Genetic Relationships Among Florida *Diaprepes abbreviatus* (Coleoptera: Curculionidae) Populations

B. BAS,[1] Z. DALKILIC,[1] T. L. PEEVER,[2] H. N. NIGG,[1] S. E. SIMPSON,[3] F. G. GMITTER, JR.,[1] AND R. C. ADAIR[4]

Institute of Food and Agricultural Sciences, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850

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ABSTRACT Genetic differentiation among six Florida populations of *Diaprepes abbreviatus* (L.) was determined using protein and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) markers. Proteins were separated by electrophoresis and stained with silver stain and for α-naphthylacetate esterase activity. No differentiation was observed among populations when egg proteins were silver stained: α-naphthylacetate esterase activity differentiated five of the six populations. RAPD-PCR data showed significant differentiation among populations, consistent with the hypothesis of three independent introductions of *D. abbreviatus* into Florida. Our data indicate that *D. abbreviatus* populations, once introduced, have generally remained in one locality with limited dispersal to new areas.

KEY WORDS *Diaprepes*, esterase, population differentiation, random amplified polymorphic DNA-polymerase chain reaction

ADULT AND LARVAL stages of *Diaprepes abbreviatus* (L.) feed on leaves, roots, and fruit of >250 agronomic and native host plants in Florida and several island nations of the Caribbean (Fennah 1942, Simpson et al. 1996). Since its introduction in 1964, *D. abbreviatus* has spread to 20 counties in Florida, where it currently infests ≈66,420 ha (164,000 acres) (Anonymous 1997). The Florida infestations of *D. abbreviatus* originated from founder insects of unknown numbers and origin. As part of an insecticide-testing program we were interested in obtaining a steady supply of feral weevils. Although weevils are available from a laboratory colony, the colony was established in 1970 and has experienced no pesticides, nor has any new genetic variation been bred into the colony. Feral weevils are potentially exposed to a variety of esterase inhibiting pesticides. For example, parathion was introduced into Florida agriculture in 1950 (Griffiths et al. 1951) and numerous organophosphates and carbamates have been and are used in Florida. Amplification of esterase genes (*Myzus persicae* Sulzer and the *Culex pipiens* L. complex) and genetic variation in esterases (e.g., *Musca domestica* L.) appear to be resistance mechanisms in insects (Brown and Brogdon 1987, Devonshire and Field 1991).

Electrophoretic banding patterns of isozymes have been used to differentiate strains in insects (Singh and Krishna 1982; Berlocher 1989; Terranova et al. 1990, 1991). Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis also has been used for insect strain differentiation (Hunt and Page 1992, Vogler et al. 1993, Mendel et al. 1994, Williams et al. 1994, Dowdy and McGaughey 1996, Garner and Slavicek 1996), and for differentiating parasitic Hymenoptera in a host (Zhu and Greenstone 1999). One study combined RAPD-PCR and esterase isozyme analysis to determine the relatedness of populations of *Tomicus piniperda* L., an exotic pine pest (Carter et al. 1996).

The purpose of these experiments was to study the genetic variation within and among populations of *D. abbreviatus.*

Materials and Methods

Insects. In September 1997, adult male and female weevils were collected at the locations shown in Fig. 1 and Table 1. Additional weevils were collected in July 1998 and for a second experiment in August 1998 from the same locations, with the exception of Tangerine, Plymouth-Apopka, and Alturas where sufficient beetles could not be found in 1998. Weevils were collected from citrus groves, except for the Homestead population that was collected in a commercial ornamental nursery and the Orlando population that was reared in the laboratory. The 1997 populations were housed separately in 30 by 30-cm aluminum screen cages (BioQuip, Gardena, CA), one population

[1] University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.
[2] Department of Plant Pathology, Washington State University, P.O. Box 6430, Pullman, WA 99164–6430.
[3] Division of Plant Industry, Florida Department of Agriculture and Consumer Services, 3027 Lake Alfred Road, Winter Haven, FL 33881.
[4] Kerr Center for Sustainable Agriculture, 7055 Cherry Lane, Vero Beach, FL 32966.

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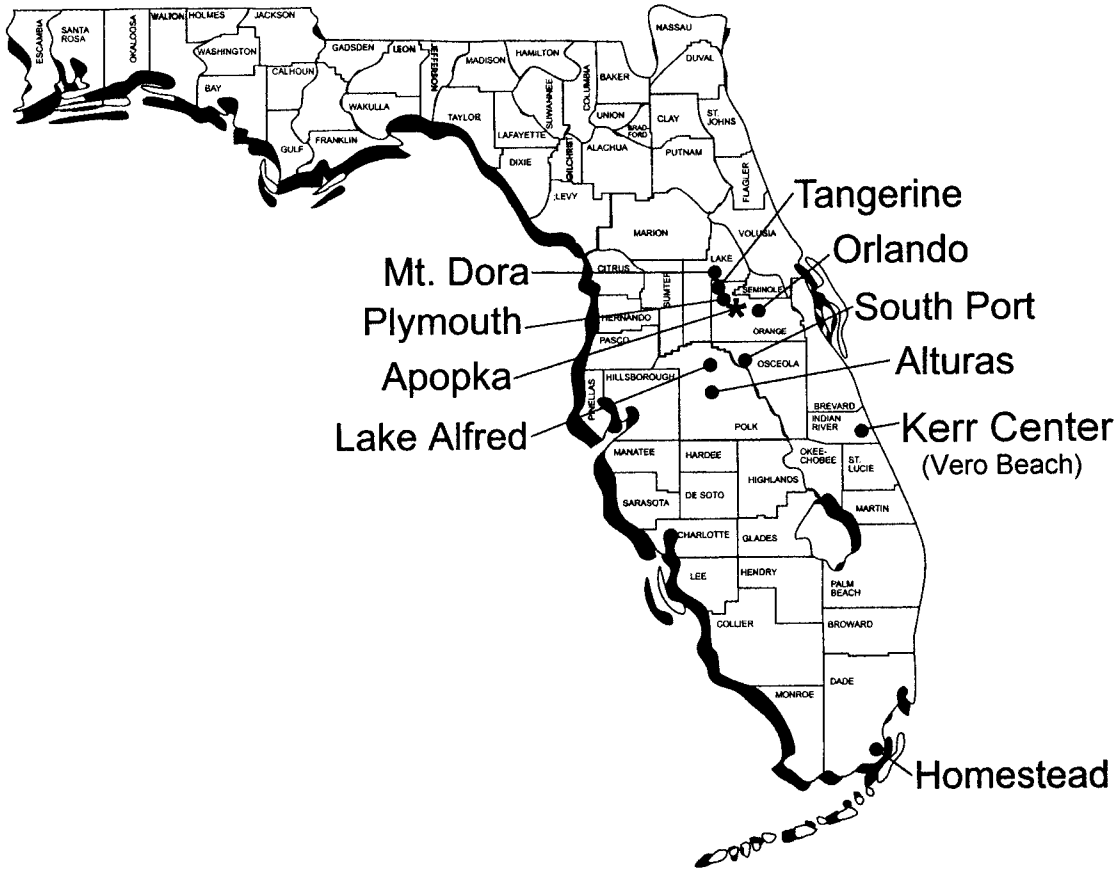

Fig. 1. Locations of *D. abbreviatus* populations sampled in this study. The original *D. abbreviatus* find was at Apopka, FL, in 1964.

per cage, and were fed young foliage of red grapefruit (*Citrus paradisi* Macfadden) for 2 wk before eggs were collected for protein and nucleic acid extraction and electrophoresis. Eggs were used for these studies because egg proteins tend to remain the same within a population (Koch 1968). For Mt. Dora and Orlando egg developmental studies, eggs were collected daily and were allowed to develop for 16, 24, 48, 72, 96, 120, 144, and 168 h at 27°C, 100% RH, and a photoperiod of 12:12 (L:D) h. This routine provided a set of eggs of known age for simultaneous analyses. When larvae hatched from eggs held until 168 h, extracts of all egg ages were prepared on the same day, equalized for protein content, and electrophoresis and staining performed. The 1997 populations contained at least 10 males and 10 females, except for the Plymouth population, which contained two females and two males. The 1998 populations contained 20–40 individuals of both sexes. The 1998 populations were caged one female and one male per 946-ml (32-oz) clear plastic container with a perforated lid. Cages were provided with young, partially expanded citrus leaves and water

Table 1. Straight line distance in kilometers between *D. abbreviatus* populations

Alturas									
98	Apopka[a]								
296	380	Homestead							
29	68	323	Lake Alfred						
109	23	394	80	Mt. Dora					
92	18	367	63	39	Orlando (Laboratory)				
95	6	380	68	18	21	Plymouth			
45	66	317	35	84	53	68	South Port		
101	16	388	77	6	35	11	77	Tangerine	
127	154	246	134	172	134	158	106	169	Kerr Center

[a] Original infestation 1964.

Table 2. Arbitrary 10-mer primers and sequences used to produce RAPD markers for *D. abbreviatus*

Marker	Sequence	Band number and estimated fragment size, bp					
		1	2	3	4	5	6
AA18	5′-TGGTCCAGCC-3′	700	600				
AH6	5′-GTAAGCCCCT-3′	800	500	500	450	400	350
AK11	5′-CAGTGTGCTC-3′	600	500				
AL3	5′-CCCACCCTTG-3′	900	800				
AN6	5′-GGGAACCCGT-3′	900	550				
D12	5′-CACCGTATCC-3′	1,150	900	850			
O5	5′-CCCAGTCACT-3′	650					
S9	5′-TCCTGGTCCC-3′	800	600				
V14	5′-AGATCCCGCC-3′	800	500				

ad libitum. The number of paired adult weevils used from each 1998 population was Mt. Dora 40 (21 laid eggs), Homestead 40 (18 laid eggs), Orlando 40 (20 laid eggs), Lake Alfred 25 (16 laid eggs), Kerr Center 20 (12 laid eggs), South Port 40 (7 laid eggs). Individual egg masses were placed in a −48°C freezer each morning as a numbered set. Each 1998 population set was processed simultaneously for DNA analysis and esterase determinations.

Sample Preparation. Cages also were provided with two waxed paper egg-laying strips (2.5 by 15 cm) at 1700 hours; eggs were collected at 0800 hours the following day. Each egg mass was removed from the waxed paper strips and eggs were physically removed from the surrounding adhesive material and weighed. Eggs were ground in 1.5-ml Eppendorf tubes with a plastic Kontes pellet pestle (Fisher, Pittsburgh, PA) in 0.1 M pH 8.2 Tris-HCl + 0.5 M NaCl buffer (Zongza and Dimitriadis 1988); 15 ml buffer per gram of eggs. Homogenates were centrifuged at 9,000 × *g* for 10 min in a model 235c microcentrifuge (Fisher), and the supernatants were used for electrophoresis. For comparison of populations, supernatants were prepared as eggs became available and were stored at −40°C for up to 9 d. Supernatants were thawed (all were stored at least 24 h), protein content determined with a Bio-Rad protein assay (Bradford 1976) (Bio-Rad, Hercules, CA), using bovine IgG (Bio-Rad) as a standard, and protein content was equalized by diluting with the buffer described above.

Protein and Esterase Staining. Native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE were performed with a 12% resolving gel and a 4% stacking gel polymerized with ammonium persulfate; the silver stain stacking gel contained SDS according to Laemmli (1970). For silver staining, wells were loaded with 5 μl of a 3:1 mixture of supernate and a solution of 2% SDS, 5% 2-mercaptoethanol, 10% sucrose and 0.002% bromophenol blue in glass distilled-deionized water (GDDI). Wells for esterase staining were loaded with 5 μl of a 1:1 mixture of supernate and 20% sucrose/bromophenol blue solution (10 ml 20% sucrose, 1.2 mg bromophenol blue). The sucrose/bromophenol blue solution was filtered with a 0.45 μm nylon 66 syringe filter before use. In 1997, two gels were run in a Bio-Rad minigel system at 20 mA per gel for 60 min. The gel running time was increased to 2.5 h in 1998 to increase the separation of esterase bands.

Nonspecific esterase activity was visualized with a modified Gomori (1952) procedure. Gels were placed in 40 ml of 0.1 M pH 7.4 sodium phosphate buffer and shaken at 100 rpm on a rotary shaker for 10 min. One milliliter of α-naphthylacetate (no stated purity, Sigma, St. Louis, MO) (20 mg/ml in methanol) and 1 ml of Fast Blue BB (dye content 75%, Sigma) (5 mg/ml in methanol, filtered with a nylon 66 filter) were added to the incubation buffer. Development time was 10 min. Esterase bands were scored as present or absent.

SDS PAGE gels were silver stained with a modified procedure of Blum et al. (1987): 16-h incubation in 50 ml 50% methanol/10% acetic acid/25 μl formaldehyde; two 10-min rinses with 50 ml 50% methanol; a 1-min rinse with 0.5 ml sodium thiosulfate (40 mg/ml) in 100 ml GDDI water; three 20-s rinses with GDDI water; impregnation for 20 min in 0.1 gm silver nitrate + 0.0375 ml 37% formaldehyde diluted to 50 ml with GDDI water; two 20-s rinses in GDDI water; development in 3 mg sodium carbonate + 0.25 ml 37% formaldehyde + 0.005 ml of 40 mg/ml sodium thiosulfate in 50 ml of water (development times varied from 45 to 75 s and depended on band intensity); 50 ml of 50% methanol/10% acetic acid for 10 min; 20-min final rinse with 50 ml 50% methanol/GDDI water.

Gels were placed between two sheets of gel drying film (Promega, Madison, WI) and dried for 72 h in a plastic rack at 27°C. After removal from the rack, gels were pressed for 48 h between sheets of weighted paper and then scanned with a Hewlett–Packard Scan Jet 4c and labeled with Desk Scan (Hewlett–Packard, Palo Alto, CA).

Preliminary esterase storage studies were conducted with the eggs and egg extracts of the 1997 Orlando and Mt. Dora populations. Eggs and egg extracts were frozen at −40°C, thawed, and assayed for α-naphthylacetate esterase activity after PAGE over a period of 9 d.

RAPD-PCR. The DNA extraction methods of Carter et al. (1996), Dowdy and McGaughey (1996), Garner and Slavicek (1996), Hunt and Page (1992), Mendel et al. (1994), Sonvico et al. (1996), Vogler et al. (1993) and Williams et al. (1994) were tested using 0.1 g eggs to 1 ml of extraction buffer. The method of Williams et al. (1994) yielded more DNA than the others, as determined by spectrophotometry (Paramacia LKB Ultraspec Plus, LKB, Biochem, England), and was used for all DNA extractions. Quantification

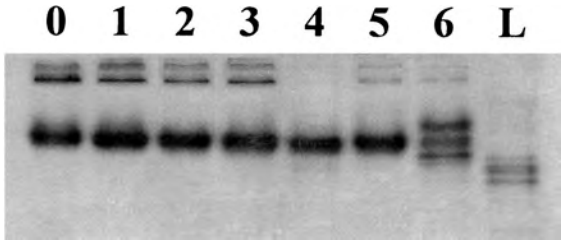

Fig. 2. Developmental esterase banding patterns of Mt. Dora *D. abbreviatus* eggs. 0, newly laid 1–6 d; L, neonate larva.

of DNA was by spectrophotometry; DNA template concentrations were equalized. Samples were treated with 1 μl of 500 mg/ml RNase (Sigma). DNA extracts were electrophoresed on a 0.8% agarose gel in 1× TAE buffer. Ethidium bromide (0.5 μg/ml) was incorporated into the gel. Samples were loaded as a mixture of 10 μl extract and 5 μl of loading buffer (0.25% bromophenol blue in 30% glycerol). Electrophoresis was for 1 h at 90 V. Bands were visualized with UV. PCR was performed according to Williams et al. (1990) on a PTC-100 thermocycler (MJ Research, Watertown, MA). Each 15-μl reaction contained 20 mM Ficoll/Dye (Idaho Technologies, Idaho Falls, ID), 200 μM dNTP mix (Idaho Technologies), 0.6 U *Taq* polymerase (Promega), 100 μM primer (Operon, Alameda, CA), 50 ng genomic DNA, and GDDI H_2O. Thermocycling conditions were as follows: 2 min at 93°C initial denaturation, 1 min at 92°C, 1 min at 35°C for primer annealing, 2 min at 72°C extension (43 cycles), and 10 min at 72°C for final extension, and 4°C storage. Primers used were 10-nucleotides in length and are listed in Table 2 (Operon). Polymorphic bands at a given location in the gel were considered to be alleles at genetic loci. Only clearly separated, reproducible bands were scored regardless of whether or not they were polymorphic in the initial sample of isolates used to screen the markers. Alleles were scored as present (positive allele) or absent (negative allele) for each locus.

Frequencies of the positive allele were estimated for each RAPD locus in each population. Allele frequencies were calculated within each population and used to estimate standard population genetic statistics using POPGENE population genetics software (Yeh et al. 1997). Heterogeneity in allele frequencies among populations was tested using the likelihood ratio chi-square statistic (G^2) for all populations and then for successive combinations by dropping one population at a time (Hamelin et al. 1994). Genetic differentiation among populations was estimated using Nei's G_{ST} (Nei 1973). Gene diversity was calculated according to Nei (1973) and Lewontin (1972). Phenograms were generated in NTSYSpc (Rohlf 1993) using the SIMGEND program where each population was treated as the operational taxonomic unit. Genetic similarity matrices were generated from RAPD allele frequencies using Nei's (1972) maximum genetic distance and clustering was performed using the SAHN program with an unweighted pair-group method with arithmetic average algorithm. Statistical support for dendrogram branches was obtained by generating a majority-rule consensus tree in PHYLIP (Felsenstein 1993) using the SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE programs with 1,000 bootstrapped samples.

Results

Preliminary experiments showed that all bands of α-naphthylacetate esterase activity were sensitive to freeze-thaw. One freeze-thaw was equivalent to a fresh extract. With two freeze-thaws, lighter bands disappeared and only the two heaviest bands stained after three freeze-thaws. The esterase developmental studies of the Orlando and Mt. Dora (Fig. 2) populations did not provide additional esterase banding differences compared with eggs a few hours old. Consequently, newly laid eggs were used for this study.

The α-naphthylacetate esterase banding patterns of the nine 1997 populations were qualitatively similar except for the faster moving esterase bands (Table 3; Fig. 3). The Alturas, Tangerine, Orlando, Lake Alfred, and Plymouth-Apopka populations shared the same fast moving band and were considered one population by this criterion. Mt. Dora and Kerr Center populations were distinct from one another and Homestead and South Port comprised another population (Table 3), four populations in total. The esterase banding patterns of the Orlando and Mt. Dora populations were different from one another at every developmental time (data not shown). The 1998 esterase banding patterns (Table 4) differ from the 1997 data (Table 3) in two methodological respects. The elec-

Table 3. α-Naphthylacetate esterase bands (1997) in nine *D. abbreviatus* populations

Band	D[1]	O[2]	HS[3]	LA[2]	PA[2]	KC[4]	A[2]	SP[3]	T[2]
1	0.03[a]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
2	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
3	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
4	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
5	—	—	0.36	—	—	0.34	—	0.35	—
6	—	—	—	—	—	0.39	—	—	—
7	—	0.44	—	0.44	0.44	—	0.44	—	0.44

Bands were scored as present or absent. Locations followed by the same number have same esterase banding patterns. D, Mt. Dora; O, Orlando; HS, Homestead; LA, Lake Alfred; PA, Plymouth-Apopka; KC, Kerr Center; A, Alturas; SP, South Port; T, Tangerine.

[a] Rf values.

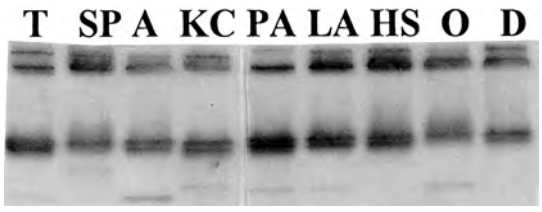

Fig. 3. Esterase banding patterns of 1997 *D. abbreviatus* populations. Composite of two gels. Note left gel contains the seven bands in Table 3. T, Tangerine, FL; SP, South Port; A, Alturas; KC, Kerr Center; PA, Plymouth-Apopka; LA, Lake Alfred; HS, Homestead; O, Orlando; D, Mt. Dora.

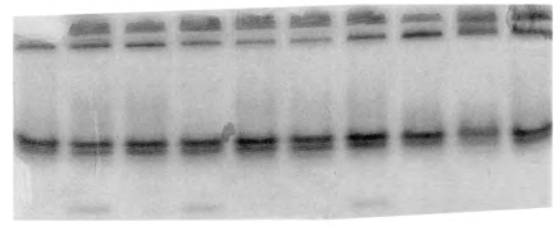

Fig. 4. Individual female egg esterase banding patterns Mt. Dora population 1998. Note three individuals with fast moving band (Table 4). Upper left lane was torn.

trophoresis analysis time was extended from 1 to 2.5 h in 1998 to increase esterase band separation and weevil eggs from individual females were examined in 1998 (Fig. 4). In fact, three additional esterase bands were available in 1998 for population segregation, presumably from the changes in electrophoresis parameters. The six 1998 populations segregated into five populations by esterase banding. Kerr Center and Homestead formed one population. South Port, Lake Alfred, Mt. Dora, and Orlando were each separate populations (Table 4).

The analyses of the RAPD-PCR results (Table 5) described the population relationships, as illustrated in Fig. 5 and in the phenogram in Fig. 6. The bootstrap analyses supported three distinct populations (Fig. 2). The Mt. Dora and Orlando populations were clearly differentiated from one another and from the other populations. Allele frequencies were significantly different among all populations for all RAPD loci except one (4B), even those that appeared most similar from the dendrogram (Homestead, South Port, Lake Alfred, Kerr Center) (Fig. 2). Overall G^2 and G_{ST} values indicated substantial genetic differentiation among all populations (Table 5). When the allele frequency data were reanalyzed by removing one population at a time, even the most closely related populations on the dendrogram (Lake Alfred and Kerr Center, Fig. 2) were significantly different. There was no evidence for "private alleles" (alleles found in only one population) (Slatkin 1985) although allele frequencies at several RAPD loci where either fixed (100%) or totally absent (0%) among populations (Table 6). The overall G_{ST} value among all populations was 0.401, which suggests that ≈40% of the variation in the RAPD markers was observed among populations compared with 60% within populations. When gene diversity statistics (Lewontin 1972, Nei 1973) were compared among populations, the same ranking of populations was obtained for both methods (Table 5). The ranking of populations from least to most genetically diverse was South Port, Mt. Dora, Kerr Center, Orlando, Lake Alfred, and Homestead.

Discussion

Previous studies have differentiated same species insect populations by PAGE and enzyme staining (Berlocher 1989; Terranova et al. 1990, 1991), by PAGE and protein staining (Coates et al. 1990), and by esterase staining plus RAPD-PCR (Carter et al. 1996). Our 1997 and 1998 six-population esterase data separated Orlando, Mt. Dora, Kerr Center, and South Port for both years (Table 7). Of these populations the most homogeneous in regard to esterases was the Orlando population (Table 4). The RAPD-PCR data also indicated the Orlando population as separate from the others.

Esterase and protein may be qualitatively and quantitatively changed by physiological development (Johnson 1977, Coyne 1982, Guérin and Kerambrun 1982, Bitondi and Mestriner 1983). Eggs laid from 1700 to 0800 hours had a theoretical age difference of ≈13 h, considering that an egg mass takes ≈1 h to oviposit (Adair et al. 1999). These 13-h periods could have resulted in the appearance (or disappearance) of any particular esterase or protein band. For the 1997 bulked samples of egg masses from different individuals this possible difference would have been blurred. We had no indication of the appropriate esterase appearance or disappearance in the developmental study (Fig. 2) or in the 1998 study (repeated twice), which used individuals. The esterase data could have been generated by subunit dissociation and association. For example, human plasma cholinesterase has at least 10 phenotypes (LaDu et al. 1991). As a four-subunit enzyme, plasma cholinesterase can disassoci-

Table 4. Percent individuals in each population displaying specific α-naphthylacetate esterase bands (1998)

Population	Band no.	1	2	3	4	5	6	7	8	9	10
Mt. Dora	21	0	100	0	100	100	0	0	0	0	30
Orlando	11	100	100	70	100	100	0	0	0	0	100
Lake Alfred	16	100	100	0	100	100	70	0	10	10	80
Homestead	18	100	100	10	100	100	0	50	30	10	0
South Port	7	100	100	40	100	100	50	10	0	0	0
Kerr Center	12	100	100	20	100	100	0	50	60	10	0

Table 5. RAPD allele frequencies in 6 Florida *D. abbreviatus* populations

RAPD Locus	Mt. Dora	Homestead	Orlando	Lake Alfred	Kerr Center	South Port	Mean	G^{2a}	$H_S{}^b$	$H_T{}^c$	$G_{ST}{}^d$
n	21	18	11	16	12	7					
1A	0.000	0.764	0.000	0.339	0.236	0.155	0.278	38.86***	0.239	0.374	0.362
1B	1.000	0.592	0.452	0.567	0.500	0.466	0.645	21.70***	0.411	0.482	0.146
2A	0.163	0.667	0.684	0.646	1.000	1.000	0.620	36.26***	0.268	0.425	0.370
3A	1.000	0.087	0.051	0.339	0.293	0.000	0.374	60.01***	0.186	0.416	0.552
3B	0.613	0.000	0.292	0.065	0.000	0.000	0.195	35.54***	0.168	0.271	0.379
4A	0.368	0.293	0.000	0.000	0.000	0.000	0.152	22.69***	0.147	0.196	0.252
4B	0.329	0.293	0.452	0.071	0.236	0.000	0.264	7.25	0.333	0.372	0.106
4C	0.000	0.764	0.684	0.388	0.087	0.622	0.388	40.97***	0.316	0.489	0.353
5A	0.078	0.667	0.452	0.750	0.423	0.622	0.476	23.60***	0.403	0.500	0.194
5B	1.000	0.376	0.684	0.000	0.000	0.244	0.426	69.11***	0.222	0.473	0.552
6A	0.553	0.376	0.051	0.293	0.134	0.000	0.297	16.76**	0.285	0.359	0.208
6B	0.000	0.150	0.000	0.000	0.293	0.074	0.081	13.31*	0.134	0.158	0.147
7A	0.225	0.473	0.225	0.567	0.043	1.000	0.384	27.50***	0.295	0.488	0.396
7B	0.051	0.028	0.684	0.441	0.087	0.000	0.199	28.46***	0.206	0.338	0.390
7C	1.000	0.118	0.684	0.000	0.043	0.000	0.355	78.22***	0.120	0.426	0.717
7D	0.000	0.333	0.293	0.134	0.000	0.000	0.133	17.59**	0.182	0.221	0.179
7E	0.553	0.000	0.000	0.000	0.087	0.000	0.146	34.35***	0.109	0.191	0.428
7F	0.452	1.000	0.106	0.750	0.592	0.466	0.608	32.98***	0.340	0.493	0.310
8A	0.106	0.473	1.000	0.000	0.355	1.000	0.384	56.57***	0.191	0.500	0.618
8B	0.051	0.118	1.000	0.250	0.000	0.000	0.207	45.41***	0.113	0.361	0.686
9A	1.000	0.473	1.000	1.000	1.000	1.000	0.886	34.10***	0.083	0.160	0.482
9B	0.000	1.000	0.000	0.500	1.000	0.622	0.510	83.56***	0.162	0.499	0.676
Overall								824.74***	0.223	0.372	0.401
h^e (rank)	0.198 (2)	0.3039 (6)	0.2452 (4)	0.2717 (5)	0.1978 (3)	0.1443 (1)					
I^f (rank)	0.2972 (2)	0.4482 (6)	0.3595 (4)	0.3967 (5)	0.3017 (3)	0.2101 (1)					

[a] Likelihood ratio chi-square test. Null hypothesis of no differences in allele frequencies among populations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[b] Genetic diversity within populations (Nei 1973).
[c] Total genetic diversity over all populations (Nei 1973).
[d] Genetic diversity among populations as a proportion of total diversity $G_{ST} = (H_T - H_S)/H_T$ (Nei 1973).
[e] Gene diversity (Nei 1973). 1 = least diverse.
[f] Gene diversity (Lewontin 1972). 1 = least diverse.

ate and reassociate and produce different banding patterns and band intensities (LaMotta et al. 1965, LaMotta and Woronick 1971). Perhaps a 2.5-h electrophoresis with a 12.5% gel could measure a similar phenomenon for *D. abbreviatus* esterases. Extensive biochemical studies would be needed to evaluate this possibility.

On average we had 14 weevils per hectare at the Mt. Dora location over 46 wk (H.N.N., unpublished data). The 40 females from Mt. Dora represented ≈20% of the total females for two of the 46 wk. However, only 21 of these weevils laid eggs over 9 d and these 21 represent ≈10% of a 2-ha population over 2 wk. The Homestead population contained thousands of weevils, as does the Orlando laboratory colony so this percentage is not calculable for these two populations. For the other populations this percentage over 2 wk was estimated as follows: Lake Alfred, 20%; Kerr Center, 10%; South Port, 2%. The failure of 33 of 40 female South Port weevils to lay eggs over 9 d may be a real physiological difference that additional experiments may define. Considering the supposed neutral nature of RAPD-PCR analyses (i.e., the assumption that DNA

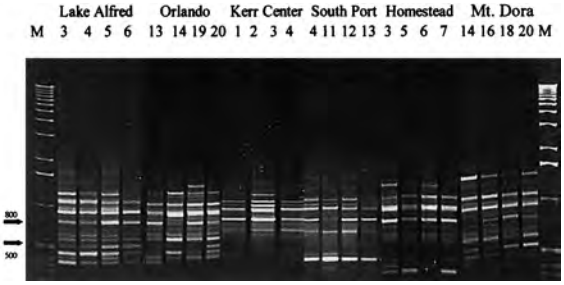

Fig. 5. RADP-PCR patterns of four individuals from six *D. abbreviatus* populations using the operon primer V14. The upper (800 bp) and lower (500 bp) arrows indicate the polymorphic fragments across populations. The first and last columns are 1-kb DNA marker lanes.

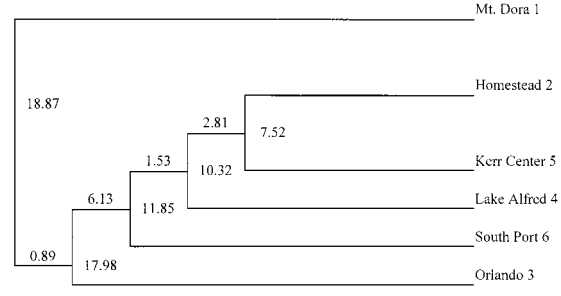

Fig. 6. Phenogram of genetic distances among six Florida *D. abbreviatus* populations based on RAPD allele frequencies. The numbers at the branches indicate the percentage occurrence of the cluster to the left of the branch in 1,000 bootstrapped samples.

Table 6. RAPD allele frequencies of 0.0 or 1.0 in 6 Florida populations of *D. abbreviatus*

RAPD Locus	Mt. Dora	Homestead	Orlando	Lake Alfred	Kerr Center	South Port
n	21	18	11	16	12	7
1A	0.0		0.0			
1B	1.0					
2A					1.0	1.0
3A	1.0					0.0
3B		0.0			0.0	0.0
4A			0.0	0.0	0.0	0.0
4B						0.0
4C	0.0					
5A						
5B	1.0			0.0	0.0	
6A						0.0
6B	0.0		0.0	0.0		
7A						1.0
7B						0.0
7C	1.0			0.0		0.0
7D	0.0				0.0	0.0
7E		0.0	0.0	0.0		0.0
7F		1.0				
8A			1.0	0.0		1.0
8B			1.0		0.0	0.0
9A	1.0		1.0	1.0	1.0	1.0
9B	0.0	1.0	0.0		1.0	

does not change very rapidly), the high percentage of populations compared with other studies of this type (Mendel et al. 1994, Carter et al. 1996, Dowdy and McGaughey 1996), the analysis of eggs from individuals, the number of loci sampled (Gorman and Renzi 1979), and the fact that genetic distance and heterozygosity estimates are hardly affected by sample size (Gorman and Renzi 1979), the RAPD-PCR data appear to be more dependable than the esterase data. Carter et al. (1996) also found that esterase and RAPD-PCR data segregated populations of *Tomicus piniperda* (L.) differently. Reasons for the differences in population segregation by the two techniques await more extensive, basic, and difficult biochemical studies.

The 1964 source of *D. abbreviatus* in the Apopka, FL, area may have been ornamental plants (Woodruff 1968) and the origin of this insect may be the Caribbean (Fennah 1942, Simpson et al. 1996). The genetic distance data (Fig. 2) and the overall G_{ST} value of 0.401 indicate a high level of differentiation among populations of *D. abbreviatus* in Florida. This differentiation could be caused by many factors (e.g., selection by pesticides, genetic drift, and high mutation rates), but we feel that the most likely cause has been independent introductions of a limited number of insects and restricted dispersal among populations. These distinct populations were suggested by genetic distance data and may indicate derivation of these populations from three founder groups of unknown size.

Table 7. *D. abbreviatus* population differentiation by esterase staining and RAPD-PCR

1997 Esterase	1998 Esterase	RAPD-PCR (1998)
Alturas, Tangerine, Orlando, Lake Alfred, Plymouth-Apopka	Orlando	Orlando
Mt. Dora	Mt. Dora	Mt. Dora
Kerr Center	Kerr Center, Homestead	Homestead, South Port, Lake Alfred, Kerr Center
Homestead, South Port	Lake Alfred	
	South Port	

Our data support the supposition that *D. abbreviatus* individuals move relatively short distances. If this weevil were able to move large distances and mate successfully, we would expect less genetic differentiation among populations. Field observations suggest that *D. abbreviatus* is a strong flier only for short distances and tends to remain in a location (Fennah 1942; Beavers and Selheime 1978; Simpson et al. 1996; H.N.N. and S.E.S., unpublished data 1996, 1997). The fact that several RAPD loci were fixed (100%) in one population and totally absent (0%) in another indicates little or no intermating between these particular pairs of populations (Tables 5 and 6). The high level of genetic differentiation among these populations also suggests limited intermating. The genetic similarity of Homestead, Kerr Center, South Port, and Lake Alfred populations, which cover a large geographic range, argues against any isolation by distance hypotheses (Fig. 1; Table 1) and suggests movement by human on or in nursery stock, vehicles, and so on. Our data support the 1964–1974 quarantine of nursery stock and the present quarantine of nurseries for this weevil. The most likely mechanism of spread of *D. abbreviatus* appears to be on infested plant material.

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