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Short Communications

Behavioral Status and Detoxifying Enzyme Activity Are Related in Worker Honey Bees

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Honey bee workers perform different tasks as they age, a process called temporal polyethism. Bees typically spend the first 3 weeks of adulthood engaged in tasks within the hive, including cell cleaning, brood and queen care ("nursing"), and food storage, and forage outside the nest during the last 2 to 3 weeks of life (reviewed by Winston, 1987). Parallel changes also occur in worker bee physiology, and age-related changes in physiological characteristics have been well documented (Allan *et al.*, 1987; Boch and Shearer, 1963; Fluri *et al.*, 1982; Harrison, 1986; Moritz and Crailsheim, 1987; Robinson, 1985).

Smirle and Winston (1988) reported that detoxifying enzyme activity varies with age in worker honey bees. Older bees, presumably foragers, exhibited elevated specific activity of glutathione *S*-transferases and mixed-function oxidases relative to younger workers that were probably engaged in tasks within the hive. These enzyme systems are known to be involved in pesticide resistance (Dauterman and Hodgson, 1978; Yu *et al.*, 1984); Smirle and Winston suggested that elevated enzyme activity in older workers may be a biochemical adaptation for foraging in the presence of environmental toxins such as pesticides and plant allelochemicals. However, it is unclear whether the observed increases in enzyme activity were related to foraging activities or to developmental processes that are independent of behavioral maturation.

Here we report an experiment designed to determine whether changes in

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glutathione *S*-transferase activity are dependent upon worker age or worker behavior. We subjected a colony of honey bees to conditions that affect temporal polyethism, including the ages at which nursing and foraging occur. An association between worker occupation and glutathione *S*-transferase activity, regardless of age, would support the hypothesis of biochemical specialization for foraging. An association between worker age and enzyme activity independent of behavioral status would indicate that increased enzyme activity is not related to foraging.

A colony of honey bees was established in an apiary at The Ohio State University with 2000 1-day-old workers, a queen, one comb containing unsealed brood, one comb of honey and pollen, and one empty comb. Bees were obtained from combs of sealed brood taken from one colony and placed in a 33°C incubator. Each bee was marked on the thorax with a paint dot to ensure that all workers sampled were residents of the experimental colony.

As expected (Ribbands, 1952), division of labor occurred in the experimental colony within a few days despite the abnormal age structure. Some young bees displayed typical nursing behavior, while other young workers foraged prematurely. New bees were prevented from emerging by the removal of all combs containing developing pupae; a few weeks later there were unusually old nurse bees and foragers of normal ages. Nurse bees and foragers were identified according to established criteria (Robinson, 1987); 50 nurses and 50 foragers were collected when they were 7, 14, and 21 days old and stored at −70°C.

Samples obtained in this fashion were shipped on dry ice to Simon Fraser University, Burnaby, B.C., and stored at −70°C until assayed for enzyme activity and protein content. All bees were frozen for the same amount of time (60 days) to ensure that sample activity was not affected by variable lengths of freezing. Groups of 10 midguts were dissected, gut contents were removed, and tissue was washed in 0.15 *M* KCl. Tissue samples were homogenized in 0.15 *M* potassium phosphate buffer, pH 6.5, centrifuged at 12,000*g* for 15 min, and filtered through glass wool. The resulting postmitochondrial supernatant was kept on ice and used immediately for enzyme assays and protein determinations.

Glutathione *S*-transferase activity was assayed with 1-chloro-2,4-dinitrobenzene as the substrate (Yu, 1984). Reactions were continued for 5 min at 22°C, and enzyme preparations were diluted to ensure a linear increase in product formation. Protein content was assayed (Bradford, 1976) using bovine serum albumin as the standard. Four or five replicates of 10 midguts were assayed for each test group. The effect of age on enzyme activity and protein content was assessed with one-way analysis of variance for both foragers and nurses. Differences between behavioral groups at each age were analyzed with *t*-tests.

There was a significant ($P < 0.001$) decrease in protein content in both nurses and foragers between 7 and 21 days of age (Fig. 1A). Glutathione *S*-transferase activity did not change with age in nurse bees ($P > 0.05$). In con-

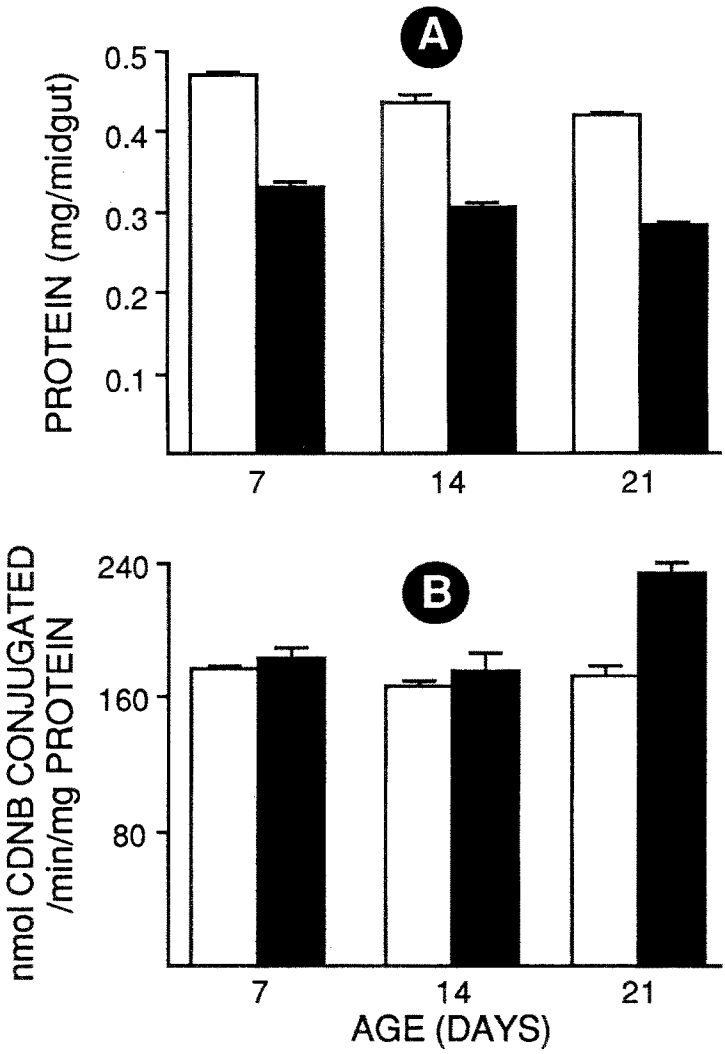

Fig. 1. Effect of age and behavioral status on (A) midgut postmitochondrial protein content and (B) glutathione *S*-transferase activity in adult worker honey bees. Mean ± SE of four or five replicates; 10 bees/replicate. Open bars represent nurse bees; filled bars, foragers.

trast, there was a significant ($P < 0.01$) age effect on glutathione *S*-transferase activity in foragers, with 21-day-old bees showing the highest specific activity (Fig. 1B). Comparisons between nurses and foragers revealed highly significant ($P < 0.001$) differences in protein content at all three ages (Fig. 1A). There was no difference in enzyme activity between nurses and foragers aged 7 and

14 days. At 21 days foraging workers had significantly higher enzyme activity than did nurse bees ($P < 0.001$) (Fig. 1B).

Freezing and/or shipping resulted in an approximately 50% decrease in glutathione *S*-transferase activity from values reported for unfrozen, fresh workers (Smirle and Winston, 1988; Yu *et al.*, 1984). However, as each sample was handled identically, the reliability of this comparative analysis is assured.

Although there was an age-related decrease in general midgut protein in nurse bees, the dramatic differences between foragers and nurse bees at all ages supports the idea that protein loss is associated with flight activity (Harrison, 1986; Smirle and Winston, 1988). Increases in enzyme activity are not a direct consequence of the loss of midgut protein because there were significant differences in protein associated with age in both nurses and foragers but an accompanying increase in enzyme activity only for 21-day-old foragers.

Behavioral status is correlated with glutathione *S*-transferase activity in worker honey bees. Enzyme activity in nurse bees did not increase despite increasing worker age. In contrast, enzyme activity was elevated in foragers but only at 21 days of age. These results suggest that changes in the activity of detoxifying enzymes may be influenced by both age and behavior. Changes may occur only after a certain age, under the stimulus of field-related duties. Alternatively, there may be a quantitative relationship between foraging behavior and enzyme activity; a certain amount of foraging may be necessary to induce changes in enzyme activity. This may be because of direct effects of foraging behavior on worker bee physiology or a consequence of enzyme induction due to exposure to environmental contaminants. Our failure to detect changes in glutathione *S*-transferase activity in 7- and 14-day-old foragers may thus be a consequence of sampling bees that began foraging recently, which is likely because there were few individuals observed foraging from this small colony on any given day.

If increases in detoxifying activity are due solely to enzyme induction, the reported differences between foragers and nurses may be a consequence of differential exposure to toxicants rather than biochemical specialization for foraging. Measurements of enzyme activity in bees that have foraged for specific amounts of time, and comparative analyses of the enzyme induction capabilities of nurse bees vs foragers, are needed to elucidate further the relationship between foraging behavior and detoxifying enzymes.

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