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Genetic Diversity Among Populations of Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), Determined by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR)

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ABSTRACT

This study reports the genetic variations between populations of the red palm weevil, *Rhynchophorus ferrugineus* in the UAE area. To test if the red palm weevil population in the UAE belongs to the same genetic group; the randomly amplified polymorphic DNA (RAPD) technique was used to assay seven populations of the insect from different locations. RAPD primer pairs detected a total 216 RAPD fragments in these populations and 111 of these showed polymorphism (51.4%). Cluster analysis of RAPD data clearly separated these populations into three groups with a genetic variability between (0.38-0.94). Genetic and geographical distances among all populations were correlated

Key Words: RAPD; Red palm weevil; *Rhynchophorus ferrugineus;* DNA finger printing

INTRODUCTION

The Red palm weevil, *Rhynchophorus ferrugineus (Olivier)* is one of the most destructive pests of date palms, *Phoenix sylvestris* Rox., and *Phoenix dactyleferous* in the Arab Gulf States (Bokhari & Abuzuhari, 1992), Egypt (Salama & Hamdy, 2001), and of coconut, *Cocos nucifera* L., in South and Southeast Asia (Sivapragasam *et al.,* 1990; Sadakathulla, 1991). During the last decade, multiple introductions of *R. ferrugineus* to the Middle East from India and Pakistan have occurred. Often, palm weevil infestations are not detected before damage caused by larval mining in the trunk is extensive and it is not possible for the tree to recover (Sivapragasam *et al.*, 1990). In date palms, the only visible sign of attack may be oozing out of palm sap from the trunk, and infestations are often not discovered until trees are blown over. Delay between detection and destruction permits emergence and migration of adult weevils prior to destruction. Transporting infested trees and offshoots for burning introduces the weevil to new areas.

Consistent introduction of new date palm varieties into the region poses a considerable chance for the insect population to change genetically. Given the high distribution capacity of the insect due to strong flight capabilities, its population is suspected to show a sort of diversity. External morphological studies would not provide enough evidence for such diversity. The RAPD technique provides a convenient and rapid assessment of the differences in the genetic composition of related individuals and has been employed in a large number of plants for the determination and assessment of genetic diversity (Williams *et al*., 1990; Welsh & McClelland, 1990).

As a result of the great genetic diversity which exists in insect species and even within populations of a same species (Apostol *et al.,* 1993; Stevens & Wall, 1995), DNA fingerprinting appears as a reliable method for typing insect cell lines. The power of the polymerase chain reaction (PCR) in insect studies is well recognized since this approach, in contrast with the analysis of restriction fragment length polymorphism, requires only nanogram amounts of DNA which can even be partially degraded; even small parts of the insects are sufficient to produce a diagnostic band pattern (Kambhampati *et al*., 1992).

Among the different nuclear marker technologies, that are currently available for monitoring genetic diversity, random amplified polymorphic DNA (RAPD) represent a rapid, simple and cost–effective tool to assess insect populations (Lou *et al*., 1998, Ross *et al*., 1999; Biron *et al*., 2000; Moya *et al*., 2000; Naber *et al*., 2000). Scataglini *et al.* (2000) used random amplification of polymorphic DNA (RAPD) markers to analyze genetic structuring and gene flow among populations of boll weevils in South America collected from native and cultivated cotton. Their analyses suggest that despite the apparent range expansion in South America beginning in the early 1980s, populations of the boll weevil were already present on native hosts before the introduction of cultivated cotton.

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. The RAPD technique has been widely used to elucidate the geographical origin of, gene flow among insect populations (Vandewoestijne & Baguette, 2002; Ayres *et al*., 2003), including curculionid weevils (Williams *et al*., 1994; Armstrong & Wratten, 1996; Taberner *et al*., 1997; Bas *et al.*, 2000; Scataglini *et al.*, 2000). 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 (Black, 1993; Lynch & Milligan, 1994; 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 (Armstrong & Wratten, 1996; Brown *et al*., 1997; Vaughn & Antolin, 1998; Pearson *et al*., 2002).

In this study, polymorphic RAPD markers were used to examine genetic diversity of red palm weevil populations from different geographical origins. We chose RAPD markers because of their potential for revealing greater genetic diversity.

MATERIALS AND METHODS

Red palm weevil (RPW) collection. Random samples were collected from seven sampling locations throughout the UAE. Sample identification numbers and their corresponding locations are shown in Fig. 1.

DNA extraction. Total genomic DNA was extracted from red palm weevils using genomic prep cells and tissue DNA isolation kit (Amersham Pharmacia Biotech, UK), according to the manufacturer's protocol. The isolated DNA was diluted in 50 ul Tris-EDTA buffer (10mM Tris-HCl, pH 8.0, & 1mM EDTA), and subsequently electrophoresed in 0.8% agarose gel following standard protocols of Sambrook *et al*. (1989).

RAPD primers. Random sequence 10 mer primers were obtained from Amersham Pharmacia (Biotech, UK).

Standardized reaction beads and PCR amplification. The Ready-to-go RAPD analysis kit (Amersham Pharmacia Biotech, UK) is supplied as microfuge tubes containing a dried bead, which includes premixed dNTPs, buffer and a mixture of Ampli Taq and Stoffel fragments. According to the manufacturer's specifications, the mixture of these two thermo stable polymerases produces a cleaner and more complex pattern than each acting alone. Each bead contains AmpliTaq DNA polymerase, stoffel fragment dNTPs, bovine serum albumin (BSA), reaction buffer, and an inert medium. They were stored in the dark at constant room temperature (25 ^{o}C). Before PCR, beads were rehydrated with sterile deionized water. Final concentration are in a total of 25 ul reaction mixture including 1.5 units of Taq, 10mM Tris-HCl (pH 9.0), 50mM KCl, 1.5 mM $MgCl_2$, 200 uM of each dNTPs and stabilizers including BSA in addition to the 25 p*M* of the primers. DNA amounts added were 50 ng aliquots. All RAPD amplifications were according to a scheme of 5 min at 95 ^{o}C for the first cycle; 1 min at 95 ^{o}C, 1 min at 36 ^{o}C and 2 min at 72 ^{o}C for the first 45 cycles; and 5 min at 72 ^{o}C for the last one. PCRs were performed using Perkin-Elmer 24000 (Techne, UK).

Electrophoresis of PCR products. RAPD products were analyzed by electrophoresis in 1.8% agarose gel pre-stained with ethidium bromide and run at 10 V/cm for 3.5 h in 1X TBE buffer. Gels were observed under UV illumination (UV Transilluminator, TFX 35 Life Technologies, CA, USA) and photographed images were acquired with Gel-Doc 1000 (Bio-Rad Laboratories, CA, USA). The molecular size of the amplified products was estimated using a Phi X 174 DNA/Hae III marker (Invitrogen, Life Technologies, CA, USA).

Scoring bands and statistical analysis Individual DNA bands were scored as present or absent (1/0) in the amplification profile of each sample. Only clear DNA bands were scored. Primers that gave either faint bands or a too high background were discarded from further analysis. Genetic similarity was calculated with the Jaccard similarity index. A dendrogram was constructed after cluster analysis of the Similarity coefficients by the un-weighted pair-group method analysis, UPGMA (Sneath & Sokal, 1973).

RESULTS

We investigated the genetic variability of seven populations of the RPW collected from UAE from various locations (Fig. 1). The RAPD profiles obtained with the RAPD primers exhibited bands between 194 and 1078 bp in length (Fig. 2). In a total of 216 bands scored only 111 were polymorphic (51.4%); 27 with primer 1, 25 with primer 2, 30 with primer 3, 61 with primer 4, 38 with primer 5, and 35 with primer 6, with an average of 36 bands per primer (Table I).

The dissimilarity values were constructed to estimate the level of DNA polymorphism among all seven populations. The calculation of the dissimilarity values was based on the presence or absence of discrete characters (RAPD bands). The dissimilarity values ranged from 0.06 to 0.6 (Table II). The dissimilarity values showed that population-4 and population-5 are most similar to each other followed by population-6 and population-7 where the dissimilarity values were 0.06 and 0.13 respectively. Genetic distance (dissimilarity) between population -2 and population -3 was 0.32, which is lower than any of them to population-1. The genetic similarity of other populations ranged between the values mentioned above. Population-1 appeared to be the most distant within this group and showed higher genetic distance to populations 4, 5, 6 and 7 than to population-2 and population-3.

Fig. 1. A map showing the major locations of RPW sampling. Random samples were taken within each location for the RAPD analysis.

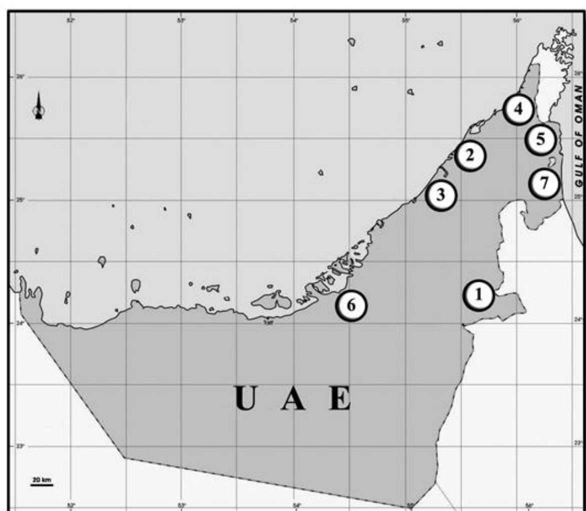
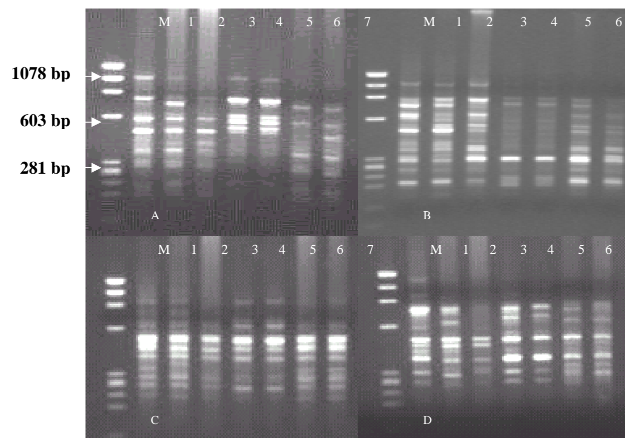


Fig. 2. Agarose gel electrophoretic patterns showing the RAPD profile for seven populations of RPW using primers 3 (A), 4 (B), 5 (C) and 6(D). M is the molecular size marker phi-X174 Hae III.



The dissimilarity value was then used to cluster the data using unweighted pair-group method with arithmetic average (UPGMA). The Dendrogram showed three clusters in addition to a single sub-cluster (Fig. 3). Clusters one and two included populations-4, population -5 and population-6, population-7; respectively, while the third cluster comprised population-1 separated from a sub-cluster enclosing population-2 and population-3.

DISCUSSION

Genetic differences among populations of RPW were detected. Variations indicated that patterns were comparable to those previously reported in other RPW species studies. Salama and Saker (2002) concluded that the major genetic variations detected among the three forms of the RPW collected from Egyptian date palm may be either due to the generation of new mutant by the weevil or the three forms may belong to different new varieties.

The dendrogram shows that there is a close relationship (0.06 dissimilarity) between population-4 and population-5 and similarly between population-6 and population-7 (0.13), whilest a wide genetic distance between population-2 and population–5 (0.6) (Fig. 3). These observations are in accordance with several other studies based on an identical method of arbitrary primed DNA fingerprinting with the M13 primer, where the genetic variation was low in local populations of air-borne basidomycetes, *Heterobasidion annosum* and *Fomitopsis*

Fig. 3. Dendrogram of the seven populations of red palm weevils derived from the genetic dissimilarity index.

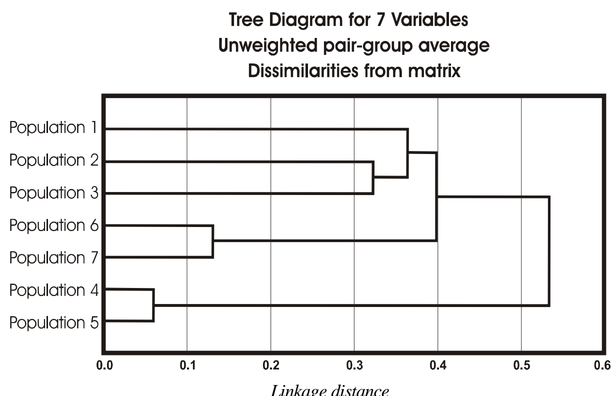


Table I. Sequence of RAPD primers, Number of bands and percentage of polymorphisms calculated from seven samples of RPW.

Primers	Sequence (5'—3')	Number of bands studied	Percentage of polymorphism
Primer 1	GGTGCGGGAA	27	84 %
Primer 2	GTTTCGCTCC	25	72.0 %
Primer 3	GTAGACCCGT	30	53.3 %
Primer 4	AAGAGCCCGT	61	65.5 %
Primer 5	AACGCGCAAC	38	7.9 %
Primer 6	CCCGTCAGCA	35	60.0 %

Table II. Dissimilarity matrix of the red palm weevil populations using Jaccard coefficient

	$Popl_1$	$Popl_2$	$Popl_3$	$Popl_4$	$Popl_5$	$Popl_6$	$Popl_7$
$Popl_1$	0						
$Popl_2$	0.404	0					
$Popl_3$	0.324	0.323	0				
$Popl_4$	0.454	0.599	0.550	0			
$Popl_5$	0.409	0.617	0.571	0.058	0		
$Popl_6$	0.390	0.394	0.444	0.523	0.511	0	
$Popl_7$	0.439	0.314	0.411	0.536	0.558	0.129	0

pinicola, although a certain degree of separation was detected in distance range over 500 km. (Karlsson, 1994; Hogberg *et al*. 1995).

The RAPD technique has been successfully applied in characterizing clones and detecting clonal diversity in parthenogenetic insects (Black *et al.,* 1992; Fong *et al.,* 1995) and in a wide variety of plants (Hsia & Rieseberg 1994; Stiller & Denton 1995; Van de ven & McNicol, 1995; Piquot *et al.,* 1996; Ayres & Ryan, 1997). Since the first reports on the discriminating power of RAPD-DNA typing in insect-related ecological questions (Hadrys *et al.,* 1993; Gawel & Bartlett, 1993), an increasing number of scientists made a quick and highly informative DNA typing method. Nowadays, endangered species (Nasser *et al.,* 1996), inbreeding in wildlife species (Shankaranarayanan *et al.,* 1997) in addition to several insects like beetles (Brown *et al.,* 1997; Apostol *et al.,* 1996); flies (Nasser *et al.,* 1996), dragonflies (Hadrys *et al.,* 1993; Hooper *et al.,* 1996) and a variety of bacterial, animal and plant species (Benecke, 1997; Veilleux *et al.,* 1995) were successfully DNA typed with RAPDs. The greatest distance (0.6) and the smallest distance (0.06) observed in this study (Table II) indicated that populations fall between 38% to 94% similarity. Previous study using RAPD-PCR analyses of whitefly species revealed that similarity ranges from 80-100% (Perring *et al.,* 1993). We concluded that there is no significant genetic variation by distance among seven population of RPW in the United Arab Emirates. Similarly, Scataglini *et al.* (2000) reported that RAPD analyses did not show significant genetic isolation by distance among neighboring populations of boll weevils in Southern Brazil, Paraguay and northern Argentina. In contrast Kim & Sappington (2004) found a significant positive correlation between genetic variation and geographical distance covering boll weevil populations in the United States. Natural variation due to beneficial mutations, chromosomal rearrangements and mitotic recombination is large enough to cause the observed difference in RAPD-PCR profiles between the RPW populations**.**

Based on this genetically derived numerical distance, it is obvious that the level of dissimilarity among all the insect populations in this study is high enough to reflect that no two insects groups may belong to the same species and this requires further attention. Meanwhile, genetic distance data showed that all tested insect groups belong to the same genus. Nonetheless, one of the important outcomes of this study is the ability to distinguish between populations of RPW using only small number of RAPD primers.

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REFERENCES

Apostol, B.L. W.C. Black, P. Reiter and B.R. Miller, 1996. Population genetics with RAPD–PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. *Heredity,* 76: 325–34

Apostol, B.L., W.C. Black, B.R. Miller, P. Reiter and B.J. Beaty, 1993. Estimation of the number of full sibling families at an oviposition site using RAPD–PCR markers: applications to the mosquito *Aedes aegypti*. *Theor. Appl. Genet.*, 86: 991–1000

Armstrong, K.F. and S.D. Wratten, 1996. The use of DNA analysis and the polymerase chain reaction in the study of introduced pests in New Zealand. *In:* Symondson, W.O.C. and J.E. Liddell, (eds.) *The Ecology of Agricultural Pests: Biochemical Approaches,* pp. 231–63. Chapman & Hall, London.

Ayres, C.F.J., M.A.V. Melo–Santos, A.M. Solé–Cava and A.F. Furtado, 2003. genetic differentiation of *Aedes aegypti* (Diptera: Culicidae), the major dengue vector in Brazil. *J. Med. Entomol*., 40: 430–5

Ayres, D.R. and F.J. Ryan, 1997. The clonal and population structure of a rare endemic plant, *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. *Mol. Ecol.,* 7: 761–72

Bas, B., Z. Dalkilic, T.L. Peever, H.N. Nigg, S.E. Simpson, F.G.J. Gmitter, and R.C. Adair, 2000. Genetic relationships among Florida *Diaprepes abbreviatus* (Coleoptera: Curculionidae) populations. *Ann. Entoml. Soc. Am.*, 93: 459–67

Benecke, M., 1997. DNA typing in today's forensic medicine and criminal investigations. A current survey. *Naturwissenschaften,* 84: 181–8

Biron, D.G., B.S. Landry, J.P. Nenon, D. Coderre and G. Boivin, 2000. Geographical origin of an introduced pest species, *Delia radicum* (Diptera: Anthomyiidae), determined by RAPD analysis and egg micromorphology. *Bull. Ent. Res.*, 90: 23–32

Black, W.C., N.M. DuTeau, G.J. Puterka, J.R. Nechols and J.M. Pettorini, 1992. Use of random amplified polymorphic DNA polymorphisms in aphids. Bull. of Entomol. Res., 82: 151–9

Black, W.C.I.V., 1993. PCR with arbitrary primers: approach with care. *Insect Mol. Biol.*, 2: 1–6

Black, W.C.I.V., C.F. Baer, M.F. Antolin and N.M. DuTeau, 2001. Population genomics: genome–wide sampling of insect populations. *Annu. Rev. Entomol*. 46: 441–69

Bokhari U.G. and R. Abuzuhari, 199.2. Diagnostic tests for red palm weevil, *Rhynchophorus ferrugineus*, infested date palm trees. *Arab Gulf J. Sci. Res.*, 10: 93–104

Brown, R.J., C.A. Malcolm, P.L. Mason and R.A. Nichols, 1997. Genetic differentiation between and within strains of the saw–toothed beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) at RAPD loci. *Insect Mol. Biol.*, 6: 285–9

Fong G., M.A. Walker and J. Granett, 1995. RAPD assessment of California *Phylloxera* diversity. *Mol. Ecol.,* 7: 459–64

Gawel, N.J. and A.C. Bartlett, 1993. Characterization of differences between whiteflies using RAPDPCR. *Insect Mol. Biol.,* 2: 33–8

Hadrys, H., B. Schierwater, S.L. Dellaporta, R. DeSalle and L.W. Buss, 1993. Determination of paternity in dragonflies by random amplified polymorphic DNA fingerprinting. *Mol. Ecol.*, 2: 79–87

Hogberg, N., J. Stenlid and J.O. Karlsson, 1995. Genetic differentiation in *Fomitopsis pinicola* (Schwarts:Fr.) Karst. Studied by means of arbitrary primed–PCR. *Mol. Ecol.*, 4: 675–80

Hooper, R.E. and M.T. Siva–Jothy, 1996. Last male sperm precedence in a damselfly demonstrated by RAPD profiling. *Mol. Ecol.,* 5: 449–52

Hsia, J.Y. and L.H. Rieseberg, 1994. Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. *Mol. Ecol.*, 7: 201–8

Kambhampati, S., W.C. Black and K.S. Rai, 1992. Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis, and applications. *J. Med. Entomol.*, 29: 939–45

Karlsson, J.O., 1994. Genetic variation in *Heterobasison annosum* detected with M13 fingerprinting and ribosomal DNA probes. *Experimental Mycology,* 18: 225–36

Kim, K.S. and T.W. Sappington, 2004. Genetic structuring of boll weevil populations in the US based on RAPD marker. *Insect Mol. Biol.*, 13: 293–303

Lou, K.F., M.J. Weiss, P.L. Bruckner, W.L. Morrill, L.E. Talbert, J.M. Martin, 1998. RAPD variation within and among geographic populations of wheat stem sawfly (*Cephus cinctus* Norton). *J. Hered.*, 89: 329–35

Loxdale, H.D., C.P. Brookes and P.J. De Barro, 1996. Application of novel molecular markers (DNA) in agricultural entomology. *In:* Symondson, W.O.C. and J.E. Liddell, (eds.), *The Ecology of Agricultural Pests: Biochemical Approaches,* pp. 149–98. Chapman & Hall, London.

Lynch, M. and B.G. Milligan, 1994. Analysis of population Genetic structure with RAPD markers. *Mol. Ecol*., 3: 91–9

Moya, A., P. Guirao, D. Cifuentes, F. Beitia and J.L. Cenis, 2000. Genetic diversity of Iberian population of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA–polymerase chain reaction. *Mol. Ecol.*, 10: 891–7

Naber, N., M. El Bouhssini, M. Labhilili, S.M. Udupa, M.M. Nachit, M. Baum, S. Lhaloui, A. Benslimane and H. El Abbouyi, 2000. Genetic variation among populations of the Hessian fly *Mayetiola destructor* (Diptera: Cecidomyiidae) in Morocco and Syria. *Bull. Ent. Res.*, 90: 245–52

Nasser, J.A., R.M. Goto, D.B. Ledig, R.C. Fleischer and M.M. Miller, 1996. RAPD analysis reveals low genetic variability in the endangered light–footed clapper rail. *Mol. Ecol.*, 5: 463–72

Pearson, C.V.M., A.D. Rogers and M. Sheader, 2002. The genetic structure of the rare lagoonal sea anemone, *Nematostella vectensis* Stephenson (Cnidaria: Anthozoa) in the United Kingdom based on RAPD analysis. *Mol. Ecol.*, 11: 2285–93

Perring, T.M., C.A. Farrar, A.D. Cooper and T.S. Bellows, 1993. Determining whitefly species. *Science,* 261: 1333–5

Piquot Y., P. Saumitou–Laprade, D. Petit, P. Vernet and J.T. Epplen, 1996. Genotypic diversity revealed by allozymes and oligonucleotide DNA fingerprinting in French populations of the aquatic macrophyte *Sparganium erectum*. *Mol. Ecol.,* 7: 251–8

Ross, K.G., D.D. Shoemaker, M.J.B. Krieger, C.J. DeHeer and L. Keller, 1999. Assessing genetic structure with multiple classes of molecular markers: A case study involving the introduced fire ant *Solenopsis invicta*. *Mol. Biol. Evol.*, 16: 525–43

Sadakathulla, S., 1991. Management of red palm weevil, *Rhynchophorus ferrugineus* F., in coconut plantations. *Planter,* 67: 415–9

Salama, H.S. and M.K. Hamdy, 2001. Some ecological parameters of the egg and larval stages of the red pal weevil, *Rhynchophorus ferrugineus* (Oliv.) (Coleoptera) (Curculionidae). *Arch. Phytopath. Pflan.*, 34: 155–64

Salama, H.S. and M.M. Saker, 2002. DNA Fingerprints of Three Different Forms of the Red Palm Weevil Collected from Egyptian Date Palm Orchards. *Arch. Phytopath. Plant Protec.,* 35: 299–306

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Scataglini, M.A., V.A. Confalonieri and A.A. Lanteri, 2000. Dispersal of the cotton boll weevil (Coleoptera: Curculionidae) in South America: evidence of RAPD analysis. *Genetica,* 108: 127–36

Shankaranarayanan, P., M. Banerjee, R.K. Kacker, R.K. Aggarwal and L. Singh, 1997. Genetic variation in Asiatic lions and Indian tigers. *Electrophoresis*, 18: 1693–700

Sivapragasam, A., A. Arikiah and C.A. Ranjit, 1990. The red stripe weevil, *Rhynchophorus schach* Olivier (Coleoptera: Curculionidae): an increasing menace to coconut palms in Hilir Perak. *Planter,* 66: 113–23

Sneath, P.H.A. and R.R. Sokal, 1973. *Numerical Taxonomy; the Principle and Practice of Numerical Clasiification.* W.H. Freeman, San Francisco.

Stevens, J. and R. Wall, 1995. The use of random amplified polymorphic DNA (RAPD) analysis for studies of genetic variation in populations of the blowfly *Lucilia sericata* (Diptera: Calliphoridae) in southern England. *Bull. Entomol. Res.,* 85: 549–55

Stiller, J.W. and A.L. Denton, 1995. One hundred years of *Spartina alterniflora* (Poaceae) in Willapa bay, Washington: random amplified polymorphic DNA analysis of an invasive population. *Mol. Ecol.*, 7: 355–63

Taberner, A., J. Dopazo and P. Castanera, 1997. Genetic characterization of populations of a de novo arisen sugar beet pest, *Aubeonymus mariaefranciscae* (Coleoptera, Curculionidae), by RAPD analysis. *J. Mol. Evol.,* 45: 24–31

Van de Ven W.T.G. and R.J. McNicol, 1995. The use of RAPD markers for the identification of Sitka spruce (*Picea sitchensis*) clones. *Heredity,* 7: 126–32

Vandewoestijne, S. and M. Baguette, 2002. The genetic structure of endangered populations in the cranberry fritillary, *Boloria aquilonaris* (Lepidoptera, Nymphalidae): RAPDs vs allozymes. *Heredity,* 89: 439–45

Vaughn, T.T. and M.F. Antolin, 1998. Population Genetics of an opportunistic parasitoid in an agricultural landscape. *Heredity,* 80: 152–62

Veilleux, R.E., L.Y. Shen and M.M. Paz, 1995. Analysis of the genetic composition of anther–derived potato by randomly amplified polymorphic DNA and simple sequence repeats. *Genome,* 38: 1153–62

Welsh, J. and M. McClelland, 1990. Fingerprinting genome using PCR with arbitrary primers. *Nucleic Acid Res.*, 18: 6531–5

Williams, C.L., S.L. Goldson, D.B. Baird and D.W. Bullock, 1994. Geographical origin of an introduced insect pest, *Listronotus bonariensis* (Kuschel), determined by RAPD analysis. *Heredity,* 72: 412–9

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.*, 18: 6531–5

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