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Expression of chemosensory proteins in the tsetse fly
*Glossina morsitans morsitans* is related to female
host-seeking behaviour

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Abstract

Chemosensory proteins (CSPs) are a class of soluble
proteins present in high concentrations in the sensilla
of insect antennae. It has been proposed that they
play an important role in insect olfaction by mediating
interactions between odorants and odorant receptors.
Here we report, for the first time, the presence of five
CSP genes in the tsetse fly *Glossina morsitans mor-
sitans*, a major vector transmitting nagana in live-
stock. Real-time quantitative reverse transcription
PCR showed that three of the CSPs are expressed in
antennae. One of them, *GmmCSP2*, is transcribed at a
very high level and could be involved in olfaction. We
also determined expression in the antennae of both
males and females at different life stages and with
different blood feeding regimes. The transcription of
*GmmCSP2* was lower in male antennae than in
females, with a sharp increase in 10-week-old flies,
48 h after a bloodmeal. Thus there is a clear relation-
ship between CSP gene transcription and host
searching behaviour. Genome annotation and phylo-
genetic analyses comparing *G. morsitans morsitans*
CSPs with those of other Diptera showed rapid evo-
lution after speciation of mosquitoes.

Keywords: chemosensory protein, tsetse fly, gene
expression, trypanosomiasis, nagana.

Introduction

Tsetse flies are a group of insect species that vector
trypanosomes, causing sleeping sickness in humans and
other animals. One species, *Glossina morsitans morsi-
tans*, has a massive economic impact on African develop-
ment because of its transmission of nagana in livestock.
It is estimated that this disease results in approximately
3 000 000 cattle deaths per year and also limits the supply
of meat and milk products and the availability of animal
labour for ploughing (Aksoy *et al*., 2005). Tsetse flies are
attracted to their hosts by a range of signals including
chemical cues, and currently one of the major control
methods for tsetse flies is the use of insect traps and
insecticide-treated targets, which use both visual and
chemical cues to lure the flies to the traps. Repellent
chemicals are also used to protect humans and animals
from being bitten. Thus there is a need to understand the
molecular basis of olfaction in tsetse flies, including
odorant binding proteins (OBPs) and chemosensory pro-
teins (CSPs) in the antennae.

CSPs and OBPs are two classes of soluble proteins
found in the sensillum lymph of insect antenna. The major
difference is that CSPs have a conserved four-cysteine
signature (C₁-X₆-C₂-X₆₁₇-C₄) and OBPs have a con-
erved six-cysteine signature (C₁-X₃₅-C₂-X₅₃-C₅-X₈₅-C₆),
resulting in very different 3D protein
structures (Campanacci *et al*., 2001; Mosbah *et al*., 2003;
Tomaselli *et al*., 2006). OBPs have been shown to be
involved in the first step of olfactory molecular recognition
and signal transduction by ferrying airborne host odorants
across the sensillum lymph to the odorant receptors.
CSPs were first discovered and named as olfactory-
specific protein D (OS-D) in *Drosophila melanogaster*
by McKenna *et al*. (1994). In fact the CSP domain
(pfam03392) used in this work is derived from the con-
served sequence alignments profiles based on a collection
of OS-D-like CSP sequences and referred to as
OS-D domain (http://www.ncbi.nlm.nih.gov/cdd?term= pfam03392). CSPs are secreted into the sensillum lymph
of insect chemosensory sensilla and it has been proposed
that they are involved in CO₂ detection, in chemical signal
transmission, in regenerating legs and in chemosensory perception (olfaction and taste), based on whether they are present in antennae, tarsi or the labarum. Indeed, although many are expressed in the antennae, others are expressed in other tissues including legs (Mameli et al., 1996; Picimbon et al., 2001), labial palps (Maleszka & Stange, 1997), tarsi (Angeli et al., 1999), brain (Whitfield et al., 2002), proboscis (Nagnan-Le Meillour et al., 2000), pheromone gland (Jacquin-Joly et al., 2001; Dani et al., 2011) and wings (Ban et al., 2003). In Apis mellifera CSPs have been reported to be involved in larval development and brood pheromone transportation (Briand et al., 2002; Forêt et al., 2007). In the cockroach Blatta germanica a CSP is involved in leg regeneration (Kitabayashi et al., 1998). One CSP of the diamond-back moth, Plutella xylostella, is able to bind nonvolatile oviposition deterrents (X. Liu et al., 2010). Several CSPs are highly expressed in the lymph of chemosensilla and exhibit binding activity towards odorants and pheromones (Pelosi et al., 2006), but there is little evidence of a role in olfaction.

In the present study we constructed and sequenced an antennal cDNA library of G. m. morsitans and searched all other available expressed sequence tags (ESTs) and genome shot-gun data. The expression of all of the CSP genes was analysed in heads, bodies and antennae. In order to associate the CSP genes with host location or sex pheromone detection, the transcription profiles of the CSP genes in the antennae were analysed separately in males and females using different starvation regimes. Phylogenetic relationships of the CSPs between G. m. morsitans and other Diptera were also used to determine the evolution of CSP genes in Diptera.

Table 1. Deduced chemosensory proteins (CSPs) identified from genome and expressed sequence tag (EST) sequences of Glossina morsitans morsitans

| Name       | Length | GenBank ID  | Closest CSP* (identities, E-value) | Sources† | Signal peptide |
|------------|--------|-------------|----------------------------------|----------|----------------|
| GmmCSP1    | 126    | FN432801    | Pebill (69%, 1e-42)              | L, A, F, G, R, S, T | 1-20aa         |
| GmmCSP2    | 158    | FN432802    | a10 (66%, 1e-50)                 | A, T     | 1-21aa         |
| GmmCSP3    | 128    | FN432803    | Pebill (70%, 3e-45)              | A, T     | 1-19aa         |
| GmmCSP4    | 123    | FN432804    | Phk-3 (65%, 9e-46)               | T        | 1-21aa         |
| GmmCSP5    | 108    | FN432805    | DmeCSP1 (73%, 2e-43)             | T        | 1-26aa         |

*The proteins in this column are all CSPs of Drosophila melanogaster.
†Sequences were identified from EST libraries of antennae (A), head (H), larvae (L), pupae (P), reproductive organs (R), fat body (F), adult gut (G) and salivary gland (S) as well as genomic trace data (T).

Results and discussion
Identification of CSPs in G. m. morsitans
Reverse position specific BLAST (RPS-BLAST) was used to search for the OS-D domains (pfam 03392) against all ESTs of G. m. morsitans in GenBank, including the antennae cDNA library data (R. Liu et al., 2010), and the genomic data sequenced (ftp://ftp.sanger.ac.uk/pub/pathogens/Glossina/morsitans/). All the sequences with an OS-D domain (with a cut-off threshold of 10^-5) were deemed to be candidate CSPs, giving 29 ESTs and 28 genomic sequences (from 75 278 ESTs and 1536 Mb of genomic sequences). The candidate genes were assembled into five contigs, all with the typical four-cysteine signature (C1-X6-C2-X6-18-C3-X2-C4) of CSPs, showing that G. m. morsitans has five independent CSP genes with from 108 to 158 amino acids (Table 1). All five predicted CSPs have a predicted signal peptide, varying from 19 to 26 amino acids and indicating that the CSP sequences are full length. The mature peptides were aligned (Fig. 1) revealing the presence of the expected four cysteines. Besides the four-cysteine signature, some hydrophobic residues are also highly conserved (Fig. 1) and these may be critical for the specific 3D structural

Figure 1. Alignment of chemosensory proteins (CSPs) of Glossina morsitans morsitans. The conserved four-cysteine signature of CSPs is shaded in dark grey. The hydrophobic positions in the alignment with the cut-off percentage >60% are shaded in light grey. The horizontal bars under the alignment represent the α-helices of Bombyx mori CSP1 (Jansen et al., 2007). The intron splice site was separated by the angle separator. The numbers below the sequences are the residue numbering. Asterisks (*) indicate positions that have a single, fully conserved residue. Colons (:) indicate conservation between groups with very similar properties – scoring >0.5 in the Gonnet PAM 250 matrix. A full stop (.) indicates conservation between groups with slightly similar properties – scoring ≤0.5 in the Gonnet PAM 250 matrix (Larkin et al., 2007). These are also indicated by the height of the dark bars in the bottom panel.

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configuration of the CSPs (Campanacci et al., 2003; Tomaselli et al., 2006; Jansen et al., 2007; Zhou et al., 2009).

Three of the G. m. morsitans CSP genes (GmmCSP2, GmmCSP4 and GmmCSP5) have an intron between conserved cysteines C2 and C3 (Fig. 1). Interestingly, the intron between C2 and C3 in GmmCSP4 is lost in the Drosophila orthologue Phk-3.

Phylogenetic analysis of the five G. m. morsitans CSPs (Fig. 2) shows that they are very diverse as seen in CSPs of other insect species (Pelosi et al., 2006; Xu et al., 2009). Only two CSPs (GmmCSP1 and GmmCSP3) are clustered together with bootstrapping support of 70%. Their amino acid sequences are 63% identical and are orthologues of the Drosophila CSP PebIII. The closest orthologues to the G. m. morsitans CSPs are found in

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Figure 2. Phylogenetic tree of chemosensory proteins (CSPs) in the dipteran species Glossina morsitans morsitans (Gmm), Drosophila melanogaster (Dmel), Aedes aegypti (Aaeg), Anopheles gambiae (Agam) and Culex quinquefasciatus (Cqui). Bootstrapping supports are indicated beside the branches at 1000 simulations. Subtrees A to C show consensuses in all the five species. Subtree D shows recent duplication of some CSPs after the divergence of the Aedes and Culex groups.
D. melanogaster with an average protein sequence identity of 69% (Table 1). ESTs of GmmCSP1 were found from cDNA libraries of larvae, antennae, fat body, adult gut, reproductive organs and salivary gland, whereas ESTs of GmmCSP3 were only present in the antennal library. This indicates that GmmCSP1 may have a range of functions, depending on where it is expressed, but GmmCSP3 may be involved in olfaction (see below), with the two genes gaining different functions during their divergence from the ancestor gene.

Tissue distribution of CSP genes
In order to examine further the potential functions of the G. m. morsitans CSPs and which CSP gene is expressed in the olfaction organs of G. m. morsitans, we carried out a real time quantitative reverse transcription PCR (qPCR) analysis to measure the transcription abundance of the CSP genes in head plus antennae, body and antennae alone (Fig. 3). Of the five CSP genes, two (GmmCSP1 and GmmCSP2) had higher transcription levels in antennae, with GmmCSP2 being about five times higher than in other tissues. Furthermore, expression of GmmCSP2 was not detected in bodies, and was very low in heads plus antennae (about 0.26 times that of β-tubulin) probably because of the presence of antennal tissues, indicating that GmmCSP2 is specifically, and highly, expressed in the antennae and could therefore play a role in olfaction. Transcription of GmmCSP1 and GmmCSP3 was detected in all the tissues tested, with GmmCSP1 having a higher transcription level in antennae relative to heads and bodies, about 0.94, 0.34 and 0.36 times higher than that of β-tubulin, respectively. GmmCSP3 was more highly transcribed in heads than in bodies and antennae, about 2.3, 0.92 and 0.68 times that of β-tubulin, respectively. The higher transcription in heads suggests that GmmCSP3 may have other possible roles apart from olfaction. Our qPCR analysis provided the expression profile information of CSP genes in the heads and bodies that was lacking in the EST data (Table 1). GmmCSP4 and GmmCSP5 were only weakly transcribed in all adult tissues and may not play any olfactory roles in adult tsetse flies.

Expression of G. m. morsitans CSPs following a bloodmeal
To characterize further the CSP genes in the antennae of G. m. morsitans, we dissected antennae tissues from male and female flies before and after a bloodmeal at a range of time intervals (Fig. 4). For males the antennae were collected from newly emerged adults, 24 and 48 h postbloodmeal (PBM). For young females the antennae were further collected 72 h PBM, and for 10-week-old females the antennae were collected 48 and 72 h PBM. Of the three CSP genes transcribed in antennae (Fig. 3), GmmCSP1 and GmmCSP3 had similar transcription levels in males and females and there were no notable differences before and after a bloodmeal up to 48 h. However, GmmCSP2 had higher transcription in females than in males, being 3.3, 1.5 and 2.5 times higher in female antennae than in male antennae for newly emerged, 24 h and 48 h PBM adults, respectively (Fig. 4). In the newly emerged flies the transcription of GmmCSP2 was 12.2 ± 1.1-fold higher than that of β-tubulin in female antennae and 3.6 ± 0.5-fold higher than β-tubulin in male antennae. Moreover, in females GmmCSP2 transcription was decreased within 24 h PBM and then increased with time from 10.1 ± 0.6-fold higher than β-tubulin transcription at 24 h PBM to 12.5 ± 0.8 and 14.9 ± 0.7-fold higher at 48 and 72 h PBM, with a further increase to 21.2 ± 0.7-fold higher in 10-week-old females. This increase may be related to the higher demand for food during starvation and the need for an increased ability to detect hosts by the female flies. Besides the relationship with starvation, GmmCSP2 transcription level was also related to age,
with older flies having a higher transcription level than with young flies at similar stages of starvation (48 h PBM).

Evolution of CSPs in dipteran insects

To characterize the molecular evolution relationships between the CSPs of *G. m. morsitans* and those of others insects in Diptera, we constructed a phylogenetic tree (Fig. 2) including five CSPs of *G. m. morsitans* identified in this study, 21 in *Culex quinquefasciatus* (Pelletier & Leal, 2011), four in *D. melanogaster* (McKenna et al., 1994; Zhou et al., 2006), eight in *Anopheles gambiae* (Zhou et al., 2006) and 18 in *Aedes aegypti* (J.J. Zhou, unpubl. data). The CSP sequences are listed in Table S2. The tree shows that each *G. m. morsitans* CSP has orthologues in *D. melanogaster* with strong bootstrapping support: GmmCSP1 and DmelPebIII, GmmCSP3 and DmelPebIII, GmmCSP2 and DmelA10, GmmCSP4 and DmelPhk-3, GmmCSP5 and DmelCSP1, consistent with the established species phylogeny that *G. m. morsitans* is more closely related to *D. melanogaster* than to mosquitoes (Arensburger et al., 2010). Interestingly, the antenna-specific CSP of *G. m. morsitans* CSP has orthologues in *D. melanogaster* with strong bootstrapping support: GmmCSP1 and DmelPebIII, GmmCSP3 and DmelPebIII, GmmCSP2 and DmelA10, GmmCSP4 and DmelPhk-3, GmmCSP5 and DmelCSP1, consistent with the established species phylogeny that *G. m. morsitans* is more closely related to *D. melanogaster* than to mosquitoes (Arensburger et al., 2010). Interestingly, the antenna-specific CSP of *G. m. morsitans* GmmCSP2 is the orthologue of DmelA10 or OS-D, a CSP from *D. melanogaster*. DmelA10 protein was found to express in the sensillum coeloconicum of antennal segment 3, which is the main olfaction organ of *D. melanogaster* (McKenna et al., 1994; Pikielny et al., 1994). GmmCSP2 was also found to be more highly expressed in female than in male tsetse flies (Fig. 4). GmmCSP1 and GmmCSP3 are expressed in heads, bodies and antennae and are orthologous to DmelPebIII (McKenna et al., 1994). DmelPebIII expression was found to be induced by viral and bacterial infections (Sabatier et al., 2003) and expressed at a high level in antennae in adult heads, eyes, crops, ejaculatory bulbs and hindguts [FlyBase: Gelbart, W.M., Emmert, D.B. (2010.10.13) FlyBase High Throughput Expression Pattern Data Beta Version], consistent with the nonspecific expression profile of GmmCSP1 and GmmCSP3 (Fig. 3).

The tree also shows three orthologous groups containing CSP members from all five species, labelled as A, B and C in Fig. 2. These appear to represent three lineages in Diptera that diverged about 210–260 million years ago (Ma), before the divergence of Brachycera and Nematocera (Arensburger et al., 2010). GmmCSP1, GmmCSP3 and DmelPebIII are clustered together in a separate branch and may have diverged after the split of Brachycera (flies) and Nematocera (mosquitoes). A gene expansion branch, labelled D in Fig. 2 shows that these CSPs diverged after the split of *Aedes* and *Culex*, and indicates a rapid CSP gene expansion around 50–54 Ma in both mosquito species.

There seems to be a correlation of the expansion of CSP genes and the number of CSPs in dipteran species. *Aedes aegypti* with 18 CSPs and *Culex quinquefasciatus* with 21 CSPs diverged from Culicinae about 50–54 Ma, whereas *G. m. morsitans* with only five CSPs and *D. melanogaster* with only four CSPs diverged from Brachycera about 210–260 Ma. *Anopheles gambiae*, with an intermediate number of eight CSPs, diverged from Culiciniae about 150 Ma (ie between the 50–54 and the 210–260 Ma period of the divergence of Brachycera and Nematocera). Thus, dipteran CSPs may have evolved from three ancestral CSP genes about 260 Ma, during the diversification of suborders Nematocera and Brachycera. The expansion of CSPs then slowed down in insect species of the Brachycera, but continued in insect species of the Nematocera about 150 Ma, with a rapid expansion in insect species of the Culicinae in the last 50–54 million years.

Experimental procedures

**Insects and tissues**

The *G. m. morsitans* colony was maintained at the Liverpool School of Tropical Medicine (colony established in 2002 from the...
Bristol colony, itself originally derived from flies from Zimbabwe). Flies were kept at 26 °C and 70% relative humidity. Male flies were fed with defibrinated horse blood every 48 h through artificial membranes (Moloo, 1971). Twenty-four hours after a fresh bloodmeal, flies were frozen at –20 °C for 5 min, and then the heads with antennae and bodies were separated and placed in 100 μl ice-cold Trizol regent (Invitrogen, Paisley, UK) for RNA extractions.

Antennae were prepared from (1) newly emerged males, (2) young males 24 h PBM, (3) young males 48 h PBM, (4) newly emerged females, (5) young females 24 h PBM, (6) young females 48 h PBM, (7) young females 72 h PBM, (8) 10-week-old females 48 h after last bloodmeal and (9) 10-week-old female 72 h after last bloodmeal. Living flies were chilled at 4 °C then antennae were detached (using sterile fine forceps) and immediately placed in an Eppendorf tube containing 100 μl ice-cold Trizol reagent. About 50–60 pairs of antennae were collected from each sample.

RNA and DNA preparation

Twenty heads with antennae weighing c. 42 mg and four bodies without heads weighing c. 92 mg were ground separately in liquid nitrogen. The powder was then mixed with Trizol regent (1 ml) and ground again. The homogenized tissue was then transferred into a 1.5-ml RNase-free tube, mixed with 200 μl chloroform and left for 10 min at room temperature. For antennal RNA isolation, about 60 pairs of antennae were ground using a plastic pestle. Total RNA was extracted using Trizol regent for 10 min and then extracted again with 250 μl chloroform, and finally DNA was precipitated with 75% ethanol and dissolved in 60 μl water (Sigma, St Louis, MO, USA). Genomic DNA samples of 20, 2, 1 and 0.2 ng/µl were used to plot standard curves for calculating the transcript abundance of each gene.

Construction of the antennal cDNA library

The antennal cDNA library was constructed using the Creator Smart cDNA Library Construction Kit (Clontech, Mountain View, CA, USA) according to the supplier’s instructions. About 700 ng antennal RNA from flies of mixed ages and sexes was used for the first strand cDNA synthesis in a reaction volume of 10 μl, from which 2 μl single strand cDNA was used for long distance PCR with pre-denaturing at 95 °C for 2 min, followed by 20 cycles of 95 °C for 15 s and 68 °C for 6 min and a final elongation cycle of 72 °C for 2 min. The purified resultant double strand cDNA was digested with Sfi, size fractionated and ligated into the pDNR-Lib vector (Clontech). The ligation mixture was desalted, electroporated into Escherichia coli XL1-blue electro-competent cells and plated on agar plates supplemented with chloramphenicol (34 μg/ml). Clones were placed into the wells of 384-well plates for sequencing of randomly selected bacterial clones in both directions using T3 and T7 primers and ABI Big Dye Terminator Cycle Sequencing kits (Life Technologies Ltd, Paisley, UK). The raw sequences were clipped in Phred to remove the unqualified ends and the vector sequences were removed with CROSS MATCH software (P. Green, unpublished). Sequence reads were assembled into clusters using PHRAP (P. Green, unpublished; http://www.phrap.org).

Identification of putative CSP cDNAs in the G. m. morsitans library

Sequences from the antennal cDNA library (this study) and of other ESTs (http://www.ncbi.nlm.nih.gov/dbEST/) as well as the whole-genome shotgun reads and the genome assembly produced by the Wellcome Trust Sanger Institute (available from ftp://ftp.sanger.ac.uk/pub/pathogens/Glossina/morsitans/) were searched with a combination of methods using MotifSearch (Zhou et al., 2004, 2008), BLASTx, on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and RPS-BLAST (Marchler-Bauer et al., 2011). The sequence hits were collected as putative CSP sequences if their BLAST scores were less than 10^{-5} compared to the OS-D domain (pfam03392) and to known CSP sequences and they had the CSP four cysteine signature, with a low molecular weight and a hydrophobic signal peptide.

qPCR

Primers were between 19 and 22 bp with the melting temperature (Tm) values ranging from 59.5 to 60.5 °C and designed using PRIMER3 PLUS (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The size of the PCR products was set within the range 120–260 bp. As genomic DNA was used to establish standard curves for quantification, the primers were optimized and designed on single exon or exons flanking short introns. Information on the primers is given in Table S1.

Invitrogen Platinum SYBR Green qPCR SuperMix-uracil DNA glycosylase (Invitrogen) was used for the qPCR reactions. An aliquot (5 μl) of total RNA ranging from 0.2 to 1 μg was first treated with RNasefree DNase in a total volume of 10 μl reaction mixture. Then, 6 μl reaction mixture was used for reverse transcription using the Improm-II Reverse Transcription System (Promega, Southampton, UK) in 20 μl reactions and then diluted to 500 μl, from which 3 μl was used as the template for each qPCR reaction. Each reaction included 1 × SuperMix, 200 nM of each of the gene-specific primer pairs, 50 nM 6-carboxy-X-rhodamine dye and 3 μl templates. The qPCRs were carried out for each of two RNA preparations from each tissue sample. The β-tubulin gene of G. m. morsitans was included for initial normalization of the template amount. The PCR reactions were carried out using a Stratagene Mx3000P qPCR system (Agilent Technologies UK Ltd, Cheshire, UK) with a thermoprofile of one cycle of 50 °C for 2 min, 95 °C for 2 min, then 45 cycles of 95 °C for 15 s, 60 °C for 45 s, followed by a melting curve analysis from 55 to 95 °C.

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References

Aksoy, S., Berriman, M., Hall, N., Hattori, M., Hide, W. and Lehane, M. (2005) A case for a Glossina genome project. *Trends Parasitol.* 21: 107–111.

Angeli, S., Ceròn, F., Scaloni, A., Monti, M., Monteforti, G., Minnocci, A. et al. (1999) Purification, structural characterization, cloning and immunocytotoxic localization of chemoreception proteins from Schistocerca gregaria. *Eur J Biochem* 262: 745–754.

Areensburger, P., Megy, K., Waterhouse, R.M., Abrudan, J., Amedeo, P., Antelo, B. et al. (2010) Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. *Science* 330: 86–88.

Ban, L., Scaloni, A., Brandazza, A., Angeli, S., Zhang, L., Yan, Y. et al. (2003) Chemosensory proteins of *Locusta migratoria*. *Insect Mol Biol* 12: 125–134.

Briand, L., Swasdipan, N., Nespolous, C., Bézirard, V., Blon, F., Huet, J.C. et al. (2002) Characterization of a chemosensory protein (ASP3c) from honeybee (Apis mellifera L.) as a brood pheromone carrier. *Eur J Biochem* 269: 4586–4596.

Campanacci, V., Mosbah, A., Boret, O., Wechselberger, R., Jacquin-Joly, E., Cammillau, C. et al. (2001) Chemosensory protein from the moth *Mamestra brassicae*. Expression and secondary structure from 1H and 15N NMR. *Eur J Biochem* 268: 4731–4739.

Campanacci, V., Lartigue, A., Hallberg, B.M., Jones, T.A., Giudici-Oriconi, M.T., Tegoni, M. et al. (2003) Moth chemosensory protein exhibits drastic conformational changes and cooperativity on ligand binding. *Proc Natl Acad Sci USA* 100: 5069–5074.

Dani, F.R., Michelucci, E., Francesc, S., Mastrobuoni, G., Capellozza, S., La Marca, G. et al. (2011) Odorant-binding proteins and chemosensory proteins in pheromone detection and release in the silkworm *Bombyx mori*. *Chem Senses* 36: 335–344.

Forêt, S., Wanner, K.W. and Maleszka, R. (2007) Chemosensory proteins in the honey bee: insights from the annotated genome, comparative analyses and expression profiling. *Insect Biochem Mol Biol* 37: 19–28.

Jacquin-Joly, E., Vogt, R.G., Francois, M.C. and Nagnan-Le Meillour, P. (2001) Functional and expression pattern analysis of chemosensory proteins expressed in antennae and pheromonal gland of *Mamestra brassicae*. *Chem Senses* 26: 833–844.

Jansen, S., Chmelik, J., Zidek, L., Padtra, P., Novak, P., Zdrahal, Z. et al. (2007) Structure of *Bombyx mori* chemosensory protein 1 in solution. *Arch Insect Biochem Physiol* 66: 135–145.

Kitabayashi, A.N., Arai, T., Kubo, T. and Natori, S. (1998) Molecular cloning of cDNA for p10, a novel protein that increases in regenerating legs of *Periplaneta americana* (American cockroach). *Insect Biochem Mol Biol* 28: 785–790.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. et al. (2007) ClustalW and ClustalX version 2. *Bioinformatics* 23: 2947–2948.

Liu, R., Lehane, S., He, X., Lehane, M., Hertz-Fowler, C., Berri man, M. et al. (2010) Characterisations of odorant-binding proteins in the tsetse fly *Glossina morsitans morsitans*. *Cell Mol Life Sci* 67: 919–929.

Liu, X., Luo, Q., Zhong, G., Rizwan-Ul-Haq, M. and Hu, M. (2010) Molecular characterization and expression pattern of four chemosensory proteins from diamondback moth, *Plutella xylostella* (*Lepidoptera: Plutellidae*). *J Biochem* 148: 189–203.

Maleszka, R. and Stange, G. (1997) Molecular cloning, by a novel approach, of a cDNA encoding a putative olfactory protein in the labial palps of the moth *Cactoblastis cactorum*. *Gene* 202: 39–43.

Mameli, M., Tuccini, A., Mazza, M., Petacchi, R. and Pelosi, P. (1996) Soluble proteins in chemosensory organs of Phas mids. *Insect Biochem Mol Biol* 26: 875–882.

McKenna, M.P., Hekmat-Scafe, D.S., Gaines, P. and Carlson, J.R. (1994) Putative D melanogaster pheromone-binding pro teins expressed in a subregion of the olfactory system. *J Biol Chem* 269: 16340–16347.

Marchler-Bauer, A., Lu, S., Anderson, J.B., Chitsaz, F., Derbyshire, M.K., DeWeese-Scott, C. et al. (2011) CDD: a Con served Domain Database for the functional annotation of proteins. *Nucleic Acids Res* 39(D): 225–229.

Moloo, S.K. (1971) An artificial feeding technique for Glossina. *Parasitology* 63: 507–511.

Mosbah, A., Campanacci, V., Lartigue, A., Tegoni, M., Cammillau, C. and Darbon, H. (2003) Solution structure of a chemosen sory protein from the moth *Mamestra brassicae*. *Biochem J* 369: 39–44.

Nagnan-Le Meillour, P., Cain, A.H., Jacquin-Joly, E., Francois, M.C., Ramachandran, S., Maida, R. et al. (2000) Chemo sensory proteins from the proboscis of *Mamestra brassicae*. *Chem Senses* 25: 541–553.

Pelletier, J. and Leal, W.S. (2011) Characterization of olfactory genes in the antennae of the Southern house mosquito, *Culex quinquefasciatus*. *J Insect Physiol* 57: 915–929.

Pelosi, P., Zhou, J.J., Ban, L.P. and Calvello, M. (2006) Soluble proteins in insect chemical communication. *Cell Mol Life Sci* 63: 1658–1676.

Picimbon, J.-F.J., Dietrich, K., Krieger, J. and Breer, H. (2001) Identity and expression pattern of chemosensory proteins in *Heliothis virescens* (*Lepidoptera, Noctuidae*). *Insect Biochem Mol Biol* 31: 1173–1181.

Pikelny, C.W., Hasan, G., Rouyer, F. and Rosbash, M. (1994) Members of a family of Drosophila putative odorant-binding proteins are expressed in different subsets of olfactory hairs. *Neuron* 12: 35–49.

Sabatier, L., Jouanguy, E., Dostert, C., Zachary, D., Dimarco, J.L., Bulet, P. et al. (2003) Pherokine-2 and -3. Two Drosophila molecules related to pheromone/odor-binding proteins...
induced by viral and bacterial infections. *Eur J Biochem* **270**: 3398–3407.

Tomaselli, S., Crescenzi, O., Sanfelice, D., Wechselberger, R., Angeli, S., Scaloni, A. *et al.* (2006) Solution structure of a chemosensory protein from the desert locust *Schistocerca gregaria*. *Biochemistry* **45**: 10606–10613.

Whitfield, C.W., Band, M.R., Bonaldo, M.F., Kumar, C.G., Liu, L., Pardinas, J. *et al.* (2002) Annotated expressed sequence tags and cDNA microarrays for studies of brain and behaviours in the honey bee. *Genome Res* **12**: 555–566.

Xu, Y.L., He, P., Zhang, L., Fang, S.Q., Dong, S.L., Zhang, Y.J. *et al.* (2009) Large-scale identification of odorant-binding proteins and chemosensory proteins from expressed sequence tags in insects. *BMC Genomics* **25**: 10–632.

Zhou, J.-J., Kan, Y., Antoniw, J., Pickett, J.A. and Field, L.M. (2006) Genome and EST Analyses and Expression of a Gene Family with Putative Functions in Insect Chemoreception. *Chem Senses* **31**: 453–465.

Zhou, J.-J., He, X.L., Pickett, J.A. and Field, L.M. (2008) Identification of odorant-binding proteins of the yellow fever mosquito *Aedes aegypti*, genome annotation and comparative analyses. *Insect Mol Biol* **17**: 147–163.

Zhou, J.-J., Robertson, G., He, X.L., Dufour, S., Hooper, A.M., Pickett, J.A. *et al.* (2009) Characterisation of *Bombyx mori* odorant-binding proteins reveals that a general odorant-binding protein discriminates between sex pheromone components. *J Mol Biol* **389**: 529–545.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2583.2011.01114.x

**Table S1.** Primers used for real time quantitative reverse transcription PCR analysis of chemosensory protein genes in *Glossina morsitans morsitans*.

**Table S2.** The GenBank ID, name and amino acid sequence of chemosensory proteins that are used to construct the phylogenetic tree (Fig. 2).

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