

Cloning and expression of PKG, a candidate foraging regulating gene in *Vespula vulgaris*

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Abstract

In honey bees, enhancement of cGMP-dependent protein kinase (PKG) expression accompanies a behavioural transition from in-hive working nursing bees towards outdoors foraging worker bees. Accordingly this gene was named *amfor* or *Apis mellifera foraging* gene. In the red harvester ant *Pogonomyrmex barbatus* a gene homologue affected food seeking behaviour as well, but in this species PKG expression decreased from the onset of foraging behaviour. Since the wasp *Vespula vulgaris* is phylogenetically positioned between ants and bees, in this paper we tried to elucidate whether the involvement of PKG in foraging behaviour can be extended to this species and if so, whether its expression is enhanced or decreased by the transition from nursing to foraging. To enable this candidate gene approach, we first had to clone the PKG homologue from the common wasp. QPCR indicated a relevantly higher expression of *Vifor* in nursing versus foraging wasps although interpretation of the results was hampered by a remarkable degree of variation as could be predicted from wasps captured in the wild as a source for mRNA extraction and quantification.

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Keywords

PKG; foraging; *Vespula vulgaris*; molecular ethology; *amfor*

Introduction

The involvement in food seeking behaviour of a cGMP-dependent protein kinase (PKG) as present in *Drosophila melanogaster* depends on allelic variation. An example of a single gene polymorphism is provided by the PKG encoded by the *foraging* (*for*) gene (Sokolowski et al., 1997). PKGs are serine/threonine kinases and are important

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in many biological processes (for a review see Wang and Robinson, 1997, and Hofmann et al., 2006). *Drosophila* larvae of the rover phenotype have higher PKG activity levels and display higher motility compared to the sitter variant (Sokolowski et al., 1997; Osborne et al., 1997). These two different alleles are selected for under different environmental conditions. In the presence of unevenly distributed food, rovers have an advantage over sitters, whereas continuously distributed food is beneficial to sitters. Under high population density conditions, the frequency of the rover allele will increase, while the sitter allele is preferentially selected for under low density conditions (Sokolowski, 1980; Sokolowski et al., 1997).

In the nematode *Caenorhabditis elegans*, *egl-4*, a gene that encodes a cGMP-dependent protein kinase, is involved in wild type motility. Two different traits in feeding behaviour have been characterised, dwelling and roaming. *Egl-4* expression will decrease roaming activity which means that the nematode will show less movement due to changes in sensory perception (Stansberry et al., 2001; Fujiwara et al., 2002). It is however clear that, in *C. elegans*, beside *egl-4* there are other genes influencing feeding behaviour and motility such as *npr-1* (de Bono and Bargmann, 1998) and *egl-21* (Husson et al., 2007), but they do not seem to be directly involved in the PKG pathway. *Npr-1* is a cGMP mediated G-protein-coupled neuropeptide receptor, but no direct link between *npr-1* and *egl-4* is found (Coates and de Bono, 2002). EGL-21 is proven to be a carboxypeptidase needed for removing the dibasic cleavage site at the C-terminus of peptides released from their precursor by furin-type proteases (Husson et al., 2007).

In contrast to the situation in *Drosophila*, in social insects such as the honey bee *Apis mellifera* and the red harvester ant *Pogonomyrmex barbatus*, the behavioural transition is also correlated with a change in expression level of the single copy gene of brain PKG but no allelic variants have been reported (Ben Shahr et al., 2002; Ingram et al., 2005). These social insects exhibit an age-related division of labour or temporal polyethism. Young ants and honey bees will perform tasks in hive and later on will switch to tasks at the entrance of the nest followed by eventual foraging, the gathering of food. This sequence of task division is well established (Robinson, 1992; Trumbo et al., 1997; Gordon et al., 2005). The observed transition in behaviour is not only age-related, but is also dependent on the needs of the colony (Robinson, 1992; Huang and Robinson, 1992). Recently, it has been shown that division of labour in honey bees is associated with widespread changes in brain gene expression levels (Withfield et al., 2003).

In honey bees, foraging workers show a higher *for* expression and PKG activity compared to in-hive active nursing bees. The results of a study on 'single cohort colonies' excludes the age-dependence of the *for* expression levels. Precocious foragers have significantly higher *amfor* expression than the same-aged nurses. Treatment with 8-Br-cGMP, a membrane permeable cGMP analogue, increased PKG activity and the possibility of precocious foraging. This study offered evidence that PKG activation can influence initiation of foraging behaviour (Ben Shahr et al., 2002). In 2003, Ben Shahr et al. reported a role for cGMP in regulating phototaxis and the age at which the bees start foraging. The opposite effect was reported on *for* gene expression in brains of the red harvester ant *Pogonomyrmex barbatus*. In this social insect, although

showing similar temporal polyethism or age-dependent task implementation, foraging individuals displayed diminished expression levels compared to in nest controls (Ingram et al., 2005).

In situ hybridisation analysis on the brain of the honey bee showed that *Amfor* is mainly expressed in the lamina of the optic lobes and the Kenyon cells in the mushroom bodies (MB) (Ben-Shahar et al., 2002). The MB are the most important centre for sensory input processing (Heisenberg, 1998). This structure is well conserved in social Hymenoptera (Gronenberg, 2001) and apparently has a role in the division of labour. Gronenberg et al., (1996) found that there are age-dependent and task-related morphological changes in the MB of the ant *Camponotus floridanus*. Beside these changes, the MB neuropile volume can additionally increase over 50% when an ant displays foraging activity. Under natural conditions, the same age- and task-dependent increase in MB volume has been found in foraging honey bees (Fahrbach et al., 1998; 2003; Farris et al., 2001). When studying the neural activity in the honey bee brain, a foraging-specific increase in neuronal gene expression activity was found in the small-type Kenyon cells, suggesting that only these cells in the MB are associated with foraging behavioural aspects (Kiya et al., 2007).

The social wasp *Vespula vulgaris* is phylogenetically situated in between bees and ants and is even more related to ants (Brothers, 1999); the division of labour found in their colonies is similar to ants (O'Donnell and Jeanne, 1992a, 1992b). In the wasp *Polybia aequatorialis*, this division of labour is also associated with the increase in volume of the MB (O'Donnell et al., 2004). Given these findings, we wanted to figure out 1) whether a PKG homologue is present in *Vespula vulgaris*, 2) whether its expression level changed according to changes in behaviour and 3) whether there is any phylogenetic rationale in the observed changes. Since for the wasp neither EST nor genomic data about the *for* cDNA sequence was available, we first had to clone the *for* homologue in *Vespula vulgaris* using the candidate gene approach in combination with RACE (Fitzpatrick et al., 2005). Furthermore, in order to obtain a validated housekeeping gene for normalisation in quantitative real time PCR (qPCR) experiments, we cloned a fragment of *Vespula vulgaris* β -actin cDNA as well. The obtained sequence information allowed us to perform qPCR to study the expression of the *Vespula for* homologue.

Materials and methods

Animals

Individuals of eight different colonies of *Vespula vulgaris* were captured in the wild in the area around Leuven, Belgium. Four colonies were captured in September and October 2005 and four in October 2006. Obtained data are the result of pooling brains from ten individuals, either in-hive nurses or approaching foragers from each colony. The wasps were collected during the day but individuals from different colonies were not sampled at the same time point and under different weather conditions because we had to rely on the presence and detection of hives in the wild. The wasps, once caught, were kept alive, transported to the lab and dissected the same day they were captured.

Sample collection

Brains were dissected directly in RNA-later® (Ambion). After one night in this solution at 4 °C, the tissue was drained and kept frozen at –80 °C. Total RNA was isolated using the RNeasy lipid tissue kit according to the protocol provided by the supplier (Qiagen). Importantly a DNase step was included in this procedure. First strand cDNA synthesis was realised using the RevertAid™ Minus First Strand cDNA Synthesis kit from Fermentas and using either random hexamer primers (qPCR) or oligo (dT)₁₈ primers (cloning) and M-MuLV reverse transcriptase.

Cloning of the *for* and β -actin homologue of *Vespula vulgaris*

RNA derived of both nursing and foraging wasps was reverse transcribed. Insect *for* homologues with accession numbers AAV65146, NP_001011581.1, XP_319605, AAL76256 and NP_477487PCR were searched for conserved regions on amino acid level to design degenerate primers in which we avoided the most degenerate amino acids like leucine, arginine and serine (RQQQHIM: degF1 5'-MGN CAR CAR CAR CAY ATH ATG-3'; KYLYMLME: degF2 5'-AAR TAY YTN TAY ATG YTN ATG GA-3'; QKHKWFDG: degR1 5'-GT NCC RCA RAA NGT CCA NGT YTT-3; KTWTF CGT degR2 5'-CC RTC RAA CCA YTT RTG YTT YTG-3'). In these primers the letters M, N, R and V denote respectively the nucleotides (A or C), (any nucleotides), (A or G) and (not T) respectively. These primers allowed cloning and sequencing of an internal fragment of about 663 bp in length. *In silico* translation and blast results proved that the cloned internal fragment was part of a *Vespula vulgaris for* gene. 5' and 3' RACE (Rapid amplification of cDNA ends) (Frohmann et al., 1988) using adapter primers supplied in the Marathon™ cDNA amplification kit (BD Biosciences) in combination with gene specific forward (*for*F1 5'-CGC ACC GGA AGT TAT TCT CAA T-3' and *for*F2 5'-AGA GGC CAC GAC ATA AGC GCC-3') and reverse (*for*R1 5'-CAT TGG ATC AGA ACC AGT GAA-3' and *for*R2 5'-TGG CGG CGC TCC AGT GAG AGG-3') primers in subsequent steps allowed cloning and sequencing a *Vfor* full length cDNA. The sequence was submitted to GenBank (ABL74445.1).

The same cDNA was used to clone an internal cDNA fragment of *Vespula vulgaris* β -actin using degenerate primers designed with the sequences with accession number CAJ14142, NP_001119726, CAA34718, AAK72124, NP_001014726, NP_001092, AAQ24838 and AAQ89578 as described previously (MVGMGQK: ActdegF1 5'-ATG GTN GGN ATG GGN CAR AAR-3', DMEKIWHH: ActdegF2 5'- GAY ATG GAR AAR ATH TGG CAY CA -3', FQQMWISK: ActdegR1 5'- TTN SWD ATC CAC ATY TGY TGR -3' and MQKEITA: ActdegR2 5'-NGC NGT DAT YTC YTT YTG CAT-3').

All fragments were cloned in the pCR®4-TOPO® vector (Invitrogen) which was used to transform One Shot® TOP10 chemically competent *E. coli* cells (Invitrogen). Plasmids were purified using the NucleoSpin® Plasmid QuickPure kit (Macherey-Nagel). Sequences from multiple clones were obtained using the BigDye Terminator Cycle Sequencing Ready Reaction kit from Applied Biosystems. All protocols were performed according to instructions provided by the supplier.

Once the full length cDNA sequence of *Vvfor* was obtained, a multiple sequence alignment using clustal W (Jeanmougin et al., 1998) was performed for the protein sequences with the available overlapping C-terminal sequence of PbFOR (AAV65146), AmFOR (NP_001011581.1) and *Nasonia vitripennis* FOR (XP_001603549.1).

Quantitative PCR

cDNA samples of *Vespula vulgaris* nurses and foragers were quantified using SYBR® Green method (Ponchel et al., 2003) in the ABI Prism 7000 detection system with gene specific primers: qForFw 5'-CGC ACC GGA AGT TAT TCT CAA TA-3', qForRv 5'-CAT TGG ATC AGA ACC AGT GAA TG-3', qActFw 5'-GGT GAT TAC CAT TGG TAA CGA AAG A-3' and qActRv 5'-ACG TCG CAC TTC AGT ATC GA-3'. Serial dilutions of control cDNA for generating standard curves were included in duplicate in every run to quantify relative differences in *Vvfor* expression, while every sample was run in triplicate in the following PCR conditions: 50°C for 2 min, 95°C for 10 min, 40x (95°C for 15 s, 60°C for 1 min). Analysis was performed with the ABI Prism 7000 SDS software. *Vvfor* quantities were normalised to the endogenous control β -actin to account for the variability in initial mRNA concentrations and differences in reverse transcriptase efficiency (For review: Bustin, 2000, and Kubista et al., 2006).

The non-parametric Wilcoxon test was used to elucidate whether the differences in gene expression between nurses and foragers from eight different colonies were statistically significant.

Results

Cloning and alignment of Vespula vulgaris cGMP dependent protein kinase

A PCR with degenerate primers resulted in the partial sequence of a PKG gene homologue, which we prolonged using 3' and 5' RACE. Since the sequencing of the inserts in multiple colonies confirmed one single sequence, we suggest only one PKG gene is expressed in the *Vespula vulgaris* brain. Our sequences give no indication for the existence of different transcripts but no other experimental tests were done to confirm or deny this. Compared to AmFOR, the *in silico* translation indicated an internal deletion of nine amino acids which is also found in the sequence of *Pogonomyrmex* FOR. VvFOR contained an insertion of two amino acids also found in AmFOR making the *Vespula* FOR protein somewhat the same length as AmFOR, being 671 and 678 AA respectively. In *Nasonia vitripennis* (a parasitoid wasp), the corresponding protein sequence has a prolonged N-terminal extension. In many other species this prolonged N-terminal extension is also present [e.g. *Drosophila* (NP_477488.1), *C. elegans* (NP_001023223.1, ...)]. The complete sequence of *Vespula vulgaris* FOR (VvFOR) is available in Genbank with accession number EF136648.1. In accordance with its function as a cGMP-dependent protein kinase inside the cell, a putative signal peptide sequence is lacking.

The alignment in fig. 1 shows that the sequences of the four Hymenoptera are very similar. VvFOR has respectively 77% , 83% and 85% identity with AmFOR, NvFOR

AmFOR 1 -----MC--TRRELQELL
PbFOR 1
NvFOR 1 MRVCFDSLFCFSSPQQRLADEEEPAQTQQQQPPPTLQTTTQPQPQMGQQQCGMABELL
VvFOR 1 -----MC--KRLRELQELL
consensus 1

AmFOR 12 RVKDEKIVLEALLCRRDAEIQELRSHLDKFFQCASILKLAFFPRN--PcPGSKGPRDFP
PbFOR 1
NvFOR 61 RARDERIAEALCQRDAEIQELRSHLDKFLSVLPKSPAPDS---PRPRKRAQGIS
VvFOR 12 RVKDRIAEALCQRDAEIQELRSHLDKFLSVLPKSPAPPATPKPRPRKRAQGIS
consensus 61

AmFOR 70 RSHRFKSLPRCQLSNKPRDRSRELTKAAIILANDFMKNLELTQIRDRDGLHVSCSFSAGSTI
PbFOR 1
NvFOR 118 AEPPEQELAPLAVQVDSKDRSRELTKAAIILNDFMKNLELTQIREIVDCMVPVTFPAGHII
VvFOR 72 AEPPEQELAPLAVVVEKSDRSRLIKRALLANDFMKHLISMAQIIEIVDCMPLIAFFFGSTI
consensus 121

AmFOR 130 IREGDVGSIVVMEEGKVEVSRDGKYLSTLAPGKVLGELAILYNCKRTATITAAATDCQLW
PbFOR 1
NvFOR 178 IREGDVGSIVFVMEEGKVEVSRDGKYLSTLAPGKVLGELAILYNCKRTATITAAATDCQLW
VvFOR 132 IREGDVGSIVFVLEEGKVEVSRDGKYLSTLAPGKVLGELAILYNCKRTATITAAATDCRLW
consensus 181

AmFOR 190 AIDRCQFQTIMMRTGLSRQAEYDFLKSVPVIFKNLPEETLIKISDVLEETFYNNGDYIIR
PbFOR 1
NvFOR 238 AIDRCQFQTIMMRTGLSRQAEYDFLKSVPVIFKALPEETLIKISDVLEETFYNNGDYIVR
VvFOR 192 AIDRCQFQTIMMRTGLSRQAEYDFLKSVPVIFKNLPEETLIKISDVLEETFYNNGDYIVR
consensus 241

AmFOR 250 QGARGDTFFIISRGQVRVTIKQPDTEEEKMIRTLKSGDFFGKALQGDLLRTANIADDP
PbFOR 1 -----GDFGKALQGDLLRTANIADDP
NvFOR 298 QGARGDTFFIISRGQVRVTIKQPDTEDEKFIRTLKSGDFFGKALQGDLLRTANIADDP
VvFOR 252 QGARGDTFFIISRGQVRVTIKQPDTEDEKFIRTLKSGDFFGKALQGDLLRTANIADDP
consensus 301

AmFOR 310 EGVSCLVIDRETFNQLISSLDEIRTRYKDSSSSVGCMENRATIPELNNEEPRDRLQDLRLP
PbFOR 25 EGVSCLVIDRETFNQLISSLDEIRTRYKDEELVRRR---LNEEPRDRLQDLRLP
NvFOR 358 EGVSCLVIDRETFNQLISSLDEIRTRYKDEELVRRR---LNEEPRDRLQDLRLP
VvFOR 312 EGVSCLVIDRETFNQLISSLDEIRTRYKDEELVRRR---LNEEPRDRLQDLRLP
consensus 361

AmFOR 370 IATLVGGGFRVVELVQIAGDSSRSFALKQMKKAQIVETROQQHIMSEKRIMGEADCFV
PbFOR 76 IATLVGGGFRVVELVQIAGDSSRSFALKQMKKAQIVETROQQHIMSEKRIMSEADCFV
NvFOR 409 IATLVGGGFRVVELVQIAGDSSRSFALKQMKKAQIVETROQQHIMSEKRIMSEADCFV
VvFOR 363 IATLVGGGFRVVELVQIAGDSSRSFALKQMKKAQIVETROQQHIMSEKRIMSEADCFV
consensus 421

AmFOR 430 KLFKTFKDRKYLMLMEACLGGELWTVLRDKGHFDDGTRFRYACVVEAFDYLHSRNIIY
PbFOR 136 KLFKTFKDRKYLMLMEACLGGELWTVLRDKGHFDDGTRFRYACVVEAFDYLHSRNIIY
NvFOR 469 KLFKTFKDRKYLMLMEACLGGELWTVLRDKGHFDDGTRFRYACVVEAFDYLHSRNIIY
VvFOR 423 KLFKTFKDRKYLMLMEACLGGELWTVLRDKGHFDDGTRFRYACVVEAFDYLHSRNIIY
consensus 481

AmFOR 490 RDLKPENLLDSQGYVKLVDFGFAKRLDHGRKTTWFCGTPEYVAPEVILNRGHDISADYW
PbFOR 196 RDLKPENLLDNOGYVKLVDFGFAKRLDHGRKTTWFCGTPEYVAPEVILNRGHDISADYW
NvFOR 529 RDLKPENLLDSQGYVKLVDFGFAKRLDHGRKTTWFCGTPEYVAPEVILNRGHDISADYW
VvFOR 483 RDLKPENLLDNEGYVKLVDFGFAKRLDHGRKTTWFCGTPEYVAPEVILNRGHDISADYW
consensus 541

AmFOR 550 SLGVLMFELLTGTPPFTGGDPMKTYNIIILKGIADIFPFRSITRNATLIIKIKLCRDNPAER
PbFOR 256 SLGVLMFELLTGTPPFTGGDPMKTYNIIILKGIADIFPFRSITRNATLIIKIKLCRDNPAER
NvFOR 589 SLGVLMFELLTGTPPFTGGDPMKTYNIIILKGIADIFPFRSITRNATLIIKIKLCRDNPAER
VvFOR 543 SLGVLMFELLTGTPPFTGGDPMKTYNIIILKGIADIFPFRSITRNATLIIKIKLCRDNPAER
consensus 601

AmFOR 610 LGYQKGGISEIQKHKWFDFGNWEGLRARTLEPPIMPRVQNAATDTNFDYPPDSDPPPPD
PbFOR 316 LGYQKGGISEIQKHKWFDFGNWEGLRARTLEPPIMPRVQNAATDTNFDYPPDSDPPPPD
NvFOR 649 LGYQKGGISEIQKHKWFDFGNWEGLRARTLEPPIMPRVQNAATDTNFDYPPDSDPPPPD
VvFOR 603 LGYQKGGISEIQKHKWFDFGNWEGLRARTLEPPIMPRVQNAATDTNFDYPPDSDPPPPD
consensus 661

AmFOR 670 DISGWDNDF
PbFOR
NvFOR 709 DISGWDNDF
VvFOR 663 DISGWDADF

Figure 1. Comparison between the amino acid sequence of cGMP-dependent protein kinases. PKG from *Apis mellifera* (AmFOR), *Pogonomyrmex barbatus* (PbFOR), *Nasonia vitripennis* (NvFOR) and *Vespula vulgaris* (VvFOR). Conserved amino acids are indicated.

and PbFOR. These results confirm that *Vespula vulgaris* is more related to *Pogonomyrmex barbatus* and they indicate a common ancestor for the *foraging* gene. When comparing these proteins to insect PKAs, only little similarity was found (results not shown).

Quantification of Vespula for gene expression in wild-captured nursing and foraging wasps

Eight colonies were studied for expression of *for* in nurses versus foragers, relative to the housekeeping gene β -actin. As there is no conclusive evidence that indeed there is only a single *for* transcript in the *Vespula vulgaris* brain, our primers were designed to match the region encoding the catalytic domain of the enzyme, since differences between transcripts most likely occur in the regulatory part. Figure 2 shows that except for one colony each assay revealed a higher expression of *for* in nurses compared to foragers. These results reflect a comparison of the *for* transcript for each colony and task phase of ten pooled individuals and this being done in triplicate for each sample in order to exclude intra-assay variation and in two separate sets of cDNA for intra and intercolony variation determination. A Wilcoxon test on the means for every colony indicated a significant difference in gene expression between nursing and foraging wasps ($p < 0.02$).

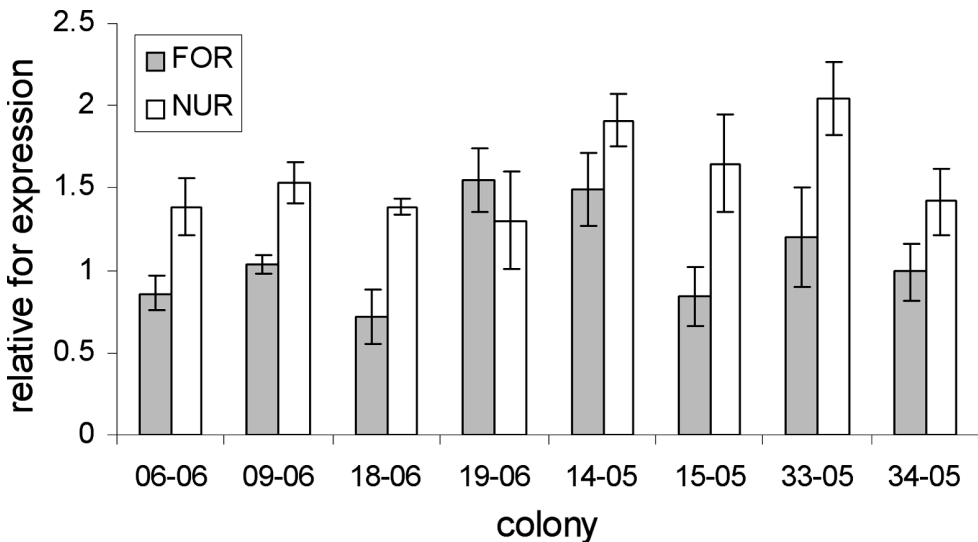


Figure 2. Quantitative PCR analysis of *Vvfor* expression in brains of nursing (NUR) and foraging (FOR) *Vespula vulgaris*. Bars represent the mean level of *Vvfor* mRNA relative to β -actin mRNA [\pm SE (converted to the same arbitrary scale as the means), $N = 2$ different cDNA sets]. Nurses have a significantly higher *Vvfor* expression (Wilcoxon test, $p < 0.02$).

Discussion

The candidate gene approach in combination with a PCR based on degenerate primers proved to be very useful for cloning a homologue cDNA in a new species. Moreover, the qPCR results confirm earlier observations that changes in cGMP-dependent protein kinase (PKG) gene expression affects food seeking behaviour in the wasp *Vespula vulgaris* as is the case in honey bee *Apis mellifera* (Ben Shahr et al., 2002), the ant *Pogonomyrmex barbatus* (Ingram et al., 2005), the fruit fly *Drosophila melanogaster* (Osborne et al., 1997) and the nematode *Caenorhabditis elegans* (Fujiwara et al., 2002). From a strictly behavioural point of view and the resemblance of temporal polyethism in bees and wasps, one would have predicted that initiation of foraging behaviour would be accompanied by an increase of PKG expression. However the opposite was observed: nursing wasps have a slightly higher expression of the *for* gene compared to foragers as is also the case in the ant *Pogonomyrmex barbatus* (Ingram et al., 2005). Although VvFOR has a high sequence identity with AmFOR (77%), a phylogenetic comparison based on Clustal W alignment (Jeanmougin et al., 1998) of the corresponding C-terminal part of the *for* genes present in the database revealed clustering with both VvFOR and PbFOR (85% identity). This is in agreement with the phylogenetic relation between wasps, bees and ants predicted from the tree of life (Brothers, 1999). Our results concerning sequence similarities, taken together with this phylogenetic relationship emphasizes the correctness of our observation at the level of behavioural regulation. The *foraging* gene and its role in foraging behaviour seem to be conserved across hymenopteran species, however its role has not been completely elucidated. It seems remarkable that although the foraging gene is conserved, the mechanism underlying the regulation of the division of labour has evolved differently. However, other examples of such remarkable differences can be found in literature. O'Donnell and Jeanne (1993) found that juvenile hormone control of age-dependent polyethism evolved independently in advanced species of Apidae and Vespidae because methoprene accelerates age polyethism in highly eusocial bee and wasp workers which is not the case in primitively eusocial species.

Our experiments had one major drawback, namely sample variability, which is inevitable when catching animals in the wild. No control for age is possible, neither could we exclude that collection of in-hive animals in some cases included a forager instead of the assumed nurses. This could have influenced the result for colony 19-06. By pooling RNA of 10 individuals, we certainly averaged these data but it is clear that a study including the results per individual would have had more confidentiality. However, when working with wasps captured in the wild, the problems with unknown age will remain. There were also differences in time of day or time of year when we caught our wasps which can influence PKG expression levels. But our overall results indicate the same trend, saying that *Vvfor* expression in nurses is higher than in foragers.

Age is a very important factor in the transition of behaviour but not the only driver of the division of labour. Other very important factors are the needs of the colony and

the availability of food (Robinson, 1992; Huang and Robinson, 1992; Schultz et al., 2001). Since the moment of foraging is not exactly predictable and clearly differs between colonies it becomes clear that, so far PKG-*foraging* gene expression is concerned, variability and averaging cannot be excluded in a study using wasps caught in the wild. On the other hand, it is clear that this quantitative study did not extend beyond the transcriptional level. Up to now no data are available about translational regulation, nor on PKG enzyme activity nor on downstream pathways in the common wasp *Vespula vulgaris*. In the context of our observations, identification of pathways downstream of PKG, as suggested for *C. elegans* (Raizen et al., 2006), is needed for elucidating the true nature of the PKG triggering mechanism.

We can conclude that the *foraging* gene was successfully cloned and quantified in both nurses and foragers of *V. vulgaris*, has a role in the foraging behaviour of the wasp, but its precise function is yet to be unveiled. To fully confirm that there is a negative correlation between foraging and *for* expression, it is necessary to extend our experiments with PKG activity measurements and a search for possible different transcripts.

After activation by the second messenger cGMP, PKG plays a role in many physiological processes by phosphorylating a variety of substrates (Wang and Robinson, 1997). Various methodologies can be addressed to track the function of PKG in an organism, or a part of it (Lohmann and Walter, 2005; Hofmann, et al., 2006). In the context of foraging behaviour, there are two interesting functions that need further attention. In vertebrate retinal photoreceptor cells, cGMP signalling is needed for the transduction of light (Zhang et al., 2005). This is necessary for the maintenance of the mammalian circadian clock (Golombek et al., 2003). Ben Shahaar et al., (2003) reported a possible role of PKG in phototaxis in *Apis mellifera*. Although a direct effect of PKG on clock entrainment is not yet found, further investigation of the (possibly indirect) effect of PKG and the relation of clock proteins with foraging behaviour is needed. A second remarkable function of PKG is found in sensory responsiveness and habituation. Rovers, with high PKG activity, were more responsive to sucrose than sitters and showed less habituation to these stimuli (Scheiner et al., 2006; Engel et al., 2006). Since the tasks of both nurses and foragers persist in food handling, this is an interesting pathway for further investigation.

If we want to fully understand the division of labour in *Vespula vulgaris*, it is important to take in account several other factors beside the *foraging* gene. It is very unlikely that this very complicated mechanism would be regulated by a single gene polymorphism, as it is in *Drosophila melanogaster* (De Belle et al., 1989).

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