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Random Arrangement of Chromosomes in a Radial Metaphase Configuration*

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Abstract. During metaphase II of spermatogenesis the chromosomes of the grasshopper, *Melanoplus femurrubrum* (De Geer), are arranged at the periphery of the metaphase plate in what has been termed the radial metaphyse configuration. Cells at this stage contain two long, three short, and either six or seven chromosomes of medium length. The arrangement of the chromosomes in the metaphase plate was analyzed by counting the number of medium-sized chromosomes which separated the two long chromosomes. In the 351 cells analyzed the frequencies of cells with the various types of arrangements agreed closely with those expected from a random arrangement of the chromosomes in the metaphase plate. The possible role of chromosome-to-chromosome connectives in the arrangement of the chromosomes in the radial configuration is discussed.

Introduction

In some organisms the arrangement of chromosomes in the metaphase plate is not random. In *Diptera* homologous chromosomes exhibit somatic pairing. In hexaploid wheat, in which somatic pairing has not been observed, the homologues have been shown to be closer to each other than would be expected by chance (Feldman *et al.*, 1966). Non-homologous chromosomes may also be arranged non-randomly. In many species the shorter chromosomes occupy the center of the plate and the longer chromosomes occupy the periphery (Wilson, 1928). In humans, satellited chromosomes may show close association (Bishun, 1966), apparently as a result of the fusion of the nucleoli formed by these chromosomes.

In many species the chromosomes are arranged radially at the periphery of the metaphase plate, and DuPraw (1970) has referred to this arrangement as the radial metaphase configuration. The radial configuration offers several advantages for studying chromosome associations. Any treatment which changes the position of the chromosomes is also expected to destroy the radial configuration. Thus, it is possible to choose for study only unaffected cells. Moreover, the association of particular chromosomes can be studied not only by measuring the distance between particular chromosomes but also by counting the

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number of chromosomes which separates any two identifiable chromosomes.

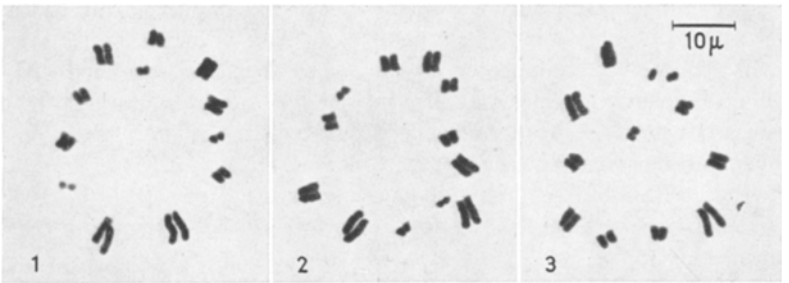
In the present study the radial arrangement of chromosomes in metaphase II of a grasshopper was utilized to determine whether two long non-homologous chromosomes tended to be arranged in the metaphase plate in any particular way.

Materials and Methods

Melanoplus femurrubrum males were collected September 23, 1971, near the University of Rochester. They were fixed in 1:3 acetic ethanol and stored in 70% ethanol. The testes were stained in alcoholic-HCl-carmine (Snow, 1963) and squashed in aceto-carmine. Only one follicle with cells in metaphase II was used per male. Cells were analyzed only when all the medium and long chromosomes were arranged near the periphery of the metaphase plate without overlap.

Observations

In *Melanoplus femurrubrum* the males are $2n = 22 + X$. Two chromosome pairs are long and three are short; the rest, including the X, are intermediate in size and will be referred to as the medium chromosomes. During metaphase II of spermatogenesis the secondary spermatocytes contained either 11 or 12 chromosomes which were arranged in a radial configuration. The arrangement of the chromosomes in the metaphase plate was analyzed by counting the number of medium chromosomes



Figs. 1—3. Secondary spermatocytes in metaphase II. × 800. Figs. 1 and 2. Each of the two long chromosomes (below) is separated from the other long chromosome by zero medium chromosomes in one direction and by seven medium chromosomes in the other direction (0–7 arrangement). Fig. 3. The two long chromosomes (upper left and lower right) in a 3–4 arrangement

which separated the two long chromosomes, first in one direction along the periphery and then in the other direction (Figs. 1–3). The three short chromosomes were not included in the analysis because they were often present inside the plate, rather than at the periphery (Fig. 3). The results of the analysis are presented in Table 1.

Table 1. The number of secondary spermatocytes in metaphase II with the various chromosome arrangements. In these cells the chromosomes are arranged in a circle and the cells were classified according to the number of medium chromosomes which separated the two long chromosomes, first in one direction and then in the other direction along the circle. Cells with six and with seven medium chromosomes are those without and with the X chromosome, respectively

Male no.	Chromosome arrangement				N	Chromosome arrangement				N
	0-6	1-5	2-4	3-3		0-7	1-6	2-5	3-4	
1	10	9	11	6	36	7	7	6	10	30
2	3	3	7	3	16	6	7	4	7	24
3	16	18	23	6	63	11	16	11	14	52
4	5	7	6	3	21	7	6	5	9	27
5	5	7	5	2	19	6	6	7	3	22
6	4	1	3	3	11	1	1	4	1	7
7	4	4	2	0	10	2	2	4	5	13
Totals	47	49	57	23	176	40	45	41	49	175

Secondary spermatocytes with 11 and with 12 chromosomes contained six and seven medium chromosomes, respectively. If in cells with six medium chromosomes the two long chromosomes were arranged randomly in the metaphase plate, the number of cells in which the two long chromosomes were separated by 0–6, 1–5, 2–4, and 3–3 medium chromosomes should be present in ratios of $1:1:1:{}^1/_2$. For cells with seven medium chromosomes the ratios should be 1:1:1:1. The observed numbers of cells with the eight types of arrangements (Table 1) agree closely with these expectations. For both cells with six and with seven medium chromosomes the departure from the expected ratios is clearly not statistically significant ($\chi^2_{(3)} = 1.32$ and $\chi^2_{(3)} = 1.16$; $P > 0.80$).

Discussion

In a recent book, Du Praw (1970, page 196) made the following statement about the origin of the radial metaphase configuration: "There is considerable evidence that the structural basis of the grouping is inherent in the chromosomes themselves, and especially in the presence of chromosome-to-chromosome connectives". He drew attention to chromosome fibers that may sometimes be seen to connect adjacent chromosomes and posed the following intriguing questions about the organization of the genome and the chromosome-to-chromosome connectives (page 204): "Is it possible, for example, that two or more (nonhomologous) chromosomes might correspond to only one very, very long DNA double helix? Might a single DNA molecule fold up in the form of several separate chromosomes connected by intermediate and un-

resolvable DNA segments? And, carrying these ideas to their logical extreme, could an entire haploid chromosome set represent linked condensations of a single gigantic and circular DNA molecule?". According to these schemes either some or all the connectives are expected to be present at all stages and to link together specific chromosomes.

In discussing these schemes DuPraw pointed out that such a supra-chromosomal organization may explain segregation of whole genomes, partial linkage between non-homologues, and other unusual aspects of chromosome behaviour. The well-known observations of the independent assortment of chromosomes, and of genes on different chromosomes, during meiosis was explained by Du Praw in the following way (page 200). "...some or all of the chromosomes in the haploid set could, in fact, be linked together by DNA molecules, yet they would segregate independently if the interchromosomal DNA segments were long enough to give 50 percent recombination and if these segments did not contain detectable genes".

At stages other than prophase I of meiosis some of the connectives were apparently assumed to be very stable, because DuPraw invoked changes in the folding of a single DNA double helix to explain how two acrocentric chromosomes may become one metacentric chromosome, and one or the other chromosomal type usually characterizes entire individuals. In some cases, however, the connectives were assumed to be fairly labile as a result of breakage and rejoining between non-homologous connectives. According to DuPraw (page 201): "Break-rejoin events in the DNA segments connecting the chromosomes would allow for a wide variety of premutations in the order of the chromosomes around the circle... Very possibly the degree of independence of different chromosomes, and the pattern of their supra-chromosomal organization varies a great deal in different species or even in different tissues." This quotation suggests that DuPraw considered the connectives to be short enough to affect the position of the chromosomes in the metaphase plate. DuPraw did not discuss the nature of the break-rejoin events but it is clear that these events must differ at least quantitatively from similar events in which the chromosomes themselves are involved. In order to observe variation in the arrangement of chromosomes among cells of the same tissue it is necessary to assume at least one break-rejoin event per cell per generation. On the other hand, the frequency of such events leading to the formation of chromosomal aberrations is usually one per one hundred cells per generation, or less (Swanson, 1957; Lewis and John, 1963).

The presence of chromosome-to-chromosome connectives of the type postulated by DuPraw may be established by studying the arrangement of the chromosomes in the metaphase plate, but only if the connectives

are relatively short and stable. Thus, if during the second meiotic division of *Melanoplus femurrubrum* males the two long chromosomes were attached to each other, or to one or more of the medium chromosomes, by relatively short and stable chromosome-to-chromosome connectives the frequencies of cells with the various chromosome arrangements should have departed significantly from those expected from a randum arrangement of the chromosomes. Since they did not, it may be concluded either that in this species specific chromosome-to-chromosome connective of the type postulated by DuPraw are absent, or that they are not short and stable enough to affect the arrangement of the chromosomes in the radial metaphase configuration.

Added in the Proof. Rcently, W. K. Heneen and W. W. Nichols, Cytogenetics (Basel) **11**, 153–164 (1972), analyzed the arrangement of three pairs of chromosomes in the radial metaphase configurations of the Indian deer, *Muntiacus muntjak* ($2n=7$). In comparing the observed frequencies of the 11 possible patterns of chromosome arrangement to those expected, however, the authors assumed erroneously that the expected frequencies of these patterns are equal. On the alternative assumption that their patterns 1, 5, 6, and 7 are expected to be twice as frequent as the others, their data indicate that the chromosomes of pair 1 exhibit a strong tendency to be adjacent, but that the other four chromosome are arranged at random.

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