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Chromosome-Sized DNA Molecules from *Drosophila*

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Abstract. Measurements of viscoelastic retardation times of detergent-Pronase lysates of *Drosophila* cells demonstrated the presence of large numbers of DNA molecules of a size commensurate with that of the chromosomes. The values estimated from the retardation times for the molecular weights of the largest molecules ranged from about 20×10^9 to 80×10^9 daltons depending on the species of *Drosophila*. The molecular weights of the DNA molecules were independent of the metaphase shapes (*i.e.*, metacentric or submetacentric), but were proportional to the DNA contents of the chromosomes in the case of translocations or deletions. It was concluded, therefore, that the DNA molecules must run the length of the chromosome and cannot be discontinuous at the centromere. When compared with the values of the DNA contents of *Drosophila* chromosomes determined by other methods, the results were consistent with the model of one, or possibly two, DNA molecules per chromosome; the simplest conclusion, that there is only one DNA molecule per chromosome (for simple chromosomes), rests on a long extrapolation of an empirical relation between retardation time and molecular weight, but is also favored by indirect evidence. Further possibilities which could not be excluded were that the large DNA molecules contained Pronase-resistant, non-DNA links, or that a fraction of smaller DNA molecules might also have been present in the chromosomes. Chromosome-sized DNA molecules were obtained almost quantitatively from unsynchronized cultured cells, suggesting that the size of the chromosomal DNA is conserved throughout much of the cell cycle. The molecules were stable for periods of up to several days at 50° C in solutions containing detergent, Pronase, and EDTA.

I. Introduction

A central problem of eukaryotic chromosome structure is the structure of the chromosomal DNA, in particular, whether the DNA consists of one or a few molecules that run the whole length of the chromosome, of whether it consists of many connected pieces. The problem has been the subject of dispute for a long time, although in recent years the concept of one fiber running the whole length has been gaining ground (see for example review by DuPraw, 1970). In this work we have approached the problem by direct measurement of the size of the DNA molecules released from the chromosomes of *Drosophila* species. Critically important to the success of this measurement was the use of a new technique, the viscoelastic technique, which is superior to conventional techniques for DNA in the expected size range.

Early evidence suggesting that a chromatid contains one piece of native DNA was provided by Taylor *et al.* (1957). They found that in cells labeled with tritiated thymidine for one generation both chromatids of each metaphase chromosome were uniformly labeled, and the label in each daughter chromosome was essentially conserved throughout subsequent generations. It was immediately apparent to them that this pattern of chromosome replication was analogous to the replication scheme proposed for DNA by Watson and Crick in 1953. However, they felt it "inconceivable" that there could exist DNA molecules as large as the chromosome (Taylor *et al.*, 1957). Their feeling was based on the few facts then known about the duplication and mechanical properties of chromosomes and DNA. For example, virtually all DNA's examined appeared to have the same low molecular weight on the order of 10×10^6 daltons; we know now that these findings were artifacts of isolation (*e.g.*, Davison, 1959). Also, virtually nothing was known about separation of the strands of DNA, especially *in vivo*, and a single growing point per chromosome, as literally proposed by Watson and Crick (1953), could not easily account for the known rate of replication. However, subsequent studies revealed that DNA replication does proceed according to the scheme of Watson and Crick (Meselson and Stahl, 1958; Rolfe, 1962), but that each eukaryotic DNA molecule replicates simultaneously at many points (Cairns, 1966; Huberman and Riggs, 1968).

The notion that one DNA molecule runs the length of the chromosome is consistent with the observation that unfixed chromsomes are fragmented by DNAse, but not by RNAse or protease (Callan and Macgregor, 1958), and with the appearance of metaphase chromosomes (DuPraw, 1970) and interphase chromatin (Ris and Kubai, 1970) in the electron microscope, although these observations alone certainly do not exclude other models.

A direct approach by measuring the size of the DNA molecules extracted from chromosomes requires that artifacts of isolation and measurement be eliminated. Known artifacts of isolation are degradation of the DNA by mechanical shear and by nuclease activity. A recently recognized source of artifacts of measurement is the sensitivity of the sedimentation coefficient of large DNA molecules to centrifuge speed (Rubenstein and Leighton, 1971), an effect which should become very serious at the size expected. We were fortunate in having available the new viscoelastic technique of size measurement, which has been recently used to measure the molecular weights of DNA molecules extracted intact from both bacteriophages and bacteria (Chapman *et al.*, 1969; Klotz and Zimm, 1972a, 1972b). The good results obtained in these experiments appeared to justify its use in the present problem. With this technique it was easy to minimize nuclease action by working at

high temperature with solutions containing high salt, proteolytic enzymes, chelating agent, and detergent, and it was easy to minimize mechanical shear by lysing the cells in the chamber of the instrument without further transfer. The technique is also advantageous in that the measurements are more sensitive with molecules of higher molecular weight.

The technique makes use of a rotating cylinder, or Couette, viscometer modified to make measurements of the elastic as well as the viscous components of the response of a DNA solution to strain imposed by rotating one of the cylinders. The rotating inner cylinder, or rotor, is suspended in the DNA solution as a Cartesian diver. It is caused to rotate by a rotating magnetic field acting on a metal ring, and its angular position is recorded as a function of time by an automatic optical and electronic system. When the rotor is caused to rurn by the magnets, the DNA molecules in the solution are stretched out, and when the magnets are turned off, the rotor recoils backward as the stretched molecules retract toward the random coils characteristic of chain molecules at rest. (The effects of momentum are negligible because of the low speeds that are used.) The recoil of the rotor decays more or less exponentially with time; the longest time constant of this decay is the principal retardation time, which is the molecular-size-dependent quantity that we measure.

It is important to point out that the viscoelastic method measures essentially the microvolumes of fluid whose flow is dominated by the macromolecules under observation, and that the measurements weight most heavily the molecules that occupy the most volume. For this reason, it is selective for large DNA molecules in a cell lysate, since these molecules, being random coils, dominate the flow of nearly all of the fluid encompassed by the coils, in contrast to proteins and membrane fragments, which, even though they may have substantial mass, do not occupy much volume. In other words, the only particles present in a cell lysate that show viscoelastic recoil with long retardation times are the large DNA molecules. This fact is demonstrated by the observation that DNAse destroys the viscoelastic recoil (see below). By measuring only the slope of the recoil curve at long times we select the largest DNA molecules for observation out of all the rest. This feature is the important strength (and limitation) of the viscoelastic method which makes it suitable for the present problem.

For the work we are reporting here, the viscoelastic technique offers two additional advantages: (1) the retardation times are insensitive to moderate amounts of molecular distortion, thereby avoiding the effects which apparently restrict sedimentation measurements to molecules on the order of 10^9 daltons (Rubenstein and Leighton, 1971; Kavenoff, 1971, 1972), and (2) the measurements do not require either large amounts of material or radioactive labeling of the DNA.

Viscoelastic experiments have been used previously by Errera (1947), by Fredericq (1962) and by Dounce (1971) on gels of nucleoprotein. We

have simply extended the technique to obtain the very long retardation times of dilute solutions which are required for the calculation of molecular sizes.

We studied *Drosophila* DNA because it was a convenient eukaryotic DNA for the first application of the viscoelastic technique to molecules larger than bacterial chromosomes. The availability of a cultured cell line (Schneider, 1972) was also critically important. *Drosophila* chromosomes are among the smallest chromosomes possessing the properties unique to the chromosomes of higher eukaryotes. *e.g.*, they contain multiple points of DNA replication (Plaut *et al.*, 1966) and condense into metaphase chromosomes with classical morphology and fine structure (Kaufmann, 1934; Gay *et al.*, 1970). The simplicity of the *Drosophila* karyotypes seemed ideal for retardation measurements because of the small number of chromosomes and because of their size distributions; the DNA contents of some *Drosophila* chromosomes had already been determined, and there are numerous well-characterized karyotypic variations both within and among species. Finally, a good deal was known about the molecular and genetic organization of *Drosophila* DNA in somatic and polytene chromosomes.

II. Materials and Methods

A. Buffers, Solutions and Enzymes

1. LET: 0.5 M Na_4EDTA, 0.01 M Tris, pH 7 at room temperature.
2. HET: LET, pH 9.5.
3. LLET: 20% (v/v) LET in 0.01 M Tris, pH 7.
4. BA: 0.01 M Na_2EDTA, 0.142 M NaCl, 0.1 M H_3BO_3, 3.4 mM NaOH, pH 8.3 (Klotz and Zimm, 1972a, b).
5. NDS: 1% (w/v) sodium decyl sulfate (Pfaltz and Bauer, Inc., Flushing, N.Y.) in HET. This detergent was used because it was more soluble than the dodecyl form, and we thank Richard Tullis for suggesting its use.
6. LNDS: 1% (w/v) sodium decyl sulfate in 1 mM Na EDTA, 1 mM CAPS (Calbiochem, La Jolla, Cal.), pH 9. We thank J.H. Taylor for suggesting CAPS.
7. BANDS: 1% (w/v) sodium decyl sulfate in BA.
8. Pronase: 20 mg/ml "nuclease-free" Pronase (Calbiochem, La Jolla, Cal.). (In preliminary experiments Pronase, B grade, heat-treated according to Hotta and Bassel (1965) was also found to be satisfactory.) Aliquots were kept frozen at 0° C and, just before use, thawed and diluted as described below. At about 50° C this enzyme was free of DNA endonuclease activity on purified T7 DNA in the presence of 0.5% (w/v) sodium decyl sulfate, but not in its absence (Angeline Douvas, personal communication).
9. LET-Pronase: Pronase diluted with 99 equal volumes of LET.
10. HET-Pronase: Pronase diluted with 9 equal volumes of HET.
11. LLET-Pronase: Pronase diluted with 99 equal volumes of LLET.
12. HLET-Pronase: Pronase diluted with 9 equal volumes of LLET.
13. BA-Pronase: Pronase diluted with 99 equal volumes of BA.
14. HBA-Pronase: Pronase diluted with 9 equal volumes of BA.

15. DNAse: 2×10^4 units/ml purified spleen DNAse II (1.2×10^4 units/mg; Worthington Biochemical Corp., Freehold, N.J.) in 0.5 M Na-acetate buffer, pH 5. This DNAse was used because it does not require divalent metal ions and is not inhibited by EDTA; we thank Bob Oshima for suggesting its use.

B. Cells and Cell Suspensions

1. Cultured cells. Wild-type *D. melanogaster* cultured cells were grown from cell line #2 established by Imogene Schneider (1972). The cells were grown at room temperature (about 22° C) in Schneider's medium (Schneider, 1964) supplemented with 0.5% (w/v) Bactopeptone (Difco) and 15% (v/v) fetal calf serum (Grand Island Biological Supply Co., San Francisco, Cal.) previously heated at 56° C for 15 minutes to inactivate complement and kill any mycoplasma. Stock cultures were grown in plastic flasks and passaged weekly. Under these conditions, the average doubling time of the cells ranged between 3 and 4 days.

Cells for lysates were replated in plastic petri dishes at cell densities between 2 and 10×10^5 cells/cm^2. The Petri dishes were left at room temperature for at least five hours before the cells were harvested for lysis. Just before lysis, cell suspensions were prepared as follows: for measurements in *high EDTA* the medium was replaced with chilled LET or LET-Pronase, the cells were detached from the plate with a rubber "policeman" and the cells were resuspended; for measurements in *lowEDTA* the medium was removed, the plate was washed with 10 ml of either LLET or BA, and then the cells were harvested in a few ml of either LLET-Pronase or BA-Pronase as described for high EDTA. The density of the cells in the resulting suspension was determined by direct hemocytometer counts and then adjusted to values between about 6 and 40×10^5 cells/ml LET or LET-Pronase for high EDTA lysates, and between 1 and 10×10^6 cells/ml LLET-Pronase or BA-Pronase for low EDTA lysates.

2. Pupal Cells. The strains of *Drosophila* used are described in Table 1. Flies were grown in glass bottles on corn meal agar in the usual way. Cell suspensions were prepared from pupae of one of the stocks in Table 1. In this procedure the developmental stage of the pupae did not appear to be critical. Pupae were collected from the walls of culture bottles with a glass rod, deposited in a small glass Dounce homogenizer with a loose fitting pestle (0.11 mm minimum clearance), washed once with chilled LET, and then disrupted in a volume of about 0.5 ml LET-Pronase per pupa. After about 10 strokes of the pestle in the case of *D. melanogaster* and 20 strokes in the case of the other larger species, the suspension was pipetted briefly to better disperse the cells and was then poured through about 10 layers of gauze to remove debris. Cell densities were determined and adjusted essentially as described for cultured cells. However, the measurements of pupal cell densities were complicated by the extreme heterogeneity in the sizes of the cells and were, therefore, only approximate.

C. Lysate Preparation

Lysates were prepared in the chamber of the instrument by one of the following procedures.

1. High EDTA. The chamber temperature was brought to 65° C and 1.8 ml NDS was added. A few minutes later 0.8 to 1.0 ml freshly prepared chilled cell suspension was added. The mixture was incubated 10 to 20 minutes in the case of cultured cells and one hour in the case of pupal cells. Then the temperature was lowered to 50° C and 1.0 to 0.8 ml freshly prepared HET-Pronase was added and incubated for at least 4 hours before retardation times were measured.

2. Low EDTA. The procedure was similar to that just described for high EDTA, with the following modifications: NDS was replaced by either LNDS or BANDS;

the volume of this solution was increased to 2.0 ml, and the incubation of the cells in detergent and Pronase at 65° C was extended to one hour; HET-Pronase was replaced by LLET-Pronase or BA-Pronase, and these were added to the chamber at 60° C and incubated at that temperature for at least four hours before retardation times were measured.

In both procedures, it was essential to have rapid mixing between the cell suspension and the detergent solution; this required careful adjustment of the solution densities, since mechanical stirring could not be used for fear of shearing the DNA. We found it convenient to make the mixing visible by adding a small amount of dye to one of the solutions. If the densities were properly adjusted, convection currents were sufficient to bring about effective mixing.

D. Determination of Retardation Times

The major part of the theory and experimental technique of our rheological method has been discussed in detail in previous papers (Klotz and Zimm, 1972a, b). To summarize that part of the theory relevant to the measurements of this paper, one can state the following: each large DNA molecule possesses a *principal retardation time*, τ, the time constant of the retarded elastic response, which is a function of the molecular weight. This τ is also a function of the solvent viscosity and the temperature, as well as DNA concentration, but these latter dependences are trivial and can be eliminated by extrapolating τ to infinite dilution and multiplying the result by the ratio of the viscosities and the absolute temperatures to yield $\tau^{\circ}_{50,\,w}$, as follows,

$$\tau^{\circ}_{50,\,w} = \frac{0.549\,T}{323\,\eta}\,\tau^{0} \tag{1}$$

where T is the absolute temperature of measurement and η the viscosity of the solvent at this temperature, 0.549 is the viscosity of water at 50° C, and τ^{0} is the measured retardation time extrapolated to infinite dilution. For linear native DNA in 0.2 M salt the resulting $\tau^{\circ}_{50,\,w}$ is related to the molecular weight by a simple empirical formula:

$$M = K(\tau^{\circ}_{50,\,w})^{q} \tag{2}$$

where K and q are constants; for DNA in 0.2 M salt, and τ in seconds, we take K to be 2.2×10^{8} and q to be 0.60 (Klotz and Zimm, 1972b). (The dependence of τ on the concentration of salt will be discussed later.)

The retardation time at infinite dilution, τ^{0}, may be obtained in principle from many different kinds of experiments; in this work we approach it through the measurement of viscoelastic recoil. The solution is placed between the concentric cylinders of a Couette-type of viscometer and deformed by applying a torque to one of the cylinders with an electromagnet. When the magnet is turned off, removing the torque, the elastic recoil of the solution is manifested in a motion of the cylinder in the opposite direction to its previous motion. This motion after the magnet is turned off can be represented by a sum of exponential functions,

$$\theta(t)-\theta(\infty) = \sum_{i} \Gamma_{i} \exp(-t/\tau_{i}) \tag{3}$$

where $\theta(t)$ is the angular position of the rotor as a function of the time t after the magnet has been turned off, $\theta(\infty)$ is its rest position after a very long time, and Γ_{i} and τ_{i} are constants. The τ_{i} are the retardation times of the solution. In general there are many constants Γ_{i} and τ_{i}, but in the case of particular interest here, where there is a unique DNA species whose molecules are larger than all the others in the

solution, there is a corresponding largest retardation time, which we denote by τ_1. This retardation time, τ_1, extrapolated to zero concentration and zero shear stress (*i.e.*, zero applied torque), is the principal retardation time, τ^0, of equation (1). The largest retardation time, τ_1, can be found by plotting $\ln[\theta(t)-\theta(\infty)]$ against t; at large values of t the plot becomes a straight line with slope $-1/\tau_1$. Alternatively Guggenheim's method (Guggenheim, 1922) may be used in the same way; in this case one plots $\ln[\theta(t)-\theta(t+\Delta)]$ against t where Δ is some convenient constant; the slope at large t is again $-1/\tau_1$.

The constant Γ_1 is obtained by extrapolating the limiting straight line of the plot of $\ln[\theta(t)-\theta(\infty)]$ back to $t=0$; the intercept of this line is Γ_1. Γ_1 is proportional to the number L_1 of DNA molecules of largest size (as defined by the value of τ) per unit volume. If the rotor has been made to turn under torque for a long time (*i.e.*, for a time equal to several multiples of τ_1) before the magnet was turned off, the relation is (Klotz and Zimm, 1972a)

$$L_1 = \frac{\eta_s \eta_{\mathrm{rel}} \Gamma_1}{k T \omega \tau_1^2} \tag{4}$$

where η_s is the viscosity of the solvent, η_{rel} the ratio of the viscosity in steady state of the solution to that of the solvent, k and T have their usual meanings, and ω is the angular velocity of the rotor before the magnets are turned off. In the present work it was frequently desirable to turn the magnets off long before a steady state had been reached because of the long times involved. If t_w (the "wind-up time"), the length of time during which the magnets were turned on, is short compared to τ_1, equation (4) is replaced by

$$L_1 = \frac{\eta_s \Gamma_1}{k T \tau_1 (\Delta\theta)} \tag{5}$$

where $(\Delta\theta)$ is the angular distance that the rotor moves during the "wind-up." Both of these expressions are strictly valid only at infinite dilution. In this work however, they were usually assumed to be sufficiently valid at finite concentrations to yield results of qualitative interest even without extrapolation to infinite dilution.

The early transients in the recoil curves correspond to the terms in equation (3) with values of τ_i smaller than τ_1; some of these arise from the presence of molecules smaller than the largest. Since the exact values of the coefficients of these terms depend on the time of wind-up, t_w, as well as on the concentrations and sizes of the various molecular species, we did not attempt a full analysis of the transients in this work. Also, we did not attempt to make use of the intrinsic viscosity because of the difficulty of reaching steady-state flow conditions in a reasonable time at convenient DNA concentrations, and because of the expected heterogeneity in size of the DNA reflecting the variety of chromosome sizes.

In this work a number of technical modifications of previous procedure (Klotz and Zimm, 1972a, b) were introduced. A gold cylinder replaced the aluminium rotor counterweight used previously because in preliminary experiments the aluminium slowly dissolved in the lysates generating troublesome gas bubbles and apparently causing the DNA to degrade. The rotor was slowly placed in a lysate shortly *after* Pronase had been added. Also, the circulation of water around the chamber was improved by increasing the diameter of the entrance for water at the top of the chamber and creating a second exit for water near the bottom of the chamber. The entrance was directed tangentially to produce a helical pattern of streamlines around the chamber. A temperature gradient resulted, with the top of the chamber warmer than the bottom by a controllable amount, usually adjusted

to about 0.2° C. This gradient tended to restrict convection in the chamber after the first few minutes after addition of solutions, and markedly increased the stability of the position of the rotor.

Values of τ were measured at 50° C, except in some cases with low EDTA, in which instability of the DNA at 50° C was encountered. In the latter case values of τ were measured at 60° C and then corrected to 50° C with the Eq. (1), where the product of the ratio of the appropriate solvent viscosities at the two temperatures multiplied by the ratio of the absolute temperatures has the value 1.22.

In preliminary experiments it was found that under the conditions reported here values of τ were independent of the length of time during which the rotor was driven by the magnets or of the rate of rotation, so long as the rotor was not driven around more than one revolution.

Since the concentration of DNA in the lysates was too low (less than 1 μg/ml) to be measurable by conventional quantitative methods, and since it was not certain that the concentration of the largest DNA molecules could be estimated reliably from the total DNA concentration, the concentration of the largest DNA molecules in a lysate was calculated from Eq. (5).

E. DNAse Treatment

Lysates were prepared from cultured cells in high EDTA as described above. After one retardation-time measurement to verify that the lysate contained chromosome-sized DNA molecules the chamber was opened and the rotor removed, the temperature was lowered to 37° C, and 0.5 ml of spleen DNAse solution was added slowly with a syringe. After 20 minutes incubation with the nuclease, the chamber was loaded and the retardation time was monitored. When short wind-ups with the magnets no longer caused the rotor to recoil, the temperature was raised to 50° C and the retardation time measured under standard conditions. In control experiments, the same procedure was repeated with 0.5 ml of the DNAse solvent added instead of the DNAse solution; no effect on the retardation time was observed.

F. Heat Treatment

A lysate was prepared from cultured cells in high EDTA as described above after one retardation-time measurement to verify that the lysate contained chromosome-sized DNA molecules, the chamber was opened, the rotor removed, and the solution was heated to 100° C for 10 minutes. Then the rotor was replaced and the retardation-time measured. In control experiments the same procedure was repeated with the heat treatment omitted. The retardation times were found to be unaffected in the unheated control, while in the heated samples the whole viscoelastic effect had disappeared.

G. Shear Treatment

1. Mild Shear. After it was verified that the lysate of cultured cells in high EDTA contained chromosome-sized DNA molecules, the chamber was opened and the rotor was removed. A piece of glass tubing (3 mm internal diameter) was lowered into the chamber and virtually the entire lysate was pumped up into the tubing at a rate of 1 ml/minute. The lysate was then pumped back into the chamber at the same rate. The chamber was reassembled for retardation measurements.

2. Strong Shear. After the effect of mild shear had been examined, the chamber was opened once more and the rotor removed. The lysate was rapidly pipeted with a Pasteur pipet (1 mm diameter at the tip) three times. Then the rotor was replaced, the chamber reassembled, and the retardation time measured.

In control experiments, the chamber was opened, the rotor removed and replaced, and the retardation time measurement was repeated; there was no observable difference in retardation time.

III. Results and Discussion

A. Retardation Times

Semi-log plots of rotor angle *vs.* time for typical measurements of each of the *Drosophila* DNA's studied are shown in Fig. 1. The deviations from linearity at short times are due in part to heterogeneity in the form of DNA molecules of sizes smaller than the largest and in part to the faster modes of relaxation of the largest molecules (Klotz and Zimm, 1972a).

The principal retardation time is 1/2.303 times the inverse of the slope of the linear portion of the semi log plots. The principal retardation time of DNA in lysates of cells of *D. melanogaster* was measured as a function of DNA concentration and the results are summarized in Figs. 2 and 3.

The retardation times of DNA solutions are dependent on the salt concentration, in consequence of the known effect of salt on the dimensions of DNA molecules as manifested by such properties as the intrinsic viscosity (Rosenberg and Studier, 1969). The magnitude of the effect apparently depends somewhat on the molecular weight of the DNA. Since we found it desirable to work at 2 M sodium ion while the calibration of Eq. (2) was carried out with 0.2 M sodium, it was necessary to determine the conversion factor. Fig. 2 shows the plots of τ against concentration obtained for lysates prepared in high and low concentrations of EDTA. The values of τ observed in low EDTA were larger and apparently more concentration dependent than those in high EDTA. Since τ^0 is proportional to the solvent viscosity, η, times the intrinsic viscosity, $[\eta]$, (Klotz and Zimm, 1972a), and the ratio of η of 0.5 M EDTA to that of 0.2 M NaCl is 2.0, these data indicate a decrease in $[\eta]$ of a factor of about 3.4 on going from 0.2 M to 2 M Na^+. This decrease is strikingly large, but seems to be compatible with published findings that the value of $[\eta]$ decreases as the concentration of salt increases and that the magnitude of the decrease increases with the molecular weight of the DNA. For example: for T2 DNA with a retardation time of about 0.5 seconds and a molecular weight of about 120×10^6 daltons, Scruggs and Ross (1964) found a 29% decrease in $[\eta]$ on going from 0.2 M Na^+ to either 1.0 or 2.0 M Na^+; for T7 DNA with a retardation time of about 0.05 seconds and a molecular weight of about 25×10^6 daltons, Rosenberg and Studier (1969) found a 19% decrease in $[\eta]$ on going from 0.2 M to 1.0 M Na^+; and for fragments of salmon sperm DNA

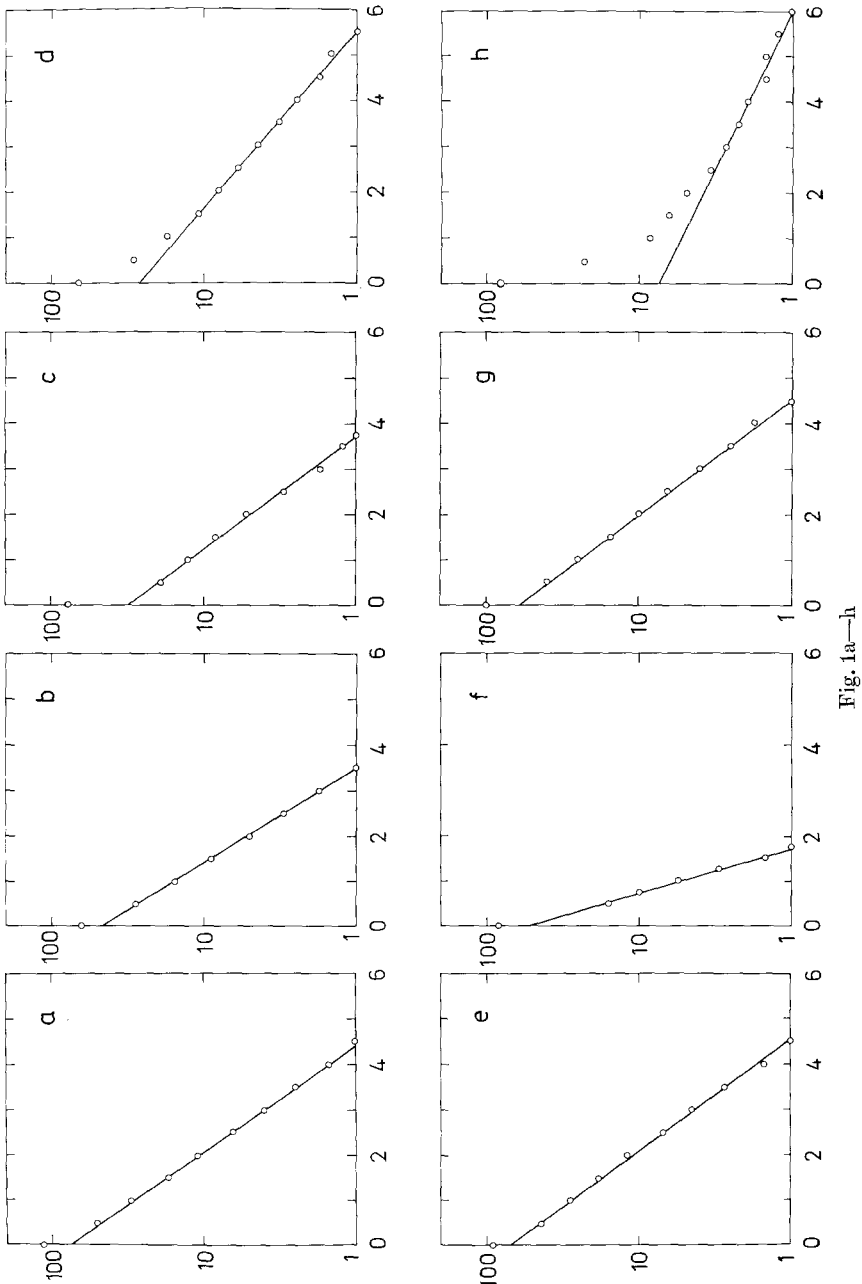

Fig. 1a—h

with average size 10 to 30×10^6 daltons, Scruggs and Ross (1964) found a decrease in $[\eta]$ between 2% and 6% on going from 0.2 M Na^+ to 2.0 M. Additional data showing that the magnitude of the effect increases with the molecular weight of the DNA have been presented by Ross and Scruggs (1968). A factor of 3.4 decrease in $[\eta]$ would imply a corresponding decrease in the amount of concentration dependence of the viscosity, which might explain the apparent lack of concentration dependence of τ in high EDTA evident from Figs. 2 and 3. The remaining small concentration dependence of τ might have been obscured by experimental error.

Since the values for the retardation times for DNA in high-EDTA lysates of cultured cells were apparently independent of shear-stress and DNA concentration in the range studied, the retardation times for all of the *Drosophila* DNA's in high EDTA were obtained from the numerical averages of the values obtained in successive measurements of each of

Fig. 1a—h. Plots of typical recoil decay curves for lysates of *Drosophila* cells. Degrees of rotor rotation are plotted vertically on a logarithmic scale against time in hours. All data represent lysates prepared in high EDTA, $T = 50°$ C. The wind-up was less than 180° of rotor rotation (equivalent to less than 5 radians of shear) and was accomplished in less than 400 seconds. — a *D. melanogaster* wild type, cultured cells: The final cell concentration in the lysate was 7×10^5 cell/ml and the measurement was made 17 hr after lysis. The retardation time calculated from the slope of the straight line is 1.0 hr and the number of molecules calculated from the y-intercept of the straight line is 5 per cell. — b *D. melanogaster* wild type, pupal cells: The final cell concentration in the lysate was about 6.5×10^5 cells/ml and the measurement was made 12 hrs after lysis. The retardation time calculated from the slope of the straight line in the plot is 1.0 hr. — c *D. melanogaster* inversion strain, pupal cells: The final cell concentration in the lysate was about 3×10^5 cells/ml and the measurement was made six hours after lysis. The retardation time calculated from the slope of the straight line in the plot is 1.1 hr and the number of molecules calculated from the y-intercept of the straight line is 4 molecules/cell. — d *D. melanogaster* translocation strain, pupal cells: The final cell concentration in the lysate was about 10^6 cells/ml and the measurement was made 24 hr after lysis. The retardation time calculated from the slope of the straight line in the plot is 1.7 hr. — e *D. hydei* wild type, pupal cells: The final cell concentration in the lysate was about 2.6×10^6 cells/ml and the measurement was made 14 hr after lysis. The retardation time calculated from the slope of the solid straight line in the plot is 1.0 hr. — f *D. hydei* deletion strain, pupal cells: The final cell concentration in the lysate was 6×10^5 cells/ml and the measurement was made six hours after lysis. The retardation time calculated from the slope of the straight line in the plot is 0.41 hr. — g *D. virilis* pupal cells: The final cell concentration in the lysate was about 2×10^6 cells/ml and the measurement was made six hours after lysis. The retardation time calculated from the slope of the straight line in the plot is 1.1 hr. — h *D. americana americana* pupal cells: The final cell concentration in the lysate was about 5×10^5 cells/ml and the measurement was made 12 hr after lysis. The retardation time calculated from the slope of the solid straight line in the plot is 3.0 hr

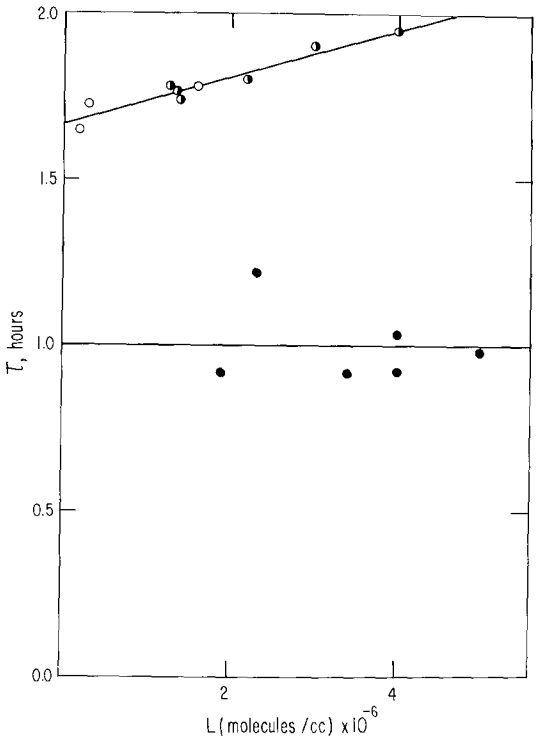

Fig. 2. Concentration dependence and retardation time in high and low EDTA at 50° C for lysates of *D. melanogaster* cultured cells. Low EDTA: (○) lysates prepared in BA buffer (0.01 M EDTA, about 0.2 M Na^+, viscosity is 1 ± 0.05 relative to H_2O; ◑ lysates prepared in LLET buffer (0.05 M EDTA, about 0.2 M Na^+, viscosity is 1 ± 0.05 relative to H_2O; ● high-EDTA lysates prepared in LET buffer (0.5 M EDTA, about 2M Na^+, viscosity is 1.9 ± 0.15 relative to H_2O)

several lysates. The resulting values for the retardation time were multiplied by a factor of 1.67 to convert from the high salt concentration of the lysates to retardation times characteristic of 0.2 M salt. The value of 1.67 was obtained from the data in Fig. 2; this should be a reasonable value to use for all of the DNA's which had about the same retardation time in high EDTA as the DNA in the lysates of cultured cells; it may be somewhat small for the largest DNA in the lysates of *americana* and somewhat large for the deletion strain of *D. hydei*. The resulting values for the times, τ^0 and $\tau^0_{50,w}$, of the different DNA's are listed in Table 2.

When we consider that the retardation times, $\tau^0_{50,w}$, of viral DNA's are of the order of seconds and those of bacterial DNA's are of the order of minutes, it is obvious that we are dealing here with much larger

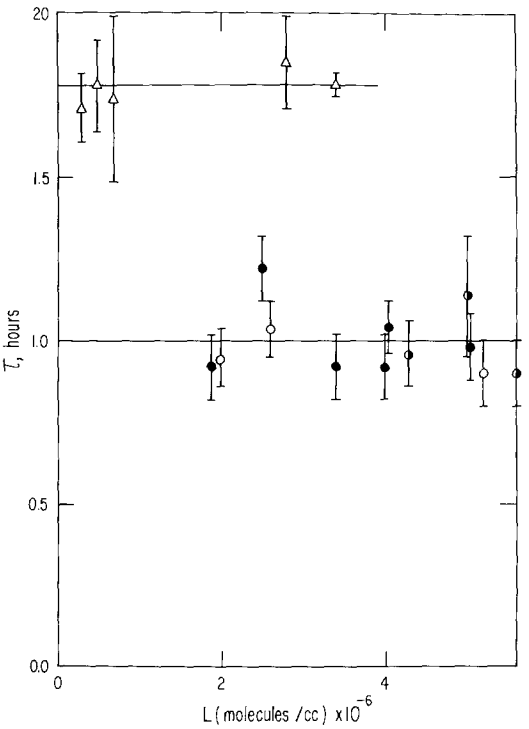

Fig. 3. Retardation times at 50° C in high EDTA plotted against concentration for lysates of *D. melanogaster*. Conditions as in Fig. 2, high EDTA. △ lysates of pupal cells from translocation strain; ◑ lysates of pupal cells from inversion strain; ○ lysates of pupal cells from wild type; ● lysates of cultured cells. The error bars show the average deviation of the recoil measurements at each concentration; only those data are shown for which at least duplicate measurements were made

molecules. We return to the question of just how large these molecules are later (Section III C). Meanwhile, we should point out that the presence of limiting straight lines at the long-time parts of the semi-log plots of recoil *vs.* time indicates that a corresponding unique largest species of chain molecule exists in our solutions. In this paper we use the term, "chromosome-sized DNA molecules" to denote this unique largest species.

By "chromosome-sized," we mean that the size of the largest DNA molecules detected in the lysates is the same as their size in the chromosomes *in vivo*, whatever that turns out to be; *i.e.*, we would like to claim that these molecules were not broken during extraction. It would seem unlikely that these largest observed molecules were breakage products

Table 1. Strains of *Drosophila*

Species and strain	Trivial designation in this paper	Obtained from
D. melanogaster		
Canton-Special	wild type	D. Lindsley
In(3LR)C269[d] (In(3LR)78C; 98F)	inversion strain	J. Merriam
T(1; 3)OR38[b] (T(1; 3)18D; 61D)	translocation strain	D. Lindsley
D.hydei		
Leiden Strain	wild type	J. Boyd
340/1[c]	deletion strain	W. Hennig
D. virilis[d]	—	W.K. Baker and J.A. Figueroa
D. americana americana[d]	—	W.K. Baker and J.A. Figueroa

[a] Chromosome 3 carries a pericentric inversion which changes the arm ratio of chromosome 3 from about 1:1 to about 7:1 without altering the length of the chromosome. The stock used contained both *In(3LR)C269/In(3LR)C269* homozygotes and *In(3LR)C269/In(3LR)TM1, Me* heterozytotes; *In(3LR)TM1, Me/In (3LR)TM1, Me* homozygotes are lethal. Since homozygous *TM1* appears to be lethal before pupation, the minimum fraction of third chromosomes in a large sample of pupae that carry *In(3LR)C269* is 2/3; to the extent that some of the parents of the pupae were *In(3LR)C269* homozygotes, the fraction will exceed this value. Therefore, at least three times the usual number of pupae were collected for each cell suspension (*i.e.*, at least 12 pupae were pooled.) — The presence of the inversion was confirmed during the course of this work by inspection of the salivary gland chromosomes kindly performed by Dan Lindsley.

[b] Chromosome 3 in *T(1; 3)OR38* carries about 60% of the metaphase X-chromosome attached to virtually the tip of one arm (3L) (Lindsley and Grell, 1968). The affected chromosome 3 is thus increased in length by about 37%. The stock in which *T(1; 3)OR38* is carried produces flies of the following genotypes: *In(1)sc^{S1} + dl-49, sc^{S1} v f car/T(1; 3)OR38, y*♀; *In(1)sc^{S1} + dl-49, sc^{S1} v f car/In (1)sc^{S1} + dl-49, sc^{S1} v f car*♀ (sterile); *T(1; 3)OR38, y/B^{S}Y* ♂ (sterile); and *In(1)sc^{S1} + dl-49, sc^{S1} v f car/B^{S}Y* ♂. – The translocation carries normal alleles of the eye color genes vermilion (*v*) and carnation (*car*) and the translocation-free genotypes are homozygous for the recessive alleles. Since eye-color can be scored in late pupae, we were able to select only translocation-bearing pupae (*i.e.*, those with normal eye color) for preparation of cell suspensions.

[c] The X-chromosomes in both males and females lack almost all of the heterochromatic arm comprising half of the length of the wild-type metacentric chromosome. Both females and males have two Y-chromosomes; males have one *X* and females have two (Hennig, 1972). Pupae were not selected with respect to sex.

[d] *D. virilis* and *americana* are closely related species; some of the acrocentric rod-shaped chromosomes of *virilis* pair with arms of the metacentric chromosomes of *americana*. Thus the largest chromosomes of *americana* are approximately twice those of *virilis* in size. See Fig. 4 and discussion in text (Section III C).

of larger molecules, because the sizes of the largest molecules obtained from the different species of *Drosophila* spanned a fourfold range and were close to the values of the total DNA contents of the largest G_1 chromosomes of the various species. We return to this point in more detail below (Section III C) after first examining the properties of the large molecules.

By DNA molecules we mean pieces of native DNA without specifying whether or not the pieces contain single-stranded nicks or gaps; such nicks should not affect retardation times, since they do not affect the viscosity (Hays and Zimm, 1970). Also we cannot exclude the possibility that some protein is still attached to the DNA at the time of measurement. However, the stability and reproducibility of the retardation times, after the first few hours of exposure to Pronase, suggest that the attached protein, if any, either is completely resistant to Pronase or has no effect on the configurations of the DNA molecule and hence has no effect on the retardation time.

B. Properties of Chromosome-Sized DNA Molecules

1. Reproducibility and Stability

a) High EDTA. All of the lysates prepared from cultured cells in high EDTA contained a measurable fraction of chromosome-sized DNA molecules which were stable at 50° C for at least eight days; no attempt was made to investigate their stability over longer times. Most of the lysates prepared from pupal cells in high EDTA also contained a measurable fraction of chromosome-sized DNA molecules which were stable at 50° C for several days. Frequently, however, this fraction was somewhat smaller than might have been expected from the karyotype of the cell. For example, in the experiment with *americana* shown in Fig. 1h the small fraction of recoil in the slowest retardation mode indicates that much less than half of the DNA is occurring as molecules of maximum size. This situation may reflect the greater sensitivity of large molecules to degrading agents. In about 10% of the lysates, in fact, absolutely no relaxation was observed, and the exact explanation for this difficulty is not known. Part of the difficulty may have arisen from variations in the preparations of pupal cells, and part may have been nuclease activity, since adding Pronase to the pupal cell suspensions and increasing the length of the incubation at 65° C significantly increased both the reproducibility and the yields, whereas lowering the EDTA concentration markedly reduced the yields and the reproducibility.

b) Low EDTA. The lysates prepared from cultured cells in low EDTA contained a smaller fraction of chromosome-sized DNA molecules than the lysates in high EDTA, and these were stable at 50° C for only about

one day, whereas at 60° C they were stable for about two days. It is likely that the major source of the diffculty in low EDTA was nuclease activity.

2. Yields

Yields of chromosome-sized DNA molecules were estimated by dividing the number of molecules per milliliter calculated from the recoil in the longest relaxation mode and Eq. (5) by the number of cells per milliliter. In the case of the cultured cells of *D. melanogaster* wild-type, for which our cell counts were most reliable, the yields obtained in high EDTA ranged from about 1 to 7 molecules of about 40×10^9 daltons per cell. The yield was usually about 4 molecules per cell when freshly replated cells were used; within a few days after replating the number frequently increased somewhat, but a few weeks after replating the number declined markedly concomittant with a decrease in plating efficiency. In the case of the pupal cells of *D. melanogaster* wild type, the yields obtained in high EDTA ranged from about 0.5 to 4 molecules of about 40×10^9 daltons per cell. The maximum expected number of DNA molecules of size about 40×10^9 daltons for G_1 cells of wild-type *D. melanogaster* is 4, since there are four large chromosomes of practically equal size in the diploid set, not counting *X* and *Y*. This number might double in G_2 and M. The same number, 4, is also expected for cells in most of S-phase if DNA synthesis proceeds as observed by Cairns (1966) and by Huberman and Riggs (1968), but the viscoelastic characteristics of such molecules are uncertain; they might well have shorter retardation times than normal molecules and might not have been counted in our measurements. Thus, in view of our yields of 1 to 7 molecules per cell, it appears that the chromosome-sized DNA molecules were obtained from most of the cells in unsynchronized cultures and, therefore, do not represent DNA molecules from any particular narrow phase of the cell cycle. This finding implies conservation of DNA size throughout most of the cell cycle, including possibly phase S.

The fact that the yields obtained from cultured cells in high EDTA were generally higher than those in low EDTA and higher than those from pupal cells in either high or low EDTA is consistent with the difficulty we have ascribed to nucleases in the previous section.

3. Sensitivity to DNAse and to Heat

Chromosome-sized DNA molecules in lysates of cultured cells were sensitive to spleen DNAse. Absolutely no recoil could be detected after a lysate containing about 0.2 μg/ml of chromosome-sized DNA molecules was incubated with highly purified spleen DNAse for 2.5 hours. Similarly, heating to 100° C destroyed the recoil.

4. Sensitivity to Shear

Chromosome-sized DNA molecules in lysates of cultured cells were sensitive to shear Mild shear (see *Materials and Methods*) degraded about half of the largest molecules present initially as judged from the decrease in the recoil after shearing. That fraction of chromosome-sized DNA molecules which remained intact after mild shear was so degraded by strong shear that no recoil could be detected.

5. Insensitivity to Pronase

The chromosome sized DNA molecules from cultured cells were insensitive to the action of Pronase. Preliminary experiments showed that Pronase is fully active on total *Drosophila* cell protein under conditions normally used for preparing lysates; moreover, under these conditions much of the activity persisted for at least several days. Neither the yield nor the stability of the chromosome-sized DNA molecules were adversely affected by either (a) increasing the initial Pronase concentration by as much as 10-fold, or (b) periodically adding more Pronase to the lysate of cultured cells.

We cannot exclude the possibility that the chromosome-sized DNA molecules contain Pronase-resistant links of material other than DNA. At least two types of link are conceivable: (a) protein and (b) RNA. It has been proposed that eukaryotic DNA's contain protein links chiefly because analyses of highly purified DNA revealed traces of protein (Bendich and Rosenkranz, 1963· Dounce, 1971). however, residual protein may be explained alternatively by the discovery that there are many proteins which bind very tightly to DNA as, for example, the "DNA-binding proteins" (Alberts *et al.*, 1968; Hotta and Stern, 1971). In the only case we know of in which there may be a protein covalently linked to DNA, *i.e.*, certain colicinogenic factors, the linkage between the protein and the DNA is quite sensitive to Pronase (Clewell and Helinski, 1969). Protein links in DNA have also been proposed on the basis of studies in which it was concluded that proteases could degrade DNA and nucleoprotein gels (Dounce, 1965) and even chromosomes (Trosko and Wolff, 1965)· however, the enzymology in these studies appears quite incomplete in that the proteases used were not assayed for the presence of contaminating nuclease activity on well characterized DNA's such as the synthetic or bacteriophage DNA's, and endogenous nuclease activity was neither measured nor eliminated. Recently it has been suggested that certain DNA molecules contain small segments of RNA (Blair *et al.*, 1972). We have tried to test for such linkages in the chromosome-sized DNA molecules from *Drosophila* and encountered many technical problems which rendered our tests inconclusive.

6. Remarks on Procedures for Handling Large DNA

Our findings concerning the extraction and stability of chromosome-sized DNA molecules may explain some of the difficulty encountered in previous attempts to obtain very large DNA molecules from eukaryotic cells.

In particular, we found it best to use relatively high concentrations of EDTA and to incubate with detergent and Pronase at relatively high temperature; these points have been discussed in section 1, above. Apparently a major problem in extracting intact DNA from cells is the activity of nucleases liberated during extraction.

It has been customary to attribute much of the difficulty in obtaining large DNA molecules to their "shear sensitivity." This practice stems from the well-known observation of Davison (1959) that the shearing forces in syringing DNA solutions fragment intact T-even DNA molecules with a molecular weight of about 120×10^6 daltons (Davison *et al.*, 1961). Hence, shear sensitivity was invoked to rationalize subsequent difficulties in obtaining intact bacterial DNA molecules with molecular weights about 10^9 daltons (*e.g.*, Cairns, 1962). To minimize shear, many practices have become popular; *e.g.*, increasing the viscosity of the lysis solution (Cairns, 1962, 1963; Huberman and Riggs, 1966). However, intact T-even bacteriophage DNA molecules are not fragmented by slow pipetting in wide-mouth pipets. Recently chromosome-sized DNA molecules have been extracted from bacteria in high yield without the use of additives to increase the viscosity of the lysis solution (Klotz and Zimm, 1972; Kavenoff, 1972) and these have been manipulated by dialysis and very slow pipetting without significant breakage (Levin and Hutchinson, 1972); in view of these and related findings, it seems likely that most of the previous difficulties with bacterial DNA were due primarily to either nuclease activity, as discussed above, or to technical artifacts (Klotz and Zimm, 1972b; Kavenoff, 1971, 1972).

C. Molecular Weights

1. Absolute Values of Molecular Weight

The molecular weights calculated from the retardation times and Eq. (2) are presented in Table 2.

The uncertainties in Table 2 represent the average deviations of the measurements; there is an additional uncertainty of 30% in the absolute values of M due to uncertainties in Eq. (2); this additional uncertainty does not apply to the ratios of the molecular weights to one another. The empirical Eq. (2) was established (Klotz and Zimm, 1972b) through the combination of a theoretical relation between retardation time and intrinsic viscosity with a well-established empirical relation between the

Table 2. Retardation times and molecular weights of *Drosophila* DNA

Species and strain	Number of determinations[a]	Number of lysates	τ^0(hr) 2 M Na^+	$\tau^0_{50,w}$(hr) 0.2 M Na^+	Molecular weight ($\times 10^{-9}$)[b]
melanogaster					
cultured cells	37	12	1.00 ± 0.11	1.67 ± 0.18	41 ± 3
wild type	7	4	1.00 ± 0.14	1.67 ± 0.23[c]	41 ± 3
inversion	9	5	1.06 ± 0.18	1.77 ± 0.30[c]	42 ± 4
translocation	10	5	1.78 ± 0.32	2.98 ± 0.54[c]	58 ± 6
hydei					
wild type	8	4	0.97 ± 0.16	1.62 ± 0.27[c]	40 ± 4
deletion	8	4	0.41 ± 0.10	0.69 ± 0.17[c]	24 ± 4
virilis	5	3	1.27 ± 0.17	2.12 ± 0.28[c]	47 ± 4
americana	3	3	3.00 ± 0.66	5.00 ± 1.1[c]	79 ± 10

[a] Number of separate measurements of retardation time, whether on the same or on different lysates.
[b] Precision measure reflects average deviation only; see text.
[c] Calculated by multiplying the values in the third column by 1.67.

latter and molecular weight, as well as by direct measurement of τ on the DNA's of bacteriophages T7 and T2. The resulting relation was confirmed to some degree by the fact that it gave reasonable molecular weights for the chromosomal DNA of the bacteria *B. subtilis* and *E. coli* (2.0×10^9 and 2.7×10^9 respectively). However, we cannot claim great accuracy for this relation, mainly because of lack of accuracy in the molecular weights of the viral DNA's on which it is based (see Freifelder, 1970), but also in consideration of possible effects of residual tightly bound protein. After the long extrapolation to the molecular-weight range of interest in this paper $\pm 30\%$ in the molecular weight seems to be a reasonable estimate of the precision.

The question of what over-all precision to quote for the values of the molecular weights is a vexing one. At the pessimistic extreme, one might simply add the average deviations of the observed numbers to the thirty percent uncertainty of Eq. (2). The result would indicate that the molecular weights were known only within a factor of two. As an optimist, one might divide the average deviation by the square root of the number of observations and combine this with the uncertainty of Eq. (2) by taking the square root of the sum of the squares. This method would suggest that the molecular weights were known within about $\pm 31\%$. Probably the most realistic assessment is somewhere between these extremes. Since we are going to attempt to decide how many molecules

of DNA there are per chromosome, the precision question is an important one. Fortunately other indirect evidence can also be brought into the discussion, as will be seen below.

The precision of the molecular weights relative to one another is much better than that of the absolute values. The precision of the relative values reflects mainly the uncertainty in the retardation times themselves and only to a small extent the uncertainty in the exponent, q, of Eq. (2). In fact, with respect to the comparison of the values of M in Table 2 with one another, the effect of the additional error from Eq. (2) may be ignored, and this is in fact ignored in the precision measures given in Table 2.

In the case of *D. melanogaster*, our values for molecular weight can be compared directly with Rudkin's values for the absolute DNA contents of individual mitotic chromosomes. The latter values, based on measurements of UV absorbance (Rudkin, 1964, 1965, and personal communication), are: 26×10^9 for the X-chromosome, 40×10^9 for chromosome 2, 43×10^9 chromosome 3, and 4×10^9 for chromosome 4. The sum of the values for the DNA contents of a haploid set of chromosomes is 113×10^9 daltons; this agrees with the value of 108×10^9 daltons determined by comparative microspectrophotometry of Feulgen-stained sperm and male somatic cells by Rasch *et al.* (1971), although a somewhat lower value, 90×10^9 daltons, was estimated from measurements of renaturation rate constants by Laird (1971). Our values for the molecular weight of the largest DNA molecules from the wild-type *D. melanogaster* are in agreement with Rudkin's values for the total DNA contents of the largest *D. melanogaster* chromosomes, 2 and 3. According to Rudkin's data, chromosomes 2 and 3 differ in DNA content by only about 5%, corresponding to a difference in relaxation time of only about 13%. Since our experimental uncertainties in relaxation time were at least 11%, we do not believe we could have distinguished between the relaxation of chromosome-sized DNA molecules from chromosomes 2 and 3.

2. Comparison with Chromosome Morphology

The comparison of DNA molecular weight with chromosome morphology offers another approach to elucidation of the relation of DNA size to chromosome structure. In this approach the uncertainty in the absolute values of the molecular weights is not involved; only the ratios are important, and these are much more accurate. Such comparisons are shown in Fig. 4.

The fact that the inversion strain gave values identical to those of the wild type indicates that the chromosomal DNA is continuous at the centromere. The inversion strain carried a pericentric inversion in

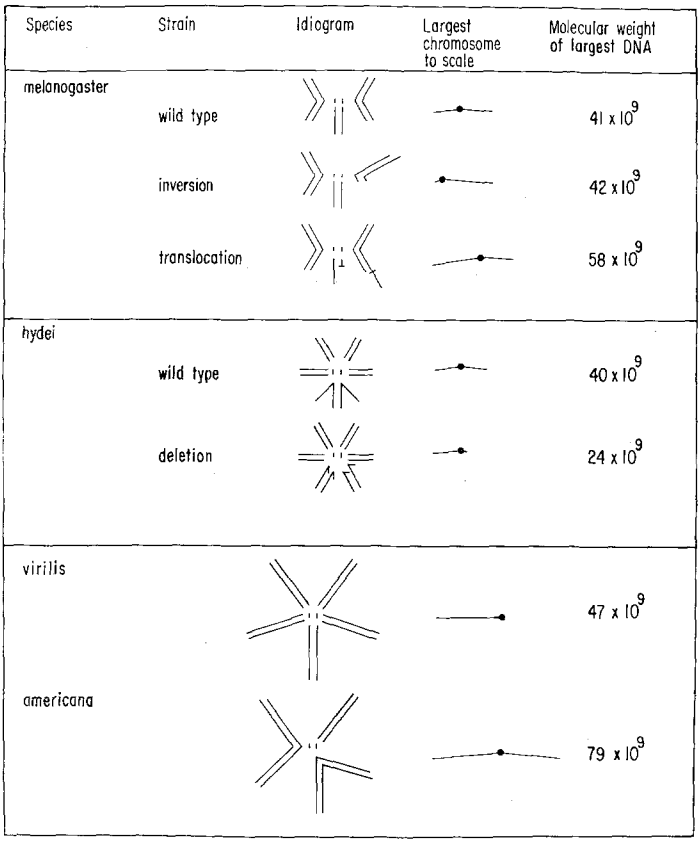

Fig. 4. Comparison of cytological chromosome size with molecular weight of largest DNA molecule for *Drosophila* species. The molecular weight of the largest DNA molecule from each species (from Table 2) is given in the last column. The idiogram, an idealized representation of the appearance of the chromosomes at metaphase, is given in the third column. In the fourth column the largest chromosome is extracted from the idiogram and drawn to scale, insofar as current information permits, so that its apparent length is proportional to its DNA content. The black dots in this column locate the centromeres. In the case of the *melanogaster* inversion and translocation strains the size was deduced from the genetic map (Lindsley and Grell, 1968). Idiograms represent females only; the X-chromosomes are shown at the bottom of each idiogram. Data on *melanogaster* from Lindsley and Grell (1968) and from Rudkin (1965, and personal communication), *hydei* from Hennig (1972, and personal communication), from E. Rasch (personal communication) and from Mulder *et al.* (1969), on *virilis* and *americana* from Patterson and Stone (1952) and from E. Rasch (personal communication); see text

chromosome 3 which changed the ratio of the arms in the metaphase chromosome from the wild-type value of about 4:4 to one which we estimate, taking account of the centromeric heterochromatin, as about 7:1. If the DNA were discontinuous at the centromere, the DNA molecules from the inversion strain should have included molecules about 75% larger than those from the wild-type, and the retardation time for the inversion should have been about 150% larger than that for the wild-type. We feel confident that we could have detected a 150% increase in retardation time, since we detected significantly larger DNA molecules from not only the translocation strain of *D. melanogaster* but also from the wild-type of *D. americana americana*.

The value for the molecular weight of the largest DNA molecules from the translocation strain also agrees with the sum of Rudkin's values for chromosome 3, allowing for the small amount of chromosome 3 translocated to the X, and for the 60% of the (metaphase) X-chromosome attached to one end of chromosome 3; on the basis of Rudkin's figures, this would be an increase in total DNA content of about 37% over that of the wild-type chromosome 3. We found that the largest DNA molecules from the translocation strain were about 40% larger than those in the wild-type. Moreover, the observed increase in the size of the DNA from this strain, 17×10^9 daltons, leads to an estimate of 28×10^9 daltons for the total DNA content of the X-chromosome, in fair agreement with Rudkin's value of 26×10^9.

The results for the other *Drosophila* species also show a correlation between the size of the largest detectable DNA molecules and the DNA content of the chromosomes, although the analysis is hindered by lack of data for the individual chromosomal DNA contents comparable to Rudkin's data for *D. melanogaster*. That good qualitative correlations can be made is evident from Fig. 4. Thus, in *D. hydei* the molecular weight obtained for the largest DNA molecules from the wild type was almost twice that for the strain in which the X-chromosome, which is the largest chromosome in the wild-type, is missing most of the heterochromatic arm. Values for the total DNA content of the *D. hydei* haploid genome are slightly higher than those for *D. melanogaster:* 120×10^9 daltons (Rasch, personal communication), 140×10^9 daltons (Dickson *et al.*, 1971) and 150×10^9 daltons (Mulder *et al.*, 1969). By comparative studies of the DNA's from the wild type and from the same strain that was used in this study, Hennig (1972) has shown that the heterochromatic arm of one X-chromosome corresponds to at least 6% of the total DNA of the genome. If we take Rasch's figures to apply, the total amount of the diploid genome would be 240×10^9 daltons, so that the deleted arm would be at least 14.4×10^9 daltons. Our data on the two strains show that the deleted arm, taken as the difference, is at least 16×10^9 daltons (more if

the X-chromosome is not the largest in the deletion strain); the two results are thus in substantial agreement.

The relation between two species of the *virilis* group of *Drosophila*, *D. virilis* and *D. americana americana*, is especially interesting. The V-shaped chromosomes in *americana* presumably arose from the rod-shaped chromosomes of *virilis* (or a common ancestor) by Robertsonian fusion, as indicated by the fact that the individual arms of the *americana* chromosomes pair with individual rods of *virilis* in hybrids (Patterson and Stone, 1952). Our values for the sizes of the largest DNA molecules extracted from these species are in agreement with the cytological observations, with the value for *americana* being about twice that for *virilis*.

In the haploid chromosome set of *virilis* there are five rods, all about equal in size. Multiplying our value for the molecular weight of the *virilis* DNA molecules by five yields an estimate for the genome size of 235×10^9 daltons, in reasonable agreement with the values of 204×10^9 (male) and 235×10^9 (female) obtained from comparative microspectrophotometry of Feulgen-stained hemocytes of this same strain by E. Rasch (personal communication). In the case of *hydei* each arm of the X-chromosome appears to be approximately equal to the whole of each of the four autosomes; thus the total haploid complement should be three times the mass of the X. Multiplying our value of 40×10^9 (which presumably corresponds to DNA from the X) by three leads to 120×10^9, in fair agreement with the values of 120 to 150×10^9 cited above for the total complement.

Thus there is substantial agreement between the sizes of the DNA molecules extracted from the various strains and the DNA contents of the largest chromosomes as determined by cytological and genetic observations.

IV. Conclusions

It remains to relate these results on the DNA to the structure of the chromosome from which it was derived. The following observations are important: (1) The size of the largest molecule of DNA changes in direct proportion to the size of the largest member of the mitotic chromosome set in *melanogaster* through an inversion and a translocation, in *hydei* through a deletion, and from *virilis* to *americana* through a fusion (Fig. 4). These results indicate that the DNA molecules run the full length of the chromosome. Further, within the rather rough precision of the numbers, we find that (2) the largest DNA molecules are equal in mass to the DNA content of the largest chromosomes in the (anaphase) mitotic set, and (3) the yield of such large molecules is approximately one per chromatid. In addition we can cite the results of Laird

and co-workers that there is in *Drosophila* only one copy of a large number of sequences of DNA per haploid DNA complement (Laird, 1971, personal communication; Dickson *et al.*, 1971).

These results lead to only one obvious conclusion, which can be summarized, subject to some disclaimers and qualifications, by the statement: *In its simplest form one chromosome contains one long molecule of DNA*. The qualification, "in its simplest form," is necessary because of the generality of the term, chromosome; here we refer to the chromosomes of normal cells from anaphase through G_1, or to the chromatids of metaphase chromosomes. Clearly the giant metaphase chromosomes of some neuroblasts (Gay *et al.*, 1970), or the polytene chromosomes of the salivary cells, or chromosomes in the process of duplication are more complicated. It must also be admitted that there may be some molecules of nuclear DNA which are much smaller than chromosome size, since with our technique it is difficult to measure such molecules in the presence of the larger ones. (It is interesting, however, that the AT-rich satellite DNA located in the centromere region of *D. melanogaster* chromosomes appears to be linked covalently to the main band (Kram *et al.*, 1972)). Thus the conclusion above obviously may not apply to all cases. Nevertheless it should have a sufficient range of validity to be useful in understanding the behavior of chromosomal genes, especially with respect to linkage, recombination, and mutation.

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