Age correlated changes in midgut protease activity of the honeybee, *Apis mellifera* (Hymenoptera: Apidae)

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Summary. Forager (older) worker honeybees typically have lower midgut activity levels of chymotrypsin and trypsin than do house (younger) worker honeybees. A relation between the age correlated enzymic change and an age correlated decrease in pollen consumption is not clearly demonstrable.

We have previously reported that pollens collected by bees possess enzymic activities appropriate for protein digestion[1]. The well known age correlated changes in behaviour of the honeybee[2] offer an opportunity to further investigate the possible physiological roles of these pollen enzymes. Newly emerged and intermediate aged individuals largely confine their activities to the hive interior; older bees forage outside the hive. Pollen eating is pursued by younger and intermediate aged individuals[3]. DeGroot[4] demonstrated that both young and old worker bees can digest protein, but he also demonstrated that older bees are lower in total body nitrogen content and lower in weight than are younger bees. We report here quantitative differences in digestive enzyme activity in 2 age correlated samples of honeybee workers.

Materials and methods. 2 colonies of Italian strain honeybees were maintained in standard hives. Samples of house (younger) bees were taken from the upper interior of the hives; forager (older) bees were collected at the hive entrances. Midguts of 5–10 collected bees were removed

Table 1. Midgut weight and midgut chymotrypsin activities. Each sample is an average for midguts of 5–10 workers. H = house (younger) bees; F = forager (older) bees

Date (1979)		Midgut weight (mg) Hive No. 1			Hive No. 2			Chymotrypsin (units/midgut) Hive No. 1			Hive No. 2			Chymotrypsin (units/mg midgut protein) Hive No. 1			Hive No. 2		
		H	F	F/H	H	F	F/H	H	F	F/H	H	F	F/H	H	F	F/H	H	F	F/H
June	5	10.5	9.3	0.89	13.2	10.5	0.80	1.87	1.70	0.91	3.70	1.50	0.41	2.57	2.40	0.93	4.47	2.48	0.55
	22	10.7	8.8	0.82	14.2	10.1	0.71	2.25	1.34	0.60	4.50	1.60	0.36	2.56	2.20	0.86	4.06	2.30	0.57
July	11	10.4	6.8	0.65	10.0	10.0	1.00	3.20	2.00	0.63	2.25	2.95	1.31	4.40	4.10	0.93	4.00	3.58	0.90
	26	11.4	8.7	0.76	14.2	9.30	0.65	4.60	3.00	0.65	5.30	3.70	0.70	4.70	4.30	0.91	4.56	4.90	1.07
August	9	12.7	9.3	0.73	11.5	10.0	0.87	5.45	2.75	0.50	4.05	3.10	0.58	4.80	4.72	0.98	3.46	3.70	1.07
	29	8.8	8.0	0.91	9.5	8.2	0.86	2.40	1.56	0.65	2.45	2.25	0.92	3.28	2.30	0.70	3.30	3.16	0.96
September	12	10.4	9.1	0.88	8.9	10.4	1.17	1.75	0.75	0.43	1.25	0.65	0.52	1.50	0.76	0.51	1.00	0.60	0.60
	28	9.2	7.8	0.85	10.5	8.6	0.82	2.50	1.70	0.68	1.70	1.90	1.12	3.30	2.50	0.76	2.54	3.00	1.18

Table 2. Honeybee midgut trypsin activities and chymotrypsin/trypsin ratios. Each sample is an average for midguts of 5 to 10 workers. H = house (younger) bees; F = forager (older) bees

Date (1979)		Trypsin (units/midgut) Hive No. 1			Hive No. 2			Trypsin (units/mg midgut protein) Hive No. 1			Hive No. 2			Chymotrypsin/trypsin Hive No. 1		Hive No. 2	
		H	F	F/H	H	F	F/H	H	F	F/H	H	F	F/H	H	F	H	F
June	5	1.15	0.20	0.17	1.22	0.23	0.19	1.60	0.60	0.38	1.50	0.37	0.25	1.63	8.50	3.03	6.52
	25	0.60	0.24	0.40	1.40	0.25	0.18	0.75	0.40	0.53	1.30	0.40	0.31	3.75	5.58	3.21	6.40
July	11	0.95	0.22	0.23	1.05	0.60	0.57	1.33	0.45	0.34	1.90	0.85	0.45	3.37	9.09	2.14	4.92
	26	1.69	0.40	0.24	1.50	0.45	0.30	1.75	0.54	0.31	1.15	0.60	0.52	2.72	7.50	3.53	8.22
August	9	1.70	0.25	0.15	1.50	0.67	0.45	1.71	0.32	0.19	1.55	0.80	0.52	3.21	11.00	2.70	4.63
	29	0.65	0.70	1.08	0.85	0.51	0.60	0.85	1.05	1.24	1.15	0.75	0.65	3.69	2.23	2.88	4.41
September	12	0.53	0.16	0.30	0.75	0.26	0.35	0.50	0.32	0.64	0.65	0.53	0.82	3.30	4.69	1.67	2.50
	28	0.65	0.19	0.29	0.46	0.25	0.54	0.88	0.28	0.32	0.55	0.40	0.73	3.85	8.95	3.70	7.60

and isolated, weighed, and then transferred to a small test tube containing 0.001 N HCl. The midguts were macerated in the acid with a glass rod, allowed to leach for 15 min at 37 °C, and then centrifuged for 5 min at room temperature at 2000×g. The supernate was used for chymotrypsin, trypsin, and protein assays as described elsewhere[5].

Results and discussion. The results are given in tables 1 and 2. Midgut weights of house bees are larger than those of forager bees in 15 of the 16 samples, reflecting the total body weight difference reported by DeGroot[4]. Both chymotrypsin and trypsin are typically more abundant in house than in forager bees, whether expressed as units per midgut or as units per mg midgut protein. However, the ratio of chymotrypsin to trypsin is greater for forager than for house bees in 15 of the 16 samples, with decreased trypsin activity being the primary component of the increased ratio. The range of increase in the 15 ratios is from 1.42 to 5.21 ($\bar{X}=2.32\pm0.97$) times greater. The data for chymotrypsin show an apparent seasonal pattern, with lowest activity values recorded on September 12; no similar seasonal pattern is apparent in the trypsin data.

Chymotrypsin is the most abundant protease in both honeybee worker midguts and in 14 surveyed pollens[1]. In our earlier study[1] we presented correlative evidence linking levels of chymotrypsin activity in honeybee midguts with the presence of that enzymic activity in pollen; no correlation could be similarly made for trypsin. The present data are similar to the earlier study in that they show an apparent seasonal pattern in chymotrypsin activity but not in trypsin activity. The consistency with which the age correlated decrease in trypsin activity is greater than that for chymotrypsin activity in these studies is thus noteworthy. An age correlated decrease in chymotrypsin activity in honeybee worker midguts could be due, at least in part, to a decrease in pollen consumption. The consistency of the greater decrease in trypsin activity is less apparently attributable to decreased pollen consumption. Question has existed as to whether proteases for digestion of pollen in honeybee midguts are endogenous, derived from pollen, or microfloral in origin[6]. That question remains unresolved.

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