Dipteran chromosomes: a simple method for obtaining high quality chromosomal preparations

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For cytogenetic studies it is necessary to develop an efficient method of chromosome preparation with well-preserved and dispersed chromosomes and little or no cell-wall debris. In this study, we describe a method for obtaining high quality chromosomal preparations of dipteran cells. This procedure combines drop method and air-dry technique and produces a cell suspension with separated and mixed cells. Suspended mitotic cells were then dropped onto a glass slide in order to distribute the cytoplasm and spread the chromosomes. We compare and evaluate different chromosome preparation methods to develop a more reliable procedure for the resolution of chromosome characteristics and chromosome bandings in blow fly mitotic chromosomes. The combined drop and air-dry technique described here is convenient for identification of sex chromosomes. Using this protocol, acceptable high-quality chromosomal spreads with flattened cells and no cell debris, and without damage and/or loss of chromosomes were obtained.

The Calliphoridae is a cosmopolitan family that includes several blow flies with great medical, forensic and veterinary importance. They are indicators of time of death, myiasis producers and pathogen vectors, and are used in maggot therapy to treat infected chronic wounds in humans and vertebrates[1–7]. Although this family has wide distribution and notable importance, very little is known about its chromosome structure, organization and morphology. The scarce cytogenetic data of a few species show a remarkably stable karyotype ($2n = 12$), generally comprising five pairs of large euchromatic autosomes and a pair of heteromorphic sex chromosomes[8–13].

Chromosome analysis requires the preparation of slides rich in suitable chromosome spreads. In general, the relative decline in the amount of data obtained from studies of insect chromosomes is due to the difficulty in obtaining cells which give good chromosome spreads. Moreover, the main problem in the work on insects is the small number of cells reachable. Even though mitotic cells do exist in many tissues, it is hard to determine the best time to carry out chromosome preparations since this varies within the same order, from one family to another, even from one genus to another[14]. Taking into account the diversity of insect species and tissues, there is no single best procedure. A high-quality, well-spread chromosome preparation is critical to enable routine work on studies of genetics, evolution, phylogeny, origin and taxonomy in animal cytogenetics. Well-spread chromosomes with a clear morphology are essential for karyotype studies and chromosome measurements.

The cytogenetics of Calliphoridae has been mainly restricted to mitotic chromosomes, as it is easy to obtain cells from neural ganglia of third instar larvae. Besides, most of mitotic karyotypes of the blow flies were studied using the squash method[9–11,15], and only two species were described by means of the spreading method[12,13].

The squash method provides a simple, fast way of visualizing chromosomes and cell nuclei in animal tissues, but can cause damage to the preparations and/or loss of chromosomes by introducing shear forces and horizontal movements[16]. During squashing, the cells may be concentrated in small areas that can show chromosome clumping with overlapping due to the failure of the cells to separate readily from one another and, thus, make it more difficult to produce well-flattened squashes and generate good spreads.

On the other hand, the spreading technique allows one to obtain flattened cells and consistent chromosomal dispersal, overcoming some of the disadvantages of the classical squash method, but cell debris are present with more or less contamination around of the chromosomes[12,13]. Therefore, considerable variation in the quality of metaphase spreads produced occurs when the cells are processed using both techniques, and the chromosome preparation becomes a limiting factor for performing classical and molecular cytogenetic studies.

Here we report a method developed for obtaining high-quality chromosomal preparations of dipteran cells without recourse to manual squashing or spreading technique. This procedure combines drop method and air-dry technique from neural ganglia of third instar larvae of *Lucilia cluvia* and *Lucilia sericata* from Argentina. This method produces a cell suspension with separated and mixed cells, allowing flattened clean chromosomal spreads with minimum overlaps and clump. We also compare and evaluate different methods of chromosomal preparation to develop a more reliable procedure for the resolution of chromosome characteristics and chromosome bandings in blow fly mitotic chromosomes.

Adult females of *L. cluvia* and *L. sericata* were obtained in open areas of the university campus near Buenos Aires City (34°36′14″S and 58°22′54″W), Argentina. Flies were collected using beef meat as baits between January and May 2014 and taxonomically identified[17]. The females were transferred to a cage for oviposition at 22 ± 2°C, and 60 ± 5% RH. We monitored daily rearing cages and blow flies to ensure their development into third-stage (L3) larvae.

We applied a modified version of the air-drying method[18] to prepare mitotic metaphase chromosomes. Forty larvae were injected with 0.01 ml of colchicines (0.1 µg/ml) and dissected under a stereomicroscope after 45 min treatment. Ganglia of L3 were dissected out using fine forceps, hipotonized at 25°C for 15 min in 3 ml 0.56% KCl, centrifuged

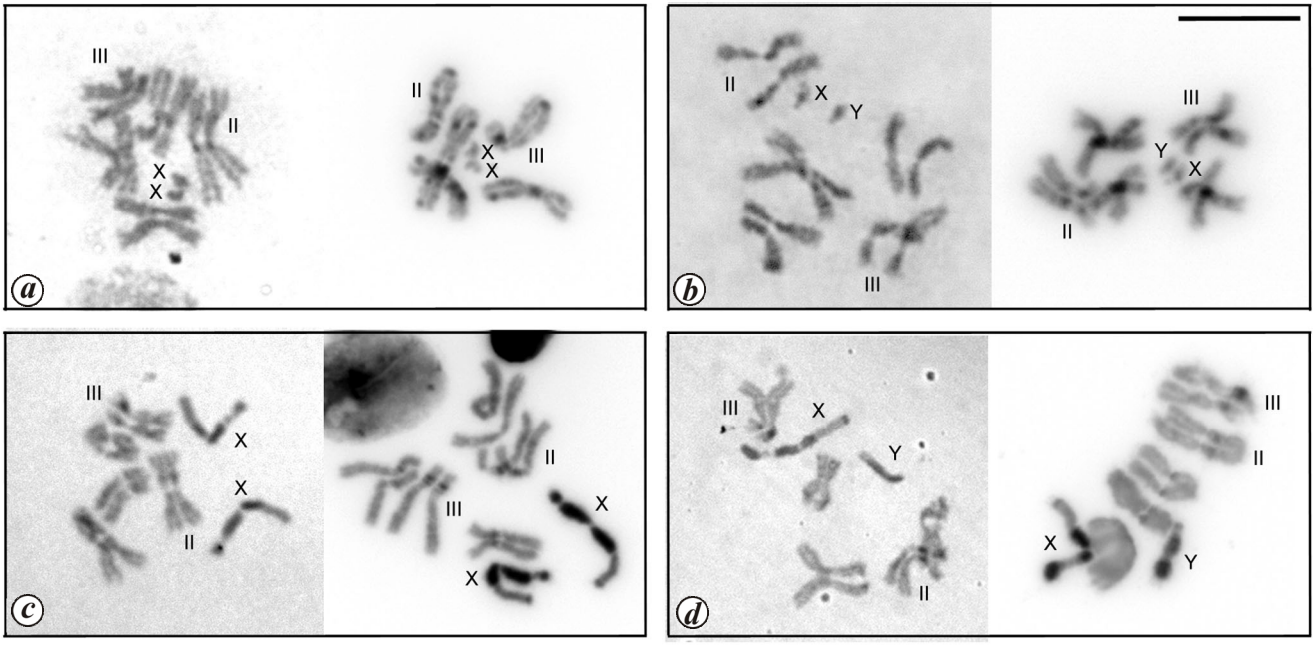

Figure 1. C-banding in females and males of (***a***, ***b***) *Lucilia cluvia* and (***c***, ***d***) *Lucilia sericata*, 2*n* = 10 + XX/XY, stained with 3% Giemsa. (Left) using by spreading technique and (right) by drop and air-dry method. X, Y, Sex chromosomes. II and III indicate chromosomes pairs 2 and 3 respectively. Bar = 10 μm.

at 500 rpm for 5 min, dispersed and fixed for 15 min in 3 ml of freshly prepared fixative (methanol : acetic acid, 3 : 1) and centrifuged at 500 rpm for 5 min. The supernatant was discarded and the cell pellet resuspended in 2 ml of cold fresh fixative. This procedure leaves majority of sediments and debris of non-disaggregated cells.

For slide-making, 4 or 5 drops of cell suspension were carefully placed onto slides which had been previously chilled in ice water for maintaining a thin film of water at the time when the drops fall on the slide from a height of about 20–30 cm. The slides were immediately flamed in a Bunsen burner at a distance of 5–10 cm. During fixative evaporation, the cells grew and flatten, splashing wider due to the fixer coating was thinner, and the cells were attached sooner by preferential evaporation of the methanol and absorption water from air of the acetic acid[19,20].

C-banding[21] with slight modifications was performed. Briefly, slides were successively treated with 0.2 N HCl at room temperature for 20 min, 5% saturated solution of $Ba(OH)_2$ at 50°C for 1–2 min and 2× SSC at 60°C for 60 min, and then stained with 3% Giemsa solution, pH 6.8 for 20–30 min.

In females and males of both species of *Lucilia*, interstitial C-blocks located in the pair of autosomes 2 and 3 were observed using the drop and air-dry method or the spreading technique (Figure 1). Both procedures showed the same C-banding pattern in *L. cluvia* (Figure 1 *a* and *b*) and *L. sericata* (Figure 1 *c* and *d*). However, the chromosome structure and the C-banding pattern had a higher resolution and sharpness by means air-dry method than spreading technique, since it could detect heterochromatin. Therefore, the total number and resolution of C-bands in the blow fly cells depend on the processing of the chromosomes. This result suggests that the C-banding pattern in dipterans depends on the type of chromosome preparation applied. The drop and air-drying technique developed here can be used for rapid and accurate cytogenetic studies because this method does not impair the appearance of the chromosomes, allowing a uniform and flattening of cells than the squash or spreading techniques. This chromosome preparation method is a more reliable, convenient and improved procedure for the resolution of chromosome characteristics and sex chromosomes are identified better. An efficient chromosomal spread with less contamination and cell debris is important for classical and molecular cytogenetics studies.

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