Mitochondrial DNA variation in geographic populations of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera; Chrysomelidae)

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Summary. This study demonstrates variability in restriction enzyme cleavage sites of mitochondrial DNA (mtDNA) among four populations of Colorado potato beetle (CPB). A suite of three enzymes (*Eco*RI, *Hpa*I, *Pst*I) was sufficient to discriminate among the populations tested. Individuals heteroplasmic for restriction enzyme patterns were found in some populations. Variability in CPB mtDNA should prove useful in efforts to trace the origin and dispersal of the species in North America.
Key words. Mitochondrial DNA; RFLP; *Leptinotarsa decemlineata*; Colorado potato beetle; population genetics.

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, was first discovered in the USA in 1811, feeding on *Solanum rostratum*[1]. In North America, CPB is found in most of the contiguous USA, southern Canada and Mexico and is known to infest 10 native and exotic Solanaceae species[2]. The beetle is a serious pest of tomato (*Lycopersicon esculentum*) and eggplant (*S. melangena*) and has become the single most destructive insect pest of potato, *S. tuberosum*, in North America and Europe[3,4]. The high reproductive capacity of CPB[5] coupled with the widespread application of pesticide used in control efforts has led to an increasing occurrence of insecticide resistance in some populations[6–9]. Basic to understanding the evolution of insecticide resistance in the species, is a thorough knowledge of the population structure and genetics of CPB. Cytogenetic studies have demonstrated the existence of three chromosomal races within CPB[10,11] and differences in host plant affinities or photoperiod response have also been described for populations from several geographic regions of the USA[2,12,13]. Isozyme studies have revealed a moderate level of variation within the species (mean genetic distance between populations of 0.022)[14], but are unable to unambiguously resolve CPB population structure.

In organisms other than CPB, comparative studies of mitochondrial DNA (mtDNA) have demonstrated extensive nucleotide sequence diversity between conspecific populations and sibling species[15–18]. The mitochondrial genome is maternally inherited and functionally haploid[17–19], features that make mtDNA less susceptible to interpopulation genetic homogenization and more susceptible to population bottlenecks and founder effects than nuclear genes[20–25]. Although Coleoptera represent the largest order within the class Insecta, mtDNA studies on the group are almost non-existent. Recently, *Pissodes* weevils were reported to have exceptionally large mtDNA (30–36 kb) and all individuals analyzed (n = 219) were heteroplasmic for length variants[26]. We report here an analysis of geographic variation in CPB mtDNA using restriction enzymes.

Materials and methods

CPB populations: Field collected beetles (larvae and adults) from four local populations of CPB, Melrose (MD), Wooster (OH), Yakima (WA) and Mission (TX), were maintained under constant and long day conditions (light:dark 16 h:8 h, constant 25 °C) on *Solanum tuberosum*[27]. The sample from Texas was collected in the field on *Solanum trinquintrum*, the primary host plant of the beetle throughout the lower Rio Grande Valley. All other samples were collected in the field on *S. tuberosum*. Eggs from the captured adults beetles were collected and the subsequent larvae and pupae were maintained for 5 to 10 days before nucleic acid extraction. A laboratory population of CPB originally from Melrose was used to provide pure mitochondrial DNA for probe production. This population has been maintained in our laboratory (RFWS) continuously for two years.

mtDNA analyses: Total nucleic acids were isolated from individual larvae, pupae or adults using a modification of methods developed for crickets[28] and honey bees[29]. One individual (larva or pupa) was gently homogenized in a 15 ml Corex tube with 1 ml of grinding buffer (10 mM Tris, 60 mM NaCl, 300 mM sucrose, 10 mM EDTA). One ml of lysis buffer (300 mM Tris, 40 mM SDS, 20 mM EDTA), and 0.7% freshly mixed DEPC were added. The resulting lysate was centrifuged at 1400 × g for 10 min. An equal volume of buffer-saturated phenol was added to the supernatant and the mixture centrifuged for 10 min at 1400 × g. The aqueous phase was retained and extracted once each with phenol:chloroform and chloroform. The final supernatant was then ethanol precipitated at − 20 °C for 2 h. The samples were spun at 1700 × g to pellet the DNA. The pellet was dried under vacuum and resuspended in an appropriate amount of 1 × TE. DNA isolated from a single individual provided enough material for 15 to 20 digests. The following 15 restriction enzymes were used: *BamH*1, *Bgl*1, *Bgl*11, *Cla*1, *Eco*R1, *Eco*RV, *Hind*111, *Hpa*1, *Kpn*1, *Pst*1, *Pvu*1, *Pvu*11, *Sac*1, *Xba*1 and *Xho*I. Restriction enzymes were obtained from Bethesda Research Labora-

tories (Gaithersburg, MD) and New England Biolabs (Beverly, MA) and the digestion conditions were those recommended by the suppliers. Digested DNA was separated on 0.7% or 1.0% agarose gels and the gels stained with ethidium bromide and photographed under UV light. Transfer of DNA to nitrocellulose filters was done by the Southern blotting technique described by Maniatis et al.[30].

To obtain pure mtDNA for probe, 8 g of larvae from the laboratory population were homogenized in mitochondrial isolation medium (MIM) buffer (220 mM mannitol, 70 mM sucrose, 2 mM Hepes, 1 mM EDTA, 1 mM EGTA; pH 7.5) on ice. The mitochondria were concentrated by differential centrifugation. After lysing mitochondria with SDS, mtDNA was purified via two equilibrium centrifugations in CsCl. The mtDNA from the second CsCl gradient was collected, ethidium bromide removed, and the sample dialyzed and resuspended in 1 × TE. The purified mtDNA was nick translated[31] to yield a 32P-labeled probe (650 Ci/mmol, ICN Radiochemicals, Inc.). Hybridization was carried out under standard conditions[30] optimized for CPB (25% formamide, 50 °C). Visualization of mtDNA fragments was conducted via autoradiography and fragment sizes were estimated from autoradiographs with the use of a digitizer and Bioscan (Bioscan Inc., Washington, DC) software.

Results and discussion

The initial screening survey revealed that three of the fifteen enzymes (*Eco*RI, *Hpa*I and *Pst*I) were especially useful in detecting mtDNA variation among the populations (figs 1 and 2). The other twelve enzymes produced invariant fragment patterns. Among the populations we found four patterns for *Eco*RI and *Pst*I and two for *Hpa*I (table). The length of *L. decemlineata* mtDNA is approximately 20 kilobases. The size of CPB mtDNA is larger than the average described for *Drosophila*[32], although smaller than the 30–36 kb reported for *Pissodes* weevils[26].

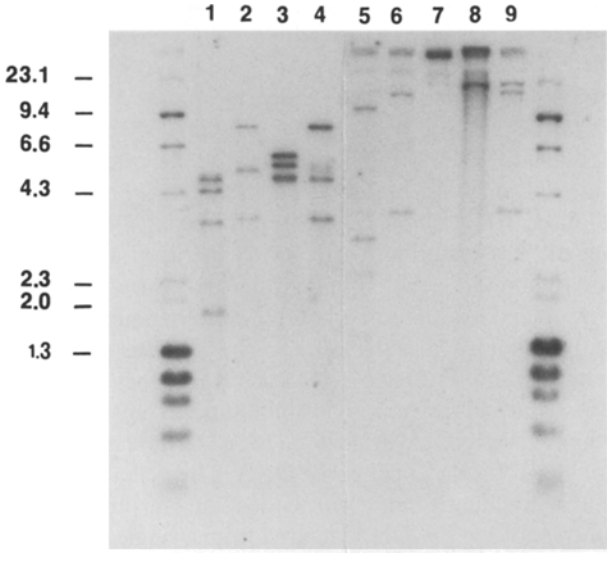

Figure 1. Autoradiograph of Southern blot showing CPB mtDNA of four populations digested with *Eco*RI (lanes 1–4) and *Pst*I (lanes 5–9). Lanes 1 and 5 = MD; lanes 2 and 6 = TX; lanes 3 and 8 = WA; lanes 4 and 7 = OH. Lane 9 = heteroplasmic individual (MD) showing duplex restriction pattern (combining site-variants seen in lane 6 and 8). Size of fragments in kilobase pairs (Kbp) on the left.

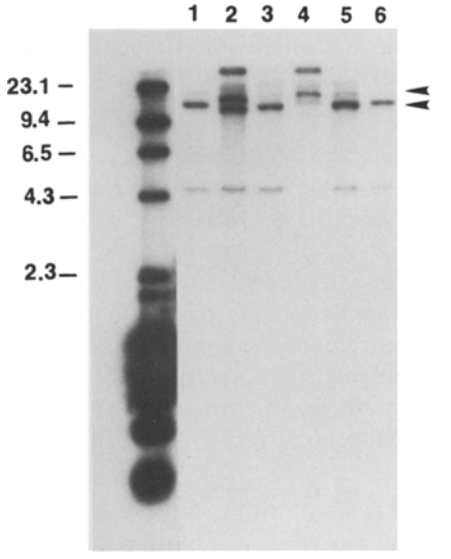

Figure 2. Autoradiograph of Southern blot showing CPB mtDNA of four populations digested with *Hpa*I: lanes 1, 5 and 6 = MD; lane 2 = TX; lane 3 = WA and lane 4 = OH. Some individuals from TX (lane 2) display two size classes (heteroplasmy) of the largest *Hpa*I digestion product indicated by arrows. Size of fragments in kilobase pairs on the left.

Based on the restriction patterns of these enzymes we are able to differentiate all CPB populations analyzed. However, recent analyses of mtDNA variation in field populations have shown that the 'Texas' *Eco*RI pattern (fig. 1), in addition to several infrequent variants, also occurs in Maryland (Azeredo-Espin, unpublished data). Although the samples from Yakima and Wooster appear monomorphic for the haplotype shown in figure 1, the limited sampling of these populations preclude such a conclusion. Heteroplasmy in restriction patterns was detected in individuals of some populations (MD, TX; fig. 1, lane 9 and fig. 2, lane 2) and confirmed by progeny analysis from isofemales lines (Azeredo-Espin, unpublished data).

Certainly the limited sampling represented by these single field collections does not allow us to estimate CPB population structure. However, characterization of mtDNA variation in CPB populations may help resolve important unanswered questions concerning population and evolutionary genetics within *L. decemlineata*. For example, CPB and its wild host plants are believed to have originated in Mexico[8,33,34]. Karyotypic analyses indicate that 3 chromosomal races exist for a pericentric inversion on the 2nd chromosome of CPB[11] in the USA. It has been suggested that the CPB population in extreme south Texas (acrocentric race), represents the original stock of the beetle that spread north and east within the

Mean estimated size (bp) of mtDNA fragments of the four populations with *Eco*RI, *Hpa*I and *Pst*I.

*Eco*RI				*Hpa*I				*Pst*I			
MD	TX	OH	WA	MD	TX	OH	WA	MD	TX	OH	WA
n = 30	n = 30	n = 15	n = 20	n = 25	n = 25	n = 10	n = 15	n = 25	n = 20	n = 10	n = 15
5930	8600	8470	5970	15420	15850	19860	14840	12560	17400	20500	20200
4980	5130	4700	5680	4350	4350		4320	2980	3410		
3280*	3420	3380	4730					2460			
1820								1840			

Note: * The length of *L. decemlineata* mtDNA is approximately 20 Kb. Digestions with *Eco*RI give an apparent length of approximately 16.5 Kb, although double digests of mtDNA from MD-CPB confirm that there are two fragments of approximately the same size class (2380 bp).

USA and ultimately across the Atlantic[12]. Comparative examination of Mexican and USA populations with mtDNA and karyotypic analysis could be used to confirm this correlation between variation in nuclear and mitochondrial genes for CPB. If this hypothesis is valid the mtDNA restriction fragment patterns found in the indigenous Texas population are then, in a practical sense, genetic markers that may be used to characterize the pattern of dispersal of CPB in North America.

It has become increasingly clear that genetic variability among and within populations of insect pests affects the success of biological control agents, resistant plant varieties and insecticide resistance management. The surprising amount of mtDNA variation found in our limited sampling of CPB populations suggests that further study of mtDNA will be valuable for resolving evolutionary, genetic and pest management questions that persist regarding this insect.

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