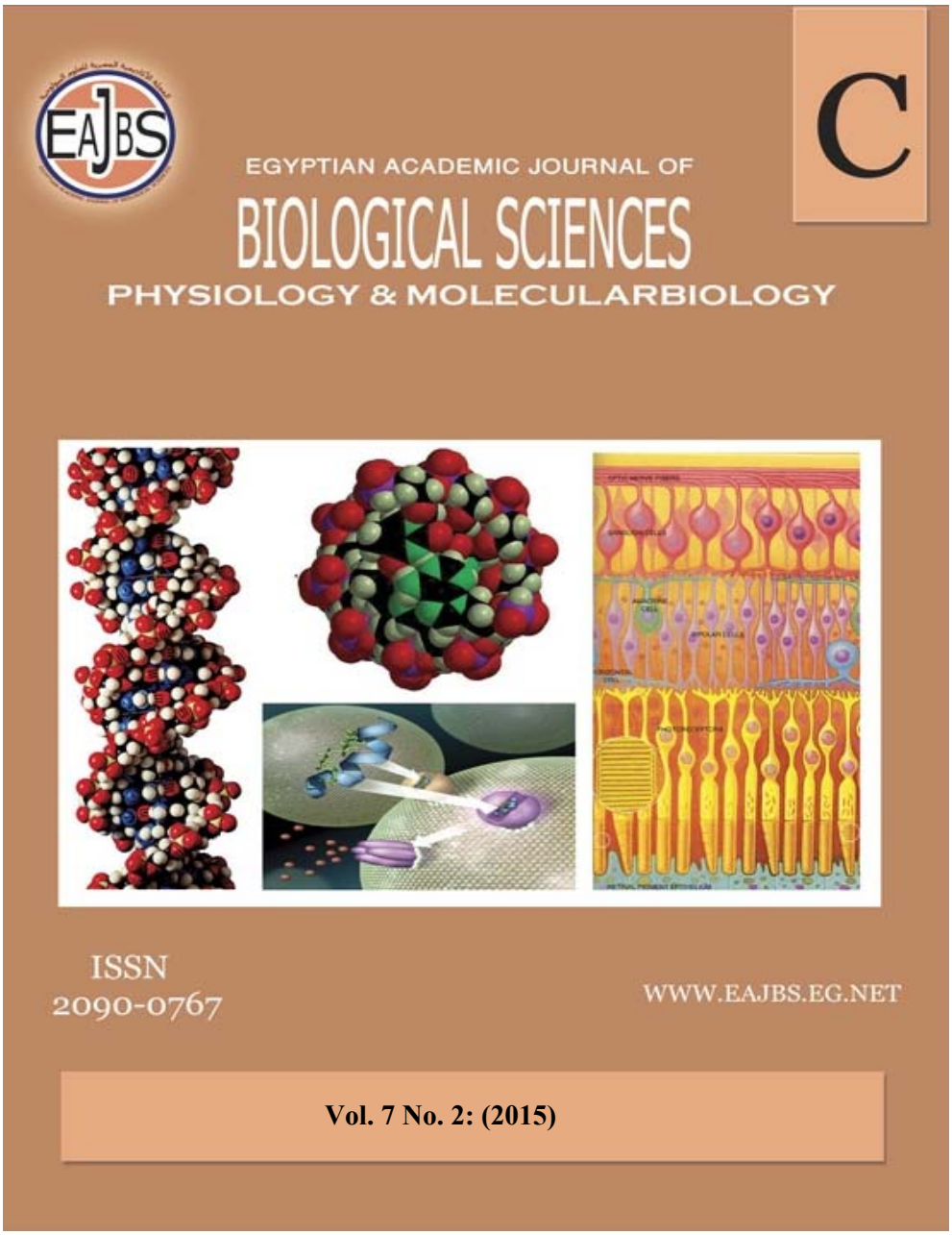

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidateimportant biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 7(2): 127 -134 (2015)

Egypt. Acad. J. Biolog. Sci.
C. Physiology & Molecular Biology
SSN 2090-0767
www.eajbs.eg.net

RAPD-Based Genetic Variances Between Female and Male Adult House Fly *Musca domestica* L.1758 (Diptera, Muscidae) From Qena, Egypt

Mohammed Bassyouni M.EL-Mahdi
Laboratory of Molecular Genetics and Molecular Biology, Zoology Department,
Faculty of Science, South Valley University, Qena, Egypt
For correspondence: E-mail address: melmahdi@svu.edu.eg (M. ELMahdi).

ARTICLE INFO
Article History
Received: 26/11/2015
Accepted: 28/12/2015

***Keywords*:**
Genetic variances
RAPD-PCR
Genotype
Diptera
Musca domestica
Qena

ABSTRACT

The genomic DNA from female and male adults house fly *Musca domestica* L. was subjected to the RAPD analysis using a set of random primers. A ten (10) selected primers produced a distinctive RAPD fingerprint of various bands ranging in size from 350 to 1700 bp. A total of 57 RAPD bands/loci were produced with an average of 5.7 bands per primer, from them 18 bands with a level of monomorphism of 31.58% were common between female and male, and 39 were polymorphic corresponding to a level of polymorphism of 68.42 %. Both female and male genotypes displayed high band frequencies, however male showed to be slightly higher than female ones. Result of this study suggested occurrence of gender-based internal genetic variations and heterozygosity of common house fly adults *Musca domestica* L. that is slightly higher in males than females. This would be necessitated for the species survival, successful environmental adaptations and resistance to chemical applications.

INTRODUCTION

The house fly *Musca domestica* L (Diptera, Muscidae) was first described by Linnaeus (1758). Adults flies have morphological similarities, grey or black thorax, four back-longitudinal dark lines, body covered with hair-like protuberance, and a pair of wings. However, females are slightly larger than males with larger distance between their compound eyes (Hewitt, 1914).

The *Musca domestica* L., are broaden flies exhibiting variety of environments and feeding adaptation on different sources. House flies are been regarded as potential carriers of microorganisms and spreader of infectious disease-causing organisms (Harwood and James, 1979; Graczyk *et al.,* 2005; Sales, *et al.,* 2002; and Al-Aredhi, 2015).

The genetic complement of house fly *Musca domestica* compose of five autosomes (I,II,III,IV and V) and two sex chromosomes (X and Y) (Milani R, *et al*, 1967).

In the natural population of house fly, maleness is due to presence of a dominant factor 'M' that is located on the Y chromosome, also can be found on any autosomes (I-V) and rarely on the X chromosome (Denholm I. *et al,* 1983). Thus, male genotype is XY^{M} and female genotype is XX that considered as the gender inherited status in house flies.

The phenotypic discrepancies in house flies are due to fluctuations in sex realiser genes actions responsible for exposing a specific sex phenotype that would be influenced by gene-environment interactions. Such interactions can trigger occurrence of mutation mechanisms that leads genetic variations (Chapman and Goulson, 2000).

Revealing polymorphism in house flies were side of attention by early and recent studies investigating allozyme polymorphisms (Black and Krafsur, 1985a; Black and Krafsur, 1986a; Krafsur, *et al*, 1992), allozyme and mitochondrial gene diversities (Krafsur *et al*., 2000), geographic differentiation based on microsatellite and mitochondrial markers (Krafsur *et al.,* 2005), esterase enzyme polymorphism (Taşkın *et al*., 2011a).

RAPD is a DNA fingerprinting assay for amplification randomly polymorphic genomic DNA fragments by PCR utilizing arbitrary primers (Williams *et al*., 1990; Welsh *et al*., 1990), these genomic fragments (RAPD markers) can be recorded directly from DNA. RAPD is characterised by its advantages in covering whole genome where the random primers can bind anywhere in the genome that will be of help to assess specific species genetic diversity (Dutta *et al*., 2012). The generated RAPD profiles can be valuable to investigating both animal and plants genotypes (Galal *et al*., 2013; Nenad *et al*., 2012).

To our best of knowledge however, rare or nothing been noticed for generating house fly gender (female/male) RAPD fingerprint profile which could facilitate revealing kind of picture for distinguish between female and male adult house flies. Therefore, this work aimed to investigate genetic variances between female and male adult house flies *Musca domestica* L. using RAPD-PCR assay.

MATERIALS AND METHODS

Samples Collection

Adults house flies, *Musca domestica* L. were randomly collected by the author from garbage near houses using a sweep net and placed into plastic container, then brought to the laboratory. Female and male adults house flies were distinguished from each other under dissecting microscope (OPTIKA Microscopes, Italy). Female and male legless thoracic region was removed, disinfected twice with commercial Detol (1/20) to remove micro-organism environmental contaminations, then washed twice with deionised water, then absolute ethyl alcohol and used for DNA extraction.

Fly genomic DNA extraction

DNA was extracted following Waldschmidt (1997) with modifications. DNA samples were stored at −20 °C till to be used. The DNA concentration and purity were determined with spectrophotometrica UV absorption at A260 and A280 and 0.8% agarose gel electrophoresis.

RAPD-PCR

Total genomic DNA (~50ng) of each sample was subjected to RAPD assay according to William *et al,* (1990) in final volume of 25 µl include 1.0 × final concentration of pre-mixed OnePCRTM 2X (GeneDireX Inc, USA), 10 pM of each 10-mere primer separately (12 deca-nucleotides; A-01, A-02, A-03, A-04, A-05, A-06, A-07, A-08, A-09, A-10, A-11 and A-12 (Bio Basic Inc, Canda) were used for amplifications. PCR reaction was carried out inside a thermocycler (Primus 25 advanced, PEQLAB Biotechnologies GmbH) under cycling settings of an initial denaturation at 95°C for 2 min, 45 cycles (94°C for 1 min, 36°C for 1 min and 72°C for 2 min), and one cycle of final extension at 72°C for 10 min.

PCR products (15 µl) were electrophoresed on 1.5% (w/v) agarose

gels using TAE buffer (0.40 mM Tris, 0.20 mM acetate, 2 mM EDTA pH 8), stained with ethidium bromide (0.5 µg/ml). Gels were photographed under UV light using the Elttrofor M20 SaS Photo-Gel System (Italy) with Nikon Coolpix LB40 digital camera. Size of DNA bands determined comparing with 100 bp DNA ladder (0.1 µg/µl, Solis BioDyne, Estonia).

Data Analysis

The amplified DNA bands were monitored and identified from RAPD images using PyElph gel image analysis software (Pavel and Vasile, 2012). DNA fingerprints were scored as presence/absence (1/0) of fingerprint bands with discrete molecular weight sizes. The number of total scored bands, polymorphic bands, and polymorphism percentage, and band sizes range were recorded for each worked primer.

RESULTS

RAPD and Genetic Polymorphism Analyses:

Twelve (12) deca-nucleotide random primers were screened for generating consistent and distinct banding patterns and to assess gender polymorphism in *Musca domestica* L. Ten (10) primers yielded distinctive RAPD patterns in both female and male house fly (Table 1), those analysed here. One primer (A-06) fail with no product being detected from female and male DNAs, and that has been omitted, while another primer (A-01) have only yielded amplified products from the male DNA that suspended from data analysis.

Table 1: Characteristic of RAPD primers used. Number of Amplified Bands per gender (NABands/Gender), Band Frequency per primer (Band Freq/Primer), Band Frequency per Gender (Band Freq/ Gender), Total number of Amplified Bands (TNABands), Number of polymorphic bands (NPBands), Number of monomorphic bands (NMBands) Polymorphism Percentage (POL%), and Range of amplified fragment in base pair (RAF [bp]).

Primer Code	Sequence	NABands/Gender		TNA Bands	Band Freq/ Primer	NP Bands	NM Bands	% POL	RAF [bp]
	5'---------3'	*M. domestica Female* ♀	*M.domestica Male* ♂						
A-02	TGCCGAGCTG	1	3	4	0.0702	4	null	100.00	500-1100
A-03	AGTCAGCCAC	2	2	2	0.0351	null	2	null	450-750
A-04	AATCGGGCTG	6	4	9	0.1579	8	1	88.89	300-1600
A-05	AGGGGTCTTG	7	6	9	0.1579	5	4	55.56	350-1700
A-07	GAAACGGGTG	3	2	4	0.0702	3	1	75.00	550-1000
A-08	GTGACGTAGG	6	4	7	0.1228	4	3	57.14	300-1600
A-09	GGGTAACGCC	3	6	7	0.1228	5	2	71.43	350-1500
A-10	GTGATCGCAG	3	3	3	0.0526	null	3	null	500-800
A-11	CAATCGCCGT	3	4	5	0.0877	3	2	60.00	450-1500
A-12	TCGGCGATAG	3	4	7	0.1228	7	null	100.00	450-1200
Total		37	38			39	18	68.42	
Band Freq/ Gender		0.6491	0.6667						

Fifty seven (57) bands were produced with an average of 5.7 bands per primer, from them 18 bands with a level of monomorphism of 31.58% were common between female and male. Out of whole amplified fragments, 39 were polymorphic with a level of polymorphism of 68.42 % and an average number of polymorphic fragments/primer of 3.9 (Table 1). The 10 primers generated banding patterns with 2-9 amplified DNA bands ranging in size from 350 to 1700 bp as compared to a 100 bp DNA Ladder (Solis Bio Dyne). Both primers A-04 and A-05 produced a maximum number of 9 fragments, while primer A-03 only amplified 2 bands. Primers A-02, A-12 exhibited percentage of polymorphism %100, primers A-03 and A-10 has no polymorphism (monomorphic bands), while for others primers the percentage

of polymorphism ranged from 55.56 to 88.89.

The highest number of polymorphic bands (8) was obtained with primer A-04 while primers A-07 and A-11 amplified a lowest number of polymorphic bands (3 bands). The band frequency per primer ranged from 0.0339 to 0.1695 as shown in Figure 1. The RAPD fingerprint banding profile generated by all 10 primers is presented in Figure 2.

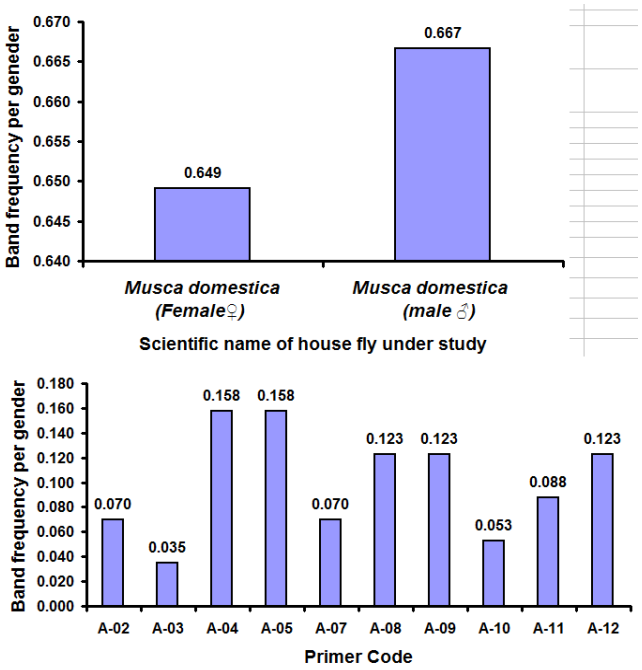

Fig. 1: The band frequencies recorded for female and male adults house fly (above) and per the 10 deca-nucleotide primers (below).

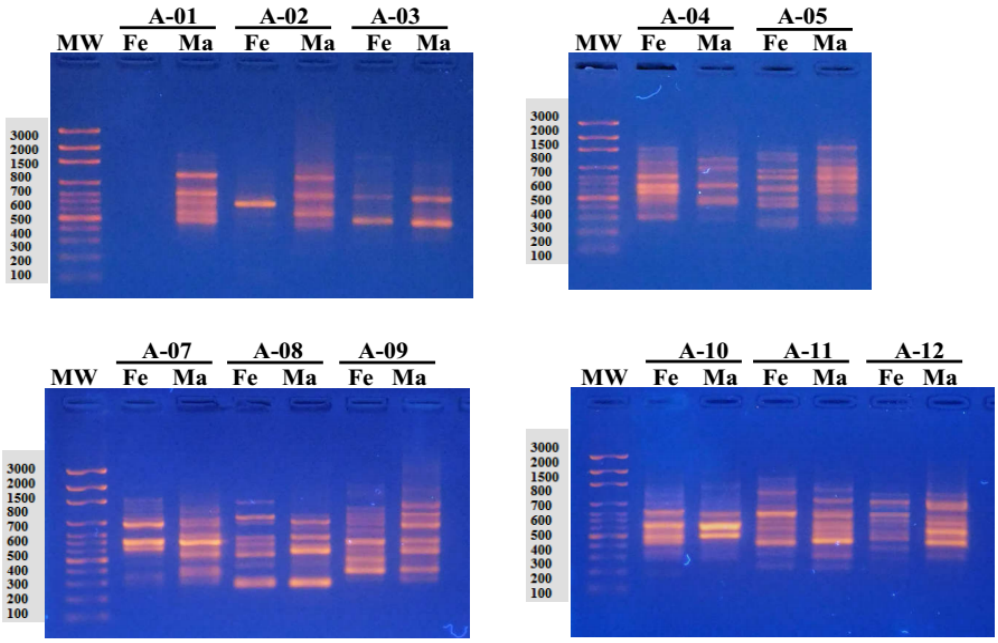

Fig. 2: RAPD-PCR fingerprints profile obtained for female and male adults house fly generated by the 10 deca-nucleotides primers. MW: Molecular weight (100-3000 base pair), Fe: Female, and Ma: Male.

Female/Male genotypes comparison:

The band frequency per gender were 0.6491 and 0.6667 for female and male adults respectively. Both genotypes displayed high band frequencies with the used primers (Table 1), however male genotypes showed to be slightly higher than that of female genotypes (Figure 1b). Primer A-05 detected highest number of 7 and 6 bands in female and male samples respectively, while primer A-03 detected the minimum number of 2 amplicons each. A high molecular weight band of ~ 1700 bp recorded for the male with primer A-05 while primer A-02 have only detected a female gender-specific band of about 650 bp.

DISCUSSION

Herein, the RAPD assay was used to detect genetic differences between female and male of adult house fly *Musca domestica L.* The RAPD techniques showed to be useful for investigating genetic relatedness among house fly populations (Malviya *et al*., 2011), genetic variability of drosophilidae species (Braganholi, *et al*., 2010), analysis of plant male and female genotypes (Ii, *et al*., 2012) as well as for taxonomic relationships to species level (Edwards and Hoy, 1993).

The RAPD fingerprint obtained here demonstrated powerful advantages for detecting sex related polymorphism in adult house fly *Musca domestica* L. The randomly primers used produced 39 polymorphic fragments revealing instructive gender-related RAPD banding patterns which also accounted for the successful experimental PCR conditions used here. Studies reported that obtaining a false bands and the assay non-reproducibility could be due to effectiveness of PCR reaction conditions (Quintaes *et al*., 2004).

Genetic variation in the genotypes of adult flies was evaluated by RAPD-PCR. Data showed that the selected primers displayed moderate to high band frequencies suggesting occurrence of gender-based internal genetic variations. Indeed, the targeting random primers used here lead to revelling the dominancy figure of distinctive patterns of amplified fragments. Such patterns would be as presence or absence of certain DNA sequences within the genetic complement of both female and male adult flies because the RAPD bands are dominantly inherited in a Mendelian fashion *(*Rothuizen and Wolferen 1994).

The genotypes of adult flies (female/male) showed higher polymorphism with the 10 tested primers that is agreed with the fact that RAPD loci have higher mutation rates comparing to other markers (Santos, *et al*., 2011). In same time, degree of polymorphism obtained here accounted for number of polymorphic DNA bands detected that actually would be subjected to internal sex-based genetic variation. Studies reported that ability of a marker to resolve genetic variation could be directly related to the degree of polymorphism recorded (Souframanien and Gopalakrishna (2004).

Data showed that the adult flies (female/male) genotypes exhibited high band frequencies reflecting a higher heterogeneity of the fly that could be due to their large population within variety of environmental conditions (Sharma *et al*., 2009). However male genotypes slightly higher (value of 0.6667**)** than female genotypes (value of 0.6491). This could be explained by the alteration flexibilities of male genotypes compared to the female ones which may suggest slightly higher heterozygosity in male housefly that could be necessitated for survive and successful environmental adaptations for sake of species growth rate, resistance to sudden exposed circumstances (Bangham *et al.,* 2008; Qingmin *et al*., 2012), particularly if the heterogeneity counted for a certain

genetic allelic variation that effect the female and male disease transmission rate and insecticide resistance.

In conclusion, result of the RAPD fingerprint obtained here demonstrated powerful advantages for detecting sex related polymorphism in *Musca domestica* L revealing instructive RAPD banding patterns. Both male and female genotypes showed high polymorphic variations, however male genotypes showed to be slightly higher than female ones. Data suggested occurrence gender-based internal genetic variations that may responsible for heterozygosity of common house flies adults *Musca domestica* L. Such polymorphic variations (slightly higher in males) would be necessitated for house flies growth rate and environmental adaptations, resistance to sudden circumstances and chemical applications. This may drive world's attention for developing new strategies against house flies transmitting disease-causing organisms. It would be wise that further work such as identification of RAPD gender-specific and gene-specific markers will be valuable for understanding the genetic complement of that significantly medical important fly.

ACKNOWLEDGMENTS

Author is thankful to the South Valley University, Qena, EGYPT for funding this research.

REFERENCES

Al-Aredhi, H.S. (2015): Role of house flies (*Musca domestica*) as vector host for parasitic pathogens in Al-Diwaniya province/Iraq. Internation Journal of Science and Research (IJSR) ISSN (Online): 2319-7064.

Bangham, J., Kim, K-W., Webster, C.L. and Jiggins, F.M. (2008): Genetic variation affecting host-parasite interactions: different genes affect different aspects of sigma virus replication and transmission in *Drosophila melanogaster*. Genetics, 178, 2191–2199.

Black, W.C. and Krafsur, E.S. (1985): Electrophoretic analysis of genetic variability in the house fly (*Musca domestica* L.). Biochem Genet 23:193–203.

Black, W.C. and Krafsur, E.S. (1986a): Temporal and spatial trends in allozyme frequencies in house fly populations, *Musca domestica* L. Theor Appl Genet 71:673–81.

Braganholi, D., Bélo, M., Bertoni, B.W., Fachin, A.L., Beleboni, R.O. and Zingaretti S.M. (2010): Genetic variability among natural populations of *Zaprionus indianus* (Drosophilidae) in the States of São Paulo and Minas Gerais, Brazil. Genet. Mol. Res. 9 (3): 1504-1512 (2010).

Chapman, J. W. and Goulson, D. (2000): Environmental versus genetic influences on fluctuating asymmetry in the house fly, *Musca domestica*. Biol. J. Linn., 70, 403-413.

Denholm, I., Franco, M.G., Rubini, P.G. and Vecchi, M. (1983): Identification of a male determinant on the X chromosome of housefly (*Musca domestica* L.) populations in South-East England. Genet Res Camb., 42: 311-322.

Dutta, S.R., Kar, P.K., Srivastava, A.K., Sinha, M.K., Shankar, J. and Ghosh A.K. (2012): Identification of RAPD and SCAR markers associated with yield traits in the Indian tropical tasar silkworm *Antheraea mylitta* drury. Genetics and Molecular Biology., 35(4):743-751. doi:10.1590/S1415-47572012005000059.

Edwards, O. and Hoy, M. (1993): Polymorphisms in two parasitoids detected using random amplified polymorphic DNA (RAPD) PCR. Contr Theor Appl Pest Manage 3:243–257.

Galal, O.A., Rehan, M. and Abd El-Karim, R.E. (2013): Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers. African Journal of Biotechnology. Vol. 12(20), pp. 2830-2839, 15 May, 2013 DOI: 10.5897/AJB2013.12332.

Graczyk, T. K., Knight, R. and Tarnang, L. (2005): Mechanical transmission of human protozoan parasites by insects. Clin. Microbiol., Rev., 18(1): 128-132.

Harwood, R.F. and James, M. T. (1979): Muscoid flies and house flies. In: Entomology in human and animal health. Macmillan Publishing Cop., N.Y. PP: 248 – 265.

Hewitt, C.G. (1914): The House-Fly, its structure, habits, development, relation to disease control. University Press, Cambridge England.

Ii, Y., Uragami, A., Uno, Y., Kanechi, M. and Inagaki, N. (2012): RAPD-based analysis of differences between male and female genotypes of *Asparagus officinalis* Vol. Hort. Sci. (Prague) 39, 2012, No. 1: 33-37).

Krafsur, E.S., Bryant, N.L., Marquez, J,G. and Griffiths N.T. (2000): Genetic distances among North American, British, and West African house fly populations (*Musca domestica* L.). Biochem Genet 38:275–84.

Krafsur, E.S., Cummings, M.A., Endsley, J.G., Marquez, J.G. and Nason, J.D. (2005): Geographic differentiation in the house fly estimated by microsatellite and mitochondrial variation. J Hered 96:502–12.

Krafsur, E. S., Helm, J. M. and Black, W.C. (1992): Genetic diversity at electrophoretic loci in the house fly, *Musca domestica* L. Biochem Genet 30:317–28.

Linnaeus, C. D.E. (1758): Systema naturse (10th ed.), vol I, p. 596; and 'Eauna suecica,' 2ed., Holmiae, 1761.

Malviya, S., Bajpai, N. and Tewari, R.R. (2011): Genetic relatedness among three populations of housefly *Musca domestica* L. using RAPD-PCR marker. International Journal of Pharma and Bio Sciences 2(4):198-204 · October 2011.

Milani, R., Rubini P.G. and Franco M.G. (1967): Sex-determination in the housefly. Genet Agaria., 21: 385-411.

Pavlović, N.V., Zdravković, J., Cvikić, D., Zdravković, M., Adzić, S., Pavlović, S. and Šurlan-momirović, G. (2012): Characterization of onion genotypes by use of RAPD markers. Genetika, 44(2):269-278.

Quintaes, B.R., Leal, N.C., Reis, E.M. and Hofer, E. (2004): Optimization of randomly amplified polymorphic DNA polymerase chain reaction for molecular typing of *Salmonella enterica* serovar Typhi. Rev. Soc. bras. Med. trop., 37: 143-147.

Rothuizen, J. and Van Wolferen, R. (1994): Randomly amplified DNA polymorphisms in dogsare reproducible and display Mendelian transmission. Anim. Genet., 25 : 13-18.

Sales, M., Costa, G.L. and Bittencourt, V.R. (2002): Isolation of Fungi in *Musca domestica* Linnaeus, (Diptera Muscidae) Captured at two natural breeding grounds in the municipality of seropedica, Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz, Rio de Janeiro; 97:1107-1110.

Santos, J.M.M., Fraga, E.C., Maia J.F. and Tadei W.P. (2011): Genetic Diversity in Dengue mosquito, *Aedes aegypti* (Diptera: Culicidae) from Amazon Region: Comparative Analysis with Isozymes and RAPD Loci, Open Trop. Med. J., 4, 11-20.

Sharma, A.K., Mendki, M.J., Tikar, S.N., Chandel, K., Sukumaran, D.,

Parashar, B.D., Veer, V., Agarwal, O.P. and Prakash, S. (2009): Genetic variability in the geographical populations of *Culex quinquifasciatus* Say (Diptera: Culicidae) from India based on random amplified polymorphic DNA analysis, Acta tropica., 112, 71-76.

Souframanien, J. and Gopalakrishna T.A. (2004): Comparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. Theor. Appl. Genet., 109, pp. 1687–1693.

Taşkın, V., Taşkın, B.G., Küçükakyüz, K. and Kence,M. (2011a): Determination of esterase enzyme polymorphism in house fly (*Musca Domestica* L.) populations in Turkey. Turk J Zool 35:869–77.

Waldschmidt, A.M., Salomão, T.M.F., Barros, E.G. and Campos L.A.O. (1997): Extraction of genomic DNA from *Melipona quadrifasciata* (Hymenoptera, Apidae, Meliponinae) Braz J Genet., 20: 421–423.

Wang, Q., Li, M., Pan, J., Di, M., Liu, Q., Meng, F., Scott, J.G. and Qiu, X. (2012): Diversity and frequencies of genetic mutations involved in insecticide resistance in field populations of the house fly (*Musca domestica* L.) from China. Pesticide Biochemistry and Physiology 102 (2012) 153–159.

Welsh, J. and McClelland, M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research, 18(24): 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18(22): 6531-6535.

RABIC SUMMERY

التباين الوراثى باستخدام تقانة التضاعف العشوائي (RAPD) بين انثى و ذكر الذبابة المنزلية من قنا، مصر

محمد بسيونى محمد المهدى
معمل الوراثة الجزيئية و بيولوجيا الجزيئيات - قسم علم الحيوان- كلية العلوم
جامعة جنوب الوادى- قنا- جمهورية مصر العربية
Correspondence Author: E-mail address: melmahdi@svu.edu.eg

تم اخضاع المادة الوراثية (DNA) لكل من اثى و ذكر الذبابة المنزلية للتنميط الوراثى بواسطة تقانة التضاعف العشوائي (**RAPD**) باستخدام مجموعة منتقاة من البادئات العشوائية. اثبتت البادئات العاملة فاعليتها و اظهار تعددية شكلية بين كل من اثى و ذكر الذبابة المنزلية حيث أن كل البادئات اظهرت حزم مؤشرة فريدة مختلفة تتراوح من 350- ١٧٠٠ زوج قاعدة.

انتج عشرة بادئات عاملة عدد من الحزم بلغ سبع و خمسون (٥٧) منها ١٨ حزمه أُحادِيُّ الشَّكْل (Monomorphic) بمعدل تباين احادى بنسبة 31.58 اما البقية و هى تسع و ثلاثون (٣٩) كانت متعددة الشكل (polymorphic) مع معدل تباينى متعدد بنسبة 68.42 و قد بلغ المتوسط الحزمى ٥.٧ حزمة للبادئة الواحدة. أظهر كل من النمطين الجينيين الأنثوي والذكري معدلات مرتفعة للتردد الحزمي و لكن النمط الجينى الذكرى اظهر ارتفاعا نسبيا مقارنا بالنمط الجينى الانثوى.

لقد اقترحت تلك الدراسة وجود اختلافات جينية داخلية والتى تعتمد على نوع الجنس و ايضا ارتفاع عدم التجانس الوراثى فى حشرة الذبابة المنزلية مع ارتفاع نسبيّ اعلى فى الذكور و الذى يعزى الى كونه ضروريا لبقاء نوع الذبابة المنزلية و تكيفها مع الظروف البيئية المختلفة.