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Cytogenetic analysis, karyotype evolution and phylogenetic study of family Scarabaeidae (Coleoptera: Insecta)

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ABSTRACT

The family Scarabaeidae comprises 25, 000 described species and is known cytologically by 404 species of 123 genera and 18 subfamilies. The present investigations recorded an account of 33 species representing 6 subfamilies, of which 17 species are new additions to this family. Scarabaeidae is conservative family in having the chromosome number 2n=20, sex determining mechanism,, Xyp‟‟andmetacentricchromosomes.Mostofthecytogeneticallyknownspecieshavea chromosome number varying from 2n=8 in Eurysternus caribaeus to 2n= 36 in Gymnopleurus miliaris race-II (present study). The most prevalent sex determining mechanism is Xyp. The most common karyotype possessed by 175 species belonging to 18 subfamilies is 9AA+Xyp male, the mostprevalentconditioninColeopteraasawhole.Asmanyas302speciesshowedahaploidnumbe r of 10. So, this can be very well designated as the „modal number‟‟ for Scarabaeidae.

Keywords
Cytogenetics, Scarabaeidae, Review, Polyphaga, Chromosomes

1. Introduction

The Polyphagan beetles possess 2n=20 as the „modal number‟‟ of chromosomes. 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. Detailed analytical studies on the lines of a large number of workers Bickham and Baker (1976), Smith and Virkki (1978) Yadav and Pillai (1979), Bengtsson (1980), Bickham (1981), Angus (1983), Bickham et al. (1983), Lyapunova et al. (1983), Vorontsov et al. (1984), Virkki (1984, 1988), Petitpierre (1987), Yadav et al. (1991), Colomba et al. (2000) and Bione et al. (2005a, b) are essential for proper understanding of interrelationships and evolutionary processes in thisgroup.

The order Coleoptera has the highest species diversity within the animal kingdom, yet cytogenetic data using specific banding techniques are still scarce. C-banding data have revealed a preferential localisation of constitutive heterochromatin (CH) in centromeric area and less so observed in interstitial and telomeric areas. Sex chromosomes also show a variable CH distribution, as it has been observed in the pericentric region or along the entire chromosome. Major contributions in using C-banding technique for the

cytological analysis of Polyphaga are of Ennis (1975), Colomba et al. (2000, 2004), Mafei et al. (2000, 2004), Rozek and Holecova (2002), Petitpierre and Garneria (2003),Vitturi et al. (2003), Petitpierre et al. (2004), Wilson and Angus (2004 a, b, 2005 a, b, 2006), Bione et al. (2005 a, b), Beauchamp and Angus (2006), Angus et al. (2007), Holecova et al. (2008), Arcanjo et al. (2009) and Oliveira et al. (2010), Carbal et al.2011.

Silver nitrate staining of meiotic chromosomes of eukaryotic species has been a very useful approach for the analysis of the structure and variability of nucleoli, nucleolar organiser region and kinetochores (Goodpasture and Bloom 1975; Virkki and Denton 1987; Virkki et al. 1991). NOR activity atthebignningofthemeioticprophaseiswidelyobservedina large number of organisms, including Coleoptera species. However, this activity was observed during a restricted period of time only, declining rapidly and disappearing in the middle of the diplotene phase. Nevertheless, the nucleolar masses produced can persist for a longer period of time, especially in species with prolonged diplotene (Virkki and Denton 1987; Virkki et al.1991).

The cosmopolitan beetle family Scarabaeidae comprises approximately 2000 genera and 25000 species (Arcanjo et al. 2009). Despite of the large number of species, there are few studies about the chromosomal diversity of Scarabaeidae representatives and approximately, only 390 species have been analysed, predominantly using conventional staining (Arcanjo et al. 2009). About 70 Scarabaeidae species have been studied using differential or molecular cytogenetic technique, such as C-banding, base specific fluorochromes, silver niterate staining or fluorescence in situ hybridisation by Moura et al. (2003), Wilson and Angus (2004 a, b, 2005 a, b, 2006), Bione et al. (2005 a, b), Angus et al. (2007) and Dutrillaux et al. (2007 a, b). The constitutive heterochromatin in this family is predominantly located in the pericentric region of the chromosomes and this genomic component shows wide heterogeneity regarding A-T richness and G-C richness. However, the nucleolar organiser region (NORs) is predominantly located either in a single autosomal pair or in the X chromosome or more than one rDNA site clustered in different chromosome pairs (Moura et al. 2003; Bione et al. 2005 a, b; Macaisne etal.2006).

2. Variation of chromosomenumber

The family Scarabaeidae comprises 25, 000 described species and is known cytologically by 397 species of 123 genera and 18 subfamilies (Table 1). The major contributors are Yosida (1949b, 1951), Virkki (1951, 1954a, 1967a), Manna and Lahiri (1972), Salamanna (1972), Kudho et al. (1973), Yadav and Pillai (1975a, 1976a, b, 1978, 1979), Vidal et al. (1977), Smith and Virkki (1978), Vidal (1984), Yadav and Dange(1988b,1989,1991),Yadavetal.(1989),Hanskiand Cambefort (1991), Colomba et al.

(1996, 2000, 2004, 2006), Moura et al. (2003), Bione et al. (2005a,b), Angus et al. (2007), Carbal de Mello et al. (2007, 2008, 2010, 2011) and Silva et al. (2009), The present investigations recorded an account of 33 species representing 6 subfamilies, of which 17 species are newadditions.

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, Colomba et al. 1996, Moura et al. 2003, Bione et al. 2005 a, b). Most of the cytogenetically known species have a chromosome number varying from 2n=8 in Eurysternus caribaeus (Carbal de Mello et al. 2007 and Arcanjo et al. 2009) to 2n= 36 in Gymnopleurus miliaris race-II (present study). The most prevalent sex determining mechanism is Xyp (Smith and Virkki 1978, Vidal 1984, Colomba et al. 2000) . The most common karyotype possessed by 175 species belonging to 18 subfamilies is 9AA+Xyp male, the most prevalent condition in Coleoptera as a whole. As many as 302 species (Table 1) show a haploid number of 10. As such this can be very well designated as the „modal number" forScarabaeidae.

All the karyologically known species of subfamilies Pleocominae and Troginae have the basic karyotype 9AA+Xyp with 2n=20 (Purcella and Virkki 1966, Virkki 1967a, Yadav and Pillai 1976b, 1978, 1979, Yadav and Dange 1988b, 1989, Yadav et al. 1989). In subfamily Geotrupinae eight species of genus Geotrupes (Virkki 1951, 1960, Smith 1960a, Salamanna 1966, 1972), Thorectes intermedius and Anoplotrupes stercosus (Colomba et al. 2004) have 2n=22, while Bolbelasmus arcuatus and Athyreus excavates (Virkki 1967a, Smith and Virkki 1978) and two species of Bolboceras viz. B. quadridens and B. indicum (Yadav and Pillai 1979, Yadav 1983, Yadav et al. 1990 and present report) possessed the modal number of scarabs. In Bolbocerus indicum both the sex chromosomes were found associated to a nocleolar body during first meiotic division. Subfamilies Orphinae, Hybosorinae, Chironinae, Glyphyrinae, Aegialiinae, Trichiinae, Acanthocerinae and Cetoniinae are uniform in having 20 chromosomes in the diploid set, while subfamily Dynamopinae with only one species Dynamopus athleta possess 2n=22: 10AA+Xyp (Yosida 1949b, 1951, Virkki 1951, 1954a, 1954b, 1967a, Smith 1960a, Kacker 1970, Manna and Lahiri 1972, Salamanna 1972, Kudho et al. 1973, Vidal et al. 1977, Yadav and Pillai 1977a, 1979, Smith and Virkki 1978, Vidal 1984 and Mascaine et al. 2006 and present reports). In the present investigations, meioformula 2n= 9AA+Xyp, lampbrush like fibres in bivalents of first prophase and high chiasma frequency of Hybosorus orientalis confirmed the earlier reports given by Kacker (1970) and Yadav et al.(1990).

Out of 40 chromosomally known species of subfamily Aphodiinae only Aphodius moestus (Yadav 1973; present report) has a diploid number of 22, whereas remaining all species possess the modal number 2n=20. 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). The other variations falling between the two extremes are 2n=12: 5+neoXy in five species of Phanaeus (Hayden 1925; Virkki 1959; Smith and Virkki 1978 and Carbal de Mello et al. 2008), 2n = 14: 6+neoXy in five species of Deltochilum (Carbal de Mello et al. 2008, 2010), Gymnopleurus mundus with 6+Xyp (present report) , Copris incertus (Virkki 1960), Copris sinicus (Angus et al. 2007), Copris species (Manna and Lahiri 1972) while Sisyphus neglectus possess 2n=16 : 7+Xyp (present report) , 32 species : Anomiopsoides heteteroclyta, Euranium arachnoids, Glyphoderus sterquilinus, Isocopris inhiata, Bubas bubalus, Gymnopleurus sinuatus, G. parvus, Copris signatus, Paracopris ramosiceps, Canthidium breve, three species of Canthon, Canthochilum spp., Catharsius sp., Onthophagus spp. and 15 species of Dichotomius have 2n=18 with 8+Xyp (Manna and Lahiri 1972; Vidal 1984; Colomba et al. 1996; Bione et al. 2005b; Angus et al. 2007; Carbal de Mello et al. 2008, 2011; Silva et al. 2009; present report), whereas Copris hispanus cavolinii has 2n=19 (Salamanna 1972) and remaining 110 species possess 2n=20 : 9+Xyp which was the most common number in this family (Yosida 1951; Virkki 1951, 1954a, 1967a; Joneja1960; Dasgupta 1963; Kacker 1970; Yadav and Pillai 1977b, 1978, 1979; Yadav and Malik 1978; Vidal 1984; Yadav and Dange 1988 a-b, 1989; Yadav et al. 1993b; Colomba et al. 1996, 2000, 2006; Bione et al. 2005b; Angus et al. 2007), while Copris fricator possess 2n=21: 10+X (Joneja1960).

Two types of diploid configurations 2n= 20 : 9+Xyp and 2n=36: 17+Xyp were encountered during the present investigations in two races of Gymnopleurus miliaris which depict dimorphic nature of this species.

Subfamily Sericinae is cytogenetically known by 14 species. All of the ten species belonging to genus Serica and Maladera depict 2n=20: 9+Xyp (Smith 1950; Joneja 1960; Virkki 1960, 1967a; Manna and Lahiri 1972; Yadav and Pillai 1974a, 1979; Yadav and Dange 1988b, 1991; Yadav et al. 1989). A single unidentified species of Aserica possessed 2n= 19 (Yadav and Pillai 1979), whereas one unidentified Autoserica species (Dua and Kacker 1975) and Ophthalmosarica karafutensis (Kudoh et al. 1973) have 2n=18. The highest diploid number of the family Scarabaeidae, 2n= 30 (Dasgupta 1977; Yadav et al. 1979; Arcanjo et al. 2009) is represented by Autoserica assemensis of this subfamily. The variation in the number of chromosomes in subfamily Melolonthinae is much less than Scarabaeinae. As many as 37 species belonging to 13 genera are known to cytology (Shaffer1920; Virkki 1951; Smith 1960a; Duff 1970; Kacker 1970; Manna and Lahiri 1972; Saha 1973; Yadav and Pillai 1974a, 1976c, 1979; Yadav and Dange 1988b; Moura et al. 2003) Whereas 33 species possess the „modal number" 2n=20:9+Xyp, three species of Apogonia (Kacker 1970; Yadav and Pillai

1974a, 1976c) show 2n=19: 9+XO and one unidentified species of Apogonia possess 2n= 21: 10+X (Saha 1973), however, Haplidia etrusca depict 2n= 18 : 8+neoXY (Salamanna 1972) . So the basic diploid number of this subfamily is 20.

In subfamily Rutelinae diploid number varies from 16 to 22 in 52 cytologically known species, out of which all 13 species of genus Adoretus and one species of Adorrhinyptia possess the higher number 2n=22: 10+Xyp (Joneja 1960; Kacker 1970, 1971; Yadav and Pillai 1975a, 1976 a, b, 1979; Mittal et al. 1987; Yadav and Dange 1988b; Yadav et al. 1989; present report), whereas one unidentified species of genus Adorrhinyptia show polymorphic nature (Saha and Manna 1971; Saha 1973). Although most of the species of genus Anomala (Yosida 1949b; Joneja 1960; Agarwal 1960, 1962; Lahiri and Manna 1969; Manna and Lahiri 1972; Kudoh et al. 1973; Yadav and Pillai 1974a, 1975a, 1979; Smith and Virkki 1978; Mittal et al. 1985) depicted basic karyotype 2n=20, yet two types of diploid number 2n=18 and 20 were reported in Anomala bengalensis (present report) and A. rufocuprea (Saha and Manna 1971; Yadav et al. 1993a; Kudoh et al. 1973; Yosida 1949b) explicating dimorphic nature of both the species.

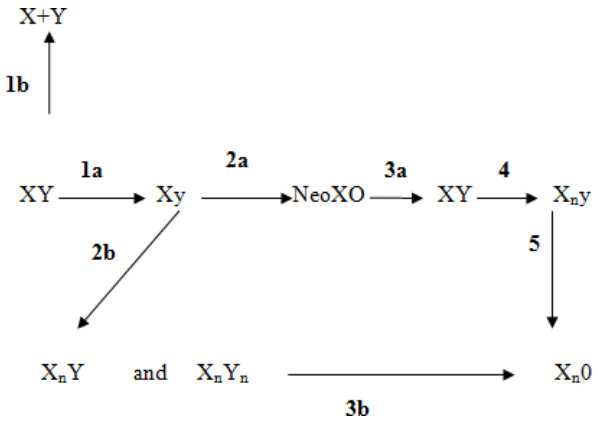
Cytological data belonging to 35 species of subfamily Dynastinae is known. The commonest number is 2n=20 possessed by 21 species belonging to the genus Cyclocephala, Dyscinetus, Diloboderus, Euetheola, Ligyrus, Bothynus, Ligyroides, Phylloganthus, Pentodon, Eophileurus, Allomyrina and Lycomedes ((Joneja 1960; Virkki 1967a; Kodoh et al. 1970, 1973; Vidal et al. 1977; Yadav and Pillai 1977a, 1979; Smith and Virkki 1978; Vidal 1984; Bione 2005a). Variations are 2n= 16 :7+neoXY in Arcophileurus vervex vervex (Vidal 1984) , 2n=18 : 8+neoXY in eight species: Cyclocephala tridentate, C. maffafa, Enema pan, Dynastea hercules hercules , Ligyrus cuniculus , Phylloganthus sinensis, Strategus syphax and Megasoma actaeon (Virkki 1951; Salamanna 1972; Vidal and Giacomozzi 1978; Vidal 1984; Dutrillaux et al. 2007 a, b), 2n=19: 9+XO in three species of Pentodon (Joneja 1960; Salamanna 1966), whereas Oryctes nasicornis depicts the dimorphic configuration with 2n= 12: 5+XY and 2n= 18: 9II (Virkki 1951, 1954b) and X-chromosome diphasism in Coeloxis bicornis was reported by Martins(1989).

3. Evolution ofkaryotype

It is very difficult to establish the evolutionary history of more remotely related cytological extremes, with certainty. But some light must be shed on how these have occurred by a study of the principles operating in the present day species, because similar processes must have prevailed in the past. On the basis of the known karyotypes found among closely related species, mainly four types of chromosome changes have been implicated in this order Coleoptera (John and Shaw 1967).

These are:

I. Centric Fusion (Chilocorus and Exochomus) and fission (Pissodes) given by (Smith 1959,1965a).
II. Pericentric Inversion (Hylobius) (Smith 1962a), Trox (Purcell and Virkki 1966) and Timarcha (Petitpierre 1970).
III. Polyploidy (Blaps) (Lewis and John1957)
IV. Erosion and replacement of the y chromosome according to the sequecnce givenbelow



Steps 3a and 4 in this sequence themselves depict upon the redeployment of autosomal material into new sex chromosome system by centric fusion (Smith 1962a).

I (a). Centric fusion- represents as a major mechanism in the karyotype evolution of many groups of animals. In the genus Drosophila (Patterson and Stone 1952, Stone 1955) acridid grasshopper (White 1951, Yadav et al. 1981, Yadav and Yadav 1990), it is strongly believed that evolution has proceeded from the higher to the lower number by centric fusion. Two types of centric fusion has been observed in Coleoptera: Autosome – Autosome fusion occurred in species, in which there is decrease in chromosome number without any change in X chromosome e.g. Gymnopleurus parvus and Copris signatus (2n=18), Sisyphus neglectus (2n=16), Copris sp. Gymnopleurus mundus, Phaenaeus yucatamus (2n= 14) and Phanaeus igneus (2n=12). Autosome – X fusion observed in species in which sex chromosome system Xy changed to neo-XY system with reduction of chromosome number e.g. Sulcophanaeus spp. (9+neoXY), Deltochilum valgum (6+neo XY), Phanaeus spp. (5+neoXY) and Eurysternun caribaeus (3+neoXY).

I (b). Centric fission- also called „dissociation" is the opposite process of centric fusion and results in increased chromosome number. But it is very difficult to envision cytologically, since it implies the formation of two telocentric chromosomes from a metacentric, or the occurrence of centromere „donors" in the form of supernumerary

chromosomes seems rarer than fusion (Mathey 1973). John and Lewis (1968) and Southern (1969) suggested that the evolutionary replacement of a metacentric chromosome by two rod-shaped elements (acrocentrics) is always due to „dissociation". These authors contend that simple fission through the centromere of a metacentric may sometimes give rise to two stable telocentrics. It is observed that the „dissociation" played a major role in the chromosome polymorphism in many beetles. Manna and Smith (1959) reported a case of polymorphism in bark weevils Pissodes. A metacentric chromosome A in Pissodes is represented by the acrocentric A^I and A^R. Though Manna and Smith (1959) tentatively suggested that the metacentric A A represents the primitive condition, they could not give any evidence to rule out the possibility of fusion of two acrocentrics. This condition is also observed in Dynamopus athleta, Geotrupes spp., Oniticellus spinipes, Thorecetes intermedius and Gymnopleurus miliaris (Bione et al. 2005 a, b, present investigations). Most frequently fission results in the supernumerary chromosomes. In certain exotic species of Chilocorus, the chromosome number has increased from the basic 18 to 22 by means of two fissions mediated by the presence of floating supernumerary chromosomes (Smith 1962b).

II.Inversions- are of two types, pericentric and paracentric- the latter has very little evolutionary significance. Stone (1955) has listed 32 pericentric inversions which have occurred during the fixation of karyotypes of the genus Drosophila, of which the best known is the one that established itself in the second chromosome of the Montana section of the virilise group, converting the elements from an acrocentric to metacentric. The exact number of paracentromeric inversions that the karyotypes have undergone in the genus is not known. The role of pericentromeric inversion in the speciation has been reported in many grasshopper species by White (1949, 1951, 1969), White and Andrew (1960, 1962), White et al. (1964), in mantids by White (1941, 1965) and in beetles by Smith (1958, 1962b, Manna and Smith (1959), Smith (1970), Petitpierre(1970).

III. Polyploidy- In Coleoptera polypoidy is common only in Curculionidae, in which a number of parthenogenetic forms are known (Suomalainin 1955, 1969, Takenouchi 1964, 1972). This phenomenon has arisen in many forms from the diploid bisexual species by automixis or a similar fusion of two diploid nuclei. The aberrant Balptinae from Tenebrionids is also reported to have undergone through polyploidisation coupled with hybridisation and successive allosome/ autosome translocation (Lewis and John 1957). In family Scarabaeidae, Gymnopleurus mundus (2n= 14 and 28), showed the polyploidy under presentinvestigations.

IV. The erosion of y chromosome and its replacement or total loss has been discussed under evolution of sex chromosome mechanism in Apogonia spp. and Coprisfricator.

In family Scarabaeidae, Of the 397 cytologically known species, sex chromosomes are known in 359 species (Table 1). The most common karyotype in Scarabaeidae is 9AA+Xyp, possessed by 175 species. A clear predominance of 2n=20 and Xyp sex chromosome system in the Scarabaeidae is a strong evidence that those possessing different complements are derived forms. A review of literature reveals that atleast four types of major changes have been involved in the evolution of karyotype which contributed to the chromosomal diversity of the family (Fig.1).

Bolobocerus quadridens and B. indicum with 2n= 9AA+Xyp (Yadav et al. 1990 and present report) link Geotrupinae which possess 2n=22. Virkki (1959) assigned the increase to the fragmentation of two metacentrics. The presence of four acrocentrics in Geotrupes spp. and Thorectes intermedius further supports this hypothesis. But presence of two pairs of acrocentrics in Bolbocerus spp. (9AA+ Xyp) and small y chromosome in B. indicum makes the present situation complicated i.e. a fresh decrease after initial increase in the number of chromosomes. Presence of a large metacentric X- chromosome suggests the neo X-Y origin of decrease followed by erosion of the y chromosome resulting in the present situation.Hybosorusorientalis,2n=20(Kacker1970,Yadavet al. 1990 and present report), represents Hybosorinae. Cytologically it shows closeness with Bolboceras, in possessing a similar diploid complement. The size and morphology of chromosomes, however vary to a great extent. Further, y chromosome is large in comparison with its Geotrupinae counterpart. Dynamopinae is chromosomally known by Dynamopus athleta having 2n=22 (10AA+Xyp) and showing a karyological kinship with Geotrupes spp. and Thorectesintermedius.

The range of diploid chromosome number in Scarabaeinae is very wide (Fig. 2). It ranges from 8 in Eurysternun caribaeus (Carbal de Mello et al. 2007) to 36 in Gymnopleurus miliaris race I (present report) which indicates a series of rearrangements in the evolution of this subfamily. Arcanjo et al. (2009) presumed that the decrease in the number of Eurysternun caribaeus is due to X-autosome fusion which brought the number from 2n= 12 in Phanaeus vindex to 2n=8, neo-XY. Whereas Virkki (1959) rightly suspected repeated neo- XY formation causing decrease from 2n=20 to 2n=12 via Haplidia etrusca and Phyllognathus silensis (8+ neo XY) Acrophileurus vervexvervex(7+neoXY) Deltochilum valgum (6+ neo XY) Phanaeus spp. (5+ neo XY) to finally Eurysternun caribaeus (3+ neo XY). The chromosomenumber21 with 9+ XO in Copris fricator, however, suggest that the karyotype evolution took place in two steps, firstly one pair of autosomes has undergone dissociation as observed in Geotrupes spp. Thorectes intermedius Dynamopus athleta, Adoretus spp. and Adorrhinyptia dorsalis and Aphodius moestus with 2n = 22 representing different subfamilies, secondly „excretion" of one of the sex chromosome, the y has taken place resulting finally XO condition. This is probable that 2n=21 (XO) in Copris fricator may have secondarily been evolved through Geotrupinae karyotype 2n=22 (Xyp). Since Geotrupinae is anatomically close to Scarabaeinae than any other subfamily, this hypothesis gets enough support (Virkki 1957).

In contrast to it Autosome – Autosome fusion occurred in species, in which there is decrease in chromosome number without any change in X chromosome e.g. Gymnopleurus parvus and Copris signatus (2n=18: 8+Xyp), Sisyphus neglectus (2n=16: 7+Xyp), Copris sp. Gymnopleurus mundus, Phaenaeus yucatamus (2n= 14: 6+Xyp) and Phanaeus igneus (2n=12: 5+Xy) (Hayden 1925, Manna and Lahiri 1972, Smith and Virkki 1978, present report).

In comparison with Scarabaeinae, Melolonthinae presents cytologically uniform picture. The variation of chromosome number is least in 37 species belonging to 13 genera known cytologically (Fig. 2). Except three species of Apogonia and Haplida etrusca, all the species possessed 2n=20. Three species of Apogonia with 2n= 19: 9+XO involves loss of y chromosome. One unidentified species of Apogonia with 2n= 21: 10+XO involves autosome dissociation followed by loss of y chromosome. This has both genetic and mechanical implications. From the gentic point of view the possibility of y being translocated on to some other member of karyotype cannot be ruled out. The mechanical properties of y are, however of considerable importance in ensuring a regular segregation of the X chromosome. But as suggested by Smith (1952),onceXhasachievedindependenceofmobility(asin XO species), it never appears to have surrendered it. This hypothesis is compatible with the discovery of two types of karyotypes, 9+Xyp (Kacker 1970) and 9+XO (Manna and Lahiri 1972) in Apogonia nigricans. It takes some time for the X chromosome to get stabilised and thus to synchronise with autosomes during Anaphase-I. So, differential behaviour of X chromosome can be observed at different stages in the congenric species of Apogonia. Therefore, the karyotype of Apogonia is not at all to be considered as rigid with regard to new genetic recombinations and reshuffling of the karyotype.

Highest diploid number 2n=36 in the family Scarbaeidae is from subfamily Scarabaeinae, in which Gymnopleurus miliaris race-I represents this diploid number (present report). This may be due to the centric fission, as acrocentric autosomes are present in this species. However, of 13 cytologically known species of this subfamily, 10 species exhibited 2n=20 (9+Xyp), the modal number of this subfamily. Whereas variation within 2n= 19 (XO) in Ascaria sp. involve the loss of y chromosome and 2n=18 (Xyp) in Ophthalmoserica karafutus showed the involvement of autosome fusion without changing the Xyp sex determining mechanism.

As we know that in subfamily Rutelinae diploid number varies from 16 to 22 in 51 cytologically known species, so the variations has taken place towards decrease and increase in chromosome number. 13 species of genus Adoretus and one species of Adorrhinyptia with higher diploid number 2n=22: 10+Xyp (Joneja 1960; Kacker 1970, 1971; Yadav and Pillai 1974a, 1976 a, b, 1979; Mittal et al. 1987; Yadav and Dange 1988b; Yadav et al. 1989; present report), suggested the autosome dissociation, whereas on the other hand one unidentified species of genus Adorrhinyptia show polymorphic nature with 2n= 16/ 18/ 20 (Xyr) (Saha and Manna 1971; Saha 1973) and Popillia japonica, Macraspis spp and Anomala corpulenta with 2n= 18 (Xyp) suggested the involvement of autosome – autosome fusion in karyotype rearrangements. Although

most of the species of genus Anomala depicted basic karyotype 2n=20, yet two types of diploid number 2n=18 and 20 were reported in Anomala bengalensis (present report) and A. rufocuprea (Saha and Manna 1971; Yadav et al. 1993a; Kudoh et al. 1973; Yosida 1949b) explicating dimorphic nature of both the species. Thus it seems probable that the trend of evolution in the genus is towards increasing asymmetry in thekaryotype.

Out of the 34 species chromosomally known from Dynastinae, 21 species with 2n=20 suggested this to be the„modal number" of this subfamily. Chromosome number 18 and 16 with the formation of neo XY sex determining mechanism in 9 species confirmed that autosome-X fusion is responsible for the reduction of diploid number (Virkki 1959) whreas alteration of chromosome number and sex determining mechanism 9+XO in Pentodon spp. involve the loss of y chromosome. Oryctes nasicornis depicts the dimorphic configuration with 2n=12 and 18, which shows chromosomal rearrangements in the species towards the increase or decrease the chromosomenumber.

Finally in subfamily Trichiinae all species represent modal number of chromosomes, whereas in Cetoniinae 19 speceis exhibited „modal number" of Scrabaeidae except Oxythyrea funesta with 2n=20 (Xy)/ 19 (XO) showed dimorphic nature and involvement of loss of y chromosome in chromosomal rearrangements.

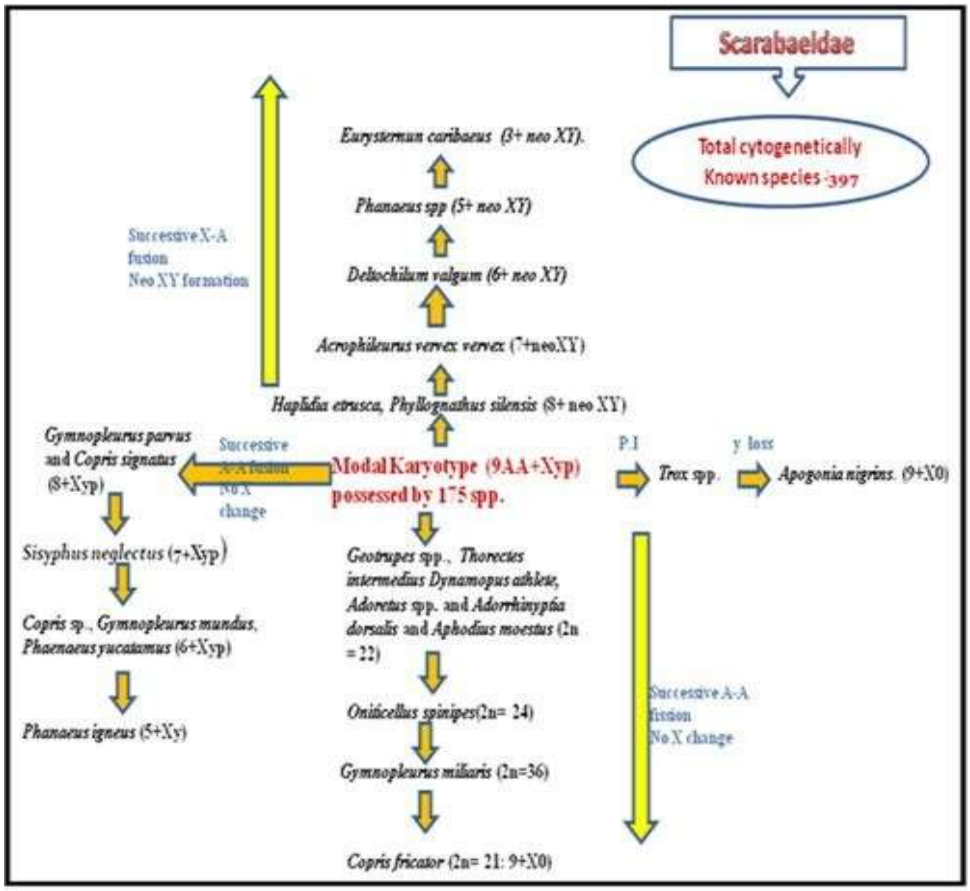


Fig. 1: Evolution of diploid number of chromosomes and sex chromosomal mechanism in family Scarabaeidae

During the present investigations cytological account of 33 species belonging to 14 genera and six subfamilies viz.: Geotrupinae, Dynamopinae, Hybosorinae, Aphodiinae, Scarabaeinae and Rutelinae of family Scarabaeidae is presentedin few papers given by Kaur and Yadav (2011, 2013, 2014 a-d). Out of these 17 species are new additions, while C- banding technique has been applied on 19 species. Constitutive heterochromatin has been localised at pericentromeric regions in two species of Bolbocerus, Aphodius testaceus, Catharcius pithecius, Copris signatus, Gymnopleurus miliaris, Oniticellus pallipes, Oniticellus pallens, Onthophagus fasciatus, Onthophagus unifasciatus and Sisyphus neglectus, agreed with the earlier reports given by Vidal and Giacomozzi (1978), while centromeric C-bands in Gymnopleurus mundus corroborated the results of Colomba et al. (2000). In other spceis C-bands were observed only at metaphase I stages with approximate two C-blocks on each autosomalbivalent.

4. Evolution of sexchromosomes

Of the 397 cytologically known species sex chromosomes are known in 359 species (Table 1 and 2). Both „Orthodox" and „Unorthodox" systems of Smith and Virkki (1978) are found in this family.

The most common male sex chromosome system in Scarabaeidae is the Xyp, possessed by 231 species amounting to about 65% of total species for which the sex chromosome mechanism is known. The other types are Xy (93 species), neo XY (13 species), XO (10 species), Xyr (8 species). This however includes many Xyp. XY is possessed by 3 species whereas X+Y is present in Gymnopleurus sinuatus (Manna and Lahiri 1972). The XY system is exhibited by Phanaeus igneus,P. vindex and Apogonia unistriata (Hayden 1925, Lahiri and Manna 1969). In P. vindex Virkki (1959), on reinvestigation, found neo-XY type of male sex chromosome system. Chironinae, cytologically represented by single species, is the only subfamily that lacks Xyp system. XO mechanism is reported for Scarabaeinae, Melolonthinae and Dynastinae only. Xy mechanism is, however, more common and is met within subfamilies Geotrupinae, Chironinae, Aphodiinae, Scarabaeinae, Melolonthinae, Rutelinae, Trichiinae and Cetoniinae.

A clear predominance of 2n=20 : 9+Xyp species in the Scarabaeidae is a strong evidence that those possessing different complements are derived forms. A review of literature reveals at least four types of major changes as follows:

i. **Centric fusion: a.** Autosome –Autosome fusion in which reduction of diploid number is there without changing the X chromosome e.g in Oryctes nasicornis, Gymnopleurus parvus, Canthochilum spp., Copirs spp. Gymnopeurus mundus,

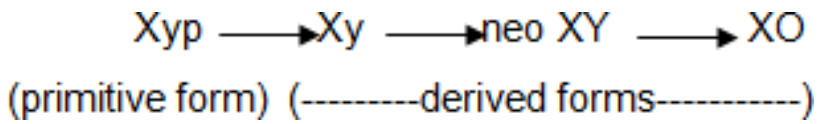
Sisyphus neglectus, Phanaeus igneus. **b.** Autosome – X fusion and formation of Neo XY, is depicted by Sulcophanaeus spp., Haplidia etrusca, Phyllognathus silensis, Acrophileusus vervex vervex, Deltochilum valgum and Phanaeus spp. Along with X- autosome fusion, autosome- autosome fission(Sulcophaenus spp.), autosome – autosome fusion (Phanaeus spp., Deltochilum valgum and Acrophileusus vervex vervex) and no autosome autosome fusion (Haplidia etrusca and Phyllognathus silensis) were observed

ii. **Centric fission** or dissociation involve increase in chromosome number without changing the sex chromosomes as studied in Dynamopus athlete, Geotrupes spp. and Adoretusspp.

iii. **Pericentric inversion:** In which the change of karyotype occurred from metacentry to acrocentry without changing the sex chromosomes e.g. Troxspp.

iv. **Elimination of y chromosome:** XO sex chromosome mechanism, in which there is elimination of y chromosome from Xy to acquire XO sex chromosom system. It is reported in Apogonia spp. and Copris fricator.

So, the sex chromosome mechanism evolved as follows in the family Scarabaeidae



5. Cytotaxomony and phylogeneticrelationships

Phylogenetic classification reflects the best estimate of the evolutionary history of organisms. It is well known that chromosomal data can be used to establish interrelationships among related species for taxonomic purpose. On the other hand, the pattern of chromosomal divergence within a group may not necessarily parallel those of morphological features.

In early 1908, Mclung in an address “Cytology and Taxonomy” stressed out the importance of chromosome studies to clarify the taxonomic relationships. Based on the difference in the morphology of a single chromosome, he divided the orthopteran species Mermeria bivittata into two groups. The correlations between morphologic and karyologic evolution are very hard to understand. Benazzi (1957) opined that some

phylogenetic trends are associated with chromosome variations, but we cannot establish at least at present, to what extent and how these two events are linked to eachother.

Helwig (1958) pointed out some interesting parallels that exist between morphological characters and chromosome morphology and the use of latter in indicating taxonomic and phylogenetic relationships. John (1981) pointed out that morphologically primitive traits do not frequently correlates with karyological ones. Stebbins (1971) infers, "the chromosomal differences have a meaning entirely different from morphological, physiological and ecological differences". The latter represents the end products of long sequences of interaction between primary, secondary genes modified by the effects of environment development.

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 separationsof 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 suitableway.

Actually a closer look makes it clear that the taxonomic grouping should ideally be based on natural relationship of several groups at different levels. Therefore, the question of speciation cannot be considered in isolation from the phylogenetic relationships. The modern or synthetic view of organic evolution regards speciation as a special and perhaps usually brief stage in evolutionary divergence, during which genetic isolating mechanisms develop to a level which makes the phyletic separation of incipient species irreversible (White, 1968).

Structure of the chromosomes and their behaviour and hybridisation experiments have been well utilised to clarify certain puzzling relationships in Cleoptera. The puzzling status of Curculinoid genera Hylobius and Pissodes was finally settled on the basis of chromosome studies by Smith (1956c, 1959, 1962b). Drouin et al. (1963) identified a weevil of the genus Pissodes as P. terminalis only on cytogenetic basis. They remark "Although specimens from Saskatwan were grossly different in elytra pattern from California, it was identified as terminalis on cytogenetic basis".

The eastern North American Ladybird beetle Chilocorus stigma of the subfamily Coccinellinae of Coccinellidae has been shown to comprise an assemblage of subunits display sequential chromosomal polymorphism through incorporation of centric fusion (Smith 1956b, 1957 a, b, 1959). Similar situation has been further clarified in genus Exochomus, from India and Pakistan, and established at least five species in an assemblage which has classified into two species on the basis of external morphology (Smith 1965a,1966)

Although Scarabaeidae is well defined group of Coleoptera yet the origin and interrelationship of scarabs had been a matter of controversy from the very beginning and the matter is stillunresolved.

Gangalbaur (1903) included all the Lamellicornia under the general term of „Scarabaeidae" in the suborder Polyphaga. Erichson (1848) on the basis of posterior abdominal spiracles divided this group into two sections- the „Scarabaeides- Laprosticti" and the „Scarabeides- Pleurosticti". Arrow (1909) pointed out that this division did not correspond with any natural line of cleavage as several intermediate forms exist. Yet Arrow (1909, 1910) allowed this distinction for Indian fauna as no intermediate forms exist and two divisions may safely be used. Arrow (1910) grouped Lucanids, Pasilids and Scarabaeidsinacommondivision„Lamellicornia"bytakinginto consideration their lamellate antennae. Many taxonomists trace the ancestry of scarabs in Lucanids (Fowler 1912). While Lameere (1900) declined to accept this view, since Lucanids possess five visible ventral abdominal segments instead of six found in Scarabs. But Gangalbaur (1903) did not attach much phylogenetic significance to the number ofabdominal segments, as it is the result of varying length of elytra. When the elytra entirely cover the abdomen, segments are five in number, but if the apex of the abdomen in uncovered, they are more than five in number.

Sharp and Muir (1912) find close affinity between the scarab genus Trox and lucanids, based on an extensive study of male genetalia. They consider Troginae as the ancestral stock from which passalids, scarabids and lucanids evolved. However, Crowson (1955) support the age old view of considering lucanids as a common ancestor for passalids and scarabaeids. Possibly, Passalidae is a direct offshoot of the luccanid stem, specialised for a peculiar mode of life. The lucanids themselves would seem to be related to the remaining scaraboids indirectly through Troginae (Crowson 1960).

The available cytological data on Lucanids presents a wide spectrum of karyotypes, the haploid number ranging from 5 in Nipponodorcus rubrofemoratus (Abe et al. 1969) to 13 in Lucans maculifemoratus (Toshioka and Yamamoto 1937, Virkki 1959, 1967a). None of the Lucanids is reported to have the basic haploid number 10 (9+Xyp), which is the only number known in different species of Trox (Purcell and Virkki 1966, Virkki 1967a, Yadav and Pillai 1976b). It can, therefore, be said with reasonable certaininty that lucanids show scarcely any karyological relationship with trogines.

Another primitive group, Geotrupinae enjoys an isolated position among scarabs. Morphologically, presence of 11 segmented antennae (only exception with Plecoma) keeps them apart from other scarabs. Anatomically, broad and septate testicular follicles are unlike other laparostictean species (Virkki 1957). Cytologically, 2n= 22 in different species of Geotrupes and Thorectes intermedius, possibly with some acrocentric chromosomes (Virkki 1960, Bione et al. 2005 a) is a notable deviation from the „modal"

karyotype 2n=20 all metacentrics. Thus, lucanids, trogines and geotrupines share species with basic scarab karyotype.

It seems that evolution has taken place in both these groups independently along different lines. In trogines, a series of pericentric inversions or „centromeric shift" changed the morphology in different species without altering the number of chromosome number (Purcell and Virkki 1966, Virkki 1967a). On the contrary in Geotrupinae, the evolution has progressed towards an increase in the number of chromosomes from 2n=20 in Bolboceras indicum and B. quadridens (present report) to Geotrupes (Virkki 1960), Thorectes intermedius (Bione et al. 2005a). Virkki (1959) assigned this increase to the fragmentation of two metacentrics. In view of the presence of acrocenrics in Geotrupes spp. and Thorectes intermedius, this seems to be a credible hypothesis. The cytological observations seem to support Medvedev (1976) who on the basis of larval characters maintains that Troginae and Geotrupinae have diverged earliest from otherLaprosticti.

Dynamopus, a primitive genus with limited species, was variously classified with Hybosorinae and Orphinae. Arrow (1911) separated these beetles and created a new subfamily Dynamopinae. On the cytological grounds, in having a common chromosome number 2n= 22, these beetles seem to be more closely related to Geotrupinae than to Hybosorinae or Orphinae. Paulian (1941) proposed Hybosorinae as a connecting link between Geotrupinae and rest of the scarabs. Since cytological knowledge of Hybosorinae is limited to only one species Hybosorus orientalis (present report), nothing definite can be said in thisregard.

The dung beetles (Scarabaeinae) include 5000 species and exhibit a diverse array of morphologies and behaviours. This variation presumably reflects the adaptation to a diversity of food types and the different strategies used to avoid competition for vertebrate dung, which is the primary breeding environment for most species. Monaghan et al. (2007), presented a molecular phylogenetic analysis of 214 species of Scarabaeinae, representing all 12 traditionally recognized tribes and six biogeographical regions, using partial gene sequences from one nuclear (28S) and two mitochondrial (cox1, rrnL) genes. Length variation in 28S (588–621 bp) and rrnL (514–523 bp) was subjected to a thorough evaluation of alternative alignments, gap-coding methods, and tree searches using model-based (Bayesian and likelihood), maximum parsimony, and direct optimization analyses. It has been suggested that the Scarabaeinae arose from mycetophagous ancestors (Scholtz and Chown, 1995). The other basal member recovered consistently was Sarophorus, thought to be a detritus feeder (old dung and carrion remains). Frolov (2004) also considered Sarophorus and Coptorhina to be sister taxa. The phylogenetic tree given by Monaghan et al. (2007) revealed that tribe Gymnopleurini is more close to Phanaeini and tribe Scarabaeini is close to Coprini whereas some members of Onthophagini are close to Oniticellini and others are close to tribe Onitini. Decrease in the chromosome number both in some species of Gymnopleurus and Phaenus support the phylogentic relationship given by Monaghan et al. (2007), similarly dominance of diploid number 20 and XO sex

mechanism in some of the species of tribes Scarabaeini and Coprini bring them more closer, whereas tribes Onthophagini, Oniticellini and Onitini have predominantly 20 chromosome number and Xyp sexmechanism.

Peringuey (1904), in the description catalogue of Coleoptera of South Africa, presented an elaborate account of subfamilies Pleurostict Scarabaeidae and retained Melolonthinae, Sericinae, Rutelinae, Dynastinae and Cetniinae as its subfamilies.

Fowler (1912) placed Melolonthinae along with Glaphyrinae and Oncerinae between the laprostict scarabids. His view is almost in conformity with the classification adopted earlier by Leconte and Horn (1883) placing the first tribe under Pleurostict Melolonthinae and last two under Laprostict Melolonthinae. Arrow (1910), however, Melolonthinae in Pleurosticti and placed it at the bottom of the group. On the basis of available data, Melolonthinae has cytological closenesswithhigherlaprostictiviz.Aphodiinaeinwhichall chromosomaly known species except Aphodius moestus (present report, Yadav et al. 1993b) possess the „modal number". The chromosomal relationships with Coprinae (Scarabaeinae), another laprostict group, is however, obscure.

Anomalini and Adoritini, the two tribes of the subfamily Rutelinae, share same common characteristics, but they are sharply divided by their mode of feeding (Arrow 1917). In old classification they were out at opposite ends of the subfamily separated by the tribe Parastasiini. Arrow (1917), however, brought these together and added a new tribe Adorhinyptini which is constituted by three species (originally described as species of Rhinayptia belonging to Anomalini) that exhibit a remarkable combination of characteristic features of Anomalini and Adoritini. In possessing a flat horizontal labrum Adorrinayptia is typically similar to anomaline, but the elytra do not have membranous margins which is an invariable mark of the Anomalini. Resemblance with Adoretini is in the presence of last abdominal spiracle being situated close to the hinder margins of penultimate segment, the sculpture of elytra and form of claws. On the cytological level, Adorrhinyptini should be considered as closely related to Adoretini, since both groups have a uniform karyotype 2n=22 (10+Xyp). The evolutionary line in these groups must have progressed towards an increase in chromosome number, probably through dissociation. Kacker (1970) ruled out the above possibility as he could not observe any acrocentrics in Adoretus incurvatus and A. versutus (Yadav and Pillai 1976a) and Adoretus sp. (present report), however, is suggestive of their origin through fragmentation. In other species secondary structural rearrangements like pericentric inversions might have accompanied dissociation. Similar observation were made in Geotrupinae genera Geotrupes and Thorectes and Dynamopinae genus Dynamopus where all cytologically known species have uniform 2n=22, but there does not exist any taxonomic closeness between these groups. This situation has a similarity in mammalian order Carnivora when Wurster and Benirschke (1968) reported these species belonging

to three different families possessing very similar karyotypes. These are cases of parallelism in karyotype evolution in completely independent groups.

Cetoniinae considered being the most advanced subfamily among Pleurosticti (Medvedev 1976). The karyotype, being composed of comparatively very small chromosomes (Yadav and Pillai 1977a), considered to be an advanced character (Stebbins 1971), supports this view. As such the modal number in this case may have secondary origin.

Phylogenetic relationships like connecting link Hybosorus orientalis between subfamilies Geotrupinae and Scarabaeinae was confirmed on the basis similarities and dissimilarities in the karyotypes of the species. 21 species are new records ions in the cytological data of the Coleoptera.

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