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Evaluation of hot saline solution and restriction endonuclease techniques in cytogenetic studies of *Cycloneda sanguinea* L. (Coccinellidae)

E.M.D. Maffei[1] and S.G. Pompolo[2]

[1]Departamento de Ciências Naturais,
Universidade Estadual do Sudoeste da Bahia,
Vitória da Conquista, BA, Brasil
[2]Departamento de Biologia Geral, Universidade Federal de Viçosa,
Viçosa, MG, Brasil
Corresponding author: E.M.D. Maffei
E-mail: maffeiemd@hotmail.com

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ABSTRACT. The goal of the present study was to determine if simple methods, especially hot saline solution (HSS) and *Msp*I and *Hae*III restriction endonucleases, which do not require special equipments, may be helpful in studies of genetic variability in the lady beetle, *Cycloneda sanguinea*. The HSS method extracted the heterochromatin region, suggesting that it is composed mostly of DNA rich in A-T base pairs. However, the X and y chromosomes were resistant to HSS banding. These bands facilitated the identification of each chromosome. In this study, we used the restriction endonucleases with different G-C base target sequences: *Msp*I C/GGC and *Hae*III GG/CC. The use of restriction enzyme *Msp*I did not show an effect on the autosomal chromo-

somes. On the other hand, the sex pair showed a pale staining, to help in the recognition of these chromosomes. *Hae*III produced characteristic bands which were identified all along the chromosomes, facilitating the identification of each chromosome. Based on these results, we can consider the heterochromatin being heterogeneous. The findings obtained here, using different chromosomal banding techniques, may be useful in the identification of intraspecific chomosome variability, specifically in Coccinellidae (Coleoptera) chromosomes, even without special equipment.

Key words: Karyotype, Animal cytogenetics, Lady beetle

INTRODUCTION

The difficulty in producing bands in insect mitotic chromosomes is generally accepted; however, C-bands and some bands produced by fluorescent techniques (Selivon and Perondini, 1997) are exceptions. C-banding was used in females of *Cycloneda sanguinea*, and the heterochromatin was mainly located in the pericentromeric region of all chromosomes and in the short arms and the sex pair XX was almost completely heterochromatic (Maffei et al., 2004), as observed for many Coleopterans studied with the C-banding technique (Ennis, 1974; Angus, 1982; Drets et al., 1983; Juan and Petitpierre, 1989). Restriction endonucleases (REs) are known to induce DNA cleavage even in fixed metaphase chromosomes (Hidas, 1995) and cause extraction of fragment DNA (Bianchi et al., 1985, apud Hidas, 1995). The goal of the present study was to determine if simple methods, especially hot saline solution (HSS) and *Msp*I and *Hae*III RE, which do not require special equipments, may be helpful in studies of genetics variability in the lady beetle *C. sanguinea*.

MATERIAL AND METHODS

Specimens of *C. sanguinea* L. were colleted on the Campus of the Universidade Federal de Viçosa, Viçosa, MG, Brazil, where it occurs naturally, and were taken to the laboratory for reproduction. Twelve adult males were used for analysis. Cytogenetic analysis of spermatogonial mitotic metaphases was performed according to the method of Maffei et al. (2000).

Banding with hot saline solution was performed according to the method of Verma and Babu (1989)

HSS banding was carried out in six-day-old slides which were incubated in Sorënsen's phosphate buffer, pH 6.8, for 5 min at 85°C, rinsed in distilled water and stained with 5% Giemsa Sorënsen's buffer for 7 min.

Restriction endonuclease analysis was performed according to the method of Gosalvez et al. (1987) with modifications

To induce RE banding, slides were conserved at 37°C for 5 h and then submitted to overnight restriction digestion with the endonucleases *Hae*III and *Msp*I (Gibco). A 30-µL solution composed of 10 U of enzyme in the buffer specified by the supplier was applied to each slide before incubation in a moist chamber at 37°C. Control slides were incubated without enzymes. After overnight incubation, the slides were washed three times with water at room temperature and air-dried. To verify the digestion effects on the chromosomes, some preparations were stained with 2% Giemsa Sorënsen's phosphate buffer for 40 min. Preparations were inspected using an Olympus BX 60 microscope and an oil immersion objective, and the best metaphases were photographed.

RESULTS AND DISCUSSION

HSS extracted the heterochromatin region described previously by C-banding, suggesting that it comprises in most part DNA rich in A-T base pairs. However, the X and y chromosomes were resistant to detection by HSS banding (Figure 1A). These bands facilitated the identification of each chromosome. According to Comings (1978), these methods can extract the heterochromatin region that is mostly DNA rich in A-T base pairs, while the DNA rich in G-C base pairs stays in the chromosomes. In this study, we used the REs with different G-C base target sequences: *Msp*I C/GGC and *Hae*III GG/CC. The use of restriction enzyme *Msp*I did not show an effect on the autosomal chromosomes. On the other hand, the sex pair showed a pale staining (Figure 1B), which helped in the identification of these chromosomes. The *Hae*III restriction enzyme showed that the DNA of this species is rich in G-C sequences, even though in some chromosomal regions (telomeres) digestion did not occur.

*Hae*III produced characteristic bands which were detected all along the chromosomes, facilitating the identification of each chromosome (Figure 1C). Based on these results, we suggest that the heterochromatin is heterogeneous. Chromosome digestion with RE has been used to induce banding in many different animal species. The digestion of chromosomes of domestic cattle (*Bos taurus*) with RE *Msp*I and *Hae*III facilitated the identification of each chromosome and the elaboration of the karyotype. These characteristics of RE bands are especially important in cattle because, in this species, several chromosomes have very similar banding patterns and consequently are easy to confuse (Cymbron et al., 2004). Many heterochromatic regions show uniform or differential specific resistance or increased sensitivity to an RE (Babu and Verma, 1990; Lopez-Fernandez et al., 1991). Therefore, this method provides a powerful tool for the study of polymorphisms and compositional heterogeneity of the heterochromatic regions (Hidas, 1995).

CONCLUSIONS

The findings obtained here, using different chromosomal banding techniques, may be useful in the determination of intraspecific chromosome variability, specifically in Coccinellidae (Coleoptera) chromosomes, even without special equipment.

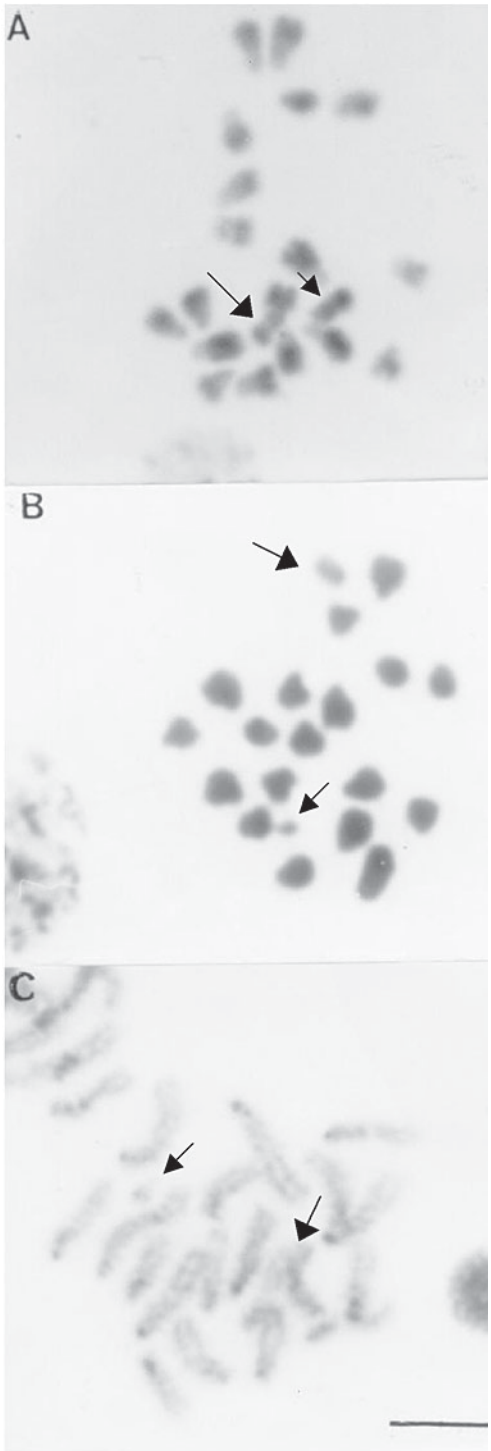

Figure 1. Spermatogonial metaphase of *Cycloneda sanguinea*. **A.** HSS banding. **B.** *Msp*I treatment and Giemsa-stained metaphase suggest resistance of heterochromatin in autosomal chromosome. Note the sex pair showing pale staining. **C.** *Hae*III produced characteristic bands which were identified all along the chromosomes. The arrows indicate the sex pair X and y (very small). Bar = 5 µm.

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