KINETOCHORE MICROTUBULE NUMBERS OF DIFFERENT SIZED CHROMOSOMES

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ABSTRACT

For three species of grasshoppers the volumes of the largest and the smallest metaphase chromosome differ by a factor of 10, but the microtubules (MTs) attached to the individual kinetochores show no corresponding range in numbers. *Locusta* mitotic metaphase chromosomes range from 2 to 21 μm, and the average number of MTs per kinetochore is 21 with an SD of 4.6. *Locusta* meiotic bivalents at late metaphase I range from 4 to 40 μm^3, and the kinetochore regions (= two sister kinetochores facing the same spindle pole) have an average of 25 kinetochore microtubules (kMTs) with an SD of 4.9. Anaphase velocities are the same at mitosis and meiosis I. The smaller mitotic metaphase chromosomes of *Neopodismopsis* are similar in size, 6 to 45 μm^3, to *Locusta*, but they have on the average more kMTs, 33, SD = 9.2. The four large Robertsonian fusion chromosomes of *Neopodismopsis* have an average of 67 MTs per kinetochore, the large number possibly the result of a permanent dicentric condition. *Chloealtis* has three pairs of Robertsonian fusion chromosomes which, at late meiotic metaphase I, form bivalents of 116, 134, and 152 μm^3 with an average of 67 MTs per kinetochore region. The telocentric chromosomes form bivalents ranging from 18 to 65 μm^3, similar to *Locusta* bivalents, but with a much higher average of 42 MTs per kinetochore region.

It is speculated that, in addition to mechanical demands of force, load, and viscosity, the kMT numbers are governed by cell type and evolutionary history of the karyotype in these grasshoppers.

KEY WORDS kinetochore · microtubule · chromosome

Kinetochore microtubules (kMTs) are acknowledged to transmit or produce force for chromosome movement at the time of cell division (6). The models on anaphase chromosome movement reviewed by Nicklas (5), however, do not predict what quantities of kMTs are required to regulate the movement of chromosomes. Observations on cell divisions in a variety of organisms show that, in general, the larger the chromosomes, the more microtubules (MTs) are attached to their kinetochores. In a survey of the available data, Fuge (2) notes that the very small chromosomes of fungi and slime molds have from 1 to several kMTs per kinetochore, the medium-sized chromosomes of HeLa, PtK, and crane fly cells have 20 to 40 kMTs, whereas the very large chromosomes of *Haemanthus* have an average of 120 kMTs per kinetochore. For a given cell, Dietz, in a discussion following a paper by Fuge (1), suggests that "in anaphase there are correlations between the number of kinetochore microtubules and the chromosome size

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(load to be transported)." Observations on metaphase chromosomes of the grasshopper *Neopodismopsis* have shown that chromosomes of 2 to 7 μm in length all have ~33 MTs at their kinetochores but that the large Robertsonian fusion chromosomes of 15 and 17 μm have ~67 MTs per kinetochore (3). Clearly, there is no strict proportionality between chromosome size and kMT numbers, but there is a general correlation to the extent that the larger chromosomes have more kMTs than the smaller ones.

This report compares the numbers of kMTs of locust chromosomes at mitosis which range in size from 2 to 21 μm^3 and the bivalents at meiosis I which range from ~4 to 40 μm^3. In addition, the number of kMTs are reported for *Neopodismopsis* chromosomes and for *Chloealtis* bivalents at meiosis I which range in size from 15 to 150 μm^3.

MATERIALS AND METHODS

Testicular material of *Locusta migratoria*, *Neopodismopsis abdominalis*, and *Chloealtis conspersa* was dissected and fixed in 2% glutaraldehyde (Fisher Scientific Co., Pittsburgh, Pa.) in 0.05 M phosphate buffer, pH 7.2, for 2 h, postfixed in osmium tetroxide, also in phosphate buffer, dehydrated through an alcohol series followed by propylene oxide, and then single seminiferous tubules were embedded in Epon. This method preserves MTs well but it is possible that some are destroyed. If the proportion of MTs destroyed is equal for all cells it does not affect the results seriously, but if there are differential artifacts the results will be confounded. Cysts of spermatogonia or spermatocytes in metaphase were found through light microscopy of thick sections and then serially thin sectioned. Ribbons of sections were collected with a loop and deposited on Formvar-covered single hole grids. Sections were stained with 2% uranyl acetate at 45°C for ½ h and then washed and briefly stained with lead citrate. Electron micrographs were taken of every section of selected metaphase cells. The volumes of chromosomes and bivalents were determined by weighing cut-outs of tracings and/or by digitizing the chromosome outlines followed by the appropriate computation. In the case of cell 2, Table I, four chromosomes were not complete at the end of the series and the volumes were calculated from their lengths relative to the lengths and volumes of the other chromosomes in the cell. Kinetochores were identified in the intermediate magnification electron micrographs and then a series of high magnification electron micrographs were made of each kinetochore region to count numbers of kMTs. Missed or obscured kinetochores are indicated by "?" in the tables. The karyotype for *Locusta* is 11 pairs of telocentric autosomes and a single X chromosome. *Chloealtis* is derived from that karyotype by three Robertsonian fusions or translocations of the large chromosomes. Consequently, there are three large metacentric pairs, five telocentric paris, and an X chromosome. *Neopodismopsis* has two pairs of Robertsonian fusion chromosomes, seven pairs of telocentrics, and an X chromosome.

RESULTS

For orientation, Fig. 1 illustrates that a chromosome at mitotic metaphase consists of two chromatids, each with one kinetochore and the kMTs directed to opposite poles of the spindle. At metaphase I of meiosis, the bivalents consist of two paired homologous chromosomes or half-bivalents. In this case the two sister kinetochores of each chromosome face the same spindle pole.

Locusta Mitosis (Table I)

The lengths of locust chromosomes were measured from light micrographs of spermatogonial metaphase squash preparations and they range from 1 to 7 μm. The volumes of the chromosomes were measured from electron micrographs of two serially sectioned spermatogonial metaphases. In cell 1, column 2, the volumes range from 3 to 27 μm^3, and in cell 2, column 4, the chromosomes are slightly more condensed, ranging in volume from 2 to 21 μm^3. The total chromosome volumes are 327 and 260 μm^3 for cells 1 and 2, respectively. The chromosomes in Table I are paired off and ordered according to volume. Real homology and karyotype position are only approximated in this manner. The 22 autosomes and the X chromosome each have two kinetochores for a total of 46 ki-

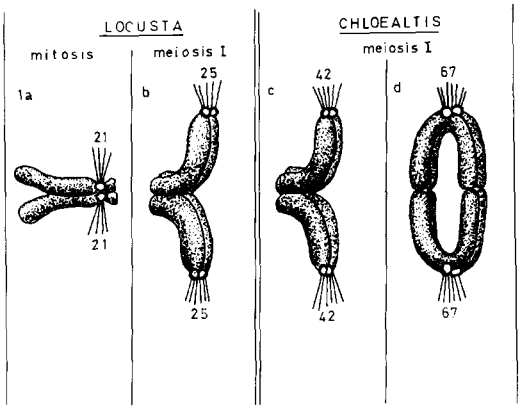

FIGURE 1 Schematic diagram of chromosome arrangements at mitosis and meiosis. (*a*) At mitosis in *Locusta* the chromosomes consist of two chromatids each with a kinetochore and kMTs, an average of 21 per kinetochore, directed to opposite spindle poles. (*b*) At meiosis I of *Locusta* the duplicated chromosomes are paired homologously and each kinetochore region has two sister kinetochores with kMTs directed to the same spindle pole. (*c*) At meiosis in *Chloealtis* the telocentrics are similar to *b* but have more kMTs, 42 vs. 25, per kinetochore region. (*d*) The *Chloealtis* Robertsonian fusion chromosomes are metacentric and have terminal chiasmata at meiosis I. The paired sister kinetochores have an average of 67 kMTs.

TABLE I

Locusta Spermatogonial Metaphase: Chromosome Sizes vs. Numbers of KMTs

Chromosomes numbered in order of volume	Cell 1 Vol	Cell 1 MTs per kinetochore	Cell 2 Vol	Cell 2 MTs per kinetochore
	μm^3		μm^3	
1	27, 24	4, 8; 12, 15	21, 21	18, 17; 20, 23
2	23, 22	11, 6; 16, 11	20, 18	26, 23; 25, 28
3	21, 20	11, 11; 23, 20	17, 17	21, ? ; 24, 25
4	19, 18	14, 8; 9, 8	17, 15	27, 25; 25, 20
5	14, 14	18, 15; 6, 7	12, 10	24, 27; 25, 17
6	14, 13	7, 13; 12, 20	10, 10	18, ? ; 20, ?
7	13, 12	9, 11; 11, 15	9, 9	16, 22; 26, 26
8	11, 11	9, 7; 8, 6	9, 8	24, 25; 23, 18
9	8, 8	7, 11; 9, 10	4, 3	12, 18; 8, 10
10	4, 4	12, 8; 2, 4	3, 3	18, 19; 18, 17
11	4, 3	7, 12; 15, 10	3, 2	21, ? ; 18, 16
X	20	12, 10	19	20, 20
	327 +	490 +	260 +	957* +
		$\bar{x}$ = 10.6		$\bar{x}$ = 20.8
		SD = 4.4		SD = 4.6
		n = 46.0		n = 42.0
		r = 0.20		r = 0.50
				$P < 0.001$

* Assuming 21 for each of the missing numbers.

netochores per cell. Cell 1 is judged to be in earlier metaphase than cell 2 by its larger chromosome volume and by the relatively small number of kMTs (sum = 490, $\bar{x}$ = 10.6, SD = 4.4). For cell 2 the values are: sum = 957 (counting 21 for each of the four missing numbers), $\bar{x}$ = 20.8, and SD = 4.6 (1).

In cell 2 but not in cell 1 there is a significant correlation between chromosome size and number of kMTs, r = 0.50, df = 40, $P < 0.001$. It is evident, however, that some of the smaller chromosomes such as Nos. 5 and 8 have as many kMTs as the larger chromosomes. Clearly there is no strict proportionality between chromosome size and kMT numbers. In interference microscope preparations of living cells the measured velocity of early anaphase chromosome movement was 1 μm/min at 21°C for large and small chromosomes alike. This value is in agreement with other such measurements (4).

Locusta Meiosis (Table II)

At metaphase of meiosis I, there are 11 bivalents each consisting of two paired homologous chromosomes and there is a single unpaired X chromosome. At anaphase, the duplicated chromosomes move to the spindle poles and each kinetochore region consists of two sister kinetochores facing the same pole (Fig. 1). In thin sections parallel to the metaphase plate, the two sister kinetochores are often discernible as two separate structures both of which have MTs attached to them, indicating that both sister kinetochores are functional.

In Table II, the bivalents are ordered and numbered according to size. The sum values of the cells, 280, 238, and 245 μm^3, are in agreement with the sum volume of the mitotic chromosomes of cell 2 (Table I), 260 μm^3. In cell 3, the chromosomes of bivalents Nos. 8–10 had completely separated while Nos. 3 and 6 were in the process of separating. The onset of anaphase I in cell 3 is associated with the highest number of kMTs, 573, as compared with 436 and 476 in cells 1 and 2. Comparison of cells 1 and 2 of Table III shows the same trend. As in mitosis, the numbers of kMTs attached to the larger and smaller chromosomes are not much different. The correlation coefficients are not significant at or below the 5% level, and the 9- to 10-fold difference in size clearly

is not reflected in the numbers of MTs attached to the kinetochore regions. The highest number of kMTs per cell at meiosis I, 573, is considerably less than the highest number observed at mitosis, 957. Taken together with the evidence that both sister kinetochores of the half-bivalents are functional at meiotic metaphase I, it follows that each connects with fewer MTs than do mitotic kinetochores (12.5 vs. 20.8, $t = 4.13$, $P < 0.001$). At anaphase I large and small chromosomes have the same velocity as mitotic chromosomes, 1 μm/min at 21°C. Apparently there is no simple relation between velocity, size, and kMT numbers within a given cell and between the two different cell types.

Chloealtis Meiosis I (Table III)

This grasshopper has a much larger total chromosome volume at meiotic metaphase I, ~600 μm^3, than *Locusta* with 240 μm^3. In addition, six pairs of the larger chromosomes have undergone Robertsonian fusions, producing three pairs of very large metacentric chromosomes (columns 2 and 4, Table III). A cyst of metaphase I spermatocytes contained cells with no or few kMTs (cell 1, Table III), presumably in early metaphase up to cells in early anaphase (cell 2, Table III) with separated half-bivalents (Nos. 1, 2, 3, and 8) and numerous kMTs.

For the three large bivalents, the average is 67 MTs per kinetochore region with an SD of 6.5 and a nonsignificant correlation coefficient. For the remainder of the chromosomes, the average is 42 MTs per kinetochore with SD = 6.2 and r = 0.62, significant at the 5% level. For cell 2 as a whole, there is a highly significant correlation between size and kMT numbers ($P < 0.001$). In comparison with locust bivalents Nos. 1 and 2, *Chloealtis* bivalents Nos. 1 and 2 are about four times as voluminous, but they have only two to three times the numbers of kMTs observed in locust. The smaller *Chloealtis* bivalents Nos. 6–8 and X, on the other hand, are similar in size to the locust bivalents, but they have more kMTs ($\bar{x} = 38$ vs. $\bar{x} = 25$, $t = 7.08$, $P < 0.001$).

Neopodismopsis Mitosis (Table IV)

No *Chloealtis* mitotic metaphases could be found and instead data are presented here for *Neopodismopsis* which has two pairs of Robertsonian fusion chromosomes and a total chromosome volume of 667 μm, roughly similar to *Chloealtis* with 580 and 641 μm^3. The *Neopodismopsis* mitotic metaphase cell has 1,534 kMTs as compared to 862 for *Chloealtis* meiosis I. If the locust data can serve as a guide, the difference reflects physiological differences of mitosis vs. meiosis rather than

TABLE II

Locusta Meiosis Metaphase I: Bivalent Sizes vs. Numbers of kMTs

	Cell 1		Cell 2		Cell 3	
Bivalent No.	Vol	No. of kMTs	Vol	No. of kMTs	Vol	No. of kMTs
	μm^3		μm^3		μm^3	
1	54	23, 20	43	24, 22	40	30, 30
2	42	? ?	40	24, 16	35	23, 25
3	31	19, 15	28	24, 20	28	20, 21
4	27	18, 21	22	17, 19	25	25, 31
5	23	20, 18	21	27, 26	22	31, 33
6	23	22, 23	16	23, 24	20	24, 27
7	21	24, 24	16	18, 15	20	21, 23
8	20	20, 20	15	23, 25	19	25, 25
9	9	10, 19	7	16, 14	8	19, 19
10	7	19, 17	7	21, 22	7	27, 35
11	6	15, 16	5	17, 18	4	22, 17
X	17/280 +	15, –/436* +	18/238 +	21, –/476 +	17/245 +	20, –/573 +
	$\bar{x}$ = 19.0		$\bar{x}$ = 20.7		$\bar{x}$ = 24.9	
	SD = 3.5		SD = 3.8		SD = 4.9	
	r = 0.43		r = 0.30		r = 0.29	

* Assuming 19 for each of the two missing numbers.

Anaphase separation has begun in cell 3 for bivalents 3, 6, 8, 9, and 10.

TABLE III

Chloealtis Meiosis Metaphase I: Bivalent Sizes vs. Numbers of kMTs

Bivalent No.	Cell 1 Vol	Cell 1 No. of kMTs	Cell 2 Vol	Cell 2 No. of kMTs
	μm^3		μm^3	
1	135	17, 22	152	69, 70
2	116	20, 24	134	75, 69
3	114	7, 7	116	58, 60
4	58	13, 10	65	52, 49
5	52	6, 7	60	50, 43
6	25	5, 5	28	36, 35
7	21	10, 3	25	40, 36
8	13	11, 7	18	38, 45
X	46/580 +	11, −/185 +	43/641 +	37, −/862 +
		$\bar{x}$ = 10.9		$\bar{x}_{(1-3)}$ = 66.8
		SD = 6.3		SD = 6.5
		r = 0.68		r = 0.72
				$\bar{x}_{(4-8+X)}$ = 41.9
				SD = 6.2
				r = 0.73
				$P < 0.01$
				r(all) = 0.95
				$P < 0.001$

Anaphase separation has begun in cell 2 for bivalents 1, 2, 3, and 8.

interspecific differences. As in *Chloealtis*, the Robertsonian fusion chromosomes have more kMTs, 67, than the telocentrics, 33.

DISCUSSION

Although kMTs are essential for anaphase chromosome movement, neither the calculated energy and force requirements for early anaphase chromosome-to-pole movement (4) nor the models for the mechanism of such movement (5), predict what quantities of kMTs are required for the chromosome movement. The general observation that small chromosomes function with few kMTs, and large chromosomes with many (2), suggests that chromosome size is a significant determinant of kMT numbers. The data presented here show, however, that the numbers of kMT cannot be predicted from size alone. *Locusta* chromosomes vary 10-fold in size while the numbers of kMTs are similar for all chromosomes. Between the three species, groups of similarly sized chromosomes have significantly different mean kMT numbers. A similar lack of correlation has been found in the crane fly (H. Fuge, personal communication) and in *Drosophila melanogaster* (K. Church, personal communication). It follows that, in addition to chromosome size, other factors are probably involved in the regulation of kMT numbers. The data reported here suggest at least two additional sources of variation.

Firstly, a cell in spermatogonial mitosis has more kMTs than a cell in meiosis, even though the total chromosomal volume is the same for the two. This is so when comparing the two types of cells within *Locusta* or when comparing *Neopodismopsis* mitosis with *Chloealtis* meiosis. Even with a much larger chromosome volume, *Chloealtis* still has fewer kMTs at meiosis than *Locusta* has at mitosis. A simple explanation might be that the meiosis I kinetochores are undivided and therefore the cell would have only half the number of kinetochores. However, evidence from precociously dividing univalents (unusual unpaired chromosomes) at anaphase I indicates that kinetochores are double at that time. It could then be assumed that only one of the two sister kinetochores is functional; but this is not supported by the observation that, where the two sister kinetochores can be seen with the EM as separate entities, both have

TABLE IV

Neopodismopsis Spermatogonial Metaphase: Chromosome Sizes vs. Numbers of kMTs

Chromosomes numbered in order of volume	Vol	MTs per kinetochore
	μm^3	
1	72, 70	75, 70; 60, 67
2	70, 70	60, 67; 65, 75
3	45, 42	40, 35; 35, 55
4	38, 35	30, 47; 30, 30
5	32, 30	22, 35; 35, 40
6	25, 23	30, 30; 45, 32
7	22, 21	42, 40; 20, 25
8	13, 12	35, 35; 30, 35
9	6, 6	17, 20; 20, 20
X	35/667 +	40, 45/1,534 +
		$\bar{x}_{(1,2)}$ = 67.4
		SD = 5.8
		$\bar{x}_{(3-9+X)}$ = 33.2
		SD = 9.2
		$r_{(all)}$ = 0.88
		$P < 0.01$
		$r_{(1-2)}$ = 0.54
		$r_{(3-9+X)}$ = 0.57
		$P < 0.01$

MTs attached to them. It must be concluded that, for unknown reasons, kinetochores at meiosis I initiate fewer MTs than at spermatogonial mitosis.

Secondly, the most pronounced variation in kMT numbers is (*a*) between species, e.g., meiosis in *Locusta* vs. *Chloealtis* and mitosis in *Locusta* vs. *Neopodismopsis*, and (*b*) between the telocentric and the Robertsonian fusion chromosomes. Both these observations suggest that the evolutionary history of the species and its karyotype is a significant determinant of kMT numbers. Observations on Robertsonian fusion chromosomes in numerous species, including *Neopodismopsis*, show that the kinetochore-associated heterochromatin is frequently serially duplicated (3). The inference is that the fusion chromosome carries two kinetochores close together (3). Such a permanent dicentric condition may account for the high number of kMT numbers in *Chloealtis* and *Neopodismopsis* metacentrics. The fact that no large Robertsonian chromosomes have the same or fewer kMTs than the telocentrics indicates that chromosome size remains a significant determinant of kMT numbers. In other words, those translocations of the large chromosomes which, in the past, produced a Robertsonian metacentric with a single kinetochore have not survived, possibly because of kinetic failure.

Karyotype evolution in general may also account for the lack of exact proportionality between chromosome size and kMT numbers. If chromosomes had exactly the number of kMTs required to perform their kinetic functions, then translocations, duplication, and other possibly advantageous evolutionary changes would be eliminated at the next cell division. Some excess of kMTs, on the other hand, permits more flexibility for rearrangement of evolutionary significance and thus an excess of kMTs may have a selective advantage.

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