A Note on the Polytene Nuclei in the Fat Body of *Drosophila* larvae

The larval fat body of *Drosophila* is a continnous tissue mass, which is composed by large cells with different cytoplasmic inclusions, most of them lipidic in nature. It is known that the nuclei in these cells contain polytene chromosomes, (see Swanson[1]), but their structure has not been largely investigated as compared with the higher polytene salivary chromosomes. In other insects, such as *Rhynchosciara*[2], the nuclei of the fat cells may be polyploid, but the chromosomes are not obviously polytene.

Normal male and female 3rd instar larvae from a wild population of *D. melanogaster* were dissected in *Drosophila* Ringer's solution, and the fat bodies were subjected to the following procedures: a) fixation in 50% acetic acid, staining with aceto-orcein and flattening in 50% lactic acid; b) fixation in 1% osmium tetroxide in *Drosophila* Ringer's solution during 1 h, dehydratation in alcohol series and embedding in Maraglass. Thin sections were stained with uranyl acetate and lead citrate, and observed in a Zeiss EM 9 A electron microscope.

Polytene chromosomes from orcein-stained fat bodies are rather difficult to analyze; however the typical banding pattern can be observed in well stained and widely spread chromosomal regions (Figure 1a). Thick sections of Maraglass-embedded fat bodies stained with Unna's blue show the nuclei of fat cells (Figure 1b) with a number of chromosomal cords (1 µm in diameter) some of which sometimes display conspicuous bands (Figure 1c). These chromosomes, and especially the central and prominent nucleolus, appear (as in the Unna sections) stained with the Aniline blue black staining technique for general protein demonstration[3], meanwhile the Sudan black B procedure[4] selectively stains the lipidic inclusions.

At low magnification, electron micrographs of the fat cell polytene chromosomes show characteristic dark banding, with lighter interband fibrillar areas (Figure 1d). The banding pattern in thin sections (Figure 1e) shows a precise correlation with that observed in the thick ones. According to size, these bands can be classified in a) thick bands (0.3µ), b) thin bands 0.05–0.1µ) and c) 'minibands'[5] formed by small transverselly oriented granules about 300 Å wide. The fibrillar material which constitutes the interband spaces appears in most of the micrographs as separate individual fibrils (60–80 Å in diameter), in agreement with the reports of Swift[6] and Sorsa and Sorsa[7]. The arrangement of interband fibrillar material, in some cases in the form of 2 fibrils running together, can easily be seen in expanded interband regions, (Figure 1f). The nucleolus appears as a compact body, which is composed of a cortex of pars granulosa and a core of pars fibrosa (Figure 1g), showing a very similar architecture to the nucleolus of salivary gland cells[6].

These observations on the ultrastructure of the fat cell polytene chromosomes show that their morphological features are in agreement with those of the salivary gland[5–9]. Light-and electron-microscopic cytogenetical analysis of polytene chromosomes in the fat cells of *Drosophila* larvae, could be of interest in relation to the presence of characteristic cytoplasmic inclusions within these cells[10].

Resumen. Los nucleos de las células grasas de larvas de *Drosophila melanogaster*, contienen cromosomas politénicos cuya ultrastructura es similar a la que presentan los

[1] C. P. Swanson, *Cytology and Cytogenetics*, (Prentice-Hall Inc. N.J. 1957), p. 596.
[2] E. Mattingly and C. Parker, J. Insect Physiol. *14*, 1077 (1968).
[3] S. R. Schulz and W. Jensen, J. Ultrastruct. Res. *22*, 376 (1968).
[4] S. M. Mc Gee-Russel and N. B. Smale, Quart. J. microsc. Sci. *104*, 109 (1963).
[5] M. Sorsa, Ann. Acad. Sci. fenn. A, IV Biologica *151*, 1 (1969).
[6] W. Swift, in *The Molecular Control of Cellular Activity* (Ed. J. M. Allen; Mc Graw-Hill Book Co. Inc. New York, Toronto, London 1962), p. 73.
[7] M. Sorsa and V. Sorsa, Chromosoma. *22*, 32 (1967).
[8] R. H. Goodman and D. Spiro, Expl Cell Res. *27*, 329 (1962).
[9] H. Berendes, Chromosoma. *29*, 118 (1970).
[10] This work was supported by a research grant from the Population Council. Inc. New York.

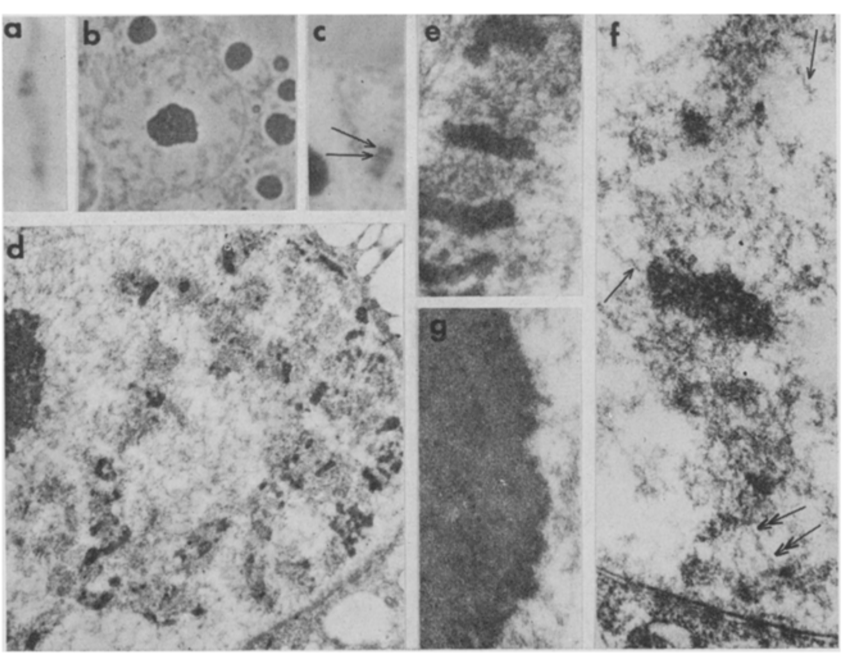

Fig. 1a. Segment of a polytene chromosome from the fat body, showing the banding pattern. Aceto-orcein, phase contrast, ×4,000.

Fig. 1b and c. Thick sections from a Maraglass-embedded fat body. Note the bands (arrows). Unna's blue, b: ×2,000; c: ×3,000.

Fig. 1d and e. Electron micrographs of fat cell polytene chromosomes, showing bands and interbands. d: ×10,000; e: ×35,000.

Fig. 1f. Section of a polytene chromosome, with dispersed interband regions. The double arrows point out a pair of interband fibrils. Single fibrils can also be observed (arrows). ×36,750.

Fig. 1g. Peripheral region of a fat cell nucleolus, ×33,250.

cromosomas en las glandulas salivales. Bandas gruesas, finas, y algunas «minibandas» se hallan separadas por regiones claras compuestas por fibrillas con un diametro de 60–80 Å. El nucleolo constituido por elementos granulares periféricos y fibrilares centrales, presenta un aspecto similar al nucleolo de glandulas salivales.

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Minimal Chromosome Number in False Spider Mites (Tenuipalpidae)

The superfamily Tetranychoidea (sub-order Prostigmata) includes three phytophagous families, the Tenuipalpidae, Tuckerellidae and Tetranychidae. Many species of Tetranychidae, spider mites, have been studied with respect to sex-determination. From cytological, rearing and genetic data, it is known that all bisexual spider mites have a haplo-diploid sex-determination. The chromosome numbers are low, ranging from $n=2$ to $n=7$[1]. The exceptionally low number of $n=2$ is rather commonly found in Tetranychidae and has also been reported from another prostigmatic mite family[2].

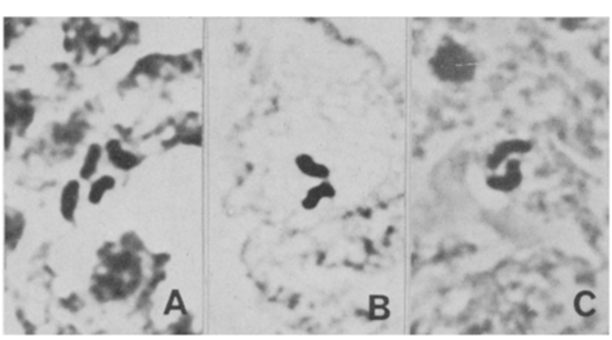
From the Tenuipalpidae, the so-called 'false spider mites', we examined the chromosome number of the bisexual species *Raoiella indica* (Hirst), occurring on date palms at Mauritius. Eggs were collected at random from infested leaves and preparations were made using the aceto-orcein squash technique. It was observed that the population consisted of both males and females in an approximate equal ratio. From 56 eggs we obtained usable chromosome preparations, being the eggs in which embryonic development was not too far advanced. 32 eggs showed exclusively 2 chromosomes, while 4 chromosomes were found in 24 eggs. The occurrence of two classes of eggs, one with 4 and the other with 2 chromosomes, supports the assumption of haplo-diploidy in this bisexual species, which is in agreement with the fact that this type of sex-determination is predominant for prostigmatic mites.

Some other species of Tenuipalpidae were investigated, which reproduce themselves by means of a thelytokous parthenogenesis. From the privet mite, *Brevipalpus obovatus* (Donnadieu), we studied two populations from different origin, one from a glasshouse in the Netherlands, one from Madagascar. Both populations were reared in the laboratory on detached leaf cultures of *Hedera helix* L. From the Dutch population, it appeared that males occurred at a very low frequency (on a total of 15,000 individuals only 12 males were scored), while the occurrence of males in the Madagascar population was even more seldom. From the Dutch population 120 eggs gave suitable chromosome preparations. All these preparations showed cells with prophases and metaphases, while other mitotic stages were also present. Two chromosomes occurred, both being small, in metaphase approximately 2 μm in length. No peculiar features could serve as a tool for a decision concerning homology of both chromosomes, despite the large amount of material examined. From the Madagascar material 20 egg preparations were made, in which constantly 2 chromosomes were found (Figure).

Besides *B. obovatus*, 2 related thelytokous species from Madagascar were examined, *viz. B. phoenicis* (Geyskes) and *B. californicus* (Banks). For both species it could be stated that also 2 chromosomes occurred.

The interpretation of the occurrence of 2 chromosomes in embryonic tissue of the 3 thelytokous *Brevipalpus* species offers difficulties in that no data concerning the number and behaviour of chromosomes in the germ line are available. Our trials to investigate the chromosome cycle during oogenesis failed. Moreover, the small size of the chromosome prevents any conclusion on the possible homology of the 2 chromosomes seen at different mitotic stages.

Despite this lack of essential information, the most simple explanation would be to assume that the females of this species are diploid in their soma and that the basic number of chromosomes is $n=1$. The assumption of female haploidy is less attractive, since this condition has never been found in any animal species under natural conditions. Also for genetic reasons it is difficult to believe that female haploidy can evolve in a group where male haploidy is the predominant type of sex-determination.



Embryonic cells of Tenuipalpidae. A) diploid complement of *R. indica*. B) haploid complement of *R. indica*. C) diploid complement of *B. obovatus*.

Zusammenfassung. Es wurde bei 3 *Brevipalpus*-Arten mit thelytoker Reproduktion festgestellt, dass Zellen in embryonalem Gewebe nur zwei Chromosomen haben und vermutlich ein einfaches Chromosom-Komplement darstellen ($n=1$).

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[1] J. GUTIERREZ, W. HELLE and H. R. BOLLAND, Acarologia *12*, 732 (1970).

[2] J. H. OLIVER and B. C. NELSON, Nature, Lond. *214*, 809 (1967).