Mem. Fac. Educ., Shimane Univ. (Nat. Sci.), Vol. 11, pp. 19—24, December 1977.

# Chromomere Arrangement in Pachytene Bivalents of Grasshopper Spermatocytes, *Acrida turrita* (Acrididae : Orthoptera)

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**Abstract :** Pachytene chromosomes of grasshopper spermatocyes were observed with squashed-Feulgen stain preparations for an analysis of the chromomere pattern in the bivalents. Eleven bivalents were identifiable in well-spread cells, in consequence the measurements of chromosome length and chromomere count of each chromosome were possible. The examination indicated that the number and pattern of chromomeres varied according as the pachytene stage proceeds. The number of chromomeres was much higher in elongated chromosomes and lower in condensed elements. Therefore the chromomere distribution, measured by the average distance between chromomeres, was a fairly good approximation in respective bivalent. Identical pattern of chromomere arrangement was detected only at advanced stage of pachytene since the contraction of chromosomes and chromomere fusion brought better visualization of the number and disposition.

## Introduction

Chromomere is the bead-like concentration of chromatin which linearly arranged along the chromosome. In meiotic prophase of both plant and animal cells, the chromomere arrangement exhibits the characteristic pattern on the elongated chromosomes.

Identification of the chromosomes in pachytene bivalents and analysis of the chromomere pattern have been carried out widely in palnt cells but rarely in animals except human (e. g. Ris 1945, Traut 1976). The majority of descriptions on the chromomere have been concerned in the giant chromosomes of Diptera and Amphibia (Strauss 1974, Vlad and Macgregor 1975). However informations on the chromomere obtained from the giant chromosomes would not be quite helpful to the morphologic interpretation of common chromosomes of meiotic prophase.

The primary purpose of the present observation was to re-examine the constancy of chromomere number and the arrangements in pachytene bivalents and to reconsider the significance of chromomere pattern in meiotic chromosomes. The pachytene chromosomes of grasshopper spermatocytes are of favorable material for the study, since they are known to be large enough for measuring chromosome length and to have sufficient visualization in their chromomere patterns.

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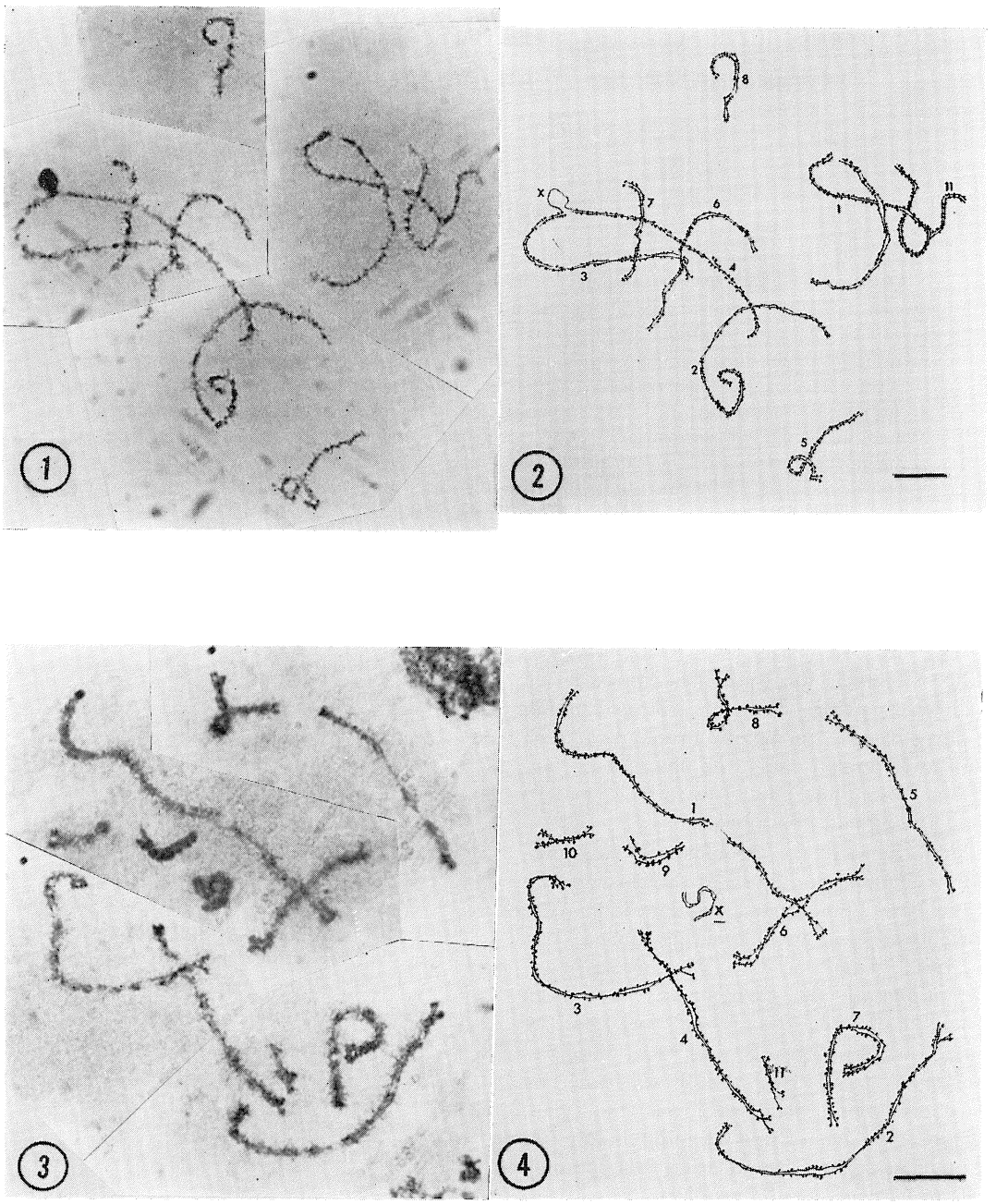
## Materials and Methods

Male grasshoppers of *Acrida turrita* Linné were collected in the Honjo experimental farm and the campus of Shimane University, Matsue. Testicular follicles were isolated from the animal and surrounded adipose tissue was taken out. Squashed preparations were made either with Carnoy-fixed material or with fresh testis. Fixed materials were placed on a slide and torn into small pieces in a drop of 45% acetic acid with a razor blade. All spermatocytes were lightly stained with acetic orcein.

For making the satisfactory slides permanent, cover glasses were removed after freezing in liquid nitrogen or dry ice and then dipped into the absolute ethanol (Conger and Fairchild 1953). The slides were refixed for 10 minutes in it and dried in air. Feulgen reaction was the most favorable stain for the permanent preparation.

Well-spread pachytene nuclei of spermatocytes were selected for making possible to identify each bivalent in the complements. Chromomere analyses, included the study of the number, size and disposition of the chromomeres in each bivalent, were made with




Figures 1 to 4. Photomicrographs and camera lucida drawings of pachytene chromosomes from grasshopper spermatocytes, *Acrida turrita*. Two cells having 11 bivalents and a condensed X chromosome were illustrated. The microscopical presentation was made by the squashed technique and Feulgen stain. Lower right scales on Figures 2 and 4 indicated 10 $\mu$m. Fig. 3 is the cell of more advanced stage of pachytene than Fig. 1.

camera lucida drawing using 100 X objective and 10 X ocular together with composite photomicrographs of pachytene complements. The average distance between chromomeres, defined as chromomere distribution, was determined from the ratio of length of the haploid chromosome set to the total number of chromomeres.

## Results

Diploid chromosome number of *Acrida turrita* was counted 23 in a spermatogonial cell, which consisted of 11 pairs of autosomes and a single X chromosome. All of them were telocentric elements. At early meiotic prophase, every pair of autosomes formed a bivalent and a X element showed heteropycnotic feature. In well-spread pachytene nuclei the expected 11 bivalents could be distinguished. Bivalents in the complement were numbered 1 to 11 in order of decreasing length.

Each bivalent was identified with its characteristic locality of chromomere knobs and/or heterochromatic segments as a marker. The markers revealed more clearly in advanced stage of pachytene. In selected cells the two strands of the paired homologues were clearly recognizable, displaying a fairly symmetrical and therefore homologous pattern of chromo-

TABLE 1 CHROMOSOME LENGTHS AND GHROMOMERE NUMBERS IN 11 BIVALENTS OF GRASSHOPPER SPERMATOCYTES AT PACHYTENE.

| Cell \ Chromosome | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. 1 | Length * | 54.0 | 55.5 | 35.5 | 24.5 | 28.5 | 27.0 | 20.3 | 19.0 | 9.5 | 9.0 | 5.0 | 287.8 |
| | Number | 37 | 43 | 27 | 21 | 24 | 21 | 17 | 18 | 9 | 9 | 6 | 232 |
| No. 2 | Length | 48.5 | 55.0 | 35.5 | 36.0 | 26.0 | 25.5 | 20.0 | 20.3 | 10.8 | 8.5 | 7.0 | 293.1 |
| | Number | 39 | 47 | 32 | 33 | 23 | 20 | 21 | 19 | 8 | 11 | 7 | 260 |
| No. 3 | Length | 53.0 | 36.0 | 21.0 | 22.0 | 31.5 | 23.5 | 16.0 | 4.5 | 8.0 | 5.0 | 5.5 | 226.0 |
| | Number | 38 | 33 | 24 | 17 | 28 | 17 | 14 | 5 | 7 | 6 | 5 | 194 |
| No. 4 | Length | 44.0 | 49.0 | 31.0 | 23.0 | 19.0 | 16.5 | 19.0 | 12.5 | 7.8 | 4.8 | 4.5 | 231.1 |
| | Number | 40 | 39 | 32 | 27 | 24 | 17 | 21 | 15 | 8 | 6 | 6 | 235 |
| No. 5 | Length | 59.5 | 50.5 | 46.5 | 35.5 | 34.0 | 25.5 | 29.0 | 23.0 | 10.5 | 9.5 | 8.0 | 331.5 |
| | Number | 48 | 38 | 31 | 31 | 28 | 28 | 32 | 22 | 8 | 11 | 6 | 283 |
| No. 6 | Length | 68.0 | 68.0 | 71.5 | 30.5 | 34.5 | 24.5 | 17.0 | 13.8 | 8.5 | 10.0 | 9.8 | 356.1 |
| | Number | 42 | 46 | 63 | 32 | 35 | 17 | 27 | 14 | 10 | 11 | 6 | 303 |
| No. 7 | Length | 32.5 | 22.8 | 23.8 | 23.0 | 21.8 | 18.0 | 19.5 | 16.0 | — | 7.5 | 7.5 | 192.4 |
| | Number | 21 | 23 | 25 | 23 | 20 | 16 | 19 | 15 | — | 8 | 8 | 178 |
| No. 8 | Length | 90.0 | 95.5 | 80.0 | 57.3 | 34.3 | 33.3 | 27.5 | 25.3 | — | — | 8.5 | 451.7 |
| | Number | 93 | 89 | 74 | 52 | 45 | 31 | 33 | 27 | — | — | 9 | 453 |
| No. 9 | Length | 92.0 | 54.5 | 55.0 | 43.5 | 28.0 | 25.5 | 21.0 | 20.0 | — | — | 11.3 | 350.8 |
| | Number | 87 | 52 | 43 | 43 | 31 | 25 | 19 | 27 | — | — | 15 | 342 |
| No. 10 | Length | 81.0 | 67.0 | 60.8 | 47.0 | 26.0 | 29.3 | 24.5 | 25.5 | 11.8 | 9.5 | 8.5 | 390.9 |
| | Number | 68 | 52 | 47 | 41 | 31 | 30 | 26 | 25 | 11 | 10 | 8 | 349 |

* Micron

TABLE 2 CHROMOMERE DISTRIBUTION BASED ON ABSOLUTE LENGTH OF THE HAPLOID COMPLEMENTS* AND TOTAL CHROMOMERE NUMBER IN 5 SELECTED SPERMATOCYTES.

| Cell | Length of the haploid chromosome set (a) | Total number of chromomeres per haploid set (b) | Chromomere distribution a/b |
|---|---|---|---|
| No. 1 | 287.8 $\mu$m | 232 | 1.24 |
| No. 2 | 293.1 | 260 | 1.12 |
| No. 3 | 226.1 | 194 | 1.16 |
| No. 4 | 231.1 | 235 | 0.98 |
| No. 5 | 331.5 | 283 | 1.18 |

* X-chromosome was excluded.

meres and interchromomeres. Pachytene chromosome length was measured on fully extended chromosomes and the chromomeres were counted in corresponding chromosomes using camera lucida drawings (Figs. 1-4). The X chromosome was excluded from the analyses because of its heteropycnotic feature.

Table 1 showed all data on chromosome length and chromomere numbers of 10 examined spermatocytes, which indicated that the number of chromomeres was much higher in longer chromosomes and lower in shorter ones. The chromomere distribution measured by the average distance between chromomeres was similar in respective bivalent. Naturally, one would expect longer chromosomes to have more chromomeres, and this has proved to be the case.

Length of the corresponding bivalents in different cells differed largely each other since the length became shorter according as the pachytene stage proceeded. Therefore five spermatocytes in the same stage were selected for more detailed analysis on similar sized bivalents. The results presented in Table 2, indicating that an average number of

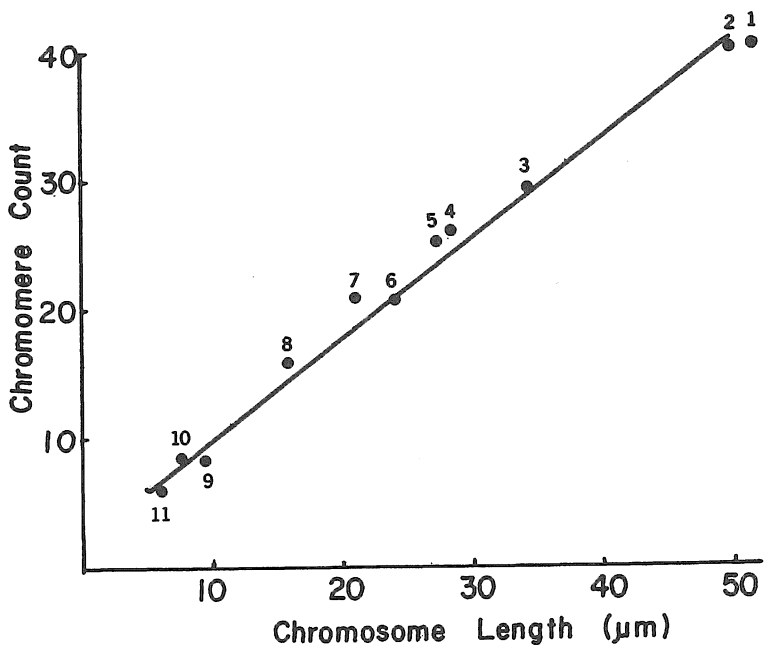



Figure 5. Correlation between the chromosome length and the number of chromomeres on each bivalent. Measurements were taken from five selected pachytene nuclei, cell No. 1 to No. 5, as shown in Table 1. Bivalent numbers (no. 1 to no. 11) were marked besides black dots.

chromomeres per total length of haploid set was a fairly good approximation. Then the linear relationship between chromosome length and chromomere number was found, that is, chromomere number increased in proportion to the chromosome length (Fig. 5). The evidence would signify that the number of chromomeres is not invariable at any time but it is conceivable to decrease as pachytene stage proceed, because of the fact that adjacent chromomeres tend to join together.

Chromomere arrangements were more conspicuous in earlier stage of pachytene, since chromomere size was much smaller and faint stain than heterochromatic segments which appeared mainly on proximal and distal part of bivalents. In advanced stage, chromomere fusion resulted the decreased number following the condensation of the bivalent. Then pattern of chromomere disposition became much clear. As shown in Figure 6, chromomeres visualized better in advanced stage. So far I observed in selected segment of certain bivalents, identical pattern of chromomere arrangement was detected between corresponding bivalent in different cells only at later stage of pachytene.

## Discussion

The morphologic interpretation of chromomeres has varied widely. While some investigators believed they represent condensations of nucleoprotein material. Others favored the view that they are regions of superimposed coils of elementary chromosome fibril which composed with nucleoprotein (DeRobertis *et al.* 1975). The later concept came from electron microscopic observations of leptonemic chromosome, which showed the strands of the chromosomes folded back and forth in the chromomeres.

For these several years, identification of pachytene bivalents has more aroused cytogenetic interest and pachytene karyotypes have been achieved using bivalent size and centromeric position as criteria (Hungerford 1971, Ferguson-Smith 1973, Luciani *et al.* 1975). These researches have been done, in human chromosomes, based on the consistency of chromomere arrangement at late pachytene. Their results demonstrated that chromomeres were conceived to be constant in number, size and position for the bivalent at any stage of prophase. However the genetic implication of chromomeres still remains obscure.

In contrast, from the chromomere number counting in lampbrush chromosomes, Vlad and Macgregor (1975) demonstrated that the inconstancy of chromomere number per haploid set in the same species of salamanders. They concluded that the term 'chromomere' defined as a discrete granule of deoxyribonucleoprotein on the axis of a lampbrush chromosome. The present observation also showed that the number varied depending on the length of the same chromosome. This examination can be explained that the chromomeres tend to fuse or condense into blocks of material as the cycle of chromosome condensation progresses from early prophase towards diakinesis, and resulted the minimal number of chromomere count in respective

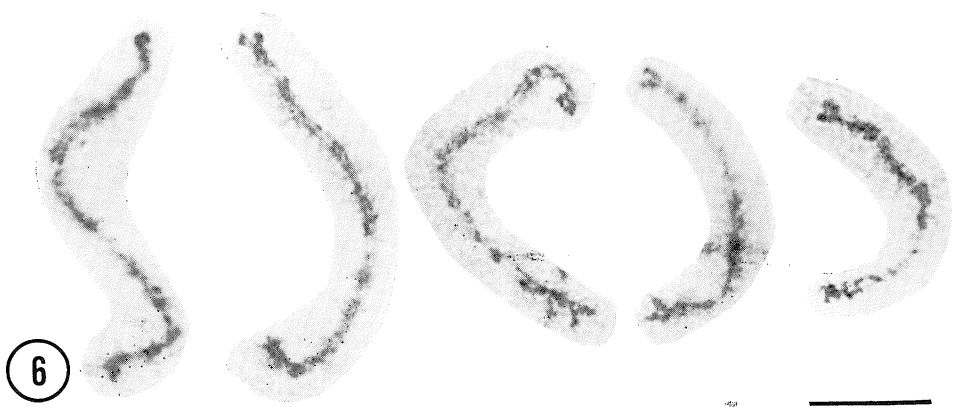



Figure 6. Bivalent no. 5 from five selected cells of pachytene. From left to right, representing chromosomes become more contracted according to proceeding the stage, and chromomeres visualized better than the earlier stage.

bivalent.

However, it would not be sufficient from the present study to conclude the chromomeres whether they represent condensations of nucleoprotein material or they are structures resulting from local coiling of a continuous thread.

*Acknowledgments* : I am indebted to Professor Masaru Akiyama for his encouragement through the study. My thanks extend to Misses Noriko Yamanaka and Keiko Iida for their help in securing materials and in laboratory works.

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