



Full Length Article

Determination of Different Geographical Populations of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) using RAPD-PCR

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ABSTRACT

This study investigated the genetic variation among thirteen geographic populations of the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), using the Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique. RAPD profiles exhibited bands between 200 to 1400 bp in length. Average genetic distances among populations ranged from 0.3 to 0.8. Genetic distances among the analyzed individuals ranged from 0.241 (between individuals from populations of Egypt & Turkey) to 1 (between individuals from populations of Japan & both Egypt, Spain & France). The Unweighed Pair Group Method with Arithmetic (UPGMA) mean categorized the tested populations into twelve clusters. Based on results, it is suggested that there are two major routes of RPW invasion, the first is at the origin of all the populations found in Egypt and Mediterranean countries and second is at the origin of the populations found in the Arabic Peninsula, Iran and Pakistan. © 2011 Friends Science Publishers

Key Words: RAPD-PCR; *Rhynchophorus ferrugineus*; Invasive species; DNA fingerprinting

INTRODUCTION

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is an invasive species that is claimed to be originated from South and Southeast of Asia, where it is considered as one of the most destructive pests of coconut, *Cocos nucifera* L. (Abraham *et al.*, 1975; Sivapragasam *et al.*, 1990; Sadakathulla, 1991; Murphy & Briscoe, 1999; Ferry & Gomez, 2002). The invasion history of RPW to the Middle East and the Mediterranean area date back to the mid 80's where it was detected for the first time in the United Arab Emirates (UAE), since then the pest is continuing its invasion to the other countries of Middle-East, including Egypt, and to most of the countries of the Northern shore of the Mediterranean sea up to Portugal (Cox, 1993; Barranco & Cabellot, 1996; Esteban-Durán *et al.*, 1998; Kehat, 1999; Karut & Kazak, 2005; Kontodimas *et al.*, 2007; EPPO, 2008; Ministry for Resources & Rural Affairs of Malta 2008; EPPO Reporting Service 2008, 2009). In those invaded countries RPW is threatening a broad host range of palms

and is considered as the most destructive pest of palms, where it spends its life cycle inside the trunk without visible sign (Nirula, 1956; Wattanapongsiri, 1966; Abraham *et al.*, 1998; Kaakeh, 2005; Faleiro, 2006a, b). The infestation of this insect is detected when trees are extensively damaged and it is not possible to recover (Gadelhak & Enan, 2005).

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 same 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).

Considering the economic importance of RPW, it is necessary to know the history of its invasion and to understand the relationships between the different geographic populations. Studying the genetic diversity of RPW will help answering several questions such as: the number of invasion routes from Asia to the West and the number of introductions that have occurred in each of the invaded countries. In addition, this study of genetic variation

among the geographic populations of invasive species is essential for designing their management strategy including biosecurity as it gives rapid and accurate identification of alien species and an accurate characterization of their populations (Armstrong & Ball, 2005; Grapputo *et al.*, 2005; Marimuthu *et al.*, 2009; Sharma *et al.*, 2009).

RAPD technique is considered as an useful tool as it need tiny amounts of DNA to give rapid and accurate identification of alien species especially in the developing countries, where DNA-based methods are unavailable due to their high cost, the requirement for complex equipments and the requirement for expertise (Williams *et al.*, 1990; Hadrys *et al.*, 1992; Bardakci, 2000).

The genetic variation of RPW was previously detected using RAPD markers. Those studies were restricted to comparisons among seven individuals of RPW from UAE, individuals of RPW from Egypt, KSA and Indonesia and different morphological forms of RPW individuals from Egypt and KSA (Abulyazid *et al.*, 2002; Salama & Saker, 2002; Gadelhak & Enan, 2005; Al-Ayied *et al.*, 2006).

In the present study RAPD was used to investigate the genetic variation and relationships among and within RPW from thirteen different countries.

MATERIALS AND METHODS

RPW samples: Samples of RPW were collected from 27 localities belonging to thirteen infested countries (Table I). In the present study, the comparison was done at both the population and individual levels. Population signified one or more individuals from the same area, while individual referred to a single individual.

DNA extraction: Total genomic DNA was extracted from RPW samples using DNeasy Tissue Kit (Qiagen GmbH) according to the manufacturer's protocol.

PCR amplification and electrophoresis of PCR products: Six different 10 mers random primers were used for the amplification of PCR products. Sequences of these primers and protocols used for DNA amplification and electrophoresis are the same as Gadelhak and Enan (2005).

Scoring bands and the binary matrix: Amplified products which were bright, reproducible and consistent in performance were chosen for scoring of shared and unshared bands. The bands were scored as present and absent where the presence of a particular band was denoted as 1 and absence as 0.

Similarity matrix: The binary matrix was subjected to the MSVP software ver. 3.12 d (Kovach Computing Services, Wales, UK) in order to detect the genetic similarity coefficient using the Nei and Li coefficient (Nei & Li, 1979) method.

Genetic distances among RPW individuals: The genetic distances were estimated to investigate the level of DNA variation among all the tested individuals. Each individual was treated as an Operation Taxonomic Unit (OTU). A matrix of genetic distances was calculated from the

similarity matrix according to Abulyazid *et al.* (2002) as follows: 1-similarity index.

Average genetic distances among the geographic populations: Average genetic distances were calculated with MEGA 4 (Tamura *et al.*, 2007) in order to compare among the thirteen geographic populations of RPW (Table I). The level of DNA variation among the Egyptian local populations was also calculated. The Egyptian individuals were divided into fifteen different populations according to their locality of origin (Table I).

Cluster analyses: Cluster analysis was performed based on the genetic distance matrix by means of the Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Sneath & Sokal, 1973) with MEGA 4 (Tamura *et al.*, 2007).

RESULTS

Scored and polymorphic bands: The genetic variation among and within 13 geographic populations of RPW (Table I) was investigated using RAPD technique. The obtained RAPD profiles exhibited bands ranged from 200 to 1400 bp in length (Table II). All of the detected bands were polymorphic. The comparisons among RAPD profiles revealed that 17 RAPD markers were unique for the Egyptian local populations.

Comparison among populations: The average genetic distances were estimated to investigate the level of DNA variation among the 13 geographic populations of RPW (Table III). The lowest average genetic distance (0.3) was found between populations from KSA and UAE (0.268). The highest average genetic distance (approximately 0.8) was found between populations from KSA and Japan (0.759).

Average genetic distances among the fifteen local populations from Egypt: The average genetic distance among the 15 local populations from Egypt (Table I) ranged from 0.2 to 0.7 (Table IV).

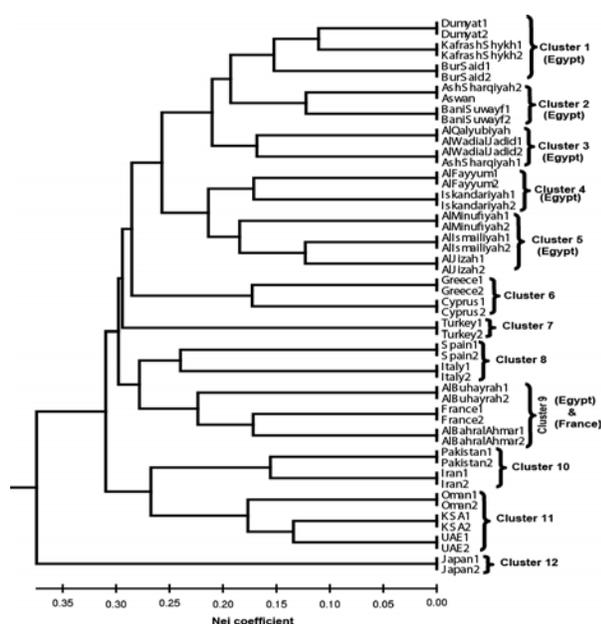
Comparison among individuals: the genetic distances among the tested individuals were estimated. The lowest genetic distance 0.241 was found between the individuals Bur Said1 and Turkey2, while the highest genetic distance 1 was observed between Al Minufiyah2 and Japan1. The genetic distances between individuals from the same country ranged from 0.154 between the two individuals of Cyprus to 1 between the two individuals of Japan. Regarding the Egyptian individuals, the lowest genetic distance 0.158 was found between AlJizah2 and AlIsmailiyah2. The highest genetic distance 0.786 was found between AlFayyum2 and Dumyat1.

Cluster analyses: The UPGMA dendrogram showed 12 clusters, out of which six clusters included the tested individuals from Egypt (clusters 1 to 5 & 9), one of these six clusters (cluster 9) contained in addition to Egyptian individuals (Al Bahr al Ahmar & Al Buhayrah) the two tested ones from France (Fig. 1).

Table I: Geographic populations and individual codes of RPW samples

Geographic populations	Individual codes
1 KSA	KSA 1&2
2 UAE	UAE1&2
3 Oman	Oman1&2
4 Turkey	Turkey1&2
5 Pakistan	Pakistan1&2
6 Iran	Iran1&2
7 Japan	Japan1&2
8 Spain	Spain1&2
9 Italy	Italy1&2
10 Greece	Greece1&2
11 Cyprus	Cyprus1&2
12 Franace	France1&2
13 Egypt	Egyptian local populations
	Al Bahr alAhmar
	Al Buhayrah
	Al Fayyum
	Iskandariyah
	Al Ismailiyah
	Al Jizah
	Al Minufiyah
	Al Qalyubiyah
	Al WadialJadid
	Ash Sharqiyah
	Aswan
	Beni Suwayf
	Bur Said
	Dumyat
	Kafr ashShykh

Fig. 1: Dendrogram of the thirteen geographic populations of RPW derived from the genetic distances



DISCUSSION

Genetic variation among 13 geographic populations of RPW was detected using RAPD. Previous studies have demonstrated genetic variation of RPW from different geographic areas using RAPD (Abulyazid *et al.*, 2002; Gadelhak & Enan, 2005). As well high genetic variation was detected among different geographical populations of other insect species using the same technique such as: *Bombyx mori* (Srivastava *et al.*, 2005); *Nebria gregaria* (Clarke *et al.*, 2001); *Culex quinquefasciatus* (Diptera: Culicidae) (Sharma *et al.*, 2009); *Aedes aegypti* (Hiragi *et al.*, 2009); *Trypanosoma cruzi* (Dib *et al.*, 2009; Lala *et al.*, 2009). In contrast, Marimuthu *et al.* (2009) found no genetic variation among different populations of shoot and fruit borer, *Leucinodes orbonalis* (Pyraustidae: Lepidoptera). This lack of genetic variation was explained by continuous gene flow between the different geographic populations.

An observed 100% polymorphism in RAPD markers has also been detected previously in the study of *Culex quinquefasciatus* (Diptera: Culicidae). 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. 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 & McInnis, 1994; Bardakci, 2000).

The average genetic distance among the thirteen geographic populations of RPW ranged from 0.3 to 0.8. Similarly, a high average genetic distance among different geographic populations was detected in *Bombyx mori* (0.140-0.513) (Srivastava *et al.*, 2005). The highest and lowest average genetic distances revealed that similarity among the analyzed populations ranged from 20% to 70%. Similarity ranges from 80 to 100% and 38 to 94% were observed previously among whitefly species and RPW populations from UAE respectively (Perring *et al.*, 1993; Gadelhak & Enan, 2005).

High values of genetic distances among the 61 individuals of RPW were observed as the genetic distance between the analyzed individuals ranged from 0.241 to 1. Similarly, high genetic distances were observed previously among individuals of RPW from UAE (Gadelhak & Enan, 2005). Also, high genetic distances (0.058-0.513) were detected in *Bombyx mori* (Srivastava *et al.*, 2005) and *Chironomus plumosus* (Diptera: Chironomidae) (Gunderina *et al.*, 2009). According to the observed genetic distances among the tested individuals there is a positive correlation between the genetic distances and the geographical distances.

RAPD partitioned RPW populations from different geographic origins as the tested populations were grouped in

Table II: Number of bands calculated from 13 geographic populations (61 individuals) of RPW

Primers	Number of bands													
	Egypt	KSA	UAE	Oman	Turkey	Iran	Pakistan	Japan	Spain	Italy	Greece	Cyprus	France	Total
Primer 1	100	5	6	6	4	5	6	4	2	6	5	5	5	159
Primer 2	69	2	7	2	3	8	7	2	2	2	7	2	3	116
Primer 3	74	8	5	5	3	7	4	3	5	5	3	4	4	130
Primer 4	59	2	3	3	4	6	4	4	3	3	3	3	4	101
Primer 5	68	4	4	4	6	6	7	4	5	5	5	6	4	128
Primer 6	87	4	4	3	5	4	4	4	2	8	7	6	4	142
Total	457	25	29	23	25	36	32	21	19	29	30	26	24	776

Table III: Average genetic distances among the 13 RPW geographic populations

Populations	KSA	UAE	Oman	Turkey	Pakistan	Iran	Japan	Spain	Italy	Greece	Cyprus	France
KSA												
UAE	0.268											
Oman	0.433	0.273										
Turkey	0.650	0.673	0.542									
Pakistan	0.607	0.607	0.590	0.468								
Iran	0.475	0.418	0.513	0.708	0.311							
Japan	0.759	0.731	0.685	0.649	0.704	0.576						
Spain	0.678	0.732	0.716	0.759	0.641	0.677	0.708					
Italy	0.643	0.689	0.659	0.649	0.662	0.665	0.810	0.479				
Greece	0.593	0.671	0.612	0.540	0.588	0.629	0.787	0.511	0.377			
Cyprus	0.707	0.666	0.647	0.627	0.700	0.659	0.815	0.653	0.691	0.345		
France	0.662	0.667	0.600	0.579	0.667	0.594	0.833	0.540	0.538	0.577	0.348	
Egypt	0.561	0.603	0.626	0.600	0.612	0.624	0.758	0.639	0.552	0.602	0.555	0.632

Table IV: Average genetic distances among the 15 Egyptian local populations of RPW

Egyptian local populations	Al Bahr alAhmar	Al Buhayrah	Al Fayyum	Iskandariyah	Al Ismailiyah	Al Jizah	Al Minufiyah	Al Qalyubiyahg	Al Wadi alJadid	Ash Sharqiyah	Aswan	Beni Suwayf	Bur Said	Dumyat
Al Buhayrah		0.349												
Al Fayyum	0.575	0.367												
Iskandariyah	0.516	0.502	0.343											
Al Ismailiyah	0.502	0.476	0.413	0.340										
Al Jizah	0.473	0.470	0.440	0.386	0.246									
Al Minufiyah	0.519	0.535	0.524	0.459	0.369	0.368								
Al Qalyubiyahg	0.628	0.457	0.522	0.436	0.535	0.485	0.300							
Al Wadi alJadid	0.585	0.624	0.581	0.434	0.461	0.508	0.446	0.195						
Ash Sharqiyah	0.570	0.661	0.629	0.474	0.546	0.529	0.535	0.480	0.372					
Aswan	0.640	0.738	0.651	0.527	0.500	0.581	0.540	0.394	0.449	0.209				
Beni Suwayf	0.540	0.724	0.583	0.435	0.542	0.580	0.591	0.371	0.497	0.288	0.156			
Bur Said	0.591	0.674	0.592	0.496	0.513	0.579	0.576	0.404	0.519	0.352	0.321	0.302		
Dumyat	0.540	0.659	0.616	0.476	0.441	0.462	0.545	0.455	0.562	0.426	0.500	0.450	0.284	
Kafr ashShykh	0.511	0.612	0.522	0.372	0.447	0.470	0.477	0.277	0.420	0.358	0.333	0.377	0.325	0.221

12 clusters (Fig. 1). The UPGMA dendrogram clearly showed three groupings: (1) the Japanese population, (2) the Egyptian and Mediterranean populations and (3) Arabic Peninsula, Iran and Pakistan populations. This result was fairly important, because it suggests that Egyptian and related Mediterranean populations have a different origin than Arabic Peninsula and Asian populations.

According to the dendrogram, not all the Egyptian individuals have direct relationships with local geographic pattern as some individuals from distant localities were clustered together: Cluster (2) Ash Sharqiyah, Aswan and Bani Suwayf; Cluster (3) Al Qalyubiyah, Al Wadi al Jadid

and Ash Sharqiyah; Cluster (4) Al Fayyum and Iskandariyah (Fig. 1). A significant positive correlation between genetic variation and geographical distance was found for boll weevil *Anthonomus grandis* (Coleoptera: Curculionidae) populations in the United States (Kim & Sappington, 2004). In contrast, Gadelhak and Enan (2005) observed that the genetic and geographical distances among RPW populations from UAE were not correlated.

Invasive populations derived from multiple introductions from different origins are expected to be genetically more diverse (Vieira *et al.*, 2007). The UPGMA analysis suggested that multiple introductions from different countries or a single introduction composed of genetically

diverse individuals may have occurred in Egypt as the Egyptian RPW individuals were clustered in six separate clusters (1, 2, 3, 4, 5, & 9) (Fig. 1), as well, the high genetic variability found within the Egyptian population (0.458) is supporting that finding.

In conclusion, our findings confirm that RAPD has the ability to distinguish among populations of RPW. The dendrogram and average genetic distances suggest that the RPW populations that invaded the Middle East and the Mediterranean area likely came from two different geographical origins. One is the source of the Egyptian and related Mediterranean populations and the other is the source of the Arabic Peninsula and Asian populations.

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