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Neuropeptide Evolution and the Analysis of Phylogenetic Relationships in Blattaria (Hexapoda)

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> Abstract

Mass spectrometric methods were used for the first time to reveal the sequences of peptide orthologs from a large number of insects species, focussing on cockroaches. Sequence data of CAPA peptides (three periviscerokinins, pyrokinin) were obtained from single specimens, which led to the reconstruction of phylogenetic trees. The results are compared with the current grouping of the respective species, based primarily on morphological characteristics. The efficacy of the mass spectrometric analyses of peptide sequences makes it possible that the described method is suitable to complement morphological and genomic analyses for the reconstruction of phylogenetic relationships.

> Key words

Insect neuropeptides, mass spectrometry, CAPA, cockroaches.

1. Introduction

Neuropeptides are the most structurally diverse messenger molecules which influence a wide range of physiological processes. They are present in all Metazoa which have developed a nervous system (see Kastin 2006). Multiple forms of peptides exist in many neuropeptide families and it is well established that the present sequences are the result of distinct differentiation processes which occurred during the evolution of the different animal taxa. In contrast to other messenger molecules such as biogenic amines, steroid hormones, and amino acids, the primary sequences of neuropeptides may even be species-specific. Molecular biological and biochemical methods made it possible to analyse neuropeptide evolution, which cannot be separated from the evolution of the respective receptors. In recent years, the introduction of highly sensitive mass spectrometer paved the way for rapid screening of the neuropeptide profile (neuropeptidome) even to the single cell level, in species as small as insects (Li et al. 2000; Predel 2001; Aebersold & Mann 2003; Predel et al. 2004).

Our approach describes the suitability of mass spectrometry for the comparison of related neuropeptides from a large number of cockroach species. CAPA peptides (see Predel & Wegener 2006), which comprise periviscerokinins (CAPA-PVKs) and a single pyrokinin (CAPA-PK), were chosen for our experiments. These neuropeptides consist of 11–17 amino acids and are coded by a single neuropeptide gene (*capa* gene). Here, we focus for the first time on the extent to which such neuropeptide sequence comparisons can be used for the reconstruction of phylogenetic relationships. To date, only adipokinetic/hypertrehalosemic hormones have been extensively compared among different insect groups (e.g. Gäde 1989; Gäde & Marco 2005). However, these peptides have a limited value for an analysis of phylogenetic relationships. Other peptide families with multiple forms such as allatostatins are certainly more significant in this context but less extensively studied, although some data exist (Belles et al. 1999).

2. Methods

CAPA peptides are typical of the abdominal neurosecretory system of all insects studied so far. They are stored in segmentally arrayed perisympathetic organs (PSOs), which are the major hormone release sites of the abdominal ventral nerve cord. This accumulation

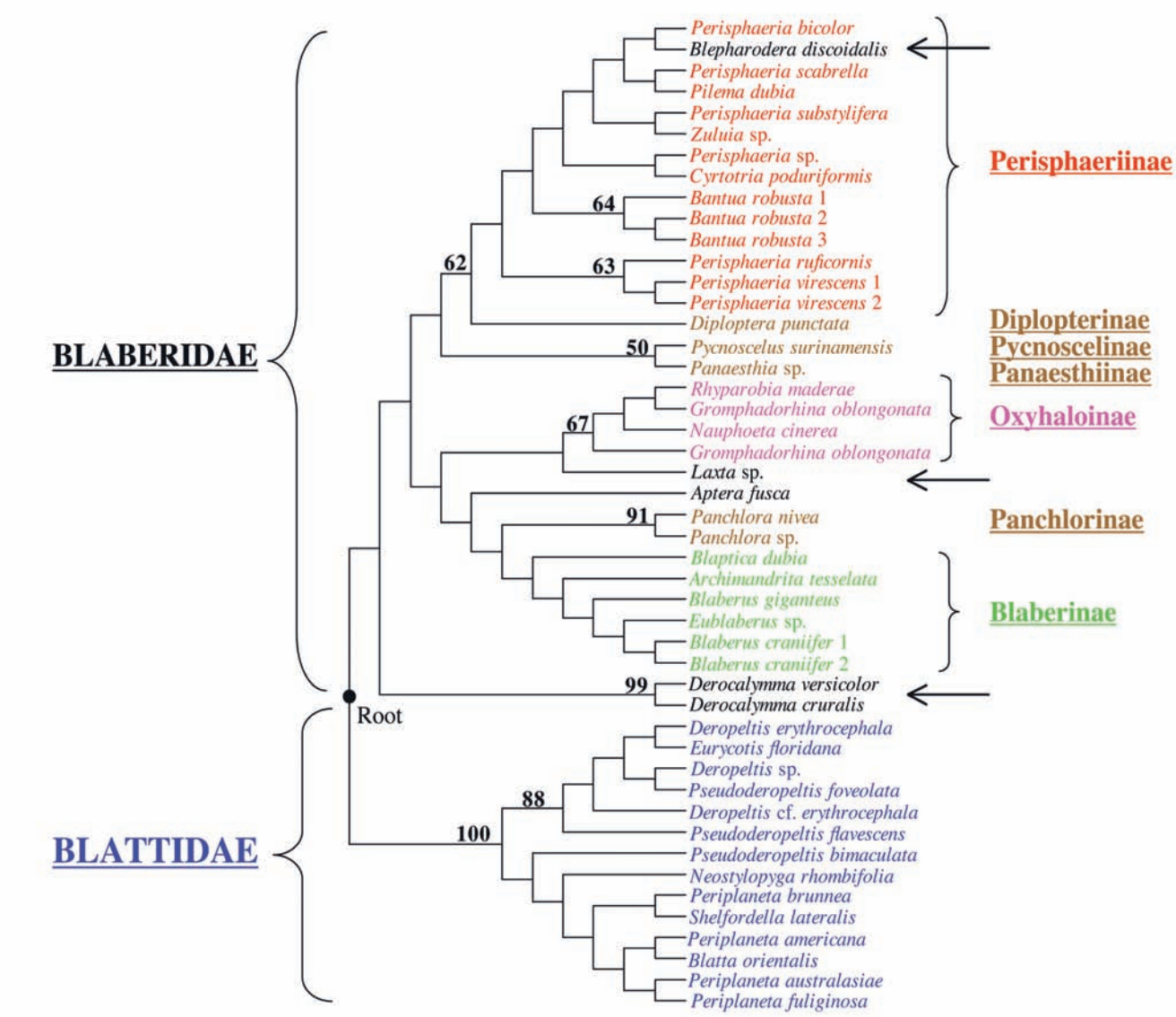

Fig. 1. Bootstrap consensus (majority-rule) tree inferred from a parsimony analysis of peptide sequences (CAPA-PVK1–3, CAPA-PK) of 45 cockroach species (only bootstrap values ≥50 are shown). Species marked by an arrow are placed in a group not identical with conclusions drawn from the analysis of morphological characters of Perisphaeriinae (GRANDCOLAS 1997).

of hormones provides a sufficient source of material for structural analysis even if only a single specimen is available. Hence, PSOs were dissected from fresh or frozen material and subsequently analysed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which included tandem mass spectrometry fragmentation techniques, to reveal the complete sequences of the CAPA-PVKs and the single CAPA-PK from each cockroach species. Resulting ambiguities in the sequences of a few of the peptides were eliminated by performing additional fragmentations using electrospray ionization quadrupole time-of-flight (ESI-QTOF) techniques. Details of the mass spectrometric methods are described elsewere (PREDEL et al. 2000, 2003). Altogether, only a single (MALDI-TOF MS) or relatively few specimens (ESI-QTOF MS) are necessary to acquire the data. Resulting fragments were manually analysed and the resulting peptide sequences aligned. Phylogenetic analyses were performed by using distance matrices, parsimony, maximum likelihood and Bayesian methods (MrBayes 2.0, PAUP*4.0, MEGA 3.1).

3. Results

About 50 cockroach species were studied in this approach. Out of the five commonly listed families (Polyphagidae, Cryptocercidae, Blattellidae, Blaberidae, Blattidae), the Blattidae (subfamilies Blattinae and Polyzosteriinae [only *Eurycotis*]) and Blaberidae (8 of 11 subfamilies) were included in our study. Before starting the sequence elucidations, it was verified

that the neuropeptide profile (PVKs, PK) of different specimens of the same species did not show any qualitative sex- or development-specific differences. The subsequent analysis of the fragmentation pattern of the neuropeptides from abdominal PSO preparations resulted in the complete sequences of the CAPA peptides in all species investigated. Amino acid substitutions are clearly restricted to certain amino acid positions. Whereas in CAPA-PVKs these substitutions can not be assigned to any particular part of the sequence, all substitutions of the CAPA-PK orthologs were exclusively found in the N-terminal portion. Three species (*A. fusca*, *Elliptorrhina* sp., *G. portentosa*) possess an additional fourth CAPA-PVK and a single species (*P. surinamensis*) expresses two CAPA-PKs.

Sequences of CAPA peptide orthologs were combined for each species and aligned. Altogether, 50 characters (amino acid positions) could be unambiguously aligned. A cladogram based on a preliminary parsimony analysis is shown in Fig. 1. The tree was rooted between the Blaberidae- and the Blattidae branches, the basal dichotomy between the Blaberidae and Blattidae is well established (e.g. Grandcolas 1996; Lo et al. 2000, 2003; Klass & Meier 2006).

Members of the Blaberinae, Oxyhaloinae and Panchlorinae were grouped in three clades identical to their current grouping in subfamilies. In contrast, monophyly was not found for Epilamprinae (with the genera *Blepharodera* and *Aptera* in Fig. 1) and Perisphaeriinae (genera *Laxta* and *Derocalymma* as well as "Perisphaeriinae" except for *Blepharodera* in Fig. 1). The subfamily Perisphaeriinae, which includes 18 genera, is particularly well represented in Southern Africa and monophyly was supported by synapomorphies in head morphology and in genitalia of both sexes (Grandcolas 1997). Our data did not support the suggested removal of *Blepharodera* from this subfamily. On the other hand, the Australian genus *Laxta*, placed in the Perisphaeriinae, did not show a close relationship with the other genera of this subfamily. The most striking difference, however, was seen with species of *Derocalymma*, which for our taxon sample appear as the sister group of the remaining Blaberidae. These results obtained from short peptide sequences were corroborated by the analysis of the respective mitochondrial cytochrome oxidase II (COII) genes (S. Roth unpubl. data).

Concerning the Blattidae our results suggest that the polyzosteriinae *Eurycotis* has a deeply subordinate position in the Blattinae, to which all our other sampled blattid species belong. Furthermore, the genera *Periplaneta*, *Deropeltis*, and *Pseudoderopeltis* appear as para- or polyphyletic. While our analysis must be considered preliminary, this may yet indicate that the generic classification of Blattidae needs extensive revision.

4. Conclusions

For the first time, mass spectrometric methods were exclusively used for a large scale, comparative peptidomic approach. Out of the approximately 100 neuropeptides expressed in insects like cockroaches, products of a single neuropeptide gene (*capa* gene) were analysed. As shown, the short sequences can be used for the reconstruction of phylogenetic relationships. The inclusion of additional neuropeptides will enhance the stability and reliability of the phylogenetic trees. On the other hand, a minimalistic approach using only two peptides (a single CAPA-PVK and CAPA-PK each), which included members of other insect orders (Orthoptera, Mantophasmatodea, Diptera) as well, resulted in nearly identical (e.g. with a basal dichotomy between the Blaberidae and Blattidae) although less robust cladograms (Predel & Roth unpubl. data). The suitability of neuropeptide sequences for the study of interordinal relationships in insects is currently under investigation.

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