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Record of Chromosomes in Giant honeybee, *Apis dorsata* Fabricius population from different geo-locations of Southern Karnataka, India

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Abstract
Cytogenetic studies were conducted to record the chromosomes in *Apis dorsata* population found at different geo-locations of Southern Karnataka by following standard methods. There were 32 chromosomes (2n=32) with long and short lengths observed. The total length of the long and the short chromosomes were measured and an ideogram was prepared based on the length of the chromosomes. However, the statistical analysis of chromosome length observed in *A. dorsata* population inhabited at different geo-locations of Southern Karnataka revealed no significant chromosome length variation and suggested no considerable variation among the individuals in *A. dorsata* population live at different geo-locations of Southern Karnataka. Surprisingly, it developed curiosity to undertake further in depth research to measure the variations, if any at molecular level. Results of such observations are published elsewhere.

Keywords: Giant honeybee, *Apis dorsata*, Chromosome, Ideogram, Southern Karnataka

Introduction
Honeybees are social insects, considered as one of the important model organisms, to study the species diversity and distribution for understanding different social behavior including biological, pharmaceutical, oncological, neurological and economical aspects and prospects. Hence, the honeybee genome is used for cytogenetic studies, karyotyping and DNA sequence studies [6]. Since, 1901 honeybee chromosomes are used widely for speciation studies. Among *Apis* species, *A. dorsata* population exhibits an altered phenotypic expression [12] and show variants. Change in chromosome structure and number could be a product of heritable changes in phenotype. However, it is difficult to detect few types of structural variation in chromosomes (e.g. small deletion and duplication), while numerical changes in the chromosomes are detectable only in some animals [12]. Further, knowledge on the chromosomes banding patterns in honeybees is considered particularly important to address questions about gene regulation and expression in caste determination and division of labour [5]. The first cytological investigations in *A. mellifera carnica* were conducted by Stanimirovc *et al*. [15] at Yugoslav regions and showed variation in biometric characters of chromosomes in honeybee ecotypes. Hoshiba and Imai [7] have recorded the morphological changes in chromosomes of *A. cerana japonica* and *A. m. ligustica* using C-banding pattern. The biometric analysis of chromosomes of ecotypes such as Banat (B) and Syenichko – Peshterski (SP) at Yugoslav territory indicated differences in relative length and centromere index (arm ratio) in chromosomes of *A. c. japonica* and *A. m. ligustica*. Further, Stevanovic [17] have investigated the chromosomes and their morphometric differences among three honeybee ecotypes namely: Banat, Timok and Syenichko–Peshterski from the territory of Serbia and revealed the existence of intra and inter population variability with respect hygienic and grooming behavior in Banat yellow honeybees and Syenichko–Peshterski honeybees. Surprisingly, many studies carried out in different parts of the world are related to *A. mellifera, A. florea and A. cerena* excepting *A. dorsata*. This shows that published reports on chromosomes of *A. dorsata* population found at different geo-locations in India in general and Southern Karnataka in particular are scanty. The changes if any in the chromosome structure and number in geographically isolated population of *A. dorsata* is necessitated. Therefore, chromosomal analysis and variations if any in *A. dorsata* populations located at different geo-locations is attempted in the present study and results of such observations are presented in this article.

Materials and Methods

The live *A. dorsata* honeybee comb (part of brood) was collected without damaging to the colony with the help of honey hunters at different geo-locations in Southern Karnataka. Collected brood was kept in a sterilized ice-box and brought to the laboratory. The pre-pupa and pupa (with pink eyes) were identified and isolated from the brood and dissected in physiological saline (N=50) to isolate the cerebral ganglia under the research Stereo Zoom Microscope (SZM). The chromosomes preparation was done as per Imai *et al*. [8] using Giemsa stain with little modification**.** The cerebral ganglia was pre-treated in a hypotonic solution (0.4% KCI and 0.01% Colchicine) for 30 min, fixed in (Acetic acid: Methanol=1:3). The cerebral ganglia were macerated completely using micro pestle to separate the tissue into cells and the resultant suspension was transferred to the sterile centrifuge tube. The cerebral ganglia suspension (CGS) was centrifuged at 3000 rpm for five minutes and later the supernatant was discarded gently. Freshly prepared 3ml Carnoy's solution (Methanol: Glacial Acetic Acid = 3:1) was added and kept undisturbed for five minutes to fix the cells. Further, the cerebral ganglia suspension (CGS) in the tube was centrifuged at 3000 rpm for five minutes and supernatant was discarded by keeping the cerebral ganglia pellets. The cerebral ganglia cells were again are suspended in the Carnoy's fluid for 5 minutes and again centrifuged at 3000 rpm for 5 minutes and repeatedly centrifuge three times at 3000 rpm for 5 minutes. After three times centrifugation the supernatant was removed and cerebral ganglia cell suspension was placed on to the sterilized micro hot slide and kept for drying for one day as per the method described by Imai *et al*. [8]. Further, the cerebral ganglia cell suspension stained with Giemsa solution for 30 minutes and excess stain was removed by rinsing in the distilled water as per Imai *et al*. [8]. The observations were made under advanced spectral confocal microscope (SCM) coupled to an image capturing system to take clear metaphase stage. The best 10 metaphase stages were observed in *A. dorsata* population belong to different geographical location in southern Karnataka. Further, by using DRAWID software the ideogram was constructed and the total chromosome length was measured as per Kirov *et al*. [10].

Results

The worker bee, *A. dorsata* have 32 chromosomes (2n=32) (Fig.1 and Table 1). Total numbers of chromosomes were similar in *A. dorsata* population found at urban locations 1 and 2, arid, semi-arid and malnad locations in Southern Karnataka (Fig.1). Moreover, the chromosomes were grouped into long and short chromosomes due to their length variation (Table 2). The length of long chromosome in *A. dorsata* found at urban location 1 was 3.78 ± 0.15 µm, while it was 3.70 ± 0.15 for urban location 2. However, the long chromosome length in *A. dorsata* population found at semi-arid location was 3.54 ± 0.22 µm and it was 3.59 ± 0.23 and 3.64 ± 0.24 µm long respectively for *A. dorsata* population found at arid and malnad geo-locations (Table 2). Although, there was a little length variation in the chromosomes, however it was statistically not significant (F=2.092; P>0.05) in *A. dorsata* population found at different geo-locations (Table 2). Similarly, length of short chromosome in *A. dorsata* population found at urban location 1 was 1.31 ± 0.14 µm, while it was 1.21 ± 0.09 µm in urban location 2. Moreover, the length of short chromosome in *A. dorsata* population found at semi-arid location was 1.20 ± 0.11 µm, and it was 1.25 ± 0.06 and 1.27 ± 0.04 µm in *A. dorsata* population found at arid and malnad locations respectively (Table 2). Statistical analysis of short chromosome length recorded in *A. dorsata* population found at different geo-location didn't show significant difference (F= 2.190; P>0.05) (Table 2). Further, the ideograms of total chromosomes observed in *A. dorsata* population obtained from different geo-locations are depicted in Figures 2 to 6.

Discussion

The *A. dorsata* worker bee population has 2n=32 chromosomes. *A. dorsata* population found at urban locations, malnad, semi-arid and arid geo-locations in Southern Karnataka indicated similar number (2n=32) chromosomes. Surprisingly, chromosomes length variation was recorded among the *A. dorsata* population found at different geo-locations that was evidenced by ideograms. Obviously, long and short length chromosomes were recorded based on their size variation. Although, the little length variation in the chromosomes was exhibited physically between the *A. dorsata* populations, it was not statistically significant. Notwithstanding to it, the length variation of chromosomes of *A. dorsata* populations found at different geo-locations mustn't be ignored. Similar types of studies were conducted in *A. mellifera, A. florea* and *A. cerena* by Asadi *et al*. and Khademir [1, 9]. Further, several published reports are made on the chromosomes variation in domesticated *Apis* species. Stanimirovic *et al*. [15] have showed biometric characters of chromosomes in honeybee ecotypes in *A. mellifera carnica* at Yugoslav regions. Similarly, Hoshiba and Imai [7] have recorded the morphological changes in chromosomes of *A. cerana japonica* and *A. m. ligustica* using C-banding pattern. The biometric analysis of chromosomes of ecotypes *viz*., Banat (B) and Syenichko – Peshterski (SP) at Yugoslav territory indicated differences in relative length and centromere index (arm ratio) in chromosomes of *A. c. japonica* and *A. m. ligustica*. Further, Stevanovic [17] has reported the morphometric differences in chromosomes among three honeybee ecotypes such as Banat, Timok and Syenichko–Peshterski from the territory of Serbia. Similar type of observations were made by Pejovic, Stanimirovic *et al*., Stanimirovic *et al*. and Cirkovic [11, 13, 16, 4] have revealed the existence of intra and inter population variability with respect hygienic and grooming behavior in Banat yellow honeybees and Syenichko–Peshterski honeybees. However, during earlier investigations, morphological traits variation was recorded in *A. dorsata* population found at different geo-locations of southern Karnataka by Bidisha and Basavarajappa, Bidisha and Basavarajappa [2, 3]. The little size variation in chromosomes indicated the possibility of development of new eco-types in *A. dorsata* population found at different geo-locations that could perhaps, due to varied ecological conditions. In this regard, still more in depth investigations are necessitated to say about the population variation in *A. dorsata*. However, the present study would become an example that how simple curiosity research may provides a clue to investigate the secrets of variations in geo-graphically distinct *A. dorsata* population in this part of the State. Further, all these reports clearly indicated the existence of intra specific variation in *A. dorsata* population to adjust to the prevailed varied agro-climatic conditions at different geographical locations in Southern Karnataka. Our observations are on par with the earlier published reports [7, 15,

[11,16,14,13, 4]. However, to confirm the intra specific variation, in depth molecular studies on mitochondrial DNA COI sequence in *A. dorsata* population living at different geo-locations studies required at greater length. Cytological investigation on chromosome size and number in *A. dorsata* worker bee population is the first of its kind in this part of the State. Since, *A. dorsata* is a wild species, very difficult to access the sample for cytological observations from the natural colonies. During the present investigation, we succeeded in this venture and obtained a hope to investigate variations if any in mitochondrial DNA sequences in *A. dorsata* population found at different geo-locations. Results of such observations will be published elsewhere.

Table 1: Chromosome number recorded in *Apis dorsata* population found at different geo-locations in Southern Karnataka.

Sl. No.	Geo-location	Chromosome number (n)	Chromosome number (2n)
1.	Urban location 1	16	32
2.	Urban location2	16	32
3.	Semi-arid	16	32
4.	Arid	16	32
5.	Malnad	16	32

Table 2: Chromosome length in *Apis dorsata* population found at different geo-locations in Southern Karnataka

Sl. No.	Chromosome	Length of chromosome Mean ± SD (µm)					
		Urban location 1	Urban location 2	Semi-arid	Arid	Malnad	'F' value
1.	Long	3.78±0.15	3.70±0.15	3.54±0.22	3.59±0.23	3.64±0.24	2.092**
2.	Short	1.31±0.14	1.21±0.09	1.20±0.11	1.25±0.06	1.27±0.04	2.190**

Note: Each value is a mean of 10 observations. ** Values are not significant

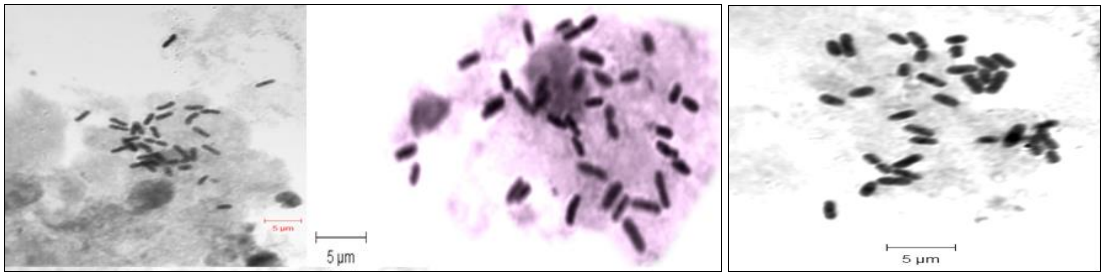

Fig 1: Chromosomes in *Apis dorsata* worker bee population (2n=32)

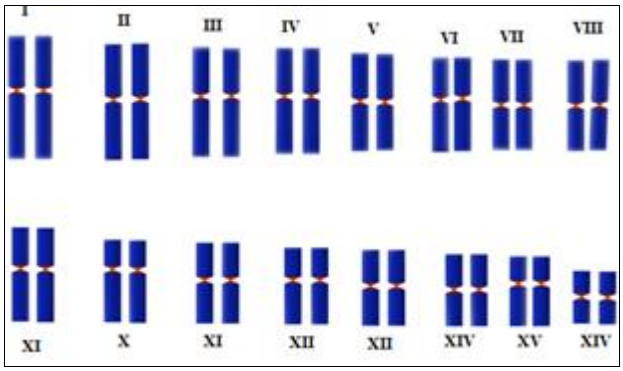

Fig 2: Ideogram of the chromosome in *Apis dorsata* population found at Urban Location 1.

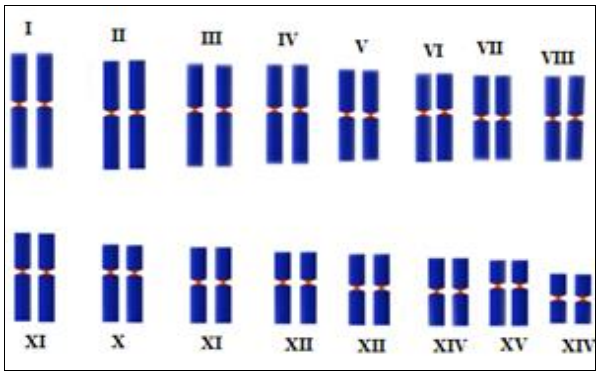

Fig 4: Ideogram of the chromosome in *Apis dorsata* population found at Arid Location.

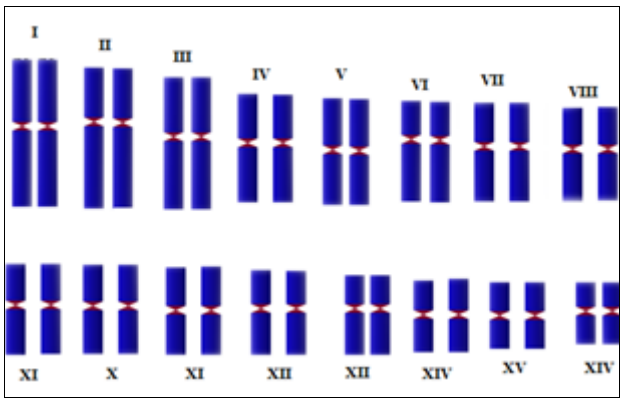

Fig 3: Ideogram of the chromosome in *Apis dorsata* population found at Urban Location 2.

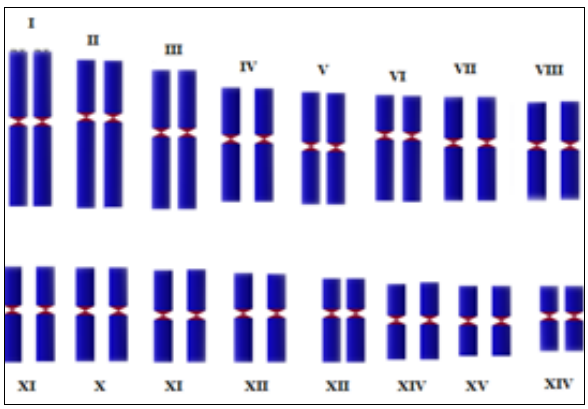

Fig 5: Ideogram of the chromosome in *Apis dorsata* population found at semi-arid Location.

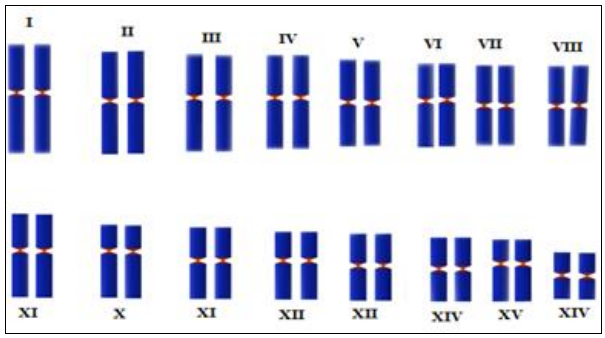

Fig 6: Ideogram of the chromosome in *Apis dorsata* population found at Malnad Location.

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References

1. Asadi N, Ghafari SM, Gharahdaghi A, Tahmasebi GHH, Khederzadeh S. Karyotype and C-banding analyses of haploid male chromosomes of *Apis florea* F. African Journal of Biotechnology 2010;9:4471-4474.
2. Bidisha R, Basavarajappa S. Fore and hind wing morphometry of wild bee *Apis dorsata* (Hymenoptera: Apidae) of geographically distinct area of Southern Karnataka, India. International Journal of Current Advance Research 2018;7:12025-12029.
3. Bidisha R, Basavarajappa S. Morphometric Analysis of Giant Honeybee *Apis dorsata* Worker Bees of Different Areas of Mysore District, Karnataka, India. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 2016;11:07-12.
4. Cirkovic D. Reproductive-productive and hygienic-grooming characterization of honey bee ecotype from Sjenica and Pester region. Ph.D. Thesis, University of Belgrade, Belgrade, 2002.
5. Drapeau MD, Albert S, Kucharski R, Prusko C, Maleszka R. Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. Genome Research 2006;16:1385-1394.
6. Honeybee Genome Sequencing Consortium. Insights into social insects from the genome of the honeybee, *Apis mellifera*. Nature 2006;443:931
7. Hoshiba H, Imai HT. Chromosome evolution of bees and wasps (Hymenoptera, Apocrita) on the basis of C-banding pattern analyses. Japan Journal of Entomology 1993;61:465-492.
8. Imai HT, Urbani CB, Kubota M, Sharma GP, Narasimhanna MN, Das BC. Rajasekarasetty. Karyological survey of Indian ants. The Japanese Journal of Genetics 1984;59:1-32.
9. Khademir TG. A review of the biological status of Iranian dwarf honeybee, *Apis florea*. Journal of Middle East Applied Science and Technology 2014;13:508-513.
10. Kirov I, Khrustaleva L, Van Laere K, Soloviev A, Meeus S, Romanov D. DRAWID: User-friendly Java software for chromosome measurements and ideogram drawing. Comparative Cytogenetics 2017;11:747.
11. Pejovic D. Investigations of hygienic and grooming behaviour of honey bee subspecies *Apis mellifera carnica* in disease resistance. M.Sc. Thesis, University of Belgrade, Yugoslavia 2001, 1-100.
12. Singh BD. Genetics. Kalyan Publishers. New Delhi. 2nd Revised Edition, 2016, 285.
13. Stanimirovic Z, Pejovic D, Stevanovic J. Hygienic behavior in disease resisteance of two honeybee ecogeographic varieties (*Apis mellifera carnica*) from Serbia. Apiacta 2002b;37:24-31.
14. Stanimirovic Z, Pejovic D, Stevanovic J, Vucinic M, Mirilovic M. Investigations of hygienic behaviour and disease resistance in organic beekeeping of two honeybee ecogeographic varieties from Serbia. Acta Veterinary. (Beograd) 2002;52:169-180.
15. Stanimirovic Z, Vucinic M, Soldatovic B, Markovic B. Genotoxicity and potential chemosterilant effects of Rosol. Acta Veterinaria (Yugoslavia) 1997:57:15-21.
16. Stanimirovic Z, Stevanovic J, Andjelkovic M. Chromosomal diversity in *Apis mellifera carnica* from Serbia. Apidiology 2005;36:31-42.
17. Stevanovic J. Investigations of morphometric and chromosomal variability in diversity preserving of Carniolan honey bee (*Apis mellifera carnica* Pollmann, 1879) in Serbia. Ph.D., thesis. Belgrade University, Yugoslavia 2002, 1-100.