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K.A. Shufran

A.A. Weathersbee III

D.B. Jones

N.C. Elliott

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K. A. SHUFRAN,[1, 2] A. A. WEATHERSBEE III,[3] D. B. JONES,[4] AND N. C. ELLIOTT[1]

USDA–ARS, Plant Science and Water Conservation Research Laboratory, 1301 N. Western Road, Stillwater, OK 74075

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ABSTRACT *Lysiphlebus testaceipes* (Cresson) is a solitary endoparasitoid of aphids and is the primary parasitoid attacking cereal aphids in the Great Plains, especially *Schizaphis graminum* (Rondani). In a previous study, it was found that a Lincoln, NE, isolate of *L. testaceipes* had a much higher survivorship at cold temperatures than isolates from Stillwater, OK, and Corpus Christi, TX. This suggested that the Nebraska isolate was locally adapted to the northern environment and perhaps genetically divergent from southern populations. We tested for genetic differentiation of the above isolates by sequencing portions of the COI and 16S mtDNA genes. We also examined a Florida isolate reared from *Toxoptera citricida* (Kirkaldy) and *L. fabarum* Marshall as an outgroup. The Great Plains isolates (Nebraska, Oklahoma, and Texas) were homogeneous with 0% and 0–0.2% sequence divergence in the COI and 16S gene fragments, respectively. The Florida isolate differed from the Great Plains isolates in nucleotide sequence by 1.4% (COI) and 0.5–0.7% (16S). Phylogenetic analysis placed the Florida isolate of *L. testaceipes* basal to the Great Plains isolates with *L. fabarum*, suggesting a possible species complex within *L. testaceipes*.

KEY WORDS parasitoid, aphid, biological control, geographic variation, cold tolerance

Lysiphlebus testaceipes (Cresson) is a solitary endoparasitoid of aphids and is native to the United States. Its geographic range is Nearctic, Neotropical, and Oceanic, and it is also found in Europe as a result of intentional introductions (Mackauer and Starý 1967). It has a very broad host range, having been reared from over 120 aphid species (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000, French et al. 2001, Tang et al. 2002). In the American Great Plains, it is a primary biological control agent of aphid pests in cereal and grain crops, especially wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and sorghum (*Sorghum bicolor*). Among the most common hosts for *L. testaceipes* in these agriculture systems are *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch), and *R. padi* L.

Despite the importance of *L. testaceipes* as a biological control agent, surprisingly little is known of its ecology or population genetics. For a species with such a wide geographic distribution and host range, we would expect to find genetic divergence. A few studies have shown diversity within *L. testaceipes.* Based on random amplified polymorphic DNA and allozyme data, constrained gene flow was found among *L. testaceipes* populations parasitizing *Aphis varians* Patch on fireweed (*Epilobium angustifolium* L.) in subalpine mountain drainages (Hopkins 1998). Variation in *L. testaceipes* has been noted in mtDNA sequence divergence between Washington and Oklahoma populations (Chen et al. 2002). Also, successful parasitism of *Aphis spiraecola* (Patch) (a synonym of *A. citricola* van der Groot) by *L. testaceipes* varies by location, presumably because of genetic differences in the wasp (Costa and Starý 1988). Incomplete development occurs in California, France, and Italy, but *A. spiraecola* is the principal host of *L. testaceipes* in Cuba (Costa and Starý 1988). Royer et al. (2001) demonstrated that a Nebraska population had a much higher survivorship at cold temperatures than populations from Oklahoma and Texas. This suggested local adaptation to the environmental extremes and possible genetic divergence of populations. Host adaptation was not an issue in this case, because all three populations originated from *S. graminum* (Royer et al. 2001).

To test the hypothesis of geographical adaptation, we explored whether there was parallel genetic variation in mtDNA genes and cold tolerance between the

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[1] USDA–ARS, Plant Science & Water Conservation Research Laboratory, 1301 N. Western Road, Stillwater, OK 74075.

[2] E-mail: kevin.shufran@ars.usda.gov.

[3] USDA–ARS, U.S. Horticultural Research Laboratory, 2001 S. Rock Road, Fort Pierce, FL 34945.

[4] Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078.

three geographic isolates of Royer et al. (2001). We also included another population from Florida, reared from *Toxoptera citricida* (Kirkaldy), to test for possible geographic and/or host variation.

Materials and Methods

Insect specimens were air-dried, laboratory-reared female *L. testaceipes* of the three geographic isolates (Lincoln, NE; Stillwater, OK; and Corpus Christi, TX) examined by Royer et al. (2001). All three isolates were originally collected from *S. graminum* on sorghum and laboratory reared for their experiment on cold tolerance. Each colony was established from 50–100 females and reared 3–4 generations (Royer et al. 2001). Specimens of another *L. testaceipes* laboratory population collected from *T. citricida* in Fort Pierce, FL, also were used (Tang et al. 2002). The Florida colony was established in November 1999 (Tang et al. 2002), and our specimens were removed from the colony in April 2000 and preserved in 95% ethyl alcohol. An asexual form of *L. fabarum* Marshall, reared from *Aphis schneideri* Börner in Germany, was the outgroup (preserved in 95% ethyl alcohol) (Belshaw et al. 1999). A wild population of *L. testaceipes* reared from *R. padi* in Stillwater, OK (six females), also was included. Voucher specimens are held by KAS.

We polymerase chain reaction (PCR)-amplified portions of the cytochrome oxidase subunit I (COI) and16S rRNA mitochondrial genes for DNA sequencing. Three females in each isolate were used. Each insect was ground in a buffer according to Black et al. (1992), and 1–2 μl of the homogenate was used as DNA template per reaction. Three to four reactions were conducted for each individual, isolate, and gene combination.

The primers used for PCR amplification and sequencing were as follows: COI, C1-J-1718 and C1-N-2191 (Simon et al. 1994); 16S, 16SWb and 16S.Sh (Dowton and Austin 1994, Chen et al. 2002). Reactions were carried out in 50-μl volumes with 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 0.4 mM dNTPs; 1.5 mM $MgCL_2$ for the COI and 3.0 mM $MgCL_2$ for the 16S; 20 pmol each primer; and 2.0 U *Taq* DNA polymerase added at the first annealing step. An MJ Research PTC-100 Thermal Controller with "Hot-Bonnet" was used with the following program steps: (1) 96°C for 3 min; (2) 94°C for 30 s; (3) 45°C (16S) or 50°C (COI) for 30 s; (4) 72°C for 1 min; (5) cycle to step 2, 29 times; (6) 72°C for 5 min; (7) 4°C hold. PCR products were gel-purified using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI) and were directly sequenced using an ABI automated sequencer at the Sequencing and Genotyping Facility (Department of Plant Pathology, Kansas State University, Manhattan, KS).

The sequences were aligned with ClustalW (Thompson et al. 1994) using the Lasergene Version 5.06 software package (DNASTAR, Madison, WI). Distance and maximum likelihood (ML) methods were used in phylogeny reconstruction with the Phylogenetic Analysis Using Parsimony (PAUP) version 4 statistical package (Swofford 2002). Distances were estimated by the method of Tamura and Nei (1993) using gamma correction factors of $\alpha = 0.0132$ (16S) and $\alpha = 0.0125$ (COI) estimated by ML in PAUP. Dendrograms were produced with neighbor-joining (NJ) analysis (Saitou and Nei 1987) based on the above estimated distances with 500 bootstrap replications. ML dendrograms were produced by the method of Hasegawa et al. (1985) using a heuristic search method. *L. fabarum* was declared the outgroup in all tests. All sequences were submitted to GenBank (accession numbers: AY207558–AY207567).

Table 1. Percent nucleotide divergence among *L. testaceipes* isolates and *L. fabarum*

	NE	OK	TX	FL	*L. fabarum*
Nebraska	*	0.2	0.0	0.5	4.4
Oklahoma	0.0	*	0.2	0.7	4.6
Texas	0.0	0.0	*	0.5	4.4
Florida	1.4	1.4	1.4	*	4.4
L. fabarum	7.4	7.4	7.4	8.1	*

Above diagonal, 16S mtDNA, 438 bp; below diagonal, COI mtDNA, 428 bp.

Results and Discussion

PCR products for the COI and 16S genes were both ≈500 bp in length. However, we were able to obtain only 428 and 438 usable nucleotides for the COI and 16S fragments, respectively, because of sequencing errors at the 5′ and 3′ ends. The nucleotides adenine and thiamine were abundant in both the COI (74.2%) and 16S (82.3%) fragments and may have contributed to sequencing error.

Each isolate was homogeneous, with no sequence variation among individuals. Therefore, only one sequence for each isolate was used. Genetic distances among *L. testaceipes* isolates and *L. fabarum* (in percent nucleotide divergence) are shown in Table 1. Despite a significant difference in *L. testaceipes* from Nebraska to withstand cold temperatures compared with those in Oklahoma and southern Texas (Royer et al. 2001), we found the mtDNA gene sequences of these three populations to be homogeneous. There were no differences in their COI DNA sequences, and their 16S DNA sequences were minimally divergent. Even though the 16S-NJ tree separated the Nebraska isolate branch from the Oklahoma and Texas clade, the separation had only 56% bootstrap support.

Despite the observed homogeneity of the Great Plains *L. testaceipes* populations, there was weak evidence provided by the NJ tree based on 16S sequences (Fig. 1) that the Nebraska population may be diverging from the Oklahoma and Texas populations. However, each geographic isolate may not represent the total variation that could be present in the wild population. In fact, homogeneity of each laboratory may have been increased by the method of establishment and rearing. However, a second population of *L. testaceipes* from Stillwater, OK, had identical 16S and COI sequences as the Royer et al. (2001) isolate from Okla-

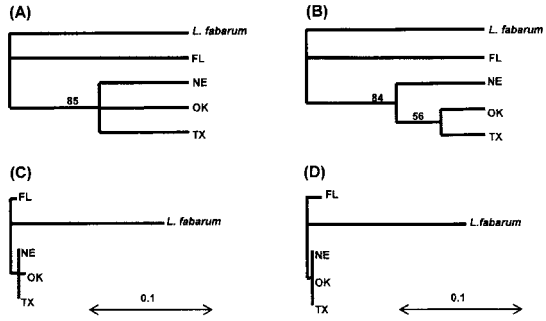

Fig. 1. Dendrograms of *L. testaceipes* isolates (Nebraska, Oklahoma, Texas, and Florida) and *L. fabarum* (declared outgroup) based on mtDNA sequences of 16S rRNA (A and C) and COI genes (B and D). Methods used were neighbor-joining (A and B) and maximum likelihood (C and D).

homa (data not shown). Mitochondrial DNA sequences may not accurately reflect local geographic adaptation caused by cold tolerance because of the maternal nature of mtDNA inheritance. Nuclear DNA markers, which are inherited bi-parentally, may be more informative.

While we found little divergence in *L. testaceipes* populations associated with cereal aphids in the Great Plains, genetic subdivision was recorded previously in *L. testaceipes* populations in Colorado, albeit in a different plant–aphid system (Hopkins 1998). Our analyses indicated that the Florida population of *L. testaceipes* was divergent from those of the Great Plains. In all of the dendrograms produced, it did not group with the Nebraska, Oklahoma, or Texas populations but was placed basal to these along with the outgroup *L. fabarum.* This may be evidence for a species complex in *L. testaceipes*, which seems a tenable hypothesis based on its broad host and geographic ranges. Indeed, many more individuals and populations from unique areas and host species need to be examined to determine if this is the case.

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