Cytogenetic Basis of Chemically-Induced Sterility in *Culex pipiens fatigans* Wiedemann

I. Chemosterilant-induced damage in the somatic chromosomes

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Studies on the potential use of induced sterility in insect control have resulted in the discovery of a large number of chemicals having effect on fertility and fecundity of many species of insects. While the cumulative effect of these compounds lead to sterility their mode of action at cellular level is not yet clear. Many chemosterilants belonging to aziridiny compounds, phosphoramides and s-triazines are known to cause cytogenetic damage in the reproductive or embryonic tissues of insects (LaChance *et al.* 1968, Morgan 1967, Cline 1968, Reinecke *et al.* 1969). In addition, a similar damage to the brain cells of *Aedes aegypti* (L.) has been observed by larval treatment of apholate and hempa (Rai 1964, Sharma and Rai 1969, George and Brown 1967). As a result of these studies a concept has evolved that cytogenetic damage caused by chemicals is some how related to sterility (LaChance *et al.* 1968).

In our earlier studies, a number of alkylating and non-alkylating chemosterilants were shown to induce sterility in *Culex pipiens fatigans* Wied. (Pillai and Grover 1969, Grover and Pillai 1969). The present investigations were, therefore, aimed to elucidate a cytogenetic basis of such chemosterilant-induced sterility in *C.p. fatigans* by detailed studies involving their effects on somatic, reproductive and embryonic tissues, and on DNA synthesis in the gonads. The present paper deals with the effects of apholate, metepa and hempa on the somatic chromosomes.

Materials and methods

The mosquitoes for the present studies were drawn from a Delhi strain of *C.p. fatigans* established in our laboratory since 1965 (Pillai and Grover 1969). The chemosterilants employed were as follows:

1) Apholate—2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis (1-aziridiny)-1,3,5,2,4,6-triazatriphosphorine, 2) Metepa—tris (2-methyl-1-aziridiny) phosphine oxide, and 3) Hempa—Hexamethyl phosphoric triamide.

Mid-fourth instar larvae (65 hrs old) were used for preparing somatic chromosomes of the brain following the squash technique of Breland (1961) modified by French *et al.* (1962). The karyotype studies were made by employing the modified mammalian chromosome technique of Lewis and Riles (1960) and chromosome measurements were made according to the procedure used by Baker and Aslamkhan (1969). Rapid cell divisions were found to occur in the brain cells of the larvae

intermittently. Therefore, counts of the total number of dividing cells (metaphases) per complete larval brain (from 65 hrs old) were made and the average of five such counts was regarded as an index of mitotic activity.

The larvae were exposed to various concentrations of apholate, metepa and hempa, in water from early second instar till pupation as described earlier (Pillai and Grover 1969, Grover and Pillai 1969). Larval treatment of 10 ppm apholate, 50 ppm metepa or 250 ppm hempa was found to induce complete sterility in *C.p. fatigans* (Pillai and Grover 1969, Grover and Pillai 1969). Brains from the treated and control mid-fourth instar larvae were dissected out and temporary squash preparations (one brain per slide) were made for studying the effects of chemosterilants on somatic chromosomes. In addition, effect of apholate and hempa on the mitotic activity and on the frequency of aberrations was studied by examining five complete brains for each concentration of the chemosterilant. An attempt was also made to study in detail various types of aberrations induced by apholate and to find out whether the breaks induced were localised or randomly distributed. Colchicine pretreatment was not used for studying the effects of chemosterilants on somatic tissue.

Results

Normal karyotype: The diploid chromosome number of *C.p. fatigans* was six ($2n=6$). The karyotype from larval brains is characterised by 3 pairs of fairly large chromosomes of different length (Table 1, Figs. 1, 2). These are designated

Table 1. Measurements of metaphase chromosomes* of *C. p. fatigans*
(Tissue: brain of IV instar larvae)

Chromosome number	Position of centromere	Length in microns			Ratio of length of I/II + III
		Arm a	Arm b	Total	
I	M	1.9	1.9	3.8	0.349
II	SM?	2.8	2.5	5.3	
III	M	2.8	2.8	5.6	

M: metacentric, SM: submetacentric.

* Ten well spread metaphases were examined and average of these was taken as chromosome lengths.

as pair I, II and III based on their length, following the convention set by Rai (1963). Chromosome I and III are metacentric, while chromosome II appears submetacentric. The ratio of chromosome pair III was only slightly larger than second, so that the difference between III and I or II and I was more than the difference between II and III. In some brain cells chromosomes show somatic pairing at metaphase, but in the majority of the cells somatic pairing is not pronounced, though the two homologues of a pair are located always adjacent to each other. Because of the large size and small number of chromosomes, *C.p. fatigans* is a good object for study of this nature.

Effect on mitotic activity: Results of the effect of apholate and hempa on the mitotic activity of the larval brain cells are shown in Table 2. Only higher doses of apholate or hempa had resulted in mitotic inhibition such that at 100 ppm apholate or 1000 ppm hempa, there was a near complete mitotic inhibition. It is evident that hempa was less effective than apholate in inducing aberrant mitosis. Only those cells where chromosome abnormality was unambiguous were counted or taken into account. Thus the values do not represent 'absolute counts'.

Table 2. Effect of apholate and hempa on the mitotic activity in the larval brain cells of IV instar of *C. p. fatigans*

Chemosterilant	ppm conc. in	Avg.** no. of dividing cells/brain
Control	—	112 (0)
Apholate	5	115 (40)
	10	118 (69)
	15*	91 (81)
	30*	50 (100)
	100*	12 (100)
Hempa	100	111 (12)
	250*	80 (30)
	500*	49 (41)
	1000*	3 (left out)

* Toxic doses.

** Average calculated by examining 5 complete brains of both sexes.

Figures in parentheses show per cent aberrant mitosis. Controversial cells were left out from counting.

Effect on chromosomes: Sterilizing doses of apholate, metepa and hempa caused both physiological as well as structural aberrations in chromosomes of brain cells during mitosis. Among the physiological aberrations, one of the common effect observed was chromosomes stickiness prevalent at the chromosome ends which in some cases gave ring-like structure due to touching of chromosome-ends between pairs (Figs. 3, 4, 12). It also resulted in few cases anaphase bridges unaccompanied by fragments.

Among the structural aberrations apholate induced partial and complete breaks in one or all chromosomes of a single cell. Partial breaks were not counted while studying the distribution of breaks on metaphase chromosomes. Some times several loci along the chromosome are seemed to show no DNA and appeared as unstained zones because of closely occurring partial breaks (Fig. 6). Chromosome I usually showed centromeric break (Fig. 5) in one of the homologues which resulted in deletion of one of the arms (Fig. 6). The frequency of such breaks was about 23.1% (Table 3). Sometimes a terminal break was also observed in chromosome I (Fig. 7, Table 3); the frequency was 10.2% of the total breaks counted. Chromosome II and III usually showed terminal breaks in one of the arms of the chromosomes (Figs. 7 and 8). The frequency of terminal

break in chromosome II was about 19.2% and in chromosome III was as high as 44.9%. Rarely chromosome III showed subterminal break in one of the homologues (Table 3). Terminally deleted fragments of chromosomes were transformed into micronuclei of various sizes depending upon the size of the deleted fragment (Fig. 8). Often chromosome arms from one, two or all the three pairs appeared fused with one another.

Dicentric chromosomal bridges during anaphase were also observed accompanied by one or few small fragments (Fig. 9). This type of fragments because of being acentric could not move on mitotic spindle and usually lagged behind, transformed into micronuclei and were not included in any of the daughter nuclei at telophase.

Table 3. Effect of 10 ppm apholate on the frequency of distribution of breaks in metaphase chromosomes of *C. p. fatigans*

No. of metaphases examined.	*Aberrant metaphases.	% Aberrant metaphases	% Frequency of breaks in**				
			Chromosome I		Chromosome II	Chromosome III	
			Centromeric	Terminal	Terminal	Terminal	Sub-terminal
500	352	70.4	23.1	10.2	10.2	44.9	2.6

* Includes both physiological and structural aberrations.

** Calculated from total number of breaks counted (partial breaks were not counted).

Metepa caused almost identical aberrations as apholate (Figs. 10–13). Polyploidy in this species was not observed in any of the preparations of untreated brains. Treatment with metepa and apholate induced polyploidy in some of the cells (Fig. 11). In case of apholate such cells were highly sticky and never spread well.

Apholate and metepa treatments also resulted in C-metaphases with chromatids spread out; such metaphases were never seen in controls except in colchicine-treated ones. This shows these chemicals interfered with spindle mechanism perhaps like colchicine.

Hempa, unlike apholate and metepa, did not cause drastic damages. It induced aberrations such as partial breaks, chromosome clumping at their ends

Figs. 1–16. Chemosterilant-induced chromosomal aberrations in the brain cells of fourth instar larvae of *C. p. fatigans*. $\times 2000$. 1, normal karyotype. 2, Normal metaphase chromosomes. 3–9. Apholate-induced aberrations. 3, metaphase chromosomes showing stickiness. 4, ring-formation due to stickiness. 5, centromeric break in chromosome I. 6, centromeric deletion in chromosome I and chromosome III with partial break or damage. 7, terminal breaks in chromosome I and III. 8, chromosome III with terminal break and terminal deletion, chromosome I forms a ring, and chromosome II with a terminal break and a micronucleus. 9, dicentric bridge at late metaphase with a micronucleus. 10–13. Metepa-induced aberrations. 10, chromosomes I and III with terminal deletions. 11, polyploidy; 12, chromosome showing stickiness. 13, chromosome II with break, the other homologue disfigured. 14–16. Hempa-induced aberrations. 14, metaphase chromosomes showing partial breaks. 15, chromosomes showing clumping at ends. 16, metaphase chromosomes with partial breaks and fusion.

and chromosomal fusions (Figs. 14–16). Rarely hempa caused complete breaks and large deletions.

Discussion

It is evident from the present studies that apholate, metepa and hempa have the ability to cause the chromosome breakage in the brain cells of *C.p. fatigans*. Similar chromosomal damage by aziridines and phosphoramides in *A. aegypti* and in human cells *in vitro* have been reported by several authors (Rai 1964, George and Brown 1967, Chang and Elequin 1967, Chang and Klassen 1968). The distribution of apholate-induced breaks was non-random. Such non-random distribution of chemically induced breaks in animal and plant cells is known (Kihlman 1963, 1966). Terminal break in one of the homologues of chromosome III was more frequent. Based on a similar finding in *A. aegypti*, Rai (1964) suggested that such localization was probably due to secondary constriction. However, this is not applicable to *C. p. fatigans* as secondary constriction is absent in chromosome III. Also there is a possibility that some of the chromosome breaks might have undergone repair during the secondary division cycle and thus in the cytological preparation only those breaks that persist over several division cycles are seen. Such repairs have been observed in WI-38 cells and in human leucocytes cultures after treatment with several alkylating agents (Chang and Elequin 1967, Chang and Klassen 1968).

Occurrence of aneuploids, polyploids and C-metaphases suggest that these compounds cause 'colchicine-like' effects. Partial or complete damage of one chromosome results in aneuploids (Fahmy and Fahmy 1964, LaChance *et al.* 1968). Polyploidy may result by arrest of metaphases and the formation of restitution nuclei. Treatment of *C. p. fatigans* with graded doses of apholate caused cytostatic, mutagenic and cytotoxic effects as categorized by Ross (1962) in case of alkylating chemicals. The sterilizing dose of apholate, however, did not show any effect on the mitotic index. Higher doses of apholate and hempa resulted in inhibition of mitosis. Such doses were found to interfere with the growth and development of this mosquito (Pillai and Grover 1969).

The aberrations induced by hempa were similar but not so severe as reported by George and Brown (1967) in *A. aegypti*. The degree of induction of sterility cannot be directly correlated to chromosome breakage effects. Chang and Klassen (1968) found that hempa in human leucocytes induced only 14% chromosomal aberrations as compared to 89% induced by apholate even at 1000-fold greater molar concentration.

The somatic damage induced by chemosterilants may cause damage to adult longevity or mating vigour or on the function of brain and mid gut (LaChance *et al.* 1968, Sharma and Rai 1969). However, sterilization with apholate (10 ppm) apparently did not reduce the mating ability, competitiveness and longevity of males of *C. p. fatigans* (Grover and Pillai 1970). This suggests that either such damages are repaired by the time adult emerges or damages to adult tissues are not to the extent that could effect its mating behaviour. Such a recovery of the damaged

midgut of *Anthonomus grandis* adults caused by Myleran has been recently reported (Reineck *et al.* 1969).

The chemosterilant-induced chromosomal aberrations in the somatic cells seem to be similar to cellular damage occurring in the gonads. Sexual sterility is a result of the induction of dominant lethals in the gametes. Such genetic damage involves drastic chromosomal structural aberrations (Rai 1964) and a reflection of these could be seen in the somatic chromosomes. Embryos formed from gametes with dominant lethals die during embryogenesis due to mitotic failure as a result of genetic imbalance (LaChance and Reiman 1964).

Summary

Treatment of larvae of *Culex pipiens fatigans* with chemosterilants viz., apholate, metepa and hempa, caused various types of chromosomal structural changes in the somatic cells. The action of hempa appeared less drastic than either apholate or metepa. The cytogenetic basis of chemosterilant-induced sterility is discussed in the light of the present findings.

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