

**CHYMOTRYPSIN-LIKE ACTIVITY IN THE HONEYBEE MIDGUT:  
PATTERNS IN A THREE-YEAR STUDY**

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Levels of activity of a chymotrypsin-like enzyme in midguts of adult worker honeybees (*Apis mellifera*) from 2 hives were measured over a 3-year period. The principal between-year pattern was one of higher levels in winter than in either fall or spring. Considerable variation in enzymatic activity was found. Spearman rank correlation analysis of the chymotrypsin-like activity showed marked concordance in changes in level of activity between the 2 hives in each of the 2 years for which complete data were available, but between-year correlations for each hive were low (or negative) and varied widely. These results suggest that levels of chymotrypsin-like activity in the midgut of worker honeybees are markedly affected by exogenous, i.e. dietary, factors.

**Introduction**

Adult worker honeybees utilize pollen as their primary source of dietary protein. Barker and Lehner (1972) reported that the contents of pollen grains appeared to be degraded enzymatically in the bees' midgut, but the source of the degradative enzymes was still unknown, possibly being the bees' midgut, the gut microflora, or the pollen grains themselves.

Grogan and Hunt (1979) noted that the presence of enzymatic activity in pollen is well known, and reported that pollens of 14 plant species visited by honeybees showed proteolytic activity indistinguishable from that of mammalian intestinal proteases in *in vitro* assays. They speculated on the possible contribution of pollens to the midgut proteolytic activity. Bi-monthly midgut assays of 2 neighbouring hives of honeybees yielded data suggestive of an exogenous origin for midgut chymotrypsin-like and trypsin-like enzymes. The present long-term study was designed as a continuation of that sampling programme to ascertain what pattern might exist in the fluctuating level of activity of midgut endopeptidases and to seek further insight on its possible exogenous origin.

**Materials and Methods**

Two swarms of an Italian strain of honeybees were hived in 1976 in standard Langstroth hives. Sampling was begun in March, 1977. Forager honeybees were netted at the hive entrances in bi-monthly sampling. In winter, samples of workers were taken monthly from the combs of the top chamber of the hives. Sampling was continued for 3 years. The midguts of 5–10 individuals were removed as described previously (Grogan & Hunt, 1977, 1980). Midguts were isolated and then taken up in 0.001-N HCl, macerated with a glass rod, leached for 15 min at 37°C, and centrifuged at 2000 G for 5 min at room temperature. The supernatant from this treatment was used for all enzyme assays.

Trypsin-like and chymotrypsin-like activities were assayed by the method of Hummel (1959), as described in detail by Grogan and Hunt (1977). Protein was determined by the procedure described by Lowry et al. (1951), using bovine albumin as a standard.

**Results**

Chymotrypsin-like enzyme was the major source of endopeptidase activity in honeybee midgut, exceeding trypsin-like activity by an average factor of 2.3 (see also Grogan & Hunt, 1980). For this reason, chymotrypsin-like activity alone was selected for correlation analysis. Fig. 1 presents average levels of activity for a 3-year period of chymotrypsin-like enzyme expressed as  $\mu\text{mol product/min/mg midgut protein}$ .

Examination of Fig. 1 reveals 2 patterns. First, the only consistent between-year pattern was one of higher levels of activity in bees in winter than in fall or spring. There was no consistent

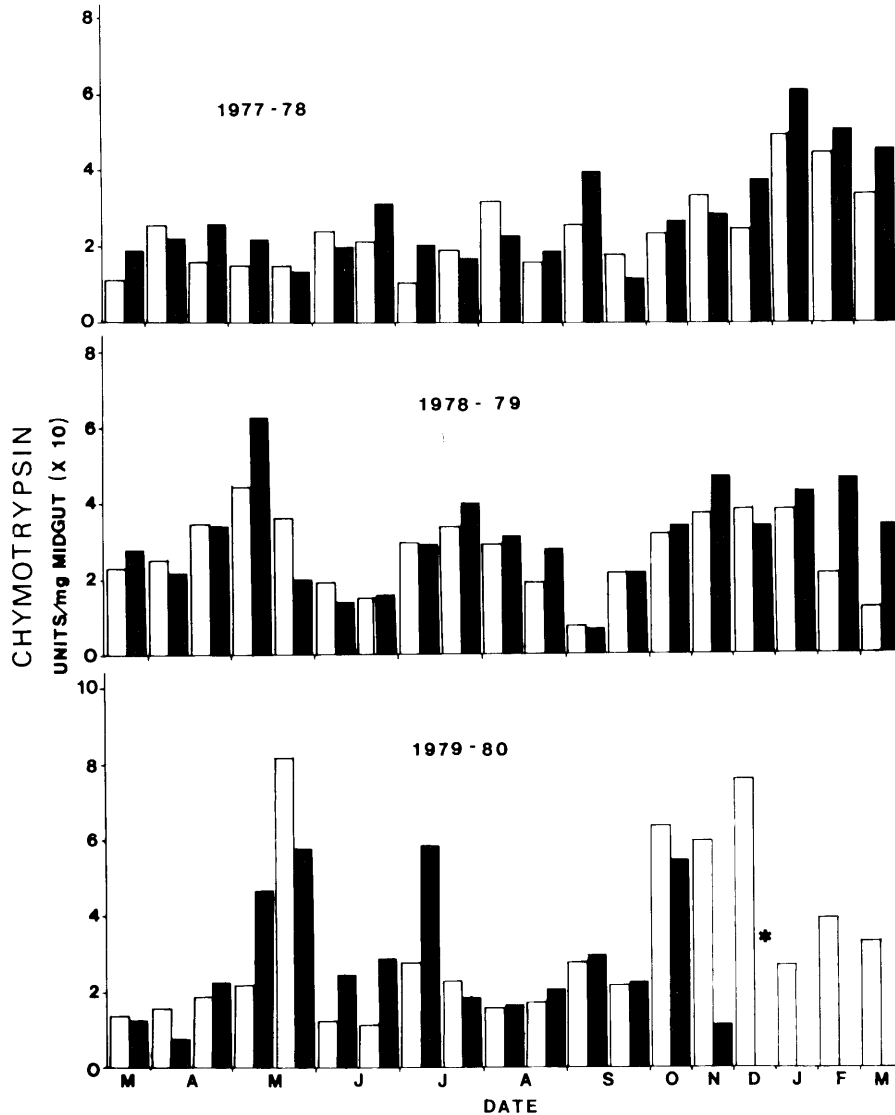


FIG. 1. Chymotrypsin activity in samples of worker honeybees from 2 colonies over 3-years. Activity is expressed as units per mg midgut protein  $\times 10$ . Open bars—Colony 1; solid bars—Colony 2. Asterisk marks death of Colony 2 during cold weather in December 1979.

pattern between years for the summer period of active foraging. Second, a between-colony, within-year trend is observable; that is, the direction of increase or decrease in enzymatic activity between sample dates is typically consistent for the 2 colonies. Spearman rank correlation coefficients ( $r_s$ ) were calculated, using the method of Siegel (1956), for both between-year and between-colony comparisons (Table 1). The results show that, for the two years for which data on colony 2 are complete, the between-colony, within-year correlations are high and nearly identical. By contrast, the within-colony, between-year correlations vary widely and are all lower than the between-colony correlation coefficients.

TABLE 1. Spearman rank correlation coefficients ( $r_s$ ) for chymotrypsin activity in worker honeybees from 2 colonies in 3 years.

Comparison	Colony	Year	$r_s$
Between colonies		1	+0.72
		2	+0.71
Between years	1	1-2	-0.74
		2-3	+0.47
	2	1-3	+0.19
		1-2	+0.65

## Discussion

Levels of activity of honeybee midgut chymotrypsin-like enzyme showed marked concordance for the between-colonies, within-year comparison but varied widely for the between-years, within-colonies comparison. We interpret this result as strongly supportive of the suggestion by Grogan and Hunt (1979) that exogenous factors play a major role in determining enzymatic activity. Grogan and Hunt (1979) suggested that pollens possess sufficiently high concentrations of endopeptidase to contribute to the activity in bees' midgut. The two colonies in this study were situated in the same bee yard and therefore probably fed synchronously on the same floral resources. The data of this study therefore do not invalidate their suggestion. Alternatively, of course, some of the total midgut protease activity may well be endogenous in origin, and the correlated activity levels between colonies may represent corresponding responses to a common exogenous stimulus. We did not assay evacuated (washed) midguts and so cannot discriminate at this time between the hypotheses of endogenous and exogenous origin. Furthermore, the age of the collected bees, whether foragers or hive bees, was not controlled (but see Grogan & Hunt, 1980 for a discussion of correlated age and midgut protease activity levels in similar samples of bees).

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