J. Egypt. Acad. Soc. Environ. Develop., 18 (1):161- 169 (2017) (D-Environmental Studies)
E.mail: easedjournal_egypt@yahoo.com ISSN 1110-8770
www.eased.byethost13.com eISSN 2314-5471 (online)

Development of specific DNA markers for two isolated populations of the desert locust, *Schistocerca gregaria* (Orthopetra: Acrididae) using RAPD technique

Hatem A. Ibrahim
Entomology Department, Faculty of Science, Ain Shams University
Email: haweish@yahoo.com, hatemibrahim@sci.asu.adu.eg
orcid.org /0000-0002-0457-8227

ABSTRACT

By amplification of pools of genomic DNA from two isolated gregarious locust populations in Africa with short decamer primers of random sequences, we identified markers which can distinguish the two major locust populations: northern *Schistocerca gregaria gregaria* and southern *Schistocerca gregaria flaviventris*. Twenty primers were examined with the genomic DNA from the two sexes of both populations. Four primers produced polymorphic patterns at low molecular weight. One primer (A2) displayed a specific amplified band with the DNA obtained from southern *S. g. flaviventris* locust which was not recognized in the northern *S. g. gregaria*. Further examination by using southern blot technique revealed that, this sequence was highly specific to the southern locusts. It is proposed that, *S. flaviventris* locusts may represent an evolutionary dichotomy which took place in the African continent. The results of this study suggest that it is possible to separate the two locusts populations into two different species.

Key words: *Schistocerca gregaria gregaria, Schistocerca gregaria flaviventris*, Locust populations, geographical isolation, RAPD-PCR, Southern blot hybridization.

INTRODUCTION

In Africa, two subspecies of the desert locust are recognized; the northern *Schistocerca gregaria gregaria* (Forskal 1775) and the southern *Schistocerca gregaria flaviventris* (Burmeister 1838) (Meinzingen, 1993; FAO, 2014). Between plagues, *S. g. gregaria* inhibits a recession area covering about 14 million $km^2$of deserts and semi deserts and extending from Sahara desert to western Asia. The invasion area of the southern *S. g. flaviventris* covers an area of about 860,000 km^2in Namibia, Angola, South Africa, Ascension Islands and Botswana. It is recognized as subspecies separated from the northern population by the degree of phase changes, colouration and morphometrics (Nolte, 1965; Botha, 1967; Waloff, 1976; Harvey, 1981; Waloff and Pedgley, 1986; Lovejoy *et al*., 2006)

In the past few years, there has been a remarkable increase in the application of the DNA techniques to problems in population genetics and systematic. There are several approaches for assaying DNA variations in population of organisms. Williams *et al*. (1990), Welsh and McClelland (1990) described a facile means of obtaining genetic markers which is not dependent on prior sequence information and which may be technically accessible to a wider range of molecular taxonomists (Lovejoy *et al*., 2006; Dushimirimana *et al*., 2012).The Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD) uses short oligonucleotoide decamer primers of random sequences to obtain patterns from repetitive units located at numerous locations throughout the genome. DNA segments amplified with RAPD-PCR are inherited in a Mendalian manner in many organisms (Williams *et al*., 1990; De Barro *et al*., 2011; Martinez- Blanch *et al.*, 2011). RAPD markers have used to generate molecular markers in a variety of insect studies for population differentiation, genetic studies and species diagnosis (Gasperi *et al*., 1993; Wilkerson *et al*., 1993; Gawel and Barlett, 1993; Hymer *et al*.,1993; Black, 1993; Favia *et al*., 1994; Sairkar *et al.*; 2013, Turki *et al.*, 2014).

This study demonstrates the use of RAPD-PCR to differentiate between two locust populations in Africa. The effect of the geographical barrier on the isolation of these populations has discussed.

MATERIALS AND METHODS

Insects

Schistocerca gregaria gregaria were collected from International Center of Insect Physiology and Ecology (ICIPE) field station located near the Red Sea (Sudan), while *Schistocerca gregaria flaviventris* were collected from the Plant Protection Institute, Pretoria, Republic of South Africa Institution. The F1 generations of both populations were collected after two weeks of maturation and frozen in 70^{o} C. for experiments.

Isolation of DNA

High molecular-weight template genomic DNA was prepared from individual locusts using method of Flook *et al*. (1992). For PCR reactions, DNA concentrations estimated by Qene-Gene II spectrophotometer (Pharmacia Inc., Piscataway, NJ, USA). DNA samples were diluted in double distilled water to final concentration of 20 ng/ ul. The diluted samples were stored at 4^{o} C. pools of DNA were prepared by mixing equal volumes of DNA from twenty individual insects of each sex.

PCR amplification

PCR reactions were performed in a total volume of 25 ul. Each reaction assembled on ice and contained 2.5 ul of 10X reaction buffer (500 mM KCL, 100 mM ris/ HCL (pH 9.1), 1.0 % Triton X-100, 2.0 mM MgCL2, 1000mm dNTPs (25 mM each) (Promega Biotech Inc., USA), 5 picomoles of each 10- base primers kit A (Operon Tech. Inc, alameda, USA), 0.5 U Taq polymerase (Promega) and 60 ng of the template DNA.. Control reactions were included and contained the reaction mixture without any DNA.

PTC-100 Programmable thermal controller (MJ Research Inc., Mass., USA) was used with the following cycles: first, the tubes heated to 95^{0}C (5 min) and then cycled 45 times using 94^{o}C (1 min), 33^{o}C (1 min) and 72 ^{o}C (2 min). A final extension was carried out at 72^{o}C (10 min). After amplification, the Samples were stored at 4^{o}C (Innis *et al*., 1990). The PCR products were separated by electrophoresis at 2.7 V/cm volts on 2% agarose gels and visualized under UV illumination after Ethidium Bromide (5 ul/ml) staining (Sambrook *et al*., 1989).

Cloning of PCR products

Following PCR amplification of DNA samples using A2 primer, the products were resolved in 1 % low melting point agarose gel. The band of interest (600 base pairs) was excited from the gel and DNA extracted by Gene-Clean II kit (Bio 101, USA). The recovered DNA was then cloned in the pMOS vector (Amersham, UK) and the recombinant plasmids transformed into pMOS cells (provided in the kit).The cloned were then sequenced by DNA sequencing kit, version 2.0 (USB, USA).

Genomic Southern blot analysis

Genomic DNA samples (10-12 ug) were exhaustively digested with EcoR I and resolved by electrophoresis in a 1 % agarose gel overnight at 1.5 V/cm. DNA fragments were transferred onto nitrocellulose membrane. The cloned vector as probe was labeled with (p 32) dCTP (Amersham, Bucks, UK) by the nick translation method (Sambrook *et al*., 1989). Hybridization was carried out over night at 65^{o} C. Following hybridization, the membrane

was washed three times (10 min each) at room temperature followed by a final washing in 0.1X SSC/ 0.1 % SDS at 65^{o} C. Autoradiography was carried out using a Fuji- RX film.

RESULS

Twenty primers were used to amplify pooled genomic DNA samples obtained from both males and females of two populations. Amplification with primers A2; A12; A18 and A20 (sequences of the twenty primers of group A listed in Table (1), produced RAPD patterns that clearly distinguished between the two locust populations (Fig.1). Typical patterns of bright bands together with faint bands detected. The fragments that differentiated between the two locust groups found in the low molecular weight region (100-600 bp).

Primer A2 which produced diagnostic bands with pooled DNA samples was subjected to further screening. This primer was used to amplify DNA samples from 10 individual locusts (5 males and 5 females) of each population in order to test its diagnostic ability to investigate the amount of the genetic variability between the two locusts. In this test, the amplification patterns obtained were consistent for any given DNA template. A distinct band (600bp), clearly visible on Ethidium Bromide stained agarose gels, was produced with DNA samples of both males and females from southern *S. g. flaviventris* only (Fig. 2). The band was absent in all samples from northern *S. g. gregaria.*

Southern blot analysis of genomic DNA

Genomic DNA samples from north and south locusts were hybridized with the cloned 600 bp product of southern locusts. The results showed strong hybridization to individual and pooled samples prepared from male (Fig. 3) and female (Fig. 4) insects. This result showed that these sequences are highly repeated in the genome of the southern population but not in the northern population.

Computer analysis of Sequence data

The DNA sequence of the 600 bp clone was compared with the other sequences available in the Gene-Bank. No homology was observed with the sequences of either male and female clones. The sequence of the clones had submitted to the Gene-Bank, accession numbers: U60233 (male) and U60266665 (female).

DISCUSSION

Pools of genomic DNA were used for PCR analysis of the two isolated populations of the desert locust. In previous studied of the identification of markers of loci controlling disease resistance in plants (Michelmore *et al*., 1991) and differentiation of two cattle species (kemp and Teale, 1994), pools of genomic DNA were constructed from individuals samples of a givens population. The justification was that; the constitution of such pools is random for all genetic regions except those contributing to the selected phenotype. The RAPD fragments which appear to be unique to pooled templates, would have high probability of being linked to the gene controlling the trait and high lighting consistent differences between the pools, while masking variability common to the pools (Lopez *et al*., 2008; Lovejoy *et al*., 2006; Dushimirimana *et al*., 2012; Amer and Elsayed, 2014). Wilkerson *et al*. (1993) also used pooled genomic DNA samples from cryptic mosquito species to study the ability of fifty-seven primers to produce RAPD patterns.

Twenty primers were screened for their diagnostic ability. Four primers (A2; A12; A18 and A20) produced polymorphic fragments that distinguished between southern from northern locust populations. One of the problems with RAPD technique is the lack of reproducibility of the bands. Three primers (A12; A18; A20) did not show consistent

amplification patterns when applied to different individuals from both populations (data not shown). This may be due to the sensitivity of the DNA templates to amplification conditions.

The A2 primer, which generated polymorphic fragments, was screened further for population specificity in RAPD- PCR of pools and individuals of the two locust populations. The 600 bp product which by screening, was apparently found in all pools and individuals (males, females) of *S. g. flaviventris* and not shown in the amplification patterns of the DNA samples of *S. g. gregaria*.

In order to examine the specificity of this 600 bp product produced by the A2 primer, the clones were used to probe Southern blot of EcoRI digested genomic DNAs of southern and northern males and females as well as pooled samples. The results revealed that this sequence was highly restricted to *flaviventris* males and females and not found in all *gregaria* individuals. This suggests that A2 detects priming sites polymorphism in southern population and that priming sites is in repetitive DNA elements. The southern blotting approach proved simple, rapid and effective. It overcomes a number of problems potentially associated with the RAPD techniques. Dot blots technique can be used also for detecting the repetitive nature of the RAPD markers. (Favia *et al.*,1994; *Teale et al.*, 1995; Sairkar *et al*., 2013; Turki *et al*., 2014).

Regions of the genome that contain repetitive sequences or inverted terminal repeats may contain a great number of sites amenable to RAPD amplification than the coding sequence regions. Repetitive DNA can change by a variety of translocation mechanisms of the sequences on the chromosome and the more important for systematic, the exact base can change (point mutation) by small deletions and insertions, but most commonly by substitutions. If two populations were isolated from one another they will accumulate independent substitutions and their DNA sequences will diverge (Posy *et al*., 1992; Gawel and Bartlett, 1993; Lopez *et al*.; 2008; Turki *et al*., 2014).

The results demonstrated that RAPD-PCR technique is able to document differences in the DNA genomes of the southern *flaviventris* and northern *gregaria* populations of the African desert locusts. These differences in the repetitive copies of the DNA are attributed to the geographical barrier (natural isolation) which prevented the genetic constitutions to flow between these locust populations where sexual mating is prohibited. F1 hybrid generation between the two populations was recorded sterile (Dcik Brown, Personal communication). Completely isolated population exists in a biotic environment that is different from any other, and this shift of the biotic environment adds another powerful selection pressure. Competition, predation and other ecological interactions are apt to be entirely different. This local condition exerts selection pressures reinforcing the steady change of gene contents and leading to the development of numerous new adjustment. On the other hand, contiguous central populations are in the midst stream of multidirectional gene flow and harbor at all times a large store of freshly added immigrant gene (Mayr, 1970). This suggests that it is possible to separate the two populations into different species.

This approach may have general use as a means of revealing DNA sequences that are characteristic of entire species and of genetically distinct populations. Additionally, this method can be used in developing molecular markers in the Acrididae locusts and other insect species.

Development of specific DNA markers for two isolated populations of the desert locust, *Schistocerca gregaria* (Orthopetra: Acrididae) using RAPD technique

Table 1:RAPD primers (kit A) used in the differentiation of the desert locust populations, *Schistocerca gragaria* .

KIT A

code	5′ to 3′	M.W.	pmoles	ug/tube
OPA-01	CAGGCCCTTC	2955	6014	18.0
OPA-02	TGCCGAGCTG	3035	5493	16.5
OPA-03	AGTCAGCCAC	2988	5192	15.5
OPA-04	AATCGGGCTG	3059	5088	15.5
OPA-05	AGGGGTCTTG	3090	5192	16.0
OPA-06	GGTCCCTGAC	2995	5742	17.0
OPA-07	GAAACGGGTG	3108	4625	14.5
OPA-08	GTGACGTAGG	3099	4892	15.0
OPA-09	GGGTAACGCC	3044	5158	15.5
OPA-10	GTGATCGCAG	3059	5088	15.5
OPA-11	CAATCGCCGT	2979	5531	16.5
OPA-12	TCGGCGATAG	3059	5088	15.5
OPA-13	CAGCACCCAC	2933	5493	16.0
OPA-14	TCTGTGCTGG	3041	5783	17.5
OPA-15	TTCCGAACCC	2939	5783	17.0
OPA-16	AGCCAGCGAA	3037	4710	14.5
OPA-17	GACCGCTTGT	3010	5654	17.0
OPA-18	AGGTGACCGT	3059	5088	15.5
OPA-19	CAAACGTCGG	3028	4988	15.0
OPA-20	GTTGCGATCC	3010	5654	17.0

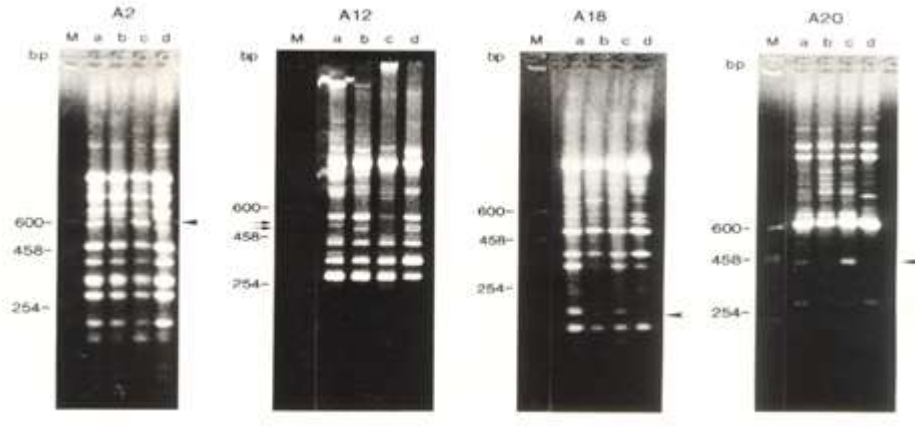

Fig. 1: RAPD implication products of DNA pools from two locust population with four primers (A2, A12 , A8 , A20) . The arrow indicates the polymorphic bands in the two locusts. a=males *S. g. flaviventris* ; b= males *S. g. gregaria* ; c= females *S. g. falviventris* ; d= females *S. g. gregaria* ; m= DNA size markers (Blue scripts sk+/ HeaIII digest)

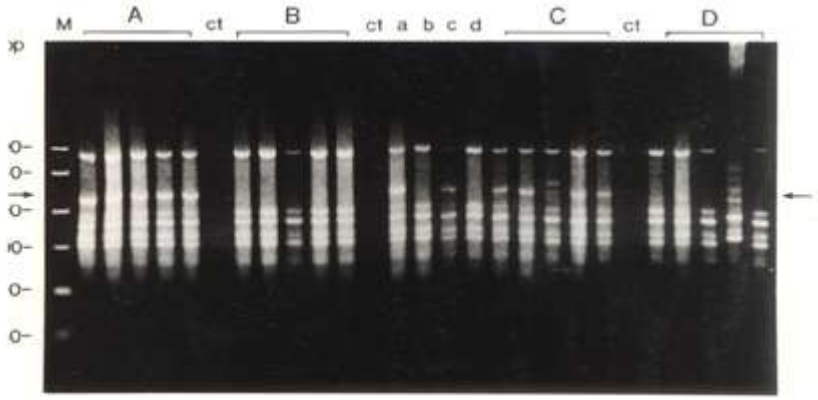

Fig. 2: RAPD amplification products from DNA individuals and pools of the two locust populations with primer A2. The arrow pointed to the 600 bp specific bands in the individuals and pools of the south locust , *S. g. flaviventris*. A, a= individual s and pools DNA of male *S. g. flaviventris*; B,b= individual s and pools DNA of male *S. g. gregaria* ; C,c = individual and pools and DNA of females *S.g. flaviventris* ; D, d = individuals and pools DNA of females *S.g. gregaria* ;ct= control samples without DNA templates , M= DNA size marker (Promega).

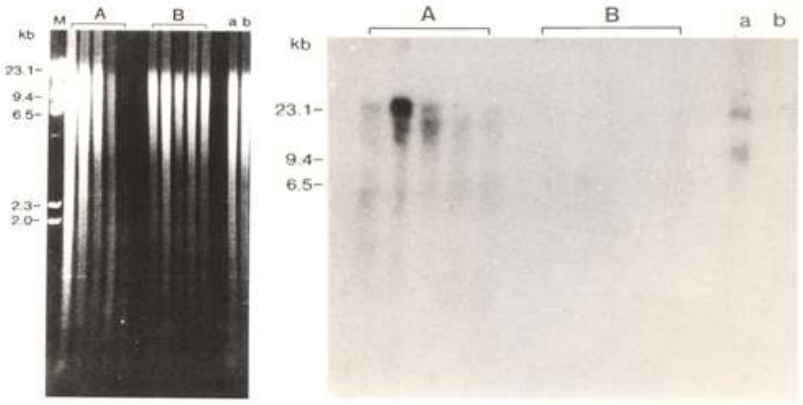

Fig. 3: Autoradiograph following a Southern blot of genomic DNA of the north and south African locust males with the cloned 600 bp RAPD- PCR south male product . The cloned product can be seen to hybridize to multiple amplifications of the males of *S. g. flaviventris* but not to males of *S. g. gregaria*. A, a= individuals and pools DNA of males *S.g. flaviventis*, B, b= individuals and pools DNA of males *S. g. gregaria.*

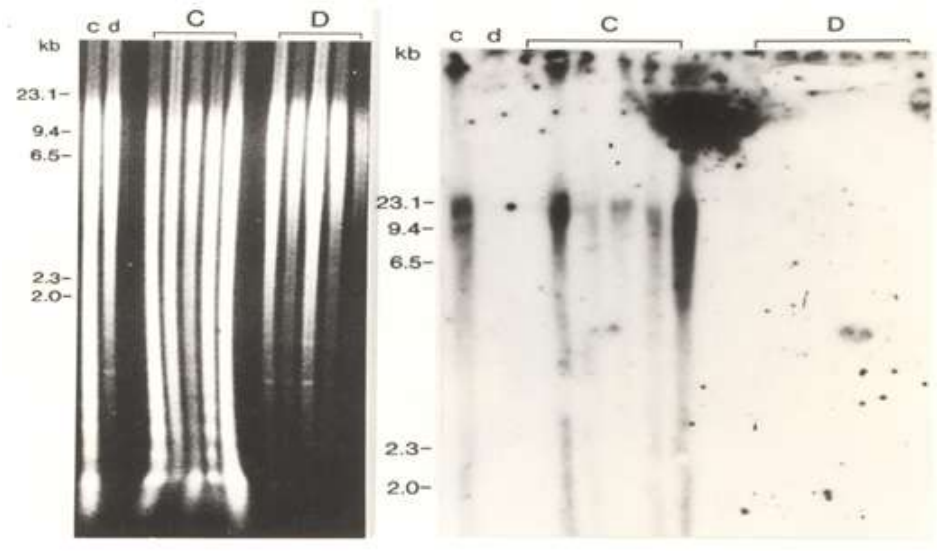

Fig. 4: Autoradiograph following a Southern blot of genomic DNA of the north and south African locust females with the cloned 600 bp RAPD-PCR south female product the cloned product can be seen to hybridize to multiple amplifications of females of *S. g. flaviventris* but not any to the females of *S. g. flaviventris*; D,d = individuals and pools DNA of females *S.g. gregaria.*

REFERENCES

Amer, S.A.M. and Elsayed, G. (2014). Comparative isozyme electrophoresis between the brown- spotted locust, *Cyrtacanthacris tatarica,* and the desert locust, *Schistocerca gregaria*. J. Insect Sci., 14:20. Available online: www. insectscience.org/14.20.

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

Botha, D.H. (1967). Some phase characteristics of the southern African from the desert locust *Schistocerca gregaria* (Forskal). S. Afr. J. Agric. Sci., 10: 61-67.

De Barro, P.J.; Lui, S.; Boykin, L.M. and Dinsdale, A.B. (2011). *Bemisis tabaci* : A statement of species status. Ann. Rev. Entomol., 56: 1-19.

Dushimirimana, S.; Hance, T. and Damiens, D. (2012). Comparison of reproductive traits of regular and irradiated male desert locust *Schistocerca gregaria* (Orthoptera: Acrididae): Evidence of last- male sperm precedence. Biology Open, 1: 232-236.

FAO (2014). Desert locust in Africa. Food and Agriculture Organization annual report.www.FAO.org

Favia, G.; Dimopoulos, G. and Louis, C. (1994). Analysis of the *Anopheles gambiae* genome using RAPD markers. Insect Mol. Biol., 3 (3): 149-157.

Flook, P.K.; Wilson, M. D. and Post, R.J. (1992)The use of repetitive DNA probes in the analysis of natural populations of insects and parasites. In Gene in Ecology. Ed. Berry, R. J., Crawford, T. J. and Hewitt, G. M., Oxford, UK.: 484-486.

Gasperi, G.; Malacrida, A.R.; Guglielmino, C.R.; Baruffi, L.; Toriti, C.; milani, R.; Damiani, G. and Bandi, C. (1993). *Ceratitis capitata*: suitable markers for population genetics and genome organization analysis. In: Management of insect pests: Nuclear and related molecular and genetic techniques. Ed. International Atomic Energy, Vienna: 251-256.

Gawel, N.J. and Bartlett, A.C. (1993) Characterization of differences between white flies using RAPD-PCR. Insect Molecular Biology 2 (1): 33-38.

Harvey, A.W. (1981). A reclassification of the *Schistocerca americana* complex (Orthopetra: Acrididae). Acrida, 10: 61-67.

Hymer, D. and McInnis, D. (1993). Polymerase chain reaction amplification of RAPD markers to distinguish populations of the Mediterranean fruit fly *Ceratitis capitata*. In: Management of insect pests: nuclear and related molecular and Genetic technique. Ed. International Atomic Energy, Vienna: 149-152.

Innis, A.M.; Celfand, D.H.; Sninsky, J.J. and White, J.T. (1990). PCR protocols. A guide to methods and applications. Academic Press, NY, USA.

Lopez, I.; Torres, C. and Ruiz-Larrea, F. (2008). Electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) of wild *Lactobacillus plantarum* and *Oenococcus oeni* wine strains. European Food Res. and Technol., 227 (2): 547- 555.

Lovejoy, N.R.; Mullen, S.P.; Sword, G.A.; Chapman, R.F. and Harrison, A. (2006). Ancient trans-Atlantic flight explains locust biogeography: molecular phylogenetics of *Schistocerca*. Proc. R. Soc., B7 273 (1588): 767- 774.

Kemp, S.J. and Teale, A.J. (1994). Randomly primered PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiate *Bosindicus* and *B. Taurus*. Animal Genetics, 25: 83-88.

Martinez- Blanch, J.F.; Sanchez, G.; Garay, E. and Eznar, R. (2011). Evaluation of phenotypic and PCR based approaches for routine analysis of *Bacillus cereus* group foodborne isolates. Leeuwenhoek, 99(3): 697- 709.

Mayr, E. (1970). Population, Species and Evolution. Belknap, Harvard Univ. Press, Cambridge, Uk.

Meinzingen, W. F. (1993). A guide to migrant pest management in Africa. FAO, AGP, Rome, Italy.

Michelmore, R.W.; Paran, I. and Kesseli, R.V. (1991). Identification of markers linked to disease resistance genes by bulked segregate analysis, a rapid method to detect markers in specific genomic regions by using segregation populations. Proc. National Academy of Science of the USA 88: 9829- 9832.

Nolte, D.J. (1965). The pigmentation of locusts. S. Afr. J. Sci., 61: 173- 178.

Post, R.J.; Flook, P.K. and Wilson, M.D. (1992). DNA analysis in relation to insect taxonomy, evolution and identification. In Insect Molecular Science. Ed. Crampton, J. M. and Eggleston, Academic Press, London, UK: 21-34.

Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning. a laboratory manual, Cold spring Harbor Laboratory Press, NY, USA.

Sairkar, P.; Shweta, C.; Batav, N. and Sharma, R. (2013). Optimization of DNA isolation process and enhancement of RAPD-PCR for low quality genomic DNA of *Terminalia arjuna*. J. Genetic Engineering and Biotechnol., 11(1): 17- 24.

Teale, A.J.; Wambugu, J.; Gwakisa, P.S.; Stranzinger, G.; Bradley, D. and Kemp, S.J. (1995). A polymorphism in randomly amplifiewd DNA that differentiate Y chromosome of differentiate *Bosindicus* and *B. Taurus*. Animal Genetics, 26: 243-248.

Turki, Y.; Mehri, I.; Fhoula, I.; Hassan, A. and Quzari, H. (2014). Comparison of five molecular subtyping methods for differentiation of *Salmonella Kentucky* isolates in Tunisia. World J. Microbiol. and Biotechnol., 30 (1): 87- 98.

Waloff, Z. (1976). Some temporal characteristics of desert locust plagues. Anti- locust Memoir, 13: 1- 36.

Welsh, J. and McClelland, M. (1990). Fingerprinting genome using PCR with arbitrary primers. Nucl. Acids Res., 18: 7213- 7218.

Development of specific DNA markers for two isolated populations of the desert locust, *Schistocerca gregaria* (Orthopetra: Acrididae) using RAPD technique

Wilkerson, R.C.; Parsons, T.J.; Albright, D.G.; Klein, T.A. and Braun, M.J. (1993). Random amplified polymorphic DNA (RAPD) markers readily distinguish cryptic mosquito species (Diptera: Calcididae: Anopheles). Insect Mol. Biol., 1 (4): 205- 211.

Williams, J.G.K.; Kubelik, A.R.; Livk, K.J.; Rafaliski, J.A. and Tingey, S.V. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531- 6535.

إنتاج علامات من الدنا لمجتمعين معزولين من الجراد الصحراوي شيستوسيركا جريجاريا (أورسوبيترا: أكريديدا) باستخدام طريقة الربيد

حاتم عبد الفتاح إبراهيم
1- قسم علم الحشرات، كلية العلوم، جامعة عين شمس، القاهرة، مصر.

تم استخدام مجموع من الدنا من مجتمعين من الجراد الصحراوي في إفريقيا لتضخيمه مع بادئات صغيرة في الطول لها ترتيب عشوائي. وقد تم تحديد علامات مميزة للتمييز بين المجتمعين، النوع الشمالي والنوع الجنوبي. تم استخدام عشرون بادئ مع الدنا للجنسين من المجتمعين. أربع بادئات فقط أنتجت اختلافات في الدنا. البادئ رقم A2 اظهر حزمه من الدنا مميزه للمجتمع الجنوبي وليست موجودة في المجتمع الشمالي. وباستخدام طريقة الطبع الجنوبي للدنا وجد أن هذه الحزمة مميزة للجراد الجنوبي ولا توجد في الجراد الشمالي. هذا يؤدى إلى إن الجراد الجنوبي حدث له تغير تام عن الجراد الشمالي وانه لا بد من فصل الجراد الجنوبي إلى نوع جديد مختلف تماما عن النوع الشمالي من الجراد.