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# A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*<sup>☆</sup>

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## Abstract

G protein-coupled receptor (GPCR) genes are large gene families in every animal, sometimes making up to 1–2% of the animal's genome. Of all insect GPCRs, the neurohormone (neuropeptide, protein hormone, biogenic amine) GPCRs are especially important, because they, together with their ligands, occupy a high hierarchic position in the physiology of insects and steer crucial processes such as development, reproduction, and behavior. In this paper, we give a review of our current knowledge on *Drosophila melanogaster* GPCRs and use this information to annotate the neurohormone GPCR genes present in the recently sequenced genome from the honey bee *Apis mellifera*. We found 35 neuropeptide receptor genes in the honey bee (44 in *Drosophila*) and two genes, coding for leucine-rich repeats-containing protein hormone GPCRs (4 in *Drosophila*). In addition, the honey bee has 19 biogenic amine receptor genes (21 in *Drosophila*). The larger numbers of neurohormone receptors in *Drosophila* are probably due to gene duplications that occurred during recent evolution of the fly. Our analyses also yielded the likely ligands for 40 of the 56 honey bee neurohormone GPCRs identified in this study. In addition, we made some interesting observations on neurohormone GPCR evolution and the evolution and co-evolution of their ligands. For neuropeptide and protein hormone GPCRs, there appears to be a general co-evolution between receptors and their ligands. This is in contrast to biogenic amine GPCRs, where evolutionarily unrelated GPCRs often bind to the same biogenic amine, suggesting frequent ligand exchanges (“ligand hops”) during GPCR evolution.

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**Keywords:** GPCR; Neuropeptide; Neurohormone; Hormone; Biogenic amine; Genomics; Insect; Honey bee; *Drosophila*

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**Abbreviations:** AKH, adipokinetic hormone; CCAP, crustacean cardioactive peptide; CHO, Chinese hamster ovary; CRF, corticotropin releasing factor; DH, diuretic hormone; DLGR, *Drosophila* leucine-rich repeats-containing GPCR; ETH, ecdysis triggering hormone; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; ic3, intracellular loop 3; LGR, leucine-rich repeats-containing GPCR; LRR, leucine-rich repeat; mAChR, muscarinic acetylcholine receptor; MIP, myoinhibitory peptide; NPF, neuropeptide F; PDF, pigment dispersing factor; PLC, phospholipase C; sNPF, short neuropeptide F; 7TM, seven transmembrane

<sup>☆</sup> Nucleotide sequence data reported here are available in the CoreNucleotide or third party annotation section of the DDBJ/EMBL/GenBank databases under the accession numbers: AF498306; AJ245824; AJ547798; AY921573; AY\_961388–AY\_961391; AY\_961393–AY\_961396; BK005219; BK005220; BK005238–BK005242; BK005257–BK005259; BK005261–BK005269; BK005271; BK005273; BK005274; BK005684; BK005712; BK005714–BK005719; BK005754; DQ151547; DQ201783; XP\_394102; XP\_394798; XP\_395101; XP\_395760; XP\_396348; XP\_396445; XP\_396491; XP\_397077; Y13429.

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## 1. Introduction

Insects are the largest animal group in the world (75% of all species are insects) and are economically and ecologically extremely important, because most flowering plants depend on insects for their pollination. The honey bees alone, for example, pollinate 20 billion dollars worth of crop yearly in the United States. But insects can also be severe agricultural pests, destroying 30% of our potential annual harvest, and can be vectors (intermediate pathogen carriers) for major diseases such as malaria, sleeping sickness, dengue fever, yellow fever, and elephantiasis.

There are, at present, highly exciting developments occurring within the field of insect research, because the genomes from the fruitfly *Drosophila melanogaster* and the malaria mosquito *Anopheles gambiae* (both belonging to the insect order Diptera, or flies) and that from the silkworm *Bombyx mori* (belonging to the order Lepidoptera, or moths and butterflies) have recently been sequenced (Adams et al., 2000; Holt et al., 2002; Xia et al., 2004). The latest published addition to insect genomics is the completion of the Honey Bee Genome Project (The Honey Bee Genome Sequencing Consortium, 2006).

Insects can be subdivided into two evolutionary lineages, the Holometabola (insects with a complete metamorphosis during development) and Hemimetabola (insects with an incomplete metamorphosis). The genomic sequences from the honey bee are especially exciting, because the honey bee belongs to an insect order (the Hymenoptera), which has very recently been shown to occupy the most basal position in the Holometabola lineage (Savard et al., 2006). In contrast, the Diptera are the most advanced holometabolous insects. A comparison between the two insect orders, as we do in the current review, therefore, will give us invaluable evolutionary information on holometabolous insects, which comprise 80–85% of all insect species. For the study of the evolution of insects it would, of course, be even more interesting to compare the genomes from holometabolous with that from hemimetabolous insects. Unfortunately, large-scale genomic information on hemimetabolous insects is not available yet.

The honey bee is extremely important for agriculture as a major pollinator and as a producer of honey. Furthermore, the

honey bee is a well-studied social insect, which has fascinating social instincts and behavioral traits that involve learning, communication, and navigation. The honey bees even have their own language known as the honey bee dance (the only known non-primate symbolic language) and research in this area has been awarded with a Nobel Prize to Karl von Frisch in 1973 (von Frisch, 1994). It is expected that the sequencing of the honey bee genome will advance our knowledge of this insect enormously, which will have a strong impact on both the applied part of honey bee research (related to agriculture) and the more basic research on social behavior and learning of the bee. Moreover, all new results obtained in the honey bee will be highly relevant for our studies of other social insects, such as ants, wasps, and termites.

G protein-coupled receptor (GPCR) genes are large gene families in all animals, sometimes making up 1–2% of the animals' genome. Also in the honey bee genome, a large number (about 240) of GPCR genes have been identified, which is about 1.5% of the total number of genes present in the bee (The Honey Bee Sequencing Consortium, 2006). GPCRs are transmembrane proteins with a characteristic topology, consisting of an extracellular N terminus, seven hydrophobic transmembrane  $\alpha$  helices and an intracellular C terminus. GPCRs are activated by extracellular signals, after which they initiate a second messenger cascade in the interior of the cell, thus transducing the signals from the outside to the inside of the cell. GPCRs can be receptors for light (the rhodopsins), odorants (olfactory receptors), or neurotransmitters/neurohormones. They can be classified into four families: rhodopsin-like (or family A), secretin receptor-like (family B), metabotropic glutamate receptor-like (family C), and atypical receptors (family D). All families have the same seven transmembrane topology, but differ by amino acid residues at certain characteristic positions. For example, family A receptors have a conserved Asp-Arg-Tyr (DRY) sequence motif just after the third transmembrane  $\alpha$  helix, whereas this motif is lacking in members of the other GPCR families (Gether, 2000).

Our research group is especially interested in neurohormone GPCRs from insects and their corresponding ligands (the neuropeptides, protein hormones, and biogenic amines), because these molecules play a central role in the physiology of these animals, *i.e.*, they occupy a high “hierarchical” position



in the steering or coordination of important processes, such as reproduction, development, growth, feeding, homeostasis, and behavior. Because the *Drosophila* Genome Project was the first insect genome project to be completed (Adams et al., 2000), most information on insect neurohormone GPCRs is available from *Drosophila*. The website of the *Drosophila* Genome Project contains a list of 41 genes predicted (“annotated”) to code for neuropeptide GPCRs, three for protein hormone GPCRs, and 21 for biogenic amine GPCRs (Hewes and Taghert, 2001) ([www.flybase.org](http://www.flybase.org)). We found one additional protein hormone GPCR and three neuropeptide GPCRs that were not annotated by flybase, thus the total number of neurohormone GPCRs in *Drosophila* is probably 69 (Hauser et al., 2006). We have further found, after cDNA cloning, that in many cases the flybase annotations were incorrect, because the predicted intron/exon organizations were wrong, or because exons from other annotated neighboring genes also were part of the correct receptor gene. Furthermore, the ligands for many of the annotated receptor genes are unknown, *i.e.*, they are orphan receptors. Therefore, proper cDNA cloning of the annotated receptor genes and subsequent ligand identification are still necessary processes.

There are several ways to identify a ligand for an orphan receptor. They all imply the heterologous expression of the receptor cDNA in cells, which, for example, can be frog oocytes, or mammalian cells in cell culture. The activation of the expressed GPCRs by a tissue extract, containing the ligand, or by a synthetic ligand from a chemical library can be measured by the second messenger responses (for example, by changes in cytoplasmic cAMP or Ca<sup>2+</sup> concentrations in mammalian cells, or cAMP- or Ca<sup>2+</sup>-induced ion currents across the cell membranes of frog oocytes). When a second messenger response occurs, the cells can be used as a bioassay and the ligand can be purified and identified (in the case of an extract) or directly be determined if the ligand comes from a library (Civelli et al., 2001; Civelli, 2005). We and others have been especially successful with a system, where we have stably transfected Chinese hamster ovary (CHO) cells in cell culture with a cDNA for an insect GPCR. These cells were also stably transfected with DNA, coding for the promiscuous G protein, G-16, and transiently transfected with DNA, coding for apoaeguorin. Three hours before the assay, we added the co-factor of apoaeguorin, coelenterazine, to the culture medium. An activation of the expressed receptor in such pretreated cells would initiate an IP<sub>3</sub>/Ca<sup>2+</sup> cascade, leading to a strong bioluminescence response (Stables et al., 1997; Lenz et al., 2001; Secher et al., 2001; Staubli et al., 2002; Cazzamali and Grimmelikhuijzen, 2002; Meeusen et al., 2002; Mertens et al., 2002). A schematic drawing of this bioassay is given in Fig. 1. The system can be improved by selecting cell clones that express the GPCRs most effectively. This can be done by using a transfection vector that, in addition to DNA coding for the insect GPCR, also contains DNA coding for green fluorescent protein (GFP). In this case, the cell clones with strongest fluorescence can be selected and used in our assay system. An example of such an assay in cloned cell lines is given in Fig. 2, where the insect neuropeptide proctolin (RYLPT) induces a bioluminescence response in CHO cells expressing the

proctolin receptor, which is 400× over background. These are robust responses that give clear answers as to the identity of the ligands for the insect orphan GPCRs.

In the last few years, our group and others have successfully cloned and identified (“deorphanized”) about 40 *Drosophila* neurohormone GPCRs, or more than half of all neurohormone GPCRs that are believed to be present in the fruitfly. This work has already given us impressive insights not only into the neuroendocrinology of *Drosophila* but also into the evolution of neurohormone receptors and co-evolution of the receptors and their ligands (Park et al., 2002; Rosenkilde et al., 2003; Mendive et al., 2005). All this knowledge gained in *Drosophila* can be used to understand the molecular endocrinology of other insects, and the first insect groups where this knowledge can be applied will, of course, be those groups for which a genome project exists.

Neurohormone receptors and their ligands are expected to play a central role in the learning and behavior of the honey bee. The releases of genomic sequencing data (December 2003–Spring 2006) prior to the current publication of the honey bee genome (The Honey Bee Sequencing Consortium, 2006), therefore, immediately raised the question about the presence of these molecules in the bee. For these reasons, we started an annotation project for neurohormone receptors right after the first genomic sequences from the honey bee were released (December 2003). In the present paper, we will describe the results of this annotation effort, where we discovered 51 novel honey bee neurohormone GPCRs in addition to five biogenic amine GPCRs characterized earlier. We will also review our current knowledge on *Drosophila* neurohormone GPCRs and describe the evolution of insect GPCRs based on our analyses of the two insect species.

## 2. Neurohormone receptor genes present in the fruitfly and honey bee genomes

We used the amino acid sequences of both the annotated and the identified (cloned and “deorphanized”) *Drosophila* neurohormone receptors ([www.flybase.org](http://www.flybase.org); Hewes and Taghert, 2001; Hauser et al., 2006) to screen (BLAST search) the genomic sequences released by the Honey Bee Genome Project ([www.hgsc.bcm.edu/projects/honeybee/](http://www.hgsc.bcm.edu/projects/honeybee/)). We started our manual annotations right after the first release of the honey bee genomic sequences in December 2003 (Amel\_1.0), where we already could assign most of the neurohormone receptor genes present in the honey bee, and finished with the release of March 2006 (Amel\_4.0). Version Amel\_2.0 (January 2005) was analyzed by several gene finding programs, the latest one being Glean3 (The Honey Bee Sequencing Consortium, 2006). We have manually curated all GPCR genes predicted by the Glean3 program and in many cases found additional sequences. We have also found several GPCR genes that were not predicted by Glean3. In addition, we compared the honey bee neurohormone GPCRs with those from *Drosophila*, using phylogenetic tree (Figs. 3–5) and gene structure (Tables 1 and 2) analyses. These combined analyses resulted in the annotation of 19 biogenic amine GPCR genes (for reasons of convenience, numbered Am 1–19 from top to bottom in Fig. 3), 35 neuropeptide receptor

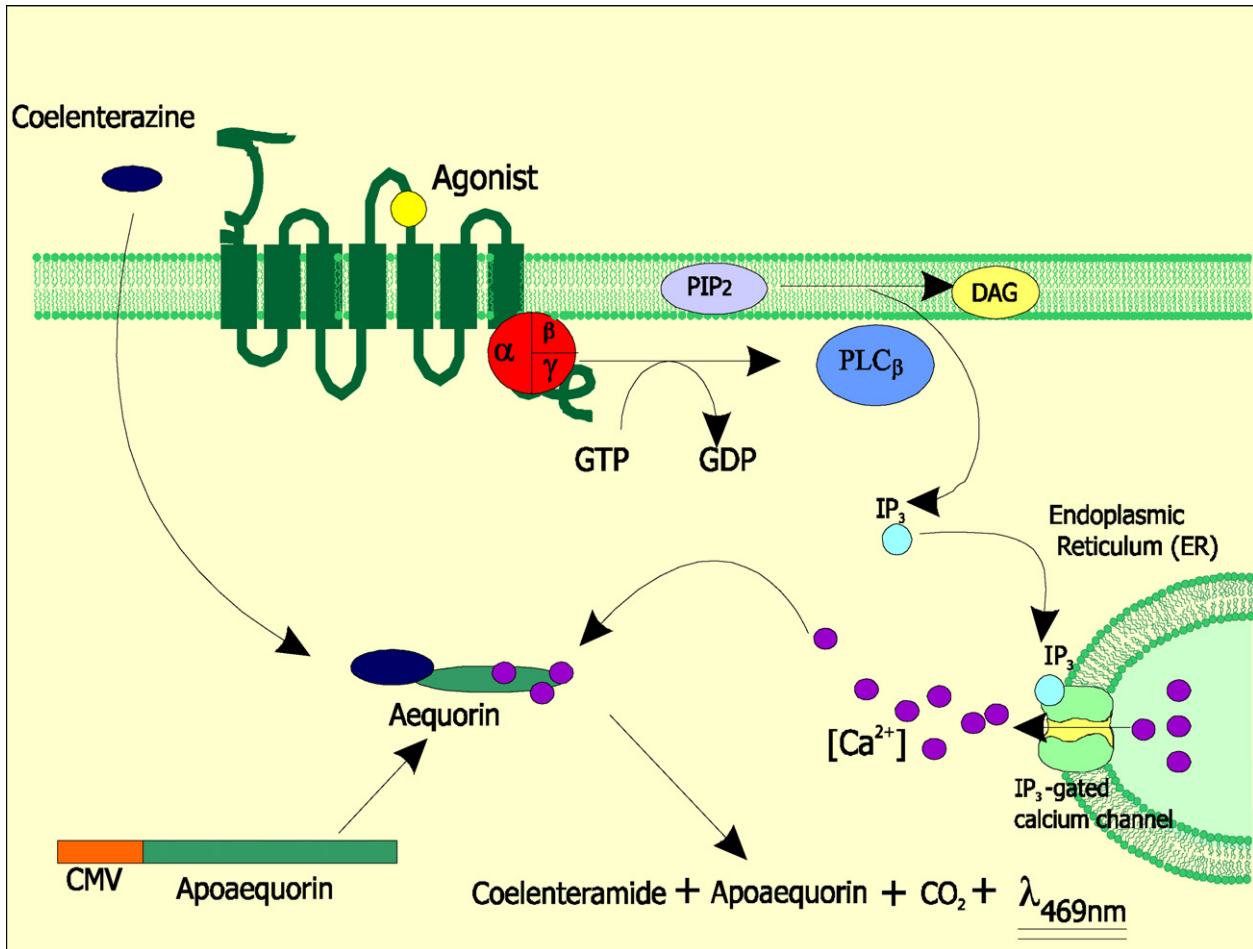


Fig. 1. Schematic drawing of the assay system used in our research group. In the upper part the CHO cell membrane is shown, expressing the insect GPCR (dark green), which binds to both the extracellular agonist (yellow) and the intracellular G protein, G-16 (red). Upon receptor activation, the  $\alpha$  subunit of G-16 dissociates from the  $\beta/\gamma$  subunits and activates phospholipase C ( $\text{PLC}_\beta$ ) which initiates an  $\text{IP}_3/\text{Ca}^{2+}$  cascade. The increased  $\text{Ca}^{2+}$  concentration stimulates aequorin to emit light of 469 nm (bioluminescence). This system was first published by Stables and coworkers for mammalian GPCRs (1997) and successfully modified and applied by us for GPCRs from insects (Staubli et al., 2002). For further details, see text.

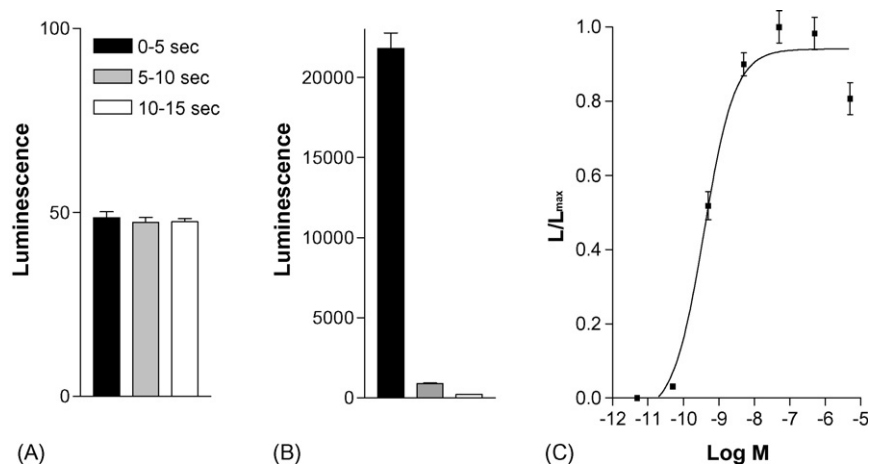


Fig. 2. An example of the experimental outcome of a bioassay schematically described in Fig. 1. The CHO cells were transfected with DNA, coding for the *Drosophila* proctolin (RYLPT) receptor (Egerod et al., 2003b). From the stably transfected cell pool, a cell line was selected that expressed the proctolin receptor most efficiently (see text). The vertical bars represent SEM, which sometimes are smaller than the symbols (or lines) used. In these cases, only the symbols (or lines) are given. (A) A control, where phosphate buffered saline (PBS) was added to the transfected cells. The cells did not react and had a background of 50 luminescence units. (B) Luminescence responses when  $5 \times 10^{-7}$  M proctolin was added to the transfected cells. There is a strong response within 5 s of over 20,000 luminescence units, which is more than 400 $\times$  over background. Furthermore, there is a clear desensitization 10–15 s after addition of the peptide. Note that the scale of the Y-axis is different from that in (A). (C) Dose-response curve of the effects seen in (B). The curve shows an  $\text{EC}_{50}$  of  $6 \times 10^{-10}$  M. Above  $10^{-7}$  M, the response decreases, because of desensitization that occurs within 5 s after addition of high concentrations of proctolin.

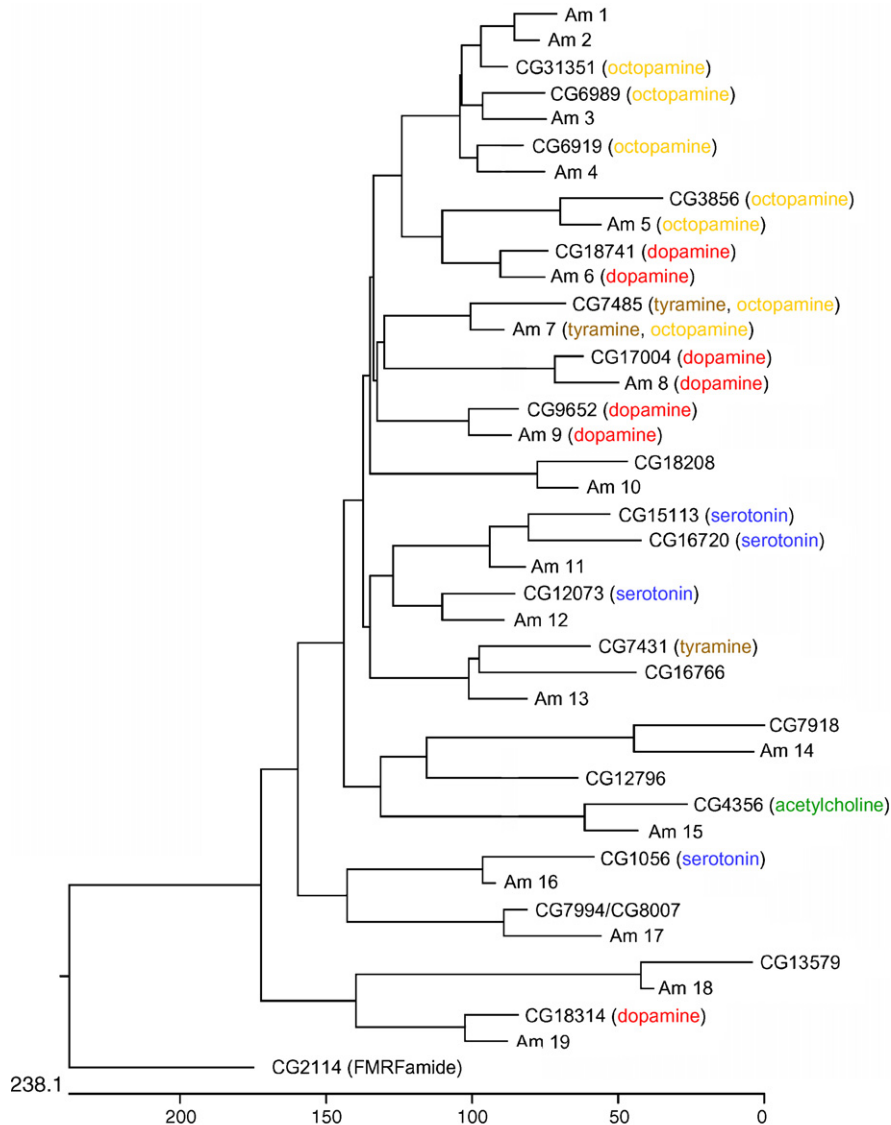


Fig. 3. Phylogenetic tree analysis of the *Drosophila* and honey bee biogenic amine GPCRs. The *Drosophila* genes are indicated by their official CG identification numbers ([www.flybase.org](http://www.flybase.org)), whereas, for reading convenience, the honey bee genes are numbered Am 1–19 (for their official GB identification numbers, see Table 1). The tree is rooted by the *Drosophila* FMRFamide receptor gene, CG2114. The length of each branch represents the distance between each receptor and the common ancestor of that receptor and its neighbor. The units at the bottom represent the number of amino acid residue substitutions, corresponding to this length.

GPCR genes (numbered Am 20–46, and Am 49–52 in Fig. 4, and Am 53–56 in Fig. 5), and two protein hormone GPCR genes (numbered Am 47–48 in Fig. 4). We have used the same numbering system in Tables 1 and 2 (first column), which also gives the official gene identification numbers (GB numbers) from the Honey Bee Genome Sequencing Consortium (fourth column). A short paragraph on our annotation efforts is given in (The Honey Bee Sequencing Consortium, 2006). In the following, we will describe our results in more detail.

### 2.1. Biogenic amine receptors

The known insect biogenic amines are dopamine, tyramine, octopamine, serotonin, acetylcholine, and histamine. There probably exists no adrenaline or noradrenaline in insects (Blenau and Baumann, 2001; Blenau, 2005; Roeder, 2005). In inverte-

brates, histamine seems to act exclusively on histamine-gated chloride channels (Roeder, 2003), which are not GPCRs and, therefore, will not be addressed in this paper. Insect biogenic amines play a crucial role in the control of a large number of behaviors, such as learning, foraging, circadian rhythm, aggression, arousal, locomotion, and defence. But they are also involved in many other physiological processes, such as diuresis and immune responses (Blumenthal, 2003; Roeder, 2005; Birman, 2005). In the following, we will give a short description of the biogenic amine receptors present in *Drosophila* and the honey bee. An overview of them is given in Fig. 3.

#### 2.1.1. Honey bee biogenic amine receptor genes that have been previously deorphanized

2.1.1.1. Am 5–9. Five honey bee receptor genes have previously been cloned and their ligands identified. These

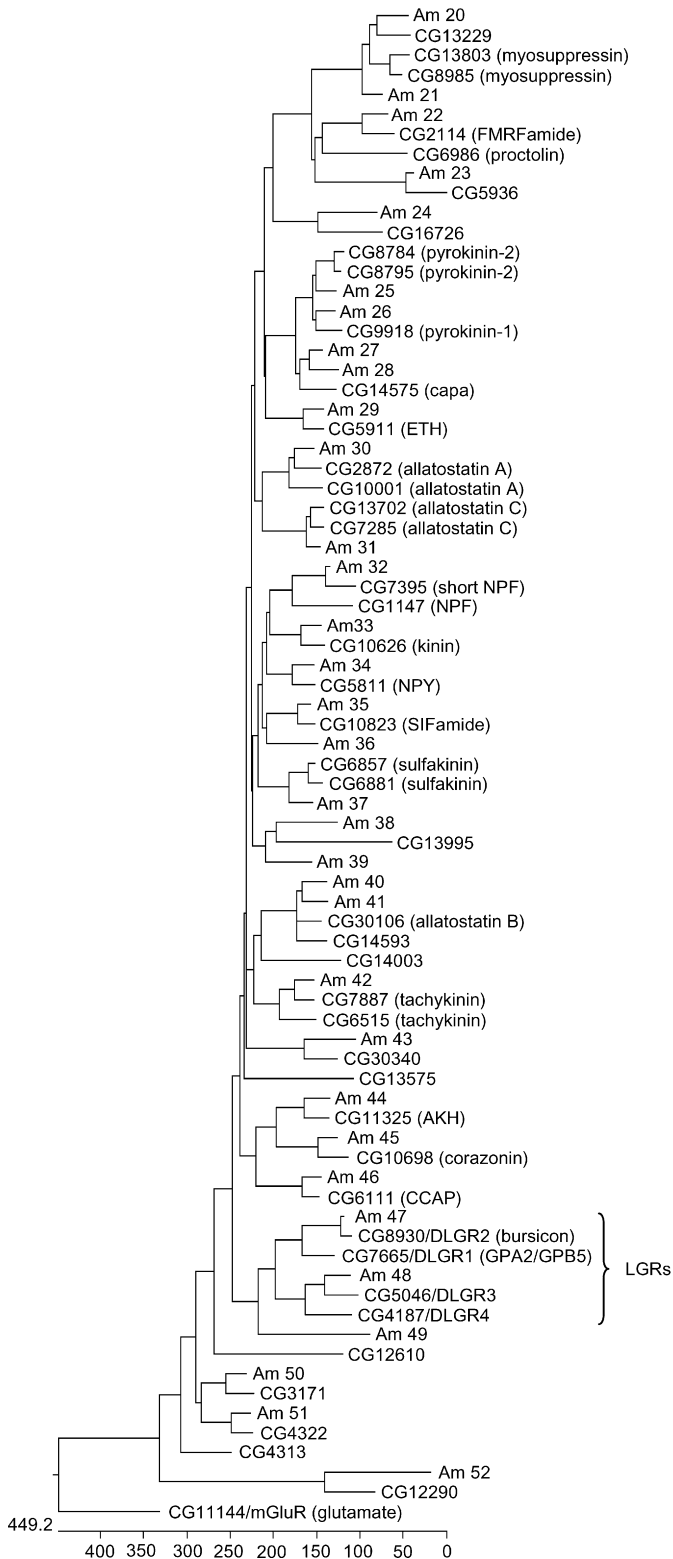


Fig. 4. Phylogenetic tree analysis of the *Drosophila* and honey bee neuropeptide and protein hormone GPCRs, belonging to family A (or family 1) (Gether, 2000). The honey bee receptors are numbered Am 20–52 (for official honey bee GB identification numbers, see Table 2). The tree is rooted by the *Drosophila* metabotropic glutamate receptor, CG11144.

genes are Am 5 (octopamine), Am 6 (dopamine), Am 7 (tyramine/octopamine), Am 8 (dopamine), and Am 9 (dopamine) (Fig. 3 and Table 1) (Blenau et al., 1998, 2000; Grohmann et al., 2003; Humphries et al., 2003; Mustard et al., 2003; Beggs et al., 2005). All five deorphanized honey bee receptors have a *Drosophila* orthologue that also has been deorphanized and that uses the same ligand (Fig. 3).

### 2.1.2. Orphan honey bee biogenic amine receptor genes that have a deorphanized *Drosophila* orthologue

The following bee receptors have not been deorphanized, but have a deorphanized *Drosophila* orthologue:

**2.1.2.1. Am 1–4. Four octopamine-like receptors (orthologues to CG31351, CG6989, and CG6919).** Four *Drosophila* genes (CG31351, CG6989, CG6919, and CG3856) have been identified as genes coding for an octopamine receptor (Maqueira et al., 2005; Balfanz et al., 2005; Han et al., 1998). Am 1 and Am 2 are clearly orthologues to CG31351 and, therefore, likely to be honey bee octopamine receptors (Fig. 3). This is supported by our finding that Am 1 has two introns and Am 2 one intron in common (*i.e.*, identical positions and intron phasings) with their *Drosophila* orthologue (Table 1). Furthermore, the Am 1 receptor protein has 61% and the Am 2 protein 65% sequence identities with the corresponding *Drosophila* receptor, when the TM1–TM7 regions were compared. All biogenic amine receptors have a large third intracellular loop (ic3) with little sequence conservation. When this loop is omitted in the sequence comparisons, the sequence identities become 70% (Am 1) and 69% (Am 2) (Table 1). All these identities strongly suggest that the two honey bee genes code for an octopamine receptor.

Am 3 is the orthologue of CG6989 and Am 4 is the orthologue of CG6919 (Fig. 3). The two honey bee genes have one or two (out of two) introns in common with their *Drosophila* orthologues. Furthermore, there are high sequence identities of 62–66% (67–69% when ic3 is omitted) between the honey bee and fly receptor proteins, arguing, again, that Am 3 and Am 4 are genes, coding for honey bee octopamine receptors (Table 1).

**2.1.2.2. Am 11, Am 12, and Am 16. Three serotonin-like receptors (orthologues to CG15113, CG16720, CG12073, and CG1056).** There are four *Drosophila* GPCRs that have been identified as serotonin receptors (Witz et al., 1990; Saudou et al., 1992; Colas et al., 1995). One honey bee gene, Am 11, is the orthologue to two of them (CG15113 and CG16720) (Fig. 3). This honey bee gene has four introns in common with CG16720 and their receptor proteins have 54% (69%) sequence identities. Am 11 is, therefore, likely to code for a serotonin receptor (Table 1). Am 12 is the orthologue of a third *Drosophila* serotonin receptor (CG12073). These genes have no introns in common, but the protein sequence identities are quite high, 60% (72%), which suggests that Am 12 also is a serotonin receptor (Table 1). Am 16 is the orthologue of the *Drosophila* serotonin receptor CG1056, with which it has four introns in common. Also, the two-receptor proteins show high



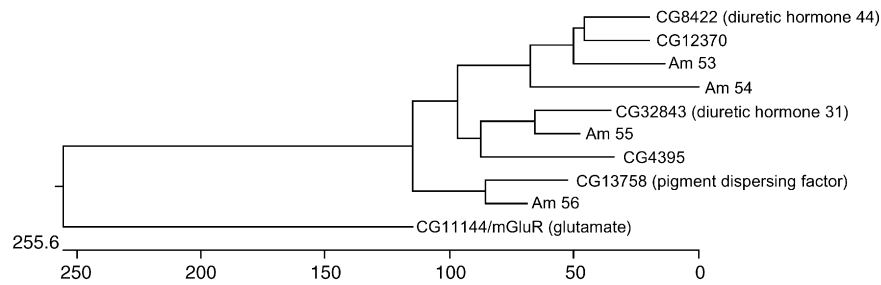


Fig. 5. Phylogenetic tree analysis of the *Drosophila* and honey bee neuropeptide and protein hormone GPCRs, belonging to family B (or family 2) (Gether, 2000). The honey bee receptors are numbered Am 53–56 (for official honey bee GB identification numbers, see Table 2). The tree is rooted by the *Drosophila* metabotropic glutamate receptor, CG11144.

sequence identities, 61% (79%), indicating that Am 16 is likely to be a serotonin receptor gene.

**2.1.2.3. Am 13. One tyramine-like receptor (orthologue to CG7431 and CG16766).** We have found that CG7431 codes for a *Drosophila* GPCR that is specific for tyramine and that does not cross-react with octopamine (Cazzamali et al., 2005a). Its paralogue CG16766 (Fig. 3) has not been characterized, so far, but might also code for a tyramine-specific receptor. Am 13 is the honey bee orthologue of the two *Drosophila* receptor genes (Fig. 3). It has only one intron in common with CG7431 (Table 1). However, the sequence identities between the two-receptor proteins, 52% (62%) nevertheless suggest that Am 13 is a honey bee tyramine receptor gene.

**2.1.2.4. Am 15. One muscarinic acetylcholine-like receptor (orthologue to CG4356).** Am 15 is the orthologue of CG4356 (Fig. 1), which has been identified as a *Drosophila* gene coding for a muscarinic acetylcholine receptor (mAChR) (Onai et al., 1989; Shapiro et al., 1989). Am 15 has six introns, but only two of them are common with the *Drosophila* gene (Table 1). Still, we believe that Am 15 is a honey bee mAChR, because the honey bee and the *Drosophila* receptor proteins have considerable sequence identities, 49% (82%) (Table 1).

**2.1.2.5. Am 19. One dopamine-like receptor (orthologue to CG18314).** Am 19 is the orthologue of CG18314, which has been identified as the *Drosophila* gene, coding for a dopamine receptor (Srivastava et al., 2005). The interesting thing is that this *Drosophila* receptor is modulated by steroids, such as ecdysone (Srivastava et al., 2005). Am 19 has one intron in common with CG18314 and the two receptor proteins show a high sequence identity of 70% (73%) (Table 1), suggesting that it also is a dopamine receptor.

### 2.1.3. Honey bee biogenic amine receptor genes that have an orphan *Drosophila* orthologue

**2.1.3.1. Am 10, Am 14, Am 17, and Am 18.** Four honey bee biogenic amine receptors (Am 10, Am 14, Am 17, and Am 18) have *Drosophila* orthologues that have not been deorphanized, so far (Fig. 3 and Table 1). This means that we cannot propose ligands for them. Thus, we know the identified or probable ligands for 15, or 68% of all honey bee biogenic amine GPCRs (Fig. 3). Furthermore, all honey bee biogenic amine-like

receptor genes found so far have a clear *Drosophila* orthologue (Fig. 3).

## 2.2. Neuropeptide and protein hormone receptors

An overview of the neuropeptide and protein hormone GPCRs present in *Drosophila* and the honey bee is given in Fig. 4 (for the family A GPCRs) and in Fig. 5 (family B).

### 2.2.1. Honey bee neuropeptide and protein hormone receptor genes that have a “deorphanized” *Drosophila* orthologue

**2.2.1.1. Am 21. One myosuppressin-like receptor (orthologue to CG8985 and CG13803).** Insect myosuppressins are neuropeptides that generally have inhibitory actions and that block both muscle and nervous system activities (Holman et al., 1986; Vanden Broeck, 2001; Nässel, 2002; Nichols, 2003). Myosuppressin also inhibits the release of ecdysone, a steroid hormone needed for growth, molting and metamorphosis, from the prothoracic glands of silkworm larvae (Yamanaka et al., 2005). Two myosuppressin receptor genes have recently been identified in *Drosophila*, CG8985 and CG13803 (Egerod et al., 2003a; Johnson et al., 2003a). There is another paralogue in *Drosophila*, CG13229, whose ligand, however, could not be identified (Egerod et al., unpublished). The honey bee has two orthologues (Am 20 and Am 21) to these three *Drosophila* receptor genes. From a phylogenetic tree analysis, it is unclear which of the two honey bee orthologues represents the myosuppressin receptor (Fig. 4). Amino acid sequence alignments, focussing on identical residues, however, suggest that Am 21 is the orthologue of the two *Drosophila* myosuppressin receptors CG8985 and CG13803, with which it has 50–51% sequence identity (40% with CG13229) (Table 2). In agreement with this assignment, the Am 20 receptor protein has 47% amino acid identities with the CG13229 protein (43% and 41% with CG13229 and CG13803). That Am 21 is the orthologue of CG13803 and CG8985, and Am 20 the orthologue of CG13229, is further supported when the genomic organizations of the five genes are compared. Am 21 has one intron in common with both CG8985 and CG13803, but none with CG13229. Am 20 has one intron in common with CG13229, but none with CG8985 and CG13803. These data, then, suggest that Am 21 is coding for a honey bee myosuppressin receptor. This conclusion has

Table 1  
Identification of 19 honey bee biogenic amine receptors

Honey bee receptor no. in the text	CG no. of the <i>Drosophila</i> orthologue	Endogenous ligand of the <i>Drosophila</i> receptor	Gene ID no. of the Glens3 annotated honey bee receptor gene	Protein region identified	Sequence identity between Tm1-Tm7 of the honey bee and the <i>Drosophila</i> receptor proteins (without ic3)	No. of introns in the honey bee gene coding region	No. of common introns between the honey bee and <i>Drosophila</i> genes (coding regions)	Accession no. for the honey bee receptor	Reference(s) for the deorphanized <i>Drosophila</i> and/or honey bee receptor
Am 1	CG31351	Octopamine	GB19803	Complete	61 (70)	3	2	XP_396445	Maqueira et al. (2005)
Am 2	CG31351	Octopamine	GB10866	Complete	65 (69)	2	1	XP_397077	Maqueira et al. (2005)
Am 3	CG6989	Octopamine	GB18869	Complete	62 (67)	2	1	XP_396348	Maqueira et al. (2005)
Am 4	CG6919	Octopamine	GB12240	Complete	66 (69)	2	2	AY_961388	Balfanz et al. (2005), Maqueira et al. (2005)
Am 5 <sup>a</sup>	CG3856	Octopamine	GB11266	Complete	56 (68)	8	2	AJ547798	Han et al. (1998), Grohmann et al. (2003), Balfanz et al. (2005)
Am 6 <sup>a</sup>	CG18741	Dopamine	GB17921	Complete	74 (82)	2	2	AF498306	Feng et al. (1996), Han et al. (1996), Humphries et al. (2003)
Am 7 <sup>a</sup>	CG7485	Octopamine/ tyramine	GB17991	Complete	62 (74)	0	0	AJ245824	Arakawa et al. (1990), Saudou et al. (1990), Bléneau et al. (2000)
Am 8 <sup>a</sup>	CG17004	Dopamine	GB14561	Complete	62 (83)	6	3	AY921573	Hearn et al. (2002), Beggs et al. (2005)
Am 9 <sup>a</sup>	CG9652	Dopamine	GB14021	Complete	72 (78)	7	0	Y13429	Goizos et al. (1994), Sugamori et al. (1995), Bléneau et al. (1998)
Am 10	CG18208	Orphan	GB18345	Complete	56 (76)	3	1	XP_394102	Saudou et al. (1992)
Am 11	CG16720	Serotonin	GB16007	Complete	54 (69)	5	3	AY_961389	
Am 12	CG15113	Serotonin	GB14021	TM1-end	52 (70)	3	3	AY_961391	Witz et al. (1990)
Am 13	CG12073	Serotonin	GB14021	TM1-end	60 (72)	3	0	AY_961390, DQ151547	
Am 13	CG7431	Tyramine	GB18047	Complete	52 (62)	6	1	AY_961391	Cazzamali et al. (2005a)
Am 14	CG16766	Orphan	GB18047	Complete	46 (58)	6	1	DQ151547	
Am 14	CG7918	Orphan	GB14509	Complete	41 (86)	3	1	BK005712	Onai et al. (1989), Shapiro et al. (1989)
Am 15	CG4356	Acetylcholine	GB12077	Complete	49 (82)	5	2	XP_395760	
Am 16	CG1056	Serotonin	GB18151	Complete	61 (79)	6	4	XP_394798	Colas et al. (1995)
Am 17	CG7994/CG8007	Orphan	GB19874	Complete	56 (83)	5	2	AY_961393	
Am 18	CG13579	Orphan	GB12670	Complete	58 (75)	5	5	AY_961394, AY_961395, AY_961396	
Am 19	CG18314	Ecdysteroids/ dopamine	GB17560	Complete	70 (73)	3	1	XP_396491	Strivastava et al. (2005)
-	CG12796	Orphan	-	-	-	-	-	-	-

<sup>a</sup> These honey bee receptors have already been deorphanized and use the same ligands as their *Drosophila* orthologues (see Fig. 3 and text).

Table 2

Identification of 37 honey bee neuropeptide and protein hormone receptors

Honey bee receptor no. in the text	CG no. of the <i>Drosophila</i> orthologue	Ligand(s) for the deorphanized <i>Drosophila</i> orthologue	Gene ID no. of the Glean3 annotated honey bee receptor gene	Protein region identified	Sequence identity between the identified parts of the honey bee and the <i>Drosophila</i> receptor proteins (TM1–TM7)	No. of introns in the honey bee receptor gene coding region	No. of common introns between the honey bee and <i>Drosophila</i> genes (coding regions)	Accession no. for the honey bee receptor	Reference(s) for the deorphanized <i>Drosophila</i> receptor
Am 20	CG13229	Orphan	GB18473	Complete	47%	3	1	BK005265	
Am 21	CG8985	Myosuppressin	GB11463	Complete	50%	3	1	BK005262	Egerod et al. (2003a), Johnson et al. (2003a)
	CG13803	Myosuppressin			51%	3	1		Cazzamali and Grimmettkhuijzen (2002), Meeusen et al. (2002)
Am 22	CG2114	FMRF-amides	GB14428	Start-TM7	54%	3	0	BK005268	
Am 23	CG5936	Orphan	GB16136	Complete	53%	4	2	BK005239	
Am 24	CG16726	Orphan	GB16565	Complete	30%	3	0	BK005684	Rosenkilde et al. (2003), Park et al. (2002)
Am 25	CG8784	Pyrokinin-2	GB18327	Complete	56%	4	2	BK005718	Park et al. (2002)
Am 26	CG8795	Pyrokinin-2			56%	4	2		Cazzamali et al. (2005b)
Am 26	CG9918	Pyrokinin-1	GB18762	Complete	55%	2	2	BK005274	Iverson et al. (2002a), Park et al. (2002)
Am 27	CG14575	Capa	GB11169	Complete	45%	5	5	BK005717	Iverson et al. (2002)
Am 28	CG14575	Capa	GB12896	Complete	46%	4	4	DQ201783	Iverson et al. (2002a), Park et al. (2002)
Am 29	CG5911	Edysis-triggering-hormone	GB13260	Complete	63%	4	3	BK005269	Iverson et al. (2002b), Park et al. (2003)
Am 30	CG2872	Allatostatins-A	GB19021	Complete	55%	3	0	BK005238	Birgüli et al. (1999), Lenz et al. (2000b), Lenz et al. (2001), Larsen et al. (2001)
	CG10001	Allatostatins-A			52%	3	1		Kreienkamp et al. (2002)
Am 31	CG7285	Allatostatin-C	GB20155	Complete	66%	1	1	BK005261	Feng et al. (2003), Merrens et al. (2002)
	CG13702	Allatostatin-C			63%	0	1		Radford et al. (2002)
Am 32	CG7395	Short-neuropeptide-F	na	Complete	68%	0	0		Li et al. (1991b)
Am 33	CG10626	Kinin	GB11188	Complete	62%	7	5	BK005263	
Am 34	CG5811	Mammalian neuropeptide-Y	GB13527	Start-TM7	62%	4	2	BK005258	Jørgensen et al. (2006)
Am 35	CG10823	SIFamide	GB16178	Complete	69%	2	2	BK005264	
Am 36	na	na	GB10679	Complete	na	5	na	BK005715	Kubiak et al. (2002), Koberup et al., unpublished
Am 37	CG6881	Sulfakinin	GB18786	Start-TM7	53%	6	2	BK005719	
	CG6857	Sulfakinin			55%	6	0		
Am 38	na	na	GB19597	TM2–TM7	na	na	na		
Am 39	na	na	GB12645	Complete	na	na	na		
Am 40	CG14593	Orphan	GB10022	Complete	52%	5	0	BK005241	Johnson et al. (2003a)
Am 41	CG30106	Allatostatins-B	GB16092	Complete	55%	5	2	XP_395101	
								BK005240	
Am 42	CG6515	Tachykinins	GB18532	Complete	50%	4	2	BK005259	Li et al. (1991a), Momnier et al. (1992), Johnson et al. (2003a), Birse et al. (2006)
	CG7887	Tachykinins			66%	4	4		

Table 2 (Continued)

Honey bee receptor no. in the text	CG no. of the <i>Drosophila</i> orthologue	Ligand(s) for the deorphanized <i>Drosophila</i> orthologue	Gene ID no. of the Glean3 annotated honey bee receptor gene	Protein region identified	Sequence identity between the identified parts of the honey bee and the <i>Drosophila</i> receptor proteins (TM1-TM7)	No. of introns in the honey bee receptor gene coding region	No. of common introns between the honey bee and <i>Drosophila</i> genes (coding regions)	Accession no. for the honey bee receptor	Reference(s) for the deorphanized <i>Drosophila</i> receptor
Am 43	CG30340	Orphan	na	Complete	43%	2	2	BK005264	
Am 44	CG11325	Adipokinetic hormone	GB16857	Start-TM7	57%	6	2	BK005716	Staubli et al. (2002), Hauser et al. (1998)
Am 45	CG10698	Corazonin	GB13022	Complete	57%	4	3	BK005273	Cazzamali et al. (2002)
Am 46	CG6111	Crustacean cardioactive peptide	GB19289	TM1-end	67%	6	4	BK005271	Cazzamali et al. (2003)
Am 47	CG8930 (DLGR2)	Bursicon	GB18043	18 LRRs-end	84%	13	8	BK005220	Ericksen et al. (2000), Mendive et al. (2005), Luo et al. (2005)
Am 48	CG5042/ CG5046/ CG31096 (DLGR3)	Orphan	GB16999	Complete	52%	18	7	BK005219	
Am 49	na	na	GB14752	Complete	na	8	na		
Am 50	CG3171	Orphan	GB16773	Complete	58%	5	2	BK005257	
Am 51	CG4322	Orphan	GB11487	Complete	51%	7	2	BK005714	
Am 52	na	Orphan	GB19647	Complete	na	5	na	BK005266	
	CG1147	Neuropeptide F	-	-	-	-	-	-	Garczynski et al. (2002)
	CG4187	Orphan	-	-	-	-	-	-	
	(DLGR4)	Orphan	-	-	-	-	-	-	
	CG4313	Orphan	-	-	-	-	-	-	
	CG6986	Proctolin	-	-	-	-	-	-	
	CG7665 (DLGR1)	GPA2/GPB5	-	-	-	-	-	-	Egerod et al. (2003b), Johnson et al. (2003b) Hauser et al. (1997), Sudo et al. (2005)
	CG12290	Orphan	-	-	-	-	-	-	
	CG12610	Orphan	-	-	-	-	-	-	
	CG13575	Orphan	-	-	-	-	-	-	
	CG13995	Orphan	-	-	-	-	-	-	
Am 53	CG14003	Orphan	-	-	-	-	-	-	
	CG8422	Diuretic hormone 44	GB10976	TM1-end	54%	9	8	BK005267	Johnson et al. (2004)
	CG12370	Orphan	-	-	52%	-	6	-	
Am 54	na	na	GB10993	TM1-end	na	10	na		
Am 55	CG32843/ CG17415/ CG17043	Diuretic hormone 31	GB12975	TM1-TM7	63%	9	7	BK005242	Johnson et al. (2005)
Am 56	CG13758	Pigment dispersing factor	GB14562	TM1-TM7	60%	8	5	BK005754	Mertens et al. (2005), Lear et al. (2005), Hyun et al. (2005)
	CG4395	Orphan	-	-	-	-	-	-	

na = not annotated or applicable.



recently been confirmed by cloning and expression of Am 21 and Am 20 in CHO cells. Am 21 could be activated by honey bee myosuppressin, whereas, Am 20 could not (A.F. Rudolf and C.J.P. Gimmelikhuijzen, unpublished).

**2.2.1.2. Am 22. One FMRFamide-like receptor (orthologue to CG2114).** The invertebrate FMRFamides are small neuropeptides with the C-terminal sequence FMRFamide (Phe-Met-Arg-Phe-amide). They play important roles in many physiological processes in invertebrates, such as feeding, reproduction, vision, and the regulation of muscle contraction and heart beat (Vanden Broeck, 2001; Nässel, 2002; Nichols, 2003). We and others have recently identified the first invertebrate FMRFamide receptor in *Drosophila*, which has the gene number CG2114 (Cazzamali and Gimmelikhuijzen, 2002; Meeusen et al., 2002). We can now find a closely related orthologue, Am 22, that probably codes for a honey bee FMRFamide-like receptor (Fig. 4). This gene contains three introns in its coding region, whereas, the coding region of the *Drosophila* orthologue gene is intronless (Table 2; Cazzamali and Gimmelikhuijzen, 2002). However, alignment of the honey bee FMRFamide-like receptor protein with that of *Drosophila* yields 54% sequence identity (Table 2), supporting our proposal that Am 22 is an FMRFamide receptor.

**2.2.1.3. Am 25 and Am 26. Two pyrokinin-like receptors (orthologues to CG9918, CG8784, and CG8795).** Pyrokinins are small neuropeptides with the C-terminal sequence FXPRLamide, which are involved in various physiological processes, among them the production and release of sex pheromones from lepidopterans and pupariation in dipterans (Nässel, 2002; Verleyen et al., 2004a). The pyrokinins can be subdivided into two groups, depending on their peptide structures, and *Drosophila* has representatives from each class, pyrokinin-1 and -2 (Cazzamali et al., 2005b). Furthermore, *Drosophila* has three pyrokinin receptors, one specific for pyrokinin-1 (CG9918), and two for pyrokinin-2 (CG8784 and CG8795) (Park et al., 2002; Rosenkilde et al., 2003; Cazzamali et al., 2005b). The honey bee has two receptor genes (Am 25 and Am 26) that clearly are the orthologues to the three *Drosophila* pyrokinin receptor genes (Fig. 4 and Table 2). Both honey bee receptor proteins have the same sequence identities (55–56%) to the three *Drosophila* receptors and their genes have the same number of introns in common with the *Drosophila* genes (2), making it difficult to decide whether they are pyrokinin-1 or -2 receptors (Fig. 4 and Table 2).

**2.2.1.4. Am 27 and Am 28. Two capa-like receptors (orthologues to CG14575).** Fluid secretion (diuresis) in insects occurs in the Malpighian tubules and reabsorption of water and other small molecules takes place in the hindgut. Several insect hormones control this important process of water and salt homeostasis. There are both diuretic and antidiuretic hormones that act on the Malpighian tubules and there is only one hormone known, so far, to act on the hindgut (Coast and Garside, 2005).

The capa peptides are small neuropeptides with the C-terminal sequence FPRVamide, which have clear antidiuretic

actions on the Malpighian tubules from the blood-sucking bug *Rodnius prolixus* and other insects (Coast and Garside, 2005; Paluzzi and Orchard, 2006), but, in contrast, have diuretic actions in *Drosophila* (Pollock et al., 2004). In addition, capa peptides have myotropic effects in a variety of insects (Wegener et al., 2002). We and others have recently cloned and identified a *Drosophila* capa receptor gene, CG14575 (Iversen et al., 2002a; Park et al., 2002). The honey bee genome contains two paralogues (Am 27 and Am 28) that are orthologues to CG14575 (Fig. 4). These honey bee genes have all five or four introns in common with the *Drosophila* orthologue and their gene products have 45% or 46% amino acid identities with the *Drosophila* capa receptor, strongly suggesting that they are honey bee capa receptors (Table 2).

**2.2.1.5. Am 29a and Am29b. Two ecdysis-triggering-hormone-like receptor splice variants (orthologues to CG5911).** Insects and other arthropods have an external skeleton (cuticle) that they need to exchange during growth and metamorphosis. The shedding of the cuticle is called ecdysis or molting. Ecdysis is initiated and regulated by a hormonal peptide, ecdysis-triggering-hormone (ETH) (Zitnan et al., 1996). We and others, have recently identified a gene in *Drosophila* (CG5911) that, by alternative splicing, gives rise to two ETH receptors (CG5911-A and -B) (Iversen et al., 2002b; Park et al., 2003). Also the honey bee genome contains an ETH-like receptor gene, Am 29, that probably gives rise to two splicing variants (see accession no. BK005269). Both honey bee ETH-like receptors have high sequence identities with their *Drosophila* counterparts (63% and 50%) and the three introns known to be present in the *Drosophila* gene do also occur at the same positions and with the same intron phasings in the honey bee gene (Table 2).

**2.2.1.6. Am 30. One allatostatin-A-like receptor (orthologue to CG2872 and CG10001).** The insect allatostatins are neuropeptides that obtained their names because of their ability to inhibit juvenile hormone biosynthesis in the *corpora allata*, two small organs (commonly fused) near the insect brain (Woodhead et al., 1989). Juvenile hormone is a terpene that plays crucial roles in insect development and reproduction. There exist three families of allatostatins that are structurally unrelated, the allatostatins-A, -B, and -C. It appears that all insects have all three types of allatostatins, but that, in each species, only one allatostatin type inhibits juvenile hormone biosynthesis, while the other allatostatins have different inhibitory functions (Lenz et al., 2000a; Williamson et al., 2001a,b; Nässel, 2002).

The A-type allatostatins are characterized by the C-terminal sequence Y/FXFGlamide (Woodhead et al., 1989). Also *Drosophila* has several A-type allatostatins (Lenz et al., 2000a; Vanden Broeck, 2001). We and others have identified two *Drosophila* allatostatins-A receptor genes, CG2872 and CG10001 (Birgöl et al., 1999; Lenz et al., 2000b, 2001; Larsen et al., 2001). We have now found that also the honey bee genome contains an orthologue (Am 30) to CG2872 and CG10001 (Fig. 4). The honey bee allatostatins-A-like receptor protein has 52–55% sequence identities to the two *Drosophila* receptors. Its gene, however, shares one intron with CG10001

and none with CG2872 and might, therefore, be somewhat more related to CG10001 (Table 2).

**2.2.1.7. Am 31. One allatostatin-C-like receptor (orthologue to CG7285 and CG13702).** C-type allatostatins are closely related, 15 amino-acid residues long cyclic neuropeptides that have originally been isolated from the moth *Manduca sexta*, where they inhibit juvenile hormone biosynthesis (Kramer et al., 1991). Also *Drosophila* has a C-type allatostatin (Williamson et al., 2001b). In *Drosophila*, two allatostatin-C receptor genes have been identified, CG7285 and CG13702 (Kreienkamp et al., 2002). The honey bee genome contains one close orthologue (Am 31) to these two *Drosophila* receptor genes (Fig. 4). There is a high sequence identity (63–66%) between the honey bee receptor protein and the two *Drosophila* receptors. Furthermore, there is one intron in the honey bee receptor gene, which has the same position and phasing as in the *Drosophila* orthologues (Table 2).

**2.2.1.8. Am 32. One short neuropeptide F (sNPF)-like receptor (orthologue to CG7395).** Short neuropeptides F (sNPF) have been sequenced from several insects and are small neuropeptides with the C-terminal sequence RLRWamide (Vanden Broeck, 2001). Several reports suggest that they are associated with reproduction and feeding in insects (Mertens et al., 2002; Lee et al., 2004). Recently, the *Drosophila* receptor gene for sNPF (CG7395) has been identified (Mertens et al., 2002; Feng et al., 2003). In the honey bee genome, we find a gene (Am 32) that is the orthologue to CG7395 (Fig. 4). This gene has only been identified manually by us and was missed by the Glean3 software. Both the honey bee and the *Drosophila* genes have no introns in their coding regions and their gene products have high (68%) sequence identities (Table 2).

**2.2.1.9. Am 33. One kinin-like receptor (orthologue to CG10626).** Insect kinins are small neuropeptides that function as diuretic hormones in the insect Malpighian tubules (Hayes et al., 1989; Coast and Garside, 2005). These peptides, which are characterized by the C-terminal sequence FXXWamide, were first isolated from the cockroach *Leucophaea maderae* (Hayes et al., 1989). Furthermore, a *Drosophila* kinin receptor, CG10626, has recently been identified (Radford et al., 2002). The honey bee genome contains a clear orthologue to CG10626 (Fig. 4). It shares five introns with the *Drosophila* gene and the honey bee receptor protein has 62% identity with its *Drosophila* counterpart (Table 2).

**2.2.1.10. Am 34. One neuropeptide-Y-like receptor (orthologue to CG5811).** Fifteen years ago, Li and coworkers (Li et al., 1991b) characterized the gene product of the *Drosophila* gene CG5811 as a neuropeptide-Y receptor, although *sensu stricto* no neuropeptide-Y exists in insects. Therefore, one might regard CG5811 as an orphan receptor. There is, however, a closely related long neuropeptide-Y-like peptide (called Drm-NPF) in *Drosophila* (Brown et al., 1999; Vanden Broeck, 2001), which might be the ligand for this receptor. Still, the situation is unclear because another receptor, CG1147 (Fig. 4), has been

identified by Garczynski et al. (2002) as the Drm-NPF receptor. It could be, therefore, that CG5811 is a second Drm-NPF receptor. CG5811 and CG1147 are evolutionarily related, albeit not very closely (Fig. 4). Drm-NPF clearly regulates feeding in *Drosophila* as does its established receptor CG1147 (Wu et al., 2005). The role of the receptor encoded by CG5811 has not been investigated yet. In the honey bee database, we could identify an orthologue to CG5811, but not to CG1147 (Fig. 4). This orthologue (Am 34) probably codes for a neuropeptide-Y-like receptor. Am 34 has two introns in common with the *Drosophila* receptor gene, and the two receptor proteins have 62% sequence identities (Table 2).

**2.2.1.11. Am 35. One SIFamide-like receptor (orthologue to CG10823).** SIFamide is the short name and also the C terminus of the *Drosophila* neuropeptide AYRKPPFNGSIFamide (Verleyen et al., 2004b). SIFamide has been isolated and predicted from various insects and crustaceans and appears to be extremely well conserved among these arthropods (Verleyen et al., 2004b). The function of this neuropeptide, however, is still enigmatic. This year, our research group has identified the *Drosophila* SIFamide receptor gene, CG10823 (Jørgensen et al., 2006). The honey bee genome contains a clear orthologue, Am 35, to this gene (Fig. 4). The two introns in the coding region are shared by both genes and the two gene products have 69% sequence identities, showing that Am 35 likely codes for a SIFamide receptor (Table 2).

**2.2.1.12. Am 37. One sulfakinin-like receptor (orthologue to CG6857 and CG6881).** The insect sulfakinins strongly resemble mammalian gastrin and cholecystokinin and are, similar to their mammalian counterparts, also involved in feeding (Wei et al., 2000). In *Drosophila*, two sulfakinin receptor genes have been identified, CG6857 and CG6881 (Kubiak et al., 2002; Kobberup, Hauser, and Grimmelikhuijzen, unpublished). The honey bee genome contains one orthologue (Am 37) to these two *Drosophila* sulfakinin receptor genes (Fig. 4). This gene clearly resembles much more CG6881 than CG6857, because it has two introns in common with the first and none with the second *Drosophila* gene. On the other hand, its gene product has 53% sequence identity with the CG6881 receptor protein and 55% with the CG6857 receptor (Table 2).

**2.2.1.13. Am 41. Two allatostatin-B-like receptors (orthologues to CG30106 and CG14593).** B-type allatostatins (also called MIPs) are neuropeptides that were initially isolated from locusts based on their myoinhibitory activities (Schoofs et al., 1991). These peptides, however, also inhibit juvenile hormone biosynthesis and can, therefore, be called allatostatins (Lorenz et al., 1995). B-type allatostatins are characterized by the C-terminal sequence W(X)<sub>6</sub>Wamide (Schoofs et al., 1991; Lorenz et al., 1995; Williamson et al., 2001a). An allatostatin-B receptor gene, CG30106, has recently been identified in *Drosophila* (Johnson et al., 2003a). There exists a close paralogue to this gene, CG14593, in *Drosophila* (Fig. 4) that has not been deorphanized, so far, but that also likely codes for an allatostatin-B receptor. The honey bee genome contains two

genes (Am 40 and Am 41) that closely resemble the two *Drosophila* receptor genes (Fig. 4). Analysis of their genomic organizations suggests that Am 41 is more related to CG30106, because it has two introns in common with this gene, whereas Am 40 has no common introns either with CG30106 or with CG14593 (Table 2). These findings indicate that Am 41 is an allatostatin-B-like receptor, whereas, the status of Am 40 is still uncertain, although it might well be a second allatostatin-B-like receptor. It is interesting to mention that Am 41 is also found in the honey bee brain EST library ([http://titan.biotech.uiuc.edu/bee/honeybee\\_project.htm](http://titan.biotech.uiuc.edu/bee/honeybee_project.htm)), showing that this honey bee allatostatin-B-like receptor is a brain receptor.

**2.2.1.14. Am 42. One tachykinin-like receptor (orthologue to CG6515 and CG7887).** The insect tachykinins are mostly known for their stimulatory actions on the insect visceral muscles, but they are also involved in other processes, such as diuresis, where they function as diuretic hormones on the Malpighian tubules (Vanden Broeck, 2001; Nässel, 2002; Coast and Garside, 2005). The *Drosophila* genome contains two tachykinin receptor genes, CG6515 and CG7887 (Li et al., 1991a; Monnier et al., 1992; Johnson et al., 2003a; Birse et al., 2006). In the honey bee genome, we could identify one orthologue, Am 42 (Fig. 4). The honey bee receptor protein has high sequence identities (50% and 66%) with its two *Drosophila* counterparts, and its gene has four introns in common with CG7887 and two with CG6515 (Table 2).

**2.2.1.15. Am 44. One adipokinetic-hormone-like receptor (orthologue to CG11325).** The insect adipokinetic hormones are a large family of structurally related neuropeptides that are involved in the mobilization of sugar (trehalose) and lipids from the insect fat body during energy requiring activities such as flight and locomotion (Gäde et al., 1997). We have recently identified a *Drosophila* adipokinetic hormone receptor, CG11325, and an adipokinetic-hormone receptor from the silkworm *B. mori* (Staubli et al., 2002). We now find that a similar adipokinetic hormone receptor gene (Am 44) also occurs in the honey bee genome (Fig. 4). This gene has two introns in common with its *Drosophila* orthologue CG11325. The honey bee adipokinetic-hormone-like receptor has 57% sequence identity with the *Drosophila* receptor (Table 2).

**2.2.1.16. Am 45. One corazonin-like receptor (orthologue to CG10698).** The insect neuropeptide corazonin (this name was derived from the Spanish word corazón, meaning heart) has originally been isolated from cockroaches, because of its cardio-excitatory actions on isolated cockroach hearts (Veenstra, 1989; Vanden Broeck, 2001; Nässel, 2002). In locusts, corazonin is involved in stress reactions and induces a characteristic black pattern in the cuticle of crowding animals that are preparing for swarming (Tawfik et al., 1999). Recently, we and others identified the *Drosophila* gene coding for a corazonin receptor, CG10698 (Cazzamali et al., 2002; Park et al., 2002). We have found that also the honey bee genome contains a corazonin-like receptor gene (Am 45), closely related to CG10698 (Fig. 4). The honey bee receptor protein has

57% sequence identity with the CG10698 receptor. Its gene has three introns in common with CG10698 (Table 2).

**2.2.1.17. Am 46. One crustacean cardioactive peptide-like receptor (orthologue to CG6111).** Crustacean cardioactive peptide (CCAP) is a small cyclic neuropeptide originally isolated from crustaceans, but that is also present, with identical sequence, in insects (Vanden Broeck, 2001; Nässel, 2002). CCAP in insects has myotropic and cardioactive actions, but also plays a role in certain motor behaviors associated with ecdysis (Cheung et al., 1992; Gammie and Truman, 1999). We and others, have recently identified a CCAP receptor gene in *Drosophila*, CG6111 (Park et al., 2002; Cazzamali et al., 2003). The honey bee genome also contains a CCAP-like receptor gene (Am 46) which has four introns in common with its *Drosophila* orthologue CG6111 (Table 2 and Fig. 4). The two receptor proteins show high sequence identities (67%) (Table 2).

**2.2.1.18. Am 47. One bursicon-like receptor (orthologue to CG8930).** *Drosophila* has four leucine-rich repeats-containing GPCRs (LGRs) that we cloned and named DLGR1–4 (Hauser et al., 1997; Eriksen et al., 2000; Bohn, Williamson, and Grimmelhuijzen, unpublished). We and others have recently also identified the natural ligand for DLGR2, which is a heterodimeric cystine-knot protein with bursicon bioactivity (Mendive et al., 2005; Luo et al., 2005). Bursicon was described more than 40 years ago as a neurohormone that causes hardening and tanning of the soft cuticle from a newly hatched fly after adult ecdysis (Fraenkel and Hsiao, 1962; Fraenkel et al., 1966). Later studies showed that bursicon also induces apoptosis of the wing epithelial cells after completed wing expansion (Kimura et al., 2004). The honey bee genome contains a clear orthologue of the *Drosophila* bursicon receptor gene (CG8930/DLGR2) (Fig. 4). This gene, Am 47, is present in one contig, which contains the information for the whole 7TM region plus 18 Leu-rich repeats (LRRs), which are located in the extracellular N terminus of the receptor and which constitute the ligand-binding site. The honey bee receptor has a very high sequence identity (84%) with DLGR-2. Its gene has eight introns in common with CG8930 (Table 2). We could also identify a gene in the honey bee genome, coding for bursicon (Mendive et al., 2005). We made a highly interesting discovery here (Mendive et al., 2005), namely that the honey bee bursicon was not, as in *Drosophila*, a heterodimeric protein, coded for by two genes, but that a single gene might yield a protein with two domains, each corresponding to the *Drosophila* monomers. Taking into account the basal evolutionary position of Hymenoptera (Savard et al., 2006), these findings suggest that, during early insect evolution, bursicon was a monomer, encoded for by a single gene.

**2.2.1.19. Am 53 and Am 55. Two diuretic hormone-like receptors (orthologues to CG8422, CG12370, and CG32843).** There are two diuretic hormones (named DH) acting on the insect Malpighian tubules that are longer neuropeptides (Coast et al., 2001; Coast and Garside, 2005). One is structurally related to mammalian calcitonin and the *Drosophila* calcitonin-like peptide is called Drome-DH<sub>31</sub>



(Coast et al., 2001), whereas the other is structurally related to mammalian corticotropin-releasing-factor (CRF) and in *Drosophila* is called Drome-DH<sub>44</sub> (Cabrero et al., 2002). Drome-DH<sub>31</sub> and Drome-DH<sub>44</sub> are not structurally related. Two *Drosophila* DH receptors have recently been identified, CG32843 (for Drome-DH<sub>31</sub>) and CG8422 (for Drome-DH<sub>44</sub>) (Johnson et al., 2004, 2005) (Fig. 5). These receptors, like all the other receptors shown in Fig. 5, belong to the family B (or family 2) type of GPCRs, in contrast to the receptors from Figs. 3 and 4, which are family A (or family 1) type GPCRs (Gether, 2000). We have now found a honey bee gene, Am 55, that is a clear orthologue to CG32843. Am 55 has seven introns in common with CG32843 and their gene products have 63% sequence identity, strongly suggesting that Am 55 is a DH<sub>31</sub>-like receptor (Table 2).

We have also found another honey bee receptor gene, Am 53, that is clearly related to CG8422. This gene has eight introns in common with CG8422 and their gene products show 54% sequence identities (Table 2). Thus, it appears that Am 53 is a Drome-DH<sub>44</sub>-like receptor. In a phylogenetic tree analysis (Fig. 5) Am 53 is about equally related to both CG8422 (the Drome-DH<sub>44</sub> receptor) and CG12370 (which is an orphan). However, the genomic organization of Am 53 suggests that the honey bee gene is more closely related to CG8422 (with which it has eight introns in common) than to CG12370 (where six introns are in common) (Table 2).

**2.2.1.20. Am 56. One PDF-like receptor (orthologue to CG13758).** Pigment dispersing factor (PDF) has first been isolated from crustaceans, because of its pigment cell dispersing activity (Riehm and Rao, 1982), but a structurally related neuropeptide has later also been found in insects (Rao et al., 1987). The best-known function of PDF in insects is its role in circadian rhythmicity, where it appears to be the principal transmitter, mediating the output of central clock neurons (Renn et al., 1999; see, however, Kula et al., 2006). Last year, three research groups independently published the identification of the *Drosophila* gene CG13758 as the one coding for the PDF receptor (Hyun et al., 2005; Lear et al., 2005; Mertens et al., 2005). Also in the honey bee genome there exists a clear orthologue of CG13758, Am 56 (Fig. 5). The honey bee receptor has 60% sequence identity with the *Drosophila* receptor and its gene has five introns in common with the *Drosophila* PDF receptor gene (Table 2). Circadian rhythms play crucial roles in the behavior and physiology of honey bees and the identification of a bee PDF receptor may represent an important advance in honey bee chronobiology.

### 2.2.2. Honey bee neuropeptide and protein hormone receptor genes that have an “orphan” *Drosophila* orthologue

The following honey bee neuropeptide and protein hormone orphan GPCR genes have *Drosophila* orthologues that also are orphans: Am 20 (orthologue to CG13229), Am 23 (orthologue to CG5936), Am 24 (orthologue to CG16726), Am 43 (CG30340), Am 48 (CG5046/DLGR3), Am 50 (CG3171), Am 51 (CG4322), and Am 52 (CG12290).

The information on the above-mentioned honey bee receptors can be found in Table 2, Fig. 4, and Fig. 5 and need little additional explanation. However, we would like to expand somewhat on Am 48 and its *Drosophila* orthologue CG5046/DLGR3.

The DLGR3 gene has been wrongly annotated by flybase ([www.flybase-org](http://www.flybase-org)), where three separate genes are proposed, CG5042, CG5046 and CG31096, which, in fact, are exons of one gene, DLGR3 (Bohn and Williamson, unpublished). Of all four *Drosophila* DLGRs, Am 48 is most closely related to the corrected DLGR3 (Fig. 4). One contig contains the information for the whole 7TM region plus three LRRs in the extracellular N terminus. This Am 48 protein has 52% sequence identity with DLGR3. Its gene has seven introns in common with the corrected DLGR3 gene (Table 2).

### 2.2.3. Honey bee neuropeptide and protein hormone receptor genes that do not have a clear *Drosophila* orthologue

We found five receptors, Am 36, Am 38, Am 39, and Am 49, belonging to family A (Fig. 4 and Table 2), and Am 54, belonging to family B (Fig. 5 and Table 2) that apparently do not have orthologues in *Drosophila*. All of them, however, do have orthologues in the beetle *Tribolium castaneum*, so they are not honey bee-specific (<http://www.bioinformatics.ksu.edu/BeetleBase/>).

### 2.2.4. *Drosophila* neuropeptide and protein hormone receptor genes that do not have a honey bee orthologue

We did not find clear-cut honey bee orthologues for the following *Drosophila* neuropeptide and protein hormone GPCR genes: CG6986 (proctolin receptor), CG1147 (NPF receptor), CG14003 (orphan), CG13575 (orphan), CG12610 (orphan), CG4313 (orphan), and CG4395 (orphan) (Figs. 4 and 5 from top to bottom). These receptors might be specific for *Drosophila* (or for Diptera in general). This, however, is probably not true for CG6986 (the proctolin receptor gene), because the proctolin neuropeptide occurs in many other insects and even in crustaceans (Vanden Broeck, 2001; Nässel, 2002). The absence of the proctolin receptor gene in the honey bee, does not come as a surprise, because the receptor and its ligand genes are apparently also absent in the genomes from the malaria mosquito *A. gambiae*, and the silkworm *B. mori* (M. Williamson, unpublished).

### 2.2.5. Neuropeptide and protein hormone receptor paralogues that are present in *Drosophila*, but that are absent in the honey bee genome

Many of the *Drosophila* neuropeptide and protein hormone GPCR genes occur in pairs, triplets or quadruplets and both their genomic organizations and gene products are structurally closely related. These genes have probably originated by recent gene duplications and are called paralogues. Very often these paralogue receptors are still using the same or closely related ligands. In many cases these paralogue receptors are lacking in the honey bee genome. In the following, we will give some examples to clarify this point

(mainly those, where the *Drosophila* orthologues have been deorphanized).

There are two myosuppressin-like receptor genes in the honey bee (Am 20 and Am 21 in Fig. 4), whereas, there are three paralogues (CG13229, CG13803 and CG8985) in *Drosophila*. There are two pyrokinin-like receptors in the bee (Am 25 and Am 26), whereas, there are three paralogues (CG8784, CG8795, and CG9918) in *Drosophila*. There is one allatostatin-A-like receptor in the bee (Am 30), while there are two in *Drosophila* (CG2872 and CG10001). There is one allatostatin-C-like receptor in the honey bee (Am 31), while there are two in *Drosophila* (CG13702 and CG7285). There is one sulfakinin-like receptor in the honey bee (Am 37), whereas, there are two in *Drosophila* (CG6857 and CG6881). There is one tachykinin-like receptor in the honey bee (Am 42), whereas, there are two in *Drosophila* (CG7887 and CG6515). Finally, there are two LGRs in the honey bee (Am 47 and Am 48), whereas, there are four in *Drosophila* (DLGR 1–4 in Fig. 4).

#### 2.2.6. Neuropeptide and protein hormone receptor paralogues that are present in the honey bee, but absent in *Drosophila*

There are two situations, where the honey bee has more neuropeptide/protein hormone GPCR paralogues than *Drosophila*. These are Am 27 and Am 28, which are both orthologues to the single *Drosophila* capa receptor gene, CG14575, and Am 38 and Am 39, which appear to be paralogues to the single *Drosophila* orphan receptor gene, CG13995 (Fig. 4).

### 3. General discussion

In this paper, we have identified 56 neurohormone GPCRs in the honey bee. Of these, 35 are neuropeptide, two are protein hormone, and 19 are biogenic amine GPCRs (Figs. 3–5). Of the 19 biogenic amine receptors, five have already been cloned and deorphanized (Am 5–9 in Fig. 3). Of the remaining 14 biogenic amine receptor genes, 10 have close *Drosophila* orthologues that have been deorphanized, so we know what the likely ligands are for the honey bee receptors. Of the two protein hormone (LGR) receptor genes, one (Am 47, Fig. 4) has a close orthologue in *Drosophila* that has been identified as a bursicon receptor gene (Mendive et al., 2005; Luo et al., 2005). Furthermore, the ligand bursicon has also been identified in the honey bee genome (GB11959; Mendive et al., 2005), so Am 47 is likely to be the bursicon receptor. None of the 35 neuropeptide receptors in the honey bee has been deorphanized and published, so far. However, 23 neuropeptide receptor genes have a close orthologue in *Drosophila* that has been deorphanized, so we know, again, what the likely ligands for these 23 honey bee receptors are. In conclusion, therefore, we know the ligands or the likely ligands for 39 of the 56 honey bee neurohormone GPCRs described in this study (70%). This is one of the major outcomes from our current analyses.

Of course, it would be very helpful for the interpretation of the honey bee neurohormone GPCRs also to add a table to this review, listing all neuropeptide and protein hormone genes present in *Apis*. However, our knowledge on these genes is still

very fragmentary. Our own group has annotated honey bee preprohormone genes for AKH, corazonin, CCAP, myosuppressin, pyrokinins and SIFamide (K. Hansen et al., unpublished). Bursicon, as mentioned earlier, has also been annotated (Mendive et al., 2005). Furthermore, the presence of allatostatin-A, corazonin, myosuppressin, tachykinins, pyrokinins, and SIFamide in the honey bee has also been experimentally confirmed by mass spectrometry (Audsley and Weaver, 2006; Verleyen et al., 2006). These neuropeptides are all ligands belonging to the 39 likely (or known) ligands mentioned earlier. In the case of own annotated honey bee neuropeptides, we have also tested them on the corresponding annotated receptors (Am 21, Am 25, Am 26, Am 35, Am 44–46; see Fig. 4) and found that they were specifically activated, thereby confirming at least some of our annotations (K. Hansen et al., unpublished).

The honey bee is an advanced insect in terms of learning, navigation, and social behavior (Elekonich and Roberts, 2005) and, thus, quite different from *Drosophila* and many other non-hymenopteran insects. A comparison between the genomes from the honey bee and the fruitfly could possibly reveal the genes that are involved in higher brain functions and sociality. Because neurohormone receptors and their ligands are expected to play a central role in learning and behavior of animals, it is especially interesting to look at their genes, using such a comparison. A comparison of the biogenic amine receptor genes between the honey bee and *Drosophila* (Fig. 3) did not show a clear difference. The numbers are about the same (19 biogenic amine receptor genes in the bee, 21 in the fruitfly) and only in one case (Am 1 and Am 2, which both are octopamine-like receptors), does the bee have more paralogues than the fruitfly (CG7078). It would be interesting to determine what the functions of these two honey bee octopamine-like receptors are and whether they are important for behavior.

There are larger differences between the honey bee and the fruitfly, when we compare their neuropeptide and protein hormone GPCRs (Figs. 4 and 5). First, there is a difference in number. *Drosophila* has 39 neuropeptide GPCRs and four protein hormone GPCRs (DLGRs) belonging to family A, and five neuropeptide GPCRs belonging to family B. For the honey bee, these numbers are 31, 2, and 4 (Figs. 4 and 5). The reasons for the larger numbers of neuropeptide and protein hormone GPCRs in the fruitfly, are probably gene duplications during fly evolution, which created a large number of paralogues. There are about 22 neuropeptide and protein hormone GPCR paralogues in the fruitfly compared to about 12 in the bee (Figs. 4 and 5). These gene duplications have probably occurred late in fly (dipteran) evolution, because an inspection of the genome from the malaria mosquito *A. gambiae* revealed fewer GPCR paralogues than in the fruitfly. Thus, although the honey bee is much more advanced than the fruitfly in terms of learning and behavior, the number of neurohormone genes is considerably less.

Still, the honey bee is not fully inferior to the fruitfly with respect to neurohormone GPCRs. There are five GPCR genes in the honey bee that do not have their counterparts in the fly. These genes are Am 36 (GB10679), Am 38 (GB19597), Am 39 (GB12645), Am 49 (GB14752), and Am 54 (GB10993).

However, all five genes have orthologues in the beetle *T. castaneum* and are, therefore, not specific for the honey bee.

During this study, we made several interesting observations with respect to neurohormone receptor evolution and the evolution and co-evolution of their ligands. For the neuropeptide and protein hormone GPCRs (Figs. 4 and 5), it appears to be such that, when needed during evolution, extra receptors were established by gene duplications. These new receptors could use the same ligand (for example, myosuppressin, top Fig. 4, is used by both CG13803 and CG8985), or, when ligand interference occurs (which would be impractical), new ligands could be formed by amino acid residue substitutions. An example of the last situation are the paralogues CG9918, CG8784, and CG8795 in the top part of Fig. 4, which use slightly different pyrokinin ligands, showing little cross-reactivity (Rosenkilde et al., 2003; Cazzamali et al., 2005a). In this way, a very large number of receptors and ligands can be created during evolution (Park et al., 2002; Cazzamali et al., 2005a).

This type of co-evolution between receptors and ligands does apparently not occur in biogenic amine GPCRs (Fig. 3). The reason for this is probably that the biogenic amine molecule is rather small and that very little structural variation is tolerated by the ligand-binding pocket of the GPCR. In short, what we see with this type of GPCR is that gene duplications also occur during evolution. Sometimes the same ligands are still being used by the paralogue receptors (for example, CG15113 and CG16720, middle of Fig. 3, both use serotonin). However, serotonin as a ligand is not confined to evolutionarily closely related GPCRs, because it is also used by a very distantly related receptor, CG1056 (bottom of Fig. 3). Another example is dopamine, which is used by two relatively closely related receptors, CG17004 and CG9652 (middle part of Fig. 3), but also by two distantly related receptors CG18741 (top part of Fig. 3) and CG18314 (bottom of Fig. 3). Thus, it seems that during biogenic amine GPCR evolution, new receptors evolved (probably by gene duplication) that needed new ligands. The only way (because of structural constraints) to obtain such new ligands was by “borrowing” from other systems. This caused a “ligand-hop” during receptor evolution. Because each *Drosophila* biogenic amine GPCR gene has a closely related honey bee orthologue, using, where identified, the same ligand (Fig. 3), these “ligand-hops” have occurred before the split of the ancestors of the present-day flies (Diptera) and honey bees (Hymenoptera), which is about 350 million years ago (Douzery et al., 2004).

#### 4. Methods

*D. melanogaster* neurohormone G protein-coupled receptor proteins were used as probes to search for similar proteins in *Apis mellifera*. tBLASTn searches were performed on the genomic sequences from various releases of the Honey Bee Genome Project (Ame1\_1.0 to Ame1\_4.0; <http://www.hgsc.bcm.tmc.edu/projects/honeybee/>). The full sequences of the candidate *Apis* receptor proteins were identified using a combination of Invitrogen Vector NTI Advance 9.0 package (InforMax), the Lasergene DNA Software package (DNASTAR), several Web-based gene prediction programs, and the

official set of Glean3 predictions (The Honey Bee Sequencing Consortium, 2006). The prediction server TMHMM (v. 2.0) from the Center for Biological Sequence Analysis, BioCentrum-DTU ([www.cbs.dtu.dk](http://www.cbs.dtu.dk)), was used for analysis of the secondary structure of the *Apis* receptor proteins. All manually annotated or Glean3 predicted but manually curated gene sequences were submitted to GenBank (Tables 1 and 2). These sequences were also fully or partially cloned by PCR. We used only the amino acid sequences from TM1 to TM7 for the construction of the phylogenetic trees (Figs. 3–5). These phylogenetic tree analyses were done, using the DNASTAR software package.

#### Note added in proof

After this review went to press, a paper was published (Schlenstedt et al., 2006), where gene Am 12 (Fig. 3) was experimentally identified (deorphanized) as a honey bee serotonin receptor gene, thus confirming our annotation.

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