cross are wild-type (heterozygous) females and non-yellow, apricot, echinus, Bar males. Complete loss of $X$ or $Y$ chromosomes will yield sterile $X0$ males phenotypically yellow, apricot, echinus, non-Bar. Deletion of either $y^+$ or $B^s$ marker from the $Y$ chromosome will yield yellow, apricot, echinus, Bar males (abbreviated to yellow males) or non-yellow, apricot, echinus, non-Bar males (or simply non-Bar males) respectively. Non-disjunction of $X$ and $Y$ will lead to the recovery of Bar females with $XXY$ constitution. Where possible, all exceptional flies were analysed genetically to confirm this classification.

Fig. 1 shows the comparative yields of $X0$ males and of non-disjunctional females while the frequencies of each deleted $Y$ chromosome class and of non-disjunctional females are given in Fig. 2. On the assumption that the determination of non-disjunction will occur only at or before anaphase in the first meiotic division, then it seems likely that the seventh brood in particular represents treated pre-meiotic stages. These results also confirm the high sensitivity of meiotic and immediately pre-meiotic stages—presumably primary spermatocytes—to the mutagenic effect of X-rays.

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## A Gene synthesizing Protease and a Pre-Viral Genome in Silkworms

Almost twenty years ago[1], we put forward a working hypothesis that viruses may originate in nucleoproteins in cells, and in 1943 [2] we discovered that the viral nuclear polyhedrosis of larvæ of *Bombyx mori* can be induced by a metabolite. The later development of work on induction has suggested[3] that the polyhedral virus would be derived from the chromosomal segment containing the genes controlling the synthesis of protease and deoxyribonuclease. We have now demonstrated that the pre-viral genome contains a gene synthesizing an alkaline protease and is located in the chromosome of healthy silkworms.

Our previous investigation[4] showed that the pupa of *B. mori* has no alkaline protease and never produces polyhedra without infection. About 200 pupæ of the Zelem × plem strain were each injected with 0·02 ml. of polyhedral solution (0·1 mgm./ml.), and polyhedra were collected in the cold by our method[5] from infected individuals before death. Viral particles were then isolated from the polyhedral crystals by the procedure previously described[6] and polyhedral protein from the crystals was also prepared at the same time. However, the first centrifugation was carried out immediately after dissolving polyhedra in a mixture of sodium carbonate and sodium chloride, without standing overnight. The proteolytic action of viral particles and polyhedral protein obtained was measured by the method previously reported[6]. The results are given in Table 1, in which the protease activity is expressed in μgm. tyrosine per hr. produced by 10 mgm. of the particles or protein.

Table 1. Proteolytic Action of Polyhedral Virus

| | *p*H of reaction mixture | 8·2 | 9·0 | 9·8 | 10·5 | 11·0 | 11·5 |
|---|---|---|---|---|---|---|---|
| μgm. of tyrosine | Viral particles | 4 | 10 | 17 | 28 | 31 | 29 |
| | Polyhedral protein | 1 | 3 | 7 | 13 | 14 | 12 |

It is thus evident that the polyhedrosis virus forms a protease which shows the highest activity at *p*H 10·5–11·0. As the polyhedral virus is of deoxyribonucleic acid nature and produced only in the nucleus, it can be concluded that there exists in the virus a gene which possesses the ability to synthesize the alkaline protease.

Our previous experiments[5,7] have further shown that the *B. mori* larva has an alkaline protease and produces polyhedra containing a protease of the same nature. The white cuticle of the $p_{44}$ strain and the black cuticle of the $p^s$ strain respectively were taken in the midst of the final diapause, namely just at the end of the formation of new cuticle during the third ecdysis, and 0·1 gm. of the cuticle consisting of epi-, exo- and endo-cuticle was ground with 10 ml. of 0·02 *N* sodium carbonate.

These two strains were then crossed and the cuticle from the descendants in the $F_1$- and $F_2$-generations was treated with the sodium carbonate solution. The proteolytic action of the suspensions obtained was immediately measured at *p*H 10·5 in the same way as before[6]. The results are given in Table 2, in which the protease activity is expressed in μgm. tyrosine produced by 10 mgm. of cuticle per hr.

Table 2. Proteolytic Action of Larval Cuticle

| Generation | $p_{44}$ | $p^s$ | $p_{44} \times p^s - F_1$ | | $p_{44} \times p^s - F_2$ | |
|---|---|---|---|---|---|---|
| Colour of cuticle | White | Black | Black | | White | Black |
| μgm. of tyrosine | 81 | 161 | 183 | | 71 | 181 |

It is thus obvious that there exists a correlation between the proteolytic activity and colour of the skin, one of the tissues in which polyhedral virus is produced. As it has already been established[8] that the plain white of the $p_{44}$-cuticle and the striped black of the $p^s$-cuticle are governed by the genes in the second chromosome in the larval cell, and that the latter is the dominant, we may conclude that the gene synthesizing the alkaline protease is located in the chromosomes, possibly in the second chromosome. Actually in an experiment the number of white and black larvæ in one group of the $F_2$-generation was 138 and 429, respectively. Similar results concerning the activity of cuticular protease and the ratio of $F_2$ larvæ were also obtained in the case of the reciprocal cross.

In view of these observations, it seems reasonable to assume that, by the inducing process, this special pre-viral genome in larval chromosomes can be activated to form the polyhedral virus.

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