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Short Communication

Mitochondrial DNA variation among host races of *Eurosta solidaginis* Fitch (Diptera: Tephritidae)

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Abstract

Eurosta solidaginis Fitch (Diptera: Tephritidae) induces galls on two species of goldenrod, *Solidago* (Compositae), in the northern regions of the United States. Recent studies have demonstrated that *E. solidaginis* is comprised of two host races that differ in adult emergence times, mate preference, and host preference. However, it is not known how much genetic variation, if any, exists among *E. solidaginis* host-associated populations west of Minnesota where the two host races occur in sympatry. Sequencing analysis was used to characterize two mitochondrial gene fragments: (1) NADH1 dehydrogenase (ND1: 539 bp) and (2) cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ (CO2KD: 396 bp) from sympatric, host-associated populations of *E. solidaginis* in North Dakota. Our results indicated that two genetically distinct lineages exist among *E. solidaginis* in North Dakota that correspond with host-plant association.

1. Introduction

Many closely related tephritids utilize more than one host-plant species (Foote et al., 1993; White and Elson-Harris, 1992). Bush (1969, 1975) suggested that adaptation to a new host plant could serve as a barrier to gene flow resulting in the formation of so-called ''host races'' (see Diehl and Bush, 1984), even if the new host-plant species is sympatric in distribution with the ancestral host. Therefore, host-race formation may be a critical intermediate step in the sympatric speciation of some phytophagous insects.

The existence of host races has been documented in several unrelated tephritid genera, including *Rhagoletis* (Bush, 1969), *Tephritis* (Romstöck-Völkl, 1997), and *Eurosta* (Abrahamson and Weis, 1997; Abrahamson et al., 1994; Craig et al., 1993; Ming, 1989). The minimal requirements for host-race formation and maintenance include a change in the preference for oviposition sites, physiological adaptations to the new host and habitat, and host-related assortative mating (Tauber and Tauber, 1989). In addition to quantifying these requirements, recent research has focused on determining the extent of genetic variation among host races using allozymes, restriction fragment length polymorphisms (RFLPs), and DNA sequencing (e.g., Brown et al., 1996; Feder et al., 1988, 1990; McPheron, 1990; McPheron et al., 1988; Waring et al., 1990).

Several recent studies (e.g., Brown et al., 1996; Waring et al., 1990) have demonstrated that host races of the gall-forming tephritid, *Eurosta solidaginis* Fitch, have genetically differentiated on *Solidago altissima* and *S. gigantea*, two species of goldenrod (Compositae) in the northern United States. Brown et al.'s (1996) analysis of DNA sequences from the 3′ ends of the mitochondrial cytochrome oxidase I and II subunits (492 bp total) revealed two distinct *E. solidaginis* haplotypes in the eastern US that correlated with host-plant source. Subsequent restriction site analysis based on these same gene regions indicated that all flies using *S. gigantea* as a

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host were of one haplotype, whereas flies reared from *S. altissima* were geographically partitioned into two different haplotypes corresponding to the east and west of the state of Michigan (Brown et al., 1996).

The genetic characterization of northeastern US populations of *E. solidaginis* by Brown et al. (1996) provided insight into the historical direction of the host shift (i.e., from *S. altissima* to *S. gigantea*) supporting earlier results by Waring et al. (1990) based on horizontal starch gel electrophoresis of protein loci. However, it is not known how much genetic variation, if any, exists among *E. solidaginis* host-associated populations west of Minnesota where the two host races occur in sympatry. Thus, the objective of the present study was to assess the amount of genetic variation among host-associated races of *E. solidaginis* inhabiting the Oakville Prairie Preserve in North Dakota using DNA sequence data from portions of the mitochondrial NADH1 dehydrogenase (ND1), cytochrome oxidase II + $tRNA_{Lys}$ and $tRNA_{Asp}$ genes (hereafter referred to as CO2KD). The Oakville Prairie Preserve is a tallgrass prairie habitat where both *S. altissima* and *S. gigantea* are approximately equally abundant and represents an extreme western zone of sympatry for *E. solidaginis* host races (J.T. Cronin, unpublished data). From a taxonomic perspective, we were also interested in assessing whether there was genetic evidence to support specific status for each of the host-associated races of *E. solidaginis*.

2. Materials and methods

A list of samples analyzed in this study along with their associated GenBank accession numbers is presented in Table 1. Flies from North Dakota were collected at the University of North Dakota's Oakville Prairie Preserve, located approximately 16 km west of Grand Forks, ND. All *E. solidaginis* specimens included in this study were field-collected as galls and reared to adulthood. *Gymnocarena mexicana* (Diptera: Tephritidae: Tephritinae) was used as the outgroup. Voucher specimens and/or genomic extracts are located in the Insect Genetics Laboratory, Department of Entomology, Kansas State University.

DNA was extracted from thoracic tissue of individual adult flies following the procedure outlined by Kambhampati and Smith (1995). The NADH1 dehydrogenase reverse primer (ND1-R) used in this study was designed by Smith et al. (1999). The NADH1 dehydrogenase forward primer was designed by aligning selected dipteran sequences obtained from GenBank. The sequences of the two primers were: ND1-F (N1-J-11861) 5′-ATC ATA ACG AAA YCG AGG TAA-3′ and ND1-R (N1-N-12530): 5′-CAA CCT TTT AGT GAT GC-3′; The primers were used to amplify a ~550 bp fragment from 13 individuals. In addition, a ~400 bp DNA fragment comprised of a portion of the 3′ end of the cytochrome oxidase II gene, all of the $tRNA_{Lys}$, and most of the $tRNA_{Asp}$ genes (CO2KD) was amplified and sequenced from 10 individuals using the primers: (C2-J-3549) 5′-CAA ATT CGA ATT TTA GTA ACA GC-3′ and (TD-N-3884) 5′-TTA GTT TGA CAW ACT AAT GTT AT-3′.

Polymerase chain reaction (PCR) amplifications were performed in 50 μl volume as described by Kambhampati et al. (1992). The temperature profile for the amplification of the two gene fragments included an initial denaturation step of 95 °C for 3 min followed by 40 cycles of

Table 1
List of *Eurosta solidaginis* samples included in the study with GenBank accession numbers

ID	Locality	Host plant	GenBank Accession ND1	CO2KD
Esol-Konza	Konza Praire, KS	*S. gigantea*	AF360517	NA
Esol-Alabama	Auburn, AL	*S. gigantea*	AF360518	AF360532
Esol-ND-10	Oakville Praire, ND	*S. gigantea*	NA	AF360540
Esol-ND-34	Oakville Praire, ND	*S. gigantea*	AF360519	NA
Esol-ND-35	Oakville Praire, ND	*S. gigantea*	AF360520	NA
Esol-ND-57	Oakville Praire, ND	*S. gigantea*	AF360521	AF360533
Esol-ND-58	Oakville Praire, ND	*S. gigantea*	AF360522	NA
Esol-ND-59	Oakville Praire, ND	*S. gigantea*	AF360532	AF360531
Esol-ND-127	Oakville Praire, ND	*S. gigantea*	AF360527	NA
Esol-ND-102	Oakville Praire, ND	*S. altissima*	AF360525	AF360537
Esol-ND-133	Oakville Praire, ND	*S. altissima*	AF360524	AF360534
Esol-ND-194	Oakville Praire, ND	*S. altissima*	NA	AF360536
Esol-ND-195	Oakville Praire, ND	*S. altissima*	AF360529	NA
Esol-ND-200	Oakville Praire, ND	*S. altissima*	NA	AF360539
Esol-ND-202	Oakville Praire, ND	*S. altissima*	NA	AF360535
Esol-ND-203	Oakville Praire, ND	*S. altissima*	AF360526	AF360538
Outgroup				
Gymnocarena mexicana	Michoacan, Mexico	NA	AF360530	AF360541

NA: not available.

95 °C for 30 s, 42–45 °C for 45 s and 72 °C for 45 s. PCR products were purified using the Wizard PCR-Preps DNA Purification System (Promega). Sequencing was conducted on an ABI 377 automated sequencer (DNA Sequencing Core Facility, University of Florida), using the D-Rhodamine Dye Cycle Sequencing Ready Reaction Kit FS (Perkin–Elmer) according to the manufacturer's specifications. Both strands of the PCR product were sequenced for all samples.

DNA sequences were read from electropherograms into a computer using the Sequence Navigator program and aligned manually. DNA and amino acid sequence variation was analyzed using parsimony analysis in PAUP* ver 4.0b6 (Swofford, 1998). Sequence alignment files in Nexus format are available from P.T.S.

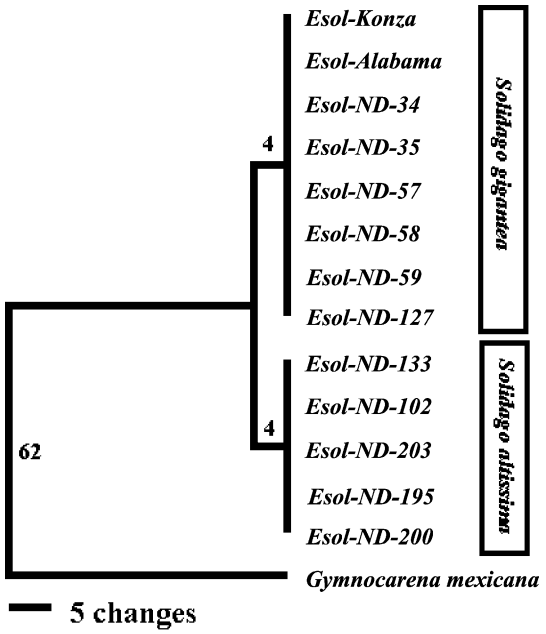

Fig. 1. Single most parsimonious tree based on unweighted parsimony analysis of a 539 bp portion of the mitochondrial NADH1 dehydrogenase gene for 13 *Eurosta solidaginis* samples and an outgroup. Tree length: 70; consistency index: 1.00; retention index: 1.00. Numbers above branches are number of substitutions.

3. Results and discussion

3.1. Genetic variation and phylogenetic analysis

The size of the amplified mitochondrial NADH1 dehydrogenase gene fragment was invariant among the included samples at 539 bp. The size of the CO2KD gene fragment ranged from 373–387 bp among the 10 individuals.

Sequence alignment was straightforward and did not necessitate the insertion of many gaps. The alignment of the ND1 fragment resulted in 596 characters. Of these, 8 (1.34%) characters were variable and separated *E. solidaginis* into two distinct groups according to host plant (Fig. 1), supporting previously published results (Brown et al., 1996; Waring et al., 1990). A list of diagnostic nucleotide sites that distinguish between the two host races is shown in Table 2. Two non-synonymous substitutions were identified at sites 521 and 524 of the alignment (Table 3).

The alignment of the sequenced CO2KD fragment resulted in 396 characters, including gaps. Of the 396, three (0.75%) characters were variable among the host races and separated *E. solidaginis* into four haplotypes (three from *S. gigantea* and one from *S. altissima*) (Fig. 2). The discovery of multiple haplotypes within the *S. gigantea* host race is in contrast to the results Brown et al. (1996) who, based on an analysis of the mitochondrial cytochrome oxidase I and II subunits, reported that all flies using *S. gigantea* as a host were of one haplotype, whereas flies reared from *S. altissima* consisted of two different haplotypes. The Esol-ND-57 sample differed from Esol-ND-59 and Esol-Alabama by a single substitution and from Esol-ND-10 by two substitutions (Fig. 2). Thus, in a pairwise comparison of DNA sequences, one to three substitutions separated the two host races, depending upon which *S. gigantea* haplotype was used (data not shown).

3.2. Taxonomic implications

Seven species are currently recognized within the genus *Eurosta*, all of which induce galls on various gold-

Table 2
Diagnostic nucleotide sites based on the alignment of a 539 bp portion of the mitochondrial NADH1 dehydrogenase gene for 13 *Eurosta solidaginis* samples

Nucleotide site	Substitution type	*S. altissima*	*S. gigantea*
1	Transversion[a]	T	G
106	Transition-synonymous	G	A
205	Transition-synonymous	G	A
391	Transition-synonymous	G	C
433	Transition-synonymous	G	A
466	Transition-synonymous	A	G
521	Transition-non-synonymous	A	G
524	Transition-non-synonymous	A	G

[a] Site number 1 is a 3rd codon position that was excluded from the amino acid translation.

Table 3
Mitochondrial NADH1 dehydrogenase amino acid comparison ($n = 179$) for the two *Eurosta solidaginis* host races

gigantea	NYISYYFSPIFSLFLSLVVWFCMPLFIKLFSFNLGLLFFLSCASLGVYSIM
altissima	NYISYYFSPIFSLFLSLVVWFCMPLFIKLFSFNLGLLFFLSCASLGVYSIM
gigantea	IAGWSSNSNYALLGGLRAVAQTISYEVSMVLILLSFVFLIGSYNIFQFFYY
altissima	IAGWSSNSNYALLGGLRAVAQTISYEVSMVLILLSFVFLIGSYNIFQFFYY
gigantea	QKYIWFLVIFLPISFVWFCICLAETNRTPFDFAEGESELVSGFNVEYSSGG
altissima	QKYIWFLVIFLPISFVWFCICLAETNRTPFDFAEGESELVSGFNVEYSSGG
gigantea	FALIFMAEYSSILFMSMLFS**VV**FLGC
altissima	FALIFMAEYSSILFMSMLFS**II**FLGC

Differences are shown in boldface text.

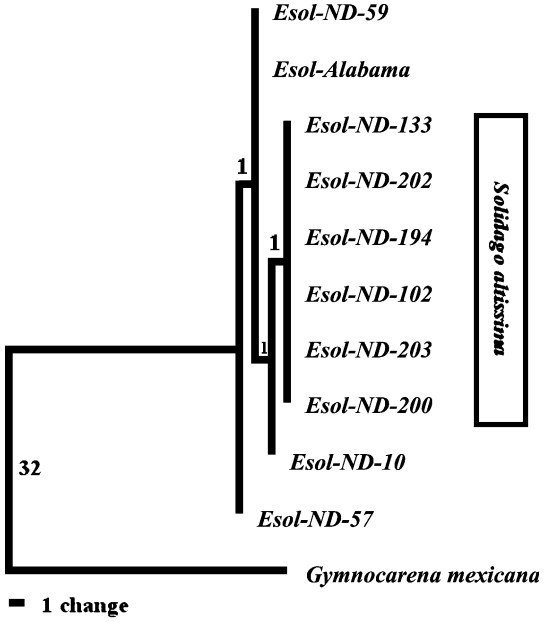

Fig. 2. Single most parsimonious tree based on unweighted parsimony analysis of a 396 bp portion (including gaps) of cytochrome oxidase II + $tRNA_{Lys}$ + $tRNA_{Asp}$ genes for 10 *Eurosta solidaginis* samples and an outgroup. Tree length: 35; consistency index: 1.00; retention index: 1.00. Numbers above branches are number of substitutions.

enrod species (Ming, 1989). Ming's (1989) revision of the genus *Eurosta* suggested that *E. solidaginis* exists as two subspecies: *E. solidaginis solidaginis* (Fitch) in the eastern US and *E. solidaginis fascipennis* Curran in the western US. The two subspecies can roughly be distinguished as adults by differences in wing pattern (Foote et al., 1993; Ming, 1989).

Brown et al. (1996) showed that all *S. gigantea*-associated flies, regardless of geographic origin, exhibited a single haplotype. In contrast, our data from the CO2KD region indicated that *S. gigantea*-associated flies exhibited three different haplotypes. Similarly, Brown et al. (1996) showed that *S. altissima*-associated flies exhibited two haplotypes that correlated with geographic origin in Michigan. One of these haplotypes (i.e., the one east of Michigan) was most similar to the *S. gigantea* haplotype, suggesting that *S. altissima* is the ancestral host and that *S. gigantea*-associated populations are derived from *S. altissima*-associated populations in the eastern US. In contrast, our data from the CO2KD region indicated that all *S. altissima*-associated flies included in represented a single haplotype. The conflicting results suggest that *E. solidaginis* may exist as a complex of host-associated sibling species. Moreover, differences in results of the present study and those of Brown et al. (1996) suggest that there may have been more than one host shift in the evolutionary history of *E. solidaginis*. However, increased sampling throughout the entire range of *E. solidaginis* is needed to shed more light on this issue.

Our analysis of the NADH1 dehydrogenase gene provided a somewhat clearer picture, as the two host races were separated by eight substitutions, two of which were non-synonymous (Fig. 1, Table 3). The *E. solidaginis* host races included in this study exhibited levels of variation similar to those detected in some well-defined *Bactrocera* species. For example, eight mutations separate *B. trilineola* and *B. frauenfeldi* and six mutations separate *B. carambolae* and *B. kirki* (Smith et al., accepted). Although it is possible that the NADH1 dehydrogenase gene evolves at different rates within the *Eurosta* and *Bactrocera* lineages, the level of genetic variation exhibited by host races of *E. solidaginis* warrant further investigation of their taxonomic status, i.e., whether each host race must be elevated to a separate species. A limitation of the present study is the small sample size within the zone of overlap where the two host races occur in sympatry. It is clear that more molecular data from additional western populations are needed to confirm the specific status of *E. solidaginis* host races. However, based on strong host-plant preference (Craig et al., 1993), differences in wing pigmentation (Ming, 1989), and consistent levels of variation within the mitochondrial NADH1 dehydrogenase gene (present study), there is evidence from multiple sources to support an elevation in taxonomic rank from sub-specific to specific for each of the two host races, i.e., *E. fascipennis* (Curran) associated with *S. altissima* and *E. solidaginis* (Fitch) associated with *S. gigantea*.

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