# KARYOTYPE AND NUCLEIC ACIDS PATTERNS OF THE CAMEL NASAL BOT FLY *CEPHALOPINA TITILLATOR* (CLARK) (DIPTERA: OESTRIDAE)

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## INTRODUCTION

The study of chromosomal and nucleic acids patterns in the larval stage of *Cephalopina titillator* clark didn't receive the necessary attention. This problem is important since *C. titillator* is a serious pest of camels as obligate parasite. It takes all its nutritional requirements for adulthood from its host during the larval stage (Zumpt, 1965). Infestation causes congestion of nasal cavity with mucus, severe inflammatory and degenerative changes, leading to extensive damage of nasopharyngeal tissues and the formation of lymphoid nodules at the site of larval attachment in the pharynx (Hussein *et al*, 1982 and Derhalli *et al*, 1989).

Chromosomal karyotype with nucleic acids patterns consider as a finger print for each living organism. The study of *C. titillator* chromosomes describes their designation, however the changes in DNA and RNA intensity, in different tissues of the 3rd larval instar,may give a clear image on the protein biosynthesis in these tissues. The amount of DNA and RNA in the cells, also, reflects the activity of gene expression, which is responsible for cell differentiation (Dignam *et al., 1989*; Dignam and Case 1990 and Gutierrez *et al.*, 2004 ) . The level of RNA transcript is one of the most convenient parameters used to monitor the gene activity in the cell lines and tissues (Nicholl, 1996 and Hobert, 2004).

Most of the previous studies on this parasitic insect in Egypt and in the Arab World were based mainly on the morphology, prevalence, incidence, monthly variations and some methods of control (Hussein *et al.*, 1983 and Fatani and Hilali, 1994). No studies concerning the nature of chromosomes of *Cephalopina* (Oestridae) have been reported. This may be due to that, the camel nasal bot fly proved to be difficult cytogenetic material. Standard techniques, which yielded excellent preparations with other species, gave poor preparations with *Cephalopina*. Presumably, the extremely long

life cycle accounts for the rarity of mitotic divisions in the larval brains where Zumpt (1965) mentioned that *C. titillator* has only two generations during the year.

The data collected in this study represent the basic informations on *Cephalopina* karyotype and nucleic acids patterns in different tissues. These studies come among biochemical, cytological and control investigations that will be published soon.

## MATERIAL AND METHODS

*Cephalopina titillator* larvae were collected from camel head (*Camelus dromedaries)* slaughtered in Giza, Egypt. The larvae were examined by a stereoscopic binocular (x100) for separating the second and third instar larvae. Larval identification was carried out according to Zumpt (1965). The partial rearing of *C. titillator* larvae in the laboratory was carried out following El-Moursy *et al.* (1993).

The analyzed chromosomes were obtained from neural ganglia of the 3rd instar larvae (La Chance, 1964). Larvae were injected with 0.05 ml/ larva of 0.1 % freshly prepared colchicine solution. The larval neural ganglia were dissected in isotonic saline of NaCl (0.7 %). A hypotonic treatment was achieved by using trisodium citrate ($Na_3C_6H_5O_7$. $2H_2O$) (0.8 %) for 18 min. The specimens were centrifuged for 5 min at 800 r.p.m. The supernatant was discarded and the pellet was fixed by the addition of 10 ml of cold and freshly mixed ethanol-acetic acid (3:1). The pellet of cells was resuspended in this fixative by using Pasteur pipette and was left for one hr in a refrigerator at 4°C. Then the suspension was centrifuged for 5 min at 800 r.p.m. The supernatant was discarded and replaced by 10 ml of fresh fixative for 10 min. After centrifugation and discarding of supernatant, the pellet of cells was resuspended and placed on clean slides and dried on hot plate (60°C). The slides were stained in 10 % Giemsa solution (pH 6.8) for 50 min, rinsed in phosphate buffer and dried on a hot plate (King and Pasteur, 1983).

Thirty well spread metaphases were examined and photographed. The total length of each chromosome was measured. Karyotype preparations were arranged in a descending order, the longest pair at first and the shortest one at last. The nomenclature of the chromosomes followed that model presented by Levan *et al.* (1964).

The nucleic acids patterns were detected according to Hassab El-Nabi *et al.* (2001). 0.03 mg of the tested organs or tissues (cuticle, fat body, gut and Malpighian tubules) were squeezed, immediately after dissection on ice, in 50µl of lysing buffer (50mM NaCl, 1mM Na2 EDTA, 10% SDS, pH 8.3) and gently stirred with micropipette. Haemolymph was centrifuged to separate haemocytes.

Gel was prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% poly vinyal pyrolidine (PVP) (Sigma). The agarose and PVP were boiled in tris borate EDTA buffer (1x TBE buffer, 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.3). Ethidium bromide (0.5 µg/ml) was added to gel at 40°C. Gels were poured and allowed to solidify at room temperature for 1hr before samples were loaded. 20µl of lysate tissue was transferred into the gel well. Lane 6 carried 2kbp ladder. 5 µl of 6x loading buffer was loaded on tissue lysate.

Electrophoresis was performed for 2hr at 50 volt in gel buffer (1x TBE buffer) with buffer level 2mm cover the gel. Gel was photographed using a polaroid camera while DNA and RNA were visualized using a 312 nm UV transilluminator. The intensity of DNA and RNA was measured as optical density by gel documentation system using Gel-Pro program.

## RESULTS AND DISCUSSION

Five pairs of biarmed chromosomes (2n=10) characterize the karyotypic macrostructure of *C. titillator*. The karyotype in which 2n=10 makes *C. titillator* an exception, along with only a few other species, to the general rule that 2n=12 in nearly all other calyptrate Diptera. The presence of diploid number of chromosomes 2n=10 in calyptrate Diptera was mentioned previously by Boyes (1958) for *Muscina stabulans* (Muscidae); LaChance (1964) for *Haematobia irritans* and *Stomoxys calcitrans* (Muscidae); Avancini and Weinzierl (1994) for *Haematobia irritans* and by El Bassiony (2001) for *Wohlfahrtia nuba* (Sarcophagidae). In contrast, LaChance (1964) found that the diploid number of chromosomes in *Hypoderma lineatum* (Hypodermatidae), which is the most related family to Oestridae, is 12.

There is no sign of heterogamety in *C. titillator* since all five pairs of chromosomes are homomorphic (Fig.1). In few calyptrate Diptera X and Y chromosomes become so useless that they could be lost, leaving sex determination under the control of formerly autosomal pairs (Boyes, 1967). The absence of heterogamety was recorded by LaChance (1964) for *Haematobia irritans* and *Hypoderma lineatum*; Loeschcke and Christensen (1987) for *Hydrotaea irritans* (Muscidae); Avancini and Weinzierl (1994) for *Haematobia irritans* and Loeschcke *et al.* (1994) for *Hydrotaea* sp.

The morphometric data of the haploid set of chromosomes (n=5) of *C. titillator* show two submetacentric (number 1 & 2) and three metacentric (number 3, 4 & 5) chromosomes. The pairs are easily identifiable except for the two metacentric pairs number 3 & 4 which are very close in size and designation.

The mean length of the longest and the shortest chromosomes are 6.31μm ± 0.18 and 3.99μm ± 0.10 respectively (Table 1). The highest and lowest mean values of the arm ratio are 1.88 and 1.25, respectively The haploid genome length of *C. titillator* measures 25.09μm.

## TABLE (I)

Morphometric data of the haploid set of chromosomes (n = 5) of *C. titillator*. Nomenclature after Levan *et al.* (1964)

| Chromosome Characteristic | 1 | 2 | 3 | 4 | 5 | Haploid genome Length (μm) |
|---|---|---|---|---|---|---|
| Total length( μm )±SE | 6.31±0.18 | 5.44±0.15 | 4.90±0.13 | 4.45±0.11 | 3.99±0.10 | 25.09 |
| Short arm( μm)±SE | 2.19±0.06 | 1.98±0.07 | 1.86±0.05 | 1.81±0.05 | 1.91±0.04 | |
| Long arm(μm)±SE | 4.12±0.14 | 3.45±0.09 | 3.04±0.09 | 2.65±0.08 | 2.40±0.07 | |
| arm ratio | 1.88 | 1.74 | 1.64 | 1.46 | 1.25 | |
| centromeric index | 34.75 | 36.36 | 37.94 | 40.58 | 47.99 | |
| designation | sm | sm | m | M | m | |

m = Metacentric sm = Submetacentric

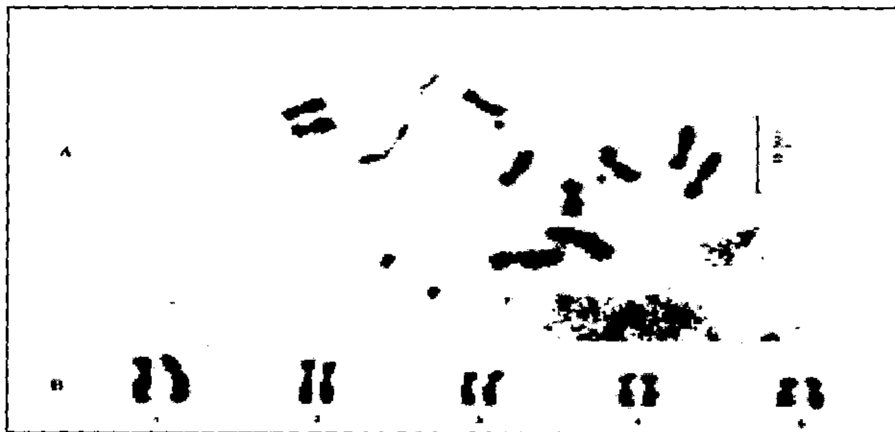

Fig (1): A- Somatic metaphase from larval brain of *C. titillator*. B- Karyotype of *C. titillator*. The five pairs are numbered 1 to 5.

The electrophoretic patterns of DNA and RNA in different tissues of 3rd instar larvae of *C. titillator* are illustrated in Figs (2), (3) and (4), where the intensity of nucleic acids is measured as optical density. Lanes 1, 2, 3, 4, 5 and 6 represent the haemolymph, cuticle, fat body, Malpighian tubules, gut and ladder respectively.

As seen in Fig (2), the released DNA is separated into one band in all tested tissues (Fig. 2). This band is located at 1991 bp. In the same manner, Tellam *et al.*

(2000) mentioned that DNA sequence of *L. cuprina* chitin synthase-like protein recorded 5757 bp in length.

Malpighian tubules showed a drastic elevation of DNA intensity; ( 90.30), with respect to all other studied tissues (Table 2 & Fig. 2). Moreover, the pattern of cuticle tissue charachterized by high level of DNA relative to fat body, haemolymph and gut. The optical density of cuticle and fat body records 20.36 and17.84 respectively. On the other hand, the electrophoretic pattern of DNA exhibits low optical density for haemolymph and gut where values of 6.98 and 8.95 were recorded respectively. This reduction in intensity of DNA level in haemolymph and gut may be due to the reduction in biosynthesis. The maintenance of a minimal DNA content in some tested tissues may, also, leads to decline mitotic index in cells of these tissues ( Qi *et al., 2004* and Wang *et al.*, 2004). The depression in mitotic index in relation to DNA level was detected by Grover *et al.* (1971).

Analysis of RNA showed that the released RNA was separated into two main bands. As seen in Table (2), the 1$^{st}$ band is located at 71 bp in both cuticle and Malpighian tubules, while the 1$^{st}$ band is located at 122 bp in fat body and haemolymph. The 2$^{nd}$ band is located at 406 bp in the four previously mentioned tissues. The RNA pattern in the gut is separated to three bands located at 71, 122 and 406 bp.

On the other hand, the intensity of RNA in Malpighian tubules tissue reflected the increasing in their contents. The values (expressed as optical density) were 15.80 and 128.85 for the 1$^{st}$ and 2$^{nd}$ bands respectively (Fig. 2). Previous studies of Datta and Antopol (1971) indicated that general decrease in the specific activity of DNAase and RNAase caused increase of DNA and RNA contents. This might explain the recorded increasing in the DNA and RNA intensities in Malpighian tubules tissue which may be attributed to the decrease in specific activity of DNAase and RNAase.

The values of optical density of RNA in the fat body, haemolymph and cuticle were varied from one to another. They were 38.32, 16.05 and 5.41 for the 1$^{st}$ band and 69.30, 34.12 and 43.60 for the 2$^{nd}$ band respectively. Supporting evidences are supplied by Monesi et al. (2001) who extended the characterization of DNA puff BhB10-1 gene of Bradysia hygida (Diptera: sciaridae) late larvae in different tissues such as gut, Malpighian tubules, fat body, cuticle and brain. The author found that the expression of BhB10-1 mRNA in larval tissues revealed different pattern from tissue to another.

The RNA electrophoretic pattern of fat body, haemolymph and cuticle, revealed a profile closely similar to that found with DNA in the same tissues, except for the cuticle, i.e. when DNA records high level in a tissue RNA records high level

too and vice versa. This may be due to that the flow of genetic information is unidirectional, from DNA to protein, with messenger RNA (mRNA) as an intermediate. The copying of DNA encoded genetic information into RNA is known as transcription, with the further conversion into protein being termed translocation. This concept of information flow is known as the central dogma of molecular biology and is an underlying them in all studies on gene expression (Nicholl, 1996).

The reduction in the optical density of DNA in the gut was accompanied with elevation in RNA level, which recorded 22.53, 54.91 and 18.09 for the $1^{st}$, $2^{nd}$ and $3^{rd}$ bands respectively. Monesi *et al.* (2001) was unable to detect BhB10-1 protein in different studied tissues (gut, Malpighian tubules, fat body, cuticle and brain) of *Bradysia hygida* while the mRNA of that protein is expressed in these tissues. The author suggested that the activation of the BhB10-1 gene in these tissues is triggered by a common factor.

## TABLE (II)

The optical density of DNA & RNA of different tissues of 3rd larval instar of *C. titillator* using gel pro-program

| tested tissues | DNA | RNA | | |
|---|---|---|---|---|
| | | $1^{st}$ band | $2^{nd}$ band | $3^{rd}$ band |
| Haemolymph | 6.98 | 16.05 | 34.12 | |
| Cuticle | 20.36 | 5.41 | 43.6 | |
| Fat body | 17.84 | 38.32 | 69.3 | |
| Malpighian tubule | 90.3 | 15.8 | 128.85 | |
| Gut | 8.95 | 22.53 | 54.91 | 18.09 |

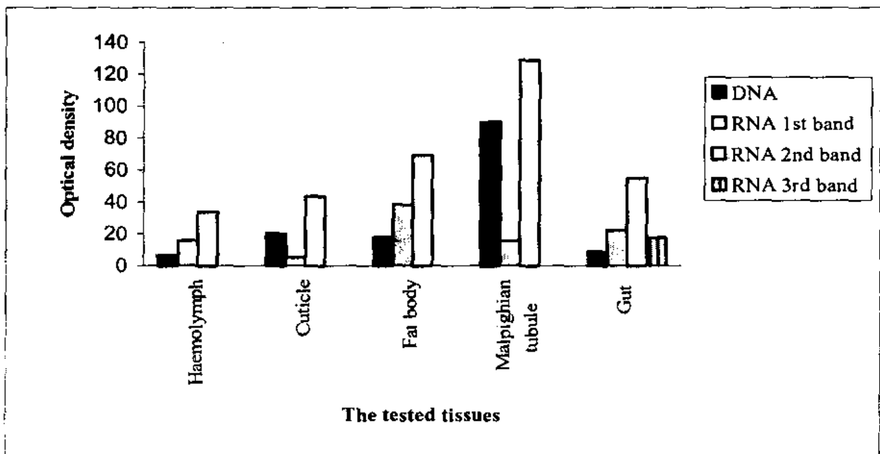


Fig (2): The optical density of DNA & RNA of different tissues of 3rd larval instar of *C. titillator*

tissues. The intensity of RNA in the cuticle showed relatively low optical density. The reduction in the density of DNA in the gut was accompanied by elevation in RNA level.

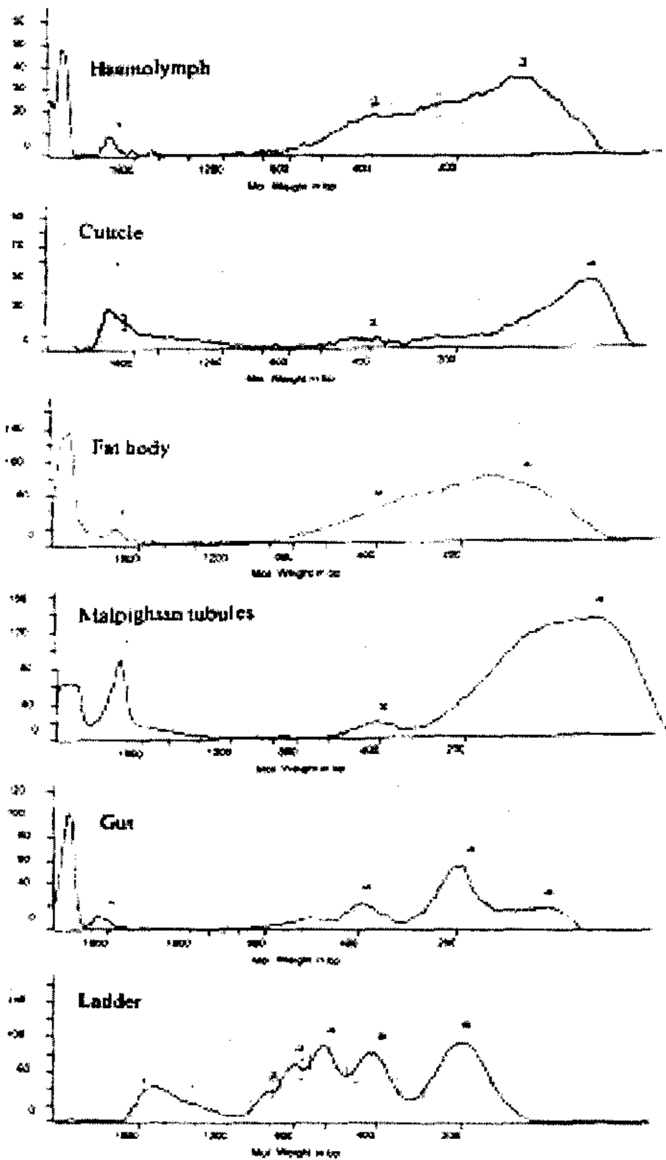


Fig (4): The intensity of DNA and RNA that has been measured as optical density by gel documentation system using Gel-Pro program. Charts 1, 2, 3, 4, 5 and 6 represent haemolymph, cuticle, fat body, Malpighian tubules, gut and ladder respectively

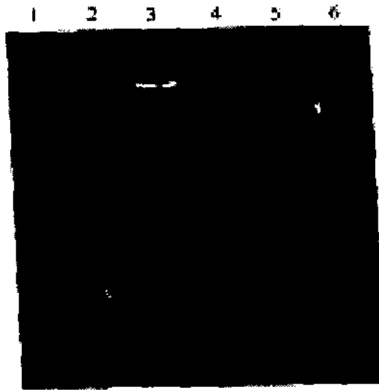


Fig (3): DNA and RNA bands of different tissues of 3rd larval instar of *C. titillator*. (Lanes 1, 2, 3, 4, 5 and 6 represent haemolymph, cuticle, fat body, Malpighian tubules, gut and ladder respectively)

## SUMMARY

Studies on the chromosome number of the camel nasal bot fly, *Cephalopina titillator* proved the presence of five pairs of biarmed chromosomes (2n=10). Among the Calyptrata, the camel nasal bot fly is one of the few species that doesn't conform to the general rule of 2n=12. The designation of the chromosomes was characterized by the presence of three metacentric and two submetacentric pairs. No sign of heterogamety was found where the all five pairs were homomorphic. The haploid genome length measures was 25.09 µm.

The nucleic acids pattern was detected in different tissues (haemolymph, cuticle, fat body, Malpighian tubules and gut) of the 3rd larval instar of C. titillator. The results showed that each investigated tissue of the insect had DNA and RNA finger print or pattern that reflects the variations of gene expression from tissue to another. The comparison for the changes in DNA content in different tissues showed that the released DNA was separated into one band in all studied tissues. On the other hand, RNA was found to be separated into two main bands in cuticle, fat body, haemolymph and Malpighian tubules, whereas in the gut RNA was separated into three bands. The intensity of DNA and RNA in different tissues was measured as optical density. Malpighian tubules showed a drastic elevation in its nucleic acids intensity in respect to the other studied tissues. The electrophoretic pattern of DNA recorded its lowest level in haemolymph and gut. The electrophoretic pattern of RNA for Malpighian tubules, fat body and haemolymph detected a profile closely similar to that found with DNA in these

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