Research Article **Cytology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHOTO-OXIDATION AND QUINACRINE FLUORESCENCE RESPONSE IN THE MITOTIC CHROMOSOMES OF THE FLESH-FLIES *SARCOPHAGA*

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ABSTRACT

Experiments have been carried out to investigate the DNA base specificity of light-induced banding (LIB) produced by photo-oxidation of chromosomes followed by Quinacrine staining to detect denatured DNA. In the present study mitotic preparations from *Sarcophaga ruficornis* and *Sarcophaga argyrostoma* were used to detect the visible light and fluorochrome interactions. Our results indicated that specific destruction of guanine residues is the main effect of photo-oxidation and LIB is a base-specific phenomenon. In addition, photo-oxidation may also cause DNA-protein cross-linking which affects the binding of some dyes, while prolonged photo-oxidation appears to cause more general damage to DNA.

KEYWORDS: **Mitotic chromosomes, *Sarcophaga* species, Quinacrine, photo-oxidation**

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INTRODUCTION

Cytogenetical studies have been immensely benefited with the use of fluorochromes, when bound to fixed chromosomes[1]. Photo-oxidation is a process which preferentially removes guanine (G) residues from chromosomal DNA. The fact that both AT and GC specific fluorochromes are available and sequential staining of chromosomes with fluorescent dyes and counterstaining with non-fluorescent DNA ligands is feasible, means that use of suitable combinations may facilitate the analysis of molecular mechanisms involved. Fluorochrome-DNA interactions help in elucidating the chemical structure of metaphase chromosomes. Quinacrine is an acridine derivative which binds to AT-rich regions of chromosomal DNA. Q-Fluorescence is enhanced by polymers containing adenine (A) and thymine (T) while polymers containing guanine (G) and cytosine (C) or simply G quench it. Different parameters like chromatin compactness, DNA conformation, could be responsible for Q-bands but Sumner[2] indicated that Q-bands do not show differential binding of quinacrine along chromosomes. Such a finding suggests that DNA base composition might be a major, but not necessarily the only factor in producing Q-bands. Characterization of the constitutive heterochromatin in the mitotic complement of sarcophagid flies with several fluorochromes such as Quinacrine, Hoechst 33258, DAPI has revealed that the pericentric regions of some autosomes and large areas of the sex chromosomes i.e. X and Y are characterized by bright fluorescence [3, 4, 5, 6,7]. To detect the significance of DNA base composition, specifically that of G interspersion in determining Q-flurescence response in mitotic chromosomes of the two *Sarcophaga* species namely *Sarcophaga ruficornis* and *Sarcophaga argyrostoma*, photo-oxidation was used that is considered capable of destroying G residues in both purified and chromosomal DNA. The cytological results we obtained seem to further suggest that Q-banding is related to G interspersion along chromosomal DNA. This technique is considered capable of detecting chromosomal areas containing A + T-rich DNA sequences[8].

MATERIALS AND METHODS

(i) Fly rearing

In the present work, two species of the flesh-flies belonging to the genus *Sarcophaga* viz., *Sarcophaga ruficornis* (Fabricius) and *Sarcophaga argyrostoma* (Robineau - Desvoidy) were used. Laboratory colonies of the two species were set up from the progenies of wild inseminated females in insect rearing cages maintained at 27 ± 2ºC. Sugar cube, protinex and water were kept in the cages for feeding the adult flies. Fresh goat kidney pieces were kept in the cages for larviposition, after which the larvae were removed and kept in glass jars covered with a cotton piece. The larvae were reared in an uncrowded condition on fresh kidney pieces for about six days. When the mature third instar larvae stop feeding and start wandering in search of dry places, they were transferred to clean glass jars with saw dust for pupation. The pupae were kept in petri-dishes in the insect rearing cage for emergence. The adult flies emerged after about eleven days of pupation. Fresh goat kidney was provided for larviposition after twelve days of emergence of the flies.

(ii) Chromosome preparation

Metaphase chromosome preparations were made from the neural ganglia of the third instar larvae of both the species. Neural ganglia were dissected out in insect saline (0.9% NaCl), incubated in sodium citrate (2%) containing colchicine solution (0.05%) for 15 min in a cavity block. The ganglia were fixed in freshly prepared Carnoy's fixative (3:1, methanol: acetic acid) for 7 min. The fixed neural ganglia was kept on a clean slide in a drop of 60% acetic acid and macerated with the help of

needles, the cell suspension was spread and air-dried.

(iii) Photo-oxidation treatment

Fixed mitotic chromosomes were incubated in 100 ml of 33.4×10^{-6} M Methylene blue solution in the presence of visible light emitted by an 18 W lamp. The distance between the light source and the surface of the solution was 12 cm at the experimental temperature of 21±2˚C and an overnight exposure was given to the prepared slides. To remove the methylene blue from the mitotic preparations, a 5 min. bath was carried out in ethanol-acetic acid (3:1) solution.

(iv) Fluoroscence staining

Air dried chromosome preparations were stained with quinacrine dihydrochloride according to the procedure of Lee and Collins[9] to visualize the heterochromatic areas in the chromosome complements of both the species. The air-dried preparations were hydrated with an ethanol series comprising 95%-, 70%-, 50%-, distilled water followed by staining in 0.5% quinacrine dihydrochloride in 45% acetic acid for 10 min. The stained slides were rinsed twice in 95% ethanol and air-dried, mounted into 1:1 Sorenson's phosphate buffer (pH 6.8): glycerol mixture and observed in Nikon 80i UV-Fluorescence microscope with the filter combination of N BV-1A.

RESULTS

The mitotic chromosome complement of the two species comprises five pairs of autosomes and a pair of sex chromosomes XX in the females and XY in the males. Staining with the fluorochrome quinacrine dihydrochloride/ quinacrine reveals that in *S. ruficornis* the pericentric region of autosome pairs II and V show fluorescent bands while the autosomal pairs III, IV and VI do not show any fluorescent regions. The distal half of telocentric X chromosome fluoresces brightly but the proximal half shows dull fluorescence while the Y chromosome fluoresces moderately with a single bright band near the telomeric region (Fig. 1 A). However, after exposure of light for 16hr with methylene blue followed by quinacrine staining of the mitotic chromosomes of *S. ruficornis*, reveals that the pericentric areas of autosomal pairs II and V show fluorescent bands. The proximal region of the X chromosome shows bright fluorescence as compared to the distal half while the Y chromosome fluoresces moderately throughout its entire length (Fig. 1B).

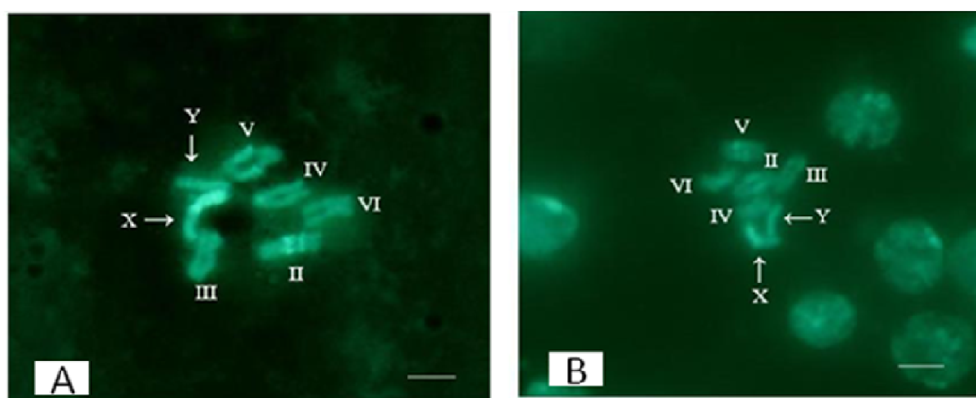

Figure 1
(A) Quinacrine staining in the mitotic chromosomes of S. ruficornis at metaphase stage used as control and (B) Mitotic plates photographed after photo-oxidation and Q-staining.

In the species *S. argyrostoma*, autosomal pairs II, V and VI show moderate fluorescence with quinacrine in the pericentric regions. The X chromosome shows differential staining, with the distal tip showing dull fluorescence while the rest of the chromosome shows bright fluorescence. The Y chromosome shows a bright fluorescence (Fig. 2 A). After exposure of light for 16hr, the mitotic chromosomes of *S. argyrostoma* reveal that the pericentric regions of all the five autosomes show moderately fluorescent bands. The X and Y chromosomes, however, show bright fluorescence as compared to the autosomal heterochromatic regions (Fig. 2 B).

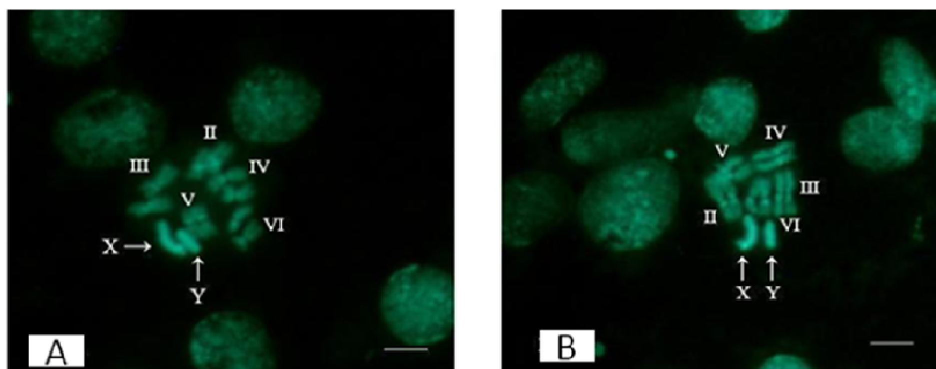

Figure 2
(A) Quinacrine staining in the mitotic chromosomes of S. argyrostoma at metaphase stage used as control and (B) Mitotic plates photographed after photo-oxidation and Q-staining. Bar in all the photographs represents 10 µm.

DISCUSSION

An enhanced fluorescence observed in the heterochromatic regions of the mitotic chromosomes of *S. ruficornis* and *S. argyrostoma* indicates that photo-oxidation, which preferentially removes guanine (G) residues from chromosomal DNA, enhances the Q-fluorescence. These results suggest that Quinacrine fluorescence is not a function of AT/GC ratio only, but is also dependent on the interspersed GC base pairs which quench the fluorescence in preparations stained with quinacrine in the absence of photo-oxidation. These results support the hypothesized primary role of DNA base composition, especially the distribution of guanine in determining the quinacrine fluorescence response in the fixed mitotic chromosome of both the sarcophagid flesh flies. Methylene blue (MB) is efficient singlet oxygen generating photoactive dye that binds to DNA and allows photosensitized reactions to be used for sequence-specific cleavage of the DNA backbone. Intercalation and groove binding are possible binding modes of the dye, depending on base sequences and environmental conditions. The increase in Q-fluorescence intensity could theoretically be due to the treatment of light on the protein components of the mitotic chromosomes. These findings are discussed in the light of previous reports and guanine destruction is hypothesized as representing the most plausible mechanism for explaining the cytological results. The fluorescence of quinacrine bound to DNA is thus base dependent as highly AT rich DNA sequences enhance and GC rich DNA sequences quench its fluorescence [10, 11, 12, 13, 14]. However, Weisblum[11] has opined that Q-

fluorescence reaction "is not strictly a function of the AT/GC ratio, but the degree of interspersion of GC base pairs among AT base pairs plays an important role". In the same context, Sumner[2] has also observed that the Q- bands may not be a reflection of the differential binding of quinacrine along the chromosomes and suggested that the DNA base composition may not necessarily be the only factor in producing the Q-bands implying thereof that the interspersion of G- also plays an important role in producing Q- bands. It is also well known that the G- residues can be destroyed by photo-oxidation treatment in light induced banding thus enhancing the quinacrine fluorescence of regions rich in AT sequences [15, 16, 17, 7, 18, 19].

CONCLUSION

It is interesting to note that autosomal pairs III, IV and VI in the complement of *S. ruficornis* and III and IV in the complement of *S. argyrostoma* do not show bright fluorescence when stained only with quinacrine. This may perhaps be due to the fact that the AT and GC rich sequences are intermingled in such a way that AT specific dyes are unable to bind to these areas. However, when the GC rich areas are suppressed by the process of photo-oxidation, only then are these AT rich sequences able to bind to quinacrine and therefore, fluoresce. The results of the present study clearly indicate that the constitutive heterochromatin in both the species is rich in AT sequences.

ACKNOWLEDGEMENTS

We would like to thank Head of Department, Department of Zoology for providing necessary laboratory facilities during the present work. I would like to express my sincere gratitude to Prof. R. R. Tewari, Department of Department of Zoology, University of Allahabad, Allahabad, for his constant motivation to accomplish this work.

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