

POPULATION ECOLOGY

Using Random Amplified Polymorphic DNA To Differentiate Strains of the Indianmeal Moth (Lepidoptera: Pyralidae)

ALAN K. DOWDY AND W. H. MCGAUGHEY

U.S. Grain Marketing Research Laboratory, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502

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ABSTRACT The movement of Indianmeal moth, *Plodia interpunctella* (Hübner), and its ability to infest stored products over large areas is not understood because of the difficulty in identifying the origins of infestations. Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) is a technique that has been used to identify genetic markers in insect populations and may be useful for differentiating populations of *P. interpunctella*. RAPD PCR was used to differentiate 6 *P. interpunctella* populations, and the genetic similarity was examined within and among populations. All individual insects were differentiated into correct population groups using only two 10-mer primers.

KEY WORDS *Plodia interpunctella*, random amplification of polymorphic DNA, polymerase chain reaction, population monitoring

THE INDIANMEAL MOTH, *Plodia interpunctella* (Hübner), is a cosmopolitan pest of stored products in both on-farm storage and commercial storage, processing and distribution. The migration of this insect and its ability to infest stored products over large areas is not understood. This lack of knowledge is caused by the difficulty in identifying the origins of infestations. If we were able to differentiate between populations of insects, we would be able to understand better how insect movement affects the infestation of stored grain.

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) is a genetic technique that has been used successfully to identify genetic markers that characterize species in several insect orders, including Diptera, Homoptera, and Hymenoptera (Black et al. 1992, Kambhampati et al. 1992, Haymer and McInnis 1994). Additionally, it has been useful in identifying the geographic origin of some introduced insect species. Mendel et al. (1994) used this technique to determine the origin of the scale *Matsucoccus josephi* Bodenheimer & Harpaz in Israel, and Williams et al. (1994) identified the geographic origin of the weevil *Listronotus bonariensis* (Kuschel) in New Zealand. Puterka et al. (1993) characterized phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko).

The application of this technique to stored-product insects eventually may be useful for determining at what point in the marketing channel a commodity became infested. This could be done

by creating a RAPD profile and identifying population-specific markers for local populations along the route traveled by the commodity. Initially this technique may be useful for differentiating insect populations in culture that originated from geographically separated origins. The objectives of this study were to determine if RAPD-PCR could be used to classify Indianmeal moth populations from different geographic areas of the midwestern United States.

Materials and Methods

Five colonies of *P. interpunctella* were established in culture in 1983 and 1 in 1988 (RC688) (Fig. 1). Colony UE343 was established from insects collected in a terminal elevator, whereas all other colonies originated from insects collected from on-farm storage. Because these are laboratory populations, some genetic variation may have been lost relative to field populations. Cultures were maintained on a standard laboratory diet (McGaughey 1985) at 25°C and 60-70% RH. Late instars were collected and preserved in 70% ethanol at -20°C.

Isolation of DNA from Individual Insects. Extraction of DNA was accomplished using a method adapted from Livak (1984). The larvae were rinsed in ddH₂O and placed on a tissue to remove excess water. Only the head capsule and prothorax were used for DNA extraction because the fat body in other tissues was difficult to remove by centrifugation. The head capsule and prothorax were removed and placed individually in 0.5-ml microcentrifuge tubes. Insects were homogenized in 75 µl of homogenization buffer (0.1 M NaCl, 0.2 M su-

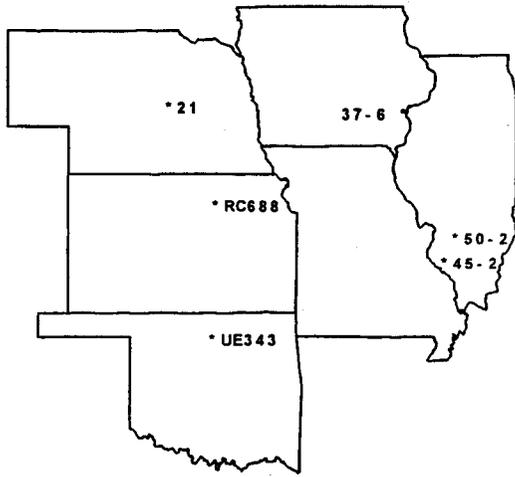


Fig. 1. Origin of *P. interpunctella* populations. 21, Merrick County, Nebraska; 37-6, Muscatine County, Iowa; 45-2, Randolph County, Illinois; 50-2, Jefferson County, Illinois; RC688, Riley County, Kansas; UE343, Grant County, Oklahoma.

crose, 0.01 M EDTA [disodium], 0.03 M Tris-HCl [pH 8.0]), and the pestle was rinsed with an additional 75 μ l of buffer. Next, 50 μ l of phage lysis buffer (0.25 M EDTA [disodium], 2.5% SDS, 0.5 M Tris-HCl [pH 9.2]) and 4 μ l diethyl pyrocarbonate were added. Samples were vortexed briefly and incubated at 65°C for 40 min. After incubation, 40 μ l of 8 M potassium acetate was added and samples were vortexed briefly and incubated at 4°C for 30 min. Samples were then centrifuged for 30 min at 4,000 \times g and the supernatant (\approx 200 μ l) was transferred to a new tube and centrifuged again for 15 min. The supernatant was again collected and transferred to a new tube. Next, 400 μ l of -20°C 95% ethanol and 5 μ l of 8 M potassium acetate was added and DNA precipitated overnight at -20°C. The next day, samples were centrifuged for 20 min at 13,000 \times g. The supernatant was decanted and pellets were washed twice in 500 μ l of cold 70% ethanol and centrifuged after each wash for 2 min to seat the pellets. The samples were finally washed in 500 μ l of cold 95% ethanol and centrifuged for 2 min to seat the pellets. The supernatant was decanted, and the pellets were dried in a rotovac. DNA was resuspended in 10 μ l of ddH₂O and stored at -20°C until used for PCR.

Polymerase Chain Reaction Protocol. All liquid transfer was done using positive placement pipettes (Benchmate, Oxford Labware, St. Louis, MO). Filtered pipette tips were used for liquids containing DNA or primers. PCR conditions were optimized as described by Rolfs et al. (1992), and amplification was replicated twice to ensure reproducibility. In a 0.5-ml microcentrifuge tube, a 50- μ l reaction was prepared containing 40.0 μ l sterile ddH₂O, 3.0 μ l MgCl₂ (1.5 mM), 2.5 μ l 10 \times PCR buffer (500 mM KCl, 100 mM Tris-HCl [pH 9.0],

Table 1. 5'-3' sequences for random 10-mer DNA primers used to detect polymorphisms in *P. interpunctella*

No.	Series OPA	Series OPB	Series OPR
1	CAGGCCCTTC	GTTTCGCTCC	TGCGGGTCCT
2	TGCCGAGCTG	TGATCCCTGG	CACAGCTGCC
3	AGTCAGCCAC	CATCCCCTTG	ACACAGAGGG
4	AATCGGGCTG	GGACTGGAGT	CCCGTAGCAC
5	AGGGGTCTTG	TGCGCCCTTC	GACCTAGTGG
6	GGTCCCTGAC	TGCTCTGCC	GCTACGGCA
7	GAAACGGGTG	GGTGACGCAG	ACTGGCCTGA
8	GTGACGTAGG	GTCCACACGG	CCCGTTGCCT
9	GGGTACGCC	TGGGGGACTC	TGAGCACGAG
10	GTGATCGCAG	CTGCTGGGAC	CCATTCCCCA
11	CAATCGCCGT	GTAGACCCGT	GTAGCCGTGT
12	TCGGCGATAG	CCTTGACGCA	ACAGGTGCGT
13	CAGCACCCAC	TTCCCCGCT	GGACGACAAG
14	TCTGTGCTGG	TCCGCTCTGG	CAGGATTCCT
15	TTCCGAACCC	GGAGGGTGT	GGACAACGAG
16	AGCCAGCGAA	TTTGCCCGGA	CTCTGCGCGT
17	GACCGCTTGT	AGGGAACGAG	CCGTACGTAG
18	AGGTGACCGT	CCACAGCAGT	GGCTTTGCCA
19	CAAACGTCCG	ACCCCGAAG	CCTCCTCATC
20	GTTGCGATCC	GGACCTTAC	ACGGCAAGGA

Source: Operon Technologies, Alameda, CA.

1.0% Triton X-100), 1.5 μ l dNTPs (200 μ M), 1.0 μ l DNA (0.01 concentration), and 1.0 μ l primer (50 pM; Table 1). The sample tubes were loaded into a thermal cycler with hot bonnet (MJ Research, PTC100-60) and heated to 95°C for 5 min. The samples were then cooled to 80°C, and 1.0 μ l Taq DNA polymerase (1 unit) was added. The reactions were then exposed to 40 cycles of 94°C for 30 s, 48°C for 30 s, ramped to 72°C at a rate of 1°C per 8 s, and 72°C for 1 min. The reactions were heated for an additional 10 min at 72°C and then held at 4°C until recovered.

Electrophoresis. An 8.0- μ l volume of PCR product was mixed with 2 μ l of 10 \times gel-loading

Table 2. Frequency of DNA bands generated by the 10-mer primer OPR-08

Band	Insect populations					
	21	37-6	45-2	50-2	UE343	RC688
2,200	0.0	0.7	0.7	0.3	0.5	0.3
2,000	1.0	1.0	1.0	1.0	1.0	1.0
1,800 ^a	0.0	0.9	0.0	0.3	0.2	0.0
1,500	1.0	0.1	1.0	1.0	1.0	1.0
1,300 ^a	1.0	1.0	0.9	1.0	0.3	0.7
1,200	0.0	0.2	0.1	0.1	0.0	0.0
1,100 ^a	0.0	0.0	0.0	0.8	0.9	0.0
850 ^a	0.9	0.0	1.0	0.0	0.7	0.2
800 ^a	0.0	1.0	0.9	0.4	0.0	1.0
650	0.0	0.6	0.2	0.0	0.2	0.0
590 ^a	0.0	0.8	1.0	1.0	0.1	0.0
520	0.2	1.0	0.9	1.0	1.0	0.9
480	1.0	1.0	1.0	1.0	0.5	0.8
440	0.2	1.0	0.2	1.0	0.4	1.0
350	0.3	0.0	0.1	0.0	0.0	1.0
320	1.0	1.0	1.0	1.0	1.0	1.0
270	1.0	1.0	1.0	1.0	1.0	1.0
180	0.6	1.0	1.0	0.6	0.4	1.0
140	0.3	1.0	1.0	1.0	1.0	0.0

^a Six most descriptive bands for differentiating individual insects into populations.

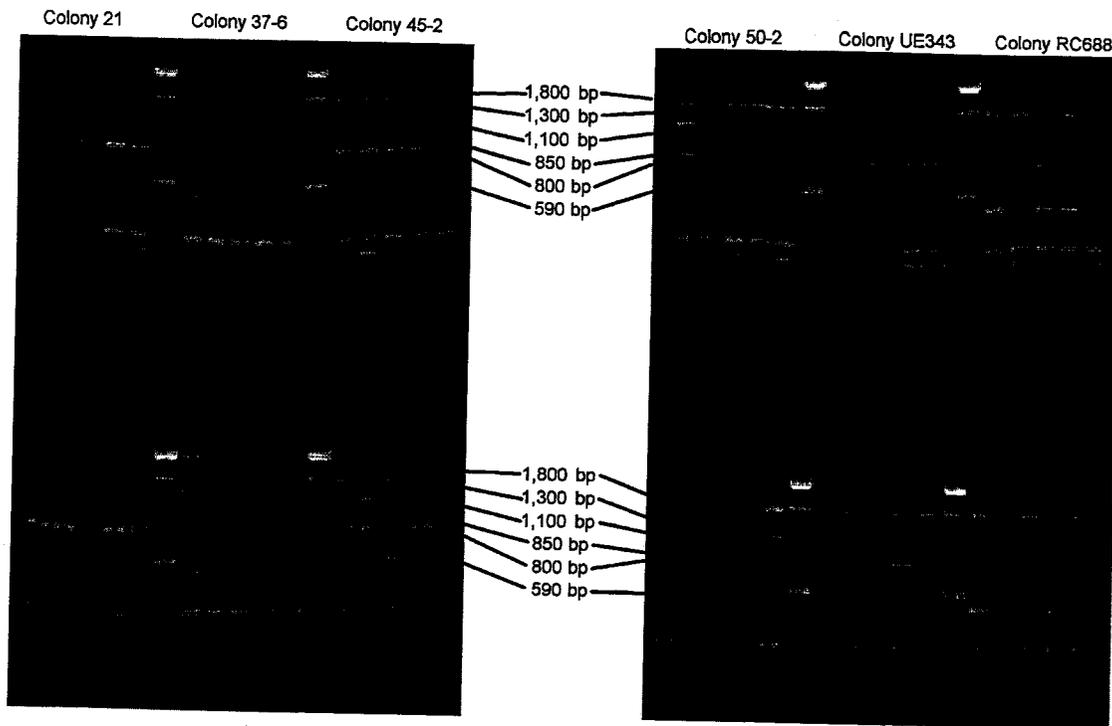


Fig. 2. Electrophoresis gel of *P. interpunctella* DNA amplified with primer OPR-08. Lanes between colonies are DNA 100-bp ladder (2,000-, 1,500-, and 600-bp bands are of higher intensity).

buffer (50% glycerol in ddH₂O, 0.1% bromophenol blue) and electrophoresed on a 2.0% agarose gel (TBE) at 70 vdc. DNA bands were stained with ethidium bromide and photographed using UV light. DNA band size was determined by comparison with a 100-bp DNA ladder (Gibco BRL, Gaithersburg, MD) run on each gel.

Data Analysis. Ten insects were examined from each colony for each of the 10-mer primers screened, for a total of 60 insects per primer. For each of the 60 primers examined, gels were examined for the presence or absence of each DNA band among the 6 populations. Only data from

primers that exhibited differences among populations were included in any statistical analyses, and their ability to classify individuals correctly into populations was tested using NEIGHBOR (Felsenstein 1994), a clustering method that implements an unweighted pair-group method using arithmetic averages (UPGMA). A data matrix was constructed in which each row contained the population and individual insect identification, and RAPD-PCR band information was scored as 1 for the presence of a band or 0 for band absence. The Nei and Li (1985) similarity index was calculated using RAPDPLOT (Kambhampati et al. 1992) which compared the similarity of all individuals to

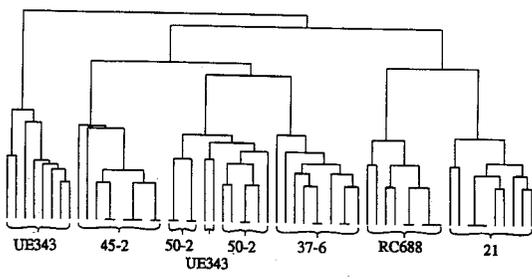


Fig. 3. Dendrogram of *P. interpunctella* individuals generated by UPGMA analysis using Nei and Li similarity based on shared fragments generated from primer OPR-08. Two individual insects not separated into correct population group.

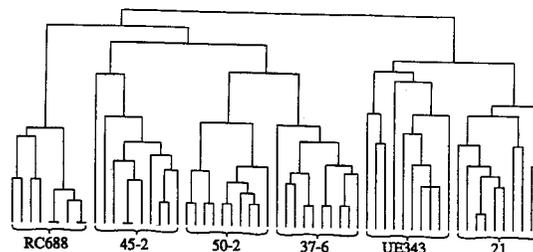


Fig. 4. Dendrogram of *P. interpunctella* individuals generated by UPGMA analysis using Nei and Li similarity based on shared fragments generated from primers OPR-08 and OPA-11. All individual insects correctly separated into 6 population groups.

Table 3. Corrected values (mean \pm SEM) of the Nei and Li coefficients of genetic similarity for DNA bands produced using primer OPR-80

Colony	21	37-6	45-2	50-2	UE343	RC688
21	0.885 \pm 0.013	0.589 \pm 0.006	0.740 \pm 0.008	0.674 \pm 0.006	0.666 \pm 0.007	0.710 \pm 0.009
37-6	—	0.935 \pm 0.008	0.793 \pm 0.005	0.805 \pm 0.005	0.619 \pm 0.012	0.716 \pm 0.005
45-2	—	—	0.932 \pm 0.009	0.798 \pm 0.005	0.713 \pm 0.008	0.737 \pm 0.006
50-2	—	—	—	0.916 \pm 0.009	0.739 \pm 0.009	0.718 \pm 0.008
UE343	—	—	—	—	0.834 \pm 0.017	0.618 \pm 0.007
RC688	—	—	—	—	—	0.925 \pm 0.069

each other. Mean similarity scores were calculated from the similarity matrix to derive the similarity matrix comparing populations. The similarity matrix calculated by RAPDPLOT was analyzed with NEIGHBOR to construct a dendrogram using DRAWGRAM (Felsenstein 1994). Significant distances between populations were identified using pseudo-F and pseudo-t² tests calculated using PROC CLUSTER (SAS Institute 1985) on the population similarity matrix.

Results and Discussion

Most of the primers tested yielded identical DNA band patterns among all populations and were therefore not useful to differentiate these populations. Because all populations were collected from a somewhat limited total geographic area, the populations were expected to yield the same banding pattern for many of the primers. There were some differences in the DNA band profile within each population because of within-population polymorphisms. Only clearly identifiable bands were included in the analysis.

Six primers (OPA-01, OPA-11, OPB-01, OPB-05, OPB-10, and OPR-08) exhibited the most differences among the populations and yielded a total of 82 bands, some of which were not consistently present in all populations. Neighbor analysis of primers OPA-01 (13 bands), OPA-11 (12 bands), OPB-01 (15 bands), OPB-05 (11 bands), and OPB-10 (12 bands) did not result in classification of individuals into correct population groups.

Analysis of primer OPR-08 (19 bands) resulted in the best classification (Table 2; Figs. 2 and 3). Classification of the populations could be accomplished using as few as 6 of the 19 bands. These are marked in Table 2 and noted on Fig. 2. These bands tended to have the least variability within a population and the greatest differences between

populations. Individuals in all populations, except for 2 from UE343, were differentiated into correct population groups (Fig. 3). The UE343 colony was the only population that originated from a commercial grain elevator rather than a farm; individuals exhibited less genetic similarity (83.4%) between individuals within the population than the individuals within the other populations (Table 3). When data from primers OPR-08 and OPA-11 were combined to construct a dendrogram, all individuals were classified into correct population groups (Fig. 4). Even with intrapopulation variability, RAPD PCR effectively separated individuals into populations.

Nei and Li coefficients of genetic similarity were calculated with data from primer OPR-08. These values are the expected proportion of shared positive bands between pairs of populations and averaged 76.5% \pm 2.4% (Table 3). If these coefficients had been calculated from data using all 60 primers that were tested, this value would have been greater because the expected proportion of shared bands was higher for the other primers than for this primer, which exhibited the most differences among populations. The most genetically similar populations were 37-6 and 50-2, sharing 80.5% of their positive bands; whereas the least similar were populations 21 and 37-6, sharing only 58.9% of their positive bands. Although populations 45-2 and 50-2 originated within 100 km of each other, only 79.8% of their positive bands were shared. There appeared to be no significant correlation between genetic similarity and the geographic distance among populations ($r = -0.21$, $P = 0.46$, $n = 15$). A similar relationship was reported by Williams et al. (1994) working with the weevil *L. bonariensis* in South America and New Zealand. Mendel et al. (1994) measured the degree of association between populations of the

Table 4. Average linkage cluster analysis using the Nei and Li coefficient of genetic similarity; *F* and *t*² values are not significant ($P > 0.05$)

No. clusters	Clusters joined	<i>F</i>	df	<i>t</i> ²	Normalized root-mean-square distance
5	21 and 37-6	1.59	4, 1	—	0.8239
4	UE343 and RC688	1.66	3, 2	—	0.8687
3	cluster 5 and cluster 4	1.46	2, 3	1.54	0.9541
2	cluster 3 and 50-2	1.35	1, 4	1.42	1.0332
1	cluster 2 and 45-2	—	—	1.35	1.0635

scale *M. josephi* by analyzing RAPD bands with the Jaccard index, which is similar to Nei and Li coefficient but more prone to bias and not as easily interpreted (Lamboy 1994). Their data, however, indicate that the Israeli and Cypriot populations are much more similar to each other than either is to the Turkish population. Our data are not as clear because all of the Indianmeal moth populations exhibit a relatively high degree of genetic similarity with each other, and the calculated Jaccard index for our data had a range of only 0.13 compared with 0.49 in the Israeli study.

Cluster analysis yielded similar results in that linkage was not indicative of the geographic proximity of population origins (Table 4). The average banding differences between groups were not significant at any level. Black (1993) and Kambhampati et al. (1992) also have reported that species belonging to the same subgroup did not cluster together when RAPD markers were used in cluster analysis. In our situation, the Nei and Li coefficient of genetic similarity and the cluster analysis may be an indicator of how difficult it would be to differentiate correctly the populations based on RAPD markers. Closely related populations with a large proportion of shared bands should be more difficult to differentiate correctly than populations with a small proportion of shared bands.

In conclusion, this study demonstrated the usefulness of RAPD-PCR for differentiating populations of the Indianmeal moth with a high degree of confidence. These 6 populations could be correctly differentiated using two 10-mer primers. Before PCR technology was available, it would have been extremely difficult to differentiate individual moths into population groups. However, we were able to differentiate populations that originated as close as 100 km easily. RAPD-PCR technology eventually may be useful for determining at what point in the marketing channel that a commodity became infested.

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