Egyptian Journal of Biology, 2003, Vol. 5, pp 138-147
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Lipase and phospholipase activities of Hymenoptera venoms (wasps and ants)

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ABSTRACT

Using polyacrylamide gel electrophoresis without sodium dodecyl sulphate (native gel), *Polistes flavis* venom has four major protein bands, one of which has lipase activity; with sodium dodecyl sulfate (SDS-PAGE), the venom had eighteen bands with molecular weights ranging from a maximum of 94 kD and a minimum of 17 kD; HPLC of the venom resulted in 122 aliquots, two of which have lipase activity. The presence of venom phospholipase activity of 23 Hymenoptera species is demonstrated. Venoms of these species migrate on yolk egg/agarose plates with five different patterns of migration. Implications for phylogenetic relationships among these 23 Hymenoptera species are discussed.

KEYWORDS: Vespidae, Formicidae, *Polistes flavis* venom, native gel, SDS-PAGE, phospholipase inhibition

INTRODUCTION

Phospholipases attack the various ester linkages in phospholipid. These enzymes have a high specificity regarding their site of action (Martin *et al.* 1983). Phospholipase A_1 attacks the ester bond in position 1 of phospholipids; phospholipase A_2 catalyses the hydrolysis of the ester bond in position 2 of phospholipids; phospholipase C attacks the ester bond in position 3; phospholipase D is an enzyme described mainly from plants that hydrolyses the nitrogenous base from phospholipids (Figure 1). Phospholipase B attacks the 1-acyl group of a lysophospholipid (e.g. lysolecithin) (Figure 2). .

Many types of animal venom exhibit phospholipase activity. Phospholipase A_2 was detected in snake venoms *(Crotalus adamanteus*, *Notechis scutatus scutatus*, *Trimeresurus gramineus*, *Trimeresurus flavoviridis*, and *Vipera russelli*) (Vargas-Villarreal *et al.* 1991; Mukherjee & Maity 1998). A phospholipase A_2 with a molecular weight of 14,000 was purified from puff-adder venom (*Bitis arietans*) (Howard 1975). Scorpion venoms (*Heterometrus fulvipes*, *Androctonus australis* and *Pandinus imperator*) also contain phospholipase A_2 (Conde *et al.* 1999). A phospholipase A_2 with a molecular mass of 12-14 kDa was isolated from the spider *Eresus niger* (Atakuziev *et al.* 1991). A phospholipase A_2 that was more than 90% pure and practically devoid of melittin was purified from venom of *Apis mellifera* (Pazos *et al.* 1993). Melittin, a cationic amphiphilic peptide (from *Apis mellifera*), has an apparent activating effect on interfacial catalysis by phospholipase A_2 of bee venom on zwitterionic vesicles of 1-palmitoyl-2-oleoylglycero-sn-3-phosphocholine (POPC) and on anionic vesicles of 1,2-dimyristoylglycero-sn-3-phosphomethanol (DMPM), as well as on covesicles of POPC/DMPM (3:7) (Cajal & Jain 1997). Phospholipase A_2 and melittin are the two main components of bee venom (Pacakova & Stulik 2000).

Apis mellifera phospholipase A_2 ($bvPLA_2$) shares some properties with other 14 kDa phospholipases A_2 (PLA_2s): (1) Ca^{2+} is required for binding ligands to the active site but not for the binding of enzyme to the interface. (2) $bvPLA_2$ does not significantly discriminate between phospholipids with different polar head groups or acyl chains. (3) $bvPLA_2$ does not bind to phosphatidylcholine vesicles, and binding occurs if anionic amphiphiles are present in the vesicle. In addition to these properties, $bvPLA_2$ exhibits unique novel features, including

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the following: (1) Neutral diluents for other 14 kDa phospholipases A_2 are not neutral diluents for $bvPLA_2$. (2) Saturation of the active site with a variety of different ligands does not completely prevent histidine alkylation by 2-bromo-4'-nitroacetophenone, and Ca^{2+} binding does not change the rate of histidine alkylation. (3) Finally, the carbohydrate portion of $bvPLA_2$ does not alter the interfacial catalytic properties of the enzyme (Yu *et al.* 1997).

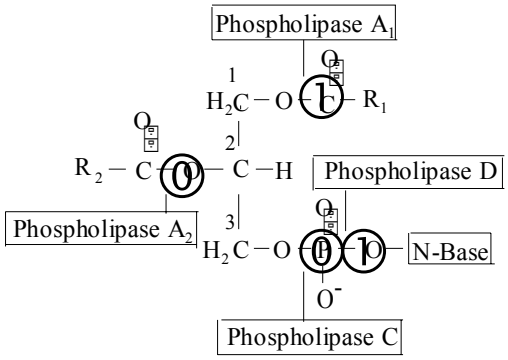

Figure 1: Sites of hydrolytic activity of phospholipases on a phospholipid substrate (Martin *et al.* 1983).

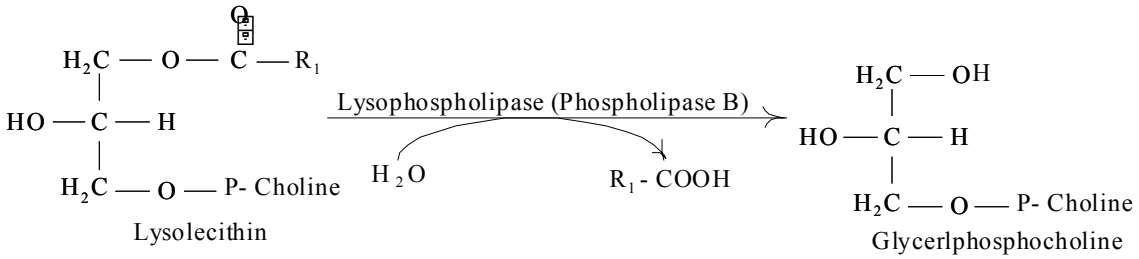

Figure 2: Effect of phospholipase B lysolecithin (Martin *et al.* 1983).

Secretory phospholipases A_2 ($sPLA_2$) found in honeybee venom are neurotoxic and bind to specific receptors in brain membranes called N-type receptors that are likely to play a role in the molecular events leading to neurotoxicity of these proteins. The surface region surrounding the hydrophobic channel of bee-venom $sPLA_2$ was identified as the N-type receptor recognition domain (Nicolas *et al.* 1997). The enzyme-rich venom of the Harvester ant (*Pogonomyrmex badius*) contains high concentrations of phospholipase A_2 and B (Schmidt & Blum 1978). Three toxins designated as verutoxin 1, 2a and 2b (VT-1, VT-2a and VT-2b) were isolated from yellow-legged hornet venom (*Vespa verutina*). VT-2a and 2b were isotoxins with similar toxicity in mice (LD_{50} = 0.87µg/g mouse). All three verutoxins possess phospholipase A_1 activity (Ho *et al.* 1999). The lethal protein of venom of the black-bellied hornet (*Vespa basalis*) is a phospholipase A_1 toxin with potent haemolytic activity (Ho *et al.* 1993). Vargas-Villarreal *et al.* (1991) purified a phospholipase A_1 from venom-sac extract of the white-faced hornet (*Dolichovespula maculata*).

The sequence of the phospholipase A_1 from venom of the white-faced hornet was determined by cDNA and protein sequencing. This protein has no sequence similarity with other known phospholipases, but it has sequence similarity with mammalian lipases. Tests with two synthetic triglycerides suggest the presence of a weak lipase activity for such white-faced hornet phospholipase A_1 (Soldatova *et al.* 1993).

Agelotoxin is a phospholipase A_2 from the venom of the Neotropical social wasp cassununga (*Agelaia pallipes pallipes)*, occurring under equilibrium of three different aggregation states: monomer, trimer and pentamer (Costa & Palma 2000).

Previously, Zalat & Schmidt (1998) showed that venoms of all social wasps (Polistinae & Vespinae) and some ants (Formicidae) have a lipase activity. Here, we aim to estimate the molecular weight of *Polistes flavis* venom lipase, and we explore the phospholipase activity of venoms among various species of Vespidae and Formicidae.

MATERIALS AND METHODS

A number of Hymenoptera species (see Table 3) belonging to the Vespidae and Formicidae were used. All insect specimens were frozen alive and stored at -80^{o}C. Wasp venoms were expressed from the sting tips, placed in small polyethylene microtubes, lyophilized and stored at -20^{o}C until used. The other venoms were obtained by micro-dissection according to the method described by Schmidt (1986) and stored desiccated at -20^{o}C in the dark.

High performance liquid chromatography (HPLC) was carried out to separate components of the venom of *Polistes flavis*. The venom components were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Weber & Osborn (1969). For non-dissociated PAGE, the conditions of Weber & Osborn (1969) were employed except that sodium dodecyl sulfate (SDS) was omitted. The gels were pre-electrophoresed, the electrodes were reversed and the lower buffer reservoir was set in iced water.

Lipase activity of *Polistes flavis* venom was assessed as follows. Olive-oil/agarose plates were prepared by dissolving 2.5 mg Rhodamine B and 0.3 g agarose in 40 ml 0.05 M Tris-HCl (pH 9.1) by heating to near boiling. The resultant solution was left to cool. Once its temperature reached 60-65 ^{o}C, 5 mg sodium azide was added. In a separate flask, 0.6 ml olive oil in 10 ml 0.05 M Tris-HCl (pH 9.1) was heated to 60-65 ^{o}C and sonicated for 1 min to form an emulsion. The two mixtures were combined. Three square Petri dishes (10 x 10 cm) were set up by pouring 16 ml of the resultant emulsion into each of them. The plates were covered, allowed to cool and stored at 4 ^{o}C (modified from Goldberg & Pagast, 1976). The use of pH 9.1 at a temperature of 25 ^{o}C in the diffusion plate results in a working pH of 8.79 at 37 ^{o}C (Goldberg & Pagast 1976). *Polistes flavis* crude venom was run into non-dissociated gels in three different lanes, using the conditions mentioned in the above section. Afterwards, the three lanes were separated from each other into three separate gel slabs using a sharp scalpel. One slab was silver stained. Another was examined for lipase activity by placing it on olive oil/agarose plates for several hr, up to as long as 24 hr, at 37 ^{o}C. By matching these two gel slabs with the third one, the position of the *Polistes flaves* venom band with lipase activity was determined. This band was cut out of the gel, squashed in a microcentrifuge tube, suspended in a few ml of distilled water, vortexed and centrifuged for 2-3 min in a microcentrifuge. The supernatant was used in SDS-PAGE according to Weber & Osborn (1969).

Phospholipase activities of the reconstituted venoms were investigated using the method of Habermann & Hardt (1972). In this method, egg-yolk used as a substrate in agoras gel. Egg-yolk/agarose plates (8 X 10 cm) were poured. After gelling, wells of 3 mm diameter were punched and the gels in the centre of the wells removed by gentle suction. Ten µl of diluted whole reconstituted venom were applied into each well. After exactly 20 hr at 25^{o}C in a moist chamber, the wells containing enzyme activity show clearing of gel. The diameter of the cleared ring was measured. The diameter is linearly proportional to the logarithm of enzyme concentration. Standards of bee venom phospholipase A were made.

RESULTS

Using SDS-PAGE, *Polistes flavis* venom was resolved into eighteen bands with molecular weights ranging from a maximum of 94 kD and a minimum of 17 kD (Table 1 & Figure 3). Non-dissociated PAGE showed four bands (Figure 4), and overlaying the gel on olive oil/ agarose plates showed that band 4 exhibited lipase activity. Separation of *Polistes flavis* venom components by HPLC resulted in 122 aliquots. Two of these aliquots showed protein bands with molecular weights of 30 and 36 kD when run into SDS-PAGE (Figure 3). These two bands were confirmed to have lipase activity. Some of the HPLC aliquots that do not shown in Figure 3 were suspected to have a very weak lipase activity.

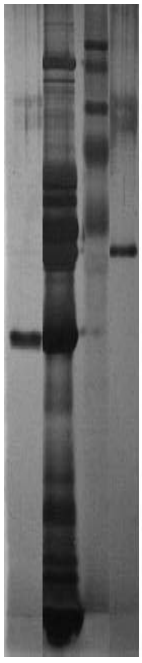

Figure 3: SDS-PAGE. Lane assignments from left to right are: (1&4) *Polistes flavis* venom fractions extracted using HPLC; (2) Whole *Polistes flavis* venom; and (3) Molecular weight standards (123, 89, 67, 50, 37 & 34 KD).

Table 1: Analysis of *Polistes flavis* venom using SDS-PAGE: major bands with their concentration with the standard molecular weight.

Deduced Molecular weight of *P. flavis* venom (kD)	*Polistes flavis* crude venom (concentration)	Molecular weight standard (concentration)
123		8.88
94	5.12	
89		8.69
81	2.83	
75	2.77	
73		
70		
67		13.53
64	2.74	
63		
59		
51	3.30	
50		26.98
43	6.33	
40	3.52	
37		29.43
36	8.02	
35		
34	5.39	12.49
30	12.11	
27	2.94	
26	3.84	
23	10.51	
21	7.71	
20	3.64	
19	3.90	
18.5	6.29	
17	9.03	
	100.00	100.00

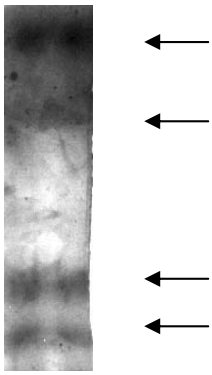

Figure 4: Behaviour of *Polistes flavis* venom in polyacrylamide gel under nondissociating conditions (native gel). Cathode is at the bottom and proteins migrated downward. Four bands are visible.

Venoms of the studied Hymenoptera species migrate on yolk egg/agarose plate following five different patterns of migration (Figure 5A–E). Within the same pattern of migration, the relative area of each circle and its degree of clarity (*i.e*. colour) vary from one species to

another. The venom migration pattern of each of the studied species is shown in Table 2. It seems that migration patterns D and E are two variants of the same migration pattern where areas 2 and 3 in pattern D are reversed in pattern E.

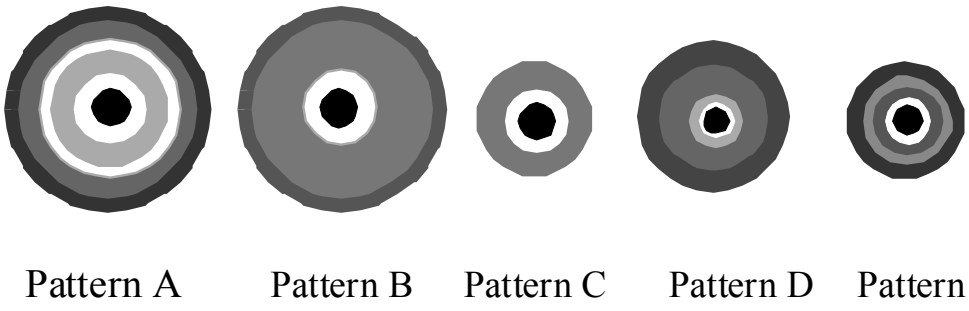

Table 2: Venom migration pattern of each of the studied species.

Pattern A	Pattern B	Pattern C	Pattern D	Pattern E
**Neoponera apicalis* **Neoponera olivaceae* **Odontomechus* sp.	**Brachygastera mellifera* **Dolichovespula arenaria* **Dolichovespula maculata* **Parachantergus fraterus* **Paraponera clavata* **Polistes dominulus* **Polistes flavis* **Polistes fuscutus* **Polistes hebraeus* **Polistes indica* **Pogonomyrmex occidentalis* **Polistes rothreyei* **Polybia smillima* **Vespa mandarinia* **Vespa pennsylvanica* **Vespa tropica*	**Pachycondyla cressiondes*	**Dinoponera grandis*	**Ectotoma tuberculatum*

Although *Neoponera olivaceae*, *Dinoponera grandis* and *Dolicovespula arenaria* have the same phospholipase activity, they exhibited two distinct patterns of migration. The same situation was recognized for *Odontomechus sp.* and *Polistes dominulus* and for *Neoponera apicalis* and *Polistes flavis* (Fig 6). It is worth mentioning that *Odontomechus* sp., *Neoponera olivaceae* and *Neoponera apicalis* have the same pattern of migration but different phospholipase activities. The same is true for the rest of the examined species (Figure 6 & Table 2). It is obvious that there is no relationship between the phospholipase activity of any given Hymenoptera venom and its migration pattern (Figs 6, 7 & 8 and Table 3).

The venom patches of different individuals of a given species can have different phospholipase activities. *Polistes flavis* is an obvious example, where one has a phospholipase activity of 7 units/mg, while another has 16 units/mg. *Dinoponera grandis* and *Dolichovespula arenaria* also have varied phospholipase activities (Table 3). However, this result needs to be investigated further using more replicates for each species in question.

Table 3: Venom phospholipase activities of Hymenoptera species (see Figs 6, 7 & 8).

Species names	Phospholipase activity (units/mg)			Mean
	Figure 6	Figure 7	Figure 8	
Neoponera apicalis	7	10		8.5
Neoponera olivaceae	1	0.8		0.9
Paraponera clavata	9	10		9.5
Pachycondyla cressiondes		0.8	0.3	0.6
Pogonomyrmex occidentalis	15			15
Dinoponera grandis	1	4		2.5
Ectatoma tuberculatum	9	10		9.5
Odontomechus sp.	5	4		4.5
Polybia smillima			8	8
Brachygastera mellifera			16	16
Parachantergus fraterus			6	6
Polistes dominulus	5			5
Polistes flavis	7		16	11.5
Polistes indica			16	16
Polistes aurifer (= fuscutus)			14	14
Polistes hebraeus			8	8
Polistes rothreyei			12	12
Vespa mandarinia		4	4	4
Vespa tropica		2	0.6	1.3
Dolichovespula arenaria	1	4		2.5
Dolichovespula maculata			4	4
Vespa pennsylvanica		2	2	2

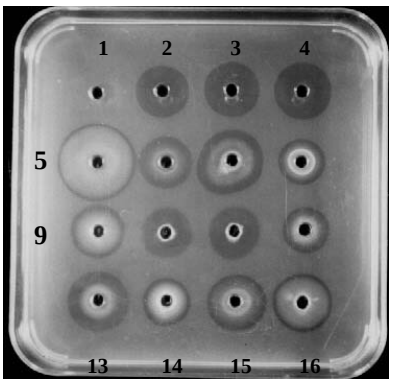

Figure 6: Migration pattern of some Hymenoptera species crude venom in egg yolk/agarose plate. Well assignments are: (1) Blank. (2) 0.15 Unit phospholipase standard. (3) 0.25 Unit phospholipase standard. (4) 0.35 Unit phospholipase standard. (5) *Pogonomyrmex occidentalis*. (6) *Odontomechus sp.* (7) *Ectotoma tuberculatum*. (8) *Neoponera olivaceae*. (9) *Polistes dominulus*. (10) 0.45 Unit phospholipase standard plus 1 M BPB. (11) 0.45 Unit phospholipase standard plus 2 M BPB.(12) *Dinoponera grandis.* (13) *Paraponera clavata.* (14) *Dolicovespula arenaria*. (15) *Neoponera apicalis*? (16) *Polistes flavis*.

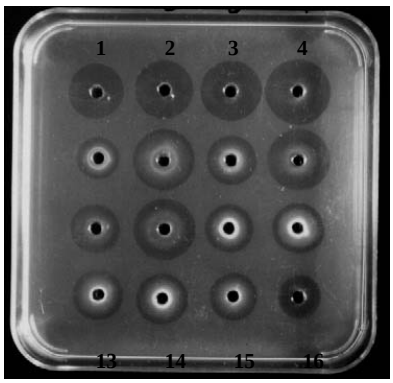

Figure 7: Migration pattern of some Hymenoptera species crude venom in egg yolk/agarose plate. Well assignments are: (1) 0.15 Unit phospholipase standard. (2) 0.20 Unit phospholipase standard. (3) 0.25 Unit phospholipase standard. (4) 0.30 Unit phospholipase standard. (5) *Neoponera olivaceae*. (6) *Neoponera apicalis*? (7) *Dinoponera grandis*. (8) *Paraponera clavata*. (9) *Odontomechus sp.* (10) *Ectotoma tuberculatum*. (11) *Vespa tropica*. (12) *Vespa mandarinia*. (13) *Dolichovespula maculata*. (14) *Dolichovespula arenaria*. (15) *Vespa pennsylvanica*. (16) *Pachycondyla cressiondes*.

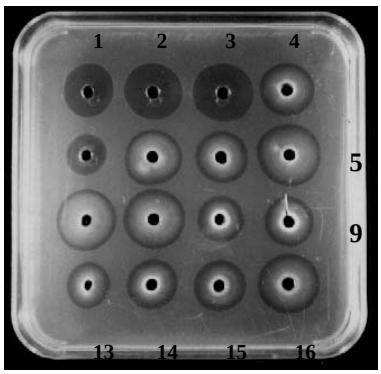

Figure 8: Migration pattern of some Hymenoptera species crude venom in egg yolk/agarose plate. Well assignments are: (1) 0.15 Unit phospholipase standard. (2) 0.25 Unit phospholipase standard. (3) 0.35 Unit phospholipase standard. (4) *Polybia simillina*. (5) *Pachycondyla cressiondes*. (6) *Polistes rothreyei*. (7) *Polistes hebraeus*. (8) *Polistes indica*. (9) *Polistes fuscutus*. (10) *Polistes flavis*. (11) *Vespa tropica*. (12) *Vespa mandarinia*. (13) *Vespa pennsylvanica*? (14) *Dolichovespula maculata*. (15) *Parachantergus fraterus*. (16) *Brachygastera mellifera*.

-phenacyl bromide) on the plate is shown in Table 3 & M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 1 M and 2 M) were tested. All concentrations were able to reduce the migration distance

(Figure 9). Using 0.1 M BPB resulted in the greatest reduction, followed by 2 M BPB: the remaining five concentrations reduced the migration distance equally. Both 1 M BPB and 2 M BPB reduced the migration distance of 0.45 units of pure phospholipase by 20% (Table 4 & Figure 9).

Table 4: Effect of phospholipase A_2 inhibitor BPB on the migrated distance of *Polistes flavis* venom on egg yolk/agarose plates.

	Migrated distance	Reduction of migrated distance (%)
P. flavis venom alone	19.5 mm	
P. flavis venom + 0.1 M BPB	13 mm	33
P. flavis venom + 0.2 M BPB	16 mm	18
P. flavis venom + 0.3 M BPB	16 mm	18
P. flavis venom + 0.4 M BPB	16 mm	18
P. flavis venom + 0.5 M BPB	16 mm	18
P. flavis venom + 1 M BPB	16 mm	18
P. flavis venom + 2 M BPB	14 mm	28

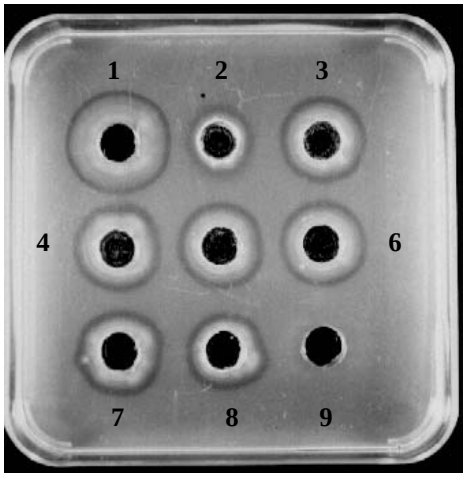

Figure 9: Effect of different concentrations of phospholipase A_2 inhibitor (BPB) on migration distance (a phospholipase activity indicator) of *Polistes flavis* crude venom in egg yolk/agarose gel plate. Well assignments are: (1) *P. flavis* venom; (2) *P. flavis* venom + 0.1M BPB; (3) *P. flavis* venom + 0.2M BPB; (4) *P. flavis* venom + 0.3M BPB; (5) *P. flavis* venom + 0.4M BPB; (6) *P. flavis* venom + 0.5M BPB; (7) *P. flavis* venom + 1M BPB; (8) *P. flavis* venom + 2M BPB; (9) blank (dist H_2O).

DISCUSSION

It seems that the chemical composition of hymenopteran venoms is biologically interwoven with the behaviour (*i.e.* ecological physiology) and biology of the producing organism. For example, the venoms causing pain and cytolytic effects (*i.e*. ants, most bee, social wasps and some solitary aculeate wasps) are characterized by the presence of low-molecular-weight basic proteins or polypeptides (Banks & Shipolini 1986; Blum 1981; Nakajima 1986; Piek 1986; Piek & Spanjer 1986; Schmidt 1986) suggesting a defence role of the venom apparatus and its venom (Leluk *et al.* 1989).

The protein composition of Hymenoptera venoms (Table 5) is a blend of protein molecules that share a reasonable degree of similarity in activity (*i*.e. biological function). The present study reveals the presence of four strongly basic proteins in the venom of *Polistes flavis* using PAGE in the absence of sodium dodecyl sulfate. Similarly Bernheimer *et al.* (1982) showed that *Polistes comanchus navajoe* venom is resolved by PAGE under nondissociating conditions into five bands. SDS-PAGE of *Polistes flavis* venom revealed eighteen bands, with molecular weights ranging from 17-94 kD. Leluk *et al.* (1989) mentioned that all of the

parasitic wasps from Ichneumonidae and Braconidae possess many high-molecular-weight proteins of M_r> 90,000, but low-molecular-weight proteins (M_r< 18,000) were uniformly absent or in very low abundance.

Table 5: Protein composition of Hymenoptera venoms. * Symbols: + = Present, 0 = Absent, No symbol = Not investigated; ** Detected in at least one but not necessarily in all species; *** Specificity unknown. From Schmidt (1994).

		Honey bees	Vespid wasps	Fire ants	Harvester ants	Bull ants	Other ants**
Enzymes	Phospholipase A_1	0	+	+***	+***	+***	+***
	Phospholipase A_2	10-12%	0				
	Phospholipase B	1%	+	+	+		
	Hyaluronidase	1-2%	+	0	+	+	+
	Acid phosphatase	1%	0	+	+		+
	Alkaline phosphatase	+	0		+		+
	Lipase	0	+		+		+
	Esterase	+	+		+		0
	Protease	0	+**	0	0		+
Peptides	Haemolysins	melittin 40%	+		barbatolysin	+	+
	Mastolytic peptides	MCD-peptide 2%	mastoparans				
	Neurotoxins	apamin 3%	+		+		+
	Antigen 5	0	+	+		?	
	Kinins	0	+		+	0	+
	Group specific allergens	+	+	+		+	

Enzymes can be identified according to their substrate(s) specificity (McGilvery & Goldstein 1979). In this sense, the fourth band of the *Polistes flavis* native gel that can digest olive oil is most likely to be a lipase enzyme, especially since two HPLC fractions of the *Polistes flavis* venom were confirmed to have a lipase activity. Do these two fractions represent two protein chains (*i.e*. subunits) of the same lipase or two separate entities (*i.e*. two enzymes)? The answer to this question awaits further investigation.

Soldatova *et al.* (1993) suggested that *Dolichovespula maculata* venom phospholipase has a weak lipase activity. The present finding on *Polistes flavis* venom lipase activity coupled with Soldatova *et al.* (1993)'s suggestion prompted us to explore for phospholipase activity of *Polistes flavis* venom. In the present study, egg yolk used as a phospholipase substrate (Habermann & Hardt 1972) and BPB as a phospholipase A_2 inhibitor. It has been found that low and high concentrations of BPB resulted in substantial reduction of the migration distance of *Polistes flavis* venom, a measure of phospholipase activity. It seems that we are dealing with at least two enzymes with two slightly different optimal pH values or two aggregated functional forms of the same enzyme. For a given enzyme there is a fixed number of binding and catalytic groups, and BPB may be bound to only some of these groups, exerting partial inhibition of the *Polistes flavis* venom phospholipase activity. At 0.1M BPB, an enzyme or an enzyme-aggregate form may be active. The increase of the *Polistes flavis* venom phospholipase activity at 0.2 M, 0.3 M, 0.4 M, 0.5 M and 1 M BPB could be a result of activation of another enzyme or another enzyme aggregate form which work side by side with the previously activated one and partially inhibited as well. The activity reduction exerted by 2 M BPB on the *Polistes flavis* venom phospholipase activity could be the result of an unfavourable change in the pH of the reaction mix. The exact mechanisms underlying these

phenomena (*i.e*. activation and partial inhibition) needed to be elucidated. Costa & Palma (2000) described a comparable case for the Neotropical social wasp cassununga (*Agelaia pallipes pallipes*) venom phospholipase. They mentioned that venom has a phospholipase A_2 which exists in three different aggregation states (monomer, trimer and pentamer) with a transition of the values of pH optimum for the substrate hydrolysis from 7.5 to 9.0, under aggregation from monomer to pentamer.

Study of the venom migration pattern in an egg yolk/agarose plate of some Hymenoptera species belonging to the Vespidae and the Formicidae revealed the presence of phospholipase in the venoms of all studied species. According to Schmidt (1994), Hymenoptera venoms have several protein compounds, and many of them are enzymes. Herein, we showed that the migration pattern of each of the studied Hymenoptera venoms seems to characterise its species. Genes express themselves in the form of protein compounds. Many of these compounds are functional proteins, i.e. enzymes. The present study showed that Hymenoptera species following the venom migration pattern B in their venom migration in egg yolk/agarose plate mainly belong to Polistinae (*Paraponera clavata*, *Polistes dominulus*, *Polistes flavis*, *Polistes fuscutus*, *Polistes hebraeus*, *Polistes rothreyei*, and *Polistes indica*) Polypinae (*Brachygastera mellifera*, *Parachantergus fraterus*, and *Polybia smillima*) and Vespinae (*Dolichovespula arenaria*, *Dolichovespula maculata*, *Vespa mandarinia*, *Vespa pennsylvanica*, and *Vespa tropica*) of the Vespidae, and Myrmicinae of the Formicidae (*Pogonomyrmex occidentalis*). Such data confirm the relatedness of the Polistinae, Polypinae and Vespinae: these subfamilies are in fact related, belonging to the Vespidae. These data also suggest that the Myrmicinae (Formicidae) may be the most plesiomorphic group, evolved from the Vespidae, or alternatively that both the Formicidae and Vespidae share a common ancestor.

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الملخص العربى

نشاط أنزيمى الليبيز والفوسفوليبيز فى سموم بعض أنواع رتبة غشائية الأجنحة (الزنابير والنمل)

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أظهرت نتائج الفصل الكهربي لسم الزنبور الإجتماعى "*بوليبستس فلافس*" - في غياب مادة الصوديوم دوديسيل سلفات (SDS) – أربع مركبات بروتينية مميزة وواضحة الفصل، أظهر المركب الرابع نشاطاً كبيراً لهضم الدهون مما يعكس دلالة على أن له نشاط يعتقد أنه لإنزيم الليبز. كما أظهرت نتائج الفصل الكهربي لسم نفس النوع من الزنابير الإجتماعية – في وجود مادة الصوديوم دوديسيل سلفات (SDS) - ثمانية عشرة مركب بروتيني، تتراوح أوزانهم الجزيئية ما بين 94 كيلو دالتون و 17 كيلو دالتون. وعند إستخدام جهاز الفصل الكروماتوجرافي السائل فائق الأداء (HPLC) لتحليل سم الزنبور فقد نتجت عمليات الفصل فى التعرف على 122 مستخلص للسم. وتم إختبارها جميعاً فى قدرتها على هضم الدهون وقد ثبت أن هناك اثنين من هذه المستخلصات لها فعالية من حيث نشاطها الأنزيمى لإنزيم الليبيز. أيضا أوضحت الدراسة وجود نشاط انزيم الفوسفوليبيز في سموم 23 نوعاً من الانواع التابعة لرتبة غشائية الاجنحة. أيضا أظهرت الدراسة ان لسموم هذه الانواع خمس اشكال مميزة للهجرة خلال الاجاروز المحمل بصفار البيض. تم مناقشة العلاقة التطورية بين تلك الانواع المختلفة بناء على التحاليل الكيميائية الأنزيمية لسمومها مما أضفى بعداً جديداً فى دراسة العلاقات التطورية بإستخدام التحاليل البيوكيميائية.