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Genetic variation among populations of the Hessian fly *Mayetiola destructor* (Diptera: Cecidomyiidae) in Morocco and Syria

N. Naber[1], M. El Bouhssini[2*], M. Labhilili[2], S.M. Udupa[2], M.M. Nachit[3], M. Baum[2], S. Lhaloui[4], A. Benslimane[5] and H. El Abbouyi[1]

[1]Faculty of Science, University of Chouaib Doukkali, El Jadida, Morocco: [2]International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria: [3]International Maize and Wheat Improvement Center (CIMMYT)/ICARDA, Aleppo, Syria: [4]Institut National de la Recherche Agronomique (INRA), Settat, Morocco: [5]Faculty of Science, University Kadi Ayyad, Semlalia, Marrakech, Morocco

Abstract

The RAPD–PCR technique was used to study genetic variation within and among geographical populations of the Hessian fly, *Mayetiola destructor* (Say), from Morocco and Syria, associated with the fly's ability to overcome resistance in three wheat cultivars containing H5, H13 and H22 resistance genes. Variation was detected both for the level of susceptibility of the cultivars and RAPD profiles of *M. destructor* populations. By the use of RAPD–PCR, high genetic variability was detected among individuals and populations of *M. destructor* within and between areas separated geographically. The DNA fingerprints of populations of *M. destructor* were area-specific with Nei's measures of genetic distance ranging from 0.156 (between Abda and Beni Mellal, Morocco) to 1.977 (between Marchouch, Morocco and Lattakia, Syria). Cluster analysis of the genetic distances among the populations, identified the Syrian population as an outlier. A highly significant correlation (r = 0.81) observed between the genetic and geographic distances among the populations, provided genetic support for dispersal of the fly from its presumed origin in West Asia to Morocco.

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a destructive pest of wheat *Triticum* spp. (Poaceae) throughout most of its production areas, including North America and western Mediterranean countries. *Mayetiola destructor* is believed to have originated in southwest Asia (the centre of diversity for *Triticum*), from where it dispersed to southern Europe and north Africa (Ratcliffe & Hatchett, 1997). It is also believed to have been introduced into the United States in the straw bedding of Hessian soldiers during the American Revolution (Black *et al.*, 1990), and was first found infesting wheat on Long Island, New York in 1779. The insect now occurs in all major wheat-growing areas of the USA.

In Morocco, losses due to *M. destructor* are estimated to represent 42% and 32% of bread wheat (*Triticum aestivum* subsp. *aestivum* L.) and durum wheat (*T. turgidum* subsp. *durum* Desf.) crops, respectively (Lhaloui *et al.*, 1992).

The most practical method of controlling *M. destructor* has been the use of resistant cultivars. In the USA, 27 genes for resistance, designated H1 to H27, which are effective against this pest have been identified in *Triticum* species and

* Author for correspondence
Fax: +963 21 222 134 90
E-mail: m.bohssini@cgiar.org

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Secale cereale L.(Poaceae) (Cox & Hatchett, 1994; Ohm *et al.*, 1997). In Morocco, only ten of these genes (H5, H7H8, H11, H13, H14H15, H21, H22, H23, H25 and H26) were found to be effective (El Bouhssini *et al.*, 1997). Resistance genes are either dominant, partially dominant, or recessive. The mechanism of resistance expressed by most of these genes is antibiosis, where first-instar larvae die after feeding on resistant plants. However, plant genotypes carrying H1H2, H7H8 and H18 genes allow some larval survival on resistant plants (El Bouhssini *et al.*, 1998).

Because of the highly specific gene-for-gene relationship between wheat and *M. destructor* (Hatchett & Gallun, 1970), biotypes of the fly have evolved as a result of selection pressure exerted by large-scale growing of resistant cultivars with the same genes for resistance. In the USA, 16 biotypes have been identified from field populations, designated Great Plains (GP) and A to O (Ratcliffe *et al.*, 1994). These biotypes are distinguished only by their ability (virulence) or inability (avirulence) to survive on and stunt wheats carrying specific genes. The evolution of new biotypes exerts continued pressure on entomologists and breeders to find and use new sources of resistance.

The geographic structure of populations at the micro- and macro-geographical scale is a fundamental component of insect ecology and evolution, which combines both demographic (e.g. variants in virulence) and genetic processes, such as gene flow/migration, genetic drift, selection and population extinction. Recent advances in molecular biology have revolutionized this field, and allow analysis of genetic relationships among populations to be coupled with the phylogeny and the distribution of genotypes within and among populations.

Isozyme electrophoresis was the first biochemical technique used to study the geographical variation in genetic structure of populations of *M. destructor* (Wellso *et al.*, 1988; Black *et al.*, 1990). However, isozyme polymorphisms were detected only at a few loci (Black *et al.*, 1990), and in some cases no variation was found among biotypes or geographic populations occurring in North America (Wellso *et al.*, 1988; Black *et al.*, 1990). Similar results have been obtained with isozymes in aphids (Loxdale *et al.*, 1983; Steiner *et al.*, 1985; Puterka *et al.*, 1993). Substantially more genetic variation in some insect species has been demonstrated by random amplified polymorphic DNA amplified by the polymerase chain reaction (RAPD–PCR technique) (Black *et al.*, 1992; Puterka *et al.*, 1993; Carter *et al.*, 1996). However, no report on the use of RAPD–PCR technique on *M. destructor* is available at the moment. In the present study we applied the RAPD–PCR technique in combination with a virulence analysis of the fly to study the geographic structure of *M. destructor* populations in Morocco and Syria. On the basis of RAPD–PCR analysis, together with information on biotypes and their distribution, genetic relationships among and within the geographic populations of *M. destructor* are inferred.

Materials and methods

Estimation of the level of susceptibility of three wheat cultivars to M. destructor

A set of three bread wheat lines, Saada, KS89H98 and BT1615*3//14-2, carrying respectively H5, H13 and H22 resistance genes and a susceptible cultivar (Nasma) were planted in five localities (Chaouia, Abda, Fes, Marchouch and Beni Mellal) representing the major wheat-growing areas of Morocco (fig. 1). The seeds were planted in single 1-m long rows (*c.* 60 seeds per row) with 50 cm between rows. A randomized complete block design was used with four replications. When the larvae were in the puparial

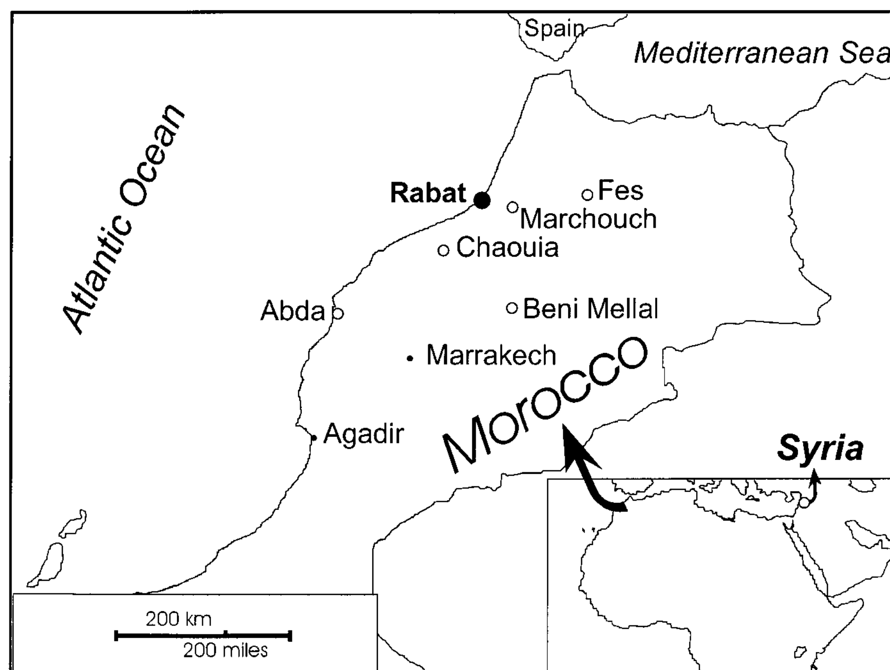

Fig. 1. A map showing sampling sites (open circles) of *Mayetiola destructor* populations from Morocco and Syria.

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stage, all the plants of each cultivar were collected from the field and taken to the laboratory for examination. Infested and uninfested plants were separated on the basis of symptoms. Infested plants were stunted and dark green, whereas resistant plants were not stunted and remained light green. In addition to these symptoms, and in order to separate resistant plants from those that escaped infestation, plants showing a resistance reaction were also examined under a stereoscopic microscope for presence of dead or live larvae.

Results were analysed using ANOVA (SAS Institute, 1989). Percentage data were arcsine transformed before analysis. Paired comparisons were made using the least significant difference (LSD) procedure.

Mayetiola destructor *sampling procedure*

From each of the five locations in Morocco and a location in Lattakia, Syria (fig. 1), 300 infested plants were randomly sampled from commercial farmers' fields of bread wheat. *Mayetiola destructor* larvae at the 'flaxseed stage' were collected from these infected plants and were taken to the greenhouse for adult emergence.

Mayetiola destructor flies were reared on cv. Nasma, a susceptible Moroccan cultivar, in standard greenhouse beds. The method of infestation was similar to that used by Cartwright & LaHue (1944). Seeds were sown in rows (*c.* 30 seeds per 34 cm row, 12 rows) in a standard greenhouse bed (54 × 36 × 8 cm) containing a mixture of soil, vermiculite and peat. Each bed containing plants at the one-leaf stage was placed under a cheesecloth tent and infested with 50 mated *M. destructor* females. These females were allowed to lay eggs on the seedlings for two days before they were removed using an aspirator. About two weeks after egg laying, 20 second-instar larvae per test bed population were randomly sampled, bulked and stored with the rest of the larvae at −80°C.

DNA extraction

The 20 cold-stored larvae per population were bulked to obtain an estimate of population genetic variability detected by RAPD analysis. These larvae were ground with a miniature pestle in 500 µl of ice cold extraction buffer (5 nM ascorbic acid, 1.4 M NaCl, 20 mM EDTA, 2% cetyltrimethylammonium bromide (CTAB) and 100 mM Tris (pH 7.5)) in a microfuge tube. Samples were extracted with one-third volume of chloroform: isoamyl alcohol (24:1) mix and centrifuged to separate phases. The aqueous phase was collected and 0.1 volume of 5 M potassium acetate and two volumes of ice cold, 100% ethanol were added. After holding at −20°C overnight, DNA pellets were obtained by centrifugation at 5000 *g* (5 min), rinsed in 70% ethanol, dried briefly, and subsequently resuspended in 50 µl TE buffer. The isolated DNA was quantified by spectrophotometer (260 nm) and quality was tested by agarose gel electrophoresis. Using a similar protocol, DNA from individual larvae was extracted by using 50 µl of extraction buffer and subsequent steps were carried out as above, with proportionate reduction in the volume of the other reagents.

RAPD–PCR analysis

One hundred and forty different primers (kits A to Y) obtained from Operon Technologies (Alameda, California), were tested on the six bulk populations. A total of 14 primers (E01, D01, D02, D16, G06, G16, J15, K01, L03, M12, N11, X14, and Y3) revealed polymorphism. These primers were also tested for polymorphism on ten individual larvae from each population to study genetic variation within populations. DNA amplification was performed in 25 µl reaction mix containing 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2 and 0.001% gelatin), 200 µM each of dATP, dTTP, dGTP and dCTP, 5 picomoles of a single 10-base primer, 25 ng of genomic DNA, and 0.75 units of *Taq* DNA polymerase (Boehringer Mannheim). Amplifications were performed in a thermocycler (Perkin Elmer, 9600) programmed for 4 min at 94°C; for 40 cycles of 30 s each at 94°C, 1 min at 36°C and 2 min at 72°C; and a final extension of 10 min at 72°C. The reaction mix without template DNA served as a control and was electrophoresed along with the samples. Amplified DNA products were loaded onto 1.5% agarose gels and electrophoresed in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA). Gels were stained with ethidium bromide and photographed under UV light.

Data analysis

The fragment pattern of each sample derived from RAPD analysis was coded in binary form, i.e. as 1s and 0s, representing the presence and absence of each fragment, respectively. Nei's genetic distance (a dissimilarity index; Nei, 1972) was calculated from the binary data for all pair-wise combinations of samples. Using the genetic distance the cluster analysis was performed to study genetic relationships among the populations of *M. destructor*. Two methods of cluster analysis, an unweighted paired-group method for the arithmetic average (UPGMA) and a neighbour joining method (NJM) were used. The UPGMA method (Sneath & Sokal, 1973) constructs a phenogram by successive (agglomerative) clustering using an average-linkage method of clustering. The neighbour joining method (Saitou & Nei, 1978) estimates a phylogram based on the idea of parsimony (Rohlf, 1993) and the tree is usually close to the true phylogenetic tree. All the data analysis was performed using the software package NTSYS-pc (Rohlf, 1993).

Results

Variation in level of susceptibility of the three cultivars to M. destructor *among geographical populations*

Table 1 summarizes the reaction of the three wheat cultivars carrying resistance genes (H5, H13 and H22) to *M. destructor* grown in the five localities. The analysis of variance of the percentage of resistant plants among the three cultivars and across the locations was highly significant ($P < 0.001$). The plants containing H13 and H22 genes showed the highest variation in the level of susceptibility to Hessian fly between locations. The level of susceptibility of plants containing H13 gene was high in Abda, Marchouch and Beni Mellal, and low in the other locations, and of the H22 gene was high in Fes and Marchouch and low in the other locations.

Genetic variation among geographical populations

Initially, 140 different primers of arbitrary nucleotide sequence were used to amplify DNA segments from the genomic DNA (bulk sample of 20 individuals) of five

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Table 1. Percentage of wheat plants showing resistance to infestation by *Mayetiola destructor* in three cultivars carrying resistance genes H5, H13, and H22 grown in different regions of Morocco.

Population	% Resistant plants			
	Saada (H5)	KS89H98 (H13)	BT1615*3//14-2 (H22)	Nasma (susceptible check)
Chaouia	67 Aa	79 Aa	79 Aa	14 Ba
Fes	90 Aa	90 Aa	57 Bc	0 Ca
Abda	80 Aa	60 Ba	90 Aa	12 Ca
Marchouch	90 Aa	58 Ba	61 Bc	10 Ca
Beni Mellal	82 Aa	58 Aa	90 Ba	0 Ca

Means followed by the same letter in horizontal rows (upper case) and in columns (lower case) are not significantly different (P = 0.05); LSD test (SAS Institute, 1996).

populations of *M. destructor* from Morocco and one population from Syria. Out of 140 primers, only 14 (10%) detected polymorphism among the six populations. Using these 14 primers, a total of 163 loci (bands) were amplified and 135 (83%) of these showed polymorphism. Figure 2 shows the primer OPK1 amplification products. The number of polymorphic bands amplified by the individual primers varied and ranged from 4 (OPE-01) to 18 (OPG-16).

Table 2 shows the pair-wise genetic distance and geographic distance (km) for the five populations from Morocco and one population from Syria. The genetic distance ranged from 0.156 (between Abda and Beni Mellal, geographic distance 200 km) to 1.977 (between Marchouch and Syria, geographic distance 3200 km). Among the Moroccan populations, the highest genetic distance, 0.364 was observed between Fes and Beni Mellal (geographic distance 600 km). The correlation coefficient of 0.81 indicated that there was a strong relationship between genetic and geographic distances.

Genetic relatedness among the populations was determined by cluster analysis (both unweighted paired-group method for the arithmetic average and neighbour joining method) based on the genetic distances (fig. 3). The two methods yielded a similar tree. The analysis grouped the populations into six distinct clusters with the Syrian population appearing as an outgroup to the Moroccan populations.

Genetic variation within geographical populations

The 14 primers that revealed polymorphism among the populations were also used to study variation within the populations from Chaouia, Fes, Abda, Marchouch, Beni Mellal and Lattakia, Syria. The analysis revealed that the RAPD patterns of individuals differed distinctly from that of the bulked samples. The primer OPX14 detected a very high degree of polymorphism within the Syrian population (fig. 4). With all 14 primers, the total number of loci amplified ranged from 101 (Chaouira) to 65 (Syria) and that of polymorphic loci varied from 77 (Chaouira) to 42 (Syria) (table 3). The individuals within each population showed different RAPD patterns, indicating a very high degree of heterogeneity (genetic diversity equal to one, Nei, 1978) within the populations. The mean genetic distance was highest for the Fes population (0.678), followed by Lattakia, Syria (0.602) and Beni Mellal (0.578), and Chaouria was the

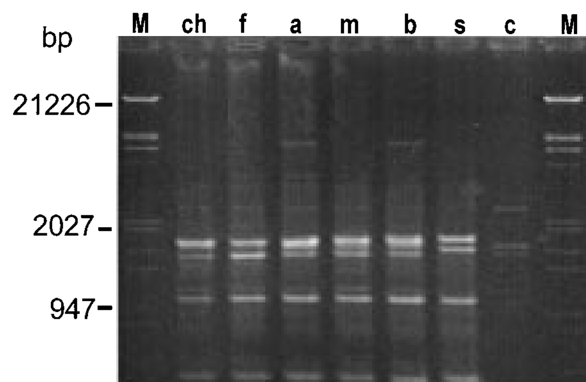

Fig. 2. RAPD–PCR analysis of *Mayetiola destructor* populations from Morocco (ch: Chaouia; f, Fes; a, Abda; m, Marchouch; b, Beni Mellal) and Syria (s), by Operon primer OPK1. Lanes, ch, f, a, m, b, and s are bulk samples of 20 individual insects from the respective populations, c, control amplification without template DNA, and M, Lambda DNA digested with *Eco*RI-*Hin*dIII.

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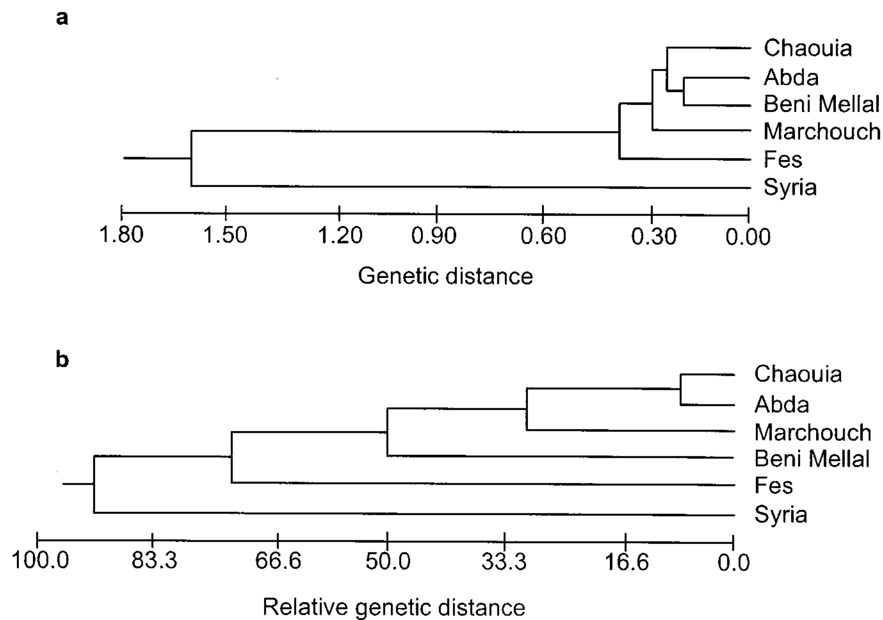

Fig. 3. Cluster analysis (a, unweighted paired-group method; b, neighbour joining method) of different populations of *Mayetiola destructor* from Morocco and Syria based on genetic distance (Nei, 1972) obtained by RAPD–PCR analysis.

lowest (0.544) (table 3). This indicated that the individuals from Fes were the most distantly related to each other, followed by those from Syria and Beni Mellal whereas individuals from Chaouria were the most closely related to each other. The coefficient of variation (CV%) based on the mean genetic distance and standard deviation within populations revealed that the population from Syria was intrinsically more variable (54.3%) than any of the populations from Morocco. Among the Moroccan populations, that from Marchouch (44.5%) was the most variable followed by Abda (38.5%), Chaouia (29.4%) and the population from Beni Mellal (22.2%).

Discussion

This study showed the existence of variation in the level of susceptibility of wheat cultivars containing different resistance genes to *M. destructor* in Morocco. Similar levels of

Table 2. Pair-wise genetic distance (upper) and geographic distance (km; lower) between six *Mayetiola destructor* populations.

Location	Chaouia	Fes	Abda	Marchouch	Beni Mellal	Syria
Chaouia	–	0.306	0.165	0.203	0.193	1.794
Fes	500	–	0.329	0.263	0.364	1.245
Abda	200	600	–	0.222	0.156	1.794
Marchouch	250	200	400	–	0.253	1.977
Beni Mellal	200	600	200	400	–	1.717
Syria	3200	3600	3800	3200	3400	–

Table 3. Number of loci amplified and genetic distance within six populations of *Mayetiola destructor* as revealed by RAPD–PCR analysis.

Location	No. of loci amplified		Genetic distance		
	Polymorphic	Total	Range	Mean ± SD	CV(%)
Chaouia	77	101	0.306–0.952	0.544 ± 0.160	29.4
Fes	73	89	0.388–1.092	0.678 ± 0.188	27.7
Abda	68	92	0.285–1.189	0.538 ± 0.207	38.5
Marchouch	52	77	0.118–1.210	0.539 ± 0.240	44.5
Beni Mellal	67	87	0.265–1.280	0.578 ± 0.128	22.2
Syria	42	65	0.164–1.832	0.602 ± 0.327	54.3

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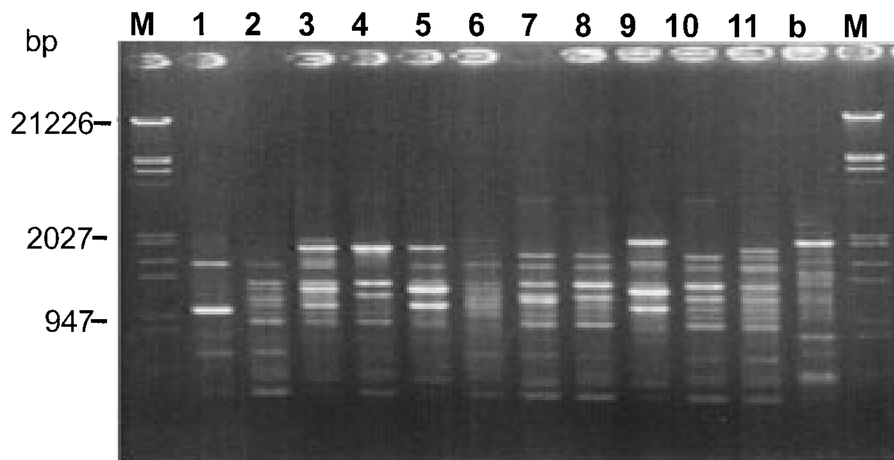

Fig. 4. RAPD–PCR analysis of the Syrian population of *Mayetiola destructor* by the Operon primer OPX14. Lanes 1–11 are amplification patterns of individual insects from the single Syrian population. Lane b is the bulk sample of 20 insects. Lane M is lambda DNA digested with *Eco*RI-*Hin*dIII.

susceptibility of the cultivars carrying H5 and H13 genes have been demonstrated, and the cultivar carrying the H13 gene was also found to be highly variable across sampled locations (El Bouhssini *et al.*, 1992). The geographic variability in the percentage of wheat plants carrying H5, H13 and H22 genes and showing susceptible reactions to *M. destructor* indicated differences in biotype frequencies across the cereal production zone of the country.

These results indicate that additional sources of resistance will be needed to stay ahead of biotype evolution of *M. destructor* in Morocco. Deployment of cultivars carrying resistance genes that allow for some avirulent larvae to survive on resistant plants may slow biotype development, since there will be less selection pressure on the fly populations (El Bouhssini *et al.*, 1998). *Mayetiola destructor* populations in all the cereal production areas should be monitored closely for changes in virulence as new resistant cultivars are deployed. Biotypes capable of overcoming specific resistance genes should be isolated in the greenhouse and used to screen for new sources of resistance.

Previous studies have revealed that the RAPD–PCR technique is a more efficient method for assessing genetic polymorphism in some insect populations than allozymes (Black *et al.*, 1992; Puterka *et al.*, 1993; Carter *et al.*, 1996). The results of the present study showed that the RAPD–PCR technique was useful in assessing genetic variation in *M. destructor*. We used 20 individual insects per population, which were bulked, to obtain characteristic amplified fragment profiles of the populations. This strategy was rapid, efficient and cost-effective in studying genetic variability of natural, out-crossing populations (Carter *et al.*, 1996). With this bulking strategy, we obtained a consensus profile of amplified fragments of DNA of a population for each RAPD–PCR reaction. The bulking of individuals minimized uninformative data arising from individual variation and maximized the chance of obtaining informative data on polymorphism among the populations (Michelmore *et al.*, 1991; Carter *et al.*, 1996).

RAPD–PCR detected genetic differences both among and within the various populations of *M. destructor*. The observed variation in the present study was much higher than the variation reported previously for *M. destructor* populations in the USA, using allozymes (Black *et al.*, 1990). Similar differences in results have been obtained in some other insects (Black *et al.*, 1992; Puterka *et al.*, 1993), as well as in some plant species (Hoey *et al.*, 1996).

A very high degree of genetic variation was observed within the populations, when RAPD–PCR analysis was carried out with ten individuals from each geographic population. All the individuals were different from each other with respect to amplified fragment profiles, yet they shared a similar pattern of overcoming resistance among different host cultivars tested. This comparison indicated that RAPD profiles were not associated with the biotype trait in *M. destructor*. This observation in *M. destructor* is obvious because only a few recessive genes control virulence in the fly (Hatchett & Gallun, 1970; Formusoh *et al.*, 1996). Furthermore, as the fly reproduces sexually, the population is both heterozygous and heterogeneous. This observation contrasts with those in aphids because aphids mainly reproduce parthenogenetically (Puterka *et al.*, 1993). Therefore, low levels of genetic diversity within geographical populations and some degree of association of RAPD pattern (clones) with biotype are possible in aphids. The extent of genetic relatedness within the different populations of *M. destructor*, estimated by mean genetic distance varied greatly. This variation could be attributed to the extent of initial colonization of the dispersed population, followed by local differentiation.

The dendrograms generated from the genetic distance matrix of RAPD data (bulking strategy) by the unweighted pair-group or neighbour joining methods showed only minor variations in the grouping of *M. destructor* populations. Based on the number of unique banding patterns associated with each population, genetic distance data and the consistency of dendrograms, it was concluded that the analysed populations were derived from at least two founder groups of *M. destructor*. Founder group 1 comprised the population from Lattakia, Syria and founder group 2 comprised all of the populations from Morocco. The strong positive relationship (r = 0.81) between geographical and the

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genetic distances among populations together with cluster analysis suggested that offspring of the founder group 1 dispersed to other parts of Morocco through the Fes region. To reach the Fes region, the flies probably dispersed from neighbouring Algeria. The uniqueness of the Syrian population in both cluster analyses, coupled with its high genetic diversity and the strong relationship between geographic distance and genetic distance, support the hypothesis that *M. destructor* originated in West Asia and dispersed to North Africa most probably via a land route. More extensive sampling of the proposed route is needed to test the hypothesis more fully.

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