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Karyological studies on established mosquito cell lines[1]

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Summary. Chromosome frequency distribution and cellular DNA estimations in different established mosquito cell lines were studied. These cell lines exhibited a wide range of cell types with a diploid stem-line comprising 50–55% and a haploid substem-line comprising 12–30% of the population. Estimation of cellular DNA contents by impulse cytoflowmetry and by Feulgen cytophotometry supported these observations. Because of their low diploid counts, these cell lines cannot be classified as diploid.

Since the first successful establishment of a mosquito cell line on hemolymph-free medium by Singh[3], several cell lines from different species of *Aedes* mosquitoes have been developed[4–6] and used for virological studies[7–15]. However, studies on characterization of these cell lines were only limited[6,16,17]. In this paper, we report the chromosome constitutions of 4 cell lines and cellular DNA contents of 5 cell lines from different species of *Aedes*.

Material and methods. Established mosquito cell lines of *Aedes aegypti* (ATC-10)[3], *A. albopictus* (ATC-15)[3], *A. vittatus* (ATC-121)[4], *A. w-albus* (ATC-136)[5] and *A. novalbopictus* (ATC-173)[6] were maintained at 28 °C on Mitsuhashi-Maramorosch medium[18] supplemented with 10% foetal calf serum and antibiotics (penicillin-streptomycin). Chromosome studies were carried out on ATC-10, ATC-15, ATC-136 and ATC-173 cell lines; DNA estimations by impulse cytoflowmetry were made on all the 5 cell lines while ATC-10 and ATC-136 cell lines were used for Feulgen cytophotometry.

2–3-day-old cultures were treated with colcemid (0.5 µg/ml) for 4–6 h. Metaphase cells were removed by shaking, treated with 0.5% KCl hypotonic solution and fixed in chilled acetic acid-methanol (1:3) fixative. Chromosomes were spread by air-drying and stained with dilute Giemsa's stain. About 400 metaphases from each cell line were screened. Chromosome preparations were also made from coverslip cultures without hypotonic pretreatment to corroborate the results obtained by the above method.

Full-grown cultures were trypsinized to obtain single-cell suspensions and were fixed in 5% buffered glutaraldehyde. These were washed 3 times with Tris buffer (0.1 M, pH 7.4), resuspended in ethidium bromide (EB) in Tris buffer (10 µg/ml) and stained in the dark for 60 min. Fluorescence intensities of these cells were recorded with Phywe ICP

a) ATC-10 (passage 210):

Chromosomes	2	3	4	5	6	7	8	9	10	11	21
% metaphases	2	30	4	1	55	1	1	1	1	2	2

b) ATC-15 (passage 110):

Chromosomes	2	3	4	5	6	7	8	9	10	12	13	14	15	17	18	24
% metaphases	2	12	2	3	55	2	3	2	1	7	2	2	2	1	2	2

c) ATC-136 (passage 180):

Chromosomes	2	3	4	5	6	7	8	9	11	12	15	21	24
% metaphases	1	20	3	1	50	3	2	2	2	11	2	2	1

d) ATC-173 (passage 105):

Chromosomes	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	19	24
% metaphases	2	15	2	3	52	2	1	2	0.5	2	15	0.5	0.5	0.5	0.5	0.5	1

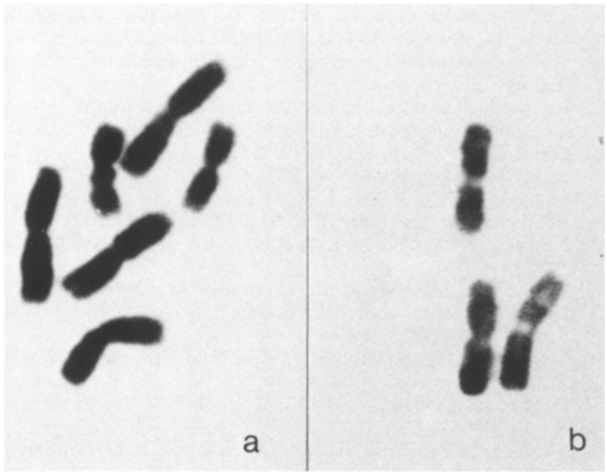

Figure 1. Giemsa-stained metaphase chromosome preparations from ATC-15 cell line showing *a* diploid chromosome compliment and *b* haploid chromosome compliment. × 800.

cytoflowmeter under optimized conditions for reading EB fluorescence and histograms of number of cells with differing fluorescence intensities were plotted. Cytophotometric estimations of DNA on Feulgen-stained cells were made on ATC-10 and ATC-136 cells; total extinction of individual cell was measured at 546 nm.

Results and discussion. As evident from the table, the number of chromosomes per metaphase ranged from 2 to 24. Metaphases with 6 chromosomes (diploids, fig. 1, a) formed a mode with about 55%, 55%, 50% and 52% of the populations, while a submode containing 3 chromosomes (haploids, fig. 1, b) formed about 30%, 12%, 20% and 15% of the populations in ATC-10, ATC-15, ATC-136 and ATC-173 cell lines, respectively. Occurrence of subdiploid, in particular haploid, and other heteroploid metaphases was also confirmed in the preparations made from coverslip cultures not pretreated with hypotonic solution. Though the stem-lines were diploid, the total percentage of diploid cells now is low; evidently, these cell lines are not diploid. Moreover, the broad spectrum encompassing chromosome configurations from 2 to 24 indicates the presence of wide heterogeneity of cell types. These observations appear to be in variance with the earlier reports on mosquito cell lines[4,16,17,19]. A majority of the Dipteran cell lines contain mostly diploid cells and a few polyploid and heteroploid cells, but no subdiploid cells (see Hink[20] for details). Profound changes in karyotypes were reported in 1 subline of *Aedes aegypti*[21] and 3 lines of *Drosophila* melanogaster[22]. An orthopteran cell line *(Blattella germanica)* on the other hand contained about 20% subdiploid cells[23].

Further, to define the state of interphasic cells, which do not reveal their chromosomes, DNA estimations were made employing cytoflowmetry (fig. 2) and Feulgen cytophotometry (fig. 3).

It is interesting to note that the frequency histograms of different mosquito cell lines showed a dominant peak with markedly skew distribution at higher fluorescence intensities (fig. 2). The skewed characteristics were not a result of a significant extent of proliferation since no distinct G_2 peak could be discovered. Instead, the histograms displayed a very broad distribution, indicative of a heterogeneous population with differing amounts of DNA. This is in contrast to the profile obtained for any normal homogeneous population. The relative proportions of cells having the modal amount of DNA, presumably diploid, and those having more than diploid amounts were determined by resolving

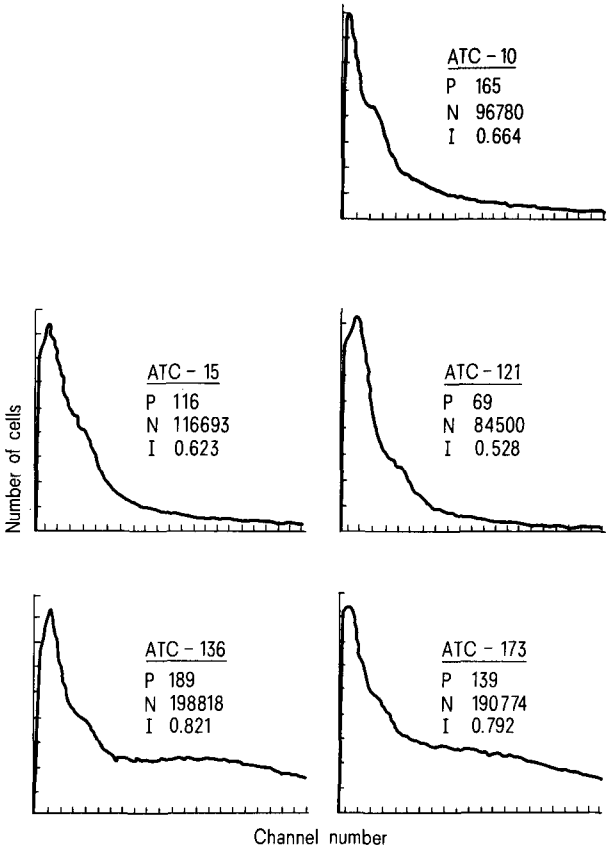

Figure 2. Estimation of cellular DNA contents by impulse cytoflowmetry. Profiles showing DNA-EB fluorescence distribution. The modes correspond to 4000 cells. P, passage number; N, total number of cells counted; I, index of heterogeneity (total number of cells with more than modal fluorescence total number of cells).

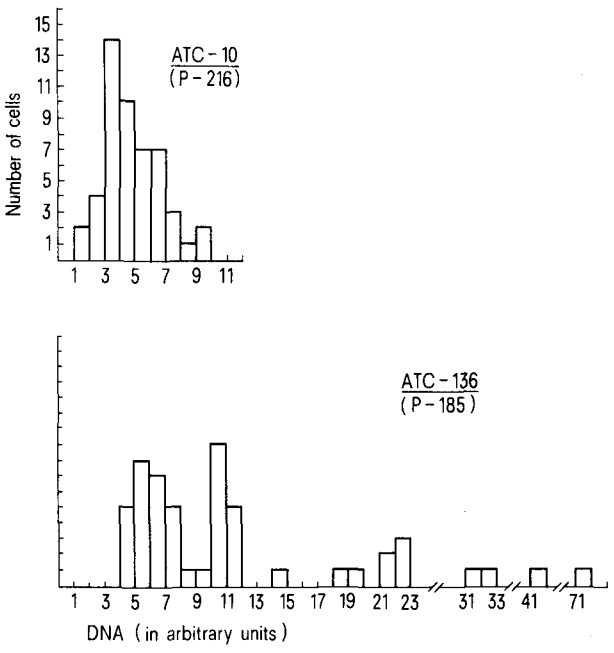

Figure 3. Estimation fo cellular DNA contents by Feulgen cytophotometry. Histograms showing distribution of total extinction values per cell, measured at 546 nm. P, passage number.

the histogram (index of heterogeneity) according to the method of Lalande and Miller[24]. These indices, together with the total number of cells required to obtain 4000 cells with mean fluorescence intensity corresponding to the modal intensity, reflect the measure of heterogeneity of cell lines.

ATC-10 and ATC-121 cell lines thus appear to be relatively less heterogeneous because these contained relatively fewer cells beyond the modal population and also because these required a lesser number of cells to generate a histogram with 4000 cells in the peak frequency channel. The histograms are a composite profile of cells in G_1, S and G_2+M phases of the cell cycle. In such a case, the larger the heterogeneity in the cell population, the more skewed is the fluorescence distribution at higher intensities. The widespread distribution of DNA values obtained for Feulgen cytophotometric estimation in the 2 lines tested also indicates the heterogeneity of these cell lines. It thus appears that data on DNA estimations by either method are comparable to those from chromosome studies.

The diploid cell lines maintained in continuous cultivation - subcultured after reaching confluency - have a finite lifespan. However, from the data presented here, it appears that the established mosquito cell lines, which have traversed more than 100 passages, are karyologically heterogeneous and contrary to all the earlier reports are shown to be not diploid. Further, it may be mentioned that of the 3 cell lines, viz., ATC-10, ATC-15 and ATC-121, the 1st 2 could induce neo-vascularization in chick chorioallantoic membrane, indicating the presence in these of an angiogenic factor; however, when inoculated in conditioned golden hamsters and albino mice, none of the 5 cell lines produced tumors[25]. Studies on agglutinability of cells after concanavalin-A treatment revealed presence of 2 cell populations in late passage mosquito cell lines: one agglutinable and the other non-agglutinable, indicating the occurrence of transformed as well as untransformed cells[26]. These findings suggest that the established mosquito cell lines exhibit characters which are generally not shown by diploid cells and thus lends support to the observation that these cell lines are not diploid.

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Enzymes involved in oxygen detoxification during development of *Drosophila melanogaster*[1]

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Summary. Activities of superoxide dismutase (SOD), catalase (CAT), and peroxidases (PER) were examined at 24-h intervals during *Drosophila* devolopment. SOD activities show a U-shaped curve from egg to adult stages whereas CAT and PER are consistently low in egg through larval stages. Male and female larvae have similar activities of SOD, CAT, and PER whereas male adults have elevated activities of these enzymes. Larvae are more sensitive to H_2O_2 and 3-amino-1,2,4-triazole (an inhibitor of CAT) than adults.

The toxicity of oxygen in living systems involves direct oxidation of thiol groups of enzymes, and production of toxic intermediates such as hydrogen peroxide, hydroxyl radical or metal oxy-compounds generated by superoxide-mediated Fenton chemistry[2,3]. The tripeptide glutathione beneficially intercedes in enzyme oxidation, while peroxidases (PER) and catalase (CAT) are effective in maintaining relatively low levels of H_2O_2. Superoxide dismutase (SOD) converts the superoxide anion in a disproportionation reaction to H_2O_2 and O_2 and may indirectly prevent the formation of the reactive ferryl ion (complex FeO_2^+) and/or an organic oxyradical $RO\cdot$[4]. Under atmospheric oxygen concentration, these defense systems protect organisms from obvious stress. In hyperoxia, these systems appear to be 'swamped' and a toxic syndrome is observed[5,6].

We recently reported on the influence of increased O_2 during development of *Drosophila melanogaster*[7]. We observed that 1st, 2nd and early 3rd instar larvae are extreme-