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Maintenance of diploid embryonic cells of Drosophila melanogaster in vitro.

Embryos of wild stock (Oregon R-C) were collected after oviposition during the night (12-14 hours).
Eggs were dechorionizated in 3 per cent sodium hypochlorite for 6 minutes. They were then rinsed, gently homogenized, centrifuged and the sediment was suspended in C-12 medium, the concentration of cells being no less than 1.5 millions per ml.

In H-5 and H-6 media of Horikawa and Fox (1964, 1966) the cells in our experiments quickly died. Therefore we used C-12 medium.

In this medium the cells quickly aggregated and adhered to the glass at 27°C. Cell growth was observed mainly in multilayer clumps of small cells adhered to the glass. Mitosis however were detected also in large flat fibroblast-like and epithelial-like cells outside of these clumps.

The number of chromosomes was counted in colchicinated cells after hypotonic treatment and staining with aceto-orcein. After 40 or more days the cells in our medium maintained diploid chromosome sets while Horikawa and Fox (1964, 1966) as well as Dolfini and Gottardi (1966) observed in H-5 medium only 20-50 per cent of normal mitosis already after 2-10 days.

| Days of culture | Number of chromosomes 4 | $6^x$ | 8 | No. metaphases analyzed |
|---|---|---|---|---|
| 18 | 1 | 5 | 138 | 144 |
| 44 | - | 2 | 167 | 169 |

[x]The fourth pair was not detected

During the period of observation (more than 40 days) the formation of multilayer clumps of small and colonies of large cells was observed as well as intensive incorporation of $H^3$-thymidine in both types of cells.

References: Horikawa, M., Fox, A.S., 1964, Science, 144: 1437.
Horikawa, M., Ling, L.N., Fox, A.S., 1966, Nature, 210: 183.
Dolfini, S., Gottardi, A., 1966. Experientia, 22: 144.

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Further evidence that exchange occurs between chromatids in X-Y detachments.

The preferential recovery of the shorter element of asymetrical dyads produced by the detachment of attached-X rejoining with Y-chromosomes from irradiated oocytes has suggested that the exchanges involved are between chromatids (Parker and McCrone 1958: Brosseau, 1964). This is based upon the observation that disjunction is not normal at the second meiotic division when there are structurally different homologs in the dyad; the shorter element being recovered more frequently (Novitski, 1951). More direct proof of the involvement of chromatids in detachment events would be the recovery of an attached-XY and a complete-Y from the same gamete which would be a rare simultaneous detachment and non-disjunction. Such a product was recovered in an experiment in which newly eclosed Drosophila females containing a reversed metacentric attached-X (y v) and a doubly marked Y-chromosome ($y^+Y \cdot B^S$) were irradiated with 3000 r and mated with y ct B (Binscty) males containing a normal Y. Subsequent testing demonstrated that the genotype of the non-disjunction and detachment female recovered was y v $\cdot$ $y^+$ / $y^+$ Y $\cdot$ $B^S$ and the y ct B paternal-X. This event was recovered in a sample of eggs collected from a single female. No unexpected non-disjunction types were observed among the offspring of the female producing the detachment and non-disjunction, therefore it is probable that no extra Y-chromosome was present in the gamete from which this event was derived. This observation is further proof of the chromatid nature of X-Y detachment in oocytes of Drosophila females.