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BIOLOGICAL IDENTIFICATIONS AND GENETIC VARIABILITY OF RED PALM WEEVIL *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER) POPULATIONS FROM DIFFERENT GEOGRAPHICAL LOCATIONS IN EGYPT

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ABSTRACT

The red palm weevil (RPW)*Rhynchophorusferrugineus*(Olivier) is one of the most destructive pests of date palms in Egypt. To study the biological identifications and genetic variability of red palm weevil populations in Egypt; Morphological characteristics (the length of the body, the width, general color and the number of spots color on the head) and randomly amplified polymorphic DNA (RAPD) technique were used to assay sevengeographical locations. Both females and males in each location were analyzed. Morphological characteristics indicated no differences between locations. RAPD primer pairs detected a total of 159 RAPD fragments in these locations and 143 of these showed polymorphism ranged from 60 -100% with average of 78.61%. Cluster analysis of RAPD data clearly separated these males and females into two major groups containing three clusters witha genetic similarity between (0.41- 0.91).

Keywords:*Genetic variability, Morphology, RAPD, Rhynchophorusferrugineus.*

INTRODUCTION

Red palm weevil (RPW) is the most widely damaging insect pest of date palm and is serious pest of palms throughout in the world. The original way of RPW was started from Malaysia and Southeast Asia. Then stretching through the countries bordering the Bengal from Sri-lank to the Malayan peninsula and Singapore; through Thailand Cambodia and Vietnam; across the South China sea to Taiwan and the Philippines; and down through the Sunda Islands. Introductions of *Rhynchophorusferrugineus*(*R.ferrugineus*) to the Arab Gulf States (Bokhari and Abuzuhari, 1992) and their spread westward into Egypt are causing the devastation of date palms throughout that region.

Adult RPW are large (30-40 mm in length) but display a high degree of color polymorphism. This color polymorphism has challenged taxonomists and other researchers for over two centuries. Currently, two color-morphs of RPW are recognized as a single species, *R. ferrugineus*: a "ferrugineus" (or orange with black markings) color morph and a "vulneratus" (or black with a red

stripe) color morph. Almost the entirety of the invaded range of RPW has been colonized by the "ferrugineus" form (Paul *et al.*, 2013).

In a given invaded country we can expect that the insect genetic diversity will be similar in case of a single introduction of insects as well as multiple successive introductions from the samAe source area. On the contrary multiple introductions from different source areas will likely increase the genetic diversity in the populations as they introduce new alleles (Grapputo*et al*., 2005).

DNA markers are especially effective tools in making inferences about movement between insect populations, because they represent selectively neutral characters (Black *et al.*, 2001). Different kinds of molecular markers can reveal different levels of genetic variation, making population genetics studies possible on a wide range of geographical scales. Among the different molecular markers, those are currently available for monitoring genetic variability and diversity, random amplified polymorphic DNA (RAPD) represent a rapid, simple and cost effective tool to assess insect populations (Biron*et al.*, 2000 and Naber*et al.*, 2000). The RAPD technique has been widely used to elucidate the geographical origin of gene flow among insect populations (Vandewoestijne and Baguette 2002 and Ayres *et al*., 2003). It has proved to be a very efficient and sensitive method for obtaining genetic markers for different kinds of organisms, demanding no prior information about genomic organization. Limitations associated with RAPD markers, like variable reproducibility, may appear unless reaction conditions are stringently controlled and a dominant mode of inheritance is lacking (Lynch and Milligan, 1994 and Loxdale*et al*., 1996). Nevertheless, properly performed RAPD analysis is a useful and reliable tool for studying the ecology and genetic structuring of many species populations (Pearson *et al*., 2002).

The objectives of this study are as follows: 1) investigate biological identifications to observe whether RPW populations collected from different geographical locations have the same morphological characteristics. 2) Study the genetic variability of red palm weevil populations collected from different seven geographical locations in Egypt.

MATERIALS AND METHODS

• *Red palm weevil (RPW) samples*

Random males and females samples from *R.ferrugineus* were collected from various 7 geographic locations in Egypt (R= Buhayra (Rashed), D= Minufiya (KaferDawood), A = Aswan, L= Luxor, B= Alexandria (Borg Elarab), M = Ismailia, K = Qina; Fig. 1).

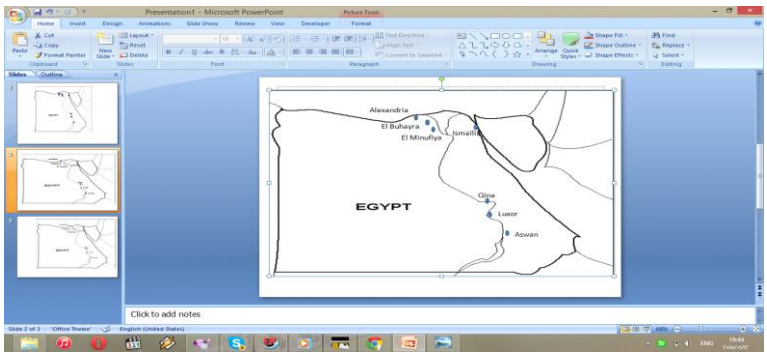

Fig. (1) Surveying locations of red palm weevil *R. ferrugineus.*

• ***Morphological characterization***

The morphological descriptions of adult males and females of *R.ferrugineus* based on the following parameters: the length of the body, the width, general color and the number of spots color on the head.

• ***Genomic DNA extraction***

Genomic DNA was extracted from leg tissues of the RPW samples (males and females) using genomic DNA extraction kit (G-Spin)^{TM} for cell / tissue (iNtRON Biotechnology, Inc.) extraction was preformed according to the manufacturer's protocol.

• ***RAPD analysis***

RAPD analysis was carried out at the random population level using ten oligonucleotide primers (Table 1) that were selected from the Operon Kit (Operon Technologies Inc., Alabameda, CA). The polymerase chain reaction mixture (25 µl) consisted of 0.8 U of *Taq* DNA polymerase; 25 pmoldNTPs; 25 pmol of primer and 50 ng of genomic DNA. PCR amplification was performed in a Biometra*T1* gradient thermalcycler for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min; annealing at 36°C for 1 min; extension at 72°C for 2 min and final extension at 72°C for 10 min (Soliman, *et al*.,2003). Amplification products were separated on 1.5% agarose gels at 80 volts for 1.30 hrs with 1X TBE buffer. To detect ethidium bromide / DNA complex, agarose gels were examined on ultraviolet transilluminator (302 nm wavelength) and photographed. Using 100 pb DNA ladder (V-gene Biotechnology Limited, shiqao, P. R. China), the lengths of the different DNA fragments were determined. For each sample, the reproducible DNA bands from two runs were scored for their presence or absence.

Table (1): Nucleotide sequences of used primers for RAPD analysis.

Primer	Sequences	Primer	Sequences
OPB-01	GTTTCGCTCC	OPH-03	AGACGTCCAC
OPB-03	CATCCCCCTG	OPO-02	ACGTAGCGTC
OPD-02	GGACCCAACC	OPO-03	CTGTTGCTAC
OPD-01	ACCGCGAAGG	OPO-04	AAGTCCGCTC
OPD-03	GTCGCCGTCA	OPO-05	CCCAGTCACT

• ***Scoring bands of RAPD's and dendrogram construction***

RAPD's fragments were scored as present/absent. Data matrices were entered into the NTSYS (Numerical Taxonomic and Multivariate Analysis System) program, version 2.1, Applied Biostatistics Inc. (Rohlf, 2000). Similarity coefficients were applied for dendrogram construction using the UPGMA (Unweighted Pair Group Method with Arithmetic Average) and the SAHN (Sequential Agglomerative Hierarchical Nested Clustering) routine in the NTSYS program.

RESULTS AND DISCUTION

• ***Morphological characterization***

The adult males and females of *R ferrugineus* were described depending on different parameters (the length of the body, the width, general color and the number of spots color on the

head). The obtained results showed that, the length of the body of males ranged from 18 to 43 mm while the females ranged from 24 to 42 mm. for the width of the thorax, the males had 8 to 16 mm width while the females had 10 to 16mm. The general color was orange with black markings. For the black spots on the pronotum it was extremely variable, there was no specific number of spots for any location neither on males nor on females (Fig. 2).

Fig. (2): Females and males of *R. ferrugineus*showing the same general color and high variability of black spots.

• ***RAPD data analysis***

In the present study, ten RAPD primers were used in screening the genetic variability between different genomic-DNA of *R. ferrugineus*. For each primer-DNA combination, the amplification was repeated at least twice. As shown in Table (2) the number of reproducible bands per primer varied between 5 for primer OPB-3 and 22 for primer OPD-2 with a total of 195 bands (Figure 3). The results in Table (2) clearly indicated that in all studied *R. ferrugineus*, 143 (78.61%) of the 159 fragments were polymorphic and 52 bands (21.39%) were monomorphic. In the meantime, all used primers generated 33 specific markers (Table 2). The largest number of these markers was specific for males and females weevils collected from Rasid, Buhayra (9 and 8 markers respectively). Furthermore, fife specific markers were observed in females of Ismailia. Also, three specific markers were reported for males of Luxor. While: females and males of Minufiya and males of Qina showed two specific markers. Finally both females of Luxor and males of Ismailia exhibited only one specific marker. The polymorphism ranged from 60% for primer OP- B3, till 100% for OP-O2, OP-O3 and OP-O4 primers (Table 2) with average of 78.61%.

Table (2): RAPD analysis of different genomic-DNA of *R. ferrugineus* populations collected from different locations.

	Name of primers									
	B1	B3	D1	D2	D3	O2	O3	O4	O5	H3
AF	14	5	14	22	20	18	16	16	20	14
P+U	12	3	10	18	19	18	16	16	20	11
Unique	2	0	1	6	6	4	4	3	6	1
PF%	85.71%	60%	71.42%	81.8%	95%	100%	100%	100%	100%	78.57%

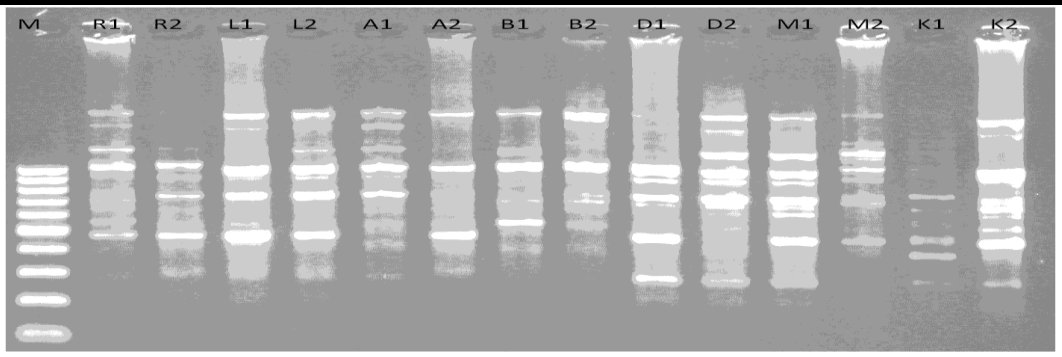

Fig. (3) Photographs showing RAPD patters from the *R. ferrugineus* females and malespopulations analyzed using OP-B1 primer. R1= females from Rashed, Buhayra, R2= males fromRashed, Buhayra, L1= females from Luxor, L2= males from Luxor, A1= females from Aswan, A2 = males from Aswan, B1= females from Borg Elarab, Alexandria, B2= males from Borg Elarab, Alexandria, D1= females fromKaferDawood, Minufiya, D2= males from KaferDawood, Minufiya, M1= females from Ismailia, M2= males from Ismailia, K1= females from Qina and K2= males from Qina.

• ***Genetic similarity and Dendogram***

Genetic similarity (GS) values generated from RAPD marker varied between 0.41 and 0.91.with an average of 0.66. Dendrogram based on similarity values (Table 4) from RAPD was constructed to reveal similarities between the different males and females populations of *R. ferrugineus*. The dendrogram (Figure 4) demonstrated that the 14 genomic samples fall into two main groups. The first one contained females and males of Buhayra (Rashed) with genetic similarity of (66%). The second one divided into two subclusters; according to similarity, the first one contained females and males of Luxor, Aswan, Alexandria and Minufiya. The second cluster contained females and males of Ismailia and Qina in similarity from 51 to 83%.

Table (3): Similarity indices calculated by NTSYS program among the *R. ferrugineus*populations based on RAPDs data.

	R1	R2	L1	L2	A1	A2	B1	B2	D1	D2	M1	M2	K1
R2	0.64												
L1	0.57	0.58											
L2	0.59	0.54	0.72										
A1	0.60	0.57	0.75	0.91									
A2	0.58	0.56	0.73	0.86	0.89								
B1	0.58	0.55	0.76	0.77	0.83	0.76							
B2	0.60	0.53	0.75	0.73	0.74	0.75	0.86						
D1	0.61	0.49	0.69	0.76	0.79	0.76	0.75	0.74					
D2	0.55	0.50	0.69	0.74	0.76	0.71	0.74	0.70	0.83				
M1	0.53	0.51	0.67	0.67	0.71	0.70	0.71	0.72	0.70	0.71			
M2	0.51	0.45	0.66	0.68	0.72	0.70	0.72	0.68	0.73	0.74	0.81		
K1	0.54	0.51	0.70	0.69	0.74	0.72	0.75	0.74	0.77	0.78	0.83	0.81	
K2	0.51	0.41	0.60	0.69	0.72	0.70	0.69	0.69	0.74	0.71	0.69	0.76	0.79

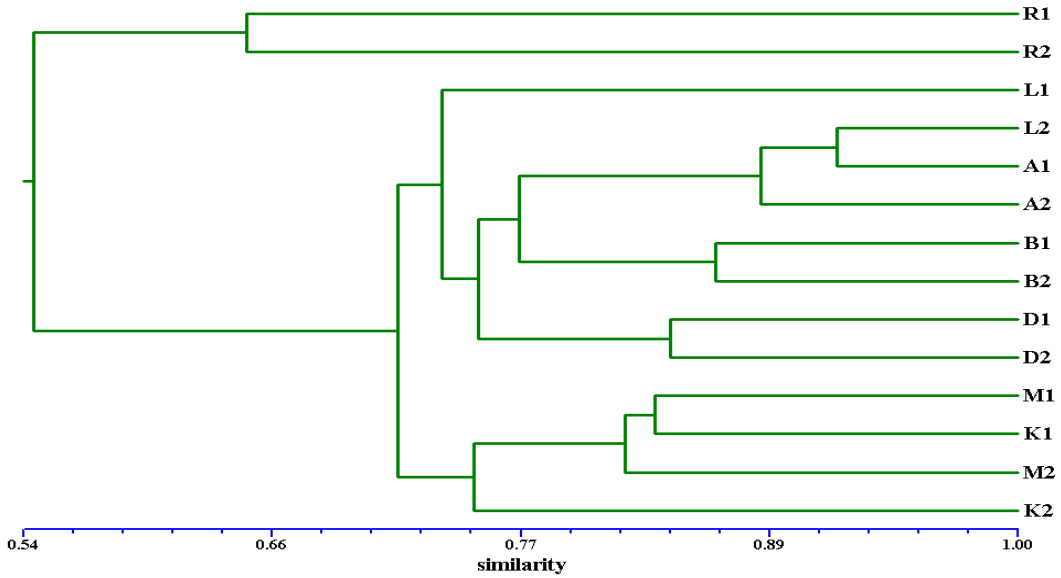

Fig. (4):Dendrograms of *R. ferrugineus* females and males population from different locations.

In this study, morphological and genetic variation among females and males of red palm weevil *R. ferrugineus*, collected from various 7 geographic locations in Egypt ware detected. Our results indicated that; there was no color variation between the locations. Where, the observed color was orange with black markings. These results are in agreement with previous studies which began by Wattanapongsiri (1966) which have demonstrated morphological variation of RPW from different geographic areas. It was found that for the color variation; two color-morphs of RPW are recognized, *R. ferrugineus*: a "ferrugineus" (or orange with black markings) color morph and an *R. vulneratus* "vulneratus" (or black with a red stripe) color morph (Paul *et al.*, 2013). The majority of the Egyptian and Middle-Eastern studies (Salama and Saker, 2002; Gadelhak and Enan, 2005; El-Mergawy*et al*., 2011a, b, c) have studied RPW specimens and populations collected from the invaded range of the pest. Their results indicated that only the orange form was found. In 2013 Paul *et al*. indicated that: all Egyptian and Mediterranean populations are belong to one phylogeographic group also, he indicated that distribution of the different RPW color-morphs was not so clear cut. Like almost all of those occurring in the invaded range, the majority of native specimens tentatively identified genetically as *R. ferrugineus* were the orange color-morph.

The genetic variation among seven locations populations in Egypt of RPW was detected using RAPD. There was variable polymorphism obtained between primers which ranged from 60-100%. An observed 100% polymorphism in RAPD markers has also been detected in PRW previously in the study of El-Mergawy*et al*., (2011a). This percentage reflects the absence of genetic homogeneity among the examined populations (Sharma *et al*., 2009). In contrast Gadelhak and Enan (2005) detected 51.4% polymorphism in RAPD markers for comparison among seven RPW individuals from UAE.

Our results observed RAPD markers especially in females and males of Rashed, Buhayra. The unique RAPD markers observed in the present study may be used to produce genetic

markers that can distinguish the geographic populations of RPW from each other (Haymer and McInnis, 1994 and Bardakci, 2000).

The average genetic distance among the seven locations populations in Egypt of RPW ranged from 0.4 to 0.91. Similarly to our results, a high average genetic distance among different geographic populations of RPW was detected (0.3 to 0.8) (El-Mergawy*et al* 2011a). The highest and lowest average genetic distances revealed that similarity among the analyzed populations ranged from 19% to 60%. Similarity range from 38 to 94% was observed previously among RPW populations from UAE (Gadelhak and Enan, 2005).

The resulted dendrogram in this study put females and males of Rashed, Buhayra in a cluster, two of the Upper Egypt governors (Luxor and Aswan) with delta governor Minufiya and the Coastal governor Alexandria are in one cluster. The rest studied governors, Ismailia which is located on the Suez Canal Gulf and Upper Egypt governor Qina were in the third cluster. The high variable locations found in each cluster, highly variable genetic distance and high observed polymorphism indicate that the Egyptian RPW individuals have no direct relationships with each other as some individuals from distant localities were clustered together. Also it can be concluded that these populations derived from multiple introductions from different origins throw flying of the RPW. Vieira *et al.,* (2007) indicated that invasive populations derived from multiple introductions from different origins are expected to be genetically more diverse. Our results are in agreement with El-Mergawy*etal*. (2011a) who indicated that, the Egyptian RPW individuals were clustered in six separate clusters. In contrast, Gadelhak and Enan (2005) observed that there is no significant genetic variation by distance among seven population of RPW in the United Arab Emirates.

CONCLUSION

In conclusion our findings confirm that morphological characterization combined with RAPD (as a molecular marker) consider good tools to distinguish among populations of RPW. The resulted dendrogram, average genetic distances and highly polymorphis suggest that the RPW populations in Egypt do not belong to each other geographically, but affected by the place of invasion andthe ability to fly from one place to another.

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