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Telomeric heterochromatin and meiotic recombination in three species of Coleoptera (*Dorcadion olympicum* Ganglebauer, *Stephanorrhina princeps* Oberthür and *Macraspis tristis* Laporte)

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Abstract. Centromeres are generally embedded in heterochromatin, which is assumed to have a negative impact on meiotic recombination in adjacent regions, a condition required for the correct segregation of chromosomes at anaphase I. At difference, telomeric and interstitial regions rarely harbour large heterochromatic fragments. We observed the presence at the heterozygote status of heterochromatin in telomere region of some chromosomes in 3 species of Coleoptera: *Dorcadion olympicum; Stephanorrhina princeps* and *Macraspis tristis.* This provided us with the opportunity to study the relationship between heterochromatin, chiasma location and meiotic recombination independently from the proximity of centromeres in this order of insects. In acrocentric chromosomes, the presence of heterochromatin in telomere region of the long arm displaces recombination near the centromere. In sub-metacentrics, recombination is almost always restricted to the other arm. This at distance effect of heterochromatin may deeply influence genetic drift.

Keywords. C-banded, telomeric heterochromatin, meiosis, recombination, Coleoptera.

INTRODUCTION

In almost all living organisms, centromeres are surrounded by heterochromatin, which harbours repetitive DNA (Nakaseko et al. 1986), whose function is not yet completely understood. In cells in mitotic growth, heterochromatin represses transcription and expression of genes located into it (Grewal and Jia 2007). During meiosis of most living organisms, recombination is necessary for the correct chromosome segregation at anaphase I, but it does not occur in heterochromatin. Consequently, recombination is repressed in centromeric regions, which harbour heterochromatin. In *Schizosaccharomyces pombe*, it was found to be approximately 200 times less in het-

erochromatin than in the genome-wide average (Ellermeier et al. 2010). A current interpretation is that recombination, thus chiasma formation in centromeric region, would lead to abnormal chromosome segregation (Lynn et al. 2004). Thus, a function of centromeric heterochromatin would be to displace recombination far from the centromeres and allow the correct chromosome segregation. Large and variable amounts of heterochromatin are present in the karyotype of many animals belonging to various taxonomic groups, but their position is not at random: frequently juxta-centromeric and rarely interstitial or terminal. In mammals, a wellknown example of terminal heterochromatin is that of the Hedgehog (Insectivora) in the karyotype of which 2 to 4 chromosome pairs are involved. However, the heterochromatic blocks are not strictly terminal because NORs (Nucleolar Organizer Regions) are located at their extremity (Mandhal 1979). It was noticed that no meiotic recombination occurred in and at proximity of heterochromatin (Natarajan and Gropp 1971). The same particularities were observed in the Primate *Cebus capucinus* (Dutrillaux 1979) but such examples remain rare. In insects, terminal heterochromatin was observed in acrocentric chromosomes of several species of grasshoppers (John and King 1982, 1985, Torre et al. 1985). These authors attributed the displacement of chiasmata to proximal position to the presence of terminal heterochromatin. In Coleoptera, large heterochromatic fragments are commonly seen in most families (Juan and Petitpierre, 1989, Correa et al., 2008, Dutrillaux and Dutrillaux, 2016), but almost always in the centromere region, as in other taxonomic groups. This preferential location may result from the amplification of DNA repeated sequence surrounding centromeres, as shown for α satellite sequences (Rudd et al. 2006, Shepelev et al. 2009). As it will be discussed, the correct segregation of chromosomes at meiosis may also depend on the embedding of centromeres in heterochromatin. Displacement of heterochromatin from centromere regions to intercalary or terminal regions would necessitate secondary events, but terminal heterochromatin may have other origins. It will be also discussed that the presence of heterochromatin in telomeric position may not confer a selective advantage, by imposing meiotic constraints. Among hundreds of species of Coleoptera we studied, karyotypes with large amounts of heterochromatin in terminal position were rarely observed. We confirm that heterochromatin terminally located on the long arm of acrocentrics may not decrease recombination, but simply displaces it near to the centromere, usually considered as a cold region (Mahtani and Willard 1998). In addition, we show that in non-acrocentric chromosomes, heterochromatin terminally located on one arm displaces recombination to the other arm. Thus, heterochromatin can suppress recombination on a whole arm and influence meiotic recombination at much larger distance than it was generally thought.

MATERIAL AND METHODS

Three examples belonging to three different families or sub-families were found among about 400 species of Polyphagan beetles:

Dorcadion olympicum Ganglebauer 1882 (Cerambycidae: Lamiinae: Dorcadionini). Two specimens were captured in May 2014 in Eastern Greece, near Alexandroupolis (40° 50'57"N and 25°52'46"E).

Stephanorrhina princeps Oberthür 1880 (Scarabaeidae: Cetoninae: Goliathini). Two specimens of African origin (Malawi) were obtained in September2007 from a private breeding.

Macraspis tristis Laporte 1840 (Scarabaeidae: Rutelinae: Rutelini). Eight adult specimenswere obtained in March 2008 from grubs captured in Guadeloupe (Basse-Terre, near Deshayes 16°18'00"N and 61°47'00"W) in December 2006.

Chromosome preparations of cells at various stages of meiosis were obtained as described (Dutrillaux and Dutrillaux 2009, Dutrillaux et al. 2010). Proliferating cells obtained from either eggs, testes or mid gut were processed as described. Chromosomes were Giemsa stained and further silver stained for localization of the Nucleolus Organizer Region (NOR) and/or C-banded for localization of heterochromatin. Image capture and karyotyping were performed using IKAROS software (Metasystems, Germany). Chromosome nomenclature: to avoid ambiguous interpretations, we will call acrocentric all chromosomes with a single euchromatic arm, whatever the size of the heterochromatin (generally C-banded) forming the other arm. Chromosomes with euchromatin (not C-banded) on both arms are either metacentric or sub-metacentric. We will focus on chromosomes with large heterochromatic blocks distally attached to euchromatic arms.

RESULTS

Dorcadion olympicum

The mitotic male karyotype is composed of 24 chromosomes. Pairs 3 and 5 are sub-metacentric and all other autosomes are acrocentric. The X chromosome is sub-metacentric and the Y is punctiform (24,XY). In one of the two specimens studied, the length of one chromo-

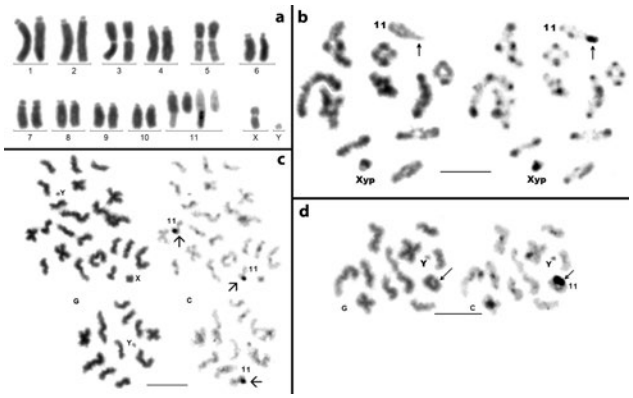

Fig. 1. Chromosomes of *Dorcadion olympicum* **:** a) Giemsa-stained karyotype and C-banded chromosomes 11 exhibiting additional heterochromatin in one homologue. b) Giemsa stained (left) and C-banded (right) spermatocyte I at diakinesis/metaphase with bivalent 11 carrying heterochromatin at one side (arrow). X and Y form a parachute bivalent (Xyp). Barr= 10 mm, as in other figures. c) Group of 3 spermatocytes II at metaphase, sequentially Giemsa stained (G) and C-banded (c). As in most other metaphases II, centromeric heterochromatin is poorly C-banded and chromosome 11 is asymmetrical, with compacted heterochromatin at the tip of a single chromatid (arrows). d) 12,Y spermatocyte II in which the 2 heterochromatin carrier chromatids of chromosome 11 remain cohesive, whereas all other chromosomes have clearly non-cohesive chromatids.

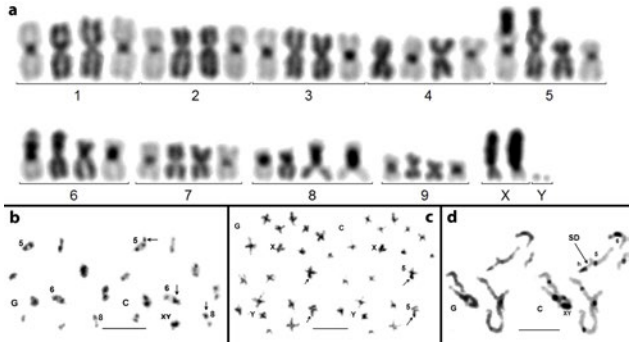

Fig. 2. Sequentially Giemsa stained (G) and C-banded (C) chromosomes of *Stephanorrhina princeps* a) Giemsa stained (center) and C-banded karyotype of a spermatogonium exhibiting additional heterochromatin on one chromosome of pairs 5, 6, 8 and on the X. Only heterochromatin of chromosome 5 is clearly separated from the centromere region. b) Diakinesis/metaphase I: heterochromatin (arrows) remains opposite to chiasmata. c) Exceptionally, in each of these 2 brother spermatocytes II, chromosome 5 is asymmetrically carrier of heterochromatin. d) Spermatocyte I at pachynema: synapsis defect (SD) of the proximal (euchromatic) part of the short arm of bivalent 5.

some 11 is enlarged by the addition of a large amount of C-banded heterochomatin at the telomeric region of the long arm. (Fig. 1a). Following a simple Giemsa staining, the chromatids of this fragment look hyper-cohesive, thin and pale. Centromeric regions are faintly C-banded, as it frequently occurs in the genus Dorcadion (personal data). At diakinesis/metaphase I of meiosis, the sex bivalent has the parachute configuration, usually found in Polyphagan Coleoptera. The compaction of the additional heterochromatin of bivalent 11 is much variable: quite elongated at early diakinesis, it becomes highly compacted at late metaphase I. Bivalent 11 remains easily identified by C-banding (Fig. 1b). The euchromatic component of bivalent 11 has the same aspect in the 131 analysed metaphases I: a cross with very uneven branches. The block of heterochromatin is always located at the tip of the longest branch. This indicates that chiasmata are systematically located near the centromere. In 90/95 spermatocytes II at metaphases, the heterochromatic block is located on a single chromatid of chromosome 11, demonstrating that one crossing-over had occurred in its long arm (Fig. 1c). In 5 instances only, the heterochromatin is present on both chromatids, which may be interpreted as either a lack of recombination in the long arm or the result of a double recombination between the centromere and the heterochromatin. Interestingly, in these chromosomes, the heterochromatin blocks remain cohesive, while euchromatic arms are well separated, as usual at this stage. This gives it a ring appearance (Fig.I d). Finally, in 4 additional pairs of sister spermatocytes II (or diploid ones), the heterochromatin carrier chromosomes remain close to each other suggesting heterochromatin remained associated at anaphase, inducing chromosome lagging.

Stephanorrhina princeps

The mitotic karyotype is composed of 18 meta- or sub-metacentric autosomes, one large X, and one punctiform Y (20, XY). Large and variable fragments of heterochromatin are C-banded on one chromosome of pairs N°5, 6, 8 and on the X. In pair N°5, the heterochromatin is distally located on the short arm (Fig. 2a). We will focus on the meiotic behaviour of the heterozygote chromosomes 5. As in the previous species, the chromatids are hyper-cohesive in their heterochromatic portion. At diakinesis/metaphase I, the heterochromatin is fuzzy and hardly detectable without C-banding. In 37/40 instances, the heterochromatin is located at one extremity of bivalent 5, which looks asymmetrical after C-banding (Fig. 2b). In 3 instances, heterochromatin is in the centre of the bivalent, which has a symmetrical appearance. Amongst 23 spermatocytes II at metaphase, chromosomes 5 have a C-band on either both chromatids (12 times), or a single chromatid (twice, fig.

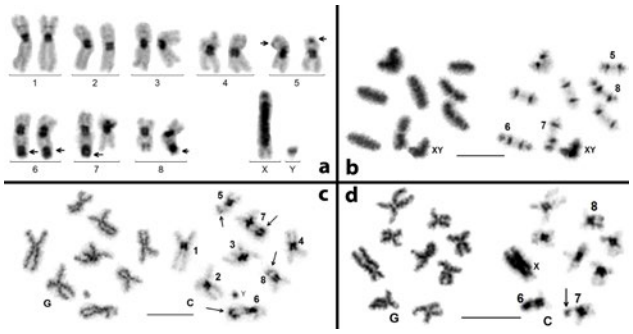

Fig. 3. Giemsa (G) stained and C-banded (C) chromosomes of *Macraspis tristis*. a) C-banded karyotype of a spermatogonium exhibiting heterozygosity for heterochromatin of pairs 7 and 8 and homozygosity for pairs 5 and 6. b) Sequentially Giemsa stained and C-banded spermatocyte I at metaphase with asymmetric bivalents 7 and 8. Heterochromatin of bivalents 5, 6, 7 and 8 is always external, opposite to chiasmata or terminal association. c) Spermatocyte II: all heterochromatin fragments (arrows)are symmetrically distributed on both chromatids. d) Unique spermatocyte II with asymmetrical chromosome 7.

2C), or had no C-band (9 times). Thus, recombination rarely occurred in the heterochromatin carrier arm. At pachynema, the bivalents with enlarged heterochromatin tend to remain close to each other, in spite of the drastic hypotonic shock to which they had been submitted. A synapsis defect of the euchromatic fragment comprised between the telomeric and centromeric heterochromatin was recurrently observed (Fig. 2d).

Macraspis tristis

The karyotype, composed of 18 chromosomes, is characterized by the frequent presence of large and variable heterochromatic fragments at telomeric regions of one arm of 3 pairs of sub-metacentrics (N°6, 7 and 8) and on the X (Fig. 3a). In all diakineses/metaphases I examined from all the specimens, the heterochromatin carrier bivalents have the same configuration: chiasma or terminal association in the euchromatic arms and opposite position of the heterochromatin (Fig.3b). This suggests that no recombination occurred in the heterochromatin carrier arms. In the specimen considered here, pairs N°7 and 8 were heterozygote for the presence or absence of a large heterochromatin fragment. Among 11/12 spermatocytes II, all carrier chromosomes had similarly heterochromatin in both chromatids (Fig. 3c). In a single one, one chromosome was asymmetrical, with heterochromatin on a single arm, indicating that recombination took place between the centromere and the heterochromatin (Fig. 3d). In a second specimen, only pair N° 8 was heterozygote for the presence of heterochromatin. No asymmetry was observed on chromosomes from 28 spermatocytes II. Finally, in a third specimen, both pairs N° 7 and 8 were heterozygote, and no asymmetry was detected among 8 spermatocytes II. Thus, in 65 analysed sub-metacentrics, recombination was almost always suppressed between the distally located heterochromatin and the centromere, and occurred in the other arm (64/65 times). As in *S. princeps*, the bivalents with enlarged heterochromatin tended to associate at pachynema.

DISCUSSION

Recombination and heterochromatin

Only few data exist on meiotic recombination and chromosome segregation in beetles, and most of them were obtain before the heterochromatin detection was possible (Smith and Virkki 1978). In both literature and our own data, a general observation is that most bivalents exhibit a single chiasma in a fairly distal, if not terminal, position at diakinesis/metaphase I. This is in agreement with the findings, in other organisms, that repression of recombination occurs not only in juxta-centromeric heterochromatin, but also in adjacent regions. Thus, recombination hot spots are rarely located near to the centromeres, but most frequently in intercalary and near telomeric regions (Lichten and Goldman, 1995). The presence of large heterochromatin fragments in chromosomes is not exceptional. They generally occur in centromeric regions, where recombination rarely occurs (Ellermeier et al., 2010). Their more exceptional occurrence at telomeric regions offers the possibility to look for the possible influence of heterochromatin on meiotic recombination and chromosome segregation, independently from the proximity of the centromere and associated repetitive DNA. Such analysis was already performed in grasshoppers, in which some species have terminal heterochromatin in acrocentric chromosomes. It showed the displacement of chiasmata to a proximal position in heterochromatin carrier, compared to other chromosomes (John and King, 1982, 1985, de la Torre et al., 1986).

Our observations in *D. olympicum* confirm these findings: at metaphase I, the heterochromatic block of chromosome 11 is almost always at distance from the chiasma. In addition, we could quantify recombination through the analysis of 95 spermatocytes at metaphase II: 90/95 chromosomes 11 carry heterochromatin on a single chromatid, which formally demonstrates an almost systematic occurrence of crossing over between the centromere and the heterochromatic block. Thus, there was

no crossing-over suppression but a displacement towards proximal regions. The 5 metaphases II with symmetrical chromosomes 11 cannot be interpreted univocally: either 2 or no crossing-over occurred or crossing-over occurred in the short (heterochromatic) arm. As discussed below, these few cells provide us with interesting information about chromosome cohesion and segregation.

In *M. tristis* and *S. princeps*, all autosomes are submetacentric, and the analysis of spermatocytes I at metaphase indicates that most bivalents form a single chiasma. For chromosomes with one heterochromatin carrier arm, chiasmata are almost systematically located on the other arm. This absence of recombination in the heterochromatin carrier arm is confirmed by the analysis of spermatocytes II in metaphase: at difference with the acrocentric of *Dorcadion*, heterochromatin is almost always either present or absent on both arms. This suggests a "choice" between the two arms, by suppression of recombination in the whole heterochromatin carrier arm. As observed in a proportion of pachytene cells of *S. princeps*, there is an asynapsis of the whole euchromatic fragment located between centromeric and telomeric heterochromatin, which may be related to the lack of recombination. These 3 examples show that large telomeric heterochromatin fragments can drastically influence meiotic recombination in euchromatin, with an effect at a long distance. It may create a hot spot of recombination fairly close to the centromere, which is quite unusual, as in *D. olympicum*. It may also generate a cold and a hot arm, as in the two other species. This effect is probably not due to the heterozygote status, because when both arms are carrier, they are generally not involved in chiasmata at diakinesis. By altering meiotic recombination, these occasional heterochromatic fragments alter the gene linkage between all the genes of the chromosome and influence genetic drift.

Cohesion, compaction and heterochromatin

At metaphase I, sister chromatids are maintained together by cohesins, a ring-shaped protein complex formed by 4 sub-units: Scc1 (Rec8), Scc3, Smc1 and Smc3. At anaphase I, cohesins are cleaved by separase all along chromatids, except in centromere regions where the sub-unit Rec8 is protected from cleavage by the protein complex Shugoshin/protein phosphatase PP2A, which counteracts its phosphorylation (Riedel et al. 2006). This permits the resolution of chiasmata, which occur in euchromatic fragments. At the following metaphase II, cohesins are no more efficient. Chromatids are well separated and chromosome cohesion is maintained at the centromere regions only. Finally, after inactivation of PP2A, centromere cleavage occurs at anaphase II, enabling the segregation of monochromatidic chromosomes. Why and how Shugoshin/PP2A complex is located in centromere regions remains unknown. Our observation may provide some information. In spermatocytes II, all chromosomes have well separated chromatid, with the exception of chromosomes 11 of *D. olympicum,* which are ring shaped when they are symmetrically carrier of terminal heterochomatin. It means that cohesin was not cleaved both at centromere and telomere, the two heterochromatic regions. Thus, Rec8 seems to be protected from cleavage by heterochromatin. This protection, necessary for the correct chromosome segregation at anaphase I, may be one of the reasons why centromeres are systematically embedded in heterochromatin.

It is commonly observed that in metaphases of mitotic cells also, heterochromatic fragments are more cohesive than euchromatin. At the molecular level, the search of a particular relationship between the protein complexes involved in cohesion and heterochromatin components could be of interest. The DNA methylation status may play a role in this context. In human cells, although juxta-centromeric heterochromatin of chromosomes 1, 9 and 16 is not G-C rich, it is strongly labelled by antibodies to 5-MdC (5-methyldeocycytidine), indicating its strong methylation status (Miller et al., 1974; Montpellier et al., 1994). In mouse germ cells, large variations of DNA methylation were reported during the progression of gametogenesis (Coffigny et al. 1999; Bernardino-Sgherri et al. 2002; Marchal et al. 2004). Drastic changes from hypo- to hyper-methylation occur in heterochromatin and euchromatin in an opposite way. For example, in early spermatogonia, hypo-methylated and elongated centromeric heterochromatin displays premature cleavage, while chromosomes remain cohesive at hyper-methylated chromatids. On the opposite, euchromatic chromatids are cleaved in spermatocytes II, when they are poorly methylated while chromosomes remain cohesive at their methylated and compacted centromere regions. Unfortunately, we could not study the methylation status of beetle chromosomes during gametogenesis, but the high similitude of variations of chromosome compaction and cohesion suggests they might correlate with DNA methylation changes.

In conclusion, the presence of large fragments of heterochromatin at telomere regions deeply alters meiotic recombination in beetles, as in other animals. Large terminal heterochromatic fragments have a suppressive effect on recombination, which seems to spreads to a much larger distance than that of centromeric heterochromatin and may depend on a different mechanism. It should have some consequences on the transmission

of genetic characters, by altering gene linkage. The variations of compaction/cohesion/recombination, opposite in euchromatin and heterochromatin during meiosis progression, suggest that the activities of the protein complexes involved are highly dependant on chromatid structure and composition.

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