# Analysis of insect holocentric chromosomes by atomic force microscopy

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In order to go in depth into the analysis of holocentric chromosome structure, atomic force microscopy (AFM) was applied to metaphase plates of the aphid *Megoura viciae*. AFM showed that aphid chromatids adhere to one another without any prominent structure detectable between them and without any evidence of chromosomal constrictions. AFM thus provided new and reliable evidences at a nanomolecular level concerning the holocentric structure of aphid chromosomes, without any of the artefacts due to sample staining or coating that are usually associated with electron microscopy.

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## INTRODUCTION

Aphid chromosomes, as well as chromosomes of several eukaryotic species, including protista, animals and plants, are holocentric. Holocentric chromosomes are present in at least some taxa of every major lineage of eukaryotes, with the exceptions of Echinodermata and Cordata (WRENSCH et al. 1994). Viewed in a phylogenetic context, holocentrism seems to be present in each principal branch of the phylogenetic tree.

These chromosomes are also termed holokinetic, because during mitotic anaphase they behave as if the spindle attachment is not localized, so that chromatids move apart in parallel and do not form the classical V-shaped figures typical of monocentric chromosomes (BLACKMAN 1987; WRENSCH et al. 1994). Moreover, ultra-structural studies on the holocentric-holokinetic chromosomes of the mite *Tetranychus urticae* showed that spindle microtubules have points of attachment extending across the entire chromosomal length (WHITE 1973; TEMPLAAR 1980). Analogous results have been obtained from the hemipteran *Agallia constricta* using both light and electron microscopy (RIEDER et al. 1990).

The holokinetic nature is confirmed by the fact that experimentally induced chromosome fragments continue to attach to the spindle and segregate correctly (HUGHES-SCHRADER and SCHRADER 1961; WHITE 1973).

Although these data are interesting, their interpretation is still controversial. Several authors have interpreted these chromosomes as polycentric or having extended centromeres rather than truly holocentric (VAARAMA 1954; SYBENGA 1981; WOLF 1996).

The advent of AFM has provided new opportunities for the study of the chromosome structure, as demonstrated by various workers (DE GROOTH and PUTMAN 1992; MCMASTER et al. 1994; WINFIELD et al. 1995). AFM has the advantage over electron microscopy that standard chromosome preparations can be examined immediately without any additional treatment, such as staining or coating (MCMASTER et al. 1994; WINFIELD et al. 1995).

Most of the data obtained by applying AFM to the analysis of chromosome structure regard human and plant monocentric chromosomes, whereas holocentric chromosomes have never been analysed with this technique. In order to fill this gap, we analyzed holocentric chromosomes of the aphid *Megoura viciae* (Hemiptera, Aphididae) using the atomic force microscope (AFM).

## MATERIAL AND METHODS

Chromosome preparations were made by spreading embryo cells, dissected from *M. viciae* parthenogenetic females, according to MANDRIOLI et al. (1999a). In brief, cells were kept in a 0.8 % hypotonic solution of sodium citrate for about 45 min and then transferred to minitubes and centrifuged at 350g for 3 min. Methanol–acetic acid 3:1 was successively added to the pellet that was broken by making it to flow up and down for 1 min through a needle of a 1 ml hypodermic syringe. Finally, the pellet was resuspended in 200 µl of fresh fixative and 20 µl of cellular suspension was dropped onto clean slides and air-dried.

*M. viciae* metaphases were analysed immediately after preparation by AFM (Park Scientific Instru-

ments, Sunnyvale, CA). Before chromosome scanning by AFM, metaphases were located, analysed and finally positioned under the tip of the AFM, using the incorporated optical microscope. AFM analysis has been performed operating in contact imaging mode. Images were obtained in air at room temperature at a constant force between the tip and the sample of 1–10 nN, using cantilevers of spring constant 0.06 N/m.

Silver staining was carried out according to HOWELL (1977) and $CMA_3$ staining was performed as described by MANDRIOLI et al. (1999b).

## RESULTS AND DISCUSSION

In order to analyze the structure of aphid holocentric chromosomes, silver staining has been performed on *M. viciae* mitotic plates. Silver staining is useful not only for the localization of active ribosomal genes (rDNA genes) but also to evidence the chromosomal axial core (HOWELL and HSU 1979). In *M. viciae*, silver-stained axial cores run parallel without points of intersection (Fig. 1a), whereas the same staining technique performed on monocentric chromosomes showed that the axial cores of the two chromatids come into contact at the centromere (BLACKMAN 1987; WRENSCH et al. 1994).

The holocentric-holokinetic behaviour of *M. viciae* chromosomes has been confirmed by $CMA_3$ staining which showed that aphid chromatids move apart in parallel during anaphase and do not form the classical V-shaped figures typical of monocentric ones (Fig. 1b).

AFM analysis has been performed on 90 *M. viciae* metaphase plates. In particular, our attention has been concentrated on sex chromosomes, not only because they are clearly recognizable but also because they are the longest and therefore the most useful for studying the junction between the two chromatids (Fig. 2a). Moreover, we focused on sex chromosomes located in clear areas of the slides as evidenced by the absence of a "skirt" around the edge of chromosomes.

AFM analysis at increasing magnification levels (Fig. 2b, c) showed that chromatids adhere to one another without any prominent structure detectable between them. Moreover, no evidence of chromosome constriction has been observed.

In order to improve structure analysis, 3D reconstruction (Fig. 2d) and pseudostaining (Fig. 2e) have been performed. In particular, 3D reconstruction clearly evidenced that the junction between the two chromatids is absolutely homogenous along the entire chromosome length and it appeared as a deep depression between the two chromatids. On the contrary, AFM analysis of monocentric chromosomes showed centromeres limited to the primary constriction (MCMASTER et al. 1994).

AFM analysis indicated that aphid chromatids adhere to one another without any interruption or prominent additional material between them, confirming the holocentric nature of aphid chromosomes (WOLF et al. 1991, 1994).

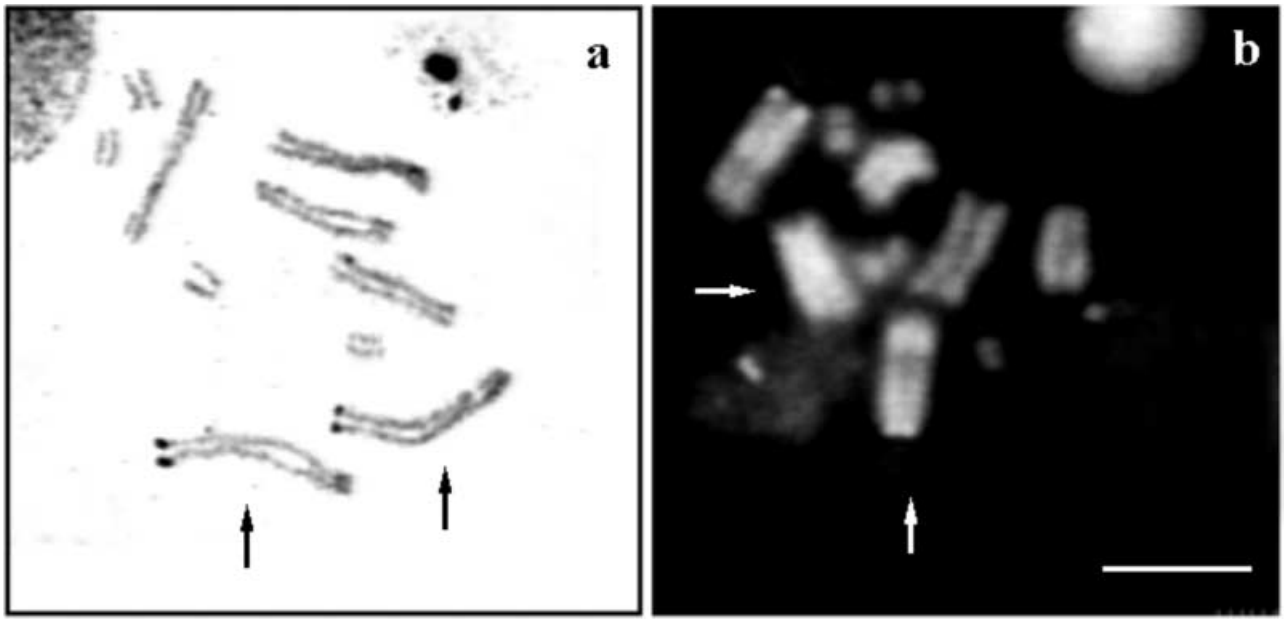


**Fig. 1.** *M. viciae* metaphase chromosomes after silver (**a**) and $CMA_3$ staining (**b**) observed at optical microscope. Arrows indicate sex chromosomes. Bar corresponds to 10 μm.

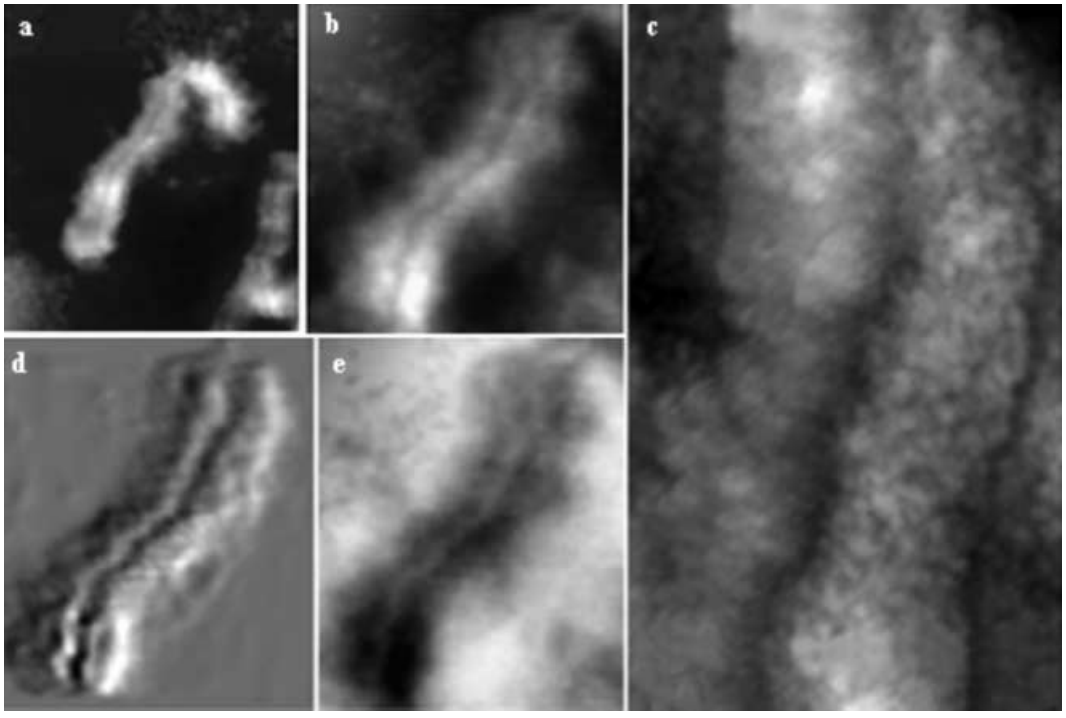


**Fig. 2.** A portion of a metaphase that was clear of cytoplasmatic material has been analyzed by AFM at increasing magnification (**a**–**c**). Successively, 3D reconstruction (**d**) and pseudostaining (**e**) have been performed. All the AFM images showed that the junction between the two chromatids is absolutely homogenous along the entire chromosome length.

AFM analysis therefore provides a new and reliable evidence at a nanomolecular level concerning the holocentric structure of aphid chromosomes without any of the artefacts due to staining or coating usually associated with electron microscopy.

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