GENETIC VARIATION AND INVASION HISTORY
OF
RED PALM WEEVIL POPULATIONS
AND
GENETIC RELATIONSHIPS
AMONG DIFFERENT
RHYNCHOPHORUS SPECIES

BY

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INTRODUCTION

The knowledge of genetic variation of the invasive species is a necessary step before investigating the genetic basis of its rapid adaptation and consequently its invasion success (Keane & Crawley 2002; Sakai et al. 2001; Wolfe 2002). Furthermore, it is an essential topic before developing an effective management strategy (Grapputo et al. 2005; Marimuthu et al. 2006; Monnerat et al. 2006; Sharma et al. 2009).

There are ten known species belonging to Rhynchophorus genus: four New World: R. palmarum (Linnaeus), R. cruentatus (Fabricius), R. ritcheri (Wattanapongsiri) and R. quadrangulus (Queden); one African: R. phoenicis (Fabricius) and five tropical Asian: R. ferrugineus (Olivier), R. bilineatus (Montrouzier), R. distinctus (Wattanapongsiri), R. lobatus (Ritsema) and R. vulneratus (Panzer) (Wattanapongsiri 1966).

The Red Palm Weevil (RPW), R. ferrugineus (Olivier, 1790) (Coleoptera: Curculionidae) is an invasive species that is originated from Southeast Asia, it has invaded Middle East and several countries of the Mediterranean Basin, Europe, Caribbean and United States of America (USA) during the last three decades (El-Mergawy 2014). The pest attacks different palm trees causing economic damages, where the annual average of date palm damage estimated by 1.9 (Zaid 1999), 90% of the attacked palms were lost in Arab peninsula (Moore 2000), also, the production dropped from 0.7 to 10 tonne/hectar (Gush 1997).

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 (El-Mergawy 2014).

In this work, I will review the genetic variation studies done on different geographic RPW and the genetic relationships between this species and other R. species.


Mitochondrial DNA was successfully used in studies for genetic variation, phylogenetic analyses, geographic distribution and DNA barcoding of different insect species. The advantages of using such molecular markers in insects are due to their maternal inheritance, haploid status and high rate of evolution. Additionally, universal primers are available for insect species that their sequences are not known (Avise et al. 1987; Behura 2006; Hebert 2003; Irwin 1991; Kambhampati & Smith 1995; Lanave et al. 2002; Lunt et al. 1996; Meyer 1994; Roehrdanz 1993; Singh 2008; Zhang et al. 2000). Different regions of the ribosomal DNA (rDNA) gene have
been used successfully to investigate the genetic variation in different insect species (Campbell et al. 1993; Fenton et al. 1994; Paskewitz et al. 1993; Schmitz & Moritz 1998). The non-functional Internal Transcribed Spacer 2 Ribosomal DNA (ITS2-rDNA) sequences were considered as a valuable molecular tool for resolving relationships between insect geographical populations because of their fast evolutionary rate (Baldwin 1992; Chen et al. 2001; Keller et al. 2006; Sota & Vogler 2001). 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 (Bardakci 2000; Hadrys et al. 1992; Williams 1990). Microsatellites considered to be important molecular markers tools due to their high variability comparing to other molecular markers, as well they follow the co-dominant Mendelian genetics (Wang et al. 2009).
1 GENETIC VARIATION AMONG RPW MORPHOLOGICAL FORMS

Genetic variation was not detected among the different prothorax forms of RPW neither using mitochondrial markers (Cytochrome b (Cytb) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b, 2011c) and Cytochrome oxidase c subunit 1 (CO1)) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b, 2011c; Rugman-Jones et al. 2013), nor using intron regions (ITS2) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b; El-Mergawy et al. 2011c; Rugman-Jones et al. 2013) 28S-D2 (Rugman-Jones et al. 2013). On the other hand, random amplified polymorphic DNA (RAPD-PCR) patterns showed that: 1) black spotted forms were more related than the non spotted forms (Salama and Sakr 2002) and 2) Brown forms with & without black spots were more related than black non spotted forms (Al Ayied et al. 2006).

2 GENETIC VARIATION OF RPW POPULATIONS


2.1 MITOCHONDRIAL GENETIC VARIATION

Mitochondrial genetic variation and invasion history of RPW were investigated using Cytb (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b) and CO1 genes (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c; Rugman-Jones et al. 2013).

2.1.1 Cytb. RPW genetic variation using Cytb gene was investigated among RPW from invaded countries belonging to different continents such as 1) Africa: Egypt; 2) Asia: KSA, and Turkey; 3) Europe: Cyprus, France, Greece, Italy and Spain (mainland and Canary Islands) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b) (Figure 1).

A genetic variation of 4.2 % nucleotide substitutions was detected dividing the tested individuals into several haplotypes. A total of 3 haplotypes (El-Mergawy-HB1, El-Mergawy-HB2 & El-Mergawy-HB3) were detected (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b). However, no intra-specific variation was detected (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b). The haplotype El-Mergawy-HB1 was the most geographic distributed one, where it was found in 8 different countries belonging to three different continents: 1) Africa – Egypt, 2) Asia
Genetic Variation & Invasion History of RPW

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- KSA, Turkey and 3) Europe - Spain (mainland and Canary Islands), Italy, Greece, Cyprus and France (El-Mergawy 2011, 2012, 2013). The wide geographic distribution pattern of this haplotype may indicate a very high invasive potential when introduced by human, accordingly this haplotype was called the invasive haplotype (El-Mergawy 2011, 2012, 2013). The invasive haplotype was fixed in RPW from different localities in each country. This can be explained by a unique introduction event, a single successful one or multiple introductions of the same haplotype. Rapid expansion of the invasive haplotype in different localities could have resulted from a series of secondary invasion events through transportation of infested young or adult date palm trees and offshoots from contaminated to uninfected areas (El-Mergawy 2011, 2012, 2013). The other haplotypes were detected in Asian countries such as: Iran (El-Mergawy-HB2), Oman, Pakistan and UAE (El-Mergawy-HB3) (El-Mergawy 2011, 2012, 2013).

2.1.2 CO1. RPW genetic variation using CO1 gene was investigated among RPW from the claimed native countries such as Cambodia, India, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam (Rugman-Jones et al. 2013). As well RPW from invaded countries such as 1) Africa: Egypt (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c); 2) Asia: KSA (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c; Rugman-Jones et al. 2013), Israel (Rugman-Jones et al. 2013), Syria, and Turkey (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c); 3) Europe: Cyprus (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c; Rugman-Jones et al. 2013), France, Greece, Italy, Spain (mainland and Canary Islands) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c) and Portugal (Rugman-Jones et al. 2013); 4) South America: Curacao and Aruba (Rugman-Jones et al. 2013) (Figure 2).

A genetic variation of 4.2 % nucleotide substitutions was detected dividing the tested individuals into several haplotypes. A total of 43 haplotypes (H1-H43) were detected (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c; Rugman-Jones et al. 2013).

CO1 intra-specific variation was detected in Cambodia (2 haplotypes), Cyprus (2 haplotypes), India (5 haplotypes), Israel (2 haplotypes), KSA (2 haplotypes), Malaysia (2 haplotypes), Oman (3 haplotypes), Pakistan (2 haplotypes), Philippines (9 haplotypes), Sri Lanka (3 haplotypes), Thailand (6 haplotypes), UAE (4 haplotypes), Vietnam (8 haplotypes) (see Table 1 for references). The presence of more than one haplotype may due to the introduction of RPW from different source populations; or only one source, either through different introduction events or from a single one containing more than one haplotype (El-Mergawy 2011, 2012, 2013). The 43 CO1 haplotypes subdivided into two sisters' phylogenetic groups: group 1 included RPW from both native and invaded area while group 2 included RPW from native countries.

Genetic distances (GDs) among all the haplotypes ranged from 0.015 (H22 & H38) to 0.046 (H12 & H43). GDs between the El-Mergawy H8 haplotype and the other haplotypes ranged from 0.035 (El-Mergawy H8 & H12) to 0.019 (El-Mergawy H8 & H38).
Among the 43 detected CO1 haplotypes, 34 haplotypes were recovered from the native area while 10 haplotypes were recovered from the invaded countries. The El-Mergawy-H8 haplotype was the most geographic distributed one, where it was found in different countries and Islands belonging to four different continents:


4) South America: Curacao (Rugman-Jones et al. 2013).

The wide geographic distribution pattern of this haplotype may indicate a very high invasive potential when introduced by human, accordingly this haplotype was called the invasive haplotype (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b, 2011c). Rapid expansion of the invasive haplotype in different localities could have resulted from a series of secondary invasion events through transportation of infested young or adult date palm trees and offshoots from contaminated to uninfected areas (El-Mergawy 2011, 2012, 2013). The invasive haplotype (as well other haplotypes) was fixed in RPW from different localities in each country (El-Mergawy 2011, 2012, 2013). This can be explained by a unique introduction event, a single successful one or multiple introductions of the same haplotype (El-Mergawy 2011, 2012, 2013). The El-Mergawy-H8 haplotype was recovered from countries belonging to both the invaded (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c) and the claimed native area (Rugman-Jones et al. 2013). It was detected in Thailand and Malaysia; accordingly, Rugman-Jones et al. (2013) suggested that the geographical origin sources of RPW populations in the Mediterranean Basin were Thailand and Malaysia. The El-Mergawy H8 haplotype was grouped with the haplotypes from Cambodia, Philippines and Vietnam (Rugman-Jones et al. 2013), so more intensive sampling in these areas might uncover the El-Mergawy-H8 haplotype (Rugman-Jones et al. 2013). Rugman-Jones et al. (2013) hypothesized that RPW invaded the Middle East through Pakistan but Pakistan was not its source origin.

The other haplotypes that were detected in the invasive area such as El-Mergawy H1-H6 (Oman, Pakistan, Syria and UAE), El-Mergawy H7 (Japan) (El-Mergawy 2011, 2012, 2013), H17 (Israel and KSA), H20 (Aruba), and H33 (Cyprus) (Rugman-Jones et al. 2013) were not detected in the native range. However, El-Mergawy H1-H7 grouped with haplotypes H9-H16 that were detected from claimed native area; India and Sri Lanka (Rugman-Jones et al. 2013). Rugman-Jones et al. (2013) mentioned that it was likely that the origin of El-Mergawy H7 from an area in the north western part of the native range, not sampled in this study; perhaps in India, Bangladesh, or Myanmar. The H20 haplotype was detected in Aruba a neighboring Island to Curacao. Rugman-Jones et al. (2013) hypothesized the presence of H20 in Aruba and not in the other invaded countries as follow: RPW was introduced once to the Caribbean, where H20 was a rare haplotype so it was not detected in the other
invaded countries, H20 was a post invasion mutation of El-Mergawy H8 haplotype as the two differed in only one nucleotide or RPW was introduced more than one time to the Caribbean from similar native area. The H33 haplotype was detected in Cyprus, there were 11 nucleotides difference than the El-Mergawy H8 haplotype, and it was grouped with the El-Mergawy H8 haplotype and the haplotypes that were detected from Cambodia and the Philippines (Rugman-Jones et al. 2013).

Demographic parameters such as Tajima's D neutral test, FST (genetic differentiation) and Nm (gene flow) values calculated among the different geographical populations showed that the tested invaded populations of RPW diverged genetically under the influence of genetic drift likely through multiple founder events (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011b, 2011c).

2.2 NUCLEAR GENETIC VARIATION


This absence of genetic variation can be explained by a strong concerted evolution (Elder and Turner 1995; Graur and Li 2000).

2.3 MICROSATELLITE MARKERS

Capdevielle-Dulac et al. (2012) isolated 15 polymorphic microsatellite markers from RPW. These markers will probably show more variability when studied on populations of the area of origin of the species. They will also help identifying the sources of the invading populations and discovering the invasion pathways.

2.4 RAPD-PCR PATTERN

In the third study genetic variation among thirteen geographic populations of RPW (61 samples) collected from Egypt, KSA, Turkey, Spain (mainland and Canary Islands), Italy, Greece, Cyprus, France, Iran, Japan, Oman, Pakistan, and UAE, was investigated using RAPD-PCR (El-Mergawy 2011, 2012, 2013) (Figures 3 and 4).

All of the detected bands (PCR products) (776) were polymorphic (100% polymorphism) and ranged from 200 to 1400 bp in length using the six RAPD primers (El-Mergawy 2011, 2012, 2013). 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 comparisons among RAPD profiles revealed that 17 RAPD markers were unique for the Egyptian local populations. These unique RAPD markers can be used as genetic markers that can distinguish the geographic populations of RPW from each other (Haymer and McInnis 1994; Bardakci 2000).
Cluster analyses of RPW populations showed that:

1) RPW subdivided according to their geographic positions into two major groups (El-Mergawy 2011, 2012, 2013) (Figure 3):

(I) The Multi-continent group: included RPW from countries belonging to three continents; Africa, Asia and Europe.

(II) The Asian group: subdivided into two Asian groups:

II-1) Included Japanese RPW and,

II-2) Included other Asian RPW.

Genetic similarities among the three groups ranged from: 30-40 % between 1) group I and II-1 and 2) group I and II-2, while it ranged from 20-30 % between group II-1 and II-2 (El-Mergawy et al. 2010; 2012, 2013).

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) (Figure 4), as well, the high genetic variability found within the Egyptian population (0.458) is supporting that finding.

Genetic similarities

Comparison among RPW from different countries:

Geographic populations (RPW from different countries). Genetic similarities ranged from 0 to 70 % among RPW geographical populations (Abulyazid et al. 2002; El-Mergawy 2011, 2012, 2013), where it was 0 % between Egypt and KSA (Abulyazid et al. 2002), 20 % (between KSA and Japan, and 70 % between KSA and UAE (El-Mergawy 2011, 2012, 2013).

Individuals (RPW individuals from different countries). Genetic similarities ranged from 0% between individuals from Egypt (AlMinufiyah2) and individuals from Japan (Japan1) to 80% between individuals from Egypt: (BurSaid1) and individuals from Turkey (Turkey2) (El-Mergawy 2011, 2012, 2013).

Comparison among RPW from the same country:

Local populations (RPW individuals from different localities in the same country). Genetic similarities ranged from 30 to 94 % among local populations (Abulyazid et al. 2002; Gadahlak and Enan 2005; El-Mergawy 2011, 2012, 2013).

**Individuals (RPW individuals from the same country).** Genetic similarities ranged from 0 % (Cyprus) to 80 % (Japan) (El-Mergawy 2011, 2012, 2013).

**Egyptian individuals.** Genetic similarities ranged from 20% (between AlJizah2 and AlIsmailiyah2) to 80 % (between AlFayyum2 and Dumyat1) (El-Mergawy 2011, 2012, 2013).

**According to the observed genetic distances:**

- RAPD analysis revealed that genetic similarities among RPW populations were higher than those among individuals. Genetic similarities ranged from 0 to 70 % among geographical populations (Abulyazid et al. 2002; El-Mergawy 2011, 2012, 2013) and 30 to 94 % among local populations (Abulyazid et al. 2002; Gadelhak and Enan 2005; El-Mergawy 2011, 2012, 2013). On the other hand, genetic similarities among RPW individuals from either same country or different countries ranged from 0 to 80 % (El-Mergawy 2011, 2012, 2013).

- At the geographic population level: there was positive correlation between the genetic distances and the geographic distances among the tested geographic populations of RPW from 13 different countries (El-Mergawy 2011, 2012, 2013). In contrast, no correlation was found among RPW from Egypt, KSA and Indonesia, where RPW from KSA was found to be related to RPW from Indonesia but not RPW from Egypt (Abulyazid et al. 2002).

- At the local geographic pattern: 1) there was no correlation between the genetic distances among individuals from different localities in UAE and the geographic ones (Gadelhak and Enan 2005) and 2) not all the Egyptian individuals have direct relationships with local geographic pattern as some individuals from distant localities were clustered together: 1) Ash Sharqiyah, Aswan and Bani Suwayf, 2) Al Qalyubiyah, Al Wadi al Jadid and Ash Sharqiyah and Al Fayyum and Iskandariyah (El-Mergawy 2011, 2012, 2013).

**Conclusions:**


2) The western area divided in the West between Middle East, where 6 haplotypes were found and the Mediterranean basin where the invasive haplotype El-Mergawy H8 (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c), in addition to H17 (Israel and KSA) H20 (Aruba) and H33 (Cyprus) (Rugman-Jones et al. 2013).

3) These three invasion roads are corresponded to three different genetic lineages of RPW populations that had independent evolutionary histories (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c).

4) 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 Arabian Peninsula (KSA, UAE & Oman) and Asian populations (Pakistan & Iran) (El-Mergawy 2011, 2012, 2013; El-Mergawy et al. 2011c).

3 GENETIC COMPARISON AMONG RPW AND OTHER RHYNCHOPHORUS SPP.

3.1 TAXONOMIC STATUS OF RHYNCHOPHORUS SPP.

Rugman-Jones et al. (2013) suggested that genetic studies using different loci and/or cross-mating studies might due to the detection of Rhynchophorus cryptic species (Figure 5).

**R. ferrugineus** and **R. vulneratus**. Hallett et al. (2004) believed that *R. vulneratus* was a color morph of RPW as the two were found to be alike in morphological characters, RAPD banding patterns, CO1 DNA, host plant preference, pheromone production and response, the lack of reproductive isolating mechanism and the existence of color inter-morphs (Hallett 1996; Hallett et al. 1993, 2004; Perez et al. 1996). Rugman-Jones et al. (2013) reported that the morphological differences were not evident to distinguish between *R. ferrugineus* and *R. vulneratus*. Accordingly, they proposed that the palm weevils from Singapore, Sumatra, Java and Bali be referred as *R. vulneratus* while those from the north and east of the Thai-Malay Peninsula is referred as *R. ferrugineus*.

**R. ferrugineus**. It was reported that the CO1 divergence level of species boundaries (3%) (Hebert et al. (2003, 2004) (as cited in Rugman-jones et al. (2013))). Rugman-jones et al. (2013) mentioned that the 43 CO1 haplotypes of RPW were divided into 3 sub-groups differed from each other by 2.5 to 3.2%. Hence, they assumed that one or more of these subgroups might represent a separate cryptic species.

**R. vulneratus**. Rugman-Jones et al. (2013) detected 3 substitutions differences within *R. vulneratus* ITS2 sequences.

3.2 GENETIC VARIATION AMONG RHYNCHOPHORUS SPP.

**CO1**. Rugman-jones et al. (2013) reported that the CO1 divergence between *R. bilineatus, R. ferrugineus* and *R. vulneratus* was >13%.

**ITS2**. Rugman-jones et al. (2013) reported that *R. ferrugineus* differed from *R. vulneratus* with 11-14 substitutions and a single base deletion, while *R. bilineatus* differed from both *R. ferrugineus* and *R. vulneratus* with several substitutions and 24 nucleotide deletions. Accordingly, they confirmed that *R. ferrugineus* and *R. vulneratus* are different species from *R. bilineatus*.

**28S-D2**. Rugman-jones et al. (2013) did not detect any differences between *R. bilineatus* and *R. vulneratus* 28S-D2 sequences. While they found two substitutions differences between *R. ferrugineus* and *R. bilineatus*.

3.3 Geographic distribution of *Rhynchophorus* spp. Haplotypes

Rugman-Jones *et al.* (2013) cited that:

1) the distribution of *R. bilineatus* haplotypes (RB1-RB8) restricted to Papua New Guinean,

2) *R. ferrugineus* haplotypes (43 haplotypes) had a northern and western distribution, and

3) *R. vulneratus* haplotypes (Rv1-Rv62) had a more southeastern distribution (Singapore, the Indonesian islands of Sumatra, Java, and Bali, and the invasive population in California).

4) *R. ferrugineus* and *R. vulneratus* haplotypes overlapped in southern Thailand and northern Malaysia on the Thai-Malay Peninsula. The geographic area of overlapping was close to Although Wattanapongsiri (1966) suggested that *R. ferrugineus* and *R. vulneratus* present in the Philippines, Rugman-Jones *et al.* (2013) did not detect the presence of *R. vulneratus* in the Philippines.

EL-Mergawy (2011, 2012, 2013, 2014) reported that the mitochondrial (CO1) genetic similarities among *R. bilineatus*, *R. cruentatus*, *R. ferrugineus*, *R. palmarum* and *R. vulneratus* ranged from 91.9 to 94.2%.

*R. palmarum* appeared as the most distantly related species to the currently analyzed ones (EL-Mergawy 2011, 2012, 2013).
FIGURES AND TABLES
**Figure (1):** Phylogenetic tree of Cytb haplotypes of RPW: the tree was reconstructed using: maximum parsimony (MP) method. Bootstrap support values (1000 replicates) are indicated above the lines (El-Mergawy 2011, 2012, 2013, 2014).
Figure (2): Phylogenetic tree of COI haplotypes of RPW: the tree was reconstructed using: Neighbor Joining (NJ) method. Bootstrap support values (1000 replicates) are indicated above the lines (El-Mergawy, unpublished).
**Figure (4):** Cluster analyses of Egyptian RPW-UPGMA dendrogram (El-Mergawy 2011, 2012, 2013, 2014).
Figure (5): Adult stage of different *Rhynchophorus* spp. Images source: (1) Giblin-Davis (2010); (2) Rugman-Jones *et al.* (2013).
**Table (1):** Geographical distribution of RPW CO1 haplotypes.

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Different shadings refer to different phylogenetic groups.

* El-Mergawy (2012, 2013); El-Mergawy *et al.* (2011c)

# Rugman-Jones *et al.* (2013)
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