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Karyological studies on bumble bee, *Bombus haemorrhoidalis* Smith in Himachal Pradesh

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ABSTRACT

Bumble bees are important pollinators and are being reared in several parts of the world. The karyological studies on bumble bees are scanty. The present studies were conducted at Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan to ascertain the chromosome number of male and female *Bombus haemorrhoidalis*. It was observed that male has n= 18 haploid chromosomes while female has n= 36 diploid chromosomes. Actual length and relative length of chromosomes of male and female of bumble bees were also studied.

Keywords: Bumble bee; castes; chromosomes; actual length; relative length

INTRODUCTION

Bumble bees are hymenopteran insects mainly known for the pollination of crops under protected conditions throughout the globe due to their qualities like higher pollination efficiency, long working hours, able to carry more number of loose pollen grains and buzz pollinating the crop. The order Hymenoptera constitutes one of the four largest orders of insects (LaSalle and Gauld 1992). Almost all the hymenopterans show haplodiploidy. Most of them reproduce by arrhenotoky ie females are diploid and develop from fertilized eggs while males are haploid as they develop parthenogenetically.

In Hymenoptera all members have haplodiploid sex determination; males are haploid (one chromosome set) and females are diploid (two chromosome sets). Arrhenotoky is most common mode of reproduction. A few members also show thelytoky mode and some others show deuterotoky in which unmated females produce both male and female. Sex determination in haplodiploids involves no heteromorphic sex chromosomes thus the only difference between male and female

is the number of chromosome sets. Drone develops from unfertilized while female develops from fertilized eggs (Dzieron 1845).

Karyological studies on *Apis mellifera* honeybee were reported by Deodikar and Thakar (1966) and Hoshiba and Kusanagi (1978). Owen (1983) found that the chromosome number of *Bombus* spp ranged from 12 to 20 and carried out the karyological studies on *Bombus* spp. Bumble bees have chromosome number typical of the members of Apoidea which have a reported range in n from 6 to 20 (Crozier 1975, Sherman 1979). Among the genus *Melipona* the stingless bees the haploid chromosome number is nine in male except in *Melipona quinquefasciata* where haploid number reported is eighteen (Kerr 1948, Kerr 1972, Kerr and Silveira 1972).

Hoshiba et al (1989) studied karyotypic evolution in the social wasps. Machida (1934) and Pardi (1947) reported chromosomes in a few species of *Polistes* with haploid number n= 6, 9, 13, 14 and 21. In the genus *Polistes* the chromosome number may vary from n= 6 to 33 (Hoshiba and Yamamoto 1985). Haploid number may vary from 2 to 32 in Parasitica (Gokhman 1985, Gokhman 1991). Two main trends prevail in karyotype evolution of parasitic wasps. First is the reduction of chromosome number which is mainly due to tandem fusion and less frequent due to centric ones and second the karyotypic dissymmetrization through an increase in size differentiation of chromosomes and in the share of acrocentric in a chromosome set (Gokhman 2006). Ichneumonines has haploid number ranges from 8 to 17 (Gokhman 1993).

The information on the cytology of bumble bees is scanty. Preliminary efforts were made to investigate and ascertain the chromosomes of different castes of *B haemorrhoidalis* Smith occurring widely in Himachal Pradesh.

MATERIAL and METHODS

The bumble bee queen, drones and workers were collected with the help of insect catching net from Nauni, Solan district of Himachal Pradesh and preserved in 70 per cent ethanol.

The chromosomal slides were made from the cerebral ganglia. The samples were dissected under dissecting microscope. Head ganglia were removed from the anterior region. For the preparation of chromosomal slide, the following procedure given by Kumari (2011) was followed:

Pre-treatment: The cerebral ganglion tissues were pre-treated in hypotonic solution having the composition of 0.01 per cent colchicine and 0.4 per cent KCl. Materials were keft in the hypotonic solution for a period of about 30 to 45 minutes. The

pre-treatment resulted in swelling of cells which was useful in better chromosomal spread.

Fixation: After the pre-treatment the materials were fixed in 1:3 acetic-methanol for 20 to 25 minutes at room temperature.

Squashing: After fixation squash preparations were made by putting a cover slip over the material with one edge extended outside the slide. Tapping was done gently with the blunt end of the forceps. The material was spread in a uniform layer on the slide by pressing it in between the folds of the filter paper. The cover slips were dislodged off the slide by sudden jerk with sharp edge of a razor blade. Most of the material remained adhered to the cover slip. Both slide and cover slip were then air dried in a dust free chamber for 1 to 2 days.

Staining: The staining of the chromosome slides was done with 2 per cent Giemsa. The stock solution of Giemsa was prepared by mixing 25 ml of methanol, 25 ml of glycerol and 1 g of Giemsa. The mixture was shaken vigorously and kept overnight in oven at 60°C The stain was filtered and stored in refrigerator at 4°C. From the stock solution working solution was prepared by mixing 3 ml of 0.2 M disodium hydrogen phosphate, 3 ml ethanol and 5 ml stock solution of Giemsa. 100 ml distilled water was added to this solution. The solution was properly shaken and pH was maintained at 6.8.

The air dried slides and cover slips were stained with the working solution for about 25 to 30 minutes. Excess stain was removed by washing the slides and cover slips under tap water and air dried. The slides and cover slips were dipped in xylene and mounted in dibutyl phthalate xylol (DPX). The slides were kept in an oven at 60°C and were observed under the research binocular microscope.

For photomicrography well spread chromosome plates were selected and observed under LEICA–DM–LS2 and photographed with the help of camera LEICA–DFC–320. The actual length of chromosomes was measured using ocular micrometer and from these total complement length was calculated for each species. From the actual length of chromosomes the relative lengths were calculated. As only conventional staining was used the centromeres were not distinctly visible thus the length of the chromosome arms could not be measured. However the total length of the chromosomes was measured that included the length of both short and long arm.

RESULTS

Haploid chromosome number of *B haemorrhoidalis* drone bee (Fig 1) was found to be eighteen (n= 18) (Table 1) (Fig 3). Actual length of the chromosome ranged from 0.36 ± 0.04 µm in the shortest to 1.52 ± 0.04 µm in the longest

chromosome. Total complement length was 15.06 ± 1.14 µm SE (Table 2). Relative length of chromosomes ranged from 2.29 ± 0.11µm SE in shortest chromosome to 10.19 ± 0.49 µm in longest chromosome (Table 3).

In queen (Fig 2) diploid chromosome number was thirty six (2n=36) (Fig 4). Actual length of chromosome ranged from 0.32 ± 0.04 µm in the shortest chromosome to 1.44 ± 0.22 µm SE in the longest chromosome. Total complement length was 29.18 ± 0.7 µm SE (Table 4) (Fig 5). Relative length of chromosome ranged from 1.09 ± 0.4 µm SE in shortest chromosome to 4.93 ± 0.2 µm SE in longest chromosome (Table 5).

Similarly some workers had thirty six (2n= 36) chromosomes of same length

Table 1. Chromosome number of different castes of bumble bee, *B haemorrhoidalis*

Caste of bumble bee	Chromosome number	Haploid/Diploid
Queen	36	Diploid (2n)
Drone	18	Haploid (n)
Worker	36	Diploid (2n)

Fig 1. *B haemorrhoidalis* male

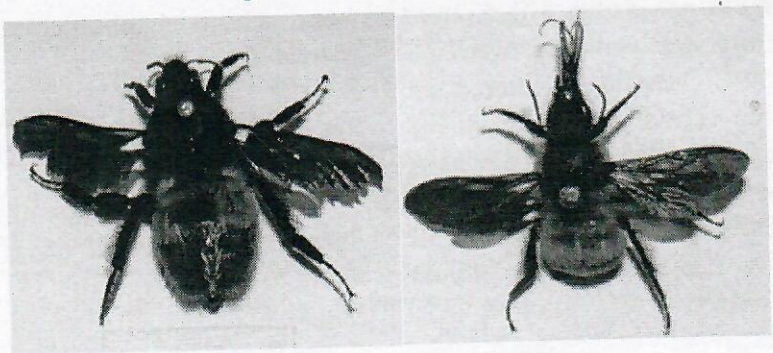

Fig 2. *B haemorrhoidalis* females

Fig 3. Male chromosomes (n= 18)

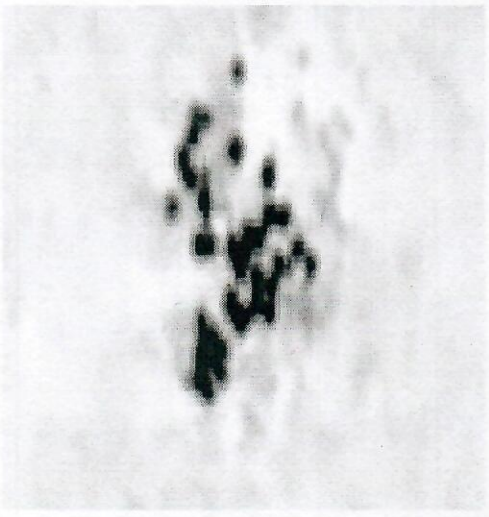

Fig 4. Female chromosomes (2n= 36)

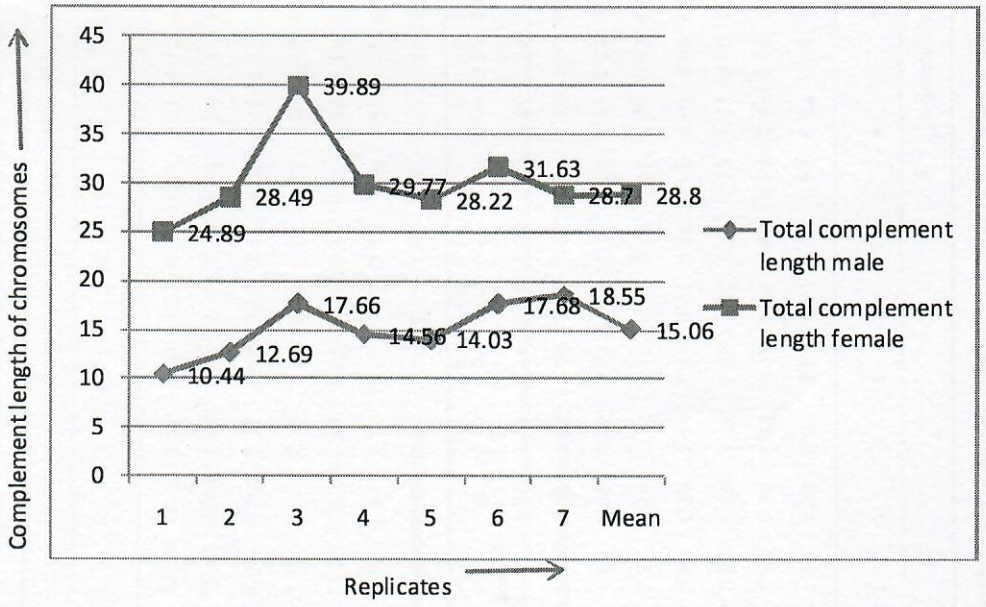

Fig 5. Actual length of somatic chromosomes of bumble bee male and female

Table 2. Actual length of somatic metaphase chromosomes of males of *B haemorrhoidalis* (n= 18)

Somatic metaphase plate	Chromosome length (in μm) of the indicated chromosome number																		Total complement length
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	1.36	1.36	0.69	0.67	0.67	0.67	0.68	0.67	0.46	0.45	0.45	0.46	0.46	0.46	0.23	0.22	0.24	0.24	10.44
2	1.36	1.36	1.12	1.13	0.91	0.89	0.67	0.69	0.68	0.68	0.69	0.68	0.44	0.45	0.24	0.23	0.24	0.23	12.69
3	1.60	1.59	1.36	1.36	1.35	1.36	1.12	1.13	0.90	0.89	0.91	0.91	0.67	0.68	0.45	0.46	0.46	0.46	17.66
4	1.36	1.35	1.14	1.14	0.91	0.89	0.90	0.91	0.69	0.69	0.68	0.69	0.68	0.69	0.46	0.45	0.46	0.47	14.56
5	1.56	1.56	1.15	1.14	0.91	0.89	0.91	0.90	0.67	0.69	0.68	0.69	0.46	0.46	0.45	0.46	0.22	0.23	14.03
6	1.59	1.59	1.36	1.35	1.36	1.36	1.14	1.13	0.91	0.91	0.89	0.91	0.66	0.69	0.46	0.45	0.46	0.46	17.68
7	1.81	1.80	1.36	1.36	1.36	1.35	1.12	1.12	0.91	0.91	0.90	0.89	0.68	0.69	0.69	0.68	0.46	0.46	18.55
Mean	1.52	1.51	1.16	1.16	1.06	1.05	0.93	0.94	0.75	0.75	0.74	0.75	0.58	0.59	0.43	0.42	0.36	0.36	15.06
±SE*	0.04	0.06	0.08	0.09	0.09	0.10	0.10	0.09	0.05	0.06	0.06	0.05	0.04	0.04	0.05	0.05	0.04	0.05	1.14

*Standard error about the mean

Table 3. Relative length of somatic metaphase chromosomes of males *B haemorrhoidalis* (n= 18)

Somatic metaphase plate	Chromosome length (in μm) of the indicated chromosome number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	12.99	13.02	6.51	6.52	6.51	6.51	6.51	6.51	4.35	4.35	4.35	4.35	4.35	4.35	2.18	2.18	2.18	2.18
2	10.51	10.69	8.90	8.92	7.13	7.13	5.34	5.36	5.36	5.36	5.36	5.36	3.58	3.58	1.79	1.79	1.79	1.79
3	9.07	8.99	7.72	7.68	7.68	7.68	6.43	6.43	5.11	5.11	5.11	5.11	3.85	3.85	2.57	2.57	2.57	2.57
4	9.23	9.52	7.79	7.82	6.26	6.26	6.26	6.26	4.69	4.69	4.69	4.69	4.69	4.69	3.11	3.11	3.11	3.11
5	11.20	11.19	7.99	8.05	6.45	6.45	6.46	6.45	4.84	4.84	4.84	4.84	3.21	3.21	3.21	3.21	1.60	1.60
6	8.40	8.37	7.19	7.10	7.13	7.13	5.96	5.96	4.75	4.75	4.75	4.75	3.58	3.58	2.39	2.39	2.39	2.39
7	9.56	9.55	7.35	7.31	7.32	7.32	6.10	6.10	4.88	4.88	4.88	4.88	3.66	3.66	3.66	3.66	2.44	2.44
Mean	10.13	10.19	7.63	7.62	6.92	6.92	6.15	6.15	4.85	4.85	4.85	4.85	3.84	3.84	2.70	2.70	2.29	2.29
±SE*	0.51	0.49	0.17	0.21	0.10	0.10	0.11	0.11	0.09	0.09	0.10	0.10	0.09	0.10	0.21	0.21	0.10	0.11

*Standard error about the mean

as in queen. Karyotype of this species of bumble bee, *B haemorrhoidalis* showed chromosomes of different length in males and females.

DISCUSSION

Haploid chromosome number of *B haemorrhoidalis* drone bee was found to be eighteen (n= 18) while in queen and workers diploid chromosome number was thirty six (2n= 36). Actual length of the chromosome in male bees ranged from 0.36 ± 0.04 µm in the shortest to 1.52 ± 0.04 µm in the longest chromosome. Total complement length was 15.06 ± 1.14 µm SE in males. In queen and worker total complement length was 29.18 ± 0.7 µm SE. Earlier Kumari (2011) has recorded 18 and 36 chromosomes in bumble bee males and females in Shimla, HP. Total complementary length was 15.17 µm in males while 29.18 µm in females which is in close confirmation with the present investigations. Similarly Owen (1983) studied the karyology of 15 species of *Bombus* and reported that their chromosome number (n) ranged from 12-20. According to Crozier (1975), Sherman (1979) and Owen et al (1995) haploid chromosomes of bumble bees (typical of the Apoidea) were found in the range of 6-20. The variation in chromosome number may possibly be due to different species of bumble bees.

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