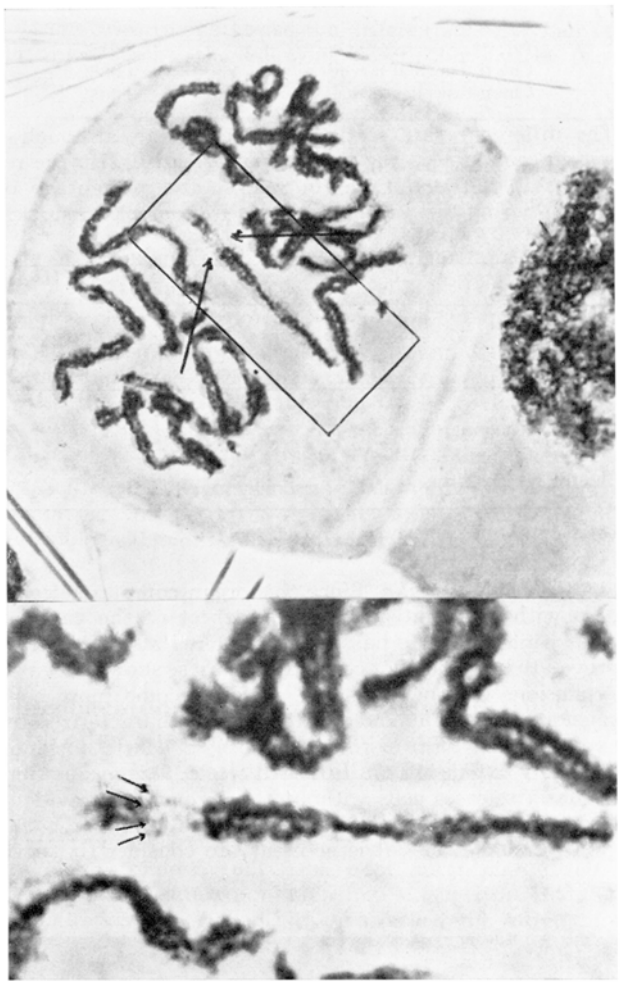
meric chromomeres generally forming a square or parallelogram in the centromeric gap. GIMÉNEZ-MARTÍN[10] has described in somatic chromosomes of *Scilla liliohiacintus* a centromeric structure made by two parallel sets of chromomeres and fibrillar zones, one for each chromatid. Each set was formed by six chromomeres and the corresponding interchromomeric fibrillar zones.



Centromere tetrapartite into four filaments.

Material and Methods. Root-tips of *Scilla non-scripta*, without any pretreatment, and the TJIO and LEVAN[11] staining technique were employed.

Results and Discussion. In the centromeric gap of the somatic metaphase chromosomes of *Scilla non-scripta*, it has been observed that the centromere present a quadruple transversal structure constituted by four filaments. These filaments form two pairs, corresponding to one pair for each chromatid. Furthermore, four sets of chromomeres are apparent, one on every filament. Four is, too, the chromomere number on each filament and all chromomeres have similar size. The said filaments are parallel, but two by two are separated by a shorter distance than the space that separates both pairs of filaments. The proximity existing between the filaments of each pair is clearly visible in certain parts of the centromeric gap.

Many authors have considered that the centromere remains undivided up to the late metaphase, and for this reason it is the unique fixed point that is able to facilitate the chromatid despiralization.

According to LIMA DE FARIA[8] the centromere is divided at least from the early prophase; but at the same time TJIO and LEVAN point out that at telophase and anaphase only two centromeric chromomeres are seen, their division not yet having been accomplished.

The present observations indicate that the centromere is tetrapartite at metaphase into four filaments and therefore it is logical to assume that each daughter chromosome has its centromere already divided. These observations agree with the quadruple chromatidic structure observed also in metaphase chromosomes of this material.

Zusammenfassung. In der Metaphase der Mitose von *Scilla non-scripta* (*Endymion non-scriptus*) besteht das Centromer aus vier Elementen, und dementsprechend hat jede Chromatide ihr Centromer geteilt.

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Sección de Citología, Instituto de Edafología y Biología Vegetal C.S.I.C. Madrid (Spain), June 4, 1963.

[10] G. GIMÉNEZ-MARTÍN, Fitón *11*, 139 (1958).
[11] J. H. TJIO and A. LEVAN, Anales Estac. exp. Aula Dei *2*, 25, 64 (1950).

Genotypical Differences Between Stocks of *Drosophila melanogaster* Revealed from Culturing *in vitro*

In a previous note, the preliminary results have been published which were obtained by culturing *in vitro* two organs of *D. melanogaster*: the lymph gland and the cephalic ganglia (CASTIGLIONI and REZZONICO[1]). The main results showed the possibility of a long survival of a number of cells which proved to be alive for their stainability and because some mitotic stages could be detected. It was thought that survival (till the 75th day for the ganglia and till the 35th for the lymph glands) could be used to test whether the reaction to culture conditions is different between stocks which can be assumed to be genetically different. The detection of differences is obviously interpretable as an indication of the phenotype at the cellular level.

The scheme of the work has been designed in the following way: (1) Cultures have been set up by placing the organ and a drop of medium, prepared according to KURODA[2], on a coverslip. A depression slide was superimposed, with the depression downwards. Cells are thus allowed to adhere to the coverslip, on which they tend to form a unicellular layer. The cultures were transferred at the end of each week into fresh medium. (2) At the end

[1] M. C. CASTIGLIONI and G. REZZONICO RAIMONDI, Exper. *17*, 88 (1961).
[2] Y. KURODA and S. TAMURA, Med. J. Osaka Univ. *7*, 137 (1956).

of each week (three weeks for the lymph glands and four for the ganglia) each culture was observed under phase contrast and an aliquot of cells stained. May Grünwald-Giemsa was used for cells of both organs adhering to the coverslip, while the pieces of ganglia which were still solid at the end of the experiment were stained with Delafield haematoxylin.

As regards the lymph gland, stocks S. Maria, Chieti *v*, and *yw* were used: 20 glands were cultured for each one. It should be noted that, in the stock Chieti *v*, some larvae (8%) are filled with hemolymph and look swollen; they die at the end of larval stage or as prepupae. Swollen larvae have an abnormal lymph gland, which lacks the anterior lobes, while the others are hypertrophic. Thus a total of four types of lymph gland have been investigated.

For each type of gland, the total cell number was determined, because all cells leave the gland within 48 h of culture and are suitable for counting:

S. Maria	$\bar{x} = 5354.50 \pm 358.18$
yw	$\bar{x} = 4276.83 \pm 290.70$
Chieti *v* normal larvae	$\bar{x} = 4648.83 \pm 440.92$
Chieti *v* swollen larvae	$\bar{x} = 8109.83 \pm 648.60$

The differences between stocks are significant: in particular, the larger glands of the swollen Chieti *v* larvae contain more cells than the smaller glands of the normal larvae.

As regards the ganglia, three stocks were used: Varese, Aspra 52 and S. Maria, all wild. Between the stocks, no differences were noted. Since ganglia remain solid in culture, and only the cells which leave the organ can be counted, the total cell number for a ganglion cannot be determined.

Results

(1) *Lymph gland.* Lymph cells show morphological differences between the stocks, which would otherwise be wholly undetectable. S. Maria shows very uniform cells (Figure 1); *yw* shows also uniform but smaller cells, with irregular contours; Chieti *v* (normal larvae) possesses larger and smaller cells; Chieti *v* (swollen larvae) is characterized by the same condition, and, in addition, by the presence of giant cells having peculiarly sharp limits (Figure 2). Cells stained with May Grünwald-Giemsa show an overall acidophily, which denotes a state of functional abnormality.

Rare mitoses have been observed.

Some quantitative data suggest that the behaviour of cultured cells varies from stock to stock. The rate of cells adhering to coverslip was calculated within the interval of 21 days. The ratio between adhering cells and released cells, expressed in percentage, gives the following values:

S. Maria	7.74%
yw	8.21%
Chieti *v* normal larvae	4.98%
Chieti *v* swollen larvae	3.30%

The differences are statistically significant, although – within each stock – the figures vary greatly. Also the relationship between time (in weeks) and percentage of cells adhering to coverslip changes from stock to stock.

Stocks	Frequencies of adhering cells	No. of cultures 1st week 5%	2nd week 0.5%	3rd week 0.25%
S. Maria		8	7	7
yw		10	10	9
Chieti *v* normal larvae		5	13	7
Chieti *v* swollen larvae		2	5	6

Chieti *v* shows a tendency to a late release.

(2) *Cephalic ganglia.* Since this organ complex releases cells without disintegrating, the aspect of the gangliar mass while releasing has been considered also. This trait proved to be typical for each stock: Varese shows irregular expansions of lobular aspect, which become more conspicuous during the 2nd week; Aspra is characterized by expansions in form of rounded lobes; S. Maria does not show any expansion until the 3rd week. This means that S. Maria releases cells without the formation of evident discontinuity of the membrane. For analysing the behaviour of single cells, it is necessary to consider that five

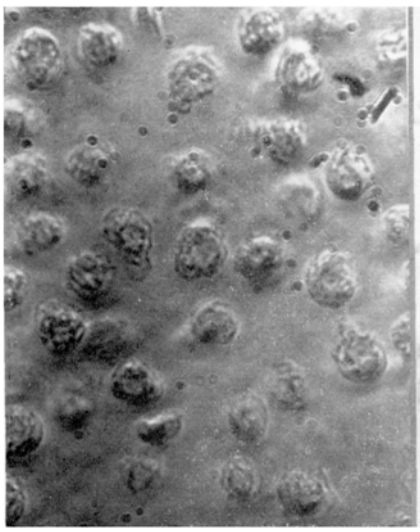


Fig. 1. Cells migrated during the first week of culture from a S. Maria lymph gland (phase contrast ×900).

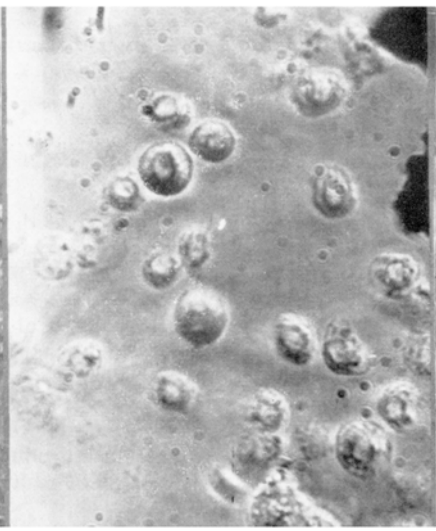


Fig. 2. Cells migrated during the first week of culture from a Chieti *v* lymph gland (swollen larvae) (phase contrast ×900).

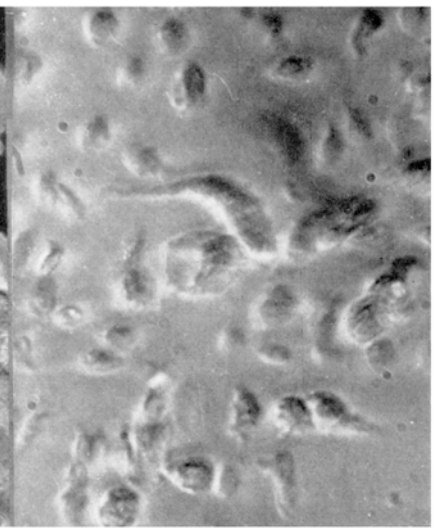


Fig. 3. Cells migrated from cephalic ganglia during the second week of *in vitro* culture (phase contrast ×900).

cell types have been found in the gangliar tissue, labelled: small, large, with a filamentous expansion, giant, elongated (Figure 3). The 4th and the 5th types have been observed only in some cultures. All types seem to correspond to cells already existing when the ganglia are still *in situ*.

The frequency of small cells released is different between stocks only at the end of the 1st and of the 3rd week: since the other cell types are very rare, their behaviour has not been considered.

If one tries to synthesize the differential behaviour of the three stocks, the following description can be proposed:

Stocks	Degree of dissolving of the ganglion	Amount of cells liberated	Total number of cells adhering to coverslip (in cultures)
Varese	++++	+++++	34.718
Aspra 52	++	++	15.279
S. Maria	+	++	19.476

The relationship between released cells and disintegration of the ganglion is obvious only in Varese.

Also the nervous cells adhering to the coverslip show an overall acidophily, denoting an abnormal functional state, but some rare mitotic stages have been observed.

Discussion. The facts referred to should require for evaluation the demonstration that they are not artefacts but true biological manifestations. Unfortunately, it is impossible at present to assert that releasing of cells is only due to an active cell migration and that all stained cells are functionally normal. Indeed, attachment to the coverslip, which is a general phenomenon, and the rare mitoses prove that at least an aliquot of cells is still living *in vitro*. Although a component of artefact is certainly present in our slides, nevertheless it seems justified to conclude that the stocks (i.e. the different genotypes) analysed so far, show a high repeatability while different stocks behave differently. Thus, one may conclude that the technique of tissue culture reveals phenotypic differences at the cellular level which are not detectable with other means, and is suitable for further genetical analysis[3].

Riassunto. Sono stati coltivati in identica condizione gangli nervosi e ghiandole della linfa di diversi ceppi di *Drosophila*, differenti genotipicamente. Si sono notate diverse peculiarità di comportamento che – sebbene in parte forse artefatti – denotano l'esistenza di differenze fra il fenotipo, che risulta così analizzato a livello cellulare. Il metodo sembra adatto a ulteriori analisi del fenotipo, in rapporto a genotipi diversi.

M. C. Castiglioni and G. Rezzonico Raimondi

Istituto di Genetica dell'Università di Milano (Italy), May 20, 1963.

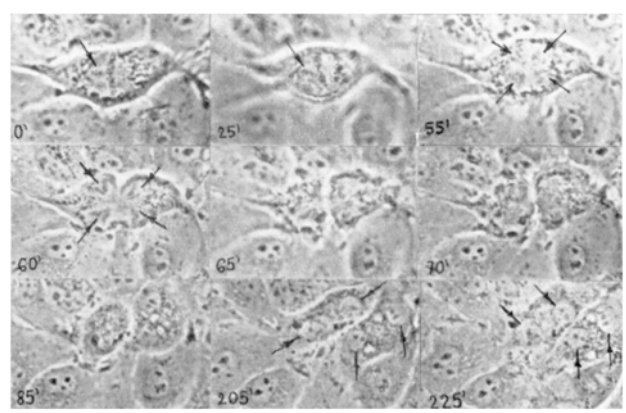
[3] This investigation was supported financially by the Rockefeller Foundation N.Y.

Beobachtung der Bildung zwei zweikerniger Zellen aus einer Zelle der Gewebekultur ohne experimentellen Eingriff

Die Bildung zweikerniger Zellen wird grundsätzlich auf zwei Arten erklärt: entweder durch amitotische Teilung des Kernes ohne Cytodiaerese, oder durch eine Mitosestörung; 1954 haben Schleich und Mayer[1] die Bildung einer zweikernigen fibroblastischen Zelle durch Adrenalineinfluss beschrieben. Wir haben in fixierten und gefärbten Kulturen eines stabilisierten Seitenstammes der PK Zellen auffallend grosse Mengen von multipolaren Mitosen und mehrkernigen Zellen gefunden, deren Ursprung wir nicht feststellen konnten. Weitere, von uns beschriebene Beobachtungen am lebenden Material könnten die Bildung eines gewissen Teiles dieser Anomalien erklären.

Die PK Zellen wurden unter gewöhnlichen Bedingungen in Medium nach Earle mit Zugabe von 20% inaktiviertem Kalbsserum und anderen Komponenten (0,001 Phenolrotlösung und Penicillin mit Streptomycin) auf Deckgläsern in Petrischalen gezüchtet. Nach der 24–48 h dauernden Züchtung wurden die Gläser in die Maximowschen Kammern eingesetzt, im Phasenkontrastmikroskop Reichert (Obj. 70:1, Ok. 15×) bei einer Temperatur von 38°C beobachtet und in 5 min Intervallen photographiert.

Die erste Aufnahme (zur Zeit 0) zeigt ein Monaster, aus welchem senkrecht auf die äquatoriale Achse etliche Chromosomen absteigen. Diese Bildung nimmt eine Ypsilonform an (25 min). In der Anaphase teilen sich die Chromosomen in vier Gruppen (Zeit 55 min – siehe Pfeile). Am Anfang der Telophase (60 min nach dem Beginn der Beobachtung – siehe Pfeile) ist zu beobachten, dass jeweils Zweiergruppen von Chromosomen in eine Tochterzelle gelangen. Die sich nachträglich bildende Spaltung hälftet die Zelle wobei jeder Teil zwei gut sichtbare Kerne mit entwickelten Kernkörperchen enthält (Zeit 205 min und 225 min, die Kerne sind mit Pfeilen bezeichnet).



Unsere Beobachtung zeigt, dass die Bildung von zweikernigen Zellen durch eine Störung der Mitose ohne Änderung der Kultivationsbedingungen möglich ist. Es ist auch wahrscheinlich, dass in den Zellen stabilisierter Zellenstämme die lange *in vitro* gezüchtet wurden, dieser Mechanismus häufig ist, ebenso wie bei entgegengesetzten Verfahren, wobei aus mehrkernigen Zellen mitotisch wieder

[1] A. Schleich und A. Mayer, Z. Krebsforsch. *60*, 47 (1954).