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Chromosome Number, Sex Chromosome Mechanism and Fluorochrome Staining Results of the *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) in the Northeast of the Iberian Peninsula, Spain

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Abstract: The alfalfa weevil *Hypera postica* (Gyllenhal) is one of the primary pests of alfalfa at southern Europe and in many regions of the world where this crop is cultivated. Morphology traits may affect the biology of one species. Detailed karyotype descriptions (chromosome morphology, size, number and banding patterns), chromosome counts and their comparative analyses are important independent tools for use in taxonomy and in understanding chromosome evolution. To test for a chromosome polymorphism among the males, a population of *H. postica* from the vicinity of Lleida (Spain) was examined. The study represents the chromosome number (2n=22), the sex chromosome mechanism (XYp) and the pattern of the fluorochrome staining (DAPI positive and CMA_3 negative) in males. The results confirm that the chromosomes cannot be a suitable marker to distinguish the species among the genus *Hypera* Germar. The information available on the chromosome number and the sex chromosome mechanism of *Hypera* spp. is presented and discussed.

Key words: alfalfa weevil, CMA_3, DAPI, differential staining, karyotype, pest

Introduction

The alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is one of the primary pests of alfalfa at southern Europe and in many regions of the world where this crop is cultivated (Goosey 2012, Hoft et al. 2002, Saeidi & Moharramipour 2017, Soroka et al. 2019, Pons & Núñez 2020). Although the weevil originates from Eurasia (Hoffmann 1963), little is known about its biology, life cycle or ecology in Europe. In spite of the recent advances in the ecology of the alfalfa weevil (Levi-Mourao et al. 2022a, 2022b), much is still unknown about its biology, e.g., aspects related with the characterization of populations from different geographical regions with different responses to the environmental conditions. Detailed karyotype descriptions (which include the chromosome morphology, size, number and banding patterns) and the comparative analyses could be important independent tools in taxonomy and in understanding chromosome evolution (Guerra 2012). The karyotype of *H. postica* was reported several times for different alfalfa weevil populations but described in details by Hsiao & Hsiao (1984) as an attempt to distinguish strains of the alfalfa weevil complex in the United States. Hsiao & Hsiao (1984) provided the only information about the heterochromatin distribution after

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Table 1. Chromosome number of species of the genus *Hypera* Germar, 1817

Species	Karyotype	Haploid number	Country of origin	Reference
Hypera adspersa var. alternans	22	10+XYp	Japan	Takenouchi (1984)
Hypera brunneipennis	22	10+XYp	USA, California	Klostermeyer (1978)
Hypera elongata		10+XYp	No data	Petryszak (1981)
Hypera eximia	22	10+XYp	USA	Klostermeyer (1978)
Hypera medicaginis	22	10+XYp	No data	Sharma et al. (1980)
Hypera medicaginis	22	10+XYp	India (on *Trifolium alexandrinum*)	Gill & Gulati (1989)
Hypera mongolicus	22	10+XYp	Japan	Takenouchi (1972)
Hypera nigrirostris	22	10+XYp	USA	Takenouchi (1965, 1972)
Hypera postica	22	10+XYp	No data	Blickenstaff (1965)
Hypera postica	22	10+XYp	USA, Nebraska	Klostermeyer (1978)
Hypera postica (as *H. variabilis*)	22	10+XYp	Spain	Gurrea Sanz (1983)
Hypera postica	22	10+XYp	USA	Hsiao & Hsiao (1984)
Hypera postica	22	10+XYp	India (on *Trifolium alexandrinum*)	Gill & Gulati (1989)
Hypera postica	22	10+XYp	Spain	Present study
Hypera punctata	Not determined	XY	No data	Stevens (1909)
Hypera punctata	20	9+neoXY	USA	Hsiao & Hsiao (1984)
Hypera rumicis	22	10+XYp	Japan	Takenouchi (1972)
Hypera viciae	22	10+XYp	Japan	Takenouchi (1972)

C-banding. There are no more data available about the differential staining application on *Hypera* spp. Different *Hypera* populations and species examined were found to share the same chromosome number, i.e. twenty-two autosomes and the XY bivalent in the so-called parachute configuration 2n=20+XYp) (Table 1). There are no data on the chromosome polymorphism reported.

In the present study, males of a population of *H. postica* from the vicinity of Lleida (Spain, Catalonia) was examined to test for a chromosome polymorphism and, especially, the sex chromosome mechanism. The study represents the chromosome number, the sex chromosome mechanism and the pattern of the fluorochrome staining in males.

Materials and Methods

Samples of *H. postica* adults were collected by net-sweeping in commercial alfalfa fields in Catalonia, NE of Spain. More than one hundred samples were collected in different fields during the period of 2019–2022. Males were identified according to the form of the last abdominal sternites (Bug Guide, https://bugguide.net/node/view/1471509/bgimage), then fixed in 3:1 fixative (96% ethanol-glacial acetic mixture). Dissected out, the gonads were squashed in a small drop of 45% acetic acid. The cover slips were removed by dry ice technique. Slides were dehydrated in fresh fixative (3:1) and air dried. Forty chromosome preparations were stained by Schiff-Giemsa method (after Grozeva & Nokkala 1996) to study the number and the behaviour of the chromosomes, whereas for the 15 other slides the DNA binding fluorochromes, GC-specific chromomycin A_3 (CMA_3) and AT-specific 4'-6' diamino-2-phenylindole (DAPI) were applied to reveal molecular organization of the chromatin according to the method of Schweizer (1976) and Donlon & Mafenis (1983), with minor modifications after Kuznetsova et al. (2001).

Chromosomes were analysed under light/fluorescent microscope Axio Scope A1 – Carl Zeiss Microscopy at 100x magnification and documented with digital camera ProgRes MFcool – Jenoptik AG. All preparations and remains of the specimens are stored at the Institute of Biodiversity and Ecosystem Research, Sofia.

Results

At spermatogonial metaphases, 22 chromosomes were found (Fig. 1). The twenty autosomes gradually decreased in size (Fig. 1b). Most of the chromosomes were metacentric (1, 4, 7, 8, 9, 10). There

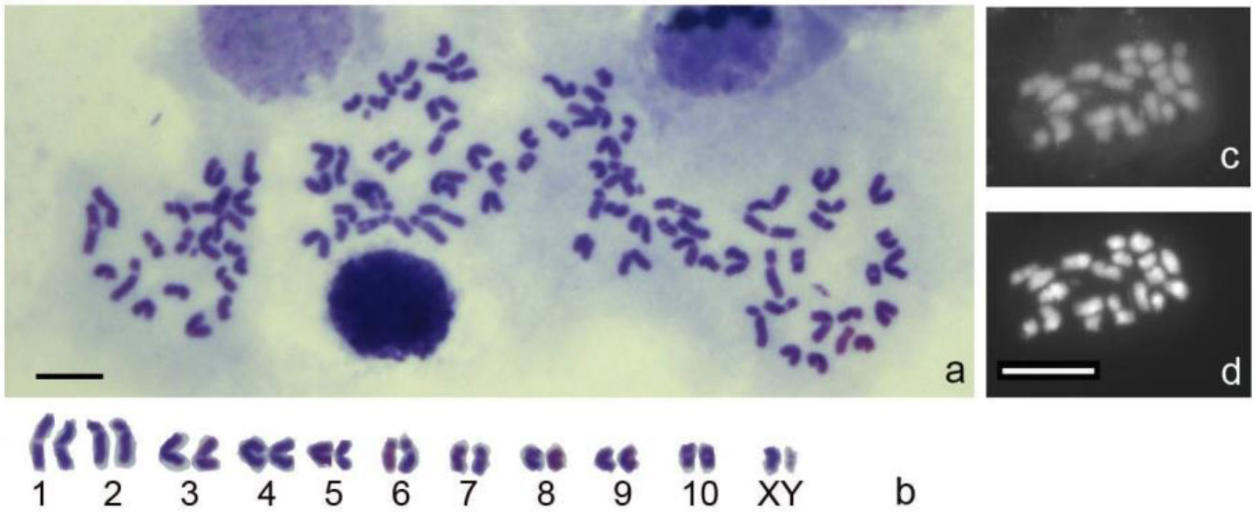

Fig. 1. Spermatogonial metaphases: a – metaphase plates after routine Giemsa staining; b – karyogram; c – metaphase plate after Chromomycin 3 (CMA_3) staining – there is no bright signals; d – metaphase plate after DAPI staining without bright signals. Scale bars: 10 μm.

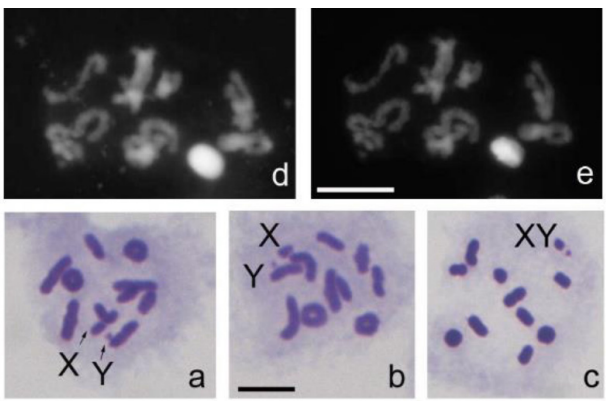

Fig. 2. Diakineses and prometaphases with one or two chiasmata: a, b, c – after routine Giemsa staining; d – after DAPI staining; e – after Chromomycin 3 (CMA_3) staining. Scale bars: 10 μm.

were at least three or four submetacentric (2, 3, 6) pairs and the Y chromosome was the smallest one in the set. At diakinesis, ten autosomal bivalents were observed and the sex chromosomes predominantly were separated. One or two chiasmata per bivalent were observed in the majority of the cells examined (Fig. 2d-e). At metaphase I, ten autosomal bivalents and the XY pseudobivalent in parachute configuration could be seen (Fig. 2a-c, Fig. 3).

After fluorochromes staining of the *Hypera* chromosomes, signals of the AT rich (DAPI positive) heterochromatin were observed at early prophase and at diakinesis at the telomeres of the chromosomes, which were not very much spiralized (Fig. 2d-e; 4). After DAPI staining, the chromosomes were bright as well in spermatogonial metaphases (Fig. 1c-d) and metaphase I (Fig. 3) but, in this stage, it was difficult

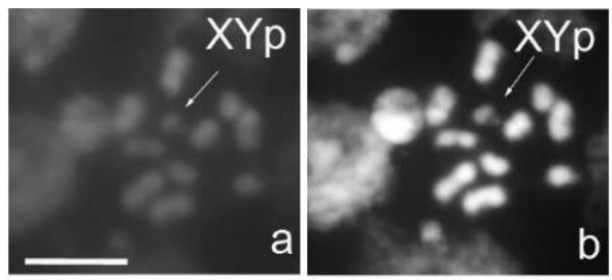

Fig. 3. Metaphase I with XYp: a – after Chromomycin 3 (CMA_3) staining; b – after DAPI staining. Scale bar: 10 μm.

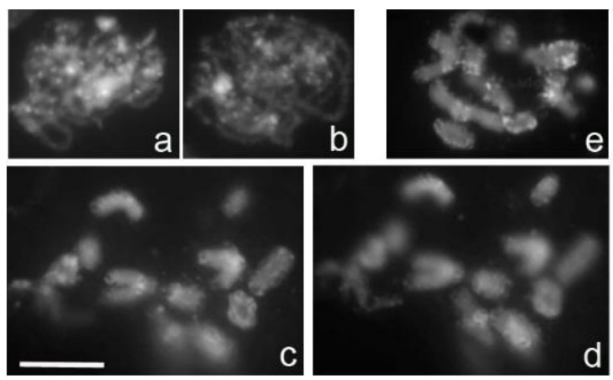

Fig. 4. Different meiotic stages after DAPI staining with bright signals in the telomeres (a, b, c, e) and after Chromomycin 3 (CMA_3) staining (d): a, b – prophases with bright dots of heterochromatin; c, d, e – diakinesis. Scale bar: 10 μm.

to localize precisely the position of the signals because of the high specialization of the chromosomes. The CMA_3 staining was repeated several times but it did not provide clear bright signals (Fig. 4). We suppose the heterochromatin in this species consists mainly of AT-rich repeats.

Discussion

The karyotype of *Hypera* species, studied several times during the years, shows a stable chromosome number, with one only exception of *H. punctata*, for which 2n=18+neoXY is reported (Hsiao & Hsiao 1984, see Table 1). In the present study, the first data on the heterochromatin distribution after fluorochrome staining are presented. Our results on the heterochromatin and its content and distribution in *Hypera postica* karyotype confirmed the information reported by Hsiao & Hsiao (1984) that after C-banding there are stable telomeric heterochromatin blocks in almost every autosome and X chromosome, no signal on the Y-chromosome, and we displayed for the first time that the heterochromatin is mainly AT-rich.

Our results contribute to the better characterization of the chromosome structure of *H. postica*, providing first chromosome data on the European population of this species as well on the structure of the heterochromatin. In present study, it was confirmed that there is no chromosome polymorphism observed in the karyotype of *H. postica* and chromosomes are not a suitable marker for distinguishing among species within the genus *Hypera*. All information available in the literature on the chromosome number and the sex chromosome mechanism of *Hypera* spp. is presented in Table 1.

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