MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXV, No. 3, Article 3 (Biology), 1958

Studies on Lethal Factors in *Drosophila*

II. Lethal Effect of *Confluent*-21 in *D. virilis*[1)]

By

Toshio SHIOMI

Zoological Institute, College of Science, University of Kyoto

(Received Nov. 25, 1958)

Confluent is a dominant character of *D. virilis*, the gene *C* being located on the 2nd chromosome at 45.0. The character of the mutant is designated as follows; second longitudinal vein of wing thickened and broadened at junction with margin, anterior and posterior crossveins also thickened, eyes slightly small and roughened; homozygous lethal (CHINO, 1936–1937, 1941).

The mutant has repeatedly been obtained in our laboratory both spontaneously and by X-ray irradiation, and the mutation is conspicuous in its frequent occurrence. Among many strains of *C*, three strains (*C*-12, *C*-19, and *C*-20) were studied by IMAIZUMI (1949) in order to analyse the lethal action of the genes which affects the developmental process of the homozygotes for *C* to death. He has reported that in respective strain the homozygotes for *C* reveal deviation from the normal developmental process in an early embryonic stage. The present work deals with a strain of *Confluent* (*C*-21) which has been found by IMAIZUMI among progenies of X-rayed flies. Notwithstanding that the venation and other phenotypic characters in the adult mutant are quite similar to those of the typical *Confluent*, the lethal stage in the homozygous individuals for C^{21} is later than that of the other strains. A part of the present work has been reported preliminarily in an abstract form (SHIOMI, 1956).

Before going further, the author expresses his sincere thanks to Professor K. NAKAMURA for constant guidance in the work and kind help in the preparation of the manuscript. Thanks are also due to Mr. T. IMAIZUMI for his kindness in providing the strain *C*-21.

Experiments

In order to confirm the stage in which the lethal action of the gene appears, the following crosses were performed:

$$C^{21}\times C^{21},\ C^{21}\times +,\ +\times C^{21},\ \text{and}\ +\times +\ .$$

For control, a wild stock *Kitakata*-24 (+) which has long been raised in our laboratory was chosen, and the experiments were carried out at 25°±1°C. Results obtained are summarized in the table:

1) Aided by a grant from the Scientific Research Expenditure of the Ministry of Education.

Crosses	Eggs		Larvae		Pupae		Imagos	
	Total	Unhatched	Total	Death	Total	Death	*C*	+
$C^{21}\times C^{21}$	846	219 *25.9%*	627	11 *1.8%*	616	3 *0.5%*	411	202
$C^{21}\times +$	637	7 *1.1%*	630	4 *0.6%*	626	2 *0.3%*	319	305
$+\times C^{21}$	825	7 *0.8%*	818	6 *0.7%*	812	2 *0.2%*	396	414
$+\times +$	664	18 *2.7%*	646	11 *1.7%*	635	3 *0.5%*	—	632

In the cross $C^{21}\times C^{21}$, nearly a quarter of eggs laid cease to develop to imagos, and imagos showing confluent characteristics are twice as many as wild ones. In the other crosses, on the other hand, the mortality during the every developmental processes is very slight and the ratio between *Confluent* and wild flies in F_1 is approximately 1 : 1. These facts clearly indicate that the zygotes carrying *C* gene in homozygous condition die during the course of development. Comparing the mortality in three stages of development, it is noticed that in the cross $C^{21}\times C^{21}$ the rate of embryos fails to hatch is remarkably high, exceeding 25% of eggs laid, while mortalities in the other stages are rather low remaining on a level with those at corresponding stages in the other crosses. Thus, it might be reasonable to conclude that the lethal action of the homozygous *C* is confined to the embryonic stage.

To find out how the C^{21} gene would exert its effect upon the developmental morphology during the embryonic stage, freshly laid eggs obtained from the crosses mentioned above were examined under microscope. After dechorionated with sodium hypochlorite (or commercial Antiformin), eggs were washed, then transferred into a watch glass containing modified RINGER's solution (NaCl 5.5 g, KCl 0.2 g, $CaCl_2$ 0.1 g in 1000 ml H_2O) and left for observation.

In the crosses other than $C^{21}\times C^{21}$, the structural development of eggs follows almost the same scheme of that in *D. melanogaster* as far as embryonic stage is concerned, and heterozygotes ($C^{21}/+$) cannot be discriminated from homozygous wild ones ($+/+$) at this stage. Contrary to these, there are two classes of eggs in the $C^{21}\times C^{21}$ cross. Up to the 12th hour after oviposition, the developmental process proceeds quite normally and no distinction can be made between these two classes; blastoderm and pole cells are formed; gastrulation is completed; germband is established and extends along the dorsal side of the embryos. In the period between the 13th and 14th hour of development, a subsequent shortening of germband is characteristic for normal embryos, but abnormality appearing in one class of embryos. The structural change of further development is arrested in such embryos, germband remains extended and no shortening takes place. Abnormal formation of embryonic structure is not observed, and they stand still till signs of cytolysis become distinct.

Remarks

Based on the results obtained from the crosses and the embryological examination, it is reasonable to conclude that the C^{21} gene is recessive in terms of lethal action; no trace of developmental retardation is manifested in heterozygous condition; while in homozygous (C^{21}/C^{21}) individuals embryonic development halts in half way and death eventually follows before hatching. So far as these features are concerned the lethal action of the gene seems to coincide with that of C^{12}, C^{19}, and C^{20} respectively (IMAIZUMI, 1949). Between *C*-21 and the other strains, however, there is a remarkable difference in the stage where cessation of embryonic development takes place. As IMAIZUMI described, in homozygous (*C*/*C*) embryos of the latter strains blocking of development appears firstly between the 2nd and 3rd hour of oviposition before the stage of blastoderm formation is reached; blastoderm and pole cells are not established and afterwards a hollow of an irregular shape appears in midventral portion of the embryo as a necrotic transformation. IMAIZUMI surmised that the abnormality may have been caused by failure of cleavage nuclei to penetrate into the peripheral cytoplasm of eggs which destined to form the blastoderm in the normal development. Contrary to these strains, the homozygous embryos of *C*-21 strain pursue the normal course of development up to the 12th hour when the extended germband is characteristic for the normal embryos of this stage. The blocking of morphogenetic movement becomes evident just before the shortening of germband commences at about the 13th and 14th hour of oviposition, during the time no abnormal transformation of embryonic structure is observed.

The most significant of these findings would be that the difference between the action of C^{21} gene and those of the other *C* genes becomes evident only in homozygous individuals which cannot be discriminated in heterozygous condition. The *C* genes occupy the same locus on the 2nd chromosome and from the genetic evidences CHINO (1936-1937) suggested the mutation to be due to a deficiency for invisible minute section.

Concerning the relation between the action of lethal factors on embryogenesis and the degree, as well as kind, of chromosomal aberration, the *C*-series lends a remarkable contrast to the *Notch* series in *D. melanogaster*. *Notch* (*N*) is a general name given to a dominant character type manifested by various mutant strains which are deficient for both *facet* (*fa*) and *split* (*spl*) gene loci, often including *white* (*w*), on the 1st chromosome. By microscopical examination of the salivary gland cells, it is revealed that some of the strains have no abnormality in the salivary chromosome bands while in the rest a section involving the bands which correspond to the loci of those genes is missing from the 1st chromosome, and the section is different in length in each of the strains. Besides these deficiencies, aberrations such as translocation and inversion in which one of the points of breakage is close to, or at the loci, are often visible (BRIDGES and BREHME, 1944). These mutants of *Notch* series are embryonic lethal in hemizygotes; hypertrophies of nervous system, lacking of ventral and cephalic hypoderm, and absence of organs from mesoderm,

are characteristic for the lethal embryos (POULSON, 1940, 1945). The present author also observed quite a similar pattern of the abnormalities in lethal embryos of some other strains in *Notch* series; N^{264-88} (normal salivary bands), N^{264-72} (deficiency), and $N^{264-112}$ (inversion) (SHIOMI, unpubl.). Another example of the same type of effects caused by different chromosomal aberrations, is the *white lethal* series (POULSON, 1945).

In the *Notch* series as well as in the *white lethal* series, the effect of chromosomal aberrations in various mutants is quite similar to each other not only in the phenotype of heterozygous flies but also in hemizygous lethal embryos, notwithstanding that the degree or kind of aberration varies according to respective mutation. About this point, HADORN (1951) opines that this becomes intelligible if one assumes that the detrimental effect of a deficiency is not quantitative in nature, but a consequence of loss of one particular gene locus. The decisive loci would be those that first enter into action in normal ontogeny. Loss of these loci, or mere inertness produced by point mutation, would therefore lead to the same effect.

The case of the *C*-series dealt with in the present work, may be explained in the same way by assuming the effect of the lethal factor to be qualitative in nature, although a quantitative interpretation seems to be more applicable than the former. Cessation of the developmental process without malformation of embryonic structure in the *C*-series seems to indicate that the phenomenon is of an event of physiological abnormality or shifting of metabolic pathway from the normal course, but not caused by morphological alteration which makes impossible of further development of embryos. In this respect, the lethal effect of the homozygous C^{21} and those of the other *C* genes are of the same category, though the stage in which the lethality is manifested is far later in *C*-21 than in the latter strains. The lethal factor in the respective strain exerts detrimental effect in the identical way shifting the metabolism towards the same direction but at different rates; in lethal embryos of *C*-21 the shifting of metabolic pathway takes place so slowly as it is insufficient to interrupt embryonic development at the early stage, the embryos surviving beyond the critical period which is lethal to the embryos of the other strains continue to develop up to the stage just before shortening of the germband. In this case, however, it would deserve notice that the lethal embryos of *C*-21 develop quite normally following a regular course and there is no tendency of slowing down of the developmental movement prior to the lethal stage. This may not necessarily indicate that the injurious effect of the lethal factor does not start early in developing embryos of *C*-21 as in those of the other strains.

Summary

1. A dominant gene, *Confluent*-21 (C^{21}, 2-45.0) of *Drosophila virilis* which has been induced by X-ray irradiation, is revealed to have recessive lethal effect in homozygous condition.

2. The lethality is confined to appear in embryonic stage; the embryo homo-

zygous for C^{21} gene persues quite normally the course of development beyond the lethal stage of other *C*-strains up to a period between the 13th and 14th hour after oviposition, when the developmental movement of the lethal embryo is arrested before the germband contraction. As no malformation of embryonic structure occurs the effect of the C^{21} gene in homozygous condition seems to be physiological in nature which makes impossible further development of the lethal embryo.

3. No chromosomal aberration is observed by microscopical examination of salivary chromosomes in heterozygotes of the *C*-21 strain as in the other *C* strains.

4. Difference in effect between the C^{21} and other *C* genes, which is only manifested in the lethal stage of homozygous embryos, is discussed in comparison with the *Notch* series of *D. melanogaster*.

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