

**SHORT COMMUNICATION**

# The honey bee tyramine receptor AmTYR1 and division of foraging labour

Ricarda Scheiner\*, Leonora Kulikovskaja and Markus Thamm

**ABSTRACT**

Honey bees display a fascinating division of labour among foragers. While some bees solely collect pollen, others only collect nectar. It is assumed that individual differences in sensory response thresholds are at the basis of this division of labour. Biogenic amines and their receptors are important candidates for regulating the division of labour, because they can modulate sensory response thresholds. Here, we investigated the role of the honey bee tyramine receptor AmTYR1 in regulating the division of foraging labour. We report differential splicing of the *Amtyr1* gene and show differential gene expression of one isoform in the suboesophageal ganglion of pollen and nectar foragers. This ganglion mediates gustatory inputs. These findings imply a role for the honey bee tyramine receptor in regulating the division of foraging labour, possibly through the suboesophageal ganglion.

**KEY WORDS:** Splicing, Tyramine, Gene expression, Pollen, Nectar, Biogenic amines

**INTRODUCTION**

Honey bees, *Apis mellifera* Linnaeus 1758, have developed a highly sophisticated division of labour. In addition to age-dependent division of labour between hive bees and foragers, honey bee foragers display a form of age-independent division of labour. Some foragers solely collect pollen, while others only forage for nectar (Pankiw and Page, 2000). Surprisingly, knowledge on the division of foraging labour is still very limited. A widely accepted model (Robinson, 1992) assumes that division of labour is based on individual differences in sensory response thresholds. Pollen foragers indeed differ from nectar foragers in sensory response thresholds (Scheiner et al., 1999). Insect biogenic amines like octopamine and tyramine are promising candidates for regulating the division of labour through the modulation of sensory response thresholds. Whereas octopamine has been studied in detail and has been shown to affect age-dependent division of labour between nurse bees and foragers, tyramine has received little attention (for review, see Scheiner et al., 2006). However, the tyramine receptor gene *Amtyr1* is a candidate gene in a quantitative trait locus correlating with different aspects of foraging behaviour (Hunt et al., 2007) and tyramine can increase the sucrose responsiveness of nectar foragers to the level of pollen foragers (Scheiner et al., 2002).

We show that the only characterized tyramine receptor of the honey bee, AmTYR1 (Blenau et al., 2000), occurs in two isoforms at the mRNA level. We studied mRNA expression of both isoforms in pollen and nectar foragers in different brain neuropils. Our results

show a prominent role for the honey bee tyramine receptor in honey bee division of foraging labour.

**RESULTS AND DISCUSSION****Differential splicing of the tyramine receptor gene**

Using the updated next-generation transcriptome data for the honey bee ([www.ncbi.nlm.nih.gov/sra](http://www.ncbi.nlm.nih.gov/sra)), we found two different short reads homologous to *Amtyr1* which reveal alternative splicing close to exon 3 (Fig. 1A). This alternative splicing was confirmed by PCR (data not shown) and both isoforms were reported to GenBank (IDs JX963632.1 and JX963633.1). One isoform (*Amtyr1*) represents the published form (Blenau et al., 2000). However, we detected an additional exon (which is exon 1 in Fig. 1A). The other isoform (henceforth referred to as *Amtyr1ΔII*), lacks exon 2 immediately before the start codon of exon 3, including a stop codon in frame. Therefore, the open reading frame (ORF) of exon 1 will not be disrupted and potentially will be continued by the ORF of exon 3 (Fig. 1A). Although we did not detect an additional start codon on exon 1, we cannot exclude the possibility that there is another start codon in the unknown 5'-part of the *Amtyr1ΔII* mRNA. Northern blot analysis showed that the tyramine receptor *Amtyr1* mRNA is about 9.5 kb (Mustard et al., 2005). Combining the known sequence information (Blenau et al., 2000) with our new sequence information from cloning PCR and RACE PCR fragments, the known *Amtyr1* mRNA comprises only about 4.5 kb. This implies a large unknown part of this gene in the 5'-direction, which might include another as-yet unknown part of the ORF. Whether both isoforms are fully functional remains an open question. The mechanism that controls the differential splicing of the *Amtyr1* mRNA is not yet known. But it is conceivable that divergence of pollen and nectar foragers at the epigenetic level causes differential splicing and differential expression of *Amtyr1*.

**Division of foraging labour correlates with differential gene expression of one isoform**

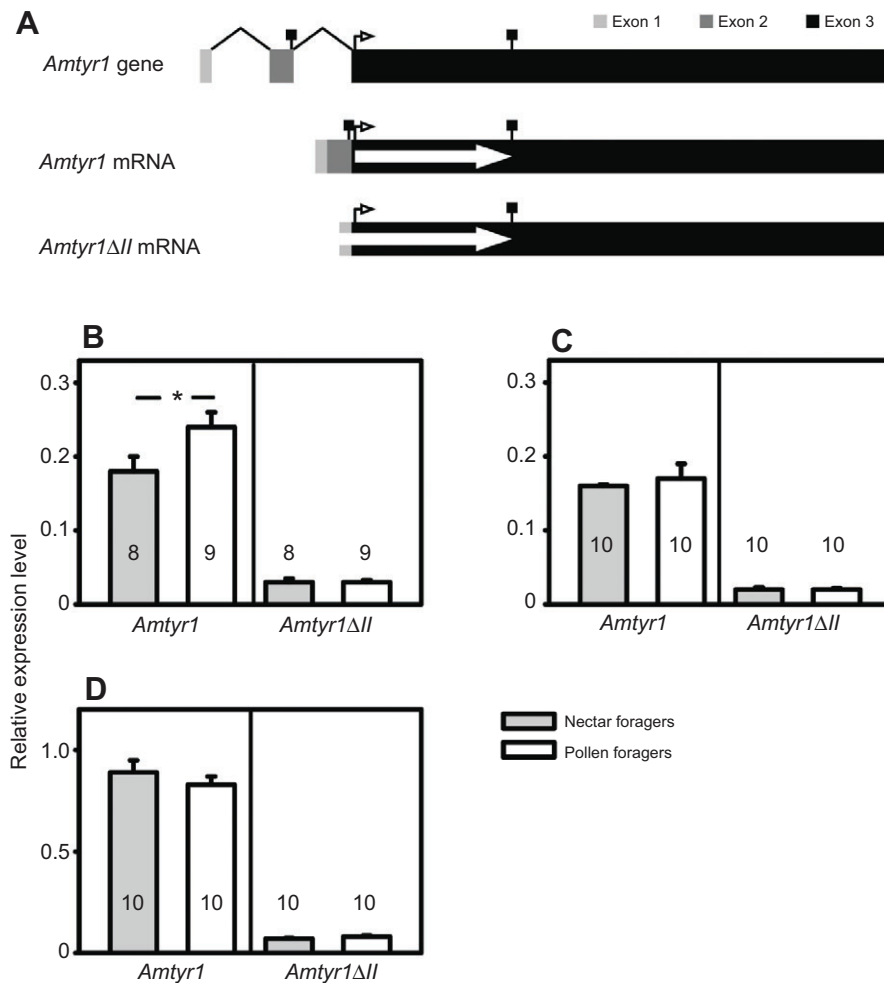
We tested whether division of foraging labour is correlated with differential expression of these isoforms. Pollen foragers displayed significantly higher mRNA expression of the *Amtyr1* isoform in the suboesophageal ganglion compared with nectar foragers (Fig. 1B;  $t=2.36$ ,  $P<0.05$ ,  $t$ -test). The other neuropils tested (i.e. optic lobes and mushroom bodies) did not differ in their mRNA expression of *Amtyr1* between foragers performing different tasks (Fig. 1C,D). The mRNA expression of the second isoform, *Amtyr1ΔII*, did not differ between pollen and nectar foragers in the different brain neuropils (Fig. 1B–D). These findings suggest an important role for the tyramine receptor AmTYR1 in honey bee division of foraging labour, possibly via the suboesophageal ganglion.

**MATERIALS AND METHODS****Honey bees and honey bee brain samples**

Bees were captured individually from a honey bee colony located at the University of Potsdam. Returning foragers were regarded as pollen foragers

Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany.

\*Author for correspondence ([rscheine@uni-potsdam.de](mailto:rscheine@uni-potsdam.de))



**Fig. 1. Gene structure and mRNA expression of the honey bee tyramine receptor AmTYR1.**

(A) The *Amtyr1* gene (structure shown in top line) appears in two mRNA isoforms (structures displayed in second and third lines). Exons are represented by boxes of different grey shades. Open reading frames are displayed as large white arrows. Start codons are represented by small arrows. Stop codons are depicted as black boxes. *Amtyr1ΔII* is the novel isoform. (B–D) Mean mRNA expression levels of both *Amtyr1* isoforms relative to the reference gene *ef1α-f1* in different brain regions. Means and s.e.m. are displayed. The significant difference in mRNA expression is indicated by an asterisk (\* $P < 0.05$ , *t*-test). The number of bees tested is indicated in each bar

when they had large pollen loads and no extended abdomen, and as nectar foragers when they had no pollen but an extended abdomen. Each bee was cooled and fixed as described elsewhere (Scheiner et al., 2013).

#### PCR, RACE PCR and quantitative real-time PCR (qPCR)

For PCR and RACE PCR, RNA preparation from bee brains was carried out using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Synthesis of cDNA, PCR and cloning of cDNA fragments was performed as described previously (Thamm et al., 2010). SMARTer RACE cDNA Amplification Kit (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) was used to amplify the 5'-UTR of the *Amtyr1* mRNAs. Primer sequences are given in

Table 1. Sequencing of cloned cDNA fragments was executed by GATC Biotech (Konstanz, Germany).

For qPCR, the following regions of the brain were dissected: suboesophageal ganglion (SOG), optic lobes (OL) and mushroom bodies (MB). Samples of the different brain tissues were collected from each bee and immediately frozen in liquid nitrogen. Total RNA extraction was carried out using peqGOLD Total RNA (Peqlab, Erlangen, Germany). From each total RNA sample, two independent cDNA syntheses were performed using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Life Technologies, Darmstadt, Germany). This was done to test the efficiency of cDNA synthesis. qPCR was carried out on a Rotor Gene Q (Qiagen, Hilden,

**Table 1. Sequences of the oligonucleotides used for PCR and qPCR**

Gene isoform	NCBI acc. no.	PCR and length (bp)	Direction	Sequence
<i>Amtyr1/Amtyr1ΔII</i>	JX963632.1/JX963633.1	PCR: 338/154	F R	CTCAGTGCCTCGTCAACG CTGTCATGTCGTAGTCCTCG
<i>Amtyr1</i> and <i>Amtyr1ΔII</i>	AJ245824	RACE PCR	R	AGCCTGTCATGTCGTAGTCCTCG
<i>Amtyr1</i>	JX963632 for F AJ245824 for R	qPCR: 102	F R TM	CAGGAAGCCAAGCGTCAATGAGG GGGAGCGTGACGTTGGA CAGCCTGTCATGTCGTAGTCCTCG
<i>Amtyr1ΔII</i>	JX963633 for F AJ245824 for R	qPCR: 97	F R TM	GATCAACCGATCTCAGAGAGCG CGCAGCCTGTCATGTCGTAG Cy5.5-GGGAATCAGGCGGGACGATGA-BBQ
<i>Ef1α-1F</i>	NM_001011628	qPCR: 394	F R TM	GAACATTTCTGTGAAAGAGTTGAGGC TTTAAAGGTGACACTCTTAATGACGC 6FAM-ACCGAGGAGAATCCGAAGAGCATCAA-BBQ

F, forward; R, reverse; TM, TagMan probe.

Germany) using TaqMan technology with different fluorescent dyes to allow duplex measurements for receptor and reference gene expression. TaqMan probes exhibit either 6-FAM-phosphoramidite (6FAM) or Cy5.5 as 5'-modifications and BlackBerry quencher (BBQ) as 3'-modification. Primer sequences are given in Table 1. The qPCR was performed with an initial step at 60°C for 1 min and 5 min at 95°C (enzyme activation), followed by 50 cycles at 95°C for 20 s and 60°C for 60 s. cDNA from each sample RNA was synthesized in duplicate. Each cDNA duplicate was examined in qPCR in triplicate. Mean copy number was calculated using Rotor Gene Q software (Qiagen). Transcript levels were normalized to *efla-fl* transcript levels (=100%) using the standard curve method ( $10^4$ – $10^8$  copies) (Reim et al., 2013).

### Statistics

Relative expression of *Amyr1* and *Amyr1ΔII* mRNA was compared between pollen and nectar foragers using two-tailed *t*-tests (SPSS 19, IBM).

### Acknowledgements

We like to thank Soojin Yi for fruitful discussions on an earlier version of this manuscript.

### Competing interests

The authors declare no competing financial interests.

### Author contributions

R.S. and M.T. outlined the experiments and wrote the manuscript. M.T. and L. K. performed the experiments.

### Funding

These experiments were supported by grants from the German Research Foundation (DFG) to R.S. [SCHE 1573/2-1, SCHE 1573/3-1, SCHE 1573/4-1].

### References

- Blenau, W., Balfanz, S. and Baumann, A. (2000). *Amyr1*: characterization of a gene from honeybee (*Apis mellifera*) brain encoding a functional tyramine receptor. *J. Neurochem.* **74**, 900–908.
- Hunt, G. J., Amdam, G. V., Schlipalius, D., Emore, C., Sardesai, N., Williams, C. E., Rueppell, O., Guzmán-Novoa, E., Arechavaleta-Velasco, M., Chandra, S. et al. (2007). Behavioral genomics of honeybee foraging and nest defense. *Naturwissenschaften* **94**, 247–267.
- Mustard, J. A., Kurshan, P. T., Hamilton, I. S., Blenau, W. and Mercer, A. R. (2005). Developmental expression of a tyramine receptor gene in the brain of the honey bee, *Apis mellifera*. *J. Comp. Neurol.* **483**, 66–75.
- Pankiw, T. and Page, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behav. Ecol. Sociobiol.* **47**, 265–267.
- Reim, T., Thamm, M., Rolke, D., Blenau, W. and Scheiner, R. (2013). Suitability of three common reference genes for quantitative real-time PCR in honey bees. *Apidologie* **44**, 342–350.
- Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**, 637–665.
- Scheiner, R., Erber, J. and Page, R. E., Jr (1999). Tactile learning and the individual evaluation of the reward in honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A* **185**, 1–10.
- Scheiner, R., Plückhahn, S., Öney, B., Blenau, W. and Erber, J. (2002). Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behav. Brain Res.* **136**, 545–553.
- Scheiner, R., Baumann, A. and Blenau, W. (2006). Aminergic control and modulation of honeybee behaviour. *Curr. Neuropharmacol.* **4**, 259–276.
- Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., Grünewald, B., Hahshold, S., Karrer, M., Koeniger, G. et al. (2013). Standard methods for behavioral studies of *Apis mellifera*. In *The COLOSS BEEBOOK: Standard Methodologies for Apis mellifera Research* (ed. V. Dietemann, J. D. Ellis and P. Neumann), pp. 1–58. Treforest: IBRA.
- Thamm, M., Balfanz, S., Scheiner, R., Baumann, A. and Blenau, W. (2010). Characterization of the 5-HT<sub>1A</sub> receptor of the honeybee (*Apis mellifera*) and involvement of serotonin in phototactic behavior. *Cell. Mol. Life Sci.* **67**, 2467–2479.