Mitochondrial Phylogeography of the Vegetable Pest *Liriomyza trifolii* (Diptera: Agromyzidae): Diverged Clades and Invasive Populations

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ABSTRACT The leafmining fly *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) is an important pest of vegetable and cut-flower crops. In recent decades, this species has become invasive, spreading from the Americas to the rest of the world. Despite substantial losses caused by *Liriomyza* leafminers, the systematics of these flies has remained poorly understood because of their small size and morphological homogeneity. Previous molecular research on other polyphagous *Liriomyza* pests has suggested that cryptic species may be present. Here, we use mitochondrial cytochrome oxidase I sequence variation to investigate phylogeographic structure within *L. trifolii*. Our results indicate that *L. trifolii* harbors distinct phylogenetic clades, suggesting the presence of cryptic species. There is also evidence of a recently derived, highly specialized pepper (*Capsicum* spp., Solanaceae)-feeding population within *L. trifolii* that may represent a host race or even a distinct species. Introduced populations from various locations contained a highly restricted subset of the mitochondrial variation present within *L. trifolii*, suggesting one or more bottlenecks during colonization.

KEY WORDS cryptic species, host-race, leafminer, invasive species, Agromyzidae

Many insect species that are of economic or medical importance belong to complexes of cryptic species that are difficult to distinguish morphologically but that may exhibit important ecological or behavioral differences (e.g., Scheffer 2005). The advent of molecular systematics has increased our ability to differentiate morphologically cryptic groups within what had been considered single species (Avise 1994). The more recent development of mitochondrial phylogeography (Avise 2000), where genealogical relationships among geographically distributed mitochondrial lineages are explored, has the additional advantage of allowing the investigation of evolutionary and biogeographic history (phylogeography) of lineages within species complexes. For species of economic or medical importance, insight from phylogeographic analysis may be critical to proper understanding of biological and ecological variation and for designing effective management strategies (Rosen 1978, Schauff and LaSalle 1998).

Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) is one of several polyphagous leafmining pests of vegetable and flower crops in the genus *Liriomyza* Mik. Although 23 *Liriomyza* species are recorded as pests or potential pests worldwide (Spencer 1973), by far the most damaging species are *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *L. trifolii* (Spencer 1973, Parrella 1982, Parrella and Keil 1984). All three of these polyphagous species are native to the Americas, but they have spread around the world in recent decades (Spencer 1973, Minkenberg 1988, Scheffer et al. 2001); introduced populations are prone to outbreaks and are notoriously difficult to control (Minkenberg 1988, Minkenberg and van Lenteren 1986, Schreiner 1995). There is a long history of taxonomic confusion regarding species boundaries and species hypotheses in all three of these species (reviewed in Spencer 1965, 1973; Parrella 1982; Parrella and Keil 1984; Spencer and Steyskal 1986).

Recent research on *L. huidobrensis* s.l. (as defined at the time) and *L. sativae* has uncovered highly diverged mitochondrial clades within these species, suggesting the presence of cryptic species (Scheffer 2000, Scheffer and Lewis 2005). In *L. huidobrensis,* the mitochondrial divergence was corroborated by fixed differences in two nuclear genes, and the name *Liriomyza langei* Frick was reinstated for the North American (California and Hawaii) clade, whereas the name *L. huidobrensis* was restricted to the South American clade (Scheffer and Lewis 2001). Currently, the highly diverged mitochondrial clades shown in *L. sativae* have not yet been corroborated by data from nuclear genes, but biological differences in the form of differences in *Wolbachia* (Rickettsiae) infection status have been observed between the two major clades of this fly species (unpublished data).

Recent research on *L. trifolii* suggests that it, too, may comprise more than one biological entity. Within

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California, behavioral and genetic data indicate that there is a genetically and reproductively distinct population specialized on peppers, *Capsicum annuum* L. (Solanaceae) in addition to a more polyphagous form (Morgan et al. 2000, Reitz and Trumble 2002). This is consistent with anecdotal reports of *L. trifolii* from different locations differing in preferred host plants. Scientists have even been warned that "flies identified as the same species . . . may not necessarily exhibit similar characteristics, even though they may be from the same host" (Parrella 1982). With *L. trifolii* continuing to spread around the world, it is important to adequately characterize the populations of this species. An understanding of the makeup and origin of invasive populations could aid management efforts and provide general information on invasion biology.

The purpose of this study was to investigate the global phylogeographic structure of *L. trifolii.* Specifically, we address four questions: 1) are highly diverged mitochondrial clades present within this species; 2) what are the geographic distributions of such lineages; 3) what are the host affiliations of such lineages, with special attention to flies feeding on peppers; and 4) what are the structures and sources of invasive populations?

Materials and Methods

L. trifolii specimens were obtained from a variety of locations and hosts around the world (Table 1). Of particular interest were flies obtained from pepper and nonpepper colonies from John Trumble's laboratory (University of California, Riverside, CA), which were the source of flies used in experiments showing genetic and behavioral differences in pepper- and nonpepper-feeding *L. trifolii* in California (Morgan et al. 2000, Reitz and Trumble 2002). Also included in this study were previously published sequences from 40 *L. trifolii* specimens obtained in the Philippines as part of a DNA barcoding study (Scheffer et al. 2006).

Larval, pupal, adult, or combinations of *L. trifolii* were preserved for study in 95% ethanol and stored at −80°C. Before DNA extraction, morphological features of specimens were checked by S.J.S. to ensure that only *L. trifolii* were included in the study. Because larval specimens of *L. trifolii* cannot be distinguished morphologically from those of its sister species, *L. sativae,* mitochondrial cytochrome oxidase I (COI) data were used to determine species identity of juvenile specimens in the study. Vouchers of adults from several populations have been deposited in the National Museum of Natural History in Washington, DC.

Total nucleic acids were extracted from single specimens by using the DNeasy insect protocol B (QIAGEN, Valencia, CA). Polymerase chain reaction (PCR) amplification of most of cytochrome oxidase I (COI) was carried out using a Mastercycler Gradient thermocycler (Eppendorf Scientific, Westbury, NY) with a touchdown amplification program: initial denaturation at 92°C for 2 min, followed by two touchdown cycles from 58 to 46°C (10 s at 92°C, 10 s at 58–46°C, 2 min at 72°C), 29 cycles of 10 s at 92°C, 10 s at 45°C, 2 min at 72°C, and a final extension step for 10 min at 72°C. A single fragment of 1,533 bp resulted from PCR amplification with primers C1-J-1535 (5′-ATTGGAACTTTATATTTTATATTTGG-3′) and TL-N-3017 (5′-CTTAAATCCATTGCACTA-ATCTGCCATA-3′) (primer names follow the system of Simon et al. 1994). PCR product was purified using the QIAquick PCR purification kits (QIAGEN). Sequencing complimentary strands of the 3′ region of COI was carried out using the internal primer C1-J-2441 (5′-CCTACAGGAATTAAAATTTTTAG TTGATTAGC-3′) as well as the external primer TL-N-3017. Occasionally, one of the primers did not sequence well, with the result that for a few individuals a small portion of the sequence data is unconfirmed. Unconfirmed sequence data were used only when the electropherograms were very clean; unconfirmed sequence data were always corroborated by identical sequences from other individuals in the study. Sequences were deposited in GenBank under accession numbers DQ516539–DQ516678. Previously published sequences from samples from the Philippines have GenBank accession numbers within the range of DQ150731–DQ150988 (Scheffer et al. 2006).

ABI Big Dye Terminator sequencing kits (Applied Biosystems, Foster City, CA) were used for all sequencing reactions with the modification that volume of all reaction components was reduced to 25% of that recommended by the manufacturer. Sequence data were obtained by analyzing samples on an ABI 377 automated DNA sequencer. Contig assembly as well as the final alignment of consensus sequences was accomplished using the program Sequencher (Gene Codes Corp., Ann Arbor, MI).

Maximum parsimony analysis of the final 529-bp data set was performed using the heuristic search feature of PAUP* 4.0b10 (Swofford 2001) with 50 random addition replicates. Identical haplotypes were removed so that each haplotype was only represented once. Uncorrected pairwise distances presented as ranges were calculated using PAUP*. Bootstrapping was performed with 500 pseudoreplications of the data set. Primary outgroups for analysis of this data set were specimens representing the three major mitochondrial lineages of *L. sativae,* the sister species to *L. trifolii* (see Scheffer and Lewis (2005) for explanation of mitochondrial lineages within *L. sativae*). The more distant congener *L. huidobrensis* also was used as an outgroup.

Results

Within *L. trifolii* 16 mitochondrial COI haplotypes were found from 178 individuals from 12 host plants in eight countries (Table 2). Parsimony analysis of the data set consisting of the 16 mitochondrial haplotypes resulted in eight equally parsimonious trees, one of which is shown in Fig. 1. Two major clades, "*trifolii*-A" and "*trifolii*-W," were recovered in all eight of the equally parsimonious trees; the trees varied only in the placement of haplotypes within the major clades dis-

Table 1. Collection information for *L. trifolii* specimens

Location	Date	*n*	Host	Collector(s)
New World				
Honduras, El Zamorano	Nov. 2000	12	Pepper	R. Cave
Mexico, Tampico Valley	Nov. 1999	12	Pepper	H. Mejia
Rio Colorado Valley	April 2001	2	Onion	J. Alling Ppq
Puerto Rico	Oct. 1999	3	Onion	J. Diaz, I. Cabrera, L. Pagen
United States, Arizona, Yuma Exp. Stn.	Sept. 2000	3	Melon (trapped)	J. Palumbo
California, Davis	1999	5	Bean	C. Black, L. Godfrey
California, Davis	April 1995	4	Greenhouse	S. Scheffer
California (northern)	Mar. 1999	11	Trumble pepper colony	D. Morgan, J. Trumble
California (southern)	Mar. 1999	16	Trumble colony	D. Morgan, J. Trumble
Florida, Bradenton	April 2001	1	Bean	S. Scheffer
Florida, Bradenton	April 2001	1	Bidens	S. Scheffer
Florida, Bradenton	April 2001	2	Tomato	S. Scheffer
Florida, Bradenton	April 2001	1	Watermelon	S. Scheffer
Florida, Bradenton	April 2001	2	Swept	S. Scheffer
Florida, Homestead	April 2001	2	Bean	S. Scheffer
Florida, Immokolee	April 2001	12	Pepper	S. Scheffer
Florida, Immokolee	April 2001	2	Swept	S. Scheffer
Florida, Orange Heights	April 2001	1	Potato	S. Scheffer
Florida, Orange Heights	April 2001	1	Zucchini	S. Scheffer
Florida, Epcot Center	Sept. 1995	3	Colony	D. Wietlisbach, F. Petitt
New York, Elbamuck	Sept. 2001	4	Onion	B. Nault
New York, Newark	Sept. 2001	2	Onion	B. Nault
North Carolina	1996	4	Coreopsis	
Old World				
Israel, Gilat Exp. Stn.	June 1999	3	Lettuce (vacuumed)	P. Weintraub
Gilat Exp. Stn.	Feb. 1999	1	Celery (vacuumed)	P. Weintraub
Gilat Exp. Stn.	May 1999	1	Carrot (vacuumed)	P. Weintraub
Italy, northern	April 2000	2	Colony	G. Burgio
Philippines, Abra Prov.	Nov. 2000	1	Tomato	R. Joshi, N. Baucas
Benguet Prov.	July 2000	6	Tomato	R. Joshi, N. Baucas
Benguet Prov.	July 2000	2	Bean	R. Joshi, N. Baucas
Benguet Prov.	Aug. 2000	1	Gobi	R. Joshi, N. Baucas, G. Sacla
Benguet Prov.	Mar. 2001	4	Pea	R. Joshi, N. Baucas, G. Sacla
Ifugao Prov.	Oct. 2000	2	Bean	R. Joshi, N. Baucas, G. Sacla
Ifugao Prov.	Oct. 2000	1	Mustard	R. Joshi, A. Bahatan
Ifugao Prov.	Oct. 2000	3	Pechay	R. Joshi, N. Baucas, G. Sacla
Mountain Prov.	Sept. 2000	4	Tomato	R. Joshi, N. Baucas, G. Sacla
Nueva Ecija Prov.	Oct. 2000	1	Bean	R. Joshi, N. Baucas, G. Sacla
Nueva Ecija Prov.	Oct. 2000	1	Cabbage	R. Joshi, N. Baucas, G. Sacla
Nueva Ecija Prov.	Oct. 2000	2	Carrot	R. Joshi, N. Baucas, G. Sacla
Nueva Vizcaya Prov.	Oct. 2000	5	Bean	R. Joshi, N. Baucas, G. Sacla
Pangasinan Prov.	Oct. 2000	2	Bean	R. Joshi, N. Baucas, G. Sacla
San Jose	Nov. 2000	8	Bean	R. Joshi, N. Baucas
San Jose	Mar. 2000	4	Onion	E. Rajotte
South Africa, Sandveld Region	Nov. 1999	12	Potato	D. Visser
Pretoria	Nov. 1999	2	Colony	D. Visser

cussed below (see Fig. 1 for branches recovered in the strict consensus). These two highly diverged mitochondrial clades had high bootstrap support values of 98 and 100%, respectively (Fig. 1). The uncorrected pairwise distances between these major clades ranged from 4.7 to 5.7%, whereas maximum pairwise distances observed within the clades was 1.7% (within *trifolii*-A, 0.95–1.7%; within *trifolii*-W, 0.19–1.3%).

In addition to the two major clades, all eight equally parsimonious trees found that the clade *trifolii*-W contains three discernible lineages, one of which is found only on pepper plants and corresponds to the reproductively isolated pepper-feeding population first identified in California (Morgan et al. 2000). All 11 of the flies sampled from the Trumble pepper colony came out in this clade as did 36 additional pepper-associated flies from Florida, Mexico, and Honduras. No nonpepper flies came out within the pepper clade; the 16 flies from the nonpepper Trumble colony all came out in the sister clade to the pepper clade, along with 91 other specimens from various nonpepper hosts. The uncorrected pairwise distances between the pepper clade, and its sister clade (both within *trifolii*-W) ranged from 0.38 to 0.95%. Although the divergence between the pepper clade and its nonpepper sister clade is very shallow, the divergence is based on two fixed nucleotide differences in the regions of COI sequenced.

The clade *trifolii*-A is present in Arizona, California, and New York. This group exhibits some mitochondrial variation, and these variants are found together within populations sampled from single fields. The clade *trifolii*-W contains samples from Florida, California, and all invasive Old World populations as well as the pepper-feeding flies from California, Florida, Mexico, and Honduras. The nonpepper-feeding portion of the *trifolii*-W clade was dominated by a single haplotype, T-9, that was present in 97 individuals (55%

Table 2. *L. trifolii* mitochondrial haplotypes from a 529-bp portion of COI (n = 178)

Hap.	Clade	N	Location (host, no. of specimens)
T-1	*Trifolii*-A	1	Arizona (melon, 1)
T-2	*Trifolii*-A	1	New York (onion, 1)
T-3	*Trifolii*-A	8	California (bean, 3); New York (onion, 2); Arizona (melon, 1); Mexico (onion, 2)
T-4	*Trifolii*-A	6	California (bean, 2); Arizona (melon, 1); New York (onion, 3)
T-5	*Trifolii*-W	6	North Carolina (Coreopsis, 4); Florida (potato, 1; zucchini 1)
T-6	Pepper	32	Florida (pepper, 12); Honduras (pepper, 12); Mexico (pepper, 8)
T-7	Pepper	4	Mexico (pepper, 4)
T-8	Pepper	11	California (Trumble pepper colony, 11)
T-9	*Trifolii*-W	97	California (Trumble colony, 14; Davis greenhouse, 4); Florida (tomato, 4; bean, 2; watermelon, 1; Bidens, 1; weed, 1; Epcot colony, 3); South Africa (potato 12; colony 2); Philippines (bean, 19; cabbage, 1; carrots, 2; gobi, 1; mustard, 1; onion, 4; pea, 3; pechay, 3; tomato, 10); Puerto Rico (onion, 3); Italy (colony, 2); Israel (swept, 4)
T-10	*Trifolii*-W	1	Florida (tomato, 1)
T-11	*Trifolii*-W	1	California (Trumble colony, 1)
T-12	*Trifolii*-W	1	Israel (lettuce, 1)
T-13	*Trifolii*-W	1	Florida (bean, 1)
T-14	*Trifolii*-W	1	Florida (swept, 1)
T-15	Pepper	4	California (Trumble pepper colony, 2); Mexico (pepper, 2)
T-16	*Trifolii*-W	3	Philippines (bean, 1; pea, 1; tomato, 1)

of all *L. trifolii* sampled). Worldwide introduced populations were comprised almost entirely of individuals carrying the T-9 haplotype; 69 of 73 (95%) flies from populations outside of the Americas (including an introduced population in Puerto Rico) carried the T-9 haplotype.

Discussion

Molecular phylogenetic analysis of *L. trifolii* indicates that this morphospecies contains two highly diverged mitochondrial clades, which may be indicative of cryptic species. Clades *trifolii*-A and *trifolii*-W are well supported with pairwise distances ranging from 4.7 to 5.7%, distances similar in magnitude to those observed between closely related agromyzid species (e.g., Scheffer and Wiegmann 2000, Scheffer 2000, Scheffer and Lewis 2001). However, whether the highly diverged lineages within *L. trifolii* represent distinct cryptic species cannot be determined at this time with evidence only from a single gene. It is possible that the observed divergences represent mitochondrial polymorphism within interbreeding populations (Avise 2000), possibly because of recent mixture of reproductively compatible but previously disjunct populations. Additionally, because the current sampling of *L. trifolii* is almost exclusively from pest populations in only a portion of the native range, it is possible that what seem to be deep splits may instead be artifacts of limited sampling.

The phylogenetic placement of the *L. trifolii* pepper-feeding flies unambiguously confirms the presence within *L. trifolii* of a genetically distinct pepper-feeding population that is broadly sympatric with other *L. trifolii.* Although feeding on pepper by *L. trifolii* has been reported from various U.S. locations previously (Stegmaier 1966; Chandler and Gilstrap 1987, 1989). it was not until recent work in California by Trumble and colleagues that a pepper-specialized population was recognized (Morgan et al. 2000, Reitz and Trumble 2002). This study extends the known distribution of the pepper-specialized population to include Florida, Mexico, and Honduras. No pepper-lineage flies were found on hosts other than pepper, and the only flies found on pepper were those belonging to the pepper lineage. Although it is probably premature to assume that all *L. trifolii* on pepper belong to the pepper-specialized population, this possibility should be considered when dealing with *L. trifolii.* Similarly, it cannot yet be concluded that all *L. trifolii* from nonpepper hosts will avoid using peppers. However, recent studies in Japan have found that a population of *L. trifolii* that does not normally use mature sweet pepper, *Capsicum annuum* L., as a host plant will avoid ovipositing on normally acceptable kidney bean, *Phaseolus vulgaris* L., leaves that have been treated with a sweet pepper extract (Kashiwagi et al. 2005). Although the mitochondrial haplotypes of the Japanese population are not yet known, it seems probable that this introduced population belongs to a nonpepper lineage, especially given the genetic uniformity of all introduced populations sampled to date (see below).

Although it was found to be consistently monophyletic, the pepper clade is only shallowly diverged from its sister clade, with pairwise distances ranging from 0.38 to 0.95%. Using a mitochondrial clock estimate for insects of 2.3% per million years (Brower 1994), this level of divergence suggests a divergence time of ≈165,000–413,000 yr. However, there is

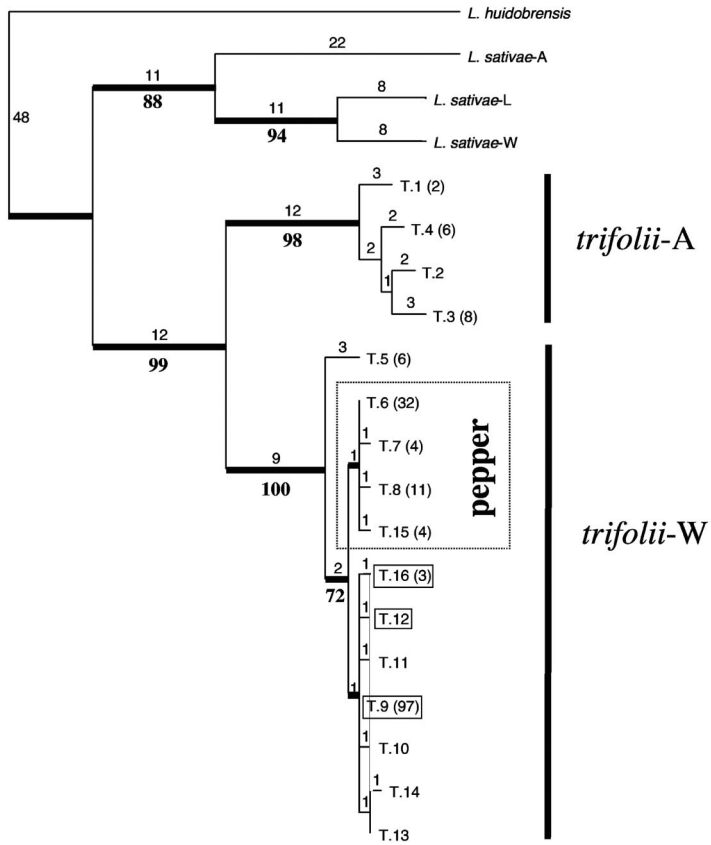

Fig. 1. One of eight equally parsimonious phylograms of relationships among *L. trifolii* haplotypes. Estimated branch lengths are shown above branches, whereas bootstrap values are shown below. Branches in bold indicate those recovered in the strict consensus of the eight equally parsimonious trees. The number of individuals carrying a haplotype (>1) is given in parentheses. Haplotypes present in geographic regions outside of the Americas are enclosed in a rectangular box. Collection locations and host affiliations of haplotypes are listed in Table 2.

likely to be considerable error associated with mitochondrial clock estimates from such shallow divergences, and this estimate should at most be taken as an upper limit of divergence time.

Reitz and Trumble (2002) raise the possibility that pepper-feeding flies may have evolved within the past decade or so in California because of competitive displacement from other hosts by *L. langei* (referred to as "*L. huidobrensis*"). Given the mitochondrial data presented here, this time frame seems unlikely. That pepper-feeding flies form a monophyletic clade containing some variation means that there must have been enough time for both lineage sorting and acquisition of mutations within this population. Even if lineage sorting had been accelerated by a bottleneck during the shift to pepper, it is likely that substantially more than a decade or so would be required for accumulation of intrapopulation mitochondrial variation. Furthermore, the current study found the pepper-feeding clade to be present in Mexico, Florida, and Honduras in addition to California; this broad distribution makes it likely that specialization of this group onto peppers predates recent selective events in California. The observation that both *L. trifolii* (Spencer 1973, Minkenberg 1988) and peppers (*Capsicum* spp.) are native to the New World means that the shift to peppers could have occurred much earlier, even before the development of modern agriculture in the Americas.

The pepper-feeding clade differs from its sister clade within *trifolii*-W by only 0.38–0.95%. That two populations so shallowly diverged exhibit reproductive isolation (Reitz and Trumble 2002) raises several questions regarding the variation observed between the more highly diverged mitochondrial clades within *L. trifolii.* If two clades, such as the pepper portion of *trifolii*-W and its nonpepper sister clade, that differ by <1% exhibit reproductive isolation, what about the major clades (*trifolii*-W and *trifolii*-A) that differ by 4.7–5.7%? Although it is not reasonable to expect that the relationship between reproductive isolation and molecular divergence necessarily will be the same for different populations, we actually know very little about this relationship for most organisms (but see Coyne and Orr 1989, 1997). This relationship seems likely to vary depending on a number of factors, in-

cluding speciation mode and degree of ecological diversification (McCune and Lovejoy 1998, Funk et al. 2006).

It seems that by most criteria, the pepper-feeding clade within *L. trifolii* could be considered a distinct species. This population exhibits reproductive isolation and both behavioral and genetic differences from other *L. trifolii* (Morgan et al. 2000, Reitz and Trumble 2002; this study). The genetic differences are concordant across two molecular marker systems (random amplified polymorphic DNA (RAPD) analysis, Morgan et al. 2000; and mitochondrial COI) and readily distinguish the pepper population. Based on the mitochondrial data reported here, elevating the pepper population to species status would render the remaining *L. trifolii* paraphyletic. A recent survey found that approximately one-quarter of all arthropod species may exhibit mitochondrial paraphyly due several causes (Funk and Omland 2003). In such an example where a specialist population is nested within a polyphagous ancestral (paraphyletic) species, we can speculate that dietary evolution has proceeded from generalism to specialization. This is especially interesting in *L. trifolii*, which is widely held (the nonpepper portion) to have actually increased its host range since becoming a crop pest (Parrella and Keil 1984, Spencer 1990). The subject of the evolution of specialization is central within evolutionary biology (Futuyma and Moreno 1988, Kawecki 1998, Hawthorne and Via 2001), and *L. trifolii* may offer an especially interesting example for further study. Additional investigation of the behavior of *L. trifolii* throughout the Americas in conjunction with expanded study of molecular variation (nuclear as well as mitochondrial) will provide critical information on the biology and systematic position of this group. *L. trifolii* offers a potentially valuable system for further investigation of species limits, speciation, host use evolution, and the relationship between molecular divergence and reproductive isolation.

Despite its agricultural importance, the original geographic range of *L. trifolii* is difficult to determine, primarily because this species is so easily confused with *L. sativae* and other *Liriomyza* leafminers. The best evidence suggests that *L. trifolii* had an early presence in both North and South America (Spencer 1973, Minkenberg 1988). In this study, California is the only sampled location to have both major *L. trifolii* lineages as well as the pepper clade, perhaps suggesting that this species is native to California. However, it has been widely held that *L. trifolii* was introduced to California from Florida in the 1970s (Spencer 1981, Parrella 1982, Parrella and Keil 1984, Zehnder and Trumble 1984, Reitz and Trumble 2002). An attempt to determine whether the *L. trifolii* lineages currently present in California are the result of repeated introductions will require additional sampling of nonpest as well as pest populations throughout California and the rest of the Americas.

It is well documented that populations of *L. trifolii* from locations outside of the Americas are the result of introductions (Spencer 1973, Minkenberg 1988). As expected, introduced populations contain only a fraction of the variation present in New World populations. Indeed, of the 73 flies sampled from introduced populations ranging across the globe, all but four carried haplotype T-9; the introduced populations are not random subsamples drawn from the mitochondrial variation present within the Americas. This suggests that some populations of *L. trifolii* may be more likely than others to be spread to new areas. This could be due to any of several causes. First, spread of only one haplotype may simply be a factor of geography; exports infested with leafminers may come primarily from a single region that contains a limited subset of haplotypes because of natural phylogeographic structuring. Second, and alternatively, infested exports could be coming from one or several regions that themselves harbor introduced populations containing reduced variation because of bottlenecks (e.g., Scheffer and Grissell 2003). And third, populations may differ in "invasiveness" for biological reasons. For example, populations that have evolved insecticide resistance may be more likely than others to reach very high numbers, which may increase the probability of their spread. *L. trifolii* is notorious for its ability to rapidly evolve resistance to new insecticides (Parrella and Keil 1984, Smith 1986, Parrella 1987, Keil and Parrella 1990, Leibee and Capinera 1995, Ferguson 2004).

In conclusion, the mitochondrial data presented here represent a first step in fully understanding the phylogeography and evolutionary history of *L. trifolii.* The present investigation establishes that well-supported, highly distinct mitochondrial lineages exist within the currently recognized morphospecies *L. trifolii*, possibly because of the presence of cryptic species. The pepper-feeding group first documented by Morgan et al. (2000) and Reitz and Trumble 2002) was found to be phylogenetically distinct from, but nested within, the other *L. trifolii.* Only the mitochondrial lineage *trifolii*-W is present in populations sampled outside of the Americas. Additional study of the phylogeography and behavior of this leafmining fly and its sister species, *L. sativae*, will provide further information on invasion processes, host use evolution, species limits, and speciation. This information will be critical for the design of more effective management and quarantine procedures to limit the damage caused by and the continued spread of these destructive flies.

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