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Cytogenetic characterization of *Mylabris pustulata* (Coleoptera: Meloidae)

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Abstract
The chromosomes obtained from meloid beetle *Mylabris pustulata* Thumb. of subfamily Meloinae was studied using standard staining and C-banding. The cytological information of beetles is useful in many ways. Theoretically, it is important to understand the mechanism governing the transmission of genetic information, hence speciation and for classification on taxonomic levels. It is also useful in the field of applied economic entomology for the improvement of species. The karyotype of *Mylabris pustulata*, is comprised of 22 chromosomes with meioformula, 10AA+Xyp. The analysis of constitutive heterochromatin (CH) revealed small blocks located in the terminal regions of dumbbell shaped bivalents in metaphase I. The increased in chromosome number from 20 (Modal number) to 22 and with conserved sex chromosome mechanism in *Mylabris pustulata* as compared to other species of this family is suggestive of the Robertsonian fission of autosomes having played some role in the evolution of karyotype in this family.

Keywords: Meloidae, Meloinae, *Mylabris*, Karyotype, Chromosomal analysis, C-banding

Introduction
The family Meloidae comprises about 2500 species belonging to 120 genera and four subfamilies (Bologna and Pinto 2001) [3]. It comprises 1% of the order Coleoptera. According to Smith and Virkki (1978) [10], 19 species of Meloidae have well characterized karyotypes, i.e. the diploid number, the chromosome morphology, and the type of the sex chromosome determination system are known (Table 1). Major contributions in the cytological account of these species have been made by Asana *et al.* (1942) [1], Smith (1953) [8], Joneja (1960) [7], Virkki (1962) [13], Yadav *et al.* (1977) [16].

Of the 19 species studied so far for karyotype, 15 possess nine pairs of autosomes and a simple Xy bivalent in the form of parachute. Thus the karyotype in this family is in accordance with the basic polyphagan type (Smith 1972) [9]. The three species of *Mylabris*, viz. *Mylabris pustulata* (present report), *M. phalerata, M. macilenta* however, possess an additional pair of autosomes, whereas the haploid number of chromosomes in *Epicauata grammica* is 12 (Virkki 1962) [13]. This suggests that the centric fission might have played some role in the evolution of the karyotype in this family, but no definite opinion in this connection can be framed before subjecting many more beetles of this family to cytological investigations and accumulating sufficient data to arrive at any conclusion.

Family Meloidae is sexually known by 21 species (Table 1), out of which 15 species belonging to 7 genera represents 'Xyp' typical Polyphagan sex chromosome mechanism. Other types of sex chromosome systems are Xy (*Meloe* sp., *Epicauta cinerea, E. pennsylvanica*) and XY (*Mylabris pustulata*). Sex chromosome polymorphism, Xyp, two or more y chromosomes like Xyyp and Xyyyp in *Epicauta atomaria* have also been reported by Drets *et al.* (1983) [6] and Zacaro *et al.* (2003) [17]. Xyp seems to be the primitive sex chromosome mechanism in this family and other types are the derived forms. Xyyp and Xyyyp may not be considered as the multiple sex chromosome system, but may have originated by precocious separation of y chromosome (Smith and Virkki 1978) [10]. But no definite judgement can be given in this connection before subjecting cytological investigations on more beetles of this family and accumulating adequate data to arrive at any conclusion.

Table 1: Chromosomal analysis in family Meloidae

Sr. no.	Species	Diploid number	Meioformula	References
	FAMILY: MELOIDAE			
	Subfamily: Meloinae			
1.	*Mylabris pustulata* Thumb.	22	10+XY	Asana et al.(1942), Yadav (1971), Y. et al. (1977), PR
2.	*M. phalerata*	22	10+Xyp	Yadav (1971), Yadav et al. (1977)
3.	*M. macilenta* Mars.	22	10+Xyp	Joneja (1960)
4.	*Sybaris paraeustus* Bedt.	20	9+Xyp	Joneja (1960
5.	*Meloe* sp.	20	9+Xy	Asana et al. (1942)
6.	*Epicauta cinerea*	20	9+Xy	Steven (1909)
7.	*E. pennsylvanica*	20	9+Xy	Steven (1909)
8.	*E. murina* Lec.	20	9+Xyp	Smith (1953)
9.	*E. isthmica* Werner	20	9+Xyp	Virkki (1962)
10.	*E. rufipedes* (Duges)	20	9+Xyp	Virkki (1962)
11.	*Epicuata* n. sp.	20	9+Xyp	Virkki (1962)
12.	*E. grammica* Fischer	24	11+Xyp	Virkki (1962)
13.	*E.atomaria*	20	9+Xyp	De Almeida et al. (2000)
		21	9+Xyyp	Zacaro et al. (2003)
		22	9+Xyyyp	Zacaro et al. (2003)
14.	*E. rosilloi* Martinez	20	9+Xyp	Zacaro et al. (2003)
15.	*E. pluvialis* Borchmann	20	9+Xyp	De Almeida et al. (2000)
16.	*Pyrota decorate* (Haag-Rutenberg)	20	9+Xyp	De Almeida et al. (2000)
17.	*Paniculolytta sanguineoquttata* (Haag- Rutenberg)	20	9+Xyp	Virkki (1962)
	Subfamily: Tetraonycinae			
18.	*Tetraonyx frontalis* (Chevrolata)	20	9+Xyp	Virkki (1962)
	Subfamily: Nemognathinae			
19.	*Zonitis tarasca* (Duges)	20	9+Xyp	Virkki (1962)

Materials and Methods

Sexually mature male specimens of *Mylabris pustulata* 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) [15].

C-bands were determined using the methods of Sumner (1972) [12]. 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). 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 and Discussion

Family: MELOIDAE; Sub family: Meloinae; Genus: *Mylabris* ; **Species**: ***M. Pustulata* Thumb.**

Observations

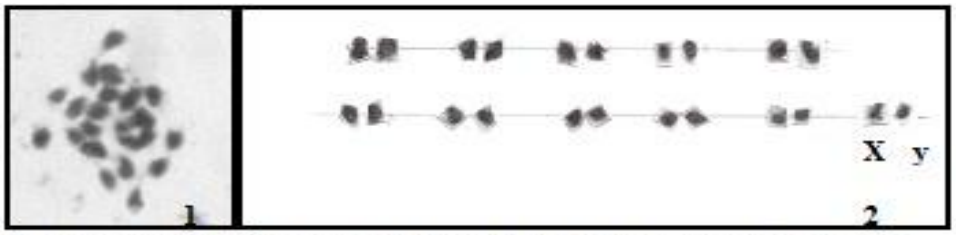
The diploid chromosome number 22 was revealed at spermatogonial metaphase (Fig. 1). During spermatogonial prophase, the chromatids under go gradual contraction. The karyotype is composed of six pairs of metacentric (Pairs 1-6) and five pairs of submetacentric (Pairs 7-11) autosomes, submetacentric X and acrocentric y chromosome (Fig. 2).

The nucleus of the interphase spermatogonium was very large, as such the cytoplasm is pushed to its periphery. The nucleus with distorted outline was found in this species (Fig. 3). Like

mitosis the interphase of the primary spermatocyte is of long duration. But for their smaller size, the primary spermatocytes were very similar to spermatogonia. There is, however, a marked increase in the nuclear volume. The nucleus was either centrally or eccentrically placed and contained an eccentrically located positively heteropycnotic body representing the sex chromosomes (Fig. 4). The two types of secondary spermatocytes with smaller size were formed (Fig. 5).

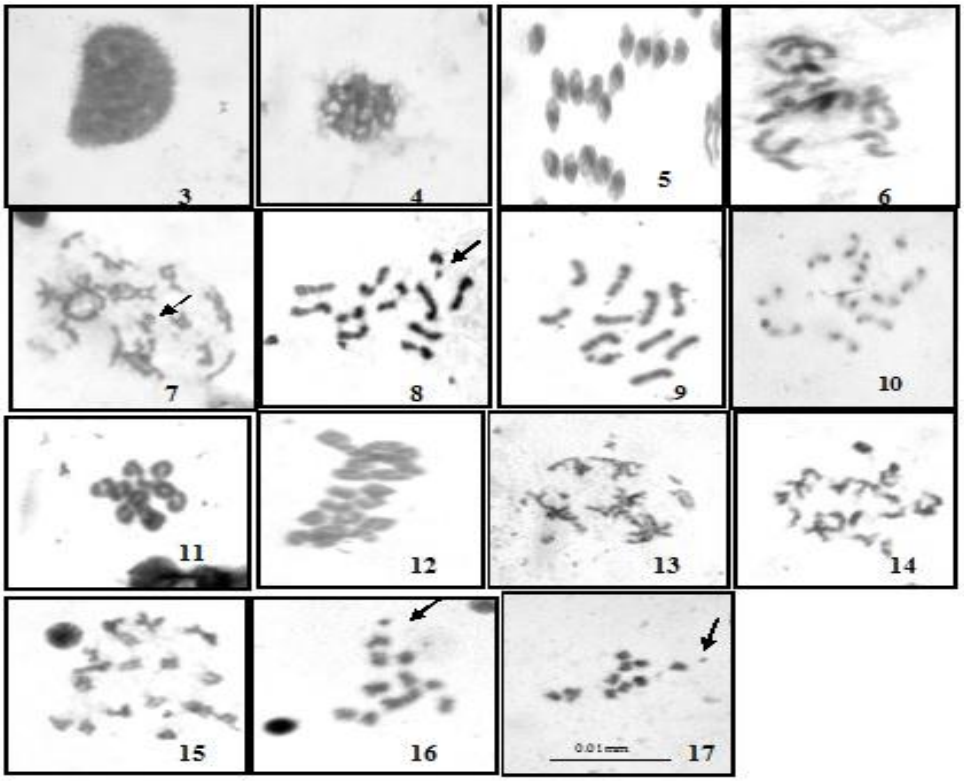
The chromosomes scattered throughout the cytoplasm (Fig.6), while some cells showed extended and decondensed chromosomes. Owing to over condensation at spermatogonial metaphase the morphology of the chromosomes was not very clear, but the centromeric position was tallied with chromosomes at metaphase-II plate.

Figures



Mylabris pustulata Thumb. Fig.1: Spermatogonial Metaphase; Fig. 2: Karyotype

Fig 1-2



Mylabris pustulata Thumb. Fig. 3: Primary spermatogonium; Fig. 4: Primary spermatocyte; Fig. 5: Secondary spermatocytes; Fig. 6: Spermatogonial prophase; Fig. 7: Diplotene with Xyp; Fig. 8: Metaphase I with Xyp; Fig. 9: Metaphase I with Xyp; Fig. 10: Metaphase I with C banding; Fig. 11: Very early Anaphase I; Fig. 12: Very early Anaphase I with extended sex bivalent; Fig. 13: Prophase II; Fig. 14: Early Metaphase II with X; Fig. 15: Early Metaphase II with y ; Fig. 16: Metaphase II with X; Fig. 17: Metaphase II with y

Fig 3-17

Condensed granulated threads forming rings, rods and crosses with one or two chiasmata per bivalent were found at diplotene stage (Fig. 7). Metaphase-I plates with ten dumb bell shaped autosomal bivalents and one sex parachute were encountered (Figs. 8 & 9). Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase-I was 1.0 and 1, respectively. The meio-formula of this species is 10AA+Xyp.

Percentage relative length of autosomes varied from 5.74 to 12.85, whereas that of X and y was 7.79 and 5.60, respectively (Fig. 18)

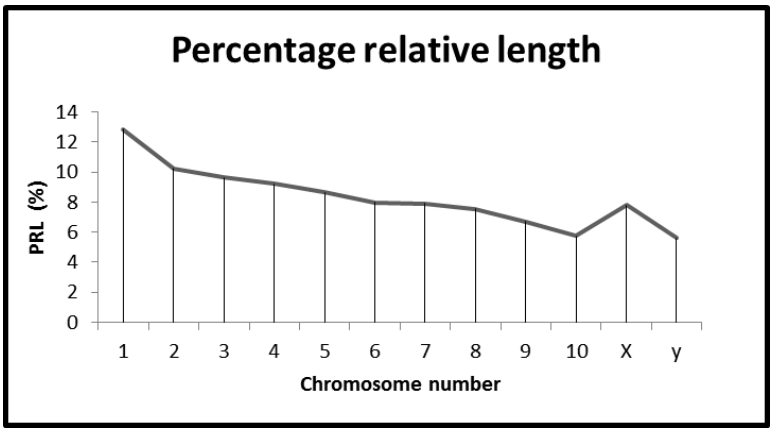


Fig 18: Percentage relative length of chromosomes

C-banding: C-banded spreads of metaphase-I revealed two well-marked heterochromatic regions in each autosomal bivalent and one in sex pseudovivalent (Fig. 10).

During anaphase-I, the homologous chromosomes began their pole ward movement. The chiasmata, which were the retaining mechanism for the adherence of homologous chromosomes, lose their retentive influence and freed the separating chromosomes (Fig. 10), while sex pseudobivalent showed precocious separation (Fig. 11 & 12).

At early prophase-II, the autosomes appeared in the form of long granulated threads which gave fuzzy appearance (Fig. 13). Two types of metaphase-II plates were encountered, one with X chromosome (Figs. 14 & 16) and the other with y chromosome (Figs. 15 & 17), in addition to ten autosomes. Finer details of chromosomal morphology were clear at this stage. The average number of spermatozoa per bundle counted for this species was 164.

Karyotypes of 19 species of Meloidae have been well characterized i.e. the diploid number, the chromosome morphology and the type of the sex chromosome determination system, by Steven (1909) [11], Asana *et al.* (1942) [1], Smith (1953) [8], Joneja (1960) [7], Virkki (1962) [13], Yadav (1973) [14], Yadav *et al.* (1977) [16] and De Almeida *et al.* (2000) [5].

Discussion

Of the Indian species, Asana *et al.* (1942) [1] described the cytological details of *Mylabris pustulata* and *Meloe* sp., Joneja (1960) [7] listed the chromosome number and sex-determining mechanism in *Mylabris macilenta* and *Sybaris paraeustus*, whereas Yadav *et al.* (1977) [16] reported the cytological details of *Mylabris pustulata* and *Mylabris phalerata*. In subfamily Meloinae the basic diploid number 2n= 20: 9+Xyp was reported in 13 species, whereas all three species of *Mylabris* viz. *M. pustulata*, *M. phalerata* and *M. macilenta* and one species of *Epicauta* possessed 2n= 22, but the maximum diploid number of this family 2n= 24 was found in *Epicauta grammica* (Virkki 1962) [13]. *Epicauta atomaria* showed the polymorphic condition by the presence of 2n = 20, 21 and 22 due to the multiple sex chromosomes like Xyp, Xyyp, Xyyyp (DeAlmeida *et al.* 2000) [5]. All the species except *Mylabris pustulata* which has XY (Asana *et al.* 1942) [1] possess Xyp type of sex- determining mechanism. The present investigations on cytology of *M. pustulata* showed chromosomal formula 2n= 10+Xyp which dose not agree with the chromosomal formula 2n=10+XY given by Asana *et al*. (1942) [1]. Subfamilies Tetraonycinae and Nemognathinae are known cytologically by one species each with diploid number 20 and Xyp sex determining mechanism (Virkki 1962) [13].

By applying the C-banding technique on *Mylabris pustulata*, during the present study well marked heterochromatin blocks were observed on autosomal bivalents at metaphase I, which confirmed the earlier reports of Pericentromeric C-bands observed in *Epicauta atomaria* by De Almeida *et al.* (2000) [5], whereas silver nitrate impregmentation with small spots at early prophase stages in *Mylabris pustulata* agreed with presence of NORs at Xyp and 7th autosomal pair in spermatogonial metaphase in *Epicauta atomaria*.

Of the 19 species studied so far, 15 possess nine pairs of autosomes and a simple Xy bivalent in the form of parachute (Table 2). Thus the karyotype in this family is in accordance with the basic polyphagan type (Smith 1972) [9]. The three species of *Mylabris*, including *Mylabris pustulata* (present report), however, possess an additional pair of autosomes, whereas the haploid number of chromosomes in *Epicauata grammica* is 12 (Virkki 1962) [13]. This suggests that the centric fission might have played some role in the evolution of the karyotype in this family, but no definite opinion in this connection can be framed before subjecting many more beetles of this family to cytological investigations and accumulating sufficient data to arrive at any conclusion.

Family Meloidae is sexually known by 21 species, out of which 15 species belonging to 7 genera represents 'Xyp' typical Polyphagan sex chromosome mechanism. Other types of sex chromosome systems are Xy (3 species) and XY (1 species). Sex chromosome polymorphism, Xyp, two or more y chromosomes like Xyyp and Xyyyp in *Epicauta atomaria* have also been reported by Drets *et al.* (1983) [6] and Zacaro *et al.* (2003) [17]. Xyp seems to be the primitive sex chromosome mechanism in this family and other types are the derived forms. Xyyp and Xyyyp may not be considered as the multiple sex chromosome system, but may have originated by precocious separation of y chromosome (Smith and Virkki 1978) [10]. But no definite judgement can be given in this connection before subjecting cytological investigations on more beetles of this family and accumulating adequate data to arrive at any conclusion

Meloid beetles are well characterised by both morphological and biological features. Previous phylogenetic hypotheses based on morphological characters assumed the repeated parallel evolution of complex biological novelties. Ontogeny, larval and adult morphology, cleaning and sexual behaviour have been used in the classification of the family (Bologna

1991) [2]. Bologna and Pinto (2001) [3] attempted to define phylogenetic relationships of meloid genera by utilizing numerous morphological and biological features in a large set of taxa. This cladistic analysis supported the monophyly of Meloidae and its division into four subfamilies (Eleticinae, Meloinae, Tetraonycinae, Nemognathinae), as well as the recognition of several of the traditional tribes.

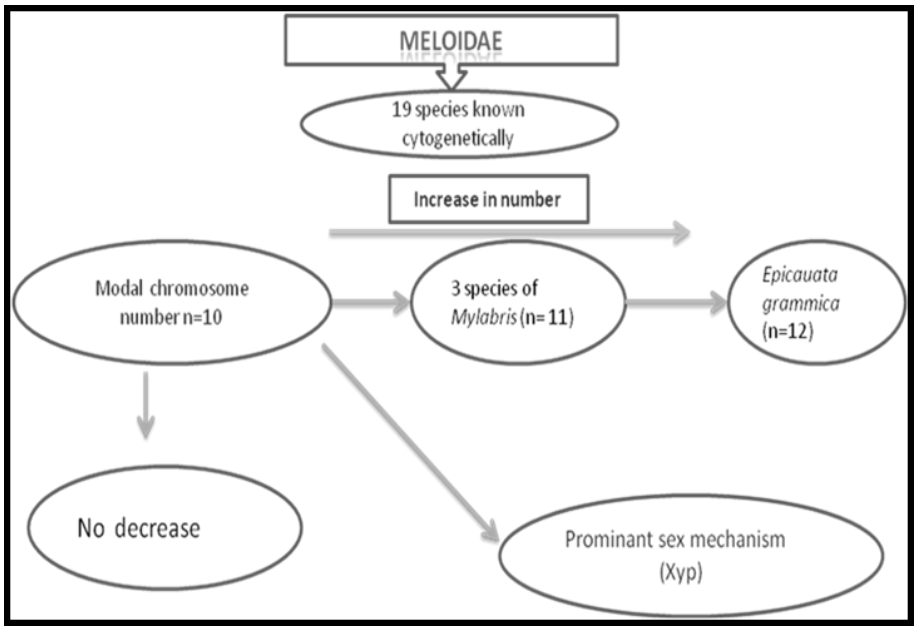


Fig 19: Chromosome number and Sex mechanism evolution in family Meloidae

Eleticinae is hypothesized as the most primitive subfamily based on both larval and adult features: first instar larva orthosomatic probable non-hypermetabolic and non-parasitic larval development, adult abdominal integument highly sclerotized and with a basal depression to receive the metacoxae; presence of an extrusible female ovipositor; probably oviposition in plant tissues. Meloinae is a subfamily traditionally divided into seven tribes with complex and differentiated sexual behaviour, correlated with morphological dimorphism, and hooked male genitalia. Their campodeiform larvae show different biological specialisations related to parasitism primarily on Apoidea, but also on Acridoidea or other Aculeata. The specialisations related to parasitism primarily on Apoidea, but also on Acridoidea or other Aculeata. The early history of Meloidae seems to have been characterised by a relatively short period of evolutionary stasis. In fact, the lineage leading to the other two subfamilies (Nemognatinae and Meloinae), which could have originated shortly later has been marked by two exceptional (and likely correlated) evolutionary events: the adoption of hypermetaboly and parasitism on other insects (primarily bees).

The relationships among Meloinae tribes are only partially overlapping with the results emerging from morphological and biological features (Bologna and Pinto 2001) [3], evidently due to the very high number of taxa (96) morphologically examined, comparing with the reduced molecular data set.

The last subfamily (Meloinae) is the most diversified, both in terms of species and higher hierarchical levels (Bologna 1991) [2]. This is also reflected in their considerable biological diversification. Furthermore, meloines include both widely distributed tribes (Meloini, Epicautini, Mylabrini) and lineages endemic to more restricted areas (Tetraonycini, Cerocomini, Pyrotini, Eupomphini).

Bologna and Pinto (2001) [3] suggested after cladistic analyses of morpho-biological characters, that larval phoresy had evolved several times independently in the blister beetles. Yet, the complicated morphological and eco-ethological larval modifications required to exploit this strategy, render this hypothesis very unparsimonious. Bologna *et al.* (2005) [4] provided strong molecular support for the polyphyly of *Meloid phoresy*, which would have evolved independently at least six times. In the Meloinae alone, the five occurrences of phoretic larvae were scored as independent events. It is probable that the strong advantages provided by being carried directly to the host nest at the larval stage, overcame the need for a repeated evolution of similar (homoplastic) adaptations. The Nemognathinae include a basal tribe (Stenoderini) with a campodeiform larva, and several plesiomorphic traits in the adult.

Conclusion

Of the 2500 species, cytological data of about 19 species is known, so to draw phylogenetic relashioship based on chromosomal information is very difficult. So, more species should be explored chromosomally to confirm the phylogeny of family Meloidae has been given on the morphological and molecular basis (Bologna *et al.* 2005) [4]. The cytological information of beetles is useful in many ways. Theoretically, it is important to understand the mechanism governing the transmission of genetic information, hence speciation and for classification on taxonomic levels. It is also useful in the field of applied economic entomology, where it may be utilized on the one hand for genetic improvement of economically useful insects and on the other hand for controlling the damage by noxious species.

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