© Comparative Cytogenetics, 2008 . Vol. 2, No. 1, P. 7-20. ISSN 1993-0771 (Print), ISSN 1993-078X (Online)

Mitotic and meiotic studies of seven Caribbean weevils: difference of sex bivalent compaction at pachynema between Curculionidae and Dryophthoridae (Insecta: Coleoptera) species

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Abstract. The mitotic and meiotic karyotypes of seven species of Caribbean weevils are reported. Three species belong to the Dryophthoridae and four to the Curculionidae families. All are considered as pests for agriculture, and were formerly classified among Curculionidae. The three mitotic karyotypes of the Dryophthidae species differ in their chromosome number and morphology while those of the four Cuculionidae species are fairly similar and can be distinguished by heterochromatin variations or NOR location only. Diakineses/Metaphases I of all species exhibit a parachute sex bivalent and the same range of autosomal bivalent compaction. At contrast, at the pachytene stage, bivalents appear less compact in Curculionidae than in Dryophthoridae species, and this difference is particularly clear for the sex bivalent: at early-mid pachytene stage, chromosomes X and Y are elongated and in end-to-end association in Curculionidae while they form a rounded dense body in Dryophthoridae species. In both conditions, the sex chromosomes are embedded in argyrophilic proteins. The different compactions of the sex chromosomes do not depend on NOR location, either on the X or autosome(s). Thus, bivalent compaction kinetic and/or structure at pachynema may be a taxonomic character, which would give arguments for the split of Curculionidae into two families.

Key words: Mitosis, meiosis, weevils, sex bivalent, compaction, pachynema.

Introduction

Curculionoidea is one of the most deleterious beetle superfamilies for agricultural plants. Their damages are particularly important in tropical regions, where they proliferate as soon as they are introduced in cultures of their host plants. A typical example is given by the banana root borer *Cosmopolites sordidus* (Germar 1824) (Dryophthoridae: Rhynchophorinae) which has now spread over all tropical regions where banana culture is developed (Koppenhöffer, 1993). Some species originated from Caribbean region, and recently invaded neighbouring continents. This is the case for the root weevil *Diaprepes abbreviatus* (Linnaeus, 1758) (Curculionidae: Entiminae) (Woodruff, 1968). Other species such as the American palm borer *Rhynchophorus palmarum* (Linnaeus, 1758) (Dryophthoridae: Rhynchophorinae), probably originated from South America, reached Caribbean islands, and are now found in Africa and tropical Asia. As the silky cane weevil *Metamasius hemipterus* (Linnaeus, 1758) (Dryophthoridae: Rhyncho-

phorinae), *R. palmarum* destroys palm trees not only by tunnelling in palm starts in the petioles, but also as vector of nematodes which cause the disease known as red ring of coconut and oil palm (Gerber, Giblin-Davis, 1990).

Chromosome studies were developed on about 500 species of weevils (Holecova et al., 1995), but none was performed on Guadeloupe fauna, which is particularly rich in these pests. While analyzing the chromosomes of some of them, *C. sordidus*, *R. palmarum*, *M. hemipterus* among Dryophthoridae and, *Exophthalmus famelicus* (Olivier 1790), *E. quadritaenia* (Chevrolat 1880), *D. abbreviatus* and *Diaprepes marginicollis* (Chevrolat, 1880) among Curculionidae, we were surprised to observe important differences in sex chromosome compaction at meiotic prophase between the two groups of species. The signification of this finding is discussed

Material and methods

All beetles were collected in Basse Terre in Guadeloupe in February 2006, and in March or December 2007. *Cosmopolites sordidus* was obtained from decayed banana pseudostems. *Rhynchophorus palmarum* and *Metamasius hemipterus* were obtained as pupae from laying trunks of decayed palm trees, and dissected as young imagines a few days later. *Diaprepes* (Schoenherr, 1823) and *Exophthamus* (Schoenherr, 1823) species were collected on the foliage of various trees in the forest. Adult males were dissected to obtain testes, which were immediately dropped in 0.88 M KCl. Testes were fragmented and remained in 0.88 M KCl for 15 min for obtaining mitotic or meiotic metaphases and one to seven hours for meiotic prophase chromosome preparations. The 30 minutes hypotonic shock was completed by 15 min treatment in diluted (1/3) calf serum or 0.55 M KCl and then treated as described (Dutrillaux et al., 2006, 2007). Chromosomes were observed after staining with Giemsa, silver (NOR staining) and C-banding. NOR (Nucleolar Organiser Region) locations were determined on chromosome bivalents at pachynema as described (Dutrillaux et al., 2007). For the nomenclature of beetle families and subfamilies, we followed Alonso-Zarazaga, Lyal (1999).

Results

Dryophthoridae

Cosmopolites sordidus. As cited by Smith, Virkki (1978) for specimens of undefined origin, its karyotype includes 30 chromosomes: 30,XY. All but two pairs of autosomes are meta- or sub-metacentric, and pairs Nos. 13 and 14 are acrocentric. The X is a small submetacentric and the Y is punctiform (Fig. 1). After C-banding, most autosomes exhibit, in addition to their juxta-centromeric staining, C-band positive heterochromatin in either their short or long arms, which allowed us to pair them with a good confidence, in spite of a polymorphism affecting the size of heterochromatic segments, as in pair No. 6 (Fig. 2). Chromosomes Nos. 13 and 14 carry a filament-like structure on their short arms, which corresponds to the NOR (see below). At metaphase I, the sex bivalent has a parachute configuration (Fig. 3) and is heavily stained by silver: 14+Xyp.

Metamasius hemipterus. 2n = 26, XY; all chromosomes are meta- or sub-metacentric (Fig. 4). C-banding is almost limited to juxta-centromeric regions, only pairs Nos. 3 and 4 have a faint staining in intercalary position of their long arms. The NOR is located at the proximal region of the long arm of a submetacentric, that we classified as No. 7 (see below). At metaphase I, the sex bivalent has a parachute configuration (Fig. 5) and is heavily stained by silver: 12+Xyp.

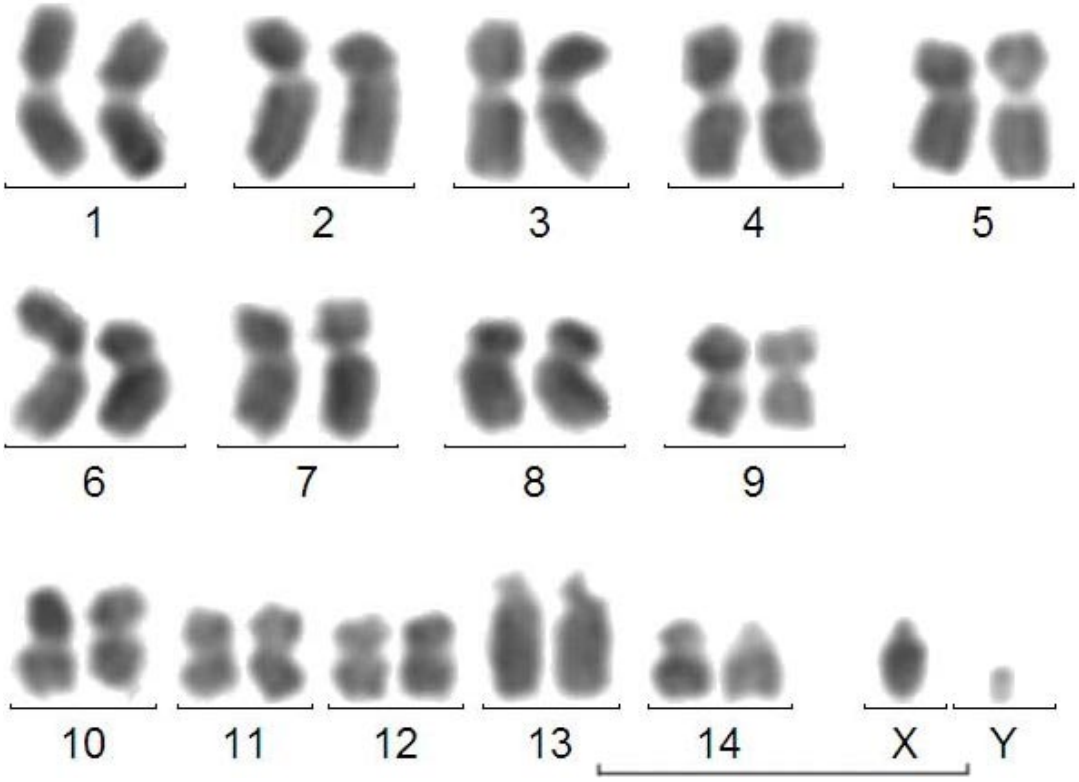

Fig. 1. Giemsa stained male karyotype of *Cosmopolites sordidus*. Bar = 10 μm.

***Rhynchophorus palmarum*.** 2n = 22, XY. Pairs Nos. 1-8 are sub-metacentric and Nos. 9-10 and the X are almost acrocentric. The Y is punctiform. After C-banding, the juxta-centromeric heterochromatin of the sub-metacentrics is double and spreads on both arms (Fig. 6). Pairs 9 and 10 carry the NOR on their short arm. At metaphase I, the parachute configuration of the sex bivalent is frequently asymmetrical: 10+Xyp (Fig. 7).

Curculionidae

Diaprepes abbreviatus. 2n = 22, XY. All autosomes are meta- or sub-metacentric, the X is acrocentric and the Y is punctiform. The staining of heterochromatin is restricted to juxta-centromeric regions. It is fairly homogeneous among autosomes, more intense on the X and absent in the Y (Fig. 8). The same staining is observed at metaphase I, in which the sex bivalent has a parachute configuration and is intensely stained by silver (Fig. 9): 10+Xyp (Smith, Virkki, 1978). The NOR could be localized on the chromosome X short arm only after analysis of spermatocytes at pachynema.

Diaprepes marginicollis. 2n = 22, XY. All the autosomes are meta- or sub-metacentric as in the previous species. The X is a large and the Y is a small acrocentric. The centromeric regions of the large meta- and sub-metacentrics are faintly stained after C-banding. One or both telomeric regions of chromosomes 3-6 are C-banded, as well as all centromeric and telomeric regions of chromosomes 7-10 and X (Fig. 10). At metaphase I, the sex bivalent has a parachute configuration and is heavily stained by silver: 10+Xyp.

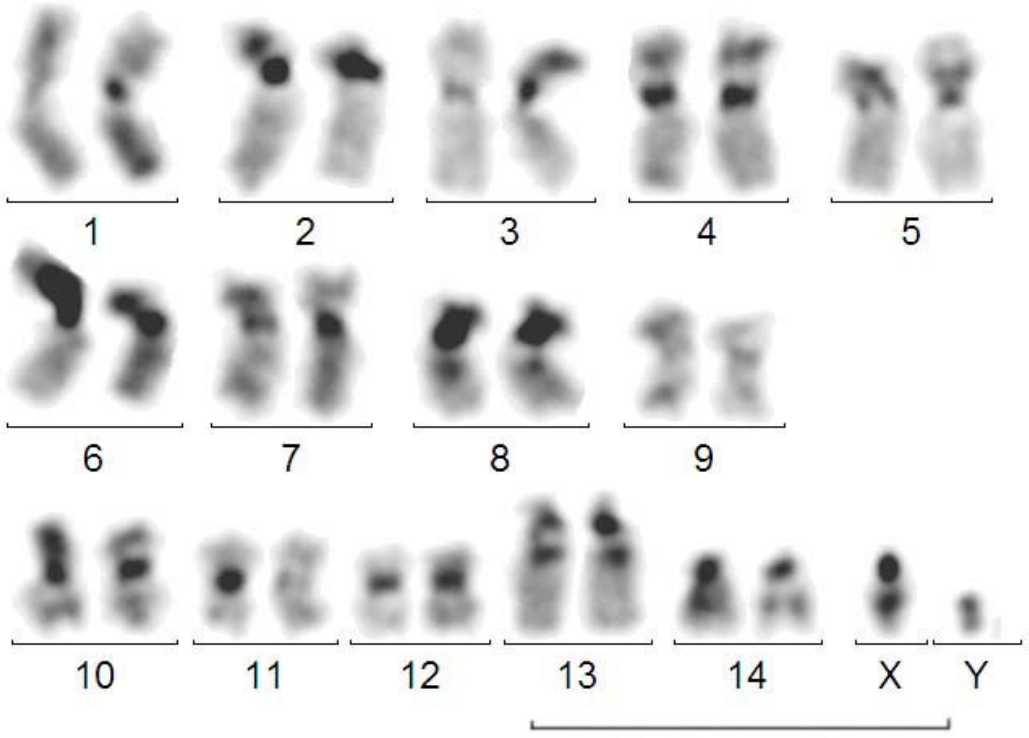

Fig. 2. Same karyotype of *Cosmopolites sordidus* after C-banding exhibiting the polymorphism of heterochromatin. Bar = 10 µm.

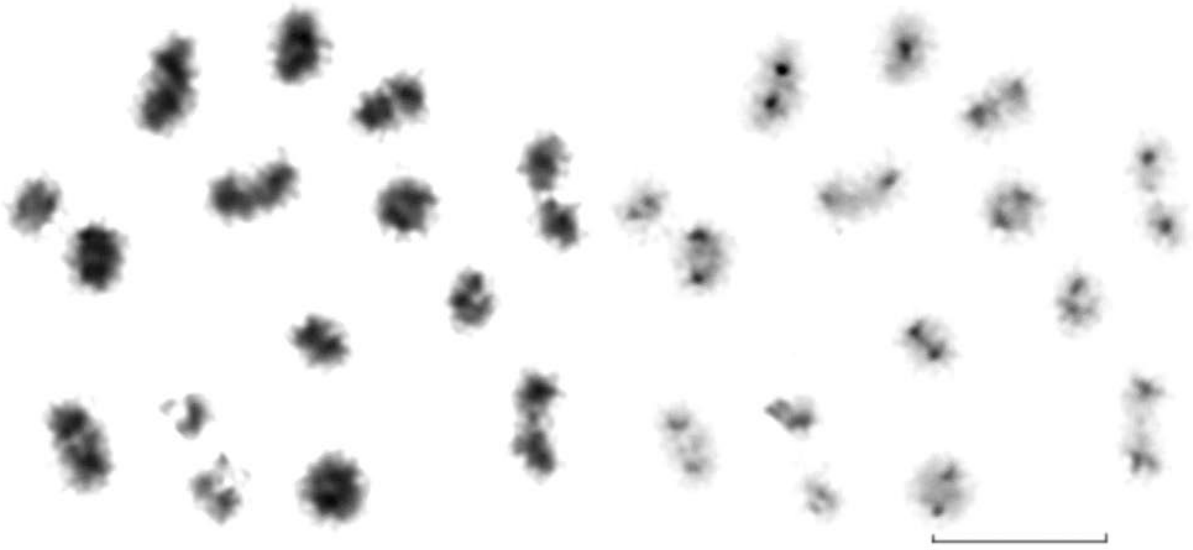

Fig. 3. Spermatocyte at metaphase I of *Cosmopolites sordidus* after Giemsa staining (left) and C-banding (right). Bar = 10 µm.

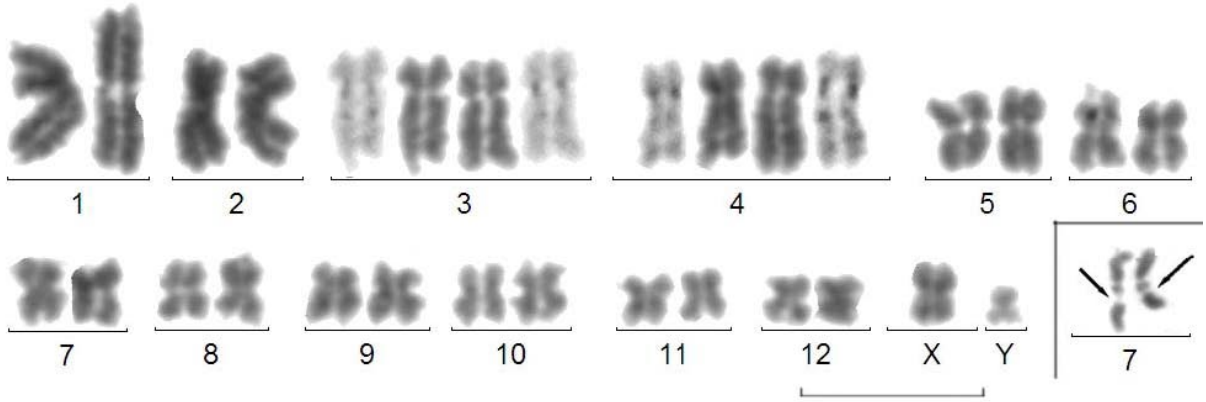

Fig. 4. Giemsa stained mitotic male karyotype of *Metamasius hemipterus*. Chromosomes 3 and 4, from the same metaphase, exhibit faint centromeric and interstitial bands after C-banding. Insert: prometaphasic chromosomes 7 exhibiting a gap (arrows) at the NOR location. Bar = 10 μm.

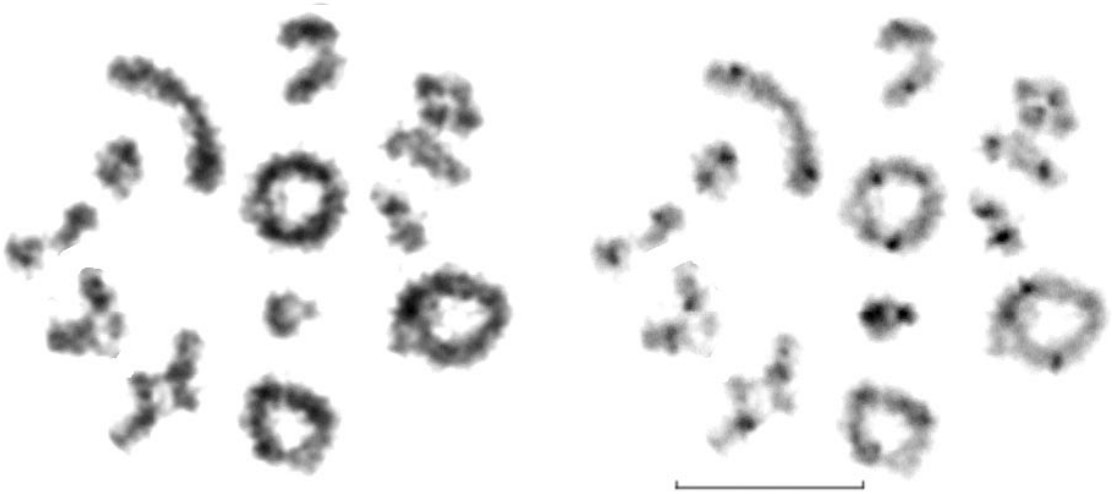

Fig. 5. Spermatocyte at metaphase I of *Metamasius hemipterus* after Giemsa staining (left) and C-banding (right). Bar = 10 μm.

Exophthalmus famelicus. 2n = 22, XY. Metaphase pictures of this species are very similar to those of *D. abbreviatus* after Giemsa staining (Fig. 11) and C-banding: 10+Xyp. The Y chromosome is however slightly larger and C-banded. In mitotic cells, the NOR could be localized on the X chromosome short arm. This was confirmed by the presence of nucleoli at one end of the sex bivalent at pachynema.

Exophthalmus quadritaenia. 2n = 22, XY. All autosomes are meta- or sub-metacentric; the X is acrocentric and the Y punctiform (Fig. 12). All the chromosomes but the Y exhibit a juxta-centromeric staining after C-banding. There is no evidence of heterochromatin in other loci. At pachynema, silver staining recurrently exhibited nucleoli at the juxta-telomeric region of a sub-metacentric, either Nos 6 or 7

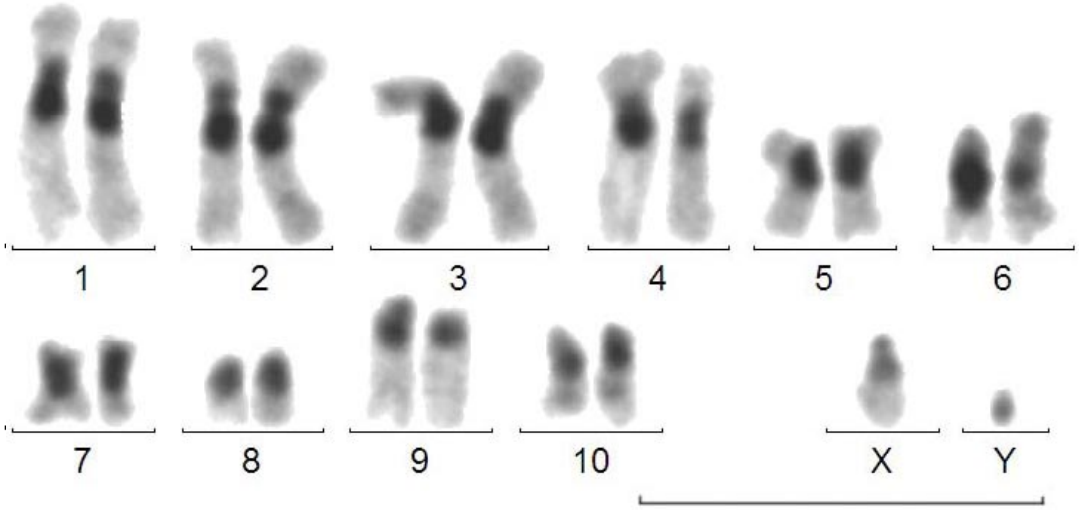

Fig. 6. C-banded male karyotype of *Rhynchophorus palmarum*. Bar = 10 µm.

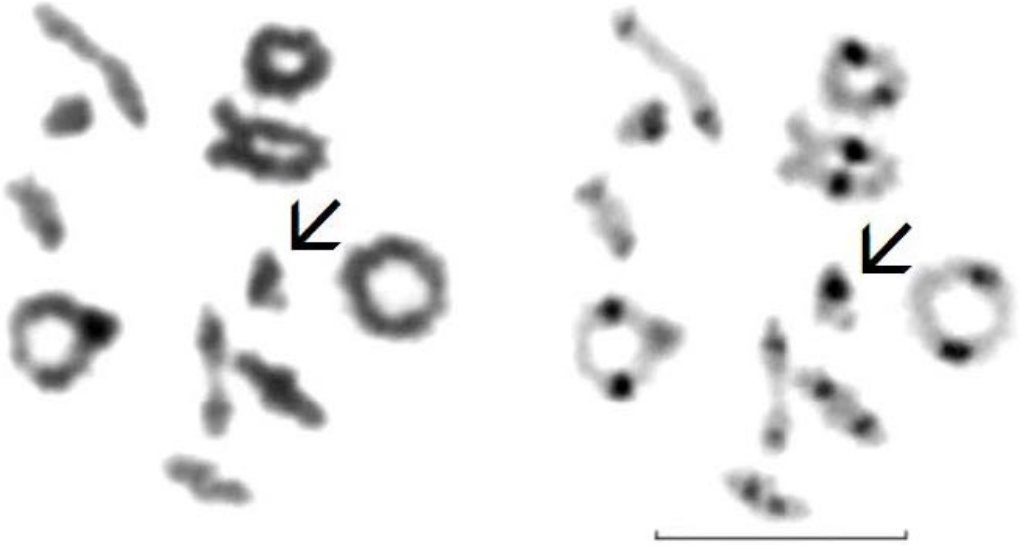

Fig. 7. Spermatocyte at metaphase I of *Rhynchophorus palmarum* after Giemsa (left) and C-banding (right). Arrow: asymmetrical parachute sex bivalent. Bar = 10 µm.

(Fig. 16, e, f). At metaphase I, the compact sex bivalent has a parachute configuration and is heavily stained by both Giemsa and silver, and is C-banded: 10+Xyp.

Sex chromosomes behaviour and their relationships with nucleoli at pachynema.

At the various steps of chromosome bivalent compaction, i.e., from early to late pachynema, the sex bivalent has the same appearance in *C. sordidus*, *M. hemipterus*

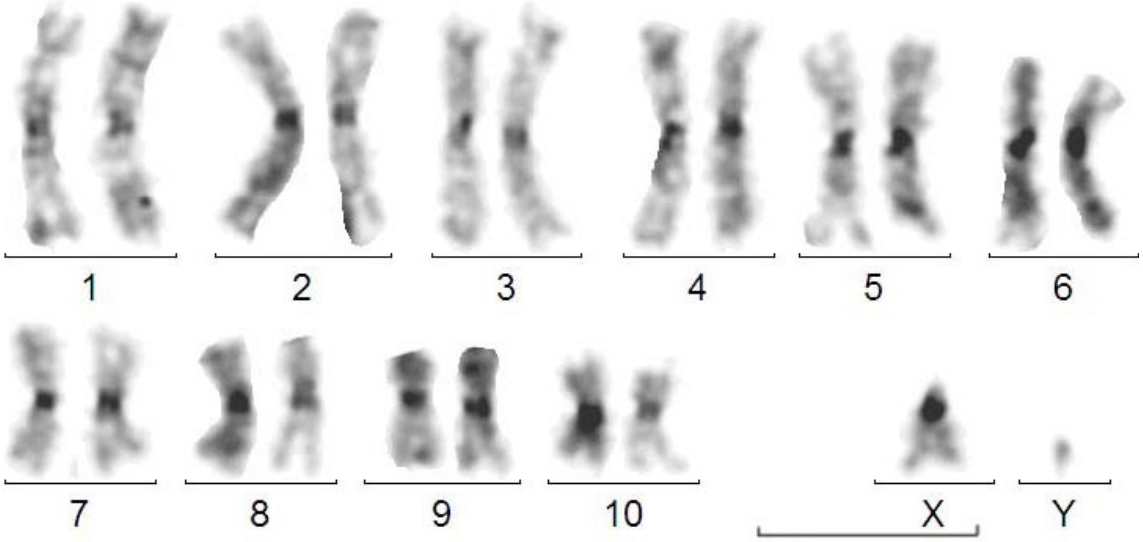

Fig. 8. C-banded male mitotic karyotype of *Diaprepes abbreviatus*. Bar = 10 µm.

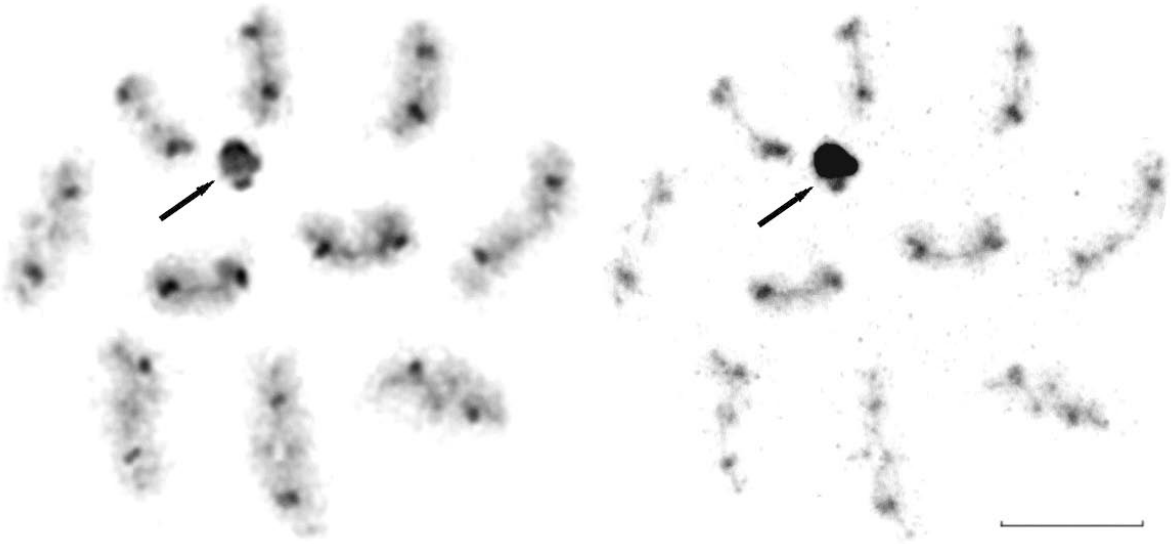

Fig. 9. Spermatocyte at metaphase I of *Diaprepes abbreviatus* after Giemsa (left) and NOR staining (right). Arrow: parachute sex bivalent. Bar = 10 µm.

and *R. palmarum*. It is compact, forming a rounded body, in which the X and the Y cannot be differentiated. It is rarely isolated but frequently located alongside heterochromatin of autosomes. We performed the analysis of 50 spermatocytes of *R. palmarum* after three consecutive stainings (Giemsa, silver and C-banding) to precise the spatial relationships between autosomes and gonosomes. The sex bivalent was in contact with bivalent 9 or 10 in 17 instances and with both of them in 15 instances (Fig. 13). This contact was always re-

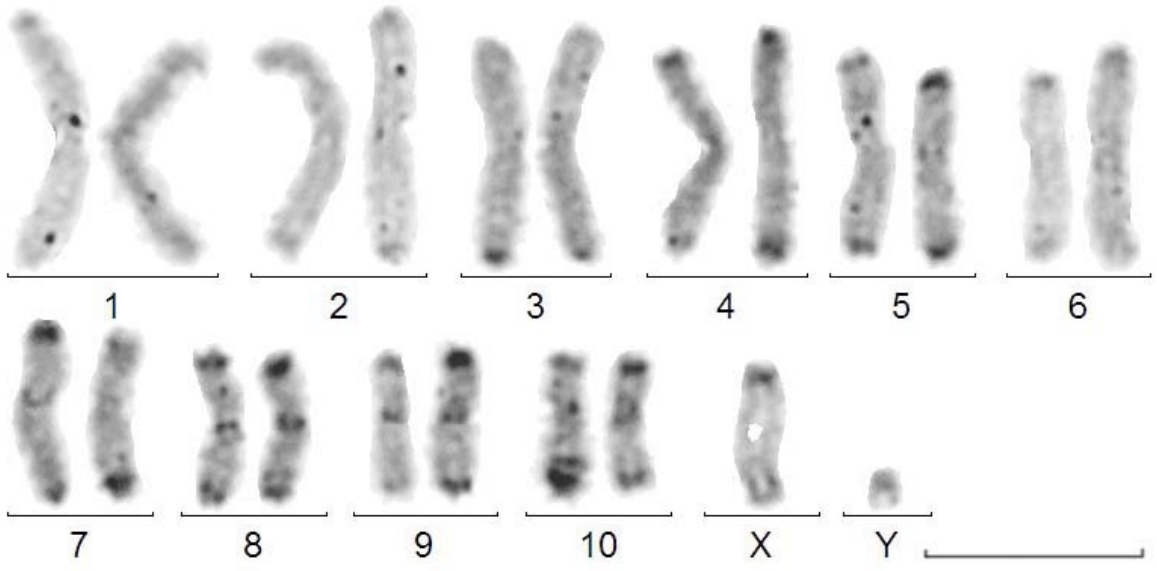

Fig. 10. C-banded male karyotype of *Diaprepes marginicollis*. Bar = 10 µm.

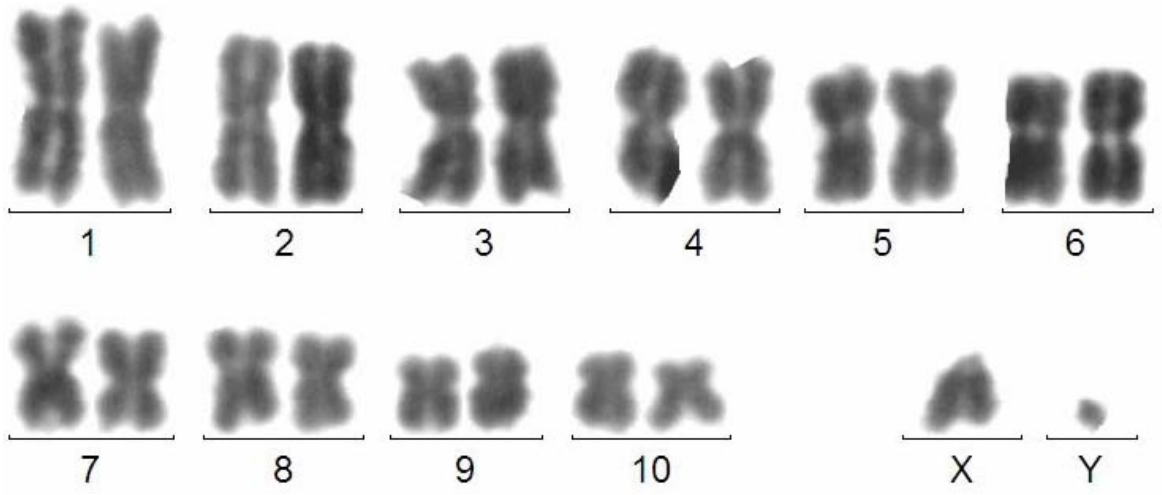

Fig. 11. Giemsa stained male karyotype of *Exophthalmus quadritaenia*. Chromosomes 3 length = 10 µm.

alized through argyrophilic material surrounding the short arms of bivalents 9 and 10, which corresponds to nucleoli in contact with the NORs (Dutrillaux et al., 2007). In most of the remaining 18 spermatocytes, the sex bivalent was alongside the centromeric heterochromatin of various other autosomes (Fig. 14), while nucleoli remained associated with bivalents 9 and 10 short arms. No detectable variations of either the size or intensity of staining of the sex bivalent paralleled autosome compaction. We performed the same analysis on 22 sper-

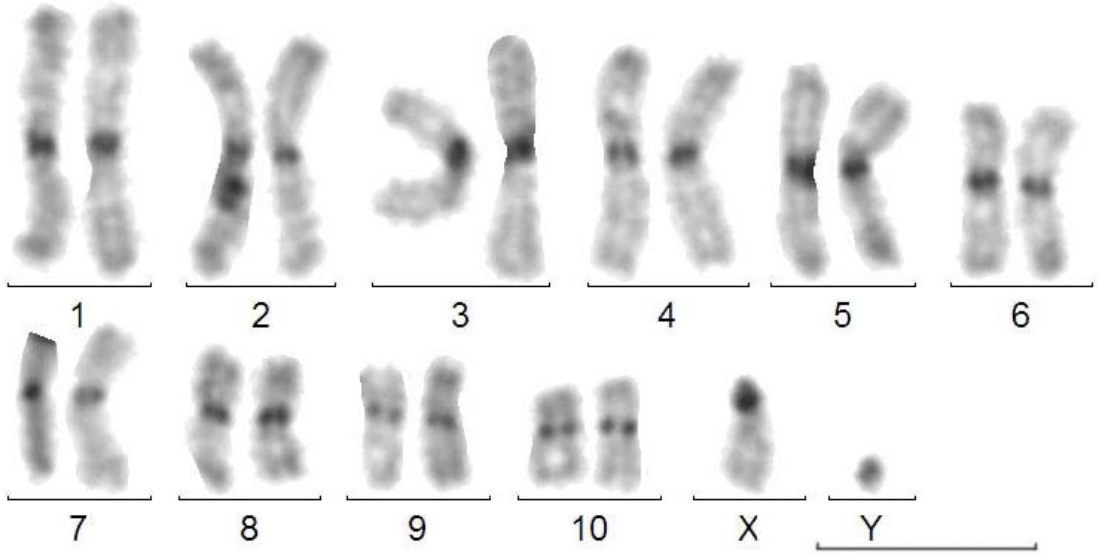

Fig. 12. C-banded male karyotype of *Exophthalmus famelicus*. Bar = 10 μm.

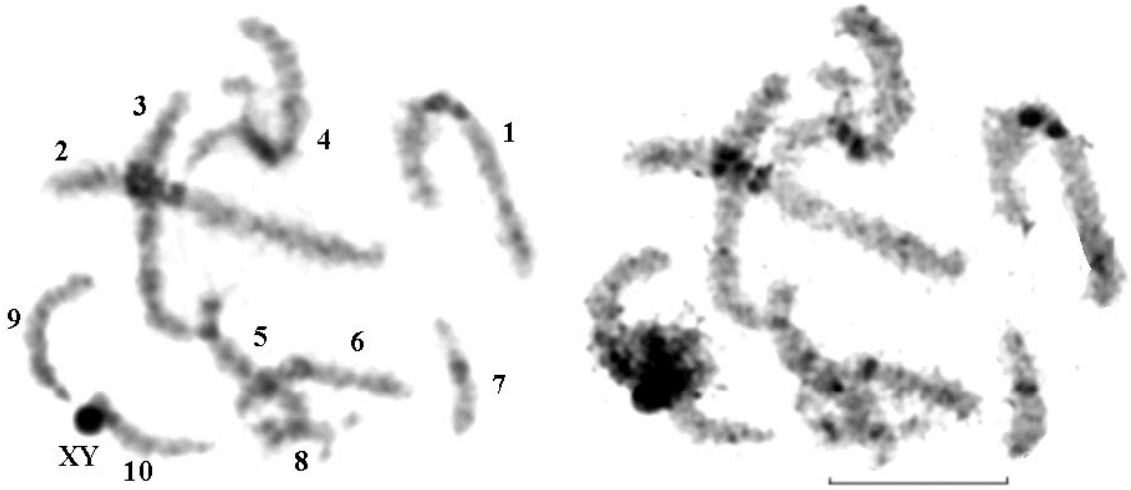

Fig. 13. Spermatocytes at pachynema of *Rhynchophorus palmarum* exhibiting spontaneous C-banding like (left) and after NOR staining (right). Two acrocentrics (Nos. 9 and 10) have NOR on their short arm. They are at contact with the sex bivalent through the nucleoli. Bar = 10 μm.

matocytes from *C. sordidus*. Nucleoli were systematically observed around the short arms of the two acrocentrics, i.e. Nos. 13 and 14, and one or both of them was always in contact with the sex bivalent (Fig. 15). In *M. hemipterus*, the relationships between the sex bivalent and nucleoli were different. Nucleolus material was always located around the proximal part of the long arms of a medium-sized bivalent, presumably No. 7, identifying the NOR. Its site was always marked by the elongation of the bivalent, as previously no-

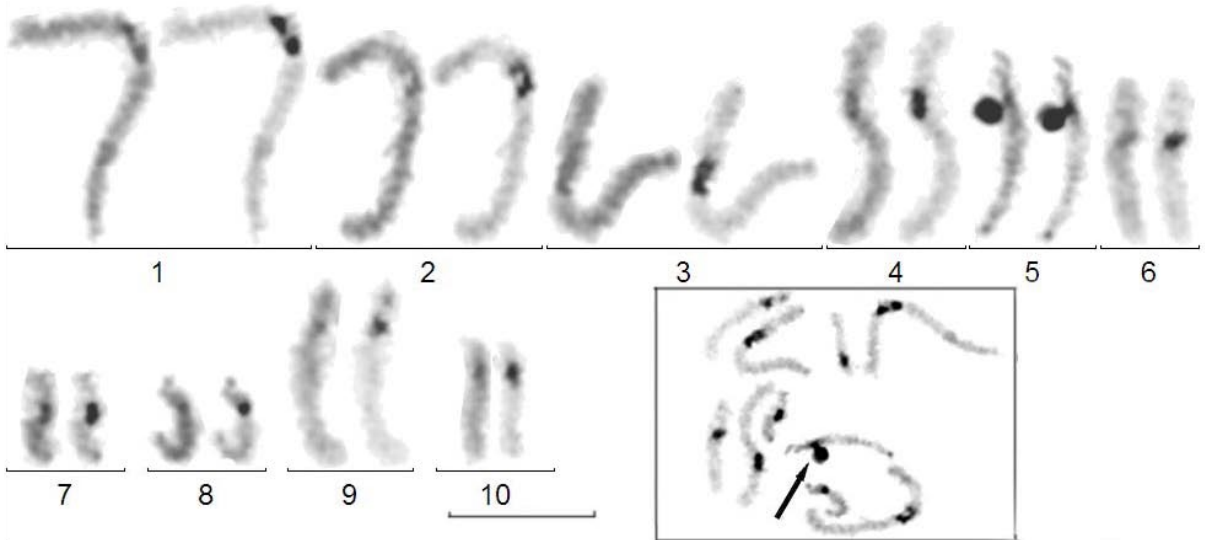

Fig. 14. Karyotype of *Rhynchophorus palmarum* established with bivalents from a spermatocyte at pachynema stained by Giemsa (left) or C-banded (Right). The rounded sex bivalent (arrow) is alongside the centromere of an autosomal bivalent, presumably No. 5. The original C-banded spermatocyte is in the inset. Bar = 10 μm.

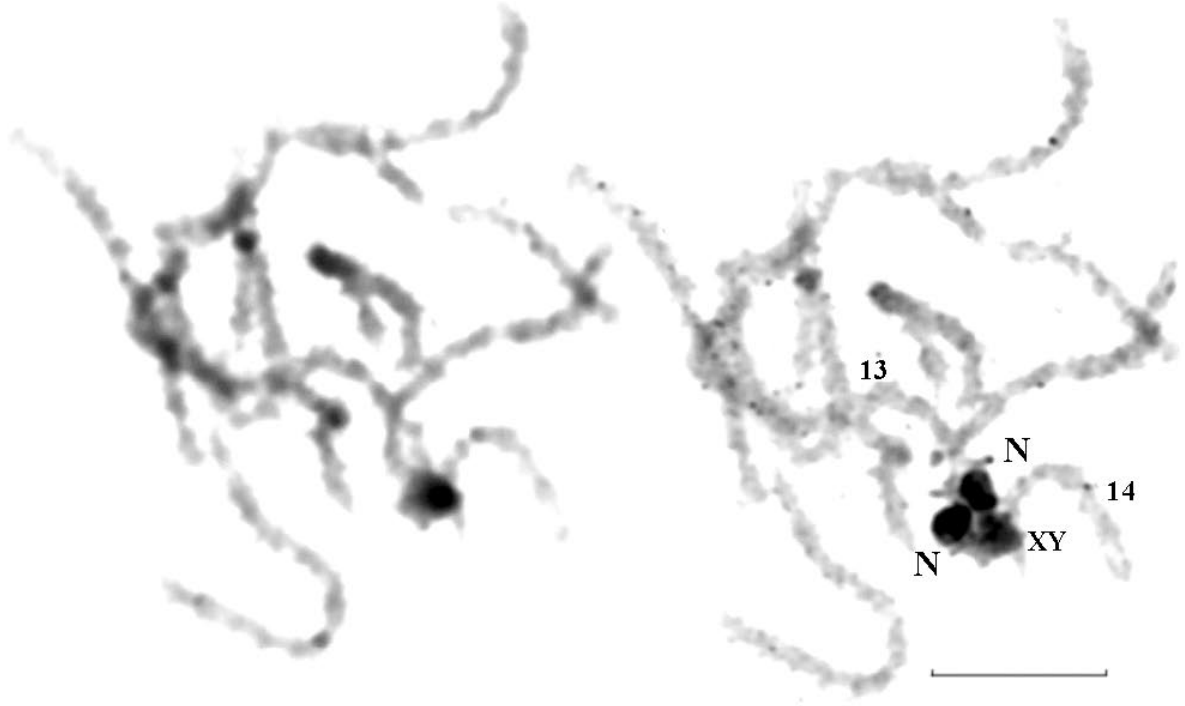

Fig. 15. Spermatocyte at early pachynema of *Cosmopolites sordidus* exhibiting the association of the two NOR carrier acrocentrics (Nos. 13 and 14) with the sex bivalent. Giemsa staining (left) and NOR staining (right). Bar = 10 μm.

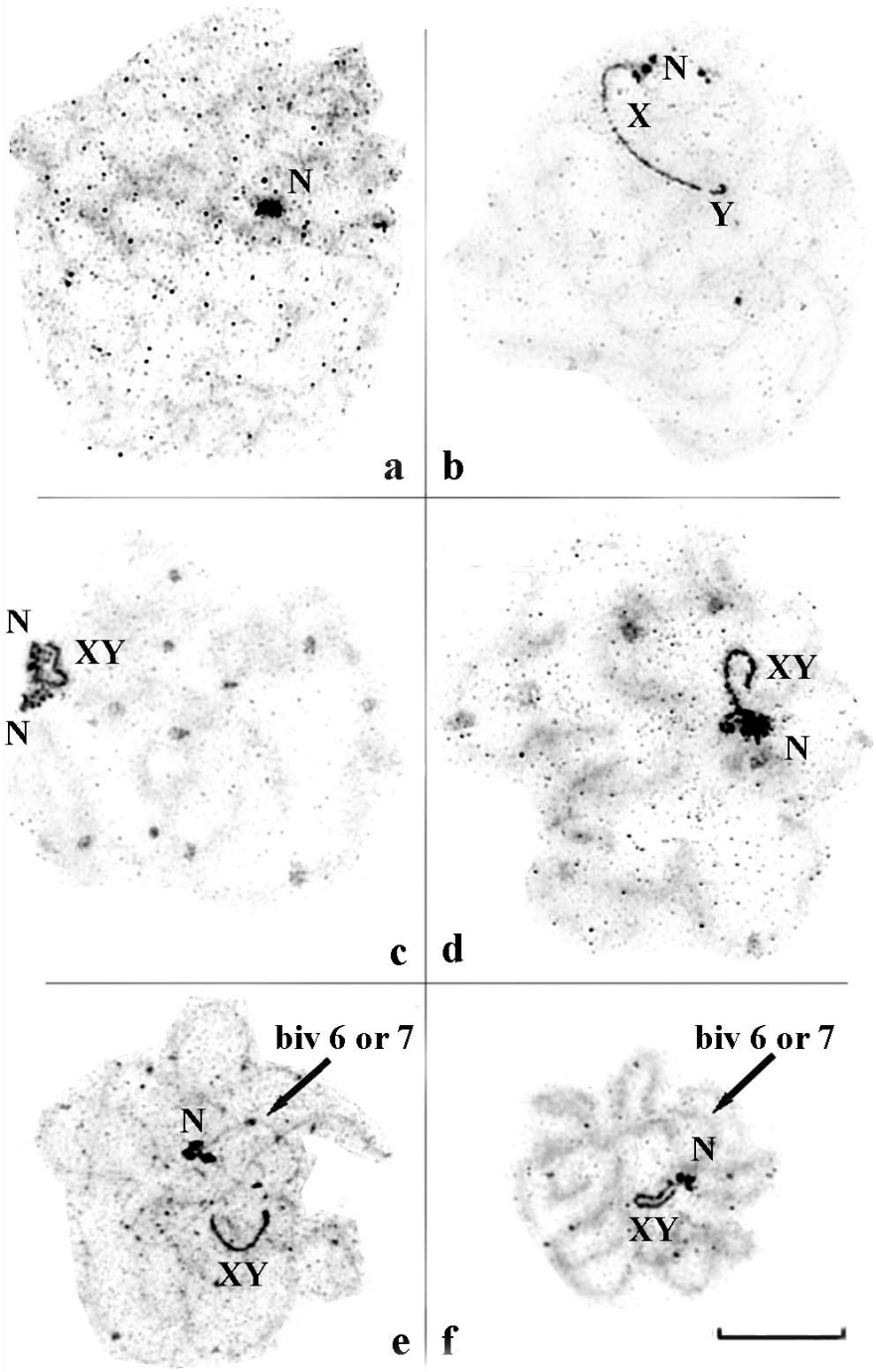

Fig. 16, a-f. NOR staining of spermatocytes at meiotic prophase of *Exophthalmus famelicus* (NOR on the X short arm, a-d) and Exophthalmus quadritaenia (NOR on chromosome 6 or 7, e, f). **a -** leptonema: only nucleolar material (N) is stained. **b -** zygonema: the linear sex bivalent starts staining. **c, d** -pachynema: the sex bivalent coils but remains distinct and becomes strongly argyrophilic. **e, f -** nucleoli on bivalent 6 or 7 may remain at distance (e) or come at contact (f) with the sex bivalent, which is argyrophilic in both conditions. Bar = 10 μm.

ticed for other beetles (Dutrillaux et al., 2007). This elongation was also visible on mitotic cells at prometaphase (Fig. 4). The sex bivalent was rarely in contact with nucleoli, i.e., in 3/35 spermatocytes at pachynema analyzed, although it was intensely stained by silver, as nucleoli (not shown).

In the two *Diaprepes* species and *E. famelicus*, the aspect of bivalents differed, although the techniques used were similar. On the average, the bivalents were less compact, and the whole spermatocytes occupied a larger surface after spreading. The most spectacular difference involved the sex bivalent. The X and Y had a linear morphology and were disposed end-to-end (Fig. 16, a-d). Their size was similar with that of autosomes. They were easily identified by their sharp appearance, contrasting with the fuzzy aspect of autosomes, whatever the staining used. After silver staining, argyrophilic granules were present at one extremity, which corresponded to chromosome X short arm, opposite to the Y chromosome. Thus, the Y was associated with the telomeric region of the X long arm. The silver staining of the sex chromosomes followed a chronology which can be deduced from the progression of autosomal bivalent morphology. At leptotene-zygotene stages, the sex chromosomes had the same pale staining as autosomes, and only the nucleoli were silver stained. At early pachynema, the sex chromosomes started staining, in contact with nucleoli. This staining progressively increased, and the sex chromosomes became as heavily stained as the remaining nucleolus. At late pachynema, the sex bivalent became a rounded dense body, as it was at pachynema in *C. sordidus*, *R. palmarum* and *M. hemipterus*. In *E. quadritaenia*, the sex bivalent followed the same morphological variations as in *Diaprepes* species and E. famelicus, but nucleoli remained in contact with bivalent Nos. 6 or 7, and their association with the sex bivalent was dispensable (Fig. 16, e-f).

Discussion

Curculionidae, the largest family among Curculionoidea, was recently subdivided into several families, including Dryophthoridae (three species of this study) and Curculionidae (four other species studied here) (Alonso-Zarazaga, Lyal, 1999). As proposed by several authors (Smith, Virkki, 1978; Sharma et al., 1980; Lachovska et al, 1998), the basic chromosome number of Curculionoidea is 22. As shown in the chromosome number list provided by Smith, Virkki (1978), however, chromosome number increases in some species, in particular among Dryophthoridae and Curculionidae. *Diaprepes* and *Exophthalmus* species have the modal number of chromosomes of their family and subfamily (Curculionidae: Entiminae), and the chromosomes numbers of the three Dryophthoridae species fall in the range of that of both families. Thus, the difference of chromosome numbers is not a criterion for discriminating between Dryophthoridae and Curculionidae.

The standard chromosome morphology remains difficult to reconstitute because it is not reported in most publications. When pictorial information is given (Holecova et al., 2002; Bailly et al., 1990), acrocentrics seem to be either rare or absent. Thus, the karyotypes described here, with many meta- and submetacentric autosomes are fairly representative of Curculionoidea. Karyotypes with two similar acrocentric pairs, both NOR carriers, are observed in two of the three Dryophthoridae species. This may indicate a common origin, but the karyotype of the third species of this family has no acrocentrics, and the NOR is located on a single autosome. Both chromosome number and morphology variations indicate that an active chromosomal evolu-

tion took place in Dryophtoridae. This is in agreement with the variations of chromosome numbers in *Sphenophorus* (Schoenherr, 1838) = *Calandra* (Clairville, Schellenberg, 1798) species (Smith, Virkki, 1978), which belong to the same family.

At contrast, the four Entiminae species kept fairly similar karyotypes, with meta- and submetacentric autosomes of gradually decreasing size and acrocentric Xs. However, the NOR location on either the X (three species) or an autosome, in *E. quadritaenia*, demonstrates that at least micro-rearrangements have occurred. This apparent karyotypic stability could be due to their fairly close geographic and phylogenetic relationships, but in Entiminae, almost all bisexual species studied so far possess 22 chromosomes, whatever their origin (Smith, Virkki, 1978; Lachowska et al., 2004). This stability may characterize Entiminae, but certainly not the whole Curculionidae family, because large variations of chromosome numbers were described in other subfamilies such as Curculioninae and Molytinae. Thus, Dryophthoridae do not differ from Curculionidae in this respect.

Finally, the most intriguing chromosomal character differentiating the two groups of species may be of epigenetic origin: the sex chromosomes undergo different compactions and relationships with nucleoli during meiotic prophase. In the Dryophtoridae species, the sex chromosomes can be clearly identified at late zygotene/early pachytene stages, and they form a compact rounded body during the rest of meiotic prophase. This sex body, frequently in contact with nucleoli during pachynema, is argyrophilic and thus, assumed to be embedded in proteins of nucleolar origin. This aspect recalls that found in other beetles such as Scarabaeidae (Dutrillaux et al., 2007). In Curculionidae species, the morphology of the sex chromosomes, forming dense filaments in end-to-end association, is quite different. They coil up and make a rounded structure only by the end of the pachytene stage. In three of the four species studied, the NOR is carried by the X, and nucleoli remain located at one tip of the sex bivalent, presumably the short arm of the X, opposite to the associated Y. Both tips of the sex bivalent come in contact with nucleoli, as it coils up. This chronological difference of sex chromosome compaction and embedding in nucleolar proteins between the two families might has been related to the location of the NORs, X-linked or autosomal, but in the fourth Curculionidae species, *E. quadritaenia*, the NOR is autosomal, as in Dryophthoridae species, and the timing of its sex chromosome compaction is comparable to that of other Curculionidae. Thus, it is probably genetically determined as a supra-specific character. The mode of X-Y association and segregation gave rise to nice works in the sixties and seventies, and figures exhibiting filament-like sex chromosomes during meiotic prophase were published by Smith and Virkki (1978). At that time, the authors signalled that "not all beetles are satisfactory for analysis at these stages". Interestingly, *Pissodes* (Germar, 1817) and *Peridinetus* (Schoenherr, 1836) species, which belong to Molytinae and Baridinae, two other subfamilies of Curculionidae, were considered as "satisfactory". The same story applies to Scarabaeoidea species, among which the sex chromosomes are either analyzable, as in *Trox* (Fabricius, 1775) (Trogidae) (Smith, Virkki, 1978) and Passalidae species or not, as in Cetoninae (Scarabaeidae) (Dutrillaux et al., 2007). Indeed, the data remain scarce, but we propose as a working hypothesis that the mode or chronology of sex chromosome and possibly autosome compaction at meiotic prophase is not uniform in Coleoptera. It varies, and its variation may be a taxonomic character. If confirmed, it would provide arguments justi-

fying the separation of Dryophthoridae from Curculionidae.

Acknowledgements

We are grateful to Claudie Pavis who permitted us to work in INRA laboratories in Domaine Duclos, Basse Terre, Guadeloupe.

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Received March 30, 2008.
Accepted by V.G. Kuznetsova and I.A. Gavrilov, May 1, 2008.
Published June 30, 2008.