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# ABSTRACTS

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### Assessing kindship through scent – the mouse model

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The ability to recognize kin has important potential fitness benefits in a variety of social contexts including parent-offspring recognition, inbreeding avoidance and cooperative breeding. While animals may recognize familiar individuals encountered during early development, using prior association during a sensitive period as a proxy for relatedness, many animals are able to recognize relatives regardless of prior familiarity. This implies the use of genetic markers to assess kinship, by phenotype matching to a recognition template learned from self and/or from familiar relatives. Scent cues have been strongly implicated in the ability to recognize unfamiliar kin across a broad range of species, although identification of the specific genetic markers used to recognize kin has proven particularly difficult. This is because, necessarily, polymorphic kinship markers must correlate strongly with sharing across the rest of the genome in normal animals. The genetic control provided by inbred laboratory mice has played a key role in identifying MHC-associated odours as one candidate marker, while our work on wild house mice has identified major urinary proteins (MUPs) as another candidate marker. Here I will critically review evidence from the different mouse models and experiments that have been used to assess the genetic markers in scent and the recognition templates involved in kinship assessment. I will also present some of our recent findings from experiments using wild mice, looking at both inbreeding avoidance and cooperation between females.

### Chemosensory signals, receptors, and behavior

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In terrestrial animals, a variety of social and sexual behaviors are regulated by chemosignals called pheromones that act via the olfactory or vomeronasal system. Mice utilize both volatile and non-volatile pheromones that are recognized by olfactory receptors (ORs) and vomeronasal receptors, which belong to the G protein-coupled receptor superfamily.

In contrast, insect chemosensory receptors are ligand-activated nonselective cation channels. In both vertebrates and invertebrates, more and more chemosensory receptors have been deorphanized. Until recently, however, little has been known about volatiles emitted from individual animals that act as ligands for ORs in natural environments. Activity-guided fractionation of exocrine gland extracts and subsequent chemical analysis resulted in identification of unsaturated aliphatic alcohol as a natural ligand for a mouse OR.

Recently, we discovered the mouse OR that specifically recognized muscone, a unique macrocyclic odor utilized as a chemosensory cue in dear musk, and also the human muscone receptor.

These studies pave the way to exploring the function of each OR in a physiological context and the molecular basis for complex chemical communication between animals. Considering the number of orphan chemosensory receptor genes, there exist more previously-unidentified signaling molecules that affect animal behaviors.

### Odor coding, identification and evaluation in insect neural networks

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Much progress has been made recently in understanding how neural networks accomplish olfactory coding. The time is ripe to pull these results into a proposed connectivity network. Here, I will propose a wiring diagram for the major steps from peripheral processing all the way to behavioral readout, using insects as models. The major players are the antennal lobe (first processing network), the mushroom bodies (most complex brain structure, crucial for learning) and the lateral protocerebrum (containing the premotor control areas). Processing steps include a sequence of: (1) lateral inhibition in the antennal lobe, (2) nonlinear synapses, (3) threshold-regulating gated spring network, (4) selective lateral inhibitory networks across glomeruli, (5) feed-forward inhibition to the lateral protocerebrum. These cover most of the experimental results from different research groups and model species

polymorphisms (SNP) across 74 genomes belonging to 14 different pig breeds. The population genomic study shows high variability of the Tas2R family linked to breeds of specific geographical origins. Thus, our data suggests that the porcine bitter taste is a plastic trait, possibly associated with the ability of pigs to adapt to diverse environments. In conclusion, pigs and chickens have a fully developed taste and nutrient sensing system. While the nutrient sensors are highly conserved, in contrast, the Tas2R repertoire shows high diversity both between species and between pig breeds. The porcine Tas2R polymorphisms may constitute additional evidence of their role in environmental/dietary adaptations.

## Relating taste receptor function to feeding ecology and evolution: a cautionary tale

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With the increased availability of genomic data, it is now possible to ask evolutionary questions about interspecific differences in taste function—e.g., how diet influences the evolution of taste receptors. This comparative approach to taste offers great promise, but one must keep in mind that (a) there are probably many as-of-yet unidentified taste receptors, and (b) not all interspecific differences in taste receptor expression reflect evolutionary adaptations. I will illustrate this point by discussing recent work on the sugar (T1r2/T1r3) and "bitter" (T2r) taste receptors. First, T1r3 KO mice are indifferent to sugars during their initial exposure, but nevertheless condition strong and enduring preferences for sugars following dietary exposure. Further, my colleagues and I recently discovered that T1r3 KO and T1r2/T1r3 KO mice display normal cephalic-phase insulin release in response to oral stimulation with sugars. Thus, one must be careful assuming that the presence of a functional T1r2/T1r3 taste receptor is necessary for a species to generate adaptive behavioral and physiological responses to sugars. Indeed, it is likely that there are additional taste receptors for simple and complex carbohydrates. Second, vertebrate species vary in the number of T2rs that they express. Because plant tissues contain a relatively high abundance of "bitter" and potentially toxic compounds, it has been hypothesized that natural selection would have favored the evolution of a more diverse repertoire of T2rs in herbivorous animals. Implicit in this hypothesis is the assumption that animals with a relatively large number of T2rs have an enhanced ability to (a) detect poisonous compounds and (b) discriminate between harmful and harmless bitter-tasting foods. I will present several lines of evidence that contradict this assumption, and suggest that at least some interspecific differences in T2r expression reflect phylogenetic constraints rather than adaptive specializations.

## Tuning breadths and receptive ranges of avian bitter taste receptors

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The ability of vertebrates to taste the numerous and frequently toxic bitter compounds present in nature is important for the quality assessment of food and hence, the survival of species. Recognition of the structurally diverse bitter compounds is facilitated by specialized G protein-coupled receptors, the Tas2rs. Interestingly, the numbers of putatively functional *Tas2r*genes deviate considerably among vertebrate species ranging from very few, e.g. in teleostean fish and some birds such as chicken and turkey, to ~50 *Tas2rs* in frog. Based on the comparatively low number of oral taste buds, low salivation, and a lack of mastication, it was hypothesized that birds may possess an inferior taste system. Indeed, the absence of a functional *Tas1r2* gene, coding for the specific sweet taste receptor subunit and the small repertoire of only three *Tas2rs* may indicate reduced importance of the tasting abilities in chicken. However, other bird species such as the zebra finch with ~7 or the white-throated sparrow with ~18 *Tas2rs* possess more putatively functional *Tas2r* genes in their genomes approaching gene numbers found in mammalian species. In order to test whether a low *Tas2r* gene number in birds may correlate with a lower importance of bitter taste and to investigate the receptive ranges of avian Tas2rs we cloned and functionally expressed selected Tas2rs of the three bird species chicken, turkey and zebra finch. After transient transfection of the Tas2r-constructs into HEK 293T-Gα16gust44 cells, calcium imaging analyses using a panel of 46 different bitter compounds were performed. Our screening of the 3 functional chicken Tas2rs representing the entire bitter taste receptor repertoire of this species revealed that all three Tas2rs represent broadly tuned receptors recognizing large spectra of bitter substances. The two turkey Tas2rs, again representing the entire Tas2r repertoire of this species, are both similarly broadly tuned. Moreover, not only the breadth of tuning is conserved among chicken and turkey receptors, the two pairs of orthologous receptors exhibited an almost identical spectrum of agonists even though the species separated ~40 million years ago. Ongoing experiments with four of the seven zebra finch Tas2rs indicate that at least some of the receptors show a narrow tuning breadth. In summary, we conclude that a low number of *Tas2r* genes does not predict a reduced importance of the bitter tasting abilities in the corresponding species, whereas a higher number of functional *Tas2rs* may allow the development of more specialized narrowly tuned receptors.

the olfactory epithelium to the olfactory bulb and further into higher brain centers to evoke the sexual behavior. To identify $PGF_{2\alpha}$-activated OSNs and central neurons along the olfactory pathway, we used immunohistochemical labeling of phosphorylated Erk as a neuronal activation marker and GCaMP calcium imaging in transgenic zebrafish. Upon $PGF_{2\alpha}$ stimulation, neuronal activation was detected in a small population of ciliated OSNs in the olfactory epithelium, two ventromedial glomeruli in the olfactory bulb, and several nuclei in the ventral forebrain and hypothalamus. In addition, double in situ hybridization using c-Fos and olfactory receptor probes revealed two candidate receptors for pheromonal $PGF_{2\alpha}$. These results provide molecular and anatomical bases for the olfactory neural circuitry mediating sex pheromone-induced reproductive behavior.

## Birth and Migration of Sensory Neurons in the Adult Zebrafish Olfactory System

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Individual olfactory sensory neurons (OSNs) express only a single olfactory receptor (OR) gene from a large and diverse genomic repertoire. Typically, OSNs expressing the same OR are confined to distinct expression domains in the olfactory epithelium (OE) and converge onto the same glomeruli in the olfactory bulb; features that are conserved in organisms as disparate as vertebrates and invertebrates. Because of their direct exposure to the environment, OSNs have a limited lifetime and need to be continuously renewed even in adult organisms. In the rodent OE newborn OSNs are supposed to be generated from stem cell populations located in the basal OE and to migrate towards more apical positions as they adopt functional maturity. In the zebrafish OE, OR expression patterns can be recognized as concentric domains with OR-specific diameters and distributions. Contrary to the rodent OE, zebrafish OSNs are not predominantly generated in the basal OE but from two distinct zones of proliferative activity located at the inner curves between olfactory lamellae and at the peripheral sensory/non-sensory boundary of the olfactory rosette. Incubation with the proliferation marker BrdU and expression analysis of proneuronal and neuronal markers allowed us to track maturation of newborn OSNs after they exit mitosis. OSNs begin to express molecular markers of OSN differentiation, including OR genes, in close proximity to the sites of active neurogenesis. As they mature, OSNs invade the sensory epithelium by active migration and form an OR-specific annular pattern, suggesting that the observed similarity of zonal OR expression patterns in rodents and zebrafish are analogous features and generated by different mechanism.

## Expression of ancestral V2Rs shifts from the main olfactory epithelium of tadpoles to the water nose of adult Xenopus laevis

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In mammals olfactory receptor families are segregated into different olfactory organs, chief among them the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. Amphibians are early diverging tetrapods compared with mammals, and occupy an intermediate evolutionary stage concerning the water-to-land transition. Consequently, their analysis may shed light on this transition from shared sensory surface to segregated subsystems. We report here that a major olfactory receptor family of *Xenopus laevis*, V2Rs, is expressed in both olfactory organs of tadpoles, with the VNO expressing more 'modern', later diverging V2Rs, whereas more 'ancient', earlier diverging V2Rs are expressed in the MOE, together with the V1R family. Furthermore, amphibians such as *Xenopus* make their own ontogenetic transition from an obligate (tadpole) to a facultative aquatic stage in adults. During metamorphosis the MOE of *Xenopus* tadpoles transforms into an air-filled cavity (principal cavity, air nose), whereas a newly formed cavity (middle cavity) takes over the function of a water nose. We report here that larval expression of 'ancient' V2Rs is gradually lost from the main olfactory epithelium as it transforms into the air nose. Concomitantly, 'ancient' V2R expression begins to appear in the newly forming water nose. Responses to amino acid odorants are present in the tadpole MOE, and show the same transition, disappearing in the transforming air nose and concomitantly appearing in the new water nose, consistent with the hypothesis that amino acid responses may be carried by V2R receptors. Interestingly, 'ancient' v2r genes are expressed in a basal expression zone both in tadpoles and adults, so this feature of V2R expression is stable during the migration of expression from one olfactory epithelium to another during metamorphosis.