Quantitative changes in lipids and carbohydrates of temporal workers and drones in *Apis cerana indica* (Fabricius)

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Abstract

Lipids and sugars from temporal castes of *Apis cerana indica* (Fabricius) were analysed qualitatively and quantitatively. Cholesterol, free fatty acids, triglycerides, methyl esters and cholesterol esters were higher in nurse and food storer but decreased in middle-aged and forager bees. The haemolymph, glucose and fructose showed significant increase in bees performing extranidal tasks while trehalose level was found to be high only in intranidal tasks. Glycogen content from thorax and abdomen was more in nurse and food storer workers but substantially decreased in guards and foragers.

Keywords: *Apis cerana indica*, lipids, sugars, worker castes, drones.

Introduction

Honeybee workers exhibit temporal division of labour. With age they shift their task from cell cleaning, comb building, food processing to outside tasks like guarding and foraging (Winston, 1987 and Johnson, 2008). This age related temporal division of labour within the worker caste in honeybees are elicited by physiological changes (Robinson, 2009). The onset of foraging for instance in honeybees are associated with decrease in abdominal lipids (Toth and Robinson, 2005), and increase in thoracic glycogen store (Harison, 1986). In *Apis mellifera*, foragers have been found to contain fewer lipids than the nurse bees (Toth and Robinson, 2005), further foragers reverting to nursing are not able to perform their task efficiently as nurse bees due to their low level of lipids. These studies showed that differences in lipids are closely related to switching of tasks in bees (Toth and Robinson, 2005).

Level of sugars in the form of trehalose, glucose and fructose in the honeybee *Apis mellifera* have been reported to vary widely (Bounias and Morgan, 1984; Bozic and Woodring, 1997; Abou-Seif *et al.,* 1993; Fell, 1990 and Leta *et al.,* 1996). This variability in haemolymph sugar composition is influenced by many factors including differences in diet (Crailsheim, 1988; Abou-Seif *et al.,* 1993 and Woodring *et al.,* 1993), metabolic rate (Moffatt, 2000 and Blatt and Roces, 2001), physiological changes and environmental conditions (Kunert and Crailsheim, 1988).

Most of these studies are based on Western honeybee, *Apis mellifera* (Winston, 1987; Seeley, 1995; Robinson, 1992 and Robinson *et al.,* 1994). However studies in the Asian bee, *Apis cerana indica* (Fabricius) is lacking, therefore the present study was carried out to determine the composition and changes in the profile of various sugars and lipids among age related temporal worker castes in the bee *Apis cerana indica*.

Materials and Methods

Colony of *Apis cerana indica* was reared in a wooden hive box. Task specific bees (n=20 bees per pool) were collected from the colony and immediately cold immobilized.

Lipid extraction and analysis: Wings and legs were removed from the thorax of each bee. Task specific bees were pooled and homogenized in a glass tissue grinder by adding chloroform/methanol/water for extraction of total lipids following the protocol of Bligh and Dyer (1959).

Separation of Lipids by Thin-layer chromatography (TLC) analysis: Various lipid classes of the lipid extract were separated by TLC on 20 x 20 cm glass plates coated with silica gel GF of thickness 0.25 mm. Plates were activated by heating in an oven at 110°C followed by cooling. Plates were run with petroleum ether (b.p. 60-70°C)/diethyl ether/acetic acid (90:10:1; v/v/v) as mobile phase according to the procedure of Mangold (1961 and 1964) and Malins and Mangold (1960). The TLC chamber was equilibrated with the mobile phase for 15 minutes before introducing the spotted plate. After running, the plates were dried with a stream of air and were visualized in iodine chamber. Bands were identified by comparing their relative mobility to authentic standards. For each detected band, the gel was scraped off into 20 ml test tubes and used for quantitative estimation of lipids.

Neutral lipids were quantified spectrophotometrically using the method of Amenta (1964). Phospholipids were determined according to the procedure of Raheja *et al.* (1973). Standards were purchased from Sigma, USA and were 99% pure. All solvents used were of HPLC grade. Data was expressed as mg of lipids/bee.

Sugar analysis: Haemolymph from the above mentioned task groups were collected by puncturing the dorsal neck membrane with microcapillary tube after ventrally deflecting the head. The collected haemolymph was immediately expelled into chilled eppendorf tube containing 4.5 ml of 80% ethanol to precipitate proteins. The sample was centrifuged at 1200 rpm for 10 minutes. The supernatant obtained was used for qualitative and quantitative analysis of sugars.

Separation and detection of sugars by TLC: Sugar standards were purchased from Merck (Darmstadt, Germany) and dissolved in 80% ethanol. TLC was performed on 5 x 20 cm glass plates coated with silica gel GF of thickness 0.25 mm. Activation and equilibration of plates were done as mentioned above. Standards and samples were applied on the TLC plates and were run to a distance of 15 cm with dimethyl formamide / n-butanol / water (3:12:2) as the mobile phase. Plates were then dried with a stream of air. Sugars were visualized in iodine chamber. Spots on plate were identified with the reference sugars run parallel.

Total soluble sugar, trehalose and fructose were determined according to the method of Arslan *et al.* (1986).

For determination of glucose, 30µl of extracted haemolymph were mixed with equal volume of water and added to 540 µl of 3% Trichloroacetic acid and analyzed by O-toluidine following the protocol of Dubowski (1962). Data was expressed as µg of sugars/µl of haemolymph.

Quantification of Glycogen in thorax and abdomen of workers and drones: Four groups of workers based on specific tasks (nurse, food storer, guard, and forager bees) and drones of unknown-age were used for analysis of glycogen separately for thorax and abdomen. Nurse bees were obtained with their head inserted in larvae cells, food storers from honey comb, guards from the entrance of the hive and foragers from the entrance of the hive during emergence. Bees were frozen on ice and their digestive

tracts were discarded. The samples were processed following the protocol of Roe and Dailey (1966) for glycogen content. Each group of sample contains 20 individuals and five such replicates were taken. One-way ANOVA was performed at 0.05 significant levels. Data was expressed as µg of glycogen/bee.

Results

Lipid analysis: Lipids detected were phospholipids, cholesterol, free fatty acids, triglycerides, methyl esters and cholesterol esters from adult worker and drones (Table 1). Nurse, middle aged, foragers of worker bees revealed the presence of the same lipid classes, but they were present in different proportions, cholesterol esters were however not detected in the food storer bees. Drones also showed similar classes of lipids.

Nurse and food storers were found to have higher lipid content than middle-aged and forager bees. Large differences in triglycerides were found among all the worker task groups, with nurse predominantly containing the highest proportion, consisting of 43% of the total. Forager bees had intermediate triglycerides comprising 21% higher than the food storer with 18% and middle-aged with 7% (Fig. 1). The level of cholesterol in nurse and food storer bees was two times greater than middle-aged and forager workers.

Free fatty acids in food storer were four times greater than forager bees and methyl esters remained more or less constant in the entire task groups.

Phospholipids were found to be the most dominant lipids in drones (Fig. 2), it comprised approximately 50% of the total, in case of the food storer it was 45% of the total, the nurse, middle-aged and forager bees contained 26%, 13% and 16% of the total respectively. Cholesterol esters were found to be the next most predominant lipids in drones comprising of 21% of the

total, in nurse and middle-aged bees it was 24% of the total. Other dominant lipids were triglycerides.

There were significant differences in cholesterol, free fatty acids, triglycerides, cholesterol esters and phospholipids in task based division of labour in worker bees with that of the drones at 0.05 significant level. However, there were no significant differences in methyl esters.

Sugar analysis: TLC analysis of haemolymph sugars reveals that trehalose, fructose and glucose were present in all the task groups (Table 2). However, the difference in concentration of these haemolymph carbohydrates among different task groups was significant (trehalose: F = 34.51, P < 0.0001; fructose: F = 286.11, P < 0.0001, glucose: F = 31.54, P < 0.0001; df = 3, 15). The descending order of sugar concentrations among task groups was found to be fructose > trehalose > glucose (Fig. 3). Total soluble sugars from different task groups also varied significantly ($F_{3, 15}$ = 40.27, P < 0.0001). Nurse bees had least amount of total soluble sugars (7.39±0.87 µg/µl) while it was significantly high in food storer (28.93±4.51 µg/µl), middle-aged (42.59± 3.11 µg/µl) and forager bees (73.87±3.00 µg/µl) (Fig. 3). The major sugar in drone was fructose (136.19±6.24 µg/µl) followed by trehalose (67.23±1.38 µg/µl). The least amount was of glucose containing 8.93±0.75 µg/µl (Fig. 4).

One-way ANOVA revealed significant changes in glycogen content in thorax (T) ($F_{3, 19}$ = 132.04, P < 0.0001) and abdomen (Ab) ($F_{3, 19}$ = 83.21, P < 0.0001) of workers performing different tasks. Glycogen concentration in the T range from 3.97±0.29 - 17.52±0.42 µg/T whereas in Ab it varies from 1.26±0.03 - 5.44±0.36 µg/Ab. Nurse bees had significantly higher level of glycogen in both T (17.52±0.42 µg/T) and Ab (5.44±0.36 µg/Ab). The level of glycogen in both T and Ab significantly

declines in food storer (T = 14.75±1.11 µg/T; Ab = 2.73±0.05 µg/Ab), guard (T = 4.14±0.24 µg/T; Ab = 1.26±0.03 µg/Ab) and forager bees (T = 3.97±0.29 µg/T; Ab = 1.51±0.09 µg/Ab) (Fig. 5a, b), with no significant difference in the latter two groups. Like workers, drones also revealed maximum amount of glycogen content in T (12.24±0.15 µg/T) and lesser amount in Ab (3.71±0.15 µg/Ab) (Fig. 6).

Discussion

The present study reveals the changes in lipids and carbohydrates among workers of specific task groups and drones of *Apis cerana indica*. Among lipids, levels of free fatty acids were found highest in the food storer bees, consistent with the findings of Toth *et al*. (2005) and Crailsheim *et al*. (1992), that nurse and foragers of *Apis mellifera* have higher and lower lipid quantity, respectively. Further, forager bees were even found to have a lower amount of free fatty acids than nurse. The highest levels of free fatty acids were found in the food storer bees; which may be due to the maximum proteolytic activity at this stage (Mortiz and Crailsheim, 1987 and Szolderits and Crailsheim, 1993).

Triglycerides were higher in the foragers; this elevation in the level of triglycerides in the foragers may be due to the effect of temperature and humidity that bees experience outside the hive while foraging. Sapcaliu *et al.* (2010) suggested that when bees are exposed to high temperature and low humidity, the level of triglycerides increases two-fold. The relatively higher amount of triglycerides in outside workers is understandable, as it is the source of energy reserve and probably supports the metabolic effort during flight to some extent. Phospholipids as a whole were the most dominant lipids.

Cholesterol was significantly high in the nurse and food storer bees, however, in the middle-aged and forager bees cholesterol dropped significantly. The decrease of cholesterol in forager could be due to a reduced hypopharyngeal gland. Ritter (1990) also pointed out that differences in diet may cause variation in cholesterol levels.

During the first week of an adult worker bee's life, consumption of pollen, rich in fats and proteins is extremely high and gradually as they become older they shift to carbohydrate rich diet for energy and metabolic processes (Crailsheim *et al.,* 1992). This alteration of diet intake could potentially influence the behavioural and physiological shifts that accompany worker honeybee's behavioural development. For instance, decrease in lipid content in adult honeybee might lead to the onset of foraging (Toth *et al.,* 2005). Depletion of nutrition has also been shown to result in increase of Juvenile Hormone (Kaatz *et al.,* 1994 and Pinto *et al.,* 2000) which in turn controls foraging (Bloch *et al.,* 2002).

The changes in the profile of sugars among various temporal worker castes showed marked differences; there is an increase in the level of glucose and fructose and decrease of trehalose in forager and other task groups. The increase in concentration of glucose and fructose and concomitant decrease of trehalose level in foragers may be due to metabolic rate, as bees involved in foraging activities have high metabolic rate than intranidal task workers. Foragers actively move in and out of the hive during their excursion for collection of nectar and pollen; this requires a constant supply of sugars in the form of trehalose, fructose and glucose in the flight muscles. As trehalose cannot be produced as fast as it is consumed and since it is obtained from conversion of fructose to trehalose via first conversion to glucose (Candy *et al.,* 1997); therefore, a lowered level of trehalose in the haemolymph of foragers may be attributed to the rapid mobilization of sugars from the

haemolymph to the flight muscles. Due to lower metabolic rate, intranidal task workers are able to maintain high level of trehalose. This result is also congruent with the finding of earlier works on *Apis mellifera* (Abou-Seif *et al*., 1993; Bozic and Woodring, 1997 and Blatt and Roces, 2001) and substantiated by the higher level of triglycerides found in the haemolymph. The changes in glycogen content are also consistent with *Apis mellifera carnica* (Panzenbock and Crailsheim, 1997) where young workers have more glycogen content than aged workers like the guards and foragers.

Table 1: TLC of lipid extract from whole adult honeybee workers (n=20), six replicates and standard lipids. Lipid extracts and standards were loaded on silica gel GF plates and were developed in petroleum ether/diethyl ether/acetic acid (90:10:1; v/v/v) according to the procedure of Mangold and Malins (1960) and Mangold (1961 and 1964). The plates were visualized in iodine chamber.

Lipid classes	Standard (R_f)	Sample (R_f)
Cholesterol	0.23±0.04	0.22
Free fatty acids	0.33±0.02	0.33
Triglycerides	0.50±0.02	0.49
Methyl esters	0.86±0.04	0.83
Cholesterol esters	0.93±0.01	0.92

Table 2: TLC of sugars of adult honeybee workers hemolymph on silica gel GF and their R_f. Plate was developed with dimethyl formamide/*n*-butanol/water (3:12:2; v/v/v) mobile phase.

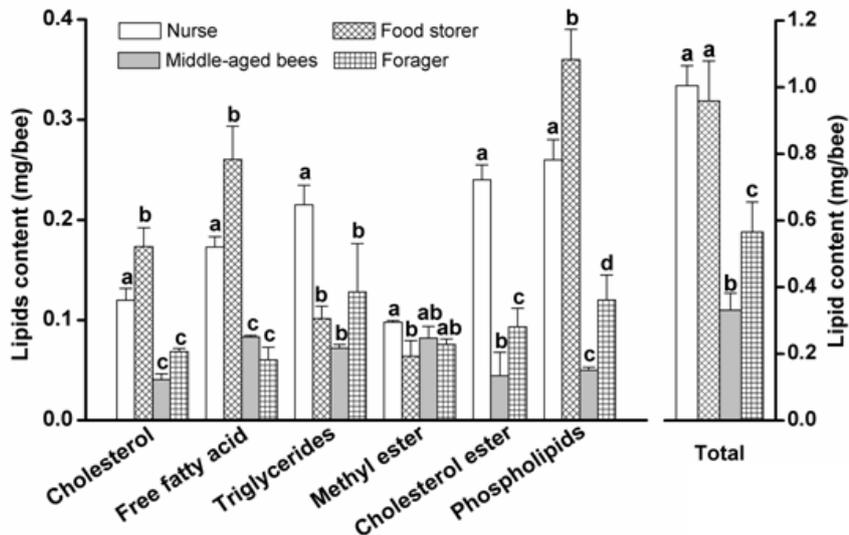| Sugars	Standard (R_f)	Sample (R_f)
Trehalose	0.34	0.35
Fructose	0.72	0.73
Glucose	0.75	0.76

Fig. 1: Lipid class composition of total homogenates from different task groups; values are mean of six determinations ± SE; different letters on the error bars indicate significant differences, same letters indicate no significant differences.
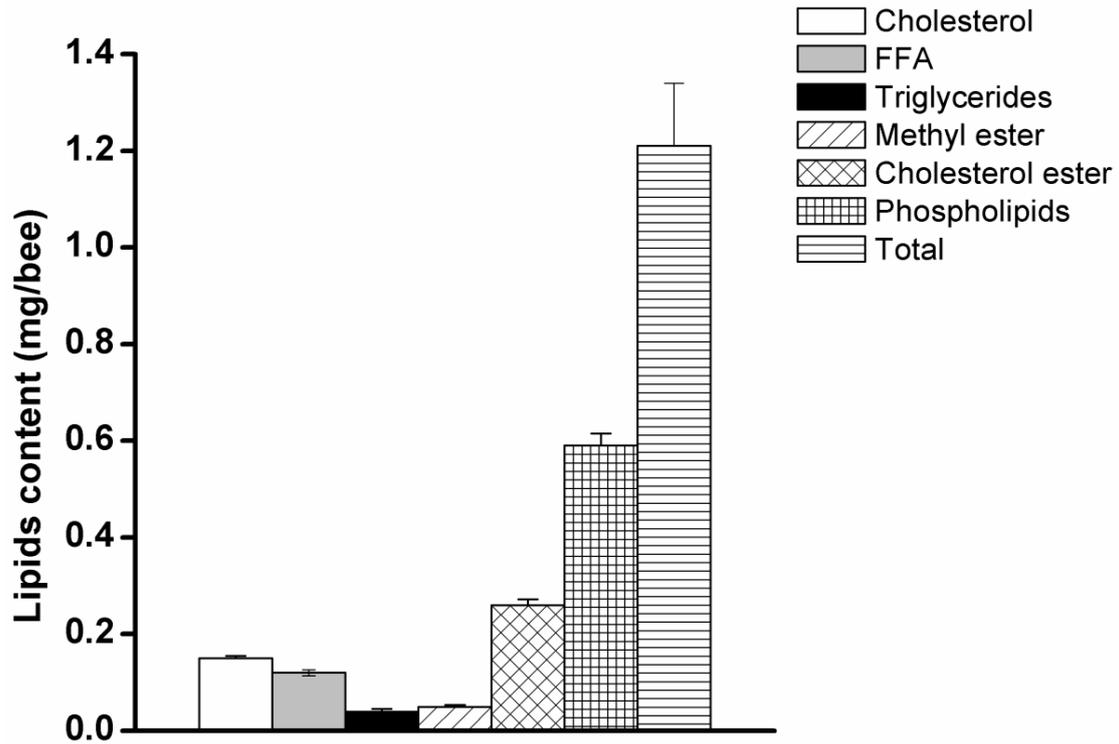
Fig. 2: Lipid class composition of total homogenates from drone; values are averages of six determinations ± SE (FFA = free fatty acids).
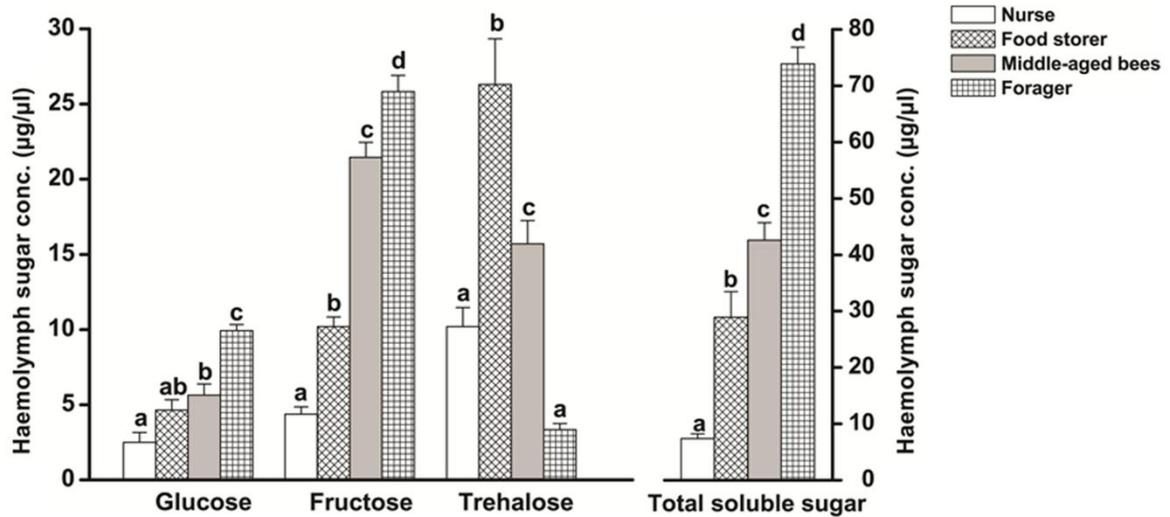

Fig. 3: Differences of sugar concentrations in different task groups of worker bee haemolymph; values are presented as mean ± SE.
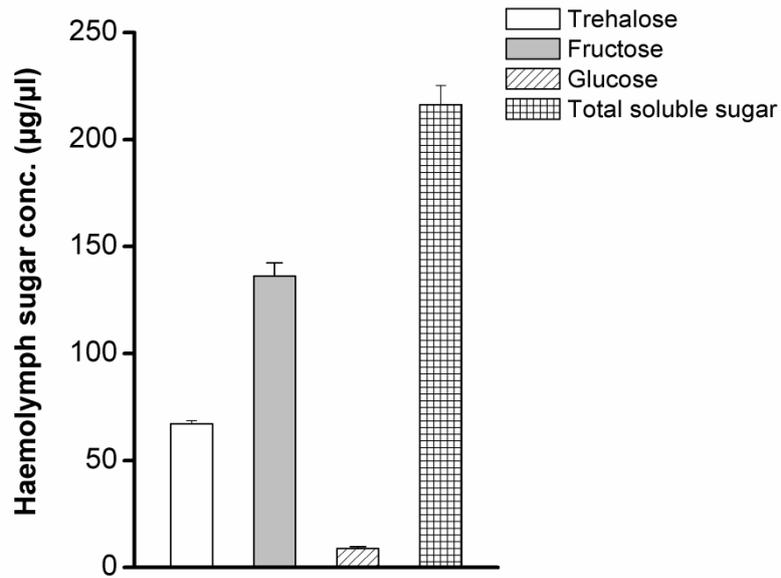
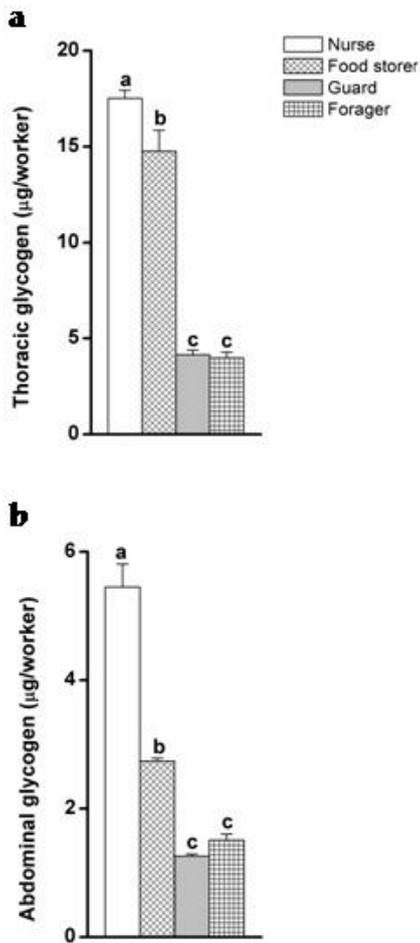**Fig. 4: Differences of sugar concentrations in drone haemolymph; values are presented as mean ± SE.**

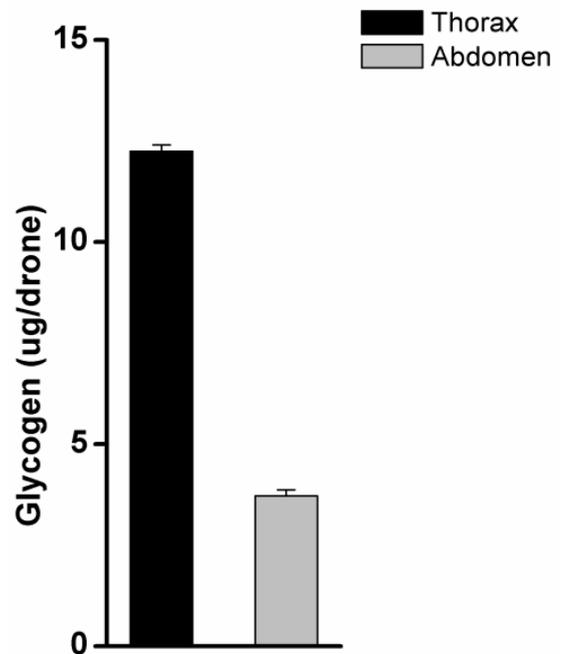**Fig. 5: Glycogen in a) thorax and b) abdomen of different task groups of workers.**

Fig. 6: Glycogen in thorax and abdomen of drones.

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