

Traction Force on a Kinetochore at Metaphase Acts as a Linear Function of Kinetochore Fiber Length

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ABSTRACT We are investigating the relation between the force pulling a kinetochore poleward and the length of the corresponding kinetochore fiber. It was recognized by Östergren in 1950 (*Hereditas* 36:1-19) that the metaphase position of a chromosome could be achieved by a balance of traction forces if such forces were proportional to the distance from kinetochore to pole. For the typical chromosome (i.e., a meiotic bivalent or mitotic chromosome) with a single kinetochore fiber extending to each pole, the resultant force (RF) would equal zero when the chromosome lay at the midpoint between the two poles. For special chromosomes that have unequal numbers of kinetochore fibers extending towards opposite poles, Östergren's proposal suggests that $RF = 0$ when the chromosome is shifted closer to the pole toward which the greater number of kinetochore fibers are pulling. We have measured the force-length relationship in living spindles by analyzing the metaphase positions of experimentally generated multivalent chromosomes having three or four kinetochore fibers. Multivalent chromosomes of varied configurations were generated by γ -irradiation of nymphs of the grasshopper *Melanoplus differentialis*, and their behavior was analyzed in living first meiotic spermatocytes. The lengths of kinetochore fibers were determined from time-lapse photographs by measuring the kinetochore-to-pole distances for fully congressed chromosomes just before the onset of anaphase. In our analysis, force (F) along a single kinetochore fiber is expressed by: $F = kL^{exp}$, where k is a length-independent proportionality constant, L represents the kinetochore fiber length, and exp is an unknown exponent. The RF on a chromosome is then given by: $RF = \sum k_i L_i^{exp}$, where kinetochore fiber lengths in opposite half-spindles are given opposite sign. If forces on a metaphase chromosome are at equilibrium ($RF = 0$), then for asymmetrical orientations of multivalents we can measure the individual kinetochore fiber lengths (L_i) and solve for the exponent that yields a resultant force of zero. The value of the exponent relates how the magnitude of force along a kinetochore fiber varies with its length. For six trivalents and one naturally occurring quadrivalent we calculated an average value for $exp = 1.06 \pm 0.18$. This result is consistent with Östergren's hypothesis and indicates that the magnitude of poleward traction force along a kinetochore fiber is directly proportional to the length of the fiber. Our finding suggests that the balance of forces along a kinetochore fiber may be a major factor regulating the extent of kinetochore microtubule assembly.

A simple hypothesis to explain meiotic and mitotic congression of chromosomes has emerged from the thoughts and observations of Rashevsky (1), Hughes-Schrader (2), and Östergren (3-5). As proposed by Östergren, congression of meiotic bivalents is produced by the pulling of homologous kinetochores towards opposite poles by traction forces, these forces increas-

ing in magnitude with increasing distance from the pole. A similar force-distance relationship would hold for mitotic chromosomes, in which kinetochores of the sister chromatids are arranged back-to-back and are pulled towards opposite poles. As a result of this force-distance relationship, chromosomes with bipolar orientation become centered on the spindle equa-

tor simply because the equator is the position where opposite kinetochore fibers are of equal length; therefore, the two kinetochores are pulled equally toward their respective poles. Should a chromosome be displaced from this equilibrium position, there would be generated a resultant force on the chromosome so as to re-establish the position of the chromosome equidistant from the spindle poles.

Forces moving chromosomes are now known to be generated along the array of kinetochore fiber microtubules which mechanically link the kinetochore to the spindle pole (6). Therefore, the force-distance relationship proposed by Östergren (3) concerns the relationship between the force applied to a kinetochore and the length of the kinetochore fiber.

In contrast to Östergren's force-balance concept of congression, chromosomes could be moved to an equilibrium position at the metaphase plate by regulation of the equilibrium length of the kinetochore fiber (7). For example, based on the dynamic equilibrium theory of Inoué and Sato (8), Dietz specifically proposed (9) that, as a consequence of achieving monomer-polymer equilibrium, kinetochore microtubule lengths change and thereby regulate chromosome position. If all kinetochores participate equally in the formation of kinetochore fibers and if tubulin subunits are freely diffusible, this proposal predicts that all kinetochore fibers will be of equal length at metaphase equilibrium. Therefore a chromosome would lie at the spindle equator due to a requirement that equal lengths of microtubules compose the two opposing kinetochore fibers. As the Dietz hypothesis predicts, movements of the kinetochores are observed to be closely coupled to changes in extent of assembly of kinetochore fiber microtubules (8, 10, 11).

The two hypotheses proposed to account for chromosome congression therefore suggest the following question: Does a requirement for balance of forces generated along microtubules of the kinetochore fiber regulate the extent of kinetochore fiber microtubule assembly (Östergren hypothesis), or does the ki-

netochore microtubule assembly mechanism directly regulate the equilibrium length of kinetochore fibers (Dietz hypothesis)? An answer to this question is possible through observation of the stable metaphase position of multivalent chromosomes that have more than two kinetochore regions and asymmetrical orientations on the spindle, i.e., unequal numbers of kinetochores oriented to opposite poles (see Fig. 1). If congression is the result of a balancing of forces, then multivalents oriented asymmetrically should be shifted off the equator, closer to the pole to which the greater number of kinetochores are attached (3, 12). Otherwise, if an equilibrium length-balance mechanism determines chromosome position, the asymmetrically oriented multivalents should lie at the spindle equator, with the lengths of all kinetochore fibers equal (Fig. 1c).

In this study, we generated multivalents in grasshoppers by γ -irradiation of nymphs, and in living-cell preparations we examined the positions of these newly arisen multivalents at congression in meiosis I. From our analysis of the positions of congressed multivalent chromosomes, we present quantitative evidence consistent with Östergren's hypothesis.

MATERIALS AND METHODS

Experimental Material and Production of Multivalent Chromosomes

Males of the grasshopper species *Melanoplus differentialis* (Acrididae, Orthoptera) were taken from a laboratory colony maintained by Dr. R. B. Nicklas at Duke University, Durham, NC.

We selected third-instar nymphs with average head width and femur length of 3.6 mm and 12 mm, respectively, and exposed them to a 480-rad dose of γ -irradiation (cobalt source operating at 160 rad/min). Both the age of nymphs when irradiated and the time postirradiation when grasshoppers were sacrificed affected the number of rearrangements per cell and general cell health. Our treatment typically yielded 1-2 rearrangements per cell, a useful number for analysis. Higher numbers of rearrangements produced multivalent chromosomes too complex for analysis.

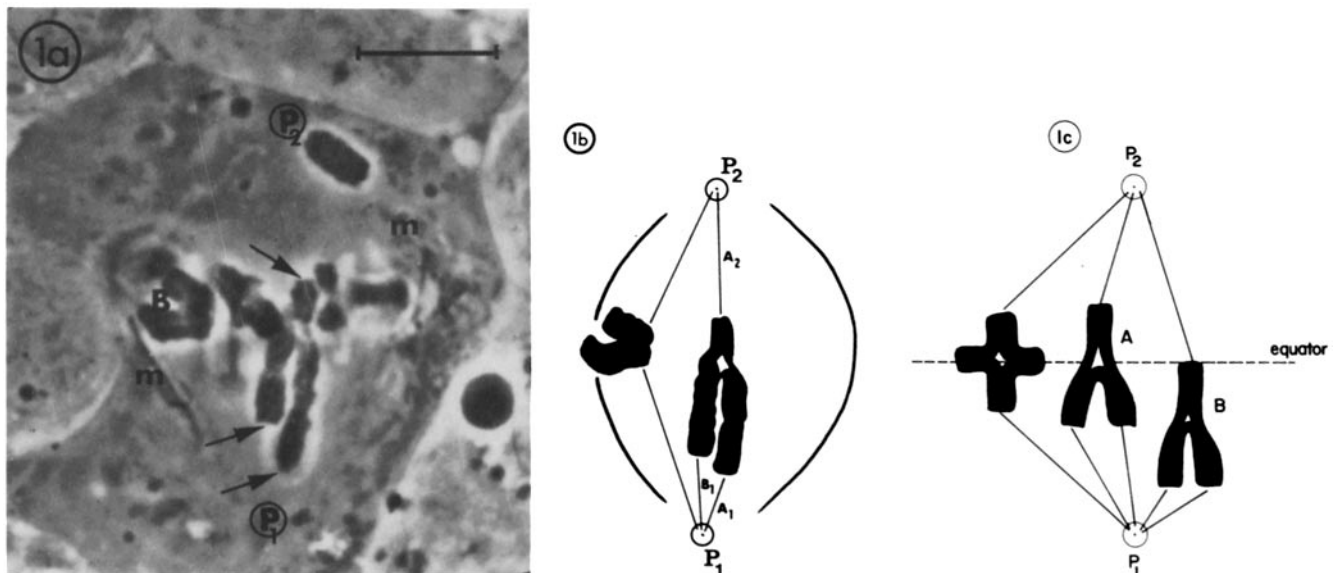


FIGURE 1 (a) Phase-contrast micrograph of a living spermatocyte in meiotic metaphase I. The spindle is outlined by mitochondria (*m*). Arrows indicate the kinetochores of a trivalent. The asymmetric orientation of kinetochores (two kinetochores attached to one pole, P_1 , and one kinetochore attached to the other pole, P_2) results in congression of the trivalent to an equilibrium position shifted off the equator. Note that typical bivalents (*B*) with symmetrical orientations lie at the spindle equator and define the "metaphase plate." Bar, 10 μ m. \times 1,800. (b) Schematic drawing of the cell shown in a. We measured the interpolar length P_1P_2 and the kinetochore fiber lengths as approximated by the kinetochore-to-pole distances A_1 , B_1 , and A_2 . The subscript identifies the pole facing a given kinetochore. A symmetrically oriented bivalent is shown for comparison. (c) Schematic drawing of the expected position of a congressed trivalent according to the Dietz hypothesis (trivalent *A*) vs. the Östergren hypothesis (trivalent *B*).

Spermatocyte Cultures and Photographic Methods

9–12 d after irradiation, living-cell preparations were made of spermatocytes explanted from the grasshoppers.

Preparation of cell cultures, phase-contrast microscopy, time-lapse cinematography, and frame-by-frame analysis of film records were done according to methods described by Nicklas and Staehly (13). The buffered saline we used for the cell preparation was described by Nicklas et al. (14). Normal, healthy cells were chosen for analysis on the criteria of spindle size and shape, mitochondrial distribution, and the successful completion of anaphase.

Measurements and Computer Analysis

The lengths of kinetochore fibers for multivalents were measured as follows. The distance between each kinetochore and the pole to which it was attached was determined just before anaphase from projected cine records (see Fig. 1). Kinetochores were identified by a slight difference in phase density between a kinetochore and the adjacent chromatid. The identity of kinetochores was later

TABLE I

Comparison of Distances between Kinetochores and Poles in the Two Half-spindles for Multivalents of Various Orientation at Metaphase

Cell	Multivalent	Structure	L_1^* μm	L_2 μm	$\Sigma L_1 - \Sigma L_2$ μm	Spindle length μm
(1)	Triv 1	2:1	6.0/4.5	10.5	0	27.5
(2)	Triv 2	2:1	8.0/8.0	10.0	6.0	25.5
(3)	Triv 3	2:1	5.5/5.0	11.5	1.0	25.0
(4)	Triv 4	2:1	6.0/6.5	13.0	0.5	27.0
(5)	Triv 5	2:1	7.5/2.0	9.5	0	25.0
(6)	Triv 6	2:1	4.0/6.0	9.5	0.5	25.0
(7)	Triv 7	2:1	6.0/5.0	9.0	2.0	24.0
$t = 1.10$ (d.f. = 6, $P > 0.05$)						
(8)	Quad 1	3:1	9.5/4.0/3.0	15.0	1.5	28.0
(9)	Quad 2†	2:1:1	7.0/8.0	12.0	3.0	24.0
(10)	Quad 3	2:2	7.5/11.0	9.0/9.5	0	25.0
(11)	Quad 4	2:2	7.0/10.0	7.5/8.5	1.0	25.0
(12)	Quad 5	2:2	7.0/8.5	6.0/9.0	0.5	27.5
(13)	Quad 6	2:2	7.0/8.0	6.0/9.0	1.0	27.0
(14)	Quad 7	2:2	6.5/11.5	8.0/9.5	0.5	25.0
(15)	Quad 8	2:2	9.0/7.0	9.0/8.0	1.0	26.0
(16)	Quad 9	2:2	12.5/4.0	13.0/3.0	0.5	27.0
$t = 0.89$ (d.f. = 6, $P > 0.05$)						
(10)	Biv 1	1:1	11.5	12.0	0.5	25.0
	2		10.5	11.0	0.5	25.0
	3		11.5	11.0	0.5	25.0
(11)	Biv 4	1:1	12.5	11.5	1.0	25.0
	5		12.0	11.0	1.0	25.0
	6		12.0	11.5	0.5	25.0
(17)	Biv 7	1:1	13.5	13.5	0	26.0
	8		9.0	8.0	1.0	26.0
	9		10.5	10.0	0.5	26.0
(13)	Biv 10	1:1	12.5	13.0	0.5	27.0
	11		10.0	10.5	0.5	27.0
	12		9.0	8.5	0.5	27.0
$t = 1.39$ (d.f. = 11, $P > 0.05$)						

* Numbers in the column labeled L_1 and L_2 are distances between kinetochores and the poles for each half-spindle (P_1 and P_2). The data are presented such that L_1 always represents the half-spindle to whose pole, P_1 , more than one kinetochore of the multivalent is oriented.

† Quadrivalent 2 orients in a 2:1:1 fashion, i.e., two kinetochore pairs orient to pole 1 (P_1), one kinetochore pair orients to pole 2 (P_2), and the fourth kinetochore pair orients amphitellically, that is, to both poles. The kinetochore-to-pole distances are given only for the monopolarly oriented kinetochores. The amphioriented kinetochore lies at the equator and has equivalent kinetochore-to-pole distances to opposite poles (see Results).

confirmed by noting that these regions of chromatin lead the way to the poles during anaphase; the attached chromatin arms trail behind these kinetochores. The polar region was identified by the phase-dense image of the centriole complex itself or was considered to occur approximately at the point of convergence of mitochondria surrounding the spindle. A total of 16 cells was analyzed: 7 trivalents and 9 quadrivalents. As controls, 12 bivalents from 4 of the 16 cells were also analyzed (Table I). Data analysis was performed as described in Results and Fig. 2 using an Apple II Plus microcomputer (Apple Computer Inc., Cupertino, CA) and a Houston Instruments Hi-Plot digital plotter (Houston Instruments, Austin, TX).

RESULTS

Trivalents

Trivalents assumed asymmetric orientations (two chromosomes oriented to one pole and only one chromosome oriented to the opposite pole) and were shifted off the equator as shown, for example, in Fig. 3. Trivalents lie closer to the pole to which two kinetochores are attached. All seven trivalents analyzed showed a similar off-equator position at metaphase; the metaphase kinetochore-to-pole distances for the seven are listed in Table I.

For each of the analyzed trivalents the sum of the lengths of the two kinetochore fibers oriented to the nearer pole approximately equaled the length of the single kinetochore fiber oriented to the far pole; the values are not significantly different based on a paired Student's t test (Table I). This result suggests that all kinetochores are nearly alike and that a linear relation-

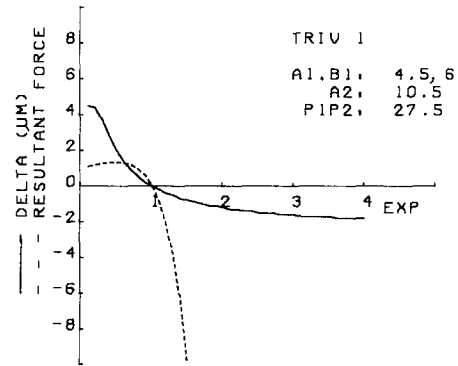


FIGURE 2 Computer analysis of force dependence on kinetochore fiber length for the trivalent shown in Fig. 1. The force (F) applied to a kinetochore is assumed to be proportional to the kinetochore fiber length (A_1 , B_1 , or A_2) to an exponential power of exp (e.g., $F = kA_1^{exp}$). Whether the forces on the trivalent are balanced is calculated by subtracting the traction forces towards pole 2 (P_2) from those towards pole 1 (P_1). For the trivalent in Fig. 1, the resultant force (RF) = $k(A_1^{exp} + B_1^{exp}) - A_2^{exp}$. The algorithm solves the equation for values of exp from 0.1 to 4.0. At metaphase the resultant force on the chromosome is assumed to be equal to zero ($RF = 0$). Therefore, the value of exp where $(A_1^{exp} + B_1^{exp}) - A_2^{exp} = 0$, best describes the force-length relationship.

The curve labeled *Resultant Force* plots the resultant force/ k (in relative units) on the chromosome, calculated assuming various values for the exponent, exp . Forces should be balanced when $RF = 0$. RF becomes zero when the value of the exponent is 1.0. For values of the exponent between 0.1 and 4, Δ gives the distance that the chromosome would need to be displaced towards pole 2 (P_2) to make the forces balance. (Negative values on x -axis indicate toward pole 2; positive values indicate toward pole 1.) For example, if force was proportional to the square of distance, then the trivalent would need to be shifted $2 \mu m$ closer to pole 2 to achieve a balance of forces. The kinetochore-to-pole distances, i.e., kinetochore fiber lengths (A_1 , B_1 , A_2), and the interpole length ($P_1 P_2$) are given in the upper right-hand corner. For $exp = 1.0$, both the resultant force and Δ values equal zero.

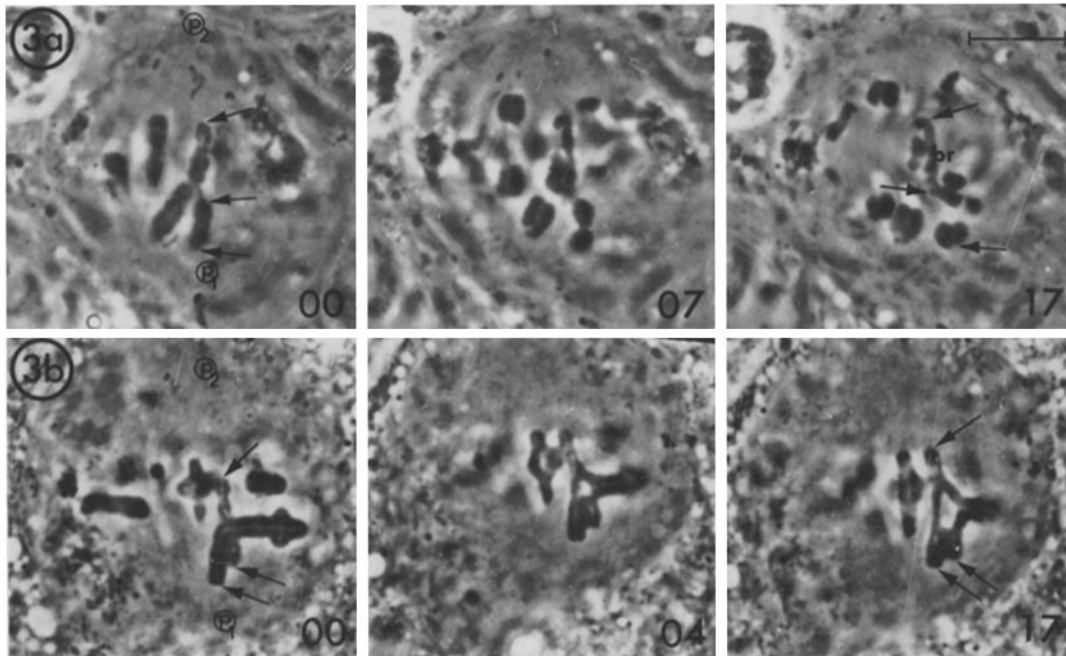


FIGURE 3 Two separate cells with single trivalents in 2:1 configuration. (a) Cell including trivalent 5, see Table I. (b) Cell including trivalent 6, see Table I. Time (T) is given on each frame in min relative to metaphase ($T = 0$). At metaphase, trivalents are shifted towards the lower pole (P_1) to which two kinetochores (arrows) are attached. The kinetochores are attached in opposite half-spindles. The kinetochores are not significantly different (see Table I). At anaphase (a, frame 07; b, frame 04), kinetochores separate, confirming that both chromosomes are trivalents. Bar, $10 \mu\text{m}$. $\times 1,290$.

ship exists between traction force and kinetochore fiber length, assuming that the measured kinetochore-to-pole distances correspond to the lengths of the kinetochore fibers (see below), and assuming, as Östergren (3) proposed, that the stable position of fully congressed chromosomes reflects isometric forces on the chromosome.

Since for these trivalents the summed lengths of the two shorter kinetochore fibers in one half-spindle usually do not equal exactly the length of the single kinetochore fiber in the opposite half-spindle, the possibility that the force-length relationship is not strictly linear must be considered. Therefore, in addition to comparing the summed kinetochore fiber lengths of opposite half-spindles, we used an algorithm to calculate the power to which these measured kinetochore-to-pole distances must be raised in order to make the difference between them exactly equal to zero. The value of the exponent relates how the magnitude of force along a kinetochore fiber varies with fiber length. If the force-length relationship is linear, then we expect the exponent value to equal 1.

In our algorithm, the force (F) applied to a kinetochore is assumed to be proportional to the kinetochore fiber length (L) raised to the power exp in the relation: $F = kL^{exp}$, where k represents a length-independent factor which in the following analysis is assumed to be the same for all kinetochores. If the metaphase position of a chromosome represents the position at which forces in opposite directions on a chromosome are exactly balanced, the resultant force (RF) will equal zero. For example, see the trivalent of Figs. 1 and 2, where $RF = 0 = A_1^{exp} + B_1^{exp} - A_2^{exp}$ (A_1 and B_1 are the kinetochore fibers to pole 1, A_2 is the kinetochore fiber to pole 2). The exponent values were derived from computer-generated plots, such as those shown in Fig. 4a and b. For six of the seven trivalents analyzed, a linear relation between force and kinetochore fiber length is indicated by the fact that $RF = 0$ when the exponent is approximately 1. The mean exponent for these six chromo-

somes (excluding trivalent 2) is 1.06 ± 0.18 (see Table II); trivalent 2 was anomalous and gave an exponent value of 3.15 (see Discussion).

We evaluated the significance of our calculated exponent values by asking how great a difference would occur in the equilibrium chromosome position for different exponent values. That is, for exponents that did not yield $RF = 0$ for the observed position of the chromosome, how much would the position of the chromosome need to be shifted for RF to equal 0. If such an analysis showed that shifts of chromosome position as small as $0.25 \mu\text{m}$ would allow exponents much greater or less than 1 to yield an $RF = 0$, then the significance of the exponent 1 would be questionable since our measurements from the ciné films could not detect such small changes with accuracy. For values of the exponent between 0.1 and 4, we determined the distance, delta (Δ), that a multivalent would have had to be displaced from its observed equilibrium position between the spindle poles to achieve an $RF = 0$. This analysis supports our conclusion that an exponent value of 1 best fits the force-balance data since a $1\text{-}\mu\text{m}$ or greater shift in the observed metaphase chromosome position is necessary to yield exponent values greater or less than 1 ± 0.4 (see Fig. 2).

A source of error in interpreting our results stems from the possibility that the kinetochore fiber ends at some distance from the pole (15, 16). For example, mechanical anchorage of a kinetochore fiber may occur at the periphery of the centrosome complex, closer to the kinetochore than we assumed by considering the centriole complex to be the terminus of kinetochore fibers. If so, our measurement of kinetochore-to-pole distances overestimates the true lengths of the kinetochore fibers. To estimate the possible contribution of such error, the measured kinetochore-to-pole distances were shortened by an amount approximately equal to the radius of the pericentriolar material ($0.5\text{--}1.0 \mu\text{m}$) and these new lengths were used to determine the exponent values. Only small differences were

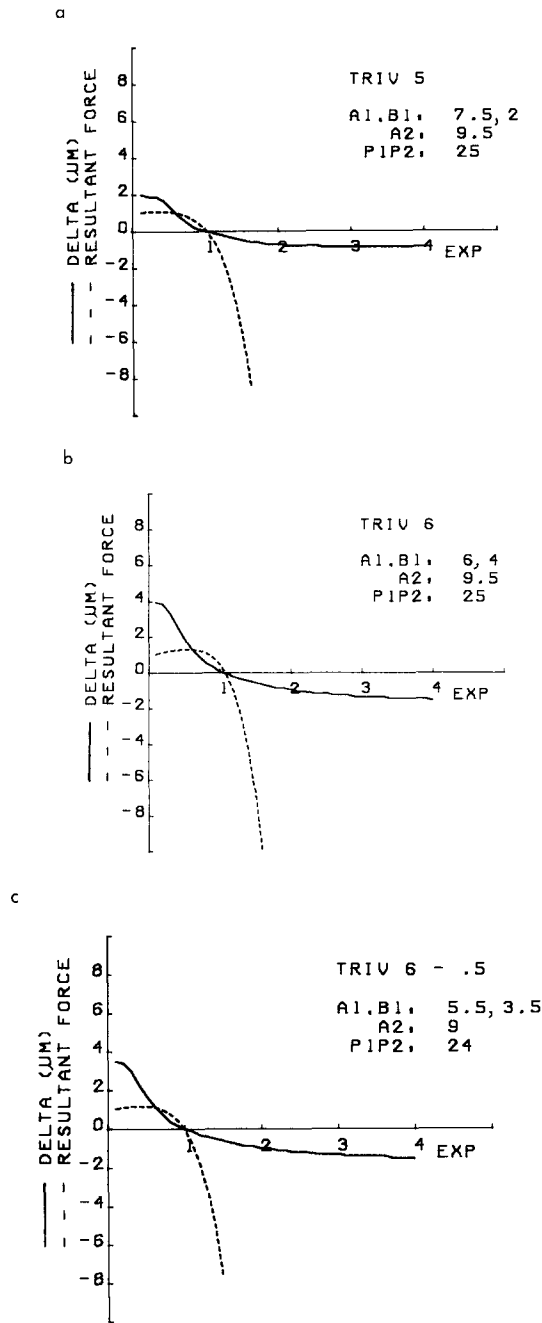


FIGURE 4 The analysis of the force balance, for (a) trivalent 5, cf. Fig. 3 a and Table I and (b) trivalent 6, cf. Fig. 3 b and Table I. Kinetochores traction force to opposite poles is balanced at the exponent value (exp) = 1.05 for both trivalents. (c) Hypothetical shortening of the measured kinetochores-to-pole distances has little effect on the exponent value.

noted between the mean exponent value derived from the measured distances (1.06 ± 0.18) and those values calculated from the hypothetically shortened distances: 0.98 ± 0.18 when shortened by $0.5 \mu\text{m}$, 0.90 ± 0.17 when shortened by $1.0 \mu\text{m}$ (see also Fig. 4 c and Table II). It must, of course, be borne in mind that any overestimation of the true kinetochores fiber lengths by our distance measurements is counteracted by spindle geometry. The spindle at the metaphase plate is elliptical in cross section due to flattening during the culture preparation. The major and minor axes of this ellipse are represented by the width and thickness of the spindle. The kinetochores-to-

pole distance measurements are accurate for kinetochores in the same optical focal plane as the spindle interpolar axis. For kinetochores on the periphery of the spindle, above or below this interpolar axis, our measurements will represent a projection of the true kinetochores-to-pole distance. Taking an average pole-to-pole spindle length of $26 \mu\text{m}$ and a $5\text{-}\mu\text{m}$ radius for the minor axis of the ellipse (17), we have calculated that our kinetochores fiber length values underestimate the correct values by $0.5 \mu\text{m}$ or less.

Quadrivalents

Symmetric orientations of quadrivalents (kinetochores oriented two to each pole) are apparently more stable than asymmetric ones (18-21). Seven of the eight experimentally generated quadrivalents had symmetrical 2:2 orientations, as shown in the example of Fig. 6. In this situation, with equal numbers of kinetochores oriented to opposite poles, the congressed quadrivalents lie on the spindle equator, on a line with normal bivalents. The length of individual kinetochores fibers within a half-spindle can be significantly unequal because of the geometry of the quadrivalent chromosome as, for example, in Fig. 6. Yet for each quadrivalent the sums of kinetochores fiber lengths in opposite half-spindles are not significantly different ($P = 0.05$) and support a linear force-length proportionality (Table I). However, the data obtained from symmetrically oriented multivalents do not yield *unique* solutions for the exponent (exp) in our computer algorithm.

One of the eight experimentally produced quadrivalents recorded was asymmetrically oriented in a 2:1:1 fashion. In this configuration the fourth kinetochores pair was separated from the other kinetochores by an extended chromatin arm ($\sim 12 \mu\text{m}$ long), and appeared to behave independently, congressing to

TABLE II
Comparison of the Exponent Value "exp" in the Unique Solution of $\Sigma F = \Sigma kl^{exp} = 0$ for Asymmetrically Oriented Multivalents

Multivalent no.	L^*	$L - 0.5 \mu\text{m}$	$L - 1.0 \mu\text{m}$
Triv 1	1.00	0.95	0.85
Triv 2	3.15	2.95	2.75
Triv 3	0.85	0.85	0.75
Triv 4	0.95	0.85	0.85
Triv 5	1.05	0.85	0.75
Triv 6	1.05	1.05	0.95
Triv 7	1.45	1.35	1.25
Mean	1.36	1.22	1.16
SD	0.74	0.73	0.66
$t = 1.19$ (d.f. = 6, $P > 0.05$)			
Excluding triv 2:‡			
Mean	1.06	0.98	0.90
SD	0.18	0.18	0.17
$t = 0.80$ (d.f. = 5, $P > 0.05$)			
Quad 1 (3:1)	1.15	1.05	0.95
Quad 2 (2:1:1)	1.45	1.35	1.35

* Exponent values are given for the solution to $\Sigma F = \Sigma kl^{exp} = 0$ using the actual measured, kinetochores-to-pole distances (L), and hypothetical lengths $L - 0.5 \mu\text{m}$ and $L - 1.0 \mu\text{m}$.

‡ The mean and standard deviation are recalculated excluding the values obtained for trivalent 2, whose behavior is anomalous (see Discussion).

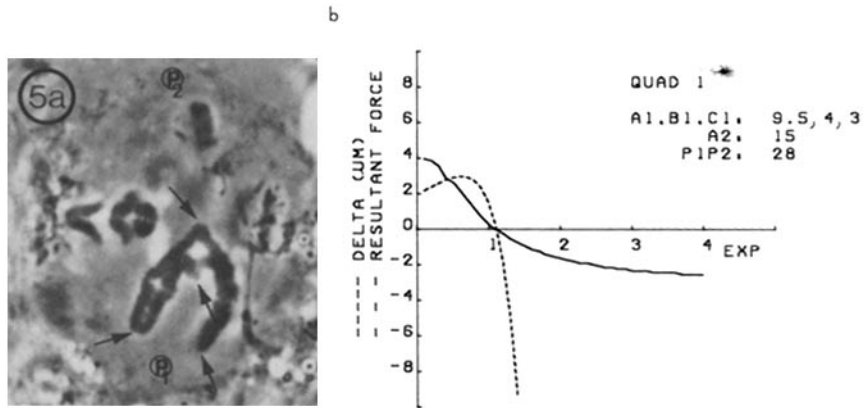


FIGURE 5 (a) Naturally occurring 3:1 quadrivalent (unpublished figure courtesy of Wise and Rickards). Kinetochores are marked by arrows. At metaphase, the quadrivalent is shifted towards pole 1. Bar, 10 μm . $\times 1,290$. (b) Analysis of quadrivalent position. The kinetochores-to-pole distances (A_1 , B_1 , C_1 , A_2) and inter-pole distance (P_1P_2) are given in the upper right hand corner of the graph. The solution of $F = A_1^{\text{exp}} + B_1^{\text{exp}} + C_1^{\text{exp}} - A_2^{\text{exp}} = 0$ (see Fig. 2) yields an exponent (exp) = 1.15.

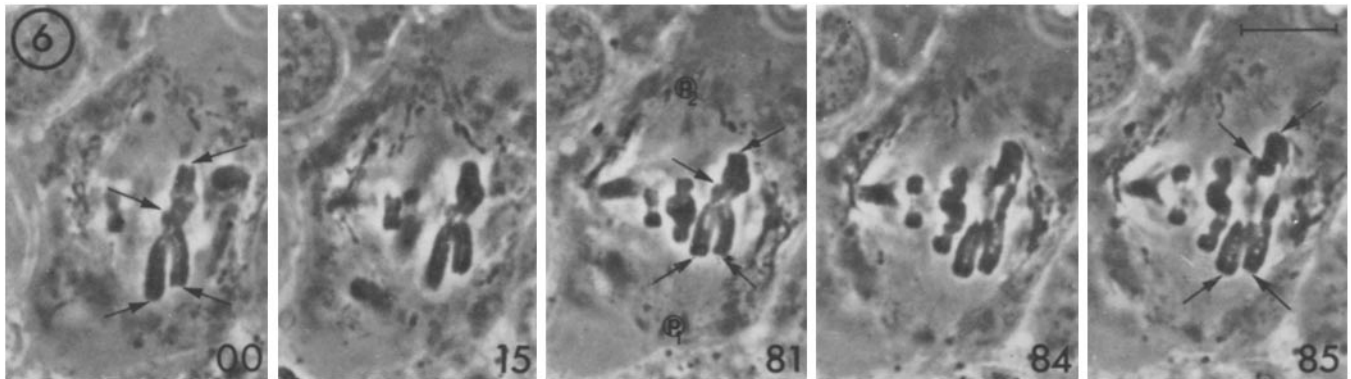


FIGURE 6 Time-lapse series of a quadrivalent in a balanced 2:2 orientation. Two kinetochores are oriented towards each pole (arrows). Time (T) in min is given on each frame. At metaphase ($T = 81$), when all bivalents lie on the equator, the balanced quadrivalent is also at the equator. Bar, 10 μm . $\times 1,290$.

the spindle equator with the normal bivalents, so that equal lengths of kinetochore fibers extended to opposite poles. Therefore, we considered the remaining three kinetochore pairs as members of a trivalent and determined that an exponent of 1.45 was required to yield an $\text{RF} = 0$ on the chromosome in its observed position. This exponent value represents a deviation from linearity. An explanation for this exception may lie in the structure of the chromatin joining the kinetochores. That is, despite the apparent independent behavior of the fourth kinetochore, the extended and folded chromatin arm connecting it to the rest of the multivalent may have presented structural constraints on the position of the rest of the chromosome.

We have also included in our analysis a naturally occurring quadrivalent described earlier by Wise and Rickards (22). Fig. 5 depicts this natural quadrivalent in the observed asymmetrical 3:1 orientation. Three chromosomes are oriented to the lower pole and the fourth is oriented to the upper pole. As noted by the authors, the chromosome is shifted off the spindle equator and closer to the pole to which three chromosomes are oriented. The extent of shift is consistent with a linear force-length relationship as indicated by the graph resulting from the computer analysis shown in Fig. 5b. Both the resultant force plot and the delta plot pass through zero at the exponent value of 1.15. Shortening all the measured kinetochores-to-pole distances by 0.5 or 1.0 μm had little effect on the computed exponent value (Table II).

Univalents

Occasionally, irradiation of the spermatocytes produced univalents. Such univalents, much like the X-chromosome (7,

15, 23), did not congress but continued to course back and forth between spindle poles. At the onset of anaphase, the univalent remained in the half-spindle in which it happened to lie. Thus, with unidirectional force applied to it, the chromosome does not achieve a stable equilibrium position. Fig. 7 shows one such trip of an induced univalent to one pole where the univalent reorients and then proceeds towards the opposite pole.

DISCUSSION

Our observations confirm some earlier observations that asymmetrically oriented multivalents are displaced off the equator (3, 18, 24–26) and strongly support Östergren's hypothesis that the position of chromosomes at metaphase is determined by a balancing of traction forces on opposing kinetochores (3–5). Moreover, our results show that the magnitude of traction force on a kinetochore fiber is a linear function of fiber length, as proposed by Luykx (27). This balance of forces is demonstrated most dramatically by the stable metaphase positions of asymmetrically oriented multivalents. These multivalents lie closer to the pole to which the greater number of kinetochore fibers, and therefore force components, are directed. The experiments reported here, like the studies of Bauer et al. (26) and Wise and Rickards (22), meet two important criteria (12): (a) by using newly arisen (i.e., experimentally induced) multivalents there is no need to consider evolutionary modifications of kinetochore activity such as might be involved in establishing stable multivalents in the genome (as is necessary, for example, in considering sex multivalents [28–30]), and (b) we can determine precisely the final metaphase position of these chromo-

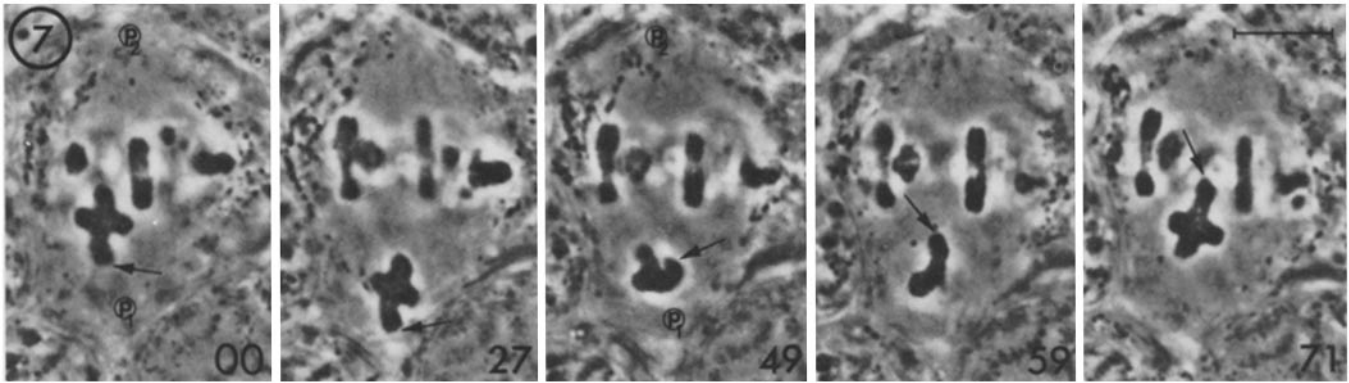


FIGURE 7 The simplest case of unbalanced forces—a univalent. Time in min is given on each frame. Irradiation has deleted one of the kinetochore pairs of a bivalent chromosome. The univalent migrates to the lower pole ($T = 0, 27$), reorients at $T = 49$, and migrates towards the upper pole ($T = 59, 71$) (arrows). The univalent never congresses. The behavior resembles that of the sex univalent X-chromosome. Bar, 10 μm . $\times 1,290$.

somes because we have followed the multivalents in living cells from late prometaphase into anaphase.

Our results do not agree with a mechanism of congression based on achieving equal lengths of all kinetochore fibers (7, 9). The shifted, asymmetrically oriented multivalents have great differences in the lengths of their individual kinetochore fibers (Fig. 3 *a* and *b*; Table I). Similarly, symmetrically oriented quadrivalents, though lying at the spindle equator, have different kinetochore-fiber lengths that depend on the geometry of the multivalent (i.e., inequality in the lengths of the chromatid arms that separate the kinetochore pairs, see Fig. 6).

Evidence that the position of chromosomes at metaphase is determined by antagonistic pulling forces on opposing kinetochores is reported by Izutsu (31). He found at metaphase that irradiating one kinetochore on a meiotic bivalent or on the adjacent kinetochore fiber caused the bivalent to move toward the pole to which its unirradiated kinetochore was attached. McNeil and Berns (32) observed similar results for mitotic chromosomes in PtK₂ cells in culture. In addition, electron microscopy showed that the kinetochore fiber had lost its attachment to the irradiated kinetochore. Thus, severing one kinetochore fiber allowed the antagonistic fiber to pull the chromosome poleward. The remaining chromosomes on the spindle were unaffected by the irradiation and moved to the metaphase plate. Our observations on univalents, chromosomes that have a single kinetochore fiber and move directly towards the attached pole, are also consistent with such behavior.

If the final metaphase position of a chromosome reflects a balance of forces on the chromosome, the relationship between traction force and kinetochore fiber length calculated for asymmetrically oriented multivalents gives a unique solution to the computer algorithm we used. Assuming that all kinetochores are functionally equivalent (have the same k value), we determined that the exponent to which the measured kinetochore-to-pole distances must be raised to yield an RF = 0 is 1. We conclude that the traction force generated along a kinetochore fiber increases linearly with length.

We attribute the linear proportionality between force and kinetochore-to-pole distance to the presence of the array of kinetochore-fiber microtubules spanning this distance. The kinetochore fiber forms a mechanical link between the chromosome and the polar complex (13, 33, 34). While all microtubules of the kinetochore fiber which terminate at the kinetochore may not run continuously to the spindle pole, a significant number do (35, 36). The simplest way to establish a linear

force-length mechanism is to distribute force-producers uniformly along the kinetochore fiber. Such forces on the chromosomes could result from cross-bridging between microtubules and microfilaments or between microtubules and the cytoplasmic matrix (6, 37–43). The magnitude of force would increase with the number of cross-bridges, which would be directly proportional to the length of the kinetochore fiber.

Alternatively, forces may be generated by the depolymerization of kinetochore microtubules, as suggested by Inoué and Sato (8; see also reference 10). The simplest interpretation of our data with this alternative would require that depolymerization occur along the entire length of the microtubules, with the rate of subunit loss per unit length being constant; total disassembly would then be greater for longer microtubules. The off-equator position of trivalents would be the position at which the amount of disassembly occurring in opposite half-spindles is equal. The plausibility of this interpretation, however, is tempered by indirect evidence *in vivo* (44, 45) and *in vitro* (46–48) that suggests that depolymerization occurs only at the ends of microtubules or spindle fibers. However, a mechanism of force production that generates forces only at the ends of the kinetochore fibers and does not sense the length of the fiber would not be consistent with our results. Such a mechanism would not achieve a balance of forces on a trivalent chromosome: instead of reaching a stable metaphase position, the trivalent would move directly to the pole with the greater number of attached kinetochore fibers.

In the relation for traction force, $F = kL^{\text{exp}}$, k represents the length-independent factor(s) that determine the magnitude of traction force applied to the kinetochore. Our assumption that k is the same for all multivalent kinetochores is justified by two results. First, for most multivalents, both symmetrically and asymmetrically oriented, the sum of the lengths of the kinetochore fibers in one half-spindle equals, within 10%, the sum of the lengths in the opposite half-spindle (Table I). Secondly, with the exception of trivalent 2 (Table II), the standard deviation of the calculated exponent values ($\text{exp} = 1.06 \pm 0.18$) for the trivalents is small. If kinetochores typically had widely different values of k , then no single force-length relationship (exponent value) would explain the observed positions of the majority of the multivalents.

The length-independent factor(s), k , may be directly related to the number of functional kinetochore microtubules. In this case, the traction force produced along a kinetochore fiber would not be simply a linear function of its length but a

function of both length and number of kinetochore microtubules (i.e., total microtubule length). Consequently, we would expect a trivalent to congress to a position where the total length of kinetochore *microtubules* (not lengths of kinetochore *fibers*) will be equal in opposite half-spindles (Fig. 8, trivalent *B*). If the number of microtubules on different kinetochores varied widely, the ultimate positions of asymmetrically oriented multivalents would show little relationship from example to example. Instead, we usually observed that the sums of kinetochore *fiber* lengths in opposite half-spindles were equal (Fig. 8, trivalent *A*). Only if all kinetochores have equal numbers of attached microtubules can both the total microtubule length and fiber length be equal in opposite half-spindles. Therefore, the observed positions of the multivalents in our study suggest that the majority of autosomes have about the same number of functional kinetochore microtubules.

Traction force, however, cannot depend only on the number of microtubules attached to a kinetochore independent of the length of the fiber. The chromosome would always move all the way to the pole towards which the greater number of kinetochore microtubules was oriented.

We have no ultrastructural information on the numbers of kinetochore microtubules attached to multivalents of *Melanoplus*, but ultrastructural analysis of several other cells has shown that the number of microtubules per kinetochore is relatively constant for a given cell type (35, 49–52). Moens (50) compared the number of kinetochore microtubules between different autosomes of primary spermatocytes from the grasshopper *Locusta*. Like the *Melanoplus* spermatocytes used in our study, *Locusta* spermatocytes contain 11 bivalents and one univalent sex chromosome. From the three metaphase I *Locusta* cells analyzed by Moens, we computed an average of 22 ± 5 microtubules per kinetochore. The low SD indicates a high degree of uniformity. However, an occasional kinetochore had substantially more or fewer microtubules than the average—as many as 35 or as few as 10.

In light of these data, consider the exceptional trivalent 2 observed in our study. Its congressed position was not consistent with a linear force-length relationship if we assumed that all three kinetochores had the same length-independent con-

stant, k . The simplest explanation would be that one kinetochore of the trivalent had a significantly different value of k . Relative values of k for each kinetochore of the trivalent can be calculated from the observed kinetochore-fiber lengths at congression (Table II), using a linear force-length relation in the force-balance equation. The value of k for the single fiber (10 μm long, extending to pole 2, Table I) could be 1.6 times greater than the value for k for the pair of fibers in the opposite half-spindle (both 8.0 μm long, extending to pole 1, Table I), or the value of k of one of the pair of fibers could be one-fourth the value of the other two fibers. It is possible that irradiation may have damaged a kinetochore in trivalent 2, thus causing a change in its normal k value. On the other hand, if k is related to the number of functional microtubules, perhaps one kinetochore in trivalent 2 had substantially more or fewer microtubules than the others. Since some kinetochores on *Locusta* chromosomes appear to have exceptionally high or low numbers of microtubules naturally, a difference in k values as would explain the position of trivalent 2 would be expected to occur now and then. Translocations between chromosomes with kinetochores of similar k values, however, would be more likely.

An important implication of our results is that, if balanced traction forces determine the position of metaphase chromosomes, traction forces may directly regulate the extent of microtubule assembly, hence the length of kinetochore fibers. Some earlier investigations also suggest force-balance regulation of microtubule assembly. Inoué (53) showed that *Chaetopterus* metaphase spindles elongated in response to interpolar tension produced by flattening the oocyte between slide and cover slip. Nicklas (12, and unpublished data) observed that chromosomes move from their congressed position in grasshopper spermatocytes when tension is applied to a meiotic bivalent by stretching a chromosome arm towards one pole with a microneedle. The kinetochores remain mechanically anchored to their poles by their kinetochore fibers, but the kinetochore fiber under tension slowly elongated while the other kinetochore fiber shortened. After tension on the stretched chromosome was released, the bivalent moved back to the metaphase plate at the normal congression velocity. These results demonstrate that the position of the chromosomes under artificially produced tension was determined by the balance of applied forces. The length of the kinetochore fibers changed in response to the applied forces. It should be noted, however, that, although these data demonstrate that chromosomes move when there is an imbalance of poleward-directed forces, the velocity at which they move is largely independent of the magnitude of the imbalance. The rate at which kinetochore fibers change length is limited by the rates at which microtubules can assemble and/or disassemble (12, 54).

In summary, our results strengthen the view that congression of chromosomes takes place as the result of a balancing of countervailing linear forces on the chromosomes. We also have suggestive evidence that this balance of forces regulates the extent of assembly of kinetochore-fiber microtubules.

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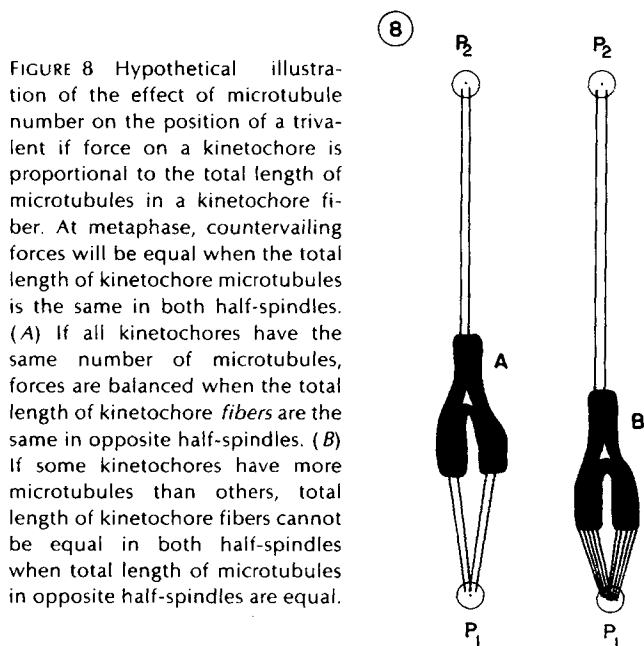


FIGURE 8 Hypothetical illustration of the effect of microtubule number on the position of a trivalent if force on a kinetochore is proportional to the total length of microtubules in a kinetochore fiber. At metaphase, countervailing forces will be equal when the total length of kinetochore microtubules is the same in both half-spindles. (A) If all kinetochores have the same number of microtubules, forces are balanced when the total length of kinetochore *fibers* are the same in opposite half-spindles. (B) If some kinetochores have more microtubules than others, total length of kinetochore fibers cannot be equal in both half-spindles when total length of microtubules in opposite half-spindles are equal.

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