Gustatory Receptor Expression in the Brain of the Bumblebee, *Bombus terrestris*.

Research into the complete characterization of gustatory receptor (Gr) expression in insects is in its early days. At present, the core of the research currently undertaken has occurred in *Drosophila spp*. due to the wealth of information on their genomes. Recent findings have identified that in adult *Drosophila* a number of Grs have been seen to be expressed in the brain (Thorne and Amrein, 2008), and that this internal expression is used to detect haemolymph nutrient concentrations thereby effecting satiation and leading to behavioural changes. (Miyamoto et al. 2012)

The purpose of this research was identify how many unique Grs *B.terrestris* has, (i.e. excluding paralogs and pseudogenes) and to characterize the expression of the unique Grs within the brain tissue.

Method

Collection

Bees collected & placed in freezer for five minutes to induce torpor.

Dissection

Five brains were dissected & placed in Trizol to prevent degradation.

Extraction

RNA isolated using Phenol:chloroform phase seperation

Amplfication

- DNase
- Reverse Transcription
- PCR (Using specific primers, see below)

Visualisation

Amplified product ran on an agarose gel via electrophoresis

Primer Design

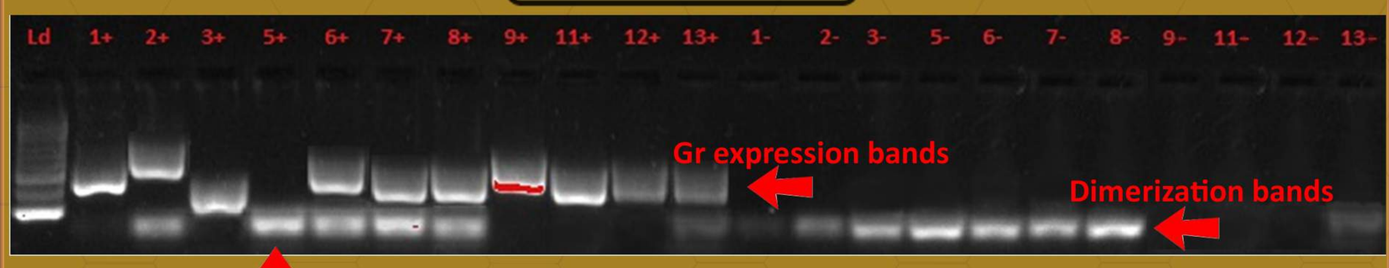
What Grs are unique in *B.terrestris*?
Specific primer design requires the identification of pseudogenes and paralogs within the genome to ensure sepcific annealing to only the Gr of interest. Professor Hugh Robertson supplied the original protein sequences & cDNA sequences for all 22 Grs with details of suspected pseudogenes and paralogs, this was further refined upon identification of Gr15 as being homologous (P=0.0) to Gr19 and Gr21. This left 11 unique Grs.

Primers were then designed using Primer3 and IDTs primer analysis tool (see table for sequences). Although every effort was made to avoid dimerization, there are still a number of primers which need resolved before quantatative analysis (qRT-PCR) can occur.

What is there predicted function?
The final step was to attempt to predict the protein function based on homology. Iterative searches were carried out using PSI-BLAST and potential functions predicted (See table)

	Direction	Primer Sequence	Ligand
BtGr1	Forward Reverse	GACCACGTTCGTATTCAATG CATCATCACTACTATGGTCACG	Trehalose
BtGr2	Forward Reverse	GCATAGACAGCAATTGCATAG GCGACCAGCATGATAAAC	Trehalose
BtGr3	Forward Reverse	GCGCACTGATACTACTGCTCG CATTACTCCAAGCACATCGC	Galactose, maltose & fructose
BtGr5	Forward Reverse	CAAGTACTCAACATGCGTC TGATCTCTTGGTATGCAGG	Unknown
BtGr6	Forward Reverse	CTACCTGATCGCTATGTACG TCCACTCTGATTAACCACC	Unknown
BtGr7	Forward Reverse	CTGATAGAGTACGCGTACACA GATCAACGTGCTTAATGTCC	Unknown
BtGr8	Forward Reverse	GAAGCCATGGTCACACTAA CCATTTGGCTTCTGGTAGT	Unknown
BtGr9	Forward Reverse	GGAATGTGGAAAAGTGAAG GCCAATGTTGATCTCGTAG	Unknown
BtGr11	Forward Reverse	CATCTCGCAGCCTATTACT GGATATGAACAGCTAGCGA	Galactose, maltose & fructose
BtGr12	Forward Reverse	GCAATGACGTTGATAGAACG GGCGTTCGTAAAGTAGCAGT	Unknown
BtGr13	Forward Reverse	GGATTATTGGATAACGATTCAAG GTTCATATCGGACATCTCAGAG	Unknown

Results



Agarose gel image shows that all Grs are expressed in the brain of *B.terrestris* except Gr5.

Discussion

TThe results indicate that, like *Drosophila, B.terrestris* also express a number of Grs in the brain.

The missing receptor Gr5 shares homology to Gr28b in *Drosophila*, suggesting the possibility that the function of Gr5 may follow that of one of the six genes on the Gr28b cluster. Although a wealth of information is available on Gr28b (See Thorne and Amrein, 2008) the complexity in expression across neurons means gaps still remains in our understanding. Only two of the six Gr28b genes have been seen to be expressed in the brain, whereas the remainder can be seen across the anatomy, some of which have various non-gustatory functions . For instance RNAi experiments have identified that Gr28b is expressed in class IV dendritic arborization neurons within the body of the larvae and is known to function as part of a phototransduction pathway (Xiung et al. 2010) whereas little expression is seen in class IV dendritic arborisation neurons in adults. Thus the unexpected function of Gr28b in *Drosophila* raises questions as to the role of Gr5 in *B.terrestris.*

What's next?
If Gr5 is not expressed in the brain, is it likely to be expressed elsewhere on the bees anatomy? If Gr28 is expressed in Drosophila larvae, can a similar function be seen in the larvae of *B.terrestris*? Can the predicted function of Gr5 be further refined using bioinformatics? What are the ligands for the remaining Grs? (Ligand screening?) And what are the relative rates of expression of each Gr? (qRT-PCR?)

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Xiang Y, Yuan Q, Vogt N, Looger LL, Yeh-Jan L, Nung-Jan Y (2010) Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. Nature 468:921-926

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