

Population variation of codling moth *Cydia pomonella* (Lep.; Tortricidae) based on molecular data from northwestern Iran

Samad KHAGHANINIA^{1,*}, Seyed Abolgasem MOHAMMADI², Ali Morad SARAFRAZI³,
Karim Haddad Irani NEJAD¹

¹Dept. of Plant Protection, Faculty of Agriculture, University of Tabriz - IRAN

²Dept. of Agronomy & Plant Breeding, Faculty of Agriculture, University of Tabriz - IRAN

³Institute of Plant Pests & Diseases Research, Tehran - IRAN

Received: 12.11.2009

Abstract: In order to study population structure in the codling moth (*Cydia pomonella* L.) using RAPD markers, 13 geographical populations from northwestern Iran were collected during 2003 and 2004. Genomic DNA was extracted from 10 overwintering larvae of each population. Out of 60 tested primers, 18 amplified 236 polymorphic bands. The total number of bands in the population varied from 169 to 206 in the Mughan and Zunuz populations, respectively. Within-population genetic diversity, based on Nei's genetic index, ranged from 0.228 to 0.281 for the Shabestar and Zunuz populations, respectively. An analysis of the molecular variance revealed significant differences within and between population variance. Between-population variation accounted for 14.44% and within-population variation accounted for 85.56% of the total molecular variance. Cluster analysis based on molecular data assigned the studied codling moth populations to 2 groups. In this grouping, Group 1 consisted of the Mughan population only. The maximum and minimum genetic distances were observed between the Mughan-Ahar and Shabestar-Mahabad populations, respectively. Canonical correlation analysis showed significant association between RAPD markers and the latitude of the studied regions. A principal coordinate analysis showed high discrimination between geographic populations and confirmed the results of the cluster analysis. A significant correlation was found between genetic and geographic distance matrices as revealed by the Mantel test.

Key words: *Cydia pomonella*, molecular data, population variation, RAPD

Introduction

The codling moth is one of the biggest pests of apple orchards, introduced as a key pest that causes direct damage. Knowledge of the genetic variation within codling moth populations is necessary for their efficient control and management. Molecular technologies provide new ways to study population diversity as well as to differentiate closely related species (Williams et al., 1990; Deverno et al., 1998).

Polymerase chain reaction (PCR) strategies offer increased sensitivity and speed for the identification and characterization of species and populations (Deverno et al., 1998). The randomly amplified polymorphic DNA (RAPD) technique has been developed to detect genetic variability by PCR amplification of arbitrary segments of genomic DNA by using short, random primers and thus does not require prior knowledge of a DNA sequence. Its

* E-mail: tabrizlisamad@yahoo.com

low cost, its efficiency in developing a large number of DNA markers in a short time, and the less sophisticated equipment that it requires has made RAPD a valuable technique (Bardakci, 2001; Delaat et al., 2005). RAPD markers are very well suited for use in insect phylogeny areas like the detection of genetic variability among populations as well as the identification of closely related species (Benecke, 1998; Lima et al., 2002).

The variability among and between populations of *Euschistus heros* (Fabricius) and *Nezara viridula* (L.) has been determined in Brazilian soybean fields using RAPD analysis (Gomez et al., 2004; Gomez et al., 2005). Bayar et al., (2006) through investigations on population variation of *Aeolothrips intermedius*, found population-specific RAPD markers for their differentiation. Deverno et al. (1998) distinguished 2 closely related sympatric species of coniferophagous moths using 17 species-specific RAPD markers. RAPD markers have also been used for studying the population variation of the Hessian fly, *Mayetiola destructor*, and the wheat stem sawfly, *Cephus cinctus*,

in Syria and America, respectively (Lou et al., 1998; Naber et al., 2000).

The objectives of the present study were: 1) to use RAPD markers for the analysis of population structure of the codling moth in a broad region of northwestern Iran, 2) to survey the relationship between genetic and geographic distances, and 3) to evaluate the relationship between genetic distance and environmental parameters.

Materials and methods

The specimens were collected from 13 regions spanning northwestern Iran: Zunuz (Zun), Shabestar (Sha), Mianeh (Mia), Marageh (Mar), and Ahar (Aha) from East Azarbayjan; Salmas (Sal), Urumieh (Uru), and Mahabad (Maha) from West Azarbayjan; Mughan (Mug) and Meshkin shahr (Mes) from Ardebil; and Mahneshan (Mahn), Zandjan (Zan), and Khoramdareh (Kho) from Zandjan, during 2003 and 2004 (Figure 1). The populations were collected from a number of sites based on the span



Figure 1. Geographic locations of sampling areas of in which codling moth populations were studied.

areas within each region. To eliminate the effect of host association in discrimination of populations, all of the specimens were collected from golden apple orchards. The sampling of fifth instar larvae was carried out by single face cardboard fastened around the apple trees at a distance of 30 cm from the ground. In each population, 15 overwintering female larvae were randomly selected for DNA isolation to minimize DNA contamination by endoparasites (Landry et al., 1999). Total genomic DNA was isolated using a modification of the procedure detailed by Zimmerman et al. (2000). The quantity and quality of extracted DNA were evaluated by 0.8% agarose gel electrophoresis as well as spectrophotometer. All of

the DNA samples were diluted to 25 ng/ μ L for use in PCR. The PCR amplification was carried out in 15- μ L reaction mixtures containing 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μ M primer, approximately 5 ng/ μ L of genomic DNA, 1.5 μ L of 10 \times reaction buffer, and 1 unit of Taq DNA polymerase (Benecke, 1998; Meena et al., 2005). Sixty primers manufactured by the University of British Columbia were used for DNA amplification (Table 1). To determine the reproducibility of the bands, amplifications were repeated 3 times using each primer (Torres et al., 1997; Deverno et al., 1998). A negative control, without DNA, was also included (Clarke et al., 2001; Lima et al., 2002).

Table 1. The sequence of primers used in the present study.

No.	Primer sequence	No.	Primer sequence	No.	Primer sequence
1	CTAATCACGG	21	GCCCCTTGAC	41	GCCCCTTGAC
2*	CCGAATCACT	22	CGGAGAGCCC	42	CGGAGAGCCC
3*	GCATAGTGCG	23*	AGGCGGAAGC	43	AGGCGGAAGC
4	TCGTCTAGCA	24	CACAAGCCTG	44	CACAAGCCTG
5	ACAGGCAGAC	25	ATCCCCAAGA	45	ATCCCCAAGA
6	CGAAAGGACT	26*	TTCGCTTCTC	46*	TTCGCTTCTC
7	TGACCTCTCC	27	CGCACGCACA	47	CGCACGCACA
8*	ACGTTGAGAC	28	CTTTCCTTCC	48	CTTTCCTTCC
9	TCATCCAGGG	29	TATACGACCC	49	TATACGACCC
10	GTGTAGAGCC	30	CACTCCTACA	50	CACTCCTACA
11*	CGCCGCTCCT	31	AATAACCGCC	51*	AATAACCGCC
12	CGGCGTTACG	32	CCCATGGCCC	52*	CCCATGGCCC
13*	AGACACCTGA	33*	CACTGCTGTC	53	CACTGCTGTC
14*	GCGCGGCACT	34	ACCTGTTCTC	54	ACCTGTTCTC
15	GAGGCGGCGA	35*	CACCTAATGG	55	CACCTAATGG
16	GTGTTTCCGG	36*	TCCCGAACCG	56*	TCCCGAACCG
17	GCAGGGTTTCG	37	GAGATCCCTC	57*	GAGATCCCTC
18*	AAACCTGGAC	38	GCGAGGTGCT	58	GCGAGGTGCT
19	AGCGTCGACT	39	ATGACGTTGA	59	ATGACGTTGA
20	CTAGTAGGGG	40	CCTGATTGCC	60*	CCTGATTGCC

*Primers that generated polymorphic bands in different populations.

Amplifications were carried out using a Biometra thermocycler. After the initial denaturation of DNA at 95 °C for 5 min, 40 cycles of 94 °C for 1 min, 37 °C for 1 min, and 72 °C for 2 min were used, along with a final extension step at 72 °C for 5 min (Clarke et al., 2001). Amplification products were resolved electrophoretically at 120 V in 1.8% agarose gel for 2 h, visualized by ethidium bromide fluorescence and photographed using Syngene gel documentation. The sizes of generated bands were verified using the SM0321 molecular weight marker (Fermentas SM0321). The banding patterns were scored as 0 (absence) and 1 (presence).

Pairwise population distance was estimated based on Nei's genetic index using POPGENE (Yeh et al.,

1997). An analysis of molecular variance (AMOVA) was performed with ARLEQUIN 2.000 (Excoffier and Schneider, 2005). Cluster analysis was performed with NTSYS-pc 2.02 (Rohlf, 1993) and canonical correlations were provided by the STATISTICA 5.5 program.

Results

Out of 60 tested primers, 18 produced 236 polymorphic bands ranging between 400 and 2700 bp with an average of 13.11 bands per primer. Figure 2 presents the band patterns of primers 3, 2, and 13 in some populations.

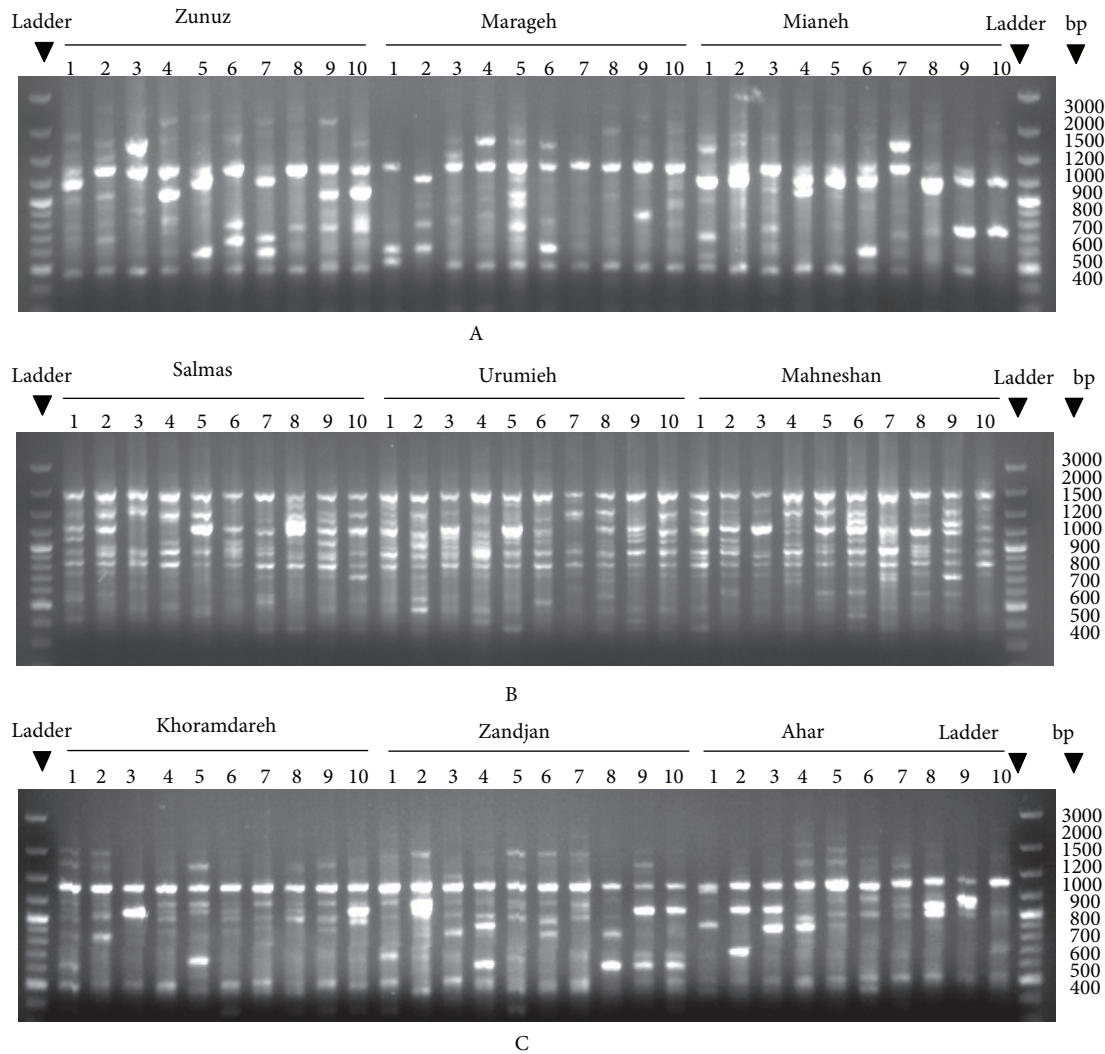


Figure 2. Band patterns of primer numbers 3 (A), 2 (B), and 13 (C) in some of the codling moth populations from Iran.

Among the studied populations, maximum and minimum numbers of polymorphic bands per primer were observed in Zunuz (206) and Mughan (169), respectively.

A molecular analysis of variation revealed significant interpopulation and intrapopulation variation (Table 2). This variation between populations was revealed to be 14.441%, while variation within populations was determined at 85.559%.

Canonical correlation analysis indicated a significant correlation between climatic parameters and molecular data (Table 3). The highest correlation was observed between elevation and molecular data. Other factors such as relative humidity, temperature, and longitude showed low correlations.

A grouping of codling moth populations based on RAPD data sorted the populations into 2 main clusters, in which Group 1 consisted solely of Mughan and the other populations were placed in Group 2, a cluster that contained 3 subgroups. These subgroups were created in accordance with the geographic

regions of the populations. Subgroup I included the Mahabad, Shabestar, Mahneshan, and Mianeh populations from regions with high temperature, medium relative humidity, and low elevation. The exception to this classification of Subgroup I was the Meshkin shahr population, which belonged to an area of high elevation, relative humidity, and low temperature that was geographically closest to the Mughan population. The populations of Urumieh, Salmas, Zunuz, Khoramdareh, Zandjan, and Ahar, featuring high elevation, low temperatures, and high humidity, were grouped into Subgroups II and III and demonstrated close relationships (Figure 3). The mean annual climate data are shown in Table 4.

The highest and lowest genetic distances were observed between the populations of Mughan-Ahar (0.1403) and Shabestar-Mahabad (0.0438), respectively. A principal coordinate analysis based on RAPD data revealed clear discrimination among populations and was largely in agreement with the results of cluster analysis, although a few differences were noted (Figure 4).

Table 2. Analysis of molecular variation for 13 populations of codling moth based on 236 RAPD markers.

Source	df	Ms	Variance	Contribution%
Between pops.	12	1141.177	6.161	14.441**
Within pop.	114	4047.981	36.501	85.559**

Table 3. Canonical correlation coefficient between environmental parameters and RAPD markers.

Climate parameter	Rc	P Value	Percentage
Longitude	0.701	0.00071	13.85
Latitude	0.601	0.00069	1.198
Temperature (°C)	0.726	0.00012	14.135
Relative humidity (%)	0.792	0.00003	20.21
Precipitation amount (mm)	0.758	0.00017	3.86
Elevation (m)	0.846	0.00000	29.63
Wind speed (km/h)	0.634	0.00022	1.159

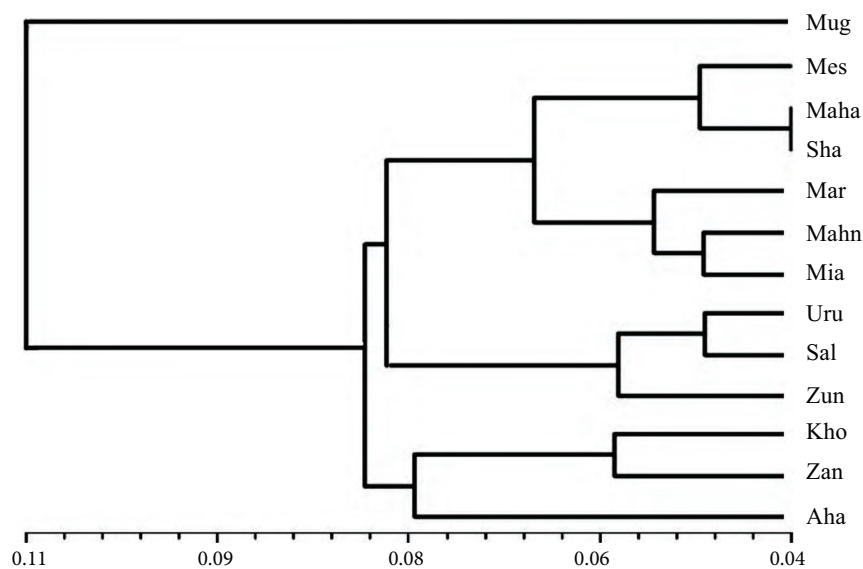


Figure 3. Dendrogram depicting genetic relationships among codling moth populations based on RAPD data using UPGMA and Nei's genetic index.

Table 4. Mean annual climate data of studied regions.

Sampling area	Longitude	Latitude	Temperature (°C)	Relative humidity (%)	Precipitation amount (mm)	Elevation (m)	Winds peed (km/h)
Salmas	38.13	44.51	12	57	215.3	1337	6.111
Zunuz	38.45	45.75	11.1	56	415.1	1710	9.863
Marageh	37.24	46.16	12.9	49	322.4	1477.7	10.503
Mianeh	37.27	47.42	13.7	51	282.1	1110	8.711
Shabestar	38.11	45.41	-	-	-	1452	-
Mahneshan	36.46	47.4	14.6	48	275.7	1282	10.558
Zandjan	36.41	48.29	11	54	313.1	1663	7.191
Khoramdareh	36.11	49.11	11.9	51	301.1	1575	11.48
Meshkin shahr	38.23	47.4	10.7	60	383.9	1568.5	5.157
Urumieh	38.13	44.51	12	57	215.3	1337	6.111
Mahabad	36.46	45.43	12.8	53	413.1	1385	6.595
Ahar	38.26	47.04	10.8	60	292.2	1390.5	9.635
Mughan	39.39	47.55	15.1	72	271.2	31.9	8.413

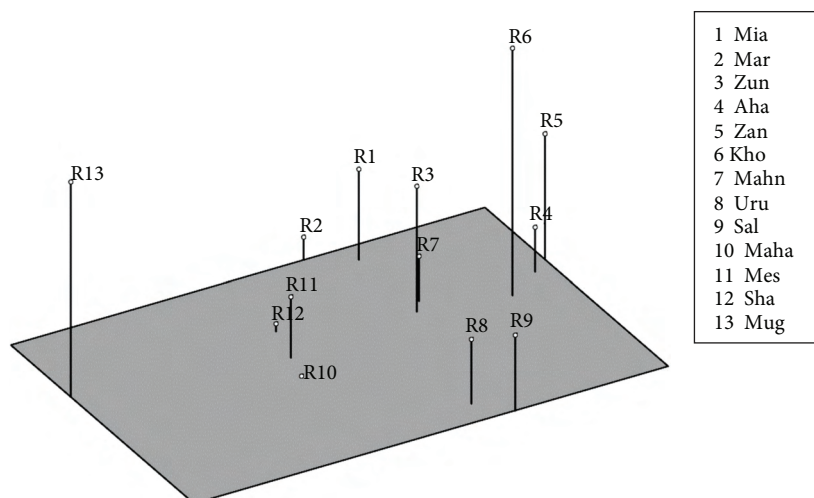


Figure 4. Three-dimensional prospectus of codling moth population differentiation based on principal coordinate analysis using RAPD data.

A positive and significant correlation ($r = 0.444$, $P < 0.012$) was observed between genetic and geographic distance matrices, as revealed by the Mantel test. Genetic distance based on RAPD data increased in relation to the increasing geographic distance of given population areas. The correlations between molecular genetic distance and morphological genetic distance in the fore- and hindwings of males and females, on the other hand, were not found to be significant.

Discussion

The low percentage of interpopulation variation, when compared with that of intrapopulation variation in the codling moth, could be the result of long migrations on the part of the adults (Mani et al., 1995; Schumacher et al., 1997; Voigt, 1999). In other words, individuals belonging to various populations could actively participate in mating in a given region, resulting in more interaction between them.

In this study, a strong association was observed between molecular variation and stable climatic parameters such as elevation, whereas population morphological variation was more closely related to variable parameters such as wind speed and precipitation. It seems, then, that genetic variability is deeper and more continuous than morphological variability (Gibert et al., 2004).

The ability of adult specimens to participate in long distance flight may be one reason for the low correlation between genetic and geographic distances. Naber et al. (2000), in their analysis of interpopulation and intrapopulation diversity in the wheat Hessian fly, *Mayetiola destructor*, reported a high correlation ($r = 0.81$) between genetic and geographic distances, which may be due to the low flight capacity of the fly.

The nonsignificant correlation between molecular and morphological distances could be explained by the differing natures of these variations. Differences at the DNA level are not always functional at the morphological level. Gibert et al. (2004) considered phenotypic plasticity as well as seasonal and environmental adaptations to be fast evolutionary parameters, whereas genetic adaptations toward divergence and speciation were considered slow, deep evolution.

The high genetic distance between Mughan and Ahar could be due to the climatic conditions of the 2 locations. Mughan is a region with an elevation of 31.9 m, 72% relative humidity, and a mean annual temperature of 15.1 °C; the Ahar area has an elevation of 1390 m, 60% relative humidity, and a mean annual temperature of 10.8 °C. Populations from the Mahabad and Shabestar regions, both located near Urumieh Lake with respective elevations of 1385 and 1452 m, showed a close genetic relationship.

Gomez et al. (2004), using 15 RAPD primers to analyze *Euschistus heros* populations in Brazilian soybean fields, detected a total of 246 polymorphic bands and reported high intrapopulation variation. Clarke et al. (2001) and Lima et al. (2002) also used RAPD markers to study population structure in *Nebria gregaria* and *Thrips tabaci*.

References

- Bardakci, F. 2001. Random amplified polymorphic DNA (RAPD) markers. *Turk. J. Biol.* 25: 185-196.
- Bayar, K., Torjak, O., Kiss, E., Gyulai, G. and Heszky, L. 2006. Genetic variation within and among populations of *Aeolothrips intermedius*. *J. Jool.* 73: 67-73.
- Benecke, M. 1998. Random amplified polymorphic DNA (RAPD) typing of necrophagous insects (Diptera & Coleoptera) in criminal forensic studies: validation and use in practice. *Forensic Science International* 98: 157-168.
- Clarke, T.E., Levin, D.B., Kavanaugh, D.H. and Reimchen, T.E. 2001. Rapid evolution in the *Nebria gregaria* group (Coleoptera: Carabidae) and the paleogeography of the Queen Charlotte Islands. *Evolution* 55: 1408-1418.
- Delaat, D.M., Carvalho, M.R.S., Acedo, M.D.P. and De Fonseca, C.G. 2005. Applicability of RAPD markers on silver-stained polyacrylamide gels to ascertain genetic diversity in *Peripatus acacioi* (Onychophora, Peripatidae). *Gen. Mol. Res.* 4: 716-725.
- Deverno, L.L., Smith, G.A. and Harrison, K.J. 1998. Randomly amplified polymorphic DNA evidence of introgression in two closely related sympatric species of *Coniferophagous choristoneura* (Lepidoptera: Tortricidae) in Atlantic Canada. *Ann. Entomol. Soc. Am.* 91: 248-259.
- Excoffier, L.G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.
- Gibert, P., Capy, P., Imasheva, A., Moreteau, B., Morin, J.P., Petavy, G. and David, J.R. 2004. Comparative analysis of morphological traits among *Drosophila melanogaster* and *D. simulans*: genetic variability clines and phenotypic plasticity. *Genetica* 120: 165-179.
- Gomez, D.R., Delpin, K.E., Almeida, M.R. and Hirose, E. 2004. Genetic differentiation among Brazilian populations of *Euschistus heros* (Fab.) (Heteroptera: Pentatomidae) based on RAPD analysis. *Neotropical Entomology* 33: 179-187.
- Gomez, D.R., De Silva, J.J., Costa, F. and Binneck, E. 2005. Population structure of the Brazilian Southern green stink bug, *Nezara viridula*. *Journal of Insect Science* 36: 216-227.
- Landry, B., Powell, J.A. and Sperling, F.A.H. 1999. Systematics of the *Argyrotaenia franciscana* (Lepidoptera: Tortricidae) species group: evidence from mitochondrial DNA. *Ann. Entomol. Soc. Am.* 92: 40-46.
- Lima, L.H.C., Campos, L., Moretzsohn, M.C. and De Oliveira, M.R.V. 2002. Genetic diversity of *Bemesia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genetic and Molecular Biology* 25: 217-223.
- Lou, K.F., Weiss, M.J., Bruckner, P.L., Talbert, L. and Martin, J.M. 1998. RAPD variation within and among geographic populations of wheat stem sawfly (*Cephus cinctus* Norton). *J. Her.* 89: 329-335.
- Mani, E., Wildbolz, T. and Riggenbach, W. 1995. Effect of pheromone trap position in large and small trees and in the open field on the catch of codling moth, *Cydia pomonella* L. males. *Mitt. Schweiz. Entomol. Gesell.* 68: 69-78.
- Meena, R.L., Ramasubramanian, T., Vekatesan, S. and Mohankumar, S. 2005. Molecular characterization of *Tospovirus* transmitting thrips populations from India. *Am. J. Biochem. & Biotech.* 1: 168-173.
- Naber, N., El Bouhssini, M., Labhilili, M., Udupa, S.M. and Nachit, M.M. 2000. Genetic variation among populations of the Hessian fly *Mayetiola destructor* (Diptera: Cecidomyiidae) in Morocco and Syria. *Bull. Entomol. Res.* 90: 245-252.
- Rohlf, F.J. 1993. Numerical Taxonomy and Multivariate Analysis System, Applied Biostatistics Inc., New York.
- Schumacher, P., Weber, D.C., Hagger, C. and Dorn, S. 1997. Heritability of flight distance for *Cydia pomonella*. *Entomol. Exp. Appl.* 85: 169-175.
- Torres, D.M., Carrio, R., Lattore, A., Simon, J.C., Hermoso, A. and Moya, A. 1997. Assessing the nucleotide diversity of three aphid species by RAPD. *J. Evol. Biol.* 10: 459-477.
- Voigt, E. 1999. Observations on the flight activity of codling moth, *Cydia pomonella* L. *IOBC Wprs Bull.* 22: 91-98.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18(22): 6531-6535.
- Yeh, F.C., Young, R.C., Timothy, B., Boyle, T.B.J., Ye, Z.H. and Mao, J.X. 1997. POPGENE, the User-Friendly Shareware for Population Genetics Analysis, Molecular Biology and Biotechnology Center, University of Alberta, Canada.
- Zimmermann, M., Wahlberg, N. and Descimon, H. 2000. Phylogeny of *Euphydryas* Checkerspot Butterflies (Lepidoptera: Nymphalidae) based on mitochondrial DNA sequence data. *Ann. Entomol. Soc. Am.* 93: 347-55.

Acknowledgements

The authors would like to sincerely acknowledge Professor M. Rahimpour (School of English, Media Studies & Art History, The University of Queensland, Australia) for the editing of this manuscript.