Parasitology Research (1994) 80:108-111
DOI: 10.1007/BF00933776

Effects of *Nosema algerae* infections on the gene activity of the salivary gland chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae)

W. Staiber

Institut für Genetik, Universität Hohenheim,
Garbenstrasse 30, D-70593 Stuttgart, Germany

Abstract

Larvae of *Acricotopus luciclus* (Diptera, Chironomidae) were successfully infected with *Nosema aIgerae* (Microsporidia, Nosematidae). Treatment of newly hatched larvae with 2-3 x 10^5 spores/ml produced a 59.7% - 83.8% rate of microsporidia-infected animals within 6 weeks. One of the host tissues infected was the polytene salivary gland; 31.3% - 35.3% of the larvae showed infections in the gland cells. This made it possible to investigate the reaction of the puffing pattern of the polytene host-cell chromosomes to the presence of an intracellular parasite. In slightly or moderately infected salivary gland cells, no change in the regular puffing patterns was observed. Only in heavily infected cells did an inactivation of cell-type-specific Balbiani rings and puffs occur, resulting in a change in the cell-type-specific genetic programme.

Introduction

Infections of larval polytene salivary gland cells carrying the so-called giant chromosomes with intracellular parasites as microsporidia or DNA viruses have been reported from chironomids and sciarids (Keyl 1960; Diaz and Pavan 1965; Pavan and Basile 1966; Wülker 1987; Wülker and Weiser 1991). Heavily infected cells were hypertrophied and exhibited in these cases special giant chromosomes resulting from parasite-induced extra replication cycles.

Less is known about the reaction of the gene activity pattern of a host cell to an intracellular parasite. Polytene cells, which show gene activity in clearly visible puffing and Balbiani ring

(BR) patterns of the giant chromosomes, represent an excellent system for investigating these interactions with the light microscope. For *Acricotopus lucidus* (Diptera, Chironomidae), exact photographic and drawn maps of the polytene salivary gland chromosomes and detailed information on their puffing patterns and developmental changes are available (Staiber and Behnke 1985). The larval salivary gland of *A. lucidus* is morphologically subdivided into three lobes. The lobes exhibit different cell-type-specific BR and puffing patterns (Mechelke 1953; Staiber and Behnke 1985). BRs represent active gene loci for the production of mRNA for the secretion proteins of the salivary gland (Beermann 1961; Grossbach 1969).

Nosema algerae (Microsporidia, Nosematidae) is a parasite of anopheline mosquitoes (Vávra and Undeen 1970). A wide variety of insects and insect cell lines, e.g. of the cabbage lopper *Trichoplusia ni,* the corn earworm *Heliothis zea,* and the cabbage moth *Mamesta brassicae,* and even pig kidney-tissue cells can be infected with *N. algerae* (Undeen 1975, Streett et al. 1980).

The purpose of the present investigation was to infect *A. lucidus* with *N. algerae* and then, if possible, to get high numbers of infected salivary gland cells for studying the effect of the intracellular parasitic protozoan on the puffing patterns of the host cells.

Materials and methods

Strains

The laboratory stock of *Acricotopus lucidus* (Diptera, Chironomidae) used for the experiments was derived from egg masses collected around Hohenheim and was bred for about 20 years at the Institute of Genetics. *Nosema algerae* (Microsporidia, Nosematidae) was kindly provided by Prof. Dr. W.A. Maier (Bonn). The spores were isolated from *Anopheles stephensi* adults that had been experimentally infected (Schenker et al. 1992).

Rearing and infection

Egg masses and newly hatched larvae of *A. lucidus* were placed in glass vessels containing 30 ml tap water with 2-3 x 10^5 *N. algerae* spores/ml. On the 3rd day after hatching, fresh tap water was added to obtain a volume of about 100 ml. The larvae were reared at room temperature in day-light and were fed with *Spyrogyra* algae.

Staining

Fourth-instar larvae and prepupae were dissected in Firling medium (Firling and Kobilka 1979). Salivary glands and bodies were fixed in ethanol acetic acid (3:1, v/v), stained in carmine acetic acid (16-24 h) and in orcein acetic-lactic acid (1 h) and then squashed in a drop of the latter stain (Staiber and Behnke 1985). Preparations were examined and photographed with a Zeiss photo-microscope II equipped with phase-contrast optics.

Results

In the first experiment, just before hatching of the larvae an egg mass of *Acricotopus lucidus* was transferred into tap water containing 2 x 10^5 *Nosema algerae* spores/ml. Within 6 weeks a total of 67 animals developed into fourth-instar larvae and into prepupae, which were then dissected and examined for microspofidia infections. In all, 40 animals (59.7%) had mature spores in their bodies and 21 (31.3%) exhibited infected salivary glands.

In a second experiment, newly hatched larvae were exposed to about 3 x 10^5 spores/ml. From a total of 136 larvae dissected within 6 weeks, 114 (83.8%) exhibited spores and 48 (35.3%) had infected salivary gland cells. As can be seen in Fig. la, both glands of a larva can have infected cells.

The salivary gland of *A. lucidus* is formed by 70-90 large cells that have diameters of about 80-120 µm and polytenic levels of their nuclei of 2048 C-8192 C in the fourth-larval instar and the prepupal stage (Speiser 1973). Each nucleus carries three giant chromosomes. The gland is subdivided into three lobes: the anterior, the main and the side lobe (Fig. la). Infected cells were found in all lobes; most frequently 1-5 cells were infected, but up to 12 cells of a gland could be found to contain parasites. Measurements of fixed and carmine/orcein-stained *N. algerae* spores resulted in lengths of 4.5-7 µm and widths of 2.25-3 µm (Fig. lb).

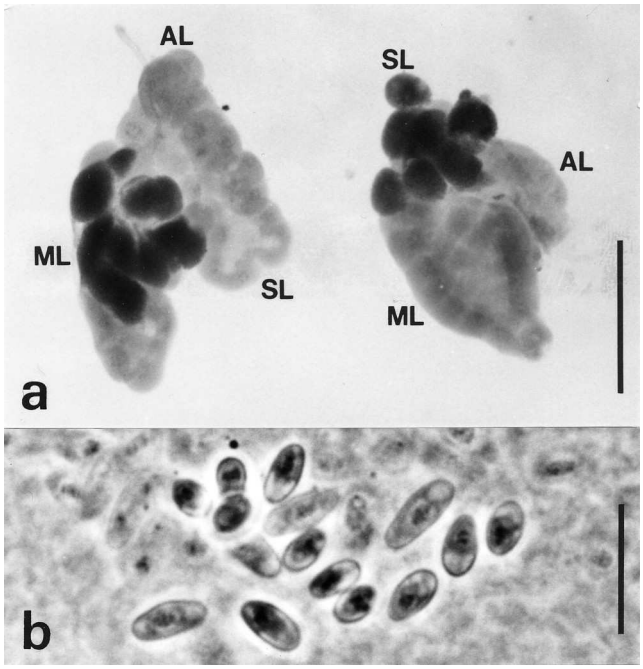

Fig. la, b *Nosema algerae-infected* larval salivary glands of *Acricotopus lucidus.* **a** Infected salivary gland pair stained with acetocarmine and orcein. Dark cells contain microsporidia. In the left gland, main lobe *(ML)* cells are infected, as are side lobe *(SL)* cells in the right gland *(AL* Anterior lobe). Bar = 500 µm. **b** Mature *N. algerae* spores from an infected salivary gland. Phase contrast. Bar = 10 µm

Cells with heavy degrees of infection, in which most of the cytoplasm is filled with spores, showed hypertrophy (Fig. la). The size of the giant chromosomes of the infected cells was the same as that of the surrounding non-infected cells, i.e. no increase in the polyteny of chromosomes of *N. algerae*-infected salivary gland cells was observed in *A. lucidus.*

In slightly and moderately infected main and side lobe cells (Fig. 2a, b) and in cells with infections, in which up to about half of the cytoplasm is filled with spores, no change in the BR and puffing patterns or, hence, in the gene activities was found. The puffing patterns were the same as those of non-infected cells (Fig.3a).

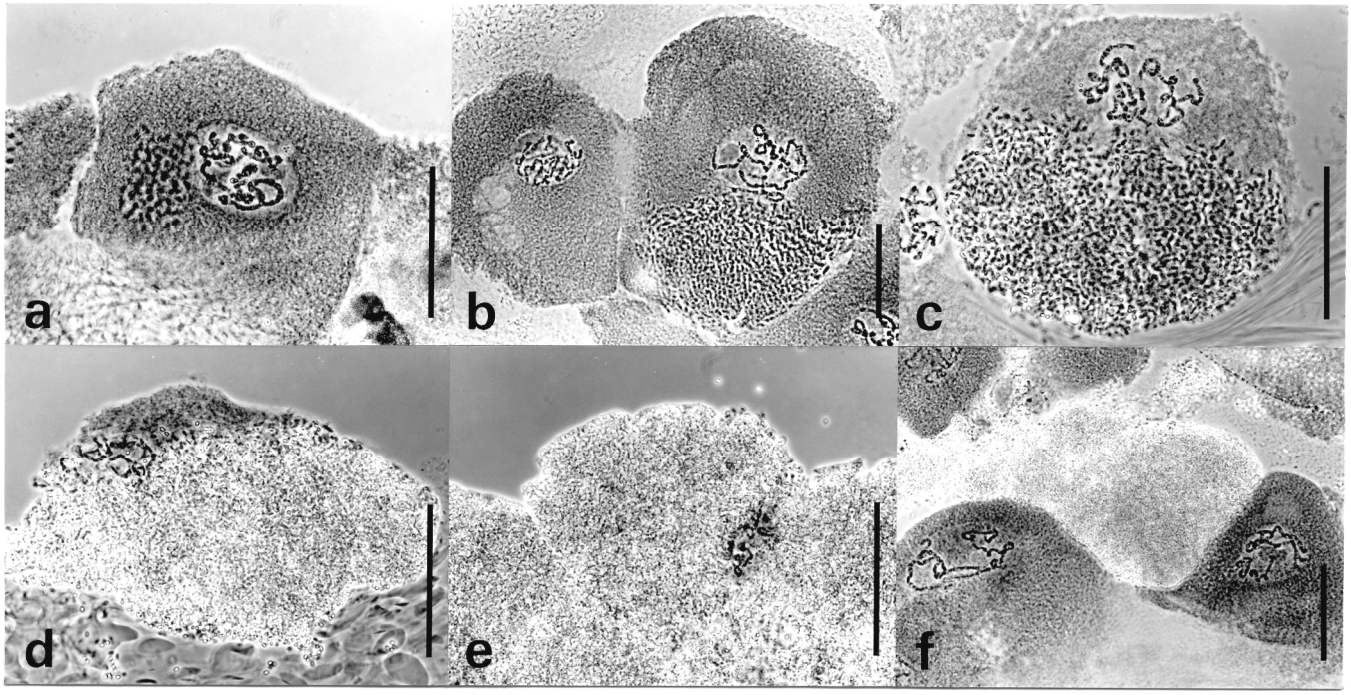

Fig. 2a-f Salivary gland cells of *A. lucidus* with different degrees of infection, **a** Slightly infected cell. **b** Moderately infected cell. **c** Strongly infected cell. **d-f** Heavily infected cells. **d** A small part of the cytoplasm, including the nucleus, is free of spores. **e** The cytoplasm is completely filled with spores. **f** A burst spore-filled cell between two non-infected cells. Phase contrast. Bars = 100 µm

In main and side lobe cells with higher degrees of infection, comparable with the strongly infected cell shown in Fig. 2c and with the heavily infected cell illustrated in Fig. 2d, first the regression of the lobe-specific BR2 (Fig. 3b) and then that of all other highly active puffs - lobe-specific and non-specific ones - such as puffs 1 (B 33-37), 2 (B 73), 6 (G 53) and 6 (H 27-31) (see Fig. 3a) was observed. Subsequent to that, the regression of the cell-specific BR1 and, finally, of the nucleolus occurred (Fig. 3c). In cells such as those shown in Fig. 2e, after the regression of all other BRs and puffs, puff 4 (A55) was activated in anterior lobe cells as well as in main and side lobe cells (Fig. 3c).

When, as was found in a few cases, hypertrophied, completely spore-filled gland cells had burst (Fig. 2f), shriveled polytene chromosomes were found, the clear banding pattern had disappeared and the chromatin was fuzzy.

Within a gland, heavily microsporidia-infected cells, in which the cytoplasm was completely filled with some thousands of spores, calculations resulted in about 0.5-1 x 10^4 spores/cell (Fig. 2e), and non-infected cells bordered each other. With one exception, no influence on neighbouring non-infected cells was noted. Only when a non-infected cell was completely surrounded by infected cells was a reduced activity of BR1 and BR2 observed.

In squash preparations of the bodies of *A. lucidus* larvae, mainly muscle cells were identified as host tissues of *N. algerae.* In larvae with non-infected salivary glands but heavy infections in other tissues, no change in the regular puffing patterns was observed on the polytene chromosomes of the salivary glands. In *A. lucidus* the vitality of the *N. algerae*-infected larvae seemed only slightly affected, if at all. No increased mortality of the animals was observed during the time lapsing from the second larval instar to the prepupal stage.

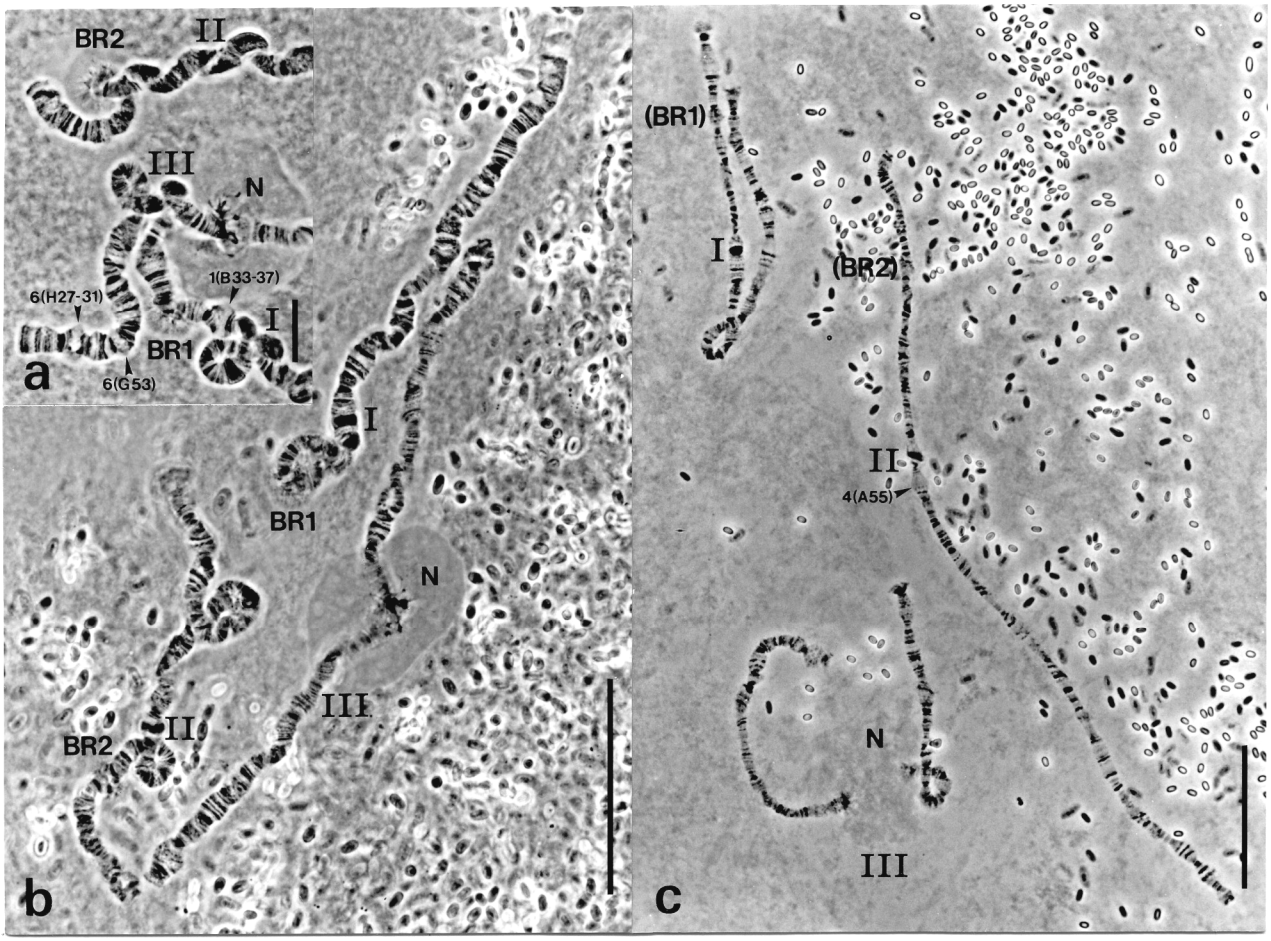

Fig. 3a-e Polytene salivary gland chromosomes of *A. lucidus.* ***a*** Section of a chromosome set of a non-infected main lobe cell. BR1, BR2, the nucleolus and some prominent puffs *(arrowheads)* are maximally expanded. Bar = 10 µm. **b** Chromosome set of a strongly infected main lobe cell as shown in Fig. 2c. BR2 and some puffs are slightly reduced in size. Bar = 50 µm. **c** Chromosome set of a heavily infected cell. BR1, BR2 and all other prominent puffs have regressed and are thus inactive. The nucleolus is in regression. Phase contrast [*BR* Active Balbiani ring, *(BR)* regressed Balbiani ring, *N* nucleolus, *I-III* chromosome I-III]. Bar = 50 µm

Discussion

The present investigation demonstrates that it is possible to infect a laboratory strain of the chironomid *Acricotopus lucidus* with the microsporidium *Nosema algerae,* resulting in high numbers of larvae having infected polytene salivary gland cells. This allows the study of the host cell-parasite interaction with respect to changes in the host gene activity.

For some time, just before the whole cytoplasm is filled with the microsporidia, the gene activity pattern of the host cell is not visibly affected. Then, in heavily infected cells, cell-specific BRs and some great puffs regress. This means that even though intracellular parasites are present, which surely affect the metabolism of the host cell, the infected cells maintain their cell-typic gene activity patterns (that is, the production of mRNA for the saliva-specific proteins) for as long as possible.

In *N. algerae-infected* larval salivary gland cells of *A. lucidus,* no special giant chromosomes could be found. In contrast to formerly reported cases of microsporidian infection (Keyl 1960, 1963; Diaz and Pavan 1965; Pavan and Basile 1966), no extra replication cycles were induced by *N. algerae.* Keyl (1960, 1963) investigated larvae of *Chironomus anthracinus* collected in the field in which an unknown microsporidian had obviously infected only salivary gland cells. The infected cells exhibited special giant chromosomes resulting from about three additional replication cycles as compared with the surrounding non-infected cells. He supposed that the highly infected hypertrophied cells produce only small amounts of saliva, if any. In highly infected cells, Keyl (1960) supposed the formation of new puffs resulting from the microsporidia-changed metabolism of the host cell. He could not say this with certainty because *C. anthracinus* is not a favourable object for such puffing pattern analyses. In *A. lucidus,* only the clear activation of one puff, puff 4(A55), was observed after the regression of all other BRs and puffs.

In an ultrastructural investigation of microsporidia-infected *C. anthracinus* larval salivary glands recently reported by Wülker and Weiser (1991), the cell-types-pecific parasite was determined as the new species *Helmichia glandulicola* (Microspora, Thelohaniidae). The observations of Wülker and Weiser that the saliva production of the host cell is "only partially reduced following infection" and that the infection does not spread from infected cells to neighbouring non-infected cells agree with the results of the present puffing pattern study of *N. algerae*-infected *A. lucidus* salivary glands.

The life cycle of *N. algerae* required 2-3 days at 28 °C as reported for infected *Heliothis zea* insect cell cultures (Streett et al. 1980). The percentages of 60%-80% found for *N. algerae*-infected larvae of *A. lucidus* varied to the same degree as those determined in infectivity studies of *N. kingi* in some *Drosophila* species (Armstrong 1976). In *C. anthracinus,* nearly 20% of the field-collected larvae exhibited *H. glandulicoIa*-infected salivary glands (Wülker and Weiser 1991). In the experiments with *A. lucidus,* 31%-35% of the larvae showed *N. algerae* infections of gland cells.

This study adds to the investigation of Keyl (1960) the observation that infections of polytene *A. lucidus* salivary gland cells with the microsporidian *N. algerae* lead to no fundamental change in the host-cell gene activity. In other words, no reprogramming of the gene activity occurs; the infected cells keep their cell-typic genetic programme constant just before their cytoplasm is completely filled with the parasite.

Acknowledgements

The author would like to thank Prof. W.A. Maier, University of Bonn, and Profs. C.U. Hesemann and F. Mechelke, University of Hohenheim, for their support. He is very grateful to Britta Schrempf-Eppstein and to Dr. Irmgard Wech for their assistance.

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