MOLECULAR CYTOGENETIC TESTING OF CHRYSOMYA BAZZIANA AND COCHLIOMYIA HOMINIVORAX (Diptera: Calliphoridae) IN AL-QADISSIYAH PROVINCE

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ABSTRACT

Chrysomya bazziana and hominivorax have 12 chromosomes, one pair is sex chromosomes and the rest are metacenteric. The karyotype morphology has no substantial differences in both species, except for the X chromosome which is subtelocentric in *Chrysomya bazziana* ,as metacentric in *C. hominivorax* and approximately, 1.3 times longer in *Cochliomyia*. Hominivorax. The presence of a C-band in the pericentromeric region was exploited to characterize autosomes. The sex chromosomes of both species were heterochromatic, with exception the final region of long arm of X chromosome. The detection of ribosomal genes was achieved by Molecular cytogenetic testing know as fluorescent In situ hybridization technique(FISH) .The position of NOR was on the sex chromosomes.

Key words:

Chrysomya - karyotype - NOR - heterochromatin - blow flies

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INTRODUCTION

Chrysomya bazziana and Cochliomyia hominivorax have hygienically and veterinary importance seeing that they constitute a risk on public health and economy..They in charge of myiasis,where they lay eggs in moist skin, orifices and existing wounds **(1,2)** .The worldwide spread and their obligation parasitism are the major reason that increases their risk. **(3)**

The cytogenetic variations of the natural populations of medical and economic insects play an important and decisive role the dexpansion of insecticide durability ,the implementation of genetic control as well as the former cytolgenetic studies pointed out that chromosome morphology is helpful in taxonomy **(4)** . The heterochromatins allotment ,the NORs position and the obscurity or entity of sex chromosomes of various species of Muscoidea was examined, where the karyotype number 2n=12 of this group, even though the description of species that they have 2n=was demostrated. the sex pair in the latter cases was abscent **(5,6,7)** There was somatic pairing intimation through prophase to metaphase in most diptera and cell types studied **(8).** The present investigation involves the examination of the karyotype of *Chrysomya bazziana and Cochliomyia hominivorax* as well as the molecular cytogenetic testing (*in situ* hybridization) was exploited to identify the location of the nucleolar organizer regions.

MATERIALS AND METHODS

Fly rearing

Chrysomya bazziana and Cochliomyia hominivorax were collected from locations of cattle and sheep in the south of Iraq(AL-Qadissiya province). A tenuous external morphological characteristic was used to identify species. The adults were kept in nylon cages (25x25x43)cm at 25±2°C and 45-55% RH. The adults had access to sugar cane 24 h/ day and to ground beef a few hours/day. Water was always available.

Chromosome preparations

The neural ganglia of L3 larvae were exploited to get mitotic chromosomes, where 0.01 ml of colchicine was used to inject larva followed by vivisection after 45 min treatment. The dissection of brains was accomplished by fine forceps, Then, dispersal in KCl(3 ml) at 25 °C for quarter of an hour, centrifugation at 600 rpm for 10 min, and fixation in methanol. Air-drying technique was followed to prepare slides **(8).**The brain of larva that do not treat by colchicine was used for cytogenetic studies .

Chromosome morphology

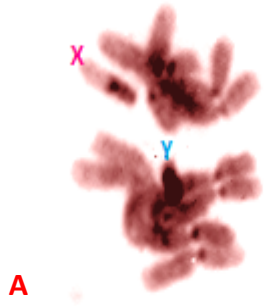
10% Giemsa was used to stain slides for shape studies. The method remembered by **(9)** was followed for the description of the chromosomes morphology.

Fluorescent *In situ* hybridization

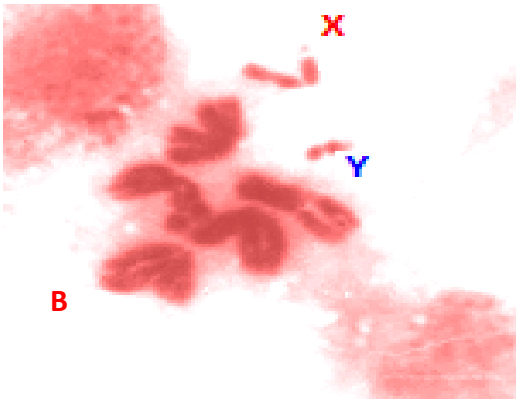
The baking of slides took 2 h at 80°C and 80% and 100%ethanol was used to dehydrate the baked slides. 25 mM of NaOH was used to denature chromosome for 60 sec, then washed for 10 sec. in 0.4 x SSC, 0.1% Tween, followed by dehydration by a series of 70% (precooled at -20°C), 80% and 100%ethanol for 2 min each. The composition of hybridization solution is 20 ng DIG-labelled DNA probe, 50%formamide (deionized) and 35% master mix (1 ml 20 x SSC, 1 ml dextran sulfate, 1 ml aqua aqua bidest. and0.5 ml salmon sperm DNA (10 mg/ml, sheared to 200 - 500 bp). 10 uJ hybridization mixture was denatured at 80°C for 8 min, cooled on ice and then applied to the slide, covered with a cover-slip and sealed with rubber cement. Hybridization came by at 37°C in a humid chamber for 12 - 18 h. After two washes in 0,4 x SSC, 0.1% Tween at room temperature, anti-DIG-antibodies (fluorescein-conjugated)were applied based on the instructions of the supplier (Boehringer). Antifade (0.233 g DABCO in 0.9 ml glycerol, 0,1 ml 0.2 M Tris pH 8) containing 0.2 ug/^il propidiumiodide was put on the slide. Photos from fluorescence microscopy were taken with Kodak Ecta Goldll (400 ASA) films.

RESULTS AND DISCUSSION

The chromosomes were metacentricm in both species as illustrated in Table1,with exception for X chromosome of *Chrysomya bazziana* ,where was subtelocentric. karyotype length showed no major difference *(*37.8) µm.The X chromosome of *Cochliomyia hominivorax* was the longest ,while Y chromosome was too short incomparison with X chromosome for the both species. In *Chrysomya bazziana,* the long arm of the X chromosome has a constriction as show in figure1(A,B).In most *Chrysomya* species that studied, the length of sex chromosome is medium, heterochromatic, and Male factor of Y chromosome is propably in charge of sex control **(10, 11,12)**.The net morphology noticed in this research was as like as that found by **(13),** where all the autosomes had been metacentric.Secondary constriction was reported by **(14)** in pairs I, III and IV *L. cluvia* and *L. sericata* autosomes are well close pairing of somatic, an identified characteristic in Diptera, where the homologous chromosomes are line in neighborhood of one and the diploid complements show as haploid set (Agrawal et al. 2010). Nonetheless, XX of females and XY of males did not give like this s somatic pairing and line separately **(15,16,17).**



Fig(1) A-C-banded Mitotic chromosomes of Cochliomyia hominivorax



Fig(1) B- Mitotic chromosomes of Chrysomya bazziana

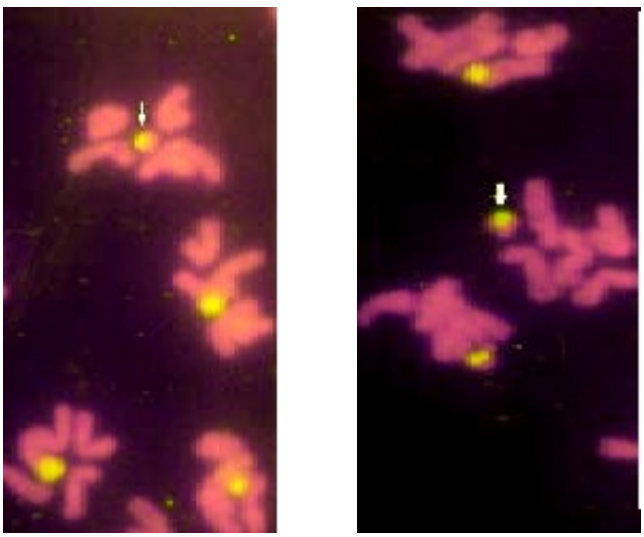
Chromosome pair	Length (μm)	Arm ratio	Relative	Designation
Chrysomya bazziana				
1	7.0	1.5	0.21	M
2	7.3	1.4	0.19	M
3	6.5	1.5	0.18	M
4	6.4	1.3	0.16	M
5	5.1	1.6	0.14	M
X	4.5	4.4	0.13	M
Y	2.7	1.2	0.06	M
Cochliomyia hominivorax				
1	7.4	1.4	0.20	M
2	6.7	1.5	0.18	M
3	6.7	1.2	0.17	M
4	5.8	1.5	0.15	M
5	5.3	1.2	0.13	M
X	6.4	1.2	0.15	M
Y	1.9	1.0	0.0	M

Table: Analysis of the somatic complements of *Chrysomya bazziana and Cochliomyia hominivorax*

M: metacentric; St: sub telocentric. The relative length of Y was expressed as a function of the length of X.

The sex chromosomes contains NOR in both species according to in situ hybridization . Fig2(A and B). The NORs are positioned on the sex chromosomes in most species that studied on the sex chromosomes **(18,19,20).** *Lucilia cuprina* karyotype showed that the nucleolus has relatedness with X and Y secondary constrictions . **(18)**characterized a confirmed marker for rDNA in the sex chromosomes of *L. cuprina* and *C. bezziana*, as well as the regions had relatedness with secondary constrictions.

Cryptic or isomorphic species are present among blowflies that bring about to taxonomic problems seeing that the similarity of outer appearance of maggot a. Based on results got , rDNA could be taken in account as basic cytological sign for the comparison of karyotypes of phylogenetically related species and sibling species. These approaches can be used to participitate in the changes analysis of karyotype associated with the evolution process and to understand of taxonomic relations**(20).**



A B

Fig(2) A- FISH in *Cochliomyia hominivorax meiotic chromosomes.*

Fig(2) B- FISH in *Chrysomya bazziana*

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