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MIDGUT ENDOPEPTIDASE ACTIVITIES OF THE HORNET
VESPA CRABRO GERMANA CHRIST
(HYMENOPTERA : VESPIDAE)

D.E. GROGAN and J.H. HUNT

Department of Biology, University of Missouri - St. Louis
St. Louis, Missouri 63121 U.S.A.

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SUMMARY

Trypsin-like and chymotrypsin-like enzyme activities are present in midguts of adult workers, gynes, and males of the hornet *Vespa crabro germana* Christ. The chymotrypsin-like enzyme is the more abundant; both enzymes have activities that are linear over time.

The chymotrypsin-like enzyme is inhibited by inhibitors specific for mammalian chymotrypsin. These results are wholly consistent with results of previously published studies on yellowjackets and paper wasps.

RESUME

Activités endopeptidases de l'intestin moyen du frelon *Vespa crabro germana* Christ (Hymenoptera : Vespidae)

Des activités enzymatiques "trypsine-like" et "chymotrypsine-like" sont mises en évidence dans l'intestin moyen des ouvrières, des fondatrices-filles et des mâles du frelon *Vespa crabro germana* Christ. L'activité "chymotrypsine-like" est la plus importante. Les taux d'activité des deux enzymes sont linéaires pendant la durée de l'expérience.

Les inhibiteurs spécifiques de la chymotrypsine des mammifères agissent contre l'activité "chymotrypsine-like". Ces résultats sont compatibles avec les données recueillies chez les guêpes *Vespula* et *Polistes*.

Midgut protease activities in wasps have been reported for yellowjackets of the genus *Vespula* (GROGAN and HUNT, 1977), paper wasps of the

genus *Polistes* (KAYES, 1978), and a swarm-founding polistine of the genus *Polybia* (HUNT *et al.*, in press). These studies were prompted in part by the work of IKAN *et al.* (1968) who reported that adult Oriental Hornets, *Vespa orientalis* F., lack digestive proteases. Differences between the studies include both study taxa and methodology. IKAN *et al.* (1968) assayed whole wasp homogenates; the other investigators utilized isolated midguts. The present report adds *Vespa* to the survey of midgut proteases based on analysis of isolated midguts.

Hornets of the genus *Vespa* are conspicuous members of the wasp fauna in the Palearctic and Oriental biogeographic realms. The European Hornet, *Vespa crabro germana* Christ, is adventive in North America, first having appeared in the mid-1800's (KROMBEIN *et al.*, 1979).

A nest of *Vespa crabro germana* was collected from within a hollow tree near Hillsborough, Orange County, North Carolina on 8 October 1981. Live adults were collected into an industrial vacuum cleaner and transferred under CO₂ anesthesia to plastic containers. The hornets were then removed individually from these containers, and midguts were removed from the living wasps by the technique of GROGAN and HUNT (1977) and immediately frozen in a testtube immersed in an acetone/dry ice mixture. Midguts were collected from 75 workers, 38 gynes, and 98 males, and were pooled according to caste. The midguts were then stored at -20°C. Endopeptidase assays, inhibition experiments, and protein analyses were performed as described by GROGAN and HUNT (1977) on 3 replicates of the sample material for each caste. Inhibition experiments were performed only on the chymotrypsin-like enzyme.

Adults of the three castes of *Vespa crabro germana* possessed both trypsin-like and chymotrypsin-like enzyme activities in their midguts. Specific activities of the trypsin-like enzyme (as units/mg midgut protein) are: worker = 0.09; male = 0.14; gyne = 0.23. Specific activities of the chymotrypsin-like enzyme (as units/mg midgut protein) exceeded those of the trypsin-like enzyme in all castes: worker = 0.66; male = 1.07; gyne = 0.45. The reaction rates for both enzymes were linear over the 5 minute assay. The chymotrypsin-like enzyme in all castes was inhibited by both phenylmethyl-sulfonylfluoride (PMSF) and L-1-tosylamine-2-phenyl-ethylchloromethyl ketone (TPCK) (*fig. 1*). These results indicate that the enzyme possesses both serine (PMSF inhibition) and a histidine group (TPCK inhibition) in its active center. These results are wholly consistent with the previously reported results on activities and characterization of midgut endopeptidases for two *Vespula* species (GROGAN and HUNT, 1977) and four *Polistes* species (KAYES, 1978) but stand in contrast to IKAN *et al.*'s (1968) reported finding for *Vespa orientalis*.

Though we feel it is likely that midgut proteases occur in most, if not all, wasps, several studies now suggest interesting patterns of variation in

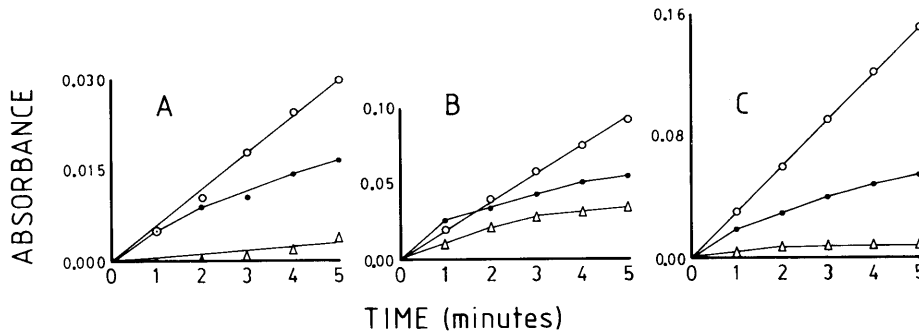


Fig. 1. — The chymotrypsin-like midgut activities of gyne (A), worker (B), and male (C) castes of the hornet *Vespa crabro germana* Christ. The activity of the enzyme over time is shown (O—O) as is the enzyme's response to TPCK (●—●) and PMSF (Δ—Δ).

Fig. 1. — Activité de l'enzyme "chymotrypsine-like" dans les intestins moyens des fondatrices-filles (A), des ouvrières (B) et des mâles (C) du frelon *Vespa crabro germana* Christ. L'activité de l'enzyme en fonction du temps (O—O) est affectée par l'action du TPCK (●—●) et du PMSF (Δ—Δ).

hymenopteran midgut protease activity. HUNT *et al.* (in press) found an age-correlated pattern of midgut chymotrypsin-like activity in *Polybia occidentalis* (Olivier), in which 8-day old females have significantly lower activities than either younger or older females. Worker honey bees, *Apis mellifera* L., have been shown to have midgut chymotrypsin activities that vary both as a correlate of age (GROGAN and HUNT, 1980) and in a circa-annual pattern (GROGAN and HUNT, 1984).

BARKER and LEHNER (1972) have questioned whether honey bee midgut proteases are exogenous or endogenous in origin. The leaf-cutter ant *Atta texana* (Buckley) has been found to apparently lack endogenous proteases (BOYD and MARTIN, 1975). Together with the report of IKAN *et al.* (1968) these reports suggest that insights into the nutrient dynamics of social hymenopterans can be gained by further searching for presence or absence of endogenous midgut proteases. We feel, however, that more informative and ultimately more enlightening data can be gained by a shift of focus to patterns of variation in midgut protease activities as a function of caste, age, nutritional status, or other behavioral or ecological variables.

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