Feulgen-DNA Duplication Degrees in the Salivary Glands of Bradysia spatitergum (Diptera, Sciaridae)

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Received July 16, 1979

Insect larval organs generally grow by increase in cell size and not by increase in cell number. Consequently, somatic polyploidy is an usual phenomenon observed in the salivary glands of this animal group (Mello 1970).

The patterns of duplication of DNA content along the salivary glands and as the insect post-embryonic development progresses vary, however, as a function of the insect Order considered, and sometimes within the same Order. That is also valid for the salivary glands of Diptera group where polyteny instead of polyploidy is exhibited (Berendes 1965, Pavan 1965, Simões 1970).

The purpose of this study is to determine the duplication degrees of the Feulgen-DNA content in the salivary glands of the Sciaridae, Bradysia spatitergum, and the comparison of their patterns with those of other insects, specially pertaining to the Diptera group. It is also an attempt to relate the duplication degrees of the Feulgen-DNA values along the salivary glands (S1 S2 and S3 regions) and at the various stages of their post-embryonic development with the activity of elaboration of cytoplasmic products previously detected with cytochemical procedures (Lima-Silva 1979).

Material and methods

Salivary glands of Bradysia spatitergum (Diptera, Sciaridae) were dissected at the 1st and early 4th larval instars and prepupal stage.

Whole mounts of the glands were fixed in ethanol-acetic acid (3: 1) for 5 min and subjected to Feulgen reaction (acid hydrolisis: 4N HCl for 1.5h at room temperature). The glands were then bleached in three changes of SO2 water, rinsed in distilled water, dehydrated through a series of alcohols and xylene and mounted in Canada balsam (nD=1.54).

Cytophotometry of the stained nuclei was carried out with a Zeiss photomicroscope equipped with a Zeiss O1 photometer and an EMI 6256 photomultiplier. The nuclei had their Feulgen-DNA values determined in arbitrary units with the two-wavelength method.

Among the cytophotometrical methods, the choice of two-wavelength method, s justified by its accuracy and adequacy to the material studied in this work (Men-
The Feulgen-DNA values \((m)\) were obtained with the formula \(m = BL_aC\), where \(B = \text{area of illuminated field at level of photometer head diaphragm}\)

\[
C = \frac{1}{2 - Q} \ln \frac{1}{Q - 1};
\]

\[
Q = \frac{L_b}{L_a} = \frac{1 - T_b}{1 - T_a};
\]

and \(T_b\) and \(T_a\) = transmittances at the wavelengths \(b\) and \(a\) which give absorptivities of 2:1 for the chromophore being studied (Ornstein 1952, Patau 1952, Mendelsohn 1966, Garcia and Iorio 1966).

In order to select the wavelengths \(a\) and \(b\) five spectral absorption curves were determined for the glandular regions \(S_1\), \(S_2\) and \(S_3\), at the above-mentioned developmental stages. The various wavelengths were obtained using a Schott monochromator filter ruler.

Operating conditions (objective and optovar magnifications, and measuring and field diaphragm diameters) were chosen as a function of the glandular regions and insect developmental phases as nuclear areas and thickness of the stained material varied at the various experimental conditions (Table 1).

<table>
<thead>
<tr>
<th>Developmental phases</th>
<th>Glandular regions</th>
<th>Number of nuclei</th>
<th>Pairs of (\lambda) chosen (nm)</th>
<th>Measuring area ((\mu m^2))</th>
<th>Objective ((\times))</th>
<th>Optovar ((\times))</th>
<th>Field diaphragm diameters (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st larval instar</td>
<td>(S_1)</td>
<td>11</td>
<td>480 e 545</td>
<td>9.71</td>
<td>63</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>(S_2)</td>
<td>7</td>
<td>480 e 545</td>
<td>49.51</td>
<td>63</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>(S_3)</td>
<td>30</td>
<td>480 e 550</td>
<td>7.91</td>
<td>63</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Early 4th larval instar</td>
<td>(S_1)</td>
<td>30</td>
<td>480 e 555</td>
<td>119.20</td>
<td>16</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>(S_2)</td>
<td>30</td>
<td>470 e 555</td>
<td>766.99</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>(S_3)</td>
<td>30</td>
<td>470 e 560</td>
<td>766.99</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Prepupal stage</td>
<td>(S_1)</td>
<td>30</td>
<td>480 e 560</td>
<td>100.00</td>
<td>16</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>(S_2)</td>
<td>30</td>
<td>470 e 525</td>
<td>766.99</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>(S_3)</td>
<td>30</td>
<td>470 e 550</td>
<td>766.99</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Measuring diaphragms were chosen in such a way that measuring area/absorbing area ratios did not exceed value 3.41 (Mello 1976, 1978). Circular measuring diaphragms were used except for the determination of Feulgen-DNA values of the \(S_1\) region where nuclei exhibit a prolato ellipsoidal shape. In this case, a rectangular slot measuring diaphragm was employed (Mello and Zanardi 1976). The homogeneity of the light and the uniformity of the photocathode response were verified according to Garcia and Iorio's criteria (1966).

Feulgen-DNA values in arbitrary units for the three different glandular regions of the various developmental phases were distributed in a scale in geometric progression such that between 50 and 100, 100 and 200, 200 and 400 etc, 10 intermediary classes were interspersed (Ferreira et al. 1967).
Results

Figures 1 to 7 show the nuclei in the various regions of the salivary glands of *B. spatitergum* subjected to Feulgen reaction.

The nuclei of the S₂ region of the 1st larval instar were demonstrated to be larger than those of S₁ and S₃ regions (Fig. 1). On the other hand, the nuclei of the three glandular regions of the 1st larval instar are smaller than those of respective regions of the early 4th larval instar and prepupal stage (Figs. 2, 3, and 4). The nuclei of the S₃ and S₂ regions of the prepupal glands have about the same size (Fig. 4). Comparing nuclei of the S₂ region of larvae in the early 4th instar (Fig. 5) with nuclei from

Figs. 1 to 7. Salivary glands of *Bradysia spatitergum* at different stages of the post-embryonic development subjected to Feulgen reaction. 1, salivary glands of the 1st larval instar, showing nuclei of the S₁, S₂ and S₃ regions. ×450. 2, salivary gland of larva in the early 4th instar, where some nuclei of S₁ and S₃ regions appear. (fb=fat body). ×450. 3, nuclei of the S₁ and S₂ regions of the prepupal stage. ×450. 4, nuclei of the S₂ and S₃ regions of the prepupal stage. ×450. 5, S₂ region showing nuclei of the salivary gland in early 4th larval instar. ×1800. 6, nuclei of the S₂ region of the prepupal stage. One of them shows degeneration signs. ×1800. 7, nuclei of the S₃ region with degeneration aspects. ×1800.
Fig. 8. Frequency histograms of Feulgen-DNA values of the three glandular regions of *Bradyisia spatitergum* at different stages of the post-embryonic development. \( f \)=frequency; dashed histograms=nuclei close to glandular outlet; dotted histograms=degenerative nuclei; shaded histogram=nuclei close to \( S_2 \) region. **A**=1st larval instar. **B**=early 4th larval instar. **C**=prepupal stage.
the same region at late 4th instar it a little increase in the size of the nuclei was observed in the latter. In the prepupae some nuclei begin to show degenerating signs in their S₃ region (Fig. 7); eventually, nuclei of the S₂ region also exhibited these signs (Fig. 6).

The Feulgen-DNA values for each experimental condition were plotted as frequency histograms (Fig. 8). In this study, the Feulgen-DNA duplication interval containing the smallest Feulgen-DNA values (S₁ and S₃ regions of 1st larval instar) was considered as 2C. By analysing the frequency histograms of the Feulgen-DNA values we can infer that the S₂ region in the salivary glands at 1st larval instar displays Feulgen-DNA values occupying duplication intervals higher than those of the S₁ region at same developmental stage. On the other hand, the Feulgen-DNA values for most nuclei of the S₂ region show duplication patterns similar to those of S₁. 2C and 4C classes occur, therefore, in S₁ and S₃ regions and 8C, 16C, and 32C classes appear in the S₂ region of the salivary glands at the 1st larval instar.

At the early 4th larval instar, the Feulgen-DNA values of the nuclei of the S₁ region close to the glandular outlet (dashed histograms of Fig. 8), are slightly lower than those near S₂ region. The former would pertain to 16C, 32C and 64C duplication classes, whereas the latter would be contained within 32C and specially 64 classes. However both nuclear types exhibit Feulgen-DNA values markedly lower than those from S₂ and S₃ regions.

In the S₃ region, the Feulgen-DNA values pertained to 256C, 512C and mostly 1024C classes. In the S₂ region, the Feulgen-DNA values were found to be contained in the 128C, 256C and 512C intervals. The distribution of the Feulgen-DNA values in the S₂ region generally reaches the highest values of this stage.

S₂ and S₃ regions of the prepupal stage continue having Feulgen-DNA values higher that those of S₁ region. Even nuclei beginning to degenerate shows this patterns Feulgen-DNA values corresponding to duplication classes 16C, 32C and 64C are found in S₁. Duplication class 16C was represented by nuclei close to the glandular outlet. Feulgen-DNA values of the S₂ and S₃ regions are included in the 512C and 1024C duplication intervals, except for nuclei at an early degenerative phase (S₃) which occupy the 256C and 512C classes (dotted histograms). In terms of distribution values, no difference was found between the S₃ nuclei constituting the centrally positioned values of this region, and those close to S₂ region (shaded histograms).

By analysing Feulgen-DNA values of the S₁ region throughout the post-embryonic, it can be assumed that the nuclei go through 3 to 4 duplications in their Feulgen-DNA content from the 1st up to the 4th instar, not taking into account that at least one additional DNA duplication had been probably undergone prior to sampling (4C nuclei). Up to the prepupal stage no other DNA replication was detected.

Nearly doublings of the Feulgen-DNA content were found in the S₂ region from the 1st up to the 4th larval instar. However, prior to the sampling of 1st larval instar, nuclei with up to 4 duplications in the Feulgen-DNA content were present. Apparently no change exists in the distribution profile of the Feulgen-DNA values when the larvae changes from 4th instar larval in to the prepupal stage. Therefore the nuclei must have undergone 8 or 9 duplications of their Feulgen-DNA content.
Six to 7 duplication degrees of Feulgen-DNA contents seem to have occurred from the 1st up to early 4th larval instar in the S₃ region. At least one additional duplication must have occurred between the 4th larval instar and the prepupal stage. Taking into account that before sampling of the 1st instar of larva, at least one DNA duplication had occurred, nuclei are found in the prepupal stage which went through 8 to 9 duplications of their Feulgen-DNA content.

Discussion

The results indicate variations in the Feulgen-DNA content along the salivary glands of Bradysia spatitergum (S₁, S₂, and S₃ regions) and at the various stages of their post-embryonic development. Similar results have been reported for the salivary glands of other Diptera, namely Drosophila hydei (Berendes 1965) and Rhynchosciara sp. (Simões 1970). In the latter case, however, data were obtained with radioautography.

Variation in Feulgen-DNA values within the same salivary glands and throughout their development have also been described in other groups of insects (Romer 1966, Cruz-Landim and Mello 1969, Mello et al. 1970). High duplication degrees of Feulgen-DNA content for some glandular regions such as those showed in this work have been reported in Drosophila hydei (Berendes 1965). Drosophila melanogaster, D. virilis, Chironomus thummi, Sciara coprophila (Rasch 1970) and Rhynchosciara sp. (Simões 1970).

The occurrence in Bradysia spatitergum, of a greater DNA content in the distal region of its glands (in this case S₃), compared to the more proximal region (S₁), resembles the pattern depicted by glands of Drosophila hydei (Berendes 1965) and Rhynchosciara sp. (Simões 1970). Meanwhile, differing from the above-cited organisms, B. spatitergum presents its highest duplication values of Feulgen-DNA contents in S₂.

In this paper it has been assumed that the phenomena of duplication of the Feulgen-DNA content is connected to the polytenization process which occurs in the salivary glands. Even though the existence of DNA puffs in Bradysia spatitergum has not still been determined, these generally occur in the salivary glands of Sciaridae during the late fourth larval instar (Breuer and Pavan 1955, Simões 1970, Saueria 1971) and would also be expected to turn up in this fly. However, the distribution of this localized additional DNA synthesis is not considered as being responsible for the increase of the Feulgen-DNA values owing to polytenization ready to duplicate them, after proofs obtained by Rasch (1970) and Lara’s coworkers (Mello 1977-personal communication) for other sciarid species.

The fact in the 1st larval instar there are S₂ nuclei with Feulgen-DNA values corresponding to 8C, 16C and 32C classes, indicates that in this region, yet in the early larval stage and prior to present sampling, there was a number of replications greater that of S₃. The fact that S₁ shows the smallest duplication intervals of Feulgen-DNA content may be related to the lower secreting activity of this glandular region. The cytochemical analysis of this material has proven that S₁ is a poor region as far as the synthesis of proteins and acid glycosaminoglycans is concerned.
(Lima-Silva 1979). On the other hand, S2, the region with the highest Feulgen-DNA values, is the richest in terms of secretion of proteins and proteoglycans.

Summary

Nuclear Feulgen-DNA values were cytophotometrically evaluated in the various zones of the salivary glands of Bradysia spatitergum at the 1st and early 4th larval instar and prepupal stage. The smallest and largest duplication intervals for the Feulgen-DNA values, both ascribed to be provided by polytenization phenomena, were found in the S1 and S2 regions of the glands, respectively. They are possibly related with different secretion activities of these glandular regions.

References


Lima-Silva, M. J. 1979. Estudo morfológico e citoquímico das glândulas salivares de Bradysia spatitergum (Hardy). Subm. for publication.


