Isolation and molecular characterization of the *Bactrocera zonata* (Saund.) sex-lethal (SxL) gene homologue of the *Ceratitis capitata*

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Emtithal, M. Abd- El-Samie
Entmology Department, Faculty of Science, Cairo University

ABSTRACT

The fruit fly Bactrocera zonata (Dacus zonata) is a serious pest of fruits in many parts of the world. B. zonata larvae feed exclusively on fruits, causing severe damage to crops. B.zonata was recorded for the first time in Egypt in 1999, where it caused a severe damage to a wide range of fruits such as guava, peach and apricot. This work includes the isolation and molecular characterization of the fruit fly B. zonata sex-lethal (Bz SxL) gene homologs to the C. capitata SxL (CcSxl) gene. Investigation of the effect of Gamma radiation on the expression of B. zonata SxL gene had been performed. Mature pupae 2 days before eclosion, were irradiated at 100, 120, 150 GY gamma irradiation. Total RNA has been isolated from every irradiated dose. Fragments of the Bz SxL were recoverd with RT-PCR and sequenced. Irradiated and non-irradiated B. zonata SxL gene (Bz SxL) express the same pattern of transcripts, which encode for a single common polypeptide in both male and female flies. The sequenced fragment of irradiated female with 150 GY dose revealed some sequence mutations. The gene shares a high degree of similarity in sequence compared to C. capitata orthologous and does not appear to play a key regulatory role in the sex determining cascade.

Keywords: *Sex determination, homology, sex- specific splicing, ionizing radiation; Tephritidae.*

INTRODUCTION

The fruit fly *Bactrocera zonata* is a serious pest of fruits and vegetables in many parts of the world. Four hundred species belonging to the genus *Bactrocera* are widely distributed in tropical Asia, South pacific and Australia, with very few species in Africa and Europe (Drew, 1989). *B. zonata* was recorded for the first time in Egypt in 1999, where it caused a severe damage to a wide range of fruits including guava, peach, apricot and mango (El-Minshawy *et al.*, 1999).

Traditional methods of pest control using chemical insecticides experienced disadvantages such as residual problems and the inability of insecticides to penetrate infested fruits to kill larval population pest. It is therefore, imperative to seek alternative and more effective methods of pest control using advanced gene technologies.

Sex determination shares some general features in all dipteran species. An initial signal governing the choice of sex is transmitted through a regulatory cascade to activate the genes ultimately required to produce the particular physiological and behavioural phenoltypes of the two sexes. In different dipteran species, the initial signal at the top of the regulatory hierarchy varies considerably.

In *Drosophila melanogaster*, the primary sex-determining signal proteins are based on the number of X chromosomes in relation to the sets of autosomes (Cline, 1993; Schütt and Nöthiger, 2000). In dipteran species, such as *Ceratitis capitata* (Family: Tephritidae), *lucilia cuprina* (Family: Calliphoridae), *Musca domestica* (Family: Calliphoridae) and *Sciara ocellaris* (Family : Sciardae), it has been shown genetically that the presence of a dominant male determiner protein, often carried on the Y-chromosome, control male sex apperance (Nothiger and Steinmann–Zwicky, 1985; Lifschitz and Cladero, 1989; Duebendorfer et al., 1992; Wilhoeftu and Franz, 1996; Ruiz *et al.,* 2003). The chromosomal structure and inheritance are similar in both the Queensland fruit fly *B. tryoni* (Family: Tephritidae) and *C. capitata* (Zhao *et al.,* 1998). Male sex in *B. tryoni* appears also to be determined by the presence of Y chromosome (Meats *et al.,* 2002). This mechanism of sex determination operates in other members of the *Bactrocera* genus, namely *B. cucurbitae* (McCombs *et al.,* 1993), *B. dorsalis* (McCombs and Saul, 1995) and *B. oleae* (Lagos et al., 2005). This suggests that the sex determination pathway in dipteran species,other than *D. melangaster*, is different in the initial signal and / or the key master switch. Sex determination in *D. melanogaster* is controlled by a cascade of enhanced genes in a specific pathway switched on primarly by the master regulatory gene sex lethal (SxL) (Bopp *et al.,* 1996). Dm SxL controls the alternative splicing of a downstream gene, transformer (Tra) which acts with Tra 2 to control alternative splicing of doublesex (dsx) (Hoshijima *et al.,* 1991). Dm SxL also controls its own splicing, creating an autoregulatory feedback loop that ensures expression of SxL in females, but not males (Bell *et al.,* 1991). *Ceratitis capitata* tra gene (Cctra) is regulated by alternative splicing and apparently controls the alternative splicing of Ccdsx. However, Cctra is not regulated by CcSxl. Instead, it appears to autoregulate in a manner similar to the autoregulation that has been seen with DmSxl gene.

Ionizing radiation technology is used to induce dominant lethal genes causing reduction of offspring which introduce a new technique for insect control called sterile insect technique (SIT) (Bushland and Hopkins,1951). It acts as a direct mutagen with minimal latency time of action. It can be used as reference to infer some kinetic parameters of chemical mutagens (Morales-Ramirez *et al.,* 1997). For the successful application of SIT, it is imperative to determine a radiation dose developing sterility in flies but otherwise remain healthy and vigorous in their mating behaviour (Qureshi and Bughio, 1969; Ashraf *et al.,* 1974).

Kattyar (1962) reported that pupae of fruit-flies *Ceratitis capitata*, when exposed to 100-130 GY from a Cs-137 source, develops sterility in adult stages without showing any deleterious effect on longevity and mating behaviour of the flies. Steiner *et al.* (1962) found that 100-120 GY from (415c-60Co) was used as the minimum dose to prevent egg laying as well as to avoid the production of sperms during the life cycle of the three species of fruit flies *Dacus dorsalis*, *C. capitat*a and *Dacus cucurbitae*. Melis and Baccetti (1960) however, reported that exposure of 80-120 GY of gamma-rays caused sterilization in *Dacus oleae*. Huque and Ahmad (1966) have reported that *Dacus cucurbitae* and *Dacus zonatus (Bactrocera zonata)* can be sterilized at a dose of 70-90Gy; and the minimum dose required to render the males of *Dacus ciliatus* fruit fly incapable of fertilizing females was 80.5-100GY when radiated in the late pupal stage.

No work has been done up to date on the effect of radiation on sex- lethal (Sxl) gene of

B. zonata. An understanding of the sex determination mechanisms in insects that are of agricultural or public health importance may help in the development of improved methods for their control using the SIT.

The objectives of this work are to: isolate the SxL gene of *B. zonata* and to compare its homology with *C. capitata* SxL and other dipteran species and to characterize the impact of ionizing radiation on SxL gene of *B. zonata* and investigate its behaviour as sex determinant gene in dipteran insects. Comparative analysis between sequenced fragments of SxL gene of *B. zonata* and irradiated genes, which induced sterility in flies, will be studied.

MATERIALS AND METHODS

The invested guava fruits were collected from Giza Governorate. The full grown larvae naturally jump to the sand where they pupate. Two days before eclosion, pupae of *B. zonata* were irradiated using gamma cell (60Co source), model 220, installed at the Department of Nuclear Physics of Atomic Energy Authority, ARE. Pupae for each radiation dose are exposed to 100, 120 and 150 GY of gamma radiation.

RNA isolation and RT-PCR analysis

Total RNA was extracted from both male and female adults using Gentra Purescript RNA Kit (Life Trade Company). One ug of total RNA was reverse transcribed with AMV (Promega) according to the manufacturer's directions and about 1/20th of the reaction was used for PCR reaction in 25 µl total volume with the following forward and reverse primers which were designed from the sequence of *C .capitata* Sex-lethal (CcSxl) at 126 bp and 422 bp and at 423 bp and 917 bp (Fig. 1a) according to Saccone *et al.* (1998).
SxL1-F(CATACGGATACAATGGTTAT), SxL2-R(TCGCGATCTGTCATATCCTG) and SxL3-F (CAGGATATGACAGATCGCGA) & SxL4-R(CCGAGCTGAGACATATAGTC) The thermal cycle program was set to 40 cycles of 94°C denaturation, 58°C annealing and 72°C elongation for 40 seconds each step (Perkin Elmer Gene Amp 9600). PCR products were recovered, and sequenced by automated DNA sequencing reactions, which were performed using sequencing ready reaction kit (Applied Biosystems, USA) in conjunction with ABI-PRISM and ABI-PRISM big dye terminator cycler.

RESULTS AND DISCUSSION

B. zonata SXL gene produces the same transcripts in xx and xy animals. Similar to *Ceratitis*, though no sex-specific size classes were detected. To confirm that transcripts in males and females are strucurally identical as in *C. capitata*, RT-PCR analysis was perfo-rmed on total RNA extracted from non-irradiated *B. zonata* adult flies. Two pairs of specific primers were used for amplification. In both cases, the amplified products were found to be of the same length in males and females 296 bp by using primers 126+/422-,(SxL1/SxL2) and 496 bp by using primers 423+/917-,(SxL3/SxL4) (Fig. 1a &b), and subs-equent sequencing of the amplified fragments did not reveal any sex-specific differences.

A 296 bp DNA fragment of the BzSxL and CcSxL was isolated by RT-PCR from total RNA of adult non-irradiated and irradiated male and female using the primers SxL1 and SxL2 (Figs. 2 and 3). Also, 496 bp DNA fragment of the BzSxL and CcSxL was isolated by RT-PCR from total RNA of adult male and female using the primers SxL3 and SxL4 (Figs. 2 and 3). The sequences of these fragments showed a 89%, 99% and 84% similarity at the nucleotide level to the corresponding fragment of CcSxL, BoSxL, DmSxL genes, respectively (Fig. 4b).

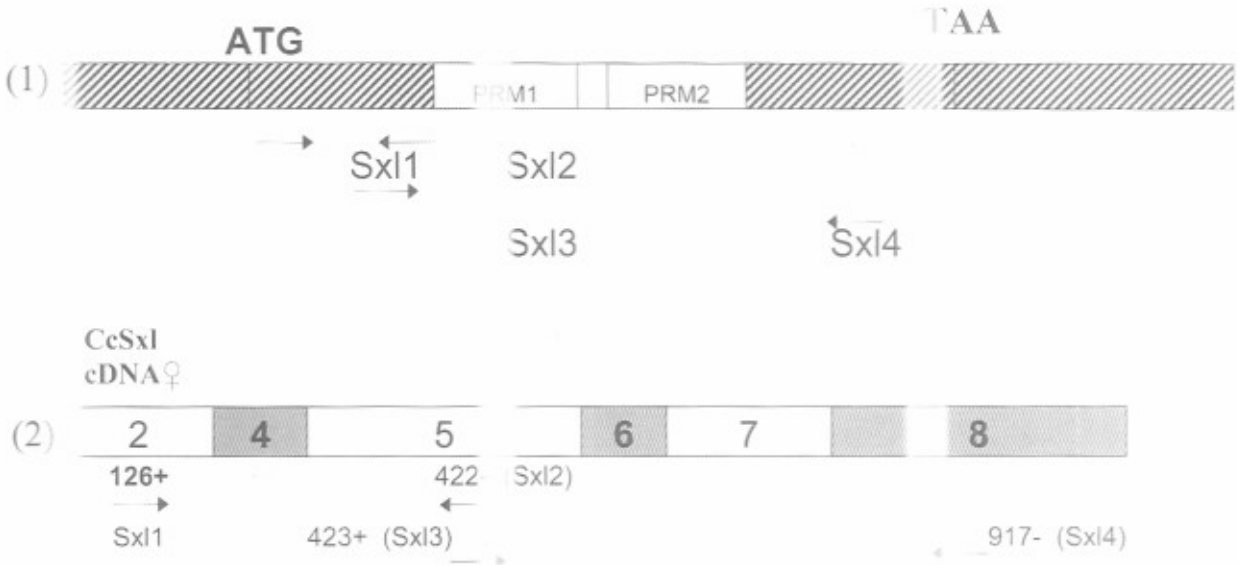

Fig.(1a): Schematic representation indicating the position of primers within the ORF of Bactrocera oleae (BoSxl) cDNA clones ♂/♀ (1) and Ceratitis capitata (Cc SxL) cDNA ♀ (2).

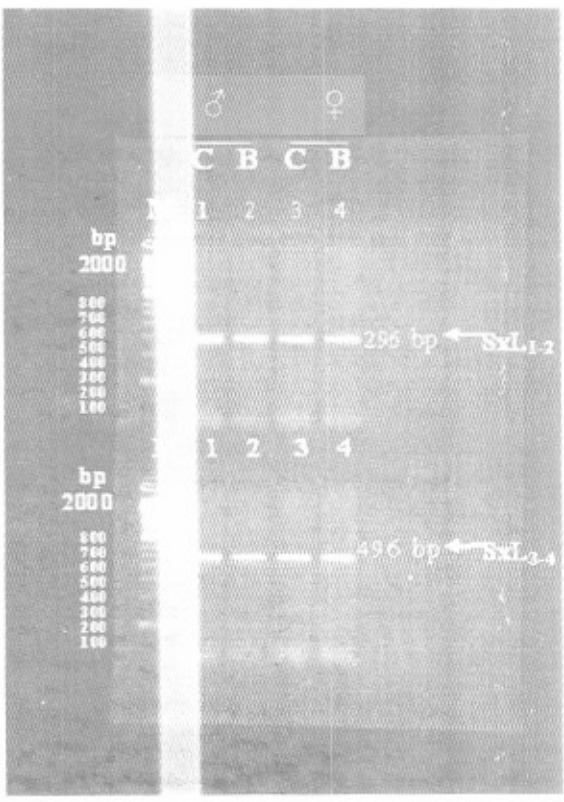

Fig.(1b):RT-PCR analysis of total RNA prepared from adult males and females of C. capitata (C), B. zonata (B), respectively. A–M (DNA marker). Lanes 1-2 C B ♂ (control), Lanes 3-4 C B ♀ (control), as arrow indicates SxL 1-2 PCR products of 296 bp B-M (DNA marker), Lanes 1-2 C B ♂ (control), Lanes 3-4 C B ♀ (control), as arrow indicates SxL 3-4 PCR products of 496 bp.

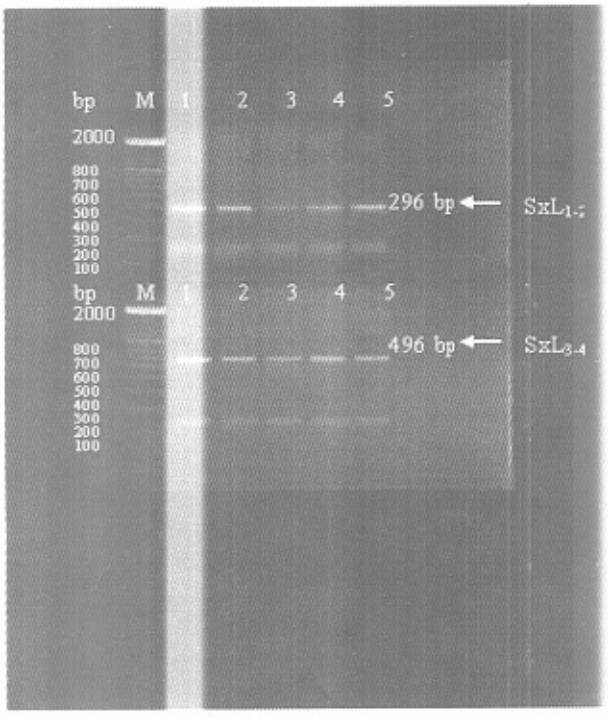

Fig.(2):RT-PCR analysis of total RNA prepared from adult males C. capitata (C) and B. zonata (B). A-M (DNA marker) Lane 1 C ♂ (control), Lane 2 B ♂ (control), lanes 3-5 (irradiated B with 100,120,150 GY) . For amplification in lanes 1-5, as arrow indicates SxL 1-2 PCR products of 296 bp. B- M (DNA marker) Lane 1 C ♂ (control), Lane 2 B ♂ (control), lanes 3-5 (irradiated B with 100,120,150 GY) For amplification in lanes 1-5, as arrow indicates SxL 3-4 PCR products of 496 bp.

Fragment of the irradiated female BzSxL gene with 150 GY was recovered with RT-PCR and sequenced. The sequences revealed that irradiation treatment caused many mutations which are known as substitution mutations because two cytosine nucleotides are replaced by a guanine nucleotide and one adenine nucleotide is replaced by thymine nucleotide (Fig. 4a). RT-PCR analysis performed on total RNA extracted from adult Bactrocera and Ceratitis males and females and their subsequent fragments sequencing in both sexes did not show any sex-specific differences. These results indicate that BzSxL gene produces the same protein in males and females. Sequence comparison of BzSxL gene to CcSxL gene revealed 89% identity at the nucleotide level and 87% identity at the amino acid level. Comparison between Bz SxL and Dm SxL genes also shows 84 % at the nucleotide level and 82% at amino-acid level (Fig. 4b).

Investigations carried out by various workers on the effective dose for sterilizing males in different species of fruit flies showed that it varied from species to species. The determination of the effective dose for sterilizing males is the first step toward adopting a sterile insect technique (SIT) as a successful method of control. It was previously reported that *Bactrocera zonata* (Saund.) can be sterilized at a dose of 90 GY when irradiated in the late pupal stage (Huque and Mal, 1967). This dose is quite realistic to make both the sexes of the fly permanently sterile. The sterilizing dose has no effect on the pupal survival, adult emergence, longevity and sexual aggressiveness. Generally, gamma radiation in the present study showed a significant effect on the expression of the Bz SxL gene. In *melanogaster* SxL acts as a binary switch that implements all aspects of sexual development. Because of its sex-specific expression in other *Drosophila* species, it has been proposed that

SxL is widely used as a master switch gene in sex determination in the genus *Drosophila* (Bopp et al., 1996). Studies of SxL orthologous in many distantly related dipterans outside the genus *Drosophila* showed that the sex-determining function of the SxL is an exception to the rule. SxL gene was studied in two different suborders of Diptera, such as Brachycera and Nematocera. Examples of Brachycera are *C. rufifacies* (Calliphoridae; Muller-Holtkamp, 1995), M. scalaris (Phoridae; Sievert *et al.*, 1997), *C. capitata* (Tephritidae; Saccone *et al.*, 1998), *M. domestica* (Muscidae, Meise *et al.*, 1998), *B. Oleae* (Tephritidae; Lagos *et al.*, 2005), *B. zonata* (Tephritidae; this work). Examples of Nematocera are *S. ocellaris* (Sciaridae; Ruiz et al., 2003), *Sciara coprophila*, *Rhynchosciara americana* and *Trichosia pubescens* (Serna et al., 2004). Previous studies showed that the above insects produce the same SxL protein in males and females.

Therefore, the sex-determining function of SxL gene has evolved recently only in the genus *Drosophila*. These results support the Wilkins hypothesis that the master genes at the top of the regulatory hierarchy can change dramatically as new species and genus evolve, while the slave genes at the bottom of the hierarchy remain the same, carrying out essentially identical functions from one species to the next (Wilkins, 1995). This work reports the isolation and characterization of SxL gene of *B. zonata*, which belongs to the suborder Brachycera, and also that the BzSxL gene transcripts in females and males are identical.

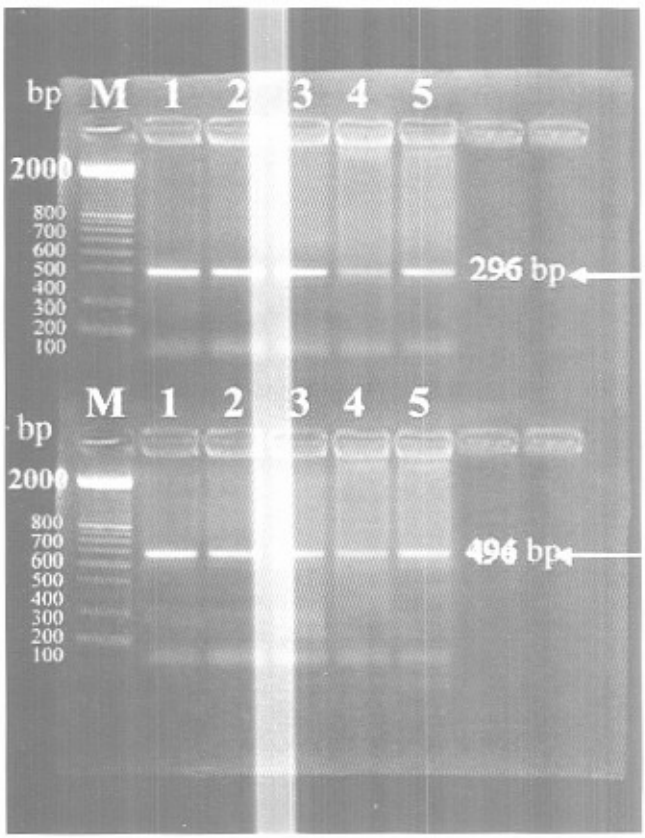

Fig. (3): RT-PCR analysis of total RNA prepared from adult females of C. Capitata (C) and B. zonata (B). A-M (DNA marker) Lane 1 C ♀ (control), Lane 2 B ♀ (control), lanes 3-5 (irradiated B with 100,120,150 GY) For amplification in lanes 1-5, as arrow indicates SxL 1-2 PCR products of 296 bp. B- M (DNA marker) Lane 1 C ♀ (control), Lane 2 B ♀ (control), lanes 3-5 (irradiated B with 100,120,150 GY) For amplification in lanes 1-5, as arrow indicates SxL 3-4 PCR products of 496 bp.

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(N)   1  TTTATTTAGGACAATCGGTCCTATAAACACTTGCAGAATAATGAGAGATTATAAGACTGG
(I)      ************************************************************
(N)  61  CTACAGTTTGGTTACGCTTTTGTTGATTTCGCTTCCGAAACGGATTCACAACGAGCGATA
(I)      ************************************************************
(N) 121  AAAAGCTTAAACGGCATTACAGTGCGCAATAAGCGTTTAAAGGTTTCATATGCTCGTCCT
(I)      ************************************************************
(N) 181  GGCGGTGAATCGATTAAGGATACCAATTTGTATGTTACGAATCTACCACGTACAATAACC
(I)      ************************************************************
(N) 241  GGTGATCAATTGGATACCATATTCGGTAAATATGGCTTGATTGTACAGAAGAATATTCTA
(I)      ************************************************************
(N) 301  AGAGACAAATTGACGGGCAAACCGCGTGGCGTTGCATTTGTCAGATTCAATAAACGCGAA
(I)      ************************************************************
(N) 361  GAAGCGCAAGAGGCAATTTCCGCTCTGAACAATGTCATACCCGAGGGTGGATCTCAGCCG
(I)      ************************************************************
(N) 421  CTCACCGTGCGCTTAGCCGAGGAGCATGGCAAAGCGAAAGCGCAGCAATACATGTCACAG
(I)      **********G************G*********************T**************
(N) 481  CTGGGATTGA
(I)      **********
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Fig.(4a): Alignment of the nucleotide sequence of the non-irradiated (N) and irradiated (I) of B. zonata female Sex-lethal (Bzsxl) fragment. The bold letters indicate the substitution mutations.

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                 .    :    .    :    .    :    .    :    .    :    .    :   900
Bzsxl                                                TTTATTTAGGACAATCGGTCCTATAAACA    29
Ccsxl          CACAGGATATGACAGATCGCGAACTCTATGCTTTATTTCGGACAATTGGCCCCATCAACA   479
Bosxl          CACAGGATATGACAGATCGCGAACTCTATGCTTTATTTAGGACAATCGGTCCTATAAACA   709
Dmsxl          CCCAGGACATGACCGATCGCGAGCTGTACGCCCTATTCAGAGCCATTGGACCCATCAACA   858
                 .    :    .    :    .    :    .    :    .    :    .    :   960
Bzsxl          CTTGCAGAATAATGAGAGATTATAAGACTGGCTACAGTTT-GGTTACGCTTTTGTTGATT    88
Ccsxl          CATGCAGAATAATGAGAGATTATAAGACTGGCTACAGTTTTGGTTATGCTTTTGTTGATT   539
Bosxl          CTTGCAGAATAATGAGAGATTATAAGACTGGCTACAGTTTTGGTTACGCTTTTGTTGATT   769
Dmsxl          CGTGCAGAATCATGCGAGACTATAAGACTGGCTACAGTTTTGGTTATGCTTTCGTGGACT   918
                 .    :    .    :    .    :    .    :    .    :    .    :  1020
Bzsxl          TCGCTTCCGAAACGGATTCACAACGAGCGATAAAAA-GCTTAAACGGCATTACAGTGCGC   147
Ccsxl          TCGCTGCCGAAACGGATTCACAACGAGCGAT-AAAGAGCTTAAACGGAATAACGGTCCGC   598
Bosxl          TCGCTTCCGAAACGGATTCACAACGAGCGATAAAAA-GCTTAAACGGCATTACAGTGCGC   828
Dmsxl          TCACATCGGAAATGGACTCGCAGCGTGCTATTAAAGTGCTGAAT-GGCATCACAGTGCGC   977
                 .    :    .    :    .    :    .    :    .    :    .    :  1080
Bzsxl          AATAAGCGTTTAAAGGTTTCATATGCTCGTCCTGGCGGTGAATCGATTAAGGATACCAAT   207
Ccsxl          AACAAGCGTTTAAAGGTTTCATATGCTCGGCCTGGCGGTGAATCGATCAAGGATACAAAT   658
Bosxl          AATAAGCGTTTAAAGGTTTCATATGCTCGTCCTGGCGGTGAATCGATTAAGGATACCAAT   888
Dmsxl          AACAAGCGGCTTAAGGTTTCCTATGCACGTCCCGGCGGAGAATCGATCAAGGACACCAAT  1037
                 .    :    .    :    .    :    .    :    .    :    .    :  1140
Bzsxl          TTGTATGTTACGAATCTACCACGTACAATAACCGGTGATCAATTGGATACCATATTCGGT   267
Ccsxl          TTGTATGTCACGAATCTACCACGTACTATAACCGATGATCAATTGGACACCATATTCGGT   718
Bosxl          TTGTATGTTACGAATCTACCACGTACAATAACCGATGATCAATTGGATACCATATTCGGT   948
Dmsxl          CTGTATGTGACCAATCTGCCGCGTACCATAACCGACGATCAGCTGGACACGATCTTCGGC  1097
                 .    :    .    :    .    :    .    :    .    :    .    :  1200
Bzsxl          AAATATGGCTTGATTGTACAGAAGAATATTCTAAGAGACAAATTGACGGGCAAACCGCGT   327
Ccsxl          AAATACGGCATGATTGTACAAAAGAATATACTCAGAGACAAATTGACGGGCAAACCACGT   778
Bosxl          AAATATGGCATGATTGTACAGAAGAATATTCTAAGAGACAAATTGACGGGCAAACCGCGT  1008
Dmsxl          AAGTACGGTTCCATTGTGCAGAAGAACATCTTGCGTGACAAGCTCACAGGTCGTCCTCGT  1157
                 .    :    .    :    .    :    .    :    .    :    .    :  1260
Bzsxl          GGCGTTGCATTTGTCAGATTCAATAAACGCGAAGAAGCGCAAGAGGCAATTTCCGCTCTG   387
Ccsxl          GGTGTTGCCTTTGTAAGATTCAATAAACGCGAAGAGGCGCAAGAAGCAATTTCCGCACTA   838
Bosxl          GGCGTTGCATTTGTCAGATTCAATAAACGCGAAGAAGCGCAAGAGGCAATTTCCGCTCTG  1068
Dmsxl          GGTGTGGCCTTTGTTCGGTACAACAAGCGTGAGGAGGCCCAGGAGGCCATTTCGGCGCTG  1217
                 .    :    .    :    .    :    .    :    .    :    .    :  1320
Bzsxl          AACAATGTCATACCCGAGGGTGGATCTCAGCCGCTCACCGTGCGCTTAGCCGAGGAGCAT   447
Ccsxl          AACAATGTCATACCAGAGGGTGCGTCCCAACCGCTCACCGTACGACTGGCCGAGGAGCAT   898
Bosxl          AACAATGTCATACCCGAGGGTGGATCTCAGCCGCTCACCGTGCGCTTAGCCGAGGAGCAT  1128
Dmsxl          AACAACGTAATACCCGAGGGCGGATCACAGCCGCTGTCCGTCCGGTTGGCTGAGGAGCAT  1277
                 .    :    .    :    .    :    .    :    .    :    .    :  1380
Bzsxl          GGCAAAGCGAAAGCGCAGCAATACATGTCACAGCTGGGATTGA                    490
Ccsxl          GGCAAGGCGAAAGCGCAACACTATATGTCTCAGCTCGGCTTGATTGGTGGAGGTGGTGGT   958
Bosxl          GGCAAAGCGAAAGCGCAGCAATACATGTCACAGCTGGGATTGATTGGTG---------GT  1179
Dmsxl           GGCAAGGCGAAGGCGGCCCACTTTATGTCGCAGATGGGC-------GTG---------GT  1321
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Fig.(4b): Multiple alignment of Sex-lethal gene sequences of B.zonata (Bzsxl) ,C.capitata (Ccsxl), B. oleoe (Bosxl),D. melanogaster (Dmsxl), female.

Hence, SxL gene does not appear to play the key discriminative role in controlling sex determination in *B. zonata* as it plays in *Drosophila*. It is worth mentioning that sex determination in *B. zonata* appears to be regulated in a similar way to *Ceratitis capitata*, but different than in *Drosophila*. In *C. capitata*, the gender does not depend on the chromosome consititution number of X chromosomes and autosomes as in *Drosophila*, but on the presence of a male-determining factor in the Y chromosome; females are XX and males are XY (Willhoeft and Franz, 1996). The male determining factor (M) holds the place of the primary signal in the sex-determination pathways in some species of the *Bactrocera* genus, namely *B. cucurbitae* and *B. drosalis* (Nöthiger and Steinmann-Zwicky, 1985; Dübendorfer *et al.*, 1992; Marin and Baker, 1998; Shearman, 2002).

Ceratitis and *Bactrocera* belong to the Acalyptratae group, as *Drosophila* does, and are the most closely related in phylogenesis to *Drosophila* species. In *Ceratitis,* the SxL gene functional divergence must have occurred quite recently during dipteran evolution within the Acalyptratae group (Saccone *et al.,* 2002). Consistent with their closer phylogenetic relationship, the *B.oleae* SxL protein revealed a high degree of conservation to that of *C. capitata* and *D. melanogaster*, particularly in the RNA Recognition motifs (RRM) that endow the SxL protein with the capacity to bind to RNA. These results agree with those found from the comparison of the SxL proteins of other Brachycera and Nematocera dipterans. In all results, a significant variation was observed in both N and C terminal domains. The great majority of nucleotide changes in RRMs were synonymous, indicating that purifying selection is acting on these domains. Transgenic studies performed by Saccone *et al.* (1998) and Meise *et al.* (1998) have shown that, in spite of the high sequence conservation, neither *Ceratitis* nor *Musca* SxL proteins are capable of replacing the endogenous SxL protein in *Drosophila*. Moreover, overproduction of both SxL proteins in transgenic flies reduced the viability of both sexes. The authors speculated that the ancient function of the SxL protein is to bind RNA, acting primarily as a translational repressor in both sexes to modulate gene activity, while in *Drosophila* it has additionally acquired the function of a master regulator for sex-specific control of genes regulating sex-determination or dosage compensation. It is not possible, however, to discard the alternative, non-mutually exclusive possibility that SxL is a general splicing factor, since both function in splicing and translation are exerted through its two RNA-binding domains.

In conclusion, the results of the present study showed that SxL gene appears to be considerably conservative in structure in *B. zonata* and in all non-drosophilid species studied. Moreover, molecular mechanisms used for regulating the expression of sex-specific and sex-determining genes in insect pests will be useful for further improvement of SIT which could be used for *Bactrocera* control. Isolating and sequencing full length gene is necessary for more understanding of the mode of action and control of SxL gene expression.

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REFERENCES

Ashraf, M.; Qureshi, Z.A.; Arif M. D. and Hussain s, S. (1974). Comparative mating ability of gamma sterilized and normal fruit

flies, *Dacus zonata.* Radiation research 60: 541-544.

Bell, L. R.; Horabin, J. I.; Schedl, P. and Cline T.W (1991). Positive autoregulation of sex-lethal by alternative splicing maintains the female determined state in *Drosophila.* Cell 65: 229-239.

Bopp, D.;Calhoun, G.; Horabin, J. I.; Samuels, M. and Schedl, P. (1996). Sex-specific control of sex-lethal is a conserved mechanism for sex determination in the genus *Drosophila.*

Bushland, R. C.; and Hopkins, D. E. (1951). Experiments with screw-Development 122, 971-982.worm flies sterilized with x-rays. J. Econ. Entomol. 44: 725-31.

Cline, T. W. (1993). The Drosophila sex determination signal how do flies count to two . Trends Genet. 9, 385-390.

Drew , R. A. I. (1989). The tropical fruit flies (Diptera: Tephritidae: Dacinae of the Australasian and Oceanian regions. Memoirs of the Queensland Museum 26, 1-521.

Dubendorfer, A.; Hilfiker-Kleiner, D. and Nöthiger, R. (1992). Sex determination mechanisms in dipteran insects: The case of *Musca domestica*. Semin. Dev. Biol. 3, 349-356.

El–Minshawy, A. M.;El–Eryan, M.A.and Awad, A.I. (1999): Biological and morphological studies on the guava fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) Found recently in Egypt. 8th Nat. Conf. of Pests & Dis. of Veg. & Fruits in Ismailia , Egypt: 71-82.

Hoshijima, K.; Inoue, K.; Higuchi, I.; Sakamoto, H. and Shimura, Y. (1991). Control of doublesex alternative splicing by transformer and transformer-2 in *Drosophila.* Science 252: 833-836.

Huque, H. and Ahmad H. (1966). Effect of gamma radiation on *Dacus zonatus* and *Dacus cucurbitae* Fd Irrad. 6: A28-A32.

Huque H. and Malik Q. R. (1967). Int. J. apple. Radiat. Isotopes 8: 658.

Kattyar K.(1962). Fourth Inter-American symposium on the peaceful Application of Nuclear Energy, pp. 211-217.Pan American Union .

Lagos, D.;Ruiz M. F.; Sanchez ,L. and Komitopoulou, K. (2005). Isolation and characterization of the *Bactrocera oleae* genes orthologous to the sex determining Sex–lethal and doublesex genes of *Drosophila melanogaster*. Gene.348:111-121.

Lifschitz, E.and Cladera, J. (1989). Cytogenetics and sex determination in *Ceratitis capitata*. In fruit flies; their biology, natural enemies and control, Chapter 6, I. (ed. A. S. Robinson and G. Hooper). New York: Elsevier.

Marin, I. and Baker, B. S. (1998). The evolutionary dynamics of sex determination. Science. 281:1990-1994.

McCombs, S. D.; Lee, S. G. and Saul, S. H. (1993). Translocation-based genetic sexing system to enhance the sterile insect technique against the melon fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 86: 651-654.

McCombs, S. D. and Saul, S. H. (1995). Translocation based genetic sexing system for the oriental fruit fly (Diptera-Tephritidae) based on pupal color dimorphism. Ann. Entomol. Soc. Am. 88, 695-698.

Meats, A.; Maheswaran, P.;Frommer, M. and Sved, J. (2002). Towards a male-only release system for SIT with the Queensland fruit fly *B. tryoni*, using a genetic sexing strain with a temperature-sensitive lethal mutation. Genetica 116:97-106.

Meise, M.; Hilfiker-Kleiner, D.;Dubendorfer, A.; Bmner,C.; Nothiger R. and Bopp, D. (1998). Sex-lethal, the master sex-determing gene in *Drosophila*, is not sex-specifically regulated in *Musca domestica*.Development125,1487-1494.

Melis A. and Baccetti B. (1960). Sterilization and competitive ability of gamma sterilized males to normal males of *Dacus oleae* Redia 45: 193-217.

Morales-Ramirez, T.; Vallarino-kelly, G.; Anguiano Orozco, R. and Rodriguer-Reyes(1997). Pharmacokinetic parameters of genotoxic activity inferred from the com-parison of the kinetics of MN-PCE induced by chemical agents and ionizing radia-tion, Mutat. Res. 391: 127-134.

Muller-Holtkamp,F.(1995). The sex-lethal gene homologue in *Chrysomya rufifacies* is highly conserved in sequence and exon-intron organ-ization.J. Mol. Evol.41: 467-477.

Nöthiger, R. and Steinmann-Zwicky, M. (1985). Sex determination in *Drosophila.* Trends Genet. I: 209-215.

Qureshi, Z. A. and Bughio, A. R. (1969). Sterilization and competitive ability of gamma sterilized males to normal males of *Dacus zonata* (Saunders). Int. j. Appl.Radiat. Isotop. 20: 473–475.

Ruiz , M. F.; Goday C.; Gonzalez , P. and Sanchez, L. (2003). Molecular analysis and developmental expression of the Sex–lethal gene of *Sciara ocellaris* (Diptera order, Nematocera suborder). Gene Expr. Patterns 3, 341–346.

Saccone,G.; Peluso, I.; Artiaco, D.; Giordano, E.;Boop D. and Polito, L.C. (1998). The *Ceratitis capitata* homologue of the *Drosophila* sex-determing gene sex-lethal is structu-rally conserved, but not sex-specifically regulated. Development 125:1495-1500.

Saccone ,G.; Pane, A. and Polito, L.C. (2002). Sex determination in flies ,fruitflies and butter-flies.Genetica 116: 15-23.

Schutt , C. and Nothiger, R. (2000). Structure, Function and evolution of sex–determing systems in Dipteren insects. Development 127: 667-677.

Shearman, D. C. A. (2002). The evolution of sex-determination systems in dipteran insects other than *Drosophila.* Genetica 116: 25-43.

Serna, E.; Gorab, E.; Ruiz, M. F.; Goday, C.; Eirin-López, J. M. and Sánchez, L. (2004). The gene sex-lethal of the Sciaridae Family (Order: Diptera, Suborder Nematocera) and its phylogeny in dipteran insects. Genetics 168: 907-921.

Sievert, V.; Kuhn, S. and Trant, W. (1997). Expression of the sex-determing cascade genes Sex-lethal and doublesex in the phorid fly *Megaselia scalaris.* Genome 40: 211-214.

Steiner, L. F.;Mrrchell, W. C. and Baumhover. A. H. (1962). Progress of fruit-fly control by irradiation sterilization in Hawaii and the Marianas Islands. Int. J. appl. Radiat. Isotopes 13: 427-432.

Willhoeftu, U. and Franz G. (1996). Identification of the sex-determing region of the *Ceratitis capitata* Y-chromosome by deletion mapping. Genetics 144:737-745.

Wilkins, A.S. (1995). Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determ-ination pathway. Bioessays 17:71-77.

Zhao, J.T.; Frommer, M.; Sved. J. A. and Zacharo-poulou, A. (1998). Mitotic and Polytene chromosome analysis in the Queensland fruit fly, *Bactrocera tryoni*: (Diptera: Tephr-itidae) Genome 41: 510-526.

الملخص العربي

عزل ودراسة جزيئية للجين Sex-lethal (SxL) لذبابة الخوخ باكتروسرا زوناتا المشابة للجين Sex-lethal لذبابة الفاكهة سيراتيتس كابيتاتا

إمتثال محمد عبدالسميع
كلية العلوم ــ جامعة القاهرة

تم تعريض عذارى ذبابة الخوخ لجرعات مختلفة (١٠٠و١٢٠و١٥٠Gy) من أشعة جاما. تم عزل الحمض الريبونيكلوتيدى (Total RNA) من كل من الحشرة البالغة لذبابة الفاكهة وذبابة الخوخ غير المعرضة والمعرضة لأشعة جاما. تم استخدام ٤ بادئات خاصة نتج عنها حزمتان الحجم الجزئيى لهما ٢٩٦و ٤٩٦زوج قاعدى فى كل من الذكور والاناث لذبابة الفاكهة وذبابة الخوخ مما يدل على درجة التشابه العالية بين هذا الجين فى الحشرتين. تم دراسة تتابع النيكلوتيدات الخاصة بالمقاطع الجينية المفصولة وقد وجد أن نسبة التشابة لهذه المقاطع الجينية89%على مستوى النيكولوتيدات و87% على مستوى الاحماض الامينية بين كل من ذبابة الفاكهة وذبابة الخوخ. وجد ان الجرعات المختلفة لاشعة جاما لها تاثير ملحوظ على مقاطع الجين المعزول من الحشرات البالغة لذبابة الخوخ وخصوصاً الجرعة 150 Gy التى تسببت فى حدوث طفرات نتيجة لإستبدال قاعدتين من Cytosine إلى Guanine و إستبدال قاعدة Adenine إلى Thymine . اثبتت الدراسة ان هذا الجين لا يلعب دورا رئيسيا فى تحديد الجنس مثلما أثبتت الدراسات السابقة فى عدد كبير من الحشرات التى تنتمى الى رتبة ثنائية الاجنحة O: Diptera بخلاف دور هذا الجين فى حشرة الدوروسوفيلا حيث انه يلعب دورا رئيسيا فى تحديد الجنس. ولذلك فاننا نوصى فى الدراسة المستقبلية بعمل دراسات خاصة بالتركيب الجينى المكتمل ودراسة الاماكن الثابتة والمتغيرة فى هذا التركيب حتى يسمح ذلك بالفهم الواضح لعمل هذا الجين وكيفية استخدامه فى مكافحة ذبابة الخوخ.