

The karyotype of *Cameraria ohridella* (Lepidoptera: Gracillariidae)

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Samenvatting. Het karyotype van *Cameraria ohridella* (Lepidoptera: Gracillariidae)

Aan de hand van materiaal verzameld te Tervuren in de herfst van 2000 werd het karyotype van *Cameraria ohridella* Deschka & Dimić, 1986 bestudeerd. Het haploïed chromosoomnummer bedraagt $n=30$, dit is het modale getal voor Gracillariidae in het algemeen. Het geslachtsdeterminerend mechanisme in *C. ohridella* is ZZ♂ - ZW♀.

Résumé. Le caryotype de *Cameraria ohridella* (Lepidoptera: Gracillariidae)

Avec du matériel réuni à Tervuren pendant l'automne de 2000 le caryotype de *Cameraria ohridella* Deschka & Dimić, 1986 fut étudié. Le nombre haploïde de chromosomes s'élève à $n=30$, le nombre modal des Gracillariidae. Le mécanisme déterminant le sexe chez *C. ohridella* est ZZ♂ - ZW♀.

Key words. *Cameraria ohridella* – karyotype – cytogenetics – chromosome number

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Introduction

Cameraria ohridella Deschka & Dimic, 1986 is a serious pest in many countries of Europe (De Prins & Puplesiene 2000, De Prins & De Prins 2001). In order to study its karyotype, leaf mines on *Aesculus hippocastanum* were collected at Tervuren (Vlaams-Brabant, Belgium) in the autumn of 2000 and in the summer of 2001.

Material and methods

The collected and reared material of 2000 was allowed to pass diapause at 2°C and 90% RH. Emergence took place during February 2001. The moths of the summer generation in 2001 emerged soon after collecting the mines. Gonads from late male pupae and ovaries from freshly emerged adult females were prepared in 1% of potassium citrate and fixed in Carnoy fixative for 72 hours. The slides were made according to the Feulgen-Giemsa staining method described by Puro & Nokkala (1977), which is especially useful for thin prophase chromosomes in oocytes with the adapted approach to Lepidoptera chromosome staining (Nokkala, pers. comm., 1999). Before preparing the squash preparation, a 2% acetic orcein staining for 1 hour was performed. The air-dried slides were embedded in Entellan.

The microphotopictures of the male karyotype and the measurements of the chromosomes were done using a Zeiss Axiodoc microscope attached to a PC, equipped with the program Zeiss Axiodoc (Zeiss©) and processed with Corel Photopaint. The microscopic images of slides are photographed using a 3CCD Toshiba digital camera connected to a Leica DMLB light microscope and equipped with the Leica IM 1000 Image Management System and Auto-Montage Syncroscopy programmes.

Results

Meiotic chromosomes in *Cameraria ohridella* males

Three cysts were found in three specimens containing spermatocytes in pachytene, diplotene, diakinesis, prometaphase I and metaphase I (Fig. 1). No cells of a later stage than *MI* were found; also the meiosis in spermatocytes was not synchronous. The chromosomes were counted in 13 *MI* cells. 30 bivalents were aligned in the metaphase I plate. The karyotype of *Cameraria ochridella* is symmetric. The bivalents show a gradually decreasing series in size from 1.95 μm^2 till 1.14 μm^2 . The measurements were done only in *MI* cells carrying the bivalents at the maximum condensation. All the bivalents in *MI* are dumb-bell shaped or elongated. The largest bivalent is situated close to the centre of the metaphase plate and shows preceding separation of homologues. Most of the bivalents, including the largest one, are homochromatic. However, the midsize bivalent in a descending series shows a distinguishable positive pycnosis. This bivalent is composed of two laterally paired univalents and is situated at almost the edge of the metaphase plate, and consists mainly of C-heterochromatin. It could be a sex chromosome. However, in the earlier stages of meiosis as pachytene, diplotene and even prometaphase I, we did not interpret this formation of bivalent as heteropycnotic. This bivalent tends to show a facultative heteropycnosis. The other of midsize bivalent is delayed in chiasmata terminalisation and in *MI* still shows two chiasmata (Fig. 2).

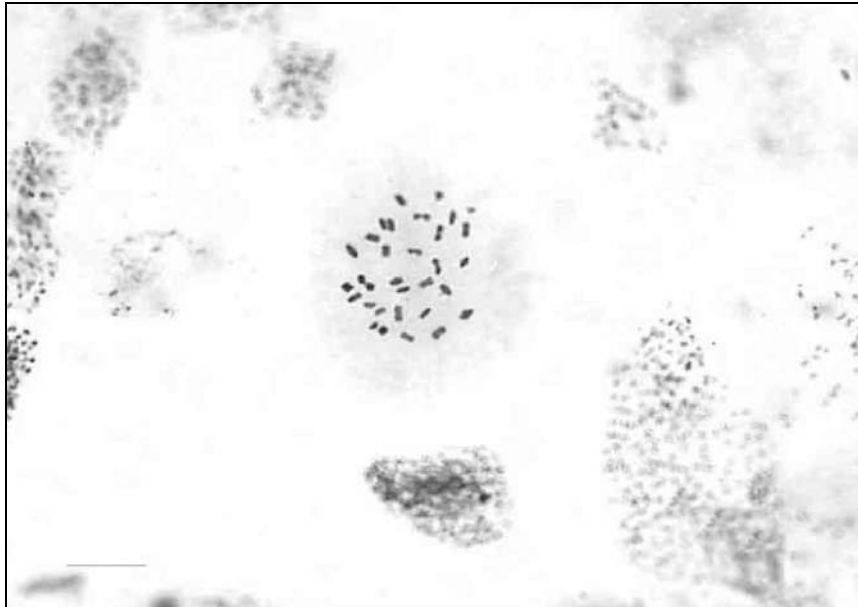


Fig. 1. A cyst containing spermatocytes in metaphase I of meiosis in *Cameraria ohridella* male pupa (Feulgen-Giemsa method). Scale bar 10 μm .

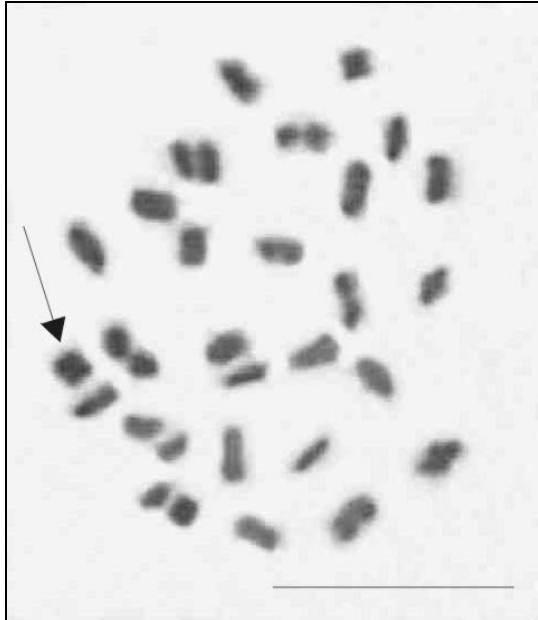


Fig. 2. Bivalents at metaphase I in male meiosis of *Cameraria ohridella* pupa, $n=30$ (Feulgen-Giemsa method). An arrow indicates a positive pycnotic bivalent. Scale bar 10 μm .

Two compacted bivalents in *MI* show a non-homologous association by their lateral parts, while the other ones are free and radially aligned, keeping a different orientation towards the axis of the meiotic plate. No B chromosomes and no polymorphism in chromosome number were observed.

Meiotic chromosomes in *Cameraria ohridella* females

Female meiosis was followed from early pachytene in medium sized eggs till metaphase I in mature eggs. 30 females and 49 oocytes in meiotic divisions were studied. During pachytene stage the bivalents consist of long lampbrush-like chromosomes aligned in parallel (Fig. 3). A bivalent chromosome having V form was found and thus we identify it as ZW. Therefore, the sex determination mechanism in *Cameraria ohridella* is $ZZ\text{♂} - ZW\text{♀}$. After pachytene, the chromosome condensation proceeded without diplotene and diakinesis stages, showing achiasmatic oogenesis, which is typical for Lepidoptera. The condensed achiasmatic bivalents in *MI* of oogenesis are isopycnotic. Non-homologous telomeric associations forming bivalent chains were found (Fig. 4). The largest bivalent showed high condensation degree in *MI*. One heteromorphic bivalent was found, which we identified as ZW. We have found two additional elements, one bivalent and one univalent, in the female karyotype, which can be considered as B chromosomes.



Fig. 3. The pachytene in female meiosis of *Cameraria ohridella*. An arrow indicates the bivalent sex chromosome. Scale bar 10 μ m.



Fig.4. Metaphase I in female meiosis of *Cameraria ohridella*, $2n=60$. Non-homologous telomeric associations forming bivalent chains are seen. Scale bar 10 μ m.

Discussion

The haploid chromosome number of *Cameraria ohridella* is determined as 30, the same as in *C. saliciphaga* (Kuznetsov, 1975) and *C. obliquifascia* (Filipjev, 1926) determined by us earlier (Puplesiene & Noreika 1993). Despite the fact that only three species of *Cameraria* are karyotyped from the Palaearctic Region, we may presume, that the karyotypes of *Cameraria* are stable and bear $n=30$ as a mode. This chromosome number is also considered as the mode for Gracillariidae (Lukhtanov & Puplesiene 1996). All three species of *Cameraria* studied, had a symmetric structure of karyotype with the bivalents forming a descending series in size, thus conforming to the ancestral condition and the mode to the Lepidoptera in general (Robinson 1971, 1990, Lorkovic 1990). Special attention should be paid to the peculiarities of the *C. ohridella* karyotype. The preceded separation of the largest bivalent in the descending series in *MI* of male meiosis is rare in our observations. Usually, in the karyotype of Microlepidoptera, the opposite trend is noticed; the largest bivalent, due to its large size, still possesses terminalised chiasmata and keeps an O form in *MI* (Lukhtanov & Puplesiene 1999). The female karyotype of *C. ohridella* shows a heteromorphic chromosome in late pachytene of oogenesis and therefore the sex determination in this species is $ZZ^{\sigma} - ZW^{\rho}$. The $ZZ^{\sigma} - ZW^{\rho}$ system is characteristic for the ditrysian clade of Lepidoptera (Lukhtanov 2000). Non-homologous telomeric associations between the bivalents observed in female meiosis were described in *Sphinx ligustri* by Nokkala (1987) and observed also in six *Yponomeuta* species (Nilsson *et al.* 1988). In general, according to our observation the *C. ohridella* karyotype shows structural stability. The recent DNA studies of this species in different populations from twelve European countries did not detect any significant differences among the populations studied (Kovács *et al.* 2000).

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