 2617–2624.

doi:10.1098/rspb.2009.0323

Freitak, D., Heckel, D.G., Vogel, H., 2009b. Bacterial feeding induces changes in immune-related gene expression and has trans-generational impacts in the cabbage looper (*Trichoplusia ni*). Front. Zool. 6, 7. doi:10.1186/1742-9994-6-7

Freitak, D., Wheat, C.W., Heckel, D.G., Vogel, H., 2007. Immune system responses and fitness costs associated with consumption of bacteria in larvae of *Trichoplusia ni*. BMC Biol. 5, 56. doi:10.1186/1741-7007-5-56

Fujii, S., Yavuz, A., Slone, J., Jagge, C., Song, X., Amrein, H., 2015. Drosophila Sugar Receptors in Sweet Taste Perception, Olfaction, and Internal Nutrient Sensing. Curr. Biol. 25, 621–627. doi:10.1016/j.cub.2014.12.058

Gaertner, L.S., Murray, C.L., Morris, C.E., 1998. Transepithelial transport of nicotine and vinblastine in isolated malpighian tubules of the tobacco hornworm (*Manduca sexta*) suggests a P-glycoprotein-like mechanism. J. Exp. Biol. 201, 2637–2645.

Galindo, K., Smith, D.P., 2001. A large family of divergent Drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla. Genetics 159, 1059–1072.

Gao, Q., Chess, A., 1999. Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. Genomics 60, 31–39.

Ghaninia, M., Olsson, S.B., Hansson, B.S., 2014. Physiological Organization and Topographic Mapping of the Antennal Olfactory Sensory Neurons in Female Hawkmoths, *Manduca sexta*. Chem. Senses. doi:10.1093/chemse/bju037

Glaser, N., Gallot, A., Legeai, F., Montagné, N., Poivet, E., Harry, M., Calatayud, P.A., Jacquin-Joly, E., 2013. Candidate chemosensory genes in the stemborer *Sesamia nonagrioides*. Int. J. Biol. Sci. 9, 481–495. doi:10.7150/ijbs.6109

Glendinning, J.I., Foley, C., Loncar, I., Rai, M., 2009. Induced preference for host plant chemicals in the tobacco hornworm: contribution of olfaction and taste. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 195, 591–601. doi:10.1007/s00359-009-0434-7

Govind, G., Mittapalli, O., Griebel, T., Allmann, S., Böcker, S., Baldwin, I.T., 2010. Unbiased transcriptional comparisons of generalist and specialist herbivores feeding on progressively defenseless *Nicotiana attenuata* plants. PLoS One 5, e8735. doi:10.1371/journal.pone.0008735

Grant, G.G., 1971. Electroantennogram Responses to the Scent Brush Secretions of Several Male Moths. Ann. Entomol. Soc. Am. 64, 1428–1431. doi:10.1093/aesa/64.6.1428

Grosse-Wilde, E., Gohl, T., Bouche, E., Breer, H., Krieger, J., 2007. Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. Eur. J. Neurosci. 25, 2364–2373. doi:10.1111/j.1460-9568.2007.05512.x

Grosse-Wilde, E., Kuebler, L.S., Bucks, S., Vogel, H., Wicher, D., Hansson, B.S., 2011. Antennal transcriptome of *Manduca sexta*. Proc. Natl. Acad. Sci. U. S. A. 108, 7449–

7454. doi:10.1073/pnas.1017963108

Grosse-Wilde, E., Stieber, R., Forstner, M., Krieger, J., Wicher, D., Hansson, B.S., 2010. Sex-specific odorant receptors of the tobacco hornworm *Manduca sexta*. Front. Cell. Neurosci. 4. doi:22 10.3389/fncel.2010.00022

Grosse-Wilde, E., Svatos, A., Krieger, J., 2006. A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem. Senses 31, 547–555. doi:10.1093/chemse/bjj059

Gunaratna, R.T., Jiang, H., 2013. A comprehensive analysis of the *Manduca sexta* immunotranscriptome. Dev. Comp. Immunol. 39, 388–398. doi:10.1016/j.dci.2012.10.004

Hallem, E. a, Carlson, J.R., 2006. Coding of Odors by a Receptor Repertoire. Cell 125, 143–160. doi:10.1016/j.cell.2006.01.050

Hallem, E.A., Ho, M.G., Carlson, J.R., 2004. The molecular basis of odor coding in the *Drosophila* antenna. Cell 117, 965–979.

Hanahan, D., 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166, 557–580. doi:10.1016/S0022-2836(83)80284-8

Hanson, F.E., Dethier, V.G., 1973. Rôle of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 19, 1019–1031. doi:10.1016/0022-1910(73)90028-0

Hansson, B.S., 1995. Olfaction in Lepidoptera. Experientia 51, 1003–1027. doi:10.1007/BF01946910

Hansson, B.S., Stensmyr, M.C., 2011. Evolution of Insect Olfaction. Neuron 72, 698–711. doi:10.1016/j.neuron.2011.11.003

Harari, A.R., Zahavi, T., Steinitz, H., 2015. Female detection of the synthetic sex pheromone contributes to the efficacy of mating disruption of the European grapevine moth, *Lobesia botrana*. Pest Manag. Sci. 71, 316–322. doi:10.1002/ps.3830

Hare, J.D., 2010. Ontogeny and season constrain the production of herbivore-inducible plant volatiles in the field. J. Chem. Ecol. 36, 1363–74. doi:10.1007/s10886-010-9878-z

Hare, J.D., Walling, L.L., 2006. Constitutive and jasmonate-inducible traits of *Datura wrightii*. J. Chem. Ecol. 32, 29–47. doi:10.1007/s10886-006-9349-8

Harvey, J.A., Van Dam, N.M., Witjes, L.M.A., Soler, R., Gols, R., 2007. Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. Ecol. Entomol. 32, 15–23. doi:10.1111/j.1365-2311.2006.00838.x

Hegedus, D., Erlandson, M., Gillott, C., Toprak, U., 2009. New insights into peritrophic matrix synthesis, architecture, and function. Annu. Rev. Entomol. 54, 285–302. doi:10.1146/annurev.ento.54.110807.090559

Hillier, N.K., Vickers, N.J., 2004. The Role of Heliothine Hairpencil Compounds in Female *Heliothis virescens* (Lepidoptera: Noctuidae) Behavior and Mate Acceptance. Chem. Senses 29, 499–511. doi:10.1093/chemse/bjh052

Hoffmann, J.A., 1995. Innate immunity of insects. Curr. Opin. Immunol. 7, 4–10. doi:10.1016/0952-7915(95)80022-0

Holland, I.B., Cole, S.P.C., Kuchler, K., Higgins, C.F. (Eds.), 2003. ABC Proteins: From Bacteria to Man. Academic Press, London, UK.

Howlett, N., Dauber, K.L., Shukla, A., Morton, B., Glendinning, J.I., Brent, E., Gleason, C., Islam, F., Izquierdo, D., Sanghavi, S., Afroz, A., Aslam, A., Barbaro, M., Blutstein, R., Borovka, M., Desire, B., Elikhis, A., Fan, Q., Hoffman, K., Huang, A., Keefe, D., Lopatin, S., Miller, S., Patel, P., Rizzini, D., Robinson, A., Rokins, K., Turlik, A., Mansfield, J.H., 2012. Identification of chemosensory receptor genes in *Manduca sexta* and knockdown by RNA interference. BMC Genomics 13, 211. doi:10.1186/1471-2164-13-211

Hung, C., 1997. Isolation and characterization of CYP6B4, a furanocoumarin-inducible cytochrome P450 from a polyphagous caterpillar (Lepidoptera: Papilionidae). Insect Biochem. Mol. Biol. 27, 377–385. doi:10.1016/S0965-1748(97)00009-X

Itagaki, H., Hildebrand, J.G., 1990. Olfactory interneurons in the brain of the larval sphinx moth *Manduca sexta*. J. Comp. Physiol. A 167, 309–320. doi:10.1007/BF00192566

Jacquin-Joly, E., Legeai, F., Montagné, N., Monsempes, C., François, M.C., Poulain, J., Gavory, F., Walker, W.B., Hansson, B.S., Larsson, M.C., 2012. Candidate chemosensory genes in female antennae of the noctuid moth *Spodoptera littoralis*. Int. J. Biol. Sci. 8, 1036–1050. doi:10.7150/ijbs.4469

Jeong, Y.T., Shim, J., Oh, S.R., Yoon, H.I., Kim, C.H., Moon, S.J., Montell, C., 2013. An odorant-binding protein required for suppression of sweet taste by bitter chemicals. Neuron 79, 725–37. doi:10.1016/j.neuron.2013.06.025

Jermy, T., Hanson, F.E., Dethier, V.G., 1968. Induction of specific food preference in Lepidopterous larvae. Entomol. Exp. Appl. 11, 211–230. doi:10.1111/j.1570-7458.1968.tb02046.x

Jiang, X.-J., Ning, C., Guo, H., Jia, Y.-Y., Huang, L.-Q., Qu, M.-J., Wang, C.-Z., 2015. A gustatory receptor tuned to d-fructose in antennal sensilla chaetica of *Helicoverpa armigera*. Insect Biochem. Mol. Biol. 60, 39–46. doi:10.1016/j.ibmb.2015.03.002

JIN, R., LIU, N., LIU, Y., DONG, S., 2015. A larval specific OBP able to bind the major female sex pheromone component in *Spodoptera exigua* (Hübner). J. Integr. Agric. 14, 1356–1366. doi:10.1016/S2095-3119(14)60849-2

Jones, W.D., Cayirlioglu, P., Kadow, I.G., Vosshall, L.B., 2007. Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. Nature 445, 86–90. doi:10.1038/nature05466

Jordan, M.D., Anderson, A., Begum, D., Carraher, C., Authier, A., Marshall, S.D.G., Kiely, A., Gatehouse, L.N., Greenwood, D.R., Christie, D.L., Kralicek, A. V, Trowell, S.C., Newcomb, R.D., 2009. Odorant receptors from the light brown apple moth (*Epiphyas postvittana*) recognize important volatile compounds produced by plants. Chem. Senses 34, 383–94. doi:10.1093/chemse/bjp010

Jørgensen, K., Kvello, P., Almaas, T.J., Mustaparta, H., 2006. Two closely located areas in the suboesophageal ganglion and the tritocerebrum receive projections of gustatory receptor neurons located on the antennae and the proboscis in the moth *Heliothis virescens*. J. Comp. Neurol. 496, 121–134. doi:10.1002/cne.20908

Kaiser, J., 2003. Hormesis. Sipping from a poisoned chalice. Science. doi:10.1126/science.302.5644.376

Kaissling, K.E., Hildebrand, J.G., Tumlinson, J.H., 1989. Pheromone Receptor Cells in the Male Moth *Manduca sexta*. Arch. Insect Biochem. Physiol. 10, 273–279.

Kanost, M.R., Jiang, H., Yu, X.-Q., 2004. Innate immune responses of a lepidopteran insect, *Manduca sexta*. Immunol. Rev. 198, 97–105. doi:10.1111/j.0105-2896.2004.0121.x

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066. doi:10.1093/nar/gkf436

Keil, T.A., 1989. Fine structure of the pheromone-sensitive sensilla on the antenna of the hawkmoth, *Manduca sexta*. Tissue Cell 21, 139–151.

Keil, T.A., Steinbrecht, R.A., 1984. Mechanosensitive and Olfactory Sensilla of Insects, in: King, R., Akai, H. (Eds.), Insect Ultrastructure. Springer US, Boston, MA, pp. 477–516. doi:10.1007/978-1-4613-2715-8_13

Kennedy, G.G., 2003. Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Annu. Rev. Entomol. 48, 51–72. doi:10.1146/annurev.ento.48.091801.112733

Kent, K.S., Griffin, L.M., 1990. Sensory organs of the thoracic legs of the moth *Manduca sexta*. Cell Tissue Res. 259, 209–223. doi:10.1007/BF00318442

Kent, K.S., Harrow, I.D., Quartararo, P., Hildebrand, J.G., 1986. An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res. 245, 237–245.

Kent, K.S., Hildebrand, J.G., 1987. Cephalic sensory pathways in the central nervous system of larval *Manduca sexta* (Lepidoptera : Sphingidae). Philos. Trans. R. Soc. Lond. B. Biol. Sci. 315, 1–36.

Kent, L.B., Robertson, H.M., 2009. Evolution of the sugar receptors in insects. BMC Evol. Biol. 9, 41. doi:10.1186/1471-2148-9-41

Kessler, A., Baldwin, I.T., 2001. Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature. Science (80-.). 291, 2141–2144.

doi:10.1126/science.291.5511.2141

Kliebenstein, D.J., Kroymann, J., Mitchell-Olds, T., 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. Curr. Opin. Plant Biol. doi:10.1016/j.pbi.2005.03.002

Klijnstra, J.W., Roessingh, P., 1986. Perception of the oviposition deterring pheromone by tarsal and abdominal contact chemoreceptors in *Pieris brassicae* J. Entomol. Exp. Appl. 40, 71–79.

Koenig, C., Bretschneider, A., Heckel, D.G., Grosse-Wilde, E., Hansson, B.S., Vogel, H., 2015a. The plastic response of *Manduca sexta* to host and non-host plants. Insect Biochem. Mol. Biol. 63, 72–85. doi:10.1016/j.ibmb.2015.06.001

Koenig, C., Hirsh, A., Bucks, S., Klinner, C., Vogel, H., Shukla, A., Mansfield, J.H., Morton, B., Hansson, B.S., Grosse-Wilde, E., 2015b. A reference gene set for chemosensory receptor genes of *Manduca sexta*. Insect Biochem. Mol. Biol. doi:10.1016/j.ibmb.2015.09.007

Koh, T.W., He, Z., Gorur-Shandilya, S., Menuz, K., Larter, N., Stewart, S., Carlson, J., 2014. The Drosophila IR20a Clade of Ionotropic Receptors Are Candidate Taste and Pheromone Receptors. Neuron 83, 850–865. doi:10.1016/j.neuron.2014.07.012

Köllner, T.G., Held, M., Lenk, C., Hiltpold, I., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2008. A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell 20, 482–94. doi:10.1105/tpc.107.051672

Krieger, J., Grosse-Wilde, E., Gohl, T., Breer, H., 2005. Candidate pheromone receptors of the silkmoth *Bombyx mori*. Eur. J. Neurosci. 21, 2167–2176. doi:10.1111/j.1460-9568.2005.04058.x

Krieger, J., Grosse-Wilde, E., Gohl, T., Dewer, Y.M.E., Raming, K., Breer, H., 2004. Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). Proc. Natl. Acad. Sci. U. S. A. 101, 11845–11850. doi:10.1073/pnas.0403052101

Krieger, J., Klink, O., Mohl, C., Raming, K., Breer, H., 2003. A candidate olfactory receptor subtype highly conserved across different insect orders. J. Comp. Physiol. a-Neuroethology Sens. Neural Behav. Physiol. 189, 519–526. doi:10.1007/s00359-003-0427-x

Kumar, P., Pandit, S.S., Steppuhn, A., Baldwin, I.T., 2014. Natural history-driven, plant-mediated RNAi-based study reveals CYP6B46's role in a nicotine-mediated antipredator herbivore defense. Proc. Natl. Acad. Sci. U. S. A. 111, 1245–52. doi:10.1073/pnas.1314848111

Kurtovic, A., Widmer, A., Dickson, B.J., 2007. A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature 446, 542–546. doi:10.1038/nature05672

Kwon, J.Y., Dahanukar, A., Weiss, L. a, Carlson, J.R., 2007. The molecular basis of CO2 reception in *Drosophila*. Proc. Natl. Acad. Sci. 104, 3574–3578. doi:10.1073/pnas.0700079104

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., Vosshall, L.B., 2004. Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43, 703–714.

Lee, J.K., Strausfeld, N.J., 1990. Structure, distribution and number of surface sensilla and their receptor cells on the olfactory appendage of the male moth *Manduca sexta*. J. Neurocytol. 19, 519–538.

Lee, K.P., Simpson, S.J., Wilson, K., 2008. Dietary protein-quality influences melanization and immune function in an insect. Funct. Ecol. 22, 1052–1061. doi:10.1111/j.1365-2435.2008.01459.x

Lee, M.H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A.K., Salen, G., Dean, M., Patel, S.B., 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat. Genet. 27, 79–83. doi:10.1038/83799

Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J.M., Hoffmann, J. a, 1995. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. Proc. Natl. Acad. Sci. 92, 9465–9469. doi:10.1073/pnas.92.21.9465

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J., Hoffmann, J., 1996. The Dorsoventral Regulatory Gene Cassette Controls the Potent Antifungal Response in *Drosophila* Adults. Cell 86, 973–983. doi:10.1016/S0092-8674(00)80172-5

Lemaitre, B., Reichhart, J.-M., Hoffmann, J.A., 1997. *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc. Natl. Acad. Sci. 94, 14614–14619. doi:10.1073/pnas.94.26.14614

Liu, S., Zhou, S., Tian, L., Guo, E., Luan, Y., Zhang, J., Li, S., 2011. Genome-wide identification and characterization of ATP-binding cassette transporters in the silkworm, *Bombyx mori*. BMC Genomics 12. doi:10.1186/1471-2164-12-491

Ma, C., Kanost, M.R., 2000. A b1,3-Glucan Recognition Protein from an Insect, *Manduca sexta*, Agglutinates Microorganisms and Activates the Phenoloxidase Cascade. J. Biol. Chem. 275, 7505–7514. doi:10.1074/jbc.275.11.7505

Maher, N., Thiery, D., 2004. Distribution of chemo- and mechanoreceptors on the tarsi and ovipositor of female European grapevine moth , *Lobesia botrana*. Entomol. Exp. Appl. 110, 135–143.

Maher, N., Thiery, D., Städler, E., 2006. Oviposition by *Lobesia botrana* is stimulated by sugars detected by contact chemoreceptors. Physiol. Entomol. 31, 14–22. doi:10.1111/j.1365-3032.2005.00476.x

Marioni, J.C., Mason, C.E., Mane, S.M., Stephens, M., Gilad, Y., 2008. RNA-seq: An assessment of technical reproducibility and comparison with gene expression arrays. Genome Res. 18, 1509–1517. doi:10.1101/gr.079558.108

Marion-Poll, F., Guillaumin, D., Claudine, M., 1992. Sexual dimorphism of tarsal receptors and sensory equipment of the ovipositor in the European corn borer, *Ostrinia nubilalis*. Cell Tissue Res. 267, 507–518.

Marriage, T.N., King, E.G., Long, A.D., Macdonald, S.J., 2014. Fine-mapping nicotine resistance loci in *Drosophila* using a multiparent advanced generation inter-cross population. Genetics 198, 45–57. doi:10.1534/genetics.114.162107

McBride, C.S., Arguello, J.R., 2007. Five *Drosophila* genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. Genetics 177, 1395–1416. doi:10.1534/genetics.107.078683

Mechaber, W., Capaldo, C., Hildebrand, J., 2002. Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to hostplant volatiles change with age and mating status. J. insect Sci. 2, 1–8.

Mechaber, W.L., Hildebrand, J.G., 2000. Novel, non-solanaceous hostplant record for *Manduca sexta* (Lepidoptera: Sphingidae) in the Southwestern United States. Ann. Entomol. Soc. Am. 93, 447–451. doi:10.1603/0013-8746(2000)093[0447:NNSHRF]2.0.CO;2

Menuz, K., Larter, N.K., Park, J., Carlson, J.R., 2014. An RNA-Seq Screen of the *Drosophila* Antenna Identifies a Transporter Necessary for Ammonia Detection. PLoS Genet. 10, e1004810. doi:10.1371/journal.pgen.1004810

Meyling, N. V., Eilenberg, J., 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113, 336–341. doi:10.1016/j.agee.2005.10.011

Missbach, C., Dweck, H.K.M., Vogel, H., Vilcinskas, A., Stensmyr, M.C., Hansson, B.S., Grosse-Wilde, E., 2014. Evolution of insect olfactory receptors. Elife 2014, 1–22. doi:10.7554/eLife.02115

Mitsuno, H., Sakurai, T., Murai, M., Yasuda, T., Kugimiya, S., Ozawa, R., Toyohara, H., Takabayashi, J., Miyoshi, H., Nishioka, T., 2008. Identification of receptors of main sex-pheromone components of three Lepidopteran species. Eur. J. Neurosci. 28, 893–902. doi:10.1111/j.1460-9568.2008.06429.x

Miyamoto, T., Slone, J., Song, X., Amrein, H., 2012. A fructose receptor functions as a nutrient sensor in the *Drosophila* brain. Cell 151, 1113–1125. doi:10.1016/j.cell.2012.10.024

Monier, J.M., Lindow, S.E., 2004. Frequency, Size, and Localization of Bacterial Aggregates on Bean Leaf Surfaces. Appl. Environ. Microbiol. 70, 346–355. doi:10.1128/AEM.70.1.346-355.2004

Montagné, N., Chertemps, T., Brigaud, I., François, A., François, M.-C., de Fouchier, A., Lucas, P., Larsson, M.C., Jacquin-Joly, E., 2012. Functional characterization of a sex pheromone receptor in the pest moth *Spodoptera littoralis* by heterologous expression in *Drosophila*. Eur. J. Neurosci. 36, 2588–96. doi:10.1111/j.1460-9568.2012.08183.x

Montell, C., 2009. A taste of the *Drosophila* gustatory receptors. Curr. Opin. Neurobiol. 19, 345–353. doi:10.1016/j.conb.2009.07.001

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Meth 5, 621–628. doi:10.1038/NMETH.1226

Mumm, R., Dicke, M., 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defenseThe present review is one in the special series of reviews on animal–plant interactions. Can. J. Zool. doi:10.1139/Z10-032

Myhre, S., Tveit, H., Mollestad, T., Lægreid, A., 2006. Additional Gene Ontology structure for improved biological reasoning. Bioinformatics 22, 2020–2027. doi:10.1093/bioinformatics/btl334

Nakagawa, T., Sakurai, T., Nishioka, T., Touhara, K., 2005. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307, 1638–1642. doi:10.1126/science.1106267

Navon, A., Hare, J.D., Federici, B.A., 1993. Interactions amongHeliothis virescens larvae, cotton condensed tannin and the CryIA(c) ?-endotoxin of *Bacillus thuringiensis*. J. Chem. Ecol. doi:10.1007/BF00980685

Nei, M., Niimura, Y., Nozawa, M., 2008. The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. Nat. Rev. Genet. 9, 951–963. doi:10.1038/nrg2480

Neuhaus, E.M., Gisselmann, G., Zhang, W.Y., Dooley, R., Stortkuhl, K., Hatt, H., 2005. Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. Nat. Neurosci. 8, 15–17. doi:10.1038/nn1371

Ni, L., Bronk, P., Chang, E.C., Lowell, A.M., Flam, J.O., Panzano, V.C., Theobald, D.L., Griffith, L.C., Garrity, P. a, 2013. A gustatory receptor paralogue controls rapid warmth avoidance in *Drosophila*. Nature 500, 580–4. doi:10.1038/nature12390

Olivier, V., Monsempes, C., François, M.-C., Poivet, E., Jacquin-Joly, E., 2011. Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol. Biol. 20, 189–99. doi:10.1111/j.1365-2583.2010.01057.x

Parr, A.J., Payne, J., Eagles, J., Chapman, B.T., Robins, R.J., Rhodes, M.J.C., 1990. Variation in tropane alkaloid accumulation within the Solanaceae and strategies for its exploitation. Phytochemistry 29, 2545–2550. doi:10.1016/0031-9422(90)85185-I

Pask, G.M., Romaine, I.M., Zwiebel, L.J., 2013. The Molecular Receptive Range of a Lactone Receptor in *Anopheles gambiae*. Chem. Senses 38, 19–25.

doi:10.1093/chemse/bjs074

Patch, H.M., Velarde, R.A., Walden, K.K.O., Robertson, H.M., 2009. A Candidate Pheromone Receptor and Two Odorant Receptors of the Hawkmoth *Manduca sexta*. Chem. Senses 34, 305–316. doi:10.1093/chemse/bjp002

Pauchet, Y., Wilkinson, P., Vogel, H., Nelson, D.R., Reynolds, S.E., Heckel, D.G., Ffrench-Constant, R.H., 2010. Pyrosequencing the *Manduca sexta* larval midgut transcriptome: messages for digestion, detoxification and defence. Insect Mol. Biol. 19, 61–75. doi:10.1111/j.1365-2583.2009.00936.x

Pelosi, P., Zhou, J.J., Ban, L.P., Calvello, M., 2006. Soluble proteins in insect chemical communication. Cell. Mol. Life Sci. doi:10.1007/s00018-005-5607-0

Pohlon, E., Baldwin, I.T., 2001. Artificial diets "capture" the dynamics of jasmonate-induced defenses in plants. Entomol. Exp. Appl. 100, 127–130. doi:10.1046/j.1570-7458.2001.00855.x

Poivet, E., Gallot, A., Montagné, N., Glaser, N., Legeai, F., Jacquin-Joly, E., 2013. A comparison of the olfactory gene repertoires of adults and larvae in the noctuid moth *Spodoptera littoralis*. PLoS One 8, e60263. doi:10.1371/journal.pone.0060263

Poivet, E., Rharrabe, K., Monsempes, C., Glaser, N., Rochat, D., Renou, M., Marion-Poll, F., Jacquin-Joly, E., 2012. The use of the sex pheromone as an evolutionary solution to food source selection in caterpillars. Nat. Commun. 3, 1047. doi:10.1038/ncomms2050

Ponton, F., Wilson, K., Holmes, A.J., Cotter, S.C., Raubenheimer, D., Simpson, S.J., 2013. Integrating nutrition and immunology: A new frontier. J. Insect Physiol. doi:10.1016/j.jinsphys.2012.10.011

Pophof, B., 2002. Moth pheromone binding proteins contribute to the excitation of olfactory receptor cells. Naturwissenschaften 89, 515–518. doi:10.1007/s00114-002-0364-5

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One 5. doi:10.1371/journal.pone.0009490

Qiu, Y., Loon, J.J.A. Van, Roessingh, P., 1998. Chemoreception of oviposition inhibiting terpenoids in the diamondback moth *Plutella xylostella*. Entomol. Exp. Appl. 87, 143–155.

Raguso, R., Henzel, C., Buchmann, S., Nabhan, G., 2003a. Trumpet Flowers of the Sonoran Desert : Floral Biology of Peniocereus Cacti and Sacred *Datura*. Int. J. plant Sci. 164, 877–892.

Raguso, R., Levin, R., Foose, S., Holmberg, M., McDade, L., 2003b. Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. Phytochemistry 63, 265–284. doi:10.1016/S0031-9422(03)00113-4

Ramaswamy, S.B., 1988. Host finding by Moths: Sensory modalities and Behaviours. J. Insect Physiol. 34, 235–249.

Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T.C.J., 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434, 732–7. doi:10.1038/nature03451

Ratcliffe, N.A., Rowley, A.F., Fitzgerald, S.W., Rhodes, C.P., 1985. Invertebrate Immunity: Basic Concepts and Recent Advances. Int. Rev. Cytol. 97, 183–350. doi:10.1016/S0074-7696(08)62351-7

Reisenman, C.E., Riffell, J.A., Hildebrand, J.G., 2009. Neuroethology of oviposition behavior in the moth *Manduca sexta*. Ann. N. Y. Acad. Sci. 1170, 462–7.

Reiter, S., Campillo Rodriguez, C., Sun, K., Stopfer, M., 2015. Spatiotemporal Coding of Individual Chemicals by the Gustatory System. J. Neurosci. 35, 12309–12321. doi:10.1523/JNEUROSCI.3802-14.2015

Renwick, J., Chew, F.S., 1994. Oviposition Behavior in Lepidoptera. Annu. Rev. Entomol. 39, 377–400. doi:10.1146/annurev.ento.39.1.377

Renwick, J.A.A., 1989. Chemical ecology of oviposition in phytophagous insects. Experientia 45, 223–228. doi:10.1007/BF01951807

Riddiford, L.M., Hiruma, K., Zhou, X., Nelson, C. a., 2003. Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. Insect Biochem. Mol. Biol. 33, 1327–1338. doi:10.1016/j.ibmb.2003.06.001

Riffell, J. a, Alarcón, R., Abrell, L., Davidowitz, G., Bronstein, J.L., Hildebrand, J.G., 2008. Behavioral consequences of innate preferences and olfactory learning in hawkmoth-flower interactions. Proc. Natl. Acad. Sci. U. S. A. 105, 3404–9. doi:10.1073/pnas.0709811105

Riffell, J. a, Lei, H., Hildebrand, J.G., 2009. Neural correlates of behavior in the moth *Manduca sexta* in response to complex odors. Proc. Natl. Acad. Sci. U. S. A. 106, 19219–26. doi:10.1073/pnas.0910592106

Robertson, H.M., Kent, L.B., 2009. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. J. Insect Sci. 9, 19. doi:10.1673/031.009.1901

Robertson, H.M., Warr, C.G., Carlson, J.R., 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. A. 100 Suppl , 14537–42. doi:10.1073/pnas.2335847100

Rund, S.S.C., Bonar, N.A., Champion, M.M., Ghazi, J.P., Houk, C.M., Leming, M.T., Syed, Z., Duffield, G.E., 2013. Daily rhythms in antennal protein and olfactory sensitivity in the malaria mosquito *Anopheles gambiae*. Sci. Rep. 3, 1–9. doi:10.1038/srep02494

Russel, F.G.M., Koenderink, J.B., Masereeuw, R., 2008. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol. Sci. 29, 200–207. doi:10.1016/j.tips.2008.01.006

Rytz, R., Croset, V., Benton, R., 2013. Ionotropic receptors (IRs): chemosensory ionotropic

glutamate receptors in *Drosophila* and beyond. Insect Biochem. Mol. Biol. 43, 888–97. doi:10.1016/j.ibmb.2013.02.007

Saina, M., Busengdal, H., Sinigaglia, C., Petrone, L., Oliveri, P., Rentzsch, F., Benton, R., 2015. A cnidarian homologue of an insect gustatory receptor functions in developmental body patterning. Nat. Commun. 6, 6243. doi:10.1038/ncomms7243

Sakurai, T., Nakagawa, T., Mitsuno, H., Mori, H., Endo, Y., Tanoue, S., Yasukochi, Y., Touhara, K., Nishioka, T., 2004. Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc. Natl. Acad. Sci. U. S. A. 101, 16653–16658. doi:10.1073/pnas.0407596101

Sánchez-Gracia, A., Vieira, F.G., Rozas, J., 2009. Molecular evolution of the major chemosensory gene families in insects. Heredity (Edinb). 103, 208–216. doi:10.1038/hdy.2009.55

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., Touhara, K., 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452, 1002–U9. doi:10.1038/nature06850

Shields, V.D.C., Hildebrand, J.G., 2001. Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. J. Comp. Physiol. A 186, 1135–1151. doi:10.1007/s003590000165

Shields, V.D.C., Hildebrand, J.G., 2001. Recent advances in insect olfaction, specifically regarding the morphology and sensory physiology of antennal sensilla of the female sphinx moth *Manduca sexta*. Microsc. Res. Tech. 55, 307–329. doi:10.1002/jemt.1180

Shields, V.D.C., Hildebrand, J.G., 1999a. Fine structure of antennal sensilla of the female sphinx moth, *Manduca sexta* (Lepidoptera : Sphingidae). I. Trichoid and basiconic sensilla. Can. J. Zool. 77, 290–301.

Shields, V.D.C., Hildebrand, J.G., 1999b. Fine structure of antennal sensilla of the female sphinx moth, *Manduca sexta* (Lepidoptera : Sphingidae). II. Auriculate, coeloconic, and styliform complex sensilla. Can. J. Zool. Can. Zool. 77, 302–313.

Singh, S.P., Coronella, J.A., Benes, H., Cochrane, B.J., Zimniak, P., 2001. Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. Eur. J. Biochem. 268, 2912–2923. doi:10.1046/j.1432-1327.2001.02179.x

Sparks, M.R., Cheatham, J.S., 1970. Responses of a Laboratory Strain of the Tobacco Hornworm, *Manduca sexta*, to Artificial Oviposition Sites. Ann. Entomol. Soc. Am. 63, 428–431.

Späthe, A., Reinecke, A., Olsson, S.B., Kesavan, S., Knaden, M., Hansson, B.S., 2013. Plant species- and status-specific odorant blends guide oviposition choice in the moth *Manduca sexta*. Chem. Senses 38, 147–59. doi:10.1093/chemse/bjs089

Stanke, M., Schöffmann, O., Morgenstern, B., Waack, S., 2006. Gene prediction in

eukaryotes with a generalized hidden Markov model that uses hints from external sources. BMC Bioinformatics 7, 62. doi:10.1186/1471-2105-7-62

Stengl, M., 2010. Pheromone transduction in moths. Front. Cell. Neurosci. 4. doi:10.3389/fncel.2010.00133

Steppuhn, A., Baldwin, I.T., 2007. Resistance management in a native plant: nicotine prevents herbivores from compensating for plant protease inhibitors. Ecol. Lett. 10, 499–511. doi:10.1111/j.1461-0248.2007.01045.x

Stewart, S., Koh, T.-W., Ghosh, A.C., Carlson, J.R., 2015. Candidate ionotropic taste receptors in the *Drosophila* larva. Proc. Natl. Acad. Sci. 112, 201503292. doi:10.1073/pnas.1503292112

Störtkuhl, K.F., Kettler, R., 2001. Functional analysis of an olfactory receptor in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. A. 98, 9381–9385. doi:10.1073/pnas.151105698

Strausfeld, N.J., Hildebrand, J.G., 1999. Olfactory systems: common design, uncommon origins? Curr. Opin. Neurobiol. 9, 634–9. doi:10.1016/S0959-4388(99)00019-7

Sturm, A., Cunningham, P., Dean, M., 2009. The ABC transporter gene family of *Daphnia pulex*. BMC Genomics 10, 170. doi:10.1186/1471-2164-10-170

Sugumaran, M., 2002. Comparative Biochemistry of Eumelanogenesis and the Protective Roles of Phenoloxidase and Melanin in Insects. Pigment Cell Res. 15, 2–9. doi:10.1034/j.1600-0749.2002.00056.x

Suh, G.S.B., Wong, A.M., Hergarden, A.C., Wang, J.W., Simon, A.F., Benzer, S., Axel, R., Anderson, D.J., 2004. A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. Nature 431, 854–859. doi:10.1038/nature02980

Swarup, S., Morozova, T. V, Sridhar, S., Nokes, M., Anholt, R.R.H., 2014. Modulation of feeding behavior by odorant-binding proteins in *Drosophila melanogaster*. Chem. Senses 39, 125–32. doi:10.1093/chemse/bjt061

Swarup, S., Williams, T.I., Anholt, R.R.H., 2011. Functional dissection of Odorant binding protein genes in *Drosophila melanogaster*. Genes. Brain. Behav. 10, 648–57. doi:10.1111/j.1601-183X.2011.00704.x

Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R., Touhara, K., 2009. Highly Selective Tuning of a Silkworm Olfactory Receptor to a Key Mulberry Leaf Volatile. Curr. Biol. 19, 881–890. doi:10.1016/j.cub.2009.04.035

Tasin, M., Betta, E., Carlin, S., Gasperi, F., Mattivi, F., Pertot, I., 2011. Volatiles that encode host-plant quality in the grapevine moth. Phytochemistry 72, 1999–2005. doi:10.1016/j.phytochem.2011.06.006

Tasin, M., Knudsen, G.K., Pertot, I., 2012. Smelling a diseased host: grapevine moth responses to healthy and fungus-infected grapes. Anim. Behav. 83, 555–562.

doi:10.1016/j.anbehav.2011.12.003

Terra, W.R., 2001. The origin and functions of the insect peritrophic membrane and peritrophic gel. Arch. Insect Biochem. Physiol. 47, 47–61. doi:10.1002/arch.1036

Theodoulou, F.L., Holdsworth, M., Baker, A., 2006. Peroxisomal ABC transporters. FEBS Lett. 580, 1139–55. doi:10.1016/j.febslet.2005.12.095

Thompson, J.N., Pellmyr, O., 1991. Evolution of Oviposition Behavior and Host Preference in Lepidoptera. Annu. Rev. Entomol. 36, 65–89. doi:10.1146/annurev.en.36.010191.000433

Thomson, R.H., 2012. Naturally occurring quinones. Elsevier.

Thomson, R.H., 1991. Distribution of naturally occurring quinones. Pharm. Weekbl. Sci. Ed. 13, 70–73. doi:10.1007/BF01974983

Tumlinson, J.H., Brennan, M.M., Doolittle, R.E., Mitchell, E.R., Brabham, A., Mazomenos, B.E., Baumhover, A.H., Jackson, D.M., 1989. Identification of a Pheromone Blend Attractive to *Manduca sexta* (L.) Males in a Wind Tunnel. Arch. Insect Biochem. Physiol. 10, 255–271.

Unruh, L.M., Xu, R., Kramer, K.J., 1998. Benzoquinone levels as a function of age and gender of the red flour beetle, *Tribolium castaneum*. Insect Biochem. Mol. Biol. 28, 969–977. doi:10.1016/S0965-1748(98)00085-X

Valencia, L., Rice, M., 1982. Contact chemoreceptors on the ovi- positor of the potato moth, *Phthorimaea operculella* (Zell.) (Lepidoptera: Gelechiidae). Int. J. Insect Morphol. Embryol. I, 121–128.

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., Boccard, F., Lemaitre, B., 2005. Drosophila host defense after oral infection by an entomopathogenic *Pseudomonas* species. Proc. Natl. Acad. Sci. U. S. A. 102, 11414–11419. doi:10.1073/pnas.0502240102

Voelckel, C., Krügel, T., Gase, K., Heidrich, N., van Dam, N.M., Winz, R., Baldwin, I.T., 2001. Anti-sense expression of putrescine N-methyltransferase confirms defensive role of nicotine in *Nicotiana sylvestris* against *Manduca sexta*. Chemoecology 11, 121–126. doi:10.1007/PL00001841

Vogel, H., Badapanda, C., Knorr, E., Vilcinskas, A., 2014a. RNA-sequencing analysis reveals abundant developmental stage-specific and immunity-related genes in the pollen beetle *Meligethes aeneus*. Insect Mol. Biol. 23, 98–112. doi:10.1111/imb.12067

Vogel, H., Musser, R.O., Paz Celorio-Mancera, M. de la, 2014b. Transcriptome Responses in Herbivorous Insects Towards Host Plant and Toxin Feeding, in: Annual Plant Reviews. John Wiley & Sons, Ltd, pp. 197–233. doi:10.1002/9781118829783.ch6

Vogt, R.G., Große-Wilde, E., Zhou, J., 2015. The Lepidoptera Odorant Binding Protein gene family: Gene gain and loss within the GOBP/PBP complex of moths and butterflies. Insect Biochem. Mol. Biol. doi:10.1016/j.ibmb.2015.03.003

Vogt, R.G., Riddiford, L.M., 1981. Pheromone binding and inactivation by moth antennae. Nature 293, 161–163. doi:10.1038/293161a0

Von Dahl, C.C., Baldwin, I.T., 2004. Methyl jasmonate and cis-jasmone do not dispose of the herbivore-induced jasmonate burst in *Nicotiana attenuata*. Physiol. Plant. 120, 474–481. doi:10.1111/j.0031-9317.2004.00269.x

von der Weid, B., Rossier, D., Lindup, M., Tuberosa, J., Widmer, A., Col, J.D., Kan, C., Carleton, A., Rodriguez, I., 2015. Large-scale transcriptional profiling of chemosensory neurons identifies receptor-ligand pairs in vivo. Nat. Neurosci. 18, 1455–1463. doi:10.1038/nn.4100

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., Axel, R., 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96, 725–736.

Vosshall, L.B., Hansson, B.S., 2011. A Unified Nomenclature System for the Insect Olfactory Coreceptor. Chem. Senses 36, 497–498. doi:10.1093/chemse/bjr022

Vosshall, L.B., Wong, A.M., Axel, R., 2000. An Olfactory Sensory Map in the Fly Brain. Cell 102, 147–159. doi:10.1016/S0092-8674(00)00021-0

Waladde, S.M., 1983. Chemoreception of adult stem-borers: tarsal and ovipositor sensilla on *Chilo partellus* and *Eldana saccharina*. Int. J. Trop. Insect Sci. 4, 159–165. doi:10.1017/S1742758400004173

Waldbauer, G.P., Fraenkel, G., 1961. Feeding on Normally Rejected Plants by Maxillectomized Larvae of the Tobacco Hornworm, *Protoparce sexta* (Lepidoptera, Sphingidae). Ann. Entomol. Soc. Am. 54, 477–485.

Wang, G., Vásquez, G.M., Schal, C., Zwiebel, L.J., Gould, F., 2011. Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. Insect Mol. Biol. 20, 125–33. doi:10.1111/j.1365-2583.2010.01045.x

Wang, J.-S., Markowitz, J.S., Donovan, J.L., Devane, C.L., 2005. P-glycoprotein does not actively transport nicotine and cotinine. Addict. Biol. 10, 127–129. doi:10.1080/13556210500122995

Wang, Q., Hasan, G., Pikielny, C.W., 1999. Preferential Expression of Biotransformation Enzymes in the Olfactory Organs of *Drosophila melanogaster*, the Antennae. J. Biol. Chem. 274, 10309–10315. doi:10.1074/jbc.274.15.10309

Wang, R., Zhang, L., Xu, L., Zong, S., Luo, Y., 2015. Sensilla on the Antennae and Ovipositor of the Sea Buckthorn Carpenter Moth , *Holcocerus hippophaecolus* Hua et al (Lepidoptera : Cossidae). Syst. Morphol. Physiol. 68–76. doi:10.1007/s13744-014-0258-1

Wanner, K.W., Anderson, A.R., Trowell, S.C., Theilmann, D.A., Robertson, H.M., Newcomb, R.D., 2007. Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. Insect Mol. Biol. 16, 107–19. doi:10.1111/j.1365-2583.2007.00708.x

Wanner, K.W., Robertson, H.M., 2008. The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. Insect Mol. Biol. 17, 621–629. doi:10.1111/j.1365-2583.2008.00836.x

Wicher, D., 2015. Olfactory Signaling in Insects. Prog. Mol. Biol. Transl. Sci. 130, 37–54.

Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., Hansson, B.S., 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452, 1007–U10. doi:10.1038/nature06861

Widmayer, P., Heifetz, Y., Breer, H., 2009. Expression of a pheromone receptor in ovipositor sensilla of the female moth (*Heliothis virescens*). Insect Mol. Biol. 18, 541–547. doi:10.1111/j.1365-2583.2009.00894.x

Winde, I., Wittstock, U., 2011. Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. Phytochemistry. doi:10.1016/j.phytochem.2011.01.016

Wink, M., Theile, V., 2002. Alkaloid tolerance in *Manduca sexta* and phylogenetically related sphingids (Lepidoptera: Sphingidae). Chemoecology 12, 29–46. doi:10.1007/s00049-002-8324-2

Xia, Y.-H., Zhang, Y.-N., Hou, X.-Q., Li, F., Dong, S.-L., 2015. Large number of putative chemoreception and pheromone biosynthesis genes revealed by analyzing transcriptome from ovipositor-pheromone glands of *Chilo suppressalis*. Sci. Rep. 5, 7888. doi:10.1038/srep07888

Xu, W., Papanicolaou, A., Liu, N.-Y., Dong, S.-L., Anderson, A., 2015. Chemosensory receptor genes in the Oriental tobacco budworm *Helicoverpa assulta*. Insect Mol. Biol. 24, 253–263. doi:10.1111/imb.12153

Yamamoto, K., Ichinose, H., Aso, Y., Fujii, H., 2010. Expression analysis of cytochrome P450s in the silkmoth, *Bombyx mori*. Pestic. Biochem. Physiol. 97, 1–6. doi:10.1016/j.pestbp.2009.11.006

Yamamoto, R.T., Fraenkel, G.S., 1960. The Specificity of the Tobacco Hornworm, *Protoparce Sexta*, to Solanaceous Plants. Ann. Entomol. Soc. Am. 53, 503–507. doi:10.1093/aesa/53.4.503

Yamamoto, R.T., Jenkins, R.Y., McClusky, R.K., 1969. Factors determining the selection of plants for oviposition by the Tobacco hornworm *Manduca sexta*. Entomol. Exp. Appl. 12, 504–508. doi:10.1111/j.1570-7458.1969.tb02548.x

Yamaoka, K., Hoshino, M., Hirao, T., 1971. Role of sensory hairs on the anal papillae in oviposition behaviour of *Bombyx mori*. J. Insect Physiol. 17, 897–911.

Yasukochi, Y., Tanaka-Okuyama, M., Shibata, F., Yoshido, A., Marec, F., Wu, C., Zhang, H., Goldsmith, M.R., Sahara, K., 2009. Extensive conserved synteny of genes between the karyotypes of *Manduca sexta* and *Bombyx mori* revealed by BAC-FISH mapping. PLoS One 4. doi:10.1371/journal.pone.0007465

Yeh, K.-W., Lin, M.-I., Tuan, S.-J., Chen, Y.-M., Lin, C.-J., Kao, S.-S., 1997. Sweet potato (*Ipomoea batatas*) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against *Spodoptera litura*. Plant Cell Rep. 16, 696–699. doi:10.1007/s002990050304

Zelcer, N., Saeki, T., Reid, G., Beijnen, J.H., Borst, P., 2001. Characterization of drug transport by the human multidrug resistance protein 3 (ABCC3). J. Biol. Chem. 276, 46400–46407. doi:10.1074/jbc.M107041200

Zhan, S., Merlin, C., Boore, J.L., Reppert, S.M., 2011. The monarch butterfly genome yields insights into long-distance migration. Cell 147, 1171–85. doi:10.1016/j.cell.2011.09.052

Zhang, D.-D., Löfstedt, C., 2015. Moth pheromone receptors: gene sequences, function, and evolution. Front. Ecol. Evol. 3, 1–10. doi:10.3389/fevo.2015.00105

Zhang, H.J., Anderson, A.R., Trowell, S.C., Luo, a. R., Xiang, Z.H., Xia, Q.Y., 2011. Topological and functional characterization of an insect gustatory receptor. PLoS One 6, 1–10. doi:10.1371/journal.pone.0024111

Zhang, J., Liu, C.C., Yan, S.W., Liu, Y., Guo, M.B., Dong, S.L., Wang, G.R., 2013. An odorant receptor from the common cutworm (*Spodoptera litura*) exclusively tuned to the important plant volatile cis-3-Hexenyl acetate. Insect Mol. Biol. 22, 424–432. doi:10.1111/imb.12033

Zhang, Y. V, Ni, J., Montell, C., 2013. The molecular basis for attractive salt-taste coding in *Drosophila*. Science 340, 1334–8. doi:10.1126/science.1234133

Zhong, X., Xu, X.-X., Yi, H.-Y., Lin, C., Yu, X.-Q., 2012. A Toll-Spätzle pathway in the tobacco hornworm, *Manduca sexta*. Insect Biochem. Mol. Biol. 42, 514–524. doi:10.1016/j.ibmb.2012.03.009

Declaration of Independent Assignment

I declare in accordance with the conferral of the degree of doctor from the School of Biology and Pharmacy of Friedrich Schiller University Jena that the submitted thesis was written only with the assistance and literature cited in the text.

People who assisted in experiments, data analysis and writing of the manuscripts are listed as co-authors of the respective manuscripts. I was not assisted by a consultant for doctoral theses.

The thesis has not been previously submitted whether to the Friedrich Schiller University Jena or to any other university.

Jena, October 23rd, 2015

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I promise: There will be delicious cakes for you!

Curriculum Vitae

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Publications

Christopher Koenig, Anne Karpinski, David G. Heckel, Ewald Grosse-Wilde, Bill S. Hansson, Heiko Vogel, "The plastic response of *Manduca sexta* to host and non-host plants" (2015) IBMB, 63, 72-85. doi:10.1016/j.ibmb.2015.06.001

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Oral Presentations

Koenig C.*, Karpinski A., Vogel H., Hansson B.S., Grosse-Wilde E. (2014). "Gene expression profiling in *Manduca sexta*." Talk presented at 9th International Workshop on Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece

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Grosse-Wilde E.*, Koenig C., Wicher D., Stengl M., Hansson B.S. (2013). "Olfaction and *Manduca sexta* gene expression." Talk presented at 13th European Symposium for Insect Taste and Olfaction (ESITO), Villasimius, Italy

Koenig C. (2012). “The ups and downs of a moth’s life: Gene expression profiling in *Manduca sexta*.” Talk presented at Chemical Ecology 2012 Mini-Symposium, Rothamsted, UK

Koenig C. (2012). “Gene Expression Profiling in the Tobacco Hawkmoth *Manduca sexta*.” Talk presented at Workshop “Molecular Tools to Study Olfaction in Arthropods”, University of Konstanz, Konstanz, Germany

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