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Pheromone Biosynthesis Activating Neuropeptide (PBAN) / Pyrokinin Family of Peptides and Fire Ants, *Solenopsis* spp.

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ABSTRACT

The fire ant, *Solenopsis invicta*, is an economically important invasive pest ant, causing over 6 billion dollars in control and repair costs each year in the United States. The fire ant is becoming a global problem increasing its importance and the need for the development of biologically-based control methods. The PBAN/pyrokinin gene is ubiquitous to insects and produces 4-5 neuropeptides that play critical roles in insect development and in reproduction. The most well studied function is regulation of moth pheromone biosynthesis through the Pheromone Biosynthesis Activating Neuropeptide, PBAN. The fire ant is one of the most studied social insects and over the last 50 years a great deal has been learned about the behaviors and chemistry of pheromone communication in this ant. However, virtually nothing is known about the regulation of these pheromone systems. We review here our research to date on the PBAN/pyrokinin gene and the fire ant in preparation for determining the function of the product neuropeptides in brood development and in adults. We discuss the following: a) PBAN/Pyrokinin peptides in fire ants; b) PBAN immunocytochemistry and the fire ant central nervous system (CNS); c) Identification of PBAN/pyrokinin neuropeptides from *S. invicta* and other *Solenopsis* species; and d) PBAN/Pyrokinin gene expression in the head, thorax and abdomen of *S. invicta*. These studies help lay the ground-work for the utilization of the PBAN/pyrokinin gene/peptide product system for novel biologically-based fire ant control.

Key words: fire ant, *Solenopsis*, PBAN, neuropeptide, gene expression, immunocytochemistry

Introduction

Of the world's 100 worst invasive alien species, ants comprise 5%, and of the 17 land invertebrates listed, 28% are ants, including the red imported fire ant,

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Solenopsis invicta (Lowe *et al.*, 2000)! In the last decade *S. invicta* has changed from an invasive pest ant in the United States to a global problem, with infestations occurring in Taiwan (Chen *et al.*, 2006), Australia (Henshaw *et al.*, 2005), mainland China (Zeng *et al.*, 2005; Zhang *et al.*, 2007), Mexico (Sánchez-Peña *et al.*, 2005), and many Caribbean Island countries (Davis *et al.*, 2001).

Solenopsis invicta, is an economically important invasive pest ant species, as has been well documented in the United States. This opportunistic omnivore occurs in very large numbers in its invasive range and prefers disturbed habitats-wherever there are human activities. In the United States the fire ant infests over 321 million acres and over $6 billion per year is spent for control and damage repair. This does not include medical costs or difficult to quantify environmental impacts. The economic sectors affected include: residential households, electric and communication systems, agriculture, golf courses, commercial businesses, schools and medical facilities, and parks and recreational areas (Lard, 2006). Of the chemical methods for controlling fire ants, toxic baits introduce the least amount of insecticide into the environment; however, they are expensive, negatively affect non-target ant species, and most have limited EPA registration. There is a need for biologically-based control alternatives.

Pheromone communication serves a range of functions in social insects, including mating, alarm, recognition, recruitment, orientation, and aggregation. In addition, social insects depend on sophisticated pheromone communication to maintain colony cohesiveness and sociality through releaser and primer pheromones. Wilson (1962) set the stage for fire ant chemical ecology for decades to come with three papers that investigated releaser pheromone communication among workers of the fire ant. Mass foraging (recruitment) and colony emigration were defined and could be fully induced by presenting extracts of Dufour's glands to workers (Wilson, 1962a, b, c). Alarm behavior (rapid erratic movement of workers) was attributed to a cephalic substance and secondarily to Dufour's gland components. Since this time other releaser pheromones have been defined behaviorally and chemically, and the probable source determined. The queen-produced recognition pheromone has been at least partly identified (Glancey *et al.*, 1984) and is biosynthesized by the poison gland and released through the sting apparatus onto deposited eggs (Vander Meer *et al.*, 1980; Vander Meer and Morel, 1995). After a long struggle a component of the fire ant alarm pheromone was isolated and identified as a pyrazine derivative (Vander Meer *et al.*, 2010). Not well defined chemically is the chemical ecology of fire ant mating flights. These are characterized by the opening of the normally closed nest tumulus and frenzied activity by workers (sterile) and male and female sexual alates, prior to the alates taking flight. This behavior is mediated by a releaser pheromone linked to the mandibular gland (Obin and Vander Meer, 1994; Alonso and Vander Meer, 1997), but the chemistry remains unknown. Brood pheromones that induce worker care have not been adequately demonstrated in fire ants or other ants (Morel and Vander Meer, 1988). Several fire ant primer pheromones have been described functionally, however, none have been isolated and identified (Vargo, 1998; Vander Meer and Alonso, 2002). The above represents considerable activity in the chemical ecology of fire ants; however, thirty-nine different glands have been described for Formicidae (Billen and Morgan, 1998) and nine major exocrine glands of fire ants have been described including pygidial, metapleural, and propharyngeal glands (Billen, 1990). This suggests that additional pheromone discoveries are yet to be made.

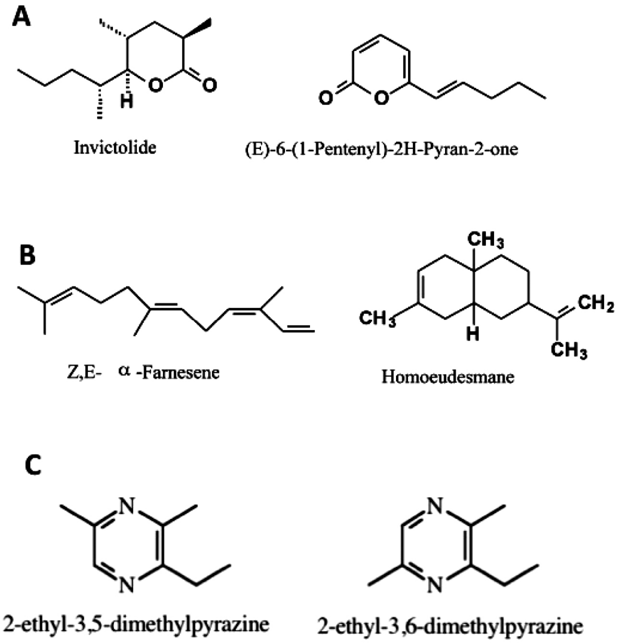


Fig. 1. Known structures for fire ant releaser pheromones: A) queen recognition (Glancey, 1986; Vander Meer *et al.*, 1980); B) recruitment (Vander Meer *et al.*, 1988); and C) alarm (Vander Meer *et al.*, 2010).

Fire ant pheromone chemistry/behavior examples

There are a number of fire ant releaser pheromones where the behavior and chemistry have been elucidated. Queen recognition pheromones were isolated in a bioassay driven separation of active compounds from large numbers of fire ant queens. One component (dihydroactinidiolide) was later thought to be an artifact of the separation. The remaining two compounds are shown in Figure 1A (Rocca *et al.*, 1983a, b).

The fire ant recruitment pheromone is a model of behavioral and chemical complexity. All components are derived from the Dufour's gland. Z,E-alpha-farnesene (Fig. 1B) is solely responsible for *worker orientation* (back and forth movement along the trail (Vander Meer *et al.*, 1981), whereas *worker attraction* (olfactometer bioassay) requires the farnesene and the homoeudesmane (see Fig. 1B) (Vander Meer *et al.*, 1988). However, non-trailing worker ants are not induced to follow a trail composed of these two components. A third bioassay was developed to measure *orientation induction*, which indicated that the complex chemistry of the Dufour's gland had to be reconstructed to elicit activity (12 components gave 85% of the activity from Dufour's gland extracts; (Vander Meer *et al.*, 1990). The three recruitment sub-categories are differentially activated by the amount of Dufour's gland material released.

The fire ant alarm pheromone has been a perplexing problem since it was first described almost five decades ago (Wilson, 1962c). The amount of material produced by the fire ant is very small and the active compounds are highly volatile. We have only recently identified one component of the alarm pheromone, 2-ethyl-3,6-dimethylpyrazine (Vander Meer *et al.*, 2010), from mandibular glands. The pheromone is available commercially as a 1:1 mixture with its isomer, 2-ethyl-3,5-

dimethylpyrazine (Fig. 1C).

Pheromone production and regulation

Although a variety of fire ant pheromones have been identified, little is known about the regulation of their production and release. Insects are known to use three hormonal signaling molecular classes of compounds to regulate pheromone biosynthesis: juvenile hormones, ecdysteriods, and pheromone biosynthesis activating neuropeptides (PBAN) (Tillman *et al*., 1999). PBAN is synthesized in the subesophageal ganglion (SG), located near the brain, and released into the hemolymph, where it acts on pheromone glands to stimulate pheromone biosynthesis in moths. It was first identified from the moth, *Helicoverpa zea* (Raina *et al*., 1989). The mechanism of PBAN control over pheromone production is well understood for sex pheromone biosynthesis in a number of lepidopteran moths. However, thus far no other insect group has been shown to regulate pheromone biosynthesis using PBAN.

PBAN/Pyrokinin peptides in insects

PBAN is one of five peptides produced by the PBAN/pyrokinin gene that represent a family of peptides that have in common a five C-terminal amino acid sequence, FXPRLamide, that represents the minimal sequence required for activity (Raina and Kempe, 1990, 1992; Fonagy *et al*., 1992; Kuniyoshi *et al*., 1992). FXPRLamide peptides will generally stimulate inappropriate sex pheromone synthesis in moths. Insects from a variety of orders have been found to have the PBAN/pyrokinin gene and have peptides with the FXPRLamide motif. Besides pheromone regulation, members of the PBAN/pyrokinin family of peptides have multiple effects on insect development and reproduction, for example: 1) stimulate pheromone biosynthesis in female moths (Raina *et al*., 1989); 2) induce melanization in moth larvae (Matsumoto *et al*., 1990; Altstein *et al*., 1996); 3) induce embryonic diapause in *Bombyx mori* (Suwan *et al*., 1994); 4) stimulate visceral muscle contraction (Nachman *et al*., 1986; Predel and Nachman, 2001); 5) accelerate puparium formation in several flies (Zdarek *et al*., 1997; Verleyen *et al*., 2004); and 6) terminate pupal diapause in heliothine moths (Sun *et al*., 2003; Xu and Denlinger, 2003). These data highlight the multifunctional roles for PBAN/pyrokinin peptides identified in insects, as well as possible avenues for novel control.

Mode of action of PBAN in insect pheromone biosynthesis

Generally, PBAN is released into the hemolymph to circulate and reach the target pheromone gland. PBAN acts directly on pheromone glands by stimulating specific receptor linked G-proteins to open a ligand-gated calcium channel to allow the influx of extracellular Ca^{2+}, which is the critical second messenger for PBAN signal transduction (Jurenka *et al*., 1991; Fo´nagy *et al*., 1992; Jurenka *et al*., 1994; Rafaeli and Soroker, 1994; Ma and Roelofs, 1995; Matsumoto *et al*., 1995a; Matsumoto *et al*., 1995b; Choi *et al*., 2003; Choi and Jurenka, 2004). The signal transduction mechanism for enzyme activation is activated quickly once PBAN binds with the receptor. The pathway remains active for a period of time. Recently, the first G-protein coupled receptor for PBAN has been identified from pheromone glands of *H. zea*, and it was demonstrated that PBAN activates the receptor at low nanomolar concentrations (Choi *et al*., 2003). Additional PBAN receptors identified from different moth species are similar based on peptide sequence identity (Hull *et al*., 2004; Rafaeli *et al*., 2007; Zheng *et al*., 2007; Kim *et al*., 2008).

PBAN/Pyrokinin peptides in fire ants

Generally, corn earworm, *Helicoverpa zea,* female moths do not produce pheromones during the photophase or when decapitated. However, injection of a) decapitated *H. zea* females, or b) females in the photophase

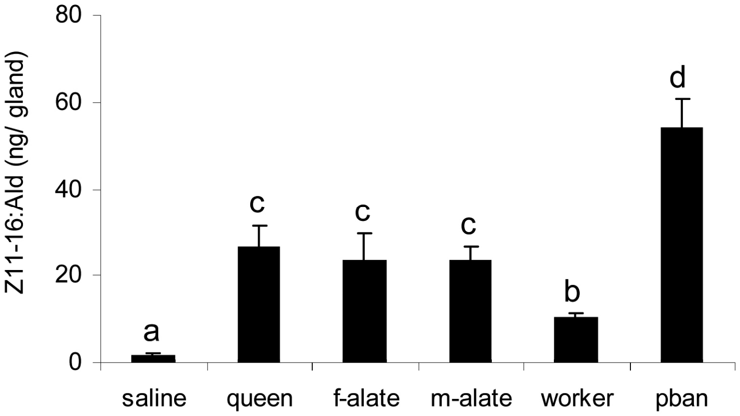


Fig. 2. The amount of Z11-16:Ald, the major sex pheromone component, produced in 2-day old *H. zea* females injected with fire ant Br-SG extracts of queen (queen), female alate (f-alate), male alate (m-alate), worker (worker), synthetic Hez-PBAN (pban), or control (saline). Fire ant nervous tissues were prepared as described previously (Choi *et al*., 2001). Error bars represent the amount of pheromone per gland ± SEM. Different letters at the top of each bar indicate that the results are statistically different (Fisher PLSD, ANOVA; $p < 0.05$) (from Choi *et al*., 2009).

with Br-SG extracts or with *H. zea* PBAN, induces, otherwise inappropriate synthesis of *H. zea* sex pheromone (Raina *et al*., 1989). If fire ants produced PBAN/ Pyrokinin family peptides, injection of these into decapitated *H. zea* females should induce some level of *H. zea* sex pheromone production. The results of injecting 2-day old *H. zea* females, which were decapitated 24-h earlier, with *S. invicta* Br-SG extract are shown in Fig. 2. All female (workers, female alates, and functional queens) and male fire ant Br-SG extracts stimulated the inappropriate biosynthesis of the *H. zea* sex pheromone, (Z)-11-hexadecenal (Fig. 2) significantly greater than the saline control. However, the amount of pheromone production was significantly lower than the result for synthetic *H. zea* PBAN injections for all fire ant samples (Fig. 2). The quantitative differences in pyrokinin/PBAN-like peptides from the fire ant sexual forms might be attributable to differences in production, gene expression related to sexual dimorphism, or functional differences between workers and sexuals (Choi *et al*., 2009). Moths are the only insects that have been shown to use a PBAN to stimulate pheromone production, although correlations of moth PBAN stimulating some pheromone biosynthesis have been reported in the Hessian fly, *Mayetiola destructor* (Foster *et al*., 1991).

PBAN immunocytochemistry and the fire ant central nervous system (CNS)

Immunocytochemical tools (anti-serum generated against a truncated C-terminal end of *H. zea* PBAN) had been developed to localize PBAN synthesis/accumulation in the central nervous system (CNS). This system was used to visualize PBAN immunoreactivity in dissected Br, SG and ventral nerve cord (VNC) of *S. invicta*, queens, female and male alates, and workers (see Fig. 3 for worker example). The number and location of PBAN-like immunoreactive neurons showed a similar pattern for all sexual forms (Choi *et al*., 2009). The distribution pattern of PBAN-like immunoreactive materials in the fire ant CNS was similar to that already shown in fly and moth species (Choi *et al*., 2001; Choi *et al*., 2004). However, unlike

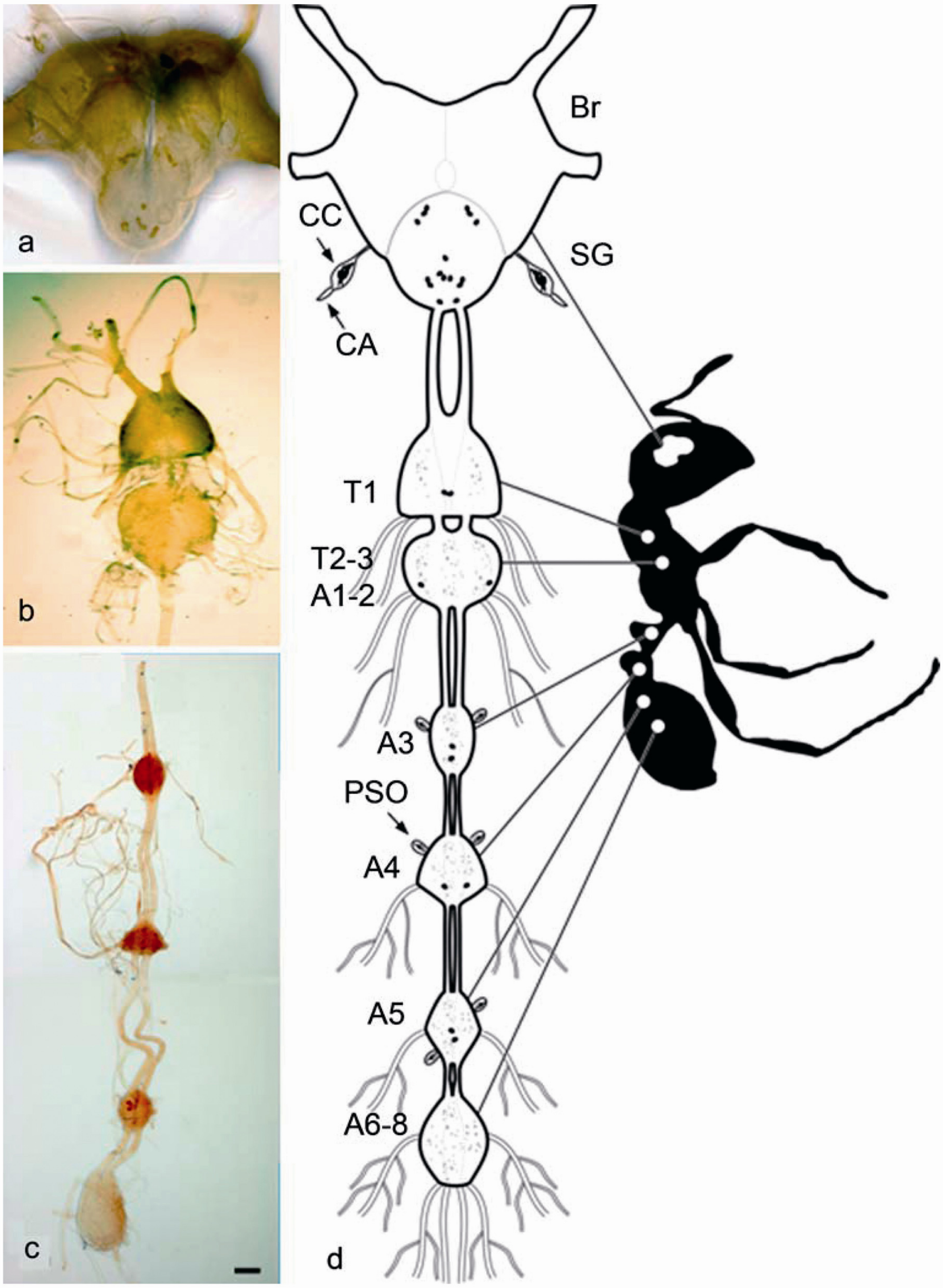


Fig. 3. Central nervous system of an adult fire ant worker. Photomicrographs of the brain and subesophageal ganglion (a), thoracic ganglia (b), and abdominal ganglia (c). (d) representation of CNS and PBAN like immunoreactivity in an adult fire ant worker (Br brain, SG subesophageal ganglion, CC corpora cardiaca, CA corpora allata, PSO perisympathetic organ, T1-T3 first to third thoracic ganglia, A1-A8 first to eighth abdominal ganglia). Bar = 50 μm (from Choi *et al*., 2009).

moths, the last fire ant abdominal ganglion does not contain PBAN immunoreactive neurons (Fig. 3).

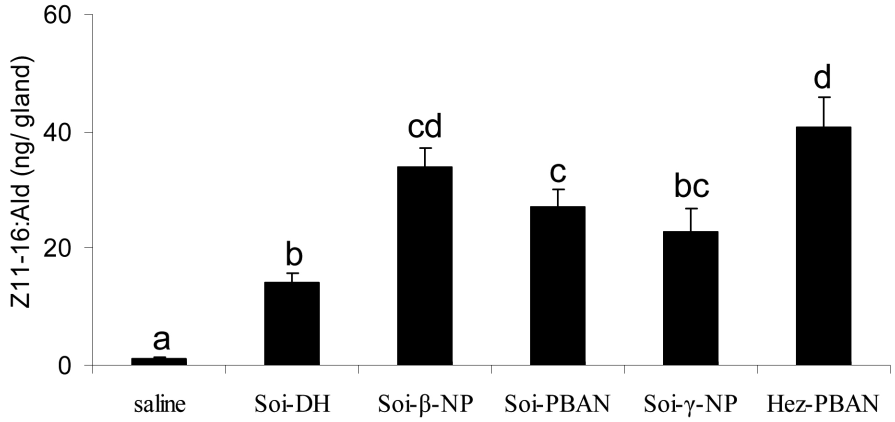
Concurrent with the pyrokinin/PBAN studies, an anatomical atlas was constructed of the entire CNS of the fire ant. This was the first report of a complete CNS atlas of an ant species. The ventral nerve cord in ancestral insects has been shown to consist of eight discrete abdominal ganglia. In evolutionarily advanced insects, the number of abdominal ganglia varies. In most cases, the first abdominal and/or the terminal abdominal ganglia are fused with one or more neuromeres; therefore, the number of discrete ganglia is reduced (Niven *et al.*, 2008). The ventral nerve structure of fire ant female alates and queens shows only two thoracic and four abdominal ganglia. The pro-thoracic ganglion (T1) is discrete, but the meso- (T2) and meta- (T3) thoracic ganglia, and the first (A1) and second (A2) abdominal ganglia are fused together to form the second structurally discrete thoracic ganglia, as found in most insects. Of the four discrete abdominal ganglia, A3, A4, and A5 are distinct ganglia; however, the last three fire ant abdominal ganglia may be fused forming the single terminal ganglion, which account for the expected eight abdominal ganglia (Choi *et al.*, 2009). A similar fusion pattern for both thoracic and abdominal neuromeres in the ventral nerve cord has been shown in Lepidoptera and Hymenoptera species (Niven *et al.*, 2008).

Demonstrating the presence of pyrokinin/PBAN neuropeptides and the localization of the immunoreactive neurons in *S. invicta* provided correlative evidence of physiological roles for these neuropeptides in fire ants.

Identification of PBAN/pyrokinin neuropeptides from *S. invicta*

Specific PBAN/Pyrokinin gene PCR primers were used to amplify and isolate a 780 bp-long full cDNA sequence that contained the entire open reading frame (ORF) of 531 nucleotides encoding 176 amino acids, including four FXPRL peptide domains. The structures of the four *S. invicta* FXPRL peptides and their homology with the known moth PBAN/Pyrokinin peptides are: (1) Diapause Hormone (DH) = 15-AA (TSQDIASGMWFGPRL); (2) β-Neuropeptide = 8-AA (QPQFTPRL); (3) PBAN = 26-AA (GSGEDLSYGDAYEVDED DHPLFVPRL); and (4) γ-Neuropeptide = 9-AA (LPWIPSPRL) (Choi and Vander Meer 2009). Unlike the five peptides, DH, α, β, PBAN and γ peptides, encoded from PBAN genes of lepidopteran moths, α-NP is not present in fire ant PBAN gene based on peptide homology. The four *S. invicta* PBAN/Pyrokinin gene products were synthesized and their pheromonotropic activities measured by their ability to induce inappropriate sex pheromone biosynthesis in decapitated *H. zea* females (Choi and Vander Meer, 2009). All four synthetic peptides were capable of activating significant sex pheromone biosynthesis in the female moths, showing the expected cross-reactivity of the conserved FXPRL-NH_2 moiety (Fig. 4).

When compared with other PBAN/Pyrokinin genes, the *S. invicta* PBAN/Pyrokinin cDNA is similar to the honeybee, but is distant from moth and beetle species (Choi and Vander Meer, 2009). Regarding PBAN, *S. invicta* PBAN (26-AA) is short and has a low degree homology with the honeybee PBAN (33-AA) even though they are both Hymenoptera. Based on amino acid sequences encoded from known insect PBAN/Pyrokinin genes, neuropeptide diversity is consistent with the taxonomic or phylogenetic classification of Insecta (Choi and Vander Meer, 2009). While we now know the amino acid sequences of the *S. invicta* PBAN/Pyrokinin peptides their function remains to be determined.



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Soi-DH  :              TSQDIASGMWFGPRL
Soi-β-NP:                     QPQFTPRL
Soi-PBAN: GSGEDLSYGDAYEVDEDDHPLFVPRL
Soi-γ-NP:                    LPWIPSPRL
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Fig. 4. Pheromonotropic activity of synthetic peptides deduced from Soi-PBAN cDNA and Hez-PBAN in *Helicoverpa zea* female moths (top) and four synthetic peptide sequences (bottom). Bars represent the means SEM of at least 5 replications. Bars with the same letters are not statistically different by analysis of Fisher PLSD (ANOVA) ($p < 0.05$) (from Choi and Vander Meer, 2009).

Identification of PBAN/pyrokinin neuropeptides from other *Solenopsis* species

The *Solenopsis* group is a large genus with 185 described species (Pitts *et al.*, 2005). The genus is difficult taxonomically due to the lack of reliable diagnostic characters. Most recently they have been re-classified into four complexes: *S. virulens*, *S. tridens*, *S. geminata* and *S. saevissima* (Pitts *et al.*, 2005). Using the same techniques used to isolate and sequence the *S. invicta* PBAN/Pyrokinin cDNA (Choi and Vander Meer, 2009) the PBAN/Pyrokinin cDNA was sequence for four additional *Solenopsis* species: *S. richteri* and a hybrid of *S. invicta* and *S. richteri* (*Saevissima* complex), *S. geminata* (*Geminata* complex), *S. pergandii* and *S. carolinensis* (members of a large group classified as thief ants that live primarily underground). *Solenopsis* PBAN genes were divided into two groups of cDNAs translating 176-AA for *S. invicta*, *S. richteri* and the hybrid, and 177-AA for *S. geminata*, *S. pergandii* and *S. carolinenesis*. The one additional amino acid residue (F) in the later group was associated with the PBAN domain (Choi *et al.*, 2010).

Comparison of the five fire ant PBAN/Pyrokinin genes, showed that *S. carolinensis* was the most distant from the other species based on nucleotide sequence homology and the *Saevissima* complex species were separated from the *Geminata* complex species (Fig. 5). This phylogenetic classification by the neuropeptide sequence is consistent with the morphological cladistic analysis of the *Solenopsis* genus (Pitts *et al.*, 2005), except that *S. pergandii* most closely resembled *S. geminata*, and both were very distant from *S. carolinensis*, indicating significant evolutionary distance between the two thief ant species (Fig. 5).

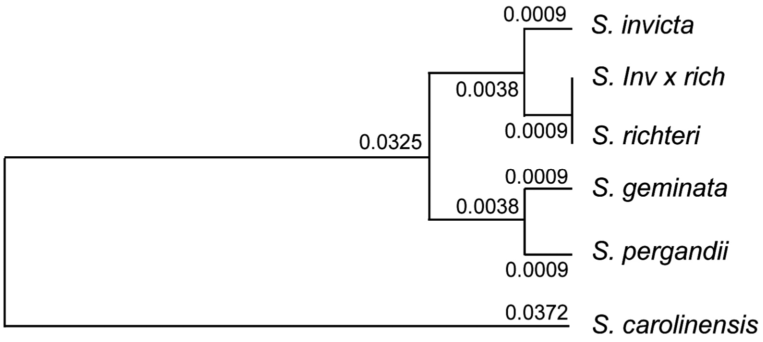


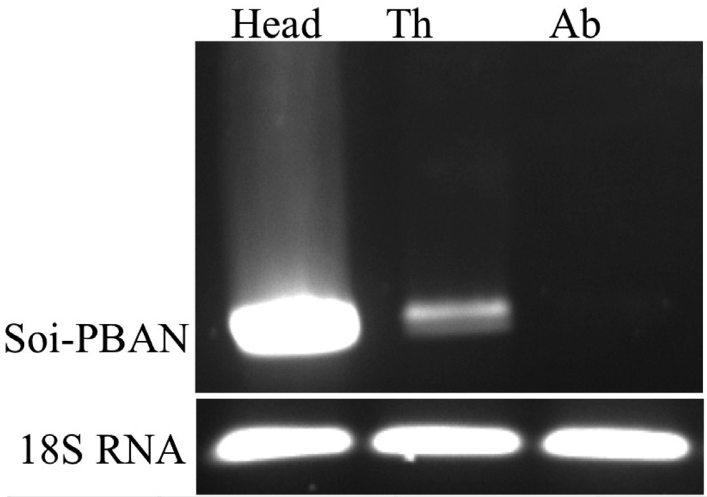
Fig. 5. Phylogenetic tree based on entire nucleotide of fire ant PBAN genes identified from current study and previous (Choi and Vander Meer, 2009). The tree was made with a fixed distance scale using the UPGMA method of Genetyx 6.0 software and multiple alignments of nucleotide sequences. The numbers indicate phylogenetic distance values in the analysis (from Choi *et al*., 2010).

PBAN/Pyrokinin gene expression in the head, thorax and abdomen of *S. invicta*

Solenopsis invicta PBAN/Pyrokinin gene transcripts from head, thorax and abdominal tissues of female adults were quantified using reverse transcription (RT)- and quantitative (Q)-PCRs (Choi *et al*., 2011) (Fig. 6). The head had the strongest transcriptional signal and maximum number of Soi-PBAN gene copies (1990 ± 280) supporting the strong PBAN immunoreactive response detected from the fire ant Br-SG (see Fig. 3) (Choi *et al*., 2009). PBAN/Pyrokinin mRNA transcription and gene copies in thoracic tissue were lower (149 ± 47 copies); however, abdominal tissue gave a non-detectable transcriptional signal and minimal number of PBAN/Pyrokinin gene copies (6 ± 2) (Fig. 6) (Choi *et al*., 2011). The latter result was not predicted based on the strong PBAN-like immuno-response detected from abdominal neurons (see Fig. 3). The most likely explanation for this apparent discrepancy is that the immuno-response assay is not specific to the PBAN peptide but responds generally to peptides with a-FXPRLamide sequence at the C-termini. Therefore, there is a gene or genes in the abdomen other than the PBAN/Pyrokinin gene that also produce FXPRL C-terminal peptides. Currently, only two gene families are known to produce FXPRL peptides: the PBAN/Pyrokinin and the capability (CAPA) genes. The CAPA gene encodes one FXPRL neuropeptide with a very conserved motif, WFGPRL at the C-termini (Predel and Wegener, 2006). Many CAPA genes encoding FXPRL peptides have been isolated from the abdominal neurohemal organs of several insect groups (Predel and Wegener, 2006). This result increases the complexity of the FXPRL neuropeptide physiology but extends the opportunities to disrupt the normal functioning of fire ant colonies.

Conclusion

The fire ant is an excellent social insect model to determine the role of PBAN and/or similar peptides in the regulation of pheromone biosynthesis and other physiological functions. Several fire ant pheromone systems have already been well defined behaviorally and chemically, thus providing measures for their disruption or enhancement. The key to utilization of the neuropeptides in control of fire ants is the identification of the physiological effect(s) of the fire ant



tissue	Head	Th	Ab
copy	1990±280	149±47	6±2

Fig. 6. Tissue expression of PBAN/Pyrokinin gene by RT-PCR (PBAN) in fire ants. 18S RNA is positive control. Same amount of total RNAs from head, thorax (Th) and abdomen (Ab) were amplified by RT-PCR. The number of PBAN/Pyrokinin gene copies was quantified by Q-PCR (N = 3) (from Choi *et al*., 2011).

PBAN/Pyrokinin peptides. The accumulation of work presented here lays the foundation for the elucidation of these physiological effects in terms of pheromone regulation, as well as the many other essential functions in fire ant development and reproduction.

References

Alonso LE, Vander Meer RK. 1997. Source of alate excitant pheromones in the red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). J Insect Behav 10: 541-555.

Altstein M, Gazit Y, Aziz OB, Gabay T, Marcus R, Vogel Z, Barg J. 1996. Induction of cuticular melanization in *Spodoptera littoralis* larvae by PBAN/MRCH: development of a quantitative bioassay and structure function analysis. Arch insect biochem physiol 31: 355-370.

Billen J, Morgan ED. 1998. Pheromone communication in social insects: sources and secretions. pp 3-33. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML, (eds). Pheromone Communication in Social Insects Ants, Wasps, Bees, and Termites. Westview Press, Boulder, Colo.

Billen JPJ. 1990. A survey of the glandular systems of fire ants. pp 85-94. In: Vander Meer RK, Jaffe K, Cedeno A, (eds). Applied Myrmecology: a World Perspective. Westview Press, Boulder. xv + 741 p.

Chen JSC, Shen CH, Lee HJ. 2006. Monogynous and polygynous red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in Taiwan. Environ Entomol 35: 167-172.

Choi M-Y, Vander Meer RK. 2009. Identification of a new member of the PBAN family of neuropeptides from the fire ant, *Solenopsis invicta*. Insect

Mol Biol 18: 61-69.

Choi M-Y, Vander Meer RK, Shoemaker D, Valles SM. 2011. PBAN gene architecture and expression in the fire ant, *Solenopsis invicta*. J Insect Physiol 57: 161-165.

Choi M-Y, Vander Meer RK, Valles SM. 2010. Molecular diversity of PBAN family peptides from fire ants. Arch Insect Biochem Physiol 74: 67-80.

Choi MY, Fuerst EJ, Rafaeli A, Jurenka R. 2003. Identification of a G protein-coupled receptor for pheromone biosynthesis activating neuropeptide from pheromone glands of the moth *Helicoverpa zea*. Proc Natl Acad Sci USA 100: 9721-9726.

Choi MY, Jurenka RA. 2004. PBAN stimulation of pheromone biosynthesis by inducing calcium influx in pheromone glands of *Helicoverpa zea*. J Insect Physiol 50: 555-560.

Choi MY, Rafaeli A, Jurenka RA. 2001. Pyrokinin/PBAN-like peptides in the central nervous system of *Drosophila melanogaster*. Cell Tissue Res 306: 459-465.

Choi MY, Raina A, Vander Meer RK. 2009. PBAN/pyrokinin peptides in the central nervous system of the fire ant, *Solenopsis invicta*. Cell Tissue Res 335: 431-439.

Davis LR, Jr., Vander Meer RK, Porter SD. 2001. Red imported fire ants expand their range across the West Indies. Fla Entomol 84: 735-736.

Fo´nagy A, Matsumoto S, Uchhiumi K, Mitsui T. 1992. Role of calcium ion and cyclic nucleotides in pheromone production in *Bombyx mori*. J Pesti Sci 17: 115-121.

Fonagy A, Schoofs L, Matsumoto S, De Loof A, Mitsui T. 1992. Functional cross-reactivities of some locustamyotropins and *Bombyx* pheromone biosynthesis activating neuropeptide. J Insect Physiol 38: 651-657.

Foster SP, Bergh JC, Rose S, Harris MO. 1991. Aspects of pheromone biosynthesis in the Hessian fly, *Mayetiola destructor* (Say). J Insect Physiol 37: 899-906.

Glancey BM. 1986. The queen recognition pheromone of *Solenopsis invicta*. pp 223-230. In: Lofgren CS, Vander Meer RK (eds). Fire Ants and Leaf Cutting Ants: Biology and Management. Westview Press, Boulder, CO.

Glancey BM, Rocca J, Lofgren CS, Tumlinson J. 1984. Field tests with synthetic components of the queen recognition pheromone of the red imported fire ant, *Solenopsis invicta*. Sociobiology 9: 19-30.

Henshaw MT, Kunzmann N, Vanderwoude C, Sanetra M, Crozier RH. 2005. Population genetics and history of the introduced fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Australia. Aust J Entomol 44: 37-44.

Hull JJ, Ohnishi A, Moto K, Kawasaki Y, Kurata R, Suzuki MG, Matsumoto S. 2004. Cloning and characterization of the pheromone biosynthesis activating neuropeptide receptor from the silkmoth, *Bombyx mori*. Significance of the carboxyl terminus in receptor internalization. J Biol Chem 279: 51500-51507.

Jurenka RA, Fabrias G, DeVoe L, Roelofs WL. 1994. Action of PBAN and related peptides on pheromone biosynthesis in isolated pheromone glands of the redbanded leafroller moth, *Argyrotaenia velutinana*. Comp Biochem Physiol Pharmacol Toxicol Endocrinol 108: 153-160.

Jurenka RA, Jacquin E, Roelofs WL. 1991. Stimulation of pheromone biosynthesis in the moth *Helicoverpa zea*: action of a brain hormone on pheromone glands involves Ca2+ and cAMP as second messengers. Proc Natl Acad Sci USA 88: 8621-8625.

Kim YJ, Nachman RJ, Aimanova K, Gill S, Adams ME. 2008. The pheromone biosynthesis activating neuropeptide (PBAN) receptor of *Heliothis virescens*:

identification, functional expression, and structure-activity relationships of ligand analogs. Peptides 29: 268-275.

Kuniyoshi H, Nagasawa H, Ando T, Suzuki A, Nachman RJ, Holman GM. 1992. Cross-activity between pheromone biosynthesis activating neuropeptide (PBAN) and myotropic pyrokinin insect peptides. Biosci Biotechnol Biochem 56: 167-168.

Lard CF, Schmidt J, Morris B, Estes L, Ryan C, Bergquist D. 2006. An economic impact of imported fire ants in the United States of America. Texas A&M University, College Station, Texas (http://fireantecon.tamu.edu).

Lowe SJ, Browne M, Boudjelas S. 2000. 100 of the World's Worst Invasive Alien Species. IUCN/SSC Invasive Species Specialist Group (ISSG) Auckland.

Ma PWK, Roelofs WL. 1995. Calcium involvement in the stimulation of sex pheromone production by PBAN in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Insect Biochem Mol Biol 25: 467-473.

Matsumoto S, Kitamura A, Nagasawa H, Kataoka H, Orikasa C, Mitsui T, Suzuki A. 1990. Functional diversity of a neurohormone produced by the suboesophageal ganglion: Molecular identity of melanization and reddish colouration hormone and pheromone biosynthesis activating neuropeptide. J Insect Physiol 36: 427-432.

Matsumoto S, Ozawa R, Nagamine T, Kim GH, Uchiumi K, Shono T, Mitsui T. 1995a. Intracellular transduction in the regulation of pheromone biosynthesis of the silkworm, *Bombyx mori*: suggested involvement of calmodulin and phosphoprotein phosphatase. Biosci Biotechnol Biochem 59: 560-562.

Matsumoto S, Ozawa R, Uchiumi K, Kurihara M, Mitsui T. 1995b. Intracellular signal transduction of PBAN action in the common cutworm, *Spodoptera litura*: effects of pharmacological agents on sex pheromone production in vitro. Insect Biochem Mol Biol 25: 1055-1059.

Morel L, Vander Meer RK. 1988. Do ant brood pheromones exist? Ann Entomol Soc Am 81: 705-710.

Nachman RJ, Holman GM, Cook BJ. 1986. Active fragments and analogs of the insect neuropeptide Leucopyrokinin: Structure-function studies. Biochem Biophys Res Commun 137: 936-942.

Niven JE, Graham CM, Burrows M. 2008. Diversity and evolution of the insect ventral nerve cord. Annu Rev Entomol 53: 253-271.

Obin MS, Vander Meer RK. 1994. Alate semiochemicals release worker behavior during fire ant nuptial flights. J Entomol Sci 29: 143-151.

Pitts JP, McHugh JV, Ross KG. 2005. Cladistic analysis of the fire ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Zool Scripta 34: 493-505.

Predel R, Nachman RJ. 2001. Efficacy of native FXPRLamides (pyrokinins) and synthetic analogs on visceral muscles of the American cockroach. J Insect Physiol 47: 287-293.

Predel R, Wegener C. 2006. Biology of the CAPA peptides in insects. Cell Mol Life Sci 63: 2477-2490.

Rafaeli A, Bober R, Becker L, Choi MY, Fuerst EJ, Jurenka R. 2007. Spatial distribution and differential expression of the PBAN receptor in tissues of adult *Helicoverpa* spp. (Lepidoptera: Noctuidae). Insect Mol Biol 16: 287-293.

Rafaeli A, Soroker V. 1994. Second messenger interactions in response to PBAN stimulation of pheromone gland cultures. pp 223-226. In: Borkovec A, Loeb MJ (eds). Insect Neurochemistry and Neurophysiology. CRC Press, Boca Raton, FL.

Raina AK, Jaffe H, Kempe TG, Keim P, Blacher RW, Fales HM, Riley CT, Klun JA, Ridgway RL, Hayes DK. 1989. Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. Science 244: 796-798.

Raina AK, Kempe TG. 1990. A pentapeptide of the C-terminal sequence of PBAN with pheromonotropic activity. Insect Biochem 20: 849-851.

Raina AK, Kempe TG. 1992. Structure activity studies of PBAN of *Helicoverpa zea* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol 22: 221-225.

Rocca JR, Tumlinson JH, Glancey BM, Lofgren CS. 1983a. Synthesis and stereochemistry of tetrahydro-3,5-dimethyl-6-(1-methylbutyl)-2H-pyran-2-one, a component of the queen recognition pheromone of *Solenopsis invicta*. Tetrahedron Lett 24: 1893-1896.

Rocca JR, Tumlinson JH, Glancey BM, Lofgren CS. 1983b. The queen recognition pheromone of *Solenopsis invicta*, preparation of (E)-6-(1-pentenyl)-2H-pyran-2-one. Tetrahedron Lett 24: 1889-1892.

Sánchez-Peña SR, Patrock RJW, Gilbert LA. 2005. The red imported fire ant is now in Mexico: documentation of its wide distribution along the Texas-Mexico Border. Entomol News 116: 363-366.

Sun JS, Zhang TY, Zhang QR, Xu WH. 2003. Effect of the brain and suboesophageal ganglion on pupal development in *Helicoverpa armigera* through regulation of FXPRLamide neuropeptides. Regul Pept 116: 163-171.

Suwan S, Isobe M, Yamashita O, Minakata H, Imai K. 1994. Silkworm diapause hormone, structure-activity relationships indispensable role of C-terminal amide. Insect Biochem Mol Biol 24: 1001-1007.

Tillman JA, Seybold SJ, Jurenka RA, Blomquist GJ. 1999. Insect pheromones--an overview of biosynthesis and endocrine regulation. Insect Biochem Mol Biol 29: 481-514.

Vander Meer RK, Alonso LE. 2002. Queen primer pheromone affects conspecific fire ant (*Solenopsis invicta*) aggression. Behav Ecol Sociobiol 51: 122-130.

Vander Meer RK, Alvarez F, Lofgren CS. 1988. Isolation of the trail recruitment pheromone of *Solenopsis invicta*. J Chem Ecol 14: 825-838.

Vander Meer RK, Glancey BM, Lofgren CS, Glover A, Tumlinson JH, Rocca J. 1980. The poison sac of red imported fire ant queens: Source of a pheromone attractant. Ann Entomol Soc Am 73: 609-612.

Vander Meer RK, Lofgren CS, Alvarez FM. 1990. The orientation inducer pheromone of the fire ant *Solenopsis invicta*. Physiol Entomol 15: 483-488.

Vander Meer RK, Morel L. 1995. Ant queens deposit pheromones and antimicrobial agents on eggs. Naturwissenschaften 82: 93-95.

Vander Meer RK, Preston CA, Choi M-Y. 2010. Isolation of a pyrazine alarm pheromone component from the fire ant, *Solenopsis invicta*. J Chem Ecol 36: 163-170.

Vander Meer RK, Williams FD, Lofgren CS. 1981. Hydrocarbon components of the trail pheromone of the red imported fire ant, *Solenopsis invicta*. Tetrahedron Lett 22: 1651-1654.

Vargo EL. 1998. Primer pheromones in ants. pp 293-313. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML (eds). Pheromone communication in social insects ants, wasps, bees, and termites Westview Press, Boulder, Colo.

Verleyen P, Clynen E, Huybrechts J, Van Lommel A, Vanden Bosch L, De Loof A, Zdarek J, Schoofs L. 2004. Fraenkel's pupariation factor identified at last. Dev Biol 273: 38-47.

Wilson EO. 1962a. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass-foraging. Anim Behav 10: 134-147.

Wilson EO. 1962b. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 2. An information analysis of the odour trail. Anim Behav 10: 148-158.

Wilson EO. 1962c. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 3. The experimental induction of social responses. Anim Behav 10: 159-164.

Xu WH, Denlinger DL. 2003. Molecular characterization of prothoracicotropic hormone and diapause hormone in *Heliothis virescens* during diapause, and a new role for diapause hormone. Insect Mol Biol 12: 509-516.

Zdarek J, Nachman RJ, Hayes TK. 1997. Insect neuropeptides of the pyrokinin/PBAN family accelerate pupariation in the fleshfly (*Sarcophaga bullata*) larvae. Ann NY Acad Sci 814: 67-72.

Zeng L, Lu YY, He XF, Zhang WQ, Liang GW. 2005. Identification of red imported fire ant *Solenopsis invicta* to invade mainland China and infestation in Wuchuan, Guangdong. [in Chinese, English abstract]. Chin Bull Entomol 42: 144-148.

Zhang R, Li Y, Liu N, Porter SD. 2007. An overview of the red imported fire ant (Hymenoptera: Formicidae) in Mainland China. Fla Entomol 90: 723-731.

Zheng L, Lytle C, Njauw CN, Altstein M, Martins-Green M. 2007. Cloning and characterization of the pheromone biosynthesis activating neuropeptide receptor gene in *Spodoptera littoralis* larvae. Gene 393: 20-30.

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費洛蒙生合成活化神經肽 **PBAN/Pyrokinin** 基因與 ***Solenopsis*** 屬火蟻

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摘　要

入侵紅火蟻 *Solenopsis invicta* 是經濟上重要的入侵害蟲，在美國每年花費超過六百萬美元進行火蟻的防治與補救。火蟻顯然已成為全球性的問題，使其重要性日益增加且亟需發展生物防治。昆蟲體內普遍存在 PBAN/Pyrokinin 基因，其產生 4-5 種神經肽對於昆蟲的發育與生殖扮演關鍵性的角色。目前關於費洛蒙生合成活化神經肽 (PBAN) 的功能，以蛾類費洛蒙生合成的調節研究最深入。而火蟻是社會性昆蟲中研究最多的物種之一，且在過去五十年對於其行為以及費洛蒙溝通的化學組成已有大量的研究，然而我們仍不清楚此費洛蒙系統對於火蟻的調節。在此我們將綜述直至今日關於 PBAN/Pyrokinin 基因與火蟻的相關研究，以確定神經肽產物在幼體發育和在成體上的功能。我們將討論以下內容：(1) 火蟻的 PBAN/Pyrokinin 肽；(2) PBAN 免疫細胞化學染色法和火蟻的中樞神經系統 (CNS)；(3) 辨別入侵紅火蟻 *S. invicta* 與其他火家蟻屬 *Solenopsis* 火蟻的 PBAN/Pyrokinin 神經肽；(4) 在入侵紅火蟻 *S. invicta* 頭部、胸部和腹部 PBAN/Pyrokinin 基因的表現。這些研究將有助於建立 PBAN/Pyrokinin 基因/肽產物系統之基礎，並將可利用在新的火蟻生物防治上。

關鍵詞： 火蟻、火家蟻屬、費洛蒙生合成活化神經肽、神經肽、基因表現、免疫細胞化學染色法。

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