

Digestive proteases in four species of *Polistes* wasps

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Adults of *Polistes exclamans*, *Polistes metricus*, *Polistes fuscatus*, and *Polistes annularis* (Hymenoptera: Vespidae) were examined for digestive protease activity. All samples analysed were found to contain chymotrypsin-like, trypsin-like, 'carboxypeptidase-A-like,' and 'carboxypeptidase-B-like' activities. The wasp chymotryptic enzyme has a serine and histidine in its active center and a specificity for aromatic amino acids and in these respects is similar to bovine chymotrypsin. The tryptic enzyme does not react like its bovine homologue, though low concentrations of this enzyme and the limit of sensitivity of the spectrophotometer may account for the differences. Enzyme comparisons are made between these *Polistes* species and three *Vespid* species.

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L'activité de la protéase digestive a été étudiée chez les imagos de *Polistes exclamans*, *Polistes metricus*, *Polistes fuscatus* et *Polistes annularis* (Hymenoptera: Vespidae). Les analyses révèlent l'existence d'activités semblables à celles de la chymotrypsine, de la trypsine et des carboxypeptidases A et B dans tous les échantillons. L'enzyme chymotryptique de guêpe possède une sérine et une histidine dans son centre actif et manifeste de la spécificité pour les acides aminés aromatiques; l'enzyme est semblable, par ces caractéristiques, à la chymotrypsine de bovin. L'enzyme tryptique de guêpe ne réagit pas comme la trypsine de bovin, mais les concentrations faibles de cette enzyme et la limite de sensibilité du spectrophotomètre peuvent être responsables des différences observées. Les enzymes de ces *Polistes* sont comparées ici à celles de trois autres espèces de vespidae.

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Introduction

In colonies of social wasps the larvae are fed solid food (insect larvae or soft-bodied arthropods) by an adult female. In return, the larvae secrete a drop of salivary fluid which is subsequently swallowed by an adult (Maschwitz 1966). This phenomenon of fluid exchange between members of a colony has been termed trophallaxis (Wheeler 1918). Maschwitz (1966) determined that this secretion is both attractive and nourishing, containing carbohydrates in concentrations approximately four times that found in larval haemolymph. Amino acids and proteins are also present but at one-fifth their haemolymph concentration. One drop of this salivary gland secretion from a very large larva is sufficient to keep an adult alive for half a day (Maschwitz 1966). When a colony of *Vespa orientalis* was deprived of access to larval secretions, the queen died within a few days (Ishay and Ikan 1968).

The work of Ikan *et al.* (1968) indicated that the larvae of *V. orientalis* were the only members of the colony capable of protein digestion. However, proteolytic activity has since been identified in the midguts of both larvae and adults and in the larval

saliva of *Paravespula germanica* by Spradbery (1973) and in the midguts of adult castes of *Vespula germanica* and *Vespula maculifrons* by Grogan and Hunt (1977).

The question of wasp nutrition appears to be a complicated one. Wasps are said to forage for two distinct food types: carbohydrates for themselves and protein for their larvae (Ikan and Ishay 1966). Floral nectars (Baker and Baker 1973, 1975), honeydew from Homoptera (Ehrhardt 1962), and insect haemolymph (Wyatt 1961), all of which are foods of adult social Hymenoptera, contain amino acids as well as carbohydrates. The larval saliva of social Vespidae contains sugars and free amino acids and so resembles these food sources (J. H. Hunt, personal communication). These readily available sources of free amino acids suggest that protein may be nonessential for adults.

There are indications, however, that protein in the form of solid food may be regularly ingested. In some species, cannibalism of larvae and pupae is not uncommon (Wilson 1971). In temperate zone *Polistes*, the dominant female devours eggs of subordinate females to assure that only her brood will be reared (Gervet 1964; Eberhard 1969). Bits of insect prey are malaxated by adults before being offered to larvae; whether adults are ingesting por-

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tions of this food has not been determined. Indeed, whether the structure of the *Polistes* digestive system is capable of dealing with solid particles is uncertain (e.g. Eisner 1957).

Objectives

The primary objective of this research was to survey *Polistes* species commonly encountered in the St. Louis, Missouri, area for the presence and activity of digestive proteases. Differential abundance patterns of the enzymes between sexes were quantified. The enzymes were characterized in terms of their cleavage specificities in order to compare them with mammalian protease enzymes.

Materials and Methods

Collection

Colonies of *Polistes exclamans*, *Polistes fuscatus*, *Polistes metricus*, and *Polistes annularis* were collected during the late summer of 1976. Males were separated from females; no external morphological features differentiate queens from workers. Adults that eclosed from nests in the lab were held in isolation and their midgut contents examined separately.

Nonspecific Protease Determination

Wasps, held at -10°C for several minutes, were placed under a dissecting microscope and their abdomens were cut open dorsally to expose their midguts. The tip of a tuberculin syringe was inserted into the lumen of the extended midgut, and its contents were removed. The extract was diluted to $100\ \mu\text{l}$ with phosphate buffer (pH 7.0) and held at 4°C for immediate analysis. Larval salivary secretions were collected by prodding the mandibular area of larvae with a capillary tube. Protease activity in such samples was assayed with Azocoll by modifying the procedure outlined by Calbiochem, La Jolla, CA, document 3805. The samples to be analysed were diluted to 0.5 ml in 0.1 M phosphate buffer (pH 7.0) and added to tubes containing 5 mg Azocoll. The solution was incubated at 37°C for 15 min; spun at $2500\ \text{g}$ for 5 min at room temperature. The supernate was read at 520 nm in a spectrophotometer.

Trypsin-like Determination

Midguts were removed from live wasps by grasping the apex of the abdomen and pulling gently. The entire digestive tract pulled free, usually breaking at the proventriculus. Midguts were dissected out and transferred to test tubes immersed in an acetone - dry ice mixture. Tissues were frozen within 15 s of removal from live wasps and stored at -60°C .

For analysis three to six midguts were incubated in 1.0 ml 0.001 M HCl for 15 min at 37°C . The extract was centrifuged at $2500\ \text{g}$ for 15 min at room temperature and the supernate was used as the enzyme source. The reaction sample had a total volume of 3.0 ml and contained 40 mM Tris-HCl (pH 8.1), 10 mM CaCl_2 , and 0.1 ml enzyme extract. The reaction was initiated by the addition of *p*-toluenesulfonyl-L-arginine methyl ester (TAME) to a final concentration of 1 mM. The rate of hydrolysis of TAME is measured by the increase in absorbancy at 247 nm (Hummel 1959). One unit is equal to the hydrolysis of $1\ \mu\text{mol}$ of TAME per minute at 25°C and pH 8.1.

Chymotrypsin-like Determination

Midguts were extracted as above. The reaction tubes contained a total volume of 3.0 ml consisting of 40 mM Tris-HCl

buffer (pH 7.8), 5 mM CaCl_2 , and 0.2 ml enzyme extract. The reaction was initiated by the addition of benzoyl-L-tyrosine ethyl ester (BTEE) in 50% methanol (w/w) to a final concentration of 0.5 mM. The rate of hydrolysis of BTEE is determined from the change in absorbancy at 256 nm. One unit is equivalent to $1\ \mu\text{mol}$ substrate hydrolyzed per minute at pH 7.8 and 25°C (Hummel 1959).

'Carboxypeptidase-A-like' Determination

Midguts were extracted in 10% LiCl by the methods described for trypsin. One-tenth millilitre enzyme extract was added to reaction tubes containing 2.9 ml freshly prepared substrate of 1 mM hippuryl-L-phenylalanine (HPA) in 25 mM Tris-HCl buffer (pH 7.5) with 500 mM NaCl. Absorbancy measurements were made at 254 nm and units of activity were defined as number of micromoles of substrate hydrolyzed per minute at pH 7.5 and 25°C (Folk and Shirmer 1963).

'Carboxypeptidase-B-like' Determination

Midgut extraction was as for carboxypeptidase A. One-tenth millilitre enzyme extract was added to 2.9 ml freshly prepared substrate containing 1 mM hippuryl-L-arginine (HA) in 25 mM Tris-HCl buffer (pH 7.65) with 100 mM NaCl (Folk *et al.* 1960). Unit of activity was defined as number of micromoles hydrolyzed per minute at pH 7.65 and 25°C .

Enzyme Characterization

Inhibitors of trypsin and chymotrypsin were added to the reaction mix: phenyl-methyl-sulfonyl fluoride (PMSF) (Gold 1965); *N*- α -*p*-tosyl-L-lysine chloromethyl ketone (TLCK) (Schoellman and Shaw 1963); and L-1-tosylamine-2-phenylethylchloro-methyl ketone (TPCK) (Shaw *et al.* 1965). One-tenth millilitre of inhibitor was added to a reaction tube to give a final concentration of 0.95 mM PMSF, 0.2 mM TLCK, or 0.2 mM TPCK. The enzyme was added to the buffer and pre-incubated for 20 min with PMSF and for 60 min with TLCK or TPCK prior to initiation of the reaction. In all cases where inhibitors were added the reaction mix was decreased by 0.1 ml buffer to maintain a constant volume.

Protein Determination

Protein determination was carried out according to methods of Lowry *et al.* (1951).

Results

Azocoll Protease Activity in *Polistes* spp.

In the four species of *Polistes*, protease activity was found in the larval saliva and in the midgut extracts from both adults living in the active colonies and from adults that eclosed in the laboratory (Table 1). Crops of newly eclosed adults were found to contain fluid but no protease activity was detected in it. No protease activity was found in wing muscle (nondigestive tissue) that was used as a control for identification of possible lysosome activity.

Digestive Protease Activity of *Polistes* Midguts

Chymotryptic and tryptic activities were found in male and female *P. exclamans*, *P. metricus*, *P. fuscatus*, and unsexed samples of *P. annularis* (Table 2).

The predominant enzyme was chymotrypsin-like. Chymotryptic activity exceeded tryptic activ-

TABLE 1. General protease activity in larval and adult *Polistes*

Species	Age	Location of activity	Activity
<i>P. annularis</i>	Larvae	Saliva	+
	Adults	Midgut extract	+
<i>P. fuscatus</i>	Larvae	Saliva	+
		Midgut	+
	Adults*	Wing muscle	-
		Midgut	+
Newly eclosed	Midgut	+	
	Crop	-	
<i>P. metricus</i>	Larvae	Saliva	+
	Adults	Midgut	+
<i>P. exclamans</i>	Larvae	Saliva	+
		Midgut	+
	Adults	Midgut	+
		Crop	-
Newly eclosed	Midgut	+	
	Crop	-	

*Adults as referred to above have been with the colony for their adult life. Newly eclosed adults were isolated in the laboratory.

ity in the same species by from 8.9 to 15 times. It exceeded 'carboxypeptidase-A-like' activity by from 7.4 to 44.2 times and exceeded 'carboxypeptidase-B-like' activity from 11.3 to 22.3 times. Its specific activity in males was from 1.1 to 2.8 times greater than that for females. The reaction rate for the chymotryptic enzyme was linear for 5 min in male *P. exclamans* (Fig. 1A), 3 min for male *P. metricus* (Fig. 2A), and 2 min for *P. fuscatus* (Fig. 3A). The reaction rates for females of these three species were linear for 7 min (Figs. 1B, 2B, 3B).

The tryptic enzyme specific activities were low in all species (Table 2). Activity rates appear to be linear (Figs. 1C, 1D, 2C, 2D, 3C, 3D).

Very few midguts of *P. annularis* were available. Specific activity values were higher for the

chymotrypsin-like enzyme than for the trypsin-like enzyme (Table 2).

Unsexed samples of *P. exclamans* possessed 'carboxypeptidase-A-like' activity (Table 3) that was between chymotryptic and tryptic activities in abundance. 'Carboxypeptidase-B-like' activity was also found in the midgut of unsexed *P. exclamans* but at very low concentrations (Table 3). 'Carboxypeptidase-A-like' specific activity in female *P. metricus* (Table 3) was only 16% of the chymotryptic specific activity noted in conspecific females, but it was greater than tryptic specific activity by a factor of 1.5. 'Carboxypeptidase-B-like' specific activity in that species (Table 3) was one-tenth that of chymotrypsin-like enzyme and about equal to that of the trypsin-like form. 'Carboxypeptidase-A-like' enzyme was studied in *P. fuscatus* males (Table 3). Amounts of 'carboxypeptidase-B-like' enzyme were so low that it was impossible to determine the content with any degree of accuracy.

Effects of Inhibitors on Endopeptidases from *P. metricus*, *P. exclamans*, and *P. fuscatus*

To compare wasp chymotryptic activity with bovine chymotrypsin, three inhibitors were used. PMSF inhibited the enzyme in all samples. The range of inhibition was from 90 to 84% for males during the period of linearity (Figs. 1A, 2A, 3A) and from 68 to 60% for females (Figs. 1B, 2B, 3B). These results indicate that the activity of the *Polistes* chymotrypsin-like enzyme has a serine residue in its active center and in this respect is similar to bovine chymotrypsin.

Figures 1A, 1B, 2A, 2B, 3A, and 3B show that inhibition by TPCK in all cases exceeded 90% dur-

TABLE 2. Midgut chymotrypsin and trypsin specific activities in adult castes of four *Polistes* species. Enzyme activity values are averages of one to six replicates

	Chymotrypsin		Trypsin	
	U/mg protein	U/mg midgut	U/mg protein	U/mg midgut
<i>P. exclamans</i>				
Female	1.18	0.095	0.095	0.0075
Male	1.34	0.12	0.09	0.007
<i>P. metricus</i>				
Female	0.68	0.05	0.07	0.007
Male	1.34	0.125	0.15	0.018
<i>P. fuscatus</i>				
Female	0.905	0.095	0.08	0.085
Male*	2.52	0.27	0.21	0.023
<i>P. annularis</i> *				
†	0.47	0.05	0.04	0.004

*Single samples.
†Unsexed.

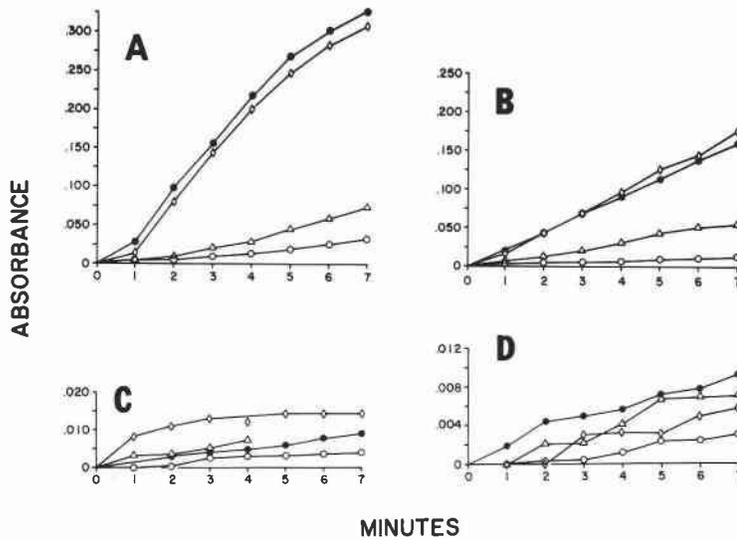


FIG. 1. The chymotrypsin-like midgut activity of male (A) and female (B) and the trypsin-like midgut activity of male (C) and female (D) *Polistes exclamans*. The response is shown of the enzyme (●) to PMSF (Δ); to TLCK (◊); and to TPCK (○).

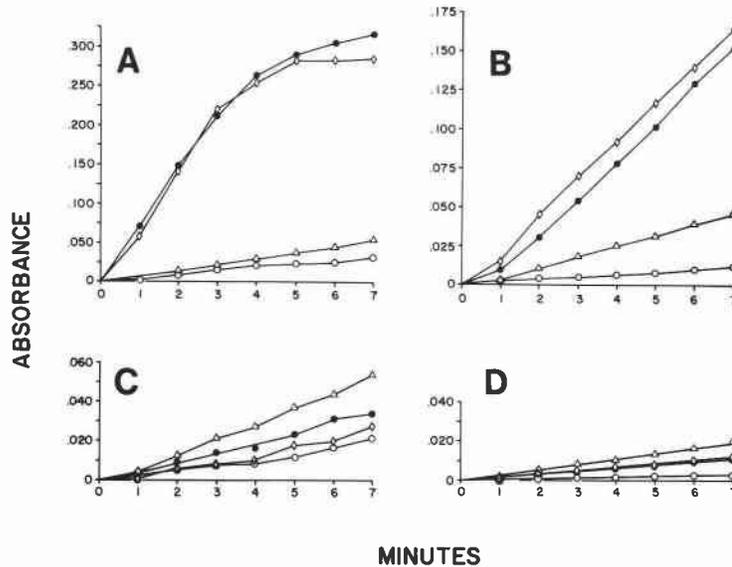


FIG. 2. The chymotrypsin-like midgut activity of male (A) and female (B); and the trypsin-like midgut activity of male (C), and female (D) *Polistes metricus*. The response is shown of the enzyme (●) to PMSF (Δ); to TLCK (◊); and to TPCK (○).

ing the period of linearity. For TPCK to be inhibitory, an enzyme must have a histidine in its active center and a specificity for aromatic amino acids. Both the *Polistes* and the bovine enzymes share these characteristics. TLCK was not an effective inhibitor of the wasp enzyme in either males or females (Figs. 1A, 1B, 2A, 2B, 3A, 3B). TLCK will inhibit an enzyme that has a histidine in its active center and a specificity for basic amino acids. This lack of inhibition represents an additional similarity between wasp and bovine enzymes.

Similar experiments were used to characterize the trypsin-like enzyme in *P. exclamans* (Figs. 1C, 1D), and *P. metricus* (Figs. 2C, 2D). The very low absorbance readings leave interpretation of the inhibition experiments open to question.

Discussion

Protease activity was found in larval saliva, in midguts of adults established in the colony, and in midguts of newly eclosed adults. Crop content of newly eclosed adults showed no proteolytic activ-

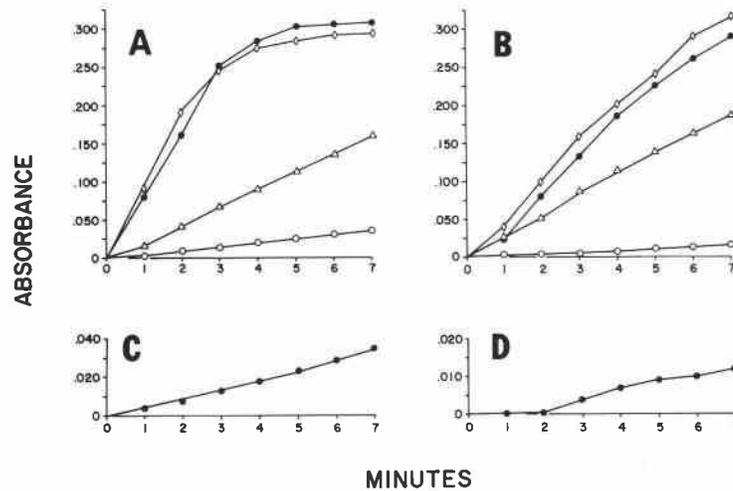


FIG. 3. The chymotrypsin-like midgut activities of male (A); of female (B); the trypsin-like midgut activity of male (C), and of female (D) *Polistes fuscatus*. The response is shown of the enzyme (●) to PMSF (△); to TLCK (◇); and to TPCK (○).

TABLE 3. Midgut carboxypeptidase A and carboxypeptidase B specific activities in adult castes of two *Polistes* species. Enzyme activity values are averages of two replicates

	Carboxypeptidase A		Carboxypeptidase B	
	U/mg protein	U/mg midgut	U/mg protein	U/mg midgut
<i>P. exclamans</i> Unsexed	0.159	0.009	0.06	Trace
<i>P. metricus</i> Female	0.110	0.008	0.06	Trace
<i>P. fuscatus</i> Male	0.057	0.005	(No results)	

ity. There was no proteolytic activity in tissue samples from wing muscle, indicating that the activity measured was that of digestive enzymes rather than lysosomal enzymes. Results indicated that digestive proteases were present in substantial quantities in four species of *Polistes*.

Enzymes similar to trypsin, chymotrypsin, carboxypeptidase A, and carboxypeptidase B were found in all instances where they were sought. Chymotrypsin-like enzyme was present in greatest abundance; 'carboxypeptidase-A-like' enzyme was apparently second most abundant; the trypsin-like enzyme was third; and 'carboxypeptidase-B-like' was least abundant. Where direct comparisons were possible, it was evident that substantially greater amounts of digestive enzymes were found in males than in females. These results indicate a high probability that the wasps studied are capable of protein digestion.

Characterization of the wasp enzymes in terms of their mammalian homologues indicates some similarities in cleavage specificities.

The results summarized in Table 2 show differences in specific activity between species of *Polistes*. Differences in specific activity were also noted between castes of *V. germanica* and *V. maculifrons* (Grogan and Hunt 1977). A higher concentration of digestive enzymes was found in male *Polistes* than in females in the three species where sex differences were noted. Grogan and Hunt (1977) noted the reverse situation in *Vespula*.

The abundance of enzyme activity found in these studies differs from the results reported by Ikan *et al.* (1968) on *V. orientalis*. Pressure on the abdomen of a hornet was used by Ikan *et al.* (1968) to collect some of their samples. Such a technique might not result in the collection of fluid from the midgut digestive region but more probably from the crop, where digestion does not take place. Passage through the muscular proventriculus of the Hymenoptera appears to be one way (Eisner 1957), although regurgitation from the midgut may occur in other insects, for example, the cockroach (Morton 1967). A further discrepancy between this study

and that of Ikan *et al.* probably arises from a difference in assay technique. The low specific activities obtained by Ikan are for total body protein assayed from whole body homogenates. The specific activity of enzymes as a percentage of total body protein would be much less than specific activity calculated on the basis of midgut protein. In light of their techniques, it is possible that even the low results presented in their tables have some positive significance.

The results of inhibition studies on the wasp trypsin-like enzyme are not easily interpreted and should be held in reserve until the enzyme can be further studied.

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