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TITLE PAGE

“Effect of Honeybee Race and Worker Age on Development and Histological Structure of Hypopharyngeal Glands of Honeybee”

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Summary

Development of hypopharyngeal glands (HPG) of native honey bee workers (*A. m. jemenitica*) and Carniolan hybrid bees reared under normal constipations was studied. The HPG development degree and acini surface were influenced with the age. The development degrees increased gradually. The maximum developmental degree was recorded at 9-days old in both two races. The development decreased after 12th days. The HPG development degree and acini surface of all ages in native bees were significantly less than Carniolan hybrid bees.

Histological studies on HPG showed that there were some differences between the glands of the two races at the maximum developmental stage (9-days). The staining of cell cytoplasm by Haematoxylin and Eosin was similar. However secretory cells numbers were more in Carniolan hybrid than the native one.

Introduction

Hypopharyngeal glands (HPG) of honeybee work produce proteinic secretion to feed larvae and queens (Crailsheim, 1991, 1992). There are many factors affecting physiological status of honeybee as race of bees, worker age, colony conditions and the time of the year. The HPG are well developed when honeybee

workers are in the nursing period. They begin to degenerate when bees start foraging. Lass and Crailsheim (1996) reported that young caged bees had smaller hypopharyngeal glands. Pollen consumption is positively correlated with gland development (Hrassnigg and Crailsheim, 1998). Yousif-Khalil (1983) clarified that the hypopharyngeal gland was the first organ that was affected by starvation, which caused a high degeneration represented by the disappearance of secretory granules, vacuoles and the much reduced size of gland acini and cells.

Al-Ghamdi, (2006) provided that there were clear differences in HPG measurements among four honeybee races. The Italian, Carniolan honeybee glands had larger acini surface than Yemeni honeybee *Apis mellifera jemenatica*.

Takenaka (1988) studied the rate of protein synthesis in hypopharyngeal glands while workers are nurse bees, and especially between 10 and 14 days old is maximal, often 14th day, protein synthesis decreases and HPG contains only small amount of protein. Also, Knecht and Kaatz (1990) reported that high rates of protein synthesis were measured in nurse bees and low protein production was recorded in foragers, secretion reservoirs are formed within the intracellular ductules.

The aim of the present work was to study the differences of morphometrical and histological structure of HPG of native and Carniolan hybrid bees and follow up the variations of gland structure during nursing period of honeybee workers.

Materials and Methods

The experimental work was carried out in Bee Research Unit apiary, College of Food Science and Agriculture, laboratory of histochemistry Faculty of Medicine, King Saud University and laboratories of Biology Department, Science College, Princess Nora Bint Abdul Rahman University. Riyadh, Saudi Arabia.

Local honey bee race *Apis mellifera jemenatica* and Carniolan hybrid bees were used in the present study to investigate the morphometrical characters and the histochemical structure of HPG in two honeybee races under normal feeding condition.

Newly emerged honeybee workers (0-24 hours) from every race were obtained each from one colony to prevent any genetic variation as possible. Four local honeybee colonies equal in strength (5 from covered with bees) with sufficient bee bread areas were selected and located in Langstroth hives. One hundred newly emerged honeybee workers were marked and introduced in every colony. Also, four Carniolan hybrid honeybee colonies at the same strength were selected and also, one hundred newly emerged honeybees were marked and introduced in every colony.

Ten honey bee workers from every colony were selected at 3, 6, 9, and 12 days old to determine the development of HPG. The HPG were dissected and put on glass slid into a droplet of ice-cold sodium chloride solution 0.85% (Isotonic to the hemolymph).

An arbitrary scale (I to IV) according to Maurizio (1954) was used to determine a development degree (grade I represented undeveloped gland and grade IV represented complete development). The diameters of five right glands were measured under binocular in mm (maximum length and width of 5 acini for each gland). Gland acinal surface was calculated according to Maurizio's formula

(1954).

$$\text{Acinal surface} = \pi \times \frac{a \times b}{2}$$

Where a=maximum length

b=maximum width

$\pi = 3.14$

One sample (five workers) from every colony was selected at 3, 6, 9 and 12 days-old to study the variation of histological and histochemical structure of HPG in both two races.

In order to obtain sections in wax, the further processes as described by Hussein et al. (1990) were used. The samples were fixed in 10% formalin for 24h and dehydrate in 70%, 90% alcohol for 1h. The material was cleared in histoclear for 2h. All previous processes were carried out by using rotary tissue processor (Leica TP 1020), then were impregnated by placing the samples in 1:1 histoclear: wax for 1h in the incubator at 60°C followed by three changes in pure paraffin wax for 45 min, 1h and 3h, respectively at 60°C. The tissue was embedding consol system (Leica EG 1150 H). The blocks were cut using automatic rotary microtome (Leica RM 2255).

For studying general structure, the hematoxylin and Eosin stains were prepared and used as described by Drury & Wallington (1980) and Bancroft Gamble (2002).

For differentiating the developmental stages of HPG of honeybee workers in sections, the staining of cell cytoplasm degree by hematoxylin and Eosin was used. The size and form of acini were recorded. The distribution of globules, number of nuclei and nucleolus were used as an indicator for gland activity.

Statistical Analysis was conducted using SAS general linear models procedure. Differences among means were determined by L.S.D at $P < 0.05$ (SAS Institute, 1990).

Result and Discussion

The differences of hypopharyngeal gland (HPG) development degree in honeybee workers reared under normal conditions in native bees *A. m. jemenitica* and Carniolan hybrid bees were studied. Data represented in Table (1) indicated that development of HPG influenced with the age increasing. The HPG development degree increased gradually from honeybee workers emergence and started to decrease after 12 days old. The maximum development degrees (3.375 and 3.925) were recorded at 9-days old when newly emerged bees were fed on sufficient bee bread area in native and Carniolan hybrid bees, respectively. At all bee

ages under study, The HPG development degrees of native bees were significantly less than Carniolan hybrid bees.

Means of acinal surface of HPG of the two honeybee races are represented in Table (2). The surface increased gradually with age and reached to the maximum at 9-days old. (0.2381 and 0.2611 μm^2) in native and Carniolan hybrid bees, respectively. In general, acinal surface was larger in imported bees than the native one.

Table 1. Means of hypopharyngeal glands development degree during nursing period of native Carniolan hybrid honeybee workers

Race	Means of hypopharyngeal glands development degree				
	3-days old	6-days old	9-days old	12-days old	Mean
Native bees	2.975	3.375	3.725	3.475	3.388
	±	±	±	±	±
	0.228 e	0.250 d	0.160 b, c	0.255 d	0.351 B
Carniolan hybrid bees	3.638	3.788	3.925	3.675	3.756
	±	±	±	±	±
	0.172 c	0.203 B	0.118 a	0.164 b, c	0.199 A

Means marked with different letters are significantly differ at 0.05 level of probability

Table 2. Means of hypopharyngeal gland acinal surface during nursing period**of native and Carniolan hybrid honeybee workers.**

Race	Means of hypopharyngeal glands acinal surface (μm^2)				
	3-days old	6-days old	9-days old	12-days old	Mean
Native bees	0.1276	0.1602	0.2381	0.1752	0.1753
	±	±	±	±	±
	0.0292	0.0305	0.0365	0.0458	0.0537
	e	D	a ,b	d	B
Carniolan hybrid bees	0.1899	0.2494	0.2611	0.2141	0.2286
	±	±	±	±	±
	0.0686	0.535	0.0504	0.0425	0.0606
	d , c	A	a	d , c	A

Means marked with different letters are significantly differ at 0.05 level of probability

Histological studies were carried out on hypopharyngeal glands of native and Carniolan hybrid honeybee workers during nursing period. Staining degree of cells cytoplasm by Haematoxylin and

Eosin was noted. Cell components and size, distribution of secretory cells, size and form of nuclei were recorded. The results indicated that there were some differences in histological structure of the HPG between the two races at the different development stage.

Fig. (1) show that the HPG acini of native and Carniolan hybrid bees at 3- days old under normal feeding conditions. Secretory cells numbers were more in Carniolan hybrid bees than native one. The nuclei were clearly observed. However, the staining of cytoplasm cells by Haematoxylin and Eosin was similar in glands of both two races.

Fig. (2) show that the acini of HPG at 6-days old become a globular. The

number and size of secretory cells were more in comparison with those of native bees. Every nucleus contained many nucleolus. The staining degree of cytoplasm by Haematoxylin and Eosin was similar in the two races.

Fig. (3) show that the acini of honeybee gland at 9-days old had a globular form. The glandular cells increased in both two honey bee races more than the other workers ages. The cells are completely loaded with cytoplasm where the glandular cells were larger in Carniolan hybrid than native bees. Large numbers of nuclei were clearly observed in both two races. The voluminous cytoplasm is crowded with more vacuoles. The acini of native honeybee glands were more stained by Haematoxylin and Eosin in comparison with the acini of Carniolan hybrid bees.

Fig. (4) represented the histological structure of HPG acini of native and Carniolan hybrid bees at 12-days old. The acini of Carniolan bees become irregular. However, the acinus of native bees changed to pear-form or incompletely circular. The numbers of secretory cells decreased in comparison with that observed at Carniolan hybrid bees. Histological structure reflected the differences between the two type of bees used by beekeeper in Saudi Arabia.

Present results were in agreement with those obtained by Al-Ghamdi (2006).

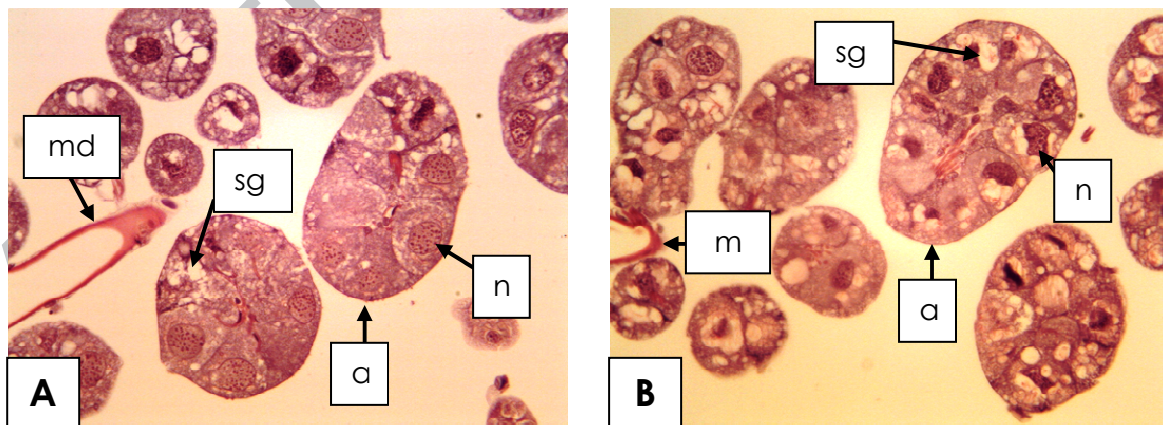


Fig. 1 Histological structure of hypopharyngeal gland acini of honeybee workers at 3-days old.

A. Native bees

B. Carniolan hybrid bees

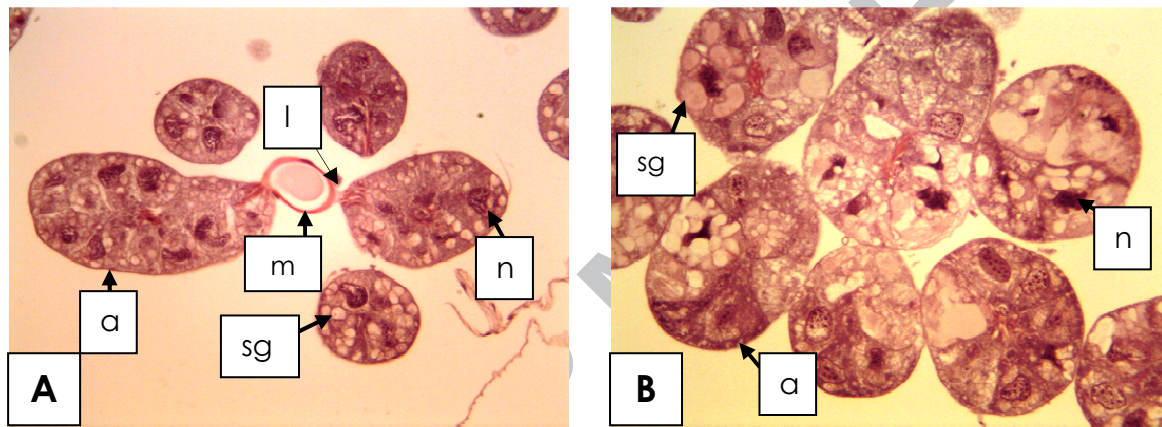


Fig. 2 Histological structure of hypopharyngeal gland acini of honeybee workers at 6-days old.

A. Native bees

B. Carniolan hybrid bees

a: acinus, ca: canaliculus, l:latra , md: main duct, n: Nucleus, sg: secretory globules

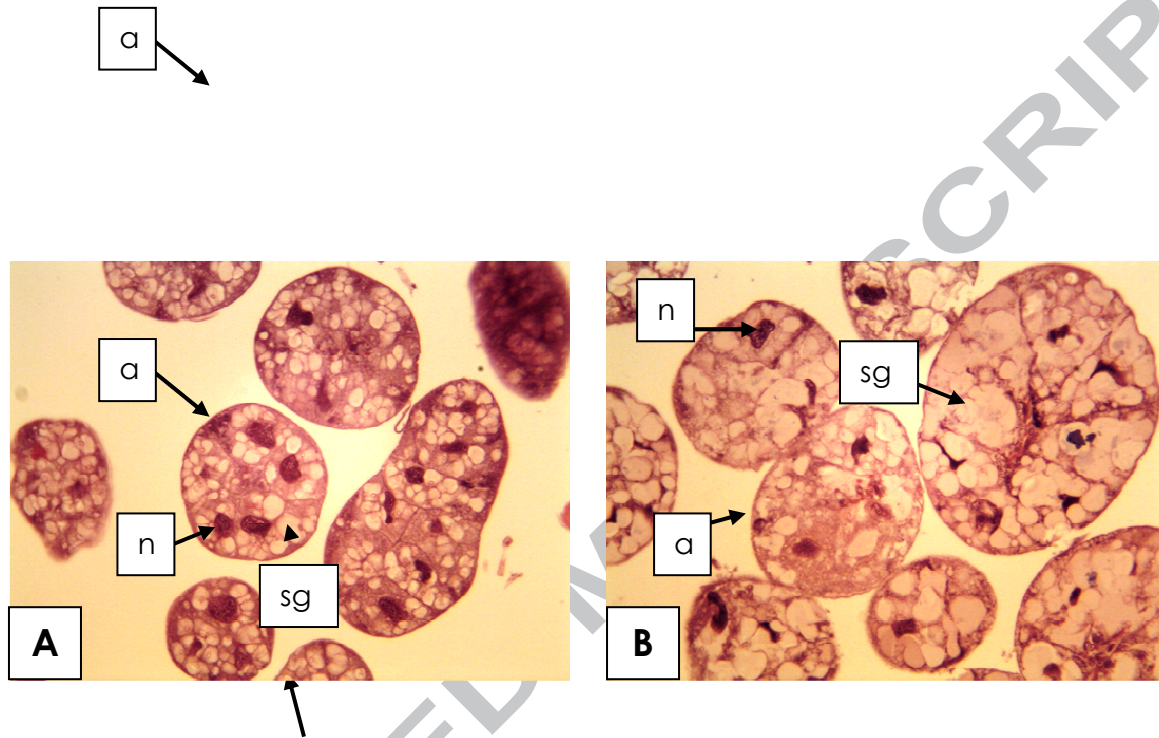


Fig. 3 Histological structure of hypopharyngeal gland acini of honeybee workers at 9-days old.

A. Native bees

B. Carniolan hybrid bees

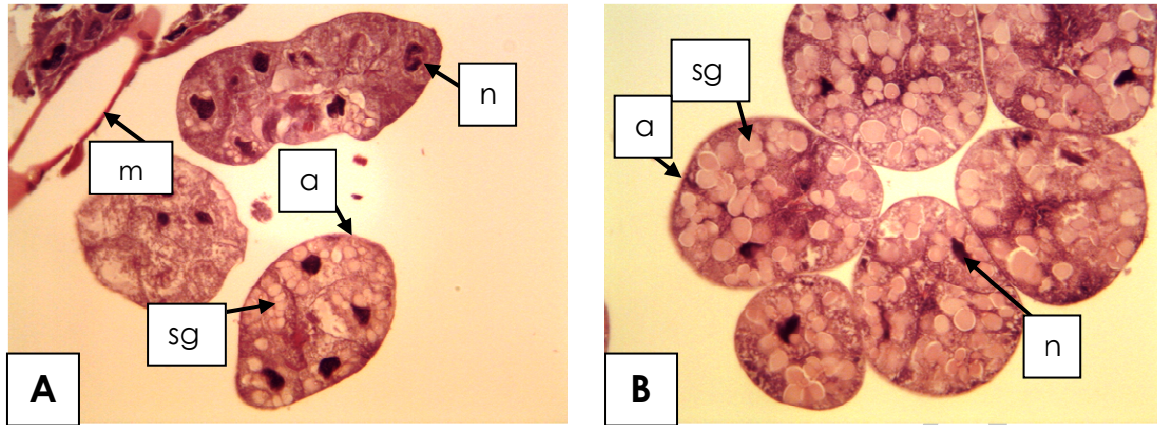


Fig. 4 Histological structure of hypopharyngeal gland acini of honeybee workers at 12-days old

A. Native bees

B. Carniolan hybrid bees

a: acinus, ca: canaliculus, l:latra, md: main duct, n: Nucleus, sg: secretory globules

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