Squash Preparation of Polytene chromosome from chironomous larvae (Drosophila) 1

Introduction - Polytene chromosomes are well known from their use in genetical, cytological and molecular studies. These chromosomes remain in a permanent interphase, but due to repeated cycles of endo reduplication and tight lateral association of all chromatids, each arm becomes thicker and distinctly visible as cable like structure with alternating light and dark regions, the bands and interbands respectively. Polytene chromosomes are most commonly found in dipteran insects, salivary glands of late 3rd stage instar larvae of ~~drosophilla~~ Drosophila provides an excellent polytene chromosome preparation due to high level of polyteny achieved by several cells in this tissue. Each salivary gland consist of abut 120 cells of these the most posterior ones cells have completed 8 or 9 rounds of endoreplication till late 3rd instar stage, This results in characteristic banding patterns consisting of more dense bands alternating with light stained interbands. For cytological studies, polytene chromosome preparations are made by the classical squashing technique following a brief fixation and staining it may be temporary or Permanent.

Materials required - Healthy late 3rd instar stage larva blotting paper, dissecting needles and fine forceps, cavity slides, clean glass slides, cover glasses 37°c incubator binocular microscope, cloth for cleaning diamond marker, marker pen, slide box razor blade, clipping jars slide tray, microscope for examination

Solutions required -

1) 1X PBS for dissection
2) 50% acetic acid
3) Aceto methanol 1:3
4. Aceto orcein 2-1-H
5. Aceto carmine
6. DPX mount
7. Absolute alcohol.

2g of orcein 50% acetic acid gives aceto orcein it is kept stirring on hot plate at 40°C for 20 min, filtered and cooled before use

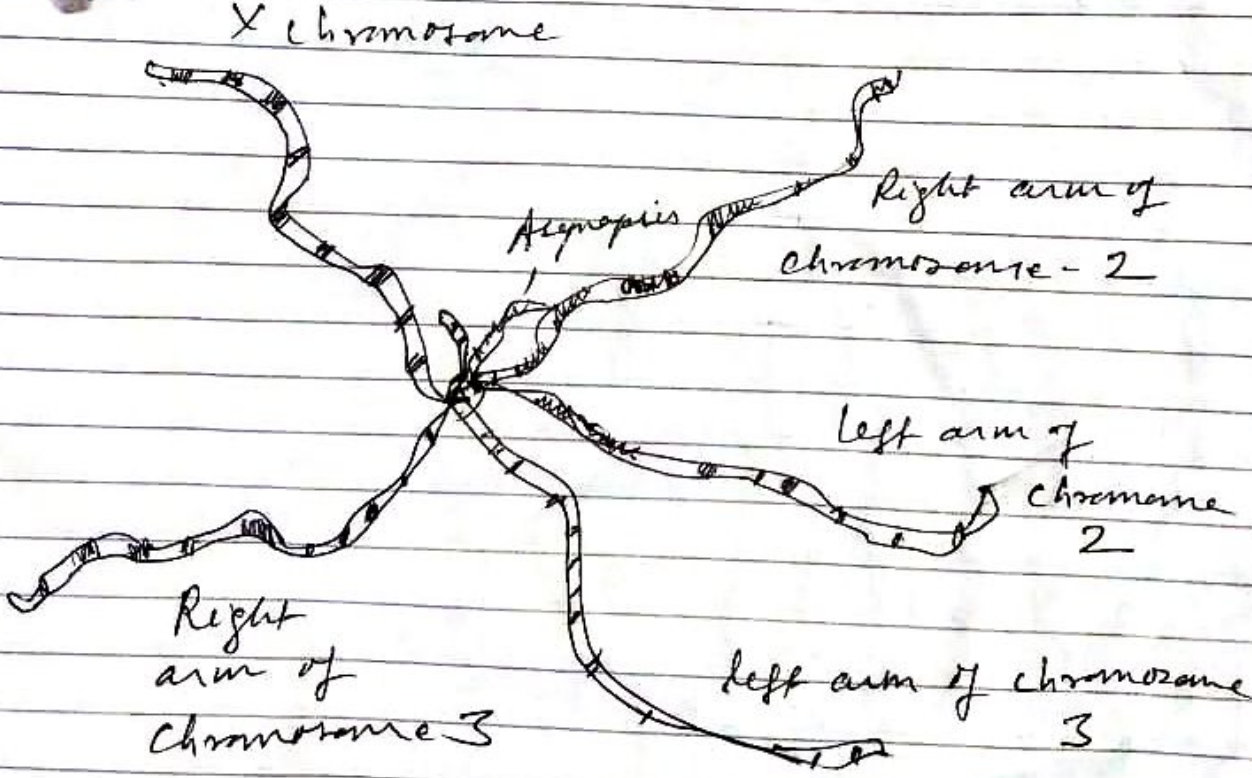
Procedure - (Temporary Preparation)

1) Take wandering late 3rd instar larvae from a healthy culture, wash it properly by transferring the larvae on a filter paper soaked in water and placed in a petridish
2) Using fine forceps or dissecting needles, pull the mouth parts of larvae to rupture larval skin. The salivary gland are seen as a pair of whitish translucent elongated structures connected ~~to~~ at their anterior ends with a common salivary duct.
3. Transfer the cleaned salivary gland to a drop of 1X PBS solution on a clean slide drain the excess PBS, Keep the slide in slanting position, add drops of freshly prepared fixative, wipe out excessive fixative, add a few drop of aceto orcein and aceto carmine Stain add 2 drops of aceto carmine and 1 drop of aceto orcein and Keep covered
4. Drain out excess stain by adding a few drops of 50% acetic acid to remove stain, cover with a clean cover glass.
5. For squashing, Put the slide with its cover glass between folds of filter paper tap the

cover glass with a needle which enables the break of nuclear membrane and release of chromosome. Apply firm pressure of thumb of the on the cover glass. This act of squashing spreads the polytene chromosomes arms of a nucleus and makes them spread in one plane.

5. After squashing seal the cover glass with DPX mount to prevent evaporation of acetic acid and drying of slide and then observe it under microscope

OBSERVATIONS



Structure of Polytene chromosome.

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