Sequence of Puff Formation in *Rhynchosciara* Polytene Chromosomes*

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Abstract. The chief characteristics of the life cycle of *Rhynchosciara* sp. are: egg stage (12 days); three larval instars of approximately 6 days each, followed by a 4th instar of approximately 40 days duration; pupation (6 days); and adult form (5—6 days). Maps of the 4 polytene chromosomes of *Rhynchosciara* sp. have been prepared, and the temporal sequence of puff formation on the chromosomes described. The cocoon is synthesized during the prepupal period, and at this time major puffs are seen on all chromosomes. The largest and most numerous puffs occur on the salivary gland chromosomes during the 24 hours prior to the last or prepupal molt. Three of the puffs that occur at this time are DNA-puffs (Summary see p. 249).

Introduction

Although the molecular mechanisms of genetic activity have been described in some detail, the operation of genes within the morphological unit, the chromosome, is less well understood. For investigation of this problem, the giant chromosomes of the larval *Diptera* have been particularly useful, since they are characterized by various transverse bands and interbands that are known to mark the positions of genetic factors. Furthermore, many bands of such chromosomes develop swollen regions, or puffs, that are thought to represent sites of localized gene action (BÜRMANN, 1957).

The use of the sciarid fly *Rhynchosciara angolae* as a favorable cytological tool was first described by NONATO and PAVAN (1951) and by PAVAN and BREUER (1952). The larval forms of this fly have exceptionally large polytene chromosomes in the salivary glands, Malpighian tubules, and intestines. Furthermore, the eggs laid by a single female hatch together, the larvae in each group are of the same sex, and the larvae develop through a larval cycle in very close synchrony. Prior to pupation, the larvae spin a common cocoon and ultimately encase themselves within its individual units. During production of this cocoon, the salivary gland chromosomes develop a series of well-defined puffs along their length. BREUER and PAVAN (1955) and PAVAN (1965) have shown that some of the puffs which appear at this time are responsible for the synthesis of

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extra amounts of DNA; they have referred to these regions as “DNA-puffs” and to the DNA synthesized in such puffs as “metabolic DNA”.

The species of *Rhynchosciara* presently under cultivation in this laboratory is not the same as the one described by Nonato and Pavan (1951). This new species is being studied at present by Pavan and Breuer (personal communication), but it has not yet been taxonomically described. The life cycle of the new species differs in several respects from that of *R. angelae* (Pavan, personal communication), but the anatomy and physiology of the two are similar.

The sequential events of the life cycle of the new species have been determined and will be presented in this paper. Furthermore, chromosome maps of the 4 polytene chromosomes of cells in *Rhynchosciara* sp. larval salivary glands have been developed, and the sequence of appearance of chromosome puffs will be described in their relation to the larval life cycle.

**Materials and Methods**

The species of *Rhynchosciara* used for these experiments was collected by R. Basile and L. C. Simões in Villa Atlantica (Praia Grande), State of São Paulo, Brazil, and sent to our laboratory in September 1965. Since then it has been cultivated in this laboratory by techniques described below.

Adult male and female flies are placed in mating chambers on the 1st day after the flies hatch from their cocoons. The mating chambers are prepared as follows: Plastic mouse cages are equipped with plate glass lids. Sterile surgical cotton is cut into pieces which cover the bottoms of the cages. A layer of heavy blotting paper is then laid over the cotton and wet thoroughly with distilled water. A 50-ce beaker containing sucrose solution (10%) is covered with a piece of blotting paper through which a cotton wick reaches the sucrose solution. This assembly, together with a handful of sterilized coconut fiber, is placed inside the cage. Adult flies will survive for 5 to 6 days in this cage, and masses of yellow eggs will be found in the interstices of the coconut fiber after 3 or 4 days. After all the flies have died, the egg masses are removed with sterile forceps and transferred to sterile non-nutrient agar.

The egg masses are kept on non-nutrient agar until a black head capsule is evident inside the eggs. The egg clutches are then transferred to individual Petri dishes that contain a mixture of cornmeal agar, Streptomycin sulfate (100 µg/ml), and Tetracycline-HCl (30 µg/ml).

The larvae from any single group of eggs will all hatch within 24 hours. This does not mean that all the eggs from any one clutch will emerge as larvae, since hatchability varies greatly from one group to another.

When a majority of the larvae have hatched, a teaspoon of food is added to the culture plate. The food used in this laboratory is the same as that described by Lara, Tamaki, and Pavan (1965). Dried sweet potato leaves imported from Brazil are crushed, and then soaked for 3 days in distilled water in a covered container. The fermented mixture is then squeezed in cheesecloth to remove excess water, and small amounts of the fermented leaves are fed to the larvae. This is the culture procedure followed throughout the 4 larval instars, except that large groups of larvae may be transferred eventually to polyethylene freezer boxes with an agar base. Also, during the long 4th instar, agar and food are renewed on alternate days.