Molecular and Biochemical Genetic Studies of Some Mutants in *Drosophila melanogaster*

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ABSTRACT

Ten mutant lines which have been included single, double and triple mutants (four stocks were built up) in addition to the wild type of Drosophila melanogaster were used to study the genetic fingerprint in nuclear DNA on the molecular and biochemical levels. The results of molecular analysis in nuclear DNA by using random amplified polymorphic DNA revealed polymorphic ratio ranged from 73.33% to 100% with eight unique bands and total fragments of 63 with an average number of 12.6 fragments /primer. The genetic similarity of nuclear DNA ranged from 44.8%(dp ,y) to 82.5%(dp,OR) for (RAPD-PCR) and the cluster analysis based on similarity matrices separated the single mutant lines from the double and triple mutant lines except white dumpy ebony. Also, the single mutant lines were separated to subgroup according to the chromosome which carries the mutant. On other hand, the biochemical analysis revealed that the different mutational load didn't affect protein concentration in different mutant lines, while some mutant lines show significant excess of amino acid polymorphism.These results led to the possibility of using molecular and biochemical DNA as a reliable and quick method for studying the genetic fingerprint in these mutant lines and other organisms.

***Key words**: Drosophila melanogaster, RAPD, fingerprinting, molecular markers, biochemical markers.*

INTRODUCTION

The genus Drosophila is famous for its contribution to genetic research. The evolutionary biology of this genus has, on the same way, been extensively studied; with morphological and molecular studies (Desalle 1992 and Kwiatowski and Ayala 1999) having decisively contributed to the establishment of the phylogenetic relationships between various species of this genus. Among Drosophila species, *Drosophila melanogaster* has been the most extensively studied organism in biology and serves as a model system for the investigation of many developmental and cellular processes common to higher eukaryotes, including humans. (Adams *et al.* 2000 and Kliman *et al.*, 2000).The annotated genome sequences of D. melanogaster, together with its associated biology, provide the foundation for a new era of sophisticated functional studies. The *Drosophila melanogaster* genome is ~ 180 Mb in size, and encodes ~ 13.600 genes with comparable functional diversity (Adams,*et al*. 2000). Characteristics at the DNA level can be accomplished by the use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). This technique represents a new method for identifying a large number of DNA polymorphisms in genome, quickly and

efficiently. In the RAPD method, a short (usually 10bp.) oligonucleotide serves as a primer by binding to complementary sites and initiating amplification of specific regions of the genome. RAPD markers have been used to resolve genetic variability between genotypes and mt.DNA. The use of different primers in the RAPD method permits detection of different levels of genotypes (Williams *et al*., 1990; Haymer and Mcinnis, 1994 and Trudy, 2010).Storage proteins from a number of species have been purified and characterized. Insect species such as those of Calliphora show a maximum content of haemolymph proteins, about 20% (Agosin, 1978), and the total content of proteins in D. melanogaster is about 23% have no match with other organisms. In general, quantitative changes of the protein patterns are less dramatic compared to the increase in total concentration (Munn and Greville, 1969; Kinnear and Thomson, 1975; Thomson *et al*., 1976; Akam *et al*., 1978; Tojo *et al*., 1978; Kramer *et al*., 1980; Baurdo Paulou *et al*., 1981; Miller and Silhacek, 1982; and Bianchi and Marinotti, 1984).

The presence of a high concentration of a wide variety of amino acids in the whole fly is a characteristic feature of insects (Anderson *et al*., 1977). The amino acids in insects provide not only the substrates for protein synthesis, but also enter into diverse metabolic pathways and participate in various physiological activities. More than 80% of Drosophila proteins with multiple runs seem to function in transcription regulation. The most frequent amino acid runs in Drosophila sequences occur for glutamine, alanine, and serine. The abundant of amino acid runs in the fly might be interpreted in terms of initiating differences in DNA-replication process, repair mechanisms, DNA-modification on systems, and mutational bases. The excess amino acid polymorphism holds across the entire mt.genome. (Rand, 2001; Karlin *et al*., 2002;Ward and Thompson,2012 and Somero *et al*., 2017).The aims of this paper are to determine the genetic variation among eleven stocks of D. melanogaster representing single, double and triple mutations using RAPD markers and to determine the genetic variation in total protein and amino acid as biochemical markers.

MATERIALS AND METHODS

Drosophila melanogaster stocks

Eleven stocks of Drosophila melanogaster have been used in this study. Six of them in addition to the wild type (OR) were supplied from the Department of Genetics, Ein Shams University. The remaining four stocks were built up by different crosses. These new stocks were used to compare single, double, and triple mutant stocks. These stocks are shown in Table (1).

Molecular Analysis of Drosophila
Extraction of Genomic DNA

Genomic DNA was extracted from eleven different genotypes of D. melanogaster according to Cabral *et al*., (2000). Half gm live adults from male and female were squashed with 320 µl of extraction buffer. The eppendorf tubes were incubated at 50°c for 20 min. following by an equal volume of a standard phenol / chloroform / iso amyl alcohol extraction, vortex and spun for 2 min. the aqueous phase was transferred to a fresh 1.5 ml tube. Then centrifuged in a microfuge at maximum speed for 10 min. the aqueous phase was transferred to a fresh tube and 120 µl of 3 M sodium acetate and absolute ethanol were added. For precipitation microfugation was made at maximum speed for 15 min., then washing the pellet with 70% ethanol and dried in a speed vacuum drier. The dried pellet was dissolved in 30 µl TE buffer depending on the size of the pellet.

Table (1): The eleven different genotype stocks of D. melanogaster.

Stocks	Genotypes	Chr. No. which carries the mutant gene
Wild type (OR)	++	
Yellow body	♀ yy, ♂ y	1(x chr.)
White eye	♀ ww, ♂ w	1(x chr.)
Vestigial wing	vgvg ♀♂	2
Ebony body	ee ♀♂	3
Vestigial ebony	vgvg ee ♀♂	2,3
Dumpy ebony	dpdp ee ♀♂	2,3
Dumpy wing	dpdp ♀♂	2 built up
White dumpy	♀ ww dpdp, ♂ w dpdp	1,2 built up
White ebony	♀ w wee, ♂ w ee	1,3 built up
White dumpy ebony	♀ ww dpdp ee, ♂ w dpdp ee	1,2,3 built up

Table (2): Sequence of a five decamer arbitrary primers used to study the genetic variability in genomic DNA of the eleven stocks of D. melanogaster.

Primer	5' Sequence 3'
OPA09	5'-GGGTAACGCC-3'
OPA10	5'-GTGATCGCAG-3'
OPB02	5'-TGATCCCTGG-3'
OPB15	5'-GGAGGGTGTT-3'
OPC07	5'-GTCCCGACGA-3'

Random Amplified Polymorphic DNA (RAPD) in Genomes

Primers used in RAPD analysis

RAPD-PCR was carried out according to the procedure given by Williams *et al*., (1990). Five random primers (10-mer) (Operon kit A, B, and C, Operon technologies, USA) were screened as shown in Table 2.

Preparation of PCR reaction

The reaction was carried out in a volume of 50µl containing 1µl of ng/µl template DNA, 4µl of 10µM decamer oligonucleotide primer from (Operon technologies, Inc., Alamada, CA, USA), 0.5Ml of 5 u / µl of Taq. DNA polymerase (Finepoll), 5Ml of 10x buffer (500 mM Kcl, 100 mM Tris-Hcl pH 9.0 and 1% Triton x-100), 2µl of 50 Mm Mgcl2, 5µl of 2 mM dNTPs (dATP, dCTP, dTTP, dGTP) and deionized d.d.H2O, followed by the addition of one drop of mineral oil. PCR amplification was performed in a thermal cycler (Perkin-Elmer/ Cetuy, Norwalk, CT, USA) programmed for one cycle of 3 min. at 94◌̊c40 cycles of 1 min. at 94◌̊c, 1 min. at 36◌̊c, and 2 min. at 72◌̊c, and a final cycle of 5 min. at 72◌̊c. The amplification products were resolved by electrophoresis in a 1.5% agarose gel (Sigma) containing 0.5 µg /ml ethidium bromide in 1x TAE buffer. RAPD markers were scored from the gels as DNA fragments present in one lane and absent in another.

Biochemical Analysis

Determination of total proteins

The protein content of different genotypes of *Drosophila melanogaster* was determined according to the method of Lowery and Randall (1977).

Procedure

A known volume of (whole flies) *D. melanogaster* extract was made up to 1.0 ml with distilled water then mixed with 5.0 ml of solution C allowed to stand for 10 min. at room temperature. An aliquot of solution D 0.5 ml was added throughly, mixed and allowed to stand for one hour. The absorbance of the solution was recorded using LKB spectrophotometer (Model 4050) at 750 nm against blank prepared in similar manner to that described above. Graphic plot of the absorbance values against various standard solution of different concentration of (BSA) was linear and used for the determination of the protein content different genotypes in *D. melanogaster*.

Determination of total amino acids

The amino acids composition of experimental samples was determined using HPLC-Pico-Tag method according to Millipore Cooperative (Cohen *et al.*, 1989). The method was performed as follows:

Acid hydrolysis of sample

A sample corresponding to mg protein was weighed into 25x150 mm hydrolyzed tube, aliquot (7.50 ml) of N Hcl was added, purged with nitrogen for 60 seconds and tube was capped immediately. The tubes was placed in 110°C oven for 24 hours, removed and allow to cool. The contents of tube were quantitatively transferred to 25 ml volumetric flask and completed to volume with HPLC grade water. About 1 ml of the solution was filtered through 0.45µm Millipore membrane filter.

Amino acid derivatization

Ten microliters of the filtered sample in 6x50 mm tube was placed into drying vial and dried in a freeze-dryer workstation for 10-15 min. Aliquot (30µl) of redray solution(consists of a mixture of 200µl methanol, 200µl, 0.2 N sodium acetate and 100µl triethylamine) was added to the sample tubes and redried again in the workstation.Aliquot (30µl) of the freshly prepared of the derivatization reagent (performed by mixing 350µl methanol, 50µl (PITC) phenyl isothio-cyanate) was added to the tube contents and allowed to react for 20 min, dried in the workstation for 15 min.

Thirty microliters methanol was added and redried again, 250µl of sample diluents (Waters,USA) were added to the dried tube, vortexed and transferred to injection vials. The standard amino acid (Sigma, USA) solution was treated the same as the sample.The amino acids were quantified by comparison of peak area with those corresponding amino acids standard solutions using the Spectra Physical Data System Program.

Statistical analysis

The test of (χ^2) and (2×2) analysis has been used aiming to clarify the significant and non-significant patterns of RAPD analysis and total protein in different stocks of *D. melanogaster* used in the present study according to Nei and Tajima (1981).

RESULTS AND DISCUSSION

To shed some light on the different genotypes of *Drosophila melanogaster* which have been used in this study, the present data may reveal some aspects for comparing single, double, and triple mutant stocks. The criteria of these comparisons include differences in the phenotypic, the variability in genomic DNA, also in the total protein content and total amino acid in all of these genotype.

Random Amplified Polymorphic DNA (RAPD-PCR) in eleven stocks of *D. melanogaster* genomes

The *Drosophila melanogaster* genome is ~180 Mb in size, and encodes ~ 13.600 genes with comparable functional diversity (Adams,

2000). The genome size is illustrated in Fig.(1). The use of RAPD-PCR provides fast and reliable discrimination of DNA variations according to Williams *et al*. (1990) who presents a new method for identifying a large number of DNA polymorphisms in the genome. In the RAPD method, a short (usually 10bp) oligonucleotide serves as a primer by binding to complementary sites and initiating amplification (Haymer *et al*., 1994). The RAPD method has already been employed to detect polymorphic DNAs that can be used as genetic markers (Black *et al*., 1992; Koller *et al*., 1993, and Kazan *et al*., 1993) and to determine linkage relationships (Dweikat *et al*., 1993) in various insect species. Therefore, when the purified genomic DNA of eleven different genotype stocks of *D. melanogaster* were used as templates for RAPD-PCR, characteristic reproducible multiple-band profiles of amplified fragments were detected. Out of the 10 tested primers, only five arbitrary primers were matched with segments of the tested genomic DNA. These primers bind to homologus sequences along the genome, and PCR amplification occurs. Bands were visualized using ethidium bromide. Within a population sample, mutations influence the base sequences of primer binding sites, allowing polymorphisms to be detected (Lóxdale and Lushai 1998) have resulted in the synthesis of discrete fragments.

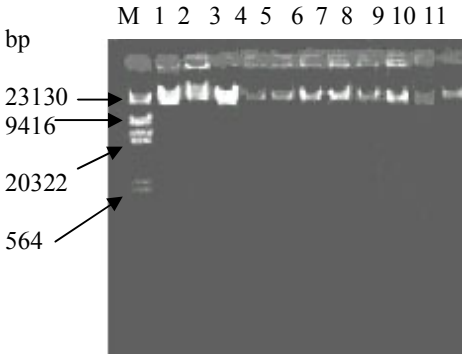

Fig.(1): Agarose gel Electrophoresis for genomic. DNA in eleven stocks of D.melanogaster. M, λ DNA cut by Hind III; lane 1,(OR); lane 2 (y); lane 3; (w); lane 4(vg); Lane 5, (dp); lane 6,(e); Lane 7, (wdp); 8,(we); Lane 9,(vge); Lane 10, (dpe), and Lane11, (wdpe).

According to Fig. (2) and Table (3) the random primer (OPA09) which amplified genomic DNA has 11 polymorphic patterns of the total 13 bands with molecular size ranging between 260bp to 2550bp, that exhibited 84.62%. It has also two monomorphic fragments which obtained at 950bp and 260bp. The results of primer (OPA10) are shown in Fig. (3) and Table (4). This primer produced 15 bands with molecular size ranging between 250bp to 3380bp. There were 11 polymorphic bands and two unique bands, one positive in stock number 7 with molecular size of 3380bp and one negative in stock number 2 with molecular size of 700bp that exhibited 73.33% polymorphism. The primer also produced 4 monomorphic bands at 2890bp, 1225bp, 930bp, and 250bp.Fig. (4) and Table (5) show that the random primer (OPB02) amplifies 9 polymorphic patterns of the total 10 bands, which exhibited 90% polymorphism. On the other hand, a band with molecular size of 1350bp was scored in stock number 10 and could be used as a positive marker. Moreover,

it has also one monomorphic band at molecular size of 750bp.

The RAPD-PCR products of the eleven genotypes of *D. melanogaster* species with primer (OPB15) are illustrated in Fig. (5) and Table (6). The total number of bands is 13 with molecular size ranging from 380bp to 2440bp. The results indicated that all the reminding bands were polymorphic, which exhibited 100%. Moreover, there were 3 negative unique bands two were present in stock number 9 with molecular sizes of 1250bp and 560bp, and one was present in stock number 6 with molecular size of 380bp and one positive unique band was present in stock number 6 at molecular size of 340bp. Fig. (6) and Table (7) show that the ratio of polymorphism in genomic DNA is 100% by using the random primer (OPC07). This primer amplifies 12 polymorphic fragments of the total 12 bands. These fragments have molecular size ranging between 100bp to 1900bp and a positive unique band was appeared in stock number 10 with molecular size of 1900bp.

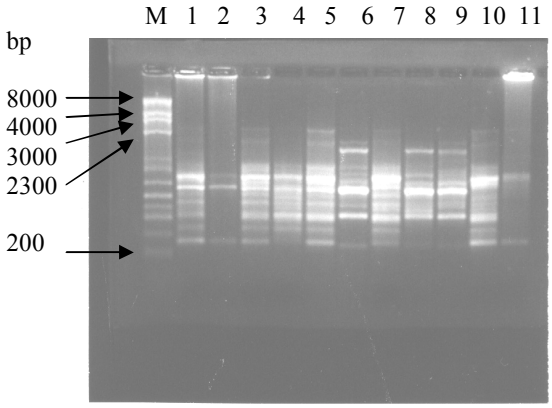

Fig.(2): RAPDs amplification of genomic DNA of D. melanogaster with primer (OPAO9). M, molecular marker-AGE-I; Lane 1, Wild type (OR); Lane 2, yellow body (y); Lane 3,white eye (w); Lane 4,vestigial wing (vg); Lane 5, dumpy wing (dp); Lane 6, ebony body (e); Lane 7, white dumpy (wdp); Lane 8,white ebony (we); Lane 9, vestigial ebony (vge); Lane 10,dumpy ebony (dpe); and lane 11, white dumpy ebony (wdpe).

Table (3): Scoring of RAPD-PCR markers for eleven genotypes of D. melanogaster using primer OPA09.

	Different genotypes of *D. melanogaster*											Molecular weights (bp)
Scoring of multiple-band profiles	1	2	3	4	5	6	7	8	9	10	11	
	+	-	+	-	+	-	+	-	-	+	-	2550
	+	-	+	-	+	+	+	-	+	+	-	1800
	-	-	-	-	-	+	-	+	+	-	-	1650
	-	-	+	+	+	+	+	-	-	+	-	1150
	+	+	+	+	+	+	+	+	+	+	+	950
	-	-	+	-	-	+	-	+	+	+	-	850
	+	+	+	+	+	+	+	+	+	-	-	750
	-	-	+	-	-	-	+	-	-	+	-	650
	+	-	+	+	+	+	+	-	+	+	-	480
	-	-	-	+	-	+	+	+	-	+	-	400
	+	-	+	+	+	+	+	+	+	+	-	380
	+	-	+	+	+	-	+	-	-	+	-	340
	+	+	+	+	+	+	+	+	+	+	+	260

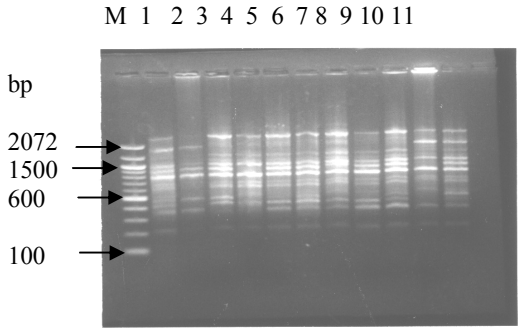

Fig.(3): RAPDs amplification of genomic DNA of D. melanogaster with Primer (OPA10). M, molecular marker-100bp DNA ladder. 1, Wild type (OR); 2, yellow body (y); 3, white eye (w); 4, vestigial wing (vg); 5, dumpy wing (dp); 6, ebony body (e); 7, white dumpy (wdp); 8, white ebony (we); 9, vestigial ebony (vge); 10, dumpy ebony (dpe); and 11, white dumpy ebony (wdpe).

Table (4): Summary of RAPD markers used to identify eleven genotypes of D. melanogaster using primer OPA10.

Scoring of multiple-band profiles	Different genotypes of *D. melanogaster*											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	-	-	-	+	-	-	-	-	3380
	+	+	+	+	+	+	+	+	+	+	+	2890
	+	+	+	+	+	-	+	-	+	+	+	2240
	+	-	+	+	+	+	+	+	+	-	+	1840
	-	-	+	-	+	-	+	+	+	+	-	1700
	+	+	+	+	+	+	+	+	+	+	+	1225
	+	+	+	-	+	+	+	+	+	-	+	1000
	+	+	+	+	+	+	+	+	+	+	+	930
	+	-	+	+	+	+	+	+	+	+	+	700
	+	-	+	+	+	+	+	+	+	-	+	640
	-	-	-	+	-	-	+	+	-	+	-	600
	+	+	+	-	-	+	-	-	+	-	+	500
	+	-	+	+	+	+	+	+	+	-	+	440
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	+	+	+	+	+	+	+	+	+	+	+	250

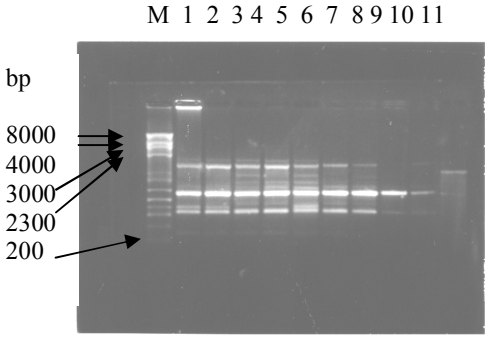

Fig.(4): RAPD amplification of genomic DNA of D.melanogaster with primer (OPBO2). M, molecular markerAGE-I.

Table (5): Diagnostic bands of eleven genotypes in D. melanogaster generated by RAPD-PCR in case of using primer OPB02.

Scoring of multiple-band profiles	Different genotypes of *D. melanogaster*											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	+	+	+	+	+	-	-	-	-	-	-	1925
	+	+	+	+	+	+	+	-	+	-	-	1650
	-	-	-	-	-	-	-	-	-	+	-	1350
	-	-	+	-	+	-	+	-	-	-	-	1200
	-	-	+	+	+	-	-	-	-	-	-	1050
	+	-	+	+	+	+	+	-	-	-	-	950
	+	+	+	+	+	+	+	+	+	+	+	750
	-	-	+	+	-	+	+	-	-	-	-	500
	+	+	-	-	+	-	-	-	-	-	-	450
	+	+	+	+	+	+	+	+	+	-	-	400

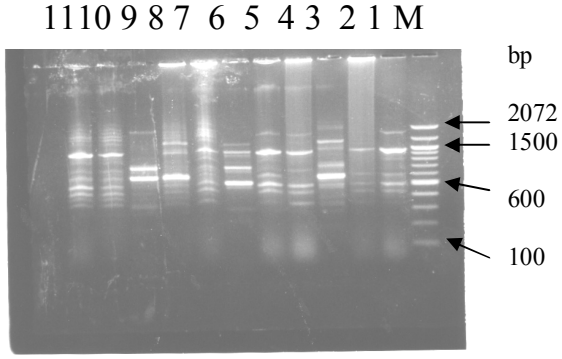

Fig. (5): RAPDs amplification of genomic DNA of D. melanogaster with primer (OPB15). M, molecular marker-100bp DNA ladd

Table (6): Scoring of RAPD-PCR markers for eleven genotypes of genomic DNA D. melanogaster using primer OPB15.

Scoring of multiple-band profiles	Different genotypes of *D. melanogaster*											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	-	-	-	+	-	-	+	+	2440
	-	-	+	-	-	-	+	-	-	+	+	2150
	+	-	-	+	+	-	+	+	+	+	+	1900
	-	-	+	-	-	+	-	+	-	+	+	1500
	+	+	+	+	+	+	+	+	-	+	+	1250
	+	-	-	-	+	-	+	-	-	+	+	800
	-	-	+	-	-	+	+	-	+	+	+	780
	+	+	+	-	+	-	+	+	+	+	+	640
	+	+	+	+	+	+	+	+	-	+	+	560
	+	+	-	+	+	-	+	+	-	+	+	440
	-	-	+	-	-	-	+	-	-	+	+	400
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	-	-	-	-	-	+	-	-	-	-	-	340

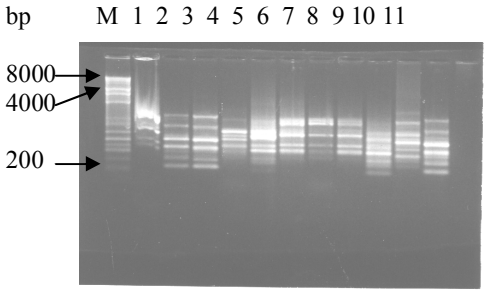

Fig. (7): RAPDs amplification of genomic DNA of D. melanogaster with prime (OPCO7). M, molecular marker-AGE-I.

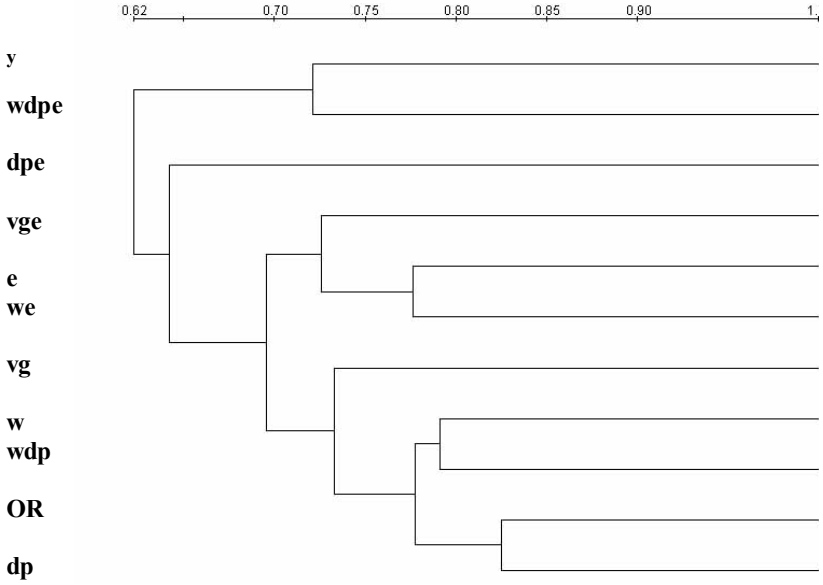

Fig.(7): Dendrogram for the eleven genotypes constructed from RAPD data using Unweighted Pair-Group Arithmetic Average (UPGMA) and genetic similarity (GS) matrices computed according to Dice coefficient from RAPD of genomes.

Table (8): Summary of RAPD markers used to identify eleven genotypes of D. melanogaster using primer OPC07.

Scoring of multiple-band profiles	Different genotypes of *D. melanogaster*											Molecular weights (bp)
	1	2	3	4	5	6	7	8	9	10	11	
	-	-	-	-	-	-	-	-	-	+	-	1900
	+	+	+	-	-	+	+	+	-	-	+	1650
	-	-	-	+	+	+	+	+	-	+	-	1450
	+	+	+	-	-	-	-	-	-	-	+	1000
	-	-	-	+	+	-	-	-	+	+	-	850
	-	-	-	+	+	-	-	-	+	+	+	650
	+	+	+	+	-	-	-	-	-	+	+	550
	-	-	-	+	+	+	+	+	+	+	-	500
	+	+	+	-	+	+	+	+	+	-	+	400
	-	+	+	-	+	-	-	-	+	-	+	340
	-	+	+	-	-	-	-	-	-	-	+	200
	-	-	-	-	+	-	-	-	+	-	-	100

Summary of RAPD analysis used in genomes

One of the most important features of the RAPD technique is detecting of high levels of polymorphism and this feature has been achieved in the present study. Five primers were screened with the genomic DNA of the 11 *D. melanogaster* genotypes. These primers generated reproducible and easily scorable RAPD profiles. These produced multiple band profiles with a number of amplified DNA fragments ranging from 10 to 15 (Table 8). The total number of fragments produced by the five primers was 63 with an average number of 12.6 fragments / primer. While the number of polymorphic fragments ranged from 9 to 13. A maximum number of 15 amplicons were amplified with primer (OPA10), while the minimum number of 10 fragments was amplified with primer OPB02. The highest number of polymorphic bands (13) was obtained with primer OPB15 that exhibited 100% polymorphism. The average number of polymorphic fragments / primer among the 11 *D. melanogaster* genotypes was (11.2).

Table (8): Summary of electrophoretic separation pattern of the RAPD-PCR products using the five primers.

Primer	Polymorphic bands		Monomorphic bands		Unique bands	Total bands
	No.	%	No.	%		
OPA09	11	84.62	2	15.38	No	13
OPA10	11	73.33	4	26.67	+ , -	15
OPB02	9	90	1	10	+	10
OPB15	13	100	No	0	- , - , - ,+	13
OPC07	12	100	No	0	+	12
Total	56		7		8	63
Average	11.2		1.4			12.6

DNA fingerprinting is a relatively new powerful method for detecting genetic variation at a variety of levels of biological organization. This technique offers a method for analyzing the genetic structure of populations, documenting genetic changes that might be occur over space or time, and for determining the genetic origin of newly

established populations (Haymer *et al*. 1992). A simple weighted average of nucleotide diversity per base pair showed *D. simulans* to be the most variable, followed by *D. mauritiana*, and *D. sechellia*. Both *D. simulans* and *D. mauritiana* have higher levels of polymorphism than reported for *D. melanogaster* (Hey and Kliman, 1993; Moriyama and powell, 1996;Trudy, 2010 and Doganlar *et al*.,2014).

Genetic relationships as revealed by RAPD markers

In the present study, to determine the genetic relationships among eleven *D. melanogaster* genotypes, the scoring data resulting from the 5 tested primers in genomic DNA was used to compute the similarity matrices according to Dice (Sneath and Sokal, 1973). The genetic similarity matrices based on the Dice coefficient were used in the cluster analysis to generate a dendrogram using the UPGMA analysis. The genetic similarity of genomic DNA ranged from 44.8% to 82.5% (Table 9). The highest genetic similarity revealed by the RAPD analysis was (82.5) between dumpy wing and wild type (OR). On the other hand, the lowest genetic similarity was (44.8) between dumpy ebony and yellow body.

Cluster analysis as revealed by RAPDs

The Dice RAPD- based coefficient of genetic similarity among the eleven genotypes of *D. melanogaster* in a dendrogram (Fig. 7). The *D.melanogaster* genotypes cluster comprised two subclusters. One subcluster includes y (chr.1) and wdpe (chr.1,2, and 3), while the other subcluster was subdivided into two groups. One includes dpe (chr.2 and 3), while the other group was divided into two subgroups. One subgroup includes vge (chr. 2 and 3), e (chr. 3), and we (chr. 1 and 3), while the other subgroup includes vg, w, wdp, OR, and dp (all are single mutants except OR and wdp). These results demonstrated that the mutants which have ebony body position in the same group except the triple mutant white dumpy ebony. Also, the mutants which have white eye position in the same group except the triple mutant white dumpy ebony.

Table (9): Genetic similarity (GS) matrices computed according to Dice coefficient from RAPD of genomes.

	OR	Y	w	Vg	Dp	e	wdp	we	vge	dpe	wdpe
OR	100										
y	78.1	100									
w	78	66.7	100								
vg	72.2	54.8	67.5	100							
dp	82.5	62.9	75	79.5	100						
e	69.4	54.8	72.5	68.6	66.7	100					
wdp	77.1	54.8	79.1	74.1	80.9	74.1	100				
we	69.6	61	62.3	68.7	69.3	77.6	74.4	100			
vge	70.4	59	68.4	63.8	77.9	72.5	67.5	72.7	100		
dpe	59.7	44.8	63.5	66.7	67.5	56	76.7	63.9	59.5	100	
wdpe	73.2	72.1	70.9	55.1	62.3	58	67.5	66.7	64.7	64.9	100

Determination of total proteins

Table (10) showed no variability in the total protein content between the eleven stocks of *D. melanogaster*. The total protein concentrations for all eleven stocks of *D. melanogaster* ranging between 16.9% to 24.1% with an average of 19.8%. In case of using the statistical analysis (χ^2) and

comparing each mutant strains with the wild type strain on the protein level encourage us to suggest that the total protein concentrations were not sufficient to detect variations among these eleven stocks. In other words there are no significant variations either in the concentration of these proteins. These results demonstrated that the gene or genes which are responsible for protein synthesis are the same in the experimental mutant strains. These data would lead to a conclusion that different mutational load did not affect protein concentration in different mutant lines. These results are in agreement with Sujkowski and Wessells (2018).

Table (10):Total protein concentrations in the eleven genotypes of D.melanogaster as determined according to the method of Lowery and Randall (1977).

Stocks	Protein conc. %	Calculated (χ^2) values
OR	19.8	
yellow body	18.4	0.0513
white eye	17.9	0.0958
vestigial wing	16.9	0.2292
dumpy wing	20.1	0.0023
ebony body	23.6	0.3327
white dumpy	18.6	0.0375
white ebony	24.1	0.4212
vestigial ebony	21.8	0.0962
dumpy ebony	19.6	0.0010
white dumpy ebony	17.6	0.1294

Determination of total amino acids

The data obtained from the present work show some characteristics for total amino acid and protein concentrations of eleven stocks of *D. melanogaster* in case of using HPLC-Pico-Tag method according to Millipore (Cohen *et al*., 1989). The concentrations of the total amino acids of the eleven stocks of *D. melanogaster* are shown in (Table 11). From the presented values, the highest amino acid ratio in wild type strain is for alanine and estimated with 19.41%, while the lowest amino acid ratio is for arginine and estimated with 1.36%. By a comparison with the single, double, and triple mutant strains which are used in this study, it has been also found that the highest amino acid ratio is for alanine and ranged between 19.44% and 22.71%. These were in agreement with wild type (19.41%) and difference is not significant. Also, it has been observed that the ratio of alanine in all single, double, and triple mutants which include dumpy wing mutant and estimated with approximately 22%. Moreover, the lowest amino acid ratio in the single, double, and triple mutant strains is for serine which is ranging between 0.58% and 1.40%. These were in agreement with wild type (1.49%), except ebony body mutant and vestigial ebony mutant which they have been significant. Also, there are some significant differences on the level of each amino acid comparing with wild type but the differences are non significant on the level of the total amino acids. On the other hand, the essential amino acid methionine was increased in the double mutant strain white dumpy and estimated with 7.57%, while it was decreased in all experimental strains and

ranging between 1.73% and 6.23%. Also, leucine was increased in the double mutant vestigial ebony and estimated with 8.15%, while it was decreased in all experimental strains and ranging between 5.19% and 4.01%. While, thereonine was decreased in the single mutant vestigial wing which estimated with 1.27% and the double mutant vestigial ebony which estimated with 2.57%.

Anderson *et al*. (1977) and Rand *et al*. (1994) found that although the significant excess of amino acid polymorphism within both *D. melanogaster* and *D. simulans* species is localized in one region of the protein. They added that mt.DNA evolution has been faster in *D. melanogaster* than in *D. simulans* and the population structure of mt.DNA is distinct in these species. A comparison within and between *D. melanogaster*, *D. simulans*, and *D. yakuba* species concerning the ratio of amino acid replacement change to synonymous change that reveals a relative excess of amino acids replacement polymorphism compared to the neutral prediction (William *et al*. 1994). Rand and Kann (1996) stated that both Drosophila and human mt.DNA show very significant excess of amino acid polymorphism. A pattern of excess amino acid (polymorphism holds across the entire mt.genome when *D. melanogaster* and *D. simulans* were compared (Rand, 2001). Also the representation of cysteine appears to correlate positively with the complexity of the organism. Miseta and Csutora (2000) investigated the occurrence of cysteine in known proteins of various species. Cesteine is a relatively rare amino acid within the proteins of investigated organisms and protein of the fruit fly contained somewhat less cysteine (1.90%) (Sujkowski and Wessells, 2018).

Table (11): Total amino acid concentrations (%).

Amino acids	OR	Y	W	vg	Dp	e	wdp	we	vge	dpe	Wdpe
	Concentration %										
Aspartic	12.15	10.64	9.84	6.88	12.50	12.18	9.85	6.85	3.48	12.32	9.69
Glutamic	3.80	4.83	3.67	1.30	3.49	5.29	4.34	2.49	7.47	3.96	3.41
Serine	1.67	0.84	1.40	0.57	0.81	17.57	1.31	0.58	4.96	0.85	0.88
Glycine	4.89	4.31	3.02	1.75	3.83	9.80	3.73	2.08	1.91	4.29	3.81
Histedine	5.63	5.03	4.92	1.52	5.92	5.72	5.15	3.25	3.55	5.27	4.89
Arginine	1.36	1.81	1.58	1.06	1.60	3.03	2.12	1.13	2.23	2.21	1.76
Therionine	3.28	4.68	4.26	0.27	4.75	3.25	4.88	2.94	2.57	4.98	4.30
Alanine	19.4	19.88	20.04	11.39	22.26	4.48	22.71	14.18	4.36	22.65	22.65
Proline	2.43	3.88	3.07	1.72	3.22	3.11	2.11	3.47	1.41	2.02	2.56
Tyrosine	9.52	8.60	10.11	19.44	11.58	4.89	9.23	14.79	8.83	8.08	10.52
Valine	5.96	4.85	5.02	9.60	5.34	4.50	4.47	6.10	4.59	4.30	4.67
Methionine	6.99	1.85	5.25	2.39	1.73	2.38	7.57	2.15	4.59	2.02	2.35
Cystine	5.65	6.95	4.39	5.95	4.00	1.39	1.79	10.06	10.24	5.09	6.36
Isoleucine	3.96	3.77	4.32	7.79	3.93	2.75	4.95	6.34	6.1	4.72	3.84
Leucine	5.49	5.19	4.77	4.01	4.53	4.19	4.18	4.09	8.15	4.14	4.81
Phenylalanine	10.3	8.04	8.78	10.91	9.26	7.29	7.07	10.20	18.35	6.57	8.29
Lysine	8.31	4.94	5.75	10.91	1.29	8.17	4.55	9.32	7.19	6.53	5.24

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الملخص العربي

دراسات وراثية جزيئية وبيوكيميائية لبعض الطفرات في حشرة الدروسوفيلا ميلانو جاستر

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تم استخدام عشر سلالات طفرية التي تمثل طفرات احادية وثنائية وثلاثية (اربعة سلالات تم بنائهم)بالاضافة للسلالة البرية لحشرة الدروسوفيلا ميلانوجاستر,و ذلك فى دراسة البصمة الوراثية فى د ن ا النواه على المستوى الجزيئى و البيوكيميائى. و قد أظهرت نتائج التحليل الوراثى الجزيئى فى ال د ن ا النووى باستخدام التكبير العشوائي ل د ن ا متعدد المظاهران نسبة تعدد المظاهر تتراوح بين ٧٣.٣٣% الى ١٠٠% و ثمانية حزم فريدة، و كان العدد الكلى للحزم ٦٣ حزمة بمتوسط ١٢.٦ حزمة/ بادئ ، كما تم تقدير العلاقات الوراثية بين التراكيب الوراثية الاحدى عشر بناءا على التشابه الوراثى باستخدام معامل Dice ، و لقد تراوحت نسبة التشابه الوراثى ما بين ٤٤,٨% الى ٨٢,٥% لل RAPD-PCR . كما أوضحت نتائج تحليلات درجات القرابة الوراثية ان الطفرات الأحادية تقع فى مجموعة متفردة عن باقى الطفرات الثنائية و الثلاثية فيما عدا طفرة ابيض العين مقضوم الجناح ابنوسي الجسم . بالاضافة الى ان الطفرات الأحادية تم فصلها الى تحت مجاميع تبعا للكروموسوم المحمول عليه الطفرة. و من ناحية أخرى فقد أوضح التحليل البيوكيميائى ان الأحمال الطفرية المختلفة لم تؤثر على تركيز البروتين فى السلالات الطفرية، بينما نجد ان بعض السلالات الطفرية أوضحت تغير معنوى فى الأحماض الأمينية. ومن هنا نجد ان النتائج المتحصل عليها من هذه الدراسة تقود الى امكانية استخدام التحليل الجزيئي والبيوكيميائي كطريقة سهلة و سريعة لدراسة البصمة الوراثية فى هذه السلالات الطفرية و فى الكائنات الأخرى.