

organisms⁴⁻⁶, asymmetrical responses to directional selection are common.

Although stress traits have rarely been selected for both resistance and sensitivity in one experiment, there are reports of quite rapid responses to selection for resistance to specific stresses such as DDT in *D. melanogaster* and other species, presumably due to the rapid rearrangement of additive genes^{7,8}. Like radioresistance, dominance has been found for resistance to various insecticides and anaesthetics such as ether^{8,9}. Therefore in conclusion, asymmetrical responses to selection for stresses may be reasonable, such that resistant strains are rapidly built up as is known for DDT. The other important conclusion is that it has been possible to show through sib-selection that the genetic variation for radioresistance present in the base populations can be selected without any contact with the selective agent¹⁰.

Zusammenfassung. Es wurde eine Selektion auf Strahlenresistenz nach Bestrahlung von *Drosophila melanogaster* mit ⁶⁰Co- γ -Strahlen während 5 Generationen mit gutem Erfolg erzielt, nicht aber eine solche auf

erhöhte Strahlensensibilität. Der asymmetrische Selektionseffekt dürfte somit auf dominanter Vererbung der Resistenz beruhen. Familienselektion kann auch ohne Bestrahlung zu resistenten Stämmen führen.

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12 January 1973.*

⁵ D. S. FALCONER, Cold Spring Harb. Symp. quant. Biol. 20, 178 (1955).

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¹⁰ This work was supported by the Australian Institute of Nuclear Science and Engineering.

Cordycepin Inhibits Induction of Puffs by Ions in *Chironomus* Salivary Gland Chromosomes

Puffs in polytene chromosomes of Diptera are inducible by inorganic¹ and organic ions². Both types of ion act directly at the chromosomal level^{3,4} and may be implicated in the control of gene activity by hormones during normal development⁵. Incubated in the absence of RNA precursors, isolated polytene chromosomes react to changes in their electrolyte milieu by a differential decondensation of bands at specific loci^{3,4}. From this it could be concluded that induction of puffs by ions is exempt from the general rule that puffs can persist or be induced only under conditions permitting RNA synthesis. This would mean that puff induction by ions exhibits biochemical characteristics different from those observed during induction of puffs by ecdysone⁶. However, in this com-

munication I present evidence that in intact cells an unimpaired capacity for RNA synthesis is required for the induction of puffs by inorganic and organic ions.

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⁴ M. ROBERT and H. N. B. GOPALAN, in preparation.

⁵ H. KROEGER, in *Metamorphosis* (Eds. W. ETKIN and L. I. GILBERT; Appleton-Century-Crofts, New York 1968), p. 185.

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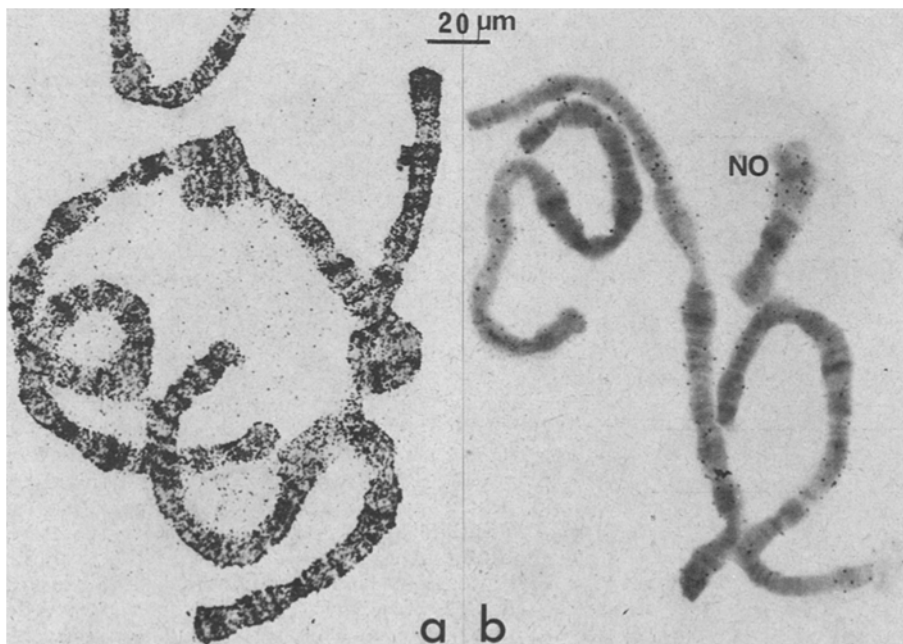


Fig. 1. Effect of cordycepin on ³H-uridine incorporation into salivary gland nuclei of *Chironomus thummi*. a) control in TM I and b) sister gland in TM I with 100 µg/ml cordycepin. NO = nucleolus organizer.

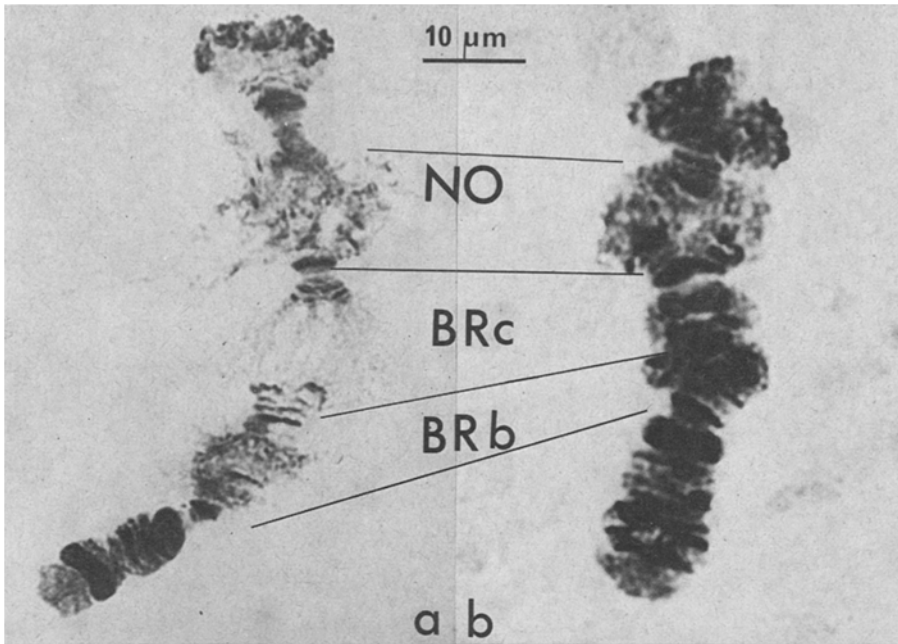


Fig. 2. Regression of Balbiani rings (BRb and BRc) caused by cordycepin. a) control in TM I and b) sister gland in TM I with 100 µg/ml cordycepin. NO = nucleolus organizer.

Material and methods. Late 4th instar larvae (eL₄) of *Chironomus thummi* were used. For staging and characteristics of the stock see KROEGER⁷.

Test media (I and II). TM I: 50 mM KCl, 50 mM MgCl₂, 5 mM Tris (pH 7.4)⁸. TM II: 2 mM KCl, 28 mM disodium fumarate, 28 mM NaCl, 5 mM CaCl₂, 7 mM magnesium succinate, 80 mM tryptophan (D or L), 5 mM TES (Tris-(hydroxymethyl)-methyl-2-aminoethane-sulfonic acid, pH 7.4)².

From each larva, the paired salivary glands were explanted separately onto siliconized slides and incubated under paraffin oil in 15 µl of medium for 1 h (TM I) or 2 h (TM II) at 20–22°C. One gland was incubated without (control) and the other with cordycepin (3'-deoxyadenosine; Sigma Chemical Co., St. Louis, Missouri). At the end of the explantation period, the glands were prepared for cytological observation in the usual manner⁹.

For autoradiography, 0.5 µl of ³H-uridine (spec. act. 27.5 Ci/mM; NEN GmbH, Frankfurt) was added to the

test media for the last 15 (TM I) or 30 (TM II) min of incubation and the glands were processed as described elsewhere¹⁰.

Results. Among the combinations of NaCl/KCl/MgCl₂ media which induce puffs in salivary gland chromosomes of *Chironomus thummi*¹¹, 50 mM KCl/50 mM MgCl₂ were chosen as the test medium; this medium induces a wide array of puffs at various sites including IIa3/4 and III d1^{8, 11} and its effects have been specifically investigated⁸. The test medium for organic ions (TM II) is based on a medium

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⁹ H. KROEGER, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1966), vol. 2, p. 61.

¹⁰ H. N. B. GOPALAN and M. ROBERT, in preparation.

¹¹ H. KROEGER and G. MÜLLER, Expl. Cell Res., in press.

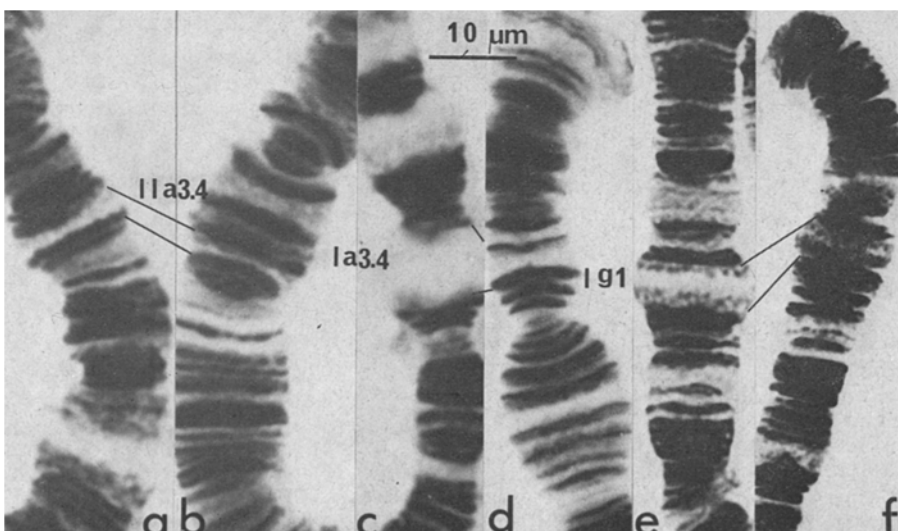


Fig. 3. Effect of 100 µg/ml cordycepin on induction of puffs by inorganic (a, b) and organic (c, d, e, f) ions in salivary gland chromosomes of *Chironomus thummi*. a) and c, e) controls from sister glands without cordycepin.

devised by POLITOFF et al.¹² in which its glutamine is replaced by tryptophan². As shown elsewhere², this medium induces puffs at the following loci: Ia3/4, Ie3, If4, Ig1, Ig2, IIId1 and IIId1/2.

At 100 µg/ml cordycepin, offered in hemolymph or Ringer solution, caused in 80–85% of the cells ($n = \text{ca. } 1000$) a nearly total inhibition of ³H-uridine incorporation into nucleoli and chromosomes (Figure 1), a general regression of all puffs and a collapse of the Balbiani rings (Figure 2). No new puffs were induced by cordycepin. At lower concentrations (10, 25, 50 µg/ml) neither inhibition of ³H-uridine incorporation nor puff regression was detectable.

In accordance with previous findings^{8,2}, exposition of salivary glands to test media I and II gave rise to the induction of the puffs listed above in about 70% (TM I) and 80% (TM II) of the cells. The puffs thus induced incorporate ³H-uridine.

Inclusion of 100 µg/ml cordycepin in TMI or TM II blocked the induction of any of the puffs listed above in 80–85% of the cells (Figure 3). In 15–20% of the cells these puffs did appear at a size approximately equal to that of the controls ($n = \text{ca. } 1000$).

Discussion. The observation that isolated polytene chromosomes incubated in simple salt solutions exhibit a differential decondensation of bands ('swelling' and 'fading')³ in conjunction with the results reported in this communication, points to a 2 step mechanism in the formation of puffs: a) an initial, highly localized, decondensation of DNP fibrils, which is independent of energy and RNA synthesis and b) a subsequent step which involves further decondensation, engulfing of neighbouring bands and an accumulation of acidic proteins¹³. This second step, which creates the 'puff-structure', seems to require energy² and RNA synthesis^{6,14}. It appears that ions are capable of effecting both steps.

That 15–20% of the cells show 'resistance' to cordycepin demonstrates a heterogeneity in the cell population of salivary glands. Such a heterogeneity was also observed by BEERMANN¹⁴ in *C. tentans* salivary gland cells in their

reaction to α -amanitin. It is possible that the 'resistant cells' are the same which were found by KROEGER et al.¹⁵ to have an 'inverted' Na/K ratio and which they assumed to be approaching DNA synthesis or to be engaged in it¹⁶.

Cordycepin seems to block RNA synthesis by preventing the addition of poly(A) residues at the 3'-terminus of the HnRNA¹⁷. Since other inhibitors of RNA synthesis like actinomycin D⁶ and α -amanitin¹⁴ also inhibit puff formation, it is probable that the effects of cordycepin reported in this paper are due to inhibition of RNA synthesis rather than any other specific facet of its action¹⁸.

Zusammenfassung. In 80–85% aller Speicheldrüsenzellen von *Chironomus thummi* bewirkt 100 µg/ml Cordycepin: 1. Fast vollständige Hemmung des ³H-Uridin-Einbaues in Chromosomen und Nukleolen. 2. Rückbildung aller vorhandenen Puffs und Kollaps der Balbiani-Ringe und 3. Hemmung der Puff-Induktion durch anorganische (K/Mg) und organische (Tryptophan) Ionen.

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Occurrence of Diploid Drones in a Neotropical Bumblebee

MACKENSEN¹ suggested that the sex determination mechanism of *Apis mellifera* is similar to that of *Habrobracon*. Diploid patches of male tissue were found² in *Apis mellifera* drones and diploid male larvae were detected³ and subsequently reared to adult diploid males; details of cytology, biological importance, etc., are gradually being accumulated⁴. The possibility of obtaining bumblebee mating under controlled conditions has already been explored⁵.

The present detection of diploid drones in *Bombus atratus* Franklin, the commonest bumblebee species in our area, is a byproduct of my main research on the reproductive biology of social bees.

Laboratory-mated bumblebee queens were placed individually into small observation cages containing pollen and honey and kept at 29°C to 30°C. One of them, mated on May 14, 1971, laid her first batch of eggs on the cage floor on June 23. In order to increase the possibility of successful nest foundation we added to the incipient colony several cocoons containing bumblebee worker pupae collected from our stock-colonies. In the meanwhile the queen oviposited 6 additional cells. At the proper time the introduced workers constructed the pockets but never provisioned them. All the necessary

protein food was added in the form of honey-moistened pollen of *Apis mellifera* poured by us into the cell pockets. Of all eggs laid by this queen 7 workers and 10 males were obtained.

The first offspring to emerge (on July 31) was a male. When the male was 13 days old he mated with his mother and the same happened on August 20th with a second male, that emerged on August 11. The durations of the copulae were respectively 60 and 53 min. After this inbreeding the whole colony was transferred to a larger cage placed inside the laboratory. Through a hole in the wall the bees could fly and collect food freely. Henceforth the queen produced 14 workers and 27 males. Two light-pink eyed male pupae were used for chromosome counting (testicular smearing, aceto-orcin staining). SILVEIRA (personal commun.) counted $n = 20$ chromosomes for *B. atratus* (Figure 1); both drones here showed

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