SIMILARITY IN BASE COMPOSITION OF HETEROCHROMATIC AND EUCHROMATIC DNA IN *DROSOPHILA MELANOGASTER*¹

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THE chromosomes of eukaryotes are generally subdivided by cytological and operational criteria into heterochromatic and euchromatic regions. Originally, the term heterochromatin was used by HEITZ (1928) to distinguish those heteropycnotic regions of the chromosomes of liverworts and mosses, which remain condensed and intensely stainable during mitotic interphase. The concept of heterochromatin acquired genetic significance when it was demonstrated that the heteropycnotic regions of *Drosophila* chromosomes corresponded to the known regions of "genetic inertness" within these chromosomes (MULLER and PAINTER 1932; HEITZ 1933; KAUFMANN 1934). Other distinctive properties of *Drosophila* heterochromatin were revealed in studies of the giant chromosomes of larval salivary glands. Portions of these specialized chromosomes corresponding to the heteropycnotic regions of mitotic chromosomes were found to be reduced in size relative to the euchromatin, and aggregated to form the chromocenter (BRIDGES 1935). In addition to the chromocentral heterochromatin there are known regions of intercalary heterochromatin whose existence has been demonstrated by a high X-ray-breakage frequency (KAUFMANN, 1939, 1946; PROKOFEIVA-BELGOVSKAYA and KHVOSTOVA 1939), and ectopic pairing (SLIZYNSKI 1945; KAUFMANN and IDDLES 1963).

During the nearly forty years that *Drosophila* heterochromatin has been subjected to critical analyses, the concept of genetic inertness due to the absence of Mendelian genes has been modified somewhat to credit heterochromatin with only relative genetic inertness, or with some specialized regulatory role in the metabolism of the cell. Recent investigations, both biochemical (RITOSSA and SPIEGELMAN 1965) and cytogenetical (HESS 1967) have clearly demonstrated loci within the major heterochromatic regions that are essential for the normal functioning of the organism. Evidence for the regulatory properties of *Drosophila* heterochromatin is based largely upon the well-documented phenomenon of heterochromatin-induced position effect (MULLER 1930; SCHULTZ 1936, 1965), wherein the transposition of a normally active locus to a position adjacent to the centric heterochromatin results in the complete or partial inactivation of the translocated gene.

While there have been considerable advances in our understanding of the functional properties of heterochromatin, there is little commensurate knowledge

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of the physico-chemical differences between heterochromatin and euchromatin. Although numerous hypotheses have been advanced to explain the manifold properties of *Drosophila* heterochromatin (reviews by HANNAH 1951; COOPER 1959), a lack of critical experimental techniques has prevented the verification or rejection of the majority of these proposals. In the present paper we examine one concept of the nature of heterochromatin and attempt to provide a reasonable test of its validity.

It is important to make a distinction at this point between heterochromatin as it is manifested in *Drosophila* by the above mentioned cytological and genetical criteria and the facultative heterochromatinization which is observed in nearly all chromosomes when condensed in mitosis or meiosis. In this paper we take the viewpoint that a molecular explanation for the properties of one may not necessarily satisfy the known facts about the other. For purposes of discussion we will refer to findings on the nature of heterochromatin in organisms other than *Drosophila* but do not wish to imply that our findings are directly applicable to other systems.

It has long been known that duplications or deletions of considerable amounts of *Drosophila* heterochromatin may be without pronounced phenotypic effect. On the other hand, some regions of heterochromatin may be additive in their quantitative effects (MATHER 1941), or distinctive in the extent to which they can control position effect variegation (KAUFMANN 1942). By analogy with the heterochromatic B chromosomes of maize, which may be absent without harmful effects, it seemed reasonable to consider large amounts (but not all) of *Drosophila* heterochromatin as "repetitious and non-genic nucleoprotein" (MULLER 1947). Essentially this same proposal, in more modern terms, was formulated by HERSKOWITZ in 1961. He suggested that heterochromatic regions of chromosomes might contain DNA which is a copolymer of two of the common DNA bases, i.e. an AT copolymer; or alternatively, heterochromatin might be composed of a GC copolymer in organisms which have a high GC content. This interesting suggestion was based upon the then newly reported isolation of an AT copolymer from certain species of crab (SUEOKA 1961), and the report of HSU and SOMERS (1961) that bromodeoxyuridine (a thymidine analog) might be preferentially incorporated into heterochromatic regions of chromosomes.

Recently, the HERSKOWITZ proposal was tested by VAN SCHAIK and PITOUT (1966) who made DNA base-ratio determinations on two stocks of maize which differed only in that one carried no heterochromatic B chromosomes, while the other contained an average of four such chromosomes per cell. Their results indicated that DNA isolated from these two stocks differs by 15% in GC content, and they have concluded that the heterochromatic B chromosomes are composed of a DNA which is mainly or entirely GC.

SCHURIN and MARMUR (1961) reported that the DNA of *Drosophila virilis* adults displays two distinct bands in density gradient centrifugation, the difference in densities corresponding to about 8% difference in GC. Since this difference might be a reflection of differences in heterochromatic and euchromatic DNA, we decided to test the hypothesis that in *Drosophila*, heterochromatin and

euchromatin differ significantly in DNA base composition. To accomplish this we took advantage of the fact that the large Y chromosome of *Drosophila melanogaster* is essentially heterochromatic. In addition, the proximal one-third of the X-chromosome and one-fifth of each limb of the second and third chromosomes have been identified as heterochromatic (KAUFMANN 1934). By making use of several compound sex chromosomes that are maintained in special stocks of this species, it is possible to obtain flies that are X0, XX, XY, XXY or XYY, while maintaining the usual number of autosomes. Each of the above karyotypes differs in the relative amount of constitutive heterochromatin. By isolating DNA from these karyotypes we are in a position to detect possible variations in DNA base composition associated with differences in amount of heterochromatin.

MATERIALS AND METHODS

Drosophila stocks used were: $Y^S \cdot X$ In EN, $\gamma \cdot Y^L$ $sc^8 \gamma^+ / \gamma^2 w^a spl bi$ (no free Y), from which the X0 and XXY karyotypes were obtained; $sc^8 \cdot Y / \gamma ac v / \gamma ac v$, which, by outcrossing with γ , permitted detection of the XYY karyotype; and wild-type Swedish-b, from which the XX, XY, and mixed XX, XY flies were obtained. Flies were raised on standard corn-meal medium in half pint bottles at 25°C. Adults were etherized, separated according to karyotype, frozen and stored at -20°C prior to extraction of DNA.

Calculation of the amount of heterochromatin per karyotype: The approximate volume of a metaphase chromosome may be calculated from its length and diameter. Multiplying the chromosome volume by the specific fraction of the chromosome that is heterochromatic (see Table 1) gives the heterochromatic volume of the chromosome. On the basis of such calculations, we were able to construct a table of amounts of heterochromatin relative to the total chromatin for each of the karyotypes used in this study (Table 1).

There are two fundamental assumptions which are made in the preceding calculations: first,

TABLE 1

*Method of calculating the relative heterochromatic volume of
Drosophila melanogaster chromosomes*

Chromosome	Dimensions** μ	Chromosome volume $\mu^3 \times 10^2$	Fraction heterochromatic	Heterochromatic volume $\mu^3 \times 10^2$
X	1.8×0.3	12.7	0.33	4.2
Y	1.9×0.3	13.4	1.0	13.4
II	2.6×0.3	18.4	0.20	3.7
III	3.2×0.3	22.6	0.20	4.5
IV	0.2×0.2	1.4	0.0	0.0
Percent heterochromatin/total chromatin of karyotypes				
Karyotype		Percent heterochromatin		
X0		21.2		
XX		22.5		
XX,XY*		26.0		
XY		30.7		
XXY		30.9		
XYY		38.0		

* denotes unseparated, approximately equal numbers of XX and XY

** KAUFMANN 1934

that the relative heterochromatic volume of a chromosome in the adult (from which the isolated DNA is obtained) is essentially the same as in the prophase or metaphase chromosome of the larval neuroblast, whose dimensions have been carefully recorded (KAUFMANN 1934); second, that the amount of DNA per unit of chromosome volume is nearly the same in heterochromatic and euchromatic areas. The first assumption, although untested, provides a reasonable working hypothesis. The second assumption is supported by the data of RUDKIN (1965) who was able to demonstrate by microphotometry that, at least for the X chromosome, the concentration of DNA is identical in the heterochromatic and euchromatic portions at metaphase. RUDKIN also independently estimated the relative amount of heterochromatin of the XX female to be 22%, a figure which corresponds to our estimate in Table 1.

DNA isolation: DNA was isolated from five-gram lots of adult flies by the method of MEAD (1964). This involves homogenization and washing in 0.15 M NaCl + 0.015 M Na-citrate (SSC) to remove RNA and other substances. The DNA was solubilized by action of an anionic detergent (Aerosol OT) and purified by repeated chloroform treatments and enzymic removal of RNA. All procedures were similar to those of MEAD with the single exception of homogenization, which was achieved by using an Oster blender.

Thermal denaturation: Determination of thermal denaturation profiles in SSC was accomplished by means of a model DU Beckman spectrophotometer equipped with thermospacers and a Haake circulating pump. Temperatures were recorded of SSC in a cuvette adjacent to the one containing DNA. Optical density measurements were corrected for thermal expansion of the solvent; and absorbance of the sample relative to the initial (25°C) value was determined at about 1°C intervals in the transition region. Determination of the GC value from the midpoint of the rise in optical density was facilitated by constructing a graph based on the data of MARMUR and DORY (1962).

Base ratio analysis: The entire yield of DNA from each five-gram lot of flies was taken up in 0.05 or 0.1 ml of 72% HClO_4 and hydrolyzed at 100°C for one hr. An equal volume of distilled water was added to the hydrolysate, and 0.02–0.05 ml was applied as a band at the origin of a paper chromatogram. Papers used were Whatman No. 1, cut into strips 4 cm by 40 cm. Development was by descending chromatography for about 24 hours in an isopropanol-HCl system (WYATT 1951). UV-absorbing bands with R_f values corresponding to the four DNA bases were cut out and eluted by overnight incubation in three to five ml of 0.1 N HCl. The optical properties of the eluted bases were measured in a Beckman DU spectrophotometer.

RESULTS

Our attempts to isolate *Drosophila* DNA utilized various modifications of the techniques developed by MARMUR (1961), KIRBY (1964), and RITOSSA and SPIEGELMAN (1965). The most reliable method in our experience is that of MEAD (1964), which was developed especially for *Drosophila*. Using this technique we consistently obtained DNA yields of about 1.0 mg from five grams of adults, corresponding to about five thousand flies.

This is similar to the recovery reported by KIRBY (1964, 1968) and on the basis of our own determinations, represents 40% of the total DNA of the adult fly. The isolated DNA is essentially free of contaminating RNA and protein as judged by orcinol and biuret methods, and by UV optical density measurements ($\text{OD } 260/\text{OD } 280 = 2$). The DNA is eluted as a single peak from a methylated albumin column (Figure 1) and demonstrates an intense hyperchromicity upon heating (Figure 2). Both of these characteristics are evidence that the DNA is in an undenatured form when isolated. No attempt has been made to determine the molecular size of the DNA.

The GC content of DNA from mixed flies (XX, XY) as determined by thermal

FIGURE 1.—Elution of *Drosophila* DNA as a single peak from a methylated albumin column. Broken line indicates molarity of NaCl solution.

denaturation data is approximately 38%. This is in agreement with the value obtained by thermal denaturation (MEAD 1964) and by buoyant density (RITROSSA and SPIEGELMAN 1965). The near perfect superimposition of the thermal denaturation profiles in Figure 2 is an indication that no gross differences exist in the base composition of DNA isolated from karyotypes with large differences in the amount of constitutive heterochromatin. Examination of this figure suggests slight differences in the profiles of some of the DNA samples. When the curves were plotted separately, and the mid-point of the transition used to determine the GC content of the DNA, we obtained the data given in Table 2. Here, slight differences in GC are noted, with a tendency toward higher GC in those karyotypes containing more heterochromatin. Since the determination of GC content by thermal denaturation data is at best an approximation of the true value, we next sought to confirm or reject this possible correlation by the more rigorous method of direct base ratio analysis.

TABLE 2

GC content of the several karyotypes as determined by thermal denaturation of isolated DNA

Karyotype	T _m *	GC (percent)
XX	84.4°	38.2
XX, XY	84.4°	38.2
XY	84.6°	38.5
XXY	84.7°	38.5
XYY	85.2°	40.0

* T_m is the temperature corresponding to the midpoint of increase in optical density.

FIGURE 2.—Thermal denaturation profiles of DNA isolated from the indicated karyotypes.

Table 3 contains a summary of these experiments. The GC content of the DNA of mixed (XX, XY) flies is 39.7%. This value is almost identical to that obtained by MEAD (1964), but is somewhat lower than values reported by FAHMY and FAHMY (1961), KIRBY (1962, 1964) and ARGYRAKIS and BESSMAN (1963). The reason for this discrepancy is not apparent, but since our value is based upon

TABLE 3

Base ratios and Guanine + Cytosine content of Drosophila DNA*

Karyotype	X0	XX	XX, XY	XY	XXY	XYY
Percent heterochromatin	21.2	22.5	26.0	30.7	30.9	38.0
Number of chromatograms	15	19	14	20	18	14
Guanine	19.7 ± 0.4	19.2 ± 0.2	19.3 ± 0.2	18.2 ± 0.1	20.9 ± 0.5	20.1 ± 0.3
Adenine	29.5 ± 0.5	28.4 ± 0.2	29.5 ± 0.5	29.0 ± 0.1	30.1 ± 0.2	28.2 ± 0.2
Cytosine	19.9 ± 0.5	20.9 ± 0.2	20.4 ± 0.2	20.5 ± 0.3	19.1 ± 0.2	19.0 ± 0.2
Thymine	30.9 ± 0.6	31.5 ± 0.2	30.8 ± 0.6	32.3 ± 0.3	29.9 ± 0.3	32.7 ± 0.5
G + C	39.6 ± 0.6	40.1 ± 0.2	39.7 ± 0.3	38.7 ± 0.3	40.0 ± 0.5	39.1 ± 0.4

* Tabulated values are the mean and standard error of the mean for the indicated number of chromatograms.

more determinations than all the others combined, and since the molar equivalence of G and C is generally better in our data, we believe it to be an accurate measure of the true GC content of the DNA.

The GC content of DNA isolated from the different karyotypes shows only a very small deviation from the value obtained for mixed flies. The grand mean value of all measurements indicates a GC content of 39.5%, and the average deviation of the different karyotypes from that mean is 0.4%. From inspection of the data in Table 3 it appears that there is a slight tendency toward lower GC values in those karyotypes with the largest amount of constitutive heterochromatin; a tendency just the opposite of that suspected from the thermal denaturation experiments.

If there is a correlation between the GC content and the amount of heterochromatin of a karyotype, then statistical treatment of these data should produce a regression line whose slope describes the direction and magnitude of the correlation. Further, it is possible to derive an equation which predicts the magnitude and direction of the regression line which would be produced by any given value for the difference in GC content of euchromatic and heterochromatic DNA's. By developing this equation graphically, and superimposing the experimental regression line onto this system we have a means of assaying these data objectively for indications of karyotype differences in GC content, as shown below.

If DNA is distributed in a nearly uniform manner throughout all the chromosomes, in both heterochromatic and euchromatic areas, and, if the sum of the mole fractions of $A+T+G+C = 1$, then an expression for the GC content of an organism's DNA is:

$$\Sigma GC = (\text{het GC}) (\text{het}) + (\text{eu GC}) (\text{eu}) \quad (1)$$

where, ΣGC is the mole fractional GC content of the total DNA, (het GC) and (eu GC) are the mole fractional GC contents of heterochromatic and euchromatic DNA, (het) and (eu) are the relative amounts of heterochromatin and euchromatin expressed as fractional equivalents of percentage (i.e. 30% het = 0.3).

If there is some difference, ΔGC , between the GC composition of heterochromatic and euchromatic DNA's, then we may substitute for the term (euGC), its equivalent, (het GC) $\pm \Delta GC$. The expression above then becomes:

$$\Sigma GC = (\text{het GC}) (\text{het}) + (\text{het GC} \pm \Delta GC) (\text{eu}).$$

By subtraction, the measurable difference in ΣGC of two karyotypes, A and B, which differ in amount of heterochromatin is then given by:

$$\Sigma GCA - \Sigma GCB = (\Delta GC) (\text{het A} - \text{het B}). \quad (2)$$

In this form, ΔGC is the slope of a regression line describing the relationship between amount of heterochromatin and expected difference in measurable ΣGC of two karyotypes. Since knowledge of any two of the above parameters will allow calculation of the third, we may use the expression to predict what effect a given ΔGC will have on the difference in measurable ΣGC of any two karyotypes, or conversely, from measurements of the $\Sigma GCA - \Sigma GCB$ we may calculate the value of ΔGC which would produce such an effect.

The usefulness of equations (1) and (2) in the interpretation of our experimental data is demonstrated in Figure 3. This figure contains the experimentally

FIGURE 3.—The experimental regression line (solid line) and the 95% confidence limits of its slope, (shaded area) are plotted together with the theoretical regression lines (dotted) which would result if the difference in GC content (ΔGC) between heterochromatic and euchromatic DNA equals the amounts indicated; fuller discussion in text. The slight regression slope obtained ($\Delta GC = -4.6\%$) does not deviate significantly from zero.

derived regression line which relates the observed value for GC composition of the isolated DNA to the difference in percent heterochromatin of the karyotypes studied. The regression line is represented by the heavy black line in the figure, and it is accompanied by the 95% confidence limits of the slope of the true regression line (shaded area). On the same axes are plotted the theoretical regression lines which result from assigning values of +20%, 0% and -20%, to the hypothetical ΔGC in equation (2). Also on this figure are two regression lines corresponding to the postulates $het = poly\ AT$ (97% AT), and $het = poly\ GC$ (97% GC). The latter two slopes were obtained by substituting into equation (1) the measured GC content of the XY male fly (38.7%), and the postulated value for $het\ GC$ (either 3% or 97%). By solving equation (1) for (eu GC) and then taking the difference between the assigned ($het\ GC$) and the calculated (eu GC), we obtain values for ΔGC which are valid for the particular set of conditions listed.

From examination of Figure 3 it is apparent that the experimental data are not compatible with the concept that *Drosophila* heterochromatic DNA consists of a poly AT or poly GC complex; or even that heterochromatic and euchromatic DNA's differ by as much as 20% in GC composition. The slope of the experimental regression line corresponds to a difference of 4.6% in the GC content of *Drosophila* heterochromatic and euchromatic DNA. However, the 95% confidence limits of the slope of the regression line extend from +3.1% to -12.3% and thus include the possibility that $\Delta GC = 0\%$. The conclusion may, therefore, be drawn that the apparent negative slope does not differ significantly from zero. Further confirmation of this interpretation is obtained by calculating the cor-

relation coefficient of the experimental data. By application of this statistic it may be shown that no significant correlation exists between GC content and amount of heterochromatin.

DISCUSSION

The data presented in the preceding section clearly eliminate the possibility that *Drosophila* heterochromatic DNA is composed of a poly GC or poly AT complex. We would note here that the argument based upon the slope of the experimental regression line need not be invoked to rule out the presence of a poly GC complex. For example, from the observed value of 38.7% as the GC content of the XY male fly, it may be calculated that if the GC content of heterochromatic DNA is 97%, the GC content of euchromatic DNA would have to be about 13%. This is an unacceptable conclusion from a functional standpoint, since it would strictly limit the linear variety of bases upon which the coding potentiality of euchromatic DNA is dependent. Similar calculations based on the premise that heterochromatic DNA has a GC content of 3% leads to a value of 54% for the GC content of euchromatic DNA in the XY male fly. In this case, there is no *a priori* reason for rejecting the concept that heterochromatic DNA might be a poly AT complex. However, this is quite improbable, since as is demonstrated in Figure 3, such a situation would have resulted in a highly significant difference in the measured GC content of the several karyotypes, leading to an experimental regression line of such magnitude that we could not possibly have overlooked it in the experimental data.

The above considerations do not exclude the possibility that long reaches of heterochromatic DNA might be composed of predominantly AT (or GC) pairs. This situation would not be detected by base ratio analysis if there are similar long reaches of GC (or AT) pairs also in the heterochromatin, the two regions serving to counterbalance each other as far as detection by base ratio analysis is concerned. That this is unlikely is shown by two methods. First, the thermal denaturation profiles of those karyotypes which contain the largest amounts of heterochromatin do not exhibit a bimodal transition region as would be expected if two species of vastly different GC content molecules were involved in the denaturation process. Second, preliminary experiments using the method of step-wise elution of DNA from methylated albumin columns (SUEOKA and CHENG 1962) did not demonstrate a population of molecules from XY males that differs in any way from those of the XX female.

We do not conclude from this study that there is no difference in the GC composition of heterochromatic and euchromatic DNA in *Drosophila*, nor do we anticipate that this should be the case. It is reasonable to expect deviation in GC content from one chromosome to another, and from one region of a particular chromosome to other regions of the same chromosome. From the results of our study, however, it does seem probable that the gross morphological and functional characteristics of *Drosophila* heterochromatin do not reside in an unusual base composition of the DNA in these regions. In fact, it is highly unlikely that the heterochromatic and euchromatic DNA's differ by more than about 15% in

overall GC composition, since this would have resulted in a highly significant difference in the measured GC content of the several karyotypes. We would like to emphasize that all of these calculations are based upon the conservative assumption that the concentration of DNA in heterochromatin is nearly the same as that in euchromatin. If, as is sometimes supposed, the concentration of DNA in heterochromatin is greater than that in euchromatin, then the model equation above would have to contain terms for the difference in DNA concentration. The net effect of this substitution would be to increase the difference in measured GC content of the karyotypes for any given value of the theoretical ΔGC . In other words, our ability to detect differences in heterochromatic and euchromatic GC content would have been enhanced rather than hindered in this circumstance.

On the other hand, it should be pointed out that recent evidence indicates that in polytene nuclei some regions of heterochromatic DNA may not replicate to the same extent as the total euchromatic DNA. The effect of this phenomenon on our data is most difficult to assess properly. If this is a general property of polytene nuclei in the adult fly rather than just in the larva where it has been described by RUDKIN (1965) and BERENDES and KEYL (1967), and if there is a significant contribution of polytene nuclei to our total isolated DNA, then our ability to detect differences in heterochromatic and euchromatic DNA's would be lessened to some extent. One significant experiment which bears on this problem is that of RITOSSA and SPIEGELMAN (1965). To obtain different doses of nucleolus organizer DNA these authors used inversions which had varying portions of the X heterochromatin associated with the NO region itself, as well as normal XY and XX flies. If heterochromatic DNA isolated from adult flies is not extracted in the same proportions represented in somatic chromosomes, then these workers would have found in their data a distinction between the saturation plateaus (the percent total isolated DNA which is capable of hybridizing ribosomal RNA) obtained with XX and XY DNA, since the XY karyotype contains a relatively larger proportion of heterochromatic DNA than the XX karyotype although each has the same amount of NO DNA.

If the interpretation of our data is correct, then there is no presently understood prohibition against the coding potentiality of heterochromatic DNA in *Drosophila*. That is, all four DNA bases must be present in these regions, and their distribution must be such that they are not absolutely clumped into AT or GC rich regions. If the data of VAN SCHAIK and PITOUT should prove to be correct, then it would appear that the heterochromatin of *Drosophila* and of maize B chromosomes differ considerably in chemical properties despite some cytogenetic similarities. It is perhaps significant that there are known genes within the heterochromatic regions of *Drosophila* whose role we may assume to be the synthesis—or at least the accumulation—of some product, which directly enters into the metabolic activity of the cell (COOPER 1959; RITOSSA and SPIEGELMAN 1965; HESS 1967). As far as is presently known there are no comparable loci in the maize B chromosome. The deleterious effect of an abundance of B chromosomes (RANDOLPH 1941) and the role of B chromosomes in the elimination of the heteropycnotic knobs of the A chromosomes (RHOADES, DEMPSEY and GHI-

DONI 1967) may, or may not, turn out to be the manifestation of some structural gene activity. It is conceivable that such effects could result from the need of the cell to maintain and reproduce a large number of chromosomes, each of which is utilizing primarily only two of the nucleoside triphosphates available in the pool for synthesis of DNA. This is purely speculative but it might prove a fruitful area of investigation if the DNA of B chromosomes is in fact mainly or entirely GC.

The results of our study are consistent with the concept of the nature of heterochromatin that has been developed in studies of facultative heterochromatinization of the mealy bug *Pseudococcus citri*. In this interesting organism the entire paternal set of chromosomes becomes genetically inactivated and heterochromatinized early in development of the males. LOEWUS and BROWN (1964) have shown that no change in GC content of the chromosomes accompanies this dramatic change in function and morphology. BERLOWITZ (1965) demonstrated cytochemically what appears to be a significant accumulation of histone protein in these chromosomes, and that this is in some way associated with their heterochromatinization. However, in a biochemical study, COMINGS reports in an abstract (1967) that he did not detect qualitative or quantitative differences in histones isolated from male and female coccids. In a similar type of analysis he reports failure to find any difference in basic proteins between X0 and XYY karyotypes in *Drosophila melanogaster*. HIMES (1967) has obtained cytochemical evidence that the distinguishing characteristic of maize B chromosomes may lie in the non-histone acid proteins, and not in the histone composition.

From these different approaches it is apparent that an understanding at the molecular level of the nature of heterochromatin must involve not only an analysis of nucleic acids and proteins but their relationship to each other. In this quest, the fact must not be overlooked that in *Drosophila* abundant genetic and cytogenetic evidence has been presented (for example, by STERN and KODANI 1955; COOPER 1959; KAUFMANN 1942; HANNAH 1951) that differences exist among heterochromatic regions within a given karyotype. For that reason, analysis at the molecular level cannot be specified in terms of heterochromatin *per se* but in the potential of specific portions thereof to control or modify in an experimentally detectable way the metabolic activity of a given genome.

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SUMMARY

DNA was isolated from a number of karyotypes of *Drosophila melanogaster*, including X0, XX, XY, XXY, and XYY. Base-ratio analysis of the DNA's revealed a mean value of 39.5% as the GC content of *Drosophila* DNA with little variation in the different karyotypes. No significant correlation was obtained between the amount of heterochromatin of the karyotype and the GC content of the isolated DNA. These data have been interpreted to mean that the distinguishing characteristic of *Drosophila* heterochromatin must reside in some property other than an unusual base composition of the DNA in these regions.

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