SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Karyotype, Heterochromatin Distribution and Meiosis of *Zabrotes subfasciatus* (Bohemann) (Coleoptera: Chrysomelidae, Bruchinae)

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Neotropical Entomology 37(5):546-551 (2008)

Cariótipo, Distribuição da Heterocromatina e Meiose de *Zabrotes subfasciatus* (Bohemann) (Coleoptera: Chrysomelidae, Bruchinae)

RESUMO - *Zabrotes subfasciatus* (Boh.) é estudada intensivamente em termos agronômicos e bioquímicos por causar danos aos grãos de leguminosas armazenados. No entanto, os dados publicados sobre o seu cariótipo são escassos e conflitantes. Assim, o objetivo deste estudo foi descrever o cariótipo e a meiose desse inseto e analisar o padrão de bandas-C de seus cromossomos. Foram analisados os gânglios cerebrais de pré-pupas e os testículos de adultos e pupas com adaptação de uma técnica que permitiu boa qualidade de preparo dos cromossomos dessa espécie. Todos os indivíduos apresentaram 26 cromossomos nas metáfases mitóticas. Esses cromossomos foram classificados em: acrocêntricos (cromossomo X); submetacêntricos (pares 4 e 5); subtelocêntricos (par 12 e cromossomo y); metacêntricos (demais pares). O cromossomo 5 apresentou uma constrição secundária. Todos os cromossomos apresentaram heterocromatina próximo ao centrômero e os cromossomos 5, 9 e X, nos braços longos. O cromossomo X mostrou-se heteropicnótico durante toda a prófase da primeira divisão meiótica. As subfases da prófase I foram pouco distintas e a meiose II de difícil identificação. Os testículos de todos os machos apresentaram poucas células. Os bivalentes apresentaram a forma de bastão na metáfase I. O seu cariótipo constitui-se de 26 cromossomos, sendo as fórmulas cariotípicas 2n = 24 + XX nas fêmeas e 2n = 24 + Xyp e n = 12 + X ou n = 12 + y nos machos.

PALAVRAS-CHAVE: Insecta, caruncho-do-feijoeiro, cromossomo, banda-C, sistema Xyp

ABSTRACT - *Zabrotes subfasciatus* (Boh.) has been extensively studied in its agronomic and biochemical aspects due to its importance as a damaging insect to leguminous grains during storage. The few cytogenetic studies published on this species yielded conflicting results. In this study, the karyotype was analyzed in order to accurately describe the chromosome C-banding patterns and meiosis. The brain ganglion at the prepupa and the adult and pupal testes were analyzed. All individuals had 26 chromosomes in both brain ganglion and spermatogonic mitotic metaphases. These chromosomes were classified as follows: the 12th pair and the Y chromosome were telocentric; the X chromosome was acrocentric; the 4th and 5th pairs were submetacentric; and the remaining pairs were all metacentric. One of the members of the 5th pair presented a secondary constriction. All chromosomes presented pericentromeric heterochromatin. The large arms of the pairs 5, 9 and X presented heterochromatin. The X chromosome showed to be heteropyknotic throughout the prophase of the first meiotic division. The subphases of prophase I were atypical and meiosis II was rarely identified. Testes of all males showed a few cells; the bivalents were rod-like shaped in metaphase I. Karyological formulae were 2n = 24 + XX in females and 2n = 24 + XYp and either n = 12 + X or n = 12 + Y in males.

KEY WORDS: Insecta, Mexican bean weevil, chromosome, C-banding, Xyp system

The Mexican bean weevil Zabrotes *subfasciatus* (Boh.) is a bruchid endemic to the tropical and subtropical regions of Central and South America (Credland & Dendy 1992, Romero & Johnson 2000). *Z. subfasciatus* is cosmopolitan due to the commerce of infested cultivated seeds (Romero & Johnson 2000, Aebi *et al*. 2004). This insect feeds on Fabaceae seeds (legume), causing expressive damage to stored grains. It is a major post-harvest pest of common bean (*Phaseolus vulgaris* L.), lima bean (*P. lunatus* L.) and cowpea seeds (*Vigna unguiculata* L.) (Macedo *et al*. 2007). Several studies on the Bruchinae subfamily have focused on the insect-plant interactions and its economic importance that include the evaluation of the effects of natural compounds of the grain on the insect reproduction and development

(Weaver *et al*. 1994), the resistance of grains to feeding and grain alpha amylase inhibitors (Ishimoto *et al*. 1995), and the coevolution of *Zabrotes* genes and plant resistance genes (Suzuki *et al*. 1994). More recently, genetic studies using molecular markers have investigated the degree of genetic differentiation among *Z. subfasciatus* populations using allozymes (Gonzalez-Rodriguez *et al*. 2002) and microsatellites (Aebi *et al*. 2004). Phylogeographic studies using nuclear and mitochondrial genes were also conducted (Alvarez *et al*. 2006). Cytogenetic studies of this species, however, remain largely unexplored.

Previous cytogenetic analyses of *Z. subfasciatus* yielded conflicting results. Minouchi (1935) described a karyotype with 2n = 24 + XO in males and 2n = 24 + XX + 0/1 B chromosomes in females. Takenouchi (1972) reported that both *Z. subfasciatus* males and females invariably presented 26 chromosomes, the female karyological formula being 2n = 24 + XX and the male 2n = 24 + XYp. Smith & Virkk (1978) described this species as presenting atypical chromosomal complement with one or more B chromosomes not homologous to those in the regular set, which behave cyclically similarly to the Yp chromosomes of the referred species.

Lack of consensus in cytogenetic data frequently occurs when conclusions are drawn based on low-resolution chromosome preparations, which usually result from the use of inappropriate techniques that render the interpretation of the results difficult. In this study, we describe the karyotype, C-banding patterns, and meiosis of *Z. subfasciatus*. We adapted the air drying technique described by Imai *et al*. (1988) to improve mitotic chromosome visualization to obtain a more reliable description of the karyotype and meiosis of *Z. subfasciatus*. To further characterize heterochromatin occurrence and distribution, we used a modified Sumner (1972) C-banding technique.

Material and Methods

Adult specimens of *Z. subfasciatus* were obtained from infested beans collected in two locations in the state of Minas Gerais, Brazil: Viçosa (20°45'S; 42°52'W) and Santa Bárbara do Leste (19°58'S; 42°08'W). The two groups, one from each location, were reared on dry bean seeds (*P. vulgaris*) in clear glass jars covered with cotton cloth under laboratory conditions (natural photoperiod, 26°C:12°C day/night, 77 ± 5% RH). The developmental stages were synchronized controlling oviposition.

Metaphasic chromosomes of 34 individuals were obtained from cerebral ganglion cells at the prepupal stage. Adults were sexed by the morphology of the gonads. Meiotic cells were analyzed in the testes of eight recently emerged adults from each population and also from four pupae sexed according to Pacheco & Paula (1995).

The mitotic and meiotic chromosome preparations followed Imai *et al*. (1988). A few modifications were introduced in the aforementioned protocol, such as the treatment of the cerebral ganglia in colchicine/hypotonic solution (0.005%) for 80 min and the treatment of the testes in a hypotonic solution without colchicine for 80 min. After the colchicine/hypotonic solution or hypotonic solution treatment, the organs were transferred to a dry slide with the aid of a Pasteur pipette and dissociated with the use of dissecting needles in three fixative solutions as described by Imai *et al*. (1988).

C-banding. The C-banding technique described by Sumner (1972), and modified by Rozec (1995), was performed with modifications to adjust the technique to the material used in this study. The slides prepared as described above were placed for hydrolysis in 0.2 N HCl for 2 min, washed in distilled water for 2 min at room temperature, incubated in 5% barium hydroxide at 40°C for 3 min, washed in 0.2 N HCl for 30s and then in distilled water for 1 min at room temperature, incubated in 2XSSC at 60°C for 13 min and washed in distilled water for 1 min at room temperature. Slides were air-dried and stained with Giemsa (8%) for 15 min.

Results and Discussion

Karyotype and C-banding patterns. The cerebral ganglia of 15 male and 19 female pupae were analyzed, enabling the observation of 21 metaphases in males and 28 in females. All individuals analyzed from both populations presented a diploid number of 26 chromosomes. The morphometric analysis (Table 1) showed chromosomes of three distinct sizes according to the total relative length (T): large, T > 10; medium, 5 < T < 10; and small, T < 5. The autosomes were classified as telocentric (12th pair), submetacentric (4th and 5th pairs) and metacentric (the remaining pairs) (Table 1). One chromosome of the 5th pair shows a secondary constriction possibly corresponding to one NOR. This constriction was evident for both homologous chromosomes in females' metaphases. In the females, the sexual pair is formed by homologous chromosomes while males present a large X chromosome and a small Y chromosome (Table 1). The sex chromosomes were classified as acrocentric (X) and telocentric (y) (Fig.1).

The karyotype of *Z. subfasciatus* is asymmetrical (Fig. 1), because the 12th pair and the allosomes showed a discrepant size in relation to the other eleven chromosome pairs that presented a gradual size variation. Smith & Virkki (1978) suggested that the symmetrical karyotypes appeared secondarily in Coleoptera. Further cytogenetic studies including other bruchids as well as a resolved phylogeny for the family will enhance our ability to elucidate whether *Z. subfasciatus* is a karyotypically conserved species.

Z. subfasciatus was first cytogenetically described by Minouchi (1935), who used tissue slices impregnated with paraffin and presented the male karyological formula 2n = 12_{II} + XO + 0/1 extra chromosome. That description was confirmed by Smith & Virkki (1978). However, Takenouchi (1972) using the smearing technique did not find any B chromosomes and observed the karyotypes 2n = 24 + XX in females and 2n = 24 + Xyp in males. In the present study, the technique applied made it possible to obtain better

Table 1. Chromosome relative length of *Z. subfasciatus* 2n♀ = 24 + XX e 2n♂ = 24 Xyp (T - total relative chromosome length; L - relative length of large arm; S - relative length of short arm; R - arm ratio, L/S)

Group*	No. pair	T	L	S	R	Classe
I	1	12.29 ± 0.59	6.19 ± 0.30	6.10 ± 0.32	1.02 ± 0.03	M
I	2	10.27 ± 0.61	5.60 ± 0.31	4.67 ± 0.31	1.20 ± 0.04	M
II	3	9.14 ± 0.20	5.41 ± 0.41	3.73 ± 0.29	1.46 ± 0.23	M
II	4	8.63 ± 0.55	5.14 ± 0.14	3.48 ± 0.50	1.50 ± 0.24	SM
II	5	7.64 ± 0.30	4.61 ± 0.57	3.03 ± 0.49	1.57 ± 0.42	SM
II	6	7.58 ± 0.55	4.00 ± 0.43	3.57 ± 0.15	1.12 ± 0.09	M
II	7	7.02 ± 0.27	3.98 ± 0.71	3.04 ± 0.59	1.39 ± 0.59	M
II	8	6.71 ± 0.35	3.67 ± 0.19	3.04 ± 0.23	1.21 ± 0.10	M
II	9	6.33 ± 0.17	3.62 ± 0.33	2.70 ± 0.23	1.36 ± 0.23	M
II	10	5.68 ± 0.57	2.93 ± 0.36	2.75 ± 0.26	1.07 ± 0.09	M
II	11	5.37 ± 0.68	2.88 ± 0.37	2.49 ± 0.33	1.16 ± 0.06	M
III	12	2.60 ± 0.20	2.60 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	T
I	X	10.24 ± 0.46	8.20 ± 0.32	2.04 ± 0.16	4.03 ± 0.19	A
III	Y	1.39 ± 0.31				T

* (I) large, T > 10; (II) medium, 5 < T < 10; (III) small, T < 5

chromosome preparations with a preserved morphology and a complete spreading (Fig. 1) and allowed an accurate karyotype description of this species.

C-banding revealed that all chromosomes presented pericentromeric heterochromatin. The y chromosome, the X long arm, and a large part of the long arms in 5th and 9th pairs are heterochromatic (Fig. 2 and Fig. 3). C-bandings corresponded to Q-bandings and DAPI markings (data not shown) indicate a possible occurrence of an AT-rich heterochromatin in this species.

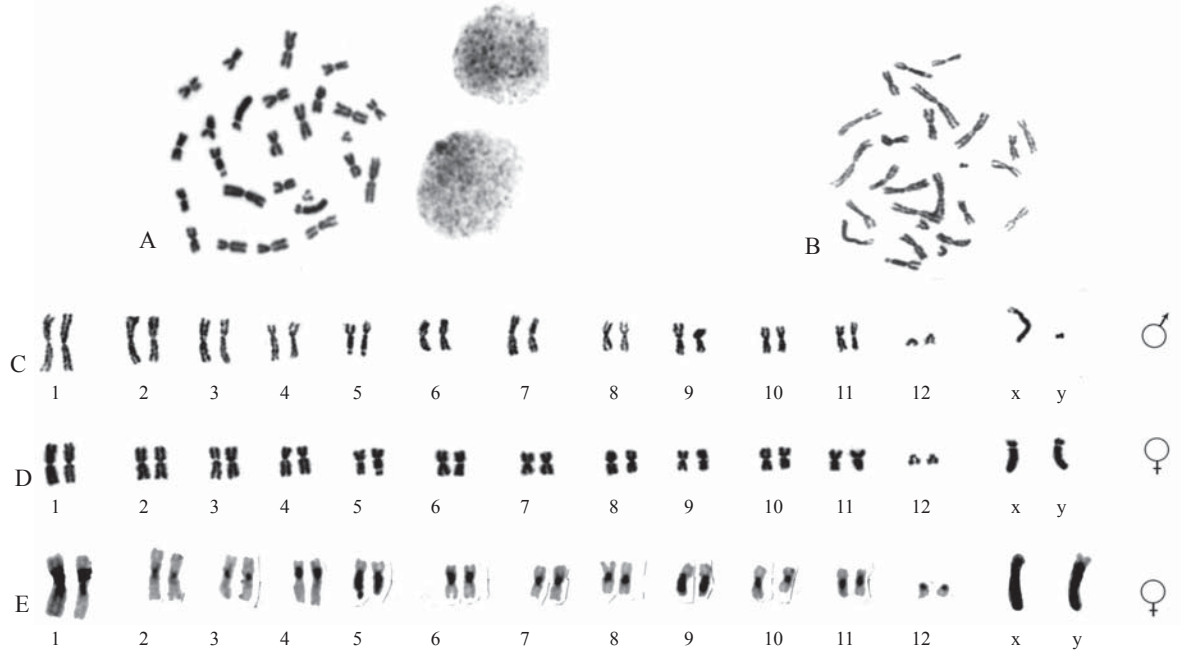

Fig. 1. *Z. subfasciatus* mitotic metaphase chromosomes and karyotype. (A) metaphasic plaque of a female; (B) metaphasic plaque of a male; (C) karyotype of a male and (D) a female, autosome pairs are numbered from 1 to 12 and sex chromosomes designated X and y; (E) female karyotype exhibiting C-bandings.

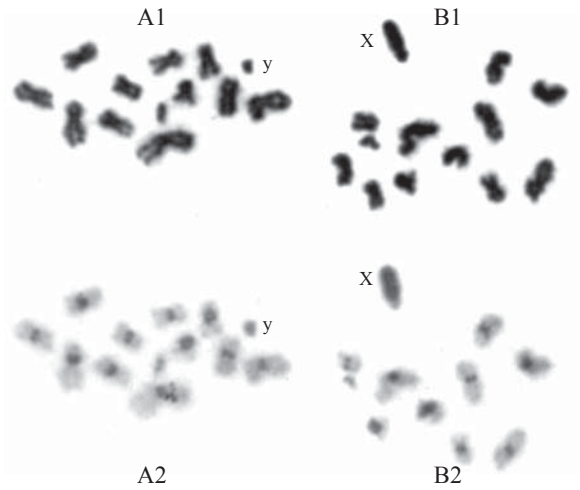

Fig. 2. Meiosis II in *Z. subfasciatus*: metaphase II exhibiting the Y-chromosome (A) and the X chromosome (B). The index 1 indicates conventional staining and the index 2 indicates C-banding. A1 and B1 are the same A2 and B2 metaphasic plaques.

Meiosis. The testes of 16 adults and four male pupae were analyzed. In three spermatogonial metaphases, 26 chromosomes were found. In the meiotic cells, the X chromosome showed to be heteropyknotic throughout prophase I. The bivalents presented a conventional form at the first metaphase (rod-like). The bivalent sex chromosome forming a "parachute" was clearly distinguished in metaphase I (Fig. 4A). Metaphases obtained at mitosis and meiosis II showed correspondent morphology, centromere sites and C-banding patterns.

In the present analysis, B chromosomes were not found, in contrast to Smith & Virkki (1978). This might be a result of increased resolution in the current technique.

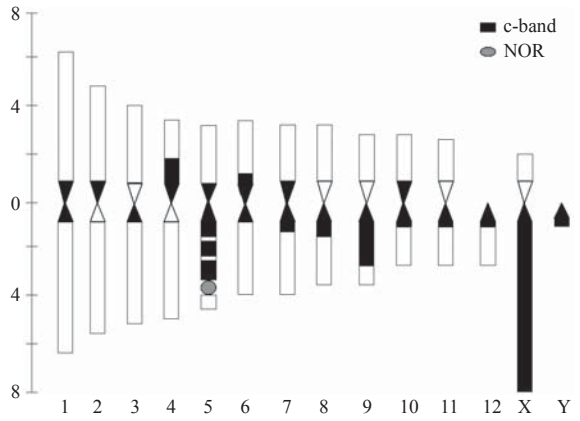

Fig. 3. Ideogram showing the asymmetrical karyotype and the heterochromatic regions according to the C-banding pattern.

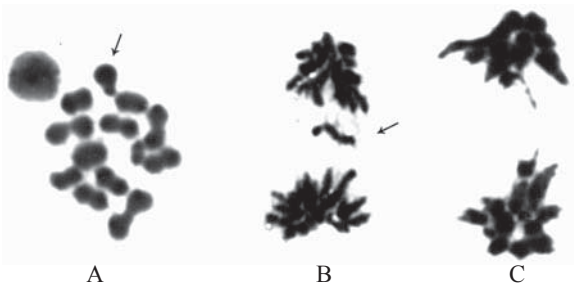

Fig. 4. Meiosis I in *Z. subfasciatus*. (A) Metaphase I with the sexual pair on parachute (arrow); (B) X chromosome undergoing a late anaphase I in relation to the autosomes (arrow); (C) A balanced chromosome segregation at the end of anaphase.

Nevertheless, it is possible that there is karyotypic variation within this species. Our data indicate that the only chromosome that was not entirely homologous behaved cyclically and presented a "parachute" structure in males that indicates a Xyp-type sex determination in this species. The most characteristic male sex-determining system of Coleoptera, the "parachute-like" Xyp, represents a non-chiasmatic association of a generally metacentric X and a small and mostly metacentric y-chromosome (Petitpierre *et al*. 2004, Palomeque *et al*. 2005). Rozec (1994) and Lachowska & Holecová (2000), analyzing the chromosomes in three beetle species in the genus *Phyllobius*, found the Xyp system of sex determination and pointed out that this is the most common form in Coleoptera. This "parachute" conformation of sexual chromosomes has been associated with the presence of the nucleolar organizing region sites (NORs) by some investigators (John & Lewis 1960, Smith & Virki 1978). Maffei *et al*. (2001) mapped rDNA genes in meiotic metaphases of *Olla v-nigrum*, using FISH and Ag-NOR staining and identified active NORs in the sex bivalent during meiosis. The "parachute" configuration of sexual chromosomes has been reported for other coleopterans such as *Eriopis connexa* Mulsant (Mafei *et al*. 2000); some species in the genus *Chrysolina* (Chrysomelidae) (Petitpierre *et al*. 2004, Palomeque *et al*. 2005); several species in the genus *Timarcha* (Chrysomelidae) (Gomez-Zurita *et al*. 2004); in *Phyllophaga* (*Phytalus*) *vestita* (Moser) and *Phyllophaga* sp. *aff. capillata* (Scarabeidae) (Moura *et al*. 2003); various species in the genus *Cyrtonus* (Chrysomelidae) (Petitpierre & Garneria 2003); in *Epicauta atomaria* (Germar) (Meloidae) and *Palembus dermestoides* (Fairm.) (Tenebrionidae) (Almeida *et al*. 2000); and also for some species in the genus *Monochamus* (Cerambicydae) (Cesari *et al*. 2004). Nevertheless, there has been some questioning of the nucleolar theory by other investigators based on evidence of the occurrence of nucleoli in a pair of autosomes in other species of Coleoptera (Maffei *et al*. 2004). The localization of NOR sites in *Z. subfasciatus* will be accomplished most readily by further studies using NOR banding. Even though these studies have not been carried out yet, the occurrence of a secondary constriction in the 5th chromosome pair indicates the presence of such sites.

Although the X and y chromosomes undergo a late anaphase I regarding autosomes (Fig. 4B), a balanced segregation follows (Fig. 4C) producing cells with the typical chromosome number (Fig. 2). Forer (1980) verified that in crane flies the autosomes separate first than the sex chromosomes, but at the end of the meiosis, the sexual chromosomes segregate normally, each going to a respective cell. In the case of *Z. subfasciatus*, our data indicate that this time-lagged phenomenon also occurs in the migration of the sexual chromosomes. However, the separation should proceed normally as in metaphase II cells with only one sexual chromosome were always observed.

The subphases of prophase I were somewhat distinct while the phases of meiosis II were difficult to identify. Testes tissue presented just a few meiotic cells as described by Takenouchi (1972).

In diplotene and diakinesis, the chromosomes occurred as a mass, so it was not possible to examine the chiasma frequency at that stage. According to Smith & Virkki (1978), the Coleoptera are not favorable for crossing over studies because the diplotene is commonly diffuse, which makes it difficult to observe the chiasma terminalization process. In metaphase I (Fig. 4A), the chromosomes were connected in the extremities.

Meiotic cells were not found in the ovaries analyzed. Observations of two female ovaries indicated that the oögonium metaphase has invariably 26 chromosomes.

All individuals exhibited the karyotypes 2n = 24 + XX in females as well as 2n = 24 + Xyp, and either n = 12 + X or n = 12 + y in males.

Acknowledgments

We would like to thank Luiz Fernando Gomes for his helpful criticisms and suggestions and other colleagues of the Laboratório de Citogenética de Insetos for their technical assistance and friendship. Thanks are also due to Cristina Esquetino Bastos for identifying the insects used in this study. We thank Carter R. Miller and an anonymous reviewer for their helpful comments on the manuscript. This research was supported by FAPEMIG. R.X.C. was awarded a CNPq fellowship.

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Received 11/IX/07. Accepted 09/IX/08.