Karyotypic Characterisation and C-banding in *Catharcius molosus*Scarabaeinae : Scarabaeidae: Coleoptera)

Paramjeet Kaur

(Department of Zoology, Kurukshetra University, Kurukshetra, Haryana, India -136119)

Abstract

The chromosomes obtained from *Catharcius molosus*of subfamily Scarabaeinaewere studied using standard staining, silver staining and C-banding. The karyotype of*Catharcius molosus*was comprised of 20 chromosomes with meioformula, 9AA+Xyp. Most of the chromosomes were metacentric and submetacentric in nature. Modal chromosome number and meta-, submeta- centric nature revealed the conservative nature of the subfamily Scarabaeinae. The analysis of constitutive heterochromatin (CH) revealed large fragments of chromosomes located on pericentric region in spermatogonial metaphase and small blocks located in the terminal regions of dumbbell shaped bivalents in metaphase I. Silver staining showed dense spots in pachytene stage of prophase I which were associated with nucleolar organizer regions. Along with this Chiasma frequency and terminalisation coefficient were also calculated which further revealed the close relationship of *Catharcius molosus* with other species of family Scrabaeinae.Structural changes in the chromosomes, distribution patterns of constitutive heterochromatin and localization of nucleolar organizer region on the chromosomes are equally important in the speciation of beetles and in other group of insects.

Keywords: *Catharcius molosus*, Karyotype, Chromosomal analysis, C-banding, Silver staining

Introduction

The order Coleoptera has the highest species diversity within the animal kingdom, yet cytogenetic data using specific banding techniques are still scarce. The scarab beetle subfamily Scarabaeinae consists of species collectively called true dung beetles. Most of the beetles of this subfamily feed exclusively on dung. Dung removal and burial by dung beetles result in ecological benefits such as soil aeration and fertilization; improved nutrient cycling and uptake by plants, increase in Pasture quality, biological control of pest flies and intestinal parasites and secondary seed dispersal. Well-known members include the genera *Scarabaeus* and *Sisyphus*, *Catharcius* and *Phanaeus*.

The Scarabaeinae constitutes a highly diverse subfamily that comprises about 5000 described species belonging to 234 genera spread widely in the world (Hanski and Cambefort, 1991). This subfamily shows maximum variation in the number, morphology and size of chromosomes. Cytologically, 162 Scarabaeinae species are known and chromosome number varies from 2n=8 in *Eurysternus caribaeus* to 2n= 24 in *Oniticellus spinipes*, with the Xyp being the most prevalent sex chromosome mechanism (Smith and Virkki 1978; Yadav and Malik 1978; Vidal 1984; Colomba *et al.* 2000).*Catharsius molossus* is one of the most widespread and abundant coprophagous species in tropical Asian regions. It is used in traditional Chinese medicine against detoxification, swelling and constipation.

Detailed analytical studies on the lines of Smith and Virkki (1978), Yadav and Pillai (1977, 1979), Angus et al. (2007), Virkki (1984), Yadav *et al.* (1990), Colomba *et al.* (2000) and Bione *et al.* (2005) are essential for proper understanding of interrelationships and evolutionary processes in this group.The present paper deals with the structure and behavior of chromosomes during spermatogenesis in *Catharsius molossus.*

Materials and Methods

Sexually mature male specimens of *Catharcius molosus* were collected from Kurukshetra University campus, Kurukshetra (Haryana, India). The beetles were sacrificed in 0.56% KCL solution. The testicular material on removal was treated with 0.001% colchicine for 20 minutes. Then it was kept in 1% sodium citrate solution for 20 minutes at room temperature. After the hypotonic treatment the material was fixed in cold 1:3 acetic-methanol for 20 minutes giving 2 or 3 changes. Fixed material was used for the preparation of slides by air drying method.

The method was as follows:

The testicular material was taken in a small amount of 50% glacial acetic acid on clean grease free slide, which was immersed in dehydrated ethanol and cleaned by a piece of muslin cloth. The testes were macerated by means of dissecting needles. The slides were then allowed to dry in air and stained in 2% Giemsa stain. This method was given by Yadav and Lyapunova (1983).

C-bands were determined using the methods of Sumner (1972). Evaluation of chromosomal morphology was based on ten spermatogonial metaphases. Selected stages were microphotographed using oil immersion objective (100X) and digital compact camera (Olympus, C-7070). The silver staining technique of Bloom and Goodpasture (1976) was followed for staining the nucleolar organizer regions.

Chiasma frequency per bivalent was calculated from randomly scored diakinetic/ metaphase I stages in each species by applying the formula as follows:

Chiasma frequency = Total number of chiasmata per cell/ no. of bivalents per cell.

Results

C. molosus L.

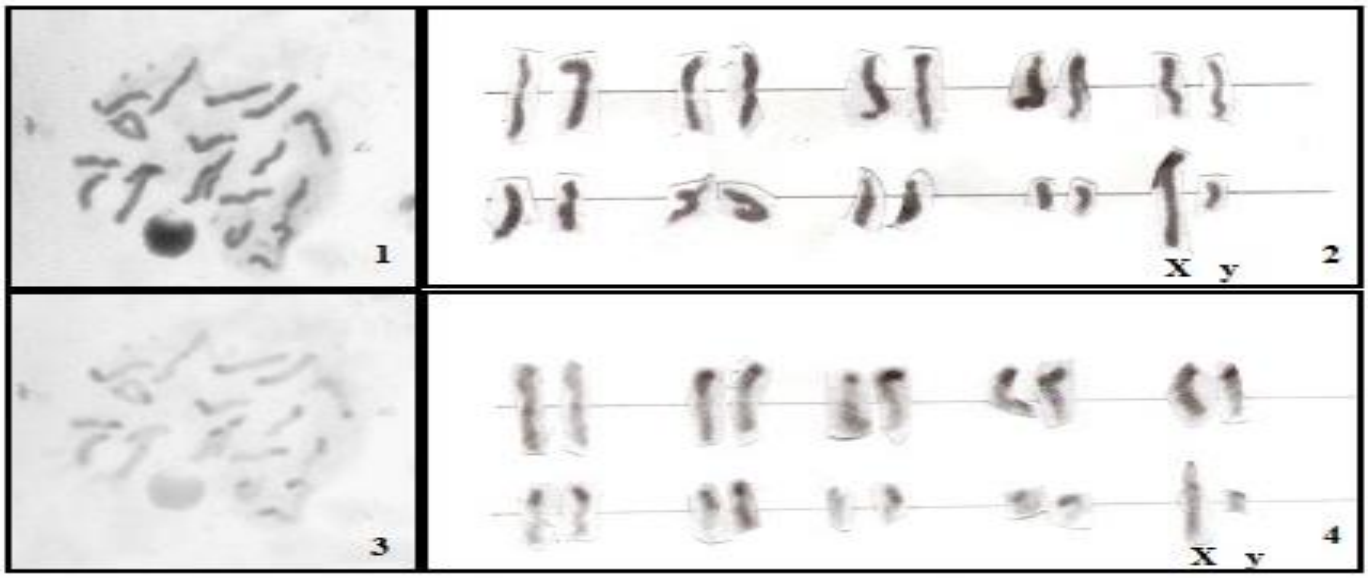
The diploid chromosome number observed in spermatogonial metaphase is 20 (Fig. 1). Chromosomes were categorised into four pairs of metacentric (pairs 2, 3, 5, 8) and five pairs of submetacentric (pairs 1, 4, 6, 7, 9) autosomes, metacentric X overlapping one of the autosome and submetacentric y which is comparatively bigger in size than those of other scarab species (Fig. 2). Percentage relative length of autosomes varied from 4.53 to 13.35, whereas that of X and y was 13.51 and 7.46, respectively (Fig. 17).Measurement of chromosomal complement revealed that the total chromosomal length is in *Catharcius molosus* is 41.09µm, whereas y chromosome is 7.46µm. X chromosome overlaps the chromosome number 1 according to the morphometric analysis, whereas y overlaps chromosome number 7.

Diffused C- bandswere observed in spermatogonial prophase (Fig. 5). Diplotene showed the extended and overlapped chromatids (fig. 7). During pachytene the chromosomes appeared as thick threads. However, heteropycnotic sex bivalent was not very clear at this stage (Fig. 6& 8). In diakinesis as many as two ringed bivalents were encountered (Fig. 9). Metaphase-I revealed nine dumb-bell shaped autosomal bivalents and the sex parachute (Fig. 10 &11). The meio-formula of this species is 9AA+Xyp.Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase-I was 1.0 and 1, respectively.

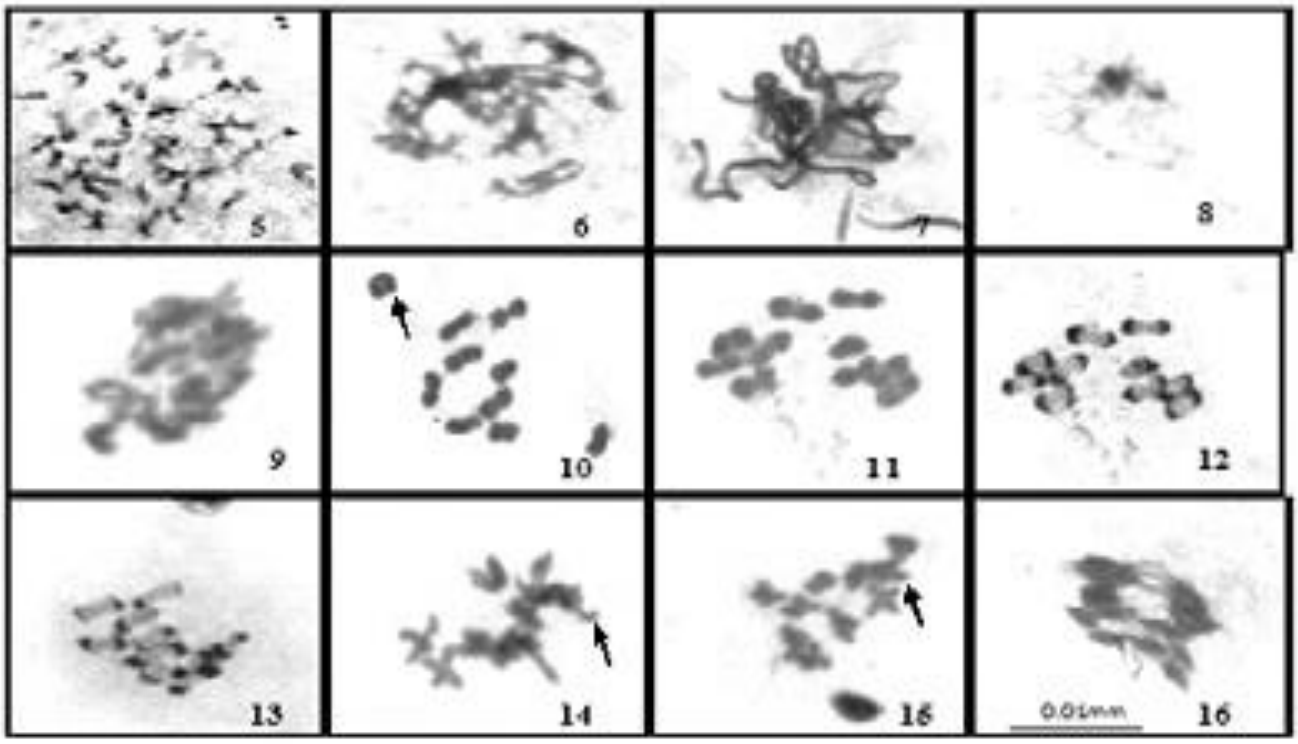
Two types of metaphase-II plates were obtained; one type possessed X chromosome (Fig. 14), whereas other type had a small submetacentric y chromosome (Fig. 15) in addition to nine autosomes. Lamp brush like fibres emanated from the autosomal bivalents and the sex chromosomes. The second meiotic division was equational for both autosomes and sex chromosomes in all the species. The autosomes and sex chromosomes separated and moved towards the opposite poles in anaphase-II stage (Fig. 16). The average number of spermatozoa per bundle counted for this species was 134.

C- banding: C- banded spreads of spermatogonial prophase (Fig. 5), spermatogonial metaphase (Fig. 3) and metaphase-I (Fig. 12 & 13) were obtained. The karyotype of C-banded spermatogonial revealed a darkly stained heterochromatic area in the middle of one pair (Pair 1) of autosomes and X chromosome, while terminally localised C-bands were observed in other eight pairs (pairs 2-9) of autosomes, y being euchromatic in nature (Fig. 4).

$AgNO_3$ banding: After silver staining, the initial stages showed the repetitive patterns and characterised by densely stained amorphous masses of sex bivalent and nucleolar organiser regions (Fig. 6 & 8).



Catharcius molosus:-Fig. 1: Spermatogonial Metaphase; Fig. 2: Karyotype; C- banded: - Fig. 3: Spermatogonial Metaphase; Fig. 4: Karyotype



Catharcius molosus:- Fig. 5: Diffused spermatogonial prophase with C- banding; Fig.6: Pachytene showing silver staining; Fig. 7: Diplotene; Fig. 8: Pachytene with silver staining; Fig. 9:Diakinesis; Fig. 10 & 11: Metaphase I with Xyp (arrow indicates Xyp bivalent); Fig. 12 & 13: Metaphase I with C- banding; Fig. 14: Metaphase II with X (arrow indicates X chromosome); Fig. 15: Metaphase II with y (arrow indicates y chromosome) Fig. 16: Anaphase II with bridge

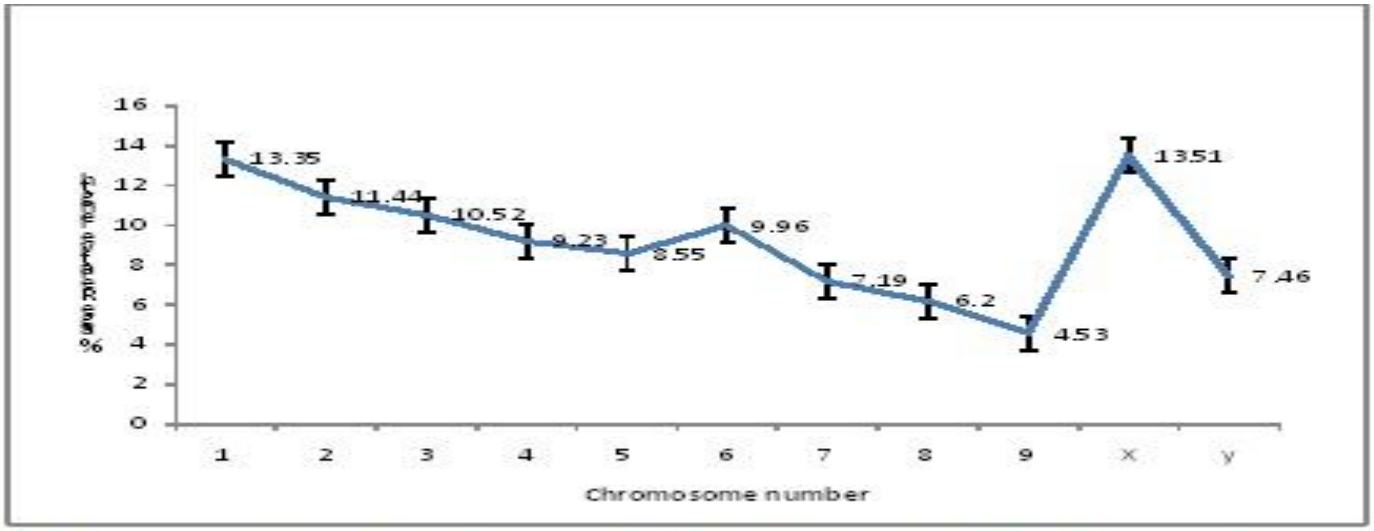


Fig. 17:- Percentage Relative length of chromosomes of *Catharcius molosus*

Discussion

Scarabaeidae is conservative family in having the chromosome number 2n=20, sex determining mechanism 'Xyp' and metacentric chromosomes (Smith and Virkki 1978, Yadav and Pillai 1979, Moura *et al.* 2003, Bione *et al.* 2005).

Unlike other scarabs there is much more variation in the form andsize of chromosomes in Sarabaeinae. Cytologically, 162 Scarabaeinae species are known and chromosome number varies from 2n=8 in *Eurysternus caribaeus* to 2n= 24 in *Oniticellus spinipes*, with the Xyp being the most prevalent sex chromosome mechanism (Smith and Virkki 1978; Yadav and Malik 1978; Vidal 1984; Colomba *et al.* 2000). So, this is the less conservative subfamily of Coleoptera.

Measurement of chromosomal complement revealed that the largerTCL (Total chromosome length) is present in *Catharcius molosus* (41.09µm) with enlarged y chromosome (7.46µm) as compare to other members of this subfamily this also agreed with the earlier reports given by Yadav and Pillai 1977. Virkki (1965) reported the instances of enlarged y chromosomesin beetles such as *Brentus enchorago* L. so, *Catharsius molossus* fallsin this category.However, in the absence of more record it is difficultto presume the manner in which this enlargement have been attained.Variations in size and structurebetween the chromosomes of one and the same karyotype in Scarabaeinae are indicative of a series of structural rearrangements.

The chiasma frequency is generally low in Scarabaeidae, a singledistally localized chiasma per bivalent being the commonest situation,hence genetic rigidity. This is the most prevalent condition in Indian Scarabaeinae too but one or two ring bivalents were often observed in*Onthophagus ramosellus* and *0. quaestus* (Yadav and Pillai 1977). As such Indian coprines seemto be less rigid for genetic recombinations.There is strong evidencethat those possessing different complements are derived forms characterized by i) loss of chromosomes, ii) increase in size of y chromosome, iii) decreasein number of autosomes by autosome-autosome fusion and iv) numericaldecrease by X-autosome fusion.

The comparison of karyotypes can be useful in establishing the phylogenetic relations within taxonomic groups. This, however, needs the assumption that divergence in karyotype structure increases with the time separations of the two species, which means that two closely related species should show less differences in number and structure of chromosomes than do two widely, separated species (Boer 1972). But, when karyological transformations are considered, it should be clear that they cannot reflect phylogenetic evolution in a suitable way. Extensive work is required to draw the exact connection among the subfamilies and their genera.

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