

Fig. 3. Electron micrographs of normal wild type chloroplast (A), of *im* mutant (B) as illustrated on the left of Figure 1 and that of the same mutant grown on  $1.5 \times 10^{-5} M$  azauracil medium (C). The technique of electronmicroscopic manipulations was given earlier<sup>21</sup>. s starch; — osmiophilic globuli; v vacuoles.

able conditions. In the control white cells of the plants, plastid differentiation is arrested at an early stage. Though the outer membrane of this organelle as well as other membranes (nuclear, mitochondrial) appear normal in this condition, the plastids in the white cells display only osmiophilic globuli and vacuoles (Figure 3B). At low concentrations of 6-azauracil the majority of the green cells produced exhibit chloroplasts undistinguishable from the normal ones (Figure 3A). At higher concentrations many of the mutant cells have green and functional chloroplasts as indicated by the large amount of starch accumulated yet the shape of the thylakoids is characteristically curved (Figure 3C). Such abnormal chloroplasts can be seen only rarely in the cells of the wild type plants grown under identical conditions, and even then, the expression of this altered form is much less conspicuous<sup>19</sup>.

In the higher plant *Arabidopsis*, mutants at a chromosomal gene locus *im* fail to differentiate normal chloroplasts under high intensity continuous illumination when grown on mineral-glucose-agar aseptic medium. Under these conditions the activity of de novo synthesis of pyrimidines is accelerated. Feeding 6-azauracil to the plants partially restores pigment production and chloroplast differentiation. On  $1.5 \times 10^{-5} M$  6-azauracil media the functional chloroplasts of the mutant cells, exhibit curved thylakoids, however. An important metabolite of the analog, 6-azauridylic acid, selectively inhibits the

activity of orotidylic acid decarboxylase and reduces the level of orotidylic acid pyrophosphorylase, and thus increases the orotic acid pool. It is suggested that the increased orotic acid supply may be one factor conducive to plastid differentiation in the mutants where normally, in the absence of the analog orotic acid is rapidly converted into nucleotides and RNA, and the pyrimidine-purine ratio deviates from normal.

**Résumé.** Les mutants au locus *im* de l'*Arabidopsis* ne réussissent pas à former de chloroplastes normaux dans certaines conditions et montrent des activités plus hautes en synthèse de pyrimidines que le témoin. La nutrition aseptique par 6-azauracile, inhibiteur spécifique de cette voie métabolique, restaure la différenciation de structure lamellaire dans la plupart de ces organites, mais aux concentrations élevées, apparaissent des chloroplastes morphologiquement déviants, quoique de fonction efficace.

S. C. CHUNG, G. P. RÉDEI and J. A. WHITE

University of Missouri, Department of Agronomy  
117 Curtis Hall, Columbia (Missouri, 65201, USA),  
13 August 1973.

<sup>19</sup> G. P. RÉDEI, *Am. J. Bot.* 52, 834 (1965).

<sup>20</sup> G. RÖBBELEN, *Z. Vererb. Lehre* 90, 503 (1959).

<sup>21</sup> G. P. RÉDEI and S. B. PLURAD, *Protoplasma* 77, 361 (1973).

## Heterochromatin Localization in the Chromosomes of *Lycosa malitiosa* (Arachnida)

This is a preliminary report on a cytogenetic study of *Lycosa malitiosa*. The main purpose of this work is to study the meiotic process and the heterochromatin localization.

Six males and 8 females from Marindia (Uruguay) were employed. The females were injected with 0.1 ml., 0.04% colchicine solution. After 20 h they were sacrificed and the hemolymph was extracted from the dorsal vessel and legs and placed in isotonic saline solution (ISS). They were pretreated with ISS and distilled water 1:1 for 25 min and then fixed in metanol-acetic 3/1. The testicular material was dilacerate and treated with 0.025% trypsin solution and all the preparations were stained with Giemsa. The heterochromatin stain procedure was made according to ARRIGHI and Hsu's<sup>1</sup> technique with slight modifications.

In the 20 metaphases studied we found identical chromosome complements with diploid number,  $2n = 20 + X_1X_2O$  in the male and  $2n = 20 + X_1X_1X_2X_2$  in the female, all chromosomes being telocentric (Figure 1).

During the meiotic prophase, the sex chromosomes were observed as strongly condensed in relation to the autosomes. The *X* elements were clearly recognized in the meiotic metaphase. Almost always they were present in an eccentric position in relation to the spindle, quite separate from the autosomes, and identified as 2 long rods lying parallel to each other. During diplonema each bivalent had 1 distal quiasma in the majority of cases and occasionally 2 proximal-distal chiasmata. In the somatic

<sup>1</sup> F. E. ARRIGHI and T. C. HSU, *Cytogenetics* 10, 81 (1971).



Fig. 1. Karyotypes of *Lycosa malitiosa* male and female.

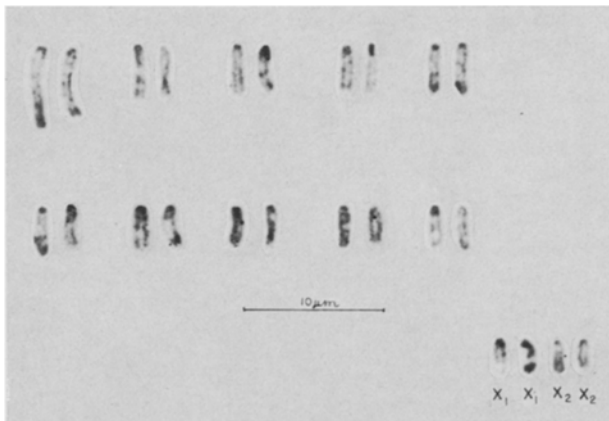


Fig. 2. Karyotype with heterochromatin blocks located in the pericentromeric regions.

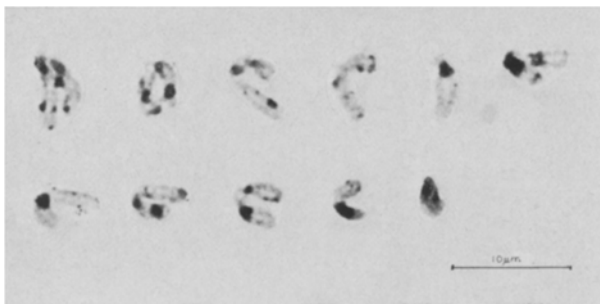


Fig. 3. Heterochromatin in the diplotene bivalents located in the pericentromeric and proximal regions of chiasmata.

metaphases the chromosomes showed heterochromatin blocks located in the centromeric regions of the chromatids.

In the female, only one  $X$  exhibited complete heterochromatinization while the others showed heterochromatin in the centric regions (Figure 2). During paquinema the bivalents showed dark bands with light interbands. The sex chromosomes remain included in a positive heteropicnotic vesicle. The heterochromatin of the diplotene bivalents is localized in the proximal regions of chiasmata and in these cases the sex chromosomes showed positive heteropicnosis (Figure 3).

The meiotic behavior is similar to that described by MONTGOMERY<sup>2</sup>, PAINTER<sup>3</sup>, HARD<sup>4</sup>, HACKMAN<sup>5</sup>, SUSUKI<sup>6</sup>, MITTAL<sup>7,8</sup> and DÍAZ and SAEZ<sup>9</sup>, in different species of *Lycosidae*. The localization of C-bands is described here for the first time in Arachnida and agrees with the studies reported in somatic chromosomes by JONES<sup>10</sup>, PARDUE and GALL<sup>11</sup>; HSU and ARRIGHI<sup>12</sup> in mammals, and POLANI<sup>13</sup> in the meiotic chromosomes of the mouse.

According to our information, there are very few investigations on C-bands localization in invertebrates; DRETS and STOLL<sup>14</sup> found in the orthoptera *Grillus argentinus* heterochromatin localized in the pericentric and telocentric regions, and GALLAGHER et al.<sup>15</sup>, in the grasshopper *Myrmeleotettix maculatus* found the centromeric regions densely heterochromatic. Further investigations are at present in progress in other species of Arachnidae.

**Resumen.** Se realizó el estudio del proceso meiótico y localización de la heterocromatina en *Lycosa malitiosa* (Arachnida). El número diploide es de  $2n = 20 + X_1X_10$  en el macho y  $2n = 20 + X_1X_1X_2X_2$  en la hembra. La heterocromatina se encuentra localizada en las metafases, somáticas, en las regiones pericentromérica, mientras que en los estadios meióticos se observan en las regiones centroméricas y próximas a los quiasmas.

N. BRUM-ZORRILLA and A.M. CAZENAVE<sup>16</sup>

Departamento de Citogenética,  
Instituto de Investigación de Ciencias Biológicas,  
Avenida Italia 3318,  
Montevideo (Uruguay),  
15 June 1973.

- <sup>2</sup> T. H. MONTGOMERY, Proc. Acad. nat. Sci. Philad. 57, 162 (1905).
- <sup>3</sup> T. S. PAINTER, Zool. Jahrb. Anat. Ont. 38, 509 (1914).
- <sup>4</sup> W. L. HARD, J. Morph. 65, 121 (1939).
- <sup>5</sup> W. HACKMAN, Acta zool. fenn. 54, 1 (1948).
- <sup>6</sup> S. SUSUKI, S. Sci. Hiroshima Univ. 15, 1 (1954).
- <sup>7</sup> O. P. MITTAL, Res. Bull. E. Panjab. Univ. 12, Part III, 271 (1961).
- <sup>8</sup> O. P. MITTAL, Res. Bull. E. Panjab. Univ. 14, Part I, 58 (1963).
- <sup>9</sup> M. DIAZ and F. A. SAEZ, Anais Congr. Lat. Am. Zool. 2, 3 (1965).
- <sup>10</sup> K. W. JONES, Nature Lond. 225, 912 (1970).
- <sup>11</sup> M. L. PARDUE and J. G. GALL, Science 168, 1356 (1970).
- <sup>12</sup> T. C. HSU and F. E. ARRIGHI, Chromosoma 34, 243 (1971).
- <sup>13</sup> P. E. POLANI, Chromosoma 36, 343 (1972).
- <sup>14</sup> M. E. DRETS and M. STOLL, Genetics 71, Suppl. 3 Part 2, 15 (1972).
- <sup>15</sup> A. GALLAGHER, G. HEWITT and I. GIBSON, Chromosoma 40, 167 (1973).
- <sup>16</sup> Acknowledgments. The authors are greatly indebted to Prof. F. A. SAEZ for his careful and critical reading of the manuscript, and Mr. R. CAPOCASSALE for contributing specimens used in the research. This research was supported in part by the grant 'Acción de Refuerzo U-15 del Programa Regional para el Desarrollo Científico y Tecnológico (OEA)'.