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Description of the karyotype of Tunisian *Mayetiola hordei* (Diptera: Cecidomyiidae) and comparison with *M. destructor*

Descripción del kariotipo de *Mayetiola hordei* (Diptera: Cecidomyiidae) de Túnez y comparación con *M. destructor*

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Abstract: We report information about the structure of the karyotype of *M. hordei*, a fly that infests barley in the south of Tunisia. The number of chromosomes was found to be (2n = 8) in somatic female cells and (2n – 2 = 6) in somatic male cells, which is similar to the karyotype of *M.destructor*. Mitotic and polytene chromosomes of *M. hordei* are described and compared with those of *M. destructor*. The karyological data are discussed in terms of genetic mechanisms underlying divergence of *M. hordei* and *M. destructor*.

Key words: Mitotic chromosomes. Polytene chromosomes. Speciation. Hessian fly. Barley stem gall midge.

Resumen: Se registra información acerca de la estructura del cariotipo de *M. Hordei*, una mosca que infesta la cebada en el sur de Túnez. El número de cromosomas fue (2n=8) en células somáticas femeninas y (2n-2=6) en células somáticas masculinas, lo cuál es similar al cariotipo de *M. destructor*. Se describen y comparen las cromosomas mitóticos y politénicos de *M. hordei* con los de *M. destructor*. Los datos cariológicos se discuten en términos de los mecanismos genéticos que explican la divergencia entre *M. hordei* y *M. destructor*.

Palabras clave: Cromosomas mitóticos. Cromosomas politénicos. Especiación. Mosca de Hess. Mosquito del tallo de la cebada.

Introduction

In North Africa, the genus *Mayetiola* includes two phytophagous species, namely the Hessian fly *Mayetiola destructor* (Say, 1817) and the barley stem gall midge *Mayetiola hordei* (Kieffer, 1909) (Gagne *et al.* 1991). In the southern arid regions of Tunisia, barley is infested by *M. hordei*, whereas in the northern sub-humid regions, the highest infestations occur on wheat and to a lesser extent on barley, by *M. destructor*.

In order to establish a controlling strategy based on the development of cereal varieties genetically resistant to *Mayetiola*, the insect populations occurring on wheat and barley in Tunisia should be studied in regard to taxonomical identity and divergence. In this context, there have been several morphological, biochemical and molecular assays trying to confirm the existence of two groups of reproductively isolated individuals corresponding to *M. destructor* and *M. hordei*. Morphologically, the two phytophageous species of *Mayetiola* can be hardly distinguished, but Gagne *et al.* (1991), using scanning electron microscopy, reported differences in the shape of the female postabdomen, the structure of male terminalia and the number of spicules covering the puparium. At the biochemical level, Makni *et al.* (2000) identified allozyme markers of taxonomic value for the two species. Molecular markers allowing an efficient and rapid distinction between the two species of *Mayetiola* were developed by Mezghani *et al.* (2002a), on the basis of Restriction Fragment Length Polymorphism (RFLP) of the cytochrome b gene. Although these approaches can be simple and reliable, inferences regarding taxonomical specificity, genetic structure and species divergence need to be further consolidated by conducting a cytogenetical analysis.

Only the Hessian fly *M. destructor* from the USA populations was karyotyped (Stuart and Hatchett 1988) and the authors found that female somatic cells have two pairs of autosomes and two pairs of sex chromosomes (2n = 8), while male somatic cells have two pairs of autosomes and two monosomic sex chromosomes (2n – 2 = 6). The complexity of the Hessian fly karyotype is also manifested by the presence of two distinct chromosome classes: eliminated (E) chromosomes and somatic (S) chromosomes (Lobo *et al*. 2006). Indeed, just after the sperm and ovules combine, each zygote contains a diploid set of S chromosomes and 30-40 E chromosomes (A1 A2 X1 X2 / A1 A2 X1 X2 + E). The E chromosomes are eliminated from the presumptive somatic nuclei during the fifth nuclear division of embryogenesis. During that division, the paternally-derived X1 and X2 chromosomes may also be eliminated from the somatic nuclei. If they are retained, the somatic cells have a female karyotype (A1 A2 X1 X2 / A1 A2 X1 X2). If they are eliminated, the somatic cells have a male karyotype (A1 A2 X1 X2 / A1 A2 O O).

The objective of the present study was to analyse for the first time the structure of the karyotype of *M. hordei* from Tunisia and compare it with Tunisian samples of *M. destructor* and with the relevant literature. Karylogical results are used to discuss genetic mechanisms underlying divergence of *Mayetiola* species.

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Materials and Methods

M. destructor and *M. hordei* flies used in this study were at third-instar and adult stages. Specimens of *M. destructor* were collected from wheat plants located in north Tunisia (Jédéida and Goubellat cities), whereas those of *M. hordei* were collected from barley plants in the southern region of Gabès. Species and sex of these samples were identified on the basis of morphological characteristics (Gagne *et al.* 1991) and diagnostic alleles at the *Pgi* and *Mdh*2 loci (Makni *et al.* 2000).

Polytene chromosome preparations were made from salivary glands of third-instar larvae. Salivary glands were dissociated, treated by acetic acid (45%) for 1 min, then fixed in a solution of acetic acid : lactic acid : water (1:2:3 ratio). The material was stained with 1% lacto-aceto-orcein for 15 min and squashed (Ashburner 1967).

Mitotic chromosome preparations were made from brain ganglia and gonads of adult flies. Brain ganglia and gonads (testes or ovaries) were dissected out in a Ringer's solution. The technique described by Guest and Hsu (1973) was used with slight modifications: tissues were treated in 0.4 colchicine-phosphate-buffered saline solution for 15 min, then in an hypotonic solution (1% sodium citrate) for 10 min, before fixing in an ethanol : acetic acid mixture (3:1) for 30 min. The fixed tissues were transferred to a drop of 60% acetic acid on a clean slide. Finally, the slide was placed on a warm hot-plate (45°C) and the drop allowed evaporation. The prepared slides were stained with 3% Giemsa in phosphate buffer (pH 6.8) for 17 min. Slides for C-banding were treated according to the technique described by Sumner (1972) with minor modifications. Twenty individuals of each *M. destructor* and *M. Hordei,* four females, and six males each were successfully karyotyped. Preparations with clear and well-distributed chromosomes were examined under a light microscope "Zeiss 63" equipped with a JVC TK-1270 colour video camera and Image Grabber, then processed with Adobe Photoshop.

Results

***Mayetiola destructor* karyotype.** Four polytene chromosomes A1, A2, X1 and X2 were easily recognized in *M. destructor* females where they had an identifiable structure, as described by Stuart and Hatchett (1988). In males, only autosomes were recognized, whereas sex chromosomes had a diffused structure (Fig. 1A). The observation of mitotic chromosomes showed a sexual chromosomal dimorphism within *M. destructor* samples. Female somatic cells have two pairs of autosomes and two pairs of sex chromosomes (2n = 8),

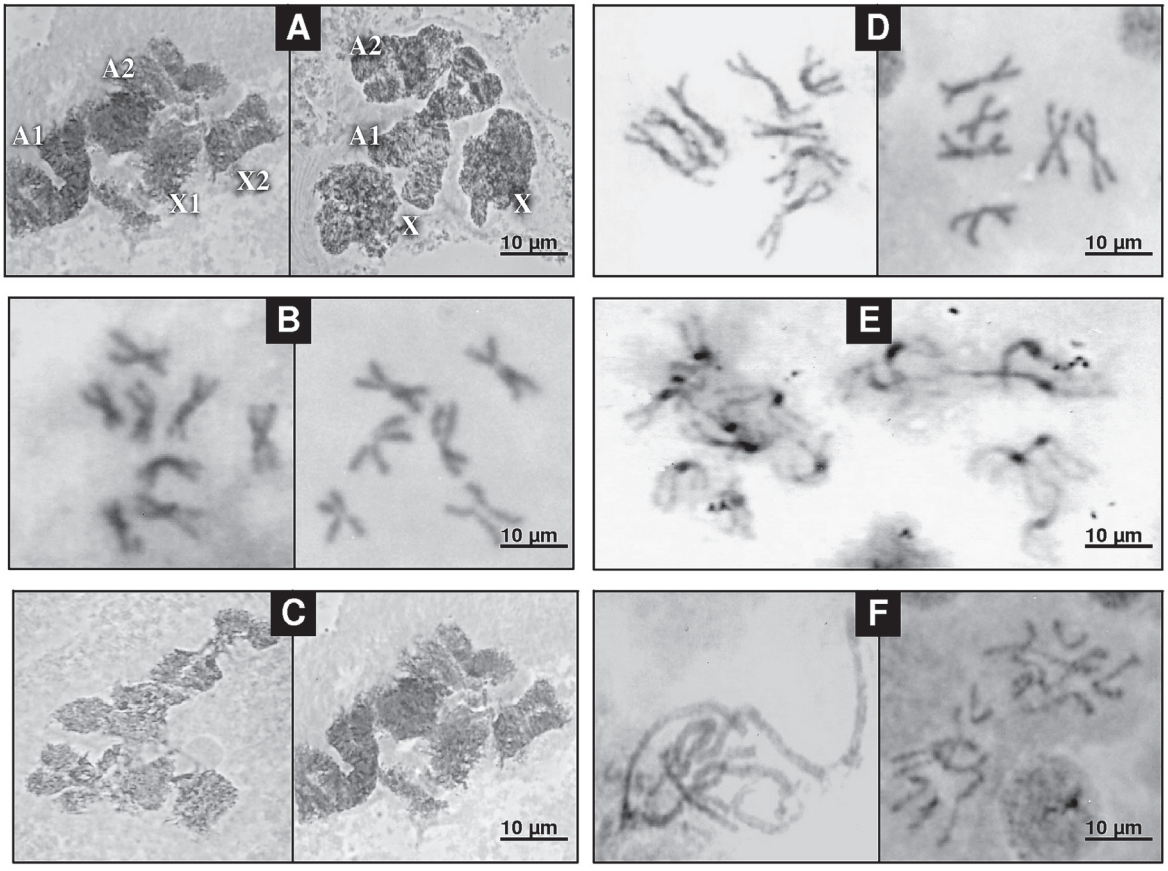

Figure 1. Polytene and mitotic chromosomes of Tunisian *Mayetiola destructor* and *M. hordei*. **A.** Polytene chromosomes in a larval salivary gland nucleus from a female (left) and a male (right) of *M. destructor*. **B.** Giemsa-stained metaphase spreads showing the eight chromosomes of the female (left) and the six chromosomes of the male (right) of *M. destructor*. **C.** Relative size of polytene chromosomes from *M. hordei* (left) and *M. destructor* (right). **D.** Giemsa-stained metaphase spreads showing the eight mitotic chromosomes of the female (left) and the six mitotic chromosomes of the male (right) of *M. hordei*. **E.** Mitotic metaphase of *M. hordei* with C-banding in the pericentromeric region. **F.** Prophase of first meiotic division of a female of *M. hordei* (left) and anaphase of second meiotic division of a male of *M. hordei* (right).

whereas male somatic cells have two pairs of autosomes and two monosomic sex chromosomes (2n – 2 = 6) (Fig. 1B).

***Mayetiola hordei* karyotype.** The salivary glands of *M. hordei* contained about 100 round-shaped cells. Only a few basal cells contained polytene chromosomes. In all preparations, polytene chromosomes of *M. hordei* were characterized by a smaller size, than those observed from Tunisian samples of *M. destructor* (Fig. 1C). The mitotic karyotype of *M. hordei* exhibited a sexual chromosomal dimorphism in chromosome number. Somatic testicular cells contained six chromosomes (2n – 2 = 6), wheareas eight chromosomes were present in somatic ovarian cells of larvae (2n = 8). That indicated a similarity with the mitotic karyotype of *M. destructor* (Fig. 1D). Metaphase cells showed two metacentric autosomal pairs in both sexes. The sexual chromosomes X1 and X2, disomic in females and monosomic in males, were also metacentric. For all chromosomes, the C-banding pattern showed a heterochromatic region that extended from the centromere, indicating that heterochromatin is mainly localized in the pericentromeric regions (Fig. 1E). Spermatogonial and oogonial meioses of *M. hordei* showed that the behaviour of chromosomes at all stages of meiotic divisions was similar to that described by Stuart and Hatchett (1988) (Fig. 1F).

Discussion

Many previous investigations were reported on the cytogenetics of insect species from different orders such as Hymenoptera (Rousselet *et al.* 2000), Orthoptera (Turkoglu and Coka 2002) or Diptera (Campos *et al.* 2007). In many cases, such studies contributed to the distinction between species with little morphological and behavioural variation (Rousselet *et al.* 1998). Concerning the two species, *M. destructor* and *M. hordei*, only the karyotype of *M. destructor* from the USA has been studied (Stuart and Hatchett 1988). The present note is the first report on the karyotype of *M. hordei,* in comparison with that of *M. destructor*. Concerning the number of chromosomes, our data demonstrate that in the two species, the karyotype was numerically stable. As the *Mayetiola* genus is characterized by low chromosome numbers, interspecific polymorphisms in number may be rare, in contrast with some insect genera with high chromosome numbers, where polymorphisms in chromosome numbers have been reported (Rousselet *et al.* 1998).

Chromosomal sexual dimorphism was also similar to that observed in *M. destructor* samples, indicating that sex in *M. hordei* was determined, as well as in other Cecidomyiidae species that have been examined cytologically (White 1973), by a sex chromosome to autosome balance system with two X chromosomes. The similarity between *M. hordei* and *M. destructor* in chromosome morphology indicates that the breaking of gene flow between two groups of *Mayetiola*, belonging initially to the same species (Mezghani *et al.* 2002b), may have resulted in independent accumulation of genic (non chromosomal) mutations in the two new species. This hypothesis is supported by the genic variability of both *Mayetiola* species, with regard to the cytochrome b, ITS1 and ITS2 genes (Mezghani *et al.* 2002a, 2002b). Nevertheless, the speciation process may also have been accompanied by chromosomal fusion or translocation, not affecting the number of chromosomes. Indeed, in the *Drosophila melanogaster* (Meigen, 1810) species complex, chromosomal variation was reported between two morphologically similar species, *D. yakuba* (Burla, 1954) and *D. teissieri (*Tsacas, 1971). Even though the karyotypes of these species were similar, the study of the puffing activities of polytene chromosomes revealed para- and pericentric inversions, enabling the establishment of their phylogeny (Ashburner and Lemeunier 1972). We, therefore, think that further cytogenetic analyses that would encompass *In situ* hybridization (ISH) and puffing activities will be needed in order to better understand the genetic mechanisms underlying divergence of *Mayetiola* species. The present study showed similarity between *M. destructor* and *M. hordei* in chromosome number, shape and sex chromosomal dimorphism, implying the absence of chromosome fission, amplification or polyploidization (Rousselet *et al.* 1998). Finally, polytene chromosomes of *M. hordei* were characterized by a smaller size, than those observed from Tunisian samples of *M. destructor*. This could be explained by a difference in the degree of polytenization or in gene transcriptional activity between both species (Lemeunier 1973).

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