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Full Length Research Paper

Karyotype and C-banding analyses of haploid male chromosomes of *Apis florea* F.

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Chromosomes, with detailed karyotype information (number, shape, total length, relative length, arm ratio and centromeric index) and C-banding patterns in the somatic division of haploid male of *Apis florae* in Iran are described. Samples were obtained from the colonies in south of Iran. Prior to the swarming season, drone-brood cells were added adjoining to the lower rows of the worker-brood cells. Testes from young larvae were removed, fixed in acetic acid methanol (1:3), and stored at -20°C. The slides pretreatment were made by usual air dry method. C-banding and staining was carried out by barium hydroxide and Giemsa solution. Sixteen chromosomes of this species were observed and divided into two groups, 4 metacentric (no.1, 4, 7, 11), 12 sub metacentric and sub telocentric.

Key words: Honeybee, *Apis florea* F., karyotype, C-banding.

INTRODUCTION

Apis florea has long been important for the pollination of crops in south Iran. The distribution area of *A. florae* is generally confined to warm climates. In the west, the species is present in the warmer parts of Oman, Iran and Pakistan, through the Indian sub-continent and Sri Lanka. Like the dwarf bees, *A. florae* and its colonies are characterized by single combs, which are usually suspended from tree branches (Morse, 1970; Seeley; et al., 1982). Less commonly, colonies nest on cliff overhangs or on human-built structures (Ruttner, 1988). It is found as far east as Indonesia, but its primary distribution centre is Southeast Asia. In Iran, currently the *A. florea* colonies are a few and can only be found in some south areas. Identification of the chromosome configuration is a helpful key in genetically resource preserving programs as the heritable substance (except cytoplasmic substance) is organized in nucleus chromosomes area. Since the chromosomes set of each species are unique, the C-banding staining has contributed immensely to understanding of chromosome structure in eukaryotes. Investigation on chromosome set of honeybee is very limited in the classical papers of Hoshiba and Kusangi (1978), and Hoshiba and Okada (1986). Therefore, the karyotype analysis of the *A. florea* has remained unstudied because of the small size of the chromosomes. The distinguish karyological and C-banding analyses of the haploid male of *A. florea* as presented in this paper were characterized by cytotaxonomical methods, including chromosome number, morphology, and C- banding patterns and their relation to the other known karyotypes of *Apis* is discussed.

MATERIALS AND METHODS

Samples collected were from south regions Iran. Prior to the swarming season, drone-brood cells are increasing, adjoining the lower rows of the worker-brood cells. Materials used included testes from drone of the young larvae (3 to 4 in star larvae). The testes were pretreated in hypotonic colchicines (0.4 KCl, 0.01% colchicin) for 30 min at room temperature and fixed in acetic acid methanol (1/3), then stored at about –20°C (Hoshiba, 1984). Small sections of the gonads were squashed on a slide in 45–80% acetic acid and the cover slip was removed after freezing in liquid nitrogen. The slides were then dried on a 60°C hot plate. Staining was carried out by the acetic-orcean and Giemsa solution for C-banding. Preparations were made by usual air-dry squash method (Imai et al., 1986, 1988). C-banding was accomplished by first treating the slide preparations for 15 min with 0.2 M HCl, then treating them with freshly prepared and filtered 2.5% barium hydroxide solution for 10 min at room temperature. After thorough rinsing in several changes of distilled water to remove as much salt deposits as possible, the slides were incubated for 2 h at 60°C in 2 x SSC (0.3 M sodium chloride contain, 0.03 M tri-sodium citrate), rinsed briefly with distilled water

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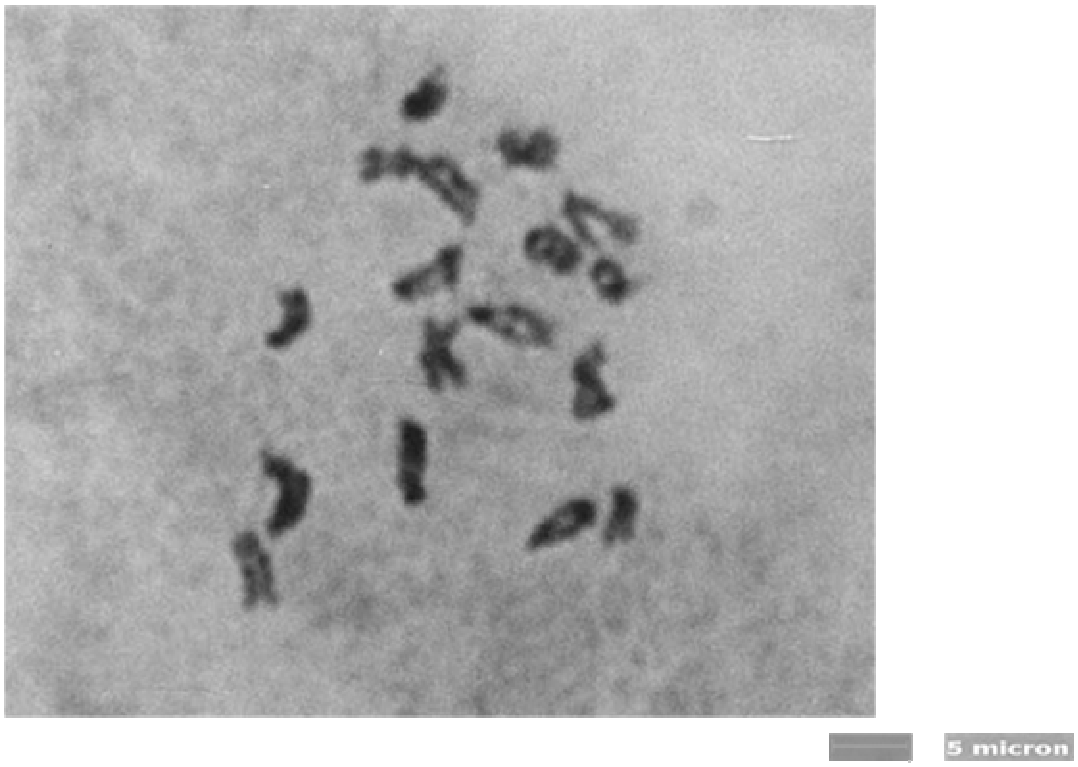

Figure 1. Haploid chromosomes complement of *Apis florea* F.

and stained with 5% Giemsa stain for 5-15 min and stained by the Giemsa solution (Sumner, 1994). The haploid chromosome number for the *A. florea* was determined by examining 30 metaphase spreads. Photomicrographs of selective chromosomes were taken under bright field illumination, using 100 X oil immersion objective and 10 X eyepiece. Nomenclature of the chromosome and the arm ratio were calculated according to the system described by Levan (levan et al., 1964).

RESULTS AND DISCUSSION

Honeybee has several advantages for cytogenetic research, including their large colony populations, their ability to take care of themselves, and their physiology, morphological, and genetics diversity. Identification of the chromosome configuration in *A. florea* is a helpful key in genetically resource preserving programs as the heritable substance (except cytoplasmic substance) is organized in nucleus chromosomes. In this research the distinct karyological and C-banding analysis of the haploid male in *A. florea* was illustrated. Also it was possible to obtain long, clear and complete 16 chromosomes of *A. florea*. We may now precisely characterize the karyotype of *A. florea.* The C-banding pattern in the mitotic metaphase of these species was characterized by the presence of paracentromeric C-bands in all autosomes. Each chromosome has unique banding patterns by this staining procedure and can be more reliably identified by the banding than by length and arm ratio (Perez et al., 1997). The analyzed individuals of the *A. florea* were found to have a chromosome number of n=16 (Figure 1). The chromosome length, relative length, centromeric index, arm ratio and chromosome morphology were measured from 10 metaphase plates and are presented in Table 1.

C-banding shows heterochromatin blocks in the centromeric regions of all chromosomes. These data are important to phylogenetic studies (Imai, 1991). The karyotype pattern was similar to previous reports about *Apis mellifera* (Hoshiba, 1984) and *Apis cerana* (Figure 2). The karyotype evolution proceeds in *A. florea* towards the enlarging of the chromosome number, with fissions and per centric inversions prevailing. Analysis of the total chromosome length (TCL) shows the longest metaphase chromosome of the *A. florea* was about 2.76 mm and the shortest 0.86 mm (Table.1). The secondary constriction was clearly seen in the longest chromosome of the species. Secondary constriction in the longest chromosome was observed. Two small acrocentric chromosomes were seen in between chromosomes. The C-banding pattern in the spermatogonial metaphase was characterized by the presence of area centromeric C-bands in all chromosomes. C-banding showed heterochromatin blocks along the chromosomes, with U chromatin restricted to the distal regions of the short arm. C-banding patterns consist of 4 metacentric (no. 1, 4, 7, 11), 12 sub meta or sub telocentric chromosome and two acrocentric. Additionally, a distal C-band is present in chromosome 1 (Figure 3). Despite the relatively large body of studies on the

Table 1. Measurements of chromosome in *Apis florea* F.

No of chromosome	Long arm (µm0	Short arm (µm)	Arm ratio L/S=r	Total length	Position of centromer
1	1.56	1.19	1.31	2.76	m
2	1.41	0.70	2.01	2.11	sm
3	1.30	0.62	2.09	1.92	sm
4	1.12	0.70	1.60	1.82	m
5	1.25	0.41	3.05	1.66	sm
6	1.20	0.41	2.93	1.61	sm
7	0.87	0.62	1.30	1.54	m
8	1.01	0.42	2.40	1.44	sm
9	0.92	0.47	1.98	1.31	sm
10	0.90	0.44	2.05	1.34	sm
11	0.84	0.42	2.00	1.26	m
12	0.62	0.61	1.00	1.23	sm
13	0.76	0.38	2.00	1.14	sm
14	0.72	0.36	2.00	1.08	sm
15	0.71	0,19	4.05	0.96	sm
16	0.66	0.20	3.30	0.86	sm

Figure 2. Karyograms of *Apis florea* F.

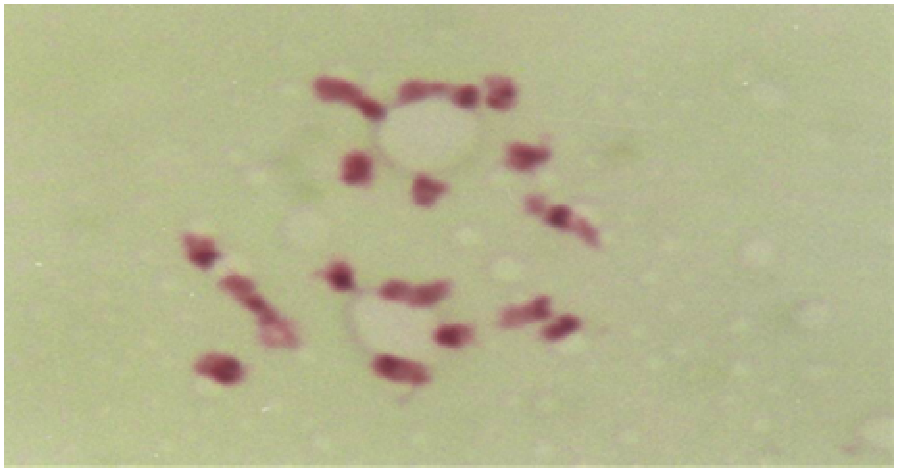

Figure 3. C-Banding patterns of *Apis florea* F.

Karyotype of honey-bee, we still have only limited knowledge on the identification of specific chromosomes (Beye and Moritz 1994). We think among the techniques used, chromo-some C-banding is of great importance for

the understanding of karyotype evolution in honeybee species.

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