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Chemo Sense

INSIDE

Scorpion Feet Smell: Chemosensory Organs in Arthropods

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Cover Image: *Hardrurus arizonensis* scorpion. The scorpion was illuminated with UV light during the night, producing a blue fluorescence that was used to take the picture (commonly known as "blacklighting" by scorpion afficionados). Total length of the animal is about 10 cm.

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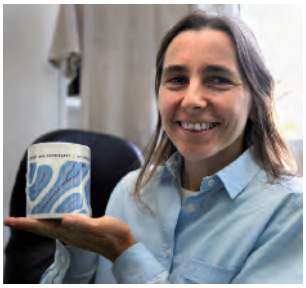

Photo: Erich Sommer

Graham Bell and Elke Weiler, Co-Editors of ChemoSense

EDITORIAL

We're Back

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If you're new to reading *ChemoSense* you'll find new and interesting information from the science of the chemical senses that will delight and inform you. Our writers are leaders in the field and their contributions serve our international community of chemosensory scientists and professionals, as well as people in business and industry, who value awareness of new knowledge. If you are an existing reader you'll know that we have had a 6 month holiday, our first break in publishing in 15 years. Such are the vagaries of cost and effort required to produce high quality information. Anyway, we're back, refreshed, with a new website for back-issues and 50% new editorial personnel: Elke Weiler, joins me as Co-Editor. Elke is pictured above with her nasal neuroanatomical work which has been used to grace the publications, letterheads and coffee mugs of Northwestern University (USA).

Do your feet smell? Or more specifically, do you smell with your feet? No? Then you are probably not an arthropod, and certainly not a chelicerat or scorpion. In this issue we bring you a fascinating mini-review on the chemosenses of scorpions by Harald Wolf. Despite being built to sniff with their feet, their chemosensory nervous system has amazing resemblance to other invertebrates and even to you, our valued mammalian readers.

Can fragrances cause allergies? Despite the molecular mechanisms of causation being poorly understood, allergies triggered by smells are more common than you might realise. A short article on the subject by Julia Kahle, will tell you more about how they manifest and what can be done to avoid serious health consequences.

Other news and announcements abound in this new edition of *ChemoSense*.

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Scorpion Feet Smell: Chemosensory Organs in Arthropods

Photo: Berlins Wirtschaftssenator Harald Wolf

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Virtually all animals possess senses of smell and taste, or chemosenses. The latter term is more appropriate, since in animals, such as aquatic species, smell and taste cannot always be distinguished. Chemoreception is indeed the oldest and perhaps most universal sensory modality, considering that even unicellular protozoans like paramecia orient with regard to chemical gradients. In higher animals, chemoreceptors are usually arranged at the anterior end of the beast in the form of noses, tentacles or antennae - the latter two including mechanosensors to probe encountered objects by touch. This is a most useful situation since it informs the animal immediately about what is coming its way, or what it has encountered on its path of locomotion. Equally important is the probing of food before it is consumed.

Arthropods – insects, crustaceans, spiders and related creatures – are no exception here. The antennae of insects, crayfish and crabs are located on the head, immediately adjacent to eyes and mouthparts (Fig.1A). The antennae are the primary chemosensory organs. Many bugs possess highly sensitive chemoreceptors, as exemplified in the well-studied silk moth Bombyx mori. Physiological measurements have demonstrated that a single molecule of its sexual pheromone bombycol may be sufficient to elicit a sensory signal in the moth brain (Schneider et al., 1968; Kaissling and Priesner, 1970; Kaissling, 1986). The silk moth thus rivals in olfactory sensitivity many mammals that are known to possess a keen sense of smell. The polar bear is a celebrity here, renowned for its ability to localise a food source by smell from more than 100m distance or under 1m of snow (Brown, 1993). Sensitivity is not all that counts, though. Distinguishing and memorising smells may be equally important for an animal's foraging success. The honeybee is a popular example arthropod here, with its ability to distinguish and learn an appreciable number of often subtly different flower odours, flower shapes and colours (Menzel and Müller, 1996).

One arthropod group appears to have been out of luck in the evolution of chemosensory organs, however. These are the chelicerats, that is, scorpions, spiders and their kin. Descending from marine ancestors, as has all animal life, these arthropods conquered terrestrial biotopes

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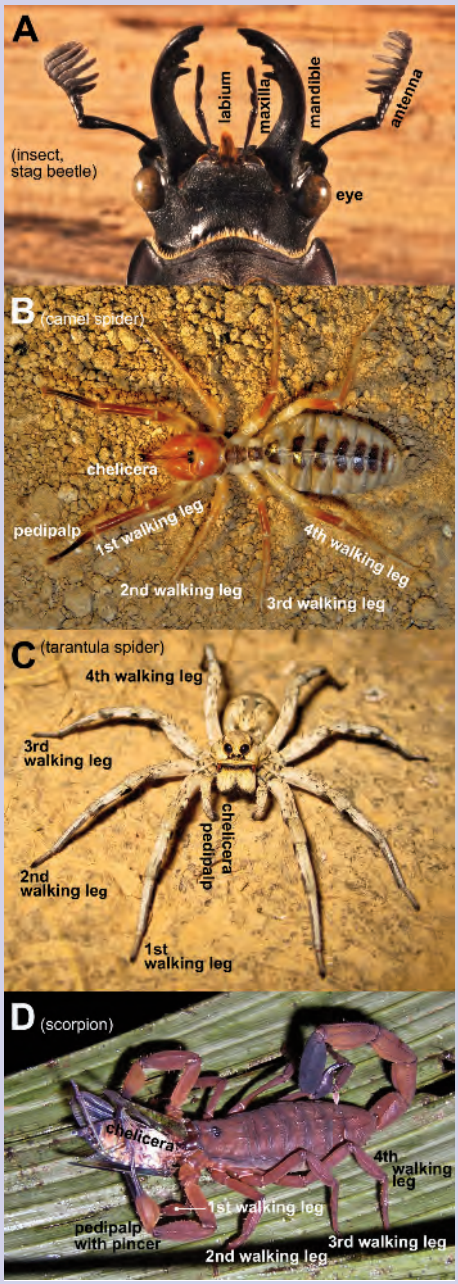

*Fig 1. Arthropod appendages. Images of an insect (**A**, head of a female stag beetle in ventral view), a camel spider (**B**), a tarantula spider (**C**), and a scorpion (**D**) illustrate the different implementations of segmental appendages as walking legs, feelers, or various mouth parts. The common origin of all these appendages is illustrated by the segmented character of the maxilla in the stag beetle, for example. The appendages are labelled according to the list in Tab.1. Note the (superficial) similarity of the stag beetle antenna (A) to the scorpion pectine in Fig.2A-C, the similarity of the (ancestral) camel spider pedipalp to a walking leg (B), the distinctly altered appearance of the pedipalp in the tarantula spider (C), and the small chelicer pincer in the scorpion, just in front of the eyes, that helps plucking apart the prey (D) for extra-intestinal digestion. Photograph in (C) by courtesy and copyright of Matthias Wittlinger.*

in the Silurian about 430 million years ago. At that time, they were already adapted to feeding with an anterior set of appendages, the set that evolved into (chemosensory) antennae in their forebears: insects and crustaceans. Thus, chelicerats do not possess antennae but instead a pair of claws or pincers eponymously called chelicers (Fig.1B, C, D).

A note on the evolution of arthropod appendages is useful here. The appendages on the arthropod body – not just antennae but walking legs, mandibles and associated mouthparts – all derive from the same originally leg-like limb type that supported both (swimming) locomotion and (filter) feeding in a distant ancestor (Budd and Telford, 2009; Scholtz and Edgecombe, 2006; Hughes and Kaufman, 2002). A pair of these limbs originally belonged to every segment of the arthropod body (Table 1). That type of appendage decorated an organism that may have looked somewhat like a brine shrimp and lived before 500 million years ago. And it explains why, as an arthropod, you cannot have the first set of appendages as antennae if you earlier decided to use them as prey processing chelicers. This is explained in some more detail in Table 1.

The original, swimming and filter feeding arthropod legs have evolved into a remarkable variety of limbs, from retaining filter feeding in barnacles to walking and jumping legs in all walking arthropods, speed-trap mandibles in some ants that feed on springtails (Gronenberg, 1995a, b), genital appendages where these are present, and poison-injecting chelicers (Fig.1C) – to name just a few.

Chelicers are generally known to form the unpleasant anterior ends in scorpions and spiders (Fig.1B-D). They may be particularly nasty in spiders where they form poison-injecting claws, functionally comparable to the tail stinger of scorpions. More unpleasantries concern the fact that these appendages do not only inject poison in spiders to incapacitate their prey, or a potential predator if defensive action is required, but in most chelicerats, enzymes are injected, too, to digest the prey tissues, allowing the chelicerate to suck up the resulting nutritious juices, a process aptly called extra-intestinal digestion. In this way, the chelicers replace the mandibles of insects and crustaceans as prey-processing structures, providing enzymatic rather than mechanical break-up of larger food items. Depending on the chelicerat group, the chelicers may also help to pluck

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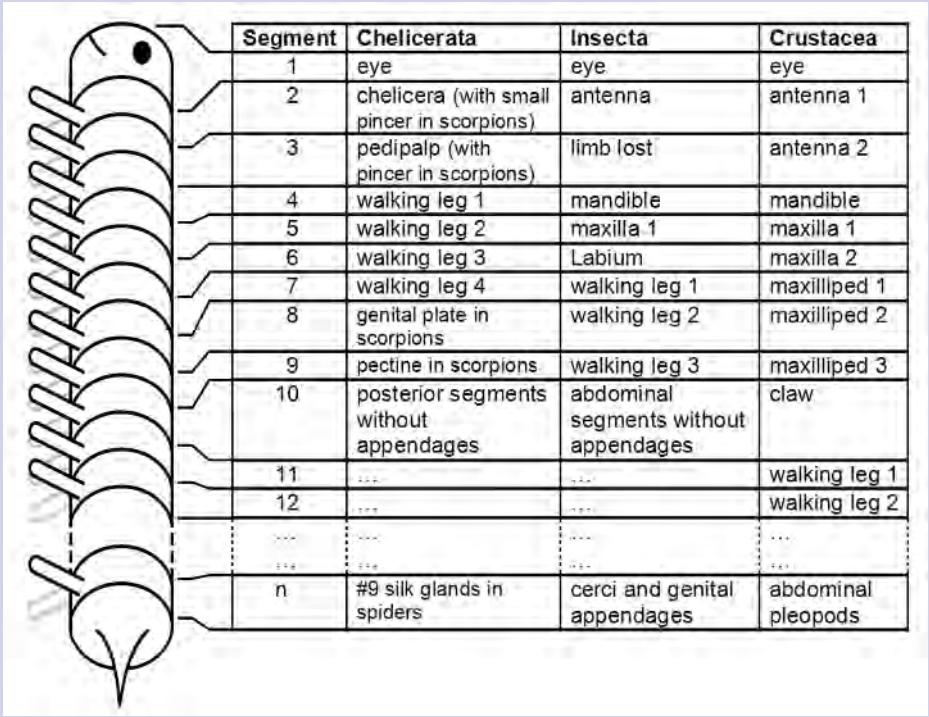

Segment	Chelicerata	Insecta	Crustacea
1	eye	eye	eye
2	chelicera (with small pincer in scorpions)	antenna	antenna 1
3	pedipalp (with pincer in scorpions)	limb lost	antenna 2
4	walking leg 1	mandible	mandible
5	walking leg 2	maxilla 1	maxilla 1
6	walking leg 3	Labium	maxilla 2
7	walking leg 4	walking leg 1	maxillped 1
8	genital plate in scorpions	walking leg 2	maxilliped 2
9	pectine in scorpions	walking leg 3	maxilliped 3
10	posterior segments without appendages	abdominal segments without appendages	claw
11	...	...	walking leg 1
12	...	...	walking leg 2
...	...	...	...
n	#9 silk glands in spiders	cerci and genital appendages	abdominal pleopods

Table 1. *Outline of arthropod appendage evolution. A highly schematised basic arthropod is shown on the left, with eyes, mouth and identical segmental appendages indicated (see text for a brief description of the ancient filter feeding organism with segmentally repeated similar appendages). The evolutionary specialisations of the appendages are listed for chelicerats, insects, and crustaceans for comparison. The 1st to the 12th appendages are listed individually, although more than 20 segments may exist. Please note that the mouth, while originally located in the frontal segment without appendages, has moved backwards in the course of evolution by one or more segments. Many details not shown in this coarse outline are still under discussion.*

away pieces of prey that have been digested not by poison but by regurgitated gut enzymes. This is true in particular for scorpions (Fig.1D). This mode of feeding and extra-intestinal digestion - perhaps a little repulsive for an educated mammal - has stayed with the chelicerats ever since, from spiders to scorpions and harvestmen. Only some mites and ticks just suck liquids out of their hosts or prey, since these are fluid anyway.

With all this feeding frenzy going on without a nose or antennae, how do chelicerats examine potential prey? After all, a chemical sense is useful for orientation and food probing in a chelicerat as much as it is in any animal. So without the anterior set of appendages available to evolve into antennae, what is the chelicerat solution to the problem of chemosenses? In fact, there are several. Spiders and camel spiders (Fig.1B) have modified their second pair of appendages (the pedipalps) into antenna-like limbs that are richly endowed with mechano- and chemoreceptors. They are used to probe objects both mechanically and chemically, much like the insect antennae are. And they may actually resemble antennae morphologically and physiologically, depending on the particular chelicerate group.

Scorpions have evolved a dedicated set of appendages for mechano- and chemoreception, too, the scorpion "antennae" (Fig.2). Intriguingly, though, these "antennae" are not located near the anterior end of the animal, as in most animals. They are instead located posteriorly, behind the four pairs of walking legs (remember, scorpions are relatives of eight-legged spiders), and they point downward, towards the floor (Fig.2A-C). Their relationship to legs is still apparent from three joints (red arrows in Fig.2B, C) that allow some movement, such as lowering onto the substrate. These appendages are called pectines according to their comb-like shape. That shape is reminiscent of insect antennae that often exhibit comb-like structures (Fig.1A) to maximise the surface available for the presentation of odorant receptors: also an obvious reason for the comb-like structure in the scorpion pectines. In male scorpions, these appendages may be equipped

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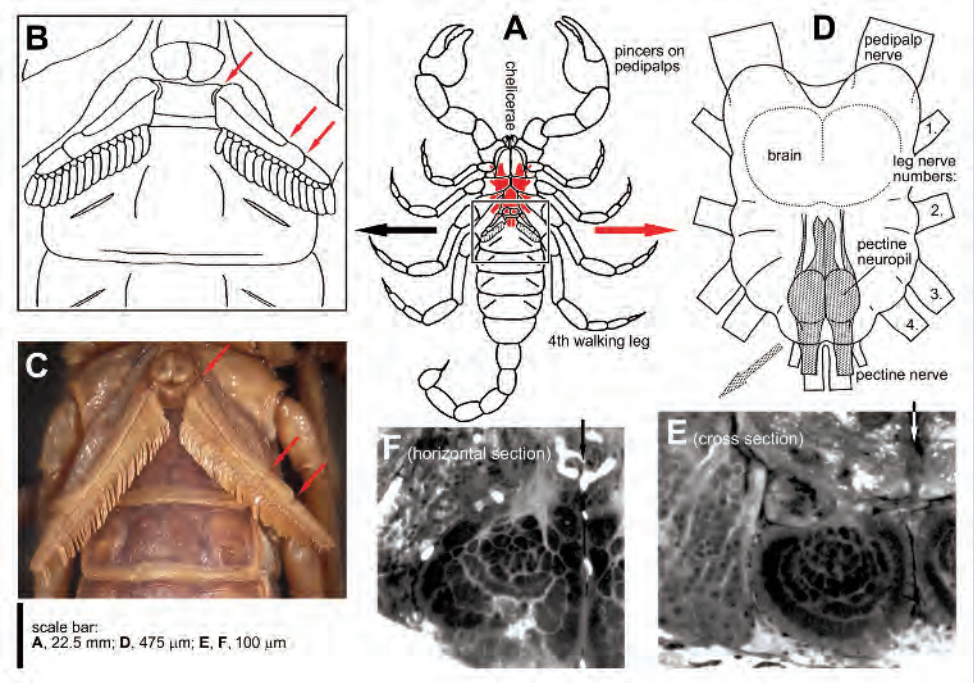

Fig 2. *Scorpions, their pectine appendages and pectine neuropils. A scorpion* ***Pandinus imperator*** *is outlined in ventral view in (**A**), with the central nervous system indicated in red. (**B**) shows an enlarged ventral view of the pectines, illustrating their comb-like appearance (compare stag beetle antenna in Fig.1A); red arrows indicate appendage joints. The photograph in (**C**) is from a different species,* ***Androctonus australis****, with distinctly larger pectines, illustrating inter-species variability. The fused, main part of the central nervous system is shown in (D) with the relevant elements labelled; pectine neuropils are emphasised by dotted shading. (**E**) and (**F**) show histological sections (10 micrometers thick) of the pectine neuropil in the frontal plane ((E), cross section) and in the horizontal plane ((F), similar orientation as in (D)); the midline of the nervous system is indicated by arrows. The glomerular structure of the pectine neuropils is visualised by a lipid stain that darkens the dense glomerular neuropil portions (details in (Wolf, 2008)). Anterior is to the top of the figure in (A)-(D) and (F); dorsal is to the top in (E).*

with more than 140,000 sensory cells, both mechano- and chemoreceptors (Wolf 2008). This number of sensory cells is quite comparable to that in highly chemosensitive insects like the silk moth mentioned above. And like in insects, scorpion males usually possess larger pectines and more sensillae because these are used for finding receptive females. A female scorpion may possess only 80,000 sensory cells on her smaller pectines, compared to those 140,000 in males (Wolf, 2008).

With their unusual position on the scorpion body, the pectines are not used to probe *air* streams for orientation or to examine prey and food items, but rather for probing the *substrate* in the context of mating, and perhaps prey or food localisation (Gaffin et al., 1992; Gaffin and Brownell, 1992, 1997). Scorpions thus indeed smell and taste with their feet, or actually with their whole legs, evolved into *substrate-oriented antenna-like organs*. This should not come as a surprise considering that many arthropods have chemoreceptors on the foot segments of their walking legs, including flies and locusts (Roessingh et al., 1997; Newland et al., 2000). Although these *contact chemoreceptors* have not yet received much attention, it is evident that such an arrangement is a useful one. After all, flies often walk across their food and in this way their feet can tell them where it tastes best and engagement of the mouthparts is warranted. Similarly in the chelicerats, chemoreceptors on walking legs, chelicerae and pedipalps allow finding and probing of food before it is consumed (Krapf, 1986).

Even more intriguing than the story of chemosensory appendages in arthropods is perhaps the similarity of these organs' nerve cell projections in the central nervous systems of virtually all animals (Strausfeld, 2012). The projections of the chemosensory nerve fibres are all organised into glomeruli, little globular compartments of nervous tissue that each receive input from one particular subtype of chemosensory cells (Hildebrand and Shepherd, 1997; Maresh et al., 2008;

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continued

Strausfeld, 2012). These glomeruli are the first stage of chemosensory signal processing (Sachse et al., 1999; Firestein, 2001). The respective brain areas in arthropods thus look almost like miniature versions of the olfactory bulbs in mammals and other vertebrates. The striking similarity of the chemosensory projection areas in vertebrates, insects, crustaceans and other invertebrates has long engaged neurobiologists. Similarities regarding the chemosensors associated with the anterior ends of the animals and their food intake were sometimes explained by a possible common origin of such structures and their association with food identification very early in animal evolution. The fact that even posteriorly located chemosensors in scorpions (and also in some other animal groups) conform to the glomerular organisation scheme (Fig.2E, F) supports another more commonly accepted interpretation (not necessarily to the exclusion of the first one, though) (Hansson, 1999; Galizia and Menzel, 2000; Maresh et al. 2008; Zou et al., 2009), as will now be described.

It appears that a glomerular organisation, with each chemosensory neuron subtype mapping in the central nervous system to a small number of glomeruli, or perhaps just an individual glomerulus, is the most suitable mode of processing chemosensory information. Selective projection of chemosensory neuron subtypes into individually defined glomeruli in a one-to-one manner implies that the number of glomeruli observed in a particular animal species reflects the number of chemosensory neuron subtypes in that animal (Vosshall et al., 1999; Galizia and Menzel, 2000; Maresh et al., 2008).

Indeed, this assumption holds for the organisms studied in sufficient detail to address this question, such as mice, honeybees and fruit flies (Firestein, 2001). The house mouse, for example, possesses about 1800 glomeruli in its left and right olfactory bulbs, respectively, with each glomerulus type represented twice in each bulb (Royet et al., 1988; Maresh et al., 2008). A corresponding number of genes, a few more than 850, codes for olfactory receptor proteins which are individually expressed in the different subtypes of olfactory receptor neurons in the olfactory mucosa of the nose (Glusman et al., 2000; Maresh et al., 2008). The honeybee, a small insect that has to be strictly conservative with the space it can allocate to its brain, has a surprising 160 glomeruli in an olfactory lobe (Galizia and Menzel, 2000), and a corresponding number of olfactory genes (Robertson and Wanner, 2006). The above scorpions have about 100 glomeruli but nothing is known yet regarding their spectrum of chemoreceptor proteins or sensory neurons.

Humans, much like the house mouse as a well studied fellow-mammal, have about 350 functional odour receptor genes, and about the same number of silent genes from earlier evolutionary time (Go and Niimura, 2008). In general, mammals may possess well over 1000 genes that code for olfactory receptor proteins, which corresponds to some 4% of the

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continued

mammalian genome being devoted to olfaction (Maresh et al., 2008). This is an appreciable investment into one sensory modality. By comparison, our visual system is content with just three receptor proteins for colour vision (Jacobs, 1996), producing the red, green, and blue visual pigments (plus one protein for scotopic vision at low light levels), and comparable numbers hold for the other senses. Why is this exceedingly large number of chemoreceptor proteins and corresponding sensory cell types necessary in chemoreception? In the visual system, the whole linear spectrum of visible light can be usefully absorbed by three different photopigments with slightly overlapping absorption spectra. Colour is extracted by comparing the relative levels of excitation in the three differently tuned photoreceptors, allowing for a smooth coding of hues across the whole spectrum (Jacobs, 1996). The situation is dramatically different for chemoreception. There is nothing like a linear spectrum of chemical compounds that an organism encounters. Biologically significant chemicals rather differ in many aspects, including possession of alcohol, aldehyde or carboxylic acid groups, aromatic or aliphatic character, and many more that may all occur in combination. Animals thus had to evolve a set of receptor proteins that are more or less specific in their binding properties for subsets of chemicals, such as monocyclic aromats or alcohol groups. And the only useful way to evaluate such a multi-receptor array appears to be an initial sorting by receptor subtype in the glomeruli. The central nervous system may then assess which pattern of glomeruli, and thus receptor subtypes, are active at all, and among those relative excitation levels may be compared to characterise a distinctive odour quality.

In summary, then, smelling scorpion feet have not just informed us about the evolution of chemosensory organs in animals, and in arthropods specifically, but they also help to develop a functional interpretation of the ubiquitous glomerular organisation of chemosensory brain areas.

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Allergy to Fragrances

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Allergy to Frangrances

continued

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ALLERGY TO FRAGRANCES

Odorants nowadays are pervasive in everyday life. As ingredients of perfumes and non-food-products such as cosmetics, oils, cleaning agents, detergents and fabric softeners they appear both as natural essences as well as synthetic fragrances.

However, odorants are not only pleasant, but can also cause severe health problems.

The first sign of an allergy to a fragrance usually is an allergic contact eczema. Typical symptoms range from itching skin erythema, weeping blisters, pruritus and scaly skin to chronic eczema in areas that had direct contact with the allergenic substance. The contact dermatitis is a so-called Type-IV-allergy, where the reaction is T-cell mediated and therefore temporally delayed. Symptoms appear 48 to 72 hours after contact with the allergen.

According to the Information Network of Clinical Centers for Dermatology, about 15-20% of the population in Germany are affected by contact allergy. Fragrances are the second most common trigger after nickel, with a prevalence of 11.5%.

DIAGNOSING FRAGRANCE ALLERGY

First there is the patient's medical history. If fragrances are suspected to provoke the allergic reaction, an epicutaneous patch test will identify the trigger substance. Allergy specialists currently use two defined mixtures of fragrances -"Fragrance Mix I" and "Fragrance Mix II". The vast majority of positive reactions are induced by isoeugenol and oakmoss. Since 2007, 26 individual fragrances can be tested which must be declared and specified on product labels of cosmetics according to the labelling regulation by the European Union. This may facilitate the search for the trigger but does not necessarily make it easier because the fragrance composition in a product usually consists of several individual substances at different concentrations and each with different and distinct allergenic potential.

TREATMENT OF CONTACT ALLERGY TO FRAGRANCES

Contact allergies cannot be cured. The therapy is solely based on the avoidance of the trigger substance and treatment of the symptoms.

To reduce the inflammatory response, externally applied corticosteroid-containing products are used. In the acute stage aqueous lotions are preferred; in a chronic stage, lipid containing emulsions are state of the art to relieve the symptoms of dry skin. In addition, urea and glycerol can increase the moisture retention of the skin. Beside the anti-inflammatory therapy, it is

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continued

particularly important to improve the basic care of the affected skin area, to regenerate the defective skin barrier.

Indispensible for successful treatment is avoiding the allergy-provoking substances, because with each new contact, the symptoms will resurge. In view of the common use of fragrances in everyday products this may be difficult, especially, when the universal terms "perfume", "fragrance", "aroma" or "flavour" are denoted on the product. The composition and concentrations of the individual ingredients are not declared – except for the 26 fragrances with a commonly high allergy potential (see above). They must be listed individually on the product label with their INCI-name (INCI = international nomenclature of cosmetic ingredients). Declaration is mandatory according to the Cosmetics Directives, if the concentration of these fragrances exceeds 0.01% in products that do not remain on the skin ("rinse-off", such as shower gels and shampoos) or is more than 0.001% in products that remain on the skin ("leave-on", for example lotions, make-up, sunscreen, and deodorants). According to the Detergent Regulation allergenic fragrances have to be marked also in detergents, fabric softeners and cleaning products if the concentration is above 0.01%.

However, labelling regulations do not yet apply to perfumed candles, incense sticks, scented toys, stationary and air scenting products.

INHALED ODORANT EXPOSURE

Many asthmatics and people with chronic obstructive pulmonary disease (COPD) have health problems due to airborne odorants which are used, for example in air-fresheners or are distributed by excessive perfume use. Experts refer to an "odor intolerance" which may significantly reduce the patients' quality of life. Odorants can induce massive breathing difficulties in these patients and may even result in an acute asthma attack.

SOME SOURCES OF ALLERGIES TO FRAGRANCES

Household products

Fragrant plants and essential oils

Popular cosmetic fragrances

NEWS

Germans Hopping for Novel Beer Flavours

By Graham Bell: *g.bell@e-nose.info*

BEER WITH MELON FLAVOUR, ANYONE? A HINT OF PEACH AND PASSIONFRUIT?

Surely the originators of lager and pils would say "nein danke!". But no, flavoured beer is taking off in Germany and it's all legally brewed according to the "beer purity law", thanks to flavour conveyed to the brew through the hops: naturally flavoured hops.

According to German law, beer can consist only of water, hops, malt and yeast. No artificial flavour is allowed. But, when the hops already have a fruity note, you can produce fruity-beer legally in the home of lager and pils. Bred in the USA and elsewhere, flavoured hops have been used for several decades "out there", even as far away as New Zealand. Now flavoured hops have been exported to Germany where beer consumers are taking a shine to the novel tastes imparted to their favourite beverage (http://www.dradio.de/dlf/sendungen/umwelt/2253137/).

The term "noble hops" traditionally refers to varieties of hops which are low in bitterness and high in aroma. European cultivars include Hallertau, Tettnanger, Spalt, and Saaz.

The game is on to breed new cultivars with interesting novel tastes. Where will it lead? Beer tasting of sausage and sauerkraut, perhaps?

Other references:

http://de.wikipedia.org/wiki/Founders_Organic_Brewery

http://en.wikipedia.org/wiki/Craft_beer#Craft_beer

http://en.wikipedia.org/wiki/Saaz_hops

http://en.wikipedia.org/wiki/Hops

AACSS 2013: Abstracts

Abstracts of the 2013 Scientific Meeting of the Australasian Association for ChemoSensory Science (AACSS) held at Phillip Island, Victoria, Australia, 16-18 August 2013.

Anandasankar Ray

PLENARY LECTURE

U.C. Riverside, USA

Insects that transmit deadly diseases to hundreds of millions of people every year and destroy nearly a third of agricultural output utilize their olfactory system to find hosts. We have developed computational and neurophysiological approaches to identify odorants that modify the responses of highly conserved olfactory receptors involved in attraction towards hosts. Using these odorants we can modify the host-seeking behavior of these deadly insects in two ways: "mask" their attraction to us and "pull" them away from us towards traps. In a second line of research we have identified highly conserved receptors that detect repellents such as DEET. Using computational and neurophysiology approaches we have identified several new repellents with greatly improved properties. The integrating the three approaches of "mask", "pull" and "push" provide the foundation for a new generation of odor-based strategies for behavior control agents that can have tremendous impact in the control of disease such as malaria and dengue globally.

1. FUNCTIONAL ANALYSIS OF NEMATODE GPCRS IN YEAST

Muhammad Tehseen, Alisha Anderson, Mira Dumancic, Lyndall Briggs and Stephen Trowell.

CSIRO Food Futures Flagship and Division of Ecosystem Sciences, Black Mountain Laboratories, Canberra, Australia.

The yeast *Saccharomyces cerevisiae* is well-known available host for human G-protein coupled receptor ligand screening due to the ease of genetic manipulation, low cost, rapid growth, and eukaryotic secretory pathway. Although the *Caenorhabditis elegans* genome was sequenced 13 years ago and encodes over 1,000 GPCRs, of which several hundred are believed to respond to volatile organic ligands, only one of these receptors, ODR-10, has been linked to a specific ligand, 2,3-butanedione. Odr-3 is potential G-protein αsubunit involved in odorant detection and activated by Odr-10. Here we report the functional coupling of the Odr-10 to yeast pheromone signalling pathway using the yeast - *C. elegans* chimeric Gα subunit (Gpa1: Odr-3) was used. Interaction between Odr-10 with Odr-3 and chimaera was confirmed using split ubiquitin yeast two-hybrid system. We also report the tailoring of a *Saccharomyces cerevisiae* strain for the analysis of *C. elegans* olfactory receptor function. In this study, a yeast *gpa1*△ *ste2*△*sst2*△*far1*△quadruple mutant was used to develop a strain that efficiently couples a nematode olfactory receptor with the yeast signalling pathway. We used two different reporter genes: green fluorescent protein (GFP) and LacZ to verify activation of the signal transduction pathway by ligand-GPCR interactions. With this heterologously engineered yeast system, we will be able to accelerate the de-orphaning of *C. elegans* GPCR proteins.

2. TASTE RECPTORS AND FOOD PREFERENCES IN *DROSOPHILA*

Anupama Arun Dahanukar

U.C. Riverside, USA

Animals rely on their taste systems to select appropriate foods for consumption. We use the model insect *Drosophila melanogaster* to understand the mechanisms by which taste neurons recognize various categories of chemicals. Sweet and bitter compounds are detected by members of a large family of Gustatory receptors (Grs), which are expressed in complex combinatorial patterns in taste neurons. Functional analysis of Grs is complicated by the observation that individual taste neurons express multiple receptors, and also by the failure to develop a suitable ectopic expression system. We have developed a novel *in vivo* expression system to "decode" taste receptors and have found that individual Grs belonging to a highly conserved clade of eight Grs are determinants of sweet ligand specificity. Interestingly, we also found that these "sweet" Grs are directly and selectively inhibited by bitter alkaloids, suggesting combinatorial mechanisms for sweet and bitter detection. We have recently tested an internal nutrient-sensing receptor, Gr43a, and its *An. gambiae* ortholog, AgGr25 in our ectopic expression system and found that both receptors show robust response to fructose and are broadly tuned to sugars. We are now poised to further investigate mechanisms of Gr function in *Drosophila* and other insects.

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3. DEVELOPMENT OF A HIGH THROUGHPUT CELL-BASED ASSAY FOR CHARACTERIZING LEPIDOPTERAN OLFACTORY RECEPTORS

Jacob A. Corcoran[1,2], Melissa D. Jordan[1], Richard D. Newcomb[1,2]

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The development of rapid and reliable assays to characterize insect odorant (ORs) and pheromone receptors (PRs) remains a challenge for the field. Previously insect ORs and PRs have been functionally characterized *in vitro* through expression in *Xenopus* oocytes, Sf9 cells and HEK293 cells. While these approaches have succeeded, these systems have inherent characteristics that prohibit them from being used in high throughput formats. We have developed an assay system whereby we can functionally characterize insect ORs and PRs in 96-well plates using a fluorescent spectrophotometer.

We began by making a T-REx HEK293 cell line capable of regulating the transcription of genes from plasmids containing the 'tetracycline operator' promoter sequence. After confirmation of Tet-Repressor function, this cell line was stably transfected with the *Epiphyas postvittana* OR co-receptor (EposORCO). The cell line was then single-cell sorted and resulting clones were evaluated for inducible EposORCO expression by RT-PCR and western blot and localization by immunofluorescence. Receptor function was also confirmed by measuring calcium flux in response to the ORCO agonist, VUAA1. The isogenic cell line with the best inducible expression and function of EposORCO was chosen for subsequent expression and characterization of *E. postvittana* ORs and PRs.

To date we have identified 72 putative olfactory receptors from genome and transcriptome libraries of *E. postvittana*. Quantitative RT-PCR analyses have revealed a subset of these genes that display male-biased antennal expression, making them likely candidates for being *E. postvittana* PRs. Here we report the responsiveness of one of the male-biased *E. postvittana* receptors to the moth's sex pheromone components to demonstrate the utility of the system.

4. LIGAND-BINDING OF THREE PHEROMONE-BINDING PROTEINS IN THE BEET ARMYWORM *SPODOPTERA EXIGUA*

Nai-Yong Liu[1,2], Fang Yang[1], Alisha Anderson[2], Wei Xu[2], Shuang-Lin Dong[1]*

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Moth pheromone-binding proteins (PBPs) play crucial roles in pheromone reception. In *Spodoptera exigua*, three SexigPBPs (PBP1-3) were cloned from the antenna, and are classified into three distinct sub-groups. Considering the sex pheromones of *S. exigua* have been identified as a four-component blend, it is hypothesized that each PBP may be tuned to a specific component (s) of the blend. To test this hypothesis, we carried out ligand-binding assays of three SexigPBPs to sex pheromones, pheromone analogs and plant volatiles. Our results show all three SexigPBPs can bind all tested sex pheromones, suggesting these SexigPBPs have no obvious discrimination among different pheromone components. However, SexigPBP1 exhibited much stronger binding affinities to sex pheromones and their analogs than the other two SexigPBPs (PBP1 >> PBP2 > PBP3), especially those ligands with a double bond in the 9th position. Plant volatiles proved to be poor ligands for all three SexigPBPs. In addition, binding of SexigPBP1 and SexigPBP2 to the main sex pheromone Z9,*E*12-14:Ac were strongly affected by pH, in contrast binding affinity of SexigPBP3 appeared to be only slightly affected. Similar results were also observed in its sibling species S. *litura*. Our results suggest that SexigPBP1 might play major roles in the process of female sex pheromone reception.

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5. SENSING SIMILARITIES: DO PIGS TASTE LIKE HUMANS?

Nadia de Jager[1] and Eugeni Roura[1]

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The exploration of the complexity of the taste system is gaining momentum, particularly in light of its role in nutrient sensing. The diverse localisation of the expression of taste receptor (TR) genes in oral and in non-oral tissues, such as in the gastrointestinal tract, has been established, predominantly as a result of rodent research. However, rodents and humans are often quite dissimilar in terms of their dietary habits and digestive physiology. In contrast, the pig has gained status as a main biomedical and nutritional model for humans. Our primary objectives are firstly to catalogue and study the TR repertoire; and secondly to explore the notion that this system is involved in mediating the nutritional status and appetite in pigs. Our results from quantitative real time PCR assays show that the pig and the human TR repertoire share high similitude, except when considering the bitter family (Tas2R). For example, while humans have 25 Tas2Rs, the pig only has 16. Expression of the genes encoding sweet and umami (Tas1R1, Tas1R2, Tas1R3), fatty acid (GPR120, GPR40, GPR41, GPR43 and GPR84), glutamic/amino acid (mGLUR1, mGLUR4, GPRC6A, and GPR92) sensing receptors have all been identified in pig tissues as a result of our research. With the exception of Tas1R2, which is not always abundantly expressed, the tissues in which we have confirmed TR expression include tongue, stomach ridge and -antrum, duodenum, jejunum, ileum, colon proximal and distal, caecum and liver. In addition, we have studied the impact of nutritional interventions on changes in the level of TR expression and preliminary results will be discussed. Overall, our results support the view that pigs have a well-developed taste system and, with the exception of the bitter taste family, that a high similarity is shared with humans.

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6. SPECIES-SPECIFIC SETS OF OLFACTORY RECEPTOR NEURONS IN INSECTS

Kye Chung Park *(New Zealand Institute for Plant and Food Research)*

Insects exhibit highly sensitive and selective peripheral olfactory sensory system. The olfactory sensillum, an independent unit housing one or a few olfactory receptor neurons (ORNs), is the key element of detecting external chemical signals, and delivers the information to the brain for further processing. Here we show the electrophysiological response profiles of ORNs in six different insect species (four species of moths and two species of weevils) to a panel of their host and non-host plant volatile compounds. Many of the ORNs were highly specialized for detecting a narrow range of volatile compounds although some ORNs showed broad spectrum responses. The profiles of ORNs were species-specific, and the differences in the ORN profiles appeared to be greater between the two different taxonomic groups (moths vs. weevils) than those between species within the same taxonomic group. The ORNs and their corresponding active volatile compounds in each species could be correlated to its host and non-host plants. Each species appeared to have two distinct sets of ORNs, one specialized for the host-plant specific volatile compounds and the other for non-host plant specific volatile compounds. It is suggested that these phatophagous insects use the combinational input from these two sets of ORNs to locate their host plants and discriminate them from non-host plants.

Key words: electrophysiology, host plant, insect, olfaction, olfactory receptor neuron, sensilla, volatile compounds

7. PHENOTYYPIC EXPRESSION OF OLFACORY RELATED GENES IN SEXULAS AND WORKERS OF THE LEAF-CUTTING ANT *A.VOLLENWEIDERI*

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Leaf-cutting ants are highly derived eusocial insects, having enormously large colony sizes. Their division of labor is mainly based on olfactory communication, where different sized workers perform distinct odor-guided behaviors [1]. Workers and sexuals (queens and males) of *A. vollenweideri* were found to exhibit different antennal lobe (AL) phenotypes, indicating differences in odor information processing [2,3]. For example large workers have one extremely large glomerulus in the AL (macroglomerulus, MG), and it is associated with trail-pheromone detection [2]. ALs fo males contain three MGs which most likely are involved in sex-pheromone detection. We investigate the molecular basis of the developmentally induced plasticity in *A. vollenweideri* by analyzing antennal transcripts and phenotype-specific microarrays. Within the antennal dataset we identified 70 odorant-receptor coding genes (OR), as well as the ortholog of the Olfactory Co-receptor (Orco). Analyzing the microarray data revealed that one OR is highly expressed in large workers compared to tiny workers (relative expression >3). In males, two ORs are highly expressed in the antenna compared to queens. Due to the one glomerulus - one receptor wiring in the AL [4], high expression levels of ORs are likely to correlate with glomerular size, making them potential trail and

sex pheromone receptors. Most of the ionotropic receptor coding genes identified in *A. vollenweideri* are highly expressed in males compared to queens. Odorant binding protein coding genes (OBPs) and chemosensory protein coding genes (CSPs) show no consistent differential expression pattern across the worker subcastes or the sexuals.

Funded by DFG: SPP 1392

8. A PHOSPHOLIPID FLIPPASE REQUIRED FOR OLFACTORY RECEPTOR NEURON FUNCTION IN *DROSOPHILA MELANOGASTER*

Michelle Pearce, Yu-Chi Liu, Takahiro Honda, Marien deBruyne, Coral G. Warr

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Drosophila olfactory perception is initiated when a chemical ligand from the environment binds to an odorant receptor (OR) localised to the dendrites of odorant receptor neurons (ORNs). In a search for new olfactory genes, we undertook a large scale EMS-induced mutant screen using electrophysiology to test for olfactory defects. We found a single mutant with a pronounced reduction in olfactory response to all odorants tested, with the exception of CO_2. Deficiency mapping identified a region consisting of twelve candidate genes of which one, *dATP8B*, was identified as containing a nonsense mutation in the mutant strain using whole genome re-sequencing. A complementation test confirmed mutations in this gene as the cause of the olfactory defect. RT-PCR and RNAi analyses indicate that dATP8B is expressed in a tissue specific manner, and is required in ORNs expressing ORs respectively. This gene encodes a phospholipid flippase, a protein subfamily thought to be essential for maintaining asymmetry of phospholipids in some lipid membranes, whereby phosphatidylserine and phosphatidylethanolamine are enriched in the cytosolic leaflet. As an otherwise uncharacterised gene, dATP8B has not been implicated in other cellular processes, and no other phospholipid flippase has been implicated in olfaction. Future work will include an investigation of the sub-cellular location of this flippase, and analyses of the other five *Drosophila* genes belonging to this family to determine their roles in olfaction and in nervous system function.

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9. MOLECULAR BASES OF OLFACTION AND OLFACTORY PLASTICITY IN LEPIDOPTERA: TRANSCRIPTOMIC APPROACHES IN THE COTTON LEAFWORM *SPODOPTERA LITTORALIS*

Emmanuelle Jacquin-Joly[1], Aurore Gallot[1-2], Arthur de Fouchier[1-3], Erwan Poivet[1], Fabrice Legeai[2], Nicolas Montagné[3]

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The sense of smell is a critical determinant of vital insect behaviours, including mate and food seeking, oviposition and predator avoidance. We have established the cotton leafworm, *Spodoptera littoralis*, as a nocturnal insect model to decipher the underlying mechanisms. We developed a transcriptomic approach: 1) to describe a large repertoire of chemosensory genes in both adults and larvae, 2) to address olfactory plasticity via digital gene-expression profiling.

Sanger and next generation sequencing strategies allowed us annotating ~ 77000 expressed contigs. Among them, we described a large repertoire of candidate odorant-binding and chemosensory proteins, ionotropic receptors and olfactory receptors, these latter being currently deorphanized via expression in the *Drosophila* empty neuron system coupled with single neuron electrophysiological recordings. Comparison between adults and larvae revealed different but somewhat overlapping expression of the chemosensory genes in the different developmental stages.

The transcriptome was also used to investigate the transcriptional changes induced by starvation in the chemosensory tissues of *S. littoralis* caterpillars, using RNAseq expression profiling. ~4 million short reads obtained from antennae and maxillary palps dissected from fed and 24 h starved larvae were mapped on the reference transcriptome and counted. We revealed both up- and down-regulated transcripts upon starvation, including genes potentially involved in olfaction.

These approaches not only establish the use of transcriptomic sequencing for the identification of divergent chemosensory receptors in a species for which no genomic data are available, but also enable investigation of chemosensory modulation via digital gene-expression profiling.

10. ANATOMICAL DEVELOPMENT OF THE HUMAN SENSE OF TASTE: COMPLETE BY MID-CHILDHOOD.

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During the past decade Laing and colleagues have shown that the anterior region of the human tongue which is rich in taste-sensitive fungiform papillae (FP) and receptor cells, ceases to grow in size by 8-10 years of age. In contrast, the posterior region, largely devoid of FP, ceases growth at 15-16 years. Importantly, the similar size of the anterior tongue of children and adults allows a direct comparison of FP density which reflects taste sensitivity. Accordingly, the present study with 30 adults and 85 7-12 year olds, aimed to determine the age at which FP density, hence sensitivity, stabilizes, and whether FP density in a small region of the anterior tongue can be used to predict total FP density/numbers. A digital camera was used to photograph the tongue and FP, whilst Adobe Photoshop software allowed analysis of the data. The results indicated that the number of papillae stabilized at 9-10 years of age, whilst the distribution and growth of FP stabilized at 11-12 years. Importantly, a single sub-population of FP predicted the density of FP on the whole anterior tongue of 7-10 year olds, whereas another was the best predictor for the older children and adults. Overall, the population, size and distribution of FP stabilized by 11-12 years of age which is very close to the age that cessation of the growth of the anterior tongue occurs. Clinically, the procedure has been used as a non-invasive assessment of taste loss during cancer, chronic kidney disease, and otitis media.

11. THE NOSE TELLS US WHAT TO SEE: MODULATING EFFECT OF ODOURS ON VISUAL PERCEPTION

Amanda Robinson, Jason B. Mattingley and Judith Reinhard

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Multisensory integration is an inherent feature of perception yet the interactions between olfaction and vision are not well understood. While it is known that vision can influence how we perceive certain odours, few studies have provided conclusive evidence that odours can impact on the visual sense. This study investigated whether odours influence selective attention towards familiar visual objects during the attentional blink (AB), a phenomenon where the second of two targets in a rapid serial visual presentation stream (RSVP) is undetected if it appears 200-500ms after the first target, due to a bottleneck in the temporal allocation of selective attention. Participants monitored a rapid stream of colour photographs of objects to discriminate which visual object with a characteristic smell (T2; lemon, orange, rose or mint) appeared after an initial target (T1). During the task, participants were exposed to an odour that was congruent, incongruent or irrelevant with respect to T2. We found that a congruent odour significantly enhanced the salience of the odour-related image and attenuated the attentional blink compared to an incongruent or irrelevant odour. This effect was not observed when the odour cue was replaced by a word cue, demonstrating that it was a sensory and not semantic effect. Indeed, a follow-up EEG study showed that congruent odours significantly enhance early neural responses to matching visual objects, implying an important influence of olfactory inputs on early visual responses. Our results provide evidence that our visual perception is significantly influenced by our sense of smell.

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12. *OSTRINIA NUBILALIS* STRAIN SPECIFIC PHEROMONE PERCEPTION: FIRST PHYSIOLOGICAL AND MOLECULAR MAP

F. A. Koutroumpa[1,2,3], Z. Kárpáti[1,4], C. Monsempes[3], S. Hill[1], B.S. Hansson[5], E. Jacquin-Joly[3], J. Krieger[6], T. Dekker[1]

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Moth pheromone communication is particularly illustrative in the study of evolution of signals for species recognition and subsequent premating isolation. We searched for the mechanism that caused Ostrinia nubilalis to display a dimorphism in pheromone preference. More precisely how does the behavioral preference and physiological differences between strains correlate with the molecular 'code' of the olfactory system? We combined whole mount double in situ hybridization and quantitative PCR on the seven pheromone-receptors of O. nubilalis, with single sensillum recordings on sensilla trichoidea. We found only one sensillum type housing sensory neurons tuned to pheromone. This sensillum contained three sensory neurons detecting the two pheromone components (Z11-14:OAc and E11-14:OAc) and the behavioral antagonist (Z9-14:OAc), respectively. We demonstrate that parental strains and hybrids differ consistently in the specificity of the 3 co-housed neurons, with transcripts' expression differences and reshuffling of sensory neuron size, not only between the E11 and Z11 neuron but among all neurons that co-inhabit this sensillum. We also demonstrate that sensory neurons mediating behavioral antagonism may co-express an unusually high number of receptors, conferring broad tuning to behavioral antagonist. This elegantly allows the 'olfactory code' to keep its simplest tripartite setup, while not affecting olfactory precision. Finally, we found an unusual dichotomous receptor expression in a single sensillum type, indicative of an intermediate step in a sensillar split. These findings are of importance in understanding coding and evolution of pheromone preference and antagonism.

13. CHEMOSENSORY GENES FROM COTTON BOLLWORM *HELICOVERPA ARMIGERA*

Wei Xu, Alexie Papanicolaou and Alisha Anderson

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The chemosensory system is critical in guiding insect behaviours. *Helicoverpa armigera* (Hübner) is one of the most polyphagous pest species in the world, so studies on its chemosensory system may give insights into the molecular mechanisms that underlie its ability to exploit a wide variety of host crops and help develop new approaches to control it. We sequenced and assembled transcriptomes from larvae antennae, larvae mouthparts, adult heads, adult tarsi and female adult abdomen. We identified fifty-eight odorant receptors (ORs), nine gustatory receptors (GRs), thirty-eight odorant binding proteins (OBPs) and nineteen chemosensory proteins (CSPs). The expression pattern of these genes were also analysed among different tissues, ages and genders. Seven candidate pheromone receptor (PR) genes were analysed by calcium imaging analysis and HarmOR13 showed significant responses to the major sex pheromone component, (z)-11-hexadecenal. This study will improve our understanding of the insect chemosensory system and assist in the development of more environmentally friendly, pheromone based control strategies.

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14. SEX PHEROMONE EVOLUTION AND SPECIATION IN THE NEW ZEALAND ENDEMIC LEAFROLLER MOTHS OF THE GENERA *CTENOPSEUSTIS* AND *PLANOTORTRIX*: THE MALE'S STORY

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The major feature of mate recognition in moths is the ability of the male to distinguish between sex pheromone blends of their own and other species. While we are beginning to understand the molecular basis for differences between species of moths in their ability to produce distinct pheromone blends, little is known of how the males' pheromone reception system evolves to track any newly evolved pheromone blend, especially at the molecular level. To address this question we are studying sibling species pairs within the New Zealand endemic leafroller genera *Ctenopseustis* and *Planotortrix*. To identify putative pheromone receptors preliminary genome assembles and antennal transcriptomes of *C. obliquana* and *P. octo* were searched and odorant receptor genes tested for male-biased expression in their antennae by quantitative RT-PCR. To date, five candidate pheromone receptors from *C. obliquana* and *C. herana* and three from *P. octo* and *P. excessana* have been identified. Functional assays reveal that OR07 from the *Ctenopseustis* species responds to (Z)-8-tetradecenyl acetate. Current work involves further functionally testing candidates in cell-based assays for the ability to detect sex pheromone components and making comparisons between species both in receptor sequence and gene expression levels to identify the differences between species in the male's side of mate recognition.

15. A MENDELIAN TRAIT FOR OLFACTORY SENSITIVITY AFFECTS ODOR EXPERIENCE AND FOOD SELECTION

Sara R. Jaeger[1], Jeremy F. McRae[1], Christina M. Bava[1], Michelle K. Beresford[1], Denise Hunter[1], Yilin Jia[1], Sok Leang Chheang[1], David Jin[1], Mei Peng[1], Joanna C. Gamble[1], Kelly R. Atkinson[1], Lauren G. Axten[1], Amy G. Paisley[1], Leah Tooman[1], Benedicte Pineau[1], Simon A. Rouse[1], Richard D. Newcomb[1,2,3].

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Humans vary in sensory acuity to many odours. However, the importance of genetic variation and how such variation also affects odor experience and food selection remains uncertain, given that such effects have been shown in bitter taste perception. Using a genome wide association approach we identified major loci for sensitivity to four odours (2-heptanone, isobutyraldehyde, βdamascenone and βionone), each located at or near clusters of olfactory receptor (OR) genes. Subsequently we focused on βionone, which shows extreme sensitivity differences. βionone is a key aroma in foods and beverages, and is added to products in order to give a pleasant floral note. Genome-wide and *in vitro* assays demonstrate rs6591536 as the causal variant for βionone odor sensitivity. Rs6591536 encodes a N183D substitution in the second extracellular loop of OR5A1, and explains >96% of the observed phenotypic variation, resembling a monogenic Mendelian trait. Individuals carrying genotypes for βionone sensitivity can more easily differentiate between food and beverage stimuli with and without added βionone. Sensitive individuals typically describe βionone in foods and beverages as 'fragrant' and 'floral', whereas less sensitive individuals describe these stimuli differently. Rs6591536 genotype also influences emotional associations, and explains differences in food and product choices. These studies demonstrate that an OR variant that influences olfactory sensitivity can affect how people experience and respond to foods, beverages and other products.

16. NEUROETHOLOGICAL BACKGROUND OF THE EUROPEAN CORN BORER PHEROMONE SYSTEM

Zsolt Karpati

The European corn borer moth (ECB, Ostrinia nubilalis; Pyralidae) is a well-known pest of the corn in the northern hemisphere. Beside its pest status the species has a pheromone polymorphism system. Two races exist which use opposite ratio of the two pheromone components causing reproductive isolation in nature.

We studied how the difference in male pheromone preference correlates with differences in wiring of olfactory input and output neurons in the primary olfactory neuropil, the antennal lobe (AL). Activity dependent neuronal staining, intracellular recording and immunocytochemistry were used to establish the structure and function of male olfactory receptor neurons (ORNs) and AL projection neurons (PNs). Both races have an indistinguishable pheromone sensitive macroglomerular complex (MGC) but a reversed topology. Apparently, the single-gene-mediated shift that causes a radical change in behavior is located upstream of the antennal lobes, i.e. at the ORN level. However, hybrids of the two races prefer intermediate ratios. How a topological arrangement of two glomeruli can accommodate for an intermediate preference was unclear. PN recordings and stainings in hybrids show a dominance of the E-type MGC topology, but volumetric measurements of the MGC and antennal response shows an intermediate preference.

We also studied how the response specificity of the males changing during the orientation flight using a wind tunnel behavioral assay. Males that have 'locked on' to a natural ratio pheromone plume readily continue plume following along off-ratio blends. This implies that males generalize pheromone quality after initial contact. The relaxation of specificity observed here may also help explain the substantial and unexpected rates of field hybridization between races. At high population densities, males that have first encountered filaments of pheromone from their own race may stray into a plume from the other race and then hybridize.

Upcoming Events

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19 – 21 November 2013	Air Quality Measurement - Methods and Technology Sacramento, California www.measurements.awma.org
28 – 31 January 2014	Australian Neuroscience Society (ANS) Adelaide, South Australia www.ans.org.au
9 – 14 April 2014	AChemS Association for Chemoreception Sciences Bonita Springs, Florida, USA www.achems.org
31 May – 3 June 2014	Odors and Air Pollutants Conference Miami Florida www.wef.org/odorsair
30 July – 1 August 2014	The Sensometric Society Chicago, USA www.sensometric.org
7-11 September 2014	ECRO: European Chemoreception Organisation Dijon, France www.ecro-online.com
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