CHROMOSOME VELOCITY DURING MITOSIS AS A FUNCTION OF CHROMOSOME SIZE AND POSITION*

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ABSTRACT

Chromosome velocity has been studied in living *Melanoplus differentialis* spermatocytes by phase contrast cinemicrography. *Melanoplus* chromosomes (and bivalents) differ in length by as much as 1:3.5. As expected, no size-dependent velocity differences were detected in anaphase, and this is also shown to be true for the less predictable movements during prometaphase congression. The size of the X chromosome can change during observation following x-irradiation, but this is equally without influence on velocity. However, an effect of position on velocity is found in both prometaphase and in anaphase: the chromosomes furthest from the central interpolar axis move 25 per cent faster than more central chromosomes. A simple mechanical model relating frictional resistance and mitotic forces to chromosome velocity is discussed in detail. Calculations from the model suggest that a significant difference in the force acting on a large, as compared with a small chromosome is necessary to account for the observed similarity in velocity. Therefore, it is concluded that the mitotic forces are so organized or regulated that velocity is, within limits, independent of load. The implications of velocity-load independence in relation to the molecular origin of mitotic forces are discussed.

INTRODUCTION

Mitotic events can be analyzed in terms of mechanical and molecular components. The mechanical problem is to describe quantitatively the relationship between the mitotic forces and the cellular mechanical properties governing the response to these forces. This problem must be solved, at least in part, before a final solution to the difficult molecular problems can be attained—problems such as the molecular basis of mitotic force production. On the other hand, a complete solution of the mechanical problem is possible without specification of any particular molecular theory, and therefore the mechanical inquiry is independent of present unsatisfactory speculations about the molecular origin of mitotic forces.

The present investigation is part of a systematic study of chromosome mechanics in which chromosome movement is viewed as a special case of the general mechanics of motion. In general mechanics, the physical properties governing the response to the applied force are identified as mass, elasticity, and frictional resistance. But in the chromosomal situation these mechanical properties are not of equal importance; indeed, the especially striking movements in prometaphase and in mid-anaphase are probably determined by the frictional resistance alone. This assertion and the following argument are defended in the discussion below; they are introduced here since they provide the rationale for this study. The argument is: (1) Chromosome velocity is a linear function of a frictional coefficient and the mitotic forces. (2) The frictional coefficient is significantly

* This paper is dedicated to Professor Hans Bauer on the occasion of his sixtieth birthday.

greater for large, as compared with small chromosomes, and this increase can be approximately calculated. (3) Hence velocity as a function of load (resistance) can be obtained by measuring the velocity of chromosomes differing in size. Furthermore, by relation (1) velocity as a function of load provides an indirect, but quantitative measure of the mitotic forces acting on chromosomes which differ in resistance to motion. A unique relationship between velocity and load has already been suggested from the indirect analysis of a different chromosomal situation (19), and the present study provides additional evidence for comparison with the earlier interpretation. The significance of the velocity-load relationship for research on the molecular basis of mitotic force production is explored in the Discussion.

It has long been known that size is without influence on velocity during anaphase, and at least one researcher has recognized that this might be significant (17). However, this relationship has not previously been examined for the equally important congression movements in prometaphase. Prometaphase and anaphase movements are similar mechanically (see Discussion), but only in prometaphase is the movement of an individual chromosome so independent of the movement of other chromosomes (Dietz, 7, and this report). Hence it was thought that an effect of size was possible in prometaphase even though absent in anaphase. In addition to this work on chromosomes differing naturally in size, experimentally induced size changes were also studied, since this permitted determination of the effect of relatively sudden size change on the velocity of one chromosome. The influence of lateral position (*i.e.*, the distance of a chromosome from the central interpolar axis) has not been studied previously, and it was studied at first to eliminate a possible complicating variable. It was soon seen, however, that the influence of position is interesting in itself, and it was made the object of a special study.

MATERIAL AND METHODS

The grasshopper *Melanoplus differentialis* (Acrididae; Locustinae) from a laboratory colony (18) was used in these studies. Living spermatocytes were cultured by methods previously described (19). The cells examined were normal in regard to the general pattern of chromosome movement, spindle size and shape, and appearance of mitochondria, for at least 1 hour after completion of data taking, and most of them lived much longer. Thus two of the cells used for observations on prometaphase and anaphase I went through anaphase II 6 to 7 hours after anaphase I. Culture temperature was controlled to within ± 0.7°C for any one cell. The over-all temperature range was 25.5 to 28.0°C.

The techniques of general observation, recording, and analysis have been previously described (19). Briefly, chromosome behavior was studied by phase contrast microscopy using a Zeiss Jena oil immersion objective of 1.25 numerical aperture. The recording was accomplished by time-lapse cinemicrography at rates of from two to thirty frames per minute. Chromosome or bivalent position was measured in the projected film with reference to an equatorial line drawn midway between the poles. In the studies on bivalent position during prometaphase, the middle of the bivalent served as the chromosomal reference point for position measurement, while in all other cases the kinetochore was used as the reference point. Length changes in *Melanoplus* prometaphase bivalents cannot be detected, and hence the midpoint of the bivalent is as good a reference point as any other. Position was measured along the actual path of motion, which is generally straight for chromosomes lying near the central interpolar axis, but curved for those near the junction of spindle and cytoplasm. The determination of pole position and the accuracy of this measurement has been previously described (19). Spindle length (distance between the poles) and spindle thickness were also measured, the latter by direct measurement during observation of the cell. These data were then plotted against time, and chromosomal velocities were determined from the plotted data. Chromosome size and shape were determined on the projected image of the film.

Chromosome position with respect to the central interpolar axis was not determined exactly; the chromosomes were simply classified into two groups: "peripheral" and "central." Peripheral chromosomes are those lying within 3 μ of the edge of the spindle as indicated by the mitochondrial sheath; all other chromosomes are "central," and lie within 5 μ of the central interpolar axis. This classification depends on knowing the vertical as well as the lateral position of a given chromosome. For example, at the upper and lower focal levels of the spindle, *all* the chromosomes in focus are within 3 μ of the spindle edge and are, therefore, peripheral. In the studies on prometaphase, the focus was shifted between each exposure (2 per minute) to a different level, and thus repeated series of the upper, middle (near the plane of the central interpolar axis), and lower focal levels were obtained. This makes possible not only the above classification, but also permits detection of vertical shifts in chromosome position. This tedious recording technique has the further advantage that the movement of nearly every chromosome in the cell

can be followed, avoiding bias in the results. Anaphase movement, however, is so uniform that this elaborate technique is unnecessary, and a focal level near the plane of the central interpolar axis was chosen in each cell, and the peripheral and central chromosomes in that plane were followed throughout anaphase.

X-irradiation was used to produce experimental alterations in chromosome size. The dosage was 500 r delivered to the whole animal 1½ to 5 days before culturing the spermatocytes. Details of the x-irradiation procedure and general observations on cultures of x-irradiated cells will be found in Nicklas (19).

RESULTS

Normal Prometaphase I

It is necessary to begin with information on bivalent size and shape needed for the interpretation of the velocity data. This is followed first by general, and then quantitative, descriptions of prometaphase movements. Forty-two bivalents from four living cells constitute the material for the following analysis.

The meiotic chromosome complement of *Melanoplus* is readily divided into four size classes, presented in Table I. The total length of the bivalent was measured even when part of an arm was curved due to the presence of proximal chiasmata.

Bivalents with the same length of chromosomal material differ greatly in shape or orientation relative to the direction of motion, due to differences in the positions of chiasmata. Three orientation-shape classes were recognized: Class I: chiasma absent or terminalized—the bivalent approximates a cylinder moving parallel to its long axis. Class II: chiasma near the kinetochore—the bivalent approximates a cylinder moving perpendicular to its long axis. Class III: chiasma near the middle of the bivalent—the bivalent is cross-shaped. It is shown below that theory predicts only relatively small differences in the frictional resistance for the two extremes (classes I and II). For reference, however, the orientation-shape classes for the bivalents examined are given in Table II. It must be noted that these considerations do not apply to anaphase, in which all the chromosomes lie with their long axes parallel to the direction of movement.

Prometaphase movements will now be generally described as background for more specialized treatment and also for comparison with the fine studies of Dietz (7) and Bauer, Dietz, and Röbblelen (3) on prometaphase in crane flies.

TABLE I

Size (in Microns) of Melanoplus differentialis Bivalents

(Based on 42 bivalents from 4 living prometaphase cells)

Size class	Number per cell	Average width × total length	Range of length
Large	2	2.5 × 12	11–13
Large medium	4	2 × 8	7–9.5
Small medium	3	2 × 5.75	5–6.5
Small	2	2 × 3	2.5–4

TABLE II

Bivalent Orientation or Shape Relative to Direction of Movement

(See text)

Size class	Bivalent orientation-shape class		
	I	II	III
Large	1	3	4
Large medium	7	7	2
Small medium	4	3	3
Small	7	0	1
Totals	19	13	10

Prometaphase in *Melanoplus*, as in the crane flies, is characterized by movements of the initially scattered bivalents parallel to the spindle interpolar axis. These movements have only statistical regularity, but center more and more precisely on the equatorial region as prometaphase proceeds. The number and duration of these movements decreases rapidly, and after the first third of prometaphase, the bivalents generally lie within 3 μ of their final prometaphase position. These movements and also the continuing, interpolar movements of the univalent X chromosome (see 18) are shown for a typical cell in Fig. 1. During the latter part of prometaphase there are long periods of no or very slow movement. Eventually, the centers of all bivalents lie within 1 to 2 μ of the equator. The lack of lateral or vertical movements is very striking; only three of the 42 bivalents showed lateral movements greater than 1 μ, and none of these exceeded 3 μ. Vertical shifts cannot be precisely measured, but shifts of 3 μ should be

easily detectable; only six of the bivalents showed such shifts. Thus we are dealing with movements as precisely one-dimensional and interpolar, as the anaphase movements generally are (ignoring the curvature of path of the more peripheral bivalents or chromosomes). The motion of individual prometaphase bivalents in *Melanoplus* is without detectable effect on the motion, or immobility, of other—even of adjacent—bivalents, but the sex chromosomes in some crane flies occasionally provide a very interesting exception to this (Dietz, 7). Changes in kinetochore orientation after the first 15 minutes of prometaphase are very rare in *Melanoplus* bivalents; delayed orientation or re-orientation was observed only twice. The duration of prometaphase varied between 2 and 5 hours in the cells studied.

The studies of bivalent velocity as a function of size and position are based on average velocity during the first 90 minutes of prometaphase. This time period is somewhat arbitrary, but it was chosen to provide a sensitive measure of any velocity differences that might obtain when the bivalents are usually moving. Velocity differences would be obscured by inclusion of the latter half of prometaphase when most bivalents are stationary. The velocity is simply the total distance traveled in the first 90 minutes after spindle formation, divided by 90 minutes. These data are presented in Table III.

The first statistical analysis was performed on data lumped into two, rather than four, size classes to provide larger numbers of bivalents in each class. A standard analysis of variance procedure was employed. The null hypothesis of no difference between the four means was tested by the F ratio test; if this could be rejected at the 95 per cent significance level, then confidence limits for various pairs of means were calculated by t test statistics. The results are given in Tables IV and V. Table IV shows that the calculated F ratio (3.09) is greater than expected (2.82), and, therefore, the null hypothesis is rejected at the 95 per cent level. In Table V, the mean for all small–small medium bivalents is compared with that for all large–large medium bivalents, without regard to position, and then the means of central and peripheral bivalents are compared without regard to size. This procedure slightly biases the statistics, since the four original classes are not equal in size, but the bias is in the direction of accepting the hypothesis of no difference in the means.

The analysis given in Table V indicates that at the 95 per cent confidence level, no effect of size is demonstrable (since the mean $\pm$ the confidence limit overlaps zero), but there *is* evidence for an effect of position on velocity: peripheral bivalents move about 25 per cent faster, on the average, than bivalents nearer the center of the spindle.

The maximal effect of size should be revealed by analyzing only the two largest and two smallest bivalents from each cell. Only sixteen bivalents are involved in this analysis which was carried out as described above for the larger group; the results are presented in Tables VI and VII. The information in Table VI permits rejection of the null hypothesis at the 99.5 per cent significance level. The confidence limits in Table VII again provide no evidence for a size effect, but clearly indicate an effect of position on velocity at the 95 per cent confidence level.

Normal Anaphase I

The uniformity of anaphase movement makes the independence of velocity and chromosome size evident even in illustrations of fixed material published in the 1870's. In living spermatocytes of *Melanoplus* this is equally obvious. But in optical sections near the plane of the central spindle axis, in which both peripheral and central chromosomes can be seen, an effect of position on velocity is evident. A portion of the cinematographic record for such an optical section is shown in Fig. 2 and graphically displayed in Fig. 3. Evidently, the velocity of individual chromosomes (the slope of the kinetochore separation curve divided by two) is dependent on position, but when this effect is allowed for, chromosome size is without detectable influence on velocity. This is particularly clear in the cell shown, in which large (chromosome 2) and small (chromosome 3) chromosomes are found side by side; their velocities differ only slightly, while both are considerably slower than peripheral chromosomes (chromosomes 1 and 5). The length of the spindle is plotted to demonstrate that spindle elongation is without significance for chromosome movement during the first half of anaphase; this is also true for the additional cells considered below. It should be emphasized that actual velocities are determined from Fig. 3. That is, kinetochore separation was measured along the actual path traversed; for peripheral chromosomes, the alternative approach of measuring the straight-line distance between kinetochores would give an artificially low value, since they travel a curved path. Mean velocities for the first half of anaphase

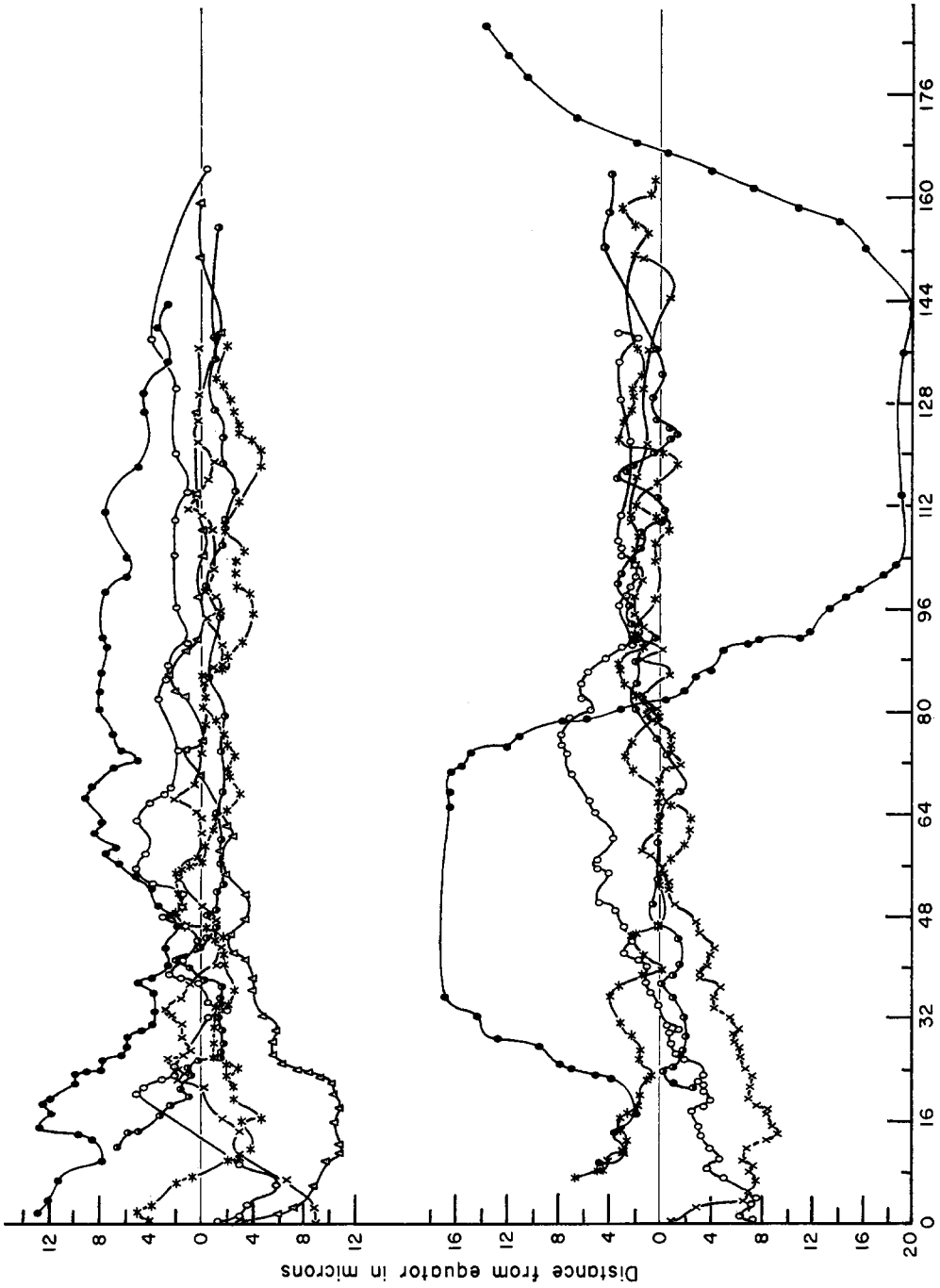

FIGURE 1 Graphical representation of bivalent and X chromosome movement in one prometaphase I cell. Two position scales are used to reduce overlapping. Ten bivalents (of a total of 11 present) are plotted; the X chromosome movements are plotted in the lower portion using closed circles. Only the first ⅔ of prometaphase is shown. The irregularity of *individual* movements of the bivalents, but their tendency to center more precisely on the equator as prometaphase proceeds is evident. The decrease in time spent in motion and speed of motion (slope of the curve) in later prometaphase is also shown.

TABLE III

Average Prometaphase Velocities

[(Micra per minute) × 10]

Size:	Large		Large medium		Small medium		Small	
Position:	Peripheral	Central	Peripheral	Central	Peripheral	Central	Peripheral	Central
Velocity of individual bivalents	3.44	2.45	3.63	2.56	3.77	3.53	4.11	4.14
	3.89	2.25	3.28	1.87	4.61	0.85	4.31	3.56
	4.00	2.06	2.83	3.77	2.58	3.04	4.08	2.34
	3.25		3.83	3.94	5.17	2.20		3.44
	2.89		3.08		2.83	1.67		2.72
			3.42		2.40	3.83		
			3.50					
			4.06					
			2.50					
			2.28					
Means	3.49	2.25	3.24	3.04	3.56	2.52	4.16	3.24

TABLE IV

F Ratio Test on the Velocity Data in Table III

(See text)

	Sum of squares	Degrees of freedom	Mean square	F ratio
Category means	6.20	3	2.07	$F = \frac{2.07}{0.670} = 3.09$
Within categories	25.45	38	0.670	$F_{0.95}(3, 38) = 2.82$*

* From Table A-7c, Dixon and Massey (8).

movement have been computed for a total of 16 chromosomes from this and two other cells. The average mean velocity for 6 peripheral chromosomes is 0.867 μ/minute; that for the 10 central chromosomes is 0.658 μ/minute. Thus, the peripheral chromosomes move about 29 per cent faster than the central chromosomes. This is essentially the same difference as that found in prometaphase.

Experimental Alteration in Chromosome Size

The behavior of X chromosomes with partial breaks induced by x-irradiation has been previously described (19). In that material, two cells were seen in which a small part of the chromosome containing the kinetochore moved independently of the rest of the chromosome. These size reductions are of particular interest here in that they

TABLE V

T Test Confidence Limits for Differences in the Means

Data from Table III (See text)

Group	Number measured	Mean (μ/min) × 10	Difference between means ± 97.5 per cent confidence limit*
Small–small medium	20	3.25	0.14 ± 0.50
Large–large medium	22	3.11	
Peripheral	24	3.49	0.71 ± 0.52
Central	18	2.78	

* This gives a 95 per cent confidence interval.

TABLE VI

F Ratio Test on Velocity of the Largest and Smallest Bivalents in Table III

(See text)

	Sum of squares	Degrees of freedom	Mean square	F ratio
Category means	6.3	3	2.10	$F = \frac{2.10}{0.258} = 8.14$
Within categories	3.1	12	0.258	$F_{0.995}(3, 12) = 7.23$*

* From Table A-7c, Dixon and Massey (8).

TABLE VII

T Test Confidence Limits for Differences in the Means

Largest and smallest bivalents in Table III (see text)

Group	Number measured	Mean (μ/min) $\times$ 10	Difference between means $\pm$ 97.5 per cent confidence limit
Small	8	3.59	0.56 $\pm$ 0.56
Large	8	3.03	
Peripheral	8	3.75	0.88 $\pm$ 0.56
Central	8	2.87	

show what happens to chromosome velocity on relatively sudden change in chromosome size. Furthermore, the magnitude of the length change is equal to, or greater than the naturally occurring length differences described above; the reduction is fourfold in cell 21-7 and threefold in cell 20-8.

The anaphase shown in Fig. 4 is from a cell in which X chromosome prometaphase movements were analyzed previously (19, see Figs. 1 and 2 and associated remarks). In this cell, the two parts of the X were joined together through all the prometaphase movements but separated at or before the start of anaphase, when the small fragment containing the kinetochore moved alone

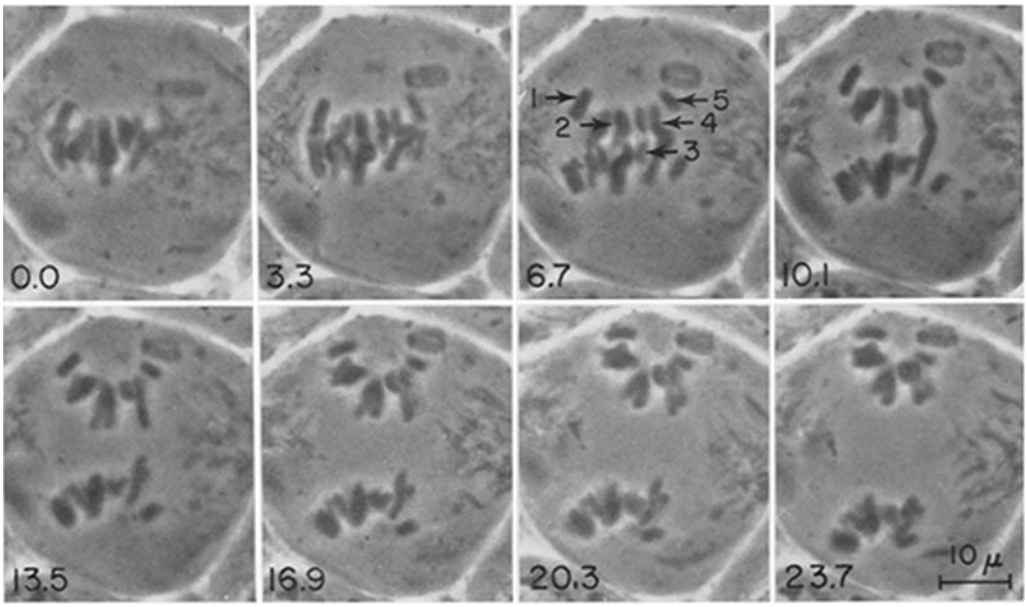

FIGURE 2 Prints from the cinematographic record of anaphase I in a *Melanoplus* spermatocyte. The chromosomes analyzed graphically in Fig. 3 are indicated by numbered arrows. It is apparent that the peripheral chromosomes (Nos. *1* and *5*) move more rapidly than the central chromosomes, particularly if allowance is made for the curved path of the peripheral chromosomes (see also Fig. 3). Time is indicated in minutes on each print.

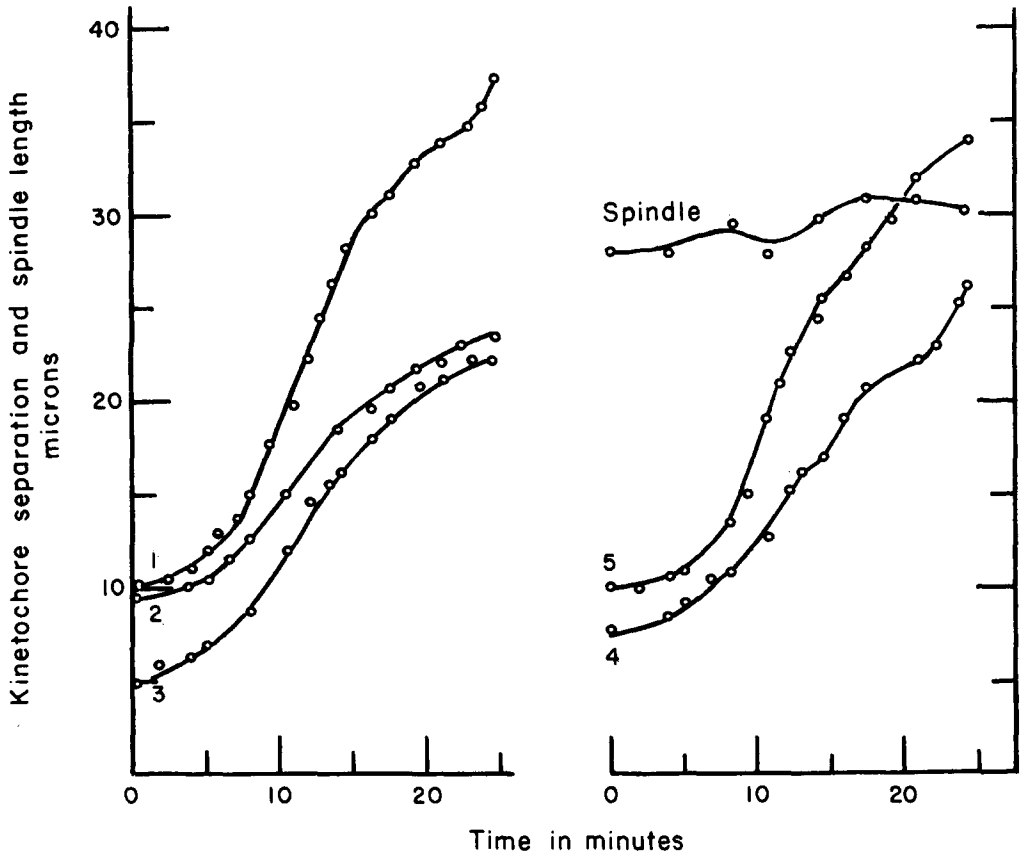

FIGURE 3 Graphical representation of spindle length and of chromosome movement for the five chromosomes identified by arrows in Fig. 2. Two separate graphs were prepared to avoid overlapping curves: the time scales of the right and left halves are identical. The speed of individual chromosomes is given by the slope of the kinetochore separation curve divided by two.

to the pole. A graph of the fragment's anaphase movement is shown in Fig. 5, from which an average velocity of 0.47 μ/minute is obtained. In Table VIII, this velocity is compared with that found during earlier movements of the whole X and with the velocity of two autosomes in anaphase. It is obvious from the table that the much shortened X moves no more rapidly than the whole X or the autosomes.

The decrease in X chromosome length in cell 20-8 was very sudden and was followed by restoration of the original length during one continuous movement. These events were the consequence of two interpolar movements occurring without a pause between them (*cf.* 18). The two parts of the X were separated at the end of the first movement (*cf.* 19), and the kinetochoric end of the X began to move back toward the pole from which it had just come before the trailing part of the X could rejoin the kinetochoric fragment (see Fig. 6). Hence, during the first part of the interpolar trip (0 to 7.3 minutes) the kinetochoric end was in free motion—the trailing portion of the X was not being pulled. After the kinetochoric end began to pass the trailing portion, the whole chromosome was again in motion. Thus, in this cell the effective length of the X chromosome was temporarily reduced. The position of the X kinetochore portion is plotted in Fig. 7, and in Table IX the velocities during this trip ("2") are compared with those in trips before ("1") and after ("3") this.

Close study of Figs. 6 and 7 will show that the decrease in velocity in the last half of trip 2 occurs 3 or 4 minutes *after* the whole X chromosome is being moved, and hence the change in effective length occurring between 7.3 and 10.4 minutes

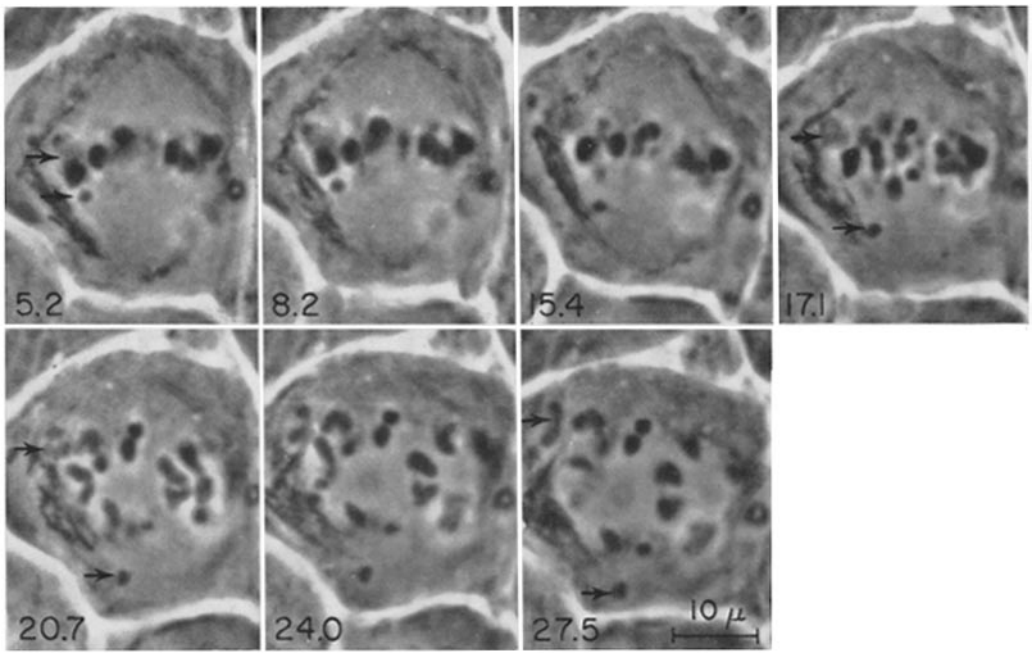

FIGURE 4 Prints from the cinematographic record of the anaphase movement of a small X chromosome fragment containing the kinetochore (lower arrows). The larger fragment, which does not move toward the pole, is indicated by the upper arrows. Cell 21-7 (*cf.* Fig. 5). The time in minutes is indicated on each print.

has no immediate influence on chromosome velocity. Furthermore, while the velocity in the early part of trip 2 is considerably higher than at other times in this cell, the velocity of 1.3 μ/minute is well within the normal range of variation for whole X chromosome velocity at this temperature (see Tables VIII above and Table 2 in reference 19).

DISCUSSION

Several conclusions can be drawn directly from the data presented:

1. Velocity is independent of chromosome size not only in anaphase, but also during the more independent movements in prometaphase. This supplies another mechanical similarity between prometaphase and anaphase. This is not the place to detail the evidence for the other similarities, but they are so important and so often ignored that a partial list follows. First, the movements are alike in direction—interpolar in both cases (7, this report). Secondly, the kinetochore is the point at which the mitotic forces act on the chromosome. This is well established in anaphase; for prometaphase, especially vivid evidence is furnished by prometaphase stretch (13) and by the behavior of the *Melanoplus* X chromosome (18). The importance of such basic mechanical information cannot be overestimated; for the point at which forces act on a body and the direction of motion resulting from these forces are the essence of the

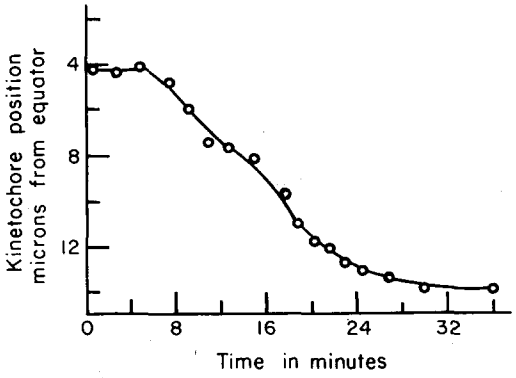

FIGURE 5 Graphical representation of the movement of the small fragment of the X chromosome in cell 21-7 (*cf.* Fig. 4).

mechanical characterization of motion and the basis for all subsequent considerations.

2. Velocity-size independence is presumably not due to inherited differences in the mitotic behavior of large *vs.* small chromosomes, since sudden experimental alterations in chromosome size have but little, if any, effect on velocity.

3. Position, on the other hand, does influence velocity. At first, it seemed surprising that the dependence of velocity on chromosome position with respect to the central interpolar axis had not been described previously. This could have been pointed out long ago from studies on anaphase in fixed material such as grasshopper spermatocytes, but an extensive search of the literature reveals no clear reference to such velocity differences. It should be mentioned that crescent-shaped chromosome distributions are seen in anaphases in salamander cells and in many other materials (see, *e.g.*, Wolf, 28, on the fly *Cloëon*). The resemblance is superficial, however, since in these other forms all chromosomes are peripheral in position and, therefore, the late anaphase appearance cannot be due to velocity differences between central and peripheral chromosomes.

A mechanical interpretation of the position effect in *Melanoplus* is possible on the basis of the model discussed below. Thus, the velocity differences could arise either from greater forces or lower viscosity at the periphery of the spindle as compared with the center. A decision between these alternatives cannot be made at present, but at least the choice is limited.

TABLE VIII

Average Chromosomal Velocities in Cell 21-7

Stage	Chromosome	Average velocity (μ/min.) × 10
Prometaphase	Whole X	5.5
		13.0
		12.5
		5.5
		5.0
Anaphase	X kinetochore fragment	4.7
	Autosome 1	7.0
	Autosome 2	12.0

Interpretation of the Independence of Chromosome Size and Velocity

A. The Model

The first step in the interpretation is to demonstrate that for size differences actually encountered, *significant* differences in the mitotic forces acting on large, as compared with small chromosomes must be postulated to account for the uniformity in velocity observed. This will eliminate the possibility that force differences are absent, but the resistance to movement for a large chromosome is only slightly greater than that for a small chromosome and, therefore, the velocity differences are not detectable. What is needed, then, is a minimum estimate of the force differences involved, and this will be obtained from a simple mechanical model. This is not difficult; it is the *justification* of the model that must be the major concern. The model describes chromosome motion at constant velocity and in the absence of visible changes in chromosome shape, and can immediately be applied to both prometaphase and mid-anaphase movements.

It is necessary first to consider which of the mechanical properties—mass, elasticity, and frictional resistance—are important determinants of the chromosomal response to the applied force. It is very easy to show simply by calculating an appropriate Reynold's number (see, *e.g.*, 23, pp. 6 to 9) that inertial forces are negligible compared with frictional forces. This has been pointed out by Jacques and Biesele (15) and also by Hughes and Swann (12), and therefore need not be labored here, particularly since the margin of error is so huge. Thus, the maximal Reynold's number is 10^{-6}, indicating that frictional forces are at least a million times as great as inertial forces. Furthermore, neither chromosomal nor spindle elasticity is important; chromosome elasticity because the chromosomes are not stretched, and spindle elasticity because only steady motion is considered. The latter point is important since cellular media *have* measurable elasticity (6). However, this will not affect steady motion, since the potential energy gain due to the deformation of spindle macromolecules as the chromosome moves is exactly balanced by the loss of potential energy in the area through which the chromosome has just passed (see, *e.g.*, Oldroyd, 20). Thus, mass and elasticity can be ignored, and the task is to relate frictional resistance, applied force, and chromosomal velocity.

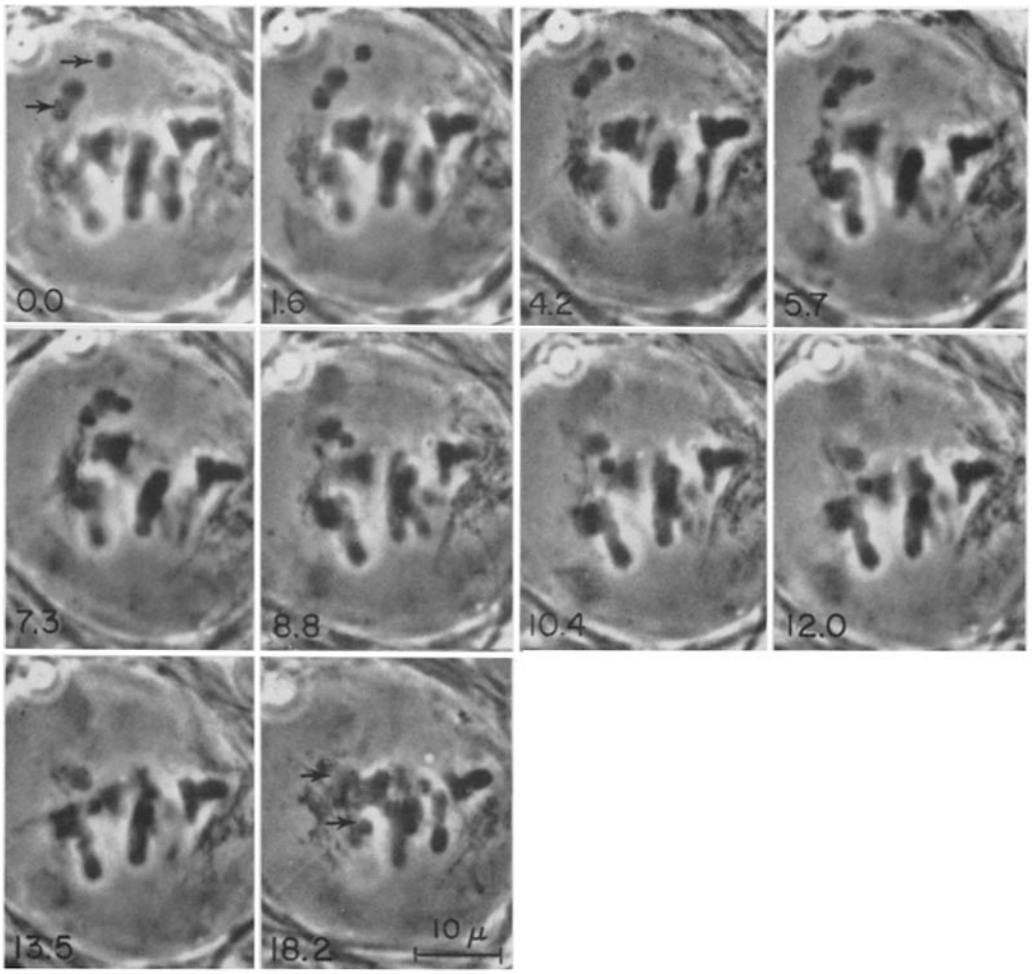

FIGURE 6 Prints showing temporary independent movement of the X chromosome kinetochore region (cell 20-8). The kinetochore region is indicated by the upper arrow in the first print, by the lower arrow in the last print. The other arrow indicates the distal end of the X (*cf.* Fig. 7). The time in minutes is indicated on each print.

The general character of this relation is indicated by equation 1:

$$F = Rv, \tag{1}$$

where F is the applied force, R is a coefficient of frictional resistance, and v is the resulting velocity. This simple relationship between force and velocity results from the extreme slowness of chromosome movement; at rapid macroscopic speeds the force is related to the square of the velocity (see, *e.g.*, 23, pp. 91 to 95). The resistance coefficient can be analyzed further;—it depends on the viscosity of the medium (η) and a factor related to the size and shape of the chromosome (s); and so we obtain,

$$F = \eta sv. \tag{2}$$

It must be emphasized that there is nothing theoretical about equation 2 so long as only slow, steady motion is considered, and, most important, if the possibility of non-constant viscosity is admitted. Fortunately, s has been exactly evaluated by Perrin (22) for objects similar in shape to chromosomes: prolate ellipsoids. This equation has been used successfully to describe the sedimentation of macromolecules in the ultracentrifuge

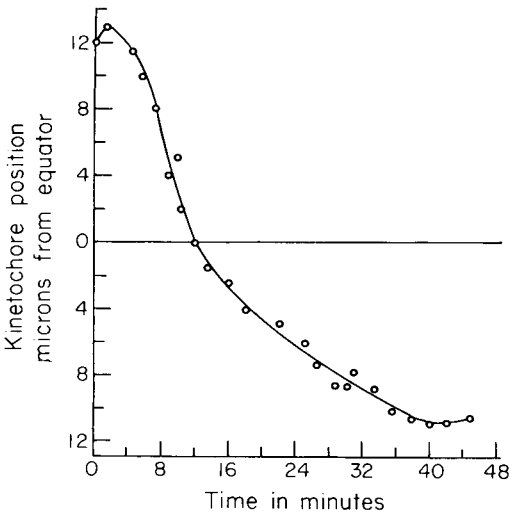

FIGURE 7 Graphical representation of X chromosome kinetochore movement in cell 20-8 (*cf.* Fig. 6).

TABLE IX

X Chromosome Kinetochore Velocities in Prometaphase

(Cell 20-8)

Trip No.	X chromosome effective length	Time (min.) (for reference to Fig. 7)	Average velocity (μ/min.) × 10
1	7.7	—	6.5
2	2.2	0–10	13.0
	7.7	10–13	12.0
	7.7	13–36	3.8
3	7.7	—	7.1

(see 25 for critical review). The best approximation to chromosome shape is obtained by using a prolate ellipsoid with a length and volume equal to that of the chromosome, and thus:

$$d = 1.64b, \text{ and } \ell = 2a,$$

where d is the chromosomal diameter, ℓ is chromosome length, and a and b are the long and short semi-axes, respectively, of the equivalent ellipsoid. Then from Perrin's equations (22),

$$s = \frac{16\pi(a^2 - b^2)}{(2a^2 - b^2)z - 2a} \tag{3}$$

for motion in which the long axis of the ellipsoid is parallel to the direction of movement, and

$$s = \frac{32\pi(a^2 - b^2)}{(2a^2 - 3b^2)z + 2a} \tag{4}$$

where the motion is perpendicular to the long axis. In both cases,

$$z = \frac{2}{(a^2 - b^2)^{1/2}} \log_e \frac{a + (a^2 - b^2)^{1/2}}{b}.$$

Using equations 3 and 4, the size-shape factor for these two different orientations has been calculated for the largest and smallest *Melanoplus* bivalents, and the results substituted in equation 2 to obtain the relative force required per micron/minute of chromosomal velocity (Table X). The term "relative force" (= relative resistance) simply means that no value for the viscosity has been specified; relative forces suffice for comparisons between bivalents (*cf.* discussion below). The result is clear: for motion parallel to the long axis, uniform velocity will result from applying 1.8 times as great a force to large, as compared with small bivalents. The data on motion perpendicular to the long axis are included, since many prometaphase bivalents have this orientation (see Table II). But an estimate of the minimal force difference necessary is the major concern here, and for this purpose the calculations based on motion parallel to the long axis suffice. This can be seen by comparing the information in Tables II and X; inclusion of the effect of perpendicular orientation would give a greater increase in the relative resistance value for the large bivalents than for the small bivalents, and hence the estimated force difference would be even greater than 1.8.

A similar estimate of force difference between the lumped small–small medium and large–large medium classes is provided by calculating the relative resistance in parallel orientation for bivalents of intermediate size and then calculating weighted average resistances for each class (Table XI). Again, a significant difference in the force required for uniform velocity is predicted; in this case, the force ratio is 1.4 for large:small bivalents.

Four assumptions underlying these calculations must be examined; these assumptions are:

1. The chromosome can be treated as a solid rod impermeable to the medium. It can be shown (*e.g.*, Tanford, 26) that even in flexible chain macromolecules in which only 1 per cent of the

volume within the domain of the molecule is occupied by macromolecular material, the internal solvent is trapped and the molecule behaves hydrodynamically like a solid particle. This applies *a fortiori* to molecules of defined shape and to chromosomes in which the internal volume occupied by material other than solvent is much greater than 1 per cent of the total volume.

2. The effects of adjacent objects or walls are negligible. The above calculations assume that the motion takes place in a volume of fluid very large relative to the size of the body. The presence of nearby objects (*e.g.*, other chromosomes and spindle fibers) will increase the frictional resistance, and this increase can be only approximately calculated. But in no case will the qualitative difference between large and small chromosomes be changed. Consider the extreme case in which the chromosome is surrounded by a solid cylinder only a micron or 2 from the chromosome. This will actually *enhance* the force difference required for uniform velocity, since resistance will then show a pronounced dependence on chromosome length: the movement considered is parallel with the long axis of the chromosome, and the additional resistance due to the presence of the cylinder results from friction between the fluid and the cylinder wall; the length of the wall being affected at any one time will depend on chromosome length. Even the quantitative results would not be changed more than a factor of 2 or 3 for small, nearly spherical chromosomes, as the computations of Crick (5, p. 513) attest. The possibility that adjacent chromosomes might influence the behavior of a given chromosome is effectively eliminated by the observed independence of prometaphase movements of individual chromosomes.

3. The fluid surrounding the chromosome has been assumed to be continuous. If the moving object is so small as to approximate the mean free path of molecules in the fluid, then the relations of Perrin will not apply, since they are based on continuum mechanics (see, *e.g.*, 16, pp. 33 ff). But chromosomes are not this small: the mean free path in liquids is of the order of a molecular diameter or about 1 mμ (10). Furthermore, the applicability of continuum mechanics to the motion of objects even smaller than chromosomes has been confirmed experimentally (reviewed by Sadron, 25). Nevertheless, this or related assump-

TABLE X

Calculated Frictional Resistance for Large and Small Melanoplus Bivalents

Direction of motion relative to bivalent long axis	Bivalent size	Size parameters in μ				Size-shape factor--*s*--in μ	Relative Resistance in dynes/poise per 1 μ/min. velocity
		ℓ*	d*	a	b		
Parallel	Large	12	2.5	6	1.52	45	7.5×10^{-9}
	Small	3	2	1.50	1.23	24	4.0×10^{-9}
Perpendicular	Large	12	2.5	6	1.52	59	9.8×10^{-9}
	Small	3	2	1.50	1.23	25	4.2×10^{-9}

* From Table I.

TABLE XI

Average Resistances for Lumped Classes of Melanoplus Bivalents

Group	Bivalent size*	Resistance/bivalent dynes/poise‡	No. of Bivs.	Total resistance	Weighted average resistance for the group
Large–Large medium	2.5 x 12	7.5×10^{-9}	2	15×10^{-9}	6.3×10^{-9}
	2 x 8	5.7×10^{-9}	4	22.8×10^{-9}	
Small–Small medium	2 x 5.75	4.9×10^{-9}	3	14.7×10^{-9}	4.5×10^{-9}
	2 x 3	4.0×10^{-9}	2	8.0×10^{-9}	

* From Table I.

‡ At a velocity of 1 μ/min.

tions might be inappropriate in the chromosomal situation if very large macromolecular aggregates were present along the path of motion. But this is not of great concern now, for the motions considered are parallel to, not across, the long axes of the spindle fibers—the only large macromolecular aggregates known to be present. Hence, it is concluded that the assumption of continuity is justifiable at this time.

4. The most difficult assumption to justify is that viscosity is constant. The difficulty is not that the spindle might be heterogeneous with regard to viscosity, since this has been controlled by explicit separation of effects of position and size on velocity. Rather, the difficulty is that viscosity in a medium as complex as the spindle will vary with stress: *i.e.*, the viscosity is non-Newtonian. Without exception, non-Newtonian liquids of biological interest show a decrease in viscosity on increased stress, but at very low stresses the viscosity is constant (see, *e.g.*, 24, chaps. 15 and 16). The viscosity decrease in a medium like the spindle would probably be due to orientation of initially random protein molecules, but it requires a finite amount of energy to produce orientation, and this explains the constancy of viscosity below a certain critical stress. Experience with many polymeric systems indicates that the critical stress will be roughly 10^4 times the concentration of polymer in gm/cc (9) or about 10^3 dynes/cm^2 for the spindle. Now, because chromosomes move so slowly, the stress on the medium is very low—about 10^{-1} dynes/cm^2, calculated from the data in Table X, using the chromosomal diameter as the area over which the force is distributed, and assuming a spindle viscosity as high as 1 poise. This stress is much lower than that expected to cause a viscosity decrease. In any event, while the greater stresses caused by the movement of large chromosomes might reduce the force difference required for similar velocities of large and small chromosomes, *some* difference in force would still be required.

B. Conclusions

It is concluded first that the above simple model is without serious objection at present: all possible objections cannot, as yet, be ruled out, but a *prima facie* case can be made. Secondly, the intuitive notion that large chromosomes are significantly more difficult to move than small ones survives rigorous scrutiny. Hence, the lack of influence of size on velocity is interpreted as reflecting an organization of mitotic forces having the unique property of velocity-load independence for at least twofold differences in load. That is, the mitotic forces are so produced or controlled (see below) that chromosome velocity is not decreased even if the hindrance to movement is doubled. This conclusion depends on a defensible, but untested mechanical model and, therefore, is tentative; but an equally economical interpretation of another situation—the stretching of chromosomes in anaphase—also leads to this conclusion (19). The argument there also depends on showing, indirectly, that the forces acting in one situation must be significantly greater than those in a second situation. But the mechanical argument in the interpretation of anaphase stretching depends on assumptions about chromosome elastic moduli, not frictional resistance as in the interpretation of size-velocity independence; and this lends additional weight to the suggestion of velocity-load independence as a general characteristic of mitotic forces. Velocity-load independence is also suggested by E. W. Taylor's demonstration (in press, 27) that anaphase velocity in newt cells is independent of tenfold intercellular differences in the viscosity of the cytoplasm. As Taylor notes, this is evidence for velocity constancy in spite of load variation (see equation 2, above); but since the viscosity could not be measured near the moving chromosomes, some uncertainty remains. Earlier workers (4, 21, 2) have used evidence like that in reference 19 to suggest that the forces acting on individual chromosomes can sometimes be much greater than those acting on freely moving anaphase chromosomes. Their evidence, particularly that of Cornman (4), did show that force differences *exist*, but they did not provide the mechanical interpretation necessary for the claim that the force difference is *significant*, nor did they clearly relate the difference in forces to the maintenance of a standard velocity in the face of an increased hindrance to motion. Bajer's study (2) supplies important evidence which can be interpreted on the model in reference 19 as indicating that velocity-load independence is present in plant cells with their rather different spindles.

It is worth noting, in passing, how small the absolute value of the force required for free motion may be. Assuming a high spindle viscosity of 1 poise, the force required for a velocity of 1 μ per minute would be about 10^{-8} dynes (*cf.* Table X). From this it is easily calculated that terminal

phosphorolysis of only 25 ATP molecules could supply the energy needed to move a large *Melanoplus* bivalent (or two chromosomes) 20 μ. Similar calculations have already been made by those with an interest in mitotic energetics (1) which gives additional importance to the defense of these calculations presented above.

Velocity-Load Independence and the Molecular Origin of Mitotic Forces

The physicochemistry of mitotic force production has usually been thought to be *the* problem of mitotic movement, and certainly there is no doubt that deeper explanation will be in these terms. The mechanical and cytological investigations reported here should serve to restrict and define the physicochemical work, for velocity-load independence implies a very unusual relationship between forces, load (resistance), and velocity which has not previously been reported for any biological system (*cf.* Hill, 11, on muscle, and Yoneda, 29, on cilia). The present aim is not speculation on the physicochemistry of force production; we already have a surfeit of such speculations. Rather, I wish to suggest the types of molecular theories which would have velocity-load independence as an automatic consequence.

The theory of "force compensation" previously proposed (19) should be mentioned, although it has velocity-load independence as an appended, not an intrinsic property. There it is maintained that velocity uniformity arises from continuous regulation of the forces acting on *each* chromosome. There is nothing to rule out force compensation, but theories which do not require continuous force adjustment are less complex and will receive greater attention here.

The first of these theories is given the inglorious title "force insignificance." The idea is that the mechanical forces account for a negligibly small fraction of the total energy required for chromosome movement. The meaning of this can be made clear by considering one specific possibility: that the gradual loss of spindle fiber material in anaphase (see reference 14) is a necessary, but *not* a sufficient condition for chromosome movement. On this view, one must supply energy for chromosome movement and also for the loss of spindle material. Put in force terms, for free motion we would have

$$F_T = R_c v + R_s v \tag{5}$$

where F_T is the total force required, v is chromosomal velocity (which equals the velocity of loss of spindle material), and R_c and R_s are, respectively, chromosomal and spindle resistance (here $R_s v$ can be identified as "internal friction"). Hence the velocity will be

$$v = \frac{F_T}{R_c + R_s}, \tag{6}$$

and if R_c is much smaller than R_s, then the velocity will be controlled by R_s and the total force required for a given velocity will not be measurably altered even if R_c is doubled. This model has the desirable property of *limited* velocity-load independence, since, if R_c were increased enough, it *would* be significant compared with R_s and the velocity would decrease (assuming F_T is constant). It must be emphasized that equations 5 and 6 and associated remarks are introduced simply as a device to make clear the implications of the "force insignificance" viewpoint. The view is made more plausible because the mechanical forces are evidently very small (see above). The above theory is identical with Taylor's (27) interpretation of the apparent independence of velocity and viscosity that he has observed; the other theories considered here would also explain his results. Taylor's discussion of force insignificance is particularly valuable for the comparison he makes between muscular contraction under no load and chromosome movement.

A final type of molecular theory would directly link mitotic force production and a standard velocity of movement. This was suggested by conversations with Dr. Andrew Szent-Györgyi of Dartmouth Medical School, and it will be called "velocity-related forcing." The idea is that built into the force-producing mechanism is a velocity-limiting "device," and as long as resistance to movement is less than the force produced by this mechanism, velocity will be constant. This partly begs the question by postulating what is to be proved, but nevertheless, this is a distinct and very plausible molecular mechanism. Again, a specific example is introduced, but here also this is done only to clarify the issue. Suppose that rearrangement of spindle fiber material produces mitotic forces. If this rearrangement occurs at a rate determined by the turnover number of an enzyme localized at the kinetochore, then the velocity of chromosome movement will be determined by

this reaction rate, unless and until the resistance to motion exceeds the available force. An interesting general corollary of this type of theory is that chromosomal spindle fibers are viewed as directly involved both in force production and in determining the position of the chromosome in space; that is, chromosomal velocity is limited to the rate of loss of the spindle fiber and the chromosome must not "overrun" its spindle fiber.

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