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Replication in *Drosophila* Chromosomes : Part VI—Relative Heterochromatin Content & Autonomous Endoreduplication Cycles of Hetero- & Eu-chromatin in Brain Cells of *Drosophila nasuta* Larvae

S. C. LAKHOTIA & MAHESH KUMAR*
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005

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Area of heterochromatic chromocentre in 6 hr old embryonic and in brain cells of late 3rd instar larvae of *D. nasuta* has been measured from Hoechst 33258 stained fluorescence preparations. The nuclear as well as the chromocentre (cc) size varies widely in 3rd instar larval brain cells and in majority of them both these areas are considerably larger than the corresponding regions in embryonic cells. However, the relative area of cc in a nucleus in larval brains is in general, lesser, although in some nuclei it is much larger, than in embryonic cells. Autoradiographic grain count data from preparations of late 3rd instar larval brains labelled chronically *in vivo* with ^{3}H-thymidine (supplemented in food) from hatching to late 3rd instar stage, show that the area of cc or non-chromocentre regions (ncc) is highly positively correlated with the degree of ^{3}H-thymidine incorporation in respective areas. It is, therefore, suggested that different nuclei in larval brains have DNA content in excess of 2C/4C levels seen in embryonic cells. Since the relative cc area and the relative ^{3}H-thymidine incorporation in cc region varies widely in different brain cells, it appears that different cells in brain of 3rd instar larvae of *D. nasuta* have varyingly disproportionate amount of hetero- and euchromatin. Late 3rd instar larval brains are labelled *in vitro* for a long period of 60 hr with ^{3}H-thymidine and autoradiographic preparations are made after different periods (0, 24, 48 and 64 hr) of chase in 'cold' thymidine supplemented medium. In different samples, in addition to unlabelled and completely labelled nuclei, a significant proportion of interphase cells are seen in which either heterochromatin only or euchromatin only was labelled. It seems that the hetero- and euchromatin regions undergo temporally dissociated independent endoreduplication cycles in different brain cells of larvae.

IN *Drosophila*, many tissues of larvae, pupae and adults contain endoreduplicated nuclei with or without the formation of typical polytene chromosomes[1,2]. In polytene nuclei of larval salivary glands the various types of heterochromatin, rDNA and euchromatin replicate differentially and thus achieve different levels of polyteny[3-9]. While extensive work has been done on underreplication of heterochromatin, etc. in polytene cells of *Drosophila*, there are fewer similar studies on non-polytene cells[10]. Earlier cytophotometric studies of Berendes and Keyl[11] on larval brain cells of *D. hydei* indicated a variable heterochromatin content in endoreduplicated but non-polytene brain cells and they suggested that this variable hetero- and euchromatin content is brought about by their independent replication cycles. We have examined this aspect in detail in brain cells of late 3rd instar larvae of *D. nasuta*. As shown earlier[12,13], the different heterochromatin regions in *D. nasuta* are relatively homogenous in their cytological and molecular properties and in all interphase nuclei the entire heterochromatin remains condensed in a single compact chromocentre region. This feature makes it easier to assess the relative heterochromatin content in different cell types by cytological methods. Our observations suggests that different brain cells have varyingly higher and disproportionate amounts of hetero- and euchromatin compared to the embryonic cells. Furthermore, results of ^{3}H-thymidine autoradiography are also presented which demonstrate autonomous endoreduplication cycles of hetero- and euchromatin in the larval brain cells. These data were briefly reported earlier[14,15].

Materials and Methods

These studies have been done on a wild type strain (Varanasi) of *Drosophila nasuta*. The flies and larvae were reared on standard Agar-Maize powder-brown sugar-yeast food at 20° ± 0.5°C.

Measurement of chromocentre and nuclear areas in embryonic and larval brain cells — Air-dried preparations (without colchicine and hypotonic pretreatments) from 6 hr old embryos and from brian ganglia (carefully freed of all adhering tissues) of late 3rd instar larvae of *D. nasuta* were made, stained with Hoechst 33258 (H) and examined for their fluorescence patterns as described earlier[12]. The heterochromatic chromocentre (cc) in H-stained interphase nuclei of *D. nasuta* fluoresces very brightly

*Present address : Biochemistry & Food Technological Division, Bhabha Automic Research Centre, Bombay 400085

compared to the euchromatic non-chromocentre (nce) regions (Fig. 1) and therefore, the ce and nce regions can be very sharply demarcated. The fluorescence patterns of interphase nuclei from embryonic and larval brain preparations were photographed on OrWo DK5 high contrast film at an initial magnification of 400x. The films were developed in an ultrafine grain developer ("Promicrol", May & Baker) to obtain maximum contrast. The developed negatives were placed in a photographic enlarger and the outlines of enlarged images (final magnification = 3200x) of nuclei and ce regions were carefully traced out on mm^2 graph paper. From these tracings, the total nuclear area as well as the ce and nce areas (total nuclear area-ce area) were calculated in terms of μm^2.

Labelling of larval brain ganglia in vivo by feeding on ^{3}H-thymidine supplemented food — The relationship between the ce and nce areas of a nucleus and the relative DNA contents have been ascertained indirectly by chronic labelling of larval brain ganglia *in vivo* with ^{3}H-thymidine and subsequent autoradiographic determination of the degree of radioactivity incorporated in the two regions in different nuclei. *D. nasuta* eggs, collected at 30 min intervals, were transferred to *Drosophila* food supplemented with 5-methyl-^{3}H-thymidine (5 μCi/g food; sp. act. of stock solution of ^{3}H-thymidine, obtained from BARC, Trombay, was 22.5 Ci/mM) and the hatched larvae were allowed to feed and grow on this food at 20°±0.5°C till late 3rd instar stage (about 200 hr after hatching). The brain ganglia from female larvae were dissected out, freed of adhering tissues and were immediately fixed in fresh aceto-methanol (1 : 3). Air-dried preparations from these brain ganglia were treated with 5% trichloroacetic acid at 4°–6°C for 5 min, washed, dehydrated and coated with Ilford L4 nuclear emulsion (1 : 1 dilution) for autoradiography. After 14 days' exposure in dark at 4°-6°C, all the slides were developed with Kodak D 19 b developer in the usual manner. The developed slides were stained with 5% Giemsa (*p*H 7) for 15–20 min. The number of silver grains present on the ce and nce regions in different nuclei were recorded. The areas of respective ce and nce regions were also measured from their camera-lucida drawings (at magnification = 1200x).

Patterns of replication of hetero- and euchromatin in 3rd instar larval brain cells — To ascertain if the hetero- and euchromatic regions in different nuclei in 3rd instar larval brains of *D. nasuta* replicate equally, the brain ganglia from late 3rd instar larvae were dissected out and cultured *in vitro* in a culture medium modified after Singh[16]. Immediately after the initiation of *in vitro* culture, ^{3}H-thymidine (sp. act. 6.5 Ci/mM) was added to the medium at a final conc. of 1 μCi/ml. The cultures were incubated for 60 hr at 20°±0.5°C, after which the radioactive medium was removed and the ganglia washed 3–4 times with isotope-free medium. One sample of ganglia was transferred to fresh medium containing colchicine (1 μg/ml) for 1 hr, after which they were processed for making air-dry preparations. Other ganglia were transferred to fresh medium supplemented with 'cold' thymidine (25 μg/ml) and incubated further at 20°C. At intervals of 24, 48 and 66 hr, ganglia were removed from the medium and processed for air-dried chromosome preparations after 1hr colchicine pretreatment as above. All the preparations were autoradiographed as above and after 15 days' exposure, they were developed, stained and examined for the patterns of ^{3}H-thymidine incorporation in hetero- and euchromatin in interphase and metaphase cells.

Results

Heterochromatin content in interphase nuclei — The interphase nuclei in 6 hr old embryos and in 3rd instar larval brains have a compact ce which fluoresces very brightly with H (Fig. 1). The brain cell nuclei show a great variation in their size as compared to the embryonic cells. The brightly fluorescing ce also shows considerable variation in brain

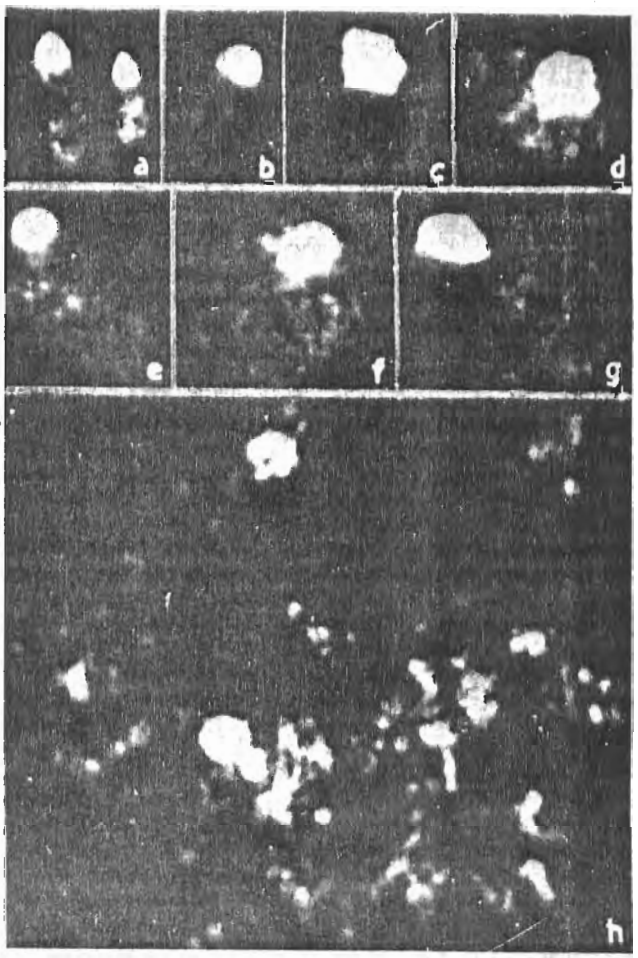

Fig. 1 — Hoechst 33258 fluorescence of non-hypotonic treated nuclei from 6hr old embryos (a) and from brain ganglia of female late 3rd instar larvae (b-h) of *D. nasuta*. In brain cells, nuclei of different sizes, including some with low level polytene chromosomes (h), are seen and in these the area of the brightly fluorescing chromocentre is highly variable. Embryonic nuclei are relatively smaller and uniform in size, × 2000

cells but the fluorescence intensity of the cc and ncc regions is not affected by their size variations (Fig. 1). The cc and ncc sizes in 107 embryonic and 125 brain cell nuclei have been measured. Nuclei were photographed randomly from at least 5 preparations of the two cell types. Data on the cc and ncc size ranges in these samples of embryonic and larval brain cells are presented in Table 1. These data clearly show that in majority of larval brain cells, the cc as well as ncc size is much bigger than in embryonic cells.

Since the cc and ncc areas in larval brain cells are considerably larger than in embryonic cells, it was of interest to know if the cc and ncc areas in a nucleus in larval brain have increased proportionately. For this purpose, the relative cc area in each of the measured nucleus, has been calculated in terms of per cent cc area $\left(\frac{\text{cc area}}{\text{nuclear area}} \times 100\right)$ and the data are presented in Table 1. In the embryonic cell sample, majority of nuclei have cc area between 9 and 23 % of total nuclear area. In 3rd instar larval brain cell sample, on the other hand, a significant proportion (35%) of nuclei have relatively smaller chromocentres with relative cc areas being only 4 to 8% of nuclear area. Interestingly, however, a few nuclei in 3rd instar larval brain sample, had relative cc area as large as 38, 44 and 68% of the nuclear area (Table 1).

Relationship between cc and ncc areas and their DNA content — The considerably larger and variable cc and ncc areas observed in the larval brain cells may be due to a variable DNA content in different nuclei. To check this possibility, the relative DNA content of cc and ncc regions in different nuclei in 3rd instar larval brain cells has been assessed indirectly by autoradiography following continuous feeding of larvae (from hatching to late 3rd instar stage) on ^{3}H-thymidine-supplemented food. The numbers of silver grains on cc (in 226 nuclei) and on ncc (in 342 nuclei) regions in autoradiographs were counted. The areas of cc and ncc regions in these same nuclei were also measured. The cc and ncc sizes vary considerably and at the same time the amount of radioactivity contained by them, as estimated from silver grain counts, also varies. In this sample of nuclei, the cc size and the number of silver grains on cc vary from 1.4 to 12.5 μm^2 and 2 to 42, respectively. The ncc size and the number of silver grains on ncc vary from 7 to 352 μm^2 and 5 to 140, respectively. A statistical analysis of the data reveals that for cc as well as ncc regions, the area is highly positively correlated with the number of silver grains over the region (r for cc area and silver grains = +0.85; r for ncc area and grain counts = +0.65). For convenience of presentation, the 225 cc measurements have been grouped into 6 groups (Table 2). The mean cc area and the mean number of silver grains present over the cc region in nuclei belonging to one group have been calculated and are presented in Table 2. Similarly, the 342 nuclei from which the ncc area was measured, have been categorised into 8 groups on the basis of their ncc size (Table 3) and the mean area and the mean number of silver grains on ncc for each of the size classes were calculated. The grouped data show

TABLE 2 — RELATIONSHIP BETWEEN AREA AND *in vivo* ^{3}H-THYMIDINE INCORPORATION OF CHROMOCENTRE IN DIFFERENT NUCLEI (GROUPS I TO VI) IN LATE 3RD INSTAR LARVAL BRAIN GANGLIA OF *D. nasuta*

Group	No. of nuclei	Chromocentre area (μm^2)		Autoradiographic grain density	
		Range	Mean	Range	Mean ± SE
I	49	1.0—2.0	1.75	2—12	6.5±0.1
II	75	2.1—4.0	3.15	2—23	9.0±0.4
III	64	4.1—6.0	4.82	5—21	13.9±0.5
IV	25	6.1—8.0	7.00	6—42	19.0±1.2
V	7	8.1—10.0	9.00	18—28	22.0±1.3
VI	6	10.1—12.6	11.30	16—33	22.7±2.4

TABLE 3 — RELATIONSHIP BETWEEN AREA AND *in vivo* ^{3}H-THYMIDINE INCORPORATION OF NON-CHROMOCENTRE REGION IN DIFFERENT NUCLEI (GROUPS I TO VIII) IN LATE 3RD INSTAR LARVAL BRAIN GANGLIA OF *D. nasuta*

Group	No. of nuclei	Non-chromocentre area (μm)^2		Autoradiographic grain density	
		Range	Mean	Range	Mean±SE
I	17	5—20	16.0	5—41	16.6±2.1
II	63	21—40	31.5	6—52	24.9±1.1
III	79	41—60	49.3	12—140	39.7±2.2
IV	66	61—80	69.4	17—84	47.0±1.9
V	34	81—100	87.4	9—106	50.4±3.8
VI	27	101—120	106.3	21—106	51.5±3.8
VII	28	121—160	139.3	28—135	73.0±4.6
VIII	28	161—352	198.8	21—126	76.0±5.1

TABLE 1 — HETERO- AND EUCHROMATIN CONTENT IN 6 HR OLD EMBRYONIC AND LATE 3RD INSTAR LARVAL BRAIN CELLS OF *D. nasuta*

Cell type	No. of nuclei	% Nuclei with different size classes of cc area (μm^2)				% Nuclei with different size classes of ncc area (μm^2)				% cc area $\left(\frac{\text{cc area}}{\text{nuclear area}} \times 100\right)$					
		0.5 to 1.5	1.6 to 2.5	2.6 to 3.5	3.6 to 8.7	2.0 to 10.0	11.0 to 20.0	21.0 to 40.0	41.0 to above	4.0 to 8.0	9.0 to 13.0	14.0 to 18.0	19.0 to 23.0	24.0 to 28.0	29.0 to 68.0
Embryonic	107	52	45	3	0	71	29	0	0	2.5	34.5	38.0	20.0	5.0	0.0
Larval brain	125	1	20	38	41	4	25	48	31	35.0	35.0	18.0	7.0	2.5	2.5

that both in ec and nec regions, there is a very high positive correlation between the area and the degree of ^{3}H-thymidine labelling and thus as the area (of ec or nec) increases, the mean number of silver grains recorded over these regions also increases (Tables 2 and 3).

From these preparations, the %ec labelling indices $\left(\frac{\text{no. of silver grains on ec}}{\text{no. of silver grains in nucleus}} \times 100\right)$ for each of the 226 nuclei in which the grains on ec as well as nec regions were counted, have been calculated. In these nuclei, the % ec labelling indices vary over a wide range from 6.2% to 71.2%, with 21% nuclei in 5 to 15% range, 60% nuclei in 16 to 25% range, 16% nuclei in 26 to 35% range and 3% nuclei from 36% to 71% range. Thus it seems that the relative uptake of ^{3}H-thymidine in ec and nec regions of different nuclei during the larval development period has varied widely

In the autoradiographic preparations of *in vivo* labelled brain ganglia, a few polytene nuclei were also seen. All of them were heavily labelled but significantly in all those polytene nuclei where the ec could be clearly identified, it was found to be completely unlabelled and relatively small.

Patterns of replication of hetero- and euchromatin in late larval brains — As mentioned in Material and Methods, in this set of experiments, the brain ganglia were labelled *in vitro* with ^{3}H-thymidine for 60 hr and were either immediately processed for chromosome preparations (0 hr sample) or were chased in isotope-free medium for 24, 48 or 66 hr before being processed for chromosome preparations. The autoradiographs of these preparations revealed different types of labelling patterns in different interphase nuclei.

The autoradiographic labelling patterns on interphase nuclei in the four samples have been characterised into 4 types, viz. (i) *unlabelled*—with less than 5 silver grains on ec or nec regions; (ii) *completely labelled*—with more than 5 grains on ec as well as nec regions; (iii) *ec labelled*—with more than 10 grains on ec but less than 5 grains on nec region, and (iv) *nec labelled*—with less than 5 grains on ec region. The data on the total number of nuclei examined and the frequency of the different patterns of labelling on interphase nuclei in the 4 samples are presented in Table 4 and some examples from the 0 hr sample are presented in Fig. 2. It is seen that in all the 4 samples among the labelled nuclei, most had labelling on their ec as well as nec regions (completely labelled type), but some nuclei were also seen in which either only the ec or only the nec region was labelled (Table 4 and Fig. 2). The frequency of completely labelled nuclei remains nearly the same except in 66 hr sample in which it goes down. Nuclei with only nec region labelled are less frequent than the ec labelled type (Table 4). Furthermore, it is also of interest to note that in some of the completely labelled type nuclei, the ec appeared to be more heavily labelled than the nec region (Fig. 2j–l).

Discussion

We have analysed the relative content of heterochromatin and euchromatin in larval brain cells of *D. nasuta*. Heterochromatin in *D. nasuta* accounts for about 40% of metaphase chromosome length and all the heterochromatic regions condense together in interphase nuclei to form a compact chromocentre which can be easily demarcated by fluorescence staining[12]. This distinct organization of hetero- and euchromatin in *D. nasuta* makes it very convenient to measure the ec and nec areas of a nucleus. The projection method used to measure the area of nuclear components gives fairly well reproducible results. The measurement of absolute and relative areas of ec and nec regions in different nuclei by this method reveals that embryonic cells have relatively less variable and smaller ec

TABLE 4 — FREQUENCIES OF DIFFERENT LABELLING PATTERNS OF INTERPHASE NUCLEI AT DIFFERENT TIME INTERVALS AFTER *in vitro* ^{3}H-THYMIDINE LABELLING OF LATE 3RD INSTAR LARVAL BRAIN GANGLIA OF *D. nasuta*

Sample (hr)	Total nuclei observed	Labelled nuclei (%)	Frequency (%) of different patterns (among labelled nuclei)		
			nec labelled	ec labelled	Completely labelled
0	2364	41.3	1.95	10.86	87.19
24	9311	36.9	4.63	14.35	81.02
48	6313	44.3	2.25	16.05	81.69
66	3021	21.9	4.07	19.63	76.28

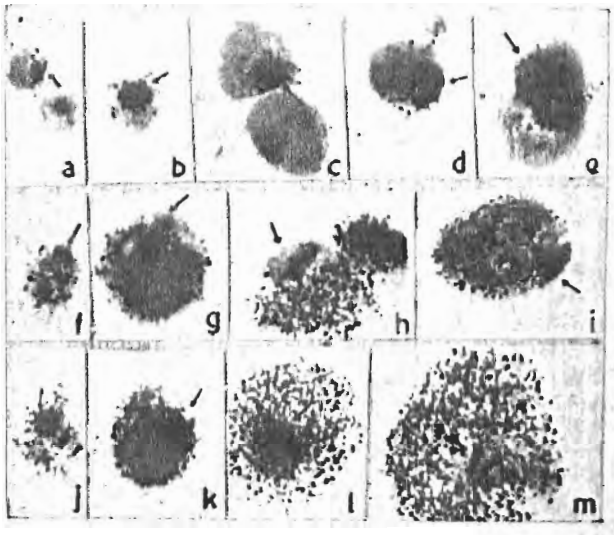

Fig. 2 — Representative examples of autoradiographic labelling patterns of interphase nuclei from the 0 hr sample after 60 hr *in vitro* ^{3}H-thymidine labelling of late 3rd instar larval brain ganglia of *D. nasuta*. Note the extensive variations in chromocentre and nuclear sizes. a-e—nuclei having silver grains only on their chromocentre region (arrows). f-i—nuclei having labelling of the non-chromocentre region only; the chromocentre (arrows) is totally unlabelled. j-m—nuclei with both chromocentre and non-chromocentre region labelled; however, note the heavier labelling of chromocentre (arrows) in j-l. ×1250

and nec areas, while larval brain cells have more variable and larger ec and nec regions. Furthermore, the relative ec area in different cells in larval brain is not the same as in embryonic cells. These characteristic variations in ec and nec areas in brain cells appear to be due to quantitative changes in DNA content in different nuclei. The chromocentre in all interphase nuclei of *D. nasuta* remains very condensed and shows similarly bright H-fluorescence irrespective of its size; this suggests that the larger ec area seen in many of the brain cells is not due to a decondensed state. In several other studies also[11,17,21] it has been suggested that the area of the heterochromatic regions in interphase nuclei is directly related to their DNA content with the two parameters very often showing a parallel increase.

The euchromatic regions of a nucleus undergo considerable variation in size in relation to metabolic activities and cell cycle dependent condensation and decondensation events. As measured in the present analysis, the size of nucleolus may also introduce variations in the nec area. Nevertheless, the extent of variation (from less than 5 μm^2 to more than 300 μm^2, Tables 1 and 3) in nec area in different brain cell nuclei is unlikely to be explainable only on the basis of degree of condensation and metabolic activities; a variable euchromatin content also appears to be a cause for the widely varying nuclear size in brain cells.

The autoradiographic data from *in vivo* labelled brain cells provide direct support for the presumption that the increases in ec and nec areas in different nuclei is, at least in part, due to increase in DNA content of the respective regions. In general, with increasing areas of ec and nec regions, the degree of autoradiographic labelling on respective regions has been seen to increase in a correlated manner. In this experiment, ^{3}H-thymidine was made available to larvae all through their development. It may be mentioned that feeding of *Drosophila* larvae on ^{3}H-thymidine- supplemented food and subsequent autoradiography has been employed in several studies and these have shown that this procedure does provide reliable information on the replicative status of a given chromatin region[7,22,23] or the relative DNA content of certain chromosome regions in different nuclei[24,25]. In the present experiment, the period of availability of ^{3}H-thymidine to different larvae and the autoradiographic processing of different preparations were kept uniform within the practical limits. It may also be noted that with increasing duration of larval feeding on ^{3}H-thymidine food, the degree of autoradiographic labelling on different nuclei was found to increase[26] and this evidences that ^{3}H-thymidine has been available in the food throughout the larval development period. The extensive variation seen in the labelling intensity of different nuclei should, therefore, be due to intrinsic differences in the cumulative uptake of ^{3}H-thymidine in these nuclei. The wide variations observed in the cumulative uptake of ^{3}H-thymidine in different nuclei of 3rd instar larval brains appear to be due to a varying number of endo-reduplication cycle undergone by them. The very high positive correlation between the ec or nec areas and their degree of labelling in this experiment provides further evidence that nuclei with larger ec and/or nec regions have undergone endoreduplication cycles and, therefore, have a greater DNA content as well as area (for ec and for nec regions) than embryonic cells which presumably show only cell cycle dependent 2C/4C variation.

The data on per cent ec area and per cent ec labelling indices in ^{3}H-thymidine-fed larvae indicate that during endoreduplication cycles, the increase in DNA contents of ec and nec areas of a nucleus has not been proportionate since these parameters show a wide range of variability in different nuclei. While the data on per cent ec area would be subject to greater methodological errors, the relative autoradiographic labelling of ec in different brain cell nuclei labelled with ^{3}H-thymidine *in vivo* for entire larval period provides more specific information on the increase in relative content of these two nuclear components during endoreduplication cycles. If the hetero- and euchromatic regions had replicated equally in all nuclei during the larval development period, the relative incorporation of ^{3}H-thymidine (expressed as per cent ec labelling indices) should have remained fairly constant in different nuclei around a value which would be related to the basic proportion of heterochromatin in typical diploid nuclei. However, the observed per cent labelling indices show a very wide variability in parallel with the per cent ec area, and therefore, these observations suggest that the ec and nec regions do not participate equally in the endoreduplication cycles in different brain cells of *D. nasuta* larvae. Nuclei with very low and very high per cent ec labelling indices are presumably related to under- or over-replication, respectively, of heterochromatin. The present cytological observations in *D. nasuta* closely parallel the earlier[11] cytophotometric data that the brain cells of late 3rd instar larvae of *D. hydei* have DNA content in excess of 2C/4C levels and in many of these nuclei, the ratio of DNA in hetero-(autonomous) and euchro matin is not the same as in typical diploid nuclei. Thus it seems that an autonomous under-or over-replication of hetero-and euchromatin in different nuclei of larval brains of *Drosophila* may be a common feature of various species.

Further evidence for independent replication of hetero-and euchromatin in larval brain cells of *D. nasuta* is provided by the analysis of autoradiographic labelling patterns in brain ganglia exposed to ^{3}H-thymidine *in vitro* for 60 hr. In these preparations interphase nuclei were seen in which either the hetero-or euchromatin alone is labelled. That the restricted ^{3}H-thymidine labelling of only hetero-or euchromatin seen in some nuclei after 60 hr *in vitro* labelling is not due to particular culture conditions is clearly shown by similar observations when the brain ganglia of 3rd instar larvae are labelled *in vivo* through food for a period of 48 hr[27]. Thus, the *in vitro* observed patterns of labelling represent the normal *in vivo* replication patterns. Since the

duration of *in vitro* ^{3}H-thymidine availability (60 hr) was much longer than the presumed average S-period[13,28,29] in mitotically dividing larval brain cells, it is expected that under normal sequence of hetero-and euchromatin replication, both the regions should appear labelled in nearly all nuclei which synthesized DNA during the 60 hr labelling period. The presence of nuclei with restricted labelling of hetero-or euchromatin after 60 hr ^{3}H-thymidine availability period may be due either to cells with very long G_1 and/or G_2 periods or to autonomous endoreduplication cycles of these two nuclear components. The first alternative appears unlikely since the larval brain ganglia appear to have mitotic cell cycles which are relatively brief. Thus it has been observed that in *D. nasuta* larval brains maintained at 24°C, some cells come to 2nd cycle metaphase within 3 to 5 hr[13]. Although this does not totally preclude the possibility that a certain population of cells in larval brain may exist with a very long cell cycle duration, the concept of autonomy of endoreduplication cycles of hetero- and euchromatin in these nuclei appears to us to more satisfactorily explain not only the partial labelling of interphase nuclei as observed in the *in vitro* labelling experiment, but also the variable relative content of hetero-and euchromatin in different brain cells as discussed above (also see ref. 11).

As in salivary gland polytene nuclei, the few polytene nuclei which occur in larval brains show an underreplication of cc region since it was seen that after feeding on ^{3}H-thymidine supplemented food for the entire duration of larval development, the small cc in these nuclei remains unlabelled while the euchromatic arms are heavily labelled. In non-polytene nuclei of larval brain, on the other hand, the present analysis and the data of Berendes and Keyl[11] suggest that in different cells the heterochromatin also participates in endoreduplication cycles and while in majority of cells, the heterochromatin lags behind euchrematin, in some cells it may be over-replicated. In non-polytene cells of wing imaginal disks of *D. nasuta* larvae also it has been observed that there is a disproportionate endoreduplication of hetero- and euchromatin[22]. It seems, therefore, that in different polytene and non-polytene cell types of *Drosophila*, the endoreduplication cycles of hetero- and euchromatin regions are autonomously regulated in a tissue specific manner. The functional significance, from the viewpoint of cellular differentiation, of this kind of autonomous regulation of heterochromatin endoreduplication remains to be understood. However, in this context it may be noted that in their review of satellite DNA and heterochromatin, John and Miklos[30] have argued that contrary to earlier assumption, each species is not necessarily characterised by a fixed amount of satellite DNA and they further state 'that it is this variability, so much of an anathema to most investigators, which is of critical importance to eukaryotes'. In the light of present discussion (also see ref. 10), it may be further suggested that different cell types, at least in *Drosophila*, also differ in their satellite DNA (or heterochromatin) and as suggested by John and Miklos[30] at species level, at cellular level also the bulk of satellite DNA may provide a modulation of function not easily achievable by regulation of conventional genes.

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