# Meiosis in Stethophyma (Mecostethus) Grossum (Orthoptera: Acrididae): An Exciting History

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Source: Journal of Orthoptera Research, 19(2) : 267-273

Published By: Orthopterists' Society

URL: https://doi.org/10.1665/034.019.0213

# Meiosis in Stethophyma (Mecostethus) grossum (Orthoptera: Acrididae): an exciting history

Submitted April 23, 2010, accepted September 6, 2010

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## Abstract

The present study is a sincere tribute from some of the cytogenetic laboratories of Madrid involved in the study of grasshopper meiosis, for the inestimable contribution of Professor Michael J.D. White to orthopteran cytogenetics, on the 100th anniversary of his birth. Here we review the knowledge accumulated over almost 90 y on the meiosis of the grasshopper *Stethophyma grossum*, especially on the male side, because of the very unusual meiotic characteristics shown by the spermatocytes, namely: whereas the three shortest bivalents achieve full synapsis and do not show chiasma localization and the single X chromosome remains asynapsed, the remaining eight bivalents show restricted synapsis and proximal chiasma localization. In addition, supernumerary segments and accessory chromosomes are present in some natural populations. Special attention is paid to the relationships among cohesin axis morphogenesis, initiation of recombination events and synapsis achievement.

## Key words

meiosis, synapsis, recombinase, chiasma localization, grasshopper, cohesin

## A historical background

Sexual reproduction depends on the success of faithful chromosome transmission during meiosis to yield viable gametes. In the meiotic process, one round of DNA replication is followed by two consecutive chromosomal divisions to halve the number of chromosomes. Several events occur during the prophase of the first meiotic division to ensure the accurate segregation of homologous chromosomes at anaphase I. First, homologues identify their partners and align along their length (pairing). Afterwards, this configuration is solidified by formation of a tripartite proteinaceous structure denominated the synaptonemal complex (synapsis).

In most organisms, homologous chromosomes interchange their genetic content, undergoing reciprocal recombination events (chiasmata), which, in combination with sister chromatid cohesion, are responsible for the correct bi-orientation of bivalents at metaphase I and the subsequent segregation of a complete set of chromosomes at anaphase I. Sister chromatids separate at the second division, generating haploid gametes. Fusion of gametes at fertilization restores the diploid chromosome number of the species and, after DNA replication, initiates zygote development.

For decades, grasshoppers were considered model organisms for studying chromosome behavior in meiosis. Among the species used for this purpose, a special interest in the cytogenetics of the genus *Stethophyma* (previously named *Mecostethus*) arose from three main fascinating characteristics: i) the tendency for chiasmata to occur close to the centomeric regions in most bivalents (Darlington 1932; White 1936; Shaw 1970, 1971a; Jones 1971, 1973); ii) occurrence of ditactic bivalents (McClung 1928; White 1951, 1973; Shaw 1970, 1971a); iii) extended polymorphisms with respect to supernumerary heterochromatic segments, in addition to a marked interpopulation variation for standard heterochromatin in a number of autosomes (McClung 1928; Carothers 1931; Shaw 1970, 1971a; Jones 1971; Santos unpub. results). From a meiotic point of view, chiasma localization has proved the most interesting feature of these species for two reasons: the causal mechanism that underlies it and its evolutionary significance in terms of recombination. The interest displayed by M.J.D. White (and ourselves) in this system leads us to review here the meiotic history of the species *Stethophyma grossum* (Fig. 1A).

## The chromosome complement

The chromosome complement of *S. grossum* includes 23 chromosomes, eleven pairs of autosomes and a single X chromosome, in males (White 1936) and 24 chromosomes in females (22+XX). All of them are telocentric (Figs 1B, C). Shaw (1970) arranged the autosomes in three size groups to include three large (L), six medium (M) and 2 small (S) chromosome pairs. Depending on the cytological stage studied, the X chromosome ranges between the fourth and fifth-largest chromosome.

The Spanish and French populations studied by Shaw (1971a) carry centromeric heterochromatic blocks on all autosomes, with M4, M6 and M9 chromosomes bearing additional blocks. The M4 carries a large block next to the centromere, with a smaller one distal to it, whereas the reverse situation occurs on the M6. M9 is the most heterochromatic chromosome, with a large block near the centromere and a small one close to the telomere.

We have observed variations in the size of the blocks of M4 and M6 chromosomes in individuals of a different Spanish population, this from the province of Guadalajara (Fig. 1D, E). In addition, this species also displays a polymorphism for extra heterochromatic segments in chromosomes S10 and S11 (Shaw 1970, 1971a, b; Jones 1971; see Fig. 1E of this work).

This variation in segment structure and the distribution of standard heterochromatin suggest that these systems have evolved independently in different populations. Also, the existence of a single B chromosome has been detected in some individuals, but detailed analyses of this possible polymorphism have not been carried out (Jones 1971, Santos unpub.).

## Chiasma frequency and distribution

*The Spermatocytes.*—Ditactic bivalents were first described in species of the genus *Stethophyma* by McClung (1928), who believed they

resulted from cases in which chiasmata are only confined to the short arms of acrocentric chromosomes, an opinion which was held and reiterated by White (1951, 1973). However, many other observations confirmed the telocentric nature of these chromosomes (Lima-de-Faria 1956; John & Hewitt 1968; Shaw 1970, 1971a; Jones 1971; Calvente *et al.* 2005). Bivalents are usually monochiasmatic, although two chiasmata were occasionally observed in the M9 bivalent (Fig. 1B). The single chiasma is not restricted to any particular region in the shortest bivalents (M9-S11), but it is exclusively located near the centromere in the rest of the bivalents (L1-M8) (White 1936; Shaw 1970, 1971a, b; Perry & Jones 1974; Calvente *et al.* 2005).

The peculiar characteristics of this meiotic system were efficiently used by Jones (1971) to analyze tritium-labelled meiotic chromosomes, and demonstrate convincingly that the origin of chiasmata was through breakage and rejoining of homologous non-sister chromatids, *i.e.*, it is a validation of the chiasmatype theory (Janssens 1924). In the same experiment he also determined the timing of meiotic and spermatogonial events at 30°C, concluding that spermatocytes take approximately 12 d to progress from the premeiotic period of DNA synthesis (S-phase) to metaphase I. The duration from metaphase I to anaphase II occupied less than 24 h, which was consistent with previous studies of meiotic timing in grasshoppers (Peacock 1970).

Shaw (1971a) observed that the structurally supernumerary heterochromatic segments of two populations from Spain and Austria produce variation in chiasma frequency between individuals and between cells within individuals, but in different ways. Thus, the increase of mean chiasma frequency produced by the Spanish supernumerary segment, located interstitially on chromosome S11, is both inter- and intrachromosomal, whereas in the Austrian population, where the segment is located very close to the centromere, its influence only affects other members of the chromosome set, *i.e.*, it is entirely interchromosomal.

The existence of supernumerary segments is a common feature in grasshoppers and, in order to explain their maintenance in natural populations, it has been proposed that the main function of such segments is the regulation of variation through the recombination process (John 1981). However, other alternatives, such as equilibrium between a nonMendelian mode of inheritance and a decrease in the fitness of the carriers, can also be proposed. At present these hypotheses await experimental validation in grasshoppers in general, and in *S. grossum* in particular.

*The oocytes.*—Do females display chiasma localization? The answer is definitively negative. Perry and Jones (1974) have reported, not only that the mean chiasma frequency per cell (14.98) is considerably higher in females than in males (11.28), but that also males and females show quite opposite patterns of chiasma distribution. Thus, in females very few chiasmata are located at proximal regions and nearly all chiasmata form either in distal or interstitial regions. Therefore, we arrive at an apparent paradox, because the effective recombination in this species occurs mainly in nonpericentromeric regions of female bivalents. In grasshoppers, the lack of a clear tendency in patterns of chiasma frequency and in distribution differences between sexes, even between related species, makes it difficult to envisage the possible adaptive value of these changes in the recombination patterns (Cano & Santos 1990).

*The synaptic process*.—The possibility of a direct relationship between chiasma localization and the extent of synapsis has engaged the attention of cytologists for many years (*e.g.*, Darlington 1935; Henderson 1969; Oakley & Jones 1982; Viera *et al.* 2009a, 2010). The extreme diffuseness of chromosomes during prophase I in *S. grossum* spermatocytes has prevented any reliable conclusion regarding the synaptic process at light-microscope level for decades. Then there were developed alternative approaches, based on determining the pattern and extent of synapsis by monitoring the assembly of the synaptonemal complex (SC) under the electron microscope (EM).

Fletcher (1977) reconstructed nuclei at four successive stages of meiosis from leptotene to pachytene, from a series of ultrathin sections, and calculated the extension of SC formation. Since the three shortest bivalents showed full synapsis, and only the pericentromeric regions of the long and medium-length bivalents were synapsed (the size of SC stretches in the longer bivalents, being about 20μm), the author concluded that the synaptic pattern observed was directly related to the distribution of chiasmata. These findings were corroborated by Wallace and Jones (1978), although they pointed out that synapsis in some L bivalents could be about 40% of the total chromosome length, exceeding considerably the localization of chiasmata (Fig. 1F-H). At this point it is necessary to mention that incomplete synapsis was recognized and described in this material more than 80 y ago by Janssens (1924), a conclusion later supported by Darlington (1931).

Three dimensional reconstructions of SCs under EM is a technically demanding procedure and consequently, only a few nuclei can be completely analyzed in a reasonable period of time. Fortunately, the development of surface-spreading techniques for making whole-mount preparations of SCs, provided the possibility of direct observations in a high number of prophase I nuclei. Counce and Meyer (1973) first applied them to orthopteran spermatocytes, and soon this methodology was revealed as a powerful tool to analyse, not only chromosomal pairing and synapsis in normal diploids, but in other situations involving structural rearrangements and changes in chromosomal numbers (Santos *et al.* 1993; del Cerro & Santos 1995; del Cerro *et al.* 1994, 1996, 1997, 1998).

A study by Jones and Wallace (1980) of *S. grossum* surface-spread spermatocytes reaffirmed the correlation previously found between the extent of synapsis at "pachytene" and the localization of chiasmata at diplotene, diakinesis and metaphase I (Fletcher 1977, Wallace & Jones 1978). It was observed that only a region comparable in length to the S11 bivalent is synapsed in each of the longest bivalents. Thus, the large bivalents would only produce a single chiasma because they behave like the short ones in respect to forming chiasmata and generating the corresponding interference signal along their length. Interestingly, "pachytene" nuclei showed a degree of polarization, with centromeric regions being localized within a restricted zone at one side of the nucleus. These authors also observed a postpachytene type of nucleus, which was identified as diffuse stage (Wilson 1925). These nuclei are very large, with absence of chromosome threads and with diffuse nucleoplasm, except for a number of prominent chromocenters thought to be the pericentromeric heterochromatin.

This stage has been described in plants, fungi and animals (Klásterská 1976, 1977, 1978; Cardoso *et al.* 1986; Zickler & Kleckner 1999, Viera *et al.* 2009b, c), but is generally overlooked in studies of meiotic prophase I, and has never been analyzed in depth. Although diffuse-stage nuclei present morphological differences depending on the species studied, it is clear that they always imply an overall change of chromosome organization between pachytene and diakinesis.

*The relationship between the processes of pairing, synapsis and recombination*.—Viera *et al.* (2004) studied (the first time by anyone) the

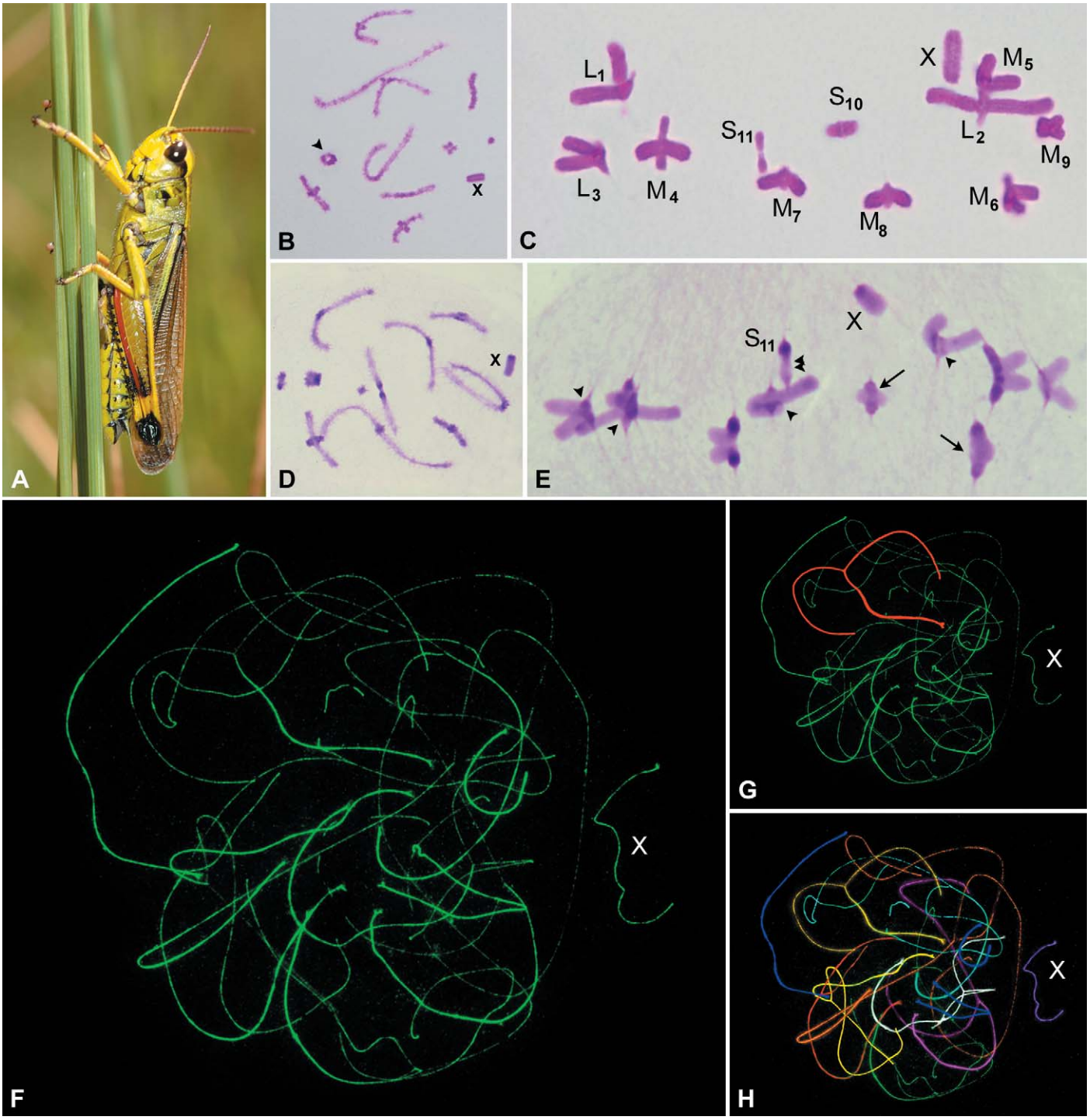


**Fig. 1.** (A) Male of *S. grossum*. (B and C) Feulgen-stained squashed spermatocytes in which the X chromosome is indicated. (B) Diplotene with a bichiasmatic M9 bivalent (arrowhead). (C) Metaphase I. (D and E) C-banded squashed spermatocytes. X chromosome is indicated. (D) Diplotene. (E) Metaphase I. Chiasmata are located at proximal (arrowheads), distal (double arrowheads) and interstitial (arrows) regions of the bivalents. Bivalent S11 is heterozygous for a supernumerary segment located near the centromeric region. (F and H) SMC3 immunolabelling of pachytene-spread spermatocytes. Paired SMC3 axes do not extend entire length of bivalents and appear polarized at the centromeric region of the nucleus. The unsynapsed X chromosome is indicated. (G) A complete bivalent showing synapsed and unsynapsed regions is indicated (red). (H) Entire nucleus reconstruction showing fully synapsed short bivalents (dark blue), partially unsynapsed large bivalents (different colors) and the asynaptic sex chromosome (purple). For color version, see Plate IV.

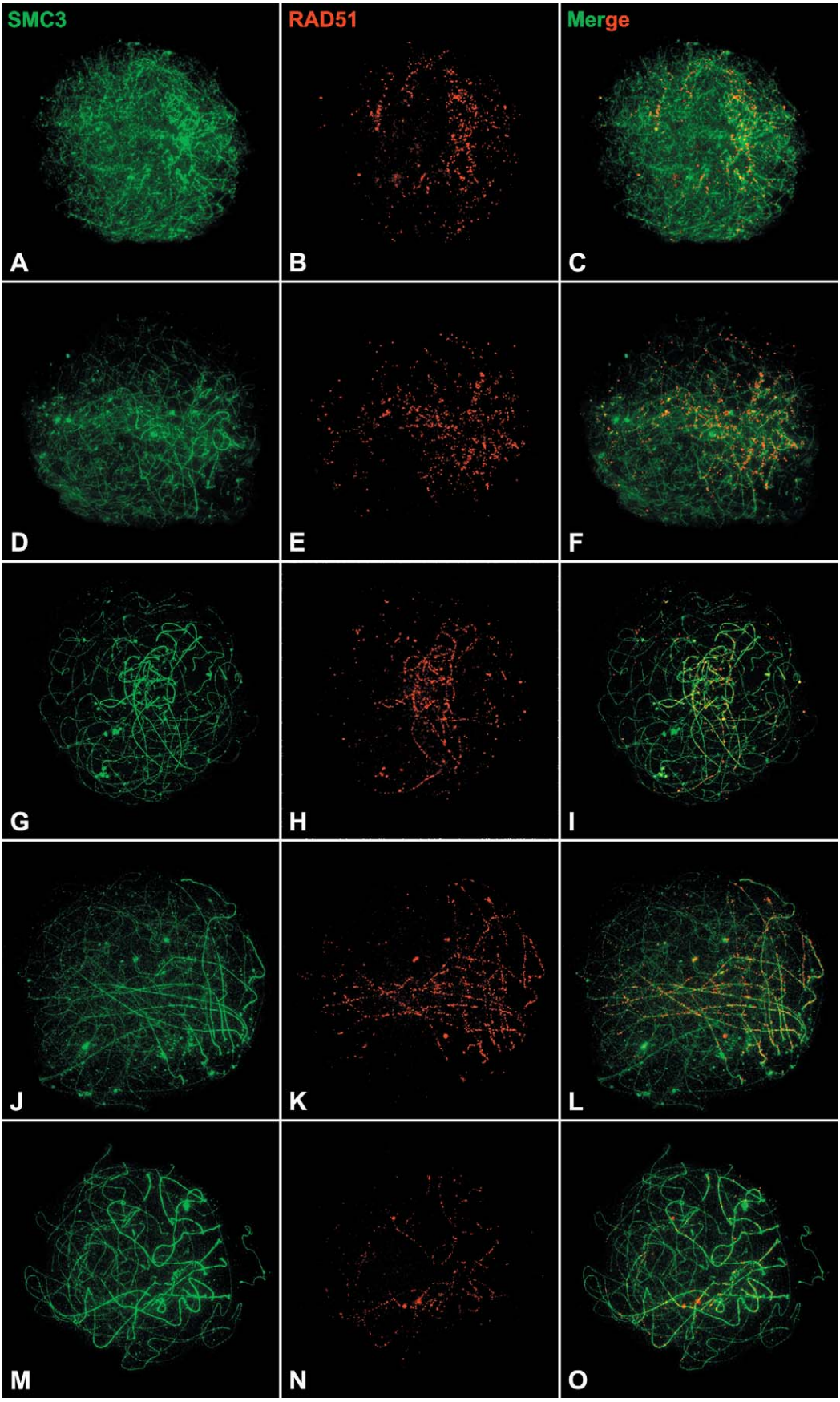


**Fig. 2.** Double immunolabelling of SMC3 (green) and RAD51 (red) proteins in *S. grossum*-spread prophase I spermatocytes. (A-C) Leptotene spermatocyte. SMC3 presents a dotted-like appearance except at a nuclear region where there are elongated and continuous SMC3 axes. RAD51 foci are partially polarized and appear over the nuclear region, which presents unpaired and elongated SMC3 axes. (D-F) At early zygotene, the SMC3 axes develop into well-defined thin lines within the entire nucleus, and they begin to pair, forming thicker axes in a restricted region of the nucleus, denoting the onset of synapsis. Although the number of RAD51 signals increase, they remain polarized in the nuclear domain in which SMC3 axes are paired. (G-L) From mid to late zygotene the length of paired SMC3 axes increases. The polarized RAD51 labelling concentrates over the paired SMC3 axes, despite some foci that are also observed along alligned, but yet unpaired, axes. (M-O) Midpachytene spermatocyte. While the SMC3 axes are paired at their full lengths in the autosomal bivalents that achieve complete synapsis, they are only partially paired in the remaining bivalents, which display incomplete synapsis. The number of discrete RAD51 foci is drastically reduced, they being exclusively located over the thicker paired SMC3 axes. See text for further details. For color version, see Plate V.

temporal relationship between DNA events of meiotic recombination and synapsis, in the grasshopper species *Locusta migratoria* and *Eyprepocnemis plorans*. They carried out the immunodetection of the phosphorylated histone H2AX variant (γ-H2AX), which marks the sites of the DNA double-strand breaks (DSB); these breaks represent the initiation of the recombination events (Madigan *et al.* 2002, Redon *et al.* 2002) and of the SMC3 cohesin subunit, which is thought to have a close relationship to the development of the axial element (AE) a SC component. This constitutes an excellent marker of meiotic timing because it allows monitoring of the synaptic process (Pelttari *et al.* 2001, Viera *et al.* 2004), and of the recombinase RAD51, which has a role in DNA strand exchange between homologous chromatids (Shinohara *et al.* 1992).

Similar results were found in both species, briefly: γ-H2AX domains appeared by late leptotene as massive accumulations at the spermatocyte nucleus, where the synapsis of autosomes begins. As synapsis progressed, the labelling of γ-H2AX was mainly detected in the remaining unsynapsed regions. In early or midpachytene spermatocytes, only a few foci were detected on the synapsed lateral elements (LE) of autosomes, and covering the chromatin of unsynapsed X chromosome. RAD51 is detectable later than γ-H2AX and localized to chromatin regions that are undergoing, or are about to undergo, synapsis. The labelling at fully synapsed chromosomes quickly decreased in late zygotene nuclei, disappearing completely by mid to late pachytene. Thus it was concluded that in these species the initiation of recombination, as detected by the formation of DNA damage signalling, occurs before the initiation of synapsis. In species such as *Saccharomyces cerevisiae, Mus musculus* and *Arabidopsis thaliana,* the strict dependence of synapsis on recombination has been demonstrated (Kleckner 1996, Mahadevaiah *et al.* 2001, Grelon *et al.* 2001). Likely, this scenario can also be applied to grasshoppers.

At this point, it is time to return to *S. grossum* because the meiotic framework is not exactly the same as that previously mentioned. In the grasshoppers *L. migratoria* and *E. plorans* all homologous chromosomes fully synapse at pachytene and chiasmata are apparently not restricted to particular chromosome regions. However, in *S. grossum* full synapsis of spermatocytes is only achieved by the three shortest bivalents, whereas the remaining bivalents show incomplete synapsis, restricted to regions near centromeres (Fig. 1F-H), and proximal chiasma localization (Fig. 1B-E). In this species, as in the previously mentioned grasshoppers, the initiation of recombination, detected by γ-H2AX labelling, occurs before synapsis, and RAD51 foci appear downstream of DSB formation (Fig. 2).

Surprisingly, in *S. grossum* the SMC3 cohesin axis formation presents a polarized and sequential maturation. Thus, it starts in a concrete polarized nuclear region, where axes begin to elongate into well-defined and continuous lines, while in the rest of the nucleus a dotted-like appearance is maintained (Fig. 2A, C). This delay of axis maturation remains throughout prophase I, differentiating two nuclear spermatocyte domains, one containing the chromosomal regions that have synapsed or are about to synapse, and the other one presenting asynaptic axes (see axis development in Fig 2).

Likewise, the recombination events appear to be restricted and polarized at the nuclear region in which cohesin axis formation was more advanced (see RAD51 labelling development in Fig 2). This polarization is observed in squashed (Calvente *et al.* 2005) and spread (Viera *et al.* 2010) spermatocytes, and is maintained throughout prophase I over those autosomal regions that are undergoing, or about to undergo, synapsis (Fig 2).

Intriguingly, in the tetrigid species *Paratetix meridionalis* a comparable situation for cohesin axis formation and maturation has been detected. This grasshopper shows incomplete pairing and synapsis restricted to the noncentromeric ends of all bivalents, and consequently distal chiasma localization (Viera *et al.* 2009a, Viera *et al.* 2010).

On these grounds, we suggest that the presence of mature cohesin axes at the time in which DSBs are generated is a prerequisite for the initiation of recombination, and subsequent synapsis achievement (Fig. 2). This hypothesis does not exclude the possibility that all processes might be influenced and modulated by additional factors such as chromatin morphology and histone modifications or epigenetic factors. Indeed, in *S. grossum* spermatocytes the modified histone H3K9me3, shows a polarized pattern of localization in the nucleus, just coincident to that shown by γ-H2AX and RAD51 (unpub. results).

*Altering male meiosis*.—There are only a few studies that address the effects of chemical and ionizing radiation on spermatocytes. The effect of methyl mercury hydroxide was analyzed by Klásterská and Ramel (1978) in the context of possible pollution effects on the environment with mercury compounds. They mainly found defects in chromosome condensation and, depending on the doses, chromosome stickiness and fragmentation. Studies of the effects of X irradiation were carried out to assess the type of chromosome aberrations produced (White 1937, Klásterská *et al.* 1976). Recently, we have tested irradiation with γ rays to induce exogenous DSBs in order to modify the chiasma frequency and distribution in the spermatocytes of *S. grossum*. As expected, the number of DSBs was largely increased but surprisingly, the recombination pattern did not substantially vary, *i.e.*, proximal chiasma localization in the L1-M8 bivalents was maintained (unpub. results).

## Concluding remarks

At present, most research on the meiotic field is carried out on just a few species considered as "model organisms", by means of either direct or reverse genetic approaches (from phenotype to genotype or *vice versa*). In our opinion, it would not be wise to put all research eggs in these few same baskets. We need to keep in mind that the study of many other natural systems, for instance species with extreme chiasma localization, holocentric chromosomes, achiasmatic conditions, presence of supernumerary segments, *etc.*, could help to gain new insights into the meiotic process. Notwithstanding this caution, the history of *S. grossum* is a continuing story.

## Acknowledgements

We would like to express our gratitude to Consejería de Medio Ambiente y Desarrollo Rural (Comunidad de Castilla La Mancha; España) for permitting animal collection in natural populations. We acknowledge Dr. José Luis Barbero for generously supplying us the anti-SMC3 anti-SMC1α antibodies. This work was supported by grants BFU2008-00459/BMC, BFU2008-00300/BMC and BFU2009-10987/BMC from Ministerio de Ciencia e Innovación of Spain.

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