EFFECT OF TEMPERATURE ON DRY MASS OF POLYTENE NUCLEI IN DROSOPHILA

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SUMMARY

Nuclei were isolated by an aqueous detergent method from Drosophila prepupal salivary glands, and measured by integrating interference microscopy. There was a highly significant correlation between nuclear volume and dry mass. Dry masses fell into 2, 3 or 4 distinct groups corresponding to polytene replication classes; the mean of a given dry mass group was between 8 and 30% less than twice that of the group below, indicating that the ratio of DNA:dry mass increases during polytene growth.

The proportion of nuclei in the higher mass groups, the mean dry mass of nuclei within a given mass group, and the percentage loss of nuclear dry mass in the first hour after isolation were all higher when animals were reared at 15 °C instead of 25 °C. Nuclear dry mass in prepupae was affected by the temperature during both the embryonic and larval periods, and also to some extent by the nutrition and degree of crowding of the cultures.

INTRODUCTION

We elsewhere (Hartmann-Goldstein & Goldstein, 1979) present microdensitometric and morphometric evidence that a higher proportion of salivary gland nuclei fall into the highest polytenic classes in Drosophila larvae cultured at 15 °C than in animals grown at 25 °C. There are no consistent differences between the 2 temperature groups with respect to the dimensions or mean Feulgen-DNA content of chromosomes within a given DNA class, but the spreading characteristics of the nuclei are affected by the culture temperature, and an experienced cytologist can frequently recognize whether a nucleus with a given Feulgen-DNA content is from a 25 °C- or from a 15 °C-reared animal. Conditions during development therefore appear to influence the number of polytene replication cycles and hence the mean size and DNA content of the nuclei, and also to affect in a relatively subtle way the morphology and behaviour of the chromosomes (see also Sokoloff & Zacharias, 1977).

Temperature-dependent chromosomal properties not attributable to differences in DNA content could conceivably be associated with qualitative or quantitative differences in the proteins, which comprise about 80% of the dry mass of the nucleus. Interference microscopy is in principle able to measure the dry mass per unit projected area and the total dry mass of a microscopic specimen (Barer & Joseph, 1954; Davies, Wilkins, Chayen & La Cour, 1954) and might therefore reveal quantitative differences between the nuclei of animals grown at different temperatures. Microinterferometric observations using conventional, non-scanning instruments have been made on
polytene nuclei, chromosomes and puffs by a number of workers (Holt, 1971; Mello, 1972; Paul & Mateyko, 1970; Vidal, 1977; Welch & DeBault, 1968), and sections of salivary glands have been studied with a scanning instrument. It is however only recently that the commercial availability of a scanning and integrating microinterferometer (Goldstein, 1977; Goldstein & Hartmann-Goldstein, 1974; Smith, 1967, 1972) has made the rapid and precise measurement of the dry mass of a heterogenous object practicable. The present article reports an investigation of the dry mass of isolated salivary gland nuclei obtained from Drosophila larvae reared under various culture conditions.

MATERIALS AND METHODS

Strains and culture conditions

D. melanogaster strain w$^6$ 258-21 (Lindsley & Grell, 1968) was reared in mass cultures at 25 ° and 15 °C. Flies of the strain Edinburgh wild type were cultured under various sets of conditions: in the first experimental design ('regimen A') standard laboratory stock cultures maintained at 25 °C were compared with cultures kept under conditions known to be favourable for cytological material, i.e. less densely stocked cultures were reared at 15 °C on a medium supplemented with a suspension of live yeast. In the second experimental design ('regimen B') 5-day-old females were allowed to lay at 25 °C on agar for periods of 1 h. After 22 h at 25 °C, larvae were picked off the agar and transferred to 40-ml vials containing 6 ml yeast medium (Alderson, 1957) to which had been added 1·5 ml of a suspension of 75 g live yeast in 30 ml water. Each vial was stocked with 30 larvae and the vials were either left at 25 ° or transferred to 15 °C for the remainder of development.

Nuclear isolation

Bulk isolation of salivary gland nuclei from strain w$^6$ 258-21 flies reared in mass cultures was performed using a simplified version of method II of Boyd, Berendes & Boyd (1968). Salivary glands were collected by hand from 150 female soft white prepupae, into 0·25 ml buffered Ringer at 3 °C. To this solution 0·025 ml of 10% Tween 80 was added, and the solution shaken gently at room temperature for 5 min. After adding 0·04 ml of 10% sodium deoxycholate the suspension was again agitated gently for a few seconds, and then pipetted violently until most of the glandular material had broken up. A small drop of the suspension was introduced into a 0·1-mm-deep haemocytometer chamber, and cleanly isolated nuclei selected for microinterferometry. In pilot experiments there appeared to be a tendency for dry masses measured a relatively long time after nuclear isolation to be somewhat less than those measured earlier, and all measurements were therefore completed within 110 min of the addition of the detergent.

The bulk isolation procedure had the drawback that dissected glands remained in the isotonic medium for varying times before the addition of detergent. Also, the final preparations were sometimes contaminated by incompletely ruptured cells and glandular detritus. Faster and cleaner isolation of nuclei from wild-type prepupae was obtained by adapting the method for use with glass microneedles and a Leitz micromanipulator (Hartmann-Goldstein, 1972). With this procedure nuclei from a single salivary gland could be isolated ready for observation and measurement in a petroleum jelly-sealed well-slide in less than 5 min. Many of the nuclei thus isolated were rounded, smooth-outlined and apparently free from adherent cytoplasm, and occasional burst, distorted or contaminated nuclei were readily recognized. Nuclei remained apparently intact for at least 1 h, and measurements were made over periods longer than 1 h only when the loss of dry mass from nuclei was studied.
Dry mass of polytene nuclei

Quantitative microscopy

A Vickers M86 scanning and integrating microinterferometer was used, generally with its x 10 objective. The optical path difference (OPD) of empty medium adjacent to the nucleus to be measured was set at about 0.1A relative to the reference area, and the integrated OPD (IOPD) of a scanned area of empty medium was subtracted as a blank from the IOPD of the nucleus. The coefficient of variation of repeated measurements of a nucleus was about ± 3%. Each nucleus was measured at least twice, but sometimes 6 or 10 times to increase the precision of measurement. In the calculation of dry mass in absolute units (pg) a mean specific refractive increment of 0.0017 cm³ g⁻¹ was assumed. The measuring, calibration and statistical procedures used have been described previously (Goldstein & Hartmann-Goldstein, 1974).

The projected areas of some nuclei were measured with a planimeter on photomicrographs of known magnification. Assuming a nucleus to be spherical, its volume \( V \) and projected area \( A \) are related by the expression

\[
V = 0.7543 A^{2/3}
\]

Fig. 1. Correlation of volume and dry mass in bulk-isolated nuclei of strain w*yw 258-21 prepupae reared at 15 °C (•) and 25 °C (△). The correlation coefficients of 0.729 and 0.820 respectively for 25 °- and 15 °C-reared nuclei were highly significant \((P < 0.001)\). The estimated slopes and intercepts of the regression lines, with their standard errors, were 12.9 ± 0.68 μm³ pg⁻¹ and 1317 ± 57 μm³ for 25°C-reared nuclei, and 17.9 ± 0.33 μm³ pg⁻¹ and 13.4 ± 73 μm³ for 15°C-reared nuclei.

RESULTS

Bulk nuclear isolation

The dry mass and volume of 22 and 39 nuclei respectively from 25 °- and 15 °C-reared larvae were measured (Fig. 1, Table 1). Nuclei fell into 2 distinct mass groups; 2 of the 25 ° and 13 of the 15 °C nuclei fell in the heavier group. In the lower mass group the mean mass of the 15 °C nuclei was significantly higher than that of the 25 °C nuclei. The mean mass of the 15 °C nuclei in the heavier group was significantly less than twice that of the 15 °C nuclei in the lighter group. At both temperatures, but especially at 15 °C, there was a statistically highly significant correlation between
nuclear volume and dry mass. The least-squares lines fitted respectively to the 15 ° and 25 °C data differed slightly in slope (Fig. 1), and the latter line did not pass exactly through the origin. There are various possible explanations for this, e.g. the smaller 25 °C nuclei may have had a slightly lower density (i.e. higher water content and hence a larger volume per unit dry mass) than the other nuclei measured.

Table 1. Volume and dry mass of bulk-isolated nuclei

<table>
<thead>
<tr>
<th>Culture temperature, °C</th>
<th>Lower mass group</th>
<th>Upper mass group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass, pg, mean ± S.E.</td>
<td>Volume, μm³, mean ± S.E.</td>
</tr>
<tr>
<td>25</td>
<td>479.3 ± 19.1</td>
<td>7283 ± 451</td>
</tr>
<tr>
<td>15</td>
<td>362.6 ± 13.9</td>
<td>10416 ± 458</td>
</tr>
</tbody>
</table>

Nuclei were isolated by a bulk method from strain w¹⁸¹ Drosophila prepupae reared in mass cultures. Nuclear dry masses fell into 2 distinct groups, respectively greater and less than 700 pg. In the lower mass group nuclei from animals reared at different temperatures differed highly significantly (P<0.001 by Student's t test) in both mean dry mass and mean volume. The mean dry mass and mean volume of the 15 °C nuclei in the heavier group were significantly (P<0.01 and P<0.05 respectively) less than twice those of the 15 °C nuclei in the lower group.

Table 2. Loss of nuclear dry mass after isolation

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass, pg</td>
<td>Mass, pg</td>
<td>Mass, pg</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>312.0 ± 7.3</td>
<td>250.6</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>250.6</td>
<td>368.5 ± 19.8</td>
<td>705.8 ± 21.4</td>
<td>2</td>
</tr>
</tbody>
</table>

In this Table 'Mass' is the initial mass after isolation, while 'Mass loss' is the percentage loss of mass after 1 h. In Mass group III nuclei from animals reared at different temperatures differed significantly (P<0.001 by Student's t test) in mean mass loss.

Loss of dry mass after isolation of nuclei from single glands

Nuclei were measured within 5 min of isolation, and then at intervals for up to 24 h. At longer times after isolation the apparent loss of nuclear dry mass was somewhat variable, possibly due in some cases to drying-out of the preparation. Reasonably consistent results were however obtained up to 1 h after isolation, by which time 27
Dry mass of polytene nuclei

nuclei from 15 °C-reared larvae had lost on average almost 6% of their initial dry mass (Table 2), while 21 nuclei from 25 °C-reared larvae had lost on average less than 2%. This difference between the temperature groups was statistically highly significant ($P < 0.01$). Smaller nuclei appeared to lose a somewhat higher proportion of their initial dry mass during the first hour than did larger nuclei, but this difference was not statistically significant.

![Graph showing dry masses of nuclei from prepupae of wild-type flies reared at either 25° (upper) or 15°C (lower section).](image)

**Effect of culture conditions on nuclear dry mass**

The period from egg collection to the prepupal stage was approximately 18 days at 15 °C (regimen A), 12 days in animals transferred from 25° to 15 °C after hatching (regimen B), and 7–8 days at 25 °C (regimens A and B). From 14 larvae reared at 25 °C, 301 nuclei were isolated and measured, of which 21 were from individuals reared according to regimen A and 280 from regimen B. From 23 larvae reared at 15 °C, 418 nuclei were measured, 152 and 266 respectively from regimens A and B.

Nuclei fell into 4 distinct mass groups, designated I (< 250 pg), II (250–500 pg), III (500–1000 pg) and IV (> 1000 pg), presumably corresponding to polytenic replication stages 6–9 respectively (Swift & Rasch, 1954). The proportions of nuclei in these
### Table 3. Dry masses of nuclei isolated from individual glands

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>25°, regimen A</td>
<td>No.</td>
<td>0</td>
<td>18</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>—</td>
<td>361.4 ± 5.7</td>
<td>684.7 ± 10.7</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_1$</td>
<td>—</td>
<td>-0.2316</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_2$</td>
<td>—</td>
<td>-0.4373</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15°, regimen A</td>
<td>No.</td>
<td>1</td>
<td>25</td>
<td>125</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>203</td>
<td>424.7 ± 9.1</td>
<td>750.4 ± 6.2</td>
<td>1063</td>
</tr>
<tr>
<td></td>
<td>$g_1$</td>
<td>—</td>
<td>-0.1948</td>
<td>-0.3763</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_2$</td>
<td>—</td>
<td>-0.8731</td>
<td>0.3055</td>
<td>—</td>
</tr>
<tr>
<td>25°, regimen B</td>
<td>No.</td>
<td>39</td>
<td>166</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>191.0 ± 3.4</td>
<td>325.6 ± 2.5</td>
<td>623.8 ± 4.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_1$</td>
<td>0.4315</td>
<td>0.6533**</td>
<td>0.0081</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_2$</td>
<td>0.7016</td>
<td>0.3616</td>
<td>-0.1133</td>
<td>—</td>
</tr>
<tr>
<td>15°, regimen B</td>
<td>No.</td>
<td>2</td>
<td>75</td>
<td>188</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>189</td>
<td>353.9 ± 4.8</td>
<td>657.7 ± 4.3</td>
<td>1363</td>
</tr>
<tr>
<td></td>
<td>$g_1$</td>
<td>—</td>
<td>0.1762</td>
<td>0.7134**</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$g_2$</td>
<td>—</td>
<td>-1.277</td>
<td>1.2785**</td>
<td>—</td>
</tr>
</tbody>
</table>

Nuclei were isolated by a micromanipulation method from single salivary glands of wild-type prepupae. The statistical parameters $g_1$ and $g_2$ are respectively the skewness and the kurtosis; all values were non-significant except those marked **, which were significant at the 0.01 probability level.
Dry mass of polytene nuclei

groups differed significantly between the two temperatures (Fig. 2, Tables 3 and 4). Almost all nuclei in group I and the majority in group II came from animals grown at 25 °C, while most in group III and both in group IV were from 15 °C-reared prepupae. Within a given mass group the statistical distribution of the dry masses was approximately normal, and moderate kurtosis and/or skewness was present in only 2 of the 8 instances where sufficient data were available for these parameters to be calculated (Table 3). In mass groups II and III, in both regimens, the mean mass was significantly higher in 15 °- than in 25 °C-reared nuclei (Table 3).

Table 4. Distribution of nuclei into mass groups

<table>
<thead>
<tr>
<th>Experimental conditions compared</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen A, 15 ° vs. regimen A, 25 °C</td>
<td>43.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regimen B, 15 ° vs. regimen B, 25 °C</td>
<td>80.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regimen A, 15 ° vs. regimen A, 25 °C</td>
<td>1.88</td>
<td>n.s.</td>
</tr>
<tr>
<td>Regimen A, 15 ° vs. regimen B, 15 °C</td>
<td>6.68</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The proportions of nuclei falling respectively into Mass groups II (250–500 pg) and III (500–1000 pg) are compared for 2 sets of experimental conditions by the $\chi^2$ test, with 1 degree of freedom.

Table 5. Failure of nuclear dry mass to double during polytenic replication

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Mass groups compared</th>
<th>Ratio of means ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen A, 25 °C</td>
<td>III:II</td>
<td>1.856 ± 0.042</td>
</tr>
<tr>
<td>Regimen A, 15 °C</td>
<td>III:II</td>
<td>1.768 ± 0.041</td>
</tr>
<tr>
<td>Regimen B, 25 °C</td>
<td>III:II</td>
<td>1.916 ± 0.021</td>
</tr>
<tr>
<td>Regimen B, 25 °C</td>
<td>II:II</td>
<td>1.705 ± 0.033</td>
</tr>
<tr>
<td>Regimen B, 15 °C</td>
<td>III:II</td>
<td>1.859 ± 0.028</td>
</tr>
<tr>
<td>Strain w^m 258-21, 15 °C</td>
<td>Upper:Lower</td>
<td>1.758 ± 0.076</td>
</tr>
</tbody>
</table>

In every case, the mean dry mass of nuclei within a given mass group was significantly less than twice that of the next lower group.

Diet and relative crowding appeared to have some effect on animals kept at 25 °C throughout development (Table 3): the mean nuclear dry mass in mass group II was significantly higher in animals grown under regimen A than in regimen B animals. In animals which had spent both the embryonic and larval periods at 15 °C (regimen A) the means of groups II and III, and the proportion of nuclei falling into group III, were higher than in regimen B animals kept at 25 °C as embryos and transferred to 15 °C only as larvae (Table 4).

For a given set of culture conditions the means of the different mass groups did not fall precisely into a 1:2:4:8 series. In every case, the mean of a mass group was significantly less than twice that of the next lower group, the discrepancy being between 8 and 30% (Table 5).
Assessment of scanning microinterferometry for nuclear mass measurement

Conventional, visual microinterferometric measurements of heterogeneous specimens such as thymus nuclei (Hale, 1957), liver nuclei (Ranek, 1976), sperm heads (Davies et al., 1957) and insect polytene nuclei, chromosomes and puffs (Holt, 1971; Mello, 1972; Paul & Mateyko, 1970) are liable to considerable error, and more sophisticated apparatus has not as yet been much used for work with nuclei. Although Davies, Deeley & Denby (1957) and Bibbiani, Tongiani & Viola-Magni (1969) measured isolated liver nuclei with integrating but non-scanning instruments of rather limited measuring range, Bahr & Wied (1971) measured bull sperm heads with a non-commercial scanning microinterferometer (Carlson, 1970) and Sumner & Robinson (1976) studied human sperm with an instrument identical with ours, the present work may be the first in which scanning and integrating microinterferometry has been applied to isolated nuclei. An evaluation of the technique therefore seems appropriate.

Nuclear dry mass measurement by microinterferometry has several advantages when compared with bulk biochemical procedures (Dounce & Ickowicz, 1969). Thus the ability to observe and measure individual nuclei permits morphologically abnormal, damaged or contaminated nuclei to be discarded, and variations or subpopulations within a population to be detected. Sufficient nuclei for microscopic measurement can be isolated in a few minutes, about an order of magnitude faster than is possible with bulk preparations. This minimizes structural changes or loss of components before measurement. The non-destructive nature of microinterferometry enables repeated measurements to be made on a single specimen, and changes in dry mass followed over a period of time. Micro-interferometry compares favourably in ease, rapidity and repeatability with another method of dry mass estimation applicable to single nuclei, X-ray microradiography (Engström & Ruch, 1951). Probably the main disadvantage of microinterferometry is the need to assume a mean specific refraction increment for the constituents of the nucleus, if the dry mass is required in absolute units: the error resulting from an incorrect choice of constant is however unlikely to exceed a few per cent.

Nuclear isolation procedure

In any nuclear isolation procedure (for reviews see Dounce & Ickowicz, 1969; Busch & Daskal, 1977) the experimental sample may be biased by elimination of certain sizes or types of nucleus. This seemed not to be a major source of error in the present work, since the observed proportions of nuclei in the various polytenic classes were very similar to those we found previously using Feulgen-stained preparations of whole salivary glands. Our nuclear isolation procedures, employing aqueous solutions of sodium deoxycholate and Tween 80, were based on methods of Boyd et al. (1968) and similar to those used for example by Cohen & Gotchel (1971) and Ristow & Arends (1968). Ristow & Arends (1968) note that deoxycholate solubilizes the outer cell membrane more rapidly than the nuclear envelope, and Tween 80 apparently stabilizes the nuclear envelope. Both substances appear to be necessary for the maintenance of
morphological integrity, which Ristow & Arends note is '...of major importance in judging the physiological state of isolated nuclei'. In this respect aqueous detergent solutions are clearly preferable to non-polar solvents, which in general give very poor morphological preservation (Hale, 1957).

We found our procedures to be simple, quick and effective, but the observed loss of mass after isolation might have been minimized or abolished by modifications to the method. For example, nonane (Hale, 1957) and a fluorocarbon oil (Paul & Mateyko, 1970) give better quantitative preservation of nuclear dry mass than do aqueous media. Mass loss in aqueous media can be reduced by the use of various additives (Barton, 1960; Ranek, 1976; Van Duijn & Van Voorst, 1971; Zweidler & Cohen, 1971) or by preliminary fixation (Abramezuk, 1972).

Effect of environment on nuclear dry mass and dry mass loss

Our previous Feulgen microdensitometry indicated that salivary gland cells tend to undergo more polytene replication cycles in cold-reared than in warm-reared *Drosophila* larvae. This finding is fully confirmed by the present microinterferometric investigation, which in addition shows that nuclei within a given polytenic class are on average significantly heavier if they come from 15 °C- rather than 25 °C-reared larvae. 'Cold' and 'warm' chromosomes with identical Feulgen-DNA contents are frequently distinguishable from each other morphologically (Hartmann-Goldstein & Goldstein, 1979), and it is tempting to suppose that the 'extra' dry mass of nuclei from cold-reared animals is due to protein incorporated in, and somehow responsible for the altered appearance of, the chromosomes. There is however no direct evidence that the additional nuclear material is actually attached to the chromosomes. The existence of substantial amounts of non-chromosomal protein in nuclei is suggested for example by evidence that in several cell types the interferometrically measured dry mass of mitotic chromosomes is significantly less than that of the interphase nuclei (Richards, 1960), and by the demonstrated passage of proteins between cytoplasm and nucleus in frog oocytes (Bonner, 1975) and dipteran polytene cells (Kroeger, Jacob & Sirlin, 1963; Paine, 1975).

In *Drosophila*, a brief high-temperature shock results in the migration of a specific non-histone protein into salivary gland nuclei (Helmsing & Berendes, 1971). If nuclei from cold-reared *Drosophila* larvae in fact contain a characteristic, relatively freely diffusible protein fraction, this could explain both their greater initial dry mass and their greater loss of dry mass after isolation. This conjecture might be tested by comparing the dry mass of whole nuclei and of isolated chromosomes; *ex hypothesi*, the mean dry mass of the chromosomes themselves ought to be similar in 15 °C- and 25 °C-reared nuclei of a given polytenic class.

Many of our experimental observations could alternatively be accounted for by differences in the nuclear envelopes. The passage of proteins across the nuclear envelope is extremely sensitive to changes both in particle size and in diameter of the nuclear pores (Paine, 1975), which may be involved in the normal regulation of cellular activity (Feldherr, 1971). Since in our experiments mass loss after isolation was greater in 15 °C- than in 25 °C-reared nuclei, the permeability of the nuclear envelope...
may have been greater in the former group. However, if the envelope permeability of the 25 °C nuclei was so great that they lost most of their freely diffusible protein immediately after isolation, before the first dry mass measurement, this could account for both their lower apparent initial mass and their relatively small subsequent mass loss.

The present work indicates that low temperatures in the embryonic and larval periods increase both the time needed to reach pupation and the mean dry mass of prepupal polytene nuclei. Other environmental factors may however affect the duration of development and the size of the nuclei in opposite directions: at a fixed temperature, favourable culture conditions result in rapid development and high percentage survival (Sang, 1956) of large larvae with large salivary gland nuclei (Wolf & Sokoloff, 1976). Surprisingly, in our experiments prepupae growing at 25 °C had a slightly lower mean nuclear dry mass if kept in uncrowded vials and given extra food (regimen B) than if cultured under supposedly less favourable conditions (regimen A). It is conceivable that the more crowded 'stock' conditions of regimen A are not as relatively unfavourable as at first appear. Alternatively, under crowded conditions there may be some selective survival of relatively large and strong larvae.

**Change in DNA:protein ratio during polyteny**

Although the dry masses of polytene nuclei fall into distinct groups, corresponding presumably to DNA replication classes, the mean dry mass of a given group is substantially (between 8 and 30%) less than twice that of the group below (Table 5). In studies reported elsewhere we have found that the mean Feulgen-DNA contents of successive polytene classes deviate significantly from a 1:2:4:8 series in only some experiments, and the apparent discrepancies are not only much smaller than in the present dry mass work, but are at least to some extent attributable to systematic measuring errors. Instrumental factors such as stray light and residual distribution error have little or no effect on scanning microinterferometry (Goldstein, 1977; Goldstein & Hartmann-Goldstein, 1974), and there seems therefore no reason to suppose that the apparent failure of nuclear dry mass to double during a polytene replication cycle is due either to technical error or to under-replication of heterochromatin (Berendes & Keyl, 1967). It also seems unlikely that loss of protein after isolation but before measurement would particularly affect larger nuclei, which have less surface area per unit volume than do small nuclei. One may conclude that during a polytene replication cycle the total dry mass of a nucleus increases by a smaller fraction than does the DNA, and that this is perhaps due to a relative under-synthesis of protein. An analogous discrepancy between nuclear DNA content and dry mass has been found in a comparison of diploid and polyploid species in *Avenae* (Bullen & Rees, 1972).

A discrepancy between polytenic dry mass and DNA content has also been reported by Mello (1972), who found that in bee Malpighian tubule nuclei the '. . . last doubling of nuclear volume and dry mass occurs in the 4th larval instar but is not accompanied by an increase in DNA content'. This dissociation between protein and DNA content is quite different from that described above, being much greater and of opposite sign.
Dry mass of polytene nuclei

Comparison of Mello's work with our own is however difficult, due to the species and tissue differences and to the fact that a non-integrating microinterferometer was used and the results reported in a form not permitting statistical analysis.

We have at present no evidence regarding the nature of the non-doubling material. It may however be noted again that surface area increases with size more slowly than does volume: if the thickness and density of the nuclear envelope and material bound to it (Stevens & Andre, 1969) remains approximately constant during a polytene replication cycle, the total nuclear volume and mass would increase less than geometrically.

REFERENCES


(Received 27 November 1978)