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Mechanisms of karyotype differentiation in Cassidinae *sensu lato* (Coleoptera, Polyphaga, Chrysomelidae) based on seven species of the Brazilian fauna and an overview of the cytogenetic data

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ABSTRACT

Among the subfamilies of Chrysomelidae, Cassidinae *sensu lato* (*s.l.*) includes 6000 species distributed in 43 tribes. Approximately 100 of these species were cytogenetically analyzed and most of them presented $2n = 18 = 16 + Xy_p$, which was smaller than $2n = 20 = 18 + Xy_p$ considered basal for Polyphaga. However, some groups of species presented maintenance of the basal diploid number and others showed increase in this number. Certain species of the latter group also exhibited variation in the type of sex chromosome system (SCS). Considering the recent taxonomic revision accomplished for the Cassidinae *s.l.* species, the existence of phylogenetic relationship for some species of this subfamily, the high diversity of species of this group in the Neotropical region, and the low number of Cassidinae *s.l.* species karyotyped so far, the aim of the present work was to establish the main mechanisms involved in the karyotype evolution of this subfamily through the study of seven species of the Brazilian fauna and overview of the cytogenetic data. The individuals were collected in southeast and south of Brazil. The chromosomal preparations obtained from embryo and testes of adult males were stained with Giemsa solution. The species *Agroiconota inedita* ($2n = 42 = 40 + Xy_p$), *Charidotella* (*s.str.*) *immaculata* ($2n = 22 = 20 + Xy_p$), *Charidotella* (*s.str.*) *sexpunctata* ($2n = 22 = 20 + Xy_p$), and *Stolas chalybaea* ($2n = 24 = 22 + Xy_p$) revealed diploid number higher than that established as basal for Polyphaga and biarmed chromosomes. The karyotype of *Cteisella confusa*, *Deloyala cruciata*, and *Metriona elatior* showed the chromosomal formulae $2n = 18 = 16 + Xy_p$ considered modal for Cassidinae *s.l.* and biarmed chromosomes. The seven species exhibited easily identified sex chromosomes due to their size and/or morphology. The analysis of meiotic cells of all the species showed pachytenes with a positively heteropycnotic block probably corresponding to the sex chromosomes; diplotenes with a high number of bivalents with two chiasmata and sex chromosomes in a parachute configuration, and metaphases II that confirmed the chromosomal morphology, the type of SCS, and the regular segregation of all chromosomes. The data regarding to the number and morphology of the chromosomes, their behaviour during meiosis, and type of SCS were inedit for the majority of these species. In relation to the all Cassidinae *s.l.* species that presented SCS of the Xy_p type, *A. inedita* was that with the highest diploid number. Furthermore, this work reported for the first time the cytogenetic information of representatives of the genera *Cteisella* and *Metriona*. Taking into account the phylogenetic and cytogenetic data of Cassidinae *s.l.* species, the karyotype differentiation of this group seems to have occurred from the basal karyotype of Polyphaga by decrease in the chromosome number and subsequent increase in this number. Pericentric inversion, centric fusion and fission seem to have been the main mechanisms that promoted the evolution of the autosomes. However, in the sex chromosome evolution, the mechanisms involved were centric fission and/or chromosomal translocation.

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1. Introduction

Chrysomelidae is the second most numerous family within Coleoptera, including more than 35,000 described species that are distributed in 16 subfamilies (Seeno and Wilcox, 1982). Among these subfamilies, 14 have representatives that were cytogenetically analyzed, whose karyotype information was compiled by Petitpierre et al. (1988). Most of these subfamilies have very interesting cytogenetic characteristics. One of them refers to the chromosome number, which can be extremely diversified in Galerucinae (*sensu* Gómez-Zurita et al., 2008), $2n♂ = 8 = 6 + Xy$ to $2n♂ = 64 = 60 + X_1 + X_2Y + B$, due to the variation in the number of autosomes and/or presence of supernumerary chromosomes, or higher than that considered basal for Polyphaga, $2n♂ = 20 = 18 + Xy_p$, in Cryptocephalinae, whose species predominantly showed $2n♂ = 30 = 28 + Xy_r$. Other feature is related to the types of sex chromosome system (SCS), with preponderance of Xy_r system in Cryptocephalinae, X0 system in Chrysomelinae, X + y and other diversified multiple systems in Galerucinae (*sensu* Gómez-Zurita et al., 2008). These characteristics indicate that different trends of karyotype differentiation occur in these subfamilies. Moreover, the knowledge of these particularities is extremely important to establish the mechanisms involved in the species karyotype differentiation during the evolutionary process.

Among the subfamilies of Chrysomelidae, Cassidinae *sensu lato* (*s.l.*) comprises *ca* 6000 species taxonomically described that are distributed in 43 tribes (Chaboo, 2007). According to this latter author, Cassidinae *s.l.* includes species originally placed by Seeno and Wilcox (1982) in the subfamilies Cassidinae and Hispinae, currently designated as Cassidinae *sensu stricto* (*s.str.*) and Hispinae *sensu stricto* (*s.str.*), respectively. Thus, Cassidinae *s.l.* is constituted by Cassidinae *s.str.* and Hispinae *s.str.* Of the species of Cassidinae *s.l.* known, only 101 belonging to 13 tribes were investigated under the cytogenetic point of view. The karyotype data of cassidines mainly refer to the diploid number that varied from $2n♂ = 16$ to $2n♂ = 51$, and types of SCS that can be Xy_p, Xy, X0, Xy_c, Xy_r, neoXY, XY, Xyy_p, $neoX_{p1}neoX_{p2}neoXneoY_p$, and other multiples, existing few records on chromosomal morphology (Table 1). Nevertheless, the majority of the species of Cassidinae *s.l.* surprisingly showed a diploid number smaller than that considered basal for Polyphaga, and maintenance of the Xy_p system. These data indicate that probably the karyotype differentiation in Cassidinae *s.l.* occurred mainly by alteration in the autosome number, considering that 87% of its species showed this kind of variation without changing the type of SCS. On the other hand, all the karyotyped species of the tribe Stolaini of this subfamily (Table 1) showed a diploid number higher than the basal, $2n = 20 = 18 + Xy_p$, and additionally, the species of *Botanochara* exhibited different types of multiple SCS. Certainly, the cytogenetic analysis of species that belong to different genera of Cassidinae *s.l.* will provide additional information concerning to the mechanisms involved in the karyotype evolution of related species.

The phylogenetic position of the Cassidinae *s.l.* species was recently proposed by Chaboo (2007) and Gómez-Zurita et al. (2008) and there is a phylogenetic relationship for 92 species of this group. Of the 101 karyotyped species of this subfamily, only 10% were from Neotropical region, in spite of their high diversity in this region (Chaboo, 2007). Considering that an evolutionary approach involving the variation of the chromosome number and morphology, types of SCS, and phylogenetic relationship among the Cassidinae *s.l.* species was not elaborated yet, the aim of the present work was to investigate the main mechanisms responsible for the chromosomal evolution of the species of this group by means of analysis of seven species of the Brazilian fauna and overview of the cytogenetic data of this subfamily.

Table 1
Species of Cassidinae *sensu lato* (Chaboo, 2007) cytogenetically analyzed, with their respective different designations of those considered currently valid (DD), diploid number (2n) and types of sex chromosome system (SCS) in males, chromosomal morphology (CM), and provenance (P).

Species	DD	2*n* and SCS	CM	P	References
Family Chrysomelidae					
Subfamily Cassidinae *sensu lato*					
Cassidinae *sensu stricto*					
Tribe Aspidimorphini					
Aspidimorpha (*s.str.*) *difformis* (Motschulsky, 1860)	*Aspidomorpha difformis*	18	–	Japan	Takenouchi and Shiitsu (1972)
Aspidimorpha (*s.str.*) *dorsata* (Fabricius, 1787)	*Aspidomorpha dorsata*	$16 = 14 + Xy$	$12M + 2A + XSM$	India	Yadav et al. (1995)
A. (*s.str.*) *dorsata*	*A. dorsata*	$18 = 16 + Xy_p$	–	India	Yadav et al. (1987)
Aspidimorpha (*s.str.*) *furcata* (Thunberg, 1789)	*Aspidomorpha furcata*	$18 = 16 + Xy_p$	$10M\text{-}SM + 6A + X(?) + y(?)$	India	Dasgupta (1973) and Manna and Lahiri (1972)
Aspidimorpha (*s.str.*) *indica* (Boheman, 1854)	*Aspidomorpha indica*	$18 = 16 + Xy_p$	–	India	Dua and Kacker (1975, 1976)
A. indica	*A. indica*	$18 = 16 + Xy$	$16M + XM$	India	Dua and Kacker (1977)
Aspidimorpha (*s.str.*) *miliaris* (Fabricius,1775)	*Aspidomorpha miliaris*	$18 = 16 + Xy_p$	–	India	Manna and Lahiri (1972)
A. (*s.str.*) *miliaris*	*A. miliaris*	$18 = 16 + Xy$	$10M + 6SM + XM$	India	Dua and Kacker (1975, 1976, 1977)
Conchyloctenia nigrovittata (Boheman, 1854)	–	$18 = 16 + Xy_p$	$16M + XM + y(?)$	India	Gill et al. (1987)
C. nigrovittata	*Aspidomorpha nigrovittata*	$16 = 14 + Xy$	$14M + XSM$	India	Yadav et al. (1995)
Laccoptera (*Laccopteroidea*) *nepalensis* (Boheman, 1855)	*Laccoptera 4-maculata*	$18 = 16 + Xy_p$	–	–	Smith and Virkki (1978)
L. (*Laccopteroidea*) *nepalensis*	*Laccoptera quadrimaculata*	$18 = 16 + Xy_p/19 = 16 + Xyy_p$	–	India (West)	Sharma and Sood (1978)
L. (*Laccopteroidea*) *nepalensis*	*L. quadrimaculata*	$18 = 16 + Xy_r/19 = 16 + Xy_r + 1B$	$16M\text{-}SM + XM\text{-}SM + y(?)$	India (Northeast)	Dey (1986)
L. (*Laccopteroidea*) *nepalensis*	*L. quadrimaculata*	$18 = 16 + Xy_p$	$10M + 6SM + XM + y(?)$	India	Gill et al. (1987)
L. (*Laccopteroidea*) *nepalensis*	*L. quadrimaculata*	$18 = 16 + Xy_p$	–	India	Yadav et al. (1987) and Kacker (1976)
L. (*Laccopteroidea*) *nepalensis*	*L. quadrimaculata*	$18 = 16 + Xy$	–	India	Dua and Kacker (1975)