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Genetic divergence among Egyptian populations of *Drosophila melanogaster* and Canton-S wild type strain

Ismael A. Khatab[*1], Antar N. El-Banna[1], Amira S. El-Keredy[2]

[1]*Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, 33516 - Kafrelsheikh, Egypt*

[2]*Department of Genetics, Faculty of Agriculture, Tanta University, Egypt*

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Abstract

Genetic divergence and interrelations of four geographic populations of *Drosophila melanogaster* (three populations from Egypt namely; Tanta, Kafr-Elshiekh and Wady El-Natron areas and Canton-S wild type strain) were analyzed by PCR Random Amplified Polymorphic DNA (PCR-RAPD). Out of twenty random primers used only 16 primers efficiently produced 79 RAPD bands, of which 42 were polymorphic. The dendrogram constructed using UPGMA based on the RAPD data showed that there was similarity among the populations collected from Egypt and do not show clear diversity or population differentiation, which refers to population growth. Moreover, used RAPD primers in this study classified the three populations from Egypt into two clusters according to their geographical origin, indicating recent population differentiation. In contrast, population Canton-S (wild type strain) was highly diverged and located in separated cluster. These results agreed with conclusions of the previous studies that *Drosophila* originated in Africa and colonized the rest of the world only recently. Moreover, *D. melanogaster* was highly differentiated.

***Corresponding Author:** Ismael A. Khatab ✉ ismael.khatab@yahoo.com

Introduction

Drosophila melanogaster has played an essential role in the development of population genetics. However, many questions regarding the demographic history and population differentiation of this species remain unresolved. Thus, the aim of the present study was to examine genetic variability and population differentiation in three *D. melanogaster* populations by employing PCR-RAPD markers, to understand the genetic variability of these populations which is fundamental to understanding their dynamics and relationships. *D. melanogaster* is particularly well suited for the identification of ecologically relevant alleles and has historically served as an important model system both in molecular and evolutionary genetics. Like humans, *D. melanogaster* originated in sub-Saharan Africa and colonized the rest of the world only recently (David and Capy 1988). The level of genetic differentiation of populations caused by selection is determined by the amount of additive genetic variation. Extensive empirical evidence indicates that the proportion of additive variation in the total phenotypic variations. Until the discovery of the high differentiation between sub-Saharan African and cosmopolitan *D. melanogaster* populations (Begun and Aquadro 1993), it was often considered a completely panmictic population. However, Yutaka (2006) mentioned that *D. melanogaster* natural populations show a high degree of polymorphism. Thus, matter of genetic diversity of *D. melanogaster* still controversial issue and *D. melanogaster* populations show evidence for considerable differentiation. Furthermore, Mutation accumulation in *D. melanogaster* has shown that spontaneous mutations are able to create abundant variation (Rifkin *et al.,* 2005). However, when comparing the levels of variation in mutation accumulation lines to the levels found in natural isolates, it can be seen that variation in natural populations is significantly lower (Denver *et al.* 2005). Additionally, divergence between closely related species was much lower than expected under a neutral model (Rifkin *et al.* 2005) and stabilizing selection plays a dominant role in shaping variation in natural populations. Studies of the genetic characteristics of marginal population make it possible to test the idea that gene flow is a cohesive force that holds a species together and allows it to evolve as a unit. Such populations may yield information on the role of heterozygosity at the chromosomal level, as in the case of many *Drosophila* species. The genetic diversity of *D. melanogaster* presents an unfailing interest for the genetics of populations and ecology studies. Recently, many results of *D. melanogaster* yielding further insight into the potential impact of natural selection on diversity across the *Drosophila* genome (Langley *et al.,* 2012; Mackay *et al .,* 2012), and connections between genetic and phenotypic variation (Mackay *et al.,* 2012). However, a large majority of the information is of North American origin, and before we can clearly understand the demographic history of that population, we must investigate genomic variation in its African and European antecedents. Furthermore, understanding of genomic variation in ancestral range populations of *D. melanogaster* will improve our ability to make population genetic inferences for worldwide populations (Pool *et al.,* 2012). Because of the divergence between authors about the differentiation in Drosophila and the absence of molecular data supporting any of these results about Egyptian populations, we decided to undertake this study to propose new hypotheses regarding the relationships between the members of these groups. Moreover, for our knowledge this is the first time to investigate the divergence between Egyptian and European as antecedents for drosophila. The purpose of this study is the investigation of genomic DNA differentiation in three natural geographically distributed populations of *D. melanogaster* from different geographical locations in Egypt compared with wild population, Canton-S strain from Germany, using RAPD markers for the analysis of genomic DNA divergence.

Materials and methods

Collection of flies

Natural populations of *D. melanogaster* flies were collected from three geographic areas from Egypt,

Tanta, Kafrelshiekh and Wady El-Natron and Canton special wild type strain (from Department of Genetics and Neurobiology, Wurzburg. Germany). The optimal medium which consisted of the ordinary cornmeal-molasses-agar- propionic acid mixture was used in all experiments as recommended by Megeed (1982). After maintaining the flies at 25°C, till DNA was isolated.

DNA extraction

DNA was extracted from individual insect by Cetyltrimethyl Ammonium Bromide (CTAB) according to Doyle and Doyle (1990). The concentrations of DNA were determined using a spectrophotometer and equal amounts of each population individuals were mixed for five individuals.

PCR and Gel electrophoresis

Twenty RAPD primers were performed using 10 random decamer primers (Table 2). Polymerase Chain Reaction (PCR) was carried out in presence of 1X *Taq* DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 100 µM dNTPs, 5 picomole single random primer, ~25 ng template DNA, 0.5 unit of *Taq* DNA polymerase in a total volume of 25 µl. PCR amplification was performed in automated thermal cycler (MJ-Mini, Bio Rad) the initial step at 95°C for 4 min followed by 35 cycles of 1 min for denaturation at 94°C, 30 sec for annealing at 36°C and 1.30 min polymerization at 72°C, followed by a final extension step at 72°C for 7 min. (one cycle); then 4°C. Amplification products were separated on 1.5 % agarose gel in 0.5 x TBE buffer against molecular size standards 1Kbp ladder. Gels were visualized under UV light and photographed using gel documentation UVITEC, UK.

Data Analysis

The amplified bands from RAPD were scored under the heading of total scorable fragments. Amplification profiles of six soybean genotypes were compared with each other and bands of DNA fragments were scored as a binary data where presence (1) or absences (0). The data were used to estimate genetic similarity on the basis of number of shared amplification products (Nei and Li, 1979). The coefficients were calculated by the following statistical equation.

$F= 2N_{xy}/ (N_x + N_y)$

Where, F is the similarity coefficient in which Nx and Ny are the number of fragments in genotypes x and y, respectively, where Nxy is the number of fragments shared by the two genotypes (Lynch, 1990). Cluster analysis was based on similarity matrix obtained with unweighted pair group method using arithmetic average (UPGMA), and the relationships between genotypes were displayed as dendrogram using the NTSYSpc 2.01 software package (Rohlf, 1998).

Results and discussion

Twenty RAPD primers were tested against the four populations of *D. melanogaster* from different geographical locations; only 16 primers successfully produced scoreable and informative bands. The sequences of these primers, the number of bands and the degree of polymorphism revealed by each primer are listed in Table (1). The RAPD profiles of the amplified products are shown in Fig. (1 *a* and *b*). Different banding patterns were obtained from different used primers. Generally, the levels of polymorphism were varied with different primers among the three populations of *Drosophila* collected from different Egyptian geographical locations and Canton-S wild strain from Germany. The percentage of polymorphism produced by each primer differed from one primer to the other; the polymorphism ranged from 33.3% using primer OPA-06 to 66.6% using primers OPK04 and OPK11 across all the studied populations. Primer OPA13 produced the highest number of bands eight bands while; primers OPA07 and OPK02 amplified lowest number of bands only two bands.

The highest similarity value (0.86) was recoded between Kafrelshiekh and Tanta populations, while the lowest value (0.41) was recoded between Tanta and Canton-S populations as shown in Table (2).

Moreover, Similarity coefficient matrices were used to generate a dendrogram of the studied four populations based on UPGMA analysis Fig. (2), the dendrogram divided the four populations into two distinct clusters. The first cluster consisted of a highly diverged population Canton-S, while the second cluster divided into two sub groups; where Tanta and Kafrelshiekh populations were grouped together and the second sub cluster consisted of the El-Wady population.

Table 1. Primers sequences, number of amplified bands, number of polymorphic bands and polymorphism percentages for the four *Drosophila melanogaster* populations.

Primer Name	Sequence (5′ → 3′)	Total No. of Bands	No of Polymorphic bands	Polymorphism %
OPA-06	GGTCCCTGAC	6	2	33.3
OPA-07	GAAACGGGTG	2	1	50.0
OPA-12	TCGGCGATAG	4	2	50.0
OPA-13	CAGCACCCAC	8	5	62.5
OPA-14	TCTGTGCTGG	7	3	39.7
OPA-16	AGCCAGCGAA	5	3	60.0
OPK-02	GTCTCCGCAA	2	1	50.0
OPK-03	CCAGCTTAGG	5	2	40.0
OPK-04	CCGCCCAAAC	3	2	66.6
OPK-05	TCTGTCGAGG	4	2	50.0
OPK-06	CACCTTTCCC	5	3	60.0
OPK-07	AGCGAGCAAG	7	3	39.7
OPK-08	GAACACTGGG	7	5	57.1
OPK-09	CCCTACCGAC	4	2	50.0
OPK-10	GTGCAACGTG	4	2	50.0
OPK-11	AATGCCCCAG	6	4	66.6
Total		79	42	

There are a little knowledge about the origin and historical structure of *D. melanogaster* populations. Moreover, for our knowledge these studies using Egyptian populations are almost rare. Previous reports of large differences in levels of variation between African and non-African populations mainly relied on data from a single African population (Begun and Aquadro 1993) or on mixed samples without knowledge of how African and non-African populations are structured (Andolfatto 2001). Here we used three populations from Egypt and Canton-S from Germany. There were many studies of the extent and distribution of the population differentiation among *Drosophila* populations using different molecular markers (Penelope *et al.,* 2005; Sharma *et al.,* 2008; Thongatabam and Nallur 2010).

Table 2. Genetic similarity among four populations based on Jaccard

KFS	Tanta	Wady	Canton-S	0
			1	Canton-S
		1	0.56	Wady
	1	0.52	0.41	Tanta
1	0.86	0.62	0.50	KFS

Results obtained from RAPD analysis could provide an easy, low cost and quick way for assessment of genetic diversify among *Drosophila* populations (James *et al.,* 1998). The variation in the number of bands amplified by different primers influenced by variable factors such as primer structure, template quantity and less number of annealing sites in the genome. RAPD technique is being used successfully to identify, characterize and estimate genetic divergence of *Drosophila* populations. The characterization of these markers in *D. subobscura* confirms its high variability (Pascual, *et al.,* 2000) and therefore makes them good candidates for the study of population differentiation and the

colonization process. Similarly, Pascual *et al.* (2001) investigated genetic variation in European and North American populations of *D. subobscura*, using microsatellites to illustrate is there genetic differentiation among Old World populations that could help us to trace the origin of the colonizers, or is gene flow and found that populations were genetically highly diverse. Furthermore, in *Drosophila* wings have been found to evolve fast in response to geographic clines, e.g. (Huey *et al.,* 2000, Gilchrist *et al.*, 2000), and they have also been found to respond well to artificial selection (Houle *et al.,* 2003, Kennington *et al.*, 2003). These rapid changes are not surprising because of the abundant genetic variation related to wing shape (Weber *et al.,* 2005).

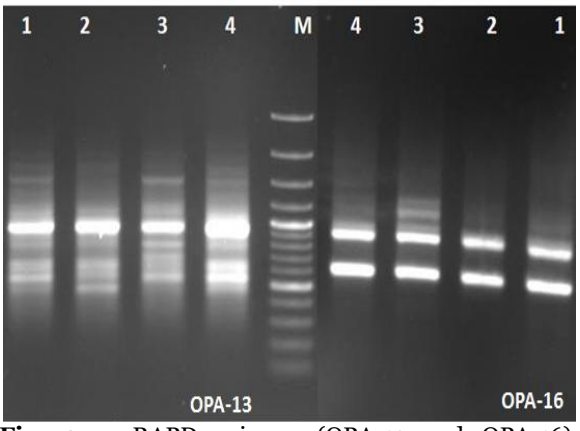

Fig. 1 a. RAPD primers (OPA-13 and OPA-16) banding patterns of the four Drosophila populations, Lanes from: 1 to 4 represent 1- KFS; 2- Tanta; 3- Wady 4-Canton S; M, 100 bp ladder.

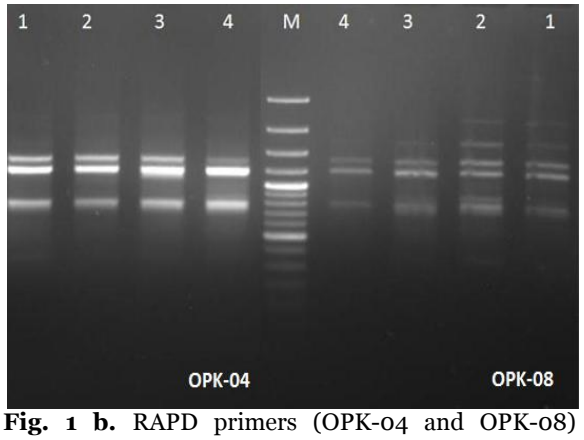

Fig. 1 b. RAPD primers (OPK-04 and OPK-08) banding patterns of the four Drosophila populations, Lanes from: 1 to 4 represent 1- KFS; 2- Tanta; 3- Wady 4-Canton S; M, 100 bp ladder.

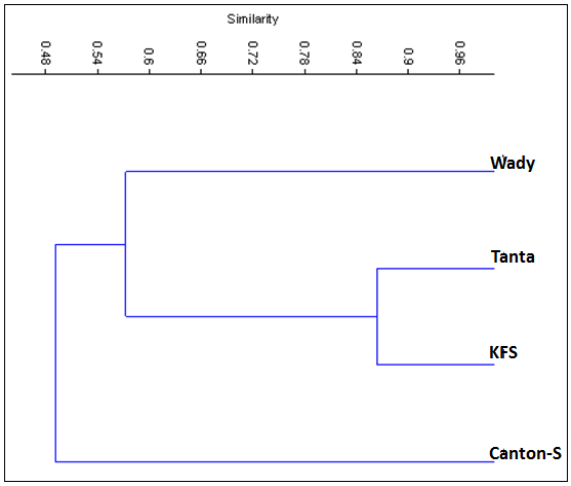

Fig. 2. Phylogenetic tree among four populations based on Jaccard.

Thus, Canton-S population cluster was distinct from all the Egyptian populations, supporting the hypothesis that this population has a unique origin (Baudry *et al.,* 2004). Within Egyptian, El-Wady population appears to be distinct from the two Kafrelshiekh and Tanta populations, though Kafrelshiekh and Tanta populations are indistinguishable. In this study, high level of genetic divergence and population differentiation were founded among *Drosophila* population from Egypt and Canton-S strain (population). So, we conclude to the geographical closeness leads to low level of genetics divergence and vice versa.

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