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Cytogenetic characterization of the holocentric chromosomes in the aphids *Myzus varians* and *Myzus cerasi*

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Abstract — Cytogenetic investigations on the holocentric chromosomes of *Myzus varians* and *Myzus cerasi* have been carried out using silver staining and C-banding followed by chromomycin A_3 (CMA_3) and DAPI staining in order to improve our knowledge of these pest crop species and facilitate their identification. In M. *varians* C-banding pattern is pecu-: liar of each chromosome pair thus allowing the identification of all homologues and the reconstruction of a reliable karyotype whereas in M. *cerasi*, C-positive regions result to be limited to both the telomeric regions of the X chromosomes. In both species, silver staining localizes rDNA genes on one telomere of each X chromosome; such telomeres are the unique brightly fluorescent C-positive regions after CMA_3 staining, whereas all other heterochro-matic bands result DAPI positive.

Key words: Cytogenetic, Heterochromatin, AgNORs, Holocentric chromosomes, Aphid.

INTRODUCTION

Aphids are lymph sucking insects and many species are serious crop pests, not only in view of their feeding action, but also because they can be important virus vectors in many crops. A wide knowledge of aphid genome organization could furnish informations useful in the layout of strategies aimed to reduce their impact on plants of agricoltural interest. Moreover, the acquisition of cytotaxonomic knowledge could allow a more precise identification in a taxon in which the species plurality and the intraspecific polymorphism together with the defectiveness of clearly distinctive morphologycal characters make, in many cases, a clear identification of species very difficult. Together with the above mentioned economic aspects, there are also theoretical reasons which could justify studies of aphid cytogenetic. In fact, aphids, because of the easiness with which mitotic chromosome could be obtained from embryonic tissues, represent an useful model to better understand the architecture of holocentric chromosomes, and to work out the differences/similarities with monocentric ones.

From the beginnings of the years 90[th], an extensive survey, concerning both cytogenetic and molecular features of holocentric chromosomes in several aphid species, has been carried out in our laboratory (MANICARDI *et al.* 1991a, b, 1992, 1994, 1996, 1998 a,b,c; MANICARDI and GAU-TAM 1994; BIZZARO *et al.* 1996; GALLI and MANICARDI 1998; MANDRIOLI *et al.* 1999 a,b; MANDRIOLI *et al.* in press).

In the present paper we have performedcytogenetic studies on *Myzus varians* Davidson and *Myzus cerasi* (Fabricius), with the aim of enhancing knowledge on genome organization of these taxa, in order to furnish further informations which could be used in improving control strategies against these pests.

MATERIAL AND METHODS

Colonies of *Myzus varians* were collected on *Prunus persica* in Piacenza whereas *Myzus cerasi* were collected on *Prunus cerasus* trees in Vignola (Modena).

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Chromosome spreads of embryo cells obtained from parthenogenetic females of both M. *varians* and M. *cerasi* were prepared as previously described by MANICARDI *et al.* 1996. C-banding treatment was performed according to the technique of SUMNER (1972); DAPI treatment was carried out according to Donlon and Magenis (1983) whereas chromomycin A_3 (CMA_3) staining was performed according to SCHWEIZER (1976).

Silver staining of NORs was performed following the technique of HOWELL and BLACK (1980).

RESULTS

All parthenogenetic females of M. *varians* examined show a chromosome number of 2n=12 (Fig. 1). C-banding and DAPI staining produce a clear-cut banding which allows a precise identification of each chromosome pair (Fig. lA) whereas CMA_3 staining of C-banded chromosomes shows a bright fluorescence exclusively limited to one telomere of the longest chromosome pair (Fig. IB). $AgNO_3$ staining put in evidence two argentophilic spots corresponding to the CMA_3 positive telomeres (Fig. 1C). Moreover both $AgNO_3$ and CMA_3 treatments evidenced a certain amount of heteromorphism between homologous NORs (see Fig. IB, C). These data as a whole allow a reliable reconstruction of M. *varians* karyotype (Fig. ID). In the absence of males, we have identified *M. varians* X chromosomes by comparison with the karyotype of related species, as they are generally the longest pair and they possess NORs.

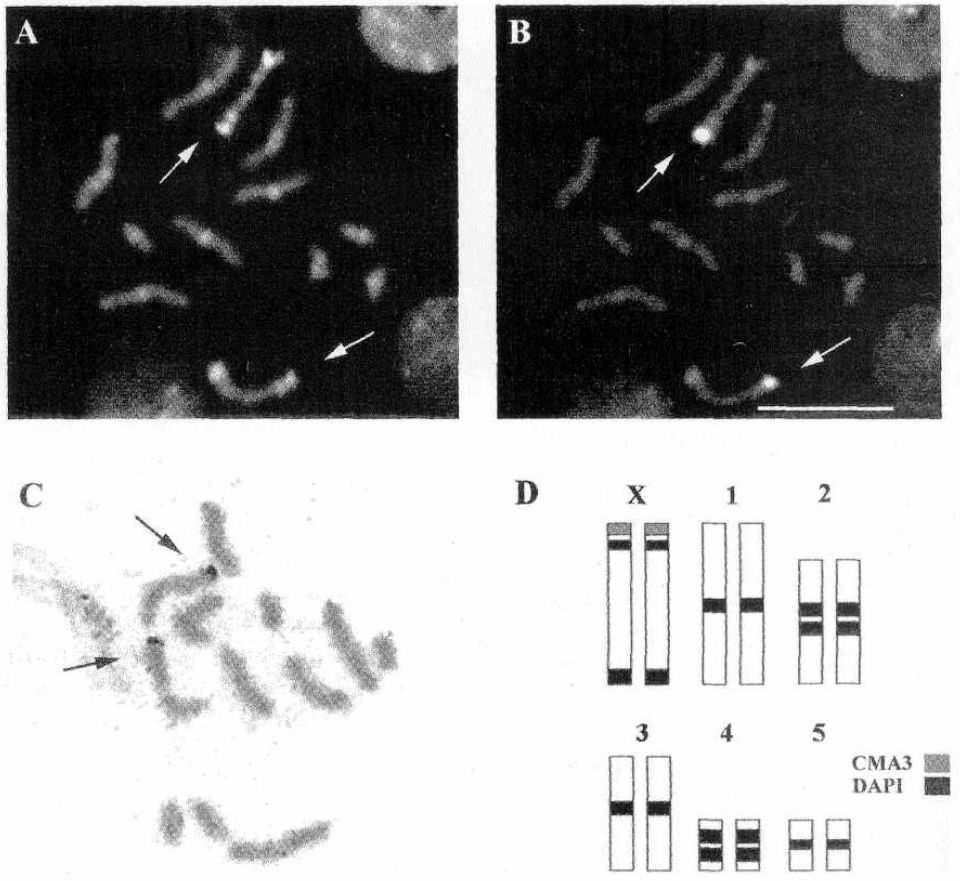

Fig. 1. — C-banded metaphase chromosomes of *Myzus varians* females after DAPI (A) and CMA_3 (B) staining; C-banding technique allows homologues pairing and karyotype reconstruction (D); silver staining (C) localises rDNA genes on one telomeric region of the X chromosome whereas the idiogram (D) illustrates C-banding pattern. Arrows indicate X chromosomes. Bar corre sponds to 10 μm.

Mitotic metaphases obtained from *M. cerasi* parthenogenetic females constantly possess 2n=10 (Fig. 2). In M. *cerasi* C-positive regions result to be limited to both the telomeric regions of the longest pair of chromosomes (Fig. 2A). Fluorochrome staining of C-banded chromosomes shows that one heterochromatic telomere is brightly DAPI positive thus demonstrating a high AT content (Fig. 2A), whereas the opposite telomere contains GC-rich DNA since it results brightly fluorescent after CMA_3 staining (Fig. 2B). This telomere results also argentophilic after silver staining (Fig. 2C). On the basis of chromosomal length we have reconstruct the karyotype of M. *cerasi* (Fig. D). In the absence of males, X chromosomes have been identified on the basis of their cytogenetical parameters (chromosomal length and presence of NORs).

DISCUSSION

Cytogenetic studies that consider both the morphology and the chromosomal number of aphids can be extremely useful to the taxonomists (BLACKMAN 1980). On the other hand, the ubiquitous condition of holocentrism, typical of aphid chromosomes, makes the proper recognition of each chromosome pair very difficult. The analysis of mitotic metaphases evidenced that the diploid chromosome number found in *M. varians (2n* =12) and M. *cerasi* (2n=10) is in accordance with literature data (BLACKMAN and EASTOP 1984). Contrary to what observed in M. *cerasi*, the use of C-banding technique on M. *varians* mitotic chromosomes considerably improved the ability to identify chromosome pair and it allows a reliable identification of all

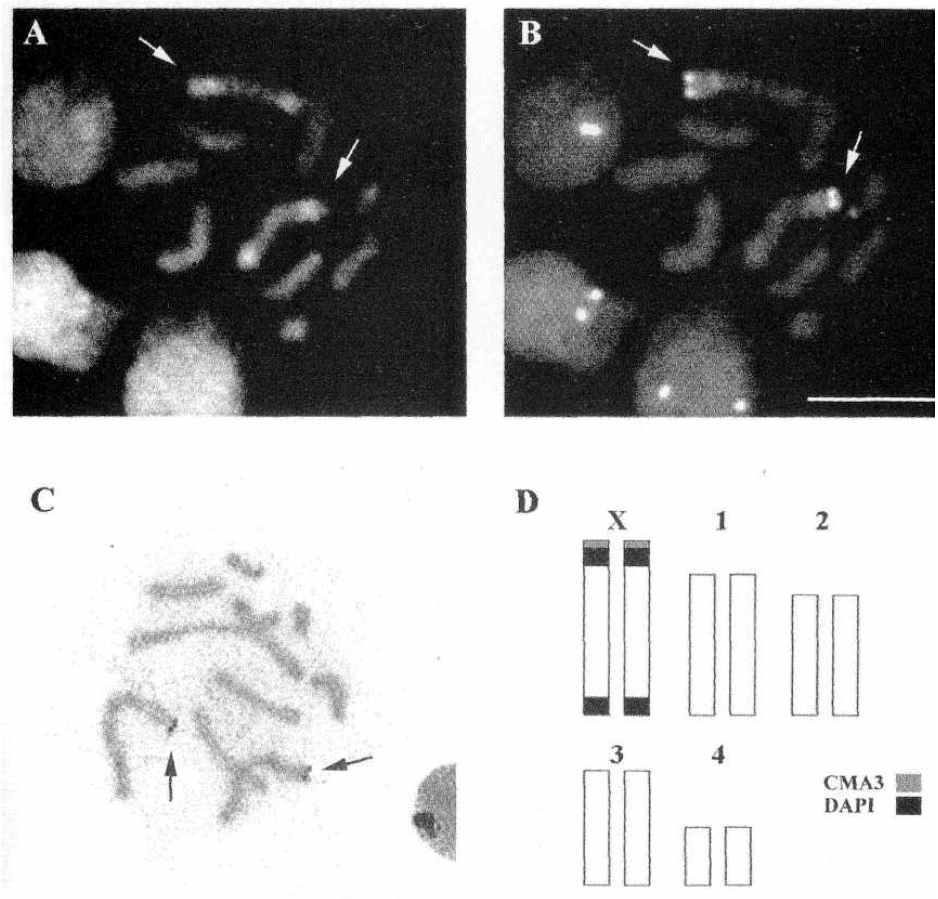

Fig. 2. — DAPI (A) and CMA_3 (B) staining of C-banded *M. cerasi mitotic* chromosomes; (C) $AgNO_3$ staining put in evidence silver positive spots on one X telomere whereas the idiogram (D) illustrates C-banding pattern. Arrows indicate X chromosomes. Bar rep^ resents 10 μm.

homologues. This banding technique will provide an useful tool for the study of karyotype evolution within this taxon.

C-banding and fluorochrome staining, used to analyse the composition of constitutive heterochromatin, have evidenced that both *M. varians* and M *cerasi* heterochromatic blocks possess AT-rich DNA whereas only the C-band located on one telomere of the X chromosome contains CMA_3 positive, GC rich DNA. Moreover, $AgNO_3$ treatment carried out on mi-totic chromosomes of both species, evidenced two argentophilic spots corresponding to the CMA_3 brightly fluorescent band located on one X telomere. These observations, while confirming data obtained in all other aphid species analysed to date (MANICARDI *et al.* 1991a, b, 1992, 1994, 1996, 1998 a,b,c; MANICARDI and GAU-TAM 1994; BIZZARO *et al.* 1996; GALLI and MANICARDI 1998; MANDRIOLI *et al.* 1999a,b; MANDRIOLI *et al.* in press), allow us to conclude that, from a cytogenetic point of view, the holo-centric chromosomes of aphids show a series of conservative characteristics which could be summarised as follows: i) constitutive hetero-chromatin is not equilocated on each chromosome; ii) C-positive areas are concentrated, and in some cases exclusively limited, to the X chromosomes whereas autosomes lack (as in M. *cerasi)* or possess few heterochromatic bands (as in M. *varians);* iii) the different response to CMA_3 and DAPI staining after C-banding points out the heterogeneity of heterochromatic DNA composition in aphid genomes, iv) excluding *Amphorophora idaei* (FENTON *et al.* 1994) and *Schoutedenia lutea* (HALES 1989), in all other aphid species rDNA genes are arranged as tandemly repeated clusters located at one telomeric region of each X chromosome (BLACKMAN and SPENCE 1996; MANICARDI *et al.* 1998c).

In M. *varians* metaphases, a certain amount of heterogeneity between homologous NORs has been observed after both CMA_3 and $AgNO_3$ staining (see Fig. 1B,C). The overlapping between silver staining, which is a marker for ribosomal gene activity (ROUSSEL and HERNANDEZ-VERDUM 1994) and CMA_3 treatment, which is only influenced by the number of rDNA genes occurring at the NORs, allows us to suggest that the above mentioned heteromorphism is due to the occurrence of a variable number of ribosomal genes, all clustered at the two X telomeres. This phenomenon, already described in several aphid species (MANICARDI *et al.* 1998c; MANDRIOLI *et al* 1999a,b), could be due to unequal crossing-over or unequal sister chrornatid exchanges (SCE). In this connection, molecular analysis of rDNA intergenic spacers of the aphid *Acyrthosiphon pisum* has shown the presence of recombination hot spots which could favour the occurrence of unequal crossing-over between rDNA genes (MANDRIOLI *et al.* 1999b).

Finally it is important to stress out that, in the absence of males, cytogenetic analysis allows a reliable identification of X chromosome since they possess the largest amount of heterochromatin and are also NORs bearing. These results are particularly useful for the reconstruction of karyotype in aphids coming from field population, in which males are generally absent.

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