

Mini Review

Insect chromosomes preparing methods for genetic researches

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Accepted 21 November, 2008

Cytogenetics are almost always based on the examination of the fixed mitotic chromosomes during the analyses of metaphase. During this phase of the cycle of cells, the DNA is folded up and chromatin is strongly condensed. The relative position of the centromere is constant, which means that the ratio of the lengths of the two arms is constant for each chromosome. Importantly, each chromosome displays a unique banding pattern. Different staining methods showed the banding pattern (g band), centromere position (c band), cytophotometric estimation and specific DNA regions (FISH). This study aims to show the important chromosome staining methods.

Key words: Fluorescence in situ hybridization (FISH), chromosome staining, g- banding, c – banding, cytophotometric estimation.

INTRODUCTION

Starting in the middle of the 1950's, much progress was accomplished by studying various aspects of the genetics of the insects. Here, we discussed the selected fields of research implying the formal genetics; structure, organization and evolution of genome at the interspecific and intraspecific level; and the genetics evolution of the groups of insects. The microdissection of chromosome is a technique in which whole chromosomes or the chromosomal segments are dissected under a taking inverted microscope of the chromosome in specific orders. Several modifications of protocol are presented during the last 15 years and reduced the number of chromosomes required for the majority of the applications. FISH is a private method in molecular cytogenetics, due to the majority of the current strongly uniform karyotypes of species, it makes the collection of the multiple copies of the same chromosome impossible. Developed probes in this manner can be employed to study homologies of chromosome in closely dependent species. This information in particular, the genetics of transmission, cytogenetics and genetics of the chromosomal resequencing, proved priceless value for the development of the theory and the evaluation of the feasibility of the genetic

order of the normal populations.

SOLID STAINING METHODS

Giemsa staining method

The technique gives only solid staining chromosomes. This technique do not show chromosome band, centromere, breaking point and NOR bands. The male insect are injected with 0.1 - 0.2 ml of 0.1% colchicine. After the tests are fixed in ethanol-acetic acid (3:1) mixture (1.5 - 3 h). Tests tissues are then minced gently in 50% acetic acid to prepare a cell suspension. A drop of the cell suspension was placed on slides. After, slides were placed in incubator on 60°C (24 h). The cell left on the slide are dried and then stained for 20 min in 2% Giemsa solution (Turkoglu and Koca, 2002).

Aceto-orcein staining method

For the photomicroscopy, cells are prepared by the standard technique; aceto-orcein or alternatively, the cells are tight out of the follicules testiculaires by soft grinding between 2 frosted slides, fixed out of methanol of 3:1: acid acetic, diffusion on plates out of glass, blaze and dried with air and then soiled with Giemsa' stain S

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(Fisher Co. scientist) diluted in the buffer solution of phosphate. To prepare the spermatogonia mitotic to measure the length of chromosome, the animals intra-abdominally are injected with 0.05 ml of a solution of colchicine (0.04 mg/ml diluted in the ringing of insect) (Drets and Stoll, 1974) and left calm for 8:00 h. Then the follicles were dissected. They are rectified between two frosted slides, and the suspension resulting is deposited with 100 t/mn in a clinical centrifugal machine, with sodium citrate for 1 - 10 min, fixed and dried as described above. Measurement of length are taken on photographs of the selected plaque of metaphase with a computer length as described for SCs. The Counce and Meyer (1973) technique of preparation for electronic microscopy was primarily employed.

Acetocarmine staining method

Numbers of chromosome are usually given in young embryos dissected out of the females insect, but from time to time in the mitotic activity in the ovary and in the prophase of oogenesis. Male and the female insect are fixed in a mixture 4 chloroform shares, 3 shares of ethanol, and 1 share of glacial acetic acid. The material is stained with the acetocarmine or access in carmine alcoholic-HCl (Snow, 1963) and then in the acetocarmine.

G banding method

The G-band is the technique used by Hillis et al. (1996) with minor modifications. Dried with the air or in incubator at 60°C (24 h), the preparations are digested within 25 - 30 s in one 0.02 trypsin solution (0.05 g/100 ml PBS solution), rinsed in cold PBS (0.15 M NaCl-0.05 M. NaHPO₄, pH 7.4) for 5 min, stained with 5% Giemsa (5 ml giemsa/100 ml Ph 6.8 Sorenson phosphate buffer) (pH 6.8), washed twice with distilled water (3 - 4 s). The preparations are examined under a photomicroscope with the optical report/ratio 100X and the plaque of mitotic metaphasic. Chromosomes are classified according to Levan et al. (1964).

C banding method

Male cells meiotic are obtained starting from the testicles and from the cells mitotic of the caecum and the ovary of the specimens injected by colchicine (0.05% in the physiological solution of insect). The material were fixed and preserved in Carnoy (3: 1, methanol and acetic acid) at -20°C and analyzed thorough. Fabrics are softened and the cells are dispersed in acetic acid of 45%, centrifuged and twice fixed after rejection of the supernatant medium. Slides are prepared as described by Henegariu et al. (2001), with modifications.

The C-band was carried out by the method described by Sumner (1990). The method could be modified, that is, dry slides are subjected to the hydrolize with 0.2 HCl for 30 min in room temperature (in darkness), washed with distilled water and incubated with the barium hydroxide of 5% at 37°C for 8 min, then washed in distilled water at the room temperature after, incubated with 2xSSC in bain-marie (also known as a water bath) at 60°C for 2 h, after, washed distilled water and stained with Giemsa 6.6% in the Sörensen phosphate buffer pH 6.8 (2 h). The morphology of chromosome is given according to White (1973).

Cytophotometric estimation methods

For the cytophotometric evaluation of Feulgen of the nuclear contents of DNA 2C the fixed testicles are hydrolized in 5 N HCl for 30 min in the room temperature and stained in the solution of Feulgen for 1 h. The soiled testicles are washed in three changes of the SO₂ water (5 g SO₂, 5 ml HCl in 100 ml of water distilled) for 10 min each one. Darkly stained tissues are squashed in a drop of acetic acid of glacial of 45%. The measurement of Cytophotometric of the DNA of the spermatid 2 C.A. was made using a microspectrophometer of Reichert-Zetopan, with a length of wave of 550 nm. (Mirsky and Ris, 1951; Turkoglu and Koca, 2002).

Fish method

Preparations of chromosome of pachytene starting from the constraint p50 of insect are carried out according to the Sahara et al. (2003b). The ovaries of the last larvae instar are dissected in a saline solution (Glaser, 1917) and treated beforehand in the hypotonic solution (KCl and NaCl) (Traut and Marec, 1999) following fixation in Carnoy fixative (ethanol, chloroform, acetic acid, 6:3: 1). Cells are dissociated in the acid 60% acetic and diffusion on a plate out of glass placed on a heat plate. The preparations were passed by an evaluated series of ethanol (70, 80, and 98%) and stored in the freezer (-30°C) until further use. Probe and labelling and the BAC-FISH: BAC-FISH is carried out according to the method described by Sahara et al. (2003b), hybridizations are performed with denatured probe DNA. Chromosomes are counterstained with DAPI. Digital images are taken with UV optic microscope.

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