Sumithione, an organophosphorus insecticide, induces a puff at the locus 43D/42A in the polytene chromosomes of the mosquito *Anopheles stephensi*

N.N. Godbole, S. Bawar and S.P. Modak*

Department of Zoology, University of Poona, Pune 411007, India

Received 29 May 1984

Abstract not received

Sumithione Anopheles stephensi *Polytene chromosome* *Puff induction* *Insecticide*

1. INTRODUCTION

In many dipteran species, appearance of puffs in the polytene chromosomes of larvae is a well studied phenomenon. Puffing involves decondensation of chromatin at localized sites on the polytene chromosomes and is accompanied in most cases by transcription of DNA in the puff region. It is known that specific puffs appear in a very regular and characteristic fashion in different species and the sequence of puffing at different loci exhibits a developmental stage-specific puff pattern. Alterations in the normal environment also result in specific puff induction, reflecting expression of genes in the responding loci. This has been well documented in cases involving the application of 'heat-shock' [1], anoxia [2], the hormone ecdysone [3], inhibitor of protein synthesis cycloheximide [4], etc., in *Drosophila* species.

A number of organophosphorus compounds and chlorinated hydrocarbons have been used to control insect populations and some such insecticides are known to be mutagenic agents. It is also noted that in response to the extensive use of such insecticides to control *Anopheline* mosquitoes a number of resistant strains have appeared in nature [5]. Yet, nothing is known about the gene(s) involved in imparting the property of resistance or sensitivity towards insecticides in either *Anopheline* or *Drosophila* species.

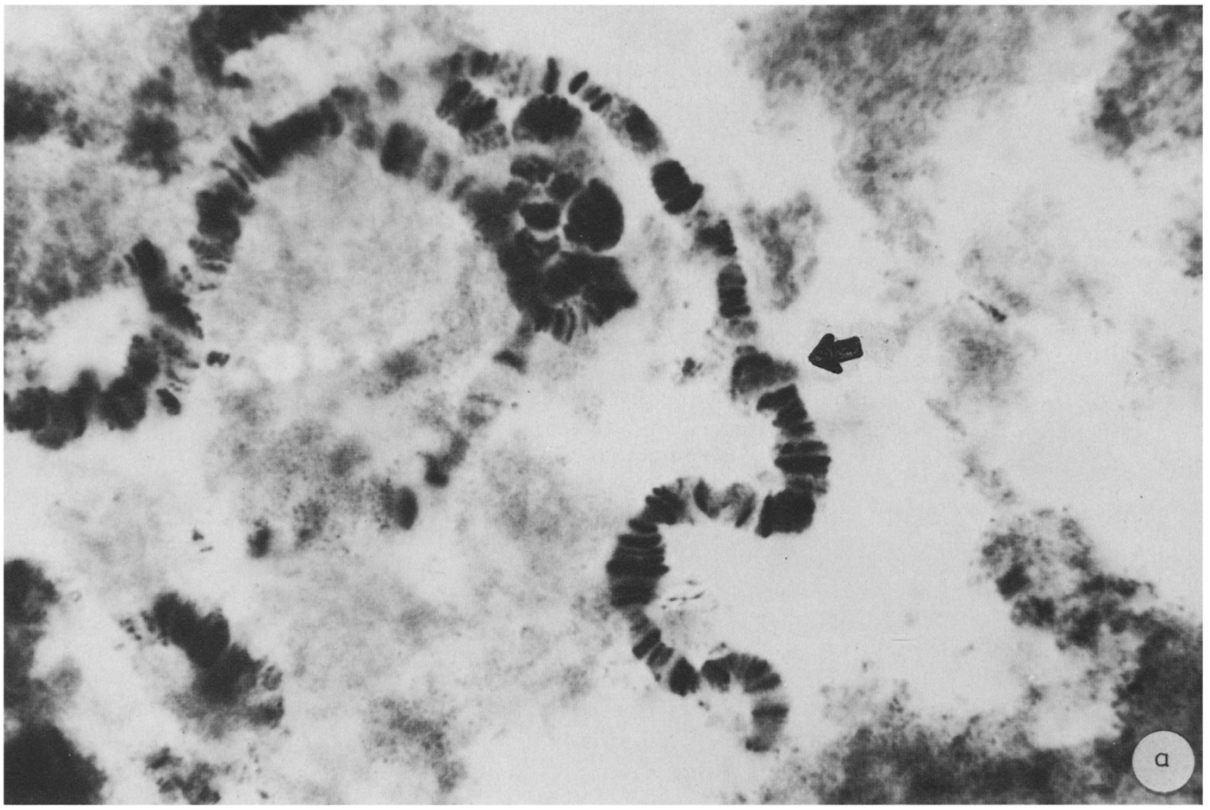
To understand whether insecticides constitute specific stimuli for 'puff-induction', the present study was initiated using *Anopheles stephensi* as the model system. We treated the larvae (fourth instar) from a pathogen-free strain of *A. stephensi* with sublethal concentrations of the insecticide sumithione and examined the time-dependent changes in morphology of polytene chromosomes in the salivary gland squashes. We found that sumithione induces a specific puff at the locus 43D/42A on the left arm of the third chromosome.

2. MATERIALS AND METHODS

Fourth instar larvae of a pathogen-free strain of *A. stephensi* were obtained from the National Institute of Virology, Pune, and maintained in dechlorinated tap water in enamel trays. Larvae were treated with various concentrations (100, 10, 1, 0.1 and 0.01 ppm) of the insecticide sumithione, which was dissolved in kerosene as a stock solution (10^7 ppm) and appropriately diluted in water. In these conditions it was found that 0.1 ppm was the sublethal concentration and all further treatments were carried out at that dose.

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00


a

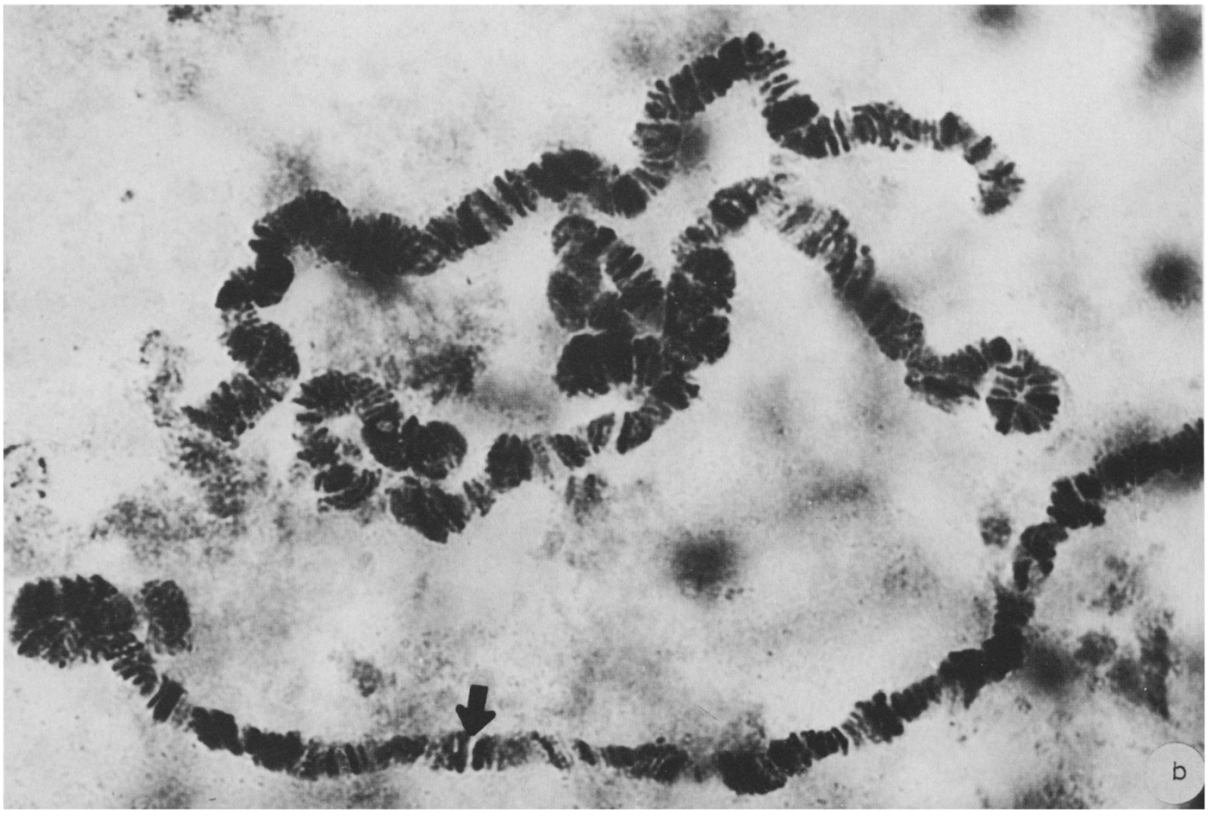

b

Fig.1. Salivary gland chromosome squashes of *A. stephensi* larvae (fourth instar). (a) Treated with 0.1 ppm sumithione, (b) control. Arrows indicate the location of the puff induced in the insecticide-treated gland (a) or the equivalent region in the control gland (b). Larvae were treated with sumithione for 120 min and then maintained for 60 min to allow maximal puff formation.

After treatment with 0.1 ppm sumithione for 120 min, larvae were washed 3–4 times in water and then transferred to fresh trays containing water. At different intervals after the removal of sumithione (15, 30, 60 and 120 min), salivary glands were dissected and squashed on glass slides to obtain chromosome preparations as in [6] and examined on a Polyvar photomicroscope (Reichert). As controls, larvae were treated with appropriate concentrations of kerosene diluted in water, but without sumithione, for 120 min and chromosome preparations made in the same fashion and observed. In a few cases chromosomes of untreated larvae were also examined but these did not show any difference from the control larvae. In all, 52 control and 67 sumithione-treated (0.1 ppm) salivary gland preparations were examined derived from 6 different experimental batches.

3. RESULTS AND DISCUSSION

The procedure of [5] allows preparation of consistently good chromosome spreads from *A. stephensi* salivary glands, as seen in fig.1. The cytogenetic map of *A. stephensi* chromosomes [7] allows identification of major loci on polytene chromosomes. However, no serious study has been made on the location of normally occurring puffs in *A. stephensi* chromosomes.

We find that in comparison with the situation in 'control' experiments (fig.1b), a unique puff appears on the left arm of the third chromosome (fig.1a) in the fourth instar larvae. Based on the cytogenetic map [7], the puff position is localized in the locus 43D/42A. As seen in fig.1a, the puff appears as maximal at 60 min after sumithione removal. The puff did not appear in this position in any of the controls. De novo appearance of this puff was also confirmed by examining chromosome preparations of normal 'untreated' larval salivary glands.

Data on 52 controls and 67 sumithione-treated larvae are tabulated in table 1. These are derived from 6 separate experiments and pooled, since in all of these 65–85% sumithione-treated larvae showed a positive response. As seen from table 1 a puff appeared 30 min after the insecticide removal in 72% of cases while none of the controls showed this phenomenon. We found that the puff which begins to appear 30 min after the removal of sumithione reaches its maximum size by 60 min and then regresses and disappears completely by 120 min. We also observed that in sumithione-responding larvae all cells in the gland show the appearance of a puff at the locus 43D/42A. We are presently unable to explain the situation concerning nonresponders, which constitute 28% of cases (table 1).

These results demonstrate that the *A. stephensi* genome contains a region which responds to sumithione treatment and that this region corresponds to the locus 43D/42A. Since the effect is observed at sublethal concentrations of the insecticide, it would be interesting to know the specific phenotype(s) involved at the molecular level. Fine cytological analysis (unpublished) revealed the puff region to cover 3 bands and 4 interbands and sug-

Table 1

Induction of a puff at the locus 43D/42A in the salivary gland polythene chromosomes of *A. stephensi* larvae (IVth instar) by sumithione

	Number of larvae treated	Number of larvae with the puff	Number of nonresponding larvae
Control[a]	52	0 (0%)	52 (100%)
Sumithione-treated[b]	67	48 (72%)	19 (28%)

[a] Larvae were treated with the appropriate concentration of the solvent kerosene dissolved in water for 120 min and then maintained in water for 30 min

[b] Larvae were treated with 0.1 ppm sumithione for 120 min and then maintained in water for 30 min

gests that a gene cluster, rather than a single gene, responds to the treatment with sumithione. It still remains to be determined whether the insecticide acts directly on the gene cluster in the locus 43D/42A or whether the effect is mediated through other metabolic steps.

Experiments are now in progress to examine the precise time course of puff induction by sumithione in salivary glands cultured in vitro and to elucidate the nature of RNA and proteins synthesized. We are also checking a number of other major insecticides for the ability to induce specific puff(s) in the same (43D/42A) or other loci. We have now produced a genomic library of *A. stephensi* [8] in order to identify the clone(s) corresponding to the locus 43D/42A with a view to studying the organization and regulation of this locus in both sensitive and resistant strains of this mosquito.

ACKNOWLEDGEMENTS

This research was supported by grants from the Department of Atomic Energy, University Grants Commission and the Department of Science and Technology, India. We are grateful to the National Institute of Virology for providing us with fourth instar larval cultures from a pathogen-free strain of *A. stephensi*. We also thank Dr M.V. Joshi and Dr A.K. Indurkar for help and useful suggestions. We are grateful to Professor Adolf Grässmann, Free University Berlin, F.R.G., for critically reviewing this manuscript.

REFERENCES

[1] Ashburner, M. (1970) Chromosoma 31, 356–376.
[2] Ashburner, M. (1970) Proc. R. Soc. Lond. 176, 319–327.
[3] Ashburner, M. (1972) Chromosoma 38, 255–281.
[4] Ashburner, M. (1972) in: Developmental Studies on Giant Chromosomes, pp. 101–151, Springer-Verlag, Berlin.
[5] Brown, A.W.A. (1967) in: Genetics of Insect Vectors of Diseases (Wright, J.W. and Pal, R. eds) pp. 505–552, Elsevier, Amsterdam, New York.
[6] French, W.L., Baker, R.H. and Kitzmiller, J.B. (1962) Mosquito News 22, 377–383.
[7] Sharma, P., Ram Prashad, Narang, S.L. and Kitzmiller, J.B. (1969) J. Med. Entomol. 6, 68–71.
[8] Rama, A., Rajadhyaksha, S.J., Therwath, A., Godbole, N.N. and Modak, S.P. (1984) Submitted.