My hypothesis is that these pulses occur when muscular activity by the male associated with the transfer of sperm and/or seminal fluid results in the release of a pulse of CO_2. If this hypothesis is correct, then it would be interesting to document the correlation between the number and magnitude of the pulses and the number of sperm and amount of seminal fluid transferred. Recordings of CO_2 pulses could be a non-invasive method for assessing male reproductive performance.

References: Berrigan, D., and L. Partridge 1997, In press, Comparative Biochemistry and Physiology; Hall, J. C., 1994, Science 264:1702-1714; Lighton, J. R. B., 1991, pp. 201-208, in P.A. Payne, Ed., Concise Encyclopedia on Biological and Biomedical Measurement Systems. Pergamon Press, Oxford; Partridge, L., and M. Farquhar 1981, Nature 294:580-582; Lighton, J. R. B., 1994, Physiol. Zool. 67:142-162; Pitnick, S., 1996, Amer. Nat. 148:57-80; Wolfner, M. F., 1997, Insect Biochem. Molec. Biol. 27:179-192.

Zhimulev, Igor F., and Natalia I. Mal'ceva. Laboratory of Molecular Cytogenetics, Institute of Cytology and Genetics, Novosibirsk 630090, Russian Federation. Action of ecdysterone on salivary gland and nurse cell polytene chromosomes of *Drosophila melanogaster otu* mutant *in vitro*.

The main purpose of this work was to investigate the reaction of polytene chromosomes from ovarian nurse cells to ecdysterone. Normally the nuclei of nurse cells (ncs) have a reticular structure because they contain dispersed chromosomes that are of little cytological value. However, in the present study we used flies of the $otu^7/y\ w\ sn^3\ otu^{11}$ genotype, since their nurse cell nuclei contain polytene chromosomes with clear-cut banding patterns. To obtain NC polytene chromosomes of best cytological quality, this fly stock was kept at 16°C (Mal'ceva *et al.*, 1995, 1997). Both salivary glands (sgs) of larvae at Puff Stages 1-3 and ovaries of 5 day old adult flies were dissected in Ephrussi-Beadle's solution and incubated in Robb's medium according to the protocol given below (Figure 1). Organs were incubated *in vitro* using 3×10^{-7}M of ecdysterone (20-OH ecdysone, Serva), according to Ashburner's technique (Ashburner, 1972).

Table 1. Changes of puffing patterns of salivary gland and nurse cell chromosomes *in vitro*.

Cell type	Experiment	Puffing pattern
Salivary gland cells	6-24h incubation without ecdysterone, 16°C	Inactivation of all ecdysterone puffs. Induction of "incubation" puffs: 47D, 50C, 60C and 93E
Ovarian nurse cells	6-24h incubation without ecdysterone, 16°C	Induction of "incubation" puffs: 47D and 50C
Salivary gland cells	2-6h incubation with ecdysterone, 25°C	Development of ecdysterone puffs to PS7
Salivary gland cells	3-24h incubation with ecdysterone, 16°C	Changes of puffing patterns till PS10-11 after 16h incubation
Ovarian nurse cells	3-24h incubation with ecdysterone, 16°C	No reaction of polytene chromosome loci to ecdysterone
Salivary gland cells	12h preincubation without ecdysterone and 8h incubation with ecdysterone, 16°C	Induction of late larval puffs (63E, 66B, 78C), induction of late prepupal puff 93F9-10
Ovarian nurse cells	12h preincubation without ecdysterone and 8h incubation with ecdysterone, 16°C	No reaction of polytene chromosome loci to ecdysterone
Salivary gland cells, ovarian nurse cells	6h preincubation without ecdysterone and 6h incubation with ecdysterone, 16°C	Induction of early late ecdysterone puffs, "incubation" puffs and puff 46F in salivary gland polytene chromosomes. No induction of puffs in nurse cell polytene chromosomes
Salivary gland cells, ovarian nurse cells	6h preincubation without ecdysterone, 6h incubation with ecdysterone and 6h preincubation with ecdysterone, 16°C	Occasionally there are "incubation" puffs and puff at 93F9-10. No ecdysterone puffs in salivary gland cells. No puffs in nurse cells

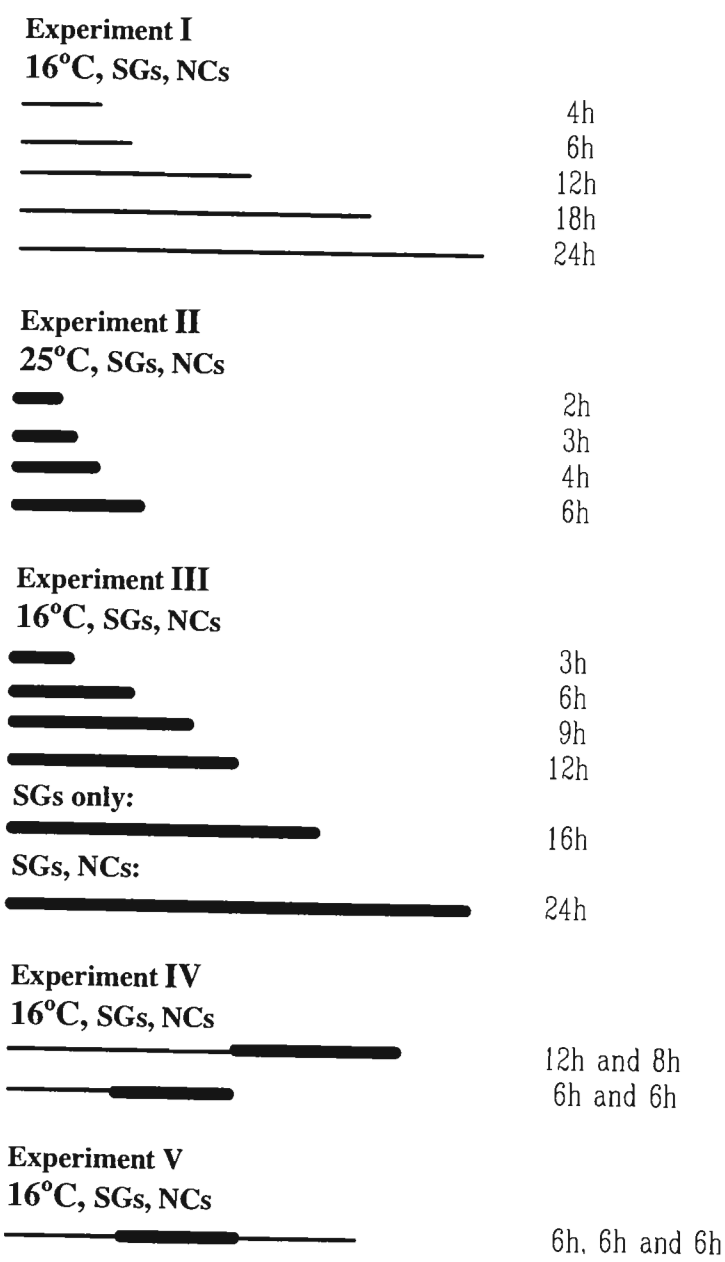

Figure 1. Scheme of incubations of salivary glands (sgs) and ovaries with nurse cells (ncs) at different temperatures. Periods (hours) of incubations with ecdysterone (thick lines) and without (thin lines) are indicated by figures.

In the first experiment, sgs were incubated in Robb's medium without hormone (experiment I of Figure 1). During the 4-24 hour incubation period obvious changes in SG chromosome puff patterns occurred. After the first 6 hour period, all the ecdysterone-stimulated puffs (2B3-5, 46F, 74EF, 75B and 85F) had disappeared. Even after 24 hours incubation *in vitro* the SG chromosomes showed very clear banding patterns and general polytene structure (Figure 2, Table 1). We know that "incubation" puffs sometimes appear on polytene chromosomes when sgs are cultured *in vitro* and that the pattern of puffs observed depends on medium used (Ashburner, 1972; Biyasheva *et al.*, 1985). In our experiments, several new puffs, namely 47D, 50C, 60C and 93E appeared during the 24 hour incubation (Figure 3). The 47D and 50C puffs also appeared in Grace's medium (Ashburner, 1972), but the 60C and 93E puffs are probably specific for Robb's medium.

Except for the development of puffs in the 47D and 50C regions, no significant changes were found in morphology of the NC polytene chromosomes during this incubation period (Table 1).

When sgs were incubated from 2 to 6 hours with ecdysterone at 25°C (experiment II, Figure 1), the development of puffing patterns stopped at PS7, as often happens with SG polytene chromosomes incubated *in vitro*. To overcome this disadvantage, we cultured sgs at a much lower temperature (16°C) for 16 hours and found that the puffing pattern had reached its final stage of development (Figure 4b). This final stage cannot be passed when SG are incubated with ecdysterone (Richards, 1976a,b). No induction of ecdysterone puffs occurred in NC polytene chromosomes during the entire incubation period (Figure 4c).

We thought that perhaps the development of ecdysterone puffs in nurse cells of *otu* mutants might be blocked by some unknown inhibitors. Therefore we preincubated sgs and ovaries in pure medium for 12 hours and then added hormone for 8 hours. In this case, in SG chromosomes, puffs appeared which are specific for late larvae (63E, 66B, 78C). Similar results were obtained after 6h preincubation without hormone and 6h incubation with ecdysterone (Figures 5,6). For unknown reasons, puff 93F9-10 appeared, which normally developed in very late prepupae (Ashburner and Berendes, 1978). No reaction of NC chromosomes to the hormone was found in these experiments.

Changes of ecdysterone concentration are very important during development of ecdysterone puffs in polytene chromosomes of sgs. The high ecdysterone titre followed by its absence can induce a new wave of puffs (Richards,

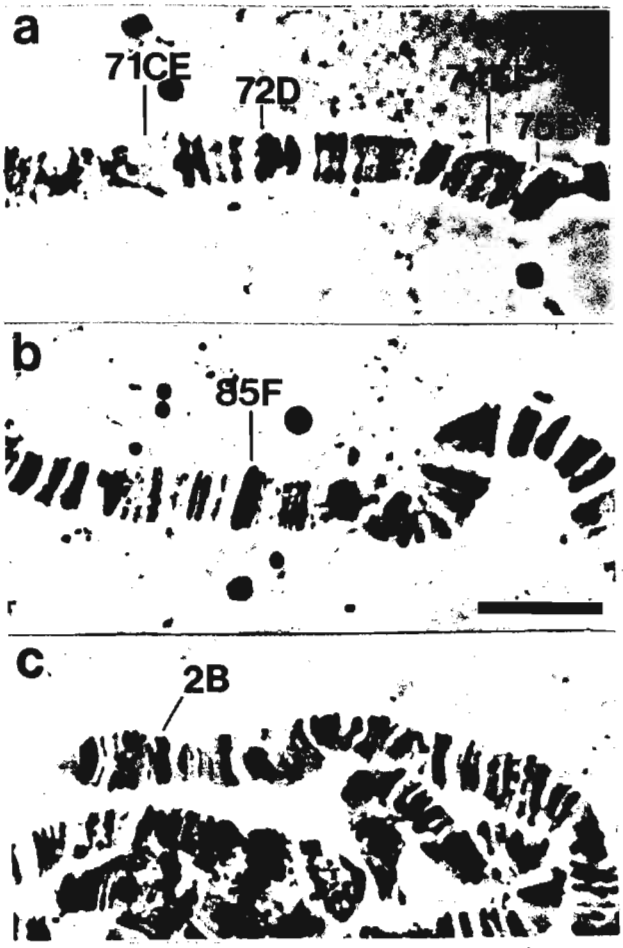

Figure 2 (above). Inactivation of ecdysterone puffs after 24 hour incubation of the salivary glands in Robb's medium without ecdysterone. Bar represents 10mm.

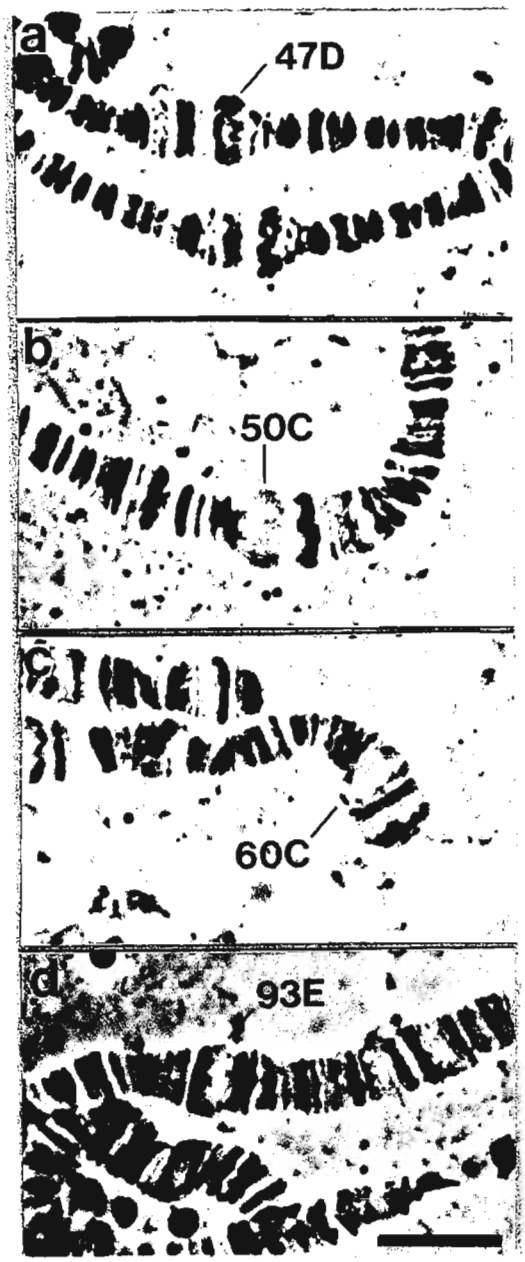

Figure 3 (at the right). Puffs induced during 24 hour cultivation of the salivary glands in Robb's medium without ecdysterone. Bar represents 10mm.

1976a,b). Therefore, we expected that such changes could be useful for puff induction in NC chromosomes. For this reason we performed experiment V (see Figure 1 and Table 1). Again no induced puffs were found in NC chromosomes.

It could be that long incubations *in vitro* will lead to the induction of heat shock puffs, which in turn could block ecdysterone induction. However, in our experiment (Figure 7) we saw only the formation of tiny heat-shock puffs on NC polytene chromosomes mainly in 63B, 67B, 87A and 93D regions. Heino also reports that it is difficult to induce heat-shock response in ncs (Heino *et al.*, 1995).

Our data show that the NC polytene chromosomes do not form puffs in response to ecdysterone, at least under the experimental conditions described above.

Acknowledgments: Authors are indebted to Drs. G. Richards, E.S. Belyaeva and R.C. King for valuable advice, to Dr. J. Fristrom for his gift of Robb's medium, and to I.P. Selivanova for her technical assistance.

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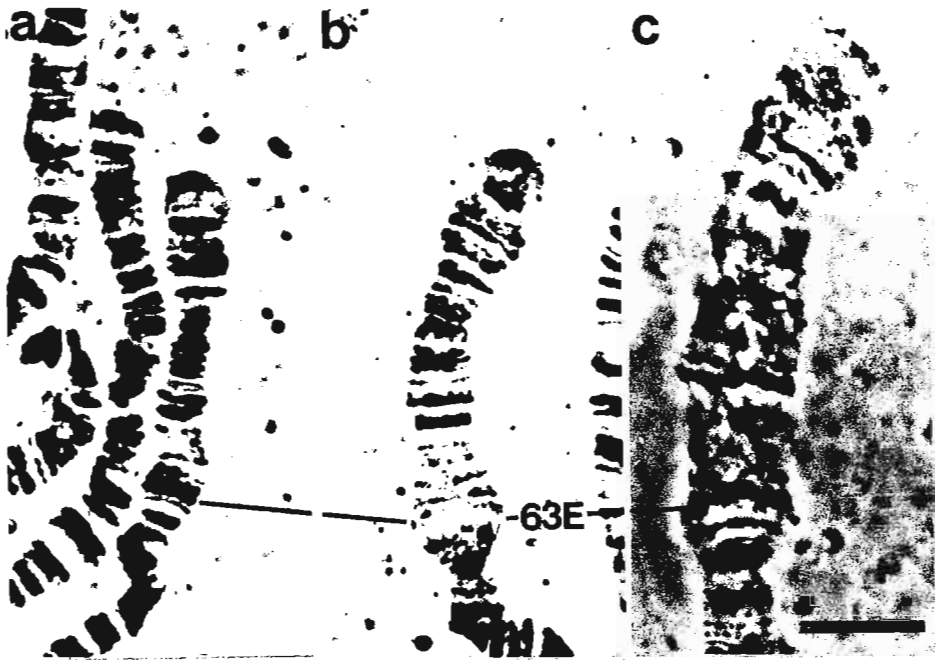


Figure 4. Morphology of the SG 3L polytene chromosome after 24 hour incubation without ecdysterone (a), 16h incubation with ecdysterone (b), and after 24 hour incubation of NC with ecdysterone (c). Bar represents 10mm.

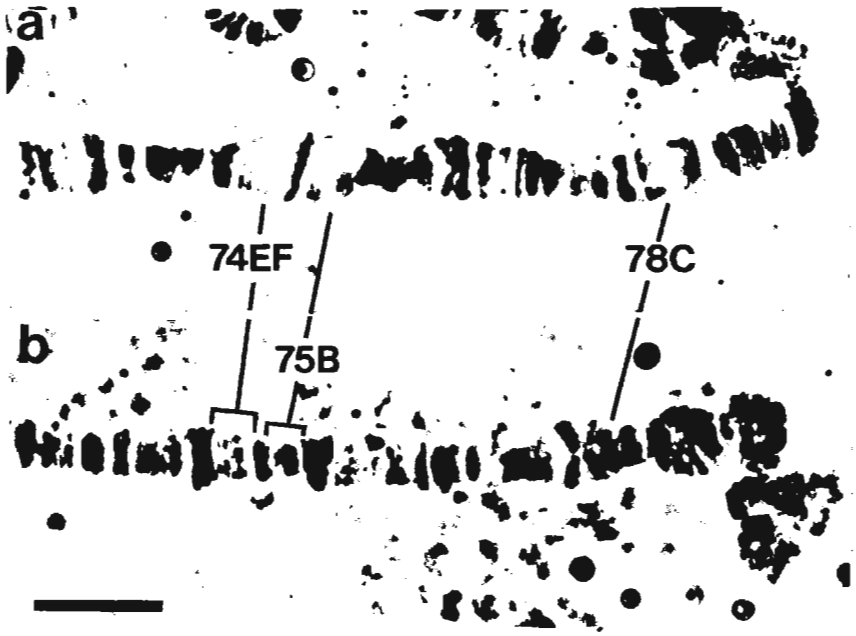


Figure 5. Puffs in 74EF - 75B and 78C regions of SG (a) and NC (b) polytene chromosomes, after 6 hour incubation at 16°C without ecdysterone and then 6 hour incubation with hormone. Bar represents 10mm.

In: *The Genetics and Biology of* Drosophila, (ed., M. Ashburner and T.R.F. Wright), 2b: 316-395. Academic Press, London; Zhimulev, I.F., 1994, Chromomeric Organization of Polytene Chromosomes. 565 p. Nauka, Novosibirsk; Heino, T.I., V.P. Lahti, M. Tirronen, and C. Roos 1995, Chromosoma 104: 44-55.

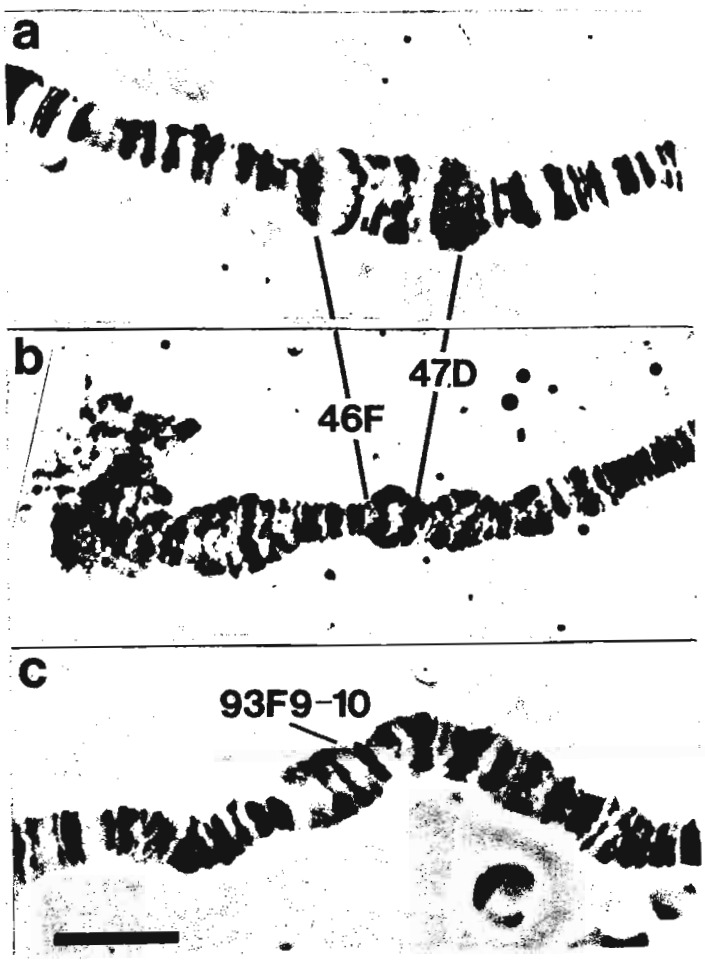
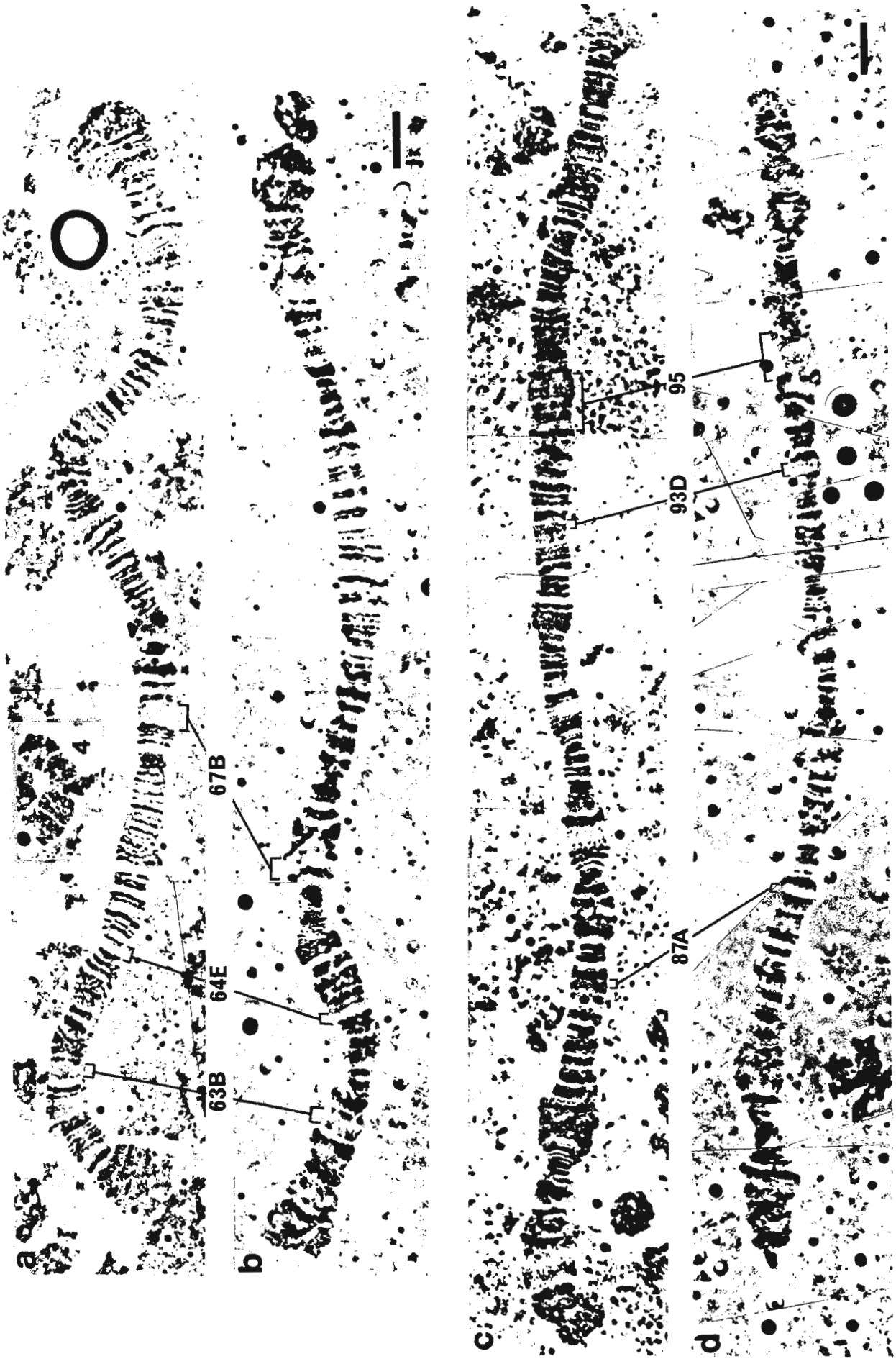


Figure 6. Induction of puffs in 46F and 47D regions in SG (a) and NC (b) after 6 hour incubation in Robb's medium without ecdysterone and 6 hour incubation with hormone. (c) - Puff in 93F9-10 region which appeared after subsequent incubations of SG for 6 hour in Robb's medium without ecdysterone, 6 hour with ecdysterone and 6 hour without ecdysterone. Bar represents 10mm.

Figure 7 (next page). 3L and 3R NC polytene chromosomes in control (a and c) and after 30 min at 37°C (b and d). Bar represents 10mm.


a
63B
64E
67B
b
c
87A
93D
95
d