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Action of the lethal combinations from T(Y;3) P 80 stock of Drosophila melanogaster during development

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Genetica. — *Action of the lethal combinations from* T (Y; 3) P 80 *stock of* Drosophila melanogaster *during development.* Nota di CARLOTTA HALFER, presentata (*) dal Corrisp. C. BARIGOZZI.

RIASSUNTO. — Due diverse combinazioni letali derivano normalmente per segregazione dal ceppo *T (Y; 3) P 80* di *Drosophila melanogaster*: una iperdiploide per la porzione distale del braccio destro del 3° cromosoma, mentre l'altra ipodiploide per la medesima.

Poiché culture in vitro a breve termine di cellule embrionali hanno dimostrato che questi cariotipi anormali sono compatibili almeno con le prime fasi dello sviluppo embrionale, si è voluto analizzare a quale fase di sviluppo subentra la letalità.

I risultati della ricerca hanno portato alla seguente conclusione: gli individui con cariotipo iperdiploide manifestano la letalità in diversi momenti dello stadio larvale, invece quelli con cariotipo ipodiploide manifestano una letalità più precoce e cioè durante lo stadio embrionale.

The aim of the present paper is to determine the stage or the stages at which the lethal caryotypes, segregated from *T (Y; 3) P 80* stock of *Drosophila melanogaster*, interrupt the developmental processes.

MATERIAL AND METHODS

The stock *T (Y; 3) P 80* is marked by the presence of a heterozygous and reciprocal translocation between the Y chromosome and the 3rd chromosome. The breakage point on the 3rd chromosome, according to data on salivary gland chromosomes kindly supplied by E. B. Lewis (personal communication), is 3 R 88 C–F approximately at the midpoint of the right arm. The Y chromosome is split into two nearly equal portions: the centric portion of the Y chromosome (Y^c), consisting of the short arm and of a segment of the long arm, is joined to the distal portion of the right arm of the 3rd chromosome. The acentric portion of the Y chromosome consists of a large segment of the long arm and is translocated onto the right arm of the same 3rd chromosome. The female chromosomes are normal.

For simplicity let us consider only the two pairs of chromosomes, that is the sex and the third chromosomes, involved in the translocation, as shown in fig. 1.

The female supplies only one type of normal gametes. In the male on the other hand, the characteristic four—chromosome complex, which appears at pachytene stage during pairing between homologous sections, can then

(*) Nella seduta del 17 aprile 1971.

disjoin in three different segregation patterns: *alternate segregation*, *adjacent—1* and *adjacent—2 segregations*. Thus theoretically it is possible to expect six different chromosomal combinations, and therefore six kinds of gametes. But, only two of these six gametes are normal the two produced by alternate segregation. In fact, each gamete has a complete complement of genes. Such germ cells produce balanced caryotypes and therefore viable zygotes, males and females, identical with the parents.

Both adjacent—1 segregation and adjacent—2 segregation (where homologous centromeres go to the same pole) produce unbalanced complement of genes, because of the presence of both a duplication and a deficiency in

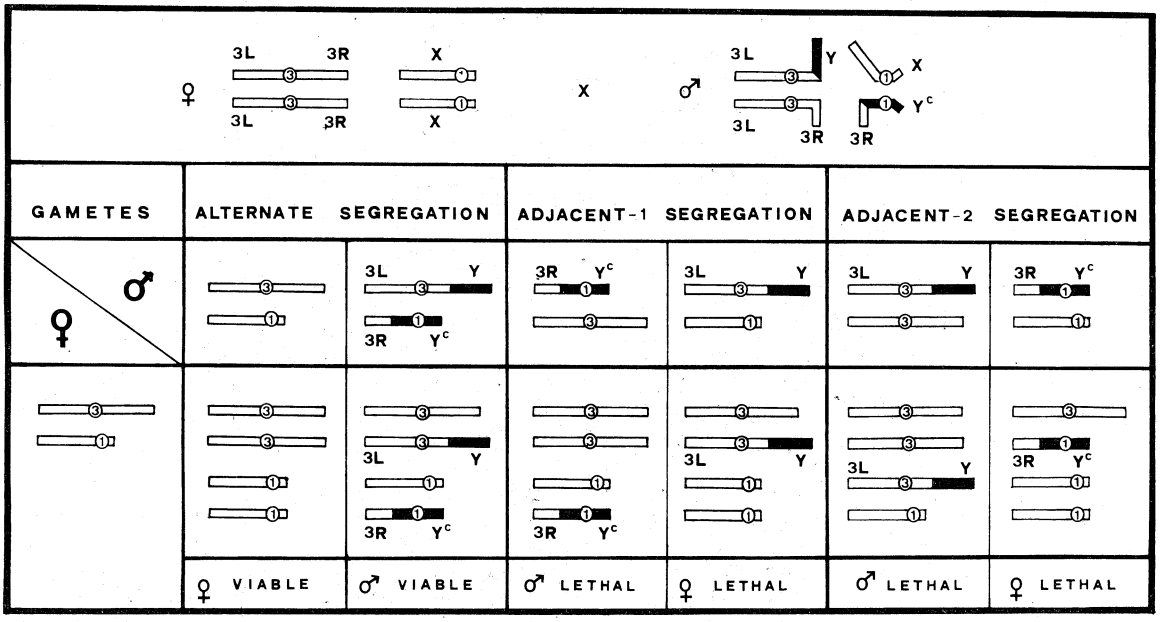

Fig. 1. – Gamete formation and offspring lethality in *Drosophila melanogaster* from males heterozygous for a reciprocal translocation between the Y and the 3rd chromosome.

each gamete. Therefore, such germ cells produce inviable zygotes with unbalanced sets of chromosomes, namely either hypodiploid or hypodiploid for some blocks of genes.

Nevertheless, from the analysis of metaphases of embryonic cells, derived from a large number of six hours old eggs, cultured in vitro for twenty hours by the technique of Horikawa and Fox [1], it was also possible to observe the presence of some unbalanced caryotypes viable since replicating, as demonstrated in a previous work [2, 3].

In fact, as illustrated in fig. 2, besides the two balanced combinations, we can observe as normally present only two of the four expected lethal combinations, precisely those derived from the adjacent—1 segregation, and no case of adjacent—2 segregation, which is known to be very rare.

All phases of the life–cycle were examined from the egg until the emergence of the imago. I have particularly considered:

1) the fertility;
2) the number of larvae which reach the pupal stage;
3) the number of pupae which develop into adults.

The unhatched eggs were analyzed only in toto, after removal of the chorion by a solution of NaClO at 3 % for 2′.

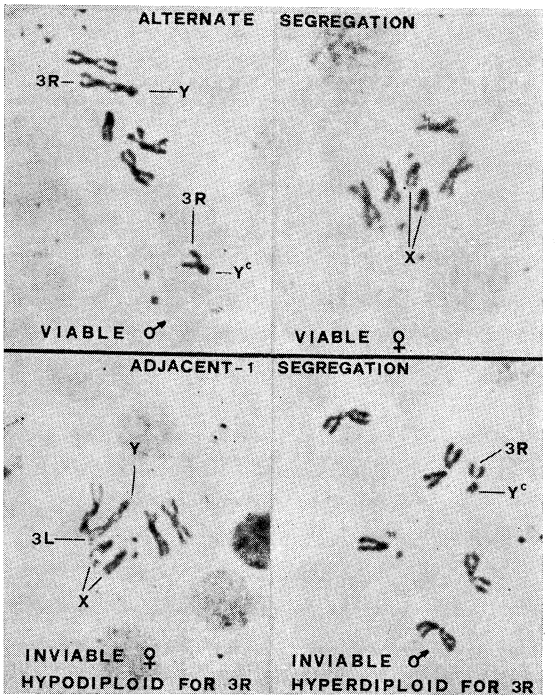

Fig. 2. – The four caryotypes derived by segregation from the stock *T(Y;3) P80*, and present in short term cultures of embryonic cells.

The cytological analysis of the larvae was carried out on the nervous ganglia by the technique of Oster and Balaban [4].

All these observations were carried out also on the wild type stock *Varese*, used as control.

Results and discussion

The data obtained from the wild stock *Varese*, and from the stock *P80* are summarized in a histogram (fig. 3), which reveals:

1) as to fertility, we can note a striking difference between the two stocks: in fact, while in the control the frequency of the hatched eggs is of 90,7 %, on a total of 1808 laid eggs, in the *P80* stock, we find a frequency of only 54,9 %, on a total of 1375 laid eggs;

2) as regards the pupal stage, in the stock *P80* only 68,5 % of the larvae reach the pupal stage, while in *Varese* the percentage is 95,9 %;

3) as to the number of pupae which develop to adults: no difference between the two stocks. In both cases the frequency of the emergence is very close to 100.0 %.

Hence, we can conclude that the total mortality in the stock *P80*, carrier of a translocation between the Y chromosome and the 3rd chromosome, mostly depends on the number of unhatched eggs and on a rate of inviable larvae, while pupal lethality practically does not exist.

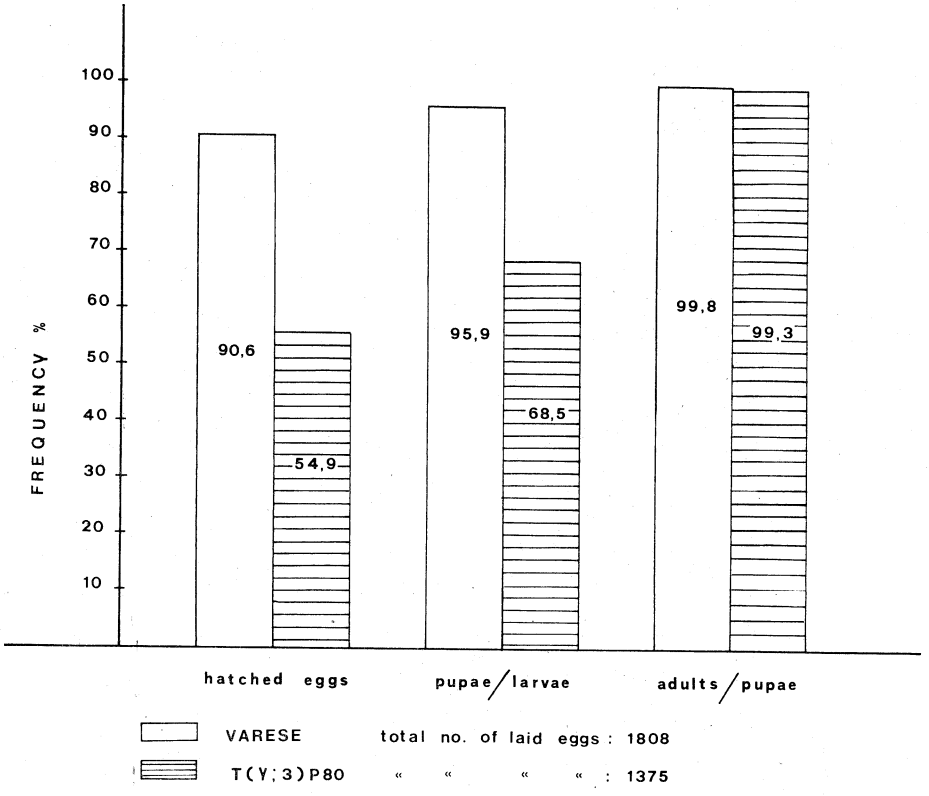

Fig. 3. – The histogram represents the frequencies (%) of the fertility, of the larvae which reach the pupal stage, and of the pupae which develop to the adults, in the stock *Varese* (control) and in the stock *T(Y;3)P80*.

Considering now lethality during the larval stage, five different categories of dying larvae were found. 238 larvae out of 756 hatched eggs died. In particular:

1) 61 larvae died just after hatching (25,6 %).

2) 69 larvae died at the first instar (29.0 %); their maximum length was mm. 3 and their survival as small larvae ranged from 5 to 9 days.

3) 19 larvae, mm. 2,5 long, died as "lethal meander"—like larvae (8.0 %). They had the tracheal trunks much too long for their dwarfed bodies and could continue as larvae for as long as 14 days.

4) 28 larvae died at the 3rd instar, after a survival ranging from 4 to 12 days (11,8 %).

5) 61 larvae died at the beginning of the prepupal stage, as pseudopupae, after 12–13 days of survival in the 3rd instar (25,6 %).

After a cytological analysis of nervous ganglia, all the abnormal larvae showed the same caryotype, corresponding to the unbalanced combination, hyperdiploid for the distal portion of the right arm of the 3rd chromosome.

Moreover, a gonad analysis showed that these abnormal larvae were males (except for a few doubful cases), evidence which is consistent with the former conclusion: in fact only one X chromosome is present (the Y is affected by the translocation).

These data prove clearly that the other unbalanced caryotype, namely hypodiploid for the distal portion of the right arm of the 3rd chromosome, which is present in embryonic cell cultures, must belong to the group of embryonic lethals, whose cytological analysis was impossible.

Nevertheless, from an analysis of unhatched eggs in toto, three different types of unhatched eggs were distinguished:

1) early embryonic death; only one central yolk mass is evident;

2) death at 10–12 hours of development, when the typical segmentation of the body is visible;

3) larvae ready to hatch, which are rare.

The above data on lethality at different developmental stages lead to a discussion of the meaning of the frequencies of various lethal groups observed.

The viability of the stock *P80* (37,4 %), in comparison with the control *Varese* (86,8 %), is very low. But, while we expect in the *P80* stock an equal proportion of deaths in the two complementary classes derived from the adjacent—1 segregation, on the other hand I noted a striking difference between the percentage of larvae and pseudopupae died (17,6 %) and the percentage of unhatched eggs (45.0 %). As regards this remarkable rate of unhatched eggs it should be borne in mind that:

a) there is an undeterminate number of unfertilized eggs;

b) there are, even if in a very low frequency, the two products derived from the adjacent—2 segregation, characterized one by a large deficiency, the other by a large duplication: for this reason they are lethal very likely at a very early embryonic stage;

c) there are a certain number of completely formed larvae in the unhatched eggs and these are not counted as larvae.

d) there is some embryonic lethality due to the weakness of carriers of unbalanced sets, which are more sensitive during development.

CONCLUSIONS

On the basis of these findings we must conclude as follows.

The caryotype hyperdiploid for the distal portion of the right arm of the 3rd chromosome, shows a poliphasic lethality, including:

1) " boundary lethals " between the completed embryo and hatched larva (E/L).

2) larval lethals, that is small larvae up to mm. 3.

3) " boundary lethals " between tha larval and the pupal stage (L/Pr).

The hypodiploid caryotype, supposing the absence of the products of the adjacent—2 segregation, could be considered diphasic. Two types of eggs, in fact, are found: in one the developmental standstill takes place in the first hours, in the other one after 10–12 hours.

On the other hand, the presence of the products of the adjacent—2 segregation may account for the existence of the two types of unhatched eggs: in fact, one could attribute a very early embryonic lethality to those caryotypes having either large deficiencies or large duplications. I must emphasize, however, that adjacent—2 segregation may be very rare or non-existent.

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